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Isosinococuline, a Novel Antitumor Morphinane Alkaloid from *Cocculus trilobus*

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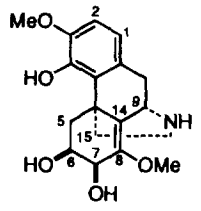
Abstract: An antitumor alkaloid, isosinococuline **2**, has been isolated from the rhizomes of *Cocculus trilobus*. Its structure was elucidated by spectroscopic methods and chemical reactions.

We have previously reported the isolation and the structure elucidation of an antitumor alkaloid, sinococuline (**1**), as an active principle of the native Japanese plant *Cocculus trilobus*.[1] Since alkaloid **1** shows promising antitumor activity against animal tumor models, we have further investigated this plant to identify the active congeners. An intensive search of the methanol extract of the rhizomes of this plant led to the isolation of the new alkaloid isosinococuline (**2**). In this communication, we describe the isolation and the structure of this alkaloid.

The alkaloid was found in the more polar fractions of the silica gel column chromatography ($CHCl_3$–Et_2NH, gradient elution) than those containing **1** and was isolated by repeated preparative-TLC (SiO_2, $CHCl_3$–Et_2NH, 3:2) and alumina column chromatography ($CHCl_3$–MeOH, 3:1).[2]

Isosinococuline (**2**) is a crystalline powder (mp 149–151 °C, decomp.; $[\alpha]^{26}_D$ –66.0 ° (*c* 0.31, MeOH)) whose molecular formula was deduced as $C_{18}H_{23}NO_5$, identical to **1**, from high-resolution EIMS (M^+ *m/z* 333.1591, Δ –1.5 mmu). The ^1H NMR (500 MHz) spectra of **1** and **2** taken in CD_3OD are similar, two characteristic methoxyl singlets (δ 3.72 and 3.83) and three methine protons (δ 3.93, 4.34 and 4.40) connected to hetero atoms are observed.[3] The main differences are in the aromatic protons. Whereas the two aromatic protons of sinococuline (**1**) appeared as a pair of doublets (δ 6.53 and 6.75, J = 8.3 Hz), the corresponding protons of the alkaloid showed two singlets (δ 6.63 and 6.71), indicating the two aromatic protons in the alkaloid being *para* to each other. Further intensive study using the 2D NMR technique (H–H COSY, HMQC[4] and HMBC[5]) revealed the connectivity of each proton and carbon, and thus established the positions of the two methoxyl groups at positions 2 and 8. The observed ring A substitution of **2** is unique and rare since the morphinane skeleton is biogenetically constructed through phenol oxidation coupling.[6,7] The above experiments also established the three methine protons at positions 6, 7 and 9. Since the chemical shifts and coupling constants of these resonances are quite similar (< Δ 0.09 ppm and < Δ 0.7 Hz) to those of **1**, the presence of two hydroxyl groups at positions 6 and 7 were deduced. The stereochemistries of these hydroxyl groups were both determined to be β-configurations by the observation of strong NOESYPH[8] correlations between H-6 and H-15$_\alpha$ and between H-6 and H-7 (Figure 1). The *cis* relationship of these two hydroxyl



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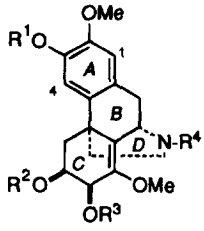

2; $R^1 = R^2 = R^3 = R^4 = H$
3; $R^1 = R^2 = R^3 = H$, $R^4 = CN$
4; $R^1 = p$-BrBn, $R^2 = R^3 = H$, $R^4 = CN$
5; $R^1 = p$-BrBn, $R^2 = CS = R^3$, $R^4 = CN$

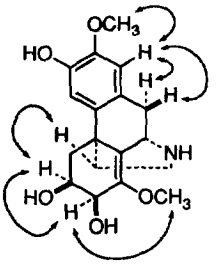

Figure 1. Selected NOESYPH correlations for **2**

groups was further supported by the formation of the cyclic thiocarbonate **5**[9] ($J_{H6,H7}$ = 6.8 Hz) which was derivatized from **2** through cyanamide formation (**3**) [BrCN (1 equiv), *i*-Pr_2NEt (1.5 equiv), MeOH, r.t., 12 h, 69%], *O*-*p*-bromobenzylation (**4**) [*p*-bromobenzyl bromide (2 equiv), K_2CO_3, acetone, r.t., 3 d, 95%] and successive treatment with 1,1'-thiocarbonyldiimidazole [2 equiv, benzene, reflux, 3 d, 55%]. The absolute configuration of **2** has been assigned to be the same (6*S*,7*S*,9*S*,13*S*) as that of **1** because of the close similarities in their CD spectra.[10]

A preliminary *in vivo* study against P-388 leukemia in mice showed *T/C* = 146% for **2** at the 25 mg kg^{-1} day^{-1} level.[11] The isolation of the alkaloid **2** possessing a different substitution pattern on ring A from **1** provides valuable information on the structure–activity relation study and for designing a more promising candidate for this class of antitumor agents.

References and Notes

1. a) *Isolation:* Itokawa, H.; Tsuruoka, S.; Takeya, K.; Mori, N.; Sonobe, T.; Kosemura, S.; Hamanaka, T. *Chem. Pharm. Bull.* **1987**, *35*, 1660. b) *Synthesis from sinomenine:* Hitotsuyanagi, Y.; Ikuta, H.; Nishimura, K.; Takeya, K.; Itokawa, H. *J. Chem. Soc., Chem. Commun.* **1994**, 2707.
2. The plant was collected in Nagano Prefecture in October, 1984. It is important to note that the content of **2** in the plant is quite variable. We have experienced the cases where no detectable amount of **2** was observed in the same species collected in other seasons or at other places in Japan.
3. R_f 0.14 (TLC SiO_2; $CHCl_3$–Et_2NH, 7:3). ^1H NMR (500 MHz, CD_3OD, ref. CD_2HOD = 3.34 ppm, *J*/Hz) δ 1.44 (1H, br d, *J* = 12.1, H-15β), 2.02 (1H, ddd, *J* = 12.1, 11.3, 6.7, H-15α), 2.06 (1H, dd, *J* = 12.6, 3.6, H-5α), 2.14 (1H, dd, *J* = 12.6, 12.6, H-5β), 2.67–2.77 (2H, m, H-16α,β), 2.95 (1H, d, *J* = 17.6, H-10$_a$), 3.15 (1H, dd, *J* = 17.6, 6.1, H-10$_b$) 3.72 (3H, s, C8-OMe), 3.83 (3H, s, C_2-OMe), 3.93 (1H, ddd, *J* = 12.6, 3.6, 3.2, H-6), 4.34 (1H, d, *J* = 3.2, H-7), 4.40 (1H, d, *J* = 6.1, H-9), 6.63 (1H, s, H-1), 6.71 (1H, s, H-4); ^{13}C NMR (126 MHz, CD_3OD, ref. = 49.0 ppm) δ 36.40 (C-10), 37.95 (C-5), 38.60 (C-13), 40.24 (C-16), 42.92 (C-15), 46.84 (C-9), 56.37 (C_2-OMe), 57.59 (C8-OMe), 67.26 (C-7), 68.68 (C-6), 111.23 (C-1), 112.52 (C-4), 123.58 (C-14), 128.38 (C-11), 137.06 (C-12), 145.96 (C-8), 146.34 (C-3), 147.56 (C-2); UV λ^{MeOH}max (log ε): 208 (4.50), 287 (3.68), 297sh (3.62).
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6. Bentley, K. W. In *The Alkaloids*; Manske, R. H. F., Ed.; Academic: New York, 1971; Vol. 13, Chapter 1.
7. Although a number of morphinane alkaloids have been reported thus far, only flavinantine and flavinine possess the same ring A substitution. Chambers, C.; Stuart, K. L. *J. Chem. Soc., Chem. Commun.* **1968**, 328.
8. Bodenhausen, G.; Koger, H.; Ernst, R. R. *J. Magn. Reson.* **1984**, *58*, 370.
9. **5**: an amorphous powder; $[\alpha]_D^{28}$ –33.2° (*c* 0.20, $CHCl_3$); FAB-MS 571.0 ($C_{27}H_{25}N_2O_5{}^{81}BrS$); δ_C 190.55 (*C*=S, $CDCl_3$).
10. The absolute configuration of sinococuline (**1**) has been established by the correlation with sinomenine.[1b] **2**: $[\theta]^{26}_{232}$ +14700 (*c* 9.55 × 10^{-5}, MeOH).
11. We are grateful to Dr. Takehiro Yamagishi of Taisho Pharmaceutical Co., Ltd. for the biological testing.

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