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THE LUPINE ALKALOIDS OF THE GENUS *BAPTISIA* (LEGUMINOSAE)*

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Abstract—The lupine alkaloids of the sixteen species comprising the genus *Baptisia* are reported. Cytisine, *N*-methylcytisine and anagyrine were found to be the major alkaloids occurring in nearly all of the species; thermopsine, lupanine, sparteine, 13-hydroxysparteine, and baptifoline occurred irregularly among the species and were usually, when present, minor alkaloids.

INTRODUCTION

THE genus *Baptisia* occurs in the eastern half of North America and is a perennial herbaceous member of the Papilionoideae, a sub-family of the Leguminosae. As part of a biochemical systematic investigation of the genus *Baptisia*, we previously described some of the flavonoid chemistry of the genus.[1–3] Flavonoids proved valuable not only for the identification of species, but also for the validation of hybrids and the analysis of population structures. In order to extend the chemosystematic data for the genus, we investigated other classes of secondary compounds and, in this connection, now report the gas chromatographic analysis of the lupine alkaloids in the sixteen *Baptisia* species.[4] The systematic implications of the alkaloid data will be described elsewhere.[5]

Although cytisine (*I*) and *N*-methylcytisine (*II*) have been reported from a number of *Baptisia* species,[6,7] detailed information on the lupine alkaloids in the genus have been described for only three species: *B. australis*[8] (*B. minor*[9] is now included as part of *B. australis*), *B. perfoliata*,[10] and *B. versicolor*.[11]

* Abstracted from the Ph.D. Dissertation of Morris F. Cranmer, University of Texas, 1965.

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[1] T. J. MABRY, J. KAGAN and H. RÖSLER, *Phytochem.* **4**, 487 (1965).

[2] H. RÖSLER, T. J. MABRY and J. KAGAN, *Chem. Ber.* **98**, 2193 (1965).

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[4] The constitution of *Baptisia* as a sixteen-species genus is based on unpublished interpretations of B. L. TURNER and R. E. ALSTON, Department of Botany, The University of Texas.

[5] M. F. CRANMER and B. L. TURNER, In preparation.

[6] For an account of preliminary paper chromatographic data on the lupine alkaloids in *Baptisia leucophaea* var. *laevicaulis*, see B. R. BREHM, Ph.D. Thesis, University of Texas (1962).

[7] For a recent review of the distribution of lupine alkaloids see: H.-G. BOIT, *Ergebnisse der Alkaloid-Chemie bis* 1960, Chap. 8. Akademie-Verlag, Berlin (1961).

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[9] L. MARION and W. F. COCKBURN, *J. Am. Chem. Soc.* **70**, 3472 (1948).

[10] L. MARION and F. TURCOTTE, *J. Am. Chem. Soc.* **70**, 3253 (1948).

[11] F. TURCOTTE, R. LEDUC and L. MARION, *Can. J. Chem.* **31**, 387 (1953). (*B. versicolor* is considered to be a *nomen nudum* in the most recent monograph of the genus, M. M. LARISEY, *Ann. Mo. Bot. Gard.* **27**, 119 (1940). B. L. TURNER has suggested privately that the plant material referred to as *B. versicolor* may have been *B. australis*.)

TABLE 1. DISTRIBUTION OF ALKALOIDS IN *Baptisia**

Species	Collection source	Alkaloids†								% dry weight
		Cytisine	Methyl-cytisine	Anagyrine	13-Hydroxy-sparteine	Thermop-sine	Bapti-foline	Sparteine	Lupanine	Unknown	
Group 1											
B. perfoliata	Johnson Co., Ga.	83	5	11			1				0·19
B. sphaerocarpa	Wharton Co., Tex.	9	74	6		11					0·64
Group 2											
B. leucantha (leaves, flowers and stems)	Jones Co., Miss.	90	10								0·31
B. leucantha‡ (seed hulls, leaves, and stems)	Taylor Co., Fla.	80	12	8							0·07
B. alba	Jackson Co., Fla.	31	16	25	5	trace				23	2·00
B. megacarpa	Gadsden Co., Fla.	19	45	36							0 94
Group 3											
B. cinerea	Richland Co., S.C.	1	3	44	1	51					2·20
B. bracteata§	Newberry Co., S.C.										
(seed hulls)		79	10	10			1				0·25
(leaves)		22	7	45	9	2	15				0·31
(stems)		60	10	28		1	1				0·15
B. leucophaea var. *laevicaulis*	Bastrop Co., Tex.										
(young plants)		77	trace	23		trace					1·02
(flowering plants)		69	3	28		trace					0 65
(old plants)		25	17	54	1	1	1	1			0·08
B. lanceolata	Franklin Co., Fla.										
(stems)		96	4								0·53
(leaves)		94	6								0·41
B. elliptica‖	Franklin Co., Fla.	7	93								0 11
B. nuttalliana	Walker Co., Tex.	76	3	3			18				0·66
B. australis	Pushmataha Co., Okla.	99	1								0·20

Group 4										
B. arachnifera	Brantley Co., Ga.	57	23	11	trace	9				2·40
B. simplicifolia	Leon Co., Fla.	26	21	32	12		trace	9		0·61
B. tinctoria	Bancombe Co., N.C.									
(stems)		84	4	8	4					0·45
(leaves)		72	9	10	9					0·08
B. lecontei	Wakulla Co., Fla.	54	46							0·46
B. calycosa	Clay Co., Fla.	97	1	2						0·21
B. hirsuta	Okaloosa Co., Fla.	60	5	28					7	0·42

* The species are arranged into four phyletic groups (cf. Ref. 5). Unless otherwise noted the data are for leaves and stems of the species collected generally during flowering.

† Column figures refer to percentage of total alkaloids.

‡ This collection represents plant material initially identified as *B. psamophila*, a taxon now treated as a regional population of *B. leucantha*.

§ *B. bracteata* is now treated as a variety or subspecies of *B. leucophaea*.

|| *B. elliptica* has been treated as a variety of *B. lanceolata* (D. HORNE, Ph.D. Dissertation, University of Texas, 1965).

METHODS

All the results described in this paper were obtained with standardized extraction and gas chromatographic analysis procedures. The species were collected during the flowering stage of development of the plants (April–May) in 1962, 1963, and 1964. The material was air-dried for all the experiments described in this paper, but similar data were obtained for one species, *B. leucophaea*, with both fresh and air-dried material.

Chloroform has been commonly employed by many investigators for extracting alkaloids from plant material but some lupine alkaloids decompose on prolonged contact with this solvent. We therefore used methylene dichloride for the extraction of the alkaloids from the dried plant material. The alkaloids were extracted from the methylene dichloride as salts with aq. citric acid and then re-extracted into methylene dichloride after making the citric acid solution basic. Concentration of the final methylene dichloride solution provided a thick syrup containing the crude alkaloid mixture. The syrup was usually sufficiently pure for direct analysis by gas chromatography. The procedure gave reproducible results which were considered to be reliable, in part, because the alkaloid fraction was never subjected to conditions known to produce alkaloid decomposition, e.g. heat, strong mineral acids, and the chloroform treatment mentioned above.

The gas chromatographic identification of the lupine alkaloids in the mixture followed, to some extent, the procedure previously described by Lloyd *et al.*[12] for the analysis of high-molecular-weight alkaloids. Their investigation included commercial samples of lupine alkaloids. Faugeras and Paris[13] recently reported the identification of cytisine (*I*) and *N*-methylcytisine (*II*) from the fruits of *Genista pilosa* L. by gas chromatography.

RESULTS AND DISCUSSION

The total yield of alkaloid material from leaves and stems of each *Baptisia* species and the relative percentage of each alkaloid present in the species are tabulated in Table 1. Gas chromatographic retention times for lupine alkaloids on two columns (3% XE-60 and 5% DC-560) are recorded in Table 2. The identification of the alkaloids in the *Baptisia* species based on their retention times on at least two different columns which differ in their polarity considerably strengthens the reliability of the data over one column analyses.

The tricyclic alkaloids, cytisine (I) and *N*-methylcytisine (II), and the tetracyclic type anagyrine (III), were present in most of the species. Baptifoline (IV), thermopsine (V, a steric isomer of III), sparteine (VI), 13-hydroxysparteine (VII), and lupanine (VIII) were found to occur irregularly in the sixteen species and usually as minor components. The tricyclic types I and II are thought to be the most biogenetically advanced members of the quinolizidine class of alkaloids.[14] Since I and II occur in all sixteen *Baptisia* species, the complete enzymatic pathway from lysine to tetra- and finally tri-cyclic types must be present in all species.[15]

The total yield of alkaloid material varied from a high of 2·4 per cent of the dry weight of *B. arachnifera* to a low of about 0·1 per cent for *B. lanceolata* (elliptica type). Some qualitative and quantitative variations were observed for the *Baptisia* alkaloids according to the

[12] H. A. LLOYD, H. M. FALES, P. E. HIGHET, W. J. A. VANDENHEUVEL and W. C. WILDMAN, *J. Am. Chem. Soc.* **82**, 3791 (1960).

[13] G. FAUGERAS and M. PARIS, *Compt. Rend.* **258**, 3791 (1960).

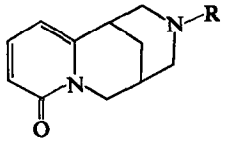
[14] (*a*) H. R. SCHÜTTE and J. LEHFELDT, *J. Prak. Chem.* **24**, 143 (1964); (*b*) H. R. SCHÜTTE and H. HINDORF, *Ann. Chem.* **685**, 187 (1965).

[15] Alternative biogenetic schemes have been proposed in which the tricyclic lupine alkaloids are intermediates to the tetracyclic types. In support of this pathway E. STEINEGGER and R. BERNASCONI, *Pharm. Acta Helv.* **39**, 480 (1964), reported lupine alkaloid data according to the stage of development of *Genista aetnensis*.

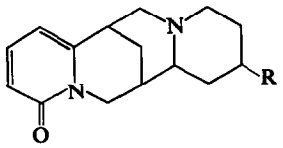
TABLE 2. GAS CHROMATOGRAPHIC RETENTION TIMES FOR LUPINE ALKALOIDS

Alkaloid	Column, 3% XE-60 (min)	Packing,* 5% DC-560 (min)
Anagyrine	12·1	87·0
Cytisine	4·90	27·0
Methylcytisine	2·80	20·5
Lupanine	2·60	26·4
Hydroxylupanine	17·4	*c.* 200
Lupinine	0·50	*c.* 2·0
Sparteine	1·00	4·6
13-Hydroxysparteine	1·40	12·3
17-Oxysparteine	1·65	14·1
Thermopsine	9·70	54·0
Baptifoline	*c.* 30	275·0

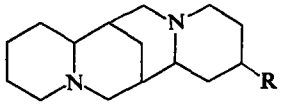
* Column conditions: 100/120 mesh silanized chromosorb W, 6 ft × 0·25 in. columns at 220° (3% XE-60) and 223° (5% DC-560) with an inlet pressure of 15 psi of argon.



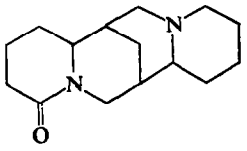
(I) Cytisine, R = H
(II) *N*-Methylcytisine, R = CH_3



(III) Anagyrine, R = H
(IV) Thermopsine, R = H
(V) Baptifoline, R = OH



(VI) Sparteine, R = H
(VII) Hydroxysparteine, R = OH



(VIII) Lupanine

stage of development of the plants. The variation in the alkaloid data for stem and leaf material from young, flowering and old *B. leucophaea* var. *laevicaulis* plants indicates the difficulty in reproducing the analyses. Such variation may account for the difference in our results for *B. australis* with those reported previously.[8,9]

EXPERIMENTAL

Isolation of the Alkaloid Fraction

All of the sixteen species of *Baptisia* were collected during flowering in the spring of 1962, 1963, and 1964, throughout the southern United States east of Austin, Texas. All the plant material was air-dried and extracted in the manner described below for *Baptisia leucophaea* var. *laevicaulis*.

Ground *B. laevicaulis* (100 g) was extracted for 24 hr with 4 l. of methylene dichloride made basic with 10 ml of conc. NH_4OH. The extraction was repeated and the two extracts combined and concentrated *in vacuo*. The concentrate was acidified with 50 ml of a 10% aq. citric acid solution. The aq. solution was extracted with methylene dichloride until a clear organic phase was obtained. The pH of the aq. solution was adjusted to 10 with NH_4OH to convert the alkaloids to their free bases. The alkaloids were then extracted with 2 × 150 ml of methylene dichloride. Concentration *in vacuo* of the methylene dichloride layer yielded a thick alkaloid residue, 438 mg, 0·44 per cent yield from the dried plant material. The alkaloid mixture could be analyzed directly by gas chromatography.

From a number of species, crystalline cytisine, m.p. 152–153°, and *N*-methylcytisine, m.p. 138°, were isolated.

Gas Chromatography

A Research Specialties model 600 dual-injection dual column instrument with an argon β-ray ionization detector was equipped with two 6 ft × 0·25 in. U-shaped stainless steel columns.

The present investigation includes data obtained on columns packed with acid-washed silanized chromosorb W coated with one of the following oils: 3% XE-60* (a General Electric high-molecular-weight copolymer containing methyl-β-cyanoethylsiloxane and dimethylsiloxane), 5% DC-560 (a Dow Corning phenylsilicone oil), or 3% SE-30 (a General Electric methyl silicone oil). Different percentage of the stationary phases as well as other support materials were used in some analyses but, in all cases, the results were essentially the same as those reported here. We found that even the best commercially available chromosorb W required an additional wash with dilute HCl in order to remove iron contaminants. This treatment reduced tailing in the peaks obtained on the gas chromatograms. Inlet pressure: 15 psi of argon. Column temperature: 220–223°. Gas chromatographic identification of the alkaloids in the plant material was accomplished by comparison of the retention times of the bases in the mixture with those observed for authentic samples.† In most instances, the authentic samples were also mixed directly with the alkaloids obtained from the plant material for final gas chromatographic identifications. The approximate relative concentrations of the alkaloids in each species were obtained by the measurement of the areas under the peaks obtained on the gas chromatograms with a polar planimeter.

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† Commercial samples of many of the lupine alkaloids were obtained from Light & Co. Ltd., Bucks., England.