

A NEW TOXIC NEOANISATIN DERIVATIVE FROM THE PERICARPS OF *ILLICIMUM MAJUS*

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A new toxic neoanisatin-derivative (1) was isolated from the pericarps of *Illicium majus*. The structure of this compound was elucidated by spectroscopic data, including the 2D COSY NMR technique. The toxicity of compound 1 is also described.

KEYWORDS *Illicium majus*; 6-deoxyneoanisatin; neoanisatin; convulsant; 2D COSY NMR

Anisatin and neoanisatin, isolated from the fruits of *Illicium anisatum* L. (Illiciaceae), are well known for their convulsive toxicity and their divergent chemical structures. Only these two compounds have been known for nearly two decades as toxic sesquiterpenes obtained from *I. anisatum* (Japanese star anise).^{1,2} A number of anisatin-like compounds, majucin, neomajucin was also isolated by us from the Chinese *Illicium* plant, *I. majus* HOOK. f. & THOMS, but only neomajucin was revealed to be toxic.³

Further investigation on the constituents of the pericarps of *I. majus* has resulted in the isolation of a new sesquiterpene lactone (1), which is the first example of another toxic neoanisatin-derivative.

The MeOH extract of *I. majus* (1.5 kg) was treated by the previously reported method.³ Fraction II obtained from counter-current distribution of this extract was chromatographed on silica gel using the solvent of CHCl₃-MeOH (97:3), and purified by a Kusano prepacked column Si-5 (*n*-hexane-AcOEt=1:1). Subsequent recrystallization from CHCl₃-AcOEt yielded compound 1 (132 mg).

Compound 1, mp 211-213°C (from CHCl₃-AcOEt); $[\alpha]_D^{25} +67.4^\circ (c=0.23)$ gave the molecular formula, C₁₅H₁₈O₇, by elemental analysis and the mass spectrum (*m/z*: 310). The IR spectrum of 1 demonstrated that the absorptions due to hydroxyl groups at 3480 and 3460 cm⁻¹, and a δ -lactone carbonyl group at 1730 cm⁻¹, along with characteristic absorptions due to a β -lactone carbonyl group at 1825 cm⁻¹ and a cyclopentanone carbonyl group at 1745 cm⁻¹. The presence of a β -lactone group was supported by the signals of AB quartet at δ_{H} 4.59 and 4.90 (each a doublet) with a small coupling constant (*J*=6.6 Hz) in the ¹H-NMR spectrum (in d₅-pyridine) of 1, which also suggested the presence of two secondary methyl groups in CD₃OD solution as two doublet signals at δ_{H} 1.05 (3H, d, *J*=7.0 Hz) and δ_{H} 1.22 (3H, d, *J*=7.3 Hz), and in d₅-pyridine solution as one doublet signal at δ_{H} 1.32 (6H, d, *J*=7.3 Hz). The signals in the ¹³C-NMR spectrum of 1 resembled those of anisatin⁴ aside from the signals due to C-2, C-3 and C-6. In the ¹H-¹H 2D COSY of 1,

Table I. ^1H -(400MHz) and ^{13}C -(100MHz)NMR Data of Compound 1 in d_5 -Pyridine (δ from TMS)

Position	^1H	^{13}C
1	3.20(q, $J=7.3$)	48.8
2	—	214.5
3 α	2.85(d, $J=17.6$)	46.2
3 β	3.63(d, $J=17.6$)	46.2
4	—	77.6
5	—	66.4
6	3.33(dq, $J=2.0, 7.3$)	35.2
7	4.56(ddd, $J=4.0, 2.2, 2.0$)	78.9
8 α	2.74(dd, $J=13.9, 2.2$)	31.2
8 β	2.14(dd, $J=13.9, 4.0$)	31.2
9	—	50.5
10	4.53(s)	69.8
11	—	174.6
12	1.32(d, $J=7.3$)	12.8
13	—	171.5
14 α	4.59(d, $J=6.6$)	64.1
14 β	4.90(d, $J=6.6$)	64.1
15	1.32(d, $J=7.3$)	8.0

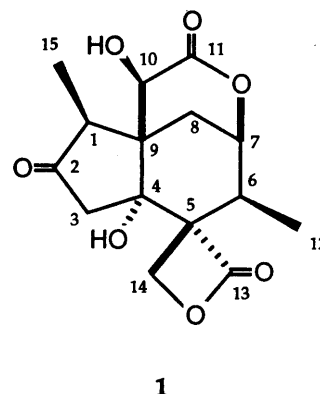


Table II. Dose Dependence Mortality Induced by Compound 1

Dose	Mortality
1.000	1/10
1.250	3/10
1.500	7/10
2.000	8/10
4.000	10/10

$\text{LD}_{50}=1.464 \text{ mg/kg}$ by Probit method.

the connectivities were clarified between H_3 -15-H-1, H_2 -3 α , β , and H-6-H-7-H $_2$ -8 α , β , respectively. These findings suggested that 1 is a 2-oxo-6-dehydroxy neoanisatin-derivative. The configuration at C-1 was confirmed as 15 β -methyl by NOE experiment, which showed enhancement between the signals due to H_3 -15 and H-10 (7%), whereas an NOE was observed between the H_3 -12 signal and one of the H_2 -14 signals ($\delta_{\text{H}}4.59$) (3%). These observations demonstrated that 1 has the same configuration as that of neoanisatin, thus the structure of 1 was determined to be 2-oxo-6-dehydroxyneoanisatin.

The toxic effect of compound 1 was examined using ddY-strain mice weighing 25-28g, with 10 animals in each dose group. When the mice were treated with this compound, the animals exhibited picrotoxin-like convulsion, in a dose-dependent manner, which is shown in Table II. The toxicity of this compound (1.46 mg/kg) is less than that of anisatin or neoanisatin (1.0 mg/kg), but nearly equivalent to that of picrotoxin.

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