Structures of Anislactone A and B; Novel Type of Sesquiterpene Lactones from the Pericarps of *Illicium anisatum*

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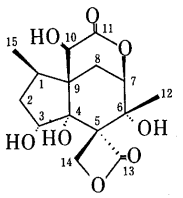
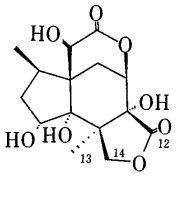
Two new sesquiterpene lactones, named anislactone A and B, were isolated from the pericarps of *Illicium anisatum*. The structure of anislactone A was established by X-ray crystallographic analysis. Anislactone B was the isomer of anislactone A, and its structure was determined by spectral data compared with those of anislactone A. They were considered to be the biological derivatives from anisatin or its derivative.

Keywords *Illicium anisatum*; Illiciaceae; anislactone A; anislactone B; X-ray analysis; Japanese star anise; anisatin; neoanisatin; NOE

Illicium anisatum L., the toxic plant belonging to Illiciaceae, is the sole species native to Japan. The convulsive constituent, anisatin, was first isolated by Lane[1] together with the non-toxic constituent, pseudoanisatin, from this plant. Hirata and co-workers elucidated the structure of anisatin in 1965,[2,3] as well as isolating another toxic sesquiterpene, neoanisatin.[3,4] The structure of pseudoanisatin was finally established by us in 1983.[5] These compounds have the same carbon skeleton, which is a unique type of sesquiterpene lactone. Our study on the constituents of Chinese *Illicium* plants for several years has resulted in the isolation of a new type of anisatin-like sesquiterpene lactones,[6–8] which we call majucin-type sesquiterpene. These results enabled us to reinvestigate the constituents of Japanese star anise, the fruits of *Illicium anisatum*, and we previously reported the isolation of 6-deoxymajucin from the seeds of this plant.[9] At the present time, our reinvestigation of the pericarps of *I. anisatum* has resulted in the isolation of a new skeletal type of sesquiterpenes.[10]



anisatin



majucin

From the dried pericarps (5.5 kg) of *Illicium anisatum*, compound **1** (18 mg), named anislactone A, and compound **2** (15 mg), named anislactone B, were isolated as crystals.

Anislactone A (**1**) was obtained as colorless prisms, mp 274—275 °C, $[\alpha]_D$ −47.7° (c=0.21, dioxane), crystallizing from AcOEt–$CHCl_3$, and has the molecular formula $C_{15}H_{20}O_6$ analyzed by electron impact mass spectrum (EI-MS) and proton and carbon counts in their nuclear magnetic resonance (NMR) spectra. Absorptions due to hydroxyl groups at 3400 and 3360 cm^{-1} and γ- or δ-lactone at 1730 cm^{-1} were shown in the infrared (IR) spectrum of **1**. The carbon-13 (^{13}C-) NMR spectrum of **1** exhibited two lactone carbonyl carbon signals at δ 176.8 and 181.0, indicating that **1** has two lactone moieties. Although its molecular formula and the presence of two lactone moieties suggested the anisatin-type structure for **1**, the spectral features of **1** indicated differences from those of anisatin- or majucin-type compounds; *i.e.* the proton (^1H-) NMR spectrum of **1** showed three singlet methyl signals at δ 1.33, 1.45 and 1.65, along with two AB type signals at δ 3.36 and 3.65 (J=17.2 Hz) and δ 3.99 and 4.54 (J=9.9 Hz), respectively. Moreover, the carbon-13–proton (^{13}C–^1H) long-range 2D (two-dimensional) correlation spectroscopy of **1** indicated that both the lactone moieties are γ. These remarkable features have not been seen in the spectral data of anisatin- or majucin-type compounds.

TABLE I. ^1H-NMR Data for Compounds **1** and **2** (400 MHz, δ from TMS in Pyridine-d_5 and J/Hz in Parentheses)

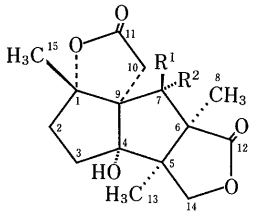
Protons	1	2
2 and 3	2.40 (1H, dd, J=13.6, 7.2) 2.03—2.17 (3H, m)	2.39—2.14 (4H, m)
7	4.65 (1H, s)	4.28 (1H, d, J=7.0)
8	1.45 (3H, s)	1.32 (3H, s)
10a	3.36 (1H, d, J=17.2)	3.45 (1H, d, J=16.5)
10b	3.65 (1H, d, J=17.2)	2.94 (1H, d, J=16.5)
13	1.33 (3H, s)	1.32 (3H, s)
14a	3.99 (1H, d, J=9.9)	3.99 (1H, d, J=8.4)
14b	4.54 (1H, d, J=9.9)	4.80 (1H, d, J=8.4)
15	1.65 (3H, s)	1.87 (3H, s)
4-OH		7.02 (1H, s)
7-OH		8.61 (1H, d, J=7.0)

Assignments were aided by the ^1H–^1H 2D COSY and NOE spectra.

TABLE II. ^{13}C-NMR Data for Compounds **1** and **2** (δ from TMS in Pyridine-d_5)

Carbons	1	2
1	95.9 s	97.0 s
2	37.8 t	39.1 t
3	36.3 t	36.9 t
4	88.4 s	91.6 s
5	55.4 s[a)]	57.7 s[a)]
6	57.6 s[a)]	62.7 s[a)]
7	72.7 d	83.4 d
8	11.9 q	15.8 q
9	68.3 s	68.5 s
10	31.7 t	38.5 t
11	176.8 s	175.7 s
12	181.0 s	179.8 s
13	17.8 q	19.0 q
14	73.7 t	75.0 t
15	21.1 q	21.6 q

a) Assignments may be interchanged. Assignments were aided by the ^{13}C–^1H long-range 2D COSY or HMBC spectra.



1 : R^1=H, R^2=OH anislactone A
2 : R^1=OH, R^2=H anislactone B

Fig. 1

TABLE III. The ^1H and ^{13}C Correlations Shown in the ^{13}C–^1H Long-Range COSY of Compound **1**, and the HMBC Spectrum of Compound **2**

Protons	**1** Carbons	**2** Carbons
H-2α,β	—	C-3 (C-1, C-4)[b]
H-3α,β	—	
H-7	C-8, C-11	C-4, C-5, C-8, C-10
H-8	C-5, C-6, C-7, C-12	(C-5, C-6, C-7, C-12)[a]
H-10a	C-1, C-11	C-1, C-4, C-9, C-11
H-10b	C-11	C-4, C-7, C-9, C-11
H-13	C-4, C-5, C-6, C-14	(C-4, C-5, C-6, C-14)[a]
H-14a	C-11, C-13	C-12, C-13
H-14b	C-13	C-4, C-13
H-15	C-1, C-2,[a] C-9	C-1, C-2,[a] C-9
4-OH		C-9
7-OH		C-9

a) These assignments were made after the structure determination. *b*) These cross peaks could not be assigned because the exact chemical shifts of H-2 and H-3 signals were defined.

Acetylation of **1** with 4-dimethylaminopyridine (DMAP), acetic anhydride and pyridine for 3 d yielded monoacetyl derivative (**3**) with the recovering of **1**, indicating the presence of a strongly hindered secondary hydroxyl group. While a part of the structure of **1** was elucidated by its ^{13}C–^1H long-range 2D COSY as shown in Table III, the connectivities around C-2 and C-3 were not clarified because no correlations were seen for C-2 and C-3. Accordingly, as the whole structure of **1**, including the stereo-structure, could not be derived from spectral and chemical data, a single crystal X-ray analysis was performed on a crystal of **1**.

Crystallographic analysis for **1**, crystallized from AcOEt–$CHCl_3$ in the orthorombic space group, was performed on a 4-circle diffractometer equipped with graphite-monochromatized CuK_α radiation using the 2θ–θ scan technique ($2\theta < 128°$).

The structure was solved by direct methods using the MULTAN84[11] series of programs, and refined by block-diagonal least-squares methods. The final refinements with anisotropic temperature factors for the non-hydrogen atoms and isotropic temperature factors for the hydrogen atoms converged the *R*-value to 0.054. The atomic positional parameters, bond lengths, and bond angles are shown in Tables IV, V, and VI, respectively.[12] An ORTEP drawing of **1** (nonhydrogen atoms) is shown in Fig. 2. In our preliminary report,[10] the authors made a regrettable failure, and wish to apologize for the critical mistake in depicting the configuration at C-7 of anislactone A. It should have been represented as 7α-OH, not 7β-OH.

The molecular formula of anislactone B (**2**), colorless

TABLE IV. Fractional Atomic Coordinates ($\times 10^4$) and Thermal Parameters ($Å^2$) for Compound **1** with Estimated Standard Deviations in Parentheses

Atom	*x*	*y*	*z*	B_{eq}
O(1)	4680 (1)	7733 (2)	7166 (2)	2.99
O(2)	3344 (1)	7915 (2)	2350 (2)	3.61
O(3)	2582 (1)	10871 (2)	5325 (3)	4.35
O(4)	3348 (1)	11423 (2)	7131 (3)	3.43
O(5)	4937 (1)	4420 (2)	4727 (3)	4.11
O(6)	4062 (1)	4813 (2)	6091 (3)	3.42
C(1)	3572 (1)	5944 (3)	6130 (4)	2.87
C(2)	3490 (1)	6426 (4)	8126 (4)	3.83
C(3)	3647 (1)	8091 (3)	8187 (4)	3.03
C(4)	4100 (1)	8312 (3)	6622 (3)	2.19
C(5)	4135 (1)	9918 (3)	5836 (4)	2.46
C(6)	3593 (1)	9969 (3)	4549 (3)	2.46
C(7)	3378 (1)	8325 (3)	4196 (3)	2.42
C(8)	3680 (1)	10884 (3)	2804 (4)	3.82
C(9)	3846 (1)	7306 (3)	5143 (3)	2.23
C(10)	4329 (1)	6593 (3)	3922 (4)	2.75
C(11)	4493 (1)	5191 (3)	4913 (4)	2.99
C(12)	3111 (1)	10783 (3)	5653 (4)	2.96
C(13)	4757 (1)	10224 (3)	4952 (4)	3.26
C(14)	4000 (1)	11149 (3)	7214 (4)	3.24
C(15)	3021 (1)	5209 (3)	5304 (5)	4.53

TABLE V. Bond Lengths (Å) for Compound **1** with Estimated Standard Deviations in Parentheses

O(1)–C(4)	1.439 (3)	C(3)–C(4)	1.544 (4)
O(2)–C(7)	1.417 (3)	C(4)–C(5)	1.552 (3)
O(3)–C(12)	1.196 (3)	C(4)–C(9)	1.524 (3)
O(4)–C(12)	1.341 (3)	C(5)–C(6)	1.533 (3)
O(4)–C(14)	1.462 (3)	C(5)–C(13)	1.545 (4)
O(5)–C(11)	1.208 (3)	C(5)–C(14)	1.530 (4)
O(6)–C(1)	1.481 (3)	C(6)–C(7)	1.566 (4)
O(6)–C(11)	1.334 (3)	C(6)–C(8)	1.542 (4)
C(1)–C(2)	1.551 (4)	C(6)–C(12)	1.526 (4)
C(1)–C(9)	1.543 (4)	C(7)–C(9)	1.546 (3)
C(1)–C(15)	1.513 (4)	C(9)–C(10)	1.536 (4)
C(2)–C(3)	1.528 (4)	C(10)–C(11)	1.497 (4)

TABLE VI. Bond Angles (°) for Compound **1** with Estimated Standard Deviations in Parentheses

Atom	Angle (e.s.d.)	Atom	Angle (e.s.d.)
C(12)–O(4)–C(14)	110.3 (2)	C(5)–C(6)–C(12)	103.1 (2)
C(1)–O(6)–C(11)	111.2 (2)	C(5)–C(6)–C(7)	108.2 (2)
O(6)–C(1)–C(2)	107.1 (2)	C(7)–C(6)–C(8)	113.3 (2)
O(6)–C(1)–C(9)	104.1 (1)	C(7)–C(6)–C(12)	109.0 (1)
O(6)–C(1)–C(15)	106.5 (2)	C(8)–C(6)–C(12)	106.5 (2)
C(2)–C(1)–C(9)	106.2 (2)	O(2)–C(7)–C(6)	114.9 (2)
C(2)–C(1)–C(15)	114.3 (2)	C(6)–C(7)–C(9)	106.0 (1)
C(9)–C(1)–C(15)	117.8 (3)	O(2)–C(7)–C(9)	108.7 (2)
C(1)–C(2)–C(3)	105.8 (2)	C(4)–C(9)–C(7)	103.0 (2)
C(2)–C(3)–C(4)	104.4 (2)	C(4)–C(9)–C(10)	114.3 (1)
O(1)–C(4)–C(3)	108.7 (2)	C(7)–C(9)–C(10)	116.2 (3)
O(1)–C(4)–C(5)	113.1 (1)	C(1)–C(9)–C(4)	105.6 (2)
C(3)–C(4)–C(5)	115.7 (2)	C(1)–C(9)–C(7)	114.7 (1)
C(3)–C(4)–C(9)	103.1 (1)	C(1)–C(9)–C(10)	102.9 (2)
O(1)–C(4)–C(9)	108.5 (2)	C(9)–C(10)–C(11)	103.1 (2)
C(5)–C(4)–C(9)	107.2 (2)	O(5)–C(11)–O(6)	120.7 (4)
C(4)–C(5)–C(6)	102.8 (1)	O(5)–C(11)–C(10)	128.2 (4)
C(4)–C(5)–C(13)	111.6 (1)	O(6)–C(11)–C(10)	111.1 (2)
C(4)–C(5)–C(14)	113.9 (3)	O(3)–C(12)–O(4)	121.3 (4)
C(6)–C(5)–C(13)	115.2 (3)	O(4)–C(12)–C(6)	111.6 (1)
C(6)–C(5)–C(14)	103.9 (1)	O(3)–C(12)–C(6)	127.1 (4)
C(13)–C(5)–C(14)	109.3 (2)	O(4)–C(14)–C(5)	106.6 (2)
C(5)–C(6)–C(8)	116.0 (1)		

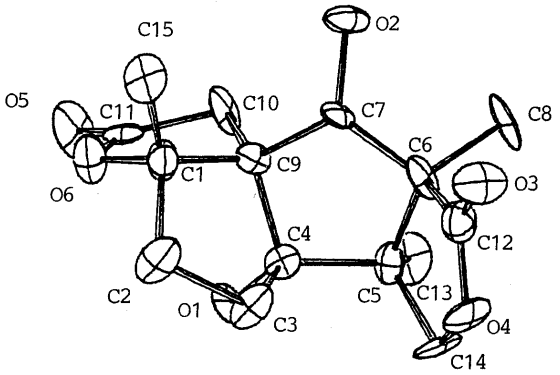


Fig. 2. Molecular Conformation for Compound **1** and Its Atomic Labelling

prisms, mp 273—275 °C, $[\alpha]_D$ −12.6° (c=0.25, dioxane), was determined as $C_{15}H_{20}O_6$, the same as that of **1**, on the basis of the EI-MS [m/z 278 ($M^+ - H_2O$)] and elemental analysis. Its IR spectrum showed the absorptions due to hydroxyl groups at 3390 and 3370 cm^{-1} together with lactone carbonyl groups at 1750 cm^{-1}, which corresponded to the signals of lactone carbonyl carbons at δ 175.7 and 179.8 in the ^{13}C-NMR spectrum of **2**. On the other hand, the features of the ^{1}H-NMR spectrum of **2** were very similar to those of **1**; *i.e.* there are three singlet methyl signals at δ 1.32 (6H) and 1.87 (3H), and two AB type signals at δ 2.94 and 3.45 (J=16.5 Hz), and δ 3.99 and 4.80 (J=8.4 Hz), respectively. In the heteronuclear multiple bond correlation (HMBC) spectrum of **2**, as is summarized in Table III, most parts of the structure of **2** revealed themselves to be similar to those of the structure of **1**. In this spectrum, more correlations were disclosed than those of **1** in the ^{13}C–^{1}H long-range COSY. Therefore, the difference between the structures of compounds **1** and **2** was considered to be due to the configuration of the 7-hydroxyl group and/or the formation of the γ-lactone moiety, which includes C-10 and C-11 carbons, with a 1- or 4-hydroxyl group. However, the arrangement of this γ-lactone formation was impossible to clarify from the HMBC spectrum of **2**, because the chemical shifts of the C-1 and C-4 signals could not be unambiguously ascribed at this stage. As the chemical shifts of the signals of C-1, C-9 and C-4 were almost similar to those of **1** in the ^{13}C-NMR spectrum of **2**, this γ-lactone should be formed with 1-hydroxyl group, the same with that of **1**. Whereas, the C-7 carbon signal of **2** was shifted to lower field from that of **1** by 10.7 ppm suggesting that the configuration at the 7-hydroxyl group of **2** was reversed from that of **1**.

To confirm this presumption, experiments of the nuclear Overhauser effect (NOE) were performed in the ^{1}H-NMR spectra of **1** and **2**. As expected from the structure of **1**, the signal at δ 4.65 (H-7) was enhanced together with one of the H-10 signals at δ 3.65, when the signal of H-15 at δ 1.65 was irradiated in the ^{1}H-NMR spectrum of **1**. However, in the case of compound **2**, when the signal of H-7 (δ 4.28) was irradiated, enhancements of H-8 and one of the H-10 signals at δ 2.94 were observed together with a small NOE of H-15 in the ^{1}H-NMR spectrum of **2**. In addition to these results, when the signal at δ 1.32 (8-and 13-methyls) was irradiated, the signals of H-10 (δ 3.45), H-14 (δ 3.99), H-7 (δ 4.28) and 4-OH (δ 7.02) were enhanced,

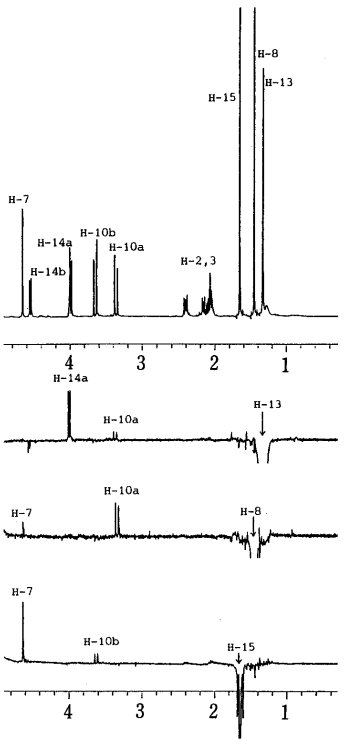


Fig. 3. ^{1}H-NMR Spectrum of Anislactone A and NOE Spectra

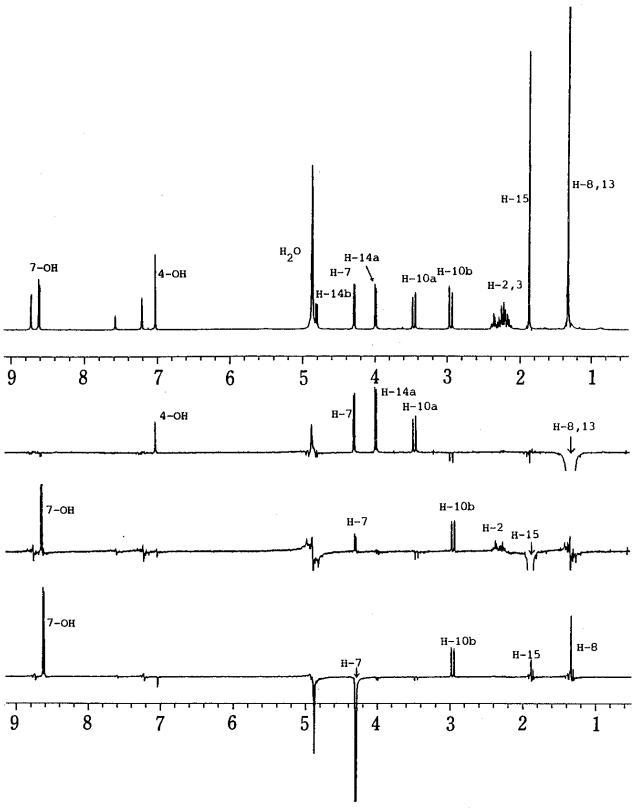


Fig. 4. ^{1}H-NMR Spectrum of Anislactone B (in C_5D_5N) and NOE Spectra

respectively, and when the signal of H-15 was irradiated, the H-2β, H-10b, H-7, and 7-OH proton signals were enhanced, respectively. These results are summarized in Tables III and IV. Consequently, the structure of anislactone B (**2**) was established as 7-epimer of anislactone A.

Although the carbon skeleton of anislactone A and B seemed to be biologically derived from the majucin-type compound, the carbon–carbon bond in which the ring contraction took place was not estimated. In our preliminary report,[10] we proposed that the ring contraction occurred between C-8 and C-9 in the structure of the majucin-type compound, because of the presence of a secondary hydroxyl group at C-7. However, the isolation of the epimers of the 7-hydroxyl group indicated that the ring contraction came about between C-7 and C-8, followed by the bond formation of C-6 and C-8, and hydroxylation at C-8. In this case, C-7 should become a methyl group. Although, this seemed to be a better explanation for converting from the majucin-type compound to anislactones, the inversion of the C-9 configuration still suggested the collapse of the C-8 and C-9 bond. Thus, we applied the numbering scheme for **1** as in Fig. 1. These compounds have a unique type of carbon skeleton, which has never been found in natural compounds.

Experimental

Melting points were determined on a Yanagimoto hot-stage apparatus and are uncorrected. IR spectra were recorded on a JASCO IR-810 spectrometer. MS were taken on a JEOL JMS-DX-303 spectrometer.

NMR spectra were recorded on a JEOL GX-400 spectrometer operating at 399.65 MHz for ^1H and 100.40 MHz for ^{13}C nuclei. NOE and 2-dimensional experiments were performed on the same apparatus. Chemical shifts are reported in ppm relative to tetramethylsilane as the internal standard.

Optical rotations were taken with a JASCO DIP-181 spectrometer. Merck Silica gel 60 (particle size 0.040—0.063 and 0.063—0.200 mm) were used for column chromatography.

Extraction and Isolation. Dried pericarps (5.5 kg) of *I. anisatum* were crushed and extracted at an ambient temperature three times (31 × 3) with MeOH. The extract was evaporated to give a brown gum (1.54 kg), which was dissolved in water and defatted with *n*-hexane, then partitioned between AcOEt and water. The AcOEt-soluble portion was subjected to counter-current distribution using the solvent system of AcOEt–water (1 : 1) to give 1—82 fractions (each 20 ml). Fractions 65—77 were combined (8.8 g) and fractionated using a column of silica gel [$CHCl_3$–MeOH (97 : 3)] to give eight fractions. Of these fractions, the residue of fraction 3 was further purified by a column of silica gel to yield **1** (18 mg). Compound **2** was initially separated as a mixture with **1**, and was hard to purify. Thus, repeated recrystallization of this mixture from AcOEt–MeOH afforded pure **2** (15 mg).

Anislactone A (1) Colorless prisms. mp 274—275 °C (from AcOEt–$CHCl_3$). $[\alpha]_D^{18}$ −47.7° (c=0.21, dioxane). EI-MS m/z: 296 (10%, M^+). IR ν_{max}^{Nujol} cm^{-1}: 3400, 3350 (OH), 1735, 1728 (CO).

Acetylation of 1 Compound **1** (2 mg) was dissolved in a mixture of dry pyridine (0.5 ml), Ac_2O (0.5 ml) and a grain of DMAP and the solution was left for 3 d at room temperature, and was then evaporated under reduced pressure. The residue was chromatographed on silica gel to yield a trace amount of monoacetylated compound **3** as an oil; m/z 338 (5%, M^+). In the ^1H-NMR spectrum, only the signals of δ_H (C_5D_5N): 1.26, 1.27 and 1.41 (each 3H, s, $-CH_3$), 2.15 (3H, s, $-COCH_3$) were identified.

Crystal Data for 1 Crystal of **1** (dimensions 0.5 × 0.5 × 0.5 mm), crystallized from AcOEt–$CHCl_3$, was used for the X-ray analysis. Intensity data were obtained on a Rigaku AFC-5R apparatus equipped with graphite-monochromatized CuK_α radiation and using the 2θ–θ scan technique ($2\theta < 128°$). Of 1431 independent reflections measured, only 1406 were considered as observed on the basis of criterion $F_O > 2\sigma(F_O)$.

Crystal data: $C_{15}H_{20}O_6$, M_w=296, orthorombic, space group $P2_12_12_1$, a=22.095(7), b=8.938(2), c=7.407(2) Å, U=1462.8 Å^3, D_c=1.35 g·cm^{-3}, μ (CuK_α)=8.8 cm^{-1}, T=297 K.

The structure was solved by direct methods using the MULTAN84 series of programs, with RATAN being used to obtained the phases and refined by block-diagonal least-squares methods. Positions of the hydrogen atoms were estimated using standard geometry. The final refinements with anisotropic temperature factors for the non-hydrogen atoms and isotropic temperature factor for hydrogen atoms were lowered R value to 0.054.

Anislactone B (2) Colorless prisms. mp 273—275 °C (from MeOH–AcOEt). $[\alpha]_D^{17}$ −12.6° (c=0.25, dioxane). EI-MS m/z: 278 (30%, $M^+ - H_2O$). IR ν_{max}^{Nujol} cm^{-1}: 3390, 3370 (OH), 1755, 1750 (CO). *Anal.* Calcd for $C_{15}H_{20}O_6$: C, 60.80; H, 6.80. Found: C, 60.52; H, 6.93.

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