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(+)-5,17-DEHYDROMATRINE *N*-OXIDE, A NEW ALKALOID IN *EUCHRESTA JAPONICA**

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Key Word Index—*Euchresta japonica*; Leguminosae; alkaloid; (+)-5,17-dehydromatrine; (+)-5,17-dehydromatrine *N*-oxide; (+)-sophoranol.

Abstract—A new lupin alkaloid, (+)-5,17-dehydromatrine *N*-oxide, was isolated from the fresh aerial parts of *Euchresta japonica*. Its structure was confirmed by spectrometric data and by direct comparison with a synthetic sample, prepared from (+)-sophoranol ((+)-5-hydroxymatrine). It was also concluded that (+)-5,17-dehydromatrine *N*-oxide and (+)-matrine *N*-oxide possess the same configuration with respect to the asymmetric nitrogen by NMR spectra.

INTRODUCTION

The legume *Euchresta japonica* is a wide spread native shrub occurring in the southern region of Japan, which has been occasionally used as an indigenous crude drug in folk remedies for neuralgic conditions and as a tonic.

Previous studies on the alkaloids have shown the presence of matrine, matrine *N*-oxide and cytisine in the roots [1, 2].

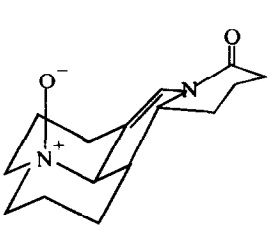
As a part of our screening for lupin alkaloids in legume species [3–7], we have further investigated the basic constituents in the aerial parts of *Euchresta japonica*. In addition to the earlier known alkaloids, a new lupin alkaloid was isolated as a very minor component and its structure was confirmed as (+)-5,17-dehydromatrine *N*-oxide, (1) by comparing the natural compound with synthetic material.

RESULTS AND DISCUSSION

(+)-5,17-Dehydromatrine *N*-oxide (**1**), obtained from the 75% MeOH extracts of the fresh aerial parts of *E. japonica*, gave hygroscopic colourless crystals, $[\alpha]_D^{28}$ + 209.3° (EtOH). The molecular formula, $C_{15}H_{22}O_2N_2$, was established by high resolution MS. Its IR spectrum showed a band at 1640 cm^{-1} due to a lactam-carbonyl group. The presence of $-\overset{|}{C}=CH-\overset{|}{N}-CO-$ unit in the molecule was assumed from the UV spectrum (λ_{max}^{EtOH} 237 nm, log ε = 4.18) [8] and the NMR spectrum in which the olefinic C-17 proton (δ 7.45, *s*, 1H) was shifted downfield by the deshielding effect of the lactam-carbonyl group.

The MS of (+)-5,17-dehydromatrine *N*-oxide showed a molecular ion peak at *m/e* 262 (10%) and fragment peaks corresponding to M^+-O, M^+-OH, and M^+-H_2O at *m/e* 246 (39%), 245 (100) and 244 (16), respectively [9, 10].

The reduction of **1** by H_2SO_3 [11] gave a deoxygenated product, which was identical to (+)-5,17-dehydromatrine prepared from (+)-sophoranol [12]. Therefore, the new alkaloid (**1**) was estimated to be (+)-5,17-dehydromatrine *N*-oxide.



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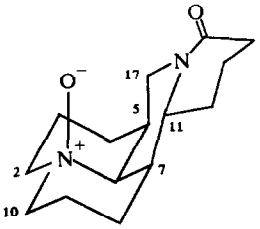

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Further confirmation of the new alkaloid as **1** was obtained by direct comparison of the natural product with a synthetic sample, prepared from (+)-5,17-dehydromatrine and *m*-chloroperbenzoic acid [13].

The configuration of the *N*-oxide nitrogen was established as the stereo-structure **1** by the ^{1}H-NMR spectrum. As shown in Table 1, the signals attributed to the C-11 and C-17 axial protons in (+)-matrine *N*-oxide

Table 1. NMR chemical shifts for C-11 and C-17 axial protons of matrine, 5,17-dehydromatrine and their *N*-oxides*

	5,17-dehydromatrine		matrine	
	Free base	*N*-oxide	Free base	*N*-oxide
C-17 H_{ax}	—	—	3.00	4.20
C-11 H^{ax}	4.15	5.13	3.81	5.12

*Values in ppm from TMS in $CDCl_3$.

*This work was presented at the 24th Meeting of the Japanese Society of Pharmacognosy, at Tokyo, 30 September 1977.

(2) resonated at lower field by 1.2–1.3 ppm compared with those of its free base. A similar relationship was observed between the chemical shifts of the C-11 proton in (+)-5,17-dehydromatrine and its *N*-oxide (1). These differences in the chemical shifts are due to the deshielding effect of the axial N—O bond [14–16]. Therefore, it was concluded that (+)-5,17-dehydromatrine *N*-oxide (1) and (+)-matrine *N*-oxide (2) possess the same configuration with respect to the asymmetric nitrogen.

EXPERIMENTAL

General Methods. Analytical TLC was performed on Si gel in the following solvents: System 1, CH_2Cl_2–MeOH–28% NH_4OH (90:9:1); System 2, CH_2Cl_2–MeOH–28% NH_4OH (86:12:2; System 3, CH_2Cl_2–MeOH (8:2) and on Al_2O_3 in System 4, C_6H_6–$(CH_3)_2CO$–MeOH (34:3:3). HPLC was carried out with System 5, 15% MeOH·Et_2O–2.5% NH_4OH (50:1), and System 6, 25% MeOH·Et_2O–H_2O–25% NH_4OH (500:20:15), using a LiChrosorb SI 100 column and UV-detector (220 nm). NMR spectra were determined using TMS as internal standard.

Isolation of (+)-5,17-*dehydromatrine* N-*oxide* (1). The basic fraction (43 g) obtained from the 75% MeOH extracts of the fresh aerial parts of *E. japonica* (6 kg), collected in July at Kagoshima, Japan, was obtained as a viscous pale yellow oil. The crude alkaloids (9.5 g) were chromatographed on a Si gel column (Merck, Type 60, 3 × 96 cm) with solvents of increasing basic content using 1.5% MeOH·CH_2Cl_2–28% NH_4OH (1000:1) to 11% MeOH·CH_2Cl_2–28% NH_4OH (1000:11) monitoring on Si gel TLC with System 1 or 2. 1 appeared in the 8% MeOH·CH_2Cl_2–28% NH_4OH (1000:8) eluates with matrine *N*-oxide (2). The fractions containing 1 and 2 were further purified by preparative HPLC, eluting with System 6; 1 was eluted from the column after 2 as hygroscopic colourless crystals (55 mg) upon crystallization from Me_2CO. 1, $[\alpha]_D^{28}$ + 209.3° (*c* = 0.15, EtOH); IR ν_{max}^{KBr} cm^{-1}: 1640 (br, lactam C=O); UV λ_{max}^{EtOH} 237 nm (log ε = 4.18); MS (70 eV): *m/e* 262.1658 (M^+, $C_{15}H_{22}O_2N_2$ requires: 262.1680); *m/e* (rel. int): 262 (M^+, 10), 246 (M^+-O, 39), 245 (M^+-OH, 100), 244 (M^+-H_2O, 16), 243 (21), 218 (22), 175 (25), 174 (48), 161 (51), 148 (21), 146 (21); NMR ($CDCl_3$): δ 7.45 (*s*, 1H, C-17), 5.13 (*dt*, 1H, *J* = 12 and 4.5 Hz, C-11). The R_f values on Si gel TLC for 1 obtained in Systems 1 and 2 were 0.12 and 0.35, respectively, whilst 2 exhibited the following R_f data: 0.16 and 0.41, respectively. 1 and 2 on Al_2O_3 TLC had R_f's of 0.26 and 0.30, respectively, in System 4.

Deoxygenation of 1 *into* (+)-5,17-*dehydromatrine.* A soln of 1 (5 mg) in H_2O (1 ml) was saturated with SO_2 gas at 0°, allowed to stand at 15–20° for 2 hr and then saturated with K_2CO_3 with cooling. The CH_2Cl_2 extract was washed, dried and evapd to dryness *in vacuo* to give a colourless oil (4 mg). The product was identical by MS, IR and TLC with (+)-5,17-dehydromatrine, prepared by the dehydration of (+)-sophoranol according to the method of Bohlmann *et al.* [12].

Synthesis of 1 *from* (+)-5,17-*dehydromatrine.* To a CH_2Cl_2 soln (5 ml) containing (+)-5,17-dehydromatrine (30 mg) was added an eq. mol. amount of *m*-chloroperbenzoic acid in CH_2Cl_2 (1 ml) at 15–20° with stirring. After a few min, the reaction mixture was washed with 5% Na_2CO_3 soln to remove the acid, dried over K_2CO_3 and evapd to dryness. The residue was recrystallized from Me_2CO to give hygroscopic colourless crystals in quantitative yield. This product, $[\alpha]_D^{28}$ +210.1° (*c*=0.14, EtOH), was identical with the natural *N*-oxide (1) by IR, MS, HPLC and TLC.

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