*Journal of Natural Products*
*Vol. 49, No. 3, pp. 424-427, May-Jun 1986*

# THE BISBENZYLISOQUINOLINE ALKALOIDS OF *STEPHANIA SUBEROSA*

AMARENDRA PATRA,[1] ALAN J. FREYER, HÉLÈNE GUINAUDEAU,[2] MAURICE SHAMMA,

*Department of Chemistry, The Pennsylvania State University, University Park, Pennsylvania 16802*

BAMRUNG TANTISEWIE, and KALAYA PHARADAI

*Department of Pharmacognosy, Chulalonkorn University, Bangkok 10500, Thailand*

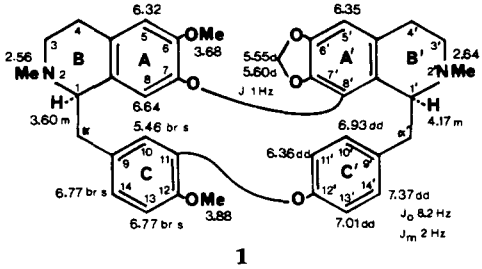
ABSTRACT.—Five new bisbenzylisoquinoline alkaloids were obtained from *Stephania suberosa*. These are (+)-2-norcepharanthine (**2**), (+)-cepharanthine 2′-β-*N*-oxide (**3**), (+)-stephasubine (**4**), (+)-norstephasubine (**5**), and stephasubimine (**6**). They are accompanied by the known (+)-cepharanthine (**1**) which is the main alkaloid.

The vine *Stephania suberosa* Forman (Menispermaceae) is a rich source of alkaloids, and the present paper will be concerned with its content of bisbenzylisoquinolines (1,2).

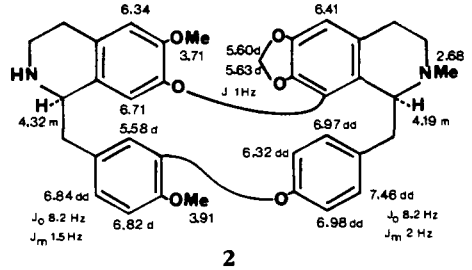
Besides the known (+)-cepharanthine (**1**), which is the major bisbenzylisoquinoline (1), five dimers were obtained, all of which are new and are structurally related to cepharanthine whose detailed $^1$H-nmr spectrum, confirmed by spin decoupling experiments, has been summarized around expression **1**.

The first new alkaloid to be characterized was (+)-2-norcepharanthine (**2**), $C_{36}H_{36}N_2O_6$. The secondary amine function was first suggested by a mass spectral molecular weight which was 14 m.u. less than for cepharanthine. A strong molecular peak *m/z* 592 (78%) was flanked by a base peak *m/z* 591—a pattern often encountered with bisbenzylisoquinolines bearing a secondary amine function (3). Another important peak, *m/z* 365 (59%), represented the upper half of the molecule. As expected for a bisbenzylisoquinoline incorporating 7-8′ and 11-12′ ether linkages, the mass spectrum also showed peaks *m/z* 486 and 485 due to the $(M-106)^+$ and $(M-107)^+$ ions (4).

The $^1$H-nmr spectrum of (+)-2-norcepharanthine, indicated around structure **2**, is very close to that for **1**. The most obvious difference was the absence of an upfield *N*-methyl singlet near δ 2.56 and the displacement of the broad H-1 singlet from δ 3.60 in cepharanthine (**1**) to δ 4.32 in the nor analog. Such a pattern is regularly observed whenever an *N*-methyltetrahydrobenzylisoquinoline in the monomeric or dimeric form is compared with the corresponding secondary amine (3). The structure of the new alkaloid was then confirmed by its *N*-methylation using formaldehyde-formic acid to (+)-cepharanthine (**1**).




**1**




**2**

[1]Permanent address: Department of Chemistry, University College of Science, 92 Acharya Prafulla Chandra Road, Calcutta 700009, India.

[2]Permanent address: Faculté de Médecine et de Pharmacie, Université de Limoges, 87025 Limoges Cedex, France; or CNRS, UA 496, Centre d'Etudes Pharmaceutiques, 99290 Chatenay-Malabry, France.

The second new alkaloid was (+)-cepharanthine 2′-β-*N*-oxide (**3**), $C_{37}H_{38}N_2O_7$. The mass spectrum showed a small molecular peak *m/z* 622 (8%), together with a somewhat stronger *m/z* 620 peak. Such an $(M-2)^+$ ion is often encountered in connection with bisbenzylisoquinoline *N*-oxides (3). Present also was a strong *m/z* 606 peak due to net loss of oxygen from the molecular ion. An intense *m/z* 379 peak represented the top half of the dimer, i.e., rings A, B, A′, and B′, while an *m/z* 190 peak corresponded to the doubly charged analog.

The $^1$H-nmr spectrum was again very close to that of cepharanthine as far as the aromatic protons and the aromatic substituents were concerned. A remarkable difference, however, prevailed with the absorptions for the right hand 2′-*N*-methyl group and the adjoining H-1′ which were both shifted downfield. The 2′-*N*-methyl singlet at δ 3.31 and the H-1′ broad singlet at δ 4.63 are characteristic of a *trans*-relationship between the *N*-oxide oxygen and H-1′ (3). This *trans*-relationship was further confirmed by an nOe study (5) which showed that irradiation of the δ 3.31 *N*-methyl singlet resulted in enhancement of the H-1′ signal at δ 4.63.

The remaining three new bisbenzylisoquinolines are closely related to each other and are all phenolic.

(+)-Stephasubine (**4**, $C_{36}H_{34}N_2O_6$, shows a strong mass spectral molecular ion *m/z* 590 (76%), while *m/z* 589 is the base peak. The only other important peak is the doubly charged molecular ion *m/z* 295 (18%). The fact that the upper part of the dimer is not observed in the mass spectrum immediately suggested that an imine or aromatic ring B (or B′) was present. This suspicion was reinforced by the uv shift suffered by the dimer upon acidification (6).

The $^1$H-nmr spectrum displayed mutually coupled signals at δ 7.48 and 8.45 ($J_o$=5.6 Hz) due to the presence of a substituted pyridine system. Conspicuously present were two doublets at δ 4.52 and 5.37, with a large coupling constant at 13.8 Hz, which represented the two geminal protons of the benzylic methylene adjacent to the pyridine ring. The presence of the H-1 broad singlet upfield at δ 3.56, accompanied by an *N*-methyl signal at δ 2.51, argued convincingly in favor of placing the pyridine system on the right-hand side of the dimer (3).

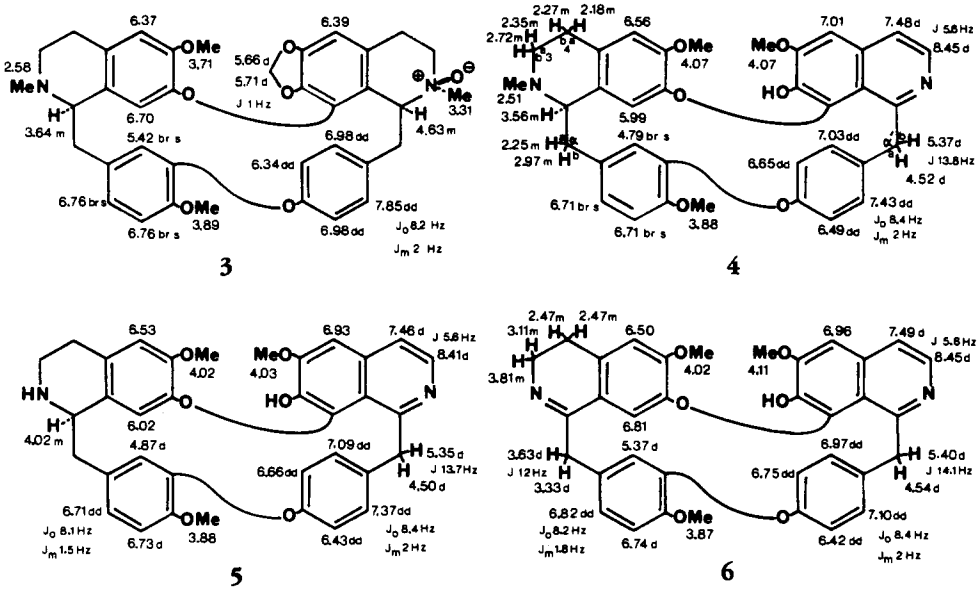
The structure assignment was then further ascertained by a complete spin decoupling and nOe analysis; the more important of the $^1$H-nmr values are quoted in the Experimental section.

Our fourth new alkaloid is (+)-norstephasubine (**5**), $C_{35}H_{32}N_2O_6$, which showed a mass spectral molecular ion 14 units less than for stephasubine (**4**), while the general fragmentation pattern was very similar to that for **4**. The $^1$H-nmr spectrum of (+)-norstephasubine (**5**) is also close to that of **4**, except for the absence of an *N*-methyl signal, and the downfield displacement of H-1 from δ 3.56 to 4.02. This shift is typical for the replacement of a *N*-methyl group by NH (3). Finally, *N*-methylation of **5** provided (+)-stephasubine (**4**).

The fifth new alkaloid at our disposal was stephasubimine (**6**) whose molecular composition, $C_{35}H_{30}N_2O_6$, indicated two hydrogens less than in norstephasubine (**5**). The $^1$H-nmr spectrum bears distinct similarities to those of dimers **4** and **5**. But a noticeable difference is the presence of two extra doublets at δ 3.33 and 3.63 ($J_{gem}$=12 Hz), attributable to the benzylic α-methylene protons of a dihydrobenzylisoquinoline. The structure assignment was then complemented by the finding that $NaBH_4$ reduction of **6** provided norstephasubimine (**5**).

Stephasubine (**4**), norstephasubine (**5**), and stephasubimine (**6**) are relatively rare examples of bisbenzylisoquinolines incorporating an aromatic isoquinoline moiety. They all possess a methoxyl at C-6′ and a hydroxyl at C-7′. The accompanying cepharanthine (**1**), norcepharanthine (**2**), and cepharanthine-2′-β-*N*-oxide (**3**) include

a tetrahydrobenzylisoquinoline as the right-hand moiety of the dimer. Interestingly, the C-6′, C-7′ substituent is now a methylenedioxy group.




## EXPERIMENTAL

ISOLATION.—The dried, powdered tuberous roots (1.8 kg) of *S. suberosa* were purchased in the Bangkok main market under the name "borapet pungchang." They are usually used in native medicine as a tonic, carminative, and expectorant. The powder was extracted with EtOH at room temperature. The solvent was evaporated and the residue (200 g) treated with 5% HOAc. The mixture was filtered. The extract was basified with $NH_4OH$ and extracted with $CHCl_3$ to give an alkaloidal fraction (22 g). This was chromatographed on a column prepared by using 1.3 kg silica gel (70-200 mesh) in $CHCl_3$. Elution of the chromatographic column using $CHCl_3$ containing increasing amounts of MeOH provided cepharanthine (**1**), 9 g; 2-norcepharanthine (**2**), 30 mg; stephasubimine (**6**), 12 mg; stephasubine (**4**), 6 mg; norstephasubine (**5**), 12 mg; and cepharanthine-2′-β-*N*-oxide (**3**), 7 mg. Final purification was by preparative tlc on silica gel plates using the system $CHCl_3$-MeOH-$NH_4OH$ (80:20:trace). All compounds are amorphous; $^1$H-nmr spectra are at 360 MHz in $CDCl_3$.

(+)-NORCEPHARANTHINE (**2**).—*m/z* 592 ($M^+$, 78), 591 (100), 486 (4), 485 (10), 365 (59), 351 (39), 349 (40), 206 (20), 192 (18), 183 (32), 160 (31); [α]D +318° (c 0.25, MeOH).

N-METHYLATION OF **2**.—The nor compound **2** (3 mg) was refluxed with an aqueous formaldehyde (1 ml) and HCOOH (2 ml) for 12 h. Work-up led to **1** in near quantitative yield.

(+)-CEPHARANTHINE 2′-β-*N*-OXIDE (**3**).—*m/z* 622 ($M^+$, 8), 621 (17), 620 (31), 606 (95), 605 (98), 592 (19), 591 (29), 516 (4), 380 (25), 379 (97), 366 (24), 365 (98), 190 (100), 183 (23), 174 (67); [α]D +152° (c 0.22, MeOH).

(+)-STEPHASUBINE (**4**).—λ max (MeOH) 240, 287, 337 nm (log ε 4.56, 3.61, 3.43); λ max (MeOH+$H_3O^+$) 235, 264, 290 sh, 321, 368, 374 nm (log ε 4.35, 4.41, 3.69, 3.39, 3.48, 3.48); *m/z* 590 ($M^+$, 76), 589 (100), 575 (26), 295 (18), 190 (5), 174 (24), 145 (13), 144 (13); Δε (nm) 0 (270), +64 (245), +4 sh (218), negative tail; [α]D +339° (c 0.09, MeOH).

STEPHASUBINE (**4**) NOEDS.—OMe-6 ⇌ H-5; H-5 ⇌ H-4a; OMe-6′ ⇌ H-5′; H-5′ ⇌ H-4′; H-8 ⇌ H-1; H-1 ⇌ 2-NMe; H-8 ⇌ H-10; H-14 ⇌ H-αb; H-8 ⇌ H-αa; OMe-12 ⇌ H-13; H-14′ ⇌ H-α′b; H-10′ ⇌ H-α′a.

(+)-NORSTEPHASUBINE (**5**).—λ max (MeOH) 240, 286, 338 nm (log ε 4.53, 3.62, 3.42); λ max (MeOH+$H_3O^+$) 235, 264, 321, 368, 375 nm (log ε 4.30, 4.36, 3.32, 3.40, 3.39); *m/z* 576 ($M^+$, 72), 575 (100), 561 (20), 545 (29), 288 (15), 190 (8), 174 (25), 146 (16), 145 (18); [α]D +309° (c 0.09, MeOH). *N*-Methylation of **5** as described above led to **4**.

STEPHASUBIMINE (**6**).—λ max (MeOH) 242, 281, 323 nm (log ε 4.64, 3.91, 3.70); λ max ($MeOH+H_3O^+$) 264, 307, 362, 368 nm (log ε 4.57, 4.02, 3.92, 3.91); *m/z* 574 ($M^+$, 100), 559 (24), 206 (43), 192 (10).

REDUCTION OF STEPHASUBIMINE (**6**).—Compound **6** (2 mg) was dissolved in MeOH (1 ml) and the solution treated with a pinch of $NaBH_4$. The mixture was stirred for 12 h and then worked up to afford **5** (tlc, $^1H$ nmr, ms) in near quantitative yield.

ACKNOWLEDGMENTS

This research was supported by grant CHE-8511984 from the National Science Foundation. A.P. was the recipient of a UNESCO/UNDP (India) fellowship.

LITERATURE CITED

1. K.P. Guha, B. Mukherjee, and R. Mukherjee, *J. Nat. Prod.*, **42**, 1 (1979).
2. P.L. Schiff, Jr., *J. Nat. Prod.*, **46**, 1 (1983).
3. H. Guinaudeau, A.J. Freyer, and M. Shamma, *Nat. Prod. Rep.* (in press).
4. J. Baldas, I.R.C. Bick, T. Ibuka, R.S. Kapil, and Q.N. Porter, *J. Chem. Soc., Perkin Trans. 1*, 592 (1972).
5. L.D. Hall and J.K.M. Sanders, *J. Am. Chem. Soc.*, **102**, 5703 (1980).
6. M. Shamma, "The Isoquinoline Alkaloids," Academic Press, New York, 1972, pp. 84-85.

*Received 5 September 1985*