Arch. Pharm. Res. Vol.21, No.5, pp. 591-594, 1998

Structural Analogues of Cumambrin B from the Flower of *Chrysanthemum boreale*

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(Received June 4, 1998)

The structural analogues of cumambrin B (**1, 2, 3, 4**) were isolated from the flower of *Chrysanthemum boreale* Makino. The structures of compounds were determined by two-dimensional ^{1}H-^{1}H COSY and ^{13}C-^{1}H COSY spectra with the aid of homonuclear and heteronuclear double resonance experiment. The stereochemistry of compounds has been verified from single crystal X-ray diffraction of cumambrin A (**2**). The antimicrobial activities of these guaianolides have been studied.

Key words : *Chrysanthemum boreale*, Cumambrin A, Cumambrin B, Angeloylcumambrin B, Tigloylcumambrin B, Guaianolide, Antimicrobial activity

INTRODUCTION

Gunianolide sesquiterpene lactones show cytotoxic, antitumoral, bacterial and allergenic properties, which cause their α,β–unsaturated lactone moiety to undergo a Micheal reaction with biological nucleophiles such as L-cysteine or thiol-containing enzymes (Hoffmann, H. M. R., 1985). Because of various biological activities, chemists have been interested in these natural products in spite of relatively high toxicity. The flowers of *Chysanthemum boreale* (Compositae) have been used as an antipyretic, and to treat vertigo (Perry, 1980) and also as common folk liquor in Korea. The isolation of flavonoides and polyacetylenes from this plant has been reported (He, 1982; Bohlmann, 1960), but there are no report on guainolide sesquiterpene lactones (**1, 3, 4**) except compound **2** (Yang, 1996). In the present paper, we describe the isolation and the total assignment of the proton and carbon NMR spectra of four antimicrobial guainolide sesquiterpene lactones, cumambrin-A (**2**), cumambrin-B (**1**), angeloylcumambrin-B (**3**), and tigloylcumambrin-B (**4**). Also we compared their antimicrobial activities.

MATERIALS AND METHODS

Mps were measured on Thomas Hoover Capillary Apparatus and is uncorrect. Specific rotation value were measured on JASCO DIP-370 polarimeter. Proton and carbon NMR spectra were measured down field relative to tetramethyl silane in $CDCl_3$; ^{1}H-NMR, ^{13}C-NMR, DEPT and COSY experiments were conducted on a Bruker AM-500 (500 MHz) spectrometer. Final solutions before evaporation were dried over anhydrous Na_2SO_4.

Plant materials

The sample of *Chrysanthemum boreale* Makino was collected in Parkjeon, Hamyang (Korea) in September, 1995 and identified by Dr. Myong Gi Chung. A voucher specimen of this raw material is deposited at Herbarium of Gyeongsang National University.

Isolation

Air dried flowers (2 kg) were extracted in Soxhelet with dichloromethane (4 l) for 48 h. The dichloromethane extract was added to 5% aq. solution of lead acetate to precipitate fatty acid, phenolics and chlorophylls, then filtered. The aqueous layer was reextracted with dichloromethane (300 ml × 5). The combined organic layers were washed with brine and dried over Na_2SO_4 and concentrated to brown mass (85 g) which was loaded on silica gel (0.5 kg) column filled with hexane. Elution was with $CHCl_3$, then with $CHCl_3$ enriched with methanol. The fractions (15 g) from $CHCl_3$ to $CHCl_3$-MeOH (20:1) were purified by repeated chromatograph on silica gel [1. hexane-EtOAc (4:1). 2. hexane-EtOAc (2:1). 3. hexane-EtOAc (1:1)] to give cumambrin B **1** (75 mg), cumambrin A **2** (580 mg), angeloylcumambrin-B **3** (45 mg) and tigloyl-cumambrin-B **4** (28 mg), respectively.

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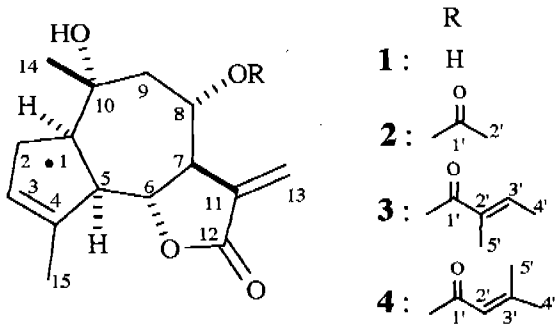

Fig. 1. Chemical structures of **1, 2, 3**, and **4**.

Cumambrin B (1): mp 177-179°C; $[\alpha]_D^{25}$=+87° (c 0.35, MeOH) ^{1}H-NMR (500 MHz, CD_3OD) δ 1.14 (3H, s, C_{15}-3H), 1.69 (1H, d, J=15.7 Hz, C_9-H_a), 1.77 (3H, d, J=1.5 Hz, C_{14}-3H), 2.09 (2H, m, C_2-2H), 2.18 (1H, dd, J=15.7, 5.1 Hz, C_9-H_b), 2.45 (1H, dd, J=15.7, 9.0 Hz, C_1-H), 2.61 (1H, dd, J=10.1, 8.5 Hz, C_5-H), 3.39 (1H, m, C_7-H), 3.79 (1H, m, C_8-H), 3.97 (1H, dd, J=10.0, 9.5 Hz, C_6-H), 5.41 (1H, s, C3-H), 5.09 (1H, d, J=4 Hz, C_{13}-H_a), 6.00 (1H, d, J=4 Hz, C_{13}-H_a); ^{13}C-NMR (125 Hz, CD_3OD) δ 17.3, 32.2, 33.9, 41.7, 51.4, 55.4 (C-1), 55.4 (C-7), 71.9, 74.1, 81.9, 120.7, 126.2, 140.9, 143.8, 171.4.

Cumambrin A (2): mp 178-180°C [177-179°C (Michael T., 1992)]; $[\alpha]_D^{25}$=+101 ($CHCl_3$, c 0.68) [+97° (c 0.6, $CHCl_3$) (Michael T., 1992)]; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 1.24 (3H, s, C_{15}-3H), 1.85 (1H, m, C_9-Ha), 1.91 (3H, s, C_{14}-3H), 2.09 (1H, m, C_2-Hb), 2.16 (3H, s, OAc), 2.23 (1H, m, C_2-Ha), 2.31 (1H, dd, J=16, 11 Hz, C_9-Hb), 2.58 (1H, m, C1-H), 2.77 (1H, dd, J=9, 8 Hz, C_5-H), 3.90 (1H, m, C_7-H), 4.00 (1H, dd, J=11, 9 Hz, C_6-H), 5.16 (1H, ddd, J=9, 6, 1 Hz, C8-H), 5.51 (2H, m, C_{13}-Hb and C_3-H), 6.18 (1H, d, J=3.5 Hz, C_{13}-Ha); ^{13}C NMR (125 MHz) δ 17.9, 21.4, 33.5, 38.9, 46.5, 54.3, 54.4, 73.4, 73.6, 80.4, 121.3, 125.5, 138.5, 143.7, 169.5, 170.2.

Angeloylcumambrin B (3): Colourless oil; $[\alpha]_D^{20}$=+96° (c 0.71, $CHCl_3$) {+100° (c 0.2, MeOH) (Haruna, M., 1981)}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 1.26 (3H, s, C_{14}-3H), 1.92 (1H, m, C_9-Ha), 1.93 (3H, d, J=Hz, C_{15}-3H), 1.94 (3H, s, $C_{5'}$-3H), 2.06 (3H, d, $C_{4'}$-3H), 2.12 (1H, m, C_2-Ha), 2.23 (1H, m, C_2-Hb), 2.36 (1H, dd, J=17, 6 Hz, C_9-Hb), 2.61 (1H, m, C_1-H), 2.78 (1H, m, C_5-H), 3.96 (1H, m, C_7-H), 4.04 (1H, dd, J=10.5, 9.4 Hz, C_6-H), 5.28 (1H, m, C_8-H), 5.52 (2H, m, C_3-H and C_{13}-Ha), 6.18 (1H, d, J=3.5 Hz, C_{13}-Hb), 6.21 (1H, m, $C_{3'}$-H); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 15.9, 17.9, 20.6, 33.6, 33.7, 39.0, 46.8, 54.2, 54.5, 72.9, 73.8, 80.0, 80.4, 121.2, 125.5, 127.2, 138.7, 143.8, 166.9, 169.5.

Tigeloylcumambrin B (4): mp 126-128°C {130°C (Bohlmann, F., *et al.* 1980)}; $[\alpha]_D^{20}$=+76.5° (c 0.36, $CHCl_3$) {+84.1 (c 0.22, MeOH)}; ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 1.24 (3H, s, C_{14}-3H), 1.85 (3H, d, C_{15}-3H), 1.89 (1H, m, C_9-H), 1.92 (3H, s, $C_{5'}$-3H), 2.06 (3H, m, $C_{4'}$-H), 2.10 (1H, m, C_2-H_a), 2.23 (1H, m, C_2-H_b), 2.34 (1H, dd, J=17, 6 Hz, C_9-H), 2.58 (1H, m, C_1-H), 3.96 (1H, m, C_7-H), 4.01 (1H, dd, J=10, 9 Hz, C_6-H), 5.20 (1H, m, C_8-H), 5.47 (1H, m, C_5-H), 5.50 (1H, d, J=5.8 Hz, C_3-H), 5.51 (1H, d, J=3.0 Hz, C_{13}-H_a), 6.15 (1H, d, 3.9 Hz, C_{13}-H_b), 6.94 (1H, m, $C_{2'}$-H); ^{13}C-NMR ($CDCl_3$, 125 MHz) δ 12.1, 14.6, 17.9, 33.5, 33.6, 38.7, 46.7, 54.0, 54.5, 73.4, 73.8, 80.3, 121.4, 125.4, 128.4, 138.5, 138.7, 143.7, 166.9, 169.6.

Test for the antimicrobial activity

All of bacterial strains were obtained from the KCCM (Korean Culture Center of Microorganism). Gram positive bacteria: *Bacillus substilis* (ATCC 9372), *Bacillus cereus* (ATCC 27348), *Staphylococcus aureus* (ATCC 13301). Gram negative bacteria: *Escherichia coli* (ATCC 15489), *Salmonella typhymurium* (ATCC 14038), *Vibrio parahaemolyticus* (ATCC, 33844), *Klebsidia pneumoniae* (ATCC 13883), *Pseudomonas aeroginosa* (ATCC, 10490).

Inhibition tests were performed by the diffusion technique of Chabbert (Chabbert, 1963) and Holt (Holt, 1975). Weighed aliquots of each dry sample were dissolved in ethanol and 20 μl portions of these solution were placed on 8 mm Whatman paper disc to give concentrations of 250 μg and 100 μg for each compounds. The discs were then placed on agar plates seeded with microorganism and after incubation for 12 h, the zone of inhibition was measured.

RESULTS AND DISCUSSION

In all four compounds, an absorption maximum at *ca.* 235 nm in ultraviolet absoption spectra and strong bands at *ca.* 1750 and 1690 cm^{-1} in the infrared absoption spectra revealed the presence of an α,β-unsaturated lactone group. The infrared (3500 cm^{-1}) and mass (M^+-18) spactra indicated the presence of a hydroxyl group in all four compounds. Additionally, the infrared (1745 cm^{-1}) and mass (M^+-60 for **2**, M^+-83 for **3**, M^+-83 for **4**) spectra indicated the presence of acetyl, angeloyl and tigloyl group, respectively. Four all compounds have similar relationships in ^{1}H-^{1}H COSY spectra because of the same skeleton. Therefore, the discussion will be paid especially on the compound **3**, which is the most intricate of all. Beginning the analysis of the COSY spectrum of cumambrin A requires that we select a starting point. A convenient entry point of the COSY is the H-13a/b vinyl protons resonating at 5.51 and 6.18 ppm, because nonequivalent methylene protons linked to the same carbon (121.2 ppm) from HMQC experiment (Table II). The H 13-a/b vinyl protons linked to their allylic H-7 resonating at 3.96 ppm which was connected to its neighboring H-6 and H-8 protons resonating at 4.04 and 5.28 ppm, respectively. The H-8

Table I. ^{13}C-NMR spectra data (125 MHz) of cumambrin B derivatives **1**, **2**, **3** and **4***

Carbon No.	**1**	**2**	**3**	**4**
C-1	55.4	54.3	54.2	54.0
C-2	33.9	33.5	33.7	33.6
C-3	126.2	125.5	125.5	125.4
C-4	140.9	138.5	138.7	138.5
C-5	55.4	54.4	54.5	54.5
C-6	81.9	80.4	80.4	80.3
C-7	51.4	46.5	46.8	46.7
C-8	71.9	73.6	72.9	73.4
C-9	41.7	38.9	39.0	38.7
C-10	74.1	73.6	73.8	73.8
C-11	143.8	143.7	143.8	143.7
C-12	171.4	169.5	166.9	166.9
C-13	120.7	121.4	121.2	121.4
C-14	32.2	33.5	33.6	33.5
C-15	17.3	17.9	17.9	17.9
C-1'		170.2	169.5	169.6
C-2'		21.4	127.2	138.7
C-3'			139.9	128.4
C-4'			15.9	12.1
C-5'			20.6	14.6

*Run in $CDCl_3$ on a Bruker AW-500 spectrometer with Me_4Si as internal standard. Assignment established by ^{1}H-^{1}H COSY and HMQC.

Table II. ^{1}H NMR (500 MHz), HMQC and ^{1}H-^{1}H COSY data for compound **3**

^{1}H-NMR	correlaled carbon	correlated proton
2.61 (m, H-1)	54.2	2.12 (H-2a), 2.23 (H-2b), 2.78 (H-5)
2.12 (m, H-2a)	33.7	2.23 (H-2b), 2.61 (H-1)
2.23 (m, H-2b)	33.7	2.12 (H-2a), 2.61 (H-1), 5.52 (H-3)
2.78 (m, H-5)	54.5	2.61 (H-1), 4.04 (H-6)
4.04 (dd, H-6)	80.4	3.96 (H-7), 2.78 (H-5)
3.96 (m, H-7)	46.8	4.04 (H-6), 5.52 (H-13a), 6.18 (H-13b)
5.28 (m, H-8)	72.9	3.96 (H-7), 1.92 (H-9a), 2.36 (H-9b)
1.92 (m, H-9a)	39.0	5.28 (H-8), 2.36 (H-9b)
2.36 (dd, H-9b)	39.0	5.28 (H-8), 1.92 (H-9a)
5.52 (d, H-13a)	121.2	6.18 (H-13b), 3.96 (H-7)
6.18 (d, H-13b)	121.2	6.18 (d, H-13b) 121.2 5.52 (H-13a), 3.96 (H-7)

Solution in $CDCl_3$ referenced to $CHCl_3$ at δ 7.26 (^{1}H) and δ 77.76 (^{13}C).

proton directly linked to the H-9a/b geminal methylene protons (1.92 and 2.36 ppm), the connectivity network terminates at H-9a/b and cannot be traced any further. Beginning from the H-6, which resonates at 4.27 ppm a strong off-diagonal response correlates with the H-5 and H-7 resonating at 2.78 and 3.96 ppm, respectively. The doublets of (J_1=11, J_2=9 Hz) at 4.04 ppm, which is an AMX system, were assigned that H-6 has anti relationship with H-5 and H-7. This spectrum shows the coupling connectivity within the molecular without any decopling being necessary. In similar fashion, H-5 was connected to H-1 resonating at 2.78 ppm which linked to H-2a/b resonating at 2.23 and 2.12 ppm, respectively. The siginal at 5.52 ppm is coupled only to H-2a resonating at 2.23 ppm . The connectivity of H-3 and H-15 is the fact that methyl of H-15 is allylic position from H-3. The isolated H-14 and acetyl protons were confirmed from DEPT and Mass data. The stereochemistry of cumambrin A was confirmed by single crystal X-ray diffraction representatively. The antibacterial activities of the angeloyl cumambrin B proved to be stronger than that of other derivatives, where-as cumambrin B having free hydroxy group at C-8 was not sensitive to all bacteria (Table III). While all compounds (**1, 2, 3, 4**) have been previously isolated from several plants (Michael, *et al.*, 1992; Haruna, 1981; Bohlmann, 1980), this is first isolation from this plant species.

ACKNOWLEDGEMENTS

This work was supported by a grant from the High-technology Development Project for Agriculture, Forestry and Fisheries.

Table III. Antibactrial activities of cumambrin B derivatives **1**, **2**, **3** and **4**

Bactria	Compounds							
	1		**2**		**3**		**4**	
	250	100	250	100	250	100	250	100
E. coli	-	-	-	-	-	-	-	-
S. typhymurium	-	-	-	-	-	-	-	-
V. parahaemolyticus	9**	-	11	9	15	10	14	10
K. pneumoniae	-	-	-	-	-	-	-	-
P. aeruginosa	-	-	9	-	13	10	11	9
B. subtilis	9	-	11	10	15	11	13	10
B. cereus	-	-	11	9	16	12	14	11
S. aureus	-	-	-	-	10	-	9	-

*Concentrations of compound (mg/paper disk).
**Diameters(mm) of clear zone.

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