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REVIEW

CHEMICAL INVESTIGATIONS OF HERBARIUM MATERIAL FOR ALKALOIDS

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Key Word Index—Rubiaceae; Papaveraceae; herbarium samples; chemical investigations; alkaloids; chemotaxonomy.

Abstract—The chemical and biological screening of plants is reviewed briefly as an introduction to the concept of the use of herbarium material for investigations into the chemical constituents of plants. Such materials have been examined for a wide range of chemicals, and in particular, examples of the extraction of alkaloids from species of Rubiaceae and Papaveraceae are discussed. The present state of the art of such physical techniques as mass and nuclear magnetic resonance spectrometry for these investigations is commented upon and the plea is made that, wherever possible, wider use be made of herbarium material for chemical studies.

INTRODUCTION

The chemical screening of plants dates back from the early 1900's but it was not until the period between 1940 and 1970 that numerous research papers dealing with both chemical and biological screening appeared in the scientific literature [1, 2]. A variety of compounds formed the subject of these endeavours and they included, for example, alkaloids, saponins, cardiac glycosides, flavonoids, coumarins, anthraquinones, cyanogenetic glycosides and essential oils [1]. Many of these papers described simple screening procedures by which plant extracts were subjected to chemical tests aimed at producing either a positive or negative response merely indicative of the presence or absence of a particular class of compound. Investigations were frequently carried out for a limited number of constituents and such investigations can be typified by reference to the search for alkaloids. Extractions were made on a whole series of plants and the extracts were tested, for example with Dragendorff's reagent, either on filter paper or in test tubes and then graded on an arbitrary scale from + to 4+. Webb screened some 1700 species [3, 4], Wall *et al.* some 4000 species [5–9] and one American pharmaceutical company screened some 25 000 species [10]. This type of investigation continued into the 1970's when it was realized, for example, that thousands of plant extracts which had been judged to be inactive during the NCI anti-cancer screening programme were not being investigated further. The results obtained from testing 1000 such extracts at a time were published in a series of articles (see, for example refs. [11, 12]). In a survey of New Guinea plants, 3700 species were tested for alkaloids but the methodology was made more rigorous by including titrations in order to estimate the alkaloid content [13]. On the basis of such screening programmes it was estimated that from random collections of angiosperms some 10% might well be expected to yield alkaloids.

Some chemical screening programmes included testing for more than one class of compound, e.g. 100 species of ferns from Trinidad were screened for alkaloids, saponins and terpenes [14] while an Indian investigation into 1200 species tested for the presence of alkaloids, saponins and flavonoids [15–17]. In some instances, extensive chemical screening has been carried out on fresh plant material by carrying out the tests in the field (e.g. ref. [18]). A smaller number of investigations have been concerned with the biological screening of plant extracts and a few examples will serve to illustrate this approach. Extracts of some 94 species of Mexican plants were tested for appetite inhibition, CNS, hypotensive, diuretic, antimicrobial, anti-inflammatory and endocrine activities [19]. In the first of another extensive series of papers, Farnsworth and his group tested 200 plants, obtained from random collections in the U.S.A., for possible antineoplastic, antimicrobial, anti-insecticidal, antiviral activities and for mouse behaviour studies [20]. In addition to performing actual biological tests, a number of literature surveys

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have been undertaken in order to seek out plants with possible biological activities, e.g. the potential of African plants for the treatment of mental health has been surveyed and data collected on plants which have been used for the treatment of amnesia, convulsions, epilepsy, hallucinations, hysteria, mental diseases and for plants which possessed narcotic and CNS stimulating activities [21]. In this type of literature survey, one series of papers by Hartwell on plants used against cancer [22] and the papers by Farnsworth *et al.* on antifertility plants [23] are outstanding.

A few workers have combined chemical and biological screening techniques. Such an approach may have been confined to a single genus; for example, some 47 species of African *Strychnos* have been investigated for their alkaloid content and also for the ability of extracts to act either as muscle relaxants or convulsants [24–29]. One investigation, however, reports the combined efforts of industrial and university personnel in testing some 89 species for the presence of alkaloids, phenols, amino acids, carbohydrates, saponins and for antimicrobial, antitumour and toxicity evaluation [30].

Herbaria may serve as sources of information on the biological activities of plants since ethnobotanical data may have been recorded on particular herbarium sheets. Compilations of such data have been made, for example, on hallucinogenic plants [31, 32]. In a painstaking search through 2.5 million herbarium sheets between 1962 and 1966, some 7000 ethnobotanic notes were unearthed and were subsequently published [33]. As part of this investigation, the recorded use of 66 species for the treatment of children's complaints of the g.i. tract, skin and respiratory tract have been reported [34]. the biological activities and therapeutic uses of some 30 Puerto Rican plants have been obtained from a study of herbarium sheets [35]. The author pointed out that this type of information is required by pharmacologists, individuals who do not normally frequent herbaria. As well as being sources of such valuable information, herbaria can under certain circumstances be sources of plant material for chemical investigations.

SCREENING PLANTS FOR THE PRESENCE OF NON-ALKALOIDAL CONSTITUENTS

Chemical screening programmes undertaken after 1970 have included the use of either chromatographic and/or spectrometric techniques for the separation and identification of plant constituents. In a number of such investigations use has been made of herbarium material.

Non-protein amino acids

A series of investigations into the non-protein amino acids of the Leguminosae has utilized a combination of two dimensional chromatography and electrophoresis. Seeds from some 300 genera have been screened for compounds of potential pharmacological interest such as canavanine as well as for the presence of new compounds [36]. As a result of surveying some 1200 species representing some 240 genera for the presence of canavanine, it has been possible to discuss the systematic significance of this amino acid in the subfamily Papilionoideae [37]. Chemotaxonomic appraisals of non-protein amino acids have been made following the investigation of some 64 species of the subtribe Caesalpinineae [38].

Flavonoids

The leaves of 128 species of 22 genera of the tribe Genisteae obtained mainly from herbarium material have been examined for the presence of flavonoids. Hydrolysis of flavonoid- and isoflavonoid-containing extracts followed by PC and TLC identifications has enabled the chemosystematics of the tribe to be studied [39]. Differences between the flavonoid content of fresh and herbarium material of *Dillenia indica* has led to the suggestion that, whenever possible, fresh plant material should be investigated when herbarium samples are examined for the presence of flavonoids [40]. Investigations of herbarium and fresh material have shown that flavonoids are valuable for chemosystematic studies in the Palmae [41]. Glycosides were identified initially on the basis of their chromatographic behaviour and UV spectroscopy. Confirmation of identity was obtained by hydrolysis and the resulting aglycones and sugars identified chromatographically. Such data, obtained mainly from phytochemical investigations of herbarium material, has been incorporated with traditional morphological features and by means of computer-assisted techniques it has been possible to assess relationships between taxa. It has been demonstrated, for example, that negatively charged flavonoids can be associated with anther structure whereas leucoanthocyanins tend to be associated with root vessel elements and floral structures [42]. Although it is estimated that there are some 6000–9000 species of Graminae, only *ca* 100 species had been examined chemically by 1976, and of these, only a handful of genera had been subjected to any detailed chemical investigation. The identification of grasses is difficult and the chemical investigations for flavonoids of herbarium and fresh leaf material of 274 species representing 121 genera has enabled some contribution to be made towards the chemosystematics of this difficult group [43].

Sweetening agents

A fascinating investigation into the sweetening properties of *Stevia* sp. has been undertaken recently by utilizing herbarium samples [44]. As many as 184 leaf samples representing 110 species were screened by tasting small squares ranging from 1–2 mm to 12–16 mm. In all, 18 species gave sweet tastes but a 62-year-old sample of *S. rebaudiana* gave the most prolonged sensation of sweetness. *S. phlebophylla* collected from Mexico in 1889 was still sweet; interestingly the authors claim that it is now possibly an extinct species.

Volatile oils

It might be expected that herbarium samples would not retain volatile oils for any length of time but in a taxonomic investigation of *Mentha* species, samples of *M. alopecuroides* collected in 1798 and in 1893 were extracted and examined by GC [45]. The results clearly indicated that there was no qualitative difference between these two old samples and one of

more recent origin. The authors pointed out, in 1967, that the technique of GC offered considerable scope for the investigation of taxonomic and population studies and could well be utilized for the correct identification of either old or fragmentary herbarium specimens.

ALKALOIDS FROM HERBARIUM MATERIAL

The detection of alkaloids in herbarium samples has been discussed previously [46] and it has been demonstrated that alkaloids were still present in a leaf sample of *Nicotiana attenuata* which was estimated to be some 1300 years old [47]. One of the most extensive chemical investigations of alkaloids from herbarium material has centred around the genus *Strychnos*. As many as 210 samples of African *Strychnos*, mainly herbarium samples, representing 69 of the 75 species from this part of the world, have been screened for tertiary alkaloids [48]. The alkaloid extracts were assessed not only by their colour reactions with Dragendorff's reagent but also by TLC and GC techniques. The results obtained were discussed in relation to the then recent botanical revision of African *Strychnos* [49]. Seven of the samples examined were isotype material, i.e. they were taken from the same collections as the specimens collected to be the nomenclatural types of the species concerned. A further 234 samples of *Strychnos*, again mainly of herbarium material and representing 36 of the 44 Asian species, were examined for their alkaloidal constituents [50]. In this instance it was possible to substantiate the chromatographic identifications by means of mass spectrometry. Nomenclatural types were also available for this chemical investigation and included three holotypes, eight isotypes, one lectotype and one isolectotype. One interesting facet of this study was the examination of a number of old samples, one being collected 300 years previously. This particular leaf sample of *S. nux-vomica* collected in 1675 by Hermann was made available by the Botany Department of the British Museum (Natural History). The leaf weighed 183 mg and yielded 2.2 mg of alkaloid (1.2%) from which strychnine and its *N*-oxide, brucine and its *N*-oxide, a colubrine, pseudostrychnine, pseudobrucine, icajine, vomicine and novacine were identified by a combination of TLC, GC and mass spectral techniques [50]. A number of old samples were investigated during the course of the same investigation and the results obtained from 12 samples collected before 1850 are summarized in Table 1. In addition to pre-1850 samples some 35 other samples which had been collected between 1851 and 1900 were also examined for their alkaloid content. Not only did this investigation point to the stability of these particular alkaloids in herbarium samples but it demonstrated that large quantities of plant material are not necessary for chemical investigation since 8 of the 12 samples examined from collections made prior to 1850 ranged from 120 to 400 mg [50].

Other workers have reported upon the presence of alkaloids in herbarium specimens (e.g. refs. [51–55]). As early as 1950, strong alkaloid-positive reactions were obtained on an extract prepared from a 124-year-old sample of *Acronychia baueri* and it was proposed that world-wide alkaloid resources could be ascertained from herbarium collections [56]. A particularly interesting example of this type of investigation is demonstrated by the work done on a sample of *Banisteriopsis caapi* collected in Amazonian Brazil by Richard Spruce in 1852. A sample of stem material was released from the Royal Botanic Gardens, Kew, for chemical analysis and some 11.5 g were extracted to yield, after 115 years of storage, some 0.4% of total alkaloid. The investigators confirmed the presence of harmine by using combined GC/MS and thus, they were able to prove that harmine was the active ingredient of the intoxicating beverage 'caapi' which is prepared from the stems of *Banisteriopsis caapi* [57]. The contribution GC/MS can make to our knowledge of alkaloid constituents obtained from only small amounts

Table 1. Alkaloid investigations of Asian *Strychnos* herbarium samples collected before 1850 [50]

Species	Collector's no.	Date collected	Country of origin	Plant* part	Alkaloid test†	No. of alkaloids identified‡
S. benthamii	Herb. Wight s.n.	<1850	Sri Lanka	l	negative	—
S. ignatii	Blume s.n.	<1830	Indonesia	s	++++	6
S. ignatii	ST/10/19/K3	<1850	—	s	++++	7
S. minor	Herb. Wight s.n.	*ca* 1850	S. India	l	negative	—
S. minor	Herb. Wight s.n.	1830	S. India	l	negative	—
S. nux-vomica	P. Hermann	1675	Sri Lanka	l	++++	10
S. nux-vomica	Sloane colln 412	1695	—	s	++++	11
S. nux-vomica	Rottler s.n.	1806	India	l	++++	10
S. potatorum	Herb. Wight 639	1836	India	l	negative	—
S. trichocalyx	Thwaites CP330	1846	Sri Lanka	s	tr	—
S. wallichiana	Herb. Wight 2286	<1850	India	l	++++	7
S. wallichiana	Sloane colln 412	1698	—	s	++++	8

*l, Leaf; s, seed.

†Classified + to + + + + on the basis of colour with Dragendorff's reagent; tr. trace.

‡Identifications made on the basis of TLC, GC and mass spectrometry.

of plant material is well illustrated in the *Erythrina* field. Following the work of Folker's group in the late 1930's and early 1940's, some 65 species of *Erythrina* have been examined for free alkaloids and glycosidic alkaloids by combined GC/MS techniques. A series of alkaloids have been identified and new ones discovered [58–64] and it has been possible to relate the alkaloids to the reviewed taxonomic position of the genus [65].

Further examples of the utilization of herbarium material for detailed chemical investigations into alkaloidal plants are provided by reference to our work on indole and quinoline alkaloids from three tribes of the tropical family Rubiaceae and of isoquinoline alkaloids from the temperate family Papaveraceae.

ALKALOIDS FROM THE TRIBES NAUCLEEAE, CEPHALANTHEAE AND CINCHONEAE OF THE RUBIACEAE

The Rubaceae comprises some 6000–7000 species distributed in some 500 genera which mainly inhabit tropical and moist environments. The delimitations and subdivision of this family have caused many problems for taxonomists and in particular instances a knowledge of alkaloidal constituents has enabled a greater understanding of generic relationships [66–68]. The current state of knowledge of the alkaloidal constituents of the family has been reviewed recently [69]. The unifying thread in the biosynthetic relationships of many of the indole, quinoline and isoquinoline alkaloids, found in the family, is their iridoid precursor, secologanin (**1**) (Fig. 1). Condensation of tryptamine (**2**) or tryptophan (**3**) with secologanin (**1**) results in strictosidine (**4**) or 5β-carboxystrictosidine (**5**) which are the precursors of a series of indole and quinoline alkaloids. Condensation of 3, 4-dihydroxyphenylethylamine (**6**) with secologanin (**1**) results in the tetrahydroisoquinoline glucoside alkaloid desacetylisoipecoside (**7**) which is the precursor of such alkaloids as emetine [69].

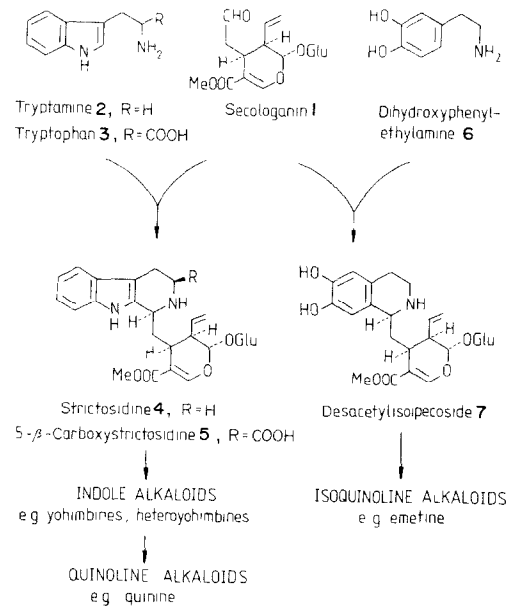


Fig. 1. Biosynthetic relationships of the indole, quinoline and isoquinolone alkaloids of the Rubiaceae.

The 10 species of the genus *Mitragyna* have been the subject of a considerable number of chemical investigations over many years and it is now recognized that four major types of alkaloids characterize the genus (**8–11**) (Fig. 2) [69, 70]. However, for many years the neighbouring genus, *Uncaria*, received relatively little attention from phytochemists and this can be attributed, at least in part, to the complexity of the genus. Botanical revision resulted in the reduction of some 120 specific names to 34 accepted species which were arranged into seven informal groups [71]; concurrent with the botanical revision, we were able to investigate, in some detail, the alkaloids of this pantropical genus by examining herbarium samples [66].

Identification of the alkaloidal-types **8–14** was accomplished by means of combined chromatographic (TLC, GC) and spectroscopic techniques (UV, mass spectra) [72]. The sequence of elution of the pentacyclic (**8**) and tetracyclic (**9**) heteroyohimbines on Si gel TLC systems is determined primarily by the ability of N-4 to hydrogen-bond to silanol hydroxyl groups [73–75]. These alkaloids separate well and can be grouped into their diastereoisomeric sets with their sequence of R_f values being in the order of allo > normal > epiallo > pseudo (defined as in Fig. 2). Similarly the corresponding oxindoles (**10**, **11**) separate in distinct sequences and can readily be identified, e.g. R_f values of alkaloids with their N-4 lone pair anti to the oxindole carbonyl > corresponding syn-isomers [75, 76]. By means of R_f values and colours produced by chromogenic reagents which are selective for particular types of alkaloids and also by means of GC it was possible to tentatively identify some 40 alkaloids [72]. Confirmation of identity was achieved by elution of the alkaloids from chromatograms followed by determination of UV and mass spectra. In particular, mass spectroscopy proved invaluable for confirmation of identity of these alkaloids and some of the diagnostic ion peaks for alkaloids of types **8–11** are given in Table 2. Within a given series it is also possible to differentiate each particular diastereoisomeric-type and the specificity of this technique for distinguishing between the pentacyclic alkaloids (**8**) is shown in Table 3. Similarly the technique distinguishes alkaloids of types **9**, **10** and **11** [72]. Other spectroscopic techniques such as ^{1}H NMR [77] and CD [78] were used but in a limited way for the identification of certain alkaloids from herbarium samples.

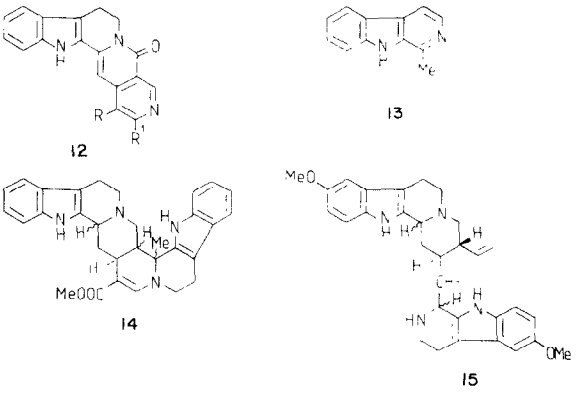


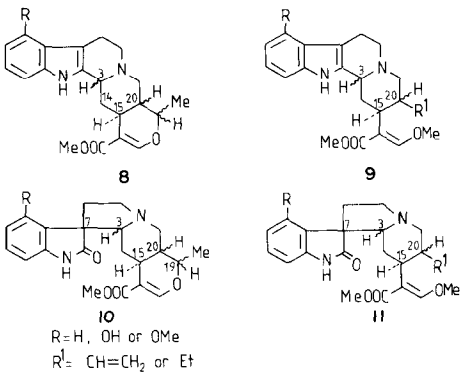
Table 2. Mass spectral fragmentation of heteroyohimbine (**8**, **9**) and oxindole (**10**, **11**) alkaloids [72]

Alkaloid-type*	$[M]^+$	Fragment ions *m/z*
Pentacyclic heteroyohimbines, **8**	352	351, 184, 170, 169, 156, 144, 143, 130
Tetracyclic heteroyohimbines, **9**	368	367, 184, 170, 169, 156, 144, 143, 130
Pentacyclic oxindoles, **10**	368	223, 144, 130, 69
Tetracyclic oxindoles, **11**	384	239, 144, 130, 69

*R = H; when R = OH or OMe, $[M]^+$ increased by 16 or 30 mass units respectively.

Table 3. Diagnostic fragmentation patterns for pentacyclic heteroyohimbine alkaloids (**8**) [72]

		$[M]^+$	$[M-1]^+$		Ratio of fragment ions	
					m/z 184/169, 170	*m/z* 209, 225/223, 251
8	R = H	*m/z* 352	351	*trans* D/E	>	>
				cis D/E	≤	<
8	R = OH	*m/z* 368	367			
8	R = OMe	*m/z* 382	381			



8 - 10 diastereoisomers, allo (C-3 Hα, C-20 Hα) epiallo (C-3 Hβ, C-20 Hα), normal (C-3Hα, C-20 Hβ) pseudo (C-3 Hβ, C-20 Hβ)

10, 11 A isomers, lactam carbonyl below plane of C/D rings, B isomers, lactam carbonyl above plane of C/D rings

Fig. 2. Heteroyohimbine and oxindole alkaloids of the Naucleeae s.l.

Application of these techniques to the alkaloid extracts of 400 herbarium samples representing all 34 species of *Uncaria* resulted in the identification of 40 alkaloids, mainly of types **8–11** (R = H) together with indoloquinolizidinones (**12**), harmane (**13**) and roxburghines (**14**) [66]. As a result of this investigation it was possible to discuss the chemotaxonomic significance of the distribution of the alkaloids of *Uncaria* species in relation to the seven informal groups recognized on the basis of botanical affinities. It was found that pentacyclic alkaloids (**8**, **10**) predominate in three of the informal groups while tetracyclic alkaloids (**9**, **11**) predominate in three other groups. Some species were remarkably constant in their alkaloid composition even over wide geographical ranges, while other species showed considerable infra-specific variation. This latter finding emphasizes the danger of making taxonomic proposals on the basis of incomplete phytochemical data. One particular variable species, *U. attenuata* [79], yielded a number of novel alkaloids and although some 17 samples were available from S.E. Asia, none was obtained from Thailand. The variability of the Thai species has since become apparent [80, 81]. Investigations of larger samples have resulted in a novel pentacyclic heteroyohimbine alkaloid (**8**) which carries a 14-hydroxyl substituent. In addition to the isolation of novel compounds it has been possible to apply a knowledge of alkaloid content as an aid to correct identification of herbarium material [79]. A more detailed knowledge of the alkaloids present in *Uncaria* species has enabled comparisons to be made

Table 4. Comparison of the alkaloids of *Mitragyna* and *Uncaria* [66]

	Mitragyna	*Uncaria*
No. of species	10	34
No. of species containing **8–11**	10	21
9-Methoxylated alkaloids	Common	Not found
9-Hydroxylated alkaloids	Common	Rare
C-19 methyl β-configuration (**8**, **10**)	Not found	Infrequent
Roxburghines (**14**)	Not found	In one species

between these alkaloids and those of the neighbouring genus *Mitragyna*—these comparisons are summarized in Table 4.

The genera *Mitragyna* and *Uncaria* have been included for many years in the tribe Naucleeae s.l. of the subfamily Cinchonoideae. Although both genera have distinctive morphological characteristics, the other members of the tribe have posed considerable taxonomic problems [82]. The most important characters which have been used for differentiating the genera are those of the ovary, stigma, calyx, placentation, seed form [71] and various authors have tended to weight these characters differently. Haviland [81] recognized *Mitragyna* and *Uncaria* as two distinct subtribes while both Haviland [83] and Schuman [84] questioned the homogeneity of the tribe and restricted it to *Nauclea* and *Sarcocephalus*; he was even doubtful as to whether *Cephalanthus* should be retained within the subfamily Cinchonoideae. In the most recent revision, Ridsdale has removed *Cephalanthus* from the Naucleeae and has placed it in a separate tribe, the Cephalantheae [86]. Furthermore, the same author in his revision of *Mitragyna* and *Uncaria* transferred these two genera from the Naucleeae to the Cinchoneae, creating a new subtribe, Mitragyninae [87]. The remaining genera within the Naucleeae s.s. have been revised and new genera created [71]. The current position recognizes some 21 genera placed within three subtribes, Adininae (16 genera, 7 being new), Naucleinae (4 genera, 1 being new) and Anthocephalinae (1 genus)—see Table 5 [71].

It was anticipated that a knowledge of the alkaloids present in these genera might be of some value in assessing relationships between genera which comprise the Naucleeae s.l. [67]. Thus, 121 small samples of herbarium material which represented 18 genera of the Naucleeae s.s., *Cephalanthus* and *Mitragyna* were examimed for the presence of tertiary alkaloids. The lowest weight of sample was 120 mg and only nine of the samples exceeded 7 g. In general, samples from the Naucleeae s.s. gave only slightly positive reactions to Dragendorff's reagent while those of the Cephalantheae and Mitragyninae gave stronger reactions. TLC indicated that many of the Naucleeae s.s. did contain small amounts of pyridinoindoloquinolizidinone alkaloids of the angustine-type (**12**) and thus probably indicate the presence of strictosidine-type (**4**) alkaloids (see Table 6).

The majority of genera from the Naucleeae s.s. did not contain significant quantities of heteroyohimbine (**8**, **9**) or oxindole (**10**, **11**) alkaloids whereas these alkaloids were prevalent in the *Cephalanthus* and *Mitragyna* samples [68, 70] and are common in *Uncaria* species [66]. The presence of these alkaloids (**8**–**11**) in *Cephalanthus* supports a relationship between the Cephalantheae and Cinchoneae and the particular alkaloids identified would suggest that chemically *Cephalanthus* is most closely related to *Uncaria*.

The majority of alkaloids isolated to date from two genera of the subtribe Adininae contain a C-5 carboxyl substituent and presumably are derived from tryptophan (**3**) rather than tryptamine (**2**). Furthermore many of these alkaloids retain glucose from the secologanin precursor. In addition to these two major characteristics, the alkaloids of the Adininae vary by a number of features, viz. aromatization of ring C, hydrolysis of the C-16 ester and subsequent lactamization with either N-1 or N-4, fusion of N-4 and C-18, lactone formation between C-19 and C-5 carboxyl, fusion of N-1 with C-19 (see Fig. 3). Only one typical heteroyohimbine alkaloid (gambirine, **9**, R = OH) has been reported from this subtribe [88].

The alkaloids of the Naucleinae indicate relationships with the Adininae (e.g. strictosidine lactam) and with the Anthocephalinae (e.g. 3α-dihydrocadambine). Glycosidic alkaloids are also present in the Anthocephalinae in which N-4 may be fused either to C-18 or C-19 with the presence of a C-3–C-19 ether link or a C-19 hydroxyl substituent. Screening of the herbarium samples indicated the presence of traces of

Table 5. Genera of the tribe Naucleeae s.l. [71]

Tribe	Subtribe	Genera
Naucleeae s.s.	Adininae	*Gyrostipula, Janotia, Breonadia, Neobreonia, Breonia, Haldina, Sinoadina, Metadina, Pertusadina, Adina, Adinauclea, Khasiaclunea, Diyaminauclea, Ludekia, Neonauclea, Myrmeconauclea*
	Naucleinae	*Ochreinauclea, Nauclea, Sarcocephalus, Burttdavya*
	Anthocephaline	*Anthocephalus*
Cephalantheae	—	*Cephalanthus*
Cinchoneae	Mitragyninae	*Mitragyna, Uncaria*

Table 6. Genera of the Naucleeae s.s. containing pyridinoindoloquinolizidinone alkaloids [67]

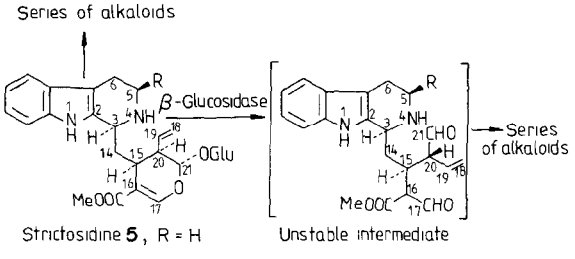
Subtribe	Genera
Adininae	*Gyrostipula, Breonadia, Breonia, Sinoadina,* Metadina, Pertusadina,* Adina, Khasiaclunea,* Ludekia,* Neonauclea, Myrmeconauclea* (11 of the 16 genera)
Naucleinae	*Ochreinauclea,* Nauclea, Sarcocephalus* (3 of the 4 genera)
Anthocephalinae	*Anthocephalus* (sole genus)

*New genus.

pentacyclic oxindoles (**10**) in two samples of one species of *Anthocephalus* demonstrating affinity of the Anthocephalinae with the neighbouring tribe Cephalantheae and further indicating that *Anthocephalus* is distinct from other members of the Naucleeae s.s. The distribution of the major types of alkaloid within the Naucleeae s.l. is summarized in Table 7. These investigations clearly demonstrate the value of chemical investigations into herbarium material particularly for assessing taxonomic relationships in problem groups.

With *Mitragyna* and *Uncaria* included in the Cinchoneae, it is pertinent to look for chemical similarities within the tribe. The alkaloid containing genera of the Cinchoneae are listed in Table 8. Morphological similarities do exist between *Uncaria–Mitragyna* and *Corynanthe–Pausinystalia* and the alkaloids of these four genera are similar. Some five (?) of the eight known species of *Corynanthe* (= *Pseudocinchona*) and five (?) out of the 13 known species of *Pausinystalia* are listed in the chemical literature; correct identification of some of these samples may well be in doubt [69]. Chemical investigation of herbarium material is one possible method which could be used for a more comprehensive view of the alkaloids of these two genera.

The barks of *Cinchona* and *Remijia* species (Cinchoneae) are well-known for their production of indole and quinoline alkaloids. Much of the information on the alkaloids of these two genera is in the early literature and the recent findings of cinchophylline-type alkaloids (**15**) in the leaves of one species of *Cinchona* [89, 90] highlights the fact that it is still possible to be surprised by the alkaloids of this genus. It is apparent that quinine-type alkaloids are present in the leaves of some species of *Cinchona* [91] but we know very little about the alkaloids from the leaves of other species of *Cinchona*. Furthermore, phy-



Subsequent reaction sequences from **5**, **6**	Tribes
(i) C-5–C-19	Naucleeae
(ii) -4H	Naucleeae
(iii) C-16 CO–N1 or N4	Naucleeae, Cephalantheae, Cinchoneae
(iv) C-18 or C-19–N-4	Naucleeae
(v) C-21–N-4	Naucleeae, Cephalantheae, Cinchoneae
(vi) C-17–C-18 or C-19	Naucleeae s.s.; Cephalantheae, Cinchoneae
(vii) Inclusion 1N	Naucleeae s.s.; Cephalantheae, Cinchoneae

Fig. 3. Major biosynthetic pathways of alkaloids from Naucleeae.

Table 7. Distribution of the major types of alkaloids within the Naucleeae s.l. [67]

Tribe	Subtribe	C-5 carboxyl	Glucoside alkaloid	PIQ*	Heteroyohimbine-oxindole
Naucleeae s.s.	Adininae	+	+	+	−
	Naucleinae	+	+	+	−
	Anthocephalinae	−	+	+	−
Cephalantheae	—	−	−	±	+
Cinchoneae	Mitragyninae	−	−	±	+

*Pyridinoindoloquinolizidinone alkaloids.

Table 8. Alkaloid containing genera of the Cinchoneae

Genus	No. of species	No. of species in chemical literature [113–115]
Mitragyna	10	10
Uncaria	34	34
Corynanthe (= *Pseudocinchona*)	8	?5
Pausinystalia	13	?5
Cinchona	40	?32
Remijia	35	4
Ladenbergia	30	4
Coutarea	7	2

tochemical work on the bark alkaloids was done before the advent of chromatographic techniques and in fact many of these early studies were discontinued when it was realised that quinine levels were low. Recently we have examined the alkaloid content of Hasskarl's collection of Peruvian *Cinchona* barks. Although these barks were collected in 1853, they are still rich in alkaloid and two of the 28 samples examined contained in excess of 4.5% total alkaloid [Anderson, L. A., Keene, A., Phillipson, J. D. and Ridsdale, C. E., unpublished observations]. The alkaloids were separated by TLC and by using HPLC, quantitative determinations of quinine, quinidine, cinchonine and cinchonidine have been made. Some of Hasskarl's samples belong to the neighbouring genus *Ladenbergia* and were misidentified as *Cinchona* species. More detailed investigation of the alkaloids from *Cinchona* and related genera such as *Remijia*, *Ladenbergia* and *Coutarea* is needed and again small amounts of herbarium material could prove to be an invaluable source of a wide range of plant material for chemical investigation. Reference to Table 8 indicates how little some of these genera have been investigated.

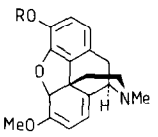
ALKALOIDS FROM THE PAPAVERACEAE

The genus *Papaver*, which comprises some 100 species, has been the subject of considerable chemical investigation for alkaloids and it might easily be assumed that there is little left to investigate. Morphine, the major alkaloid obtained from the Opium poppy *P. somniferum* L. is used for alleviating pain but because of its addictive properties its use is restricted mainly to cases of intense pain associated with terminal illness. Some 80% of the world's legal supply of morphine is converted into the more widely used codeine which is a milder analgesic without addictive properties. The gigantic illegal use of opiates and in particular the facile conversion of morphine to heroin means that the Opium poppy is a dangerous plant to cultivate on such a large scale. To date there is no plant which produces codeine in sufficiently high yields to be of any commercial value but some strains of *P. bracteatum* Lindl. do produce high yields of thebaine which can readily be converted into codeine but not so readily into morphine. Our lack of knowledge about *Papaver* and its alkaloids can be illustrated by reference to recent work in two of the nine sections of the genus, namely *Oxytona* and *Miltantha*.

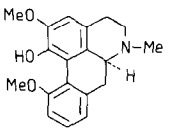
Section Oxytona

P. bracteatum is listed in the chemical literature as producing either thebaine (**16**) or isothebaine (**18**) as major alkaloid whereas the closely related species *P. orientale* is said to have thebaine or isothebaine or oripavine (**17**) as the major alkaloid [92]. Both species are also said to have a series of minor alkaloids, including morphinane-, morphinandienone-, aporphine-, proaporphine-, protoberberine-, rhoeadane- and papaverrubine-types. In 1974, the genus was revised by Goldblatt [93] who recognized three species—*P. bracteatum*, Lindl., *P. orientale* L. and *P. pseudo-orientale* (Fedde) Medw. Each of these species can be distinguished morphologically by its petal markings, by the shape of the capsules and by the chromosome numbers which are $n = 7$, $n = 14$ and $n = 21$, respectively. Furthermore, each species

seems to be characterized by its major alkaloid, as follows: *P. bracteatum*, thebaine (**16**); *P. orientale*, oripavine (**17**); and *P. pseudo-orientale*, isothebaine (**18**). It thus appeared that the chemical literature could be explained simply in terms of incorrect identification of plant material and thus it seemed obvious that at last there was a semblance of order out of chaos. This view was not only too simplistic but it was also short-lived. The major alkaloids of a Turkish sample of *P. pseudo-orientale* proved to be none of these three alkaloids (**16–18**) but to be salutaridine (**19**) and the then novel alkaloid macrantaline (**20**) [92]. The latter alkaloid is a likely biogenetic precursor of the protoberberine-type alkaloids mecambridine (**21**), aryapavine (**22**) and orientalidine (**23**) known from this section and also of the phthalideisoquinoline alkaloid narcotine which has not been reported from *Oxytona* species.



16 R=Me
17 R=H



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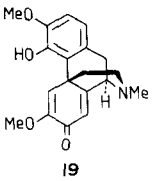

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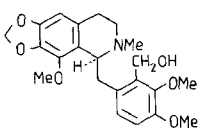

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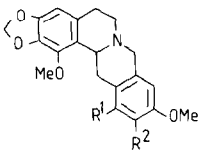


	R^1	R^2
21	CH_2OH	OMe
22	CH_2OH	OH
23	CH_2OCH_2O	

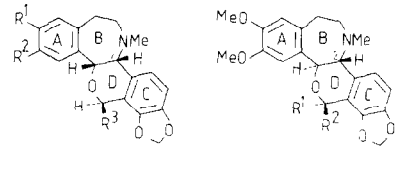
These findings prompted us to investigate other samples of Turkish *Oxytona* and to this end herbarium material was utilized. *Papaver* alkaloids separate well by TLC and different chromogenic reagents can be used for tentative identifications of some of the different structural types [94]. Confirmation of identities was readily obtained by mass spectrometry. Five samples of *P. orientale* capsules and 15 samples of *P. pseudo-orientale* capsules representing collections from central and mainly eastern Anatolia were investigated. Wherever possible, chromosome numbers were determined for some of the samples. A summary of the findings is given in Table 9. Some of the samples of *P. orientale* contained oripavine as major alkaloid and their diploid chromosome number was 28 as expected, but one sample contained no oripavine and mecambridine (**21**) was the major alkaloid. Further evidence for the presence of different chemical races was obtained from the samples of *P. pseudo-orientale*. The majority of samples possessed a diploid chromosome number of 42 with isothebaine (**18**) as major alkaloid but some samples contained mecambridine (**21**) and orientalidine (**23**) as major alkaloids. One sample of *P. pseudo-orientale* had a diploid chromosome number of 14 with salutaridine (**19**) and thebaine (**16**) as major alkaloids whereas another sample had a diploid chromosome number of 28 and only salutaridine as major alkaloid [94].

Section Miltantha

The section contains some nine species which are listed in Table 10. When the alkaloids from the species of this section were reviewed in 1981, four of the nine species had not been reported in the chemical literature [95]. Thebaine (**16**) has been reported as the major alkaloid of Turkish *P. fugax* which also contained narcotine and rhoeadine as minor alkaloids [96]. These findings were different from those of previous investigations into the species and indeed morphinane-, phthalideisoquinoline- and rhoedane-type alkaloids had not been reported previously from the section. Species of section *Miltantha* are not always readily distinguishable and nomenclatural difficulties do exist, particularly for chemists (see Table 10), e.g. *P. tauricolum* Boiss. listed in the *Flora of Turkey* may possibly be a synonym for *P. persicum* Lindl. of N. Iraq and Iran. Some of the species do show considerable variation in their morphological characters as exemplified by *P. tauricolum* in which the capsules of some plants bear long white adpressed bristles whereas the capsules of other plants are glabrous.

In order to see whether a knowledge of the alkaloids of these species might be of some value in determining relationships within the section *Miltantha*, the alkaloid literature was reviewed. The alkaloids said to be present in *P. persicum* included 1-benzyltetrahydroisoquinoline- (armepavine), proaporphine- (mecambrine, pronuciferine), aporphine- (nuciferine, mecambroline, *O*-demethylnuciferine, roemerine), protoberberine- (coptisine, palmatine), protopine-, benzophenanthridine- (sanguinarine) and papaverrubine-type alkaloids [97]. *P. tauricolum*, listed separately in the chemical literature, was said to contain five of the same alkaloids reported from *P. persicum* (namely, armepavine, mecambrine, nuciferine, sanguinarine and papaverrubines). Our investigations into the alkaloids of Turkish *P. tauricolum* revealed that the major alkaloids, from three different geographical sources, were all rhoeadanes, but different ones in each case [97]. The capsules from Malatya contained the *cis* B/D ring alkaloids oreogenine (**24**) and oreodine (**25**) as major alkaloids, Gulek capsules only oreogenine and Kayseri capsules the *trans* B/D ring alkaloids glaucamine (**26**), glaudine (**27**) and epiglaudine (**28**). None of these alkaloids had been reported previously from this species. Further investigations revealed that at least three different races of the closely-related species *P. fugax*, *P. tauricolum* (*P. persicum*) and *P. armeniacum* exist in which the major alkaloidal types are A. morphinane, B. proaporphine, aporphine and C. rhoeadane (Fig. 4) [95].

There were no reports on the isolation or identification of alkaloids from Iraqi species of Papaveraceae and to obtain samples for chemical



	R^1	R^2	R^3
24	OMe	OMe	OH
25	OMe	OMe	OMe

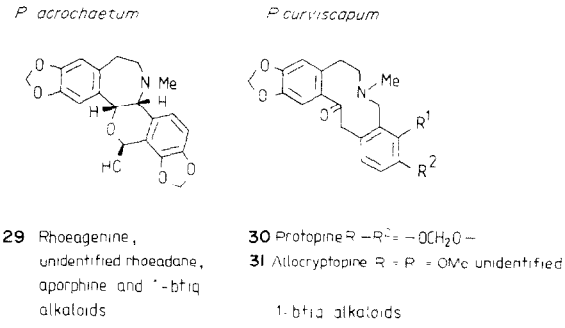
	R^1	R^2
26	OH	H
27	H	OMe
28	OMe	H

analysis could be expensive and difficult to accomplish. The National Herbarium of Iraq and the College of Agriculture Herbarium, University of Baghdad, kindly made available replicate collections of herbarium material of *Papaver*, *Glaucium* and *Roemeria* for chemical investigation. Twenty-six samples of *Papaver*, 15 of the actual samples being cited in the new *Flora of Iraq*, Vol. 4, were investigated [98]. Some of the findings for the alkaloids identified in *Papaver* species are given in Table 11.

In the investigation of the Iraqi herbarium samples four species of *Miltantha* (*P. persicum*, *P. armeniacum*, *P. acrochaetum* and *P. curviscapum*) were examined. The latter two species had not been chemically investigated previously. *P. acrochaetum* yielded rhoeagenine (**29**) as the major alkaloid and mass spectrometry indicated the presence of unidentified rhoeadanes, aporphines and 1-benzyltetrahydroisoquinoline alkaloids. The major alkaloids of *P. curviscapum* were protopine (**30**) and allocryptopine (**31**) with minor quantities of unidentified aporphine and 1-benzyltetrahydroisoquinoline alkaloids [98] (Fig. 5). The major alkaloid of Iraqi *P. persicum* (syn. *P. tauricolum*) and of *P. armeniacum* was identified as the rare glycosidic alkaloid floripavidine (**32**) which has been reported once previously by Russian workers who obtained it from *P. floribundum* (a synonym of *P. fugax*). Floripavidine (**32**) obtained from the herbarium samples was identified by UV, ^{1}H NMR, mass spectrometry and by hydrolysis to rhamnose and *N*-methylasimilobine (**33**).

Other sections of Papaver

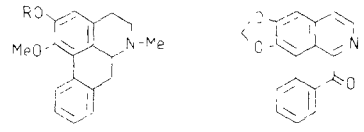
Chemical investigation of the herbarium samples of Iraqi *Papaver* spp. indicated that chemical races exist in other sections of the genus. Six samples of *P. glaucum* of the *Papaver* section were examined. In two samples no alkaloids were detected; one contained rhoeagenine (**29**) as a major alkaloid, one contained unidentified rhoeadanes, one an unidentified aporphine, while the remaining sample yielded aporphine alkaloids including liriodenine (**34**) (UV, ^{1}H NMR, mass spectrometry), not previously reported from the genus, roemerine (**35**) and its *N*-oxide (**36**) isolated for the first time together with dehydroroemerine (**37**) [98]. Not all species of the section *Papaver* have been investigated chemically as evidenced by the recent work on *P. lacerum*. The four closely-related alkaloids roemerine (**35**), *N*-methylasimilobine (**33**) and the two corresponding proaporphines mecambrine and pronuciferine were identified [99]. Until recently, it was believed that *N*-methylasimilobine (**33**) was a rare alkaloid in the genus *Papaver* but not only has it been identified as occurring in *P. lacerum* but also as the rhamnoside in *P. armeniacum* and *P. persicum*. Surprisingly it is also the major alkaloid of the common field poppy *P. rhoeas* obtained from Egypt [100].



29 Rhoeagenine, unidentified rhoeadane, aporphine and 1-btiq alkaloids

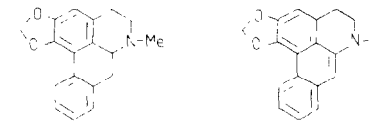
30 Protopine R–R' = –OCH$_2$O–
31 Allocryptopine R = R' = OMe unidentified
1-btiq alkaloids

Fig. 5. *Papaver* species not previously in the chemical literature [98].



32 R = Rhamnose
33 R = H

34



35

36 *N*-Oxide of 35

37

Alkaloids from other genera of the Papaveraceae

The tribe Papaverae contains a number of alkaloid-bearing genera including *Meconopsis* (41 spp.), *Glaucium* (21 spp.), *Argemone* (9 spp.) and *Roemeria* (6 spp.). Limited chemical examination of *Glaucium* (two of the five Iraqi species) and of *Roemeria* (one of the two Iraqi species) indicated that small samples of herbarium material would yield information on the alkaloids of these genera [98].

Meconopsis, the closest genus to *Papaver*, contains 40 species native to the Himalayas and China and one

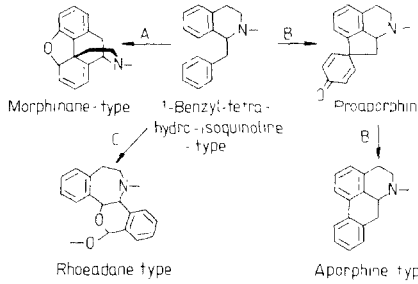


Fig. 4. Chemical races of *P. fugax*, *P. tauricola* and *P. armeniacum* [95].

Table 9. Chromosome numbers and alkaloidal-types isolated from capsules of Turkish samples of *P. orientale* and *P. pseudo-orientale* [94]

Sample	ISTE no.	Chromosome no. (2*n*)	Route A			Route B	Route C		
			Promorphinane	Morphinane		Aporphine	Protoberberine		Rhoeadane
			Salutaridine	Thebaine	Oripavine	Isothebaine	Mecambridine	Orientalidine	Alpinigenine
P. orientale									
01	43084	28	—	—	+++	+	+	+	+
03	42663	—	—	—	—	—	+++	+	—
04	42723	28	+	+	+++	+	—	—	+
P. pseudo-orientale									
P2	31915	28	+++	—	—	+	+	+	—
P3	30402	14	+++	+++	—	—	—	—	—
P10	43047	42	+	+	—	+++	+++	+++	—
P14	41239	42	+	+	—	+++	+++	+++	+
P16	41285	42	++	—	—	+++	++	++	+

Table 10. Section Miltantha Bernh. [95]

P. achrochaetum Born.*
syn. *P. fugax* Poir. *var. microcarpum* (Boiss.) Fedde
P. tauricolum Boiss. *var. microcarpum* Boiss.

P. armeniacum (L.) DC.
syn. *P. caucasicum* M.B. *var. stenocarpum* Boiss.
P. roopianum (Bordz.) Sosn.

P. curviscapum Nab.*

P. cylindricum Cullen*

P. fugax Poir.
syn. *P. caucasicum* M.B.
P. floribundum Desf.

P. libanoticum Boiss.*

P. persicum Lindl. possibly identical with *P. tauricolum* Boiss.†
syn *P. hyoscyamifolium* Boiss. et Haussk n.

P. polychaetum Schott et Kotschy

P. triniifolium Boiss.
syn. *P. urbanianum* Fedde

*Not in chemical litaeature.
†In *Floras of Iran and Iraq*, *P. persicum* Lindl. syn *P. tauricolum* Boiss. In *Flora of Turkey*, *P. tauricolum* (*'tauricola'*) possibly identical with Iranian *P. persicum*.

Table 11. Alkaloids from herbarium samples of Iraqi species of *Papaver* [98]

Section	Species*	Major types of alkaloid†
Miltantha	*P. persicum* Lindl.	(A) Rhoeadane; (B) aporphine
	P. acrochaetum Bornm.‡	Rhoeadane
	P. armeniacum (L.) DC	Aporphine, rhoeadane
	P. curviscapum Nabk.‡	Protopine
Papaver	*P. glaucum* Boiss. et Haussk n.	(A) Aporphine; (B) rhoeadane
Caratinae	*P. macrostomum* Boiss. et Huet	Protopine, aporphine, tetrahydroportoberberine
	P. bornmuelleri Fedde‡	Negative
Orthorhoeades	*P. rhoeas* L.	(A) Protopine, aporphine; (B) proaporphine, aporphine
	P. dubium L.	Negative
Argemonor-rhoeades	*P. hybridum* L.	Rhoeadane

*26 samples, 15 cited in *Flora of Iraq*, Vol. 4.
†19 alkaloids identified, numerous unidentified minor alkaloids.
‡Not previously in chemical literature.

European species. The alkaloids obtained from species of *Meconopsis* are summarized in Table 12. Protopine and sanguinarine are reported from five of the Asian species, whereas *M. cambrica*, the one species indigenous to Europe, is reported to contain mecambrine (proaporphine), mecambroline (aporphine) and mecambridine (protoberberine). A reinvestigation of *M. cambrica* grown in England [101] revealed that the major alkaloid was the quaternary aporphine magnoflorine. Also present were proaporphine- (mecambrine, pronuciferine), protoberberine- (mecambridine), promorphinane- (amurine, flavinantine) and benzophenanthridine- (sanguinarine) type alkaloids. Thus chemical races also exist in *Meconopsis*. It would be expensive and time-consuming to collect the Asian species and a more comprehensive view of *Meconopsis* alkaloids might conceivably be obtained from chemical investigations of herbarium material.

CONCLUSIONS

There is a fundamental lack of knowledge about the constituents of many plants, even those which have received considerable attention from phytochemists as exemplified here by reference to the alkaloids of tropical species of Rubiaceae and temperate species of Papaveraceae. In fact some species are becoming extinct before their chemistry has received any attention, e.g. one of the species of *Stevia* examined for sweetening agents is now believed to be extinct and only remains as a herbarium specimen [44]. A recent article has claimed that nothing is known about the chemical composition of over 99% of the Brazilian flora [102] and that the next century may be characterized by an enormous rate of extinction for these plants. The frightening thought has emerged that it is doubtful whether 5% of the world's organisms can be investigated chemically before the remaining 80% become extinct [103]. This has led to the view that if no register is made of the molecules perfected by nature over 3 billion years of evolution then not only are we destroying a powerful telescope for looking into our past but also we are endangering our future [102].

Herbaria are traditionally the stores of reference plant material and obviously should continue as such. However, it is possible, as illustrated here, to make limited use of herbarium material for chemical investigations. Such investigations have the advantage that identification of the plant samples are ensured and that as subsequent revisions are undertaken the actual samples investigated chemically will still remain. Mounting expeditions for plant material is costly and in order to investigate chemically a wide range of plant samples, as for example the pantropically distributed genus *Uncaria*, it would be impossible for all the samples to be collected during one person's lifetime. Furthermore, it is advisable for any chemosystematic studies that as wide a range as possible of samples be investigated. Therefore it could seem highly desirable to carry out chemical work on herbarium material and in particular where abundant plant material is available. Examples have been quoted to illustrate that a wide range of secondary plant products are stable in herbarium samples over periods of many years.

The physical, chemical and biological techniques now available to phytochemists are more sensitive and more specific than at any previous time. It is possible to obtain considerable information about the presence of specific chemicals from small fragments of plant material and such a technique as radioimmunoassay (RIA) which is highly sensitive in the picomole range has been applied for the analysis of indole alkaloids in *Catharanthus* sp. [104] and cardenolides in *Digitalis* sp. [105]. There are two spectroscopic techniques in particular, mass spectrometry and NMR, which have revolutionized phytochemistry. The application of mass spectrometry to natural products has been the subject of numerous publications and extensive review articles [106–108]. The linking together of mass spectrometry to such separatory techniques as GC and HPLC provides powerful analytical tools for the investigation of biological samples. Newer techniques are constantly being introduced into mass spectrometry and their applications to Biology has been the subject of a recent volume [109]. Although many problems can be solved effectively by EIMS there are many which cannot, particularly if the substances being investigated are involatile or thermolabile. The techniques of field desorption mass spectrometry and laser-assisted flame ionization detection, chemical ionization, californium-25 plasma (Cf) and fast atom bombardment are currently being developed and look particularly promising for solving biological problems. Thus such intractable molecules as glycopeptides and polysaccharides are being structurally

Table 12. *Meconopsis* alkaloids [101]

40 species indigenous to Himalayas and China:	
M. aculeata	protopine, sanguinarine
M. betonicifolia	protopine, sanguinarine
M. horrida	protopine, sanguinarine
M. latifolia	protopine, sanguinarine
M. rudis	protopine, sanguinarine, allocryptopine, magnoflorine
M. paniculata	coptisine
M. dhwojii	sanguinarine
1 species indigenous to Europe:	
M. cambrica	mecambrine (proaporphine)
	mecambroline (aporphine)
	mecambridine (protoberberine)

elucidated by this methodology, even the sequencing of polypeptides at picomole levels is being determined by mass spectral techniques.

Tandem mass spectrometry, MIKES (mass analysed ion kinetic energy spectrometry), collision-induced spectrometry and multiple quadrupole spectrometry, can separate complex mixtures without combination with chromatographic techniques. Thus it is possible to overcome many of the problems associated with the link-up between a chromatographic technique and mass spectrometry. The sensitivity of the method ensures that sub-picograms can be detected [110]. In fact, the sensitivity of tandem mass spectrometry is comparable with RIA and is a faster method of analysis which has obvious applications for chemotaxonomic studies [111]. Some applications of this technique for the analysis of natural products have been reviewed and two examples are included to illustrate the advantages of the method [112]. Samples of 1 mm^2 of Coca leaf have been analysed specifically for cocaine, without prior extraction of the alkaloid [112]. Tandem mass spectrometry of pyrrolizidine alkaloids in *Senecio* has been demonstrated to be 100-times more sensitive and 30-times faster than routine LC. One major problem with these sophisticated techniques is the high cost of instrumentation but it has been predicted that the cost of computerized tandem mass spectrometry may well come down to *ca* $50 000 [111].

Some examples of the application of ^1H NMR to the identification of alkaloids in herbarium samples have been given here but all the examples quoted were of spectra obtained from either 60 or 100 MHz spectrometers. With the more powerful 250 or 400 MHz instruments which are now becoming more routinely available, it is possible to obtain satisfactory ^1H NMR spectra using only a single scan with as little as 0.3 mg of a sample with a MW in the range of 300–500 [Hawkes, G. E., personal communication]. However, with the use of FT overnight, amounts as small as 100 μg can give meaningful spectra. Currently, ^{13}C NMR normally requires samples in the order of 40 mg (MW's 300–500) for reasonable spectra but the use of a microprobe will lower this requirement to 10 mg and FT overnight to 2 mg. Increased sensitivity for ^1H NMR can be expected from the use of high field strength magnets (e.g. 600–1000 MHz) but this does not apply for ^{13}C NMR spectrometry. One particularly interesting development is the application of ^{13}C NMR to solids, e.g. coal, soil, crystalline chemicals. Newer techniques such as 'inept' spectrometry adds further sophistication allowing for an increase in sensitivity of four-times for ^{13}C spectra and 10-times for ^{15}N spectra. This method is particularly useful for picking out tetravalent carbon, CH, CH_2 and methyl groupings within molecules [Hawkes, G. E., personal communication].

The development of such mass spectral and NMR techniques may well lead to methods of physical analysis which will leave the original herbarium sample undamaged. However, there is no doubt that the sophisticated methodology which is available today represents a formidable tool for the investigation of small samples of herbarium material. It is to be hoped that co-operation between taxonomists and phytochemists in choosing particular groups of plants will allow for rapid increases in our knowledge of secondary metabolities and the plants from which they are obtained.

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