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DE-*O*-ETHYLSALVONITIN AND SALPRIONIN, TWO FURTHER DITERPENOIDS FROM *SALVIA PRIONITIS*

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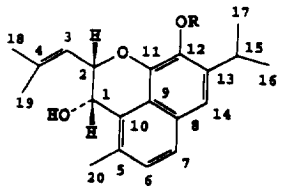
Key Word Index—*Salvia prionitis*; Labiatae; diterpenoid; de-*O*-ethylsalvonitin; salprionin.

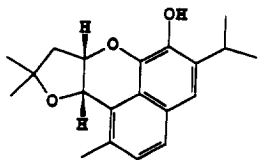
Abstract—From *Salvia prionitis* two new diterpenoids, de-*O*-ethylsalvonitin and salprionin, were isolated, and their structures and NMR data were assigned by spectral analysis and computer modelling calculations.

INTRODUCTION

Besides about 25 diterpenoids reported previously [1–7], two additional new diterpenoids were isolated from *Salvia prionitis* Hance (Labiatae), a plant native to the southern provinces of the People's Republic of China, where it is used as an antibacterial, antitubercular and antiphlogistic drug in folk medicine. In this paper, we present the isolation, structure elucidation and NMR assignments of these two new compounds.



1: R = H
3: R = CH_2CH_3



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RESULTS AND DISCUSSION

Dried roots of *S. prionitis* were extracted with ethanol, followed by column chromatography on silica gel, preparative TLC and crystallization, to afford the isolates, **1** and **2**.

Isolate **1**, $C_{20}H_{24}O_3$ [high-resolution mass spectrometry (HRMS)], was obtained as yellowish crystals and was determined to be de-*O*-ethylsalvonitin (**1**), a diterpenoid with C_2H_4 less than that of salvonitin (**3**) [7] through the analysis of their UV, ^1H and ^{13}CNMR, COSY, HETCOR, NOED and mass spectra. As shown in Table 1, the results of selective INEPT irradiation further deduced the structure for **1** and led to a complete NMR data assignment. The remaining relative configuration of H-1 and H-2 of **1** was suggested to be *cis* by computer modelling calculations [8] (calc.: $J_{1\alpha,2\alpha} = J_{1\beta,2\beta} = 0.93$ Hz, $J_{1\alpha,2\beta} = J_{1\beta,2\alpha} = 8.9$ Hz; obs.: $J_{1,2} = 2.4$ Hz).

Isolate **2**, $C_{20}H_{24}O_3$ (HRMS), was obtained as a yellowish powder. This compound showed very similar UV, ^1H and ^{13}CNMR data to those for **1**, and the two isolates possessed the same molecular formula. Compounds **1** and **2** showed differences only in the chemical shifts of the remaining six carbons (C-1, C-2, C-3, C-4, C-18 and C-19), as well as the attached protons to these carbons. One of the reasons is that **2** lacks a double bond at C-3 and C-4, since its C-3 is a methylene carbon. As a quaternary carbon, C-4 of **2** is also connected to C-3 and two methyl groups (C-18 and C-19), but this carbon showed a very downfield chemical shift (δ80.3), suggesting that it might also be connected to an oxygen atom. Furthermore, the chemical shift of C-1 was moved more downfield (δ73.3), suggesting that the hydroxyl function at C-1 had formed an ether linkage between C-1 and C-4. Thus, **2** has an additional saturated furan ring attached to the saturated pyran ring as shown.

Analysis of the COSY, HETCOR and selective INEPT spectra of **2** led to the determination of its structure and afforded the complete proton and carbon NMR assignments. Computer modelling calculations [8] suggested that H-1 and H-2 of **2** should have the same configuration (calc.: $J_{1\beta,2\beta} = J_{1\alpha,2\alpha} = 2.12$ Hz, $J_{2,3a} = 5.42$ and $J_{2,3b} = 1.38$ Hz, $J_{1\beta,2\alpha} = J_{1\alpha,2\beta} = 9.07$ Hz, $J_{2,3a} = 10.05$ Hz and $J_{2,3b} = 6.99$ Hz; obs.: $J_{1,2} = 2.4$ Hz, $J_{2,3a} =$ 4.8 Hz and $J_{2,3b} < 1.0$ Hz).

Compounds **1** and **2** were subjected to cytotoxicity tests [9, 10], but neither displayed activity.

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Table 1. Summary of the major results of selective INEPT spectra of de-*O*-ethylsalvonitin (**1**) and salprionin (**2**)

Proton	**1** Selective INEPT (carbon)	**2** Selective INEPT (carbon)
1	(2), 5, 9, (10)	(2), 5, 9, (10)
2	(1), (3), 4, 10, 11	(1), 4
3a	1, (2), (3), (4), 18, 19	(4), 18, 19
3b		1, (2), (4), 18, 19
6	8, 10, 20	(5), 8, 10
7	5, 9, 14	5, (8), 9, 14
14	7, 9, 15,	7, 9, 12, (13), 15
15	12, (13), 14, (16), (17)	12, (13), 14, (16), (17)
16	13, (15), 17	13, (15)
17	13, (15), 16	13, (15)
18	3, 19	3, (4)
19	3, 18	3, (4)
20	(5), 6, 10	(5), 6, 10

*Two-bond correlations between ^{1}H and ^{13}C are shown in parentheses.

EXPERIMENTAL

Mps (uncorr.) were determined on a Kofler hot-stage apparatus. The optical rotations were measured with a Perkin-Elmer 241 polarimeter. UV spectra were recorded in MeOH on a Beckman DU-7 spectrometer. IR spectra were recorded in a KBr pellet on a MIDAC FT-IR interferometer. ^{1}H, ^{13}C, APT NMR, NOED and COSY spectra were recorded with a Varian XL-300 spectrometer. 1D heteronuclear ^{1}H–^{13}C shift correlation [11] and selective INEPT spectra [12, 13] were obtained with a Nicolet NMC-360 instrument. Data sets of 16K covering a spectral width of 10 kHz were acquired. For aromatic protons $^3J_{C-H} = 8$ Hz, and aliphatic protons $^3J_{C-H} = 6$ Hz were usually used for selective INEPT experiments. Mass spectra and HR mass spectra were recorded on a Finnigan MAT-90 instrument. Computer modelling calculations were carried out with the software of PCMODEL for Windows [8].

Plant material. The plant material of *S. prionitis* Hance was collected in Jiangxi Province, China, in June, 1986, and identified by Dr X.-L. Huang. Voucher samples are deposited in the herbarium of Shanghai Institute of Materia Medica, Chinese Academy of Sciences, Shanghai.

Extraction and isolation. The air-dried powdered roots of *S. prionitis* (11 kg) were percolated with EtOH at room temp. and the EtOH extract concd *in vacuo* at 50° to afford a thick dark syrup, which was distributed between $CHCl_3$ and H_2O. The organic layer was subjected to CC on silica gel, eluting with $CHCl_3$ [1–4]. Frs containing **1** and **2** were subjected to repeated prep. TLC, using cyclohexane–CH_2Cl_2 (1:4) as solvent, to afford pure **1** and **2**.

De-O-ethylsalvonitin (**1**). Compound **1** was obtained as yellowish crystals (15 mg, 0.00014%) from Me_2CO, mp 100–102°; UV (MeOH) λ_{max} nm (log ε): 219.5 (4.47), 245.5 (4.65), 309.5 (3.65) and 342 (3.49); IR (KBr) ν_{max} cm^{-1}: 3437, 2961, 2930, 1435, 1408, 1373, 1219, 1175, 1055, 1001, 760; ^{1}H NMR ($CDCl_3$) δ: 1.32 (*d*, $J = 6.6$ Hz, 3H, H-16), 1.36 (*d*, $J = 6.6$ Hz, 3H, H-17), 1.57 (*br.s*, 3H, H-19), 1.87 (*br.s*, 3H, H-18), 2.54 (*s*, 3H, H-20), 3.42 (sep., $J = 6.6$ Hz, 1H, H-15), 4.86 (*dq*, $J = 1.5$, 9.0 Hz, 1H, H-3), 4.89 (*d*, $J = 2.4$ Hz, 1H, H-1), 5.39 (*dd*, $J = 2.4$, 9.0 Hz, 1H, H-2), 7.18 (*d*, $J = 8.1$ Hz, 1H, H-6), 7.23 (*s*, 1H, H-14), 7.63 (*d*, $J = 8.1$ Hz, H-7); ^{13}C NMR ($CDCl_3$) δ: 17.1 (C-20), 18.7 (C-18), 22.3 (C-17), 22.9 (C-16), 25.1 (C-19), 27.7 (C-15), 67.3 (C-1), 77.6 (C-2), 116.2 (C-14), 119.1 (C-9), 120.0 (C-3), 124.3 (C-10), 126.3 (C-8), 126.9 (C-6), 127.6 (C-7), 132.7 (C-4), 132.7 (C-5), 136.7 (C-13), 139.4 (C-11), 140.0 (C-12); EIMS: *m/z* (rel. int. %): 312 (M^+, 38), 296 (25), 295 (100), 255 (5), 253 (5), 244 (9), 243 (54), 240 (7), 237 (5), 227 (12), 201 (5); HRMS: *m/z* 312.1725, for $C_{20}H_{24}O_3$, calc. 312.1734.

Salprionin (**2**). Compound **2** was obtained as a yellowish powder (10 mg, 0.0001%), UV (MeOH) λ_{max} nm (log ε): 219 (4.27), 245 (4.52), 308.5 (3.42), 341.5 (3.32); IR (KBr) ν_{max} cm^{-1}: 3450, 2958, 1437, 1368, 1338, 1209, 1171, 1140, 1049, 1030, 998 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ: 1.33 (*d*, $J = 6.6$ Hz, 3H, H-16), 1.36 (*d*, $J = 6.6$ Hz, 3H, H-17), 1.44 (*s*, 3H, H-18), 1.48 (*s*, 3H, H-19), 2.37 (*dd*, $J = 4.8$, 14.4 Hz, 1H, H-3a), 2.41 (*br. d*, $J = 14.4$ Hz, 1H, H-3b), 2.54 (*s*, 3H, H-20), 3.42 (sep., $J = 6.6$ Hz, 1H, H-15), 4.63 (*br. dd*, $J = 2.4$, 4.2 Hz, 1H, H-2), 5.15 (*d*, $J = 2.4$ Hz, 1H, H-1), 7.16 (*d*, $J = 8.4$ Hz, 1H, H-6), 7.23 (*s*, 1H, H-14), 7.63 (*d*, $J = 8.4$ Hz, H-7); ^{13}C NMR ($CDCl_3$) δ: 17.4 (C-20), 22.3 (C-16), 22.3 (C-17), 27.5 C-15), 28.7 (C-19), 29.7 (C-18), 46.0 (C-3), 73.3 (C-1), 79.3 (C-2), 80.3 (C-4), 115.9 (C-14), 119.2 (C-9), 121.5 (C-10), 126.0 (C-8), 126.3 (C-6), 127.2 (C-7), 132.7 (C-5), 134.3 (C-11), 135.9 (C-13), 140.0 (C-12); EIMS *m/z* (rel. int. %): 312 (M^+, 29), 254 (6), 244 (15), 243 (100), 227 (7), 165 (5), 152 (5), 115 (5), 43 (24); HRMS: *m/z* 312.1703 for $C_{20}H_{24}O_3$; calc. 312.1734.

Cytotoxicity assay. The biological evaluation for cytotoxic activities of these compounds was carried out according to established protocols [9, 10].

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