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A LUPANE-TRITERPENE AND A 3(2 → 1)ABEOLUPANE GLUCOSIDE FROM *HOVENIA TRICHOCAREA*

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Key Word Index—*Hovenia trichocarea*; Rhamnaceae; triterpenic acid; lupane; 3(2 → 1)abeolupane; 2α,23-dihydroxybetulinic acid; hovetrichoside; hovenic acid; ceanothetric acid.

Abstract—A new lupane-triterpene, hovenic acid and 3(2 → 1)abeolupane glucoside, hovetrichoside H were isolated from the fresh bark of *Hovenia trichocarea*, together with ceanothetric acid, (+)-lyoniresinol-3a-*O*-β-D-glucopyranoside, (−)-lyoniresinol-3a-*O*-β-D-glucopyranoside, and citrusin B. The structures of the new compounds were established by extensive NMR experiments and chemical methods. Hovenic acid was established to be 2α,3β,23-trihydroxylup-20(29)-en-28-oic acid (2α,23-dihydroxybetulinic acid). Hovetrichoside H was identified as 2α-carboxy-3β-hydroxy-3(2 → 1)abeolup-20(29)-en-27,28-dioic acid (ceanothetric acid) 28-*O*-β-D-glucopyranoside.

INTRODUCTION

Two *Hovenia* species occur in Japan and the leaves of both *Hovenia* species are used as a remedy for crapulence [1]. We have already reported the isolation and structural elucidation of seven phenolic glycosides, named hovetrichosides A–G, from the fresh barks of *Hovenia trichocarea* [2]. In continuation of our studies on constituents of this plant, two more new triterpenoids, designated as hovenic acid and hovetrichoside H, and four known compounds have been isolated and characterized. Their structures were elucidated by chemical and spectral methods, especially 2D-NMR techniques.

RESULTS AND DISCUSSIONS

The ethylacetate-soluble portion of the ethanol extract of the fresh bark of *Hovenia trichocarea* Chun et Tsiang was separated using silica gel chromatography. Further purification of each fraction by reversed-phase high-pressure liquid chromatography gave hovenic acid (**1**) and hovetrichoside H (**2**), together with ceanothetric acid (**3**) [3], (+)-lyoniresinol-3a-*O*-β-D-glucopyranoside (**4**), (−)-lyoniresinol-3a-*O*-β-D-glucopyranoside (**5**) [4] and citrusin B (**6**) [5]. The four known compounds were identified as **3**, **4**, **5** and **6** by comparison with published data [3–5].

Hovenic acid (**1**) was obtained as colourless needles, m.p. 180–182°C, $[\alpha]_D^{25}$ −30.3° (MeOH; *c* 0.2), whose molecular formula $C_{30}H_{48}O_5$ was established by its HR-EI mass spectrum. Its IR spectrum indicated the absorptions for hydroxyl (3415 cm^{-1}) and carboxyl groups (1715 cm^{-1}). The ^{13}C NMR spectrum revealed 30 carbon signals which were shown by a DEPT experiment to be five methyls, nine methylenes, five methines, five quaternary carbons, one alcoholic methylene, two alcoholic methines, one carboxylic acid and two olefinic carbons, revealing that **1** is a triterpenic acid having five rings. The ^1H NMR spectrum of **1** revealed the signals for four tertiary methyl groups at δ 0.97, 0.98, 1.04, 1.07, one isopropenyl moiety at δ 1.76, 4.75 and 4.92, one hydroxymethyl group at δ 3.71 and 4.21 and a 1,2 glycol at δ 4.21 and 4.24. ^1H–^1H COSY and ^{13}C–^1H COSY experiments revealed the partial structures **a** (CH_2CHCH), **b** ($CHCH_2CH_2$), **c** ($CHCH_2CH_2CHCHCHCH_2CH_2$), **d** (CH_2CH_2) and **e** ($CH_2{=}CCH_3$). An HMBC experiment revealed that the H_3-25 methyl protons which appeared at δ 0.98 were coupled to C-1, C-5, C-9 and C-10 and the H_2-23 methylene protons to C-3, C-4, C-5 and C-24. This suggested a linkage among partial structures **a**, **b** and **c**. Furthermore, the HMBC spectrum showed that the H_3-26 methyl protons were coupled to C-7, C-8, C-9 and C-14 and the H_3-27 methyl

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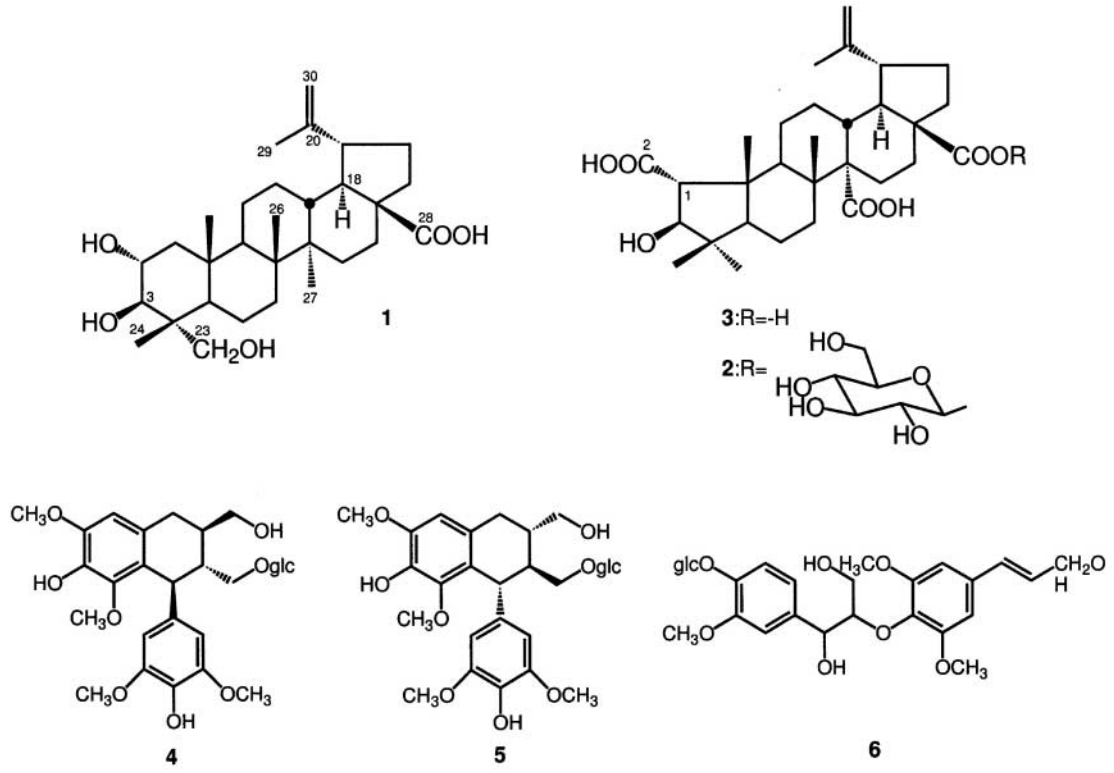

protons were coupled to C-8, C-13, C-14 and C-15. These data established the connectivities between the partial structures **c** and **d**. The HMBC spectrum also showed couplings between H_3-29 and C-19, H-18 and C-28. Thus, the planar structure of **1** was determined (Fig. 1). The α-OH at the C-2 position and β-OH at the C-3 position were assigned from the splitting pattern of the H-2 (δ 4.24) and H-3 (δ 4.21). The stereochemistry of the hydroxymethyl at C-4 was deduced as the α-configuration from an NOE between the C-25 methyl group and C-24 methyl group in a ROESY experiment. The α-isopropenyl group at the C-19 position was proven by the NOE between H-13 (δ 2.72) and H-19 (δ 3.52). Thus, the structure of hovenic acid (**1**) was formulated as 2α,3β,23-trihydroxylup-20(29)-en-28-oic acid (2α,23-dihydroxybetulinic acid).

Compound **3** was obtained as an amorphous powder (m.p. 145–147°C). The molecular formula was established as $C_{30}H_{44}O_7$ on the basis of HR-CI mass spectrum. Analysis of the various NMR data of **3** showed that **3** was 2α-carboxy-3β-hydroxy-3(2 → 1)abeolup-20(29)-en-27,28-dioic acid (ceanothetric acid), which was recently isolated from *Ceanothus americanus* [3].

Hovetrichoside H (**2**), $[\alpha]_D^{25}$ +11.9° (MeOH; *c* 2.2) had the molecular formula $C_{36}H_{54}O_{12}$ (negative FABMS, *m/z* 677 $[M-H]^-$), i.e. 162 mass units ($C_6H_{10}O_5$) higher than that of **3**. The ^{1}H NMR and ^{13}C NMR spectra of **2** showed that it was a ester glycoside of ceanothetric acid. Alkaline treatment of **2** gave **3**, which was shown to be identical with an authentic sample by TLC and NMR spectroscopy. On acid hydrolysis, **2** afforded D-glucose as the sugar part and this was confirmed by its specific rotation using chiral detection in HPLC [6]. In the ^{1}H NMR spectrum of **2** an anomeric proton signal appeared at δ 6.49 (1H, *d*, J = 8.5 Hz), disclosing the glucose had the β-configuration. The location of the glucosidic linkage was proved by the long range couplings between C-28 (δ 176.8) and H-1 (δ 6.49) of glc, H-16α (δ 1.90), H-18 (δ 2.20) and H_2-22 (δ 1.42 and 2.20) in the HMBC experiments. Hence, **2** was formulated as 2α-carboxy-3β-

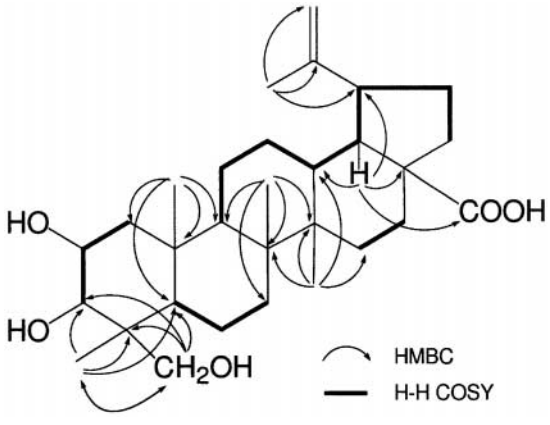

Fig. 1. HMBC correlations and H–H COSY for **1**.

hydroxy-3(2 → 1)abeolup-20(29)-en-27,28-dioic acid (ceanothetric acid) 28-*O*-*β*-D-glucopyranoside.

EXPERIMENTAL

General

M.p.s points were measured with a Yanagimoto micromelting point apparatus and were uncorrected. Optical rotations were taken on a JASCO DIP-360 polarimeter. IR spectra were recorded on a JASCO FT/IR-5300, NMR spectra on Varian UNITY 600 and/or JEOL GSX-400 spectrometer in pyridine solution using TMS as an internal standard. NMR experiments included ^{1}H–^{1}H COSY, ^{13}C–^{1}H COSY, HMBC, TOCSY and ROESY. Coupling constants (*J* values) are given in Hz. The FABMS (Xe gun, 10 kV, triethylene glycol as the matrix) was measured on a JEOL JMS-PX303 mass spectrometer and JEOL JMS-HX-100 mass spectrometer. HPLC separations were performed with an Hitachi HPLC system (L-7100 Pump, L-4000 UV).

Plant material

The bark of *Hovenia trichocarea* Chun et Tsiang was collected in Tokushima Prefecture, Japan, in April 1995. The voucher specimen (TB 5421) is deposited in the Herbarium of the Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Tokushima, Japan.

Extraction and isolation

The fresh bark (10 kg) of *Hovenia trichocarea* was extracted with absolute EtOH at room temperature for 6 weeks. The EtOH extract was partitioned between H_2O and EtOAc. An aliquot (70 g) of the EtOAc soluble portion (140 g) was subjected to CC on silica gel eluting with CH_2C1_2–MeOH–H_2O (25:4:0.1–25:10:0.1) to afford frs. 1–6. Fr. 3 was subjected to CC on silica gel eluting with CH_2C1_2–MeOH–H_2O (30:1:0–25:6:0.1) to afford frs. 3–1′–4′. Frs. 3–3′–4′ were further purified by prep. HPLC (C_8, 55% MeOH) to afford hovenic acid (**1**, 15 mg) and ceanothetric acid (**3**, 120 mg). Fr. 5 was subjected to CC on silica gel eluted with CH_2Cl_2–MeOH–EtOAc–H_2O (4:2:4:1, lower layer) to give 5 fractions (frs. 5–1′–5′). Fr. 5–4′ was purified by prep. HPLC (ODS S-5, 8–6% CH_3CN) to afford (+)-lyoniresinol-3a-*O*-*β*-D-glucopyranoside (**4**, 30 mg), (−)-lyoniresinol-3a-*O*-*β*-D-glucopyranoside (**5**, 30 mg) and citrusin B (**6**, 20 mg). Fr. 5–5′ was purified with HPLC (ODS S-5, 17% CH_3CN) to give hovetrichoside H (**2**, 210 mg).

Hovenic acid (**1**)

Colorless needles, m.p. 180–182°C. $[\alpha]_D^{25}$ −30.3° (MeOH; *c* 0.2). FT-IR (dry film) ν_{max}: 3415 (OH), 1715 (C=O) cm^{-1}. HREIMS *m/z* 488.3495 $[M]^+$ $C_{30}H_{48}O_5$ required 488.3502. ^{1}H NMR (600 MHz, pyridine-d_5): δ 0.97 (3H, *s*, Me-27), 0.98 (3H, *s*, Me-25), 1.04 (3H, *s*, Me-24), 1.34 (1H, *dd*, *J* = 12.0, 12.0 Hz, H_α-1), 1.50 (1H, *m*, H_β-22), 1.71 (1H, *dd*, *J* = 12.0, 12.0 Hz, H-18), 1.76 (3H, *s*, Me-29), 1.84 (1H, *ddd*, *J* = 13.0, 13.0, 4.5 Hz, H_β-15), 1.90 (1H, *br d*, *J* = 12.0 Hz, H_β-12), 2.14 (1H, *m*, H_α-22), 2.36 (1H, *dd*, *J* = 12.0, 4.5 Hz, H_β-1), 2.58 (1H, *ddd*, *J* = 13.0, 3.5, 3.5 Hz, H_β-16) 2.72 (1H, *ddd*, *J* = 12.0, 12.0, 4.5 Hz, H-13), 3.52 (1H, *m*, H-19), 3.71 and 4.21 (each, 1H, *d*, *J* = 10.5 Hz, H_2-23), 4.21 (1H, *d*, *J* = 10.0 Hz, H-3), 4.24 (1H, *dd*, *J* = 10.0, 4.5 Hz, H-2), 4.75 (1H, *br s*, H_2-30), 4.92 (1H, *br d*, *J* = 2.0 Hz, H_2-30). ^{13}C NMR data, see Table 1.

Hovetrichoside H (**2**)

Colorless needles, m.p. 212–214°C. $[\alpha]_D^{25}$ +11.9° (MeOH; *c* 2.2). FT-IR (dry film) ν_{max}: 3380 (OH), 1740, 1710, 1695 (C=O), 1080 (OH) cm^{-1}. FABMS *m/z*: 677 $[M–H]^-$, 515 $[M–Glc–H]^-$. ^{1}H NMR (600 MHz, pyridine-d_5): aglycone moiety: δ 1.23 (3H, *s*, Me-24), 1.28 (3H, *s*, Me-23), 1.35 (3H, *s*, Me-26), 1.38 (1H, *m*, H_α-6), 1.40 (1H, *m*, H_α-21), 1.42 (1H, *m*, H_β-22), 1.44 (3H, *s*, Me-25), 1.50 (1H, *dddd*, *J* = 12.0, 12.0, 12.0, 3.0 Hz, H_β-6), 1.72 (1H, *dddd*, *J* = 12.0, 12.0, 12.0, 4.0 Hz, H_β-11), 1.78 (3H, *s*, Me-29), 1.81 (1H, *ddd*, *J* = 13.0, 3.0, 3.0 Hz, H_β-7), 1.90 (1H, *ddd*, *J* = 12.0, 12.0, 3.0 Hz, H_α-16), 2.02 (1H, *m*, H_β-12), 2.11 (1H, *m*, H_β-15), 2.13 (1H, *m*, H_α-7), 2.13 (1H, *m*, H_β-21), 2.18 (1H, *m*, H_α-5),

Table 1. ^{1}H and ^{13}C NMR data of compounds **1**–**3** in pyridine-d_5

Position	1	2	3
C-1	48.3	178.3	178.0
2	69.3	67.2	67.2
3	78.3	84.8	84.7
4	43.8	43.9	43.9
5	48.3	57.3	57.3
6	18.7	19.3	19.3
7	34.6	33.7	37.9
8	41.3	41.8	41.8
9	51.1	46.3	46.2
10	38.7	50.2	50.2
11	21.6	24.4	24.5
12	26.2	27.0	27.2
13	38.7	40.5	40.7
14	43.0	60.5	60.5
15	30.4	28.9	29.0
16	33.0	34.9	35.6
17	56.7	57.2	56.8
18	49.8	52.5	52.4
19	47.8	47.7	48.0
20	151.3	150.8	151.0
21	31.3	31.0	31.4
22	37.7	37.1	37.9
23	66.5	20.5	31.6
24	14.3	31.6	20.5
25	18.3	19.6	19.6
26	16.6	18.2	18.3
27	15.0	178.9	178.5
28	178.9	175.6	179.3
29	19.6	19.6	19.6
30	110.0	110.5	110.1

2.20 (1H, *m*, H_α-18), 2.20 (1H, *m*, H_α-22), 2.24 (1H, *br d*, J = 12.0 Hz, H_α-11), 2.50 (1H, *br d*, J = 13.0 Hz, H_α-15), 2.60 (1H, *br d*, J = 12.0 Hz, H_α-9), 2.78 (1H, *dddd*, J = 12.0, 12.0, 12.0, 4.0 Hz, H_α-12), 2.93 (1H, *ddd*, J = 12.0, 12.0, 4.0 Hz, H_α-13), 2.93 (1H, *ddd*, J = 12.0, 4.0, 4.0 Hz, H_β-16), 3.23 (1H, *s*, H_β-1), 3.54 (1H, *ddd*, J = 11.0, 5.0, 5.0 Hz, H_β-19), 4.63 (1H, *br s*, H_2-30), 4.80 (1H, *s*, H_α-3), 4.93 (1H, *br s*, H_2-30). ^{13}C NMR data of aglycon, see Table 1. Sugar moiety: δ 4.07 (1H, *m*, H-5), 4.21 (1H, *dd*, J = 8.5, 8.5 Hz, H-2), 4.33 (1H, *dd*, J = 9.0, 8.5 Hz, H-3), 4.39 (1H, *dd*, J = 9.0, 9.0 Hz, H-4), 4.41 (1H, *dd*, J = 12.0, 4.5 Hz, H-6), 4.47 (1H, *dd*, J = 12.0, 2.0 Hz, H-6), 6.49 (1H, *d*, J = 8.5 Hz, H-1). ^{13}C NMR (125 MHz, pyridine-d_5): δ 62.3 (C-6), 71.2 (C-4), 74.5 (C-2), 79.0 (C-3), 79.6 (C-5), 95.6 (C-1).

Acid hydrolysis of hovetrichoside H (**2**)

A solution of **2** (3 mg) in 5% H_2SO_4 in EtOH was heated at 100°C for 3 h. The reaction mixture was extracted with Et_2O. The aq. layer was neutralized with Amberlite IRA-45 and evaporated *in vacuo* to dryness. The sugar was determined by using RI detection (waters 410) and chiral detection (Shodex OR-1), respectively, in HPLC (Shodex RSpak DC-613, 75% CH_3CN, 1 ml/min, 70°C) by comparison with authentic sugars (10 mM each of D-glc and L-glc). The sugar part gave a peak indicating positive optical rotation at 7.38 min (D-glc, 7.38 min).

Alkaline hydrolysis of hovetrichoside H (**2**)

A solution of **2** (30 mg) in 0.6 N NaOH (20 ml) in MeOH (5 ml) was heated at 30°C under N_2 for 1 day. The reaction mixture was adjusted to pH 1.0 with 10% H_2SO_4 and extracted with *n*-BuOH. The *n*-BuOH layer was subjected to HPLC (ODS, 54% CH_3OH) to afford ceanothetric acid (**3**, 5 mg). Compound **3**: amorphous powder, m.p. 145–147°C. $[\alpha]_D^{25}$ +27.6° (MeOH; *c* 4.2). FT-IR (dry film) ν_{max}: 3430 (OH), 1695 (C=O), 1035 (OH) cm^{-1}. FABMS *m/z* 515 $[M-H]^-$. ^{1}H NMR (400 MHz, C_5D_5N) δ: 1.23 (3H, *s*, Me-26), 1.27 (6H, *s*, Me-23 and -26), 1.44 (3H, *s*, Me-25), 1.81 (3H, *s*, Me-29), 3.22 (1H, *s*, H-2), 4.80 (1H, *s*, H-3), 4.66 and 4.99 (each 1H, *s*, H_2-30). ^{13}C NMR data see Table 1.

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