CONVULSANTS FROM STAR ANISE (*ILLICIUM VERUM* HOOK.F.)

Emi OKUYAMA,* Tomonori NAKAMURA, and Mikio YAMAZAKI*

Faculty of Pharmaceutical Sciences, Chiba University, 1-33 Yayoicho, Inage-ku, Chiba 263, Japan

Convulsants veranisatin A and B, isolated from Star Anise (*Illicium verum* Hook.f.), were structurally elucidated by spectroscopic data and compared with anisatin-related compounds.

KEYWORDS veranisatin A; veranisatin B; *Illicium verum*; Star Anise; convulsive component

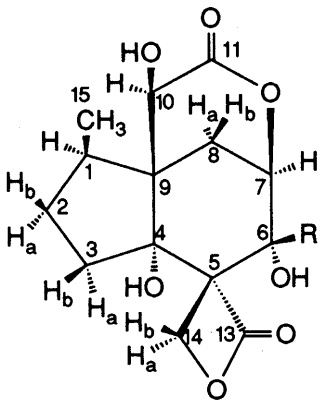
During our research on neurotropic components from medicinal plants,[1] the methanol extract of Star Anise (*Illicium verum* Hook.f., Illiciaceae) was observed to have a hypothermic effect (ΔTmax -5.2 °C, $p<0.001$, 3g/kg,*po*) in mice, and the ethyl acetate extract caused severe convulsions and was lethally toxic depending on the dose. *I. verum* has been in use as a basic spice, and is also used in the treatment of stomach disease, pain, etc., in traditional Chinese and Japanese medicinal systems. Genus Illiciaceae also contains *I.anisatum* L., which is known to have toxic components such as anisatin.[2] This paper reports the first isolation of the convulsants from *I. verum*.

Oral administration of the ethyl acetate extract after being defatted decreased the body temperature (ΔTmax -5.6 °C, $p<0.001$) at a dose of 100 mg/kg, while 500 mg/kg caused severe convulsions and death in mice (4/4). While following the convulsive effects and lethal toxicity of the ethyl acetate extract from the dried fruit (20 kg) of *I. verum*,[3] separation was performed by silica gel (*n*-hexane/ethyl acetate at 10:1~2:1 and $CHCl_3$/methanol at 1:0~10:1), Sephadex LH-20 (methanol), and ODS (water/methanol at 2:1) column chromatographies. Then, one convulsive fraction was further purified through repeated HPLC (Aquasil) eluted with $CHCl_3$/methanol/water at 100:5:0.1 to give two convulsants, veranisatin A (33 mg) and veranisatin B (20 mg).

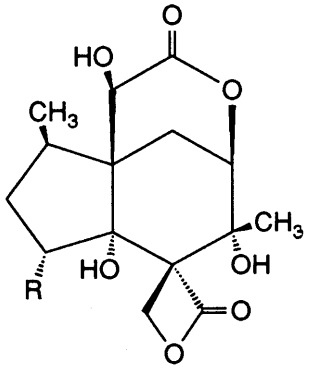
Veranisatin A (**1**), mp 181~182 °C (colorless prisms from ethyl acetate), $[\alpha]_D^{22}$ -14.8° (c=1.0, methanol), had the molecular formula of $C_{16}H_{22}O_8$ on the basis of HR-FAB-MS [m/z 364.1209 $(M+Na)^+$, Δε -0.3 mmu]. The IR spectrum showed hydroxyl (3380 cm^{-1}, br.), β-lactone (1830 cm^{-1}) and δ-lactone (1750 cm^{-1}) groups. The ^1H and ^{13}C-NMR spectra including ^1H-^1H and ^{13}C-^1H COSY indicated the partial structures of C(15)H_3-C(1)H-C(2)H_2-C(3)H_2-, -C(8)H_2-C(7)H-O- and -C(12 or 14)H_2-O-, and also showed the existence of one methoxyl, two ester carbonyl and three hydroxyl groups and four quaternary sp^3 carbons, which were further connected by COLOC.[4] Deuterium-induced differential isotope shifts[5] in the ^{13}C-NMR of **1** were observed at δ71.01, 78.86, 86.10 and 32.88 (0.12, 0.08, 0.12 and 0.07 Hz, respectively) in acetone-d_6 with one drop of CD_3OD-CD_3OH at 1:1, indicating that the carbons at positions 10, 6 and 4 contain hydroxyl groups. The NOEs of δ1.10/δ1.59, δ1.10/δ4.30, δ1.59/δ2.43~2.51, δ2.43~2.51/δ4.30, δ3.53/δ4.03 and δ3.53/δ4.26 indicated the stereochemistry shown in the figure. The absolute stereochemistry was obtained by direct comparison of the CD ($[\theta]_{236}$ -1950, methanol) with those of anisatin (**3**) and neoanisatin (**4**) ($[\theta]_{233}$ -2720 and $[\theta]_{238}$ -1940, respectively). Veranisatin B (**2**), mp 212~213 °C (colorless prisms from ethyl acetate), $[\alpha]_D^{22}$ -14.5° (c=1.0, methanol) exhibited spectral data similar to those of **1**. HR-FAB-MS gave the molecular formula of $C_{16}H_{20}O_9$ [m/z 379.1003 $(M+Na)^+$, Δε -0.2 mmu]. Comparison of the IR

Table. ^{1}H- and ^{13}C-NMR Data of 1 and 2 in Acetone-d_6

	1		2	
No.	C	H	C	H
1	39.50	2.43~2.51(m)	39.36	2.46~2.50(m)
2	31.16	a 2.07~2.14(m)	31.19	a 2.12~2.18(m)
		b 1.59(dddd,12.4,11.7,8.2,2.5)		b 1.65(dddd,12.1,11.7,8.6,2.6)
3	32.88	a 1.67(ddd,12.4,9.3,2.5)	32.87	a 1.75(ddd,13.0,9.0,2.6)
		b 2.43~2.51(m)		b 2.54(ddd,13.0,11.7,6.4)
4	86.10	5.52(OH,s)	87.05	5.82(OH,brs)
5	64.29		63.87	
6	78.86	6.05(OH,d,1.5)	80.67	6.85(OH,brs)
7	79.49	4.26(dd,3.9,2.2)	79.00	4.88(dd,3.9,2.2)
8	27.80	a 2.43(dd,14.7,2.2)	27.51	a 2.47(dd,14.9,2.2)
		b 2.07(dd,14.7,3.9)		b 2.18(dd,14.9,3.9)
9	51.97		51.48	
10	71.01	4.30(d,3.4)	71.06	4.36(d,3.7)
		5.07(OH,d,3.4)		5.25(OH,d,3.7)
11	174.84		174.50	
12	76.36	3.53(d,11.0)	170.06	
		4.05(dd,11.0,1.5)		
13	169.04		168.66	
14	65.15	a 4.30(d,6.1)	65.85	a 4.36(d,5.9)
		b 4.03(d,6.1)		b 4.08(d,5.9)
15	14.22	1.10(3H,d,7.0)	14.11	1.12(3H,d,7.1)
16	60.54	3.41(3H,s)	53.59	3.82(3H,s)



1 : R=C(12)H$_2$OC(16)H$_3$
2 : R=C(12)OOC(16)H$_3$



3 : R=OH
4 : R=H

spectrum of **2** with that of **1** showed one more ester carbonyl absorption at 1740 cm^{-1} together with 1825 cm^{-1} and 1760 cm^{-1}. ^{13}C-NMR also indicated the ester carbonyl at δ170.06, but no methylene carbon which was observed at δ76.36 in **1**. These data indicated the presence of methoxy carbonyl group at C-6. The assignment shown in the table was confirmed by the 2D-NMRs. The CD spectrum ($[\theta]_{237}$ -2370) suggested the same stereostructure as **1**.

Veranisatin A and B presented convulsive effects and lethal toxicity (3/3) at a dose of 3 mg/kg, *po*, while at 1 mg/kg , both compounds showed hypothermia (ΔTmax -4.5 °C, $p<0.001$) but no convulsions.

ACKNOWLEDGEMENTS We thank Professor K.Yamada (Nagoya University) for gifts of anisatin and neoanisatin, Mr.S.Terabayashi (Tsumura Co., Ltd.), Miss Y.Watabe and Miss C.Fukai for their technical assistance, and the Chemical Analysis Center of this University for running the spectra.

REFERENCES AND NOTES

1) E.Okuyama, S.Nishimura, M.Yamazaki,*Chem.Pharm.Bull.*, **41**, 926 (1993); E.Okuyama, L.-H.Gao, T.Nakamura, S.Nishimura, M.Yamazaki, *J.Pharmacobio-Dyn.*, **15**, s-11 (1992); E.Okuyama, L.-H.Gao, M.Yamazaki, *Chem. Pharm. Bull.*, **40**, 2075 (1992); E.Okuyama, S.Nishimura,.M.Yamazaki, *ibid.*, **39**, 405 (1991).

2) J.F.Lane, W.T.Koch, N.S.Leeds, G.Gorin, *J.Am.Chem.Soc.*, **74**, 3211 (1952); K.Yamada, S.Takada, S.Nakamura, Y.Hirata, *Tetrahedron*, **24**, 199 (1968); I.Kouno, M.Hashimoto, S.Enjoji, M.Takahashi, H.Kaneto, C.-S.Yang, *Chem.Pharm.Bull.*, **39**, 1773 (1991).

3) The material was purchased from a commercial outlet of traditional medicines in Japan, and was kindly confirmed by Mr.S.Terabayashi, Tsumura Co. Ltd.

4) ^{1}H-^{13}C long-range correlations derived from the COLOC data, δ1.10 (H_3-15): δ31.16, 39.50, 51.97; δ1.67 (Ha-3): δ86.10; δ2.05~2.14 (Ha-2, Hb-8): δ51.97, 78.86, 79.49, 86.10; δ2.43, 2.43~2.51 (Ha-8, H-1, Hb-3): δ39.50, 51.97, 71.01; δ 3.41 (H_3-16): δ76.36; δ3.53 (Ha-12); δ60.54, 64.29, 78.86; δ4.03 (Hb-14): δ78.86, 86.10, 169.04; δ4.05 (Hb-12): δ60.54, 79.49; δ4.26 (H-7): δ51.97, 64.29, 78.86; δ4.30 (H-10, Ha-14): δ51.97, 86.10, 169.04, 174.84; δ5.07 (10-OH): δ51.97; δ5.52 (4-OH): δ32.88, 51.97, 86.10; δ6.05 (6-OH): δ64.29, 76.36, 78.86.

5) P.E.Pfeffer, K.M.Valentine, F.W.Parrish, *J.Am.Chem.Soc.*, **101**, 1265 (1979).

(Received June 10, 1993)