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THE PROTOBERBERINE ALKALOIDS OF *STEPHANIA SUBEROSA*

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Key Word Index—*Stephania suberosa*; Menispermaceae; roots; protoberberines; alkaloids.

Abstract—Six new protoberberines were found in *Stephania suberosa* root extracts: (−)-tetrahydrostephabine, (−)-stephabinamine, stephabine, 8-oxypseudopalmatine, (−)-*trans*-xylopinine *N*-oxide and (−)-*cis*-xylopinine *N*-oxide. Ten known alkaloids were also detected: (−)-tetrahydropalmatine, (−)-tetrahydropalmatrubine, (−)-stepholidine, (−)-kikemanine, (−)-capaurimine, (−)-coreximine, (−)-corytenchine, (−)-discretine, pseudopalmatine and (−)-xylopinine.

INTRODUCTION

The vine *Stephania suberosa* Forman, native to Thailand, is commonly used in that country for the treatment of a variety of ailments under the local name 'borapet pungchang'. We have found that the basic extracts yield a wide variety of isoquinolines of different structural types. This paper, however, will be concerned solely with those alkaloids of *S. suberosa* which incorporate the tetracyclic protoberberine skeleton.

RESULTS

Two types of protoberberines were found in the plant. The more common one is substituted at C-2,3,9,10 and is represented by the known bases (−)-tetrahydropalmatine, (−)-tetrahydropalmatrubine, (−)-stepholidine, (−)-kikemanine and (−)-capaurimine. The last named alkaloid also bears an extra phenolic hydroxyl at C-1 [1, 2].

The second type is of the 'pseudo' variety, being substituted at C-2,3,10,11. Included among this group are the known (−)-coreximine, (−)-corytenchine, (−)-discretine, pseudopalmatine and (−)-xylopinine (**1**). (−)-Xylopinine is, in fact, the major protoberberine of the plant, and its NMR chemical shift assignments (360 MHz, $CDCl_3$) (see **1**) were confirmed by a detailed NMR nOeds study (**1** − **NOE**). In particular, H-9 (δ6.58) and H-8β (δ4.02) show reciprocating enhancements. H-12 (δ6.67) and H-13α (δ3.26) also exhibit reciprocating nOes. The chemical shift for H-9 is, therefore, upfield from that for H-12.§

The above compounds were accompanied by six new alkaloids, all of the pseudo variety, which will be discussed below. Our first new natural product is the monophenolic (−)-tetrahydrostephabine (**2**), $C_{21}H_{25}NO_5$. The MS molecular peak, *m/z* 371, is 16 mass units greater than that for xylopinine (**1**). This difference also extends to the important peak *m/z* 208, as compared to *m/z* 192 for xylopinine (**1**). These data indicated the presence of an extra hydroxyl group in rings A or B of the alkaloid. The NMR spectrum of tetrahydrostephabine (**2**) was somewhat similar to that for xylopinine (**1**), but the absence of one of the aromatic proton absorptions present in xylopinine indicated that the hydroxyl function of tetrahydrostephabine (**2**) resided on ring A. Furthermore, the doublet of doublets absorption due to one of the two C-13 protons, which appeared at δ 3.26 in xylopinine (**1**), suffered a significant downfield shift in tetrahydrostephabine (**2**), appearing at δ 3.86. This indicated that an oxygenated substituent was located in the immediate vicinity, more specifically at C-1.

In order to settle conclusively the location of the phenolic function on ring A, the NMR spectrum of tetrahydrostephabine (**2**) was recorded first in DMSO-d_6 and then in DMSO-d_6 + NaOD. It is known that under such circumstances an aromatic proton *para* to a phenolic function will undergo an upfield shift in basic solution greater than 0.55 ppm, while protons *ortho* or *meta* to the phenol will experience smaller upfield movements [4, 5]. In the present instance, the upfield shift of H-4 from δ6.27 to 5.56 (Δ0.71 ppm) clearly indicated that the phenolic function was at C-1. Finally, diazomethane *O*-methylation of **2** provided (−)-*O*-methyltetrahydrostephabine.

The second new alkaloid, (−)-stephabinamine (**3**), is structurally related to (−)-tetrahydrostephabine (**2**). However, the MS and the NMR spectra indicated that one of the methoxyls of ring D in **2** had been replaced with a hydroxyl in **3**. Very significantly, the downfield shift of H-12 from δ6.62 in tetrahydrostephabine (**2**) to 6.69 in stephabinamine (**3**) pointed to the ring D phenolic group of stephabinamine being located at C-11 rather than at C-

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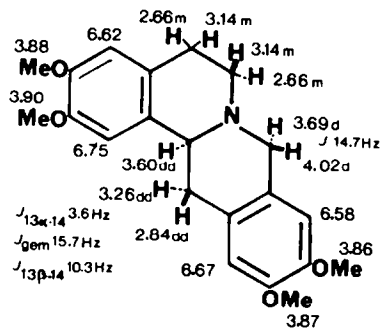
§These assignments of chemical shifts are at variance with those made in Ref. [3] which were not supported by nOe experiments.

10. A parallel downfield shift of H-12 is also observed when going from xylopinine (**1**) to corytenchine (**4**).

The yellow protoberberinium salt stephabine (**5**), $C_{21}H_{22}NO_5^+$, isolated as the chloride, was our third alkaloid. Sodium borohydride reduction of this species led to racemic tetrahydrostephabine (**2**). Conversely, iodine oxidation of **2** furnished **5**.

The orange coloured 8-oxypseudopalmatine (**6**), $C_{21}H_{21}NO_5$, was also obtained from the chromatographic column. This aromatic species gives a blue spot with the Dragendorff spray reagent characteristic of 8-oxyprotoberberines in general; and displayed an IR carbonyl band at 1635 cm^{-1} in chloroform. The NMR spectrum, (see **6**) presents the characteristic apparent broad triplets at δ2.95 and 4.37 diagnostic of an 8-oxyprotoberberine. Four methoxyl and five aromatic singlet absorptions were also in evidence. As expected, the MS molecular ion, m/z 367, was also the base peak. The identity of **6** was then confirmed by direct comparison with a sample of 8-oxypseudopalmatine obtained from the accompanying pseudopalmatine by disproportionation under alkaline conditions. Compound **6** had been previously known synthetically [6, 7]. There is a possibility that in the present instance, 8-oxypseudopalmatine (**6**) could be an artifact of isolation, formed from pseudopalmatine during the chromatographic process.

The remaining two compounds obtained from the column are (−)-*trans*-xylopinine *N*-oxide (**7**) and the (−)-*cis* analogue **8**. Their structures were proven through direct comparison with the known (±)-*trans*- and (±)-*cis*-xylopinine *N*-oxides which had been originally obtained by *in vitro* oxidation of (±)-xylopinine (**1**) [7].



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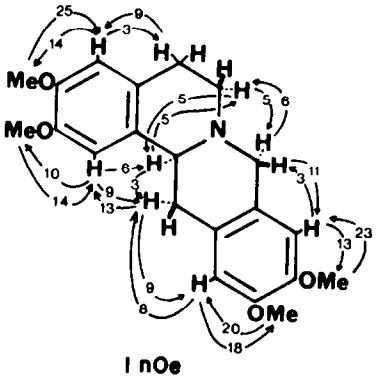
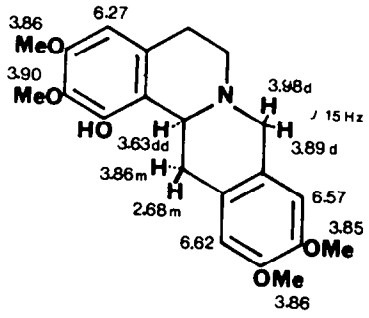


1 nOe



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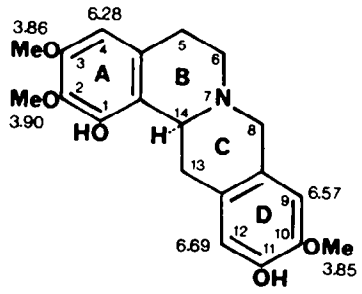

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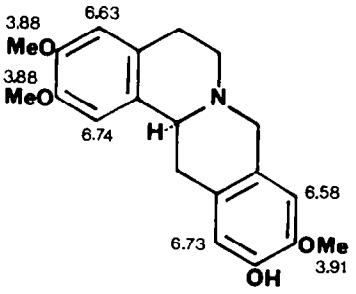

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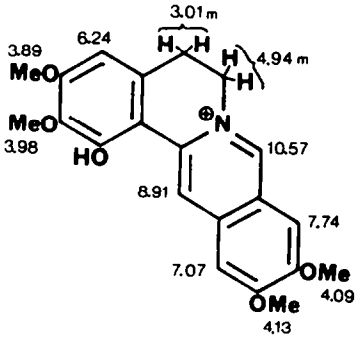

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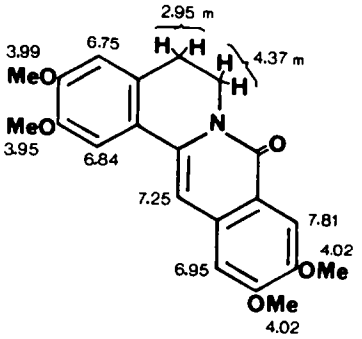

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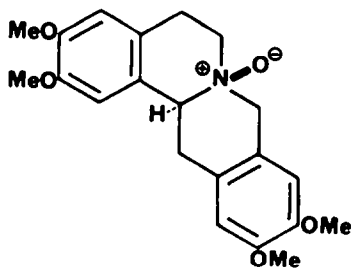

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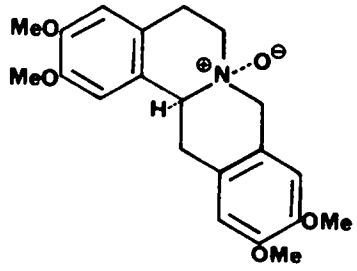

8

An interesting feature of *S. venosa* protoberberines is that normal as well as pseudo type protoberberines are produced, substituted at C-2,3,9,10 and 2,3,10,11, respectively. Both types may also be oxidized at C-1, as in (−)-capaurimine and (−)-stephabinamine (**3**), in which case ring A bears three oxygenated substituents.

EXPERIMENTAL

NMR spectra were obtained at 360 MHz in $CDCl_3$ soln. UV and CD spectra are in MeOH.

Isolation. The air-dried powdered tuberous roots (1.8 kg) were extracted with EtOH at room temp. 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 (72–200 mesh) in $CHCl_3$. Elution was first with $CHCl_3$, and then with $CHCl_3$ containing increasing amounts of MeOH. Alkaloidal mixtures from the column were further separated by TLC using such solvent systems as $CHCl_3$–MeOH (19:1), C_6H_6–MeOH (9:1), $CHCl_3$–MeOH–NH_4OH (19:1:trace), and $CHCl_3$–MeOH (17:3).

The following alkaloids, as amorphous materials, were obtained roughly in the order of their elution from the column: (−)-tetrahydropalmatine, 12 mg; (−)-xylopinine (**1**), 3.51 g; (−)-tetrahydropalmatrubine, 27 mg; (−)-discretine, 6 mg; (−)-tetrahydrostephabine (**2**), 28 mg; (−)-kikemanine, 18 mg; (−)-coreximine, 6 mg; (−)-stephabinamine (**3**), 5 mg; (−)-corytenchine (**4**), 4 mg; (−)-capaurimine, 6 mg; (−)-stepholidine, 2 mg; 8-oxypseudopalmatine (**6**), 22 mg; (−)-*cis*-xylopinine *N*-oxide (**8**), 8 mg; (−)-*trans*-xylopinine *N*-oxide (**7**), 10 mg; pseudopalmatine, 600 mg; and stephabine (**5**), 2 mg. Known alkaloids were characterized either by comparison with authentic samples or through their spectral characteristics [2].

(−)-*Tetrahydrostephabine* (**2**). *m/z* 371 (M^+, 36), 206 (24), 164 (100); λ_{max} 228 sh, 286 nm (log ε 4.10, 3.66); CD Δε (nm) 0 (285), −1.7 (275), −0.58 (258), −10.9 (230); $[\alpha]_D^{25}$ −277° (c 0.59, $CHCl_3$).

(−)-O-*Methyltetrahydrostephabine.* Base **2** (5 mg) was dissolved in MeOH, and the soln treated with excess ethereal diazomethane for 48 hr at near 0°. Work-up supplied the pentamethoxylated derivative, $C_{22}H_{27}NO_5$, *m/z* 385 (M^+, 36), 220 (27), 164 (100); δ 3.86 (6H), 3.87 (3H), 3.88 (3H), 3.91 (3H), 6.46 (*s*, H-4), 6.59 (*s*, H-9), 6.62 (*s*, H-12); $[\alpha]_D^{25}$ −272° (*c* 0.28, $CHCl_3$).

(−)-*Stephabinamine* (**3**). *m/z* 357 (M^+, 53), 208 (100), 206 (62), 150 (66); λ_{max} 228 sh, 284 nm (log ε 4.14, 3.63); Δε (nm) 0 (295), −1.7 (276), −0.44 (252), −5.74 (230); $[\alpha]_D^{25}$ −212° (c 0.13, $CHCl_3$).

Stephabine (**5**) *chloride.* λ_{max} 243, 294, 312, 325, 383 nm (log ε 4.21, 4.45, 4.12, 3.97, 3.65). Reduction of **5** with $NaBH_4$ in methanol afforded (±)-**2**.

8-*Oxypseudopalmatine* (**6**). Orange crystals from MeOH, mp 198–199° [4, 5].

(−)-trans-*Xylopinine* N-*oxide* (**7**). *m/z* 371 (M^+, 13), 164 (100); Δε (nm) 0 (298), −1.2 (285), −0.6 (255), −7.5 (236), −1.9 (217), −16.7 (210); $[\alpha]_D^{25}$ −200° (*c* 0.44, MeOH).

(−)-cis-*Xylopinine* N-*oxide* (**8**). *m/z* 371 (M^+, 15), 164 (100); Δε (nm) 0 (250), −10 (237), 0 (228), +2.2 (224), 0 (217), negative tail; $[\alpha]_D^{25}$ −96° (*c* 0.41, MeOH).

Pseudopalmatine chloride. mp 212–213°, yellow needles from $CHCl_3$; λ_{max} 265, 288, 310 sh, 339, 379 nm (log ε 4.16, 4.48, 4.34, 4.10, 3.66); δ 3.15 (2H, *br t*, H-5), 3.98 (3H), 4.06 (3H), 4.09 (3H), 4.13 (3H), 5.00 (2H, *t*, H-6), 6.72 (1H, *s*, H-4), 7.45 (2H, *s*, H-1 and H-12), 7.67 (1H, *s*, H-9), 8.40 (1H, *s*, H-13), 10.34, (1H, *s*, H-8). Reduction of the salt with $NaBH_4$ in MeOH provided (±)-xylopinine (**1**). Alternatively, pseudopalmatine (6 mg) was treated with hot aq. KOH soln at 80° for 10 hr. The solid product was filtered and purified by TLC. The main band showed a blue fluorescence under UV light and corresponded to 8-oxypseudopalmatine (**6**) (1.5 mg).

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