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IRIDOID GLUCOSIDES FROM *VIBURNUM TINUS*

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Abstract—Five new iridoid glucosides, named viburtinosides I–V, together with suspensolide F, were isolated from leaves and branches of *Viburnum tinus*. All the iridoids contain a β-D-glucopyranosyl moiety linked to the oxymethylene at C-11 and an isovaleroyl at position 1, but differ for other acylating groups. The structures have been elucidated mainly by spectroscopic means.

INTRODUCTION

Viburnum species are used in folk medicine for their diuretic, antispasmodic and sedative properties, mainly on uterine excitability [1, 2]. In the last 15 years, the study of some species has led to the isolation of a number of iridoid glycosides, characterized by a sugar moiety at C-11 and an acyl group at C-1 [3–5]. Now we report a complete study on the iridoid glycosidic constituents of *V. tinus* L., an evergreen shrub widely distributed in Southern Europe. Previously, one paper reported on the isolation of a pyrane iridoid formed during acid treatment of the extract of the plant [6].

RESULTS AND DISCUSSION

Extraction of fresh leaves and branches and preliminary purification gave a glycoside-containing fraction, from which six iridoid glucosides, **1**–**6**, were isolated by CC. Each of the compounds was obtained as a syrup endowed with the typical smell of isovaleric acid. As evidenced by the ^{1}H NMR data and in accordance with the pattern of *Valeriana*-type iridoids found in other *Viburnum*, the isovaleric acid was linked to position 1, whereas β-D-glucopyranoside moiety was bound to the oxymethylene at C-11. Five of the isolated iridoids, viburtinosides I–V, are new compounds, whose structure determination is now reported.

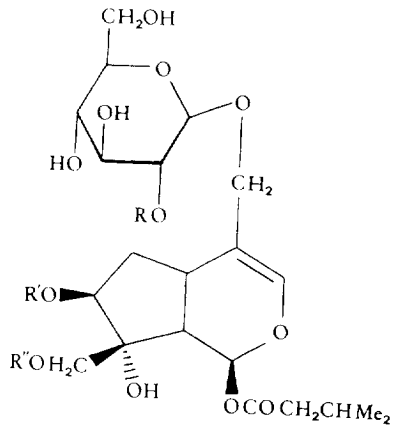
The ^{1}H NMR spectrum of **1**, $C_{25}H_{38}O_{13}$, viburtinoside I, was almost identical to that reported for valerosidate (**7**) [7]. The only significative differences were the low field shift of two double doublets at δ4.68 and 4.79, assignable to H-2′ and H-7, respectively, on the basis of a ^{1}H–^{1}H COSY experiment. These effects, in accordance with the presence of two acetyl groups (δ2.02 and 2.04), were also present in the ^{13}C NMR data (Table 1). Acetylation at C-8 must be discarded in the light of the clear effect (*ca* 10 ppm) reported for acylation of tertiary hydroxy groups. Concerning the stereochemical assignments, all the data, including NOE experiments and ^{13}C NMR chemical shift values of C-9 and C-10, were consistent with a 7-β-OH/8-β-Me configuration [8].

Compound **2**, viburtinoside II, $C_{32}H_{42}O_{15}$, showed in the ^{1}H NMR spectrum signals very similar to those of **1**, with three important differences: (a) the lack of the sharp singlet at δ1.27 (Me-8) and the presence of a broad singlet at δ4.18 (2H) accounting for an acylated oxymethylene; (b) the presence of an H-7 signal at δ3.92; (c) the presence of only a single acetyl group (δ2.01) and of additional signals of a *trans*-*p*-coumaroyl. In accordance with the ^{13}C NMR data, the last two groups were esterified to hydroxyl groups at positions 2′ and 10, and the isovaleroyl was linked to the hydroxyl at C-1. The acetyl group was unambiguously assigned to position 10, on the basis of the long-range coupling between the carboxylic carbon of the acetyl and the H_2-10 observed in the ^{1}H–^{13}C COLOC experiment. The presence of the *p*-coumaroyl in the glucose moiety was also confirmed by the isolation of 1-methyl, 2-*p*-coumaroyl-β-D-glucopyranoside from the methanolysis products of **2** (Experimental).

The NMR spectra of **3**, viburtinoside III, $C_{32}H_{42}O_{15}$, were very similar to those of **2**, except for the coumaroyl moiety which now showed the signals of a *cis* form.

In viburtinoside IV (**4**) and V (**5**) (both with molecular formula $C_{25}H_{38}O_{14}$) the *p*-coumaroyl unit present in **2** and **3**, was replaced by an acetyl group, which was linked to C-2′, while a second acetyl group was linked to C-10 (H_2-10 at δ4.22) in **4** and to C-7 (H-7 at δ4.94) in **5**, respectively.

Finally, **6**, $C_{21}H_{34}O_{12}$, proved to be the dideacetyl derivative of the two latter compounds and, therefore, it was identified as suspensolide F, previously isolated from *Viburnum suspensum* Lindley [9].



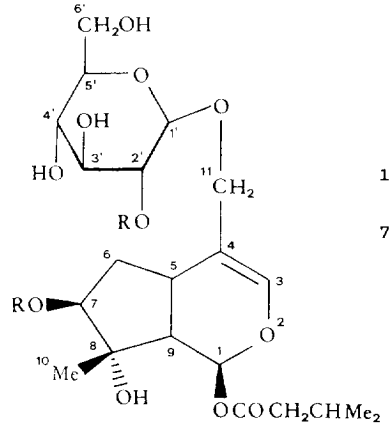
2 R = *trans-p*-coumaroyl; R'= H; R"= Ac

3 R = *cis-p*-coumaroyl; R'= H; R"= Ac

4 R = R"= Ac; R'= H

5 R = R'= Ac; R"= H

6 R = R'= R"= H



1 R = Ac

7 R = H

The isolation from *V. tinus* of glucoside iridoids structurally analogous to those found in many other species of *Viburnum* and in some species of *Valeriana*, confirms the supposed close relationship between this genus and members of the family of Valerianaceae [10]. Furthermore, all the new iridoids reported in this study are characterized by an acyl group bound at position 2'. This feature seems to increase the stability of these compounds by protecting the glucosidic linkage from attack by enzymes, probably as a result of steric hindrance, as demonstrated by the fact that viburtinosides are not hydrolysed by β-glucosidase.

Table 1. ^{13}C NMR spectral data of **1**–**5** (CD_3OD, TMS as int. standard)*

C	1	2	3	4	5
1	90.4	91.5	91.7	91.6	91.7
3	139.3	140.1	140.6	140.1	140.6
4	114.5	115.5	116.0	116.0	115.3
5	31.3	32.4	32.2	33.0	33.1
6	35.2	38.4	37.2	38.4	36.0
7	82.9	78.9	79.1	79.2	81.4
8	80.4	82.5	81.8	82.7	79.3
9	48.0	45.5	45.2	45.4	45.3
10	22.8	68.9	69.0	68.8	65.6
11	69.2	69.7	69.8	69.6	69.4
1'	100.0	101.1	101.3	100.7	100.5
2'	74.3	75.2	74.6	75.2	75.2
3'	75.3	76.0	76.1	76.0	76.1
4'	70.9	71.7	71.9	71.5	68.8
5'	76.8	77.9	78.1	77.8	77.9
6'	62.0	62.6	62.7	62.5	62.6
1"		127.2	127.4		
2"		131.2	133.9		
3"		116.8	115.9		
4"		161.1	160.2		
5"		116.8	115.9		
6"		131.2	133.9		
α		115.8	115.3		
β		146.8	145.5		
COO		167.6	168.2		
No. Ac groups	2	1	1	2	2

*All of the compounds had additional signals arising from the isovaleroyl group at *ca* δ173.0, 44.0, 26.5 and 22.5.

EXPERIMENTAL

^{1}H NMR: 500 MHz (the CHD_2OD peak is assigned to δ3.3); ^{13}C NMR: 125 MHz (the CD_3OD peak is assigned to 49 ppm).

Extraction and isolation. The plant material was collected in Rome and identified by Prof. B. Anzalone, *Università di Roma "La Sapienza"—Dipartimento di Biologia Vegetale* (voucher HHR-2988). Fresh leaves and young branches (1 kg) of *V. tinus* were exhaustively extracted with MeOH. The extract was concd to dryness, the residue diluted with H_2O and successively extracted with EtOAc and *n*-BuOH. The EtOAc extract, on CC on silica gel with MeOH–$CHCl_3$ (1:9), afforded viburtinosides I, II and III (120, 75 and 68 mg, respectively) as pure compounds. The *n*-BuOH extract was subjected to CC on silica gel with MeOH–$CHCl_3$ (1:4) to give viburtinosides IV (38 mg), V (15 mg) and the known suspensolide F (20 mg), which was identified by comparison of its NMR data with those reported in literature.

Viburtinoside I (**1**). Amorphous powder, $[\alpha]_D^{20} = -37.4$ (MeOH; *c* 1.0); IR ν_{max}^{KBr} cm^{-1}: 3400, 1750, 1670, 1460, 1370, 1250, 1100; ^{1}H NMR (CD_3OD): δ0.90 (6H, *d*, *J* = 6.6 Hz, $\underline{Me}_2CHCH_2$-), 1.27 (3H, *s*, H_3-10), 1.84 (2H, *m*, H-6), 1.99 (3H, *s*, Ac), 2.02 (3H, *s*, Ac), 2.04 (1H, *m*,

$Me_2\underline{CH}CH_2$-), 2.16 (2H, *d*, J=7.8 Hz, $Me_2CH\underline{CH_2}$-), 2.20 (1H, *dd*, J=3.8 and 10.0 Hz, H-9), 2.79 (1H, *m*, H-5), 3.22 (1H, *, H-5'), 3.36 (H, *t*, J=9.0 Hz, H-4'), 3.46 (H, *t*, J=9.0 Hz, H-3'), 3.67 (1H, *dd*, J=5.3 and 12.0 Hz, H-6'a), 3.81 (1H, *dd*, J=2.0 and 12.0 Hz, H-6'b), 3.98 (1H, *d*, J=12.0 Hz, H-11a), 4.14 (1H, *d*, J=12.0 Hz, H-11b), 4.40 (1H, *d*, J = 8.0 Hz, H-1'), 4.68 (1H, *dd*, J = 8.0 and 9.0 Hz, H-2'), 4.79 (1H, *dd*, J=2.6 and 4.3 Hz, H-7), 6.12 (1H, *d*, J=3.8 Hz, H-1), 6.22 (1H, *bs*, H-3).

Viburtinoside II (**2**). Amorphous powder, $[\alpha]_D^{20}$= −39.7 (MeOH; *c* 2.7); UV λ_{max} nm (log ε): 311 (4.1), 299 (3.9), 226 (3.8); IR ν_{max}^{KBr} cm^{-1}: 3400, 1740, 1700, 1630, 1600, 1510, 1240, 830; ^{1}H NMR (CD_3OD): δ0.93 (6H, *d*, J = 6.6 Hz, $\underline{Me}_2$ $CHCH_2$-), 1.89 (2H, *m*, H-6), 2.01 (3H, *s*, Ac), 2.04 (1H, *m*, $Me_2\underline{CH}CH_2$-), 2.15 (2H, *d*, J = 8.0 Hz, $Me_2CH\underline{CH_2}$-), 2.26 (1H, *dd*, J = 4.5 and 9.6 Hz, H-9), 2.90 (1H, *bq*, H-5), 3.27 (1H, *, H-5'), 3.39 (1H, *t*, J = 9.0 Hz, H-4'), 3.58 (1H, *t*, J = 9.0 Hz, H-3'), 3.70 (1H, *dd*, J = 5.4 and 12.0 Hz, H-6'a), 3.88 (1H, *dd*, J = 1.8 and 12.0 Hz, H-6'b), 3.92 (1H, *bs*, H-7), 4.05–4.25 (2H, *m*, H-11), 4.18 (2H, *bs*, H_2-10), 4.55 (1H, *d*, J = 8.2 Hz, H-1'), 4.81 (1H, *dd*, J = 8.0 and 9.0 Hz, H-2'), 6.18 (1H, *d*, J = 4.0 Hz, H-1), 6.30 (1H, *bs*, H-3), 6.40 (1H, *d*, J = 16.3 Hz, H-α), 6.80 (2H, *d*, J = 8.0 Hz, H-3'' and H-5''), 7.47 (2H, *d*, J = 8.0 Hz, H-2'' and H-6''), 7.64 (1H, *d*, J = 16.3 Hz, H-β).

Methanolysis of **2**. Compound **2** (50 mg) was dissolved in dry MeOH (5 ml) containing 2 drops of conc. HCl. The soln was stirred at 40°, under a stream of N_2; after 30 min the soln was diluted with H_2O (5 ml) and then extracted with Et_2O. The aq. phase was neutralized with Na_2CO_3, concd to dryness and chromatographed on silica gel with $CHCl_3$–MeOH (9:1), to give 1-methyl, 2-*p*-coumaroyl-β-D-glycopyranoside (4 mg), identified by analysis of its NMR data.

Viburtinoside III (**3**). Amorphous powder, $[\alpha]_D^{20}$= −40.1 (MeOH; *c* 2.3); UV λ_{max} nm (log ε): 312 (4.1), 299 (3.9), 226 (3.8); IR ν_{max}^{KBr} cm^{-1}: 3400, 1740, 1705, 1625, 1600, 1515, 1240, 835; ^{1}H NMR (CD_3OD): δ0.92 (6H, *d*, J =6.6 Hz, $\underline{Me}CHCH_2$-), 1.85 (2H, *m*, H-6), 2.03 (3H, *s*, Ac), 2.05 (1H, *m*, $Me_2\underline{CH}CH_2$-), 2.15 (2H, *d*, J=8.0 Hz, $Me_2CH\underline{CH_2}$-), 2.33 (1H, *dd*, J=4.0 and 9.6 Hz, H-9), 2.84 (1H, *bq*, H-5), 3.32 (1H, *, H-5'), 3.42 (1H, *t*, J=9.0 Hz, H-4'), 3.57 (1H, *t*, J=9.0 Hz, H-3'), 3.68 (1H, *dd*, J=5.1 and 12.0 Hz, H-6'a), 3.88 (1H, *dd*, J=1.8 and 12.0 Hz, H-6'b), 3.88 (1H, *bs*, H-7), 4.05–4.25 (2H, *m*, H-11), 4.17 (2H, *bs*, H_2-10), 4.50 (1H, *d*, J=8.0 Hz, H-1'), 4.81 (1H, *dd*, J=8.0 and 9.0 Hz, H-2'), 5.84 (1H, *d*, J=12.0 Hz, H-α), 6.13 (1H, *d*, J=4.0 Hz, H-1), 6.32 (1H, *bs*, H-3), 6.74 (2H, *d*, J =8.0 Hz, H-3'' and H-5''), 6.87 (1H, *d*, J=12.0 Hz, H-β), 7.68 (1H, *d*, J=8.0 Hz, H-2'' and H-6'').

Viburtinoside IV (**4**). Amorphous powder, $[\alpha]_D^{20}$= −68.9 (MeOH; *c* 2.0); IR ν_{max}^{KBr} cm^{-1}: 3400, 1730, 1660, 1100; ^{1}H NMR (CD_3OD): δ0.94 (6H, *d*, J=6.6 Hz, $\underline{Me}_2CHCH_2$-), 1.93 (2H, *m*, H-6), 2.05 (3H, *s*, Ac), 2.08 (3H, *s*, Ac), 2.06 (1H, *m*, $Me\underline{CH}CH_2$-), 2.20 (2H, *d*, J =7.2 Hz, Me_2CHCH_2-), 2.32 (1H, *dd*, J=4.5 and 10.0 Hz, H-9), 2.93 (1H, *bq*, H-5), 3.29 (1H, *, H-5'), 3.36 (1H, *t*, J=9.3 Hz, H-4'), 3.53 (1H, *t*, J=9.3 Hz, H-3'), 3.69 (1H, *dd*, J=5.4 and 12.0 Hz, H-6'a), 3.88 (1H, *dd*, J=2.4 and 12.0 Hz, H-6'b), 3.94 (1H, *bt*, J = 3.4 Hz, H-7), 4.07 (2H, *d*, J=11.2 Hz, H-11a), 4.22 (2H, *bs*, H_2-10), 4.23 (1H, *d*, J=11.2 Hz, H-11b), 4.49 (1H, *d*, J=8.0 Hz, H-1'), 4.70 (1H, *d*, J=8.0 and 9.3 Hz, H-2'), 6.17 (1H, *d*, J=4.5 Hz, H-1), 6.32 (1H, *bs*, H-3).

Viburtinoside V (**5**). Amorphous powder, $[\alpha]_D^{20}$ = −45.7 (MeOH; *c* 0.7); IR ν_{max}^{KBr} cm^{-1}: 3400, 1730, 1650, 1100; ^{1}H NMR (CD_3OD): δ0.95 (6H, *d*, J=6.6 Hz, $\underline{Me}_2CHCH_2$-), 1.98 (2H, *m*, H-6), 2.05 (3H, *s*, Ac), 2.08 (3H, *s*, Ac), 2.10 (1H, *m*, $Me_2\underline{CH}CH_2$-), 2.22 (2H, *d*, J =6.9 Hz, $Me_2CH\underline{CH_2}$-), 2.33 (1H, *dd*, J=4.9 and 9.7 Hz, H-9), 2.86 (1H, *bq*, H-5), 3.27 (1H, *, H-5'), 3.34 (1H, *, H-4'), 3.51 (1H, *t*, J=9.3 Hz, H-3'), 3.67 (1H, *dd*, J=5.1 and 12.0 Hz, H-6'a), 3.69 (2H, *bs*, H_2-10), 3.86 (1H, *dd*, J=1.9 and 12.0 Hz, H-6'b), 4.12 (2H, *d*, J=11.6 Hz, H-11a), 4.25 (1H, *d*, J=11.6 Hz, H-11b), 4.50 (1H, *d*, J=8.0 Hz, H-1'), 4.71 (1H, *d*, J=8.0 and 9.3 Hz, H-2'), 4.94 (1H, *dt*, J =3.3 Hz, H-7), 6.13 (1H, *d*, J=4.9 Hz, H-1), 6.35 (1H, *bs*, H-3).

*Partially masked by the solvent signal.

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