Phytochemistry, 1979, Vol. 18, pp. 645–647. ©Pergamon Press Ltd. Printed in England. 0031-9422/79/0401-0645 $02.00/0

(+)-5α,9α-DIHYDROXYMATRINE, A NEW LUPIN ALKALOID FROM *EUCHRESTA HORSFELDII**

SHIGERU OHMIYA†, KIMIO HIGASHIYAMA†, HIROTAKA OTOMASU†, ISAMU MURAKOSHI‡ and JOJU HAGINIWA‡

† Hoshi College of Pharmacy, Ebara 2-4-41, Shinagawa-ku, Tokyo, Japan 142; ‡ Faculty of Pharmaceutical Sciences, University of Chiba, Yayoi-cho 1-33, Chiba, Japan 260

(*Received* 8 *May* 1978)

Key Word Index—*Euchresta horsfeldii* (*E. formosana* cv *riukiuensis*); Leguminosae; alkaloids; (+)-5α,9α-dihydroxymatrine; sophoranol; sophoranol *N*-oxide; matrine: matrine *N*-oxide; anagyrine; *N*-methylcytisine; *N*-formylcytisine; cytisine.

Abstract—A new lupin alkaloid (+)-5α,9α-dihydroxymatrine has been isolated from the leaves of *Euchresta horsfeldii* (*E. formosana* cv *riukiuensis*) together with matrine, matrine *N*-oxide, sophoranol, sophoranol *N*-oxide, anagyrine, *N*-methylcytisine, *N*-formylcytisine and cytisine.

INTRODUCTION

As a continuation of our screening for lupin alkaloids [1–6], the basic constituents of *Euchresta horsfeldii* (*E. formosana* cv *riukiuensis*) were examined. This plant, belonging to the Leguminosae, is a perennial shrub and is native to the Ryukyu Islands in Japan. In previous work on this species, the alkaloid, cytisine, has been isolated by Plugge [7].

Further chemical examination of the constituents has resulted in the isolation of a new alkaloid (**1**) and 7 more alkaloids of known structure apart from cytisine. This report deals with the structural eludication of **1** as (+)-5α,9α-dihydroxymatrine and also with the distribution of the alkaloids in the stems, roots and leaves of *E. horsfeldii*.

RESULTS AND DISCUSSION

All 9 alkaloids were isolated by preparative HPLC from the basic fraction of the leaves of *E. horsfeldii*. The known 8 alkaloids, matrine, matrine *N*-oxide, sophoranol, sophoranol *N*-oxide [8], anagyrine, *N*-methylcytisine, *N*-formylcytisine and cytisine were identified by comparison with authentic samples (co-TLC and co-HPLC). The amounts of alkaloid in the fresh stems, roots and leaves of *E. horsfeldii* are listed in Table 1. The R_f (TLC) and R_t (HPLC) values used in the identification of these alkaloids are shown in Table 2.

The new alkaloid (**1**), mp 192–193°, $[\alpha]_D^{20}$ + 40.6° (EtOH), gave a molecular formula of $C_{15}H_{24}O_3N_2$ by high resolution MS. Its IR spectrum (KBr) showed absorptions for two OH groups (3400 and 3250 cm^{-1}), a lactam-CO (1602 cm^{-1}) and weak *trans* bands (2700–2800 cm^{-1}).

The PMR spectrum ($CDCl_3$) of **1** suggested a structure very similar to that of sophoranol ((+)-5α-hydroxymatrine) in the relative configuration (Table 3), viz. a double-doublet at δ 4.31 (1H, J = 13.5 and 1 Hz), a doublet at δ 3.17 (1H, J = 13.5 Hz), a multiplet at δ 3.79, C-17 H_{eq} (δ 4.34, *dd*, J = 13.5 and 1.5 Hz), C-17 H_{ax} (δ 3.21, *d*, J = 13.5 Hz) and C-11 H (δ 3.77, *m*), respectively. In addition, these signals both in **1** and sophoranol showed the same pyridine-induced chemical shifts [9]. From the above results, (**1**) was concluded to possess a sophoranol structure but having an extra OH group.

The additional OH group was identified as secondary because in the PMR ($CDCl_3$) of **1**, a multiplet at δ 3.67 overlapping with C-11 proton, assigned to the carbinyl proton from its chemical shift, and the presence of a carbinyl function in the molecule was further confirmed by ^{13}C NMR of **1**, e.g. a doublet centred at δ 62.7 changed into a singlet by the irradiation of H (δ 3.67, *m*). In the PMR of **1** with C_5D_5N, the multiplet separating from the C-11 proton, appeared as a broad triplet at δ 4.15 with J = 12 Hz. The magnitude of the coupling constant is 12 Hz, which indicated that the carbinyl proton and its immediate neighbouring two protons are oriented axially to each other and the orientation of OH group is accordingly equatorial.

The difference of chemical shifts (Δδ) attributable to the C-2 H_{eq} (δ 2.78) and the C-10 H_{eq} (δ 3.05) was 0.27 ppm on the PMR ($CDCl_3$) of **1**, while the Δδ from sophoranol (δ 2.86 and 2.75, in $CDCl_3$) was 0.11 ppm.

Table 1. Alkaloid content (% fr. wt) in stems, roots and leaves in *Euchresta horsfeldii* (*E. formosana* cv *riukiuensis*)

Alkaloids	Stems	Roots	Leaves
Matrine	0.041	0.051	0.114
Sophoranol	0.023	0.019	0.120
Anagyrine	0.013	—	0.038
N-Methylcytisine	0.020	0.009	0.094
5α,9α-Dihydroxymatrine (**1**)	—	—	0.015
N-Formylcytisine	0.009	0.004	0.022
Cytisine	0.164	0.155	0.280
Matrine *N*-oxide	0.136	0.279	0.583
Sophoranol *N*-oxide	0.008	0.007	0.107

* This work was presented at the 98th Annual Meeting of the Pharmaceutical Society of Japan, 4 April, 1978.

Table 2. Chromatographic properties of the nine standard alkaloids*

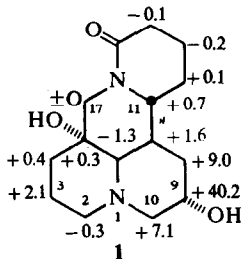
Alkaloids	R_f TLC† 1	2	3	4	5	R_t (min) HPLC† 6	7
Matrine	0.81	0.79	—	0.77	—	6.0	—
Sophoranol	0.67	0.76	—	0.52	—	7.7	—
Anagyrine	0.73	0.63	—	0.70	—	8.5	—
N-Methylcytisine	0.66	0.61	—	0.59	—	10.2	—
5α,9α-Dihydroxymatrine	0.29	0.48	0.58	0.06	0.40	12.5	—
N-Formylcytisine	0.49	0.59	0.67	0.20	0.39	36.0	—
Cytisine	0.41	0.31	0.70	—	—	47.0	—
Matrine *N*-oxide	0.21	—	0.59	*c* 0.09	0.44	—	20.2
Sophoranol *N*-oxide	0.11	—	0.31	0.03	0.24	—	35.8

* All alkaloids were detected on TLC by Dragendorff's reagent and I_2.
† See Experimental.

Moreover, the lower-field signal (δ 3.05) of 1 was affected by pyridine and shifted downfield and the $\Delta\delta$ increased to 0.58 ppm. A similar downfield shift, however, was not observed in sophoranol. These data indicated that the additional OH group could be situated either at C-3 or C-9.

To determine the position of the OH group, the ^{13}C NMR spectrum of 1 was analysed; the ^{13}C NMR assignment of sophoranol has been previously reported by Bohlmann *et al.* [10]. In 1 the signals for C-8 (δ 26.0), C-9 (22.5) and C-10 (56.9) of sophoranol were absent but other signals at δ 35.0, 62.7 and 64.0 were observed. The other ^{13}C NMR signals in 1 were very similar to those observed for sophoranol. The differences of chemical shift are presented in Fig. 1. Among the significant signals, the peak at δ 62.7 is that for the carbinyl group. Considering the expected substituent effect of an equatorial OH group [11], the 3 signals shifted downfield were reasonably assigned to C-8, C-9 and C-10 of 1, respectively.

The structure of the new alkaloid can consequently be designated as (+)-5α,9α-dihydroxymatrine (1).



1

Fig. 1. Structure of (+)-5α-9α-dihydroxymatrine and differences in ^{13}C NMR values (ppm) from sophoranol.

EXPERIMENTAL

The high and low resolution MS were measured at 70 eV. The PMR (100 MHz) and ^{13}C NMR (25 MHz) were recorded using TMS as internal standard.

Analytical TLC was performed on Si gel in the following systems: (1) $CHCl_3$–MeOH–28% NH_4OH (90:9:1); (2) $CHCl_3$–MeOH (4:1); (3) $CHCl_3$–MeOH–28% NH_4OH (43:6:1) and on Al_2O_3 in (4) C_6H_6–Me_2CO–MeOH (18:1:1); (5) C_6H_6–Me_2CO–MeOH (6:1:1). Analytical HPLC was carried out with solvents (6) [15% MeOH–Et_2O]–2.5% NH_4OH (50:1) and (7) [25% MeOH–Et_2O]–H_2O–25% NH_4OH (100:4:3) at a flow rate of 1 ml/min, using a LiChrosorb SI-100 (Merck, 10 μm, 0.3 × 50 cm) column and UV detector (220 nm). Preparative-HPLC was performed on LiChrosorb SI-100 (10 μm) monitoring with a RI or UV (220 nm) detector.

Table 3. PMR chemical shifts for 5α,9α-dihydroxymatrine (1) and sophoranol ((+)-5α-hydroxymatrine) in $CDCl_3$ and in C_5D_5N*

	1 (in $CDCl_3$)	Sophoranol (in $CDCl_3$)	1 (in C_5D_5N)	Sophoranol (in C_5D_5N)
C-17 H_{eq}	4.31 *dd*	4.34 *dd*	4.88 *dd*	4.82 *dd*
C-17 H_{ax}	3.17 *d*	3.21 *d*	3.27 *d*	3.27 *d*
C-11 H	3.79 *m*	3.77 *m*	3.72 *m*	3.75 *m*
C-2 H_{eq}	2.78 *bd*	2.75 *bd*†	2.76 *bd*	2.67 *bd*†
C-10 H_{eq}	3.05 *bdd*	2.86 *bd*†	3.34 *m*	2.77 *bd*†
C-9 H	3.67 *m*		4.15 *bt*	

* δ Values in ppm from TMS as internal standard.
† Assignments could be reversed.

Isolation of alkaloids. E. horsfeldii was collected in February, in Iriomote-jima, Ryukyu Islands, Japan and divided into stems, roots and leaves. The alkaloid fractions (2.1, 0.7 and 1.0 g) were obtained from the 75% MeOH extracts of the fresh leaves (140 g), stems (160 g) and roots (195 g), respectively. The basic fraction (2.1 g) from the leaves was extracted with boiling C_6H_6. The C_6H_6 soluble fraction (1.2 g) was analysed by preparative-HPLC using solvent 6. The alkaloids were eluted in the following order; matrine, sophoranol, anagyrine, *N*-methylcytisine, the new alkaloid (1), *N*-formylcytisine and cytisine.

1 was obtained as colourless crystals from Me_2CO, mp 192–193° (uncorr.) $[\alpha]_D^{20}$ +40.6° (*c* = 0.17, EtOH); λ_{max}^{KBr} cm^{-1}: 3400 and 3250 (2 OH), 2700–2800 (*trans* quinolizidine), 1602 (lactam C=O); MS (70 eV): *m/e* 280.1803 (M^+ $C_{15}H_{24}O_3N_2$, requires; 280.1787), *m/e* 280 (M^+, 42%), 263 (M^+ − OH, 54), 221 (31), 112 (87), 55 (100). PMR ($CDCl_3$): δ 4.31 (*dd*, 1H, *J* = 13.5 and 1 Hz, C-17 H_{eq}), 3.79 (*m*, 1H, C-11 H), 3.67 (*m*, 1H, C-9 H), 3.17 (*d*, 1H, *J* = 13.5 Hz, C-17 H_{ax}), 3.05 (*bdd*, 1H, *J* = 10 and 4 Hz, C-10 H_{eq}), 2.78 (*bd*, 1H, *J* = 10 Hz, C-2 H_{eq}); PMR (C_5D_5N): δ 4.88 (*dd*, 1H, *J* = 13 and 1 Hz, C-17 H_{eq}), 4.15 (*bt*, 1H, *J* = 12 Hz, C-9 H), 3.72 (*m*, 1H, C-11 H), 3.34 (*m*, 1H, C-10 H_{eq}), 3.27 (*d*, 1H, *J* = 13 Hz, C-17 H_{ax}), 2.76 (*bd*, 1H, *J* = 12 Hz, C-2 H); ^{13}C NMR ($CDCl_3$) (assignments were made by the aid of off-resonance and selective proton decoupling technique): carbon atom (chemical shift in ppm) 2 (δ 56.3), 3 (22.5), 4 (36.9), 5 (68.0), 6 (67.2), 7 (38.4), 8 (35.0), 9 (62.7), 10 (64.0), 11 (53.8), 12 (26.8), 13 (18.6), 14 (32.6), 15 (171.2), 17 (46.5).

Matrine *N*-oxide and sophoranol *N*-oxide were isolated from the C_6H_6 insoluble fraction by preparative HPLC.

Identification and estimation of alkaloids. The 8 known alkaloids were identified by co-TLC and co-HPLC with authentic samples, as described in previous papers [1–6]. The alkaloid contents in the stems, roots and leaves were determined by HPLC.

REFERENCES

1. Ohmiya, S., Otomasu, H., Murakoshi, I. and Haginiwa, J. (1974) *Phytochemistry* **13**, 643.
2. Ohmiya, S., Otomasu, H., Murakoshi, I. and Haginiwa, J. (1974) *Phytochemistry* **13**, 1016.
3. Murakoshi, I., Sugimoto, K., Haginiwa, J., Ohmiya, S. and Otomasu, H. (1975) *Phytochemistry* **14**, 2714.
4. Murakoshi, I., Fukuchi, K., Haginiwa, J., Ohmiya, S. and Otomasu, H. (1977) *Phytochemistry* **16**, 1460.
5. Murakoshi, I., Kakegawa, F., Toriizuka, K., Haginiwa, J., Ohmiya, S. and Otomasu, H. (1977) *Phytochemistry* **16**, 2046.
6. Ohmiya, S., Otomasu, H., Haginiwa, J. and Murakoshi, I. (1978) *Phytochemistry* **17**, 2021.
7. Plugge, P. (1895) *Arch. Pharm.* **233**, 430.
8. Ohmiya, S., unpublished results.
9. Demarco, P. V., Farkas, E., Doddrell, D., Mylari, B. L. and Wenkert, E. (1968) *J. Am. Chem. Soc.* **90**, 5480.
10. Bohlmann, F. and Zeisberg, R. (1975) *Chem. Ber.* **108**, 1043.
11. Grover, S. H. and Stother, J. B. (1974) *Can. J. Chem.* **52**, 870.