PERGAMON

Phytochemistry 60 (2002) 765–768

PHYTOCHEMISTRY

www.elsevier.com/locate/phytochem

Triterpenoids from *Viburnum suspensum*

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Received 29 January 2002; received in revised form 17 April 2002

Abstract

Three triterpenoids, 3-oxo-11,13(18)-oleanadien-28-oic acid, 24-hydroxy-3-oxo-11,13(18)-oleanadien-28-oic acid, 6β-hydroxy-3-oxo-11,13(18)-oleanadien-28-oic acid have been isolated together with the previously known virgatic acid, vibsanin B and 3-hydroxyvibsanin E from the leaves of *Viburnum suspensum*. Their structures were determined by spectroscopic methods and by comparison of their NMR spectral data with those of the previously known 11,13(18)-oleanadien-3β-ol.

Keywords: *Viburnum suspensum*; Caprifoliaceae; Leaves; Triterpenoid; 11,13(18)-Oleanadiene; Vibsane diterpenoid

1. Introduction

The genus *Viburnum* (Caprifoliaceae) contains over a hundred species, only 21 of which have been studied chemically (Kuo, 1992). *Viburnum* species characteristically contain iridoids, triterpenoids, coumarins and flavones (Iwagawa et al., 1994; Machida and Kikuchi, 1997; Kuroyanagi et al., 1986). The rare vibsane diterpenoids e.g. vibsanin B (**5**) (Fukuyama et al., 1997), however, have been isolated from the leaves of *Viburnum awabuki* and *Viburnam odoratissimum* (Kawazu, 1980; Kubo et al., 2001a). As part of a search for new vibsane diterpenoids (Kubo et al., 2001b) in *Viburnum* species, the chemical constituents of the leaves of *Viburnam suspensum* Lindl. have been studied. Three oleanane triterpenoids **1**–**3** were isolated together with the previously known virgatic acid (Ulubelen and Ayanoglu, 1976), and vibsane diterpenoids, vibsanin B (**5**) and 3-hydroxyvibsanin E (Fukuyama et al., 1999).

2. Results and discussion

The leaves of *V. suspensum* were extracted with methanol and the methanol extract was fractionated by repeated CC on silica gel, octadecyl silica gel (ODS) and Sephadex LH-20 to give three new oleanane triterpenoids **1**–**3** along with the previously known virgatic acid, vibsanin B (**5**) and 3-hydroxyvibsanin E.

Compound **1** was assigned the molecular formula $C_{30}H_{44}O_3$ (HR–EI MS, m/z 452.3279 $[M]^+$). Its spectral data showed the presence of a carboxylic group (3209 and 1710 cm^{-1}; δ_C 180.8), a ketonic carbonyl group (1703 cm^{-1}; δ_C 217.5) and a conjugated diene moiety (257, 249 and 242 nm). The ^1H NMR spectrum of **1** contained the signals due to seven tertiary methyl groups [δ_H 0.81, 0.83, 0.96, 0.99, 1.03, 1.04, and 1.10 (each 3H, *s*)], a disubstituted olefin [δ_H 5.63 (1H, *dd*, $J=10.6$, 1.8 Hz) and 6.47 (1H, *dd*, $J=10.6$, 3.2 Hz)] which were found not only to be long-range coupled to H-9 at δ_H 2.05 (1H, *dd*, $J=3.2$, 1.8 Hz) by H–H COSY but also to be conjugated to a $\Delta^{13,18}$ tetrasubstituted double bond on the basis of the expected HMBC correlations of H-12. Thus compound **1** is a 11,13(18)-oleanadiene derivative (Caldwell et al., 2000). In fact, the ^{13}C NMR spectral data (Table 1) of **1** were very similar to those of 11,13(18)-oleanadien-3β-ol (**4**) isolated from *Phyllanthus flexuosus* (Tanaka and Matsunaga, 1988) except for the presence of the C-3 and C-28 carbonyl resonances. The positions of the ketone function and the carboxylic acid were confirmed to be C-3 and C-28, respectively, by HMBC correlations. Thus **1** was assigned as 3-oxo-11,13(18)-oleanadien-28-oic acid.

Compound **2** had the molecular formula $C_{30}H_{44}O_4$ (HR–EI MS, m/z 468.3262 $[M]^+$) and its NMR spectra resembled those of **1** apart from a tertiary methyl group and appearance of a hydroxymethylene [δ_H 3.44 (1H, *d*, $J=11.3$ Hz) and 3.95 (1H, *d*, $J=11.3$ Hz); δ_C 65.1].

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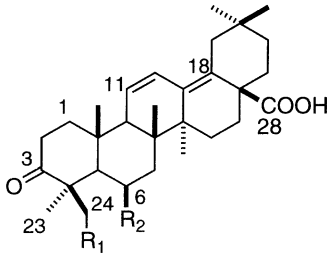
0031-9422/02/$ - see front matter
PII: S0031-9422(02)00153-X



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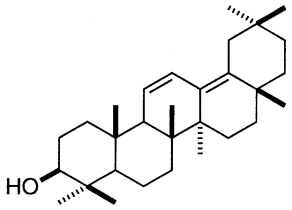

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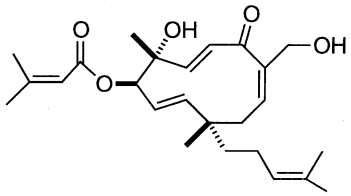

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It was clear from the HMBC spectrum that either Me-23 or Me-24 had been oxidized. A clear NOE from the hydroxymethylene protons to Me-25 (δ_H 0.96) indicated that the hydroxymethylene group was at C-24. Thus compound **2** is 24-hydroxy-3-oxo-11,13(18)-oleanadien-28-oic acid.

Compound **3** was found to have the same molecular formula $C_{30}H_{44}O_4$ (HR–EI MS, m/z 468.3263 $[M]^+$) as **2**. The IR and UV spectra of **3** showed the presence of hydroxyl (3511 cm^{-1}), carbonyl (1694 cm^{-1}) and carboxylic (3191 and 1734 cm^{-1}) groups, and a conjugated diene system (242, 249 and 257 nm). The NMR spectroscopic data for **3** were similar to those of **1** except for the presence of an oxygen-bearing methine resonating at δ_H 4.52 (1H, *ddd*, $J = 3.3$, 2.7, 2.5 Hz) and δ_C 69.7. This methine signal was coupled to the H-5 doublet signal as observed in the H–H COSY spectrum and also showed correlations to C-5, C-7 and C-10 signals in the HMBC spectrum indicating that the hydroxyl group is attached to C-6. The hydroxyl group was clearly β in view of the small coupling (2.5 Hz) of H-6 and the NOESY interaction of H-6 and H-5. Thus compound **3** is 6β-hydroxy-3-oxo-11,13(18)-oleanadien-28-oic acid.

A number of iridoids and iridoid glycosides (Iwagawa et al., 1994) and labdane diterpenoids (Iwagawa et al., 1993) have been reported from the leaves of *V. suspensum*, but this is the first report of 11,13(18)-oleanadiene triterpenoids. The presence of virganic acid, structurally related to 1-oxo-erythrodiol (Kagawa et al., 1998), as well as typical vibsane diterpenoids suggest that *V. suspensum* is taxonomically close to *V. awabuki*. Thus oleanane triterpenoids, iridoids and vibsane diterpenoids may be regarded as characteristic constituents of *Viburnum* species.

3. Experimental

3.1. General

Optical rotations were measured on a Jasco DIP-1000 digital polarimeter. IR spectra were measured on a Jasco FT–IR 5300 infrared spectrophotometer. NMR spectra were recorded on a Varian Unity 600 or 400 instrument. Chemical shifts were given as δ (ppm) with TMS as internal standard. The MS were recorded on a JEOL AX-500 instrument. Column chromatography (CC) was carried out on Kiselgel 60 (70–230 mesh and 230–400 mesh), and Sephadex LH-20.

3.2. Collection, extraction and isolation

The leaves of *Viburnum suspensum* Lindl. were collected in the medicinal plant garden, Tokushima Bunri

Table 1
^{13}C NMR spectral data of **1–3**[a]

C	1	2	3
1	38.7	38.4	41.4
2	33.9	34.1	34.1
3	217.5	220.4	216.1
4	47.5	51.4	49.1
5	54.7	55.0	56.2
6	19.5	19.2	69.7
7	31.5	31.7	40.3
8	40.5	40.3	39.6
9	53.7	53.4	54.2
10	36.4	36.1	36.2
11	125.6	125.7	125.6
12	126.6	125.8	126.1
13	136.8	136.0	136.4
14	42.0	41.9	42.5
15	24.9	24.9	24.8
16	32.5	32.5	32.5
17	48.0	48.0	47.9
18	131.6	132.6	132.0
19	40.6	40.4	40.5
20	32.6	32.5	32.6
21	36.8	36.7	36.7
22	35.5	35.4	35.4
23	26.3	21.5	24.3
24	20.8	65.1	23.3
25	17.7	18.2	18.7
26	16.1	15.8	17.1
27	19.7	19.6	19.7
28	180.8	179.1	180.7
29	24.0	24.0	24.0
30	32.3	32.1	32.2

[a] 150 MHz in $CDCl_3$.

University (TBU) in September 1998 and a voucher specimen has been deposited in the Institute of Pharmacognosy, TBU.

The dried leaves of *V. suspensum* (1.3 kg) were powdered and extracted with MeOH at room temperature to give the MeOH extract (20 g). This extract (20 g) was then subjected to silica gel chromatography (70–230 mesh) eluted with EtOAc/hexane (1:3) to yield eight fractions (A–H). Fraction B (1.3 g) was applied to a Cosmosil 75 C_{18} (ODS) column eluted with MeOH/H_2O (3:1) to afford vibsanin B (**5**) (12 mg) and 3-hydroxyvibsanin E (25 mg). Fraction C (300 mg) was further divided by a Sephadex LH-20 CC with MeOH as eluent giving 3 fractions. Fr. 3 gave 3-oxo-11,13(18)-oleanadien-28-oic acid (**1**) (11 mg) as colorless crystals. Fr. 2 (74 mg) was applied to a silica gel column with hexane/EtOAc/AcOH (7:2:0.1) to afford 24-hydroxy-3-oxo-11,13(18)-oleanadien-28-oic acid (**2**) (13 mg), 6β-hydroxy-3-oxo-11,13(18)-oleanadien-28-oic acid (**3**) (15 mg), and virgatic acid (**5**) (7 mg).

*3.2.1. 3-Oxo-11,13(18)-oleanadien-28-oic acid (**1**)*

Colorless prisms; mp 223–225 °C; $[\alpha]_D^{20}$ −68° ($CHCl_3$, *c* 0.24); IR (film, cm^{-1}): 3209 (COOH), 1710 (CO), 1703 (CO); UV λ_{max} EtOH nm (ε): 242 (26 700), 249 (29 800), 257 (23 400); HR–EI–MS *m*/*z* 452.3279 $[M]^+$ (calc. for $C_{30}H_{44}O_3$: 452.3291); EI–MS *m*/*z* (rel. int.): 452 $[M]^+$ (100), 407 (45); ^1H NMR (600 MHz, $CDCl_3$): δ 0.81 (3H, *s*, H-29), 0.83 (3H, *s*, H-26), 0.96 (3H, *s*, H-30), 0.99 (3H, *s*, H-27), 1.03 (3H, *s*, H-25), 1.04 (3H, *s*, H-23), 1.10 (3H, *s*, H-24), 1.11 (1H, *ddd*, *J* = 14.0, 13.3, 3.0 Hz, H-15), 1.51 (1H, *ddd*, *J* = 13.2, 10.4, 7.1 Hz, H-1α), 1.70 (1H, *d*, *J* = 14.8 Hz, H-19), 1.72 (1H, *dd*, *J* = 14.4, 3.0 Hz, H-16), 2.02 (1H, *d*, *J* = 14.8 Hz, H-19), 2.05 (1H, *dd*, *J* = 3.2, 1.8 Hz, H-9), 2.12 (1H, *ddd*, *J* = 13.2, 7.4, 3.8 Hz, H-1β), 2.14 (1H, *ddd*, *J* = 13.2, 7.4, 3.8 Hz, H-22), 2.46 (1H, *ddd*, *J* = 16.9, 7.1, 3.8 Hz, H-2α), 2.54 (1H, *dd*, *J* = 14.4, 3.3 Hz, H-16), 2.60 (1H, *ddd*, *J* = 16.9, 10.4, 7.4, H-2β), 5.63 (1H, *dd*, *J* = 10.6, 1.8 Hz, H-12), 6.47 (1H, *dd*, *J* = 10.6, 3.2 Hz, H-11); ^{13}C NMR: see Table 1.

*3.2.2. 24-Hydroxy-3-oxo-11,13(18)-oleanadien-28-oic acid (**2**)*

Colorless prisms; mp 217–218 °C; $[\alpha]_D^{20}$ −86.5° ($CHCl_3$, *c* 0.38); IR (film, cm^{-1}): 3463 (OH), 3191 (COOH), 1734 (CO), 1694 (CO). UV λ_{max} EtOH nm (ε): 242 (21 000), 249 (23 800), 257 (17 000). HR–EI–MS *m*/*z* 468.3262 (calc. for $C_{30}H_{44}O_4$: 468.3239). EI–MS *m*/*z* (rel. int.): 468 $[M]^+$ (37), 438 (41), 392 (100); ^1H NMR (600 MHz, $CDCl_3$): δ 0.77 (6H, *s*, H-26, 29), 0.92 (3H, *s*, H-30), 0.95 (3H, *s*, H-27), 0.96 (3H, *s*, H-25), 1.24 (3H, *s*, H-23), 1.05 (1H, *m*, H-15), 1.25 (1H, *m*, H-21), 1.30 (1H, *m*, H-7), 1.32 (1H, *m*, H-21), 1.35 (1H, *m*, H-22), 1.36 (1H, *m*, H-7), 1.46 (1H, *ddd*, *J* = 13.2, 13.2, 3.6 Hz, H-6β), 1.55 (1H, *m*, H-6), 1.58 (1H, *dd*, *J* = 13.2, 8.8 Hz, H-1α), 1.60 (1H, *dd*, *J* = 13.2, 3.2 Hz, H-5), 1.61 (1H, *m*, H-15), 1.69 (1H, *d*, *J* = 14.6 Hz, H-19), 2.03 (1H, *dd*, *J* = 3.0, 1.6 Hz, H-9), 2.11 (1H, *ddd*, *J* = 13.2, 8.2, 4.7 Hz, H-1β), 2.49 (1H, *d*, *J* = 14.6 Hz, H-19), 2.51 (1H, *ddd*, *J* = 15.9, 8.5, 4.7 Hz, H-2β), 2.57 (1H, *dd*, *J* = 15.9, 8.2 Hz, H-2α), 3.44 (1H, *d*, *J* = 11.3 Hz, H-24), 3.95 (1H, *d*, *J* = 11.3 Hz, H-24), 5.55 (1H, *dd*, *J* = 10.7, 1.6 Hz, H-11), 6.45 (1H, *dd*, *J* = 10.7, 3.0 Hz, H-12); ^{13}C NMR: see Table 1.

*3.2.3. 6β-Hydroxy-3-oxo-11,13(18)-oleanadien-28-oic acid (**3**)*

Colorless prisms; mp 235–237 °C; $[\alpha]_D^{20}$ −60.4° ($CHCl_3$, *c* 0.31); IR (film, cm^{-1}): 3511 (OH), 3191 (COOH), 1734 (CO), 1694 (CO). UV λ_{max} EtOH nm (ε): 242 (32 800), 249 (35 000), 257 (29 000). HR–EI–MS *m*/*z* 468.3263 (calc. for $C_{30}H_{44}O_4$: 468.3239). EI–MS *m*/*z* (rel. int.): 468 $[M]^+$ (100), 392 (33); ^1H NMR (600 MHz, $CDCl_3$) δ 0.81 (3H, s, H-29), 0.95 (3H, *s*, H-27), 0.96 (3H, *s*, H-30), 1.10 (1H, *ddd*, *J* = 14.0, 2.7, 2.7 Hz, H-15), 1.16 (3H, *s*, H-26), 1.17 (3H, *s*, H-23), 1.21 (3H, *s*, H-24), 1.21 (1H, *d*, *J* = 2.5 Hz, H-5), 1.32 (1H, *m*, H-21), 1.36 (1H, *ddd*, *J* = 14.7, 13.2, 4.6 Hz, H-1α), 1.36 (1H, *dd*, *J* = 13.5, 4.5 Hz, H-22), 1.41 (1H, *m*, H-21), 1.51 (3H, *s*, H-25), 1.57 (1H, *dd*, *J* = 14.3, 2.7 Hz, H-7), 1.61 (1H, *dd*, *J* = 14.3, 3.3 Hz, H-7), 1.72 (1H, *ddd*, *J* = 13.7, 13.5, 2.7 Hz, H-16), 1.72 (1H, *d*, *J* = 14.6 Hz, H-19), 1.80 (1H, *ddd*, *J* = 14.0, 13.7, 2.7 Hz, H-15), 2.01 (1H, *dd*, *J* = 3.0, 1.8 Hz, H-9), 2.01 (1H, *ddd*, *J* = 14.0, 2.7, 2.7 Hz, H-16), 2.17 (1H, *ddd*, *J* = 13.2, 6.3, 2.7 Hz, H-1β), 2.28 (1H, m, H-22), 2.30 (1H, *ddd*, J = 14.7, 4.6, 2.7 Hz, H-2), 2.56 (1H, *dd*, *J* = 14.3, 2.2 Hz, H-19), 2.82 (1H, *ddd*, *J* = 14.7, 14.7, 6.3 Hz, H-2), 4.52 (1H, *ddd*, *J* = 3.3, 2.7, 2.5 Hz, H-6), 5.70 (1H, *dd*, *J* = 10.4, 1.8 Hz, H-11), 6.51 (1H, *dd*, *J* = 10.4, 3.0 Hz, H-12); ^{13}C NMR: see Table 1.

Acknowledgements

We thank Dr. Masami Tanaka and Miss. Yasuko Okamoto for measuring NMR and MS spectra.

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