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(+)-9α-HYDROXYMATRINE FROM *SOPHORA MACROCARPA*

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Key Word Index—*Sophora macrocarpa*; Leguminosae; leaves; alkaloids; 9α-hydroxymatrine; structural determination.

Abstract—A new quinolizidine alkaloid, (+)-9α-hydroxymatrine, was isolated from the leaves of *Sophora macrocarpa*. Its structure was determined by a combination of spectroscopic methods, among which nuclear Overhauser enhancement experiments played a crucial role. Some generalizations are made regarding the mass spectra of matrinoids.

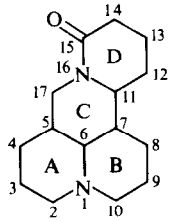
INTRODUCTION

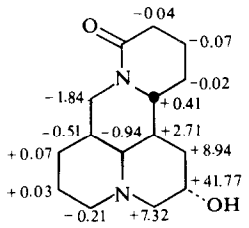
Sophora macrocarpa Sm. ('mayo' or 'mayu') is a fairly common leguminous shrub of central Chile, growing in open places with adequate soil moisture and at forest edges. Its leaves are a rich source of matrine, accompanied by smaller amounts of matrine *N*-oxide, 5α-hydroxymatrine (sophoranol), *N*-methylcytisine and cytisine [1]. This report deals with the structure elucidation of a new alkaloid which was shown to be 9α-hydroxymatrine (**1**).

RESULTS AND DISCUSSION

The new base (**1**), mp 158–159°, $[\alpha]_{590}^{20}$ + 25.4° (MeOH) has the molecular formula $C_{15}H_{24}N_2O_2$ and its mass spectral fragmentation exhibits features suggestive of a matrinoid skeleton, viz. an abundant $[M]^+$ (100%) and a strong $[M-1]^+$ peak, a medium-intensity signal at *m/z* 96 and a weaker one at *m/z* 98 which would be expected to be stronger in the sparteine series [2, 3]. Its IR spectrum showed hydroxyl absorption, *trans*-quinolizidine bands and a lactam carbonyl peak.

Acting on the hypothesis that the new substance was a hydroxylated matrinoid, its ^{13}C NMR spectrum was recorded and compared with that of matrine. Most of the resonances showed a good fit and the differences could be attributed to an equatorial hydroxyl group located at C-9 (ring B) (**1**) or C-3 (ring A) (**2**), as shown in Fig. 1. The ^1H NMR spectra of **1**, in deuterochloroform and in deuteropyridine, supported these assignments but could not distinguish between them. The slightly broadened downfield doublet of doublets (J = 12.6 and 4.5 Hz) at δ 4.39 in deuterochloroform is shifted to 4.65 in deuteropyridine due to the formation of a charge–transfer complex of the solvent with the lactam function and is assigned to H-17e, *peri* with respect to the carbonyl group [4]. The multiplet at δ 3.84 in deuterochloroform is shifted to 4.08 in deuteropyridine, whereas the similar signal at 3.64 is essentially unaffected, indicating that the former corresponds to the carbinyl proton and the latter to H-11 [4]. H-17a gives a triplet (J = 12.6 Hz) at δ 2.98 ($CDCl_3$) or 3.04 (C_5D_5N) [4]. H-2e and H-10e give





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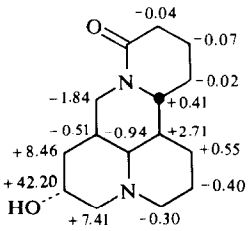

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Fig. 1. Numbering of the matrinoid skeleton and differences in ^{13}C NMR chemical shifts relative to matrine for 9α-hydroxymatrine (**1**) and the hypothetical 3α-hydroxymatrine (**2**).

‡Most of this work was carried out while B.K.C. was employed by the University of Chile.

complex signals centred at δ 2.83 and 3.03 ($CDCl_3$) or 2.70 and 3.23 (C_5D_5N), respectively [4].

Nuclear Overhauser enhancement (NOE) difference spectra showed unambiguously that the structure is 9α-hydroxymatrine (**1**). Irradiation at the resonance frequency of the carbinyl proton enhanced the H-11 signal by 10.5%, showing that both atoms lie very close to each other and that, therefore, the carbinyl proton is H-9a; in addition, the signals of the neighboring H-8e and H-10e were enhanced by 2.5% and 3.0%, respectively. When the sample was irradiated at the H-11 resonance frequency, the intensities of the signals due to H-9 and H-17a increased by 11.5% and 5.2%, respectively, the latter showing that H-11 and H-17a lie on the same (β- or *endo*-) side of the molecule; an NOE of 4.5% was also observed for a multiplet centred at δ 1.50 which could be attributed to H-12β. Irradiation at the H-17a frequency gave NOEs of 21.7% for H-17e, 5.2% for H-11 and 4.6% for a multiplet centred at δ 1.60 attributed to H-3a.

The ^{1}H NMR spectral assignments, based on chemical shift values, spin–spin couplings, decoupling and NOE experiments, are depicted in Fig. 2.

With the position of the hydroxyl group in 9α-hydroxymatrine secured, its high resolution mass spectrum, that of 5α-hydroxymatrine, and the published low resolution data for 5α,9α-dihydroxymatrine [5] shed some light on the electron impact-induced fragmentation of matrinoids (Scheme 1), which has been reviewed by Vul'fson and Zaikin [6].

In the mass spectrum of 5α-hydroxymatrine, which has not been described before, a very stable $[M]^+$ (87%) and a fairly intense $[M-1]^+$ peak (29%) are observable. The base peak at *m/z* 247 corresponds to the loss of a hydroxyl radical from the $[M]^+$, as is confirmed by the metastable ion signal at *m/z* 231.1. There is also an abundant fragment at *m/z* 246 (24%) due to the direct loss of water from the $[M]^+$, as is shown by the metastable ion peak at *m/z* 221 (26%), whose formula, $C_{12}H_{17}N_2O_2$ (**a** and/or **b**) corresponds to the net loss of C_3H_7 from the $[M]^+$. This fragmentation is typical of the matrinoid skeleton, but if it occurred as postulated by Iskandarov and Yunusov [2], the hydroxyl group would necessarily be lost together with C-5 yielding a relatively abundant ion with *m/z* 205, which is not apparent in the spectrum. On the other hand, the retention of the oxygen atom is to be expected if this process occurs as postulated by Vul'fson *et al.* [7].

The hydroxyl group also persists in the fragments at *m/z* 208 (4%), 193 (10%) and 166 (15%), corresponding to the peaks at *m/z* 192, 177 and 150 of matrine and sophocarpine [2]. A $C_6H_{10}NO$ fragment, **e**, *m/z* 112 (35%) is formed directly from the $[M]^+$, as is confirmed by the metastable ion peak at *m/z* 47.5. A weak signal (3%) accompanies the previous one and corresponds to the formula $C_7H_{14}N$, arising from the $[M-1]^+$ ion, as the metastable peak at *m/z* 50.8 shows. The signal at *m/z* 98 is in fact a doublet due to two fragments with formulae (**d**) $C_6H_{12}N$ (4%) and C_5H_8NO (6%); the former is a characteristic of quinolizidine alkaloids, and the latter is probably related to $C_6H_{10}NO$. The low relative abundance of the $C_6H_{12}N$ ion contrasts sharply with the usual values in matrinoids (*ca* 50%) [2], and it can be ascribed to competition between the processes leading to **d** ($C_6H_{12}N$) on one hand, and to **e** ($C_6H_{10}NO$, *m/z* 112), **f** ($C_6H_{10}N$, *m/z* 96) and C_5H_8NO (*m/z* 98) on the other. Ion **c** was not observed at *m/z* 114, but ion **f** was fairly abundant (36%).

In the mass spectrum of 9α-hydroxymatrine (R^5 = H, R^9 = OH), the $[M]^+$ is the base peak, and the $[M-1]^+$ ion is nearly as intense as in the unsubstituted matrine.

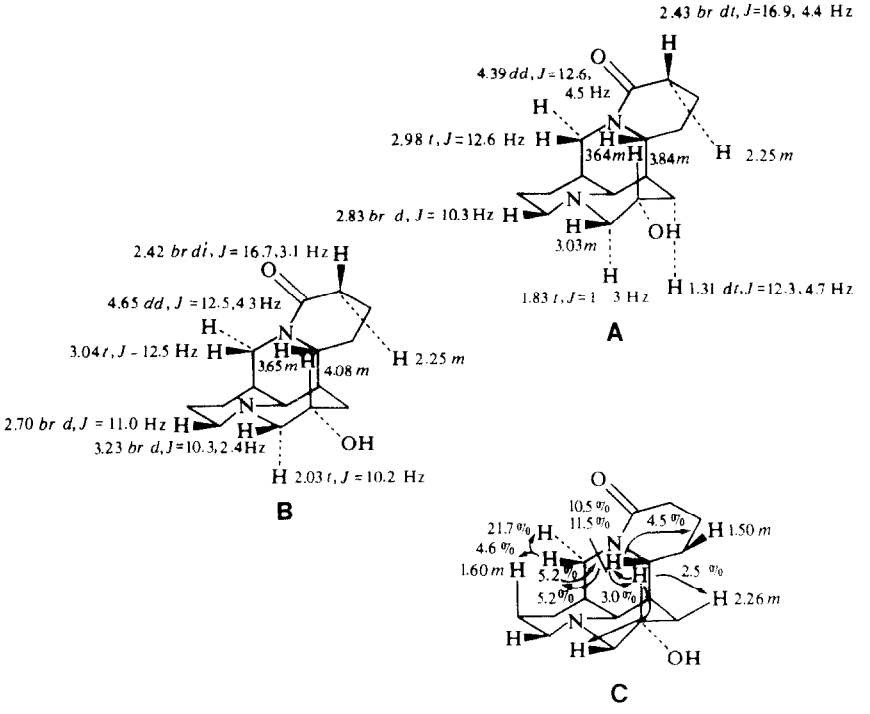
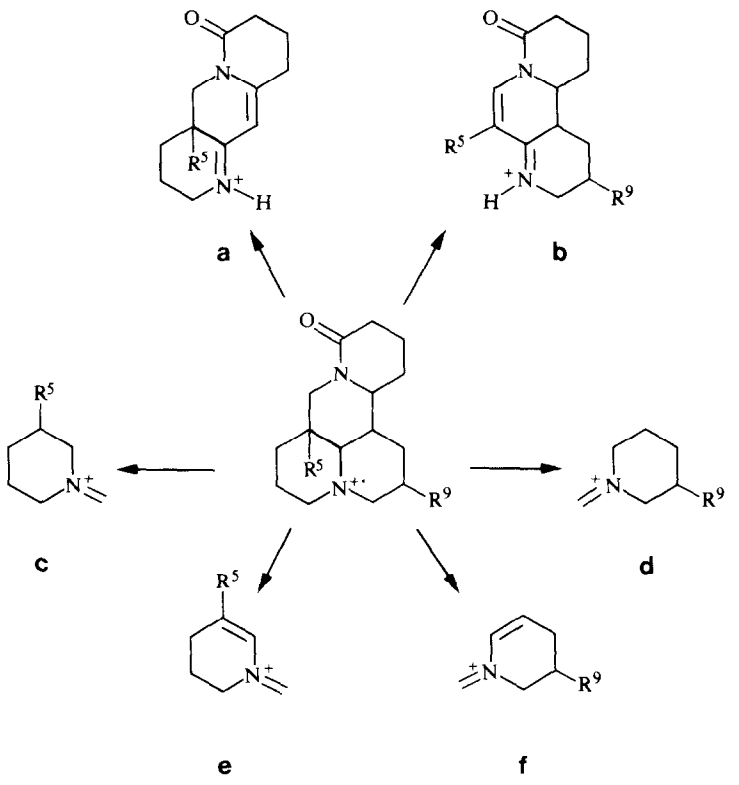


Fig. 2. ^{1}H NMR assignments for 9α-hydroxymatrine (**1**), **A** in $CDCl_3$, **B** in C_5D_5N and **C** NOEs in C_5D_5N.



Scheme 1. Characteristic mass spectral fragments of matrinoids.

The ions due to the losses of hydroxyl and water from the $[M]^+$ occur at *m/z* 247 (19%) and 246 (7%), respectively. A weak signal at *m/z* 221 (9%) represents the net loss of C_3H_7 to afford ion **b**, and an intense one at *m/z* 205 (79%) corresponds to the more probable cleavage of the oxygenated ring to give ion **a**. As in the 5α-hydroxy isomer, ions with the expected compositions are found at *m/z* 208 (11%), 193 (13%) and 166 (45%). The $C_6H_{12}NO$ fragment, **d**, *m/z* 114 (11%) and the $C_6H_{10}NO$ fragment, **f**, *m/z* 112 (14%) are also present in the spectrum, as are the less abundant competing *m/z* 98 ions **c** ($C_6H_{12}N$, 2%) and C_5H_8NO (7%), and the more abundant **e** (*m/z* 96, 41%).

Comparison of the intensities of the mass spectral peaks of 5α- and 9α-hydroxymatrine shows some large differences which can be explained by the relative lability of the tertiary carbon–oxygen bond in the former isomer. Thus, the $[M]^+$ and $[M-1]^+$ peaks are weaker in the spectrum of 5α-hydroxymatrine and the $[M-OH]^+$ and $[M-H_2O]^+$ peaks are much stronger. The fragments with *m/z* 208, 193 and 166, in which the alcohol function is intact, give weaker signals in the spectrum of the 5α-hydroxy isomer.

Few mass spectral peaks have been reported for 5α,9α-dihydroxymatrine [5]. These, however, fit into the above pattern in a satisfactory manner. There is a medium-intensity $[M]^+$ at *m/z* 280 (42%) and a stronger one for $[M-OH]^+$ at *m/z* 263 (54%). The signal at *m/z* 221 (31%) corresponds to ion **a**; the presumably weak signal for ion **b**, due to the loss of the unoxygenated C_3 chain of ring A, expected at *m/z* 237, was not mentioned. The peak at *m/z* 112 (**e** + **f**), on the other hand, is quite intense (87%) and, not surprisingly, no signals warranted reporting at *m/z* 98 or 96.

Two hydroxylated matrinoids have been isolated from *S. alopecuroides*, and some features of their low resolution mass spectra have been published [8]. One of these substances, designated 'base IV', was claimed to be 3α- hydroxysophoridine on the basis of its IR, ^1H NMR and mass spectra. These lead unequivocally to the conclusion that 'base IV' is in fact a sophoridine analogue with a 3α- or a 9α-hydroxy group. The authors' choice of the first of these two possibilities, however, is based on the presence of a mass spectral peak at *m/z* 205 (31%) [8], and the acceptance of the Iskandarov-Yunusov structure for this fragment [3]. Since our work shows that structures **a** and **b**, originally proposed by Vul'fson *et al.* [7], are to be preferred for the *m/z* 205 fragment of matrine and its stereoisomers, the fact that no *m/z* 221 peak was mentioned for 'base IV' is only a reflection of the greater ease with which an oxygenated C_3 fragment is lost from rings A or B. We, therefore, believe that the 9α-hydroxysophoridine structure cannot be ruled out for 'base IV' until more appropriate evidence is adduced.

Occurrences of hydroxylated matrinoids have seldom been documented in the Leguminosae: 5α-hydroxymatrine (sophoranol) in *S. flavescens* [9], *S. macrocarpa* [1], *Euchresta horsfeldii* [5] and *E. japonica* [10]; 3α- (or 9α-) hydroxysophoridine in *S. alopecuroides* [8]; 5α,9α-dihydroxymatrine in *E. horsfeldii* [5]; and now 9α-hydroxymatrine in *S. macrocarpa*. Recent work on the biosynthesis of matrine [11] supports the idea that the unoxygenated matridine and its stereoisomers are formed first from Δ^1-piperideine, and that C-15 is then oxidized to afford matrine and its stereoisomers. *S. alopecuroides* incorporates labeled sophoridine efficiently into 3α- (or 9α-) hydroxysophoridine [12]. It is tempting to speculate

that hydroxylation at certain preferred positions on the less hindered α-side of the molecule is an important first step in the catabolism of matrine and sophoridine, and that the hydroxymatrinoids are not accumulated to any great extent but are further oxidized and degraded.

EXPERIMENTAL

The ^{1}H NMR (360 MHz) and ^{13}C NMR (20 MHz) spectra were recorded using TMS as int. standard or (in C_5D_5N) with the signal at δ 7.55 as the reference. The NOE expts were carried out by FT-NOE difference spectroscopy and each NOE was reported as the percentage enhancement, summarized in Fig. 2. NOEs were obtained using the phase alternating pulse sequence: eight readings were acquired with the decoupler set exactly at a given resonance and eight readings with the decoupler off-resonance were then subtracted; this procedure was repeated until an adequate signal–noise ratio was achieved. EIMS were obtained at 70 eV. Analytical TLC was carried out on Si gel using $CHCl_3$–MeOH (1:1), or on Al_2O_3 using C_6H_6–EtOH–H_2O (49.3:50:0.7).

The crude alkaloids (130 g) from air-dried leaves of *S. macrocarpa* (9 kg, see ref. [1]) were fractionated by extensive open CC and TLC on Si gel and Al_2O_3 to afford several known compounds [1]. 9α-Hydroxymatrine (**1**) was obtained as a minor component of fractions containing mainly *N*-methylcytisine.

(+)-5α-Hydroxymatrine. Isolated as colorless rectangular parallelepipeds, mp 172–173° (C_6H_6–petrol) (lit. [5] 171°), $[\alpha]_D$ +70° (MeOH; *c* 1.0) (lit. [5] +66°); IR ν_{max}^{KBr} cm^{-1}: 3236 (O–H), 2933, 2857, 2796, 2747 (*trans*-quinolizidine), 1629 (lactam C=O); ^{1}H NMR as in ref. [5]; ^{13}C NMR as in ref. [13]; EIMS (probe) 70 eV, *m/z* (rel. int.): 264.1840 (87) $[M]^+$ ($C_{15}H_{24}N_2O_2$ requires 264.1838), 263.1761 (29) $[M-H]^+$ ($C_{15}H_{23}N_2O_2$ requires 263.1759), 247.1815 (100) $[M-OH]^+$ ($C_{15}H_{23}H_2O$ requires 247.1811), 246.1728 (24) $[M-H_2O]^+$ ($C_{15}H_{22}N_2O$ requires 246. 1732), 222.1850 (6) ($C_{14}H_{24}NO$ requires 222.1857), 221.1289 (26) (**a** and/or **b**, $C_{12}H_{17}N_2O_2$ requires 221.1289), 208.1569 (4) ($C_{12}H_{20}N_2O$ requires 208.1576), 193.1345 (10) ($C_{11}H_{17}N_2O$ requires 193.1341), 166.1235 (15) ($C_{10}H_{16}NO$ requires 166.1232), 112.1129 (3) ($C_7H_{14}N$ requires 112.1126), 112.0768 (35) (**e**, $C_6H_{10}NO$ requires 112.0763), 98.0962 (4) (**d**, $C_6H_{12}N$ requires 98.0969), 98.0606 (6) (C_5H_8NO requires 98.0605), 96.0813 (36) (**f**, $C_6H_{10}N$ requires 96.0813).

(+)-9α-Hydroxymatrine (**1**). Obtained as colorless needles, mp 158–159° (petrol), $[\alpha]_{590}^{20}$ +25.4° (MeOH; *c* 1.0); IR ν_{max}^{KBr} cm^{-1}: 3257 (O–H), 2590, 2778 (*trans*-quinolizidine), 1609 (lactam C=O); ^{1}H NMR (360 MHz, $CDCl_3$): δ 1.31 (1H, *dd*, *J* = 12.3, 4.7 Hz, H-8), 1.83 (1H, *t*, *J* = 10.3 Hz, H-10a), 2.25 (1H, *m*, H-14α), 2.43 (1H, *br dt*, *J* = 16.9, 4.4 Hz, H-14β), 2.83 (1H, *br d*, *J* = 10.3 Hz, H-2), 2.98 (1H, *t*, *J* = 12.6 Hz, H-17a), 3.03 (1H, *m*, H-10e), 3.64 (1H, *m*, H-11), 3.84 (1H, *m*, H-9), 4.39 (1H, *dd*, *J* = 12.6, 4.5 Hz, H-17e); ^{1}H NMR (360 MHz, C_5D_5N): δ 2.03 (1H, *t*, *J* = 10.2 Hz, H-10a), 2.25 (1H, *m*, H-14α), 2.42 (1H, *br dt*, *J* = 16.7, 3.1 Hz, H-14β), 2.70 (1H, *br d* *J* = 11.0 Hz, H-2a), 3.04 (1H, *t*, *J* = 12.5 Hz, H-17a), 3.23 (1H, *br dd*, *J* = 10.3, 2.4 Hz, H-10e), 3.65 (1H, *m*, H-11), 4.08 (1H, *m*, H-9), 4.65 (1H, *dd*, *J* = 12.5, 4.3 Hz, H-17e); ^{13}C NMR (20 MHz, $CDCl_3$): δ 18.95 (*t*, C-13), 20.82 (*t*, C-3), 27.32 (*t*, C-4), 27.78 (*t*, C-12), 32.83 (*t*, C-14), 34.88 (*d*, C-5), 35.71 (*t*, C-8), 41.42 (*t*, C-17), 44.17 (*d*, C-7), 53.62 (*d*, C-11), 57.04 (*t*, C-2), 62.90 (*d*, C-6), 62.99 (*d*, C-9), 64.66 (*t*, C-10), 169.33 (*s*, C-15); EIMS (probe) 70 eV *m/z* (rel. int.): 264.1840 (100) $[M]^+$ ($C_{15}H_{24}N_2O_2$ requires 264.1838), 263.1773 (72) $[M-H]^+$ ($C_{15}H_{23}N_2O_2$ requires 263.1760), 247.1814 (19) $[M-OH]^+$ ($C_{15}H_{23}N_2O$ requires 247.1811), 246.1746 (7) $[M-H_2O]^+$ ($C_{15}H_{22}N_2O$ requires 246.1732), 222.1868 (6) ($C_{14}H_{24}NO$ requires 222.1857), 221.1285 (9) (**b**, $C_{12}H_{17}N_2O$ requires 221.1290), 219.1490 (19) ($C_{13}H_{19}N_2O$ requires 219.1497), 208.1571 (4) ($C_{12}H_{20}N_2O$ requires 208.1576), 205.1342 (79) (**a**, $C_{12}H_{17}N_2O$ requires 205.1342), 193.1346 (13) ($C_{11}H_{17}N_2O$ requires 193.1341), 166.1229 (45) ($C_{10}H_{16}NO$ requires 166.1231), 114.0919 (11) ($C_6H_{12}NO$ requires 114.0919), 112.0769 (14) (**f**, $C_6H_{10}NO$ requires 112.0762), 98.0962 (2) (**c**, $C_6H_{12}N$ requires 98.0970), 98.0606 (7) (C_5H_8NO requires 98.0606), 96.0812 (14) (**e**, $C_6H_{10}N$ requires 96.0813).

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