Phytochemistry, Vol 22, No 5, pp 1167–1171, 1983
Printed in Great Britain

0031-9422/83/051167-05$03 00/0

FREE AND ESTERIFIED NECINES IN *HELIOTROPIUM* SPECIES FROM MEXICO AND TEXAS*

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(*Received* 3 *July* 1982)

Key Word Index—*Heliotropium*, Boraginaceae, pyrrolizidine alkaloids, necines, trachelanthamidine, lindelofidine, supinidine, retronecine, epoxypyrrolizidine.

Abstract—Fourteen species of *Heliotropium* from Mexico and Texas contained 3–7 necines per species. In most cases there were no qualitative differences in the necine pattern of a given species depending on the sample origin Retronecine or trachelanthamidine were the dominant, and supinidine was the very minor necine in the examined species, except for *H angiospermum* in which a nonesterified aminoalcohol, with a mass spectrum and optical rotation corresponding to that of 1β,2β-epoxy-1α-hydroxymethyl-8α-pyrrolizidine, accounted for 90% of the total necine content; as a minor component this base was also detected in *H ternatum* and *H molle*. Besides lindelofidine, found as a minor necine in most samples, one or two of three different saturated diols were detected in some species; a saturated triol with a mass spectrum similar to that of croalbinecine was found in *H. racemosum* The proportion of nonesterified retronecine or trachelanthamidine ranged from 0 to 60 or 90%, respectively, depending on the species. The necine patterns are discussed in relation to chemotaxonomy

INTRODUCTION

The necine patterns of *Heliotropium* species collected in Mexico and the U S A differ significantly from those found in this genus on other continents [1] After hydrolysis of their pyrrolizidine alkaloids (PA), all of the species exhibited the presence of the aminoalcohols trachelanthamidine, supinidine and retronecine; lindelofidine was detected in most species, whereas heliotridine only occurred in four Qualitative differences depending on the collection locality were found in *H curassavicum*

This study presents the necine patterns in nine previously examined species, but collected at different localities, and also in new species, namely *H sessei*, *H racemosum*, *H. wigginsii*, *H molle* and *H angiospermum*. All species, except for *H curassavicum* and *H indicum*, were collected in 1981 In order to ensure a full extraction of esterified and nonesterified aminoalcohols from aqueous solutions after *N*-oxide reduction, a several fold chloroform extraction (fraction I) was followed by an exhaustive chloroform–ethanol extraction (fraction II).

RESULTS AND DISCUSSION

The life habits and habitats of the plants collected at flowering and/or fruiting are presented in Table 1. The amounts of PAs (Table 2), in most cases were significantly higher than those previously reported for *Heliotropium* species from Mexico and the U S A. [1, 2] The portion of PAs recovered in fraction II ranged between 1 and 38% of the total content, the higher values being observed in samples with a relatively high content of free diols, especially of retronecine. However, in several samples not only free but also esterified necines were found in fraction II.

All species from all localities contained trachelanthamidine and retronecine, ranging from trace amounts to 99%, and supinidine ranging from trace amounts to 4% of the total Lindelofidine was found in 11 of the 14 species. However, in *H curassavicum* and *H gregii* it would not be detected if present below 1% of the total due to the occurrence of trachelanthamidine as the dominant necine Lindelofidine was not detected in *H. foliosissimum* (2311) in which trachelanthamidine was only a minor necine, although it was present in *H. foliosissimum* (2150) collected in 1978 at a distance of 300 miles from the plants reported here [1]. None of the examined species revealed the presence of heliotridine.

A series of additional aminoalcohols was found in some of the species A free aminoalcohol with a mass spectrum corresponding to 1-hydroxymethyl-1,2-epoxypyrrolizidine, previously detected only in *Crotalaria trifoliastrum* [3] and *C grantiana* [4], was found in all samples of *H. ternatum*, *H. molle*, and *H. angiospermum* (Table 2). In the latter species it accounted for *ca* 90% of the total necine content, its mass spectrum and specific optical rotation were similar to those reported for 1β,2β-epoxy-1α-hydroxymethyl-8α-pyrrolizidine isolated from *C. trifoliastrum*. The specific rotation of the base, detected in *H. ternatum* and *H. molle* as a minor aminoalcohol was not determined PA extracted from two previously analysed samples of *H ternatum* [1] were re-examined without hydrolysis. Traces of the epoxy compound were detected in *H. ternatum* (1980) from Yucatan, but not in *H ternatum* (2161.2) collected in San Luis Potosi.

A very significant loss of the free epoxypyrrolizidine, observed after PA exposure to hydrolysis, was always accompanied by the appearance of a base with M^+ *m/z* 173 and base peak *m/z* 83, apparently a hydration product.

*Part 4 in the series "Aminoalcohols of Pyrrolizidine Alkaloids in *Heliotropium* Species" For Part 3 see [1].

Table 1 *Heliotropium* species collected in Mexico and Texas, U S A *

Section Subsection	Species†	Life habit	Development stage‡	Sample origin: State	Sample origin: Habitat	Number of analysed plants/ clones
Halmyrophila	*curassavicum* L (1502)	geophyte	Fl–Fr	Baja California Sur	desert, sea shore, sand	1
	(1434)	geophyte	Fl–Fr	Sinaloa	moderately dry	1
Tiaridium	*indicum* L (1406)	annual	Fl–Fr	Nayarit	moderately dry, mud soil	4
	(1636)	annual	Fl–Fr	Chiapas	wet tropics, disturbed soil	2
Orthostachys						
Ebracteata	*procumbens* Mill (2306)	annual	Fl–Fr	Tamaulipas	moderately wet tropics, mesic clay/shale	2
	(2315)	annual	Fl–Fr	Jalisco	semi-desert, highland disturbed soil	3
	queretaroanum Johnston (2307)	shrub	Fl–Fr	Tamaulipas	moderately wet tropics, mesic clay/shale	4
	(2308)	shrub	Fl–Fr	Tamaulipas	moderately wet tropics, mesic clay/shale	2
	sessei Johnston (2312)	shrub	Fl–Fr	Hidalgo	desert; limestone soil	2
Bracteata	*confertifolium* (Torr) Gray (2323)	shrub	Fl–Fr	Coahuila	desert, limestone soil	3
	(2324)	shrub	Fl–Fr	Tamaulipas	moderately dry zone, exposed limestone	3
	foliosissimum MacBride (2311)	geophyte	lFr	Hidalgo	semi-desert, disturbed lime stone soil	4
	fruticosum (=*texanum*) L (2300)	annual	Fl	Texas (south)	moderately wet, disturbed, rich soil	5
	greggii Torr (2319)	geophyte	Fl–Fr	Durango	desert, white soil	3
	(2322)	geophyte	Fl	Chihuahua	Larrea desert	3
	racemosum Johnston (2301)	annual	Fl–Fr	Texas (south)	moderately wet, sandy soil	8
	ternatum Vahl (2307A)	shrub	Fl	Tamaulipas	moderately wet, tropics, mesic clay/shale	4
	(2317)	shrub	Fl–Fr	Nayarit	dry, clay soil	10
	wigginsii Johnston (2318)	annual	Fl–Fr	Sonora	desert, flat mud soil	7
Cochranea	*molle* (Benth) Johnston (2320)	geophyte	Fl	Durango	desert, mud soil near irrigation pond	1
	(2321)	geophyte	Fl	Chihuahua	Larrea desert	2
Schobera	*angiospermum* Murray (2309)	geophyte	Fl–Fr	San Luis Potosi	disturbed soil in wet tropic forest	3
	(2310)	geophyte	Fl–Fr	Hidalgo	desert, white limestone	3

*All species, except for *H curassavicum* and *H indicum*, were collected in 1981, *H curassavicum* (1434) and *H indicum* (1406) were collected in 1971, *H curassavicum* (1502) and *H indicum* (1636) were collected in 1972

†In parentheses Michael W Frohlich collection numbers are indicated, vouchers are deposited in the Union College Herbarium The distances between two collections of *H curassavicum*, *H indicum*, *H procumbens*, *H queretaroanum*, *H confertifolium*, *H greggii*, *H ternatum*, *H molle* and *H angiospermum* were 200, 950, 300, 45, 210, 180, 400, 80 and 75 miles, respectively

‡Fl, flowering, Fr, fruiting, lFr, late fruiting

Table 2. Nitrogen, alkaloid, and aminoalcohol contents of *Heliotropium* species

Species*	Nitrogen (% dry wt)	Alkaloids† Total (% dry wt)	Alkaloids† Fraction II‡ (% total)	Aminoalcohols§ (% total) T	L	S	R	E	M^+ 157 (Base peak) M^+ 173 82 R_t (min) 9·2	9·6	83 5·9	98 11·5
curassavicum (1502)	0·54	0·37	2	60 (2)	?	3	37 (2)	—	—	—	—	—
(1434)	2.39	1·12	1	71 (<1)	?	2	27 (2)	—	—	—	—	—
indicum (1406)	3.04	0·56	17	4 (2)	tr	tr	93 (18)	—	—	—	3 (2)	—
(1636)	0.61	0·61	15	3 (1)	tr	tr	95 (16)	—	—	—	2 (1)	—
procumbens (2306)	1·45	0·17	20	10 (1)	tr	tr	90 (25)	—	—	—	tr	—
(2315)	1.29	0·03	38	16	2	tr	82 (47)	—	—	—	—	—
queretaroanum (2307)	1.06	0·02	23	12	2	tr	86 (20)	—	—	tr	—	—
(2308)	1.68	0·02	28	28 (1)	9 (tr)	tr	53 (24)	—	—	8 (3)	—	—
sessei (2312)	1.32	0·13	23	42 (12)	11 (tr)	tr	47 (28)	—	—	—	—	—
confertifolium (2323)	0.96	0·57	11	2	<1	<1	97 (5)	—	—	—	—	—
(2324)	0.75	0·06	10	6 (<1)	6	tr	88 (10)	—	—	—	—	—
foliosissimum (2311)	3.28	0·36	16	9 (2)	—	tr	90 (22)	—	<1	—	—	—
fruticosum (2300)	2.87	0·73	9	9	<1	<1	90 (2)	—	—	—	—	—
gregii (2319)	1.62	0·89	2	99 (91)	?	tr	<1	—	—	—	—	—
(2322)	3.81	2·11	2	99 (90)	?	tr	<1	—	—	—	—	—
racemosum (2301)	1.07	0·50	32	3 (1)	<1	tr	86 (28)	—	3 (2)	5 (2)	—	2 (1)
ternatum (2307A)	0·49	0·12	11	27 (3)	2 (tr)	4 (tr)	61 (13)	6 (6)	—	—	—	—
(2317)	1·69	0·37	3	69 (1)	26 (1)	tr	3 (1)	2 (2)	—	—	—	—
wigginsii (2318)	1.52	0·13	7	59 (5)	18 (1)	3	20 (5)	—	—	—	—	—
molle (2321)	2.50	1·85	20	1	tr	3	94 (22)	2 (2)	—	—	—	—
(2320)	3.27	1·00	28	1	<1	2	93 (34)	3 (3)	—	—	—	—
angiospermum (2309)	1.46	0·52	6	3 (1)	1	<1	tr	90 (90)	—	—	5 (3)	—
(2310)	1·26	0·63	7	1	tr	4	tr	91 (91)	—	—	5 (2)	—

*Except for *H. queretaroanum* and *H. sessei*, all remaining samples included roots

†Calculated as monocrotaline (MW 325)

‡$CHCl_3$–EtOH (2·1) extract following extraction with $CHCl_3$

§Trachelanthamidine, T, lindelofidine, L, supinidine, S, retronecine, R; 1,2-epoxy-1-hydroxymethyl-pyrrolizidine, E; tr, trace (below 0·5%) In parentheses, amounts of nonesterified necines

Such a compound was not detected in any extracts prior to hydrolysis Epoxides are often found as intermediates in hydroxylation by mono-oxygenases [5]

In *H angiospermum*, an additional aminoalcohol with a mass spectrum suggesting a pyrrolizidine-2,9-diol [6, 7] has also been found A necine, with a similar mass spectrum and R_t, was previously detected in field collected *H. indicum* (2112) but not in the greenhouse grown plants originating from seeds of the latter [8] The same necine has been found in two new *H indicum* samples collected at a distance of 900 miles from each other It has now also been detected as a trace in the unhydrolysed fraction II upon re-examination of the greenhouse grown plants Traces of the necine have been found in *H procumbens* (2306) but not in *H procumbens* (2315)

Two other saturated diols with mass spectra characteristic of a pyrrolizidine-7,9-diol [6, 9] were detected in some species One exhibited a R_t value identical to that of platynecine, not previously found in Boraginaceae, it was detected as a minor or trace necine in *H queretaroanum* from both localities and in *H racemosum* The other diol was found in *H foliosissimum* as well as in *H racemosum* Its R_t value was similar to that of pyrrolizine-7,9-diol, detected as a major necine in the field sample of *H curassavicum* (2138) from Oaxaca [1] and corresponded to the R_t value of turneforcidine This necine could not be detected in greenhouse grown plants originating from *H curassavicum* (1502) collected in Baja California Sur or in *H curassavicum* (2303) collected in Tamaulipas [1, 8] Neither could it be found in the field sample of *H curassavicum* (1434) from Sinaloa

Except for *H curassavicum* and *H indicum* [1, 7] saturated diols have not been previously detected in any *Heliotropium* species Of the four saturated diols found in PA-bearing plants only turneforcidine was shown to occur in Boraginaceae, namely in *Tournefortia sibirica* [4], in addition, hastanecine ethochloride was found in *Lindelofia macrostyla* [10] A triol necine, the only one ever detected in Boraginaceae, has been recently found in *H ovalifolium* from India and identified as croalbinecine [11]. A search for a triol in *H procumbens*, closely related to *H ovalifolium*, gave negative results However, a saturated necine triol with a mass spectrum similar to that of croalbinecine has been detected as a minor component in *H racemosum*

In previous studies [8], no organ- or age-dependent differences in the qualitative composition of necines were found in *H curassavicum*, *H spathulatum*, or *H indicum* The differences in the ratios between individual necines were relatively small and no shifts from a minor to a major status or vice versa were observed with any aminoalcohol during plant development If this is true also for other *Heliotropium* species the qualitative and quantitative composition of necines in the flowering plants, here and previously [1] analysed, may be considered as representative for a given population

In most cases there were no qualitative differences in the necine patterns of a given species depending on the collection site in Mexico or the U S A The fact that a very minor saturated diol was not detected in one of four *H. ternatum*, or one of two *H procumbens* or *H foliosissimum* samples, does not necessarily indicate its absence in those populations However, the significant qualitative differences between the *H curassavicum* population from Oaxaca and the populations from other localities in Mexico seem to be real

No striking quantitative differences in the aminoalcohol composition depending on the sample origin were observed in any species except for *H ternatum* in which lindelofidine, trachelanthamine, or retronecine was found as the dominant necine, this species exhibits extreme morphological variability. Among the remaining 25 species, the dominant aminoalcohol retronecine was found in 17 species, trachelanthamidine in four and supinidine, heliotridine and 1,2-epoxy-1-hydroxymethylpyrrolizidine in one species each, in *H sessei* both trachelanthamidine and retronecine were major aminoalcohols

Secondary products, such as alkaloids, have proved useful in chemotaxonomic studies on interspecies relationships [12] However, the present results show that the occurrence of particular necines could hardly be indicative of species relations within the genus *Heliotropium*. This is true for the most common aminoalcohols, as well as for those detected only in few species but belonging to very distant sections of *Heliotropium*. One should, however, bear in mind that the optical rotations of trachelanthamidine, lindelofidine and supinidine have been determined only in five, two and one species, respectively [1], the occurrence of laburnine [(+)-trachelanthamidine], (−)-isoretronecanol and/or (+)-supinidine in some other species is not excluded Among the 350 examined PA-bearing plant species belonging to 14 different families, laburnine has been reported in one, two and 15 species belonging to Leguminosae, Sapotaceae and Orchidaceae, respectively, (−)-isoretronecanol was found in several species of Sapotaceae and Santalaceae and (+)-supinidine in *Cynoglossum australe* and *C. lanceolatum* (Boraginaceae) [4]

Exposure of PA extracts to hydrolysis, followed by fractional extraction of necines, may facilitate detection of trace amounts of an aminoalcohol The search for necines other than retronecine in hydrolysed PA extracts from *Senecio longilobus*, collected in southern Arizona and New Mexico with total PA content of 0 01 and 2 8 % respectively, and from *S riddellii* from New Mexico with PAs amounting to 1 5 % yielded negative results

The relative accumulation of particular necines might, perhaps, be of chemotaxonomic value, especially if the rates of their synthesis and transformation decomposition and the enzymes involved were known In this respect our knowledge is negligible In young *H. spathulatum* plants the turnover rates of trachelanthamidine, supinidine and retronecine appeared to be very low [13]

The presence of nonesterified necines in PA-bearing plants has been rarely reported Free laburnine has been detected in *Cytisus laburnum* and in five species belonging to the Orchidaceae [4], two species of the latter also contained free lindelofidine Nonesterified trachelanthamidine was found in *Eupatorium maculatum*, *Heliotropium strigosum* [4] and, recently, in *H curassavicum* [7, 8] and *H spathulatum* [8], free retronecine was reported in *Crotalaria retusa* [4], *H ovalifolium* [11], *H curassavicum* and *H spathulatum* [8] A nonesterified saturated 2,9-diol was found in *H curassavicum* [7], and besides the free 1,2-epoxy-1-hydroxymethylpyrrolizidine in *Crotalaria*, saturated and unsaturated 1-methylene- and 1-methoxymethylpyrrolizidines were also found in several *Crotalaria* species [4] Very recently, free turneforcidine was identified in *Crotalaria candidans* [14]

As the data in Table 2 indicate, nonesterified necines have been detected in all *Heliotropium* samples, 1,2-epoxy-

1-hydroxymethylpyrrolizidine being the only necine that occurred exclusively in a free form. The detection and/or assessment of lindelofidine and supinidine in a free form was in most instances impossible due to their extremely low concentration. In species with a significant amount of these necines, the nonesterified portion accounted for less than 10% of the total. The ratios between the free and esterified forms of trachelanthamidine and retronecine varied greatly depending on the species and, to a lesser extent, on the collection site. The proportion of free trachelanthamidine ranged from 0 to 90% of the total and did not seem to be related to the total PA level in a given species. The same is apparently true for retronecine. The proportion of its free form ranged from 2 to over 50% of the total depending on the species.

The observed ratio between the free and esterified forms of a given aminoalcohol might have been affected by the drying process after the plants were collected and/or by the analytical procedures. No significent differences were found in the ratios of free and bound forms of trachelanthamidine, supinidine and retronecine between air-dried and fresh samples of greenhouse grown *H. spathulatum* plants in which the nonesterified necines accounted for *ca* 12% of the total. Neither were any differences in this respect found between air-dried and fresh samples of greenhouse grown *H. angiospermum* plants. Alkalization of the PA aqueous solutions within a pH range of 9–11.0 followed immediately by PA extraction, did not affect the proportion of nonesterified necines when tested on samples of several plant species.

Very significant differences in the total content of nitrogen were found between species as well as between two samples of the same species. These differences could be due to differences in the nitrogen supply and also, to some extent, to differences in the ratios between the weights of various organs in a given sample. No significant correlation was found between the PA and nitrogen contents of the examined plants

EXPERIMENTAL

Plant material Most of the plants were collected in the summer of 1981 at flowering and/or fruiting· *H curassavicum* (1434) and *H. indicum* (1406) were collected in 1971, *H curassavicum* (1502) and *H indicum* (1636) in 1972 The plants were air-dried The analysed samples represented whole plants including roots, except for *H queretaroanum* and *H. sessei* whose roots were not collected

Analysis Ground samples were extracted exhaustively with boiling MeOH under reflux, the solvent removed *in vacuo*, the residue extracted with 2 N H_2SO_4 and *N*-oxides reduced in the presence of excess Zn After basification PAs were extracted with $CHCl_3$ (fraction I) and then with $CHCl_3$–EtOH (2 1) (fraction II). Each PA fraction was hydrolysed with 7% aq. NaOH at 100° for 3 hr The necines from fraction I were extracted with $CHCl_3$ and then with $CHCl_3$–EtOH Necines from fraction II were similarly extracted, but both extracts were combined. Aliquots were taken before and after hydrolysis for quantitative determination and GC/MS The analytical methods were as described previously [8, 13]

MS of 1,2-epoxy-1-hydroxymethyl-pyrrolizidine (R_t 4.1 min) M^+ 155 (17), 126 (4), 124 (13), 96 (10), 80 (4), 71 (5), 70 (100), 68 (10), 67 (5), 56 (6), 55 (65) R_f value TLC 0 34, $[\alpha]_D^{20}$ of base from *H angiospermum* −61 3° (EtOH, *c* 0.85) MS of turneforcidine (R_t 9 2 min, R_f 0 11) obtained after hydrolysis of crocandine M^+ 157 (15), 113 (20), 82 (100), 68 (21), 67 (6), 55 (32)

MS of platynecine (R_t 9 6 min, R_f 0 08) obtained after hydrolysis of platyphylline M^+ 157 (14), 113 (18), 82 (100), 68 (18), 67 (4), 55 (27)

Necines isolated from *H. racemosum* and *H queretaroanum* with R_t 9.2 and/or 9 6 min showed MS similar to those of turneforcidine and/or platynecine, respectively

MS of base R_t 5 9 min and R_f 0 09 isolated from *H angiospermum* and *H indicum* M^+ 157 (18), 140 (8), 126 (6), 124 (5), 104 (6), 98 (10), 84 (8), 83 (100), 70 (18), 55 (78) MS of base R_t 11 5 min, isolated from *H racemosum* M^+ 173 (18), 155 (7), 129 (28), 112 (7), 99 (45), 98 (100), 82 (54), 70 (8), 55 (12)

Acknowledgements—We are grateful to Dr R J Molyneux for supplying us with plants of *Senecio riddellii* and *S longilobus* from New Mexico and to Dr. R J. Huxtable for *S longilobus* from southern Arizona We are also grateful to Dr. C K Atal and Dr. J. M Roitman for their gifts of crocandine and platyphylline, respectively

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