

ALKALOIDS OF *CORYDALIS GOVANIANA*

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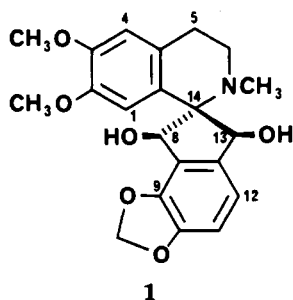
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*Corydalis govaniانا* Wall (Fumariaceae) is a stout herb native to the western Himalayas at altitudes in the range of 2,500 to 3,500 m. Ethnomedically, the roots have found use by the people of the Kurram valleys in the treatment of syphilitic, scrofulous, and cutaneous infections (1). The alkaloid fraction acts as a uterine stimulant and stomach muscle depressant *in vitro*, and *i.v.* injection of the alkaloid fraction produces a hypotensive effect in anesthetized cats (2).

There have been only two previous phytochemical examinations of this species, the first of which yielded the isoquinoline alkaloids protopine, columine, bicucculine, and isocorydine, together with choline and fumaric acid from the roots (3). Subsequently, (-)-govadine and (-)-govanine were obtained from the leaves and stems (4).

Our own studies of the alkaloid fraction of the whole plant have afforded protopine, ochotensine, 13-*epi*-yenusomine,<sup>1</sup> and isocorydine. The structures of these compounds were determined through examination of their spectroscopic properties, and the principal details of these data are available from the authors. In the course of this work heteronuclear correlation experiments were conducted on the spiro-benzylisoquinoline alkaloid 13-*epi*-yenuso-

mine [1]. MacLean *et al.* had previously (7) conducted <sup>13</sup>C studies on compounds in this series. Our work independently verifies the majority of these assignments as detailed below and also provides more explicit verification of the structure of 1 than was hitherto available.



Two pairs of aromatic protons were observed, the *ortho*-coupled protons, H-12 and H-11, at  $\delta$  6.89 and  $\delta$  6.82 and the *para*-coupled protons, C-4 and C-1, at  $\delta$  6.66 and  $\delta$  6.16, respectively. The corresponding carbon atoms appeared in the region 108-116 ppm. Assignment of these was achieved through a heteronuclear correlation experiment which led to the signals at  $\delta$  115.6, 112.1, 109.8, and 108.5 being assigned to C-12, C-4, C-1, and C-11, respectively. Because of the substantial proton chemical shift difference between the C-2-OCH<sub>3</sub> ( $\delta$  3.40) and the C-3-OCH<sub>3</sub> ( $\delta$  3.83), the carbon frequencies for these groups could be assigned with certainty to  $\delta$  55.1 and  $\delta$  55.4, respectively. Other assignments of protonated carbons were confirmed to be in agreement with those reported previously. In and of itself the hetcor experiment does not unequivocally establish the assignment of C-8 and C-13 inas-

<sup>1</sup>The alkaloid 1 was originally given the name "raddeanine" (5) based on its isolation from *Corydalis ochotensis* var. *raddeana*. The structure is identical to that of 13-*epi*-yenusomine. A quite different "raddeanine" (imperialine) was obtained earlier from *Fritillaria raddeana* (6). We, therefore, suggest that the name 13-*epi*-yenusomine be used for the spirobenzylisoquinoline alkaloid.

much as the chemical shift of their attached protons is ambiguous. This situation was clarified through a series of SINEPT experiments (8), which we have successfully applied in other studies (9,10).

Irradiation of the signal at  $\delta$  5.43 with  $J=5$  Hz enhanced the signal at  $\delta$  122.6; when  $\delta$  5.22 was irradiated with  $J=5$  Hz, the signal at  $\delta$  137.6 was enhanced, together with the signal at  $\delta$  147.9. On the other hand, irradiation with  $J=3$  Hz at  $\delta$  5.43 enhanced  $\delta$  142.5 indicating that this proton is H-

13 showing coupling with C-8a ( $\delta$  122.6) and C-10 ( $\delta$  142.5). Thus, the proton at  $\delta$  5.22 is H-8. Irradiation of the methylenedioxy protons with delay times set for  $J=4$  Hz led to the selective enhancement of the signals at  $\delta$  142.5 (C-10) and  $\delta$  147.9 which must be attributed to C-9. Two remaining signals in this region at  $\delta$  146.3 and  $\delta$  147.7 remained to be assigned, and this was achieved through irradiation of the 2-OCH<sub>3</sub> and the 3-OCH<sub>3</sub>. Thus, irradiation at  $\delta$  3.83 led to selective enhancement at  $\delta$  147.7 (C-3), and a negative

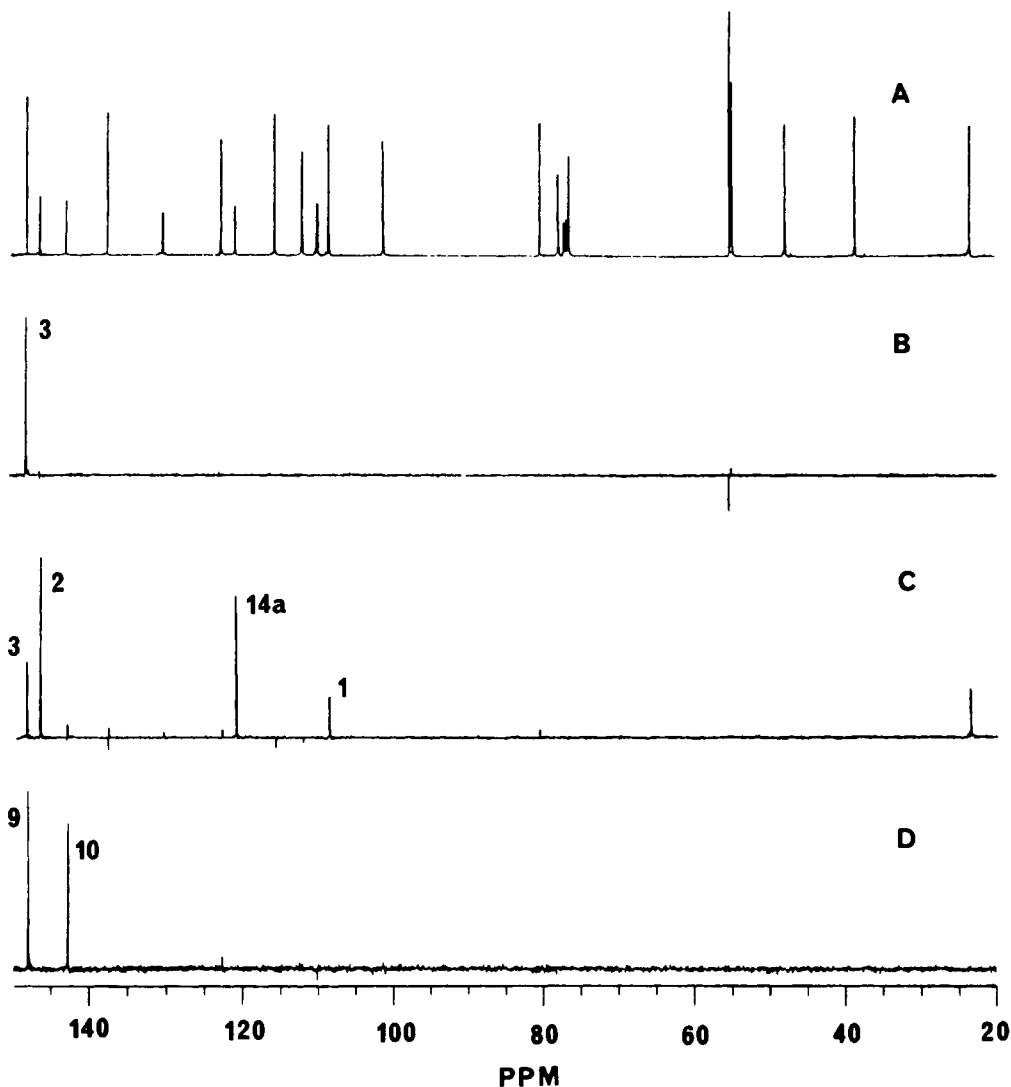


FIGURE 1. SINEPT experiments on 13-*epi*-yenhusomine [1]. (A) Decoupled <sup>13</sup>C nmr spectrum, (B) irradiation of 2-OCH<sub>3</sub> ( $J=4$  Hz), (C) irradiation of H-1 ( $J=8$  Hz), and (D) irradiation of -OCH<sub>2</sub>O- ( $J=2$  Hz).

enhancement was noted at  $\delta$  55.4. On the other hand, irradiation of  $\delta$  3.40 (2-OCH<sub>3</sub>) led to enhancement of the signal only at  $\delta$  146.3.

The question of the respective assignment of H-11 and H-12 and H-1 and H-4 is worthy of comment. Irradiation of the signal at  $\delta$  6.16 led to the enhancement of signals at  $\delta$  80.6 (C-14) and  $\delta$  130.6 (C-4a) indicating that this proton must be H-1. Irradiation of the proton at  $\delta$  6.66, on the other hand, led to enhancement at  $\delta$  23.6 (C-5) and  $\delta$  120.2 (C-14a) when the delay times were set for  $J=8$  Hz confirming that this is H-4. Some examples of these experiments are shown in Figure 1.

The assignment of H-11 and H-12 was based on the assignment of the corresponding carbon atoms from literature values (i.e., C-12 is more downfield than C-11) and could not be independently verified.

## EXPERIMENTAL

**PLANT MATERIAL.**—The plant material used in this study was obtained from the upper regions of the Kashmir (3,200 m) in August 1983. A herbarium specimen is deposited in the herbarium of the Regional Research Laboratory, Jammu, India.

**EXTRACTION AND FRACTIONATION.**—Powdered, dried, whole plant material (3 kg) of *C. govianiana* was extracted by percolation with MeOH (5×5 liters), and the solvent was evaporated. The residue (150 g) was stirred with 2% aqueous tartaric acid solution (3×2 liters) and filtered. The filtrate was basified to pH 9 with NH<sub>3</sub> and extracted with CHCl<sub>3</sub>. Evaporation of the dried, filtered solution afforded a dark brown oil (50 g, 1.7%).

The C<sub>6</sub>H<sub>6</sub>-soluble portion of the extract (40 g) was chromatographed over neutral alumina (1.5 kg) eluting successively with C<sub>6</sub>H<sub>6</sub>, C<sub>6</sub>H<sub>6</sub>/EtOAc mixtures, EtOAc, and EtOAc/MeOH mixtures. A total of 40 fractions (250 ml each) were collected. From fractions 6-10 eluting with C<sub>6</sub>H<sub>6</sub>-EtOAc (1:1) protopine (100 mg, 0.03%) was obtained, and fractions 14-15 eluted with EtOAc afforded ochotensine (25 mg, 0.008%). Fraction 16, also eluted with EtOAc, yielded 13-*epi*-yenusomine [1] (50 mg, 0.17%), and fractions 20-21 eluted with EtOAc-MeOH (5:1) gave isocorydine (200 mg, 0.07%). The physical

properties of protopine, ochotensine, and isocorydine were in agreement with those reported previously.

**IDENTIFICATION OF 13-EPI-YENUSOMINE [1].**—13-*epi*-Yenusomine [1] was obtained as colorless needles, mp 208-209 °; ir  $\nu$  max (KBr) 3450, 2900, 1420, 1220, 1190, 1040, 980 cm<sup>-1</sup>; uv  $\lambda$  max (MeOH) (log  $\epsilon$ ) 285 nm (3.85); <sup>1</sup>H nmr  $\delta$  (CDCl<sub>3</sub>) 6.89 (1H, d,  $J=7.8$  Hz, H-12), 6.82 (1H, d,  $J=7.8$  Hz, H-11), 6.66 (1H, s, H-4), 6.16 (1H, s, H-1), 6.00 and 5.96 (1H each, s, -OCH<sub>2</sub>O-), 5.43 (1H, br s, H-13), 5.22 (1H, br s, H-8), 3.83 (3H, s, 3-OCH<sub>3</sub>), 3.40 (3H, s, 2-OCH<sub>3</sub>), 3.34 (2H, m, H<sub>2</sub>-6), 2.79 (2H, m, H<sub>2</sub>-5), 2.59 (3H, s, NCH<sub>3</sub>); <sup>13</sup>C nmr  $\delta$  (CDCl<sub>3</sub>) 147.9 (C-9), 147.7 (C-3), 146.3 (C-2), 142.5 (C-10), 137.6 (C-12a), 130.6 (C-4a), 122.6 (C-8a), 120.2 (C-14a), 115.6 (C-12), 112.1 (C-4), 109.8 (C-1), 108.5 (C-11), 101.3 (-OCH<sub>2</sub>O-), 80.6 (C-14), 78.3 (C-13), 76.8 (C-8), 55.4 (3-OCH<sub>3</sub>), 55.1 (2-OCH<sub>3</sub>), 48.0 (C-6), 38.7 (NCH<sub>3</sub>), 23.6 (C-5); ms  $m/z$  (%) 385 (M<sup>+</sup>, 1), 367 (63), 252 (40), 339 (22), 338 (86), 336 (15), 324 (42), 309 (29), 308 (100), 206 (60), 204 (12), 191 (16), 190 (20), 162 (21), 154 (17), 147 (14), 132 (11), 91 (12), 77 (18), 65 (19).

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