CHEMOTAXONOMY OF THE RHOEADALES

by

Monica Peter

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Department of Botany
McGill University
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CHAPTER I

INTRODUCTION

A. An Introductory Note

Taxonomy has been defined as "the study of classification, including its bases, principles, procedures and rules;" (Simpson 1961). An alternative definition (of systematics) is "the scientific study of the kinds and diversity of organisms and of any and all relationships among them." Taxonomy is therefore, one of the oldest fields of biological science; because in order to classify, even at the most elementary levels, man had to identify organisms. This necessitated observing, and making comparisons, integrating specific data and developing generalizations from these. Since this is the case, one may suggest that taxonomy is an outdated science, as almost everything has been named and 'pigeon-holed' already. It must be borne in mind, however, that early scientists were merely concerned with writing descriptions and giving names, while in modern days taxonomists are interested in more than describing and naming species. Now they attempt to establish relationships and affinities with more accuracy.

Any attempt by man to categorize natural variations must by necessity result in differences depending on the approach used in distinguishing various observable criteria. Terms (categories)

like species, genus, tribe, family, order, etc. may be regarded as highly arbitrary. For example, one worker might recognize ten or fifteen genera as a given family, while another might designate a single genus for the same group, and place ten or fifteen species within this major taxon. While both agree on the number of biological entities involved, they differ as to their rank within the larger group. Since the early 1900's taxonomists have been employing new methods to gather data and make their interpretations regarding the limits and degrees of existing relationships. These methods include cytology, serology, genetics, anatomy, embryology, statistics and chemistry.

Chemo-taxonomy has a vital role as a complementary method to taxonomy. Chemistry enlarges the scope of taxonomic research, and many more characters are available for comparison and evaluation through its use. While leaf shape, flower colour and other obvious morphological characters are very valuable, the infinite number of chemical constituents of plants, which may be significant in illustrating relationships, provide an increased basis for finding affinities. These "secrets" of nature can be revealed by the use of various chemical tests, in which, not only the abundance of occurrence, but also the restricted presence or lack of certain constituents become important. For example, the alkaloid protopine is considered an indicator of the family Papaveraceae, since it is rarely found elsewhere.

In this case the restricted occurrence makes the constituent significant in a study of affinity. On the other hand, sucrose occurs commonly throughout the plant kingdom, and is therefore chemo-taxonomically not important. However, if it were discovered that a certain group of plants was entirely sucrose-free, then this character would assume prominence in regard to its relationship to other groups. Such possibilities exist and it was through chemo-taxonomy, that researchers found new clues and followed them up with a new surge of interest. This paper is concerned with some aspects of this type of investigation. The order it is dealing with is well known and of importance far beyond its mere botanical aspects. The order Rhoeadales is composed of families growing all over the world. Members of the Cruciferae (cabbage, turnip, radish, rape, etc.) serve as food for man and animals. Spices (mustard from the Cruciferae, and capers from the Capparidaceae) and narcotics (opium, morphine, etc. from the Papaveraceae) are produced within this order. Besides these useful commodities are some other products which are suspected of being dangerous, for example, the goitregenic substances of the Brassicae, which are of great interest to researchers for their medical implications.

B. The Purpose of this Research

Several years ago, (at least ten) Prof. R. Darnley Gibbs realized that the order Rhoeadales presented an interesting taxonomic problem by virtue of the many and diverse opinions which had been expressed on its classification. He also felt, that having only seven families (according to Engler and Diels) this order could be worked on from many points of view more readily than a larger, less manageable one. With this in mind, he did some preliminary research, and later passed the problem on to a former student, who investigated the cyanogenetic glycosides[1]. The findings of both these workers are incorporated in this paper. In 1961 Dr. Gibbs suggested that I should investigate the family relationships of the same order in the light of a wider array of chemical constituents.

Despite a recent work of Hegnauer[2] on the classification of the order Rhoeadales, it was felt that further research was necessary, as his survey considered only a few chemical characters. However, his paper served as a spring-board for this work, in which the purpose was to investigate the order Rhoeadales

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[1] J.M. Honeyman, (1956) "On the occurrence of cyanogenetic glycosides in the O. Rhoeadales". Taxon Vol. V, No. 2, pp. 33-34.

[2] R. Hegnauer, (1961) Die Gliederung der Rhoeadales sensu Wettstein im Lichte der Inhaltstoffe. Planta Medica 9: 37-46.

using chemical tests with a view to add some research findings about: (a) further chemical characters of the families, (b) affinities and/or absence of affinities between the families generally considered to constitute the order, (c) gaps in the knowledge of these families, which might facilitate any further work done on this group. It was hoped that these investigations would lend support to, or aid in refuting some of the more recent classifications of the order. Hutchinson's "Families of flowering plants", (1959) was used as a general frame of reference for a critical review of orthodox taxonomy as opposed to the older views of say Engler and Diels.

C. Historical Background

Taxonomy was first based on characters identifiable by the human sensorium. The earliest classification grouped plants according to form and size into trees, shrubs, and herbs. In the years between 1686 - 1704 a step forward was made by John Ray, who in "Historia Plantarum", divided plants into two groups: '"Imperfect" - apparently without flowers or seeds and springing up spontaneously (Fungi, Algae and some kind of Mosses)"', and '"More nearly perfect" - with flowers and seeds"'. He further divided the second group on the basis of their seeds[1].

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[1]R. Darnley Gibbs, Botany (Philadelphia: Blakiston Company, 1950), p. 338.

Later, gradually also content matter of plants was considered, chiefly in relation to drug plants. In those days the physician was a botanist, with his indispensible herb garden, and his interest was the grouping of plants having similar "virtues" or medicinal properties. For example[1], in 1699 James Petiver reported that .... "the Herbae Umbelliferae" were generally found to be endowed with a 'Carminative Taste and Smell', powerful expellers of Wind, and therefore good in all flatulent Diseases, and of great use in the chollick, etc. To cite a few for example, as Aniss, Caraway, Cummin, Angelica, Smallage, Parsley, Lovage, etc." Petiver also mentions the Cruciferae as follows:

> "3. We proceed to those herbs which have a Tetrapetalose Regular Flower ... the most Essential Vertue and use of the Herbs of this class I observe are more particularly in the leaves and seeds, and next them the roots, and if any part is slighed it is the Flowers and Podds. The leaves are more particularly used in the Water and Garden Cresses, Sea and Garden Scurvy-grass, Hedge-Mustard, Iberis ... Others of this family that are more peculiarly eminent for the Vertue contained in their seed, are the common Mustard and Rape ... I am certain the effects of many of these herbs ... are by most, if not all Physitians, as well Antient as Mordern, allowed to be extraordinary Diureticks and Antiscorbuticks."

The history of the various classifications of Rhoeadales by eminent taxonomists over the years is rather interesting[2].

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[1]R. Darnley Gibbs, "History of Chemical Taxonomy", in Chemical Plant Taxonomy, Edit. T. Swain, (London. New York: Academic Press, 1963), pp. 42-44.

[2]H. Harms, Die natürlichen Pflanzenfamilien, (Leipzig: Engelmann, 1936) Vol. 17b, pp. 1-4.

According to Linné's sexual system (1738), the largest group of the Cruciferae was the 15th Class Tetradymannia, which was divided into Siliculose and Siliquose; also Cleome was considered to belong to them. In the draft of his "natural systems", Papaveraceae were in Ordo XXX, Capparidaceae in the next one, Hypecoum and Fumaria in Ordo XXVIII; the Cruciferae were far removed in Ordo LVII.

Earlier systematists had already some recognition of the "natural" system: Caesalpinus (1583) included Cruciferae in his group VII: Herbaceae bin's conceptualis. Morison (1680) considered the main relatives of his Siliquosae to be the Cruciferae and some of the typical Papaveraceae. Joh. Rajus (1682) and H. Boerhaave (1710 - 1720) had somewhat similar opinions. Their group Tetrapetalae, Cruciformes, Siliculosae, et Siliquosae comprised Cruciferae, Papaver and Cleome (as Sinapistrum). Tournefort (1694) related Cruciferae under the name Cruciformes, together with Hypecoum, Chelidonium and Cleome; he made some quite unique deviations too. A.L. de Jussieu (1789) places Papaveraceae after Ranunculaceae followed by Cruciferae, Capparides and Sapindales. Resedales he grouped under "Genera Capparidibus affinia" whereas Marcgravia, Norantea, Drosera, and Parnassia, were placed in far removed areas of the system. Moringa he included in Leguminosae. Bartling's (1830) Rhoeadeae comprised Tremandreae, Polygaleae, Fumariaceae, Papaveraceae, Cruciferae, and Capparidaceae. Endlicher's (1839) Rhoeadales

consists of Papaveraceae, Cruciferae, Resedaceae, Datiscaceae; the last one now belonging to the Parietales. Moringeae are still under Leguminosae. Lindley (1836) combined Alliance I, Crucíales of the Parietosae, the Cruciferae or Brassicaceae, Capparidaceae and Resedaceae; on the other hand, Papaveraceae with its suborder Fumarieae, are assigned to Ranales and Moringaceae to Violales. In a later work (1853), he separated Fumariaceae and included them in Berberales. This list of early systematists could be prolonged and great variances shown. Some of the more controversial ones were A. Brongniart (1850), Bentham and Hooker (1862), Baillon (1872), A.W. Eichler (1878), A. Kerner etc., until via the "Konigsberger Stammbaum", Wettstein in his handbook (1924) concludes about Rhoeadales (Papaveraceae, Tovariaceae, Capparidaceae, Cruciferae, Resedaceae and Moringaceae) that ....

> "the relationship of the families comprising this order is without any doubt; as was recently proven serodiagnostically in clear reactions by Alfred Preuss and morphologically by thorough investigations of Murbeck."

Also in 1924, Engler and Gilg classified Rhoeadales. Their system was as follows:

Suborder 1. Rhoeadineae. Flowers heterochlamydic; mostly only two petals. Papaveraceae.

Suborder 2. Capparidineae. Flowers heterochlamydic; four or more petals. Capparidaceae, Tovariaceae, Cruciferae.

Suborder 3. Resédineae. Flowers spirocyclic, heterochlamydic. Resedaceae.

Suborder 4. Moringineae. Flowers cyclic, heterochlamydic, zygomorphic. Moringaceae.

Suborder 5. Bretschneiderineae. Flowers slightly zygomorphic, heterochlamydic. Bretschneideraceae.

The same order is followed in the second edition of Engler and Prantl's "Die Natürlichen Pflanzenfamilien" (1936).

Engler recognized that morphological criteria alone are insufficient, and studies in floral anatomy (Saunders, Eames, Wilson, Dicksen) in the early 30's called for newer and more differentiated criteria for taxonomical considerations. Engler was not alone in this feeling, because around this time other taxonomists began to realize that "the connection between the natural relationships of plants and their chemical composition" could be significant. These were the beginnings of "comparative phytochemistry". Between 1917 and 1945 McNair[1] published several papers in which he attempted (unsuccessfully) to apply comparative chemistry generally, to taxonomy. Since that time, the concept has gained ground, and increasing research is being done to solve problems of disputed relationships using this method.

Consider the order Tubiflorae, for example. According to the Engler and Diels classification (1936, 11th edition) the Tubiflorae (6th order of the Sympetalae) is an order of twenty-

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[1] Gibbs, op. cit. p. 47

two families, including more than 1000 genera and about 20,000 species. In this order are such well known families as the Solanaceae, Labiatae, Convolvulaceae, etc. One would think that relationships of such economically important families would have been fully established long ago. However, this is not the case. Hutchinson (1959) splits some of Engler and Diels families, resulting with twenty-eight families instead of twenty-two; and these he distributes in four major groups as end lines of evolution[1]. If Hutchinson is right, there should be considerable differences between the groups; but on the other hand, if Engler and Diels were right, these families should be a homogeneous group, and therefore of similar chemical characteristics. Dr. Gibbs[2] worked on this order using comparative chemistry, and concluded that the group was homogeneous, more in agreement with Engler and Diels.

Another disputed order is the Geraniales, in which Engler and Diels put twenty-one families. Hutchinson[3] splits them up into woody and herbaceous lines; and instead of twenty-one, his order Geraniales[4] has only three families, developing from

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[1]R. Darnley Gibbs, "Comparative Chemistry of Plants as applied to a Problem of Systematics: The Tubiflorae," Trans. Royal Society of Canada, Vol. LVI : Series III : June, 1962. Sect. III. pp. 144, 145.

[2]Ibid. p. 158.

[3]J. Hutchinson, The Families of Flowering Plants, (Oxford: 2nd ed., Vol. I, 1959) p. 120.

[4]Ibid. pp. 108, 117.

the herbaceous Ranales. The other families are distributed along branches from the woody Magnoliales. Currently research is underway at McGill, investigating the chemotaxonomy of this order.

A third example of controversy is to be found in the order Rhoeadales, with which this paper is concerned. Engler and Diels (1936) place seven families in this order: Cruciferae, Capparidaceae, Resedaceae, Papaveraceae, Moringaceae, Bretschneideraceae and Tovariaceae. Hutchinson[1] (1959) considers these families as being representatives of two distinct lines of development.

In his 1959 edition of "Families of flowering plants", he made a significant rearrangement of the order Rhoeadales (sensu Wettstein 1935). In the scheme of Wettstein and many other taxonomists, the Rhoeadales (= Brassicales according to Pulle) is a natural unit deriving directly from the Polycarpicae. In this order they place the families Papaveraceae, Capparidaceae, Cruciferae, Moringaceae, Tovariaceae. In 1956 Moritz and Rohn[2], using improved serological methods, showed that the families

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[1] Ibid. pp. 108, 117.

[2] O. Moritz and H.L. Rohn, "Untersuchungen zum Problem der serologischen Fernreaktionen", Planta, 47: (1956) pp. 16-46.

combined as Rhoeadales form a natural group. Hutchinson on the other hand, believes that in the old Rhoeadales there are representatives of two entirely different lines of development (herbaceous and woody). Accordingly, he has made a new classification of the order. Capparidaceae, Tovariaceae, and Moringaceae form for him the order Capparidales; which derives from the Pittosporales. The remaining families he includes in three monofamilial orders, deriving from the Ranales. (See Fig. 1).

Figure 1. The position of Families of Rhoeadales (according to Wettstein), in the System of Hutchinson (1959).

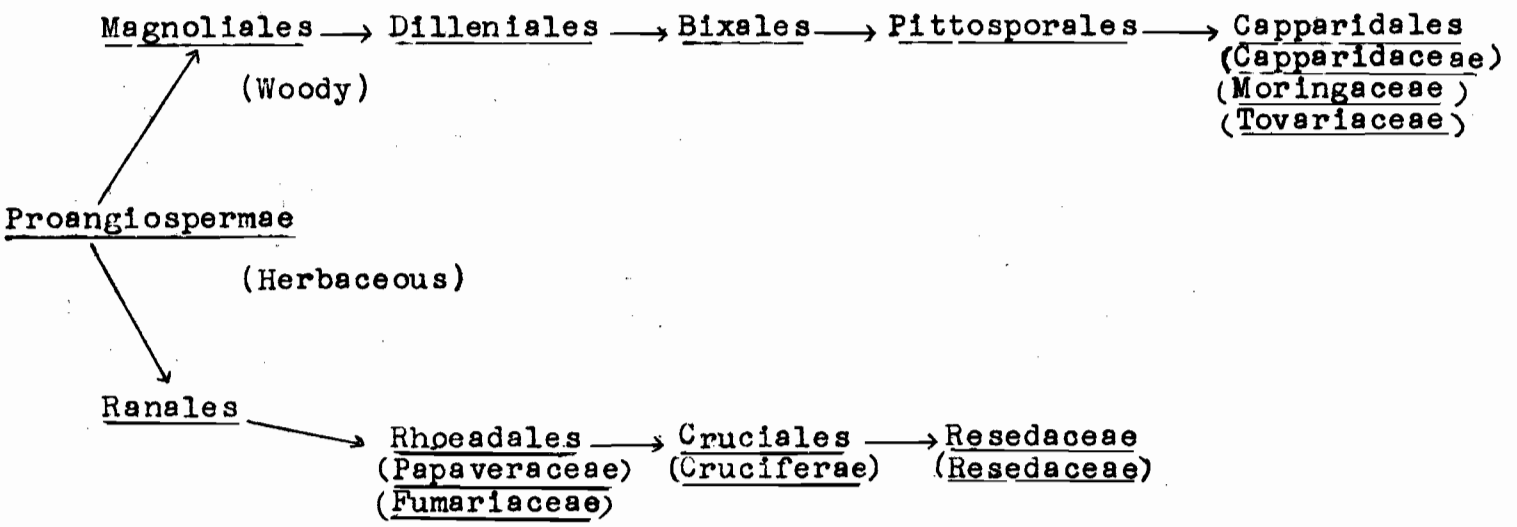
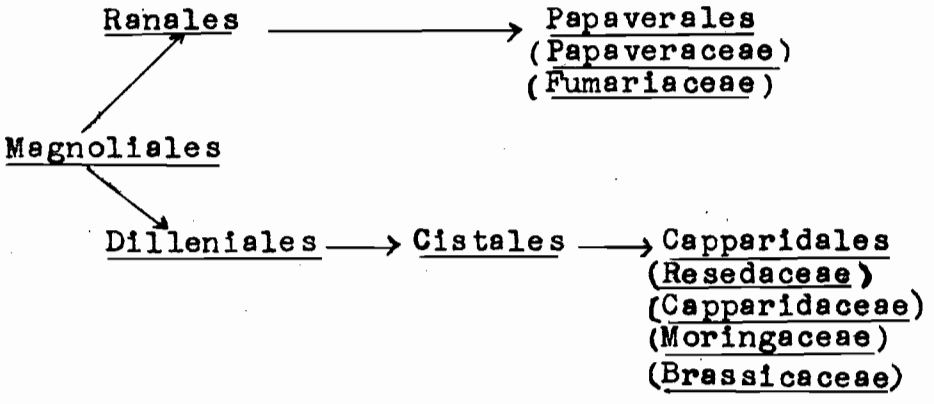


Figure 2. The Position of the Families of the Rhoeadales (according to Wettstein), in the System of Takhtajan (1959).



Also in 1959, Takhtajan made a new classification of the Rhoeadales. He split off Papaveraceae, and included them in the order Papaverales (as Papaveraceae s. stricto + Fumariaceae). The remaining families he combined in the order Capparidales; deriving from the Cistales (possibly Flacourtiaceae).

Hallier had made a similar limitation of the Rhoeadales in 1912. He too included the Papaveraceae in his Ranales. The Moringaceae he placed in the Caesalpinioideae of the Leguminosae; and in an order Cruciales, deriving directly from the Ranales, he has Cruciferae, Resedaceae, and Capparidaceae (including Koeberlinia and Tovaria). In Table I. these views and some others are shown.

Table I. The Families which have composed the Order Rhoeadales, in some classifications.

Families \ Taxonomists	Engler & Diels (1936) Pulle (1950) Skottsberg (1940, 1955)	Cronquist (1957) Pulle (1952)	Bessey[1] (1915) Soó (1933) Wettstein[3] (1935)	Gundersen (1950)	Rendle (1938)	Hutchinson (1948, 1959) Boivin (1956)
Papaveraceae*	+	+	+	+	+	+
Fumariaceae		+				+
Capparidaceae	+	+	+	+	+	
Cruciferae	+	+	+	+	+	
Tovariaceae	+	+	+	+		
Resedaceae	+	+	+	+		
Moringaceae	+	+	+			
Bretschneideraceae	+					

* Papaveraceae includes Papaveroideae, Fumarioideae. and Hypecoideae.

[1]Bessey also includes in his order the Nymphaeaceae (excluding Nelumbonoideae and Cabomboideae).

[2]Soó includes Bretschneideraceae in the family Moringaceae.

[3]Wettstein's opinion on classification is shared by Hegnauer (1961).

D. The Problem

The preceeding paragraphs of the background serve to illustrate the widely divergent views on classification of the order Rhoeadales which exist. In this research project, efforts were directed to answering some of the questions which arose from these disputes. The problem was considered as follows:

Does chemical evidence indicate the families of the order Rhoeadales (according to Engler and Diels,1936) to be an homogeneous group, or does it rather support splitting off some members into other orders?

Are the chemical characters of the Capparidaceae, Moringaceae and Tovariaceae distinct enough to warrant the hypothesis that they stem from a line of development different from the Resedaceae, Cruciferae and Papaveraceae?

Are the characters of the Papaveroideae and Fumarioideae sufficiently distinct to merit their being classified as separate families?

In pursueing the previous questions we were led to consider aspects that only accentuated the complexity of our problem. For instance, similar characters may be used to support different arguments depending on the frame of reference used. Can conclusions based on chemical characters, therefore, be considered valid enough to justify changing hitherto acceptable and workable taxonomic schools of thought?

CHAPTER II

LITERATURE SURVEY

A. Comparative Phytochemistry

"We are impressed, each time we look into the history of a particular topic, with the difficulty of discovering the real beginnings." This statement by Gibbs[1] in his "History of chemical taxonomy" was made with reference to phytochemistry, and is a very apt one. Alston and Turner[2] report that as early as 1909 Greshoff used the term "comparative phytochemistry" and defined it as: "the knowledge of the connection between the natural relationship of plants and their chemical composition". Greshoff further suggested, that a short chemical description should be part of the "formal description" of a new genus or species. Such a chemical description Alston and Turner would like to be called the "biochemical profile" of the plant. At present such a step does not seem imminent, and this may be due to the fact that communication between various branches of science (especially chemistry and botany) is still minimal. Chemists may be interested to isolate and identify specific compounds produced by plants, but

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[1]R. Darnley Gibbs, "History of Chemical Taxonomy", in Chemical Plant Taxonomy, Edit. T. Swain, (London, New York: Academic Press),1963, p. 41.

[2]Ralph E. Alston and B.L. Turner, Biochemical Systematics (N.J.: Prentice-Hall, Inc.,)1963, pp. 46-47.

it is unlikely that they would undertake a survey of the complement of constituents present, which would be of more concern to the phytochemist. Robinson[1] makes the keen and relevant observation, that although chemistry is a very essential tool in biological sciences, the approach must of necessity be different since "the kinds of things he (the biologist) needs to know are not necessarily the same things which a chemist needs to know." Robinson further points out, and his viewpoint is shared by Hegnauer[2] and others, that the literature on plant chemistry extends across several special fields, each with a different view. However, in spite of these difficulties, phytochemistry is increasingly being employed.

E.C. Bate-Smith[3] has reviewed the subject recently, and feels that not only are the chemical constituents of plants important, but also their variations and the processes by which these might arise. He believes that two kinds of research are needed:

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[1]Trevor Robinson, The Organic Constituents of higher plants, (Minn.: Burgess Pub. Co.), 1963, preface, i.

[2]R. Hegnauer, ChemoTaxonomie der Pflanzen, (Birkhaüser, Basel und Stuttgart), Vol. 1, 1962, preface pp5-7.

[3]E.C. Bate-Smith, "Plant Biochemistry", in Vistas in Botany, Edit. W.B. Turril, (Pergamon Press), 1959, pp. 100-122.

1. Extensive survey, employing indicative reactions; and

2. intensive research, detailed studies of restricted groups of plants with reference to specific, identified constituents. In the area of extensive survey, he states that Gibbs has made "the most determined attempt to date to bring chemical considerations to bear upon the Taxonomic situation."

Over the years Gibbs has published several papers on the role of comparative chemistry in systematics and he emphasises the point that chemistry is invaluable "to supplement" but not "to replace" morphological characters.[1] Another worker who gives attention to survey of many characters is Hegnauer[2]. When in 1962 the first volume of his Chemotaxonomy of Plants[3] appeared, it covered the lower plants and Gymnosperms. Hegnauer felt that chemical compounds may be of more value to the systematists after their biosynthesis has been clarified. On the other hand, Plouvier[4] expresses the opinion that "la présence ou l'absence d'un composé determiné constitue un caractère chimique simple et précis, pouvant servir à distinguer des groupes."

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[1]R. Darnley Gibbs, "Comparative chemistry of plants as applied to a Problem of Systematics: The Tubiflorae." Trans. Royal Society of Canada, Vol. LVl : Series lll : June, 1962. Sect. lll. p. 143.

[2]R. Hegnauer, "Chemotaxonomic matters 11: Phytochemical indications of the position of the Aristolochiaceae in the system of Dicots." Pharmazie 15 (11): (1960) pp. 634-642.

[3]R. Hegnauer, op. cit.

[4]Victor Plouvier, "Le caractère chimique en taxonomie végétale". Rev. Gen. Sci. Pures Appl. Bull. Associ. Franc. Avan. Sci. 69, (1962) pp. 331-46.

These few examples should suffice to show that the approaches and methods of phyto-chemistry are quite varied, although the aims are the same; i.e. to obtain more reliable bases for plant classification.

B. Chemotaxonomic Methods

Advances have been made in chemotaxonomy, due to the improvement of old techniques along with the development of new ones. For example in Serology, Alston and Turner[1] give a good summary of the historical developments in this technique. They recall the early work of Nuttall (1901). Alston and Turner also summarize a series of papers by Chester (1937) on the controversy between the Berlin and Königsberg schools of thought on Serology. Interest in this method waned until the work of Moritz and Rohn[2] (1956). Their work is especially significant for this paper, as they applied serological techniques to the order Rhoeadales, and on that basis pronounced the order to be "a natural group". In a later paper Moritz and Frohne criticized

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[1] R.E. Alston and B. L. Turner, op. cit. pp. 68-90.

[2] O. Moritz and H.L. Rohn, "Untersuchungen zum Problem der Serologischen Fernreaktionen." Planta 47: (1956) pp. 16-46.

the primary interest of taxonomists in the quantitative statement of serological method. They suggested that the qualitative approach was essential. We translate from their paper as follows: "No systematist would assume that three kinds of plants of which A would yield a result of 100 grams, B of 50 grams and C of 25 grams of apocarp fruits of pod character, that B would for this reason be closer related to A than to C. He would, however, include A,B and C as pod-bearing fruits." Therefore, they argue, it is equally false to apply only quantitative results from serology to answering questions in biological taxonomy[1]. Of even greater interest is the most recent paper by Frohne[2] (1962) where he discusses the relation of comparative serobotany to comparative phytochemistry, exhibited by serological investigations in Rhoeadales.

A most widely used method in phytochemistry, is Chromatography. A good summary of the history of this technique has been made by Gibbs[3]. The role of paper chromatography in taxonomy has been discussed by Hagen[4], who is optimistic of the

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[1]O. Moritz and D. Frohne, "Form and Basis of quantitative statements in serological taxonomy", Flora, Vol. 146, (1958) pp. 442-443.

[2]D. Frohne, "Relation of comparative serobotany to comparative phytochemistry, exhibited by serological investigations in Rhoeadales". Planta Medica, 10. (1962), pp. 283-97.

[3]R. Darnley Gibbs, "History of Chemical Taxonomy", in Chemical Plant Taxonomy, edit. T. Swain, (Lond., N.Y.: Academic Press, 1963),pp 69-70.

[4]C.W. Hagen, Jr., "The role of paper chromatography in Taxonomy", Proc. Indiana Acad. Sci., 70: (1960), p. 207.

importance of this technique to the taxonomist, since far more attributes can be determined from a single specimen using this method, than can be conveniently assessed by any other. He does caution, however, that serious errors may be made if rigid evaluation of the attributes revealed is not made. This pitfall however, is ever present for any method employed.

Bate-Smith[1] has attempted to solve a taxonomic problem in the Rosaceae by using chromatography, while Kjaer[2] and his co-workers continue to investigate the sulphur compounds by this method. Other types of chromatography (besides paper) are also employed by some researchers.

Increasing need for quick detection of various chemical constituents (especially in the field) has led to the development of methods requiring little equipment and time. Such a technique has been applied to alkaloid determination by Kraft[3]. He removes a few drops of plant juice from the leaf by means of a special pressure plier, and these are tested immediately with Dragendorff's reagent (Potassium bismuth iodide) paper. The breath and depth of colouration as compared with standards,

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[1] E.C. Bate-Smith, "Chromatography and taxonomy in the Rosaceae, with special reference to Potentilla and Prunus." J. Linn. Soc. (Bot.) 58, 370, (Nov. 1958) p. 39.

[2] A. Kjaer and H. Thomsen, " Isothiocyanates. XLIV. The isothiocyanate glucoside (glucocapparin) in Crataeva roxburghii," Acta Chem. Scand. 16, (1962), pp. 783-784.

[3] D. Kraft, "A simple field method for alkaloid determination", Pharmazie, 8 (2) : (1953), pp. 170-173.

gives quantitative information on the alkaloid strength of the plant. He applied this method to Papaver, Datura, Nicotiana, Conium, Atropa, and other species. There is also an added advantage, in that the juice obtained by this method may be studied later by paper chromatography. Kraft recommends this method for other components (e.g. Vitamins, Tannins, etc.,) using different reagent papers. Results for species of Rhoeadales, tested for tannic acid by a similar method will be reported later in this paper. Other simple methods such as the ones carried out during this research will be discussed later too.

C. The Order Rhoeadales

The families included in the order Rhoeadales (Engler and Diels) have been placed in several different arrangements and grouped under various names over the years. Table 1A. shows the families and orders as included by some taxonomists. This table does not show all the other families which some of the systematists include, but rather gives the more common ones and the names of the orders under which they have been grouped. Another question in dispute has been the derivation of the group and the affinities of the families. A few examples follow which should illustrate these points.

Pulle (1952) included all the seven families of the Englerian Rhoeadales in an order Brassicales, deriving from the Clusiales and adjacent to the Batidales. While Nakai (1943) includes in his order Brassicales only Resedaceae, Capparidaceae, and Brassicaceae. The Papaveraceae, Fumariaceae, Hypecoaceae and Pteridophyllaceae form his Papaverales. On the other hand, Benson (1957) calls the order Rhoeadales of Engler and Diels the Papaverales. Bentham and Hooker's Parietales are essentially the Rhoeadales, but they include also, the Sarraceniaceae, Cistineae, Canellaceae, Bixineae and Violarieae. Caruel (1881) and Lindley (1833) had also placed the Bixaceae, Cistaceae, Frankeniaceae and Sauvagesiae in a relationship with the Resedaceae and other members of the Rhoeadales. The Datiscaceae have

Table 1A

Families	Taxonomists	Orders
Capparidaceae +	Reichenbach (1827)	Amphispermae
Moringaceae +	Pulle (1952)	Brassicales
Resedaceae +	Nakai (1943)	Brassicales
Tovariaceae +	Boivin (1956)	Capparidales
Cruciferae +	Takhtajan (1954)	Capparidales
or	Hutchinson (1959)	Capparidales
Brassicaceae + +	Davy J. Burtt(1937)[1]	Capparidales
Papaveraceae + + *	Hallier (1912)	Cruciales
Fumariaceae + + *	Hutchinson (1959)	Cruciales
Hypecoaceae	Lindley (1836)	Cruciales
Bretschneideraceae +	Drude (1886)	Cruciferae
Violaceae	Drude (1887)	Cruciferae
Tetradynamae	Dumortier (1829)	Cruciferarieae
Erythrospermaceae +	Boivin (1956)	Cruciferales
Pteridophyllaceae	Caruel (1881)	Cruciflorae
Asterocarpaceae	Klotzsch & Garcke(1862)	Cruciflorae
	Reichenbach (1828)	Cruciflorae
	Gundersen (1950)	Papaverales
	Van Tieghem & Constantin (1950)	Papaverales
	Benson (1957)	Papaverales
	Takhtajan (1954)	Papaverales
	Nakai (1943)	Papaverales
	Dumortier (1829)	Papaverarieae
	Klotzsch & Garcke (1862)	Papaveriflorae
	Lindley (1833)	Parietales
	Bentham & Hooker (1862)[3]	Parietales
	Hallier (1912)[4]	Ranales
	Lindley (1833)	Ranales
	Caruel (1881)	Raniflorae
	Hutchinson (1948)	Resedales
	Boivin (1956)	Resedales
	Kerner (1891)	Resedales
	Dumortier (1829)	Resedarieae
	Copeland (1957)	Rhoeadeae
	Endlicher (1836)	Rhoeades
	Grisebach (1854)	Rhoeades
	Martius '1835)	Siliquosae
	Lindley (1836)	Violales
	Horaninoro (1843)[6]	Violastra

Table 1A. Some alternative arrangements of the Rhoeadales (Engler and Diels) as made by other taxonomists.

* Papaveraceae including Fumariaceae.

[1] Cruciferae is not treated as a family. Burtt includes Cruciales in Capparidales.

[2] Capparidaceae includes Koeberlinia and Tovaria.

[3] Bentham, Hooker & Lindley include other families in their Parietales

[4] Hallier places Moringa and Bretschneidera in Leguminosae of the order Aesculinae but includes several other families in the Ranales.

been mentioned in this connection too. Other taxonomists have considered the group to have affinities with the Violales. For example, Horaninow (1843) included in an order Violastra, the families Capparidaceae, Moringaceae, Resedaceae, Cruciferae, Papaveraceae and Violaceae, as well as several other families.

Bessey[1] (1915) and Boivin[2] (1956) and others believed that the Rhoeadales were derived from the Ranales. Pulle thought them to be derived from a line between the Ranales and the Hamamelidales. Hutchinson believed that the Papaveraceae and Fumariaceae derived from the Ranales but the other families (Capparidales) he thought came from the Pittosporales. Takhtajan considered the Capparidales deriving from the Cistales. Wettstein considered the Rhoeadales to be derived from the Polycarpicae and Hegnauer[3] supports this point of view. However, Hegnauer also discussed various other possibilities, such as connections with the Centrospermae and Passiflorales. An interesting opinion was expressed by Norris[4] (1941). He made an anatomical study of the

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[1] C.E. Bessey, "The Phylogenetic Taxonomy of Flowering Plants" Ann. Missouri Bot. Gard. 2: (1915), pp. 106-164.

[2] B. Boivin, "Les familles de tracheophytes, "Bull. Soc. Bot. France, 103: (1956), pp. 490-505.

[3] R. Hegnauer, "Die Gliederung der Rhoeadales sensu Wettstein im Lichte der Inhaltstoffe". Planta Medica 9: (1961), pp. 37-46.

[4] T. Norris, "Torus anatomy and nectary characteristics are phylogenetic criteria in the Rhoeadales." Amer. Jour. Bot. 28: (1941), pp. 101-113.

nectaries of these families, and on this basis concluded that "in general, it appears that the Resedaceae and the Capparidaceae are the most primitive families of the existing Rhoeadales" .... and concluded that "the Papaveraceae, the Fumariaceae and the Cruciferae, by subsequent parallel evolution derived from a common ancestral group, somewhat resembling the existing Resedaceae and Capparidaceae ..." He continued ... "Considering the absence of nectaries in the tori of the Papaveraceae, it seems clear that this family cannot be regarded as having given rise to any other family of the Rhoeadales."

Another aspect of study has been whether the Rhoeadales is a natural group or not. Moritz and Rohn (1956) decided on the basis of serological studies that the Rhoeadales were a natural group. Copeland[1] (1957) reported an order Rhoeadeae (9) composed of the families Papaveraceae, Tovariaceae, Fumariaceae, Capparidaceae, Cruciferae, Moringaceae and Violaceae which was derived from the Multisiliquae. Order 10 in this classification was the Centrospermae, but Copeland expressed doubts about these placements.

As pointed out earlier in this paper, Hutchinson and Takhtajan using morphological criteria disagree with the idea of a natural order, and favour instead a splitting of the group into the Papaverales and Capparidales. In this context a recent study by

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[1] H.F. Copeland, "Forecast of a system of the dicotyledons," Madrõno, 14: (1957), pp. 1-9.

Frohne[1] (1962) deserves special attention. He demonstrated in the order Rhoeadales, the relationship between serobotany and comparative phytochemistry, and his results indicated that a separation of the order into Papaverales (Papaveraceae s. str. and Fumariaceae) and Capparidales (Cruciferae, Capparidaceae, Resedaceae, and perhaps also Moringaceae and Tovariaceae) was desirable. He found the Papaveraceae and Fumariaceae closely related serologically, and also that a clear relationship of the Papaverales to the Ranunculaceae existed. With this he seemed to support Hegnauer's[2] findings in his investigation of the biochemical characters of the order.

D. The Taxonomic Significance of Some Constituents

Alkaloids

Substances too narrowly restricted in their distribution, or those too generally present can be of little use as taxonomic indices. As yet the information about the classification of biochemical substances and processes is limited and not too well established. However, for some time certain substances have been used for chemical research with only marginal consideration of their taxonomical significance. The alkaloids fall into this category. For years they have been of economic importance, and as such much was done on their chemistry.

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[1] D. Frohne, "Das Verhaltnis von vergleichender Serobotanik zu vergleichender Phytochemie, dargestellt an serologischen Untersuchungen im Bereiche der "Rhoeadales", Planta Medica, Vol. X, No. 3, (Sept. 1962), pp. 283-297.

[2] R. Hegnauer, Op. Cit.

Recently (1963) Hegnauer[1] reviewed the taxonomic significance of alkaloids, and he stated that alkaloid chemistry began about 140 years ago with Serturner, who recognized morphine as the effective principle in opium. Since 1950, Manske and Holmes[2] have contributed greatly to the information on alkaloids. Willaman and Schubert[3] have done much on the taxonomic distribution of these compounds; and earlier (1935) McNair[4] made valuable contributions in the field. Recent publications on alkaloid chemistry have been made by Boit[5].

Hegnauer[6] defines alkaloids in the context of chemical plant taxonomy as "more or less toxic substances which act primarily on the central nervous system. They have a basic character, contain heterocyclic nitrogen, and are synthesized in plants from amino acids or their immediate derivatives. In most cases they are of limited distribution in the plant kingdom." He considers that since they are present in about

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[1]R. Hegnauer, "The taxonomic significance of alkaloids", in Chemical Plant Taxonomy, Edit. T. Swain, (Lond., N.Y. : Academic Press) 1963, chap. 14.

[2]R.H.F. Manske and H.L. Holmes, (Edits.) The Alkaloids, (N.Y. : Academic Press) Vols. 1-VI, 1950-1960.

[3]J.J. Willaman and B.G. Schubert, "Alkaloid-bearing plants and their contained alkaloids", Agric. Res. Ser. U.S.D.A. Tech. Bull. No. 1234, (Washington), 1961.

[4]J. B. McNair, "Taxonomic and climatic distribution of alkaloids", Bull. Torrey Club, 62: (1935), pp. 219-226.

[5]R. Hegnauer, op. cit.

[6]R. Hegnauer, ibid.

1/6 of vascular plants, they are most useful chemotaxonomically if their biosynthesis is studied. He noted that Hakim et al (1961) showed that coptisine and sanguinarine are equally well distributed as protopine in the Papaveraceae, and felt that the alkaloids are strong evidence for removing the Papaveraceae from the Rhoeadales, to the Polycarpicae, with Nymphaeaceae and Berberidaceae as close relatives. However, Hegnauer does point out the very important fact, that although alkaloids have an important role, more information is required about their chemistry, biogenesis and distribution.

It may be significant that certain workers have found changes in the alkaloid content of plants under varying conditions. Heydenreich and Pfeifer[1] report diurnal variations in the production of alkaloids. Tests made four times a day showed that morphine was produced in the roots especially at night. Aksanowski, Jurzysta et al[2] found that alkaloids in Papaver somniferum vary during vegetation and also throughout the plant. Ignorance of such variations could account for significant discrepancies in analyses for alkaloids made by different workers.

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[1]K. Heydenreich and S. Pfeifer, "Alkaloid metabolism in Papaver somniferum V. Changes in alkaloid content dependent on time of day." Sci. Pharm. 30. (1962), pp. 164-73.

[2]R. Aksanowski, M. Jurzysta, et al. "Alkaloids of Papaver somniferum during vegetation", Dissertationes Pharm. 14, (1962), pp. 47-58.

Henry[1] (1949) refers to alkaloids of Papaveraceae as the "Opium alkaloids", and includes them under the heading of "Isoquinoline group of alkaloids". A similar arrangement is made by Alston and Turner[2] also. These alkaloids are derivatives of isoquinoline and are grouped as follows:

1. Tetrahydroisoquinoline derivatives.
2. Benzylisoquinoline derivatives.
3. Cryptopine type
4. Morphine type
5. Alkaloids of unknown constitution.
6. Synthetic isomerides of Laudanine.
7. Phthalide isoquinoline derivatives.
8. α-Naphthaphenanthridine derivatives.
9. Tetrahydroprotoberberine derivatives.
10. Aporphine type.
11. Minor Corydalis alkaloids. (Not assigned to chemical groups). (Fig. 3 shows some basic formulae of these alkaloids).

A list compiled from various sources, but chiefly from

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[1]T.A. Henry, The Plant Alkaloids, 4th ed. (Phil., Tor.: Blakiston Co.), 1949, p. 178.

[2]R.E. Alston and B.L. Turner, Biochemical systematics, (N.J.: Prentice-Hall, Inc.), 1963, pp. 158-160.

Manske and Holmes[1] and Willaman and Schubert[2] shows the distribution of the groups of alkaloids in the Papaveroideae and Fumarioideae. (See Appendix Table 5.)

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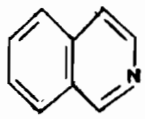
[1] R.H.F. Manske and H.L. Holmes, ibid.

[2] J.J. Willaman and B.G. Schubert, ibid.

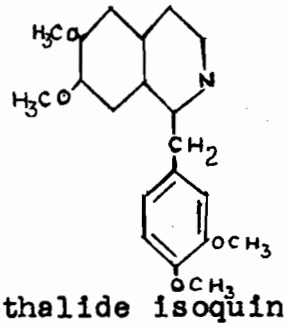
Fig. 3

Some basic structural formulae of alkaloid groups in Papaveraceae (including Fumarioideae and Hypecoideae).

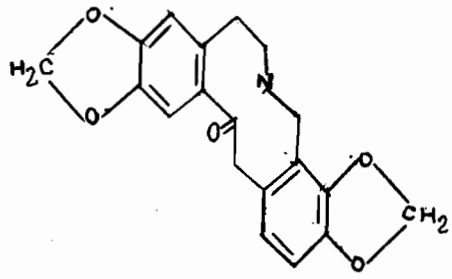
(1) Isoquinoline



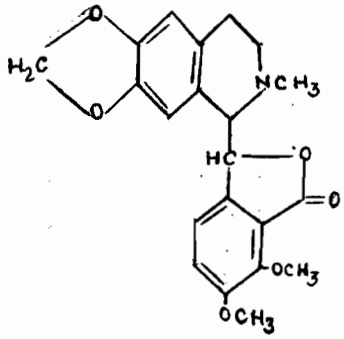
(2) Benzylisoquinoline, e.g. Papaverine



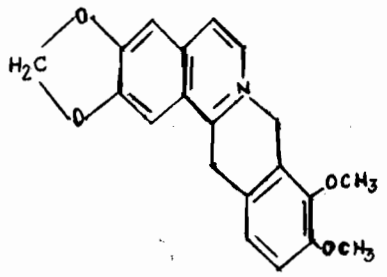
(3) Cryptopine type, e.g. Protopine



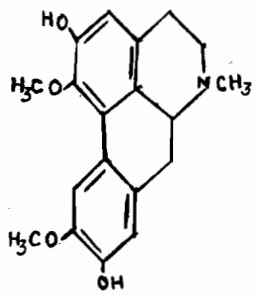
Phthalide isoquinoline derivs. e.g. Hydrastine



Berberine type. e.g. Berberine



Aporphine type e.g. Boldine



When the alkaloids contained in these families are considered as chemical groups, some interesting variations are revealed (see Table II). There are five groups of alkaloids restricted to the Papaveroideae (including Hypecoideae). These are, the benzylisoquinoline derivatives occurring in Papaver somniferum and a variety; the morphine type, also found mostly in Papaver spp.; alkaloids of unknown constitution, of which the chief ones are rhoeadine and rhoeaginine, are spread mainly among Papaver spp.; and lastly, the α-Naphthaphenanthridine serivatives, spread in Papaveroideae, but with a notable exception: Two of these alkaloids occur in Dicentra spectabilis of Fumarioideae.

In the Fumarioideae there are three exclusive groups. The Phthalideisoquinoline derivatives, spread throughout this sub-family, (only narcotine occurs in Papaver paeoniflorum and P. rhoeas). The tetrahydroprotoberberine sub-group is predominantly Fumariacious, except for berberine which is spread in the Papaveroideae as well; and dehydrothalictrifoline found in Glaucium flavum and G. serpieri. Finally, the minor Corydalis alkaloids are restricted to the genus Corydalis, the only exception noted being roemeridine, occurring in Papaver pavonium.

The three groups common to both sub-families are, the tetrahydroisoquinoline derivatives; with hydrocotarnine reported only in Papaver somniferum var. polycepharum, and corypalline reported in Corydalis aurea and C. pallida. Secondly, the cryptopine type are the most ubiquitous. Protopine is not absent

Table II. Summary of Alkaloids in Papaveraceae (including Hypecoideae and Fumarioideae).

Alkaloid group	Papaveroideae	Fumarioideae
1. Tetrahydroisoquinoline	+	+
2. Benzylisoquinoline	+	
3. Cryptopine	+	+
4. Morphine	+	
5. Unknown constitution	+	
6. Isomerides of laudanine	+	
7. Phthalide isoquinoline		+
8. α-Naphthaphenanthridine	+	
9. Tetrahydroprotoberberine		+
10. Aporphine	+	+
11. Minor Corydalis alkaloids		+

from any genus, and allocryptopine is also of wide distribution. The other alkaloids of this group are not so widely spread, but occur in both sub-families. Thirdly, the aporphine group is common to both sub-families, although some of these alkaloids are more prevalent in Fumariacious species, and bulbocapnine occurs only in the Fumarioideae.

Alkaloids are quite scarce in the other families of the Rhoeadales, and those few which do occur are not of the same chemical group as the ones of the Papaveraceae. This feature tends to separate the Papaveraceae from the other families. A detailed list of distribution of alkaloids of Cruciferae and Capparidaceae appears in the appendix.

Fatty Acids

Alston and Turner[1] quote Hilditch (1956) as saying that fatty glyceride compounds could be made the basis of a system of classification of plants. They, however, felt this proposition to be rather difficult due to the wide distribution of fatty acids in quite unrelated families (with some exceptions, e.g. the Flacourtiaceae and their cyclic unsaturated acids) as well as the large number of acids occurring within the same group or family. While fatty acids alone may not be a good "basis of a system of classification", they can act as

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[1] R.E. Alston and B.L. Turner, op. cit. p. 119.

indicators of relationships. The chief drawback to this approach is the lack of application of these constituents to taxonomic problems by competent workers; since fatty acids were of interest primarily to chemists, from their viewpoint. Hopkins and Chisholm[1] have made several analyses for fatty acids, while Eckey[2] and Hilditch[3] provide a wealth of information on the chemistry of fatty acids. They all mention the occurrence in plants and in some cases make comparisons between families. For example, Hilditch[4] noted that erucic acid forms a large proportion of the component acids of seed fat of Cruciferae and Tropaeolaceae. He stated that this acid had not been detected with certainty in any other seed fats. Surely this kind of approach is taxonomic; not a classification in itself, but certainly a recognition of a taxonomic relationship from the viewpoint of fatty acids.

A comparison of fatty acid composition of rape seed and mustard seed oils was made by Craig[5] in 1956. He analyzed

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[1]C.Y. Hopkins and M.J. Chisholm, "Identification of conjugated triene fatty acids in certain seed oils", Can. J. of Chem. 40, (1962), 2078.

[2]E.W. Eckey, Vegetable fats and oils, (N.Y.: Reinhold) 1954.

[3]T.P. Hilditch, The chemical constitution of natural fats, (London, Chapman and Hall,) 3rd. edit. 1956.

[4]Ibid.

[5]B.M. Craig, "Comparison of a fatty acid composition of rape seed and mustard seed oils;" Canadian Jour. Technol. 34 (5): 1956, pp. 335-339.

different commercial stocks of seeds from various parts of the world, and found that iodine values ranged from about 101 in Argentina to 123 for Turkish mustard seed oils. Small variations were found in the total amounts of C_{16} and C_{20} acids; and large variations in C_{18} and C_{22} acids. The variations in the content of palmitic, stearic, hexadecanoic, arachidic and behenic acids were small. The linolenic acid contents of Turkish rape seed and mustard seed oils were 6% higher than for other oils, which were different from one another by less than 2%. Linoleic, oleic, and erucic acids showed maximum variations of 16 to 27; 7 to 27; 18 to 52% respectively. This work indicates that significant differences can occur in the same species from different sources. Perhaps this might be worth considering when making distinctions at the genus and species levels.

In his examination of the Rhoeadales in the light of content matter, Hegnauer[1] paid some attention to the fatty acid content of the families Capparidaceae, Resedaceae, as well as Limnanthes douglasii. He felt that the Capparidaceae, Cruciferae and Resedaceae particularly, seemed closely related biochemically. "They all have oily seeds, without endosperm. In these seed oils occur oleic acid, erucic acid, linoleic

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[1]R. Hegnauer, "Die Gliederung der Rhoeadales sensu Wettstein im Lichte der Inhaltstoffe. Planta Medica 9: (1961), pp. 37-46.

acid and linolenic acid as the main fatty acids." This example is another one in the application of fatty acids to a taxonomic problem.

An examination of the distribution of fatty acids in the Rhoeadales reveals that the families have all moderately oily seeds. The saturated acids do not account for more than 25% in them. Unsaturated acids of more than one double bond are found to be linoleic and linolenic acids. In the Papaveraceae linoleic forms the greater percentage, composing up to 70% of the total fatty acid content. This family is very poor in linolenic acid. In the other families, linoleic and linolenic acids occur in almost equal amounts.

Unsaturated acids of only one double bond, show significant differences. The Cruciferae stand apart from the others in having erucic acid as the major fatty acid. Oleic and eicosenoic also occur in this family. In the other families, eicosenoic is absent, and the major acid is oleic. It is particularly interesting to note that the pattern of fatty acids in the Tropaeolaceae is very similar to that of the Cruciferae. This point has been noted by Hilditch[1] and other workers and shows up clearly in Table III.

At the genus level there are some striking results too.

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[1] T.P. Hilditch, op. cit.

Table III. A summary of the distribution of fatty acids in the families of the Rhoeadales.

Family	Seed Protein	Seed Oils	Sat'd. Acids	Unsat'd Acids (one double bond) OLEIC C_{18}	EICOSENOIC C_{20}	ERUCIC C_{22}	Unsat'd (hydroxy acids) RICINOLEIC C_{18}	C_{20}	Unsat'd (two double bonds) LINOLEIC C_{18}	Unsat'd (three double bonds) LINOLENIC C_{18}
Papaveraceae	++	++	+	++					+++	
Capparidaceae	++	++	+	++					++	
Moringaceae	?	++	+	+++					(+)	
Resedaceae	?	++	?	++					++	++
Tovariaceae	?	?	?	?					?	?
Cruciferae	++	++	+	+	+	+++	++*	+++*	++	++
Tropaeolaceae	++	++	(+)	+	+	+++			(+)	

Key to amounts (average approximations)

(+) trace, less than 5%

\+ poor, less than 25%

++ moderate, less than 50%

+++ rich, more than 50%

? no record

* Applicable only to genus Lesquerella.

In the Cruciferae, Lesquerella is different in having no erucic acid, but a large amount of unsaturated hydroxy acids, mainly the C_{20} acid, and also the C_{18} ricinoleic occur. Also a few species are worth mentioning due to the absence of erucic acid. These are Capsella bursa-pastoris Hesperis matronalis, Lepidium montanum var. angustifolium, and Merisyrenia camperum. In the Papaveraceae, Argemone mexicana stands out in having palmitoleic (6%) and ricinolic (10%) acids. These results were compiled from various sources, but a large part was taken from Mikolajczak[1] et al. They also analysed the protein of seeds, and found that these families all have moderate amounts of it.

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[1] K.L. Mikolajczak, et al, "Search for new industrial oils. Oils of Cruciferae." Jour. Amer. Oil Chem. Soc. 38 (12): 1961, pp. 678-681.

Myrosin and Myrosin Cells

Myrosin was first described by Bussey[1] in 1840. Later it was discovered that this enzyme was capable of catalysing the hydrolysis of naturally occurring thioglucosides (a rare group of glycosides), and it was called "thioglucosidase". Now it is usually designated as myrosin, myrosinase or sinigrase, the latter being named after its best-known substrate, the mustard oil glucoside sinigrin.

At first the secretory cells were called "Proteid-sacs" due to the proteid reaction of their contents, and later when these contents were found to be myrosin, the name was changed to "myrosin cells". As early as 1893 such cells and cell layers were isolated mechanically from the pericycle of Cheiranthus stems. The anatomical features of these idioblasts were dealt with in detail by Metcalfe and Chalk[2], and they also discussed their distribution in the Rhoeadales. They can be demonstrated histochemically be staining with Millon's reagent, iodine or orcinol in hydrochloric acid. I found during another project, that these cells may also be demonstrated by a method using alcohol, iodine and aniline blue solution. Kjaer[3] mentions that

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[1] A. Bussey, "Sur la formation de l'huile essentielle de moutarde," J. Pharmac. Chim., 26. (1840), p. 39.

[2] C.R. Metcalfe and L. Chalk, Anatomy of Dicotyledons, (Lond.: Oxford Clarendon Press.) 1950, pp. 74-97.

[3] A.Kjaer, "Naturally Derived Isothiocyanates (Mustard Oils) and their Parent Glucosides", Fortschritte d. Chem. org. Naturstoffe, 18, (1960), p. 136.

Peche employed a technique whereby a precipate of barium sulphate was formed inside the enzyme containing cell, and thus they could be identified. Kjaer[1] has given an interesting and informative summary of the history of myrosin.

A great surge of interest in myrosin arose about 1953 and has continued since. For example, workers[2] in India investigated how the yield of volatile mustard oil is related to the amount of myrosin present in seeds. They found that by mixing black or brown mustard seeds (Sinapis nigra and S. juncea respectively) with white mustard seeds (S.alba var.) the yield of volatile oil could be increased considerably. They concluded that the amount of myrosin in Sinapis nigra and S. juncea is insufficient to effect complete hydrolysis of the sinigrin present. Another team[3] there, considered methods for the removal of myrosin during the refining of mustard oils. In Finland, Virtanen and Gmelin[4] investigated the chemistry of the enzymatic cleavage performed by myrosin. They agreed that the cleavage

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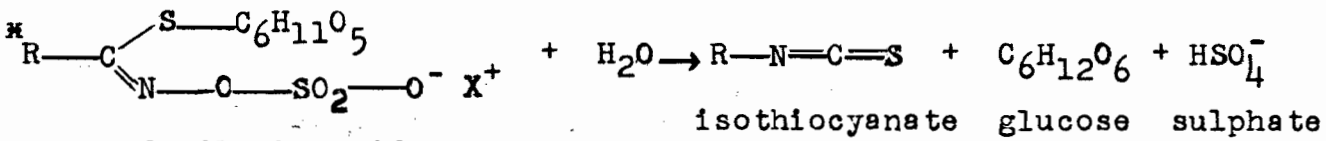
[1] A. Kjaer, ibid, 136-138.

[2] A.B. Datta, "Interesting variations in the volatile-oil yield of mixed Indian mustard seeds," Sci. and Culture, 24, (1958), pp. 182-184.

[3] Lal Bahadur Mathur and Rajeshwar Sahai, "Refining of Oils", Indian, 56, (March 12, 1958), p. 859.

[4] Rolf Gmelin and Artturi I. Virtanen, "A new type of Enzymatic Cleavage of Mustard Oil Glucosides. Formation of Allyl thiocyanate in Thlaspi arvense L. and Benzylthiocyanate in Lepidium ruderale and L. sativum L, "Acta. Chem. Scand., 13, (1959), p. 47.

of mustard oil glucosides to isothiocyanate, sulphate and glucose, was considered to be a specific enzymatic process responsible for the characteristic pungent odour and taste of many Crucifers and other families. They found that efforts to separate a thiocyanate-forming enzyme led in every case to myrosinase activity; and concluded that this new type of cleavage of mustard oil glucosides to thiocyanates is common in the Cruciferae. Ettlinger[1] and his co-workers in Texas studied the chemical formula of mustard oil glucosides in an effort to discover the way the enzyme really acts. They also established convincingly the structure of sinigrin, thus improving the earlier structure proposed by Gadamer. The following reaction has been suggested by them.



Mustard oil glucoside

isothiocyanate glucose sulphate

* Where R= CH_2=$CHCH_2$, and X= K,
the compound is sinigrin.
If R= (p) $HOC_6H_4CH_2$ and X= sinapine,
the compound is sinalbin.

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[1] M.G. Ettlinger and A.J. Lundeen. "The structure of sinigrin and sinalbin; an enzymatic rearrangement." J. Am. Chem. Soc., 78, (1956), p. 4172.

In another investigation, Ettlinger[1] et al found that Sinapis alba contains at least two enzymes that catalyse the same reaction, viz. the hydrolysis of mustard oil glucosides or glucosinolates (a name they have suggested and prefer). One enzyme is, like fungal sinigrase[2], indifferent to vitamin C and is the classical myrosin. The other enzyme requires the vitamin C as cofactor and this they have called an "ascorbate-activated glucosinolase". This says Kjaer[3] is the first clearly demonstrated physiological function of ascorbic acid. They have found this second enzyme to be a "specific thioglucosidase", and their paper describes the reaction in detail, illustrating the report with formulae and equations. Gaines[4] et al also studied this enzyme system, and concluded it was a two enzyme system. On the other hand, some Japanese workers, Zenji Nagashima[5] and M. Uchiyama, who set out to examine Neuberg's hypothesis that myrosinase was a mixture of two enzymes, tended

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[1]M.G. Ettlinger et al, "Vitamin C as a coenzyme: The hydrolysis of mustard oil glucosides," Proc. Nat. Acad. Sci., Vol. 47, No. 12, (Dec. 1961), pp. 1875-1880.

[2]E.T. Reese, et al, "A thioglucosidase in fungi", Arch. Biochem. and Biophys., 75 (1) : (1958), pp. 228-242.

[3]A. Kjaer, "The distribution of sulphur compounds,"in Chemical plant taxonomy," (Lond.:Pergamon Press),(1963),p. 463.

[4]R.D. Gaines and K.J. Goering, "Myrosinase II. The specificity of the myrosinase system," Arch. Biochem. and Biophys., 96 (1); (1962), pp. 13-19.

[5]Zenji Nagashima and Masaaki Uchiyama, "Studies on myrosinase part III," Nippon Nogei-Kagaku Kaishi, Vol. 33, (1959), pp. 881-885.

to conclude that it was a single enzyme. They considered the activity ratios of myrosulfatase and thioglucosidase and reported that their findings were contrary to the hypothesis of Neuberg. Although they did not prove conclusively that the system was a single enzyme, their work is significant for this paper, because during their investigation they considered the distribution of myrosin in 110 species of plants from thirty-seven families besides the Cruciferae. They found all twenty-one species of the Cruciferae tested contained myrosin, regardless of the part of the plant. It was also identified in Tropaeolum, but in none of the other species, which included two species of Papaveraceae, five species of Liliaceae, six of the Leguminosae, and one from the Euphorbiaceae. They concluded that myrosin could be a useful criterion in the classification of plants.

Hegnauer[1] reports that Prof. Van Stenis was alerted to the probable relationship between the Cruciferae and Capparidaceae when he became aware of the occurrence of myrosin cells in the Capparidaceae. Hegnauer concluded that the fact that myrosin cells were spread in the Cruciferae, Capparidaceae, Resedaceae and Moringaceae indicated a close relationship between them. He also raised the question of homology of the lactiferous cells of the Papaveraceae with the myrosin cells of the other families of the Rhoeadales. The alkaloids of the Papaveraceae are localized

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[1]R. Hegnauer, Planta Medica, Vol. 9, (1961), pp. 37-46.

in segmented milk canals or alkaloid idioblasts, Hegnauer points out. It was with great interest, therefore, that I noted during an anatomical survey of members of the Rhoeadales, (for another project) that the myrosin idioblasts appear to be arranged in rows, as along a canal. This feature can be seen in figure 4 which is a photograph of a section of root of Cochlearia armoracia L. taken with polarized light. The section was unstained, but had been treated with 50% sulphuric acid for half an hour.

Table IV gives some indication of the distribution of myrosin outside the Rhoeadales. Early reports of myrosin occurrence in some families are questioned, since techniques in the past were not as accurate or discriminate as those of recent times. For instance, Wehmer and Hadders[1] (1933) list Allium sativum var. vulgare (garlic), and A. cepa (onion), members of the Liliaceae, as producing myrosin in their bulbs. They also report a "myrosin-like substance" in the leaves of Bocagea dalzelii Hook. (Anonaceae). No recent reports of myrosin in these families have been noted. However, Mazelis[2] (1963) found an enzyme (L-3-alkyl sulphinylalanine) in members of the genus Brassica which degrades cysteine sulfoxides, and this

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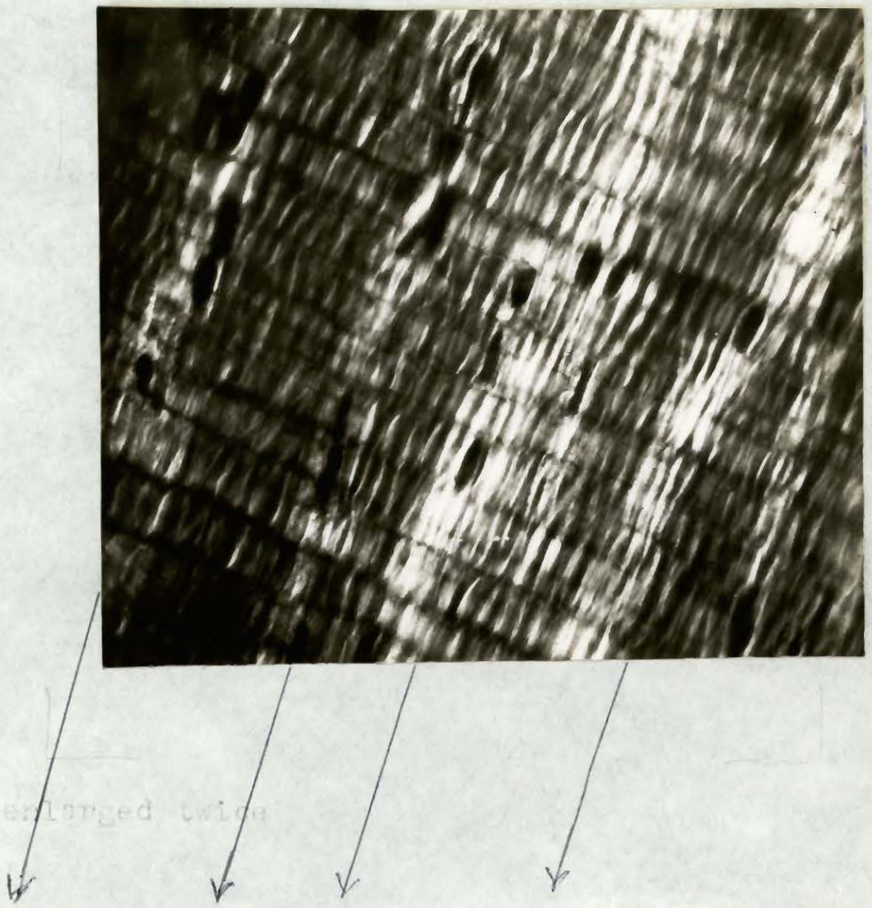
[1]C. Wehmer and M. Hadders, "Systematic occurrence and distribution of Enzymes," Hand. der Pflanzenanalyse. Edit. Klein (Berlin, 1933) Vol. IV, pt. 2, p. 867.

[2]Mendel Mazelis, "Demonstration and characterization of cysteine sulfoxide lyase in the Cruciferae," Phytochemistry, Vol. 2, (1963), pp. 15-22.

Fig. 4

Photograph of Cochlearia Armoracia L. (root) taken with polarized light and showing linear arrangement of Myrosin cells. (Longitudinal section). Low power, enlarged twice.



Myrosin cells represented by the black spots in "linear arrangement" in parenchyma tissue.

Table IV. The reported occurrence of Myrosin and Myrosin Cells, in Families Outside the Rhoeadales.

Species	Seeds	Roots	Stems	Leaves	Reference
Anonaceae					
* Bocagea dalzelli Hook.				+	Wehmer[1] & Hadders
Caricaceae					
* Carica papaya L.	+	+	+	+	Wehmer & Hadders
Euphorbiaceae					
Manihot sp.					Wehmer & Hadders
Putranjiva roxburghii Wall.					Kjaer[2]
Leguminosae					
Scorodophloeus sp. Harms.					Wehmer & Hadders
Liliaceae					
Allium cepa				bulb	Wehmer & Hadders
Allium sativum v. vulgare				bulb	
Limnanthaceae					
Limnanthes douglasii R.Br.	+			+	Wehmer & Hadders
Phytolaccaceae					
Codonocarpus cotinifolius (Desf.)					Kjaer
Plantaginaceae					
Plantago majus L.				+	Kjaer
Salvadoraceae					
Salvadora oleiodes Den.	+				Kjaer

* Reported as a "myrosin-like enzyme".

[1] Wehmer & Hadders, Hand. der Pflanzenanalyse. Vol. IV, pt.2, p.867,(1933).

[2] Kjaer, Fortschritte d. org. Chem. Natur., 18, p. 168, 1960.

Table IV. (cont'd.)

Species	Seeds	Roots	Stems	Leaves	Reference
Tropaeolaceae *Tropaeolum majus* L.	+		+		Wehmer & Hadders
Violaceae *Viola tricolor* L.	+				Wehmer & Hadders
Fungi *Aspergillus sydowi*					Reese[1]

[1] Reese, *et al*, Arch. *Biochem. & Biophys.* 75 (1): pp. 228-242, (1958).

enzyme is similar in its mode of action to alliinase which has been described in Allium species. Kjaer[1] in his discussion of sulphur compounds also mentions that a thiol (1-propanethiol) was present in Allium cepa. In another paper[2] he refers to (-)-β-amino isobutyric acid isolated from the bulbs of Iris tingitana, which is very similar to the non-hydroxylated isopropyl mustard oil. These examples suggest that the identity of compounds with such slight chemical differences may have lead to erroneous conclusions.

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[1] A.Kjaer, "Distribution of sulphur compounds", in Chemical plant taxonomy, Edit. Swain (Lond. New York : Academic Press) 1963, p. 456-462.

[2] A. Kjaer, "Mustard oils and their Parent Glucosides," Fortschritte d. Chem. Org. Naturstoffe. 18, (1960), p. 153.

Mustard Oil Glucosides

"Even the mere knowledge of the distribution pattern of a given compound, if critically evaluated and presented with due consideration of evidence provided by entirely different approaches, may frequently give considerable help in taxonomical problems. The organic sulphur compounds in plants provide an example." This statement by Kjaer[1] is applicable particularly to the isothiocyanates and families of the order Rhoeadales. Contrary to myrosin which is located in idioblasts, mustard oil glucosides are scattered throughout the parenchyma tissue. Myrosin usually accompanies thioglucosides or mustard oil glucosides in the plant, but they only interact when the tissues are crushed, then the characteristic pungent odour and sharp taste of, for example radishes, mustard and other Crucifers becomes evident. Thioglucosides undergo hydrolysis by myrosin to glucose, sulphuric acid and isothiocyanates. (The equation was given earlier). The classical representatives of the group are sinigrin and sinalbin. Kjaer[2] maintains that all mustard oil glucosides have the same basic structure, that of sinigrin; their individuality depends on the side chains. The simplest side chain is a methyl group, found in the compound glucocapparin. Kjaer[3] lists these groups in order of increasing complexity.

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[1] A. Kjaer, "The distribution of sulphur compounds", in Chemical plant taxonomy, Edit. Swain, (Lond. New York, : Academic Press), (1963), p. 454.

[2] _____ ibid, p. 463.

[3] _____ ibid, p. 465-466.

Ettlinger[1] et al suggested a form of nomenclature for these compounds of a systematic nature, and Kjaer supports this idea. In this new system, sinigrin becomes identical with potassium allylglucosinolate, and sinalbin with sinapine 4-hydroxybenzylglucosinolate.

Kjaer and his co-workers have done much research on the chemistry and distribution of mustard oil glucosides, and have published many papers. Especially noteworthy is his review of the subject which appeared in 1960. In this comprehensive survey, Kjaer[2] discusses the historical development and chemical methods used in isolating and identifying these compounds. He mentions about 200 species of plants tested and the constituent thioglucosides present in them. He concludes that isothiocyanates are regular constituents of the Cruciferae, but are not restricted to this family. Another constant source is the Capparidaceae, as well as the limited number of species of Resedaceae and Moringaceae tested. He noted however, that the thioglucosides in about 40 species of Capparidaceae[3] which were investigated differed significantly from those encountered

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[1] M. G. Ettlinger et al, "Vitamin C as a coenzyme: The hydrolysis of mustard oil glucosides," Proc. Nat. Acad. Sci., Vol. 47, No. 12, (1961) pp. 1875-1880.

[2] A. Kjaer, "Mustard oils and their parent glucosides," Fortschritt d. Chem. org. Naturstoffe, 18, (1960), pp. 123-169.

[3] A. Kjaer and H. Thomsen, "XLV. Isothiocyanate-producing glucosides in species of Capparidaceae," Phytochemistry, Vol. 2 (1963), pp. 29-32.

in the Cruciferae. Glucocapparin has not been detected with certainty in any Crucifers, but is widely distributed in Capparidaceae. The glucoside with an ethyl side chain - glucolepidiin - has been found only once; in Lepidium Menziesii DC., a North American species. In contrast, the thioglucoside with an isopropyl-grouping appears rather widely distributed, and is often accompanied by glucocochlearin.

Over twenty years ago, Hopkins[1] made an investigation of a sulphur containing substance of Conringia orientalis seed. He found that this Crucifer had a bitter taste instead of the usual sharp one known in mustards. From his analysis he concluded that the bitter principle was a sulphur compound, but one different from the isothiocyanates, and he suggested it was 2 mercapto-5, 5-dimethyloxazoline, with an empirical formula C_5H_9ONS. In 1950 Ettlinger's[2] work led to the correction of Hopkins' formula, and recently Kjaer[3] et al isolated the parent glucoside - glucoconringin. Kjaer stated that Schultz and Wagner also reached conclusions similar to his about the identity of the glucoside. Glucoconringin has also been found

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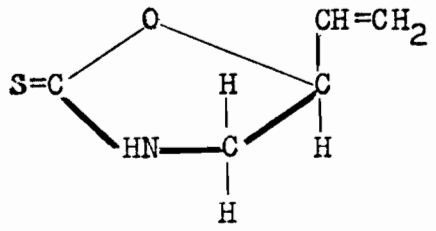
[1]C.Y. Hopkins, "A sulphur-containing substance from the seed of Conringia orientalis," "C'dian. Jour. of Research, B, 16, (1938), pp. 341-344.

[2]M.G. Ettlinger, "Infrared Spectra and Tautomerism of 2-Thiooxazolidone and Congeners," J. Amer. Chem. Soc., 72, (1950), p. 4699.

[3]A. Kjaer, "Mustard oils and their parent glucosides," Fortschritte d. Chem. org. Naturstoffe, 18, (1960), p. 150.

in various species of Cochlearia.

Another interesting development reported by Kjaer[1] is the establishment of the chemical configuration of goitrin and progoitrin (glucorapiferin). He states that Astword in 1949 isolated from yellow turnip and other Brassica sp. an anti-thyroid factor, (-)-5-vinyl-2-oxazolidinethione, which they believed was desended from an isothiocyanate glucoside; and later Greer isolated the glucoside (progoitrin) from rutabaga seeds. Progoitrin is a precursor of goitrin and occurs predominantly in the seeds of Brassica spp.. It is remarkable that little is found in fresh cabbage. The structure of goitrin is shown below.



In this same paper just mentioned, Kjaer reports that experiments in Australia have indicated that cheirolin isolated from the ripe fruits and leaves of Rapistrum rugosum (L.) All. was goitrogenic in rats. This could be significant in animal feeding. Another discovery of potential interest to farmers

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[1]A. Kjaer, ibid, p. 151.

is the fact that certain insects could be induced to feed on leaves which they normally refuse, by spraying the leaves with sinigrin or sinalbin solutions, but not allyl isothiocyanate.

Recently two new glucosides have been reported. In Hesperis matronalis seed, a glucoside for which Kjaer[1] suggested the name glucohesperin, was found. He mentions that in 1956 Wagner and Schultz on the basis of chromatograms, concluded that Hesperis matronalis contained one minor and three major thioglucosides; and one of them (glucomatronalin) formed a crystalline heptoacetate. The second one, glucolesquerellin, was discovered by Daxenbichler[2] et al in the seeds of Lesquerella lasiocarpa.

Chemotaxonomic differentiation at the genus level is much less common than other studies. However, Kjaer[3] attempted to use data of isothiocyanate glucosides for this purpose. He

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[1] A. Kjaer. "A mustard oil of Hesperis matronalis seed, 6-Methylsulphinylhexyl isothiocyanate," Acta. Chem. Scand. 17, No. 3 (1963), pp. 846-847.

[2] M.E. Daxenbichler, "Isothiocyanates from enzymic hydrolysis of Lesquerella seed meals," J. Am. Oil Chemists' Soc., 39 (1962), pp. 244-245.

[3] A. Kjaer and S.E. Hansen, "Isothiocyanates XXXI: The distribution of mustard oil glucosides in some Arabis species. A chemotaxonomic approach," Saertryk Af Botanisk Tidsskrit, 54, (1958), pp. 374-378.

and his co-workers chose for investigation the complicated genus Arabis, and they were able to observe distinctly different patterns in the various strains of Arabis. From their results they concluded that their method was potentially useful at that level.

A somewhat similar study was made by Delaveau[1] on Brassica. He collected the essential oils liberated by myrosinase from mustard seed and analysed it chromatographically. He found that the oil from Brassica nigra contained allyl isothiocyanate and a small amount of phenethyl isothiocyanate. On the other hand, B. Juncea produced mostly allyl isothiocyanate as well as some butenyl, pentenyl and phenethyl isothiocyanates. These results led him to regard B. juncea as an amphidiploid hybrid between B. nigra and B. rapa sylvestris.

From surveying the literature it was evident that the thioglucosides are predominantly characteristic of the Cruciferae, but also occur in the Capparidaceae, Resedaceae and Moringaceae. None have been reported in any members of the Papaveraceae or Fumariaceae. There are some cases of occurrence of thioglucosides outside the families of the Rhoeadales, and perhaps they will prove significant when further studies have been made. At present such occurrences are considered atypical for the

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[1] P. Delaveau, "Chromatographic study of a case of amphidiploidy in Brassica," Compt. rend. Soc. Biol., 153, (1959), pp, 579-581.

Caricaceae, Euphorbiaceae, Limnanthaceae, Phytolaccaceae, Plantaginaceae, Salvadoraceae and Tropaeolaceae. Very common among these families is glucotropaeolin and glucocochlearin.

Table V shows a brief summary of the chemical groups of thioglucosides found in the families of the Rhoeadales. A plus sign indicates that a certain family has one or more glucosides of a particular chemical group; which may be present in one or several species. The detailed occurrence in various species is given in Table 4 of the appendix. Kjaer[1] has pointed out the interesting fact that the keto substituted side chain compounds, e.g. glucocappasalin, glucocapangulin and gluconorcappasalin are found mainly in the South American species of the Capparidaceae, in which glucocapparin is far less prominent.

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[1]Kjaer, "The distribution of sulphur compounds", in Chemical plant taxonomy, (Lond.: Pergamon Press,)(1963),p. 470.

Table V. A Summary of the Chemical Groups (of R of derived Isothiocyanates R-NCS) Represented in Families of the Rhoeadales.

Chemical groups of R.	ALKYL Compounds	ALKENYL	THIOETHERS	SULFOXIDES	SULPHONES	ARYLALKYL	PHENOLS & ETHERS	ESTERS	KETO	ALIPHATIC HYDROXY	HETEROCYCLIC
Cruciferae I											
Cruciferae II	+	+	+	+	+	+	+	+		+	+
Cruciferae III			+								
Cruciferae IV	+	+	+	+	+		+	+		+	
Capparidaceae I	+								+	+	
Capparidaceae V	+									+	
Moringaceae						+					
Resedaceae						+			+		
Papaveraceae											

Miscellaneous Compounds.

Amino acids are the building blocks of proteins and as such are rather common metabolites of organisms. They become taxonomically useful only when unusual ones are involved, as for example in the work on the genus Lathyrus (Leguminosae) which Alston and Turner[1] attribute to Bell (1962). An earlier study was made by Reuter[2] (1957). He investigated the principal forms of soluble nitrogen in various parts of 166 species from 48 families. Reuter tried to interprete the relative quantities, rather than presence or absence only.

In his presentation of patterns of amino acids of storage organs of several species not closely related, some results of interest to this paper were noted. Dicentra eximia and Nymphaea hybrida both have glutamic and aspartic acids, as well as alanine and glutamine. Altogether their patterns are very similar. This point should not be over-stressed, however, as there were other species, e.g. Bowiea volubilis and Carya amara which also had patterns similar to Dicentra eximia. δ-acetyl ornithine was found by Reuter to be restricted to the Fumarioideae, where it formed the chief amino acid in 19 species from 4 genera. It did

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[1] R.E. Alston and B.L. Turner, Biochemical systematics, (N.J.: Prentice-Hall), 1963, p. 100.

[2] G. Reuter, "Die Hauptformen des löslichen Stickstoffs in vegetativen pflanzlichen Speicherorganen und ihre systematische Bewertbarkeit", Flora 145,(1957-58) pp. 326-338.

not occur as the principal acid in any of the species of Papaveroideae tested. Hylomecon, Chelidonium majus and Glaucium flavum have small amounts; but there is no mention of occurrence in other families of the Rhoeadales.

Kjaer[1] discussed the non-protein sulphur amino acids, and an interesting point is brought out. (+) - S-methyl-L-cysteine sulphoxide was isolated from cabbage juice and turnip root, but also occurred outside the genus Brassica. It has been detected in Allium cepa and Capsella bursa-pastoris, as well as in Cheiranthus cheiri L. and Sinapis alba. It is believed that S-propenyl-L-cysteine sulphoxide gives rise to the lachrymatory principle in onions.

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[1] A. Kjaer, "The distribution of sulphur compounds," in Chemical Plant Taxonomy, Edit. Swain, (Lond., New York: Academic Press), 1963, p. 459.

Crystals have been used as taxonomic indices in various families, e.g. in the Liliaceae, where the shapes of Calcium oxalate crystals are considered significant in Allium[1]. One type of crystal which has been used frequently is the raphide. Raphides are bundles of needle-shaped crystals of calcium oxalate which occur in special sacs and are visible through the microscope. The history and significance of these in taxonomy have been well discussed by Gibbs[2] in a recent paper. In an earlier article[3] he described a method by which the distribution of raphides and syringin could be investigated. Alston and Turner[4] also review this subject in their book. Not much significance has been attached to crystals from the point of view of the Rhoeadales. However, it is interesting that gypsum crystals have been recorded in several members of the Capparidaceae[5], while other crystals have been noted in species of the Cruciferae. Raphides have never been recorded in any of the families concerned here.

[1] R. Darnley Gibbs, "History of Chemical Taxonomy", in Chemical Plant Taxonomy, (Lond.: Pergamon Press), (1963), p. 55

[2] _____, ibid, pp. 51-57.

[3] _____, "Comparative Chemistry of Plants as Applied to a Problem of Systematics:" Trans. Roy. Soc. Canada, Vol. LVI: Series III, (June 1962), p. 146.

[4] R.E. Alston and B.L. Turner, Biochemical Systematics, (N.J. : Prentice Hall), 1963, p. 271.

[5] Gibbs, op. cit. p. 57.

Glycosides are defined by Paris[1] as "organic compounds in which there is usually a semi-acetal linkage between the reducing group of a sugar and an alcoholic or phenolic hydroxyl group of a second molecule called an aglycone. This link, being effected through oxygen, gives rise to the O-glycosides which are most common in plants." There are several different types of glycosides, and one type - mustard oil glucosides - have already been discussed.

Cardiac glycosides are compounds related to the steroids, having in addition a lactone ring and a sugar (often a tetrasaccharide) attached to carbon 3 of the cyclopentanophenanthrene skeleton. The aglycones are rarely found in a free state but they can be divided into two classes: cardenolides which have a five-membered ring, and bufanolides with a six membered ring. Cardenolides have been detected in the Cruciferae. For example alleoside A (also called helveticoside and erysimin) has been reported in Erysimum and Cheiranthus. Cheiranthus also contains cheiroside A or cheiroside H, cheirotoxin and corchallin. Erysimoside and syreniotoxin occur in Erysimum. Bufanolides have been detected in the Ranunculaceae. Strophanthidin occurs in Adonis, and also in Cheiranthus and Erysimum.

The sugar moiety varies widely, but Paris[2] says that normal hexoses like glucose are rare. However, cheiroside A is hydrolysed

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[1] R. Paris, "The distribution of plant glycosides," in Chemical Plant Taxonomy, Edit. Swain, (Lond, New York: Academic Press), 1963. p. 337.

[2] _____ ibid, p. 351.

to D-glucose and desglucocheiroside A. Perhaps this could prove to be of taxonomic value. Alleoside A is hydrolysed to D-digitose and Strophanthidin (also called erysimidin).

Cyanogenetic glycosides are produced by many plants, and they yield hydrocyanic acid on treatment with enzyme or acid. These glycosides are difficult to obtain in crystalline state, and it is therefore impossible to state with certainty that the compound indicated by a colour reaction is really a cyanogenetic glycoside. Hegnauer[1] investigated the distribution of cyanogenesis in cormophytes and the taxonomic significance of this product. He examined 400 species using the sodium picrate test and 29 were found to be cyanogenetic. Among those he found positive were some members of the Rhoeadales. He reported it for the first time in Cardamine pratensis L, and Lepidium latifolium L.. From his survey, Hegnauer estimated 3-5% of cormophytes to be cyanogenetic and considered this character to be of limited taxonomic significance until the chemical nature of the parent substance is known. He believed that a genus or tribe may contain the same cyanogenetic compound, while a family may produce different ones, and felt that phytochemical research did not seem to support Hallier's (1912) idea that different phyletic lines of dicotyledons contained similar cyanophoric compounds, although more investigation was still needed to disprove the point.

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[1]R. Hegnauer, "Over de verspreiding van blauwzuur bijvaatplanten," Pharmaceutisch Weekblad, Vol. 93, (Sept.1958), pp.801-819.

Alston and Turner[1] agree with Hegnauer's opinion basically, but believe that the systematic importance cannot be denied, in spite of the limited chemical knowledge about the group. They cite the work of Dilleman (1958) who found cyanogenetic substances to consist of a sugar, a cyanhydric acid and a third substance whose nature is variable. He classified true cyanogenetic heterosides in three groups, which are discussed fully. They doubted the suggestions that their role was perhaps that of protective agents, wastes or reserve energy sources. In the same review, Alston and Turner report on the recent findings of Trione (1960) that hydrogen cyanide was sensitive to environmental conditions. He observed diurnal variations, and reactions to light, soil moisture and temperature.

Indican is a chromogenic glycoside found in the Cruciferae and other families. It is the glucoside of indoxyl, and it is hydrolysed by the enzyme indemulsin. The essential dye-stuff of this chromogenic glycoside is indigo, formerly of great economic importance. In 1961 Berkley[2] made a study of the content of Woad (Isatis tinctoria) and reported it to be very low compared with the poorest Indigofera leaf (viz. Indigofera sumatrana). Rich Indigofera leaves yield 30-70% of indigotin, whereas Isatis tinctoria yielded only 0.05%. Indican occurs in 3 other families, Leguminosae (Indigofera tinctoria), Polygonaceae

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[1] R.E. Alston and B.L. Turner, op. cit. p. 182.

[2] C. Berkley, "Indigotin content of Woad," Nature, Vol. 191, No. 4796, (Sept. 1961), pp. 1414-1415.

(Polygonum tinctorium), and Apocyanaceae (Wrightia tinctoria). Paris[1] discusses these and other chromogenic glycosides, concluding that the present state of knowledge does not permit them to be of much value in comparative phytochemistry.

Saponins are another type of glycoside which are detected mostly through their ability to haemolyse blood. They have been found in about 70 families, but their complete distribution is not known. Steroidal saponins are less widely spread but have been discovered in the Papaveraceae, Ranunculaceae and Violaceae, apart from other unrelated families.

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[1] R. Paris, op. cit. p. 356.

Phenolic substances.

Phenolic substances are a part of the primitive metabolic pattern, associated with, but not essential to the woody habit of growth. Phenolic compounds appear to be metabolically inert and in living cells are recognized as stable characteristic end products. They are present universally and are of extraordinary diversity. To gain significance as taxonomic indicies, their pattern of distribution could be considered from various points of view, e.g. a small number of common phenolic constituents in a large number of families, or a particular uncommon constituent could be traced for its limited distribution.

Leuco-anthocyanins are considered to be phenolic substances. Bate-Smith[1] (1954) found them more common in plants of woody, rather than herbaceous habit; and he considers the ability to produce these compounds to be a primitive character which the herbaceous groups have lost. He correlates it with the trend from woody to herbaceous habit. Bate-Smith[2] tested several members of the Rhoeadales, and found them without leuco-anthocyanins in their leaves, although he found that frequently leuco-anthocyanins were present in their seed coats. In another

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[1] E.C. Bate-Smith, and N.H. Lerner, "Leuco-anthocyanins 2. Systematic distribution of leuco-anthocyanins in leaves," Biochem. Jour., Vol. 58, No. 1, (1954), pp. 126-132.

[2] E.C. Bate-Smith, "Plant phenolics as taxonomic guides," Proc. Linn. Soc. Lond., Session 169, (Dec. 1958) Pt. 3. pp. 198-211.

article Bate-Smith[1] suggests that many woody families without leuco-anthocyanins are atypical in the phyletic series, and that apparent conflicts occur in large families where many species may not have been tested. The Limnanthaceae, a herbaceous family has leuco-anthocyanins. Swain[2] reported that Masquerlier et al found that leuco-anthocyanins stimulate cell division, and they attributed the increase in toughening of the testa of broad beans on ageing to the formation of leuco-anthocyanins and not to lignification as is the case in other vegetables. Such an effect they believed indicated that the leuco-anthocyanins are polymeric molecules capable of binding the polysaccharides in cell walls very firmly.

Another type of phenolic compound is the group of hydroxy acids, for example caffeic and ellagic, di- and tri- hydroxy acids respectively. These are fairly wide-spread, so their absence becomes taxonomically significant. Bate-Smith[3] found many members of the Cruciferae without hydroxy acids. Methoxy acids, for example ferulic and sinapic, are generally absent, therefore their presence in the Capparidaceae, Cruciferae and Papaveraceae may be significant. Bate-Smith remarks also, that sinapic

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[1] E.C. Bate-Smith and Lerner, op. cit.

[2] T. Swain and E.C. Bate-Smith, "Leuco-anthocyanins", in The Chemistry of vegetable tannins, (1956) pp. 109-120.

[3] E.C. Bate-Smith, "Plant phenolics as taxonomic guides", Proc. Linn. Soc. Lond., session 169, (Dec. 1958), Pt. 3. pp. 198-211.

acid is especially interesting because it is the twice methylated derivative of trihydroxycinnamic acid missing in nature. Furthermore, "ferulic and sinapic acids provide .... a strong chemical link between the cellular phenolic constituents and lignin, and it is therefore important that these acids are found especially in herbaceous plants, where the lignification of the cell walls and vessels is least in evidence."

Tannins are another class of phenolics of interest. Bate-Smith and Lerner remark on the "congruence between tannins and leuco-anthocyanins": e.g. leuco-anthocyanins react with ferric salt reagents used for tannin detection. It is believed that leuco-anthocyanins sometimes interfere with the production of good leather. In a study of the occurrence of leuco-anthocyanins and tannins, Bate-Smith[1] was able to recognize three categories of plant families. Firstly, those which were tanniferous, secondly those which were mostly negative, and thirdly, those which were completely negative. In the last group were the Capparidaceae, Cruciferae, Fumariaceae and Resedaceae. Their tannin content was determined on the basis of anatomical examination, and cells with tannins were identified by their colour. Petioles were examined mostly, but

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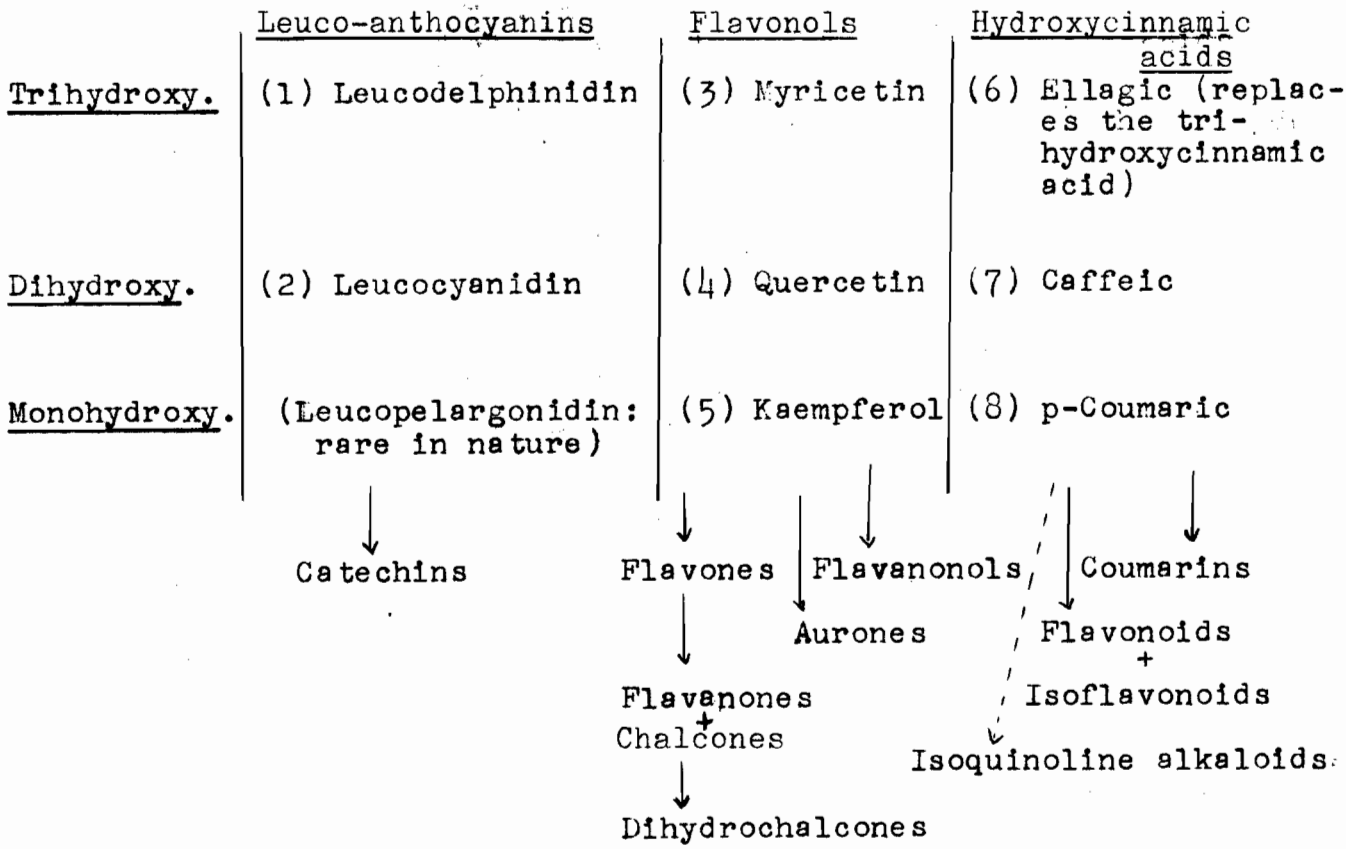
[1]E.C. Bate-Smith and C.R. Metcalfe, "Leuco-anthocyanins. 3. The nature and systematic distribution of tannins in dicotyledonous plants," Jour. Linn. Soc. Lond. (Bot.). Vol. LV, No. 362, (1957) pp. 669-705.

they suggested that the tannin content of a plant may vary with the organ.

It is interesting to note that from the eight most common phenolics of dicots several different types of compounds are derived by various substitutions. Such derivates include isoquinoline alkaloids like laudanine and the methylated derivative of the phenolic prototype - berberine. A specific constituent - nudicaulin - is the yellow flower pigment of Papaver nudicaule and other members of the family. Nudicaulin has a N-containing anthocyanin-like structure, which is not completely known. In the Cruciferae, sinapin - an ester of sinapic acid with choline - is present, frequently in combination with the mustard oils. In the Resedaceae the mustard oil in the root of Reseda officinalis does not appear to contain sinapic acid. Luteolin is a characteristic constituent of Reseda luteola. (Sinapin, choline and acetyl choline are often discussed as alkaloids; they are aliphatic quarternary bases).

Figure 5 shows the common phenolic compounds and some of their derivatives produced by certain chemical transformations. To some of these transformations members of the Rhoeadales owe their economic importance. For example, in Capparis spinosa flower buds form "capers", the much used spice, and these contain flavonols and sinapic acid. In the Cruciferae, leaves of cabbage (Brassica sp.) cress (Lepidium sativum), water cress (Nasturtium officinale) contain sinapic acid.

Fig. 5 The eight common phenolic constituents of dicotyledons, and some of their derivatives.



Geissmann[1] also reports anthocyanins in many Cruciferae. Seeds of mustard (Brassica nigra) may have leuco-anthocyanins in the seed coat, while roots like turnip, rutabaga and radish are also economically important on the basis of their flavonoid compounds. Hattori[2] reports a glycoside of a flavone - diosmin or diosmetin-7-rhamnoglucoside - which is found in the leaves of Capsella bursa-pastoris.

Coumarins are formed by additional oxidative ring closure in the ortho-position of the cinnamic acids. Dean[3] remarked what he considered a striking fact: ".. apart from coumarin, hydrocoumarin and dicoumarol, all naturally occurring coumarins .... can be regarded as derivatives of umbelliferone, which is one of the most widely distributed compounds of this class." These compounds are not very important as yet in considerations of the Rhoeadales.

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[1] The chemistry of flavonoid compounds, Edit. Geissmann, (New York: MacMillan), 1962, Chap. 16.

[2] Shizuo Hattori, ibid, Chap. 11.

[3] F.M. Dean, "Naturally occurring coumarins", Prog. Chem. Organic Nat. Prod., 9: (1952), pp. 225-291.

CHAPTER III

EXPERIMENTAL

A. Methods

The samples tested throughout this research were mainly leaves, with small bits of petiole or stem attached. In some cases (where stated) seedlings and seeds were also examined. Specimens were obtained from Botanical gardens all over the world. A large proportion of the plants investigated were cultivated in McGill greenhouses from seeds obtained chiefly from Kew and the Montreal Botanical Gardens. Tests were carried out on freshly picked material, while imported specimens were shipped airmail in plastic bags to maintain their freshness.

Species from all families of Rhoeadales were tested, except for material of Bretschneidera sp. which was unavailable. Five tests were employed and some additional ones where applicable. The following description gives the highlights of each method; but the details appear in appendix A (p.107).

(1) The Leuco-anthocyanin test A, is carried out according to the method of Bate-Smith and Lerner[1] (1954). In this process, colourless water soluble Leuco-anthocyanins (which are considered phenolics) are hydrolysed and oxidized to the corresponding

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[1] E.C. Bate-Smith and N.H. Lerner, "Leuco-anthocyanins (11). Systematic distribution of leuco-anthocyanins in leaves." Biochemical Journal, Vol. 58, No. 1 (1954) pp. 126-132.

colored anthocyanidins. These coloured substances, for example, Pelargonidin, Cyanidin and Delphinidin, are soluble in iso-amyl alcohol; thus becoming identifiable. In 1933 Robinson[1] proposed structure (1) for leuco-anthocyanins. Later, Bate-Smith[2] (1953) suggested the structure (11) to be that of an oxidized "Flavandiol" instead of a "Flavantriol" as proposed by Robinson. The most recent structure (111) is given by Clevenger[3] (1964). This structure demonstrates the marked similarity to cathechins and gallocatechins, as is shown in Alston and Turner[4] (1962). (See Fig. 3).

The Leuco-anthocyanin test is carried out on finely chopped leaves and a positive result is the formation of a cherry red colour, soluble in the amyl alcohol layer. Bate-Smith[5] found Leuco-anthocyanins more common in woody than herbaceous families

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[1]G.M. Robinson and R. Robinson, "XXXI. A survey of anthocyanins. 111 Notes on the distribution of Leuco-anthocyanins". Biochem. Jour. 27: (1933) pp. 206-212.

[2]E.C. Bate-Smith, "Colour reactions of flowers attributed to (a) flavonols and (b) carotenoid oxides"; Jour. Exper. Bot. 4: (1953) pp. 1-9.

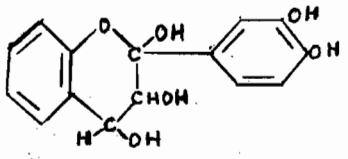
[3]Sarah Clevenger, "Flower Pigments" Scientific American, Vol. 210, No. 6 (June 1964), p. 88.

[4]Ralph E. Alston and B.L. Turner, Biochemical Systematics (N.J.: Prentice-Hall), Inc., 1963, p. 280.

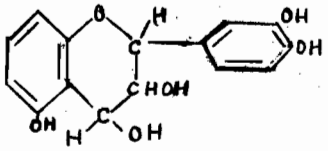
[5]E.C. Bate-Smith and N.H. Lerner, "Leucoanthocyanins 11. Systematic distribution of leuco-anthocyanins in leaves." Biochemical Journal, Vol. 58, No. 1, (1954) pp. 126-132.

and believes that the ability to produce these compounds is a primitive character which herbaceous groups have lost. He correlates this ability with the trend from the woody to herbaceous habit.

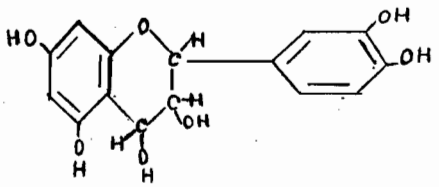
Fig. 6. Structural Formulae of Leuco-anthocyanidins and some related compounds.

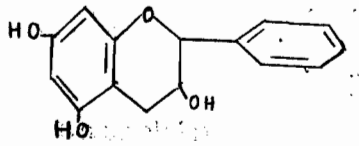


(1) "Flavantriol" According to Robinson.

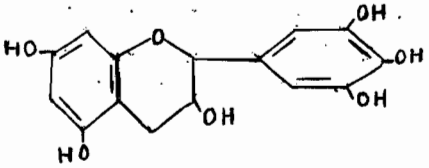


(11) "Flavandiol" According to Bate-Smith.



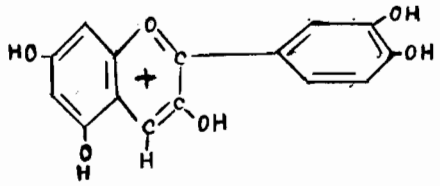


Catechin



(111) Leuco-anthocyanin (from Clevenger)

Gallocatechin



(IV) Anthocyanidin (from Clevenger)

As an additional test the HCl/Methanol test was used. It was first introduced by Isenberg and Buchanan[1] (1945). It is applicable only to woody material, and as the species in the order Rhoeadales are mainly herbaceous, it was used rarely in this project. Wood shavings are treated with a mixture[2] of HCl and methanol. A positive result is the development of a magenta colour in the wood. The colour of the solution is noted also. What compounds cause the development of this purple colour is not definitely known, but it has been suggested that catechol tannins may be responsible. These condensed tannins yield polyphenols on hydrolysis, and may be condensation products of compounds such as catechin or gallocatechin. Therefore they are closely related to the leuco-anthocyanins. A positive HCl/methanol test is very closely correlated with a positive leuco-anthocyanin. test.

(11) The Ehrlich test, for Aucubin and Aucubin-like substances, is performed also on leaves from which an alocholic extract is prepared. Spots of the extract are made on filter paper and the development of a deep blue colour on the spot after treatment with Ehrlich reagent (p-dimethylamino-benzaldehyde: HCl: 95% ethanol) is a positive reaction. This reaction seems to be caused by Aucubin; although it is suspected that other compounds may give the blue colour as well. Recently

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[1] I.H. Isenberg and M.A. Buchanan, "A colour reaction of wood with methanol-hydrochloric acid." Journal of Forestry (Wash., D.C.), 43: (1945), pp. 888-890.

[2] The mixture is of 25 ml. conc. HCl: 1000 ml. methanol, and a few ml. are used to steep shavings of freshly cut sap-wood.

the structure of aucubin (Fig. 7(a)) has been elucidated[1].

(111) The HCN Test (for Cyanogenetic Glycosides). The occurrence of Prussic acid or Hydrogen cyanide was first reported[2] in 1803 (by Böhm). He found it in the water after bitter almonds had been steeped. Cyanogenetic Glycosides of which over twelve are known, yield Hydrogen cyanide when hydrolysed by water, emulsin and chloroform. Amygdalin (Fig. 7 (b)) was the first glycoside found. A positive reaction to this test is the formation of an orange brown or red colour on sodium picrate paper, suspended in a sealed test tube above the material being examined.

(IV) The Juglone Test. Juglone is a naphthoquinone (Fig. 7 (c)) which may occur in plants as the glycoside of hydrojuglone (1, 4, 5 trihydroxynaphthalene). A filtered chloroform extract from leaves is evaporated to dryness, and the residue is dissolved in ether, and then shaken with an equal amount of ammonium hydroxide solution. If a brilliant purple colour develops at once in the ammonia layer, the reaction is positive. A bright yellow colour in the aqueous layer instead of purple may be due to flavones (Fig. 7 (d)); and in rare cases a blue or blue-green colour may develop on standing. These are referred to as Test B. Sometimes this layer is fluorescent when

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[1] R. Darnley Gibbs. "Comparative chemistry of plants as applied to a problem of systematics;" Trans. Roy. Soc. Can., Vol. LVI : Series III : Sect. III (June 1962), p. 148.

[2] E. Shaw. "Comparative chemistry and taxonomy of the "Hamamelidales". A thesis, McGill University, April, 1960, p. 69.

examined by ultra-violet light and may indicate coumarins (Fig. 7(c). Such an examination is referred to as Test C.

(5) Hot Water and Cigarette Tests were first described by Miss Dagmar Dykyj-Sajfertova[1], and are believed to indicate the presence of polyphenolases. (These are respiratory enzymes which act upon suitable substrates in leaves). A fresh leaf is dipped part-way into water at 85°C and held there for 5 seconds. Rapid darkening along the water-line or in the dipped portion is a strongly positive reaction. Darkening after sometime is a weak positive; no colour after 30 mins. is negative. Dykyj-Sajfertova noted that leaves with acid cell-saps gave a yellow colour in this, as well as the Cigarette Test, and called the phenomenon the "oxalis reaction" because it was given by species of Oxalis which she tested. The cigarette test is very simple to perform. A glowing cigarette is applied to the back of a leaf for 3 seconds. In positive species, a dark ring appears rapidly.

Two further tests used were the tannic acid test and chromatograms for phenolic acids. Both of these were carried out by Mrs. P. Bahr and the results are included in this paper.

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[1] R. Darnley Gibbs, "Comparative Chemistry of plants as applied to Problems of Systematics." Recent Advances in Botany, 1961. P. 68.

The Tannic Acid Test was performed by Bates[1] method. Tannins are best considered as phenolics. Hydrolysable tannins yield gallic or ellagic acid and glucose when heated with mineral acid. Leaves are placed between two pieces of filter paper moistened with ferric chloride solution, and squeezed with a pair of pliers. The development of a dark blue-grey spot indicates a positive reaction.

Phenolic Acids were sought by chromatography. The method of Ibrahim and Towers[2] was used. The solvents were benzene: acetic acid: water and formic acid. The chromatograms were sprayed with ferric chloride and sulfanilic acid.

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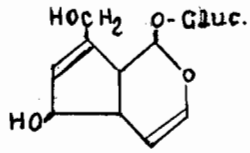
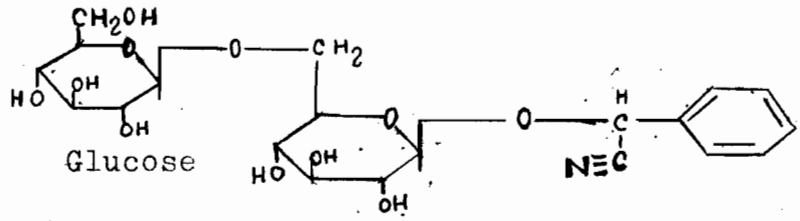
[1] R.P. Bates and P.R. Henson, "Studies of inheritance, photoperiodic response, and determination of tannin content of Lespedeza cuneata Don." J. Agron., Vol. 47, No. 11 (Nov. 1955), p. 503.

[2] R.K. Ibrahim and G.H.N. Towers, "The identification, by chromatography, of plant phenolic acids." Arch. Biochem. and Biophys. 87: (1960), pp. 125-128.

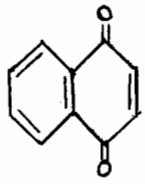
Figure 7. **Structural Formulae of some Compounds.**

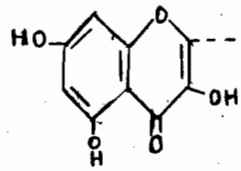


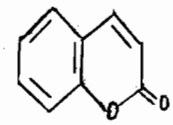
(a) **Aucubin** (revealed by the Ehrlich test.)



(b) **Amygdaloside** (revealed by the HCN test.)







(c) **Naphthoquinone (Test A)**

(d) **Basic Flavonol Structure (Test B)**

(e) **Coumarin (Test C)**

(revealed by the Juglone tests.)

B. Results

The present writer's results using members of the families and subfamilies studied are listed in the tables following; the individual species are shown in the appendix.

(1) Leuco-anthocyanin (test A)

All specimens tested from the six families gave consistently negative results. Only in the Cruciferae were there some doubtful specimens; these were Isatis glauca and I. tinctoria which developed a purple-brown colour, and so the true reaction to the L.A. test was undeterminable. On the other hand, Diplotaxis catholica and Peltaria allicea gave a pale pink colour. It was not a positive result, and its cause was uncertain. The other species tested gave either yellow or green colours with this test.

(2) HCl/Methanol Test.

Few species of the families examined were woody, hence this test was not applicable in most cases. However, a few woody members were obtained and as would be expected, all proved to be negative to the HCl/Methanol test.

(3) Ehrlich (test A).

General agreement among all the families tested was found for this test also. No member was found to be positive. The colours with Ehrlich reagent were mostly yellows, but a few

greys and greens were also noted. With ammonium hydroxide the specimens remained colourless or turned to a pale yellow. None suggested even a limited presence of Aucubin or related compounds.

(4) Hydrogen cyanide (HCN test A)

The results indicate that Hydrogen cyanide can be produced by three families; although they are predominantly negative to this test. In the Capparidaceae leaves of two or three species of Capparis and the tuber of Courbonia sp. were cyanogenic. In the Papaveraceae, very strongly positive species were observed in the genera Dendromecon, Eschscholtzia and Papaver; all of the Papaveroideae. Only Dicentra spectabilis was found to be positive in the Fumarioideae. In the Cruciferae, more positive species occurred in the Sinapeae than in the Hesperideae; and on the whole the Papaveraceae gave more strongly positive reactions than did the other families. One species of Reseda was positive, and none from Moringaceae or Tovariaceae, but only few specimens of these families were examined.

(5) Juglone (tests A-C).

Quinones and naphthoquinones appear to be completely absent from all of these families. In no case was an immediate or delayed development of a purple colour observed. Neither were any brilliant yellows noted. Nothing remarkable showed up on standing either. With ultra-violet light a few species produced

a pale blue fluorescence. This result was observed in Capparis cynophallophora of the Capparidaceae, Dicranostigma lactucoides, Eschscholtzia sp., Romneya coulteri, Corydalis sempervirens of the Papaveraceae, and Stanleya pinnata inyvensis, two Aethionema spp., Turritis glabra, Alyssum argenteum, Cheiranthus cheiri, Erophila verna, of the Cruciferae; as well as in Reseda luteola of the Resedaceae. In all cases the fluorescence was pale, though unmistakable. It is noteworthy that the ~~substance causing the~~ fluorescence can be produced by all these families. It was also observed in some seedlings.

(6) The Hot Water Test.

A slight difference between the Papaveraceae and the other families was revealed by this test. In the Papaveraceae several positive species were found (some of them gave an immediate reaction). In the other families there were a few doubtful positives (class lll reactions) but in no case was an immediate reaction observed.

The results of this investigation, were similar to those of other workers for all tests. It was found too, that the reactions of seedlings were in accordance with those of mature plants. Seeds tested for HCN did not indicate any greater frequency of cyanogenesis than was evident among the leaves of these plants. In general these tests appear to indicate no marked variations between the six families examined.

Tannic Acid Test.

From the survey of Tannic acid made by Mrs. P. Bahr, the Papaveraceae stands apart from the others. All members of the Papaveraceae tested were positive or contained at least a trace of Tannic acid. The other families tested were all negative, except for two species of the Capparidaceae; Crataeva sp., and Capparis jamaicensis.

Phenolic Acids.

Protocatechuic, Vanillic, Phenyl lactic and Syringic acids were absent from all the six families, except for a few traces (e.g. Eschscholtzia californica and Dicentra canadensis had a trace of Protocatechuic; while some Vanillic occurred in Aubrietia tauricola and Lepidium sativum. Phenyl lactic was observed in Eschscholtzia californica). On the other hand, all the families contained some Gentisic, Ferulic, Sinapic, Caffeic, Paracoumaric and Ellagic acids. These results of Mrs. P. Bahr compiled from chromatograms also indicate general similarity between the families being considered.

CHAPTER IV.

DISCUSSION

A. Analysis of Results

Bate-Smith has said that the distribution of leuco-anthocyanins seemed to substantiate somewhat Hutchinson's division of plants into 'Lignosae' (woody) and 'Herbaceae' (non-woody). We were therefore very curious (in view of his arrangement of the Rhoeadales) to see whether our results of leuco-anthocyanins suggested two classes of plants. In accordance with Hutchinson's hypothesis, Capparidaceae, Moringaceae, and Tovariaceae, which are developed along the woody side, should be positive for leuco-anthocyanins. Contrary to this expectation, however, all specimens tested from all six families were negative to the leuco-anthocyanin test, and no evidence to support two phyletic lines of development was observed. The only two questionable results, viz. Diplotaxis catholica and Peltaria alliacea, have not been reported as positive elsewhere. What caused the pale pink colour noted is still in doubt. Similarly in the Ehrlich test, all results obtained were negative. No pink colour was ever noted in any of the species tested, as might have been predicted for those species placed by Hutchinson in the Lignosae. The results of the Ehrlich test were consistent with those of the leuco-anthocyanin test, as was also true for the few HCl/Methanol tests performed. Development of a magenta colour in the Ehrlich test is closely correlated with a positive leuco-anthocyanin test.

Cyanogenetic glycosides are rather widely distributed in dicotyledons. Our results for seeds, seedlings and leaves indicated that hydrogen cyanide can and does occur to a limited extent in these families. Hegnauer found HCN in 12 species from 3 genera of Papaveraceae, and 15 species from 3 genera of the Cruciferae. Our results show that 6 species from 3 genera of the Papaveroideae were strongly positive. Only one (Dicentra spectabilis) from the Fumarioideae was observed to be positive. It was remarkable too, that members of the Papaveroideae gave more immediate and stronger positive reactions than did any of the others.

Juglone - a hydroxynaphthoquinone included in the class of phenolic substances - also was found to be absent from the species tested. This does not seem to suggest two groups of families. The pale blue fluorescence observed was not intense or brilliant enough to be caused by coumarins. Neither did coumarins appear on the chromatograms for phenolics. It is uncertain what trace compound was responsible for the fluorescence, however, all families, including seedlings showed an ability to produce it.

Differences between the Papaveraceae and the other families were noted in the Hot Water Test (and the Cigarette Test when it was applied). None of the other families examined gave a strongly positive (i.e. class I or II) reaction. In the tannic acid test the Papaveraceae and 2 species of Capparidaceae were positive. This is noteworthy, since Bate-Smith reported that all families of the Rhoeadales were without tannins. A few

Table VI Experimental Results of the Writer.

Families and Subfamilies	Leuco-anthocyanin test Gen/Sp. +	?	-	Ehrlich test Gen/Sp. +	?	-	HCN test Gen/Sp. +	?	-	Juglone test Gen/Sp. +	?	-	Bl Fl	Hot water test Gen/Sp.	I	II	III	IV	?	Tannic acid (Bahr's results) Gen/Sp. +	?	-
Capparidaceae																						
A.1 Dipterygioideae (1/1)																						
11 Capparidoideae (25/385)	4/5		4/5	4/5		4/5	4/5	1/1	4/4	4/5		4/5	1/1	3/4			1/1	2/3		2/2	2/2	
111 Koeberlinioideae (1/1)																						
1V Roydsioideae (1/1)																						
V Emblingioideae (1/2)																						
V1 Cleomoideae (12/273)	3/3		3/3	3/3		3/3	3/3		3/3	3/3		3/3		1/1				1/1		1/2		1/2
Moringaceae (1/3)	1/1		1/1	1/1		1/1	1/1		1/1	1/1		1/1		1/1				1/1		1/1		1/1
Tovariaceae (1/2)	1/1		1/1	1/1		1/1	1/1		1/1	1/1		1/1		1/1				1/1				
Cruciferae																						
A.1 Thelypodieae (28/155)	2/2		2/2	2/2		2/2	2/2		2/2	2/2		2/2	1/1	1/1				1/1				
11 Sinapeae (62/296)	29/50	1/2	28/48	27/52		27/52	27/57	5/5	20/50	28/47		28/47	2/3	20/39			1/1	20/38		25/35	3/4tr	23/31
111 Schizopetaleae (3/258)																						
IV Hesperideae (31/816)	19/25		19/25	17/22		17/22	17/26		17/26	18/23			18/23	16/17				16/16	1/1	17/36	1/1tr	16/35
Resedaceae (2/60)	3/8		3/8	3/8		3/8	3/6	1/1	3/5	3/4		3/4	1/1	3/5				3/4	1/1	1/2		1/2
Papaveraceae																						
1 Hypecoideae (2/16)	1/1		1/1	1/1		1/1	1/1		1/1	1/1		1/1										
11 Papaveroideae (24/356)	15/26		15/26	15/32		15/32	15/28	3/5	13/23	15/27		15/27	4/4	12/23	6/6	4/7	6/8	1/1	1/1	11/19	4/4	8/14tr	1/1
111 Fumarioideae (15/127)	4/9		4/9	4/9		4/9	4/9		4/9	4/9		4/9	1/2	4/6	1/1	1/1		2/3	1/1	6/12	3/8	3/3tr	1/1
Tropaeolaceae (1/50)	1/1		1/1	1/1		1/1	1/1		1/1											1/1		1/1

Bracketed numbers beside the subfamilies are the approx. genera and species included. (Engler & Diels)
Under the name of each test, is the number of genera and species tested. + are positive
? are doubtful
- are negative
Bl. Fl. Blue florescence

Table V11. Experimental Results (Bahr).

The occurrence of Phenolic acids, Acid hydrolyzate.

	Group A								Group B			
	Gen Sp. Tested	Gentisic acid	Ferulic acid	Sinapic acid	Caffeic acid	p-Coumaric acid	Ellagic acid	Protocatechuic acid	Vanillic acid	Phenyl lactic acid	Syringic acid	Phloretic acid
Capparidaceae												
11 Capparioideae	2/2	1/1	2/2	2/2	1/1	-	1/1				-	-
V Cleomoideae	1/1	-	1/1	1/1	1/1tr	-	-				-	-
Moringaceae	1/1	-	-	-	1/1tr	-	-	-	-	-	-	1/1
Tovariaceae	1/1	1/1	1/1	-	1/1	1/1	1/1	-	-	-	-	-
Cruciferae												
11 Sinapeae	13/16	5/5	10/10	7/7	6/6	1/1tr	5/5	-	1/1tr		-	-
IV Hesperideae	8/10	7/8	4/4	2/2	2/2tr	2/2	-	-	-	-	-	-
Resedaceae	3/5	2/3	3/3	3/3	-	2/2	3/3tr	-	-	-	-	-
Papaveraceae												
11 Papaveroideae	6/7	1/1	3/3	1/1	5/5	1/1tr	3/3	1/1	-	1/1	-	-
111 Fumarioideae	3/3	-	3/3	-	3/3	1/1tr	1/1	1/1	-	-	-	-

Table VIII

Summary of Appendix B (Table I)

List of all plants tested (by writer, Honeyman, Gibbs and others.)

	PLANTS																				SEEDS		
	HCL/Meth			L.A.			Ehrlich			HCN			Juglone			Hot Water					L.A.			Ehrlich			HCN			Juglone			HCN		
	+	?	-	+	?	-	+	?	-	+	?	-	+	test c bl. fluor.	-	I	II	III	IV	?	+	?	-	+	?	-	+	?	-	+	?	-	+	?	-	+	?	-
Capparidacae (40/660)																																					
A.2. Capparidoideae		3/4			4/5			4/5		2/2	1/2	8/9	1/1		4/6		1/1	2/3																		1/1
B.5. Cleomoideae					3/3			3/3				3/3						1/1			1/1			1/1			1/1			1/1					5/9
Total nos. tested		3/4			7/8			7/8		2/2	1/2	11/12	1/1		4/6		1/1	3/4			1/1			1/1			1/1			1/1						6/10
Moringaceae (1/3)		1/1			1/1			1/1				1/1			1/1			1/1																		1/1
Tovariaceae (1/2)					1/1			1/1				1/1			1/1			1/1																		1/1
Papaveraceae (28/600)																																					
1. Hypecoideae					1/1			1/1				1/1			1/1																						1/3
2. Papaveroideae		2/4			15/26			15/27		3/6		13/23	3/3		15/27	6/6	4/8	5/9	3/4	1/1		1/1	4/5		4/5		3/3			* 3/5						9/31
3. Fumarioideae					4/8			4/9		1/1		4/9	1/1		4/9	1/1	1/1	1/1	4/7				1/2		1/2					1/2						4/10
Total nos. tested		2/4			20/35			20/37		1/7		18/33	4/4		20/37	7/7	5/9	6/10	7/11	1/1		1/1	5/7		5/7		3/3			4/7						14/44
Cruciferae (200/1900)																																					
1. Thelypodieae					1/1			1/1				1/1	1/1		1/1			1/1				1/1		1/1			1/1			1/1			1/1			2/2
2. Sinapeae		2/3			29/49			28/46		6/6	1/1	25/36	3/3		26/45			21/39				18/34			18/34	1/1		17/33		18/28	9/9	1/1	24/64			
3. Schizopetaleae																																				1/1	
4. Hesperideae		1/1			18/22			15/20				19/26	3/3		16/21			12/16				9/13			8/12			7/10		7/11			34/53			
Total nos. tested		4/4			48/72			44/67		6/6	1/1	45/90	7/7		43/67			34/56				28/48			27/47	1/1		25/44		26/40	10/10	1/1	61/120			
Resedaceae (6/60)					3/4			3/7		1/1		3/5	1/1		3/4			3/5				3/7			3/7	1/1		3/4		3/7			1/4			
Tropaeolaceae (1/50)					1/1			1/1				1/1	1/1					1/1				1/1			1/1			1/1		1/1			1/3			

Fraction beside each family indicates the genera/species included by Willis.

* Blue fluorescence in 2/2 genera and species. No doubtfuls in juglone test of plants.

Table IX. Chemical Constituents and Family Relations of the Rhoeadales.

The families are grouped according to Hutchinson (1959), but the strong line indicates a more desirable division.

Families	LICNOSAE			HERBACEAE			
	Capparidales			Rhoeadales			
Constituents	Capparidaceae	Moringaceae	Tovariaceae	Cruciales		Resedales	
				Cruciferae	Resedaceae		Papaveraceae	Fumariaceae
Cyanogenetic glycosides	-	-	-	-	-	-	-	-
Catechol tannins	-	-	?	-	-	-	-	?
Leuco-anthocyanins	-	-	-	-	-	-	±	-
Aucubin + rel'd compd.	-	-	-	-	-	-	-	-
Naphthoquinones	-	-	-	-	-	-	+	+
Polyphenolases	-	-	-	-	-	-	(+)	(+)
Tannic acid	+	+	+	+	-	-	+	+
Gr. A. Phenolic acids	+	+	+	-	-	-	(+)	(+)
Gr. B. Phenolic acids	-	-	-	-	-	-	+	+
ALKALOIDS								
Hydroisoquinoline	-	-	-	-	-	-	+	+
Benzyl isoquinoline	-	-	-	-	-	-	+	-
Cryptopine	-	-	-	-	-	-	+	+
Morphine	-	-	-	-	-	-	+	+
Unknown constitution	-	-	-	-	-	-	+	+
Synthetic isomerides	-	-	-	-	-	-	-	-
Phthalide isoquinoline	-	-	-	-	-	-	+	-
-Naphthaphenanthrene	-	-	-	-	-	-	+	+
Hydroprotoberberine	-	-	-	-	-	-	+	+
Aporphine	-	-	-	-	-	-	+	+
Minor Corydalis	-	-	-	-	-	-	+	+
FATTY ACIDS								
Sat'd acids	?	+	-	+	+	+	+	+
Oleic	+	+	-	+	+	+	+	+
Eicosenoic	-	+	-	-	-	-	-	-
Erucic	-	+	-	-	-	-	-	-
Ricinoleic	-	+*	-	-	-	-	-	-
Hydroxy C_{20}	-	+*	-	-	-	-	-	-
Linoleic	+	+	-	+	+	+	+	+
Linolenic	+	+	-	(+)	+	+	+	+
Seed oils	+	+	-	+	+	+	+	+
Seed proteins	?	+	-	+	+	+	+	+
Myrosin	+	+	-	-	-	-	+	-
Thioglycosides	+	+	-	-	-	-	+	+
Crystals	-	+	-	-	-	-	-	-
Raphidesids	-	-	-	-	-	-	+	+
δ-acetyl ornithine	-	-	-	-	-	-	-	-
Saponins	-	-	-	-	-	-	-	-

* Applies to Lesquerella species only.

Key

+ occurrence

(+) trace occurrence

\- absent

? no record

traces were noted also in the Cruciferae. However, all families except for Papaveraceae and the two species of Capparidaceae mentioned above were negative to the tannic acid test. There is a possibility that the colour reaction which formed the basis of our results was affected by some compounds other than tannic acid, for example alkaloids.

For easy discussion of the phenolic acids, I have grouped them as follows: A, includes gentisic, ferulic, sinapic, caffeic, para-coumaric and ellagic acids. Group B has protocatechuic, vanillic, phenyl lactic and syringic. Phloretic acid occurred abundantly in Moringa oleifera, but as this was the only occurrence, this acid has not been included in either group. Group A acids were present in all six families, while group B ones were for the most part absent. The importance of biosynthesis is revealed especially in considering phenolic acids, tannins and other phenolic compounds. The presence of sinapic acid for instance, assumes more significance in this respect as the derivative of trihydroxycinnamic acid. Also the interrelationships of the compounds needs careful study as to their probable interference in chemical reactions. Table VI shows a summary of results of tests from all sources, that contributed to this paper.

B. Chemical Characters of the Families

Table IX shows the occurrence of various constituents in these families. An examination of this table suggests that all six families are similar due to the absence of condensed tannins and leuco-anthocyanins, the rarity of hydrocyanic glycosides, the absence of the naphthoquinones and group B phenolic acids. The presence of seed proteins, oily seeds, and amounts of saturated acids, oleic and linoleic acids also make them appear similar. Discrepancies occur in the hot water test where the Papaveraceae show a tendency to be positive, and may therefore contain polyphenolases. The Papaveraceae also are distinguished by positive tannic acid test, alkaloids, amino acids and saponins.

Now we may consider some of the questions posed in the introduction as problems.

How does chemical evidence of such complexity influence the older opinions of family relationships? Table IX shows the families arranged according to Hutchinson. In it Papaveraceae and Fumariaceae differ in their chemical characters from the rest of the group. Cruciferae and Resedaceae show a similarity to the Capparidaceae, Moringaceae and Tovariaceae. The Papaveraceae and Fumariaceae are set apart from the other families by their alkaloids, and the absence of thioglucosides. Frohne reported that in his "gel diffusion" technique, (electrophoresis) the Papaveraceae and Fumariaceae were positive, but members of the Cruciferae,

Capparidaceae, Resedaceae, Moringaceae and Tovariaceae, also Tropaeolum majus and Viola tricolor were all negative. The strong line in Table IX would therefore indicate a more desirable division of the families. There seems to be little evidence of dissimilarity between the woody and herbaceous groups according to Hutchinson. However, Bate-Smith[1] has expressed the opinion, that the groups at the ends of two lines of development could be similar, despite their different paths of evolution, and if there were no transitional forms, the different phylogeny would be unrecognizable. He felt that the association of sinapic acid with a reduction in woodiness of plants could be interpreted as an example of a retention in a transitional form. He felt that this type of evidence might be useful in tracing the affinities of the Cruciferae and Capparidaceae, which contained abundant sinapic acid, but were not associated with woody families. Another question which we raised was whether the chemical characters of the Papaveroideae and the Fumarioideae were sufficiently distinct to merit their being classified as separate families. Contrary to the Englerian idea of homogeneity, Hutchinson states that the relation between the Fumariaceae and Papaveraceae is "more apparent than real", and accordingly he made them separate families of

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[1]Bate-Smith, "The phenolic constituents of plants & their taxonomic significance", J. Linn. Soc. (Bot.), 58, 371, P. 170.

his order Rhoeadales. He included Hypecoum and Pteridophyllum in Fumariaceae. Manske found no Papaveraceae (in the wide sense) investigated to be completely devoid of alkaloids, and protopine occurred in every papaveraceous plant he investigated. In the Papaveroideae 16/32 genera and species had protopine, while in the Fumarioideae 4/46 genera and species had it. Our results showed the Papaveroideae to be positive for tannic acid, and the Fumarioideae had only traces. Also in the HCN test 3/5 genera and species of Papaveroideae were strongly positive, but only one species of Fumarioideae (Dicentra spectabilis) was observed to be positive. These points could be used in favour of a separation. δ-acetyl ornithine has been thought to be a taxonomic index ranking with protopine. Reuter found this compound restricted to the Fumarioideae. Frohne reported that from his serological data, Papaveraceae and Fumariaceae were closely related, and should be grouped together in an order Papaverales. Further investigation is still needed to clarify the closeness of the relationship between the Papaveraceae and Fumariaceae, but at present indications favour separation of families. Hegnauer suggested that phytochemistry supports inclusion of the Papaveraceae in the Polycarpicae. Protopine, chelidonine and sanguinarine are the alkaloids characteristic of the Papaveraceae as a whole, and their closest relatives would be the Nymphaeaceae and Berberidaceae.

A discussion of affinities must also include mention of the great similarity between the Cruciferae and Tropaeolaceae.

These families, one in the Geraniales and the other in the Rhoeadales both have large amounts of erucic and eicosenoic acids, mustard oil glycosides, and are similar due to absence of certain constituents, like leuco-anthocyanins, tannic acid, polyphenolases. This congruence warrants a detailed investigation, as there is still the possibility of misplacement of the family of Tropaeolaceae in Geraniales, although most taxonomists consider this to be a classical case of parallel evolution.

The use of chemical characters as supplementary aids in comparative taxonomy is becoming more recognized as research goes on. The abundance of criteria available is likely to surpass those of a morphological taxonomy. Its application is still limited however, as both taxonomists and chemists are still groping to pool their knowledge and improve communication between them. The increasing literature on the subject indicates a growing interest in this field of study. Chemotaxonomy offers a challenge by its very magnitude and complexity, and although IBM machines and numerical systems[1] of classification may produce results more quickly, the lure of greater knowledge and understanding of nature and the reward of some discovery, however small, will continue to fascinate and thus stimulate this kind of research.

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[1]R. Sokal and P.H.A. Sneath, Principles of Numerical Taxonomy, (San Francisco, Lond.: Freeman & Co.), 1963.

CHAPTER V

CRITICISM

Perusal of the literature has revealed that communication between the various branches of scientific research is inadequate. Genetical findings, chemical advances in technology and botanical data are garnered often without thought of their usefulness in other fields of study. Hence duplication of effort and slower progress result.. The sciences are divided too, by different terminology,and specialization often loses sight of its holistic frame of reference. Hegnauer describes the dilemma this way[1].

Linne's opinion (1751) that plants related through form are as a rule also similar as to their content matter was hitherto inadequately considered in taxonomy. For this there are different reasons, mainly methods in systematics and phytochemistry are entirely different, each has its own terminology and literature. Chemical institutes usually have no taxonomical periodicals and herbariums only little phytochemical literature. Phytochemical works are of lesser usefulness to the systematist because they usually report facts with inadequate interpretation and chemotaxonomical discussion. Chemical analysis of plants is younger, more time-consuming and expensive than morphological

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[1] R. Hegnauer, Chemotaxonomie der Pflanzen, (Birkhauser, Basel und Stuttgart,) 1962, Vol. 1, Vorwort. (Preface).

analysis. Knowledge of the former is therefore much more incomplete. The systematist is able to distinguish sharply between analogy and homology. This is more difficult on phytochemists.. Structurally similar matter may develop in different ways in different families. Compounds of little relationship to the structural chemist may be produced in plants in similar biosynthetical ways. Conclusions as to their value in systematics is often only possible after clarifying the biogenesis of the compounds.

The scope of this research was limited by the time available for completion, as well as the fact that many of the plant species were unavailable. It was necessary therefore to make assumptions on the basis of relatively small samples. Such evidence though useful for specific purposes may be inconclusive as to its general validity. A survey of chemical characters by a few simple tests, such as was carried out in this project is a very useful method of approach. However many times it would have been invaluable to pursue further some compound revealed by a colour or other reaction. For example, in the case of the questionable reaction of Diplotaxis catholica and Peltaria alliacea to the leuco-anthocyanin test a follow-up by chromatography or other method of analysis could have provided valuable information like the possibility that the colour reaction could perhaps have been produced by some unknown compound. A clarification why some of our results for tannic acid conflicted with those of Bate-Smith would also have been very interesting.

Similarly, the compounds which produced positive reactions to the Hot Water Test may have merited further investigation.

There still remain some gaps in the knowledge of the comparative chemistry of the Rhoeadales. Little is yet known about Bretschneidera [illegible]. No specimens were available to me and no recent results from others were noted. Also, the anatomical homology (if any) between myrosin cells and the lactiferous cells and canals of the Papaveraceae may need further clarification. An histochemical survey of the members of the Rhoeadales would help toward that goal, and it was unfortunate that I could not make such a study concurrently. A study of the biosynthesis of sinapine[1] has already been done, but further examination of other compounds might help to increase the scope of comparative phytochemistry of this order.

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[1] A. Tzagoloff, "Metabolism of sinapine in mustard plants. I Degradation of sinapine into sinapic acid and choline," Plant Physiol. 38, (1963), pp. 202-206.

CHAPTER VI

CONCLUSIONS

Hegnauer's opinion about the classification of the order Rhoeadales was supported by our results. He suggested that the separation of the Papaveraceae from the Rhoeadales and their direct derivation from the Ranales (according to Hutchinson) appeared to be justified, since the Papaverales were close to the Ranales through oily seeds with endosperm, and tetrahydroisoquinoline bases. Their alkaloids were another factor which favoured a separation. The other families, Capparidaceae, Cruciferae, Resedaceae (perhaps also Moringaceae and Tovariaceae) appeared chemically closely related, having similar seed oils, myrosin and mustard oils spread throughout them. The lack of leuco-anthocyanins in the species of Capparidaceae and in the Moringaceae tested seems to militate against including these families in the Lignosae (according to Hutchinson) where leucoanthocyanins are widely spread. The chemical characters are in the best harmony with the delimitation of Takhtajan, viz. Papaverales (with Papaveraceae, Hypecoaceae and Fumariaceae; or Papaveraceae s.l.), and Capparidales (with Capparidaceae, Moringaceae, Resedaceae, Tovariaceae, Cruciferae). Further clarification is needed on the derivation of these groups.

SUMMARY

A review of the historical and recent literature revealed a diversity of opinions about the family relationships and hence classifications of the order Rhoeadales.

The purpose of this paper was to determine some of the chemical characters of the families concerned, by some simple chemical tests plus a literature survey, in an effort to gather more data on the problems of divergent views about the classification of the families of this order, and to contribute some findings to the already existing knowledge about the comparative chemistry of the Rhoeadales.

Phytochemistry was found to be valuable as a supplementary aid to taxonomy, and in this paper, its use has provided data substantiating differences between the Papaveraceae, Fumariaceae and the other families of the Rhoeadales, as suggested by Takhtajan. An arrangement into two orders, Papaverales and Capparidales, as proposed by him, seems to be supported by the findings of this study. Further research may help to clarify the ancestry of these groups.

APPENDIX A.

Methodology

1. Leuco-anthocyanin Test A.

Fresh material from mature leaves was used. In the case of seedlings and species with tiny leaves, the stem and bits of petiole were also included. The leaves were chopped finely with scissors. When the leaves were reduced to pieces of approximately a millimeter in size, about 0.5 grams of this material was put into a small test tube and 5 mls of 2N HCl were added. The tube and contents were heated in a boiling water bath for 20 minutes. The tube was then removed from the bath and allowed to cool. Then 5 mls of amyl alcohol were added and covering the tube with a thumb, the tube was shaken vigorously so that the contents were well mixed. The colours, if any developed, were noted, after which the tube was covered with aluminum foil and left overnight. The next day the colour of the amyl alcohol layer was recorded. A bright cherry red colour was a positive result, while browns and other colours were negative.

In order to check the reagents and technique, as well as to have a colour reference, a known positive specimen (e.g. Grevillea robusta) was included in each batch of tests as a control.

2. Ehrlich Test.

Fresh leaves were chopped very finely and about 0.5 grams were placed in a small test tube. A few drops of 50% aqueous ethanol were added and the material stirred with a glass rod. This was done carefully to avoid removing too much chlorophyll from the leaves. The tube was then placed in a boiling water bath and the contents stirred occasionally. Additional alcohol was added to replace that lost by evaporation, but the liquid was maintained at a minimum. When a concentrated extract was obtained, the tube was removed from the bath, cooled, covered with foil and left overnight, or for some hours. The next day the tube contained a moist mass, with liquid visible only when pressed with a glass rod.

For each specimen tested, a piece of filter paper (Whatman's No. 1; 7 cms. diameter) was needed. On this paper the identity of the plant was written. Then it was supported around the edges by clean inverted test tubes, and three spots of extract, about dime size, were made with a glass rod along the diameter of the paper. The spots were labeled "1, 2, 3" and the paper hung up to dry.

Test A. Spot No. 1 was left untouched, but to No. 2 a drop of Ehrlich control reagent (conc. HCl and 95% ethanol in the same proportions as in Ehrlich reagent) was added. Ehrlich reagent (a mixture of 1 gram p-dimethylamino-benzaldehyde: 5 mls conc. HCl: 200 mls 95% ethyl alcohol) was added to the third

spot in the same amount as for the control. The filter paper was allowed to dry, and any colours which developed were noted. To intensify these colours, or bring out some which did not appear in the cold, the paper when dry was placed in an oven at 100°C for one minute. Plants which are positive for this test, i.e. have aucubin or similar compounds, develop a deep blue colour in spot No. 3, which has the Ehrlich reagent. A brown colour develops in the control spot (No. 2). A magenta colour usually develops in spot 3 if leucoanthocyanins are present.

Test B. After the paper had been removed from the oven and allowed to cool, a drop of ammonium hydroxide was added to spot No. 1 and the colour noted. In species giving a positive reaction to Ehrlich's reagent, a bright yellow colour developed, as for example in Globularia spp. and Plantago spp. which were used as controls.

3. HCN Test A.

Special tubes with tightly fitting ground glass stoppers were used in this test. Picric acid paper, prepared by soaking Whatman's filter paper No. 1 in picric acid and hanging it to dry, was cut into small strips of wedge shape (1.25 x 0.5 inches). A stock of these were kept in a slightly moisturized brown glass bottle. To test for hydrogen cyanide, a wedge of picric acid paper was attached to the base of the glass stopper by means of melted wax. A small amount of leafy material (about 1 gram) was ground in a mortar with a few drops of water. Then a pinch

of commercial emulsin was added and grinding continued. Finally two or three drops of chloroform were added to the mixture. When a smooth consistency was obtained, the semi-liquid mass was poured into the test tube. Care was taken to direct the mixture into the tube without contaminating the sides and mouth of it. The mouth of the tube was then wiped clean with a little water as the wet surface ensured a tight seal. The picric acid paper was dipped horizontally into a petri dish of 10% sodium carbonate solution, and the excess liquid was removed by bringing the paper into contact with some dry filter paper. The stopper was then inserted. In a strongly positive reaction the yellow test paper should change to orange or dull red-brown within a few minutes. A negative reaction was recorded when no change had occurred after a week. Passiflora caerulea was used as a control with each batch of specimens tested.

4. Juglone Test A.

About 2 grams of chopped leaves, petiole and stems (in some cases bark was also tested) were placed in a large test tube with a ground glass stopper, and chloroform was added, so that it just covered the leaf material. The tube was covered and the steeping proceeded overnight. The next morning the mixture was filtered and the filtrate evaporated to dryness in a boiling water bath. The tube was inverted and the residue allowed

to cool. After this, the residue was dissolved in 5 ccs of ether, and then 5 ccs of aqueous ammonium hydroxide were added and the tube well shaken. The colour in the aqueous layer was noted. An immediate development of a purple colour would indicate the presence of juglone or related naphthoquinones and is therefore a positive reaction. A bright yellow colour may be due to flavones.

Test B. The mixture was allowed to stand for a few hours after Test A and any colour which then developed was noted.

Test C. The aqueous layer was examined for fluorescence under ultra-violet light. Brilliant fluorescence may be indicative of coumarins and other substances.

5. The Hot Water and Cigarette Tests.

The temperature of a water bath was brought to 85°C and care was taken to maintain it between 85°C and 90°C while tests were being made. A leaf was plunged part way into the water, and held there for about 5 seconds and then withdrawn. A dark band which formed immediately along the water line dividing the immersed and exposed parts, was classed as strongly positive, and recorded as I. A slower development is II, while a doubtful colour or very slow reaction was noted as III. No colour development within 30 minutes indicated a negative reaction and was classified as IV. For the oxalis reaction, refer to page 84.

The Cigarette Test is very similar to the Hot Water Test, and the results are graded and recorded in like manner. In this test, however, a glowing cigarette was held against the back of a leaf for about three seconds. A dark ring develops around the area of contact in positive specimens. Hedera helix was used as a control for these tests.

APPENDIX B, Table 1. A list of all plants tested (by the writer, Honeyman, Gibbs and others.)

	PLANTS																				SEEDLINGS												SEEDS		
	HCL/Meth			L.A.			Ehrlich			HCN			Juglone			Hot Water					L.A.			Ehrlich			HCN			Juglone			HCN		
	+	?	-	+	?	-	+	?	-	+	?	-	+	?	-	I	II	III	IV	?	+	?	-	+	?	-	+	?	-	+	?	-	+	?	-	+	?	-
Capparidaceae																																					
A.1 Dipterygiodeae																																					
11 Capparidoideae																																					
Apophyllum anomalum												-																									
Boscia foetida												-																									
Cadaba juncea												-																									
* Capparis cynophallophora			-			-			-			-			**				-																		
ferruginea			-								?				-					?																	
flexuosa																																					-
* jamaicensis						-			-	+					-				-																		
lasiantha												-																									
mitchelli											?																										
nobilis										+																											
Courbonia sp.																																					
* Crataeva sp.			-			-			-			-			-			+																			
* Maerua sp.						-			-			-			-				-																		
* Steriphoma elliptica			-			-			-			-			-																						
111 Roydsioideae																																					
1V Emblingiodeae																																					
B.V Cleomoideae																																					
Cleome aculeata																																					-
gigantea																																					-
spinosa																																					-
* sp.						-			-			-			-				-																		
trachysperma																																					-
* violacea																					-		-		-		-									

* Plants tested by the writer

** Blue fluorescence in the Juglone test

\+ positive

? doubtful

\- negative

(+) weak positive

c result of the Cigarette test

Table 1. (cont'd)

	PLANTS										SEEDLINGS					Seeds
	HCl/Meth	Leucoantho	Ehrlich	HCN	Juglone	Hot Water					L.A.	Ehrlich	HCN	Juglone	HCN
	+ ? -	+ ? -	+ ? -	+ ? -	+ ? -	?	I	II	III	IV	+ ? -	+ ? -	+ ? -	+ ? -	+ ? -
Dactylaena micrantha															–
* Gynandropsis gynandra		–	–	–	–										–
speciosa															–
Pedicellaria pentaphylla															–
Polanisia graveolens															–
* icosandra		–	–	–	–										
Papaveraceae															
1 Hypecoideae															
* Hypecoum grandiflorum		–	–	–	–										–
Leptocaroum															–
procumbens															–
11 Papaveroideae															
Argemone alba															–
* mexicana		–	–	–	–					–	–	–	–	–	–
" v. ochroleuca															–
platyceras															–
var. rosea															–
Bocconia frutescens	–		–	–	–					+					
*Cathcartia villosa		–	–	–	–		+								
*Chelidonium majus		–	–	–	–		+			–	–	–	–		–
majus v. laciniatus															–
* Dendromecon harfordii															
rhamnoides		–	–	+	–					+					
* rigidum		–	–	+	–					+					
* Dicranostigma lactucoides		–	–	–	** –		+								
Eschscholtzia caespitosa				+		?									
* californica				+							–	–		–	
* sp.		–	–	+	** –										
* Glaucium corniculatum		–	–	–	–					+					–
" v. rubrum		–	–	–	–					+					
flavum										+					
* Macleya cordata		–	–	–	–		+								–
Meconopsis betonicifolia															–
cambrica															–
grandis															–
* horridula		–	–	–	–										

Table 1. (cont'd.)

	PLANTS																						SEEDLINGS										Seeds		
	HCl/Meth			Leuco-antho			Ehrlich			HCN			Juglone			Hot Water						L.A.			Ehrlich			HCN			Juglone			HCN		
	+	?	-	+	?	-	+	?	-	+	?	-	+	?	-	?	I	II	III	IV		+	?	-	+	?	-	+	?	-	+	?	-	+	?	-
* Meconopsis nepalensis						-			-			-			-			+																	
* regis						-			-			-			-			+																	
Papaver aculeatum																																			-
* alpinum						-			-			-			-																				-
argemone																				-															
atlanticum																																			-
commutatum																																			-
* dubium						-			-			-			-				+																-
glaucium																																			-
heldreichii																																			-
hybridum																																			-
monanthum																																			-
* nudicaula						-			-	+					-		+																		
* orientale						-			-			-			-			+						-			-							-	-
pavoninum																																			-
* pilosum						-			-			-			-			+																	-
* pyrenaicum						-			-			-			-				+				-											**	
radicatum																																			-
rhoeas																		+																	-
rupifragum																																			-
* somniferum						-			-			-			-					-		?			-			-						**	-
" v. polycephalum															-																				-
triniifolium																																			-
* Platystemon californicus						-			-			-			-	+																			
* Romneya coulteri			-			-			-			-			-	**		+																	-
* hybrida			-			-			-			-			-			+																	
* trichocalyx			-			-			-			-			-				+																-
* Sanguinaria canadensis						-			-			-			-			+																	-
* Stylophorium diphyllum						-			-			-			-	+																			-
III Fumarioideae																																			
* Adlumia fungosa						-			-			-			-			+																	-
Corydalis capnoides																				-															
cava																			+																-
cheilanthifolia																																			-
* glauca						-			-			-			-					-			-			-			-					-	-
" v. alba																																			-

Table 1. (cont'd.)

	PLANTS																		SEEDLINGS								Seeds		
	HCl/Meth			Leuco-antho			Ehrlich			HCN			Juglone			Hot Water					L.A.			Ehrlich			HCN			Juglone			HCN		
	+	?	-	+	?	-	+	?	-	+	?	-	+	?	-	?	I	II	III	IV	+	?	-	+	?	-	+	?	-	+	?	-	+	?	-
Corydalis lutea						-			-			-			-		+																	-
* " ochroleuca						-			-						xx -					-				-										-
* " sempervirens																																		-
" siberica						-			-			-			-																			-
* " thalictrifolia												-																						-
Dicentra canadensis						-			-			-			-																			-
* " cucullaria						-			-			-			-																				
* " eximia										+																								-
" formosa						-			-			-			-																				
" spectablis										+																								-
* Fumaria capreolata						-			-			-			-																				
* " muralis						-			-			-			-																				
" officinalis																																		-
Cruciferae																																		
A. 1 Thelypodieae																																		
* Heliophila amplexicaulis L.f																						-			-			-			-	+		
" longifolia D.C.																																		-
* Stanleya pinnata inyvensis					-			-			-			xx -					-														
Streptanthus cordatus Nutt.																																		-
- 11 Sinapeae																																		
* Aethionema antitaurus					-			-			-			xx -																				
* " grandiflorum					-			-			-			xx -																				
" stylosum D.C.																																		-
Alliaria officinalis																																		-
Arabidopsis thaliana																																		-
Armoracia lapathifolia																																	(+)	
* Barbarea intermedia					-			-			-			-					-			-			-			-			-			
* " longirostis					-			-			-			-					-			-			-			-			-			
* " vulgaris R.Br.					-			-			-			-					-			-			-			-			-			-
" " v. arcuata																																		-
" v. sylvestris																																		-
Biscutella auriculata L.																																		-
* " ciliata					-			-			-			-					-			-			-			-			-			
* " didyma					-			-			-			-					-			-			-			-			-			
* " laevigata					-			-			-			-					-			-			-			-			-			

Table 1. (cont'd.)

		PLANTS										SEEDLINGS								Seeds	
		HCl/Meth			Leuco-antho			Ehrlich			HCN			Juglone			Hot Water				L.A.			Ehrlich			HCN			Juglone			HCN		
		+	?	-	+	?	-	+	?	-	+	?	-	+	?	-	?	I	II	III	IV	+	?	-	+	?	-	+	?	-	+	?	-	+	?	-
	Brassica alba Boiss.																																		-
	campestris L.										-																								-
	cheiranthos																																		-
	chinensis										-																								
	erucastrum																																		-
	fructiculosa																																		-
	hirta																																		-
	juncea										-																								-
	" v. crispifolia				-			-			-			-			-					-			-			-						
	kaber																																		-
	" v. pinnatifida																																		-
	napobrassica										-																								
*	napus L.										-																								
*	napus L. v. oleifera				-			-			-			-			-					-			-			-			-			-
*	nigra Koch.				-			-			-			-			-					-			-			-			-			-
*	oleracea L.				-			-			-						-					-			-			-			-			-
	" L.v. acephala										-																								
	" L.v.bullata										-																								
	L.v. botrytis										-																								
	"L.v. capitata										-																								
	" v. gemmifera										-																								
	" v. gongylodes										-																								
	" L.v. italica										-																								
	" v. sylvestris																																		-
	" v. tronchuda										-																								
	oxyrrhina																																		-
	pekinensis										-																								
*	rapa				-						-						-					-			-			-			-			
	" L.v. esculenta																c																		
	" L.v. oleifera																																		-
	sinapistrum Boiss.																c																		
	sylvestris																																		-
	verna										-																								

Table 1. (cont'd.)

	PLANTS																SEEDLINGS										Seeds		
	HCl/Meth			Leuco-antho			Ehrlich			HCN			Juglone			Hot Water					L.A.			Ehrlich			HCN			Juglone			HCN		
	+	?	-	+	?	-	+	?	-	+	?	-	+	?	-	?	I	II	III	IV	+	?	-	+	?	-	+	?	-	+	?	-	+	?	-
* Cakile edentula (Bigel.) Hook.																																+	?	
v. lacustra																																		
* maritima						–			–			–			–																			–
Calepina corvini																																		
* Cardamine aliara						–			–			–			–				–															
bulbifera (R.Br.)																			c–															
* bulbosa						–			–						–																			
dictyosperma Hook.										+																								
* hirsuta L.																					–			–			–							–
impatiens																			c–															
* lyrata submerga									–			–			–																			
* pensylvanica						–			–						–																			
* pratensis L.						–			–		?				–				+		–			–			–			–				
Cochlearia armoracia L.																			e+															
* danica						–			–			–			–				–		–			–			–			–				
groenlandica L.																																		–
officinalis						–						–							–		–			–			–							–
Coronopus procumbens																																		–
* squamatus						–			–	(+)																								
* Crambe abyssinica						–			–			–			–				–		–			–			–			–				
cordifolia																																		–
* hispanica Linn.						–			–			–			–				–															–
maritima Linn.												–																						
tatarica v. pinnatifida																																		–
Dentaria diphylla Mich.										(+)																								
* Diplotaxis catholica						–			–			–			–						–			–			–			–				
erucoides																																		–
* tenuifolia DC.						–			–			–			–				–															
* Eruca sativa Mill.						–			–			–			–				–		–			–			–			–	(+)			
* Erysimum aurantiacum						–			–			–			–				–		–			–			–							
* cheiranthoides L.						–			–			–			–				–															–
hieraciifolium																			c–															–
inconspicuum																																		–
linifolium																																		–
perofskianum																																		–

Table 1. (cont'd.)

	PLANTS					SEEDLINGS					Seeds
	HCl/Meth	Leuco-antho	Ehrlich	HCN	Juglone	Hot Water	L.A.	Ehrlich	HCN	Juglone	HCN
	+ ? -	+ ? -	+ ? -	+ ? -	+ ? -	? I II III IV	+ ? -	+ ? -	+ ? -	+ ? -	+ ? -
Erysimum pumilum											-
* repandum		-	-	-	-	-	-	-	-	-	
* Hirschfeldia incana		-	-	-	-	-	-	-	-	-	-
Iberis affinis				-							
amara						c -					-
coronaria											-
correaefolia											-
gibraltarica				-		-					
pinnata											-
* sempervirens	-	-	-	-	-	-					
welwitschii											-
* Ionospidium acaule		-	-	-	-		-	-	-	-	
Isatis djurdjurae											+
* glauca		-	-	-	-	-	-	-	-	-	
* tinctoria L.	-	-	-	-	-	-	-	-	-	-	-
Lepidium affina											-
* campestre (L.) R.Br.							-	-	-	-	-
densiflorum Schrad.											-
draba L.				+		c -					
graminifolium L.											(+)
* latifolium		-					-	-	-	-	-
menziesii											-
perfoliatum											-
* sativum		-	-	-	-	-	-	-	-	** -	+
* virginicum		-	-	-	-	-	-	-	-	-	
* Lunaria annua L.		-	-	-	-	-					-
rediva L.						c -					-
* Myagrum perfoliatum		-	-	-	-	-					-
* Peltaria alliacea		-	-	-	-	-					(+)
* turkmena		-	-	-	-	-					
* Raphanus caudatus L.							-	-	-	-	-
raphanistrum L.				-		c -	-	-	-	-	-
sativus L.v. longipinnatus											-
" v. niger						c -					
* Roripa nasturtium-aquaticum		-	-	-	-	-	-	-	-	-	

Table 1. (cont'd.)

	PLANTS					SEEDLINGS					Seeds
	HCl/Meth	Leuco-antho	Ehrlich	HCN	Juglone	Hot Water	L.A.	Ehrlich	HCN	Juglone	HCN
	+ ? -	+ ? -	+ ? -	+ ? -	+ ? -	? I II III IV	+ ? -	+ ? -	+ ? -	+ ? -	+ ? -
⁎ Roripa sylvaticum		−	−		−						
⁎ Sinapis alba		−	−	−	−	−	−	−	−	−	
Sisymbrium altissimum L.				−							−
assoanum											−
austriacum											−
loeselii						c−					
⁎ officinale Scop.		−	−	+	−	−	−	−	−	−	
⁎ Succowia balearica		−	−	−	−	−					(+)
Texiera glastifolia											−
Thlaspi alpestra L.											−
⁎ arvense L.		−	−	+	−	−	−	−	+	−	+
⁎ Turrtiis glabra		−	−	−	⁎⁎ −	−					−
⁎ Vella pseudo-cytisus	−	−	−	−	−	−					
spinosa Boiss.											−
3. Schizopetaleae											
Schizopetalon walkeri											−
4. Hesperideae											
Alyssum alpestre Linn.											−
⁎ argenteum		−	−	−	⁎⁎ −		−	−		⁎⁎ −	−
granatense											+
maritimum											−
⁎ saxatile L.		−	−	−	−	−	−	−	−	−	−
spinosum											−
Anastatica hierochuntina											−
⁎ Arabis alpina L.		−	−	−	−	−	−	−	−	−	−
bellidifolia											−
⁎ diversicarpa		−	−	−	−	−	−	−	−	−	
glabra											−
⁎ holboellii											−
laevigata											−
verna											−
⁎Aubrietia deltoides		−		−		−					−
⁎ hybrids							−	−	−	−	
⁎ Berteroa incana (L.) DC.		−	−	−	−	−	−	−	−	−	−
Braya linearis											−

Table 1. (cont'd.)

	PLANTS					SEEDLINGS									Seeds
	HCl/Meth	Leuco-antho	Ehrlich	HCN	Juglone	Hot Water					L.A.	Ehrlich	HCN	Juglone	HCN
	+ ? -	+ ? -	+ ? -	+ ? -	+ ? -	?	I	II	III	IV	+ ? -	+ ? -	+ ? -	+ ? -	+ ? -
Bunias erucago L.															–
orientalis L.															–
Camelina sativa				–											–
* Capsella bursa-pastoris		–	–	–	–					–	–			** –	–
Cardaminopsis arenosa															–
* Cheiranthus allionii		–		–						–					
* cheiri		–		–	** –					–					
muralis															–
Chorispora tenella															–
* Clypeola jonthlaspii		–	–	–	–										–
* Descurainia sophia (L.) Webb.		–	–	–	–										–
* Draba aisoides		–	–	–	–					–					
aizoon															–
alpina L.															–
* arabisans															–
* siliquosa		–	–	–	–										
* Erophila verna		–	–	–	** –										
Erucastrum gallicum															–
Farsetia clypesta R.Br.															–
* eriocarpa DC.		–	–	–	–					–	–	–	–	–	–
* Hesperis matronalis L.		–	–	–	–					–					–
* Hutchinsia alpina											–	–	–	–	–
Jondraba auriculata Webb. & Berth.															–
Kernera boissieri															–
saxatilis															–
Lobularia libyca															–
* maritima (L.)				–											
v. benthamii															–
* Malcomia africana		–	–	–	–					–	–	–	–	–	–
* maritima		–	–	–	–					–	–	–	–	–	–
Matthiola arborescens															–
glabra															–
* incana R.Br.			–	–	–										
* sinuata		–	–	–	–					–	–	–	–	–	
* Moricandia arvensis		–	–	–	–					–	–	–	–	–	–
Nasturtium ~~nasturtium~~-aquaticum officinale R.Br.															–

	PLANTS							SEEDLINGS				Seeds
	HCl/Meth	Leuco-antho	Ehrlich	HCN	Juglone	Hot water		L.A.	Ehrlich	HCN	Juglone	HCN
	+ ? -	+ ? -	+ ? -	+ ? -	+ ? -	? I II III IV		+ ? -	+ ? -	+ ? -	+ ? -	+ ? -
Neslia paniculata (L.)												-
Pringlea antiscorbutica R.Br.				-								
Psychine stylosa												-
Ptilotrichum spinosum												-
Ricotia lunaria												-
Rhytidocarpus moricaridioides												-
Schivereckia bornmuelleri												-
* Schivereckia doerfleri		-	-	-	-							
Stenophragma thalianum												-
Vesicaris graeca Benth.												-
* utriculata	-	-	-	-	-	-						-
Wilckia littorea Druce												-
patula D.C.												-
Moringaceae												
* Moringa oleifera Lam.	-	-	-	-	-	-						-
Tovariaceae												
* Tovaria pendula Ruiz. et Pav.		-	-	-	-	-						
Tovaria virginiana (L.) Raf.												-
Resedaceae												
* Astrocarpus clusii		-	-	-	-	-		-	-	-	-	
* Astrocarpus sesamoides			-					-	-	-	-	
* Caylusea abysinica		-	-	-	-	-		-	-	-	-	
* Reseda alba			-	+				-	-	+	-	-
* Reseda chrystallena			-									
* Reseda lutea				-		-		-	-	-	-	-
* Reseda luteola		-	-	-	-	-		-	-	-	-	-
* Reseda odorata		-	-	-	-	-		-	-	-	-	-
* Tropaeolaceae												
Tropaeolum aduncum												-
* Tropaeolum majus L.		-	-	-	-	-		-	-	-	-	-
Tropaeolum minus L.												-

Table 2. Bahr's results of the tannic acid test for Capparidaceae and Papaveraceae.

Species	Pos.	?	Neg.
Capparidaceae II.			
Capparis jamaicensis	+		
Crataeva sp.	+		
Cleome sp.			-
Cleome violacea			-
Papaveraceae II.			
Argemone mexicana		tr	
Chelidonium majus L.*		tr	
Dicranostigma lactucoides	+		
Escholtzia californica		tr	
Glaucium corniculatum		tr	
Glaucium corniculatum v. rubrum		tr	
Macleya cordata*		tr	
Meconopsis aculeata			-
Meconopsis cambrica	+		
Meconopsis dhwojii	+		
Meconopsis horridula		tr	
Meconopsis nepalensis		tr	
Meconopsis regia		tr	
Papaver alpinum		tr	
Papaver dubium		tr	
Papaver nudicaule		tr	
Papaver orientale		tr	
Romneya coulteri*	+		
Sanguinaria canadensis		tr	
III			
Adlumia fungosa	+		
Corydalis glauca			-
Corydalis ochroleuca	+		
Corydalis rupestris	+		
Corydalis sempervirens		tr	
Corydalis thalictrifolia	+		
Dicentra exima		tr	
Dicentra formosa	+		
Dicentra hybrid	+		
Dicentra oregana	+		
Dicentra rosea	+		
Dicentra sp.		tr	

* Species tested by Bate-Smith also and where conflicts occur.
tr trace occurrence
\+ positive
\- negative

Table 3. Alkaloids of the Cruciferae and Capparidaceae (Compiled from Willaman and Schubert).

B- bark
Fl - flower
Fr - fruit
s - seed
St - stem
G - green parts
L - leaves
W - whole plant
Wo - wood

	Cheiroline	Erysoline	Lunarine	Lunaridine	Lunarimine	Narcotine	Sinapine	Stachydrine	3 hydroxystachydrine	Tyramine	Unknown
Cruciferae II											
Aethionema elongatum Boiss.											+
Brassica nigra Koch.							s+				
Brassica oleracea L.						+					
Brassica rapa							s+				
Erysimum arkansanum Nutt.	s+										
Erysimum aureum Bieb.	+										+
Erysimum crassipes Eisch & Mey.											+
Erysimum feodorovii-kassumovii											+
Erysimum nanum Boiss.	+										
Erysimum perofskianum		+									
Iberis amara L.											+
Lepidium sp.							+				+
Lepidium hyssopifolium Desv.											+s W
Lepidium virginicum L.											+G Fl
Lunaria annua L.			+								
Lunaria biennis Moench.			+s	+s	+s						
Rapistrum rugosum All.											L +St
Sinapis alba L.							+				
IV. Capsella bursa-pastoris										+	

Table 3.(cont'd.)

	Cheiroline	Erysoline	Lunarine	Lunaridine	Lunarimine	Narcotine	Sinapine	Stachydrine	3 hydroxystachydrine	Tyramine	Unknown
Capparidaceae											
11. Apophyllum anomalum F.Muell.											+
Capparis canescens Banks ex DG.											L B+
Capparis lasiantha R.Br.											+B
Capparis lucida Banks.											+L
Capparis mitchelli Lindbl.											+B
Capparis nobilis F.V.											L B+
Capparis nummularia DC.											S L+
Capparis persicaefolia											+
Capparis sarmentosa A.Cum.											Fl +
Capparis sola Macbride											+B
Capparis spinosa L.											+
Capparis tomentosa Lam.								Fr+			
Courbonia virgata Brongu.									Fr+		+
Crataeva sp.											+
V. Cleome ciliata Schum & Thom.											+ L
Gynandropsis gynandra (L.)											S W+
Polanisia graveolens Raf.											Wo+
Polanisia viscosa DC.											

Table 4 The occurrence of Myrosin and Mustard Oil Glycosides

	Alkyl					Alkenyl		Thio-ethers	Sulfoxides							Sulfones + Aryl-Alkyl				Phenols Ethers			Esters			Keto			Aliphatic-hydroxy				Hetero cyclic
	Glucocapparin 1	Glucolepidiin 2	Glucoputranjivin 3	Glucocochlearin 4	Glucojiaputin	Sinigrin 5	Gluconapin 6	Glucobrassicanapin 7	Glucoibervirin 8	Glucoerucin 11	Glucoberteroin 15	Glucolesquerellin	Glucoiberin 9	Glucoraphanin 12	Glucoraphenin 13	Glucoalyssin 16	Glucohirsutin 17	Glucoarabin 18	Glucocamelinin 19	Glucocheirolin 10	Glucoerysolin 14	Glucotropaeolin 20	Gluconasturtiin 24	Sinalbin 21	Glucoaubrietin 22	Glucolepigramin	Glucolimnanthin 23	Glucohesperin	Glucoerypestrin 25	Glucomalcolmiin 26	Glucobenzosisymbrin	Glucobenzsisaustricin	Glucococappangulin	Glucococappasalin	Gluconorcappasalin	Glucoconringiin 28	Glucocleomin	Glucosisymbrin 30	Glucosisaustricin	Glucobarbarin 29	Progoitrin 27 *	Glucobrassicin	Neoglucobrassicin
Cruciferae II.																																								
Alliaria officinalis andrz.					S +																																		
Barbarea sp.																						S R +																	
vulgaris R.Br.																						S +																	
praecox R.Br.																						+																	
Brassica napus L.						S +																																	
nigra Koch.					S +																																		
oleracea L.																																							+
sp.						S G +		S G +	+				+ G	+ G								SRG +	S +															SRG +	
Cardamine sp.						S +	S G +																																
Cardamine pratensis L.							+ G																																
Cochlearia sp.							S G +																																
Cochlearia officinalis L.							S +																																
Coronopus didymus (L.) Sm.																					+ G																		
Coronopus sp.																					+																		
Dentaria sp.																																							
Diplotaxis tenuifolia (L.)DC.																																							

(+) doubtful S seeds

L latex R roots

G green parts

W wood

B bark

* Progoitrin is also called Glucorapiferin

The numbers beside the glucosides are those used by Kjaer (1960) and indicate increasing complexity.
Unnumbered glucosides have been published since 1959.

Table 4 (cont'd. The occurrence of Myrosin and Mustard Oil Glycosides

	Alkyl					Alkenyl		Thio-ethers	Sulfoxides				Sulfones+ Aryl-Alkyl		Phenols Ethers			Esters		Keto	Aliphatic-hydroxy			Hetero-cyclic		
	Glucocapparin 1	Glucolepidiin 2	Glucoputranjivin 3	Glucococlearin 4	Glucojaputin	Sinigrin 5	Gluconapin 6	Glucobrassicanapin 7	Glucoibervirin 8	Glucoerucin 11	Glucoberteroin 15	Glucolesquerellin	Glucoiberin 9	Glucoraphanin 12	Glucoraphenin 13	Glucoalyssin 16	Glucohirsutin 17	Glucoarabin 18	Glucocamelinin 19	Glucocheirolin 10	Glucoerysolin 14	Glucotropaeolin 20	Gluconasturtiin 24	Sinalbin 21	Glucoaubrietin 22	Glucolepigramin	Glucolimnanthin 23	Glucohesperin	Glucoerypestrin 25	Glucomalcolmiin 26	Glucobenzosisymbrin	Glucobenzsisaustricin	Glucocapangulin	Glucocappasalin	Gluconorcappasalin	Glucoconringiin 28	Glucocleomin	Glucosisymbrin 30	Glucosisaustricin	Glucobarbarin 29	Progoitrin 27*	Glucobrassicin	Neoglucobrassicin
Diplotaxis sp.						S R G +				S G +				+																											
Eruca sativa Mill.																				S +								S +													
Eruca sp.																																									
Erysimum rupestra DC.									R +	S +			S +															S +													
Erysimum perofskianum Fisch. & May									S +	S +				+																											
Erysimum sp.						S +																S G +																			
Iberis amara L.														G +																											
Iberis sempervirens L.																				S R G +																					
Isatis tinctoria L.																																									
Lepidium campestre (L.) R.Br.																						S G +																			
Lepidium draba L.																																									
Lepidium menziesii DC.																																									
Lepidium sp.																																									
Lunaria annua L.																																									
Lunaria biennis Mnch.																																									
Lunaria rediva L.																																									
Nasturtium officinalis R.Br.																						S G +																			
Nasturtium sp.																																									
Raphanus rugosum (L.) All.																				S G +																					
Raphanus sativus L.														S +																											
Raphanus sp.																																									
Roripa sp.																																									
Sinapis alba L.																						S +																			
Sinapis nigra L.						S +																																			
Sisymbrium austriacum Jacq.																																								S +	

The occurrence of Myrosin and Mustard Oil Glycosides

Table 4 (cont'd.

	Alkyl					Alkenyl			Thio-ethers		Sulfoxides					Sulfones Aryl-Alkyl				Phenols Ethers				Esters			Keto	Aliphatic-hydroxy				Hetero-cyclic	
	Glucocapparin 1	Glucolepidiin 2	Glucoputranjivin 3	Glucocochlearin 4	Glucojiaputin	Sinigrin 5	Gluconapin 6	Glucobrassicanapin 7	Glucoibervirin 8	Glucoerucin 11	Glucoberteroin 15	Glucolesquerellin	Glucoiberin 9	Glucoraphanin 12	Glucoraphenin 13	Glucoalyssin 16	Glucohirsutin 17	Glucoarabin 18	Glucocamelinin 19	Glucocheirolin 10	Glucoerysolin 14	Glucotropaeolin 20	Gluconasturtiin 24	Sinalbin 21	Glucoaubrietin 22	Glucolepigramin	Glucolimnanthin 23	Glucohesperin	Glucoerypestrin 25	Glucomalcolmiin 26	Glucobenzosisymbrin	Glucobenzsisaustricin	Glucocapangulin	Glucocappasalin	Gluconorcappasalin	Glucoconringiin 28	Glucocleomin	Glucosisymbrin 30	Glucosisaustricin	Glucobarbarin 29	Progoitrin 27*	Glucobrassicin	Neoglucobrassicin
Sisymbrium sp.			S+	S+			S+																																		
Thlaspi arvensis L.												S+																														
III																																									
Lesquerella lasiocarpa																																									
IV																																									
Alyssum argenteum Vitm.						S+	S+		S+				S+			S+																										
Alyssum sp.						S+	S+		S+																																
Arabis hirsuta (L). Scop.																S+																									
Arabis alpina L.																	S+																								
Arabis sp.	S+		S+			S+																																			
Aubrietia sp.																								S+		S+															
Berteroa incana (L.) DC.											S+																														
Berteroa sp.																S+																									
Bunias sp.																								S(+)																
Camelina sativa (L.) Crantz.																			S+																					
Camelina sp.																			S+																					
Capsella sp.						S+																																		
Cheiranthus cheiri L.									R+										S+																					
Conringia orientalis (L.) Andrz.																																				S+				
Draba sp.		S+	S+																																					
Farsetia sp.									R+																															
Hesperis matronalis L.									S+																			S+												
Malcolmia maritima R.Br.																			S+										S+											
Matthiola bicornis DC.																S+																								
Matthiola sp.									S+																															
Vesicaria sp.									S+																															

Table 4 (cont'd.) The occurrence of Myrosin and Mustard Oil Glycosides

	Alkyl				Alkenyl		Thio-ethers	Sulfoxides								Sulfones Aryl-Alkyl				Phenols Ethers				Esters			Keto		Aliphatic-hydroxy			Hetero-cyclic	
	Glucocapparin 1	Glucolepidiin 2	Glucoputranjivin 3	Glucocochlearin 4	Glucojiaputin	Sinigrin 5	Gluconapin 6	Glucobrassicanapin 7	Glucoibervirin 8	Glucoerucin 11	Glucoberteroin 15	Glucolesquerellin	Glucoiberin 9	Glucoraphanin 12	Glucoraphenin 13	Glucoalyssin 16	Glucohirsutin 17	Glucoarabin 18	Glucocamelinin 19	Glucocheirolin 10	Glucoerysolin 14	Glucotropaeolin 20	Gluconasturtiin 24	Sinalbin 21	Glucoaubrietin 22	Glucolepigramin	Glucolimnanthin 23	Glucohesperin	Glucoerypestrin 25	Glucomalcolmiin 26	Glucobenzosisymbrin	Glucobenzsisaustricin	Glucocapangulin	Glucocappasalin	Glucnorcappasalin	Glucoconringiin 28	Glucocleomin	Glucosisymbrin 30	Glucosisaustricin	Glucobarberin 29	Progoitrin 27*	Glucobrassicin	Neoglucobrassicin
Capparidaceae																																									
I. Boscia fischeri Pax.	W +																																								
Capparis spinosa L.	S +																																		S +						
Capparis sp.	S +		WG +	WG +																											S +	S +								
Crataeva tapia L.	S +																																								
Crataeva Roxburghii R.Br.	S +																																								
V.																																									
Cleome spinosa Jacq.	S +																																		S +						
Cleome sp.	S +																																		S +						
Gynandropsis gynandra (L.)	S +																																								
Gynandropsis speciosa DC.	S +																																								
Maerua hoehnelii Schweinf.	BRG +																																		BRG +						
Ritchiea albersii Gilg.	BR +																																								
Thylachium africanum Lour.	R +																																								
thomasii	BGR +																																								
Moringaceae																																									
Moringa pterygosperma Gaertn.																				R +																					
Resedaceae																																									
Reseda lutea L.																				R +																					
Reseda alba L.																					R +																			
Reseda luteola L.																					R +																			
Reseda odorata L.																					R +																			

The occurrence of Myrosin and Mustard Oil Glycosides

Table 4 (cont'd.)

	Alkyl					Alkenyl			Thio-ethers	Sulfoxides							Sulfones Aryl-Alkyl			Phenols Ethers			Esters			Keto			Aliphatic-hydroxy				Hetero-cyclic	
	Glucocapparin 1	Glucolepidiin 2	Glucoputranjivin 3	Glucococchlearin 4	Glucojiaputin	Sinigrin 5	Gluconapin 6	Glucobrassicanapin 7	Glucoibervirin 8	Glucoerucin 11	Glucoberteroin 15	Glucolesquerellin	Glucoiberin 9	Glucoraphanin 12	Glucoraphenin 13	Glucoalyssin 16	Glucohirsutin 17	Glucoarabin 18	Glucocamelinin 19	Glucocheirolin 10	Glucoerysolin 14	Glucotropaeolin 20	Gluconasturtiin 24	Sinalbin 21	Glucoaubrietin 22	Glucolepigramin	Glucolimnanthin 23	Glucochesperin	Glucoerypestrin 25	Glucomalcolmiin 26	Glucobenzosisymbrin	Glucobenzsisaustricin	Glucocapangulin	Glucocappasalin	Gluconorcappasalin	Glucoconringiin 28	Glucocleomin	Glucosisymbrin 30	Glucosisaustricin	Glucobarbarin 29	Progoitrin 27*	Glucobrassicin	Neoglucobrassicin
Caricaceae																																			
Carica papaya L.																				S +															
Euphorbiaceae																																			
Jatropha multifida L.																				L +															
Putranjiva roxburghii Wall.	S +	S +																																	
Limnanthaceae																																			
Limnanthes douglasii R.Br.																									S +										
Phytolaccaceae																																			
Codonocarpus cotinifolius (Desf.)																				(+)															
Plantaginaceae																																			
Plantago majus L.													S +																						
Salvadoraceae																																			
Salvadora oleiodes Den.																				S +															
Tropaeolaceae																																			
Tropaeolum majus L.																				S S +															
Tropaeolum peregrinum	S +	S +																																	

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APPENDIX B

Table 5

Alkaloids in the Papaveraceae

	Tetrahydro. & Benzylisoquinoline 1		2												Cryptopine 3									
	Corypalline	Hydrocotarnine	Papaveramine	Papaverine	Xanthaline	δl-Laudanine	Laudanidine	Codamine	Laudanosine	Narcotoline	L-Narcotine	Gnoscopine	Oxynarcotine	Narceine	Protopine	Fumaramine	Cryptopine	Cryptocavine	Ochrobirine	Allocryptopine	Hunnemannine	Corycavamine	Corycavidine	β-homochelidonine
I Hypecoum leptocarpum															+									
procumbens															+									
trilobum																								
II Argemone alba																								
hispida																				+				
mexicana															+					+				
Bocconia arborea															+					+				
cordata															+					+				+
frutescens															+					+				
pearcei															+					+				
Chelidonium majus															+					+				+
Dendromecon rigidum															+					+				
Dicranostigma sp.															+					+				
lactucoides															+									
Eschscholtzia californica															+					+				
Glaucium corniculatum															+					+				
fimbrilligerum															+					+				
flavum															+					+				
luteum															+									
serpieri															+									
Macleya cordata															+					+				
microcarpa															+		+			+				
Papaver argemone															+									
arenarium																								
armeniacum																								
atlanticum															+									
bracteatum																								
caucasicum																								
dubium																								
floribundum																								
gracile															+									
hybridum																								
macrostomum															+									
monanthum															+									
orientale															+									
paeoniflorum				+							+													
pavoninum															+					+				
pilosum															+									
rhoeas											+				+									
setigerum																								
somniferum				+		+			+		+			+										
" v.polycephalum		+	+	+	+		+	+	+	+	+	+	+	+	+		+			+				
Sanguinaria canadensis															+					+				
Stylophorum diphyllum															+									
franchetianum															+									
lactucoides															+									
III Adlumia fungosa															+					+				
Corydalis ambigua															+									
aurea	+														+					+				
bulbosa															+									
caseana															+					+				
cava															+							+	+	
cheilanthifolia															+					+				
claviculata															+									
cornuta															+									
crystallina															+									
decumbens															-									
fabacea																								
incisa															+									
lutea															+				+					
micrantha															+									
montana															+									
nobilis															+		+							
ochotensis															+			+						
ophiocarpa															+			+		+				
ochroleuca															+				+					
pallida	+														+									
platycarpa															+									
scouleri															+		+			+				
sempervirens															+		+							
sibirica															+		+		+					
solida															+					+				
tenata															+					+				
thalictrifolia															+									
tuberosa															+							+	+	
vernyi															+									
Dicentra canadensis															+									
chrysantha															+		+	+						
cucullaria															+		+			+				
eximia															+									
formosa															+					+				
ochroleuca															+		+							
officinalis																								
oregana															+					+				
pusilla															+									
spectabilis															+									
Fumaria agraria															+									
capreolata															+									
densiflora															+									
micrantha															+	+								
muralis															+									
officinalis															+			+						
parviflora															+									
schleicheri															+	+								
vaillantii															+	+								

	Morphine 4						Unknown constitution 5							Isomerides of Laudamine 6				Phthalide isoquinoline 7								
	Morphine	Codeine	Neopine	Thebaine	Isothebaine	Porphyroxine	Aporeidine	Aporeine	Rhoeadine	Rhoeagenine	Meconidine	Papaveramine	Lanthopine	Floripavidine	Floripavine	Armepavine	Orepavine	Meconin	Bicuculline	Bicucine	Adlumine	Adlumidine	Corlumine	Corlumidine	Cordiastine	Capnoidine
I Hypecoum leptocarpum																										
procumbens																										
trilobum																										
II Argemone alba																										
hispida																										
mexicana	+	+																								
Bocconia arborea																										
cordata																										
frutescens																										
pearcei																										
Chelidonium majus																										
Dendromecon rigidum																										
Dicranostigma sp.																										
lactucoides																										
Eschscholtzia californica	+	+																								
Glaucium corniculatum																										
fimbrilligerum																										
flavum																										
luteum																										
serpieri																										
Macleya cordata																										
microcarpa																										
Papaver argemone									+	+																
arenarium									+	+																
armeniacum																+										
atlanticum	+	+																								
bracteatum				+	+												+									
caucasicum																+										
dubium							+	+																		
floribundum														+	+	+	+									
gracile									+																	
hybridum									+																	
macrostomum									+																	
monanthum									+																	
orientale				+	+												+									
paeoniflorum		+		+																						
pavoninum																										
pilosum									+																	
rhoeas	+			+					+	+																
setigerum	+																									
somniferum									+																	
" v.polycephalum	+	+	+	+		+		+	+		+		+													
Sanguinaria canadensis																										
Stylophorum diphyllum																										
franchetianum																										
lactucoides																										
III Adlumia fungosa																			+	+	+	+				
Corydalis ambigua																										
aurea																			+	+					+	
bulbosa																										
caseana																			+							
cava																			+							
cheilanthifolia																										
claviculata																										
cornuta																										
crystallina																			+							+
decumbens																										
fabacea																										
incisa																						+				
lutea																										
micrantha																										
montana																										
nobilis																			+				+	+		
ochotensis																										
ophiocarpa																					+					
ochroleuca																			+							
pallida																										
platycarpa																			+							
scouleri																			+		+		+	+		+
sempervirens																			+	+	+					+
sibirica																			+				+			
solida																										
tenata																										
thalictrifolia																					+	+				
tuberosa																										
vernyi																										
Dicentra canadensis																										
chrysantha																			+							
cucullaria																			+	+			+	+		
eximia																										
formosa																										
ochroleuca																			+							
officinalis																										
oregana																										
pusilla																										
spectabilis																										
Fumaria agraria																										
capreolata																										
densiflora																										
micrantha																										
muralis																										
officinalis																										
parviflora																										
schleicheri																										
vaillantii																										

	Naphthaphenanthridine 8			Hydroprotoberberine 9												Aporphine 10							Corydalis 11				
	Chelerythrine	Chelidonine	Sanguinarine	Berberine	Corydaline	Dehydrocorydaline	Corytulbine	Isocorytulbine	Coptisine	Stylopine	Scoulerine	Aurotensine	Isocorypalmine	Corypalmine	d-tetrahydropalmatine	Bulbocapnine	Corydine	Isocorydine	Corytuberine	Dicentrine	Glaucine	Glaucentrine	Cularine	Ochotensine	Cheilanthifoline	Capauridine	Capaurine
I Hypecoum leptocarpum																											
procumbens																											
trilobum			+																								
II Argemone alba				+																							
hispida		—																									
mexicana	+		+	+					+																		
Bocconia arborea	+																										
cordata	+		+																								
frutescens	+		+																								
pearcei	+		+																								
Chelidonium majus	+	+	+	+					+	+																	
Dendromecon rigidum																											
Dicranostigma sp.	+	+	+	+					+	+								+									
lactucoides	+		+															+									
Eschscholtzia californica			+																								
Glaucium corniculatum	+	+	+	+													+	+			+						
fimbrilligerum	+		+														+				+						
flavum	+		+								+							+			+	+					
luteum	+		+																		+	+					
serpieri											+										+	+					
Macleya cordata	+		+	+					+																		
microcarpa																											
Papaver argemone																											
arenarium																											
armeniacum																											
atlanticum																											
bracteatum																											
caucasicum																											
dubium																											
floribundum																											
gracile																											
hybridum																											
macrostomum																											
monanthum																											
orientale																											
paeoniflorum																											
pavoninum																											
pilosum																											
rhoeas									+																		
setigerum																											
somniferum																											
" v.polycephalum																											
Sanguinaria canadensis	+		+																								
Stylophorum diphyllum	+	+	+																								
franchetianum	+	+	+							+																	
lactucoides	+		+															+									
III Adlumia fungosa																											
Corydalis ambigua					+	+	+		+						+												
aurea					+	+						+			+											+	+
bulbosa																+											
caseana											+		+	+	+												
cava					+	+	+	+	+	+			+	+		+	+		+								
cheilanthifolia				+						+				+											+		
claviculata										+													+				
cornuta										+																	
crystallina																											
decumbens						+									+	+											
fabacea					+																						
incisa														+													
lutea										+			+		+		+	+									
micrantha											+				+											+	+
montana					+	+					+				+											+	+
nobilis					+					+			+		+												
ochotensis												+												+			
ophiocarpa				+										+													
ochroleuca													+	+	+												
pallida											+				+											+	+
platycarpa					+		+	+		+	+	+	+		+		+	+									
scouleri											+														+		
sempervirens																											
sibirica											+													+	+		
solida					+					+		+			+												
tenata																	+	+			+	+					
thalictrifolia										+				+													
tuberosa					+	+	+	+			+		+	+	+	+	+		+		+						
vernyi																											
Dicentra canadensis					+		+									+		+	+								
chrysantha																											
cucullaria					+		+									+	+	+	+				+	+			
eximia																	+			+	+	+	+				
formosa																	+		+	+	+	+	+				
ochroleuca																											
officinalis											+																
oregana														+			+			+	+	+	+				
pusilla																				+							
spectabilis	+		+						+																		
Fumaria agraria																											
capreolata																											
densiflora																											
micrantha																											
muralis																											
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parviflora																											
schleicheri																											
vaillantii																											