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A NEW BISBENZYLISOQUINOLINE-*N*-OXIDE ALKALOID FROM SEEDS OF *ANISOCYCLA CYMOSA*

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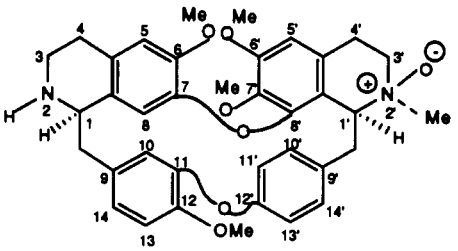
ABSTRACT.—A new bisbenzylisoquinoline-*N*-oxide, (+)-2-norobaberine-2′-β-N-oxide [**1**], along with six known alkaloids, 2-norobaberine, daphnandrine, coclobine, anisocycline, palmatine, and remrefidine, has been isolated from seeds of *Anisocycla cymosa* (Menispermaceae). The structure of **1** was determined by spectral data and its reduction into (+)-norobaberine.

Numerous benzylisoquinoline alkaloids have been recently isolated from *Anisocycla cymosa* Troupin (Menispermaceae) roots and leaves (1). This woody climber growing in Zaire is used in Zairian traditional medicine as a tonic, antipyretic, analgesic, and antirheumatic. On continuation of our phytochemical studies on the different parts of the plant, we now report the isolation and structure elucidation of a new bisbenzylisoquinoline-*N*-oxide alkaloid, (+)-2-norobaberine-2′-β-*N*-oxide [**1**], isolated from the seeds, along with six known alkaloids: 2-norobaberine, daphnandrine, coclobine, anisocycline, palmatine, and remrefidine.

Compound **1** was isolated as an amorphous white powder; the uv spectrum displayed maxima at 212 nm and 284 nm indicative of bisbenzylisoquinoline alkaloids (2). This identification was further supported by the eims and ^{1}H-nmr spectra. The eims of **1** showed a molecular ion of medium intensity at *m/z* 624 consistent with $C_{37}H_{40}N_2O_7$, accompanied by five strong peaks at *m/z* 608, 396, 381, 368, and 191 and two peaks of medium intensity at *m/z* 499 and 303. The *m/z* 608 $[M-16]^+$ (100), which was attributed to the loss of an oxygen atom from the molecular ion, is diagnostic of an *N*-oxide (3–5). The *m/z* 381 represented the upper half of the molecule (5–8), while an *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* 499 $[M-107]^+$, characteristic for the D ring loss (6). The ^{1}H-nmr data for (+)-2-norobaberine-2′-β-*N*-oxide [**1**] (Table 1) are closely related to those for (+)-2-norobaberine with regard to the aromatic protons and the aromatic substituents. The most obvious differences were the chemical shifts of the 2′-*N*-Me groups (δ 3.39) and the asymmetric proton H-1′ (δ 4.70), which were both shifted downfield in **1**. The shifts of less than 0.8 ppm downfield for the absorption for the asymmetric proton (H-1 or H-1′) and the shift of 0.7 to 0.9 ppm for the absorption of the *N*-Me group of the *N*-oxide indicate that H-1 (or H-1′) is located on the side opposite to the *N*-oxide oxygen (5,10). Therefore, the asymmetric proton H-1′ and the 2′-*N*-Me signal of **1** appearing respectively at δ 4.70 (Δδ 0.5) and 3.39 (Δδ 0.7) suggest a *trans* relationship between the *N*-oxide oxygen and H-1′ (5,9–11). Finally, **1** was reduced with zinc in HCl to afford the known alkaloid (+)-2-norobaberine (12).



1

(+)-2-Norobaberine-2′-β-*N*-oxide [**1**] with a moderate positive specific ro-

TABLE 1. ^{1}H-nmr Data[a] for (+)-2-Norobaberine-2′-β-*N*-oxide [**1**] and (+)-2-Norobaberine (250 MHz).

Proton	Compound	
	(+)-2-Norobaberine[b]	**1**[c]
H-1	4.22 m	4.25 m
H-1′	4.22 m	4.70 m
H-5	6.36 s	6.41 s
H-5′	6.35 s	6.38 s
H-8	6.69 s	6.73 s
H-10	5.62 brs	5.54 brs
H-10′	6.86 dd (2.1, 8.3)	6.88 dd
H-11′	6.39 dd (2.5, 8.3)	6.27 dd (2.4, 8.2)
H-13	6.78 brs	6.78 brs
H-13′	7.00 dd (2.5, 8.3)	7.00 dd (2.4, 8.3)
H-14	6.78 brs	6.78 brs
H-14′	7.47 dd (2.5, 8.2)	7.90 dd
2′- NMe	2.67 s	3.39 s
6-OMe	3.62 s	3.63 s
6′- OMe	3.78 s	3.81 s
7′- OMe	3.23 s	3.27 s
12-OMe	3.90 s	3.90 s

[a]*J* values are in parentheses and reported in Hz; chemical shifts are given in δ units (downfield from TMS).

[b]Assignments taken from Tantisewie *et al.* (12); data recorded in $CDCl_3$.

[c]Data recorded in $CDCl_3$-CD_3OD (4:0.04).

tation, $[\alpha]^{20}$ D+158°, exhibits the same 1*R*,1′*S* absolute configuration as (+)-2-norobaberine (12–16). The other isolated alkaloids, (+)-2-norobaberine, (+)-daphnandrine, coclobine, anisocycline, palmatine, and remrefidine, were identified by direct comparisons of their tlc behavior as well as their uv, ^{1}H nmr, and eims with those of authentic samples previously isolated (1,11–13,15,17).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Uv spectra were recorded on a Shimadzu UV-265 FS spectrophotometer and ir spectra on a Perkin-Elmer 177 spectrophotometer. Mp's were measured with a Gallenkamp mp apparatus. Optical rotations were measured on a Perkin-Elmer 141 polarimeter. Eims were recorded with a VG Micromass 7070F apparatus (70 eV). All nmr spectra were recorded at 250 MHz for ^{1}H nmr on a Brüker WP 250 spectrometer, using TMS as internal reference. Ion exchange resin Amberlite® IRA 400 (Cl^-) (Aldrich, Milwaukee, WI), silicic acid (Union Chimique Belge, RPL, Belgium), 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) for preparative tlc. The tlc chromatograms were visualized under uv at 254 nm and/or sprayed with Dragendorff's and potassium iodoplatinate reagents.

PLANT MATERIAL.—Seeds of *A. cymosa* were collected near Yangambi, Zaire, in April 1990 and identified by Mr. Tentula, Department of Botany, Institut de Recherches Agronomiques de Yangambi. A voucher specimen has been deposited in the Herbarium of the Insitut de Recherche en Sciences de la Santé, Kinshasa, Zaire.

EXTRACTION AND ISOLATION.—Powdered dry seeds (90 g) were extracted exhaustively with MeOH (500 ml) by percolation. The MeOH extract was evaporated to dryness under reduced pressure, and the residue was taken up with 5% aqueous HCl (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 A was extracted five times with $CHCl_3$ (30 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 B (5 g). The aqueous solution A was acidified by 3 N aqueous HCl, and the quaternary alkaloids were precipitated by Mayer's reagent. The precipitate was centrifuged

and dissolved in Me_2CO-MeOH-H_2O (6:2:1); the solution was passed through an ion-exchange resin column. The eluate and the column washings were combined and evaporated to dryness to afford the quaternary alkaloidal fraction C (1 g).

TREATMENT OF FRACTION B.—Fraction B (5 g), fixed on cellulose, was transferred on the top of a neutral Al_2O_3 (activity III, 150 g) column. Elution was performed with $CHCl_3$ containing increased amounts of MeOH. Ultimate purification was obtained by preparative tlc on Si gel, using the following mobile phases: S_1 [$CHCl_3$–MeOH–2-butanone–petroleum ether (20:10:4:7)] for the separation of the quaternary alkaloids and S_2 [$CHCl_3$-MeOH-Me_2CO-petroleum ether-25% aqueous NH_4OH (30:4:3:4:0.5)] for the separation of the tertiary alkaloids. This procedure allowed the isolation of **1** (450 mg, S_2), daphnandrine (25 mg, S_2), norobaberine (20 mg, S_2), coclobine (21 mg, S_2), anisocycline (35 mg, S_1), and palmatine (15 mg, S_1).

TREATMENT OF FRACTION C.—Fraction C (200 mg) was resolved by cc on Si gel (30 g), eluted with $CHCl_3$ containing increasing percentages of MeOH. Further purification by preparative tlc on Si gel, using as mobile phase $CHCl_3$-MeOH-H_2O-25% aqueous NH_4OH (5:3:1:1) afforded remrefidine (100 mg).

(+)-2-Norobaberine-2′-β-N-oxide [**1**].—White amorphous powder: $[\alpha]^{20}D$ +158° (*c*=0.31, $CHCl_3$); uv λ max (MeOH) 284, 212 nm; ^1H nmr ($CDCl_3$, 250 MHz) see Table 1; eims *m/z* (rel. int.) $[M]^+$ 624 (36), 608 (100), 499 (23), 396 (43), 381 (51), 368 (80), 303 (33), 191.

REDUCTION OF **1**.—Compound **1** (4 mg) was stirred at room temperature for 2 h with powdered zinc (20 mg) in 10% HCl (5 ml). Workup afforded a compound identical (uv, ^1H nmr, eims, tlc) to (+)-2-norobaberine previously isolated (12).

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