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# SOME UNUSUAL PROAPORPHINE AND APORPHINE ALKALOIDS FROM *STEPHANIA VENOSA*

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ABSTRACT.—New proaporphines found in Thai *Stephania venosa* include (+)-*N*-carboxamidostepharine [**2**], (−)-*O*-methylstepharinosine [**3**], and (−)-stepharinosine [**5**]. Alkaloid **2** is the first proaporphine incorporating a urea functionality, while **3** and **5** are the only proaporphines oxygenated at C-12. The plant also produces the new aporphines (−)-sukhodianine-β-*N*-oxide [**6**], (−)-ushinsunine-β-*N*-oxide [**7**], and (−)-stephadiolamine-β-*N*-oxide [**8**]. All three *N*-oxides are hydroxylated at C-7, but (−)-stephadiolamine-β-*N*-oxide [**8**] is also hydroxylated at C-4. Thus, this is the first aporphine hydroxylated at both of these sites; 17 known isoquinoline alkaloids were also obtained.

The Southeast Asia vine *Stephania venosa* Spreng. (Menispermaceae), whose rhizomes are sometimes used as a bitter tonic and which is known in Thailand under the name of "sabu-le-ad" or blood-soap due to its red latex, is rich in isoquinoline alkaloids. Previous studies on this plant have revealed the presence of several aporphines, oxoaporphines, and protoberberines (1-3).

In a continuing study of the rhizomes of this plant, we have identified 23 alkaloids, six of which are new and are somewhat unusual. Of these six, three are proaporphines and three are 7-hydroxylated aporphines.

The major alkaloid found is actually the known norproaporphine (+)-stepharine [**1**]. A detailed $^1$H-nmr spin decoupling and nOe study in $CDCl_3$ led to the complete assignment of chemical shifts for the protons of this base, as indicated around expression **1** (4).

The mass spectrum of our first new proaporphine, namely the urea derivative (+)-*N*-carboxamidostepharine [**2**], exhibited an even molecular ion, *m/z* 340, pointing to the presence of two nitrogen atoms in the molecule. Facile loss of the carboxamide entity, $CONH_2$, from the molecular ion resulted in formation of the important ion *m/z* 296 (66%), flanked by an even stronger *m/z* 297 ion (99%). The base peak, *m/z* 268, corresponded to loss of the elements of carbon monoxide from the *m/z* 296 ion. Each of these assignments was confirmed by hrms. The ir spectrum displayed a band at 1660 $cm^{-1}$ representing the dienone system and two other absorptions at 1620 and 1650 $cm^{-1}$ due to the carboxamido portion of the molecule.

The $^1$H-nmr spectrum of (+)-*N*-carboxamidostepharine [**2**] was useful in the structure elucidation, especially by comparison with the spectrum of **1**. No major changes in the absorption pattern in the vinylic-aromatic region could be observed. The main difference between the two spectra resided in the downfield shift of H-6a which appeared at δ 5.03, as compared to δ 4.33 for the free base **1**. Another difference was the presence of a broad two-proton singlet at δ 4.58 due to the hydrogens of the carboxamido function.

The remaining two new proaporphines, (−)-*O*-methylstepharinosine [**3**] and (−)-

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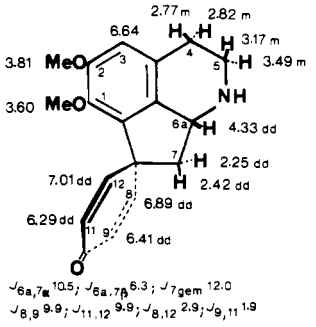
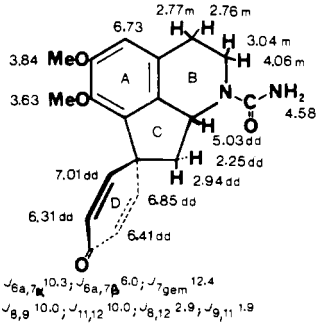
stepharinosine [**5**], are of more than passing interest because they represent the first examples of proaporphines oxygenated at C-12.

The mass spectrum of (−)-*O*-methylstepharinosine [**3**] showed a relatively small molecular ion, *m/z* 329 (9%). The base peak, *m/z* 298, could be accounted for by loss of methoxide from the molecular ion.



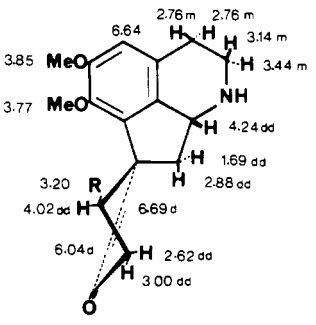

$J_{6a,7\alpha}$ 10.5; $J_{6a,7\beta}$ 6.3; $J_{7gem}$ 12.0
$J_{8,9}$ 9.9; $J_{11,12}$ 9.9; $J_{8,12}$ 2.9; $J_{9,11}$ 1.9

**1**



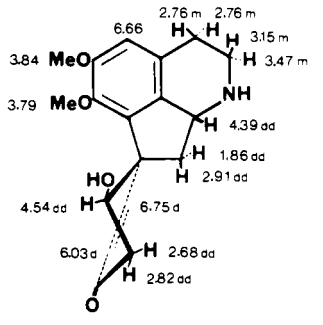

$J_{6a,7\alpha}$ 10.3; $J_{6a,7\beta}$ 6.0; $J_{7gem}$ 12.4
$J_{8,9}$ 10.0; $J_{11,12}$ 10.0; $J_{8,12}$ 2.9; $J_{9,11}$ 1.9

**2**




$J_{6a,7\alpha}$ 9.5; $J_{6a,7\beta}$ 6.6; $J_{7gem}$ 12.4
$J_{11\psi ax,12}$ 10.5; $J_{11\psi eq,12}$ 4.3; $J_{11gem}$ 16.5
$J_{8,9}$ 10.0

**3** R=OMe ($^1$H nmr)
**4** R=H




$J_{6a,7\alpha}$ 9.8; $J_{6a,7\beta}$ 6.6; $J_{7gem}$ 12.6
$J_{11\psi ax,12}$ 11.6; $J_{11\psi eq,12}$ 4.6; $J_{11gem}$ 16.6
$J_{8,9}$ 10.0

**5**

The $^1$H-nmr spectrum of (−)-*O*-methylstepharinosine [**3**] was somewhat related to that for the recently characterized (−)-11,12-dihydrostepharine [**4**] (4). Chemical shift assignments for the top parts of these two molecules, involving rings A and B, are similar. The difference in chemical shifts between the two vinylic protons of ring D for (−)-*O*-methylstepharinosine [**3**], $\Delta\delta$=6.69-6.04=0.65 ppm, argues strongly in favor of an *anti* relationship between H-6a and the ring D unsaturation (4). The most unusual feature of the spectrum was the three-proton singlet at δ 3.20 and the one-proton doublet of doublets at δ 4.02. The former must be due to the C-12 aliphatic methoxyl. The relatively upfield shift of this substituent was indicative of a 1,3-relationship to the carbonyl function, because in the case of the ketonic reduced proaporphine (−)-roehybrine the C-9 aliphatic methoxyl absorbs further downfield at δ 3.50 (5). H-12 (δ 4.02), which is geminal to the aliphatic methoxyl, must be pseudoaxial since its coupling constants with the two C-11 protons are substantially different in magnitude ($J_{11\psi ax,12}$=10.5 Hz, $J_{11\psi eq,12}$=4.3 Hz).

It is known that (−)-11,12-dihydrostepharine [**4**] shows a distinct nmr nOe between H-6a and H-12$_{\psi eq}$ (4). Such an interaction is absent in (−)-*O*-methylstepharinosine [**3**], which confirms the C-12 pseudoequatorial disposition of the aliphatic methoxyl. Again, the lack of an nOe interaction between H-12 and H-7β is indicative of the specific conformation for ring D of species **3**. Complementing these

data were reciprocating nOe's between the C-1 methoxyl and the vinylic protons at C-8 and C-9. It should also be pointed out that the conformation of ring D indicated in expression **3** is also the one found to prevail in the related dihydroproaporphine (±)-dihydroglaziovine hydrobromide, as indicated by X-ray analysis (6).

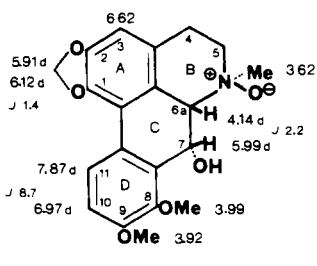
The mass spectrum of (−)-stepharinosine [**5**] showed a strong molecular ion, *m/z* 315 (98%), with facile loss of $H_2O$ to provide base peak *m/z* 297.

The nmr spectrum of **5** was generally related to that of **3**. The main difference was the downfield shift of H-12 to δ 4.54 and the lack of a three-proton singlet absorption due to an aliphatic methoxyl. This nmr spectral information, together with the facile loss of $H_2O$ observed in the mass spectrum, pointed to the presence of a hydroxyl rather than a methoxyl substituent at C-12. The equatorial configuration of the hydroxyl was given by the coupling constants for H-12 with the C-11 methylene protons which paralleled those observed for H-12 in the spectrum of (−)-*O*-methylstepharinosine [**3**].

The positive specific rotation for *N*-carboxamidostepharine [**2**], as well as the negative specific rotations for the two *anti*-dihydroproaporphines **3** and **5**, were all indicative of the C-6a *R* configuration (4).

Perusal of structures **3** and **5** makes it apparent that these species must be formed from (+)-stepharine through addition of MeOH in one case and $H_2O$ in the other. A relevant question at this stage is whether such additions could occur during the isolation-purification process. Although many proaporphines are presently known, such solvent additions have never been detected. Additionally, and in our hands, substantial quantities of (+)-stepharine [**1**] were isolated during the present work and were subjected to extensive chromatography under different conditions including contact with MeOH and $H_2O$. In no instance could compounds analogous to **3** and **5** be isolated. We, therefore, conclude that in all probability we are dealing with true natural products. Significantly, enone **3** and, even more so, enone **5** are unstable and tend to decompose in solution.

Our three new 7-hydroxylated aporphines are all *N*-oxides of identical configuration and are (−)-sukhodianine-β-*N*-oxide [**6**], (−)-ushinsunine-β-*N*-oxide [**7**], and (−)-stephadiolamine-β-*N*-oxide [**8**].

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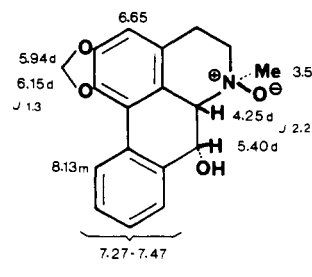


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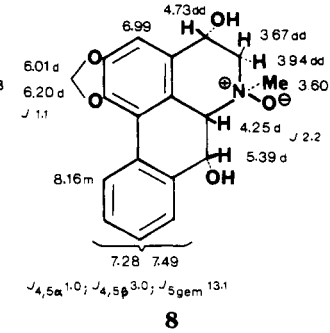


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The mass spectrum of (−)-sukhodianine-β-*N*-oxide [**6**] included very small molecular ion *m/z* 371 (0.8%). Such a weak ion is characteristic of an *N*-oxide inasmuch as facile loss of 16 a.m.u. results in formation of the corresponding ionized free base—in the present instance represented by ion *m/z* 355 (48%). The *m/z* 355 ion can now lose hydroxide to furnish base peak *m/z* 338. Such ready loss of hydroxide is typical of 7-hydroxylated aporphines (7).

Except for the protons affected by the *N*-oxide function, the general features of the nmr spectrum of (−)-sukhodianine-β-*N*-oxide [**6**] paralleled those for (−)-sukhodianine (2). In particular, the presence of the two one-proton doublets at δ 4.14

and 5.99 ($J$=2.2 Hz) confirmed the *syn* relationship between H-6a and H-7. Furthermore, a partial nmr nOe study clarified the configuration of the *N*-oxide function. Reciprocating nOe's were detected between the *N*-methyl signal (δ 3.62) and H-7 (δ 5.99), while no nOe's were seen between the *N*-methyl and H-6a (δ 4.14). An *anti* arrangement must, therefore, exist between H-6a and the *N*-methyl group.

(−)-Ushinsunine-β-*N*-oxide [**7**] displayed a pattern in the mass spectrum similar to that for (−)-sukhodianine-β-*N*-oxide [**6**]. A very small molecular ion, *m/z* 311, was accompanied by base peak *m/z* 295 due to loss of oxygen. A strong *m/z* 278 peak was also present, accounted for by loss of hydroxyl from the base peak.

The by now familiar pattern of two one-proton doublets at δ 4.25 and 5.40 with $J$=2.2 Hz attested to the presence of an alcohol function at C-7 with a *cis* relationship between H-6a and H-7. The downfield shifts of the *N*-methyl singlet (δ 3.58) and the H-6a doublet (δ 4.25) pointed to the same relative configuration as in *N*-oxide **6**.

(−)-Stephadiolamine-β-*N*-oxide [**8**], our third new aporphine, showed a weak mass spectral molecular ion, *m/z* 327 (1.2%). Loss of oxygen from the molecular ion furnished ion *m/z* 311 (31%). The *m/z* 311 ion could decompose by either of two routes. Loss of hydroxide, typical of C-7 hydroxylated aporphines, provided ion *m/z* 294 (13%). Alternatively, loss of the elements of $H_2O$, characteristic of 4-hydroxylated aporphines, gave rise to ion *m/z* 293 (12%) (7).

The nmr spectrum of (−)-stephadiolamine-β-*N*-oxide [**8**] appeared initially to present a complex picture. In addition to the two one-proton doublets at δ 4.25 and 5.39 denoting H-6a and H-7, there was also a narrow one-proton doublet of doublets at δ 4.73 assigned to H-4 which is geminal to a hydroxyl. The shape of the H-4 absorption, together with its relatively upfield shift, denoted a *syn* relationship between H-4 and H-6a (8). Significantly, the H-5 methylene protons appeared as two downfield doublet of doublets at δ 3.67 and 3.94. In analogy with the two preceding aporphine *N*-oxides, the *N*-methyl singlet absorption appeared downfield at δ 3.60 indicating the identical relative configuration with regard to H-6a. Finally, the negative specific rotations for aporphines **6-8** testified to the *R* configuration at C-6a.

It has already been noted that the occurrence of C-7 oxygenated aporphines is limited to the four botanical families Annonaceae, Lauraceae, Magnoliaceae, and Menispermaceae and that aporphines oxygenated at C-7 inevitably belong to the C-6a *R* configuration (8). Aporphines oxygenated at both C-4 and C-7 have also been found among the Annonaceae (7), but (−)-stephadiolamine-β-*N*-oxide [**8**] is the first aporphine known to be hydroxylated at both C-4 and C-7 and with a *cis* relationship between H-6a and H-7. The two previously recognized 4,7-dioxygenated aporphines, namely norpachystaudine and pachystaudine, originate in the Annonaceae and bear a *trans* relationship between H-6a and H-7 (7). Additionally, they incorporate a methoxyl at C-7 rather than a hydroxyl.

## EXPERIMENTAL

GENERAL ISOLATION PROCEDURE.—The dried, powdered rhizomes of *S. venosa* (5 kg) were first defatted with petroleum ether and were then extracted with cold EtOH. The EtOH extracts were concentrated to a syrup (530 g) that was extracted with 10% HCl. The acidic aqueous layer was washed with $CHCl_3$. The organic solution was separated, and the solvent evaporated. The residue, Fraction A, weighed 111 g. The acidic aqueous layer was basified with $NH_4OH$ and extracted with $CHCl_3$. Separation and evaporation of the organic phase left a residue, Fraction B, weighing 20 g. This crude basic extract was placed on a Si gel column. Elution was with $CHCl_3$ containing increasing amounts of MeOH. Further purification was by column chromatography using Si gel for tlc and by tlc on Si gel glass plates.

The major alkaloids obtained were (−)-crebanine and (+)-stepharine [**1**] (each 5% by weight). Minor alkaloids (1% or less) included (−)-anonaine, (−)-asimilobine, (−)-nuciferoline, (−)-apoglaziovine, (−)-tuduranine, (−)-mecambroline, (−)-stesakine, (−)-ushinsunine, (−)-sukhodianine, (−)-4-α-hydroxycrebanine, dehydrocrebanine, (−)-tetrahydropalmatine, (−)-kikemanine, (+)-reticuline, and (+)-thal-

rugosamine, together with (+)-*N*-carboxamidostepharine [**2**], (−)-*O*-methylstepharinosine [**3**], (−)-stepharinosine [**5**], (−)-sukhodianine-β-*N*-oxide [**6**], (−)-ushinsunine-β-*N*-oxide [**7**], and (−)-stephadiolamine-β-*N*-oxide [**8**]. All compounds obtained are amorphous. Their nmr spectra were reported in $CDCl_3$ solution at either 360 or 200 MHz.

(−)-*N*-CARBOXAMIDOSTEPHARINE [**2**].—$C_{19}H_{20}N_2O_4$; ms *m/z* 340 ($M^+$, 22), 297 (98), 296 (66), 282 (17), 268 (100); hrms *m/z* $M^+$ 340.1452 (calcd for $C_{19}H_{20}N_2O_4$ 340.1423), 297.1338 (calcd for $C_{18}H_{19}NO_3$ 297.1364), 296.1323 (calcd for $C_{18}H_{18}NO_3$ 296.1286); ir ν max ($CHCl_3$) 1660, 1650, 1620, 1580 $cm^{-1}$; cd (MeOH) Δε (nm) 0 (310), +1.7 (272), 0 (235), −0.8 (220), +0.5 (218), negative tail near 215 nm; $[\alpha]_D$ −22° (*c* 0.2, $CHCl_3$).

(−)-*O*-METHYLSTEPHARINOSINE [**3**].—$C_{19}H_{23}NO_4$; ms *m/z* 329 ($M^+$, 10), 315 (1), 314 (5), 299 (22), 298 (100), 297 (21), 282 (13), 270 (10), 269 (12); hrms *m/z* $M^+$ 329.1632 (calcd for $C_{19}H_{23}NO_4$ 329.1627), 298.1417 (calcd for $C_{18}H_{20}NO_3$ 298.1443); ir ν max ($CHCl_3$) 1665 $cm^{-1}$; $[\alpha]_D$ −26° (*c* 0.11, $CHCl_3$). Principal nmr nOe's are MeO-2 to H-3 (18%), H-3 to MeO-2 (9%), H-4 to H-3 (8%), H-5β to H-6a (5%), H-6a to H-5β (7%), H-6a to H-7β (5%), H-7β to H-6a (4%), H-7α to H-8 (6%), H-8 to H-7β (2%), H-8 to MeO-1 (4%), MeO-1 to H-8 (2%), H-9 to MeO-1 (1%), MeO-1 to H-9 (4%), H-12 to H-$11_{eq}$ (3%), H-$11_{eq}$ to H-12 (4%), H-12 to MeO-12 (6%), MeO-12 to H-12 (6%).

(−)-STEPHARINOSINE [**5**].—$C_{18}H_{21}NO_4$; ms *m/z* 315 ($M^+$, 98), 314 (74), 298 (77), 297 (100), 296 (75), 286 (70), 268 (85). The compound decomposed too rapidly to allow for an exact determination of specific rotation.

(−)-SUKHODIANINE-β-*N*-OXIDE [**6**].—$C_{20}H_{21}NO_6$; ms *m/z* 371 ($M^+$, 0.8), 355 (48), 354 (35), 353 (61), 340 (56), 338 (100), 336 (25), 324 (12), 323 (21), 322 (21), 254 (19), 190 (21); uv λ max (MeOH) 217, 247 sh, 279, 300 sh, 307, 322 sh nm (log ε 4.42, 3.91, 4.15, 3.91, 3.76, 3.63); $[\alpha]_D$ −13° (*c* 0.07, MeOH). Principal nmr nOe's are H-6a to H-7β (16%), H-7β to H-6a (20%), NMe to H-7β (36%), H-7β to NMe (15%), H-7β to MeO-8 (20%), H-10 to MeO-9 (20%), H-10 to H-11 (32%).

(−)-USHINSUNINE-β-*N*-OXIDE [**7**].—$C_{18}H_{17}NO_4$; ms *m/z* 311 ($M^+$, 3), 295 (100), 278 (42), 277 (26), 252 (91), 251 (64), 236 (30); $[\alpha]_D$ −52° (*c* 0.06, MeOH).

(−)-4-α-HYDROXYUSHINSUNINE-β-*N*-OXIDE [**8**].—$C_{18}N_{17}NO_5$; ms *m/z* 327 ($M^+$, 1.2%), 311 (31), 294 (13), 293 (12), 291 (10), 290 (22), 275 (25), 250 (100); $[\alpha]_D$ −37° (*c* 0.06, MeOH).

## ACKNOWLEDGMENTS

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