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A NEW PIPERINE-TYPE AMIDE FROM *PIPER GUINEENSE*

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Key Word Index—*Piper guineense*; Piperaceae; $\Delta^{\alpha\beta}$-dihydrowisanidine; wisanidine; alkaloids.

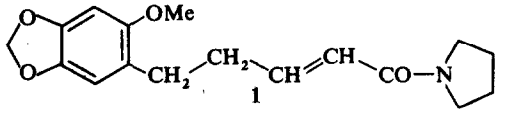
INTRODUCTION

Piper guineense grows in forest regions of Cameroon. The seeds are commonly used as an ingredient in medicinal preparations and as a spice in foods. The genus *Piper* has been extensively investigated [1–4] and the fruits have been shown to vary in their chemical composition with geography. In the present communication, we wish to report the isolation and structure elucidation of a new alkaloid $\Delta^{\alpha\beta}$-dihydrowisanidine 1 from *P. guineense*.

RESULTS AND DISCUSSIONS

Chromatography of the powdered seeds of *Piper guineense* over Si gel with hexane–ether gave $\Delta^{\alpha\beta}$-dihydrowisanidine 1 (yield 0.02%) and wisanidine 2 (yield 0.01%) in succession. 1, $C_{17}H_{21}NO_4$, M^+ 303 gave a positive alkaloid test with Meyer's reagent. It revealed in its IR spectrum bands at 1655 cm^{-1} and 1610 cm^{-1} indicating conjugation of the double bond with an amide carbonyl group; 1260 cm^{-1} the methylenedioxy group and 2870 cm^{-1} the methoxy group. The NMR spectrum indicated signals for the methylenedioxy group (δ 5.88, 2H); two aromatic protons (δ 6.52, 1H and 6.63, 1H) and the methoxy group (δ 3.76, 3H). The splitting constants of the protons attached to the unsaturated carbon atoms (δ 6.10 and 6.97, J_{HH}, 16Hz) indicated a *trans* configuration and the signals of the protons on the pyrrolidine ring were in agreement with those observed on the pyrrolidine ring of trichostachine [5]. Structure 1 proposed was in agreement with the mass fragmentation pattern.

In a series of reactions carried out with 1 we were able to confirm the proposed structure. Hydrolysis of the alkaloid with 20% alcoholic KOH gave pyrrolidine and a new acid, $\Delta^{\alpha\beta}$-dihydrowisaninic acid mp 136–138°. Catalytic hydrogenation of the new alkaloid with 10% Pd/C gave tetrahydrowisanidine, mp 58° previously unknown. The Zn–Cu couple reduction of the double bond of the alkaloid confirmed that it was conjugated with the carbonyl group of the amide [6, 7]. The Zn–Cu couple reduction product was identical (TLC, UV, IR, NMR, MS, mp, mmp) with tetrahydrowisanidine obtained by catalytic hydrogenation of 1. Attempts to oxidise the alkaloid to known products in order to establish the position of the methoxy group were unsuccessful and an indirect method was therefore used. The alkaloid was dehydrogenated with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) by refluxing in dry C_6H_6 for 6 hr, after which the product was chromatographed over neutral alumina. The dehydrogenation product mp 170–172° was identical (IR, UV, NMR, MS, mp, mmp) with wisanidine 2. Oxidation of the dehydrogenation product gave 2-methoxy-3,4-methylenedioxybenzaldehyde mp 110° (Lit. [8] 111.5–112°) thus confirming that the methoxy group is at the 2-position.



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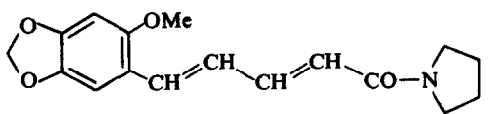

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EXPERIMENTAL

Mp's uncorrected, were recorded on a Kofler banc; IR spectra in KBr discs; NMR spectra in $CDCl_3$ solution with TMS as internal standard were recorded in a 100 Hz spectrometer; column chromatography was on neutral alumina and Merck Si gel (0.063–0.200). Seeds of *Piper guineense* collected from Buea road in Cameroon were dried at ambient temp. and powdered. Powdered material was Soxhlet extracted for 48 hr. The hexane soln was concnd under red. pres. to give a crude extract (300 g). Chromatography of the crude extract over neutral alumina eluting with hexane–Et_2O gave a mixture of oily substances followed by a mixture of solids. The solids were rechromatographed over Si gel and elution with hexane–Et_2O (2:3) gave a mixture of minor compounds followed by 1 and 2 in succession. 1 was recrystallised from C_6H_6–hexane (1:1) to give colourless crystals mp 82–84°. λ_{max}^{EtOH} nm (log ε) 204 (3.88), 303 (4.78), ν_{max}^{KBr} cm^{-1} 2950, 2870, 1660, 1600, 1500, 1290, 1260, 1145, 1075, 1030, 1000, 970, 925, 855, 810, NMR: δ 1.90 (4H, *m*) 3.50 (4H, *t*) 6.10 (H, *d*, *J* 16Hz) 6.97 (1H, *m*, *J* 16Hz), 2.47 (2H, *t*), 3.76 (3H, *s*), 6.52 (1H, *s*), 5.88 (2H, *s*) MS: *m/e* M^+ 303, 205, 166, 135 (Found: C, 67.69; H, 7.06; N, 4.19; $C_{17}H_{21}NO_4$ requires C, 67.33; H, 6.93; N, 4.62.

Hydrolysis of 1. A mixture of $\Delta^{\alpha\beta}$-dihydrowisanidine (320 mg) and KOH (10 g) in EtOH (50 ml) was refluxed for 23 hr. The solvent was evaporated under red. pres. and residue diluted with H_2O (250 ml). The mixture was extracted with $CHCl_3$ (500 ml) and the alkaline soln was acidified to pH 2 and extracted with Et_2O (500 ml). The ethereal layer was washed and dried. Solvent was evaporated and the solid crystallised as pale yellow crystals mp 136° (C_6H_6–hexane ν_{max}^{KBr} cm^{-1} 1700, 1500, 1155, 1035, 1000, 935, 873, 805. NMR: δ 2.51 (2H, *t*), 2.71 (2H, *t*), 3.75 (3H, *s*), 5.82 (1H, *d*, *J* 16Hz), 7.14 (1H, *m*, *J* 16Hz), 5.88 (2H, *s*), 6.52 (1H, *s*, aromatic H), 5.63 (1H, *s*, aromatic H), MS M^+ 250, 165, 135 (Found C, 63.25; H, 5.96 $C_{13}H_{14}O_5$ requires C, 62.40; H, 5.60).

Catalytic hydrogenation of 1. A mixture of $\Delta^{\alpha\beta}$-dihydrowisanidine (502 mg) and 10% Pd/C (100 mg) in EtOH was shaken with hydrogen at atmospheric pressure until absorption stopped (1.5

hr). Removal of the catalyst and solvent left a residue, tetrahydrowisanidine (400 mg), which was crystallised from hexane–Et_2O mp 58–60°, ν_{max}^{KBr} cm^{-1} 2960, 2940, 1635, 1500, 1470, 1430, 1295, 1212, 1180, 1150, 1075, 1030, 990, 920, 855, NMR: δ 1.63 (4H, *m*), 2.28 (2H, *t*), 2.56 (2H, *t*), 3.44 (4H, *t*), 3.73 (3H, *s*), 5.87 (2H, *s*), 651 (1H, *s*), MS M^+ 305, 274, 175, 165, 135 (Found C, 66.71, H 7.45, N 4.52, $C_{17}H_{23}NO_4$ requires C, 66.89; H, 7.54; N, 4.59).

Reduction of **1** *with Zn–Cu couple.* Preparation of the Zn–Cu couple [6, 7]. Zn powder (35 g) was washed (4 × 20 ml) with dilute HCl (3%) each washing taking 5 min. The mixture was stirred during each washing and the acid removed by decantation. The Zn was then washed with water 3 × to eliminate traces of acid and (4 × 20 ml) with 2% $CuSO_4$. The couple was finally washed (3 × 20 ml) with the solvent, MeOH and rapidly poured into the reaction vessel. Alkaloid **1** (1.00 g) in MeOH–H_2O (3:1) was refluxed with the couple prepared above for 52.5 hr. The reaction mixture was taken up in more MeOH (50 ml) and filtered. The filtrate was evaporated to dryness under reduced pressure and the residue crystallised from Et_2O–hexane (1:1) mp 58–69°. This product was identical (IR, UV, NMR, MS, mp, mmp) with tetrahydrowisanidine.

Dehydrogenation of **1** *with DDQ.* A mixture of alkaloid **1** (1.420 g) and 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) (1.40 g) in dry C_6H_6 (100 ml) was refluxed for 5 hr after which the mixture was chromatographed over neutral alumina eluting with hexane–C_6H_6. A yellow solid was obtained (500 mg) which was recrystallised from Me_2CO mp 170°. This compound was identical (IR, UV, NMR, MS, mp, mmp) with wisanidine.

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NUPHAROPUMILINE, A NEW QUINOLIZINE ALKALOID FROM *NUPHAR PUMILA*

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Key Word Index—*Nuphar pumila*; Nymphaeaceae; quinolizine alkaloids; (+)-nupharopumiline.

Abstract—A new quinolizine alkaloid, (+)-nupharopumiline, has been isolated from the rhizomes of *Nuphar pumila*. It has been shown by spectroscopic and chemical methods to possess the stereostructure **6** (1R, 4S, 7S).

INTRODUCTION

Several *Nuphar* species produce alkaloids having a furyl group attached to a quinolizine or piperidine ring system [1–3]. Similar compounds have been isolated from the scent gland of the Canadian beaver [4].

In connection with our investigation of the alkaloid contents of *Nuphar pumila* (Timm.) DC. (Nymphaeaceae), a perennial rhizomatous herb with a wide distribution in the temperate zone of the northern hemisphere [5–7], we isolated several basic compounds (total alkaloids obtained represent about 0.5% of the air dry material). In addition to the earlier known [1–3] (−)-deoxynupharidine **1**, (−)-7-*epi*-deoxynupharidine **2**, (+)-nupharidine **3**, and (+)-7-*epi*-nupharidine **4**, a new unstable base, for which the name (+)-nupharopumiline is proposed, was isolated in low yield.

RESULTS

(+)-Nupharopumiline, $[\alpha]_D^{20}$ + 27° ($CHCl_3$), mn 195–197° (CCl_4), represents about 0.15% of the total alkaloids of *Nuphar pumila* (*vide supra*), from which it was separated by column and preparative layer chromatography (*vide infra*). The ^1H NMR spectrum ($CDCl_3$) of nupharopumiline reveals the presence of two methyl groups (δ 0.93, 3H, *d*, 6 Hz: δ 1.03, 3H, *d*, 6 Hz), a vinyl proton (δ 4.48§, 1H, *dd*, 12 Hz, 4 Hz), and a furyl group (δ 6.60, 1H, *m*: δ 7.45, 1H, *m*: δ 7.64, 1H, *m*).

The MS (70 eV: 200°) of nupharopumiline shows an

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§ The corresponding signal in the ^1H NMR spectrum ($CDCl_3$) of Δ^3dehydrodeoxynupharidine **5** is claimed [8] to appear at δ 4.96.