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Platelet-Activating Factor Antagonistic Activity and ^{13}C NMR Assignment of Pregomisin and Chamigrenal from *Schisandra chinensis*

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In the course of searching for PAF receptor antagonists, pregomisin (**1**) and chamigrenal (**2**) were isolated from the fruits of *Schizandra chinensis* Baill by the bioactivity-guided isolation. Both compounds showed PAF antagonistic activity and the IC_{50} values were 4.8×10^{-5} M and 1.2×10^{-4} M, respectively. In addition, the ^{13}C NMR assignments of **1** and **2** using DEPT, HMQC, COLOC and HMBC were reported for the first time.

Key words : *Schisandra chinensis* Baill, Schisandraceae, PAF receptor antagonist, pregomisin, chamigrenal, ^{13}C NMR

INTRODUCTION

Platelet activating factor (PAF) with chemical structure of a 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholine is a product of IgE-sensitized basophils (Hanahan, 1986). PAF exerts a myriad of physiological and pathological roles such as the aggregation, degranulation and chemotaxis of neutrophils, asthma, increase of vascular permeability, hypotension, cardiac anaphylaxis, thrombosis, gastrointestinal ulceration, acute-inflammation, allergic skin disease and transplanted organ rejection (Braquet *et al.*, 1987; Saito *et al.*, 1988).

Recently, in order to search for PAF antagonists, we have screened the extracts of various natural products and reported the active substances, schisandrin A, B and C, from *Schisandra chinensis* which is used in tonic and cough remedies (Jung *et al.*, 1997). In a countinuing study, we found that two compounds, pregomisin (**1**) and chamigrenal (**2**), also possessed an *in vitro* inhibitory effect on PAF binding. Previousely, these compounds were reported as the inhibitior of cyclic adenosine 3',5'-monophosphate phosphodiesterase (Sakuri *et al.*, 1992) and the remedy agent of liver failure (Hiroshi *et al.*, 1985), respectively. However, no report of the PAF antagonistic activity of these compounds have been published, thus we report herein these PAF antagonistic activity. In addition, the ^{13}C NMR assignments of **1** and **2** using DEPT, HMQC, COLOC and HMBC were reported for the first time.

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MATERIALS AND METHODS

General experimental procedures

For TLC, Si-HPT-Silica gel (J. T. Baker) and Whatman $KC_{18}F$ plates were used. The column chromatography was performed using a Merck Kieselgel 60 (No. 9538). The ^1H (300 MHz) and ^{13}C NMR (75 MHz) spectra were obtained on a Varian Unity 300 spectrometer using TMS as an internal standard. COLOC and HMBC data were recorded on a Bruker DRX-600 spectrometer. The radioactivity was counted by a liquid scintillation spectrophotometer (Beckman LS 6000TA, USA).

Plant material

The air-dried fruits of *S. chinensis* B. were purchased at a herbal drug store in Taejon, Korea. The voucher speciman was kept in the sample chamber of our laboratory (NDC-014).

Extraction and isolation

Fractionation and isolation were carried out along bioassay results. The crushed fruits (6 kg) were extracted with MeOH (15 l) three times at room temp. The

combined MeOH extracts were evaporated under reduced pressure, to give a brown residue (1,485 g). This was partitioned with *n*-hexane and water. The *n*-hexane extract (200 g) was separated to seven fractions by silica gel chromatography (*n*-hexane-EtOAc, 100:0 →0:100, step gradient). From the fifth fraction (150 mg), **1** (68 mg) was isolated by prep. TLC (silica gel, *n*-hexane-EtOAc, 3:1, ×8) and HPLC (*n*-hexane-*i*-PrOH, 98:2). The second fraction was distilled *in vacuo* (150°C) and the distillate (33 g) was separated to thirteen subfractions by silica gel chromatography (*n*-hexane-EtOAc, 24:1). From the third subfraction, **2** (30 mg) was isolated by prep. TLC (silica gel, *n*-hexane-EtOAc, 24:0.5, ×5).

Pregomisin (1): ^{1}H NMR ($CDCl_3$) 0.86 (6H, *d*, *J*=6.6 Hz, 2Me), 1.77 (2H, *m*, H-2 and H-3), 2.25 (2H, *dd*, *J*=13.3, 9.2 Hz, H-1a and H-4a), 2.70 (2H, *dd*, *J*=13.3, 9.2 Hz, H-1b and H-4b), 3.84 (6H, *s*, 2OMe), 3.88 (6H, *s*, 2OMe), 6.26 (2H, *d*, *J*=1.8 Hz, H-2' and H-2"), 6.43 (2H, *d*, *J*=1.8 Hz, H-6' and H-6"); ^{13}C NMR ($CDCl_3$) see Table I.

Chamigrenal (2): ^{1}H NMR ($CDCl_3$) 0.79 (3H, *s*, H-12), 0.83 (3H, *s*, H-13), 4.26 (1H, *s*, H-14a), 4.78 (1H, *s*, H-14b), 6.71 (1H, *dd*, *J*=4.2, 3.6 Hz, H-4), 9.29 (1H, *s*, H-15); ^{13}C NMR ($CDCl_3$) see Table I.

Preparation of reagents solutions and buffers

ACD solution (trisodium citrate 2.5%, citric acid 1. 37%, glucose 2% in water) was used as an anticoagulant. Bovine serum albumin (BSA) and ginkgolide B were from Sigma Co. (St. Louis, USA). Tris-BSA Buffer (10 mM Tris, 10 mM $MgCl_2$, 30 mM KCl, 1 mM EGTA, 0.1% glucose, 0.25% BSA, pH 7.0) was used for washing platelets, preparing platelet suspension, dilution of sample and washing the filters. Radiolabelled PAF(1-*O*-[^{3}H]octadecyl-2-acetyl-*sn*-glycero-3-phosphocholine) with a sp. act. of 142 Ci/mmol and unlabelled PAF were purchased from Amersham (Little Chalfont, UK) and Sigma Co., respectively. For the scintillation fluid, Lumagel®-safe was purchased from Lumac*LSC B.V. Co. (Olen, Belgium).

Table I. ^{13}C NMR data of pregomisin (**1**) and chamigrenal (**2**) ($CDCl_3$)[a]

1		**2**	
No.	δ	No.	δ
1	39.4 (CH_2)	1	25.0 (CH_2)
2	39.0 (CH)	2	19.1 (CH_2)
3	39.0 (CH)	3	140.8 (C)
4	39.4 (CH_2)	4	151.1 (CH)
1', 1"	133.6 (C)	5	31.0 (CH_2)
2', 2"	104.9 (CH)	6	46.0 (C)
3', 3"	138.2 (C)	7	148.4 (C)
4', 4"	152.1 (C)	8	31.9 (CH_2)
5', 5"	149.0 (C)	9	23.5 (CH_2)
6', 6"	108.6 (CH)	10	36.8 (CH_2)
OCH_3	55.8 (CH_3)	11	37.2 (C)
	61.0 (CH_3)	12	23.1 (CH_3)
CH_3	16.3 (CH_3)	13	25.0 (CH_3)
	16.3 (CH_3)	14	110.7 (CH_2)
		15	193.7 (CH)

[a]Each carbon character was determined by DEPT.

Preparation of samples for PAF receptor binding assay

Each sample was dissolved in dimethyl sulfoxide (DMSO) and diluted with buffer (final concentration of DMSO, 0.8%) and 0.8% DMSO in buffer was used as control.

Preparation of washed rabbit platelet suspension

Five volumes of blood of a rabbit was collected by heart puncture into 1 volume of ACD solution. The blood was centrifuged at 270 g for 10 min, and the top platelet-rich plasma (PRP) was carefully ladeled out. PRP was recentrifuged at 1250 g for 10 min, the platelets were then washed by recentrifugation in the buffer. The final platelet concentration was adjusted to 4×10^8 cells/ml buffer by means of hematocytometer (Brand 717810, Germany).

Determination of PAF antagonistic activity

PAF antagonistic activity was determined as described in literatures (Valone *et al.*, 1982; Yang *et al.*, 1995) with some modifications. The reaction mixture consisted of 100 μl of rabbit platelet suspension (4×10^8 cell/ml), 90 μl of [^{3}H]PAF (0.9 nM, 70,000 dpm) with or without unlabelled PAF (500-fold of radioactive form), and 60 μl of sample or control solution. The reaction mixture was incubated at room temperature for 30 min. The free PAF was separated from bound PAF by filtration of the reaction mixture and radioactivity was then measured. The difference between total radioactivities of bound [^{3}H]PAF in the absence and the presence of excess unlabeled PAF is defined as specific binding of the radiolabeled ligand. In a set of experiments, [^{3}H]PAF was incubated with 5 different concentrations of samples and the antagonistic effect of samples on the specific binding was expressed as percentage inhibition of the control. The activity assay was carried with triplicate at one concentration of a sample. The IC_{50} value was defined as the final concentration of the inhibitor required to block 50% of the specific [^{3}H]PAF binding to rabbit platelet receptors.

RESULTS AND DISCUSSION

By the bioactivity-guided isolation, two compound, pregomisin (**1**) and chamigrenal (**2**), were isolated from the fruits of *S. chinensis* as effective components. These

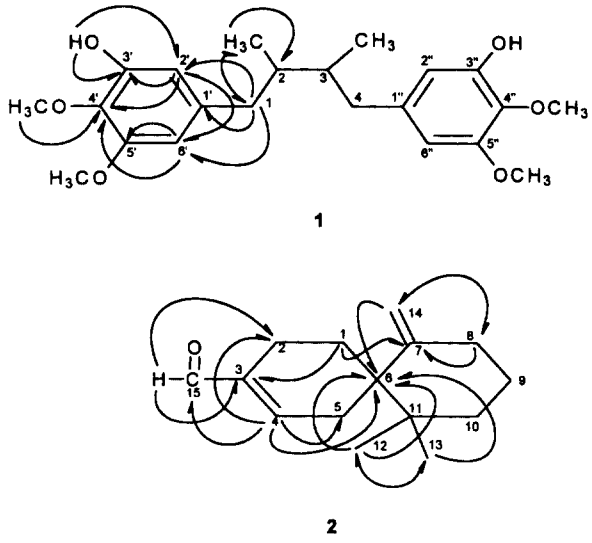

Fig. 1. The longe-range correlations of pregomisin (**1**) observed in COLOC and chamigrenal (**2**) in HMBC.

compounds were identified by spectral analyses and the comparison of their reference data (Ohta *et al.*, 1968; Ikeya *et al.*, 1979). But the ^{13}C NMR assignment of both compounds had not yet been reported, so that we assigned ^{13}C NMR data by 2D NMR techniques as following.

In the COLOC of **1**(Fig. 1), the methyl proton signal at δ 0.86 was correlated to the carbon signals at δ 39.0 (C-2) and 39.4 (C-1) which were coupled with the proton signals at δ 1.77 (H-2), 2.25 (H-1a) and 2.70 (H-1b) in HMQC, respectively. Besides, the carbon signals at δ 133.6 (C-1'), 104.9 (C-2') and 108.6 (C-6') were correlated with the methylene proton signal of H-1 and the methine proton signal at δ 6.26 (H-2') was correlated to the carbon signals at δ 138.2 (C-3') and 152.1 (C-4'). Also the methine proton signal at δ 6.43 (H-6') was correlated to the carbon signals at δ 149.0 (C-5') and 152.1 (C-4'). The carbon signals at C-2' and C-3' were correlated with the hydroxyl proton signal at δ 5.74. On the basis of the data, the ^{13}C NMR data of pregomisin (**1**) was completely assigned as shown in Table I.

In the HMBC of **2** (Fig. 1), the olefinic proton signal at δ 6.71 (H-4) was correlated to the carbon signals at δ 31.0 (C-5) and 193.7 (C-15) which were coupled with the proton signals at δ 2.10 (H-5) and 9.29 (H-15) in HMQC, respectively. Also, the olefinic proton signal was correlated to the carbon signal at δ 19.1 (C-2) and 46.0 (C-6), and the carbon signal at C-6 was correlated with the methyl proton signals at δ 0.79 (H-12) and 0.83 (H-13) and methylene proton signal at δ 4.26 and 4.78 (H-14). The carbon signal at δ 148.4 (C-7) was correlated with two methylene proton signals at δ 1.31 (H-1a) and 2.03 (H-1b) and 2.10 (H-8) which was correlated to the carbon signal of C-14. As the above findings, the ^{13}C NMR signal of 2 were completely assigned as shown in Table I.

In the [^{3}H]PAF receptor binding assay of pregomisin (**1**) and chamigrenal (**2**), the IC_{50} values of them were 4.8×10^{-5} M and 1.2×10^{-4} M, respectively. In previous studies, many series of lignans and sesquiterpenes have been reported from natural sources as PAF antagonists. In particular, bistetrahydrofuran and butanolide type lignans ($IC_{50}=1.2\times10^{-6}$ M~4.2×10^{-7} M) from *Forsythia suspensa, Arctium lappa* and *Magnolia biondii*, dibenzo [*a,c*]cyclootene derivatives (schisandrin A and B, $IC_{50}=1.7\times10^{-5}$ M and 8.9×10^{-5} M, respectively) from *Schisandra chinensis* and plenolin type sesquiterpenes ($IC_{50}=6.7\times10^{-6}$ M~2.5×10^{-7} M) from *Centipeda minima* were reported (Iwakami *et al.*, 1992; Jung *et al.*, 1997; Pan *et al.* 1987).

Though PAF antagonistic activity of pregomisin (**1**) and chamigrenal (**2**) are weak, these compound were found to be new type PAF antagonist. The present results suggested that pregomisin and chamigrenal may be useful components of the Schisandrae Fructus for the treatment of PAF-related inflammation, *e.g.* asthma, allergy, atopy's dermatitis and other inflammatory diseases together with schisandrin A and B.

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