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PYRROLIZIDINE ALKALOIDS OF *HELIOTROPIUM* FROM MEXICO AND ADJACENT U.S.A.

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Key Word Index—*Heliotropium*; Boraginaceae; Mexico; saturated pyrrolizidine alkaloids.

Abstract—The levels of alkaloids were determined in leaves and stems of 24 species of *Heliotropium* collected in Mexico and adjacent U.S.A. All species contained unsaturated pyrrolizidines, their content in leaves ranging from 0.003 to 0.18%. Saturated pyrrolizidine alkaloids were detected in nine species. Greenhouse-grown plants of five species had a significantly higher alkaloid content in their leaves than the corresponding field samples. In all these species, except for *H. indicum*, the differences were due to higher levels of saturated pyrrolizidines.

INTRODUCTION

Pyrrolizidine alkaloids occur as both tertiary bases and *N*-oxides in many genera of Compositae, Boraginaceae and Leguminosae. Those containing an unsaturated 1,2-dehydro, 1-hydroxymethylpyrrolizidine system are toxic to man and animals mainly due to the alkylating capacity of their pyrrolic derivatives; the degree of toxicity also depends on other structural features of these compounds [1-4]. A high ratio between the tertiary bases and their *N*-oxides increases the toxicity of pyrrolizidine alkaloid-containing plants, especially to monogastric animals [5].

Hepatotoxic, pulmotoxic, hemolytic, antimitotic, teratogenic, mutagenic, and carcinogenic effects of unsaturated pyrrolizidine alkaloids have been found; heliotrine and lasiocarpine showing the strongest action [2, 4, 6–11]. Aerial parts of *Heliotropium ternatum* and extracts from *H. angiospermum* induced esophagus lesions and carcinomas in hamsters and sarcomas in rats [12, 13]. Esters of heliotridine (lasiocarpine and heliosupine) and of retronecine (monocrotaline, seneciphylline, and indicine *N*-oxide) revealed an antitumor activity [6, 14]; platyphylline, an ester of the saturated platynecine with senecic acid, showed mydriatic and antispasmodic activities [2]; semisynthetic esters of heliotridine and platynecine had local anaesthetic effects [15]; and heliosupine and a number of semisynthetic quaternary pyrrolizidines exhibited an hypotensive action [16, 17].

Some pyrrolizidine-containing plants, such as *H. indicum*, *H. angiospermum*, *H. ternatum*, *H. curassavicum*, *H. polyphyllum*, *H. steudneri* and species of other genera, especially when dried, are attractants for numerous male butterflies [18–21]. Among the alkaloid baits tested, heliotrine and indicine were the most powerful attractants [19, 21]. There is evidence that the butterflies use the 1,2-dehydropyrrolizidines to produce dihydropyrrolizines with pheromone/allomone activity. Lycopsamine, viridifloric ester of retronecine, isolated from *H. steudneri* has been shown to be a precursor of the pheromone present in hairpencil secretion of male *Danaus* butterflies [20]. Volatile products from necic acids, such as (+)-trachelanthic and (−)-viridifloric acids, are thought to attract males to the plants, whereas intact alkaloids are thought to act as phagostimulants [21].

Heliotropium is a large genus of over 200 species found predominantly in the warmer parts of both hemispheres. Their life habits range from annual herbs and geophytes to perennial shrubs. Some species are weeds of cultivated crops and grazing lands, others are members of climax communities, especially in desert regions. Some species, e.g. *H. procumbens*, *H. ternatum*, *H. fruticosum*, *H. confertifolium*, *H. torreyi*, *H. curassavicum*, *H. angiospermum*, *H. greggii* and *H. indicum*, are abundant in Mexico and adjacent U.S.A. The genus comprises a number of sections of which section *Orthostachys* is the largest. The taxonomy of the Mexican species of *Orthostahys* has been discussed elsewhere [22].

This preliminary study presents the levels of unsaturated and saturated pyrrolizidine alkaloids in *Heliotropium* species collected in Mexico and adjacent U.S.A. in the summer of 1978.

RESULTS AND DISCUSSION

The life habits, developmental stages, and sample origins of the 24 *Heliotropium* species that were analysed are presented in Table 1. The plants, from which leaves and stems were collected and preserved by drying, were at the stage of flowering and/or fruiting; i.e. at the stage at which in many species the alkaloid content is very high or the highest [23, 24].

Table 1. *Heliotropium* species collected in Mexico and adjacent U.S.A.

Section and Subsection	Species*	Life habit	Developmental stage	Sample origin	
				State	Habitat
Schobera	*angiospermum* Murray (2165.6)	Geophyte	e Fl	Queretaro	Larrea desert
Coeloma	*glabriusculum* Gray (2170.5)	Geophyte	f Fl	Coahuilla	desert
Halmyrophila	*curassavicum* L. (2138)	Geophyte	Fr	Oaxaca	thorn forest; weed
	curassavicum L. (1502)	Geophyte	f Fl (g)	Baja Calif. Sur (seeds)	sea shore
	spathulatum Rydb. (2175)	Geophyte	e Fl	Calif.	stream bank
	spathulatum Rydb. (2175)	Geophyte	e Fl (g)	Calif. (rhizomes)	
Tiaridium	*indicum* L. (2112)	Annual	l Fl	Mexico	hot wet tropics
	indicum L. (2112)	Annual	e Fl (g)	Mexico (seeds)	hot wet tropics
Orthostachys					
Ebracteata	*calcicola* Fern (2164)	Shrub	Fl-Fr	Tamalipas	semi-desert
	calcicola Fern (2030)	Shrub	S (g)	Tamalipas (seeds)	semi-desert
	calcicola Fern (2153-3)	Shrub	Fr	Puebla	semi-desert
	fallax Johnston (2135-4)	Shrub	Fl-Fr	Oaxaca	dry forest
	fallax Johnston (2135-1)	Shrub	Fl-Fr	Oaxaca	dry forest
	procumbens Mill. (2118-1)	Annual	f-l Fr	Tamalipas	semi-forest
	procumbens Mill. (1538 or 1850)	Annual	f-l Fr (g)	Baja Calif. Sur. or Oaxaca (seeds)	sandy arroyo or tropic weeds
	queretaroanum Johnston (2159)	Shrub	f Fl	Queretaro	desert
	torreyi Johnston (2161)	Shrub	Fl-Fr	Tamalipas	dry hillside
Bracteata	*confertifolium* Torr. ex Gray (2166)	Shrub	f Fl	Coahuilla	desert
	convolvulaceum (Nutt.) Gray (2113)	Annual	f Fl	Texas	sandy desert
	cremnogenum Johnston (2114)	Annual	f Fl	Guerero	hot tropics
	filiforme Lehm. (2136)	Annual	l Fr	Oaxaca	hot wet tropics; weed
	foliosissimum McBride (2105-2150)	Geophyte	Fl-Fr	Oaxaca	grazed hillside
	fruticosum L. (2117)	Annual	f Fl	Puebla	limestone hills
	greggii Torr. (2168)	Geophyte	f Fl	Coahuilla	desert
	huehuetoca (2154)†.	Annual	f Fl	Mexico	disturbed soil
	huehuetoca (2154)†.	Annual	Fl-Fr (g)	Mexico (seeds)	disturbed soil
	karwinskyi Johnston (2162)‡.	Shrub	f Fl	Tamalipas	semi-desert
	karwinskyi Johnston (2162-2)	Shrub	f Fl	Tamalipas	semi-desert
	karwinskyi Johnston (2031)	Shrub	S (g)	Tamalipas (seeds)	semi-desert
	limbatum Benth (2148)	Geophyte	f Fl	Oaxaca	grazed hillside
	tenellum (Nutt.) Torr. (2171a)	Annual	f Fl	Texas	desert
	ternatum Vahl (2137)	Shrub	f Fl	Oaxaca	roadside; weed
	ternatum Vahl (2161.2)	Shrub	f Fl	San Luis Potosi	dry forest
	ternatum Vahl (1980)	Shrub	f Fl (g)	Yucatan (seeds)	disturbed soil
Axillaria	*axillare* Greenman (2156)	Annual	f Fl	Queretaro	Larrea desert
	pringlei Robins (2116)	Annual	Fl-Fr	Puebla	limestone hills
	pringlei Robins (2147)	Annual	Fl-Fr	Oaxaca	disturbed soil
	pringlei Robins (2147)	Annual	Fl-Fr (g)	Oaxaca (seeds)	disturbed soil

* In parentheses, Michael W. Frohlich collection numbers are indicated; vouchers are deposited in the Union College Herbarium.

† Huehuetoca refers to the collection locality. The species assignment is not certain.

‡ *H. karwinskyi* (2031) and (2162) are from the same locality.

§ e = early; f = full; l = late; Fl = flowering; Fr = fruiting; S = seedling; (g) = greenhouse.

Nine species were grown in the greenhouse from collected seeds or rhizomes, namely *H. curassavicum*, *H. spathulatum*, *H. procumbens*, *H. calcicola*, *H. karwinskyi*, *H. ternatum*, *H.* huehuetoca, *H. pringlei* and *H. indicum*. The leaves were sampled at flowering, except for *H. calcicola* and *H. karwinskyi* which were at the seedling stage. No significant differences between the fresh and dried leaf samples were found either in the unsaturated and total alkaloid contents or in the alkaloid spectrum after chromatography. Neither were any significant differences in the alkaloid content found when dried samples of some species were re-examined after a period of 2–3 months.

Bull *et al.* [2] have reported a loss of 50–80% of alkaloid during drying of *H. supinum* plants which contain esters of heliotridine and supinidine and free supinidine [25]. At the same time they report no significant losses for *H. europaeum* which contains five heliotridine and two supinidine esters [26, 27]. There is evidence that drying may affect the ratio between tertiary base forms and corresponding *N*-oxides without changing the total content of pyrrolizidine alkaloids [28]. This artifact might apply to the levels of *N*-oxides of the unsaturated fraction reported in Table 2; the total content determination and chromatographic analysis of the alkaloids were performed after reduction of *N*-oxides.

The alkaloid composition is known only for two of the 24 examined species, namely: (a) *H. indicum*, in which esters of the unsaturated retronecine (indicine, indicinine and acetylindicine) were identified [29]. Yet, plants collected in Bangladesh revealed the presence of indicine, supinine, heleurine, heliotrine, lasiocarpine and echinatine, thus esters of retronecine, supinidine and heliotridine [30]; and (b) *H. curassavicum*. In some populations, collected in India, heliotrine, lasiocarpine and 7-angelylheliotridine were found [31]; in some others esters of the saturated trachelanthamidine (curassavine, coromondalin and heliovicine) were identified [32]. Very closely related to *H. curassavicum* is *H. spathulatum*. They are frequently not distinguished at the species level.

The total alkaloid content in the collected leaves of *H. curassavicum* and *H. spathulatum* was 0.14 and 0.004%, respectively. Both species revealed the presence of saturated and unsaturated pyrrolizidines in their leaves and stems. Leaves sampled from plants grown in the greenhouse showed a total alkaloid content of 0.74–0.87%. However, the level of the unsaturated fraction remained very low. Analytical and preparative TLC revealed the presence of two major and three minor saturated pyrrolizidines as well as three minor unsaturated alkaloids. None of the unsaturated components present showed the pattern of development by Mattocks' reagents, characteristic of rosmarinine which contains a saturated ring hydroxylated at C-3 [33].

A preliminary analysis of the compounds isolated from the greenhouse-grown *H. curassavicum* and *H. spathulatum* was performed by GC–MS. In both species four major saturated alkaloids (assigned by their peak at *m/e* 124 as being of the trachelanthamidine type), several minor saturated alkaloids and several minor unsaturated ones (assigned by their base peak at *m/e* 138) were detected. Three of the major saturated components have been tentatively identified as curassavine (M^+ 299), acetyl curassavine (M^+ 341), and possibly a mixture of acetyl coromondalin and acetyl heliovicine (M^+ 327). The compounds identified as acetates show a M^+-43, M^+-44 and an intense peak at *m/e* 43. No assignment has as yet been made for the fourth major component. The two species, while showing approximately the same distribution of the major alkaloids, did differ in the distribution of the minor ones. *H. spathulatum* showed more and a greater variety of the unsaturated alkaloids.

The levels of unsaturated and saturated alkaloids reported in Table 2 are burdened with errors, sometimes perhaps even substantial. They may result from differences between various alkaloids in extinction coefficients when determined spectrophotometrically, molecular weights when determined by titration, and in the degree of recovery from aqueous solutions during extraction [34]; they may also result from impurities in the extracts, especially from plants with a low alkaloid content.

Unsaturated alkaloids were detected in leaves and stems of all 24 species. Except for *H. indicum*, *H. filiforme*, and *H. karwinskyi*, their level in the leaves ranged from traces to 0.05%. In 15 species TLC did not indicate the presence of alkaloids with saturated necines. In six of these, namely *H. torreyi*, *H. cremnogenum*, *H. filiforme*, *H. foliosissimum*, *H. fruticosum* and *H. tenellum*, no differences were found between the spectrophotometric and titrimetric determinations. In eight other species with a total alkaloid content below 0.03%, the values for the unsaturated fraction amounted to 30–60% of those determined by titration. In leaves of *H. indicum* with a total alkaloid content of 0.09 or 0.69% the values obtained by spectrophotometry were about 35% lower. In *H. glabriusculum*, *H. greggii* and *H. axillare*, whose leaves revealed unsaturated alkaloids in amounts between 3 and 11% of the total, TLC did indicate the presence of saturated pyrrolizidines as major alkaloids. The same can be said about *H.* huehuetoca, *H. pringlei* and *H. ternatum*.

When grown in the greenhouse, leaves of *H.* huehuetoca did not show any significant difference in the total alkaloid content or spectrum as compared to those collected in Mexico. Leaves of *H. pringlei*, *H. procumbens* and *H. calcicola* revealed only a slightly higher level of these compounds. However, leaves of *H. ternatum* and especially of *H. karwinskyi* plants showed a much higher content of total alkaloid as compared to those collected from plants in their natural habitats. Like in *H. curassavicum* and *H. spathulatum*, the leaves and stems of both species revealed the presence of saturated necines as major alkaloids and the higher total content in leaves from greenhouse grown plants was due to this fraction. Moreover, *H. karwinskyi* seedling leaves showed an even lower level of the unsaturated fraction than did leaves collected from the shrub in the desert. Only in *H. indicum* was a very significant increase in unsaturated alkaloids found in leaves of plants grown in the greenhouse.

The alkaloid content in plants is known to depend on genetic control, plant organ and age, as well as on environmental factors [23, 24, 35–37]. Information about the variation in the amount and composition of pyrrolizidine alkaloids in plants, in particular of *Heliotropium* species, is very scarce. The reported

Table 2. Pyrrolizidine alkaloids in *Heliotropium* species

Species*	Number of plants per sample†	Alkaloid content (% dry wt $\times 10^3$)‡ Unsaturated N-oxides (Leaves)	Unsaturated Total (Leaves)	Total	Stems
angiospermum	3	1	3	6	24
glabriusculum	25	3	8	71	101
curassavicum	5	3	5	133	145
curassavicum (g)	1		11	869	
spathulatum	3	tr	tr	4	8
spathulatum (g)	4		12	738	
indicum	4		62	91	
indicum (g)	3		451	688	
calcicola	5	2	4	8	23
calcicola (g)	3		9	27	
calcicola	1	tr	tr	2	
fallax	1	3	3	6	31
fallax	1	tr	tr		
procumbens	1	1	2	4	7
procumbens (g)	1		6	16	
queretaroanum	15	4	8	25	23
torreyi	1	4	6	5	19
confertifolium	5	5	22	41	56
convolvulaceum	3	40	51	105	103
cremnogenum	15	2	8	8	7
filiforme	3	60	178	190	227
foliosissimum	5	1	8	7	43
fruticosum	4	3	4	5	12
greggii	20	3	12	406	302
huehuetoca	20	tr	3	29	66
huehuetoca (g)	1		2	31	
karwinskyi	1	19	103		
karwinskyi	1		112	171	103
karwinskyi (g)	6		58	708	
limbatum	6	3	6	16	17
tenellum	20	3	5	6	3
ternatum	3	1	3		
ternatum	3	3	14	38	27
ternatum (g)	1		23	274	
axillare	15	7	19	316	278
pringlei	9	2	3		
pringlei	1	2	2	11	39
pringlei (g)	9		5	65	

* (g) indicates plants grown in the greenhouse, from which fresh and dried samples were analysed. No significant differences between the samples were found either in the alkaloid content or in the alkaloid spectrum after chromatography.

† *H. glabriusculum*, *H. curassavicum*, *H. gregii* and *H. foliosissimum* are probably represented by one clone each.

‡ Amounts $<0.001\%$ are indicated as trace. The contents of unsaturated alkaloids in stems of *H. curassavicum* and *H. procumbens* were 0.004 and 0.002%, respectively. Stems of other species were not analysed for unsaturated alkaloids.

values vary from 0.004–0.005% in leaves of *H. undulatum* and *H. peruvianum* [38] to 4.9% in *H. dasycarpum* [39]. The total alkaloid in the aerial parts of *H. europaeum* ranged from 0.58 to 3.08% depending on the region in Australia where they were collected, the *N*-oxides amounting to 90% of the total [40]. The reported alkaloid contents in *H. curassavicum* areial parts are 0.45 [32] and 0.66% [2].

The plants grown in the greenhouse had an optimal supply of water and mineral nutrients. The insignificant or small changes of the alkaloid levels in leaves of *H.* huehuetoca, *H. procumbenens*, *H. calcicola* and *H. pringlei* would indicate that in these species the genetic control might be dominant. In low alkaloidal lupine plants the limiting factor is rather a low rate of alkaloid synthesis than a high rate of transformation into non-alkaloidal compounds [41]. On the other hand, the behavior of *H. curassavicum*, *H. spathulatum*, *H.*

karwinskyi and *H. ternatum* would indicate that their alkaloid level might be controlled to a significant extent by external factors.

Among 20 species of *Heliotropium*, besides *H. indicum*, in which pyrrolizidine alkaloids were identified [5, 27, 32, 38, 40, 42–45], only two species, namely *H. strigosum* and *H. curassavicum* revealed the presence of alkaloids containing a saturated necine. No unsaturated alkaloids were reported to accompany the saturated ones. In 11 of the 20 species heliotrine was the major alkaloid.

The simultaneous occurrence of saturated and unsaturated pyrrolizidines and a significant increase in the level of the former in some species under certain conditions could be of importance not only from the point of view of plant toxicity but also in regard to alkaloid synthesis, interconversions and metabolism. In lupine plants the transformation of quinolizidine alkaloids showed the direction towards an increasing degree of oxidation [35, 36, 41).

In their natural habitats in Mexico and adjacent U.S.A., the *Heliotropium* species under study revealed in most cases a very low level of alkaloids in leaves as well as in stems.

EXPERIMENTAL

Plant material. Heliotropium plants were collected during August, September and December 1978, air-dried and stored at room temp. Greenhouse plants were grown from seed or rhizomes in a rich general purpose soil mixture and fertilized with N, P and K. Sampled leaves were dried with low heat in the laboratory.

Extraction. Dry material was finely powdered and extracted 4× with MeOH at 65° under reflux. Fresh leaves were homogenized in MeOH and similarly extracted. Extracts were filtered, combined, dried *in vacuo* and the alkaloids transferred into 0.5 N H_2SO_4. Reduction of *N*-oxides and extraction of alkaloids with $CHCl_3$ was performed according to ref. [2]. In order to achieve better purification the alkaloids in many cases were reextracted from acid.

Determination. Total alkaloid content was determined by titration using 0.001 N rather than 0.01 N *p*-toluenesulphonic acid [2]; heliotrine was used to standardize the solution. The unsaturated fraction was determined by Mattocks' method [34] measuring *A* at 565 nm and using the extinction coefficient for heliotrine. Alkaloids after reduction with Zn in 2 N H_2SO_4 were separated on Merck Si gel G plates using BuOH–HOAc–H_2O (4:1:5) as the solvent system. Alkaloids were developed with Dragendorff reagent [46] and Ehrlich's reagent [33]. Preliminary tests before and after reduction of *N*-oxides were performed using paper disc chromatography and Dragendorff reagent. MS were obtained using Hewlett–Packard 5992 GC–MS system equipped with a 74 cm × 2 mm glass column packed with 2% OV-101 plus 0.2% Carbowax 20 M on 100/120 mesh Chromosorb W-HP. The injection port temp. was 200° and column temp. 180°. A membrane separator (at 180°) was used for the GC–MS interface with a helium flow of 22 ml/min. Samples were injected directly onto the glass column as MeOH solutions.

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REFERENCES

1. Mattocks, A. R. (1972) in *Phytochemical Ecology* (Harborne, J. B., ed.) pp. 179–200. Academic Press, London.
2. Bull, L. B., Culvenor, C. C. J. and Dick, A. T. (1968) in *Frontiers of Biology* (Neuberger, A. and Tatum, E. L., eds.) Vol. 9, pp. 1–293. North-Holland, Amsterdam.
3. Culvenor, C. C. J., Edgar, J. A., Jago, M. V., Outteridge, A., Peterson, J. E. and Smith, L. M. (1976) *Chem. Biol. Interactions* **12**, 299.
4. Shumaker, R. C., Raczniak, T. J., Johnston, W. D. and Allen, J. R. (1977) *Proc. Soc. Exp. Biol. Med.* **154**, 57.
5. Culvenor, C. C. J. (1973) in *Chemistry and Biochemistry of Herbage* (Butler, G. W. and Baily, R. W., eds.) Vol. 1, pp. 375–446. Academic Press, London.
6. McLean, E. K. (1970) *Pharm. Rev.* **22**, 429
7. Schoental, R. (1975) *Cancer Res.* **35**, 20.
8. Green, C. R. and Christie, G. S. (1961) *Br. J. Exp. Pathol.* **42**, 369.
9. Hooper, P. T. (1975) *J. Comp. Pathol.* **85**, 341.
10. Rao, M. S. and Reddy, J. K. (1978) *Br. J. Cancer* **37**, 289.
11. Johnson, W. D., Robertson, K. A., Pounds, J. G. and Allen J. K. (1978) *J. Natl. Cancer Inst.* **61**, 85.
12. O'Gara, R., Lee, C. W., Morton, J. F., Rapadia, G. J. and Dunham, L. J. (1974) *J. Natl. Can. Inst.* **52**, 445.
13. Dunham, L. J., Sheets, R. H. and Morton, J. F. (1974) *J. Natl. Cancer Inst.* **53**, 1259.
14. Kugelman, M., Wen-Chih-Liu, Axelrod, M., McBride, T. J. and Rao, K. W. (1976) *Lloydia* **39**, 125.
15. Suri, O. P., Sawney, R. S. and Atal, C. K. (1975) *Indian J. Pharm.* **37**, 36.
16. Rakshain, R. V. and Mats, M. N. (1973) *Rastit. Resur.* **9**, 419.
17. Gupta, O. P., Ali, M. M., Ghatak, B. J. and Atal, C. K. (1977) *Indian. J. Exp. Biol.* **15**, 220.
18. Rothschild, M. (1972) in *Phytochemical Ecology* (Harborne, J. B., ed.) pp. 1–12. Academic Press, London.
19. Pliske, T. E. (1975) *Environ. Entomol.* **4**, 455.
20. Schneider, D., Boppré, M., Schneider, H., Thompson, W. R., Boriack, C. J., Petty, R. L. and Meinwald, J. (1975) *J. Comp. Physiol.* **97**, 245.
21. Pliske, T. E., Edgard, J. A. and Culvenor, C. C. J. (1976) *J. Chem. Ecol.* **2**, 255.
22. Frohlich, M. W. (1978) Ph.D. Dissertation, Harvard University.
23. Birecka, H. (1963) *Acta Soc. Bot. Pol.* **32**, 131.
24. Waller, G. R. and Nowacki, E. K. (1977) *Alkaloid Biology and Metabolism in Plants.* Plenum Press, New York.
25. Willaman, J. J. (1961) *U.S. Dep. Agric., Agric. Res. Ser. Techn. Bull. No.* 1234.
26. Culvenor, C. C. J. and Smith, L. W. (1969) *Tetrahedron Letters* **41**, 3603.
27. Culvenor, C. C. J., Stanley, R. J. and Smith, L. W. (1975) *Aust. J. Chem.* **28**, 2319.
28. Pedersen, E. (1975) *Arch. Pharm. Chem. Sci. Ed.* **3**, 55.
29. Mattocks, A. R. (1967) *J. Chem. Soc.* **5**, 329.
30. Hoque, M. S., Ghani, A. and Rashid, H. (1976) *Bangladesh Pharm. J.* **5**, 13.

31. Rajagopalan, T. R. and Batra, V. (1977) *Indian J. Chem. (B)* **15,** 494.
32. Subramaniam, M., Subramanian, S., Culvenor, C. C. J., Edgar, J. A., Frahn, J. L., Smith, L. W. and Cockrum, P. A. (1978) *J. Chem. Soc. Chem. Commun.* 423.
33. Mattocks, A. R. (1967) *J. Chromatogr.* **27**, 505.
34. Mattocks, A. R. (1967) *Analyt. Chem.* **39,** 443.
35. Birecka, H., Szklarek, D. and Mazan, A. (1960) *Bull. Acad. Pol. Sci., Ser. Sci. Biol.* **8**, 167.
36. Birecka, H. and Scibor-Marchocka, A. (1960) *Bull. Acad. Pol. Sci., Ser. Sci. Biol.* **8,** 449.
37. Mothes, K. (1960) in *The Alkaloids, Chemistry and Physiology* (Manske, R. H. F., ed.). Vol. 6, pp. 1–29. Academic Press, London.
38. Delorme, P. (1976) Ph.D. Dissertation, Université Claude Bernard, Lyon.
39. Akramov, S. T., Kijamitdinova, F., Yunusov, S. Yu. (1961) *Dokl. Akad. Nauk Uz. USSR*, 30; (1964) *Chem. Abstr.* **60,** 19209 e.
40. Crowley, H. C. and Culvenor, C. C. J. (1956) *Aust. J. Appl. Sci.* **7**, 359.
41. Birecka, H. and Sebyta, T. (1960) *Bull. Acad. Pol. Sci., Ser. Sci. Biol.* **8**, 183.
42. Warren, F. (1970) in *The Alkaloids, Chemistry and Physiology* (Manske, R. H. F., ed.). Vol. 12, pp. 245–331. Academic Press, London.
43. Suri, P. D., Sawney, R. S. and Atal, C. K. (1975) *Indian J. Chem.* **13**, 505.
44. Akramov, S. T., Shodmanov, Z., Samotov, A. and Yunusov, S. Yu. (1968) *Khim. Prir. Soedin.* **4**, 258.
45. Zalkow, L. H., Gelbaum, L. and Keinan, E. (1978) *Phytochemistry* **17**, 172.
46. Munier, R. (1953) *Bull. Soc. Chim., Biol.* **35**, 1225.