This fraction (1 g × 2) was subjected to prep PC with solvent I and a band corresponding to that of hexasaccharides was extracted with H_2O. This extract was concd *in vacuo* and lyophilized to give a white powder (336 mg), which was applied to a carbon–Celite column (1:1; 5.5 × 62 cm; pre-washed with HCl) which, after washing with H_2O (1.0 l.), was eluted successively with 13% (5.0 l.), 15% (5.0 l.) and 17% EtOH. Two fractions eluted with 17% EtOH of volumetric ranges 0.8–2.0 l. and 2.4–4.0 l. were pooled, desalted with Amberlite IR 120B and Amberlite IRA 410, concd and lyophilized to afford two white powders (95 and 110 mg). They were termed saccharide A and saccharide B in order of emergence.

Methylation and methanolysis. Methylation of the isolated saccharides was conducted by the method of Hakomori [11] as described in the previous paper [2], and the methylated saccharides were methanolysed by heating with 1.5% MeOH–HCl at 92° for 5 min. The reaction mixture was treated with Amberlite IR 120B and IRA 410 to remove HCl, and evapd *in vacuo* to dryness.

GLC of methanolysates. The methanolysates were dissolved in a small quantity of MeOH and injected onto a stainless steel column (3 mm × 1 m) packed with 15% butane-1,4-diol succinate polyester on acid-washed Celite. The flow rate of carrier nitrogen gas was 40 ml/min.

Hydrolysis. (1) Partial hydrolysis: the isolated saccharide (20 mg) was dissolved in 0.025 M $(COOH)_2$ (5 ml) and partially hydrolysed by heating at 60° for 15 min. (2) Complete hydrolysis: the isolated saccharide (2 mg) was dissolved in 0.1 M HCl (0.5 ml) and hydrolysed by heating at 100° for 30 min. (3) Enzymatic hydrolysis: the isolated saccharide (2 mg) was hydrolysed by incubating with β-fructofuranosidase (0.2 ml; 0.4 mg of Sigma VI yeast β-fructofuranosidase in 0.2 ml of McIlvaine buffer, pH 5.5) at 30° for 15 hr.

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ANGELOYLCUMAMBRIN-B, AN ANTIMICROBIAL SESQUITERPENE LACTONE FROM *CHRYSANTHEMUM ORNATUM* VAR. *SPONTANEUM*

MITSUMASA HARUNA, MAKIKO KATO, KAZUO ITO, TOSHIAKI NIKAI,* HISAYOSHI SUGIHARA* and HIROYUKI MURATA†

Division of Natural Products Chemistry, Faculty of Pharmacy, Meijo University, Yagoto, Tempaku-ku, Nagoya 468, Japan; *Division of Microbiology, Faculty of Pharmacy, Meijo University, Yagoto, Tempaku-ku, Nagoya 468, Japan

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Key Word Index—*Chrysanthemum ornatum* var. *spontaneum*; Compositae; angeloylcumambrin-B; guaianolide; new sesquiterpene lactone; microbial growth-inhibitor.

Abstract—Angeloylcumambrin-B, a new antimicrobial guaianolide sesquiterpene lactone, was isolated from *Chrysanthemum ornatum* and the structure was determined by a combination of chemical and physical methods.

In the continuing research for physiologically active sesquiterpene lactones of the Compositae [1–3], we have examined the fresh whole herbs of *Chrysanthemum ornatum* Hemsl var. *spontaneum* (Makino) Kitam. [≡*C. japonense* (Makino) Nakai] gathered in November 1977 in Kagoshima, Japan. In the present paper, we describe the isolation and structure determination of a new antimicrobial guaianolide sesquiterpene lactone, angeloylcumambrin-B (**1**) (0.01%), together with the previously known sesquiterpenoids, cumambrin-A (**2**) (0.05%) [4], cumambrin-B (**3**) (0.0075%) [4], and handelin (**4**) (0.075%) [5].

Angeloylcumambrin-B [(**1**); colourless oil; $[\alpha]_D^{20}$ +100° (c = 0.2, MeOH); UV λ_{max} nm (ε): 211 (13 300); CD: $[\theta]_{223}$ +13 200, $[\theta]_{260}$ −2000] showed the MS molecular ion at m/z 346, in agreement with the molecular formula $C_{20}H_{26}O_5$. The presence of an α-methylene-γ-lactone moiety was confirmed by IR bands ($CHCl_3$) at 1770 and 1670 cm^{-1} and also by the presence in the ^1H NMR spectrum of a characteristic pair of low-field doublets at δ 6.13 (1H, J = 3.0 Hz) and δ 5.47 (1H, J = 2.5 Hz) and in the ^{13}C NMR spectrum of a triplet at

†Present address: Junicho, Ibuski 891-04, Japan.

δ 121.1 (C-13). In the ^{1}H NMR spectrum the signals attributable to methyl groups were observed. A singlet at δ 1.23 could be assigned to a methyl group attached to a carbon further substituted by an oxygen function. A broadened singlet was observed at δ 1.94, and it was assigned to a vinyl methyl group (C_4-Me). The presence of the *tert*-OH group was indicated by an IR band at 3650 cm^{-1} and by resistance to the formation of an acetate. The ^{13}C NMR spectrum of **1** was similar to that of cumambrin-A (**2**) except for the presence of the ester side-chain and the absence of the acetate (cf. Table 1).

Table 1. ^{13}C NMR spectral data (25.05 MHz) of lactones **1** and **2***

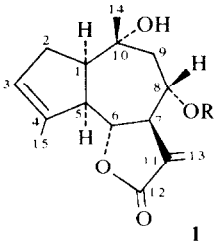
Carbon No.	1	2
1	54.2 *d*	54.2 *d*
2	38.8 *t*	38.9 *t*
3	125.2 *d*	125.4 *d*
4	143.3 *s*	143.7 *s*
5	54.2 *d*	54.4 *d*
6	73.2 *d*	72.9 *d*
7	46.3 *d*	46.8 *d*
8	80.1 *d*	80.3 *d*
9	33.4 *t*	33.5 *t*
10	73.3 *s*	73.6 *s*
11	138.2 *s*	138.7 *s*
12	169.1 *s*	169.4 *s*
13	121.0 *t*	121.1 *t*
14	33.2 *q*	33.5 *q*
15	17.8 *q*	17.8 *q*
1′	—	166.8 *s*
2′	—	127.1 *s*
3′	—	139.7 *d*
4′	—	15.8 *q*
5′	—	20.6 *q*
OCOMe	169.8 *s*	—
OCOCH$_3$	21.3 *q*	—

*Run in $CDCl_3$ on a Jeol FX-100 spectrometer with Me_4Si as internal standard. *s*, singlet; *d*, doublet; *t*, triplet; *q*, quartet. Assignment established by single frequency off-resonance decoupling.

The nature of the ester side-chain of **1** was revealed by its IR band (1718 cm^{-1}) and the ^{1}H NMR spectrum, which showed characteristic signals for the methyl [δ 2.03 (*dd*, 3H, J = 1.5, 7.0 Hz, 4′-Me) and δ 1.92 (*d*, 3H, J = 1.5 Hz, 5′-Me)] and vinyl [δ 6.18 (*m*, 1H, 3′-H)] protons of an angeloyl residue.

From the above data, the new guaianolide appears to be the angeloyl derivative of cumambrin-B (**3**). This assumption was confirmed as follows: alkaline hydrolysis of (**1**) with 10% KOH in dioxane followed by acetylation with Ac_2O–pyridine afforded the guaianolide, which was completely identical with cumambrin-A (**2**) by comparison of the IR and ^{1}H NMR spectra. Thus, the structure of the new guaianolide was established to be **1**.

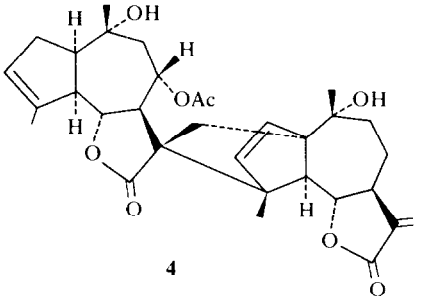
Angeloylcumambrin-B (**1**) showed antimicrobial activity against *Escherichia coli*, *Staphylococcus pygenes*, *Mycobacterium smegmatis* and *Candida albicans* at *ca* 500 ppm.



1 R = CO-C(Me)=CH-Me (angeloyl)
1′ 2′ 5′ 3′ 4′

2 R = Ac

3 R = H



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