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ALKALOIDS OF *STEPHANIA SINICA*

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Key Word Index—*Stephania sinica*; Menispermaceae; hasubanan alkaloid; runanine; cepharanthine.

Abstract— A new hasubanan alkaloid named as runanine was isolated from *Stephania sinica*. On the basis of spectral analysis, four methoxyl groups could be located at C-2, C-3, C-7 and C-8, respectively. Its structure was determined to be **1**. Two other known compounds, cepharanthine and sitosterol, were also obtained from this plant.

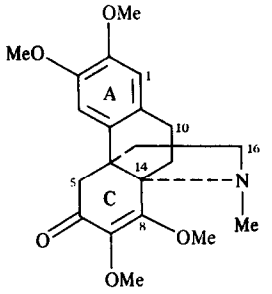
INTRODUCTION

Stephania sinica Diels (Menispermaceae), which is called Runan in China, is distributed in the Hubei, Gueizhou and Yunnan provinces of China. Runan was mistakenly identified as growing in Guangxi province [1]. Several isoquinoline alkaloids have been reported in *Stephania* spp. One of them is a hasubanan alkaloid which was isolated from *S. japonica* [2]. The chemistry of *S. sinica* has not been studied previously. We report here the preliminary chemical study of this plant; a new alkaloid (**1**) named runanine was isolated from the roots together with two other known compounds, cepharanthine (**2**) and β-sitosterol.

RESULTS AND DISCUSSION

Runanine (**1**) mp 100–105°, $[\alpha]_D^{18}$ −400°, M_r 373, $C_{21}H_{27}O_5N$, is a white crystalline solid. The UV (λ_{max}^{EtOH} 207, 230, 270 nm) and IR (ν_{max}^{KBr} 1660 cm^{-1}, α,β-unsaturated ketone) spectra showed that it could be an alkaloid of either the sinomenine or hasubanan type [3]. The 360 MHz ^{1}H NMR spectrum showed two singlet peaks: δ6.64 (1H) and 6.47 (1H) at low field. Signals for four methoxyl groups were at δ3.61, 3.79, 3.81 and 4.05, respectively. The signals at 2.51 belonged to an N-methyl group. There were no signals from δ4.10 to 6.40. The α,β-unsaturated ketone (–CR^2=CR^1–C=O) should be assigned to the C ring and the methoxyl group of 4.05 to R^2. The positions of the other methoxyl groups were established by NOE techniques. On irradiation at δ3.79 and 3.80, the intensities of the signals at both 6.64 and 6.47 increased by 10%; on the other hand the same phenomena were not observed on irradiation at 3.61 and 4.05. Therefore, the two methoxyl groups at δ3.79 and 3.80 were assigned to C-2 and C-3, and the signals of 6.64 and 6.47 were aromatic protons. Both R^1 and R^2 were substituted by methoxyl groups because of the absence of signals for ethylene protons. The structure of the α,β-unsaturated ketone should be –C(MeO)=C(OMe)–C=O. The geminal protons of an AB system were confirmed by an INDOR experiment to be at δ3.00 and 2.60 (J = 13.2 Hz), respectively. A molecular model showed that the distance between H-5_{eq} and H-4 was *ca* 1.8 Å to give NOE. On irradiation at 3.00, the intensity of the signal at 6.64 increased by 22.6%; the signal at 6.47 did not change. Therefore, the geminal protons could be assigned to those at H-5; H-4 was at 6.64 and H-1 at 6.47. In highfield ^{1}H NMR (360 MHz) there were no proton signals from δ3.2 to 4.2 except for the four methoxyl signals. In another range of the spectrum, the two pair signals of A_2B_2 systems were exhibited, one of which was assignable to an ethylamine bridge, the other to four protons for C-9 and C-10. The structure **1** showed the absence of an H-9 which appears in sinomenine type alkaloids at *ca* δ3.60–3.80 [4]. Consequently, the location of the ethylamine bridge was determined as shown in **1**, which is an alkaloid of the hasubanan type. To confirm the partial structure of ring C, **1** was reduced with potassium borohydride. The ^{1}H NMR of the reduced product showed a triplet at δ4.25, the proton bearing the hydroxyl group coupling with those of C-5. The carbonyl of **1** should be located at C-6. As the optical activity of **1** was similar to that of hasubanonine ($[\alpha]_D^{27}$ −214° [5]), **1** must have the same configuration as hasubanonine (C-13: *R*, C-14: *S*).

In the same fraction as **1**, β-sitosterol (mp 139–140°) was obtained and in other fractions, alkaloid **2** was obtained. Alkaloid **2** was identified as cepharanthine [6] by spectral evidence (see Experimental).



1 Runanine

EXPERIMENTAL

Extraction and isolation. Dried roots of *S. sinica* Diels (13 kg) were extracted with EtOH and the non-phenolic alkaloids extract (45 g) obtained according to usual extraction methods. The extract was packed on top of an Al_2O_3 column (neutral, 200 g, 100–200 mesh, Type IV), and eluted with cyclohexane–EtOAc. The fraction (2.6 g) from 100:17 was a mixture of alkaloid **1** and β-sitosterol. This mixture was dissolved in 0.1 N HCl and filtered. The ppt was washed with H_2O until neutral and recrystallized from EtOH to yield β-sitosterol (2 g). The aq. soln was treated with NH_4OH (pH 10) and extracted with $CHCl_3$. The $CHCl_3$ extract was evapd to give crude alkaloid **1** (0.6 g), which was recrystallized from cyclohexane–Et_2O to give white prisms. Alkaloid **2** (7.5 g) was obtained from the fraction of 100:20–45.

Runanine (**1**). White crystals, mp 100–102°, $[\alpha]_D^{18}$ −400° (*c* 0.8, $CHCl_3$), UV λ_{max}^{EtOH} nm: 207, 230, 270. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1660 (α,β-unsaturated ketone), 1590, 1518 (Ar), 1450, 1328, 1220, 1140, 1050, 1042, 925. ν_{max}^{KBr} cm^{-1}: 1660, 1595, 1480, 1440, 1320, 1220, 1100, 1020, 925. ^{1}H NMR ($CDCl_3$): δ2.51 (3H, *s*, NMe), 1.8–2.2 (4H, *m*, C-15H_2, C-16H_2), 2.7–2.9 (4H, *m*, C-9H_2 and C-10H_2), 4.05 (3H, *s*, C-8OMe), 3.61 (3H, *s*, C-7OMe), 3.79, 3.80 (3H, each *s*, C-2,3OMe), 2.60 (1H, *d*, *J* = 13.2 Hz, H-5$_{ax}$), 3.00 (1H, *d*, *J* = 13.2 Hz, H-5$_{eq}$), 6.47 (1H, *s*, H-1), 6.64 (1H, *s*, H-4). MS *m/z* (rel. int.): 377 $[M]^+$ (36.6), 342 (6.4), 315 (100), 314 (44.3), 283 (18.6), 258 (20.3), 245 (14.8, M−$C_6H_8O_3$), 230 (17.6), 59 (93.6). Calc. for $C_{21}H_{27}O_5N$: C, 67.45; H, 7.29; N, 3.57: Found: C, 67.41; H, 7.37; N, 3.70%.

Reduction of **1**. Compound **1** (20 mg) dissolved in Et_2O (5 ml) was added to a suspended Et_2O soln containing KBH_4 (20 mg) and left overnight at room temp. The soln was evapd to dryness and a few drops of aq. acid added. The ppt was filtered off and to the aq. soln, NH_4OH was added to give pH 9–10. The soln was then extracted with $CHCl_3$ to afford the reduced compound as a colourless oil. ^{1}H NMR ($CDCl_3$): δ2.5 (3H, NMe), 3.6 (3H, OMe), 3.7 (3H, OMe), 3.81 (3H, OMe), 3.9 (3H, OMe), 4.25 (1H, *t*), 6.5 (1H, *s*), 6.7 (1H, *s*).

Cepharanthine (**2**). Mp 145–152°, yellow crystals (C_6H_6–Me_2CO), $[\alpha]_D^{19}$ + 280.9° (*c* 1, $CHCl_3$); UV λ_{max}^{EtOH} nm: 210.5, 218.5. IR ν_{max}^{KBr} cm^{-1}: 1610, 1500, 1360, 1260. MS *m/z*: 606 $[M]^+$ (100%), 381, 379, 365, 349, 192, 190, 110. The IR spectrum of **2** was identical to that of cepharanthine.

β-Sitosterol. White crystals (EtOH), mp 139–140°, $[\alpha]_D^{14}$ −40° (*c* 1, $CHCl_3$), positive to Liebermann–Burchardt reaction. IR ν_{max}^{KBr} cm^{-1}: 3350, 1460, 1380, 1050, 1020, 960, 840, 800. Identical with an authentic sample of β-sitosterol.

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