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# Cumambrin A in *Chrysanthemum boreale* Makino Preparation, X-ray Crystal Structure and $^{13}C$- and $^{1}H$-NMR Study of Cumambrin A

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**Abstract** – Cumambrin A has been isolated from the dried flowers of *Chrysanthemum boreale* Makino. The complete $^{1}H$ and $^{13}C$ NMR assignment of cumambrin A was achieved from two-dimensional $^{1}H$-$^{1}H$ COSY and $^{13}C$-$^{1}H$ COSY spectra with the aid of homonuclear and heteronuclear double resonance experiments. The its structure has been verified by single crystal X-ray diffraction.

**Key words** – *Chrysanthemum boreale* Makino; cumambrin A; α,β-unsaturated sesquiterpenoid lactone.

In the course of a continuing search for tumor inhibitors from plant sources, cumambrin A from *Chrysanthemum boreale* Makino was found to show significant cytotoxicity against L1210, K562 and A549 cells.[1] *Chrysanthemum boreale*[2] is medicinal herb whose flower has been used as common folk liquor in Korea. Cumambrin A (Fig.1) has been isolated from several Compositaes[3,4] since the first isolation from *Ambrosia cumanenis*.[5] The value of two-dimensional NMR techniques in the spectral assignment of complex sesquiterpenoid lactones has been the object of increasing interest. In the present study we wish to report the isolation and the total assignment of the proton and carbon NMR spectra of antitumoral cumambrin A through two dimensional NMR techniques.

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## EXPERIMENTAL

**General** – Column chromatography was carried out 230~400 mesh silica gel and spots were visualized by spraying with 10% $H_2SO_4$ in MeOH and then heating on a hot plate. Mp was measured on a Thomas Hoover Capillary Apparatus and is uncorrect. Specific rotation value was measured on a JASCO DIP-370 polarimeter. Proton and carbon NMR spectra were measured down field relative to tetramethyl silane in $CDCl_3$; $^{1}H$-NMR and $^{13}C$-NMR were conducted on Brucker AM-500(500 MHz) spectrometer. The X-ray study was performed on a Rigakaku AFC7R diffracto- meter. The elemental analysis were carried out at the Korea Research Institute of Chemical Technology.

**Plant materials**–The flower of *Chrysanthemum boreale* Makino were collected on September, 1992 at Hamyang suburb, Korea. A voucher specimem was identified by prof. Myong Ki Jung of department of biology, Gyengsang National University, and a pictorial book[6] for wild plants of Korea.

**Extraction and isolation**–Air dried flowers (1 kg) were extracted in Soxhlet with dichloromethane (2 l) for 48 hr. The dichloromethane extract was added to 5% aqueous solution of lead acetate to precipitate fatty acid, phenolics and chlorophylls, then filtered. The aqueous layer was extracted with dichloromethane (200 ml×5). The Organic layers were dried over $Na_2SO_4$ and concentrated. The crude residue was chromatographed on silica gel [chloroform-acetone (100/1, 10/1, 1/1)] to give cumambrin A (1.6 g, 0.16%). The crystalline residue was recrystallized from dichloromethane-diethyl ether for analysis. mp 181~183°[lit.[5] 178°]: $[\alpha]D^{25}=+101°$($CHCl_3$, c 0.68) [lit.[5] +97°]: $^1H$ NMR (500MHz) δ ($CDCl_3$; assignment made by $^1H$-$^1H$ COSY and $^{13}C$-$^1H$ NMR) 1.24 (s, 3H, H-15), 1.85(dd, 1H, H-2a), 1.91 (s, 3H, H-4), 2.09 (m, 1H, H-2b), 2.16 (s, 3H, OAc), 2.23 (m, 1H, H-2a), 2.31 (dd, 1H, J=16, 11 Hz, H-9b), 2.58 (m, 1H, H-1), 2.77 (dd, J=9, 8 Hz, H-5), 3.90 (m, 1H, H-7), 4.00 (dd, J=11, 9 Hz, H-6), 5.16 (ddd, 1H, J=9, 6, 1 Hz, H-8), 5.51 (d, 2H, H-13b and H-3), 6.18 (d, 1H, J=3.5 Hz, H-13a); $^{13}C$ NMR (125 MHz) δ 17.9 (C-14), 21.4 (OAc), 33.5 (C-15 and C-2), 38.9 (C-9), 46.5 (C-17), 54.3 (C-1), 54.4 (C-2), 73.4 (C-8), 73.6 (C-10), 80.4 (C-6), 121.3 (C-13), 125.5 (C-3), 138.5, 143.7, 169.5, 170.2.

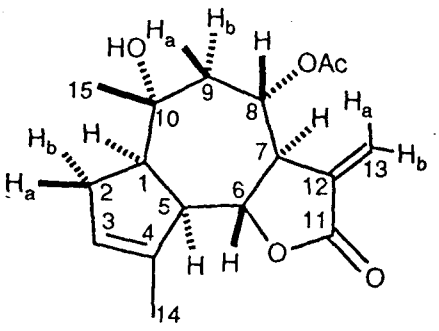


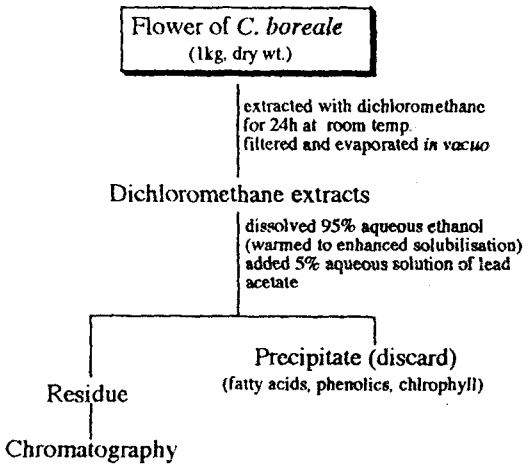

**Fig. 1.** Cumambrin A (1).

**X-ray crystallography**–A colorless prismatic crystal of $C_{17}H_{22}O_5$ having approximate of 0.30×0.25×0.15 mm was mounted on a glass fiber. All measurements were made on a Rigaku AFC7R diffratometer with graphite monochromated Cu-K radiation and a 12 kW rotating anode generator. The structure was solved by the direct method and expanded using Fourier techniques. The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included but not refined. The crystallographic data of cumambrin A are as follows.

$C_{17}H_{22}O_5N$, M=306.36, $P2_12_12$(#18), a=16.524(2) Å, b=9.679(1) Å, c=10.029(2) Å, V=1603.9(8) Å3, z=4, Dcalc=1.269 g/cm$^3$, R=0.042.

## RESULTS AND DISCUSSION

The isolation method is shown in Scheme 1.




**Scheme 1.**

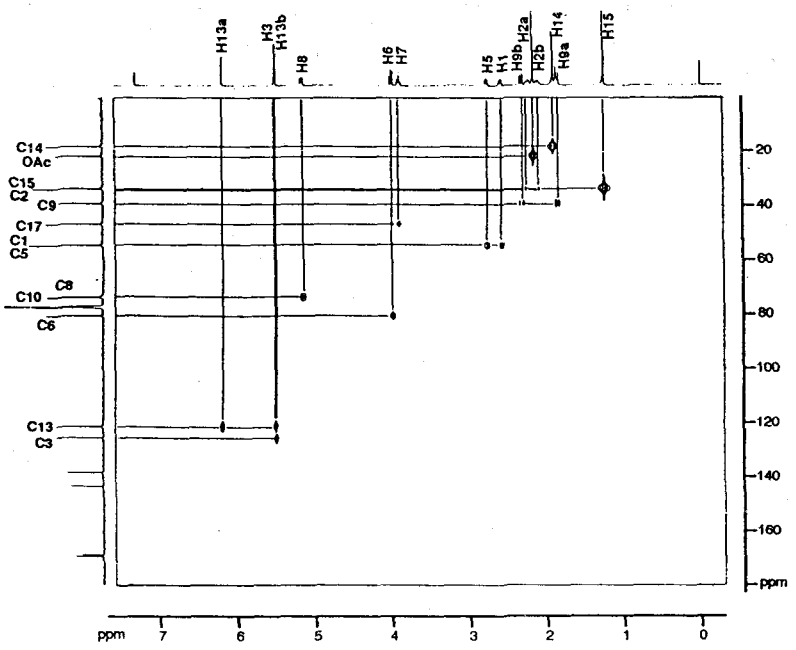



**Fig. 2.** Two-dimensional $^{13}C$-$^{1}H$ COSY spectrum of 1.

The dried ground flowers were extracted with dichloromethane and the dichloromethane extracts was added to 5% aqueous solution of lead acetate to precipitate fatty acid, phenolics and chlorophylls, then filtrated. The filtrate was reextracted with dichloromethane. The organic layer was concentrated. The crude residue was chromatographed on silica gel to give cumambrin A.

The molecular formular $C_{17}H_{22}O_5$ was determined on basis of the elemental analysis and high resolution mass spectrometry [Anal. Calcd. for $C_{17}H_{22}O_5$: C, 66.65; H, 7.24; $M^+$, 306.1467. Found: C, 66.63; H, 7.21; $M^+$, 306.1452]. An absorption maximum at 235 nm in the ultraviolet absorption spectrum and strong bands at 1750 and 1690 $cm^{-1}$ in the infrared absorption spectrum revealed the presence of a lactone group and an α,β-unsaturated cyclopentanone group. The infrared (3500 $cm^{-1}$) and mass ($M^+$, 18, 288)[1] spectra indicated the presence of a hydroxyl group. Additionally, the infrared (1745 $cm^{-1}$) and mass ($M^+$-60, 246) spectra indicated the presence of an acetate group.

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 (121.3 ppm) from $^{13}C$-$^{1}H$ COSY experiment (Fig. 2). The H-13a/b vinyl protons linked to their allylic H-7 resonating at 3.90 ppm which was connected to its neighboring H-6 and H-8 protons resonating at 4.00 and 5.16 ppm, respectively (Fig. 3). The H-8 proton directly linked to the H-9a/b terminal methylene protons (1.58 and 2.31 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 2.77

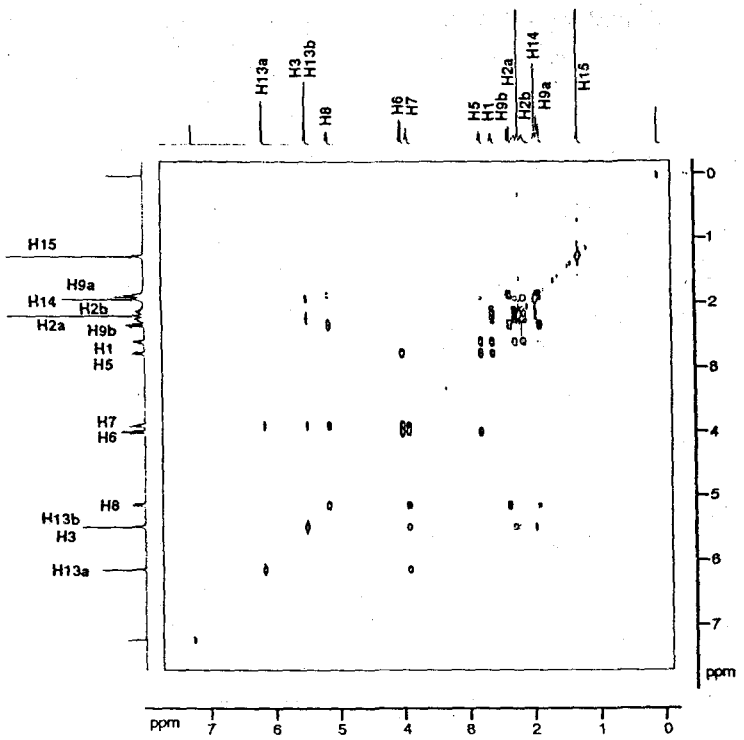



**Fig. 3.** Two-dimensional $^1H$-$^1H$ COSY spectrum of 1.

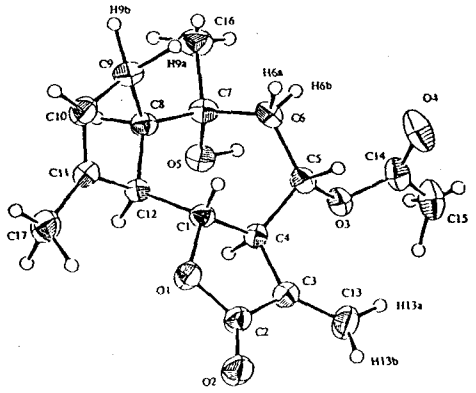



**Fig. 4.** Perspective view of the crystallographic structure of 1. The atom labelling is different from the chemical nomenclature of 1.

and 3.90 ppm, respectively. The doublets of ($J_1$=11, $J_2$=9 Hz) at 4.27 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 decoupling being necessary. In similar fashion, H-5 was connected to H-1 resonating at 2.58 ppm which linked to H-2a/b resonating at 2.23 and 2.09 ppm, respectively. The signal at 5.51 ppm is coupled only to H-2a resonating at 2.23 ppm. The connectivity between H-3 and H-14 is the fact that methyl of C-14 is allylic position from H-3. The isolated H-15 and acetyl protons were confirmed from DEPT and Mass data. The sterochemistry has been confirmed by X-ray analysis (Fig. 4). We have isolated antitumoral cumambrin A from *C. boreale*. Also we confirmed its fine structure with 2D-NMR techniques and single crystal X-ray diffraction.

## ACKNOWLEDGEMENTS

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