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THE ALKALOIDS OF *SOPHORA VELUTINA*

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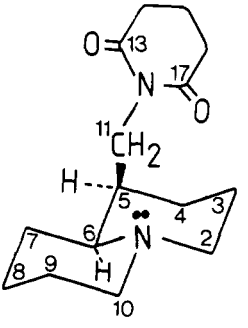
ABSTRACT.—(+)-Lamprolobine (**2**), the novel alkaloid (+)-9β-hydroxylamprolobine (**3**), and cytisine were isolated as the major alkaloids from the leaves of *Sophora velutina* var. *zimbabweensis*. The alkaloids were characterized on the basis of ir, ms, $[\alpha]_D$, ^1H and ^{13}C nmr, and by two-dimensional nmr spectral assignments.

Although more than twenty species of *Sophora* (tribe Sophoreae: Leguminosae) have been investigated for their alkaloidal constituents, there is no previous report concerning *Sophora velutina* Lindl. Matrine- and anagyrine-type alkaloids are typical of the genus, and although other alkaloids such as sparteine- and dipiperidine-types do occur, their distribution is more limited. Recently, the lipinane-type alkaloids (−)-epilamprolobine (**1**) and its *N*-oxide have been reported as very minor alkaloids of *Sophora tomentosa* (1). In this paper, lamprolobine-type alkaloids are reported as being major constituents of *S. velutina* Lindl. var. *zimbabweensis* Gillett and Brummitt.

Three major alkaloids, (+)-lamprolobine (**2**), (+)-9β-hydroxylamprolobine (**3**), and the well-known cytisine were isolated from the leaves of *S. velutina*. The known alkaloid (+)-lamprolobine (**2**) was identified on the basis of its spectral properties ($[\alpha]_D$, ir, nmr, ms), which were compared with those of (−)-epilamprolobine (**1**) (1,2). The complete assignment of the nmr spectra was achieved by means of mono- and two-dimensional techniques, and, in particular, the following differences were noted between the spectra of (**1**) and (**2**): (a) The carbon signals observed for **2** were almost identical to those reported for **1**, with the exception of those signals attributed to 3-C, 5-C, and 11-C (Table 1). (b) The chemical shifts of the 11-C protons of **1** have been reported as being



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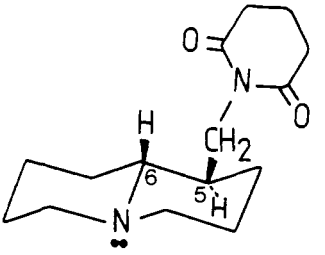

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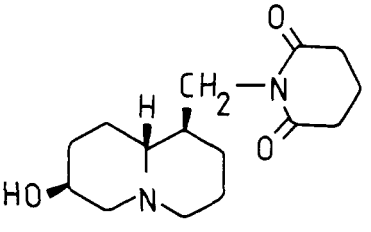

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TABLE 1. Comparison of the ^{13}C-nmr Spectra of (−)-Epilamprolobine (**1**) (1), (+)-Lamprolobine (**2**)[a], and 9β-Hydroylamprolobine (**3**)[a]

Carbon No.	Compound		
	1	**2**	**3**
2	57.2	56.7	56.2
3	21.2	24.4	24.3
4	26.7	27.9	28.0
5	37.1	39.3	39.3
6	65.2	66.5	66.6
7	29.6	29.4	27.7
8	25.1	24.6	33.6
9	25.6	25.4	65.5
10	57.6	56.3	63.1
11	37.8	41.5	41.7
13	172.6	172.7	172.8
14	33.1	33.0	33.1
15	17.3	17.2	17.4
16	33.1	33.0	33.1
17	172.6	172.7	172.8

[a]Chemical shifts are in ppm from TMS in $CDCl_3$.

well separated, occurring at δ 3.77 and 4.28 (1), whereas in the ^1H nmr of **2**, these signals nearly coalesced at δ 3.7 (Table 2).

The ir spectrum of the other major alkaloid (**3**) indicated the presence of a *trans* fused quinolizidine ring system (strong Bohlmann bands at 2800-2700 cm^{-1}) and an imide function [1670 (s), 1720 (w) cm^{-1}]. In addition, strong absorption bands at 3600 and 3300 cm^{-1} indicated the presence of a hydroxyl group. The molecular formula of $C_{15}H_{24}N_2O_3$ was obtained from ms measurements ($M^{+\cdot}$, *m/z* 280.1787; calcd 280.1783), and the fragmentation indicated a lamprolobine-type structure (3) with one additional hydroxyl group present in ring A (C_5H_9NO, *m/z* 99.0686; calcd 99.0686). The presence of the hydroxyl substituent was confirmed by preparation of an *O*-acetyl derivative, and the position of substitution at 9-C was determined by ^1H nmr (Table 2).

The ^1H-nmr spectrum of (**3**) (Figure 1) displayed a series of signals integrating for three protons in the 3.7 ppm range; two of these signals were attributed to the 11-C protons (see above), whereas the remaining signal was assigned to the proton at the carbon substituted by the hydroxyl group. The chemical shift of the signals attributed to the methylene protons of 11-C coincided with those of the corresponding signal obtained for (+)-lamprolobine (**2**), indicating that both alkaloids possessed the same relative configuration at 5-C. Because the chemical shifts were known for the two methylene protons at 11-C, it was possible to analyze the correlation pattern as revealed by the cross peaks laying off the diagonal in the 2-D proton-proton contour map (Figure 1), thus leading to the complete assignment of the ^1H-nmr spectrum of (**3**) (Table 2).

From these assignments, it is obvious that 9-C is the position of hydroxyl substitution. The spin-spin analysis of the 9-CHOH-10-CH_2N fragment provided the information needed for the assignment of the hydroxyl configuration. The coupling constants $^3J_{9\text{-}10e}$=4.3 Hz and the $^3J_{9\text{-}10a}$=11 Hz correlating with dihedral angles of 49° and 180° respectively, consistent with an equatorial hydroxyl substituent at 9-C. The $[\alpha]_D$ of the new alkaloid was determined as +24°, and hence, it is identified as (+)-9β-hydroxylamprolobine (**3**). Additional support for our conclusions came from the assignment of the ^{13}C spectrum of (**3**). Thus, where the carbon shifts of (**3**) were com-

TABLE 2. Assignment of the ^{1}H-nmr Spectra of (+) Lamprolobine (**2**) and (+)-9β-Hydroxy-lamprolobine (**3**) Isolated from *Sophora velutina*.

Proton No.[a]	Compound			
	2		**3**	
	δ[b]	*J*[c]	δ[b]	*J*[c]
4-H, u	1.01	$J_{4u\text{-}4d}$=12.0 $J_{4u\text{-}5}$=12.0 $J_{4u\text{-}3d}$=12.0 $J_{4u\text{-}3u}$=3.52	1.05	$J_{4u\text{-}4d}$=12.1 $J_{4u\text{-}5}$=12.1 $J_{4u\text{-}3d}$=12.1 $J_{4u\text{-}3u}$=4.6
7-H, u	1.23		1.34	
8-H, u	1.28		1.23	
4-H, d	1.42		1.62	$J_{4d\text{-}3d}$=4.2
3-H, u	1.55		1.46	$J_{3u\text{-}2d}$=3.6 $J_{3u\text{-}2u}$=3.2
3-H, d	1.58		1.62	$J_{3d\text{-}2u}$=11.0 $J_{3d\text{-}2d}$=3.6
9-H, u	1.60		—	
9-H, d	1.65		3.78	$J_{9\text{-}10d}$=4.3
6-H	1.68		1.62	
5-H	1.74		1.68	
7-H, d	1.78		2.07	
8-H, d	1.91		2.07	
15H-H_2	1.91		1.95	
10-H, u	2.01		1.95	$J_{10u\text{-}10d}$=11.0 $J_{10u\text{-}9}$=11.0
14-H_2/16-H_2	2.63	$J_{14\text{-}15}$=$J_{15\text{-}16}$=6.5	2.65	$J_{14\text{-}15}$=$J_{15\text{-}16}$=6.5
2H, d[d]	2.77		2.80	
10-H, d[d]	2.83		3.02	$J_{10d\text{-}8u}$=2.0
11-H, u	3.67	$J_{11d\text{-}11u}$=12.7 $J_{11u\text{-}5}$=9.5	3.67	$J_{11d\text{-}11u}$=12.8 $J_{11u\text{-}5}$=9.5
11-H, d	3.77	$J_{11d\text{-}5}$=5.0	3.76	$J_{11d\text{-}5}$=5.0

[a]u=upfield; d=downfield.

[b]Chemical shifts are expressed in ppm and are relative to TMS in $CDCl_3$.

[c]Coupling constants are in Hz.

[d]For assignments of protons at C-2 and C-10 in quinolizidines see Bohlmann *et al.* (7,8).

pared with those of (**1**) and (**2**) the following facts were noted: (a) C-9 undergoes a large chemical shift variation in (**3**) (40 ppm) consistent with hydroxyl substitution; (b) the chemical shift of C-5 and C-11 is virtually identical in (**2**) and (**3**), indicating that there is identical configuration at C-5 for both compounds.

Lamprolobine-type alkaloids have been reported previously from *Lamprolobium* (tribe Bossiaeeae) (2) and from *Lupinus* (tribe Genistae) (4). This is the first report of these alkaloids as major constituents of *Sophora*, and the results show that there is a strong chemical relationship between the tribes Sophoreae, Bossiaeeae, and Genistae, supporting their botanical affinities (5).

EXPERIMENTAL

Preparative tlc was carried out using silica gel GF_{254} (Merck) and one of the following solvent systems: A. $CHCl_3$-MeOH-NH_4OH (90:10:1); B. iPrOH-EtOAc-$CHCl_3$-NH_4OH (55:20:20:5); C. $CHCl_3$-Et_2NH (90:10). The ir spectra were recorded on a Perkin-Elmer 298 spectrometer from $CHCl_3$ solutions. Nmr spectra were obtained on a Varian XL-300 instrument utilizing $CDCl_3$ solutions, and chemical shifts reported are relative to TMS. Optical rotations were measured with a Perkin-Elmer 1541 polarimeter. Mass spectra were recorded on an Analytical ZAB-IF high resolution mass spectrometer

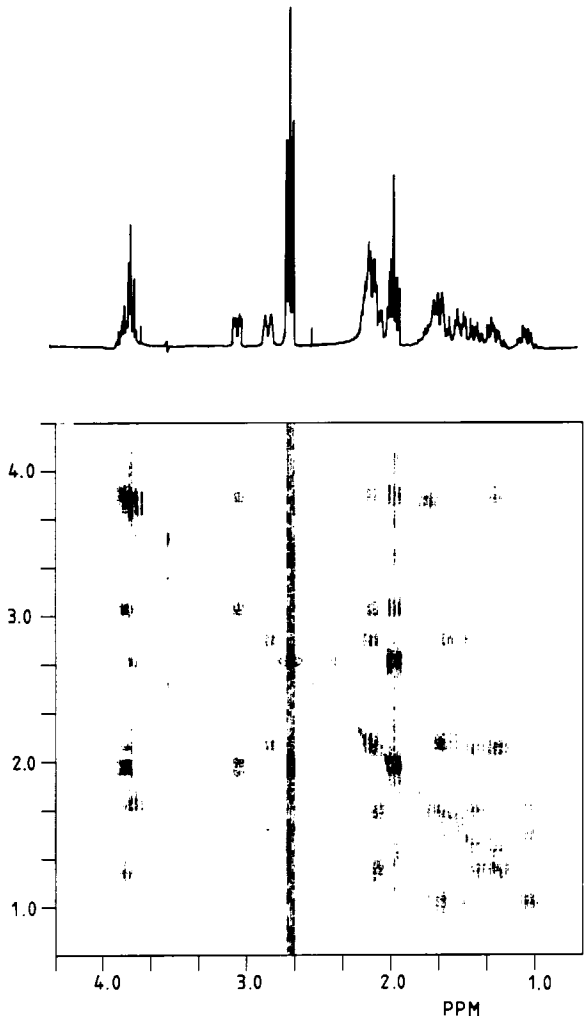

FIGURE 1. ^{1}H nmr and 2-D proton-proton nmr of (+)-9β-hydroxylamprolobine (**3**).

operating at 70 eV. The plant material was collected and identified as *S. velutina* var. *zimbabweensis* by Dr. T. Muller of the Zimbabwe National Herbarium and National Botanic Garden, where a voucher specimen is retained.

ISOLATION OF ALKALOIDS.—Finely powdered leaves (100 g) were extracted by maceration with MeOH, the filtered extract concentrated to low volume under reduced pressure and extracted with 2% H_2SO_4. The filtered acid extract was washed with Et_2O, basified with NH_4OH, and extracted into $CHCl_3$ (3×30 ml). The combined $CHCl_3$ extracts were washed with H_2O, dried over anhydrous Na_2SO_4 and taken to dryness under reduced pressure to yield 0.45 g (0.45%) of total crude alkaloid. (+)-Lamprolobine (**2**, 180 mg), (+)-9β-hydroxylamprolobine (**3**, 26 mg), and cytisine (31 mg) were isolated by preparative tlc using solvent systems A, B, and C.

(+)-LAMPROLOBINE (**2**).—[α]D +23° (EtOH); ir ν max $CHCl_3$ 2800-2700 cm^{-1} (Bohlmann bands), 1720 cm^{-1} (w) and 1670 cm^{-1} (s) imide; ms *m/z* (r.a.) 264.1838 ($M^{+\cdot}$, calcd for $C_{15}H_{24}N_2O_2$ 264.1835, 26), 222 (6), 152 (50), 138 (100), 110 (52), 97 (54), 83 (72); Rf (system A) 0.68. ^{13}C- and ^{1}H-nmr spectral assignments are given in Tables 1 and 2, respectively.

(+)-9β-HYDROXYLAMPROLOBINE (**3**).—[α]D +24° (EtOH); ir ν max ($CHCl_3$) 3600 cm^{-1} (OH), 3400-3200 (bonded OH), 2800-2700 cm^{-1} (Bohlmann bands), 1720 cm^{-1} (w) and 1670 cm^{-1} (s) imide); ms *m/z* (r.a.) 280.1787 ($M^{+\cdot}$, calcd for $C_{15}H_{24}N_2O_3$ 280.1783, 33), 263 (10), 262 (7), 168 (67), 154 (100), 126 (40), 113 (33), 99.0686 (calcd for C_5H_9NO 99.0686, 53), 96 (87); Rf (system A), 0.45.

^{13}C- and ^{1}H-nmr spectral assignments are given in Tables 1 and 2, respectively. A ^{1}H- ^{1}H-correlation spectrum was obtained using the 90-τ-90-Acq. pulse sequence (6); 256 increments of 2K points each were recorded, and 80 scans were collected for each increment. The ^{1}H-nmr spectrum and 2-D proton-proton nmr spectrum are shown in Figure 1. The assignment of the ^{13}C spectrum was made using a DEPT experiment.

(+)-9-*O*-ACETYL-LAMPROLOBINE.—(+)-9β-Hydroxylamprolobine (1 mg) was dissolved in 0.5 ml pyridine/Ac_2O (1:1) and the mixture allowed to stand at ambient temperature for 14 h. Excess Ac_2O was decomposed by addition of H_2O and the pH adjusted to 9.0 by addition of NH_4OH. The aqueous mixture was extracted with $CHCl_3$ (3×2 ml), the combined $CHCl_3$ extracts washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated to dryness under N_2 to leave a pale yellowish oil (1.1 mg); ms *m/z* (r.a.) 322 (1.5), 321 (3), 279 (3), 252 (100), 210 (18), 196 (20), 178 (11), 150 (18), 136 (25); Rf (system A) 0.74.

CYTISINE.—The isolated alkaloid had identical tlc, ms, and ^{1}H-nmr properties to an authentic sample; $[\alpha]_D$ −115° (MeOH).

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LITERATURE CITED

1. I. Murakoshi, E. Kidoguchi, M. Nakamura, J. Haginawa, S. Ohmiya, K. Higashiyama, and H. Otomasu, *Phytochemistry*, **20**, 1725 (1981).
2. N.K. Hart, S.R. Johns, and J.A. Lamberton, *J. Chem. Soc. Chem. Commun.*, 302 (1968).
3. N. Neuner-Jehle, H. Nesvadba, and G. Spiteller, *Monatsh. Chem.*, **95**, 687 (1964).
4. W.J. Keller, *Phytochemistry*, **19**, 2233 (1980).
5. R.M. Polhill, in: "Advances in Legume Systematics, Part I." Ed. by R.M. Polhill and P.H. Raven, Royal Botanic Gardens Kew, Richmond, Surrey, M.A.F.F. 1981, p. 393.
6. W.P. Ave, E. Bartholdi, and R.R. Ernst, *J. Chem. Phys.*, **64**, 2229 (1976).
7. F. Bohlmann, D. Schumann, and H. Schulz, *Tetrahedron Lett.*, 2705 (1965).
8. F. Bohlmann, D. Schumann, and C. Arndt, *Tetrahedron Lett.*, 2705 (1965).

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