STRUCTURE OF PACHYGONINE, A NEW QUATERNARY ALKALOID FROM *PACHYGONE OVATA*

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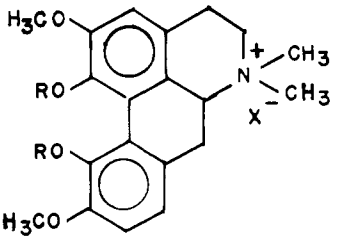
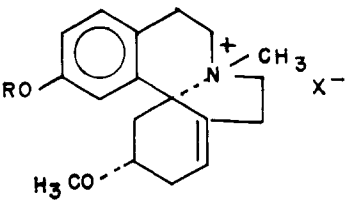
ABSTRACT.—A new quaternary alkaloid, pachygonine, and two known alkaloids, magnoflorine (**1a**) and 0,0'-dimethylmagnoflorine (**1b**), were isolated from the roots of *Pachygone ovata* Miers (Menispermaceae). The structure of pachygonine was established as **2a** on the basis of spectroscopic evidence and its conversion to cocculidine methoiodide (**2c**).

Pachygone ovata Miers (Menispermaceae) is a large evergreen scandent shrub found in abundance on the sandy shores of the Coromandel coast, in Southern Deccan, Madras, Mysore, and Andhra Pradesh. The dried fruits of the plant are reported to be used as a vermicide and as a fish poison (1). No chemical work on the plant has previously been reported.

In the present work, we report the isolation and characterization of two known alkaloids, magnoflorine (**1a**) and *O,O'*-dimethylmagnoflorine (**1b**), and a new quaternary alkaloid, pachygonine (**2a**), from the roots of the plant.



1a R=H, X=OH
1b R=CH_3, X=OH



2a R=H, X=OH
2b R=$Si(CH_3)_3$, X=OH
2c R=CH_3, X=I

The methanolic extract of the dried and ground roots was fractionated into a non-quaternary alkaloid fraction (fraction A) and a quaternary alkaloid fraction (fraction B). Fraction A was set aside for future analysis. Fraction B, which displayed interesting cardiovascular properties in anesthetized animals, was chromatographed over a silica gel column to yield, in sequence, coimpound **1a**, a pachygonine-containing fraction, and compound **1b**. The pachygonne-containing fraction was further purified by column chromatography over alumina to provide pachygonine. Compounds **1a** and **1b** were readily identified by comparison of their iodides with authentic samples of iodides of magnoflorine and *O,O'*-dimethylmagnoflorine, respectively (2).

Pachygonine (**2a**), mp 265–267° (dec.); $[\alpha]_D$+196.61 (c 4.7, MeOH). Its uv spectrum depicted bands at λmax (MeOH) 205, 228 and 285 nm (log ε 4.33, 3.91 and 3.36, respectively) and λmax (MeOH+NaOH) 205, 251 and 307 nm, establishing the presence of a phenolic hydroxy group. In the pmr spectrum, bands were observed at δ 7.16 (1H, d, J=8 Hz), 6.8 (1H, dd, J=8 Hz and 2 Hz), 6.63 (1H,d, J=2 Hz), indicating the presence of a 1,2,4-trisubstituted benzene ring. The presence of a trisubstituted double bond was evident from the multiplet centered at δ 6.2 (1H, which disappeared in the pmr spectrum of dihydropachygonine, **3**,

obtained by catalytic hydrogenation of pachygonine. Singlets at δ 3.36 and 3.2 (br) indicated the presence of O–CH_3 and N–CH_3 groups.

The mass spectra of pachygonine and especially of its trimsyl derivative (**2b**) were particularly revealing. Accurate mass measurement (HRMS) of the M–15 ion (343.1962) of the trimsyl derivative **2b** led to the molecular formula $C_{21}H_{32}NO_2Si$ for **2b**. The fragmentation pattern of the trimsyl derivative was reminiscent of that of the $\Delta^{-1,6}$olefinic group of *Erythrina* alkaloids (3). The constitutions of the major ions, established by accurate mass measurements, at *m/e* 285($C_{17}H_{23}NOSi$), 284($C_{17}H_{22}NOSi$), 213($C_{14}H_{15}NO$), 212($C_{14}H_{14}NO$), 196($C_{14}H_{14}N$), 147(C_9H_9NO) and 73(C_3H_9Si) were, indeed, in agreement with those for fragments arising from a compound with structure **2b** (figure 1). For pachygonine, too, the fragments (see experimental) were completely in accordance with the structure **2a** assigned to pachygonine (4).

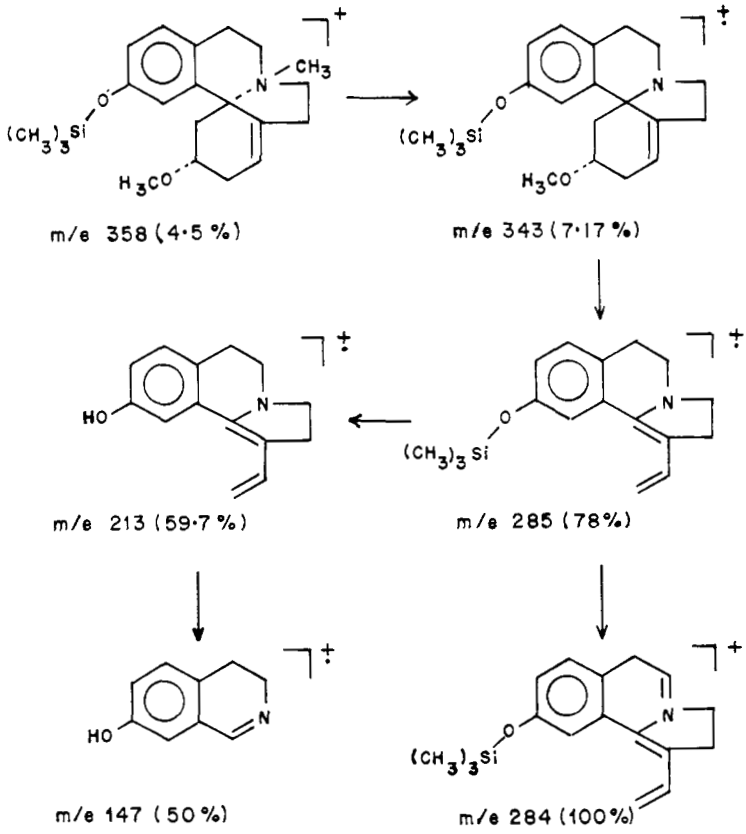


FIG. 1. Major mass spectral fragments of **2b**.

Further confirmation for the structure **2a** was obtained by treatment of pachygonine with diazomethane to give a monomethyl ether which, on treatment with HI, gave cocculidine methoiodide, (**2c**), identical in all respects with an authentic sample (5, 6).

Reports on the natural occurrence of quaternary alkaloids with an erythrinane skeleton like pachygonine are uncommon (7, 8). The isolation of pachygonine

from *Pachygone ovata* extends the occurrence of the *Erythrina* group of alkaloids to the *Pachygone* genus of the Menispermaceae family of plants. Pharmacological studies with pachygonine showed that it is a potent negative chronotropic agent. The pharmacological properties of pachygonine are the subject of a separate publication.

MATERIALS AND METHODS[1]

PLANT MATERIAL.—The plant material was collected at Coimbatore in March 1977 and was identified at the Southern Circle of the Botanical Survey of India, Coimbatore. A herbarium specimen is deposited in the Herbarium of the Research Centre of Hoechst Pharmaceuticals Limited, Bombay 400 080, India.

EXTRACTION AND ISOLATION.—Dry and finely ground roots (30 kg) of *Pachygone ovata* were extracted repeatedly with methanol. The combined methanol extracts were concentrated *in vacuo* and the residue (1.4 kg) was triturated with 3M aqueous hydrochloric acid. The combined acid extracts were extracted with chloroform. The aqueous phase was made alkaline by the addition of ammonium hydroxide and extracted thrice with ethyl acetate. The ethyl acetate layer was washed with water, evaporated to dryness (fraction A), and set aside for future analysis. The aqueous phase was brought to pH 2 by the addition of concentrated hydrochloric acid and a saturated aqueous solution of ammonium reineckate was added. The precipitate of alkaloid reineckate (0.40 kg) was filtered, dried *in vacuo* and dissolved in 50% aqueous acetone. The resulting solution was passed over an anion exchange resin column of seralite SRA-400 (1 kg), and the column was eluted with 50% aqueous acetone. The eluate was evaporated *in vacuo* and the residue (fraction B) was chromatographed on a column of silica gel; ethyl acetate, ethyl acetate-methanol mixtures, methanol and methanol-ammonium hydroxide mixtures were used as eluents. The fraction eluted with methanol gave magnoflorine, **1a**, yield 3.0 g; the fraction eluted with methanol-ammonium hydroxide (97:3) provided the pachygonine-containing fraction, yield 2.0 g; and the fraction eluted with methanol-ammonium hydroxide (1:1) gave *O,O'*-dimethylmagnoflorine, **1b**, yield 3.4 g.

MAGNOFLORINE.—Compound **1a** was crystallized from methanol-acetone, mp 234–35° (dec.); $[\alpha]_D$+200.5 (c 1.05, MeOH). The iodide was prepared by treatment of magnoflorine with HI according to the usual procedures and had mp 250–54° (dec.). The mixture mp with an authentic sample of magnoflorine iodide remained undepressed. The ir and uv spectra were identical with those of the reference sample.

O,O'-DIMETHYLMAGNOFLORINE.—Compound **1b** was crystallized from acetone, mp 221–24° (dec); $[\alpha]_D$+197.67 (c 1.03, MeOH). The iodide was prepared according to the usual methods and had a mp of 244–48° (dec.). The mixture mp with an authentic sample remained undepressed. The authentic sample was prepared by methylation of magnoflorine iodide and gave ir and uv spectra identical with those of the sample.

PACHYGONINE **2a**.—The pachygonine-containing fraction was further purified by column chromatography over alumina with ethyl acetate and ethyl acetate-methanol mixtures as eluents. The ethyl acetate-methanol (1:1) fraction provided pachygonine. It was crystallized from methanol-ethyl acetate (1:3) to give colorless needles, mp 265–267°; $[\alpha]_D$+196.61 (c 4.7, MeOH). The uv and nmr spectral bands are described in the text. It gave a ms: *m/e* 286 (M^+), 285 (M^+-H), 271 (M^+-CH_3), 270 (M^+-H-CH_3), 254 (M^+-H-CH_3O), 227 (M^+-H-C_3H_6O), 213 (M^+-CH_3-C_3H_6O), 212 (100%, M^+-H-CH_3-C_3H_6O).

PACHYGONINE TRIMETHYL SILYL ETHER, **2b**.—Compound **2a** (2 mg) was dissolved in acetonitrile (0.6 ml), and *N*-methyl-*N*-trimethyl-silyltrifluoroacetamide (0.1 ml) was added. The solution was allowed to stand at 30° for 24 hours; the excess reagent and solvent were then distilled off *in vacuo*. The residue was used for HRMS measurements. For the M^+ and fragments, refer to the text.

PACHYGONINE METHYL ETHER, **2c**.—Compound **2a** (40 mg) in methanol was methylated on prolonged (6 days) treatment with diazomethane. The product was treated with HI to give the iodide. It was crystallized from methanol-ether, mp 241–42°. The mixture mp with a sample of cocculidine methoidide, prepared from an authentic sample of cocculidine, remained undepressed. The Rf, ir and uv of the compound and the reference sample were identical.

[1]Melting points were determined on a Kofler heating bench-type 7841 apparatus and are uncorrected. Uv spectra were obtained on a Carl-Zeiss UV-VIS Specord Spectrophotometer. Ir spectra were obtained on a Perkin-Elmer Spectrophotometer, model 521, using KBr pellets. Nmr spectra were recorded on a Varian T-60 Spectrometer, using tetramethylsilane as an internal standard. Mass spectra were obtained on an AEI MS-9025 Spectrometer. Optical rotations were measured with a Carl-Zeiss 31841 Polarimeter.

DIHYDROPACHYGONINE, **3**.—Compound **2a** (50 mg) in methanol was hydrogenated in the presence of platinum oxide. One molar equivalent of hydrogen was absorbed. The reaction was worked up in the usual manner to give a gummy product which resisted all attempts at crystallization. Silica gel tlc of the gum showed two discrete spots. In the ir spectrum, no band was observed at 897 cm^{-1}. In the pmr spectrum, no bands were observed for the olefinic proton in the region of δ 6.2.

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