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TWO PIPERIDINE ALKALOIDS FROM *SIPHOCAMPYLUS VERTICILLATUS*

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Key Word Index—*Siphocampylus verticillatus*; Campanulaceae; lobelidiol; piperidine alkaloid.

Abstract—From *Siphocampylus verticillatus*, two novel piperidine alkaloids have been isolated, namely *meso*-8,10-di-*n*-propyllobelidiol[*meso*-2,6-bis(2′-hydroxypentyl)-*N*-methylpiperidine] and 8-ethyl-10-*n*-propyllobelidiol [6-(2′-hydroxybutyl-2-(2″-hydroxypentyl)-*N*-methylpiperidine].

INTRODUCTION

Siphocampylus verticillatus (Cham.) G. don. (Campanulaceae) is a genus related to *Lobelia* [1] and grows abundantly in wasteland between 1000 and 2000 m altitude in the Paraná State, Brazil. Leonart *et al.* [2] described a preliminary comparative investigation of *Siphocampylus verticillatus* and *S. sulfureus* Wimmer, showing that it is possible to differentiate the two species because of the alkaloids present only in *S. verticillatus*, where Contin [3] demonstrated the presence of ursolic acid, a flavonoid (luteolin) and a coumarin (scoparone). Studies on the tincture of *S. foliosus* have shown toxic activity in rabbits [4], whereas hexane extracts of *S. verticillatus* inhibit *in vitro* growth of nine species of bacteria, although its alkaloid fraction inhibits only *Shigella boydii* and *S. flexneri* [5]. The present paper deals with the isolation of two piperidine alkaloids from *S. verticillatus* and the elucidation of their structures as further homologues of the lobelidiol series [6].

RESULTS AND DISCUSSION

Alkaloid structures

We propose for the major alkaloid m.p. 31–32, $[\alpha]_D$ 0° obtained as described in the Experimental, the structure of *cis*-8,10-di-*n*-propyllobelidiol, **1** [7]. There was no significant UV absorption and the IR spectrum had a broad peak at 3377 cm^{-1} which the formation of an *O*,*O*-diacetate showed to be due to OH and not NH absorption. A wealth of structural information was afforded by the EI/CI mass spectra (Fig. 1). There was a strong molecular ion at *m/z* 271, which analyzed for $C_{16}H_{33}O_2N$ (271.2501). The base peak at *m/z* 184 analyzed for loss of $C_5H_{11}O$, whereas a pair of ions at *m/z* 98 ($C_6H_{11}N$) and 96 (C_6H_9N) corresponded to a second loss of the same fragment with hydrogen transfer to leave singly or doubly unsaturated *N*-methylpiperidines; a peak at *m/z* 170 was attributable to loss of an *N*-methyl group from *m/z* 184. This fragmentation behaviour is characteristic for 2,6-disubstituted piperidine alkaloids where cleavage α to N is favoured. Another important fragment at *m/z* 228 analyzed for loss of C_3H_7, and since it can be attributed to another favoured cleavage α to O, indicated clearly the location of the OH groups.

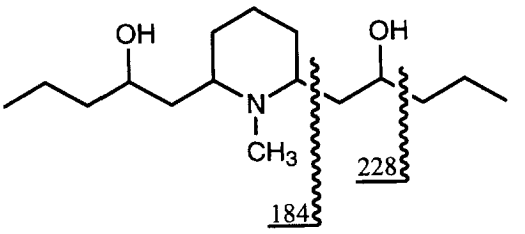

Fig. 1.

^{13}C and ^{1}H NMR spectra (75 and 300 MHz in $CDCl_3$) together with appropriate decoupling and COSY experiments confirmed the gross structure and, furthermore, gave important information about the stereochemistry. The ^{13}C NMR spectrum had only 9 carbon signals for the 16 carbon atoms known to be present from the mass measurement, which is only consistent with a *meso* structure having a plane of

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symmetry through N and C-4 of the piperidine ring, and also explains the zero optical rotation. Hence the C-2 and C-6 substituents must be *cis*, and the chiral centres at C-8 and C-10 must be enantiomeric. This symmetry was also corroborated by the ^{1}H NMR spectrum of **1**, where a distorted triplet at δ 0.8 (6H, $J = 7$ Hz) corresponded to the identical pair of terminal methyl groups (H-13/16). A multiplet at δ 1.1–1.7 was attributed to the methylenes in C-3/5, 4, 7/9, 11/14 and 12/15. A singlet at δ 2.10 indicated the presence of *N*-Me, and a two-proton multiplet at δ 3.05 was due to H-2 and H-6 of the piperidine ring. A broad signal at δ 3.75 (2H) was assigned to two protons geminal to hydroxyl groups, which were confirmed as secondary by the chemical shift of 1.1 ppm on acetylation. Couplings and connectivity were elucidated from decoupling experiments on the diacetate derivative of **1**. Thus, on irradiation at δ 4.36 (H-8/10), a signal at δ 1.79 (H-7a/9a) changed to a clear double-doublet with $J = 13$ and 6.5 Hz due to coupling with H-7b/9b and H-2/6 respectively, whereas the quartet at δ 1.41 (H-11/14) became a triplet with $J = 7.5$ Hz due to coupling with H-12/15, in accordance with the connectivity shown by the COSY spectrum.

Hence we had established that the major alkaloid is *cis*-8,10-di-*n*-propyllobelidiol, and that it could be one of the two possible *meso* diastereoisomers—2*R*,6*S*,8*R*,10*S* **1a** or 2*R*,6*S*,8*S*,10*R* **1b** [7]. The most convenient way to distinguish between these would have been by an X-ray structure, but unfortunately the crystals of **1** were not suitable for diffraction. Recently, Miguel *et al.* [8] have reported the X-ray structure of the hydrochloride salt of **1**, which shows it to correspond to **1b**.

On the other hand, the minor alkaloid **2** proved to be an interesting novel unsymmetrical homologue of **1**. Its mass spectrum had a M^+ 257.2360 analyzing for $C_{15}H_{31}O_2N$, i.e. CH_2 less than **1**, and similar major

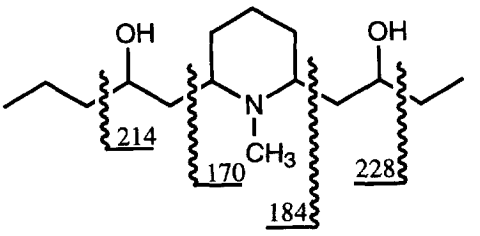
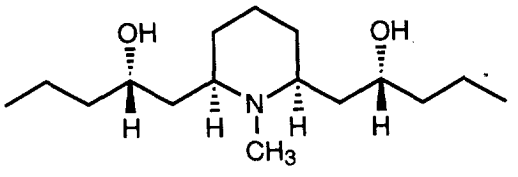
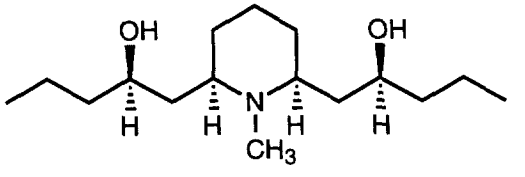


Fig. 2.

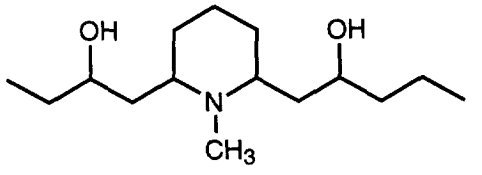
peaks at *m/z* 228.1968 ($C_{13}H_{26}O_2N$) and 214.1819 ($C_{12}H_{24}O_2N$) corresponded to ready losses α to O of one ethyl and one propyl group respectively (Fig. 2). Furthermore, there was a base peak at *m/z* 170.1550 ($C_{10}H_{20}ON$) and another large peak at 184.1703 ($C_{11}H_{22}ON$) attributable, as with **1**, to cleavages α to N of $C_5H_{11}O$ and C_4H_9O fragments. Again, the same ions due to loss of both side chains at *m/z* 98 and 96, characteristic of a 2,6-disubstituted *N*-methylpiperidine alkaloid were also present. The presence of two OH groups and a tertiary N was indicated by an IR band at 3380 cm^{-1} and the formation of an *O,O*-diacetate with M^+ 341 and an ester C=O band at 1750 cm^{-1}. Importantly, the ^{1}H NMR spectrum showed two *different* methyl triplets at δ 0.88 and 0.92, thus establishing terminal ethyl and *n*-propyl (rather than *iso*-propyl) groups. A singlet at δ 2.53 confirmed the *N*-Me. Significantly, two *different* secondary CH—O signals were present at δ 3.68 and 3.77, together with a two-proton multiplet at δ 3.53 for two CH—N. Four-proton multiplets at δ 1.60 and 1.80 were attributable to the 3/5 and 7/9 methylene groups respectively, whereas a broad eight-proton peak between δ 1.3 and 1.6 accounted for those at 4, 11, 12 and 14. Hence it may be concluded that the gross structure of the minor alkaloid is 8-ethyl-10-*n*-propyllobelidiol **2**. Although one could speculate that the



1a



1b



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stereochemistry might be similar to that of the major alkaloid, there is no firm evidence.

EXPERIMENTAL

General procedures

For analytical and preparative TLC Merck silica plates precoated with Kieselgel 60 (F_{254}) were used and visualization was effected by UV light and by spraying with Dragendorff's reagent. Column chromatography was carried out on Merck Kieselgel 60. Melting points were determined on a Kofler block without correction. Optical rotation $[\alpha]_D$ was determined at 20°. Nuclear magnetic resonance spectra were recorded at 200 MHz and 300 MHz. Molecular formulae were determined from accurate mass measurement. Solvents and reagents were purified when necessary by standard methods. Acetylation was performed by standard procedures using Ac_2O/pyridine at room temp. for 12 h.

Plant material

The whole plant was collected in the State of Paraná, near Curitiba, Brazil, during the summer period and identified by R. Leonart. A voucher specimen is deposited in the Herbarium of the Botany Department, Universidade Federal do Paraná, Brazil, under the number 14702.

Extraction, isolation and identification of alkaloids

The dried plant material (rhizomes and aerial parts, 1 kg) was extracted with 1% HCl until the reaction with Dragendorff's reagent was negative, the acid extract filtered and concentrated to 1/3 of its initial volume by distillation. It was then made alkaline (pH 9–10) with dil. ammonia and the free base extracted with $CHCl_3$. Evaporation of the solvent *in vacuo* yielded an amorphous brown residue (3.4 g) which was chromatographed on silica, elution with $CHCl_3$:*i*-PrOH (7:3) yielding an alkaloid **1** (107 mg). Further fractionation by preparative TLC on silica with EtOAc:*i*-PrOH:NH_4OH (16:3:1) as eluent, gave another alkaloid **2** (10 mg).

8,10-*Di*-n-*propyllobelidiol* (**1**). White fluffy needles from $CHCl_3$ m.p. 31–32, $[\alpha]_D$ 0° (MeOH, *c* 2.0 and 4.0); TLC R_f 0.60; IR ν_{max} cm^{-1} 3377, 2932, 1466, 1346, 1158, 844, 755; *m/z* (%) $[M^+]$ 271.2501 ($C_{16}H_{33}O_2N$ requires 271.2512), 228 [M−43]; 184 [M−87] (100), 170 [M−101], 166 [M−105], 140 [M−131], 98 [M−173], 86, 84, 70 and 55; ^1H NMR (300 MHz, $CDCl_3$, TMS) δ 0.81 (6H, *t*, J = 7 Hz, H-13, 16), 1.10–1.60 (10), *m*, J = 7, H-12, 15, 3, 5, 4), 1.42 (4H, *q*, J = 7, H-11, 14), 1.78 (2H, *q*, J = 13 and 7, 5, H-7a, 9a), 2.20 (3H, *s*, *N*-Me), 3.05 (2H, *bs*, H-2, 6) and 3.75 (2H, *t*, J = 13 and 6, 5, H-8, 10); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.29 (C-13, 16), 18.78 (C-4), 23.12 (C-12, 15), 25.10 (C-11, 14), 26.16 (*N*-Me), 39.62 (C-3, 5), 40.36 (C-7, 9), 62.23 (C-2, 6), 71.16 (C-8, 10). Acetylation of **1** gave a diacetate as a gum; TLC R_f 0.80, IR ν_{max} cm^{-1} 2932, 1752, 1465; *m/z* $[M^+]$ 355.2732 ($C_{20}H_{37}O_4N$ requires 335.2724); ^1H NMR (300 MHz, $CDCl_3$, TMS) δ0.80 (6H, *t*, J = 7 Hz, H-13, 16),1.10–1.35 (12H, *m*, H-3, 4, 5, 7b, 9b, 12, 15), 1.41 (4H, *q*, H-11, 14), 1.79 (2H, quintet, J = 13 and 7.5, H-7a, 9a), 1.93 (6H, *s*, 2Ac), 2.10 (3H, *s*, *N*-Me), 2.20–2.40 (2H, *m*, H-2, 6) and 4.86 (2H, quintet, J = 13 and 6.5 Hz, H-8, 10) [8].

8,*Ethyl*-10-n-*propyllobelidiol* (**2**). Amorphous mass; TLC R_f 0.50, *m/z* $[M^+]$ 257.2360 ($C_{15}H_{31}O_2N$ requires 257.2356), 228 (23), 214 (21), 184 (86), 170 (100), 112 (15), 110 (12), 98 (93), 96 (62); IR ν_{max} cm^{-1} 3380, 2930; ^1H NMR (300 MHz, $CDCl_3$, TMS) δ 0.88 (3H, *t*, J = 7 Hz, H-13/15), 0.92 (3H, *t*, J = 7 Hz, H-15/13), 1.35–1.55 (8H, *m*, H-4, 11, 12, 14), 1.60 (4H, *m*, H-3, 5), 1.80 (4H, *m*, H-7, 9), 2.53 (3H, *s*, *N*-Me), 3.53 (2H, *m*, H-2, 6), 3.68 (1H, *bs*, H-8/10) and 3.77 (1H, *bs*, H-10/8). Acetylation gave a diacetate as a gum: IR ν_{max} cm^{-1} 2930, 1750; *m/z* CI (%) 342 (100), 282 (5), 226 (21), 212 (23); Found: 342.2641 $[M^+ + H]$ ($C_{19}H_{36}O_4N$ requires 342.2646); 226.1806 ($C_{13}H_{24}O_2N$ requires 226.1809); 212.1641 ($C_{12}H_{22}O_2N$ requires 212.1645).

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