A NEW TOXIC NEOANISATIN DERIVATIVE FROM THE PERICARPS OF *ILLICIUM 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 $CHCl_3$-MeOH (97:3), and purified by a Kusano prepacked column Si-5 (*n*-hexane-AcOEt=1:1). Subsequent recrystallization from $CHCl_3$-AcOEt yielded compound **1** (132 mg).

Compound **1**, mp 211-213°C (from $CHCl_3$-AcOEt); $[\alpha]_D$ +67.4°(*c*=0.23) gave the molecular formula, $C_{15}H_{18}O_7$, 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 δ4.59 and 4.90 (each a doublet) with a small coupling constant (*J*=6.6Hz) in the ^{1}H-NMR spectrum (in d_5-pyridine) of **1**, which also suggested the presence of two secondary methyl groups in CD_3OD solution as two doublet signals at δ_H1.05 (3H,d, *J*=7.0Hz) and δ_H1.22(3H,d,*J*=7.3Hz), and in d_5-pyridine solution as one doublet signal at δ_H1.32(6H,d,*J*=7.3Hz). The signals in the ^{13}C-NMR spectrum of **1** resembled those of anisatin[4] aside from the signals due to C-2, C-3 and C-6. In the ^{1}H-^{1}H 2D COSY of **1**,

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

Position	^{1}H	^{13}C
1	3.20(q,*J*=7.3)	48.8
2	—	214.5
3α	2.85(d,*J*=17.6)	46.2
3β	3.63(d,*J*=17.6)	
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
8α	2.74(dd,*J*=13.9,2.2)	31.2
8β	2.14(dd,*J*=13.9,4.0)	
9	—	50.5
10	4.53(s)	69.8
11	—	174.6
12	1.32(d,*J*=7.3)	12.8
13	—	171.5
14α	4.59(d,*J*=6.6)	64.1
14β	4.90(d,*J*=6.6)	
15	1.32(d,*J*=7.3)	8.0

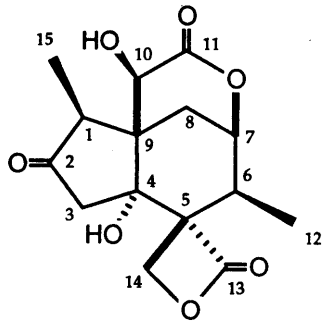

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Table II. Dose Dependence Mortality Induced by Compound 1

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

LD_{50}=1.464 mg/kg by Probit method.

the connectivities were clarified between H_3-15-H-1, H_2-3α,β, and H-6-H-7-H_2-8α,β, respectively. These findings suggested that **1** is a 2-oxo-6-dehydroxy neoanisatin-derivative. The configuration at C-1 was confirmed as 15β-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(δ_H4.59) (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.

REFERENCES

1) J.F.Lane, W.T.Koch, N.S.Leeds, and G.Gorin, *J.Am.Chem.Soc.*, **74**, 3211 (1952).
2) K.Yamada, S.Takada, S.Nakamura, and Y.Hirata, *Tetrahedron*, **24**, 199 (1968).
3) I. Kouno, N. Baba, M. Hashimoto, N. Kawano, M. Takahashi, H. Kaneto, C.-S. Yang, and S. Sato, *Chem,Pharm.Bull.*,**37**, 2448 (1989); and references cited therein.
4) S. Manabe, K. Wakamatsu, Y. Hirata, and K.Yamada, *Tetrahedron*, **35**, 1925 (1979).

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