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BENZYLISOQUINOLINE ALKALOIDS FROM *ANISOCYCLA JOLLYANA* LEAVES

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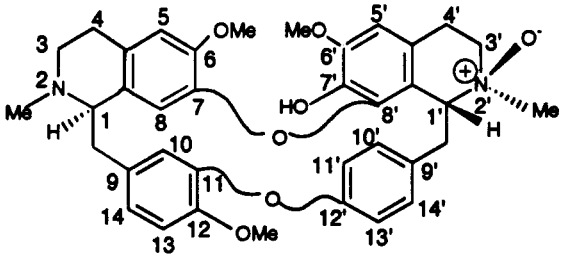
ABSTRACT.—A new bisbenzylisoquinoline alkaloid, (+)-limacusine-2′-β-*N*-oxide [**1**], was isolated from the leaves of *Anisocycla jollyana*, along with nine known alkaloids: (−)-2′-norlimacine, (−)-2-norlimacine, (−)-limacine, (−)-limacine-2′-β-*N*-oxide, (+)-homoaromaline, (+)-trilobine, (+)-isotrilobine, (+)-dehydro-1,2-telobine, and (+)-remrefidine. The structure of **1** was established by spectral methods.

As part of our ongoing investigation of alkaloids from *Anisocycla* species (Menispermaceae), we reported recently the isolation and the identification of bisbenzylisoquinoline, protoberberine, aporphine alkaloids, and quinones from *A. cymosa* Troupin (1–4). Among these compounds, cocsoline and its derivatives showed antiprotozoal activity against *Plasmodium falciparum, Giardia lamblia,* and *Entamoeba histolitica* (2). The results of a phytochemical investigation of the leaves of *A. jollyana* (Pierre) Diels, another potential source of bisbenzylisoquinoline alkaloids, are described herein. This plant is the second *Anisocycla* species found growing in southwestern Zaire; it has no known uses in traditional medicine.

Combined cc and prep. tlc on Si gel and on Al_2O_3 of a MeOH extract of *A. jollyana* leaves resulted in the isolation of ten alkaloids.

Compound **1** was obtained as colorless needles from $CHCl_3$/MeOH. Its uv spectrum displayed maxima at 230 and 282 nm, indicative of being a bisbenzylisoquinoline alkaloid (5). This observation was further supported by eims and ^{1}H-nmr spectrometry. The eims of **1** showed a molecular ion of medium intensity at *m/z* 624 consistent with the elemental composition, $C_{37}H_{40}N_2O_7$, accompanied by five prominent peaks at *m/z* 608, 381, 367, 191^{++}, and 174. The *m/z* 608 $[M-16]^+$ (78) ion, which was attributed to the loss of an oxygen atom from the molecular ion, is diagnostic for an *N*-oxide (6–8). The *m/z* 381 fragment represented the upper half of the molecule (7–10), while a *m/z* 191^{++} ion corresponded to the doubly charged upper half of the *m/z* 608 species. As expected for a bisbenzylisoquinoline incorporating 7–8′ and 11–12′ ether linkages (oxyacanthine-type alkaloids), the eims also showed an ion at *m/z* 501 $[M-16-107]^+$, characteristic for D-ring loss (9). The ^{1}H-nmr data of **1** are similar to those reported for (+)-limacusine (11–13) with regard to the aromatic protons and the substituents. A remarkable difference, however, was found in the signals corresponding to the 2′-*N*-methyl



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group and the asymmetric proton H-1′, which were both shifted downfield. The 2′-*N*-methyl singlet at δ 3.19 and the H-1′ proton signal at δ 4.63 are characteristic of a trans-relationship between the *N*-oxide oxygen and H-1′ (8,10,14). The presence of an nOe effect between H-1′ (δ 4.63) and the 2′-*N*-methyl singlet (δ 3.19) confirmed this configuration (15) as expected for a bisbenzylisoquinoline incorporating 7–8′ and 11–12′ ether linkages. Finally, **1** was reduced with zinc in HCl to afford the known alkaloid (+)-limacusine (11,12). These results established **1** as limacusine-2′-β-*N*-oxide. This new dimer, belonging to the oxyacanthine subgroup, exhibited a moderate positive specific rotation, $[\alpha]^{20}D$ +157° (*c*=1.1, $CHCl_3$), and, therefore, the same *RR′* absolute configuration as (+)-limacusine.

The other alkaloids were identified by direct comparison of their tlc behavior as well as their uv, ^{1}H-nmr, and eims data with those of alkaloids isolated previously (1–4, 16). Three dimers, trilobine (0.036%), isotrilobine (0.044%), and dehydro-1,2-telobine (0.090%) belong to the trilobine subgroup; one dimer, (+)-homoaromaline (0.720%) to the oxyacanthine subgroup; four dimers, (−)-2′-norlimacine (0.010%), (−)-2-norlimacine (0.292%), (−)-limacine (0.382%), and (−)-limacine-2′-β-*N*-oxide (0.012%) to the berbamine subgroup; and one, remrefidine (0.172%) to the aporphine subgroup of benzylisoquinoline alkaloids.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Uv spectra were recorded on a Shimadzu UV-265 FS spectrophotometer. Mps were measured with a Gallenkamp mp apparatus. Optical rotations were measured on a Perkin-Elmer 141 polarimeter. Eims were recorded with a VG Micromass 7070 F apparatus (70 eV). All nmr spectra were recorded at 250 MHz for ^{1}H on a Brüker WP 250 spectrometer, using TMS as internal reference. Si gel 60 (E. Merck, Darmstadt, Germany) and neutral Al_2O_3 (M. Woelm, Eschwege, Germany) were used for cc, and Si gel 60 PF_{254} (E. Merck, Darmstadt, Germany, layer thickness 1.0 mm) and neutral Al_2O_3 (M. Woelm, Eschwege, Germany) for prep. tlc (layer thickness 1.0 mm). The tlc chromatograms were visualized under uv at 254 nm and/or sprayed with Dragendorff's and potassium iodoplatinate reagents.

PLANT MATERIAL.—Leaves of *Anisocycla jollyana* were collected in 1992 near Kivuza Kiasi-Kole, in the province of Bas-Zaire, Zaire, and identified by Mr. Bavukinina-Ngoma and Mr. Menavanza, Institut de Recherches en Sciences de la Santé, Kinshasa, Zaire, where a voucher specimen has been deposited.

EXTRACTION AND ISOLATION.—Powdered dry leaves (300 g) were extracted exhaustively with MeOH (3 liters) by percolation. The MeOH extract was evaporated to dryness under reduced pressure, and the residue was taken up with 5% aqueous HCl (2×100 ml). After filtration, the solution was extracted several times with petroleum ether (300 ml). After alkalinization with aqueous 25% NH_4OH, the aqueous phase was extracted five times with $CHCl_3$ (5×50 ml). The combined $CHCl_3$ extracts were washed with H_2O and dried on anhydrous Na_2SO_4, then evaporated to dryness yielding the alkaloidal fraction (6 g). A part of this extract (1 g) was chromatographed on a column packed with neutral Al_2O_3 with a mixture of $CHCl_3$-MeOH (4:1). The less polar tertiary bases were further separated on a Si gel column (70–200 mesh, 100 g) eluted with $CHCl_3$ containing increasing amounts of MeOH; their final purification was achieved by prep. tlc on Si gel, using the following mobile phases: $CHCl_3$-MeOH-Me_2CO-25% NH_4OH (27:3:8:0.3) and $CHCl_3$-toluene-MeOH-Me_2CO-EtOAc-25% NH_4OH (10:10:3:5:3:0.3) to afford eight known alkaloids: trilobine (18 mg), isotrilobine (22 mg), dehydro-1,2-telobine (45 mg), homoaromaline (360 mg), 2′-norlimacine (5 mg), 2-norlimacine (146 mg), limacine (191 mg), and limacine-2′-β-*N*-oxide (12 mg). The polar *N*-oxide and quaternary bases were directly purified by prep. tlc on Al_2O_3 with toluene-$CHCl_3$-MeOH-25% NH_4OH (10:15:4:0.3) as solvent. This procedure allowed the isolation of the one new alkaloid [**1**] (6 mg) and the known base remrefidine (86 mg).

(+)-*Limacusine-2′*-β-N-*oxide* [**1**].—Colorless needles; mp 215°; $[\alpha]^{20}D$ +157° (*c*=1.1, $CHCl_3$); uv λ max (MeOH) (log ε) 218 (4.72), 283 (3.90) nm; ^{1}H nmr ($CDCl_3$, 250 MHz) δ 2.53 (3H, s, N-Me-2), 3.19 (3H, s, N-Me-2′), 3.50 (1H, m, H-1), 3.56 (3H, s, OMe-6), 3.81 (3H, s, OMe-6′), 3.89 (3H, s, OMe-12), 4.63 (1H, m, H-1′), 6.36 (1H, s, H-5), 6.38 (1H, s, H-5′), 6.71 (1H, s, H-8), 6.79 (1H, br s, H-10), 6.79 (1H, br s, H-13), 6.86 (1H, dd, *J*=2.0 and 8.0 Hz, H-10′), 6.86 (1H, dd, *J*=2.1 and 6.6 Hz, H-11′), 6.89 (1H, br s, H-14), 7.78 (1H, dd, *J*=2.1 and 4.5 Hz, H-13′), 7.80 (1H, dd, *J*=2.0 and 7.9 Hz, H-14′); eims *m/z*

$[M]^+$ 624(6), $[M-16]^+$ 608(78), $[M-16-107]^+$ 501(7), 381(88), 367(49), 191^{++}(100), 174(41).

REDUCTION OF **1**.—Compound **1** (5 mg) was stirred at room temperature for 2 h with powdered zinc (20 mg) in 10% HCl (5 ml). Work-up afforded a compound identical (uv, ^{1}H nmr, eims, tlc) to (+)-limacusine isolated previously (11,12).

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