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A LABDANE DITERPENE GLYCOSIDE FROM FRUIT OF *VITEX ROTUNDIFOLIA*

MASATERU ONO,* YASUYUKI ITO and TOSHIHIRO NOHARA†

Research Institute of General Education, Kyushu Tokai University, Choyo 5435, Aso, Kumamoto 869-14, Japan;
† Faculty of Pharmaceutical Sciences, Kumamoto University, Oe-honmachi 5-1, Kumamoto 862, Japan

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Key Word Index—*Vitex rotundifolia*; Verbenaceae; Viticis trifoliae Fructus; labdane-type diterpene glycoside; viteoside A.

Abstract—A new labdane-type diterpene glycoside, viteoside A, was isolated from the fruit of *Vitex rotundifolia*. The structure was characterized as (8*R*,10*S*)-6β-acetoxy-3β,9α-dihydroxy-labda-13*Z*-en-16,15-olide 3-*O*-β-D-glucopyranoside on the basis of spectroscopic data. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Vitex rotundifolia L. is widely distributed in Asia, and its fruit, Viticis trifoliae Fructus, is used as a folk medicine for headaches [1]. During the course of our studies on natural antioxidants, the methanol extract of this fruit was found to show strong antioxidative activity [2].

We earlier reported the isolation and structure elucidation of eight iridoid derivatives, two phenyl propanoids, one flavanone and three lignane derivatives in the methanol extract of Viticis trifoliae Fructus; and further, that seven phenolic compounds among these compounds were found to have a stronger antioxidative activity than 3-*tert*-butylhydroxyanisole [2, 3]. In a continuation of our studies on this fruit, we now report the isolation and structure determination of a new labdane-type diterpene glycoside, named viteoside A.

RESULTS AND DISCUSSION

The methanol extract of the fruit of *Vitex rotundifolia* L. was purified by MCI gel CHP20, Sephadex LH20 and silica gel column chromatography as well as HPLC on ODS to afford a new labdane-type diterpene glycoside, viteoside A (**1**).

Compound **1** exhibited an $[M-H]^-$ ion peak at *m/z* 555 in the negative FAB-MS and an $[M+Na]^+$ ion peak at *m/z* 579 in the positive FAB-MS indicating its molecular weight to be 556. The molecular formula was determined as $C_{28}H_{44}O_{11}$ by high resolution positive FAB-MS. The IR spectrum of **1** showed strong absorption bands due to hydroxyl groups (3410 cm^{-1}) and an α,β-unsaturated lactone (1732 and 1647 cm^{-1}). The ^1H NMR spectrum showed the signals of three tertiary methyl groups (δ 1.46, 1.37, 1.37), one secondary methyl group (δ 1.13), one acetyl group (δ 2.08) and one anomeric proton (δ 4.86). The ^{13}C NMR spectrum gave 28 carbon signals including one acetyl group (δ 170.3, 21.8) and one glucopyranosyl group (δ 106.9, 75.8, 78.8, 71.8, 78.2, 63.0) [4], whose glycosidic linkage was shown to be β by the coupling constant ($J = 7.3$ Hz) of the anomeric proton signal. These ^1H NMR and ^{13}C NMR signals were assigned with the aid of ^1H-^1H COSY, HMQC and HMBC spectra as shown in Table 1, and they suggested **1** to be a labdane-type diterpene glucoside. The connectivities of the glucosyl unit and acetyl group were determined by the HMBC spectrum (Fig. 1).

Acidic hydrolysis of **1** gave glucose which was identified as the D-form by GC analysis according to Hara *et al.* [5]. The relative stereochemistry of the aglycone moiety of **1** was determined by the NOE experiments, which were mainly carried out in pyridine-d_5, but the NOE correlation between the signals of H_3-19 and H_3-20 was measured in methanol-d_4, owing to both signals giving the same chemical shifts in pyridine-d_5 (Fig. 2). The absolute configuration at C-3 was established to be *S* by the comparison of the chemical shifts of the signals due to C-2, C-3, C-4 and C-1′ (anomeric carbon) between **1** and dammarenediol-I 3*S*-*O*-β-D-glucopyranoside (**2**), because it is known that the chemical shifts of α-, β- and β'- carbons of secondary alcohols to which β-D-glucopyranose was attached, and anomeric carbon reflect the absolute configuration of alcohols [4, 6] and the chemical shifts of C-2, C-3, C-4 and C-1′ of **1** were superimposable on those [δ 26.8

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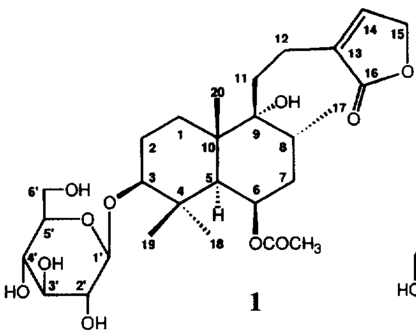
* Author to whom correspondence should be addressed. Fax: +81(0)9676-7-2659; e-mail: mono@as-1.ktokai-u.ac.jp



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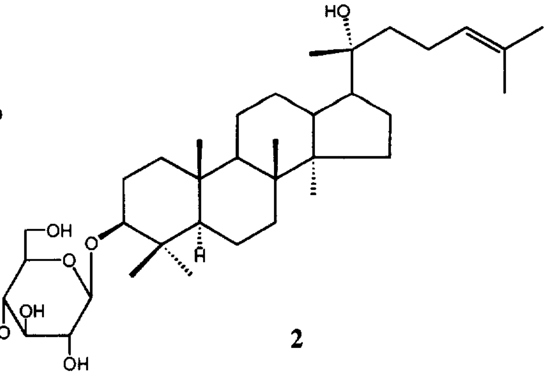

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(C-2), 88.8 (C-3), 39.7 (C-4), 106.9 (C-1′)] of **2**, while different from those [δ 23.8 (C-2), 84.8 (C-3), 38.6 (C-4), 102.3 (C-1′)] of dammarenediol-I 3*S*-*O*-β-L-glucopyranoside [4].

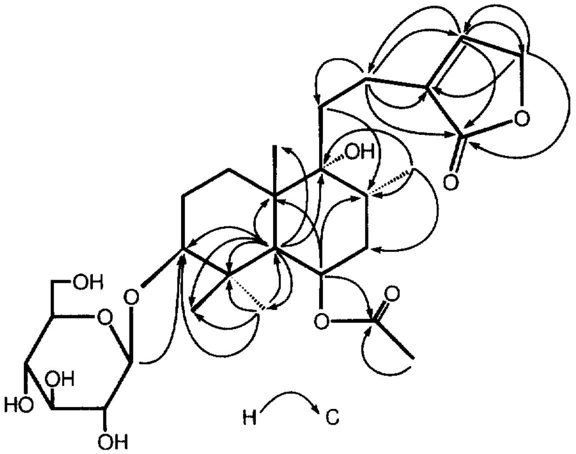

Fig. 1. ^{1}H-^{13}C long range correlations observed for compound **1** in the HMBC spectrum.

Consequently, the structure of **1** was determined as (8*R*,10*S*)-6β-acetoxy-3β,9α-dihydroxy-labda-13*Z*-en-16,15-olide 3-*O*-β-D-glucopyranoside.

Table 1. ^{1}H and ^{13}C NMR spectral data for viteoside A (**1**) (in pyridine-d_5)

H/C	δ_H*	δ_C
1	1.53 *ddd* (12.8, 3.7, 3.7) ca 2.24	31.9
2	ca 2.02 2.42 *dddd* (13.7, 3.7, 3.7, 3.7)	27.0
3	3.45 *dd* (11.9, 3.7)	88.8
4		40.3
5	2.14 *d* (3.7)	47.4
6	5.77 *ddd* (3.7, 3.4, 3.4)	70.5
7	1.75 *ddd* (14.0, 3.4, 3.4) ca 2.06	37.0
8	ca 2.23	32.4
9		76.6
10		43.9
11	1.90 *m* ca 2.10	33.0
12	2.70 *t* (7.9)	23.0
13		134.7
14	7.25 *t* (1.8)	145.2
15	4.76 *d* (1.8)	70.8
16		174.9
17	1.13 *d* (6.7)	16.4
18	1.46 *s*	28.2
19	1.37 *s*	18.1
20	1.37 *s*	19.5
$COCH_3$	2.08 *s*	170.3 21.8
1′	4.86 *d* (7.3)	106.9
2′	4.05 *dd* (8.6, 7.3)	75.8
3′	4.23 *dd* (8.6, 8.6)	78.8
4′	4.25 *dd* (8.6, 8.6)	71.8
5′	3.95 *ddd* (8.6, 4.9, 2.4)	78.2
6′	4.43 *dd* (11.6, 4.9) 4.56 *dd* (11.6, 2.4)	63.0

* *J* values (in Hz) in parentheses.

EXPERIMENTAL

General

^{1}H NMR: 500 MHz; ^{13}C NMR: 125 MHz; NOE (400 MHz); HMQC (500 MHz); HMBC (500 MHz) (TMS as int. standard). CC: Diaion HP 20 (Mitsubishi Chemical Industries Co., Ltd.), Sephadex LH 20 (Pharmacia Fine Chemicals) and silica gel 60 (230–400 mesh, Merck). HPLC: YMC pack S-5 120A ODS (250 mm × 20 mm i.d., YMC Co., Ltd.). GC: silicone OV-1 (30 m × 0.32 mm i.d., Ohio Valley Specially Chem.)

Plant material

Fruit of *Vitex rotundifolia* L. was purchased from Uchida Wakanyaku Co., Ltd. (Lot. 232418).

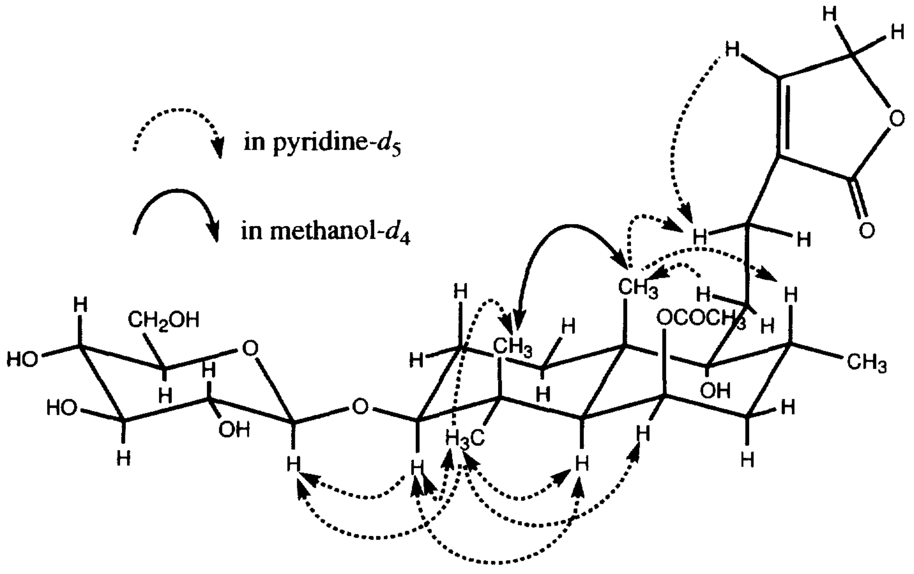

Fig. 2. NOEs observed for **1** in the NOE difference spectra.

Extraction and isolation

Powdered fruit of *Vitex rotundifolia* L. (2.914 kg) was extracted with MeOH. The MeOH extract (185.2 g) was partitioned between hexane and MeOH. The MeOH layer was filtered through absorbent cotton. The filtrate was evaporated under red. pres. and crude material (158 g) was successively subjected to Diaion HP 20 (40% MeOH, 70% MeOH, 90% MeOH, MeOH, Me_2CO), Sephadex LH 20 (60% MeOH), silica gel ($CHCl_3$–MeOH, 40:1, 30:1, 20:1, 10:1) and HPLC (60% MeOH) to yield viteoside A (**1**) (13 mg).

Viteoside A (**1**). Powder, $[\alpha]_D^{27}$ $-20.4°$ (MeOH; c 0.5). IR ν_{max}^{KBr} cm^{-1}: 3410 (OH), 1732, 1647 (α,β-unsaturated lactone). FAB-MS (negative) m/z: 555 $[M-H]^-$; FAB-MS (positive) m/z: 579 $[M+Na]^+$; HR FAB-MS (positive) m/z: 579.2778 $[M+Na]^+$ (Calcd for $C_{28}H_{44}O_{11}Na$: 579.2781). ^1H NMR (pyridine-d_5): see Table 1; ^1H NMR (methanol-d_4): δ 7.34 (1H, t, $J = 1.5$ Hz, H-14), 5.40 (1H, ddd, $J = 3.4, 3.4, 3.4$ Hz, H-6), ca 4.87 (2H, H_2-15), 4.31 (1H, d, $J = 7.3$ Hz, H-1′), 3.84 (1H, dd, $J = 11.6, 2.2$ Hz, Ha-6′), 3.67 (1H, dd, $J = 11.6, 5.5$ Hz, Hb-6′), ca 3.34 (2H, H-3′ and H-4′), ca 3.31 (1H, H-5′), 3.19 (1H, dd, $J = 8.5, 7.3$ Hz, H-2′), 3.11 (1H, dd, $J = 11.3, 4.0$ Hz, H-3), 2.39 (2H, m, H_2-12), 2.13 (1H, m, H-8), 2.04 (3H, s, $COCH_3$), ca 2.01 (1H, Ha-2), 1.72 (1H, d, $J = 1.8$ Hz, H-5), ca 1.47 (2H, Hb-7 and Hb-11), 1.30 (3H, s, H_3-20), 1.11 (3H, s, H_3-18), 1.03 (3H, s, H_3-19), 0.94 (3H, d, $J = 6.7$ Hz, H_3-17). ^{13}C NMR (in pyridine-d_5): see Table 1.

Acidic hydrolysis of **1**. Compound **1** (3 mg) in 2 N HCl (0.3 ml) was heated at 85° on a water bath for 90 min and the reaction mixture was neutralized with 1 N NaOH. After removal of the solvent under red. pres., the residue was extracted with MeOH (1 ml). The MeOH extract was chromatographed over silica gel ($CHCl_3$–MeOH–H_2O, 14:2:0.1, 10:2:0.1, 7:3:0.5, 6:4:1) to give a monosaccharide (0.5 mg).

This monosaccharide was converted into trimethylsilyl ether of the methyl thiazolidine 4 (R)-carboxylate derivatives and subjected to GC analysis (detector: FID, column temp.: 230°C, injector temp.: 270°C, detector temp.: 270°C, carrier gas: He) [5]. The retention time of this product was identical with that of an authentic sample of D-glucose derivative.

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