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Chemical Constituents in *Aloe barbadensis*

Man Ki Park[1], Jeong Hill Park[1], Young Geun Shin[1], Su Mi Choi[1], Yong Seok Choi[1], Kyeong Ho Kim[2] and Seung Ki Lee[1]

[1]College of Pharmacy, Seoul National University, Seoul 151-742, Korea and [2]College of Pharmacy, Kangwon National University, Chuncheon 220-701, Korea

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Two compounds were newly isolated from the leaves of *Aloe barbadensis* Mill. Their structures were identified as 3,4-dihydro-3,9-dihydroxy-8-methoxy-3-methyl-1(2H)-anthracenone(**1**) and 10-β-D-glucopyranosyl-1,8,10-trihydroxy-3-(hydroxymethyl)-(R)-9(10H)-anthracenone(**2**) by chemical and spectral evidences.

Key words : *Aloe barbadensis* Mill., Liliaceae, 3,4-Dihydro-3,9-dihydroxy-8-methoxy-3- methyl-1(2H)-anthracenone, 10-β-D-glucopyranosyl-1,8,10-trihydroxy-3-(hydroxymethyl)-(R)-9(10H)-anthracenone

INTRODUCTION

In our previous paper (Park *et al.*, 1995; 1996), we described the structure elucidation of C-glycosylchromones and C-glycosylanthrones from *Aloe* species. Further examination on chromones or anthrones in this plant was carried out to afford a tetrahydroanthracene derivative and a C-glycosylanthrone. This paper describes the structure elucidation of these two compounds (Fig. 1) from *A. barbadensis*.

MATERIALS AND METHODS

Materials

Leaves of *A. barbadensis* were obtained from the Nam Yang Aloe Co., Korea.

Instruments

Melting point was recorded on a Gallenkamp melting point apparatus (UK) and was uncorrected. UV spectra were measured on a Shimadzu UV-2100 UV/VIS spectrometer (Japan). ^{1}H- and ^{13}C-NMR spectra were recorded on Jeol JNM-LA300 spectrometer (Japan). IR spectra were obtained on Perkin-Elmer 1710 spectrometer (USA). Mass spectra were obtained using VG TRIO-II GC/MS system (UK) and Jeol AX505WA mass spectrometer (Japan). Silica gel 60 and TLC plates were purchased from Merck (Germany). HPLC was carried out on Samsung SLC-100 system (Samsung, Korea) using Alltech Econosphere C18 column (10 mm×250 mm, 10 μm) (Alltech, USA).

Extraction and isolation

Lyophilized leaves (3 kg, dry weight) of *A. barbadensis* were extracted with EtOH for 3 days at room temperature. The solvent was evaporated *in vacuo* to yield 340 g of EtOH extract. The EtOH extract was suspended in H_2O (1.5 L) and extracted with Et_2O (1 L×3). The aqueous layer was further extracted with water-saturated *n*-BuOH (1 L×3). The Et$_2$O extract (46 g) was successively chromatographed on silica gel column chromatography (500 g, 230-400 mesh, column : 5×70 cm) using EtOAc/MeOH=30/1 (2.5 L) → 5/1 (1 L) using stepwise gradient elution. Six fractions (E1-E6) were obtained and the Fr. E-3 (8.3 g) was further chromatographed on silica gel column (100 g, 230-400 mesh, column : 3×30 cm) using Hexane/EtOAc=2/1 (1.2 L). Compound **1** (5 mg)

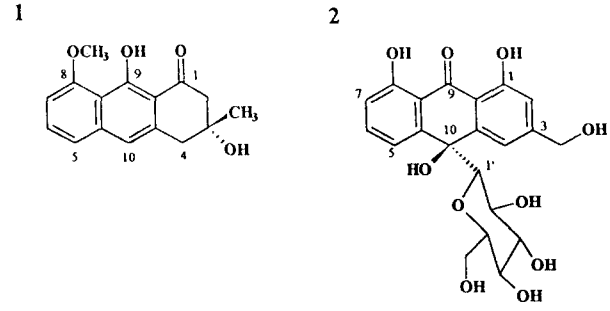

Fig. 1. Structures of compound 1 and 2 from *Aloe barbadensis*.

Correspondence to: Jeong Hill Park, College of Pharmacy, Seoul National University, Seoul 151-742, Korea

was isolated from the Fr. E-3-2 (120 mg) by recrystallization in Benzene/EtOAc. The *n*-BuOH fraction (70 g) also was chromatographed on silica gel column chromatography (800 g, 230-400 mesh, column : 80 mm × 1 m) using EtOAc/MeOH/H_2O=20/2/1 (5 L) → 10/1/1 (2 L) → 2/1/0 (2 L). Four fraction (B1-B4) was obtained and Fr. B1 was chromatographed on silica gel column (100 g, 230-400 mesh, column : 3 × 50 cm) using $CHCl_3$/MeOH/H_2O=10/1/0 (1 L) → 70/30/4 (0.5 L) to make 7 fractions. Among them, Fr. B1-5 was chromatographed using a semi-prep HPLC (column : Alltech Econosphere C18 (10 mm × 250 mm, 10 μm), mobile phase : linear gradient from 10% MeOH → 46% MeOH for 36 min, 46% MeOH → 46% MeOH for 10min, 46% MeOH → 100% MeOH for 10 min, flow rate : 2.0 ml/min, detection : UV 293 nm). Compound **2** (7 mg, Rt=47.7 min) was obtained in 2.3×10^{-4}% yield.

Compound 1: amorphous solid, mp: 161-163°, $[\alpha]_D^{18}$: -26° (MeOH; c=0.1), R_f : 0.25 (benzene/EtOAc=4/1; Kieselgel 60F_{254}), UV λ_{max} (MeOH) : 229, 252, 292, 306, 392 nm, IR ν_{max} (KBr) : 3480, 2950, 1625, 1580, 1460, 1400, 1380, 1280 cm^{-1}, ^{1}H-NMR (300 MHz, $CDCl_3$, δ ppm) : 1.45 (3H, s, 3-CH_3), 2.87 (2H, s, 2-H), 3.11 (2H, s, 4-H), 4.01 (3H, s, 8-OCH_3), 6.82 (1H, d, *J*=8.0 Hz, 7-H), 7.01 (1H, s, 10-H), 7.22 (1H, d, *J*=8.1 Hz, 5-H), 7.50 (1H, t, *J*=8.1 Hz, 6-H), 14.2 (1H, s, 9-OH). Mass [EI$^+$, m/z] (rel.int. %) : 272 [M^+] (100), 254 (58), 239 (34), 214 (68), 190 (49), 163 (21).

Compound 2: amorphous solid, mp : 138-140°, $[\alpha]_D^{18}$: +5.1° (MeOH; c 0.2); R_f : 0.34 (EtOAc/MeOH/H_2O= 10/1/0.5; Kieselgel 60F_{254}), UV λ_{max} (MeOH) : 269, 300, 315, 368 nm, IR ν_{max} (KBr) : 3397, 1638, 1616, 1455, 1285 cm^{-1}, ^{1}H-NMR (300 MHz, DMSO-d_6, δ ppm) : 11.90 (1H, s, 8-OH), 11.80 (1H, s, 1-OH), 7.59 (1H, dd, *J*=7.8, 8.2 Hz, 6-H), 7.42 (1H, d, *J*=7.8 Hz, 5-H), 7.28 (1H, s, 4-H), 6.91 (1H, d, *J*=8.2 Hz, 7-H), 6.88 (1H, s, 2-H), 6.80 (1H, s, 10-OH), 5.57 (1H, brs, 2'-OH), 5.44 (1H, brs, 3-CH_2OH), 4.79 (1H, brs, 4'-OH), 4.89 (1H, brs, 3'-OH), 4.58 (2H, s, 3-CH_2OH), 4.08 (1H, brs, 6'-OH), 3.13 (1H, d, *J*=9.5 Hz, 1'-H), 3.5-2.5 (5H, sugar-H), ^{13}C NMR (75 MHz, CD_3OD, δ ppm) : 163.29 (C-1), 115.35 (C-2), 151.72 (C-3), 116.90 (C-4), 118.07 (C-5), 137.12 (C-6), 117.93 (C-7), 162.61 (C-8), 194.52 (C-9), 76.72 (C-10), 148.88 (C-11), 117.29 (C-12), 116.50 (C-13), 146.92 (C-14), 64.64 (C-3CH_2), 85.31 (C-1'), 72.95 (C-2'), 79.59 (C-3'), 71.70 (C-4'), 81.76 (C-5'), 63.30 (C-6'), Mass [EI$^+$, m/z] (rel. int. %) : 434 [M^+] (0.1), 272 (100), 241 (39), 225 (18)

Synthesis of compound 2

Aloin (100 mg), isolated from *A. barbadensis,* was added to a solution of MeOH (10 ml)/NH_4OH (5 ml)/H_2O (10 ml) and was stirred for 18 hrs at room temperature. The solution was neutralized with 1 M HCl and concentrated *in vacuo* to remove MeOH. This solution was extracted with *n*-BuOH (10 ml × 3) and the solvent was evaporated *in vacuo,* the residue was subjected to semi-prep HPLC (column : Alltech Econosphere C18 (10 mm × 250 mm, 10 μm), mobile phase : linear gradient from 10% MeOH → 46% MeOH for 36min, 46% MeOH → 46% MeOH for 10 min, 46% MeOH → 100% MeOH for 10 min, flow rate : 2.0 ml/min, detection : UV 293 nm). compound 2 (31 mg) was detected at ca. 48 min.

RESULTS AND DISCUSSION

Compound **1**, $C_{16}H_{16}O_4$, gave greenish fluorescence under long wavelength UV (365 nm). The UV spectrum of **1** have maxima at 292, 306 and 392 nm, which is similar to that of torosachrysone (Takido *et al.*, 1977), a tetrahydroanthracene derivative. EI-MS spectrum showed [M^+] peak at m/z=272. The IR spectrum showed a carbonyl bands at 1625 cm^{-1} and a hydroxyl group at 3480 cm^{-1}. The ^{1}H-NMR spectrum of **1** showed a similar pattern to that of tetrahydroanthracene (Takido *et al.*, 1977) and singlet at δ 14.2 suggested the presence of a hydrogen-bonded aromatic hydroxy group. Two doublets at δ 7.22 (*J*=8.1 Hz) and δ 6.82 (*J*=8.0 Hz), and one triplet at δ 7.50 (*J*=8.1 Hz) arised from H-5, H-7 and H-6, respectively. The H-10 proton resonance peak was observed at δ 7.01. Two peaks arised from aliphatic protons were observed at δ 3.11 and δ 2.87. Singlets at δ 4.01 and δ 1.45 indicated the presence of a methoxy and a methyl group. All above data were coincided with that of 3,4-dihydro-3,9-dihydroxy-8-methoxy-3-methyl-1(2H)-anthracenone, or aloechrysone in literature (Dagne *et al.*, 1992), which was reported from *Aloe berhana* but not from *Aloe barbadensis.*

Compound **2**, $C_{21}H_{22}O_{10}$, gave red fluorescence under long wavelength UV (365 nm) and showed a typical absorption pattern of anthrone at 269, 300, 368 nm in UV spectrum (Conner *et al.*, 1989; 1990). EI-MS spectrum showed [M^+] peak at m/z=434, which suggested the substitution of a hydroxyl group in aloin (Farah *et al.*, 1992), a main component of C-glycosylanthrone in *A. barbadensis.* The ^{1}H-NMR spectrum of **2** showed two phenolic proton at δ 11.90 (1H, s), 11.80 (1H, s) and five aromatic protons at δ 7.59 (1H, dd, *J*=7.8, 8.2 Hz), 7.42 (1H, d, *J*=7.8 Hz), 7.28 (1H, s), 6.91 (1H, d, *J*=8.2 Hz), 6.88 (1H, s) and chemical shifts and coupling constants of **2** in phenolic proton or aromatic proton's region showed almost identical to that of aloin. These indicate the hydroxylated position of **2** is not the region above. Anomeric proton of **2** appeared at δ 3.13 (1H, d, *J*=9.5 Hz) and its coupling constant indicated β-glycoside linkage (Overend, 1972).

The ^{13}C-NMR spectrum of **2** showed 21 carbon peaks and also showed almost same pattern to that of aloin (Farah *et al.*, 1992). However, comparing chemical shift of C-10 in **2** with that of aloin, it showed ca. 30 ppm down-field shift in **2**. This indicates the hydroxylated position in **2** is C-10. Hexose carbon of **2** at δ 85.31 (C-1'), 72.95 (C-2'), 79.59 (C-3'), 71.70 (C-4'), 81.76 (C-5') and 63.30 (C-6') suggested that compound **2** has a C-glycosyl linkage and its sugar moiety is glucose (Markham, 1982).

Other assignments were decided mainly by comparison of chemical shifts and coupling constants with those of C-glycosylanthrones (Conner *et al.*, 1989; 1990; Farah *et al.*, 1992) and by ^{1}H-^{1}H COSY, DEPT experiments. To confirm the structure of compound **2**, it was also synthesized from aloin in NH $_{4}$OH/MeOH solution and was identical with 10-hydroxyaloin A of literature (Rauwald, 1990).

By these facts, compound **2** was identified as 10-β-D-glucopyranosyl-1,8,10-trihydroxy-3-(hydroxymethyl)-(R)-9(10H)-anthracenone or 10-hydroxyaloin A, which was reported from *Rhamnus purshianus* (Rauwald, 1990) but not from *Aloe* species.

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REFERENCES CITED

Conner, J. M., Gray, A. I., Reynolds, T. and Waterman, P. G., Anthracene and chromone derivatives in the exudate of *Aloe rabaiensis*. *Phytochemistry*, 28, 3551-3553 (1989).

Conner, J. M., Gray, A. I., Reynold, T. and Waterman, P. G., Anthrone and chromone components of *Aloe cremnophila* and *A. jacksonii* leaf exudates. *Phytochemistry*, 29, 941-944 (1990).

Dagne, E., Casser, I. and Steglich, W., Aloechrysone, a dihydroanthracene from *Aloe berhana*. *Phytochemistry*, 31, 1791-1793 (1992).

Farah, M. H., Andersson, R., Samuelsson, G., Microdontin A and B : Two new aloin derivatives from *Aloe microdonta*. *Planta Medica*, 58, 88-93 (1992).

Markham, K. R., *Techniques of flavonoid identification*, Academic Press, New York, 1982, p.81.

Overend, W. G., Glycosides, In Pigman, W. and Horton, D., (Eds.), *The carbohydrates : chemistry and biochemistry*, Academic Press, New York, 1972, pp. 306-309.

Park, M. K., Park, J. H., Kim, K. H., Shin, Y. G., Myoung, K. M. and Lee, J. H., Chemical constituents on Aloe capensis. *Saengyak Hakhoechi*, 26, 244-247 (1995).

Park, M. K., Park, J. H., Shin, Y. G., Kim, W. Y., Lee, J. H. and Kim, K. H., Neoaloesin A : A new C-glucofuranosyl chromone from *Aloe barbadensis*. *Planta Med.*, 62, 363-365 (1996).

Rauwald, H. W., 16th *Annual Symposium on Pharmacognosy and Natural Products Chemistry*, on 27, Oct, 1989 in Utrecht/Netherlands, Pharm. Ztg. Wiss., 3, 169 (1990).

Takido, M., Takahashi, S., Masuda, K. and Yasukawa K., Torosachrysone, a new tetrahydroxyanthracene derivative from the seeds of *Cassia torosa*, *Lloydia*, 40, 191-194 (1977).