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FONTANESIOSIDE AND 5-HYDROXYSECOLOGANOL FROM *FONTANESIA PHILLYREOIDES*

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Key Word Index—*Fontanesia phillyreoides*; Oleaceae; secoiridoid glucosides; fontanesioside; 5-hydroxysecologanol; verbascoside.

Abstract—The iridoid content of *Fontanesia phillyreoides* was investigated by reverse phase chromatography of the ethanolic extract of the aerial parts. Five known iridoid compounds, i.e. loganic acid, secologanoside, secologanic acid, secologanol and swertiamarin, besides verbascoside were isolated. In addition, two novel secoiridoids, named 5-hydroxysecologanol and fontanesioside, were isolated and characterized. Their structures were determined by NMR spectroscopy and by chemical evidence.

INTRODUCTION

In a review of the Oleaceae, Johnson [1] segregated Fontanesieae as a monotypic tribe only including the genus *Fontanesia*, which consists of two species (*F. phillyreoides* and *F. fortunei*), both with a discontinuous distribution in the Mediterranean region, and East Asia (China). Furthermore, the genus is considered a rather specialized relic [1].

Seed protein serological data have been used to study the systematics of Oleaceae [2]; three *Fraxinus* species, six genera of Oleoideae, two *Forsythia* species and *F. fortunei* were examined with respect to reciprocal affinity by double diffusion immunoprecipitation and immunoelectrophoresis of seed extracts revealing some degree of similarity between *Forsythia* species and *F. fortunei*.

No naturally occurring iridoid glucosides have been reported from *F. phillyreoides* Labill. but a paper [3] describes the isolation of two alkaloids, fontaphilin (**1**) and gentianin (**2**) from this species. Compound **2** is known [4] to be formed from swertiamarin (**3**) and gentiopicroside (**4**) during the extraction procedure commonly used to obtain alkaloids (successive treatment with acid and ammonia). In our present investigation of Oleaceae, we now wish to report our results from the investigation of *F. phillyreoides*. Two novel secoiridoids were isolated in addition to five known iridoids and secoiridoids.

RESULTS AND DISCUSSION

Aqueous solutions of the ethanolic extracts (from a small and large batch of foliage collected in July and August, respectively) of the aerial parts of *F. phillyreoides* were delipified with diethyl ether and then extracted successively with *n*-butanol and ethyl acetate. Fractionation by reverse phase chromatography of the EtOAc extracts allowed the isolation of a secoiridoid, named fontanesioside (**5**), in addition to verbascoside. The major compounds similarly isolated from the *n*-butanol-extracts (treated with alumina) were **3**, secologanic acid (**6**), secologanol (**7**) and 5-hydroxysecologanol (**8**), which is a new secoiridoid. The acidic constituents, loganic acid (**9**) and secologanoside (**10**), were obtained by chromatography of a bicarbonate solution of the water extract from the large batch of plant, and subsequent rechromatography of the polar part dissolved in 10% aqueous acetic acid to give retention of the iridoid acids.

The ^{1}H NMR spectrum of **8** was similar to that of **7** [5] (within 0.1 ppm), except for the absence of a signal from H-5, a minor downfield shift (+0.3 ppm) of H_a-6 and an upfield shift (−0.3 ppm) of H-8. In accordance with a hydroxylation of C-5, the ^{13}C NMR spectrum of **8** (Table 1) exhibited downfield shifted signals of C-5, C-6 and C-9 (+39.4 ppm, +6.2 ppm and +8.2 ppm, respectively) relative to the corresponding values observed for **7**. Moreover, the expected upfield γ-effects on C-7 and C-8 (−2.6 ppm and −1.9 ppm) were observed.

Due to the marked lability of **8**, the compound was not further characterized as the native glucoside. In the presence of traces of both weak acids (e.g. silica gel or acetic acid) and bases (bicarbonate), it rapidly underwent ring closure to give **3**. This proves **8** to be the methyl ester of the opened form of **3**.

Acetylation of **8** yielded a pentaacetate (**8a**), even under forced conditions (DMAP catalysis), and swertiamarin tetraacetate (**3a**) was obtained as a by-product. As expected, lactonization competes with acetylation under

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Table 1. ^{13}C NMR data of secoiridoids

Carbon	7 (D_2O)	8 (D_2O)	11 (CD_3OD).	8a ($CDCl_3$)	5 (CD_3OD)	5 (D_2O)	5a ($CDCl_3$)
1	98.4	99.4	97.8	96.4	97.6	99.4	96.4
3	153.4	154.7	153.1	151.4	153.2	155.0	151.5
4	111.4	111.9	—	112.9	111.5	111.5	112.9
5	30.3	69.7	69.2	67.5	68.7	70.4	67.6
6	32.6	38.8	39.0	34.8	35.5	36.1	35.1
7	60.7	58.1	57.7	60.2	60.6	61.7	60.9
8	134.6	132.7	132.6	131.7	132.3	131.9	131.6
9	44.3	52.5*	48.7	51.1*	51.9	53.1	51.1*
10	120.5	122.3	119.5	121.0	120.0	122.8	121.2
11	—	169.6	—	166.7	167.1	169.4*	166.7
11-OMe	52.7	52.8*		51.6*	50.7	52.5	51.6*
1′	99.8	100.2	98.9	97.6	98.8	100.2	97.7
2′	73.4	73.4	73.3	70.6	73.3	73.4	70.7
3′	76.5	76.5	76.7	72.1	76.7	76.5	72.1
4′	70.3	70.4	70.3	68.0	70.3	70.3	68.0
5′	77.2	77.3	77.2	72.3	77.3	77.3	72.3
6′	61.5	61.5	61.5	61.5	61.5	61.5	61.5
COMe				170.9			170.6
COMe				170.6			170.2
COMe				170.1			169.8
COMe				169.7			169.4
COMe				169.3			165.7
MeCO				21.0			21.2
MeCO				20.7			20.8
MeCO				20.6‡			20.6‡
MeCO				20.4			20.4
1″					121.6†	121.9	127.9†
2″/6″					132.5‡	132.9‡	131.2‡
3″/5″					116.1‡	116.3‡	121.5‡
4″					163.8†	161.8	154.2†
C-α					168.4	169.1*	168.9†

*Interchangeable.
†Low intensity.
‡Double intensity.

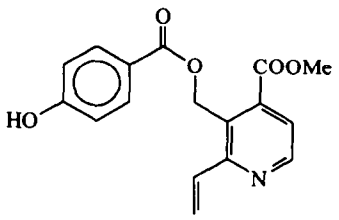
these conditions. In the ^{1}H NMR spectrum of **8a** a two-proton triplet at δ4.22 (seen at δ3.69 in the glucoside) could be attributed to an acylated 7-hydroxymethyl group. The ^{13}C NMR data for **8a** confirmed the presence of an acetoxy group at C-7, as this carbon was shifted downfield by 2.1 ppm, and both C-6 and C-5 showed upfield shifts when compared to those of the glucoside (−4.2 and −2.2 ppm, respectively).

In the ^{1}H NMR spectrum of **5**, doublets at δ7.82 and 6.80 were assignable to an aromatic *p*-substituted system. Another set of signals could be assigned to a secoiridoid moiety with close resemblance to **8**, except for the downfield positions (δ4.4 instead of 3.7) of the protons at C-7. This indicated that **5** was the 7-*p*-hydroxybenzoate of **8**. Comparison of the ^{13}C NMR data for **5** and **8** showed a downfield α-effect (+3.6 ppm) on C-7, and smaller β- and γ-shifts of C-6 and C-5 (−2.7 and +0.7 ppm, respectively) in **5**, supporting the suggested acylation of the primary 7-OH group. Final proof was obtained by alkaline hydrolysis of **5**, which yielded 5-hydroxysecologanolic acid (**11**) and *p*-hydroxybenzoic acid. Methylation of the acid, **11**, gave **8**, which in turn underwent a slow spontaneous elimination of methanol to give **3** [6].

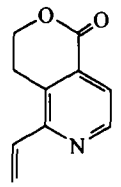
Usual acetylation of **5** afforded the pentaacetate (**5a**), which was obtained in pure form only after repeated preparative TLC, since its phenolic acetyl group is particularly labile, owing to the *p*-carbonyl functionality. As expected, the ^{13}C NMR signals from the iridoid nucleus of **5a** were almost identical with those of **8a**.

The presence of **5** and **8** in *F. phillyreoides* explains the earlier report [3] of **1** and **2** from this plant, since treatment with acid followed by ammonia would indeed be expected to transform **5** and **8** into **1** and **2**, respectively.

Compound **6** was acetylated to give a 4:1 mixture of the 7-epimeric pentaacetates (**6a** and **6b**), of which the most abundant epimer was obtained by repeated TLC. According to conformational work on sweroside (**12**) [7], a pseudo-chair conformation would impose small coupling constants (1–5 Hz) between H_α-7 and both the protons at C-6, whereas H_β-7 should have a large (10–15 Hz) and a small (1–3 Hz) coupling to the respect-



1



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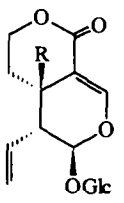
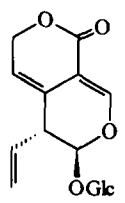


3 R = OH
12 R = H



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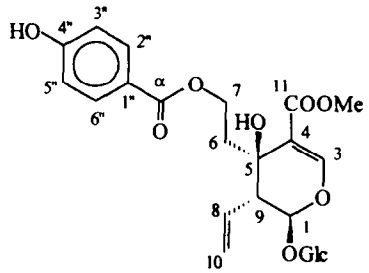
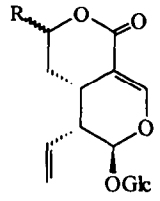
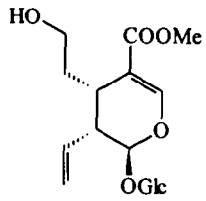


5



6 R = OH
6a R = α-OAc
6b R = β-OAc



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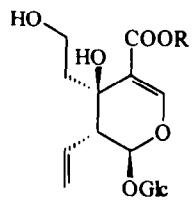
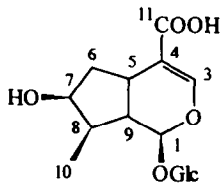


8 R = Me
11 R = H



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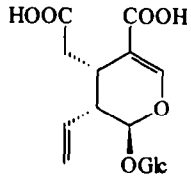

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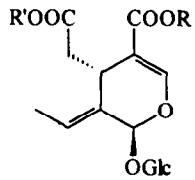

13

Scheme 1.

ive protons at C-6. The most abundant 7-epimer (**6a**) has in fact the coupling constants (10.5 and 3 Hz) corresponding to an acetoxy group in the 7α-position; the least abundant 7-epimer (**6b**) is also consistent with a 7β-configuration of the acetoxy group (H-7 a triplet with $J = 1.5$ Hz).

Identification of **3** and its tetraacetate (**3a**) was performed by NMR [6, 8, 9], but it is noticeable that several crystalline forms of **3a** seem to exist: one of mp 190–193° [10, 11], another of mp 176–178° [12] and the form we obtained with mp 165–166°; the latter crystallized from EtOH with extreme ease. However, **3** might well be an artefact in *F. phillyreoides* being formed from **8** during work-up (or on storing of the extract), since **8** must be an intermediate in the biogenesis of **5**. However, owing to its inherent instability, **8** is converted to **3** by a non-

enzymatic, spontaneous reaction. Moreover, **8** was only isolated from the plant batch which was fractionated immediately after extraction.

Small amounts of two acidic iridoids were obtained, i.e. **9** which is widespread among the orders having secoiridoids [13] and **10**. The latter is only reported as naturally occurring in Caprifoliaceae (Dipsacales) [14, 15].

Consequently, **9**, **6**, **8** and **5** could constitute the major biosynthetic sequence in *F. phillyreoides*, whereas a minor secondary, oxidative pathway leads to **10**.

Compound **6** has been reported from a few natural sources: *Vinca rosea* (Apocynaceae) [16], *Anthocleista vogelii* (Potaliaceae) [17], *Lonicera coerula* and *L. etrusca* (Caprifoliaceae) [15, 18]. Monoterpenoid ester derivatives of **6** occur in *Menyanthes trifoliata* (Menyanthaceae) [19–21]; however, these compounds have the less favourable 7β-configuration of the acyloxy group.

Previously, **7** has been found in *Gentiana verna* (Gentianaceae) [5]; in addition **7** is known as part of bisiridoids in *Abelia grandiflora* (Caprifoliaceae) [22] and in *Dipsacus sylvestris* (Dipsacaceae) [23].

The distribution of **3** is so far limited to Gentianaceae (many genera) [24] and Potaliaceae (*Anthocleista procera* [25]) and frequently co-occurs with **12**. In view of our investigation, it seems unlikely that *Fontanesia* should have close affinity to *Forsythia*, as suggested by serological data [2], since the iridoids in the latter are exclusively of the carbocyclic type. However, the conspicuous similarity of the secoiridoids found in *F. phillyreoides* and members of Gentianaceae could reflect a closer relationship between Fontanesieae and Gentianaceae. On the other hand, the co-occurence of secologanin-type compounds and verbascoside also indicate an affinity to other tribes of Oleaceae (Jasmineae, Fraxineae and Oleineae) where oleoside-type compounds (**13**) are usually found together with verbascoside. The latter has so far never been found in species of Gentianaceae (or in the order Gentianales at all) [26], so from a chemotaxonomic point of view, Fontanesieae might constitute the link between Oleaceae and Gentianaceae. In fact the Oleaceae has been related either to Scrophulariales/Lamiales (where verbascosides are commonly found [26]) or to Gentianales by various taxonomists, e.g. Dahlgren and Takhtajan [27, 28]. Our results support the latter taxonomic placement.

EXPERIMENTAL

General procedures. Mps: uncorr.; ^{1}H NMR (250 MHz): D_2O or CD_3OD (HOD signal at 4.75 ppm or MeOD signal at 3.31 ppm, respectively, were used as int. standards); acetates were recorded in $CDCl_3$ with TMS as int. standard; ^{13}C NMR (62.5 MHz): C-6′ was set to 61.5 ppm as a standard [29]; prep. TLC: 20 × 40 cm plates coated with 1 mm layers of silica gel PF_{254} (Merck); bands were detected in UV light (254 nm); reverse phase MPLC: Merck Lobar C-18 columns size B and C. H_2O–MeOH mixts were used as eluents and peaks were detected by UV at 240 nm.

Plant material. Fresh foliage from *F. phillyreoides* was collected at the beginning of July 1991 (small batch) and at the beginning of August 1991. The plant material was supplied by The Botanical Garden of Copenhagen and voucher specimens (No. IOK-14-92) have been deposited at The Botanical Museum, Copenhagen.

Work-up of batch 1. Fresh foliage (61 g) was blended with EtOH (500 ml). Filtration and evapn *in vacuo* gave a syrup (3.59 g), which was partitioned in H_2O–Et_2O (3:5; 200 ml). The aq. layer was concd *in vacuo* yielding a brownish syrup (2.36 g). A methanolic soln of this crude extract was passed through a layer of activated C (1.5 g); subsequent evapn *in vacuo* gave a yellowish foam (2.30 g). Dissolution in H_2O (50 ml) and extraction with EtOAc (3 × 100 ml) gave, after removal of the solvent, an EtOAc extract, A (0.61 g), and a water-soluble residue, which was redissolved in H_2O (30 ml) and reextracted with *n*-BuOH (2 × 60 ml). The combined *n*-BuOH layers were washed with H_2O (2 × 10 ml) and taken to dryness giving B (0.79 g). The remaining aq. phase gave C (0.77 g). Fractionation by MPLC (C-column; 3:1 to 1:1) of extract A yielded only one major iridoid fr., i.e. crude **5** (227 mg; 0.37%).

The H_2O extract (C) was also subjected to MPLC (B-column; 10:1 to 1:1). Three frs were obtained by successive elution with 10:1 (C-1; 33 mg), 4:1 (27 mg impure **6**) and 3:1 (C-2; 50 mg), respectively. Rechromatography of C-1 (in 5% HOAc) on a RP-18 B-column (H_2O to 1:1) gave secologanoside (**10**; 3:1; 3 mg; 0.005%) [14].

The *n*-BuOH extract (B) was treated with Al_2O_3 (2 g) eluting with H_2O (100 ml). Evapn *in vacuo* of the filtrate yielded a syrup (0.31 g), which was combined with C-2 (50 mg) and fractionated (B-column; 5:1 to 1:1). Impure secologanic acid (**6**; 18 mg) was eluted with 5:1, whereas impure swertiamarin (**3**; 40 mg; 0.066%) [6, 8] was followed by almost pure 5-hydroxysecologanol (**8**; 28 mg; 0.046%) when eluting with 4:1. Then came slightly impure secologanol (**7**; 2:1; 10 mg; 0.016%) [5] and **5** (1:1; 33 mg; 0.054%). The impure **6** (45 mg) was dissolved in 5% HOAc and applied to a RP-18-B-column (H_2O to 1:1) giving almost pure **6** (21 mg; 0.034%) upon elution with 2:1.

Work-up of batch 2. Fresh foliage (175 g) was similarly worked-up to give 3 residues: an EtOAc-soluble residue, D (1.92 g), a *n*-BuOH extract, E (1.31 g) and a water-soluble extract, F (3.44 g).

MPLC (B-column; 2 portions; 5:2 to 1:1) of D afforded first impure **6** (2:1; 44 mg). Crude verbascoside (89 mg) was eluted by 3:2, whereas crude fontanesioside (**5**; 1.04 g) was obtained upon elution with 1:1. Rechromatography (B-column; 2:1 and 3:2) afforded pure verbascoside (56 mg; 0.032%) and slightly impure **5** (0.85 g yellowish foam; 0.49%) was likewise obtained (C-column; 3:2 and 1:1).

Fractionation of the H_2O *extract.* Extract F (3.44 g) in satd $NaHCO_3$ was subjected to MPLC (C-column). Elution with H_2O gave a large fr. of polar components F-1 (2.66 g), while elution with 6:1 gave F-2 (0.42 g). Continued elution with 3:2 afforded F-3 (291 mg).

To F-1 was added 10% HOAc (2 ml) and MeOH (50 ml); insoluble material was filtered off and the filtrate passed through Al_2O_3 (3 g), which was further eluted with H_2O (50 ml). The combined eluates were evapd giving a syrup (2.37 g), which was applied to a RP-18 C-column (in 10% HOAc; H_2O and 1:1). Elution with 1:1 gave a syrup (91 mg) which was rechromatographed twice (B-columns) to give loganic acid (**9**; 8 mg; 0.005%) [30] and secologanoside (**10**; 7 mg; 0.004%) [14]. F-2 and F-3 were rechromatographed (B-columns) to give **6** (4:1; 229 mg; 0.13%) and **3** (3:1; 164 mg; 0.094%) [6, 8], respectively.

Fractionation of the n-BuOH *extract.* The *n*-BuOH-soluble residue, E (1.31 g), and F-3 were treated with Al_2O_3 (2 × 20 g) eluting with H_2O (600 ml). The eluate was evapd *in vacuo* giving a residue (0.78 g); MPLC (B-column; 2 portions; 5:1 to 1:1) gave impure **3** (4:1; 78 mg) [6, 8], slightly impure **7** (2:1; 22 mg) [5] and crude **5** (147 mg; 0.084%).

Purification of crude fontanesioside (**5**). The above crude **5** (0.85 g) was subjected to prep. TLC ($CHCl_3$–MeOH, 4:1; 4 plates; R_f 0.53). Work-up with MeOH and treatment with activated C (0.5 g) gave a foam (0.76 g) that was applied to an Al_2O_3-column (6 g) eluting with H_2O (75 ml). Evapn *in vacuo* of the eluate treated with activated C (0.3 g) gave a white foam (0.56 g). MPLC (C-column; in 10% HOAc; 3:2 to 1:1) gave pure **5** (413 mg) as a hygroscopic foam, $[\alpha]_D^{20}$ −118.7° (MeOH; *c* 0.60).

^{1}H NMR (CD_3OD): δ7.82 (*d*, 2H, *J* = 8.8 Hz, H-2″ and H-6″), 7.55 (*s*, H-3), 6.80 (*d*, 2H, *J* = 8.8 Hz, H-3″ and H-5″), 5.69 (*d*, *J* = 5.7 Hz, H-1), 5.67 (*dt*, *J* = 17.2 Hz and 2 × 9.4 Hz, H-8), 5.37 (*dd*, *J* = 17.2 and 1.8 Hz, H_a-10), 5.29 (*dd*, *J* = 10.2 and 1.8 Hz, H_b-10), 4.42 (*m*, 2H, 2 × H-7), 3.61 (*s*, 3H, 11-OMe), 2.83 (*dd*, *J* = 8.9 and 5.7 Hz, H-9), 2.58 (*dt*, *J* = 14.1 and 2 × 7.1 Hz, H_a-6), 2.03 (*ddd*, *J* = 14.1, 7.7 and 6.4 Hz, H_b-6), 4.68 (*d*, *J* = 7.9 Hz, H-1′), 3.92 (*dd*, *J* = 12.0 and 1.6 Hz, H_a-6′), 3.67 (*dd*, *J* = 12.0 and 5.6 Hz, H_b-6′), 3.40 (*t*, *J* = 9.0 Hz, H-3′), 3.35–3.27 (*m*, 2H, H-4′ and H-5′), 3.21 (*br t*, *J* = 8.5 Hz, H-2′). Found: C, 53.12: H, 6.19. $C_{24}H_{30}O_{13}\cdot H_2O$, requires: C, 52.94: H, 5.92%.

Fontanesioside pentaacetate (**5a**). Acetylation (pyridine–Ac_2O 5:4, 4.5 ml; 2 hr at room temp.) of **5** (350 mg) gave a mixt. of acetates (332 mg). Repeated prep. TLC (Et_2O–pentane 10:1; twice) yielded pure **5a** as a foam (153 mg), $[\alpha]_D^{20}$ −85.3° ($CHCl_3$; *c* 0.80). ^{1}H NMR ($CDCl_3$): δ8.02 (*d*, 2H, *J* = 8.7 Hz, H-2″ and H-6″), 7.45 (*s*, H-3), 7.14 (*d*, 2H, *J* = 8.7 Hz, H-3″ and H-5″), 5.55 (*ddd*, *J* = 17.0, 10.0 and 8.8 Hz, H-8), 5.42 (*d*, *J* = 4.2 Hz, H-1), 5.39 (*dd*, *J* = 17.0 and 2.0 Hz, H_a-10), 5.33 (*dd*, *J* = 10.0 and 2.0 Hz, H_b-10), 4.50 (*t*, 2H, *J* = 7.4 Hz, 2 × H-7), 3.70 (*s*, 3H, 11-OMe), 2.89 (*dd*, *J* = 8.8 and 4.2 Hz, H-9), 2.72 (*dt*, *J* = 14.3 and 2 × 7.3 Hz, H_a-6), 1.94 (*m*, obscured by AcOs, H_b-6), 5.25 (*t*, *J* = 9.5 Hz, H-3′), 5.10 (*t*, *J* = 9.5 Hz, H-4′), 5.02 (*dd*, *J* = 9.5 and 8.0 Hz, H-2′), 4.86 (*d*, *J* = 8.0 Hz, H-1′), 4.28 (*dd*, *J* = 12.6 and 4.4 Hz, H_a-6′), 4.15 (*dd*, *J* = 12.6 and 2.3 Hz, H_b-6′), 3.74 (*ddd*, *J* = 10.0, 4.4 and 2.3 Hz, H-5′), 2.31 (*s*, 3H, phenolic AcO) 2.10, 2.03, 2.01, 1.98 (each *s*, 3H, 4 × AcO). Found: C, 55.20: H, 5.54, $C_{34}H_{40}O_{18}$, requires: C, 55.43; H, 5.47%.

Secologanic acid (**6**). Crude **6** (249 mg) was applied to a RP-18 C-column (6:1 to 3:1). The central part of the fr. eluted with 3:1 was evapd *in vacuo* to give pure **6** (103 mg; 0.044%) as a hygroscopic foam, $[\alpha]_D^{20}$ −187.1° (H_2O; *c* 0.57), lit. −115° (MeOH; *c* 1.0) [16]. ^{1}H NMR (D_2O): δ7.58 (*d*, *J* = 2 Hz, H-3), 5.53 (*d*, *J* = 2 Hz, H-1), 5.46 (*dt*, *J* = 17.5 and 2 × 9.5 Hz, H-8), 5.27 (*dd*, *J* = 17.5 and 2 Hz, H_a-10), 5.23 (*dd*, *J* = 10 and 2 Hz, H_b-10), 3.10 (*m*, ^{2}H-coupling to the H-6's, which undergoes rapid exchange, H-5), 2.71 (*m*, H-9), 2.10–1.60 (*m*, 2H partially exchanged with ^{2}H, 2 × H-6), 4.77 (*d*, partly obscured by the HOD-signal, H-1′). ^{13}C NMR (D_2O): δ98.3 (C-1), 154.6 (C-3), 132.0 (C-8), 42.4 (C-9), 121.8 (C-10), 99.1 (C-1′), 73.4 (C-2′), 76.3 (C-3′), 70.4 (C-4′), 77.1 (C-5′), 61.5 (C-6′), signals for C-4, C-5, C-7 and C-11 were not seen without relaxation delay, whereas the signal for C-6 was broadened by ^{2}H coupling. Calcd for $C_{16}H_{22}O_{10}\cdot 1/2\ H_2O$: C, 50.13%: H, 6.05%, found: C, 49.69%: H, 6.40%.

Secologanic acid pentaacetates (**6a**, **6b**). Acetylation (pyridine–Ac_2O, 1:1; 4 ml; 2 hr at room temp.) of **6** (92 mg) gave a crude product (153 mg; 4:1 mixt. of **6a** and **6b**) [16, 17], which was fractionated by prep. TLC (Et_2O–pentane, 10:1; 3 developments). Only one major band (R_f 0.60), strong at the bottom and diffuse at the top, was seen. Work-up (2 × 75 ml EtOAc) of the band (divided into 2 parts) afforded a 5:1 mixt. and a 1:1 mixt. of **6a** and **6b** (60 mg and 40 mg, respectively). The 5:1 mixt. of pentaacetates (60 mg) was further purified by prep. TLC (Et_2O–pentane, 10:1) giving the most abundant 7α-acetate **6a** (R_f 0.29; 28 mg) as a syrup, $[\alpha]_D^{20}$ −172.5° ($CHCl_3$; *c* 0.80). ^{1}H NMR ($CDCl_3$): δ7.58 (*d*, *J* = 2.5 Hz, H-3), 6.48 (*dd*, *J* = 10.5 and 3 Hz, H-7), 5.42 (*dt*, *J* = 17.5 and 2 × 9 Hz, H-8), 5.36 (*d*, *J* = 2 Hz, H-1), 5.36–5.26 (*m*, 2H, 2 × H-10), 2.88 (*m*, H-5), 2.71 (*ddd*, *J* = 9, 5.5 and 2 Hz, H-9), 2.06–1.91 (1H obscured by AcOs, H_a-6), 1.63 (*dt*, *J* = 2 × 13.5 and 10.5 Hz, H_b-6), 5.23 (*t*, *J* = 9.5 Hz, H-3′), 5.08 (*t*, *J* = 9.5 Hz, H-4′), 4.98 (*dd*, *J* = 9.5 and 8 Hz, H-2′), 4.89 (*d*, *J* = 8 Hz, H-1′), 4.29 (*dd*, *J* = 12.5 and 4.5 Hz, H_a-6′), 4.13 (*dd*, *J* = 12.5 and 2 Hz, H_b-6′), (*ddd*, *J* = 10, 4.5 and 2 Hz, H-5′), 2.13, 2.10, 2.04, 2.00 and 1.96 (*s*, 5 × 3H, 5 × AcO); ^{13}C NMR (incl. DEPT) ($CDCl_3$): δ96.0* (CH, C-1), 152.1 (CH, C-3), 103.6 (*q* C, C-4), 23.7 (CH, C-5), 28.9 (CH_2, C-6), 92.4 (CH, C-7), 130.3 (CH, C-8), 41,7 (CH, C-9), 121.7 (CH_2, C-10), 96.3* (CH, C-1′), 70.2 (CH, C-2′), 72.0 (CH, C-3′), 67.9 (CH, C-4′), 72.1 (CH, C-5′), 61.5 (CH_2, C-6′), 170.5, 169.9, 169.3, 169.1, 168.4 and 163.0 (*q* C, C-11 and 5 × Ac-CO), 20.7, 20.6, 20.5† and 20.4 (5 × Ac). Compound **6b**: ^{1}H NMR ($CDCl_3$): δ7.63 (*d*, *J* = 2.5 Hz, H-3), 6.59 (*t*, *J* = 1.5 Hz, H-7), 3.24 (*m*, H-5), 2.65 (*m*, H-9), 1.85 (*m*, H_b-6).

Swertiamarin (**3**) *and its tetraacetate* (**3a**). Impure **3** (242 mg) was treated with activated C (0.3 g) and was further purified by prep. TLC ($CHCl_3$–MeOH, 4:1) giving a syrup (R_f 0.39; 186 mg). After rechromatography (RP-18 C-column; 5:1 and 4:1) pure **3** (99 mg) was obtained as a foam, $[\alpha]_D^{20}$ −140.2° (EtOH; *c* 0.55). Calcd

* Interchangeable.

† Double intensity.

for $C_{16}H_{22}O_{10}\cdot H_2O$:C, 48.98; H, 6.17. Found: C, 49.03; H, 6.37%. ^1H and ^{13}C NMR as in refs [6, 8].

Acetylation (pyridine–Ac_2O, 1:1; 2 ml; 2 hr at room temp.) of **3** (81 mg) gave **3a** as a syrup (121 mg). Crystallization from EtOH yielded needles (79 mg) of mp 162–165°. Recrystallization twice from EtOH afforded needles of mp 166°. Prep. TLC (Et_2O; R_f 0.39) gave a crystalline residue that upon recrystallization had the same mp. ^1H NMR ($CDCl_3$): δ7.49 (*s*, H-3), 5.47 (*d*, J = 1.5 Hz, H-1), 5.40–5.30 (*m*, 3H, H-8 and 2 × H-10), 4.84 (*m*, obscured by H-1', H_a-7), 4.36–4.26 (*m*, obscured by H_a-6', H_b-7), 3.75 (*br s*, 5-OH), 2.96 (*br d*, J = 7.1 Hz, H-9), 1.83–1.74 (*m*, 2H, 2 × H-6), 5.28 (*t*, J = 9.7 Hz, H-3'), 5.10 (*t*, J = 9.7 Hz, H-4'), 5.03 (*dd*, J = 9.7 and 8.0 Hz, H-2'), 4.83 (*d*, J = 8.0 Hz, H-1'), 4.32 (*dd*, J = 12.5 and 4.5 Hz, H_a-6'), 4.15 (*dd*, J = 12.5 and 2.6 Hz, H_b-6'), 3.76 (*m*, obscured by 5-OH, H-5'); ^{13}C NMR as in ref. [9].

Secologanol (**7**). The impure portions of **7** (32 mg) were subjected to MPLC (B-column; 4:1–2:1). The main fr. was **7** (20 mg; 0.008%) eluted by 5:2. Prep. TLC ($CHCl_3$–MeOH, 3:1) gave almost pure sweroside (12 mg; 0.005%), i.e. the weakly acidic silica gel brought about lactonization. ^1H NMR as in ref. [15]; ^{13}C NMR: Table 1.

5-Hydroxysecologanol (**8**). ^1H NMR (D_2O): δ7.56 (*s*, H-3), 5.61 (*dt*, J = 17.0 and 10.0 Hz, H-8), 5.59 (*d*, J = 5.3 Hz, H-1), 5.36 (*dd*, J = 17.0 and 1.7 Hz, H_a-10), 5.32 (*dd*, J = 10.0 and 1.9 Hz, H_b-10), 3.72 (*s*, 3H, 11-OMe), 3.69 (*t*, 2H, J = 7.5 Hz, 2 × H-7), 2.80 (*dd*, J = 9.5 and 5.3 Hz, H-9), 2.33 (*dt*, J = 14.3 and 7.8 Hz, H_a-6), 1.83 (*dt*, J = 14.3 and 7.1 Hz, H_b-6), 4.74 (obscured by HOD signal, H-1'), 3.88 (*dd*, J = 12.2 and 2.0 Hz, H_a-6'), 3.69 (*dd*, J = 12.2 and 4.5 Hz, H_b-6'), 3.5–3.2 (*m*, 4H, H-2', H-3', H-4' and H-5'); ^{13}C NMR: Table 1.

5-Hydroxysecologanol pentaacetate (**8a**). Almost pure **8** (28 mg) was acetylated (pyridine–Ac_2O, 1:1) to give a 2:1 mixt. of the expected **8a** and swertiamarin tetraacetate (**3a**) [9] (due to lactonization on storage or acetylation).

Crude **5** (0.56 g) was hydrolysed with NaOH (2 M; 20 ml) for 24 hr at room temp. The reaction mixt. was neutralized with 10% HOAc (10 ml) and after addition of further HOAc (1 ml; to pH 6), Na_2CO_3 was added till pH 8 (to avoid extensive lactonization). The soln was concd to a small vol. (10 ml) and HOAc (1 ml) was added, and the soln applied to a RP-18 C column (25:1–3:1). Upon elution with 5:1, 5-hydroxysecologanolic acid (**11**; 309 mg) and *p*-hydroxybenzoic acid (106 mg) were obtained; crystallization (H_2O–MeOH, 1:1 + 1% HOAc) of the latter gave crystals of mp 212° compared with an authentic sample of mp 211–212°.

5-Hydroxysecologanolic acid (**11**). ^1H NMR (CD_3OD): δ7.59 (*br s*, H-3), 5.8–5.6 (*m*, obscured by H-1, H-8), 5.70 (*d*, J = 6 Hz, H-1), 5.37 (*br d*, J = 18 Hz, H_a-10), 5.32 (*br d*, J = 11 Hz, H_b-10), 4.70 (*d*, J = 8 Hz, H-1'), 3.94 (*br d*, J = 12 Hz, H_a-6'), 3.83–3.65 (*m*, 3H, H_b-6' and 2 × H-7), 3.48–3.19 (*m*, 4H, H-3', H-4', H-5' and H-2'), 2.82 (*br t*, J = 7 Hz, H-9), 2.46 (*m*, H_a-6), 1.89 (*m*, H_b-6); ^{13}C NMR: Table 1.

A methanolic soln (10 ml) of **11** was added to an excess of MeOH–$CH_2N_2\cdot Et_2O$ (1:1, 20 ml) in order to avoid acid catalysed lactonization of the labile **8**. The solvent was removed and the residue was immediately acetylated (pyridine–Ac_2O, 1:1, 4 ml) for 1 hr, at which point DMAP (10 mg) was added and the mixt. left overnight. Work-up gave a yellow syrup, which was subjected to prep. TLC (Et_2O) yielding **3a** (R_f 0.31; 57 mg) [9] and almost pure **8a** (R_f 0.35; 286 mg). Repeated prep. TLC (Et_2O) gave pure **8a** (213 mg), $[\alpha]_D^{20}$ −74.6° ($CHCl_3$; *c* 1.0). ^1H NMR ($CDCl_3$): δ7.41 (*s*, H-3), 5.48 (*dt*, J = 17.4 and 2 × 9.5 Hz, H-8), 5.38 (*d*, J = 4.2 Hz, H-1), 5.37 (*dd*, J = 17.4 and 2.5 Hz, H_a-10), 5.30 (*dd*, J = 9.8 and 2.5 Hz, H_b-10), 4.22 (*t*, 2H, J = 7.4 Hz, 2 × H-7), 3.74 (*s*, 3H, 11-OMe), 2.84 (*dd*, J = 8.7 and 4.2 Hz, H-9), 2.58 (*dt*, J = 14.4 and 2 × 7.3 Hz, H_a-6), 1.80 (*dt*, J = 14.4 and 2 × 7.4 Hz, H_b-6), 5.23 (*t*, J = 9.5 Hz, H-3'), 5.08 (*t*, J = 9.5 Hz, H-4'), 5.00 (*dd*, J = 9.5 and 8.0 Hz, H-2'), 4.83 (*d*, J = 8.0 Hz, H-1'), 4.27 (*dd*, J = 12.2 and 4.4 Hz, H_a-6'), 4.14 (*dd*, J = 12.2 and 2.3 Hz, H_b-6'), 3.72 (*m*, obscured by 11-OMe, H-5'), 2.08, 2.01, 1.99, 1.98 and 1.96 (each *s*, 3H, 5 × AcO); ^{13}C NMR: Table 1. Found: C, 52.49; H, 6.05%. $C_{27}H_{36}O_{16}$ requires: C, 52.60: H, 5.89%.

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