A Phenolic Glycoside From *Artemisia Sacrorum*

Yaeko Konda, Nobuyuki Funato and Yoshihiro Harigaya*

School of Pharmaceutical Sciences, Kitasato University,
Minato-ku, Tokyo 108, Japan

Xian Li, Dezhi Zhang and Masayuki Onda

Shenyang College of Pharmacy, Wenhua-Lu, Shenyang, China

Received June 13, 1991

A phenolic glycoside has been isolated from *Artemisia sacrorum* grown in the Northeast of China. This compound is identified to be 4-*O*-β-D-glucopyranosyl-2-*O*-methylphloroacetophenone by means of nmr spectroscopy and synthesis.

J. Heterocyclic Chem., **28**, 1949 (1991).

In the course of the studies on biologically active natural products, we previously reported three *ent*-kauranoid diterpenes from *Artemisia sacrorum* Ledeb. *(Compositae)* grown in the Northeast of China, which is known as a Chinese folk medicine for hepatitis [1]. Our further investigations have led to the isolation of a phenolic glucoside, whose structural elucidation and synthesis are described in this paper.

The ethanol-soluble portion of the water extract of this plant was successively extracted with petroleum ether, chloroform, ethyl acetate and butanol. The chromatographic separation of the ethyl acetate soluble protion afforded compound **1**.

Compound **1**, $C_{15}H_{20}O_9$, colorless needles, mp 185-188° (methanol) exhibited a positive Molisch color reaction. The 2D nmr data are listed in Table 1. It was shown that seven protons appeared at δ 5.62-4.03 and their corresponding carbons arise from a β-D-glucopyranoside by comparison with those of methyl β-D-glucopyranoside [1] and that the remaining proton resonances are attributed to an acetyl (δ 2.40), a methoxyl (δ 3.57), two *meta*-protons (δ 6.59, 6.25, J_{meta} = 2.5 Hz) and a hydroxyl (δ 14.19), suggesting aglycone to be a phloroacetophenone monomethyl ether. The ^1H-^{13}C COSY experiments and nOes linking H-1' to H-3 and H-5 pointed out 4-*O*-β-D-glucopyranosyl-2-*O*-methylphloroacetophenone for **1**.

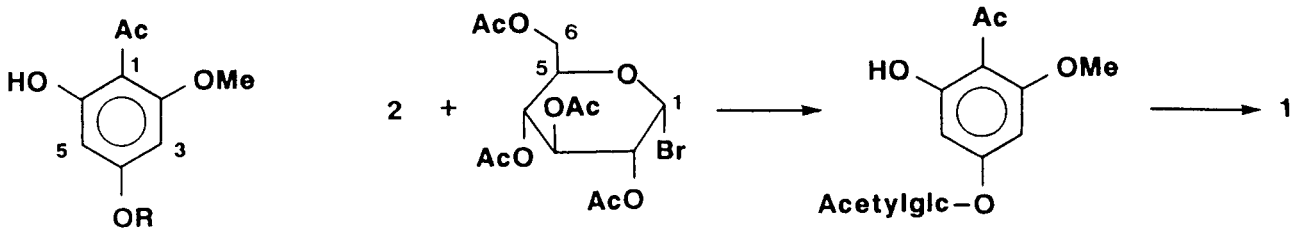
Table I

MNR Data for Compound **1** [a]

Carbon No.	Carbon δ	Correlated H [b]	Correlated H δ	H coupled with C [c]	H coupled with H [d]
1	107.19 s			H-3, H-5, 6-OH	
2	165.06 s			H-3, 2-OMe	
3	92.36 d	H-3	6.25 d (2.2)	H-5	
4	167.49 s			H-3, H-5	
5	97.58 d	H-5	6.59 d (2.2)	H-3, 6-OH	
6	163.58 s			H-5, 6-OH	
1-Ac	33.10 q	Me	2.40 s		
	203.67 s			Me	
2-OMe	55.82 q	Me	3.57 s		
6-OH			14.19 s		
1'	101.58 d	H-1'	5.62 d (7.3)		H-2'
2'	74.93 d	H-2'	4.24 m		H-1'
3'	78.62 d	H-3'	4.27 m		
4'	71.35 d	H-4'	4.20 t (8.5)		H-5'
5'	79.39 d	H-5'	4.03 ddd (8.5, 5.5, 2.0)		Ha-6'
6'	62.43 t	Ha-6'	4.24 m		
		Hb-6'	4.44 br d (12.0)		

[a] Spectra were taken at 400 MHz (^1H) and 100.6 MHz (^{13}C) in C_5D_5N. [b] ^1H-^{13}C (one-bond) COSY. Figures in parentheses are coupling constants (Hz). [c] ^1H-^{13}C (long-range) COSY. [d] ^1H-^1H COSY.

Scheme 1



1 R = Glc

2 R = H

3

4

Glc = β-D-glucopyranosyl

Acidic methanolysis of **1** furnished compound **2** and a mixture of methyl α- and β-D-glucopyranosides [1]. Two nonequivalent *meta*-protons (δ 6.02, 5.94, J_{gem} = 2.5 Hz) in addition to an acetyl (δ 2.55), a methoxyl (δ 3.90) and two hydroxyls (δ 13.92, 9.47) observed in nmr experiments showed **2** to be 2-*O*-methylphloroacetophenone. Finally, **2** was identical with an authentic sample prepared by the procedures described in literature [2-4].

The synthetic route to **1** is shown in Scheme 1. The synthesis of compounds **1** and **4** were already reported [5]. However, it seemed to us that their glycosidation positions still remain unreliable. Since the authentic sample for identification should be perfectly standardized, these compounds were prepared in order to clarify their structures by means of nmr spectroscopy (2D, nOe).

Treatment of **2** [2-4] with 2,3,4,6-tetra-*O*-acetyl-α-bromo-D-glucopyranoside (**3**) [6] in the presence of sodium hydroxide gave **4** (48%). The structure of **4** was unambiguously ascertained by an intramolecular hydrogen-bonding (OH---O=C) at 3201 cm^{-1} [7] and nOes linking H-1′ to H-3 and H-5. Ammonolysis of **4** afforded **1** (93%) which was identical with the natural product.

To our knowledge, this is the first report of **1** as natural substance.

EXPERIMENTAL

Melting points (uncorrected) were determined on a micro hot-stage apparatus. Spectra were recorded on the following spectrometers: ir, Hitachi 260-30; ^{1}H nmr, Varian XL-400 (400 MHz) or VXR-300 (300 MHz) (reference, tetramethylsilane); ^{13}C nmr, Varian XL-400 (100.6 MHz) (reference, tetramethylsilane); hrms, JEOL JMS DX-300; elemental analysis, Perkin-Elmer 240B.

All nmr spectra were taken at a probe temperature, 20°, using a 5 mm tube. The measuring conditions employed are shown.

The DEPT spectra were recorded using the θ = 90° and 142° pulses to separate the CH/CH_3 and CH_2 lines phased 'up and down', respectively. Acquisition data were number of scans, 16-32K; relaxation delay for protons, 2 sec; and 90° pulse widths, 30.0 μsec and 9.6 μsec for ^{1}H and ^{13}C, respectively. The delay between pulses (3.57 msec) was set to 1/2J(CH), where J(CH) was taken to be 140 Hz.

The ^{1}H-^{1}H COSY was done with a ^{1}H single probe; relaxation time 1 sec; 90° (^{1}H) = 14.3 μsec; 90° mixing pulse; F_1 = F_2 = 1768 Hz; data matrix 1024 x 128; 16 scans during 128 time increments (zero filling in F_1); 2 dummy scans; spectra were symmetrized about a diagonal axis using the FOLDT command after 2D transformations.

The ^{1}H-^{13}C COSY was done under the following conditions: ^{13}C, 30 ~ 105 MHz probe; relaxation time, 1 sec; 1 dummy scan; 90° ^{1}H and ^{13}C pulses were calibrated at 31.0 and 9.6 μsec, respectively. One-bond correlation: F_1 = 1786 Hz, F_2 = 12346 Hz; data matrix 2048 x 40; 256 scans during 40 time increments; acquisition time 0.083 sec; $^1J_{CH}$ (average) 140 Hz; size of final data points, 2K. Long-range correlation: F_1 = 5227 Hz, F_2 = 18018 Hz; data matrix 2048 x 32; 1280 scans during 32 time increments (zero filling in F_1); 1 dummy scan; acquisition time 0.057 sec; $^{LR}J_{CH}$ (average) 7.0 Hz; size of final data points 2K.

The nOe spectra were recorded by means of nOe difference spectroscopy. The pre-irradiation time of each resonance was 4.0 sec for **1** and 2.0 sec for **2** and **4**. The interpulse delay was 0 sec. The irradiation data sets were interleaved to cancel drift and changing magnet homogeneity. These spectra were transformed by the difference between two free induction decays.

Extraction and Isolation.

The details were described in literature [1]. The ethyl acetate-soluble portion (23 g) was chromatographed over silica gel (2.5 kg) eluting with a mixture of chloroform and acetone. After sugeroside (46 mg) was eluted with chloroform-acetone (5:1) [1], **1** (35 mg) was obtained from the chloroform-acetone (1:1) eluate.

4-*O*-β-D-Glucopyranosyl-2-*O*-methylphloroacetophenone (**1**).

The colorless needles had mp 185-188° (methanol); ir (potassium bromide): ν cm^{-1} 3386, 3227 (OH), 1629 (C=O); nOe (400 MHz) (pyridine-d_5): % H-5 6.4⇌13.6 H-1′ 8.6⇌7.9 H-3 3.7⇌19.3 2-OMe; hrms: m/z M^+, 344.1105 (344.1108 for $C_{15}H_{20}O_9$).

Anal. Calcd. for $C_{15}H_{20}O_9$: C, 52.32; H, 5.85. Found: C, 52.31; H, 5.83.

Acidic Methanolysis of **1**.

A mixture of **1** (5.0 mg), 5% hydrochloric acid (1 ml) and methanol (2 ml) was refluxed for 8 hours. The reaction mixture was concentrated *in vacuo*, and the aqueous residue was extracted with chloroform (5 ml x 2). Work-up of the organic layer, followed by recrystallization from benzene, afforded **2** (3.1 mg) as colorless needles, mp 204-205°; ir (chloroform): ν cm^{-1} 3569, 1625; ^1H nmr (300 MHz) (acetone-d_6): δ 13.92 (1H, s, 6-OH), 9.47 (1H, s, 4-OH), 6.03 (1H, d, J = 2.3 Hz, H-3), 5.94 (1H, d, J = 2.3 Hz, H-5), 3.90 (3H, s, 2-OMe), 2.55 (3H, s, 1-Ac); nOe (300 MHz) (acetone-d_6): % 2-OMe 9.4 ⇌ 3.6 H-3, 4-OH 3.2 → H-5 9.5 ← 6-OH; hrms: m/z M^+, 182.0575 (182.0579 for $C_9H_{10}O_4$).

This compound was identified with an authentic sample [2-4] by direct comparison.

The aqueous layer was neutralized with silver carbonate, filtered and concentrated *in vacuo* to dryness, giving a mixture of methyl α- and β-glucopyranosides (1.4 mg) (2:1 ratio) which were identified with authentic samples [1] by comparison of ^1H nmr and tlc.

2-*O*-Methyl-4-2′3′4′6′-tetra-*O*-acetyl-β-D-glucopyranosylphloroacetophenone (**4**).

To a solution of **2** (50 mg, 0.27 mmole) and **3** (187 mg, 0.45 mmole) in acetone (2 ml) were added 10% aqueous sodium hydroxide (0.2 ml) and water (0.1 ml), and the whole was stirred at room temperature for 4.5 hours. The reaction mixture was concentrated *in vacuo*, and the residue was extracted with ethyl acetate (10 ml x 3). Work-up of the organic layer, followed by preparative tlc (silica gel, chloroform-methanol = 100:1), afforded **4** (67 mg, 48%), Rf 0.5, as colorless needles, mp 169-171° (methanol); ir (chloroform): ν cm^{-1} 3531, 1755, 1620; ir (c = 1 x 10^{-3}, carbon tetrachloride): ν cm^{-1} 3201; ^1H nmr (300 MHz) (chloroform-d) δ 13.83 (1H, s, 6-OH), 6.13 (1H, d, J = 2.5 Hz, H-5), 5.98 (1H, d, J = 2.5 Hz, H-3), 3.85 (3H, s, 2-OMe), 2.61 (3H, s, 1-Ac); nOe (300 MHz) (chloroform-d): % 2-OMe 16.7 ⇌ 8.0 H-3 1.1 ⇌ 1.2 H-1′ 7.6 ⇌ 6.9 H-5 1.7 ⇌ 1.2 6-OH, 2-OMe 2.2 ⇌ 3.3 1-Ac 1.7 → 6-OH; hrms: m/z M^+, 512.1523 (512.1529 for $C_{23}H_{28}O_{13}$).

Anal. Calcd. for $C_{23}H_{28}O_{13}$: C, 53.91; H, 5.50. Found: C, 53.59; H, 5.49.

Synthetic **1**.

To a solution of **4** (16 mg, 0.03 mmole) in absolute methylene chloride (0.4 ml) and methanol (0.2 ml) was added absolute 24% ammonia/methanol (2.4 ml). The whole was stirred at room temperature for 5 minutes and allowed to stand at 4° for 22 hours. Work-up of the reaction mixture, followed by preparative tlc (silica gel, chloroform-methanol = 10:1), gave **1** (11 mg, 93%), Rf 0.15, as colorless needles, mp 185-188° (methanol); hrms: m/z M^+, 344.1121 (344.1108 for $C_{15}H_{20}O_9$).

This compound was identified with the natural product by direct comparison.

REFERENCES AND NOTES

[1] X. Li, D. Zhang, M. Onda, Y. Konda, M. Iguchi, and Y. Harigaya, *J. Nat. Prod.*, **53**, 657 (1990).

[2] K. C. Gulati and K. Venkataraman, *J. Chem. Soc.*, 267 (1936).

[3] G. R. Nagarajan and V. S. Parmar, *Indian J. Chem. (B)*, **16B**, 439 (1978).

[4] A. C. Jain, S. M. Gupta, and P. Bambah, *Indian J. Chem. (B)*, **24B**, 393 (1985).

[5] G. Zemplen, R. Bognar and K. Thiele, *Chem. Ber.*, **77**, 446 (1944); V. K. Ahkluwalia and N. Rani, *Indian J. Chem.*, **14B**, 594 (1976).

[6] M. Barczai-Martos and F. Korösy, *Nature*, **165**, 369 (1950).

[7] Intramolecular hydrogen-bondings (c = 1 x 10^{-3}, carbon tetrachloride): cm^{-1} 2-hydroxyacetophenone 3208, 2-hydroxy-6-methoxyacetophenone 3204, 2,4-di-*O*-methylphloroacetophenone 3205.