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2′-APIOSYLGARDOSIDE, AN IRIDOID GLUCOSIDE FROM *VERBENOXYLUM REITZII*

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Key Word Index—*Verbenoxylum reitzii*; Verbenaceae; iridoid glucosides; theviridoside; ipolamiide; 2′-apiosylgardoside; verbascoside.

Abstract—A new iridoid glucoside, 2′-apiosylgardoside together with the known compounds theviridoside, ipolamiide and verbascoside were isolated from leaves of *Verbenoxylum reitzii*. The structure of the new compound was determined mainly by NMR spectroscopy. © 1998 Published by Elsevier Science Ltd. All rights reserved

INTRODUCTION

Verbenoxylum (Verbenaceae subfamily Verbenoideae) is a monotypic genus of the tribe Citharexyleae, segregated from *Citharexylum* mainly because of its schizocarpic fruits [1]. *Verbenoxylum reitzii* (Moldenke) Troncoso is a tree 5–8 m high restricted to the coastal forest of Rio Grande do Sul and Santa Catarina in southern Brazil [2]. The leaves have been reported to contain the iridoids theviridoside (**1**) and ipolamiide (**2**) [3]. We have now reinvestigated the plant.

RESULTS AND DISCUSSION

The water-soluble part of an ethanolic extract of the leaves of *V. reitzii* was fractionated by reverse phase chromatography to give as the major compounds **1** and **2** and verbascoside together with a new, minor iridoid glycoside (**3**).

The ^{1}H NMR data (D_2O) of **3** showed an overall similarity with those published for gardoside [4], particularly the significant signals arising from H-1 (δ 5.70), the exocyclic C-10 methylene group (δ 5.4) and the methine proton at C-7 (δ 4.55). However, it was evident that additional signals were present in the spectrum.

The ^{13}C NMR spectrum (D_2O, Table 1) of **3** showed 21 signals of which ten could be assigned by comparison with the spectrum of gardoside methylester (**5**) [5], allowing for the differences arising from the former being an acid and the latter an ester. Going from an ester to an acid is known to cause variable upfield shifts for C-3 and downfield shifts for C-4 and C-11, strongly depending on the degree of ionisation of the carboxyl group [6, 7]. Of the remaining 11 signals, one set of six signals could be assigned to a β-glucopyranosyl moiety substituted at the 2-O-position as seen from the unusually high field signal of the anomeric carbon atom (δ 97.7 vs 99–100 for the unsubstituted iridoid glucosides [8]) together with the signal from C-2′ found at δ 78.1 as compared with the usual value of *ca*. 73.5. This left five signals of which the one found at δ 110.0 suggested the presence of an apiofuranosyl group in **3**. Comparison with the spectra (in CD_3OD; Table 1) reported [9] for the inerminosides A1 (**6**) and C (**7**) supported on the one hand the presence of a 2′-O-substituted gardoside moiety and on the other that of the disaccharide moiety shown. The β-configuration of the terminal apiofuranosyl unit was ascertained by the low field position (δ 110) of the anomeric carbon signal as opposed to the α-analogue which would be expected to appear 5 ppm more upfield [10]. In conclusion, **3** has been assigned the structure 2′-O-(β-apiofuranosyl)-gardoside.

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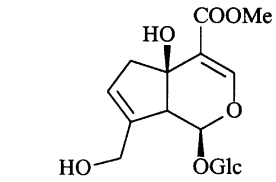

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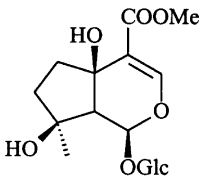

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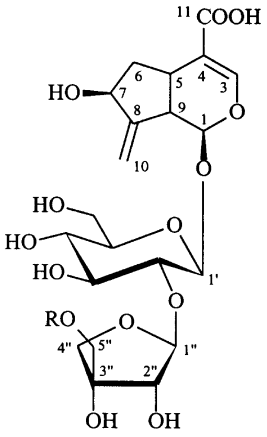
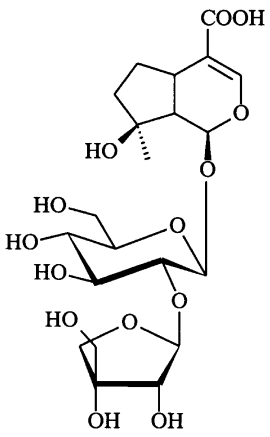


3; R = H
7; R = dihydrofolio-menthoyl



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Iridoid glycosides incorporating an apiose moiety appear to be rare in the literature. In addition to the above-mentioned [9] inerminosides A1 and C, additional apiosyl-derivatives have been isolated from *Clerodendron inerme* (Verbenaceae) [11]. The first apiose containing iridoid to be published was 6′-O-apiosylebuloside from *Sambucus ebulus* (Caprifoliaceae) [12]. Patriscabroside III [13], isolated from *Patrinia scabra* (Valerianaceae), is a 6′-(*β*-apiofuranosyl)-*β*-glucopyranosyl derivative of patriscabrol.

The two other genera in tribe Citharexyleae investigated, namely *Citharexylum* and *Duranta*, are both characterized by the presence of iridoids of the lamiide type [14, 15] and the finding of ipolamiide (**2**) in *Verbenoxylon* is consistent with this. However, in Verbenaceae, theviridoside (**1**) has so far only been reported from *Lantana* and *Lippia* from the same subfamily (Verbenoideae) but from the tribe Lantaneae [15]. The minor constituent **3** is probably of no taxonomic significance since the other source of similar compounds, *Clerodendron*, belongs to another subfamily, Viticoideae, which is mainly (but not solely) characterized by decarboxylated iridoids [15–17].

EXPERIMENTAL

General

^{1}H and ^{13}C NMR spectra were recorded in D_2O (standards δ 4.80 and the C-6′ signal (δ 61.5) [8]) or in CD_3OD (δ 49.0). A potted specimen of *V. reitzii* grown from seeds of an earlier authenticated specimen [3] was obtained from the experimental station of The Botanical Garden of Copenhagen in Taastrup near Copenhagen.

Table 1. ^{13}C NMR data for **3** and model compounds

C	**3** (D_2O)	**5** (D_2O)[a]	**3** (CD_3OD)	**7** (CD_3OD)[b]	**6** (CD_3OD)[b]
aglycone					
1	95.8	96.8	96.0	96.1	94.4
3	148.7	153.5	152.8	151.6	149.8
4	116.4	111.3	112.0		
5	30.3	30.9	31.7	33.2	31.9
6	38.6	39.3	40.3	41.3	30.6
7	73.2	73.1	73.8	74.0	41.2
8	152.1	151.2	153.4	153.8	80.9
9	44.4	44.1	45.0	45.1	52.5
10	111.9	113.9	112.9	113.6	24.4
11	175.0	170.3	176.8	173.3	
glucosyl					
1′	97.7	99.3	98.3	98.3	98.2
2′	78.1	73.5	78.3	78.0	77.8
3′	77.0	76.5	78.3	78.3	78.2
4′	70.3	70.4	71.7	71.8	71.9
5′	77.0	77.2	78.4	78.5	78.5
6′	61.5	61.5	62.8	62.8	63.0
apiosyl					
1″	110.0		110.7	110.2	110.5
2″	78.4		78.8	78.9	79.1
3″	80.5		80.8	79.2	80.3
4″	74.5		75.3	75.4	75.4
5″	64.6		66.2	68.6	66.1

[a]Data from [5].
[b]Data from [9].

Isolation of glucosides

Fresh leaves (165 g) were blended with EtOH (500 ml) and filtered. The concd extract was partitioned in Et_2O–H_2O and the aq. phase taken to dryness (6.8 g). This was dissolved in satd $NaHCO_3$ soln (10 ml) and subjected to reverse phase chromatography on a Merck Lobar C18 column (size C) eluting with H_2O–MeOH (25:1 to 1:1) to give first a polar fraction, then theviridoside (**1**, 520 mg), ipolamiide (**2**, 100 mg) and verbascoside (**4**, 180 mg). The above polar fraction was concd and redissolved in 20% AcOH (10 ml) and chromatographed as above to give 2′-apiosylgardoside (**3**, 14 mg).

2′-*Apiosylgardoside* (**3**)

Amorphous. FAB MS (neg; HEDS-matrix): *m*/*z* 505 $[M–H]^-$ (calc. for M, $C_{21}H_{30}O_{14}$, 506); ^1H NMR (500 MHz, D_2O): aglucone moiety: δ 7.20 (*d*, J = 1.5 Hz, H-3), 5.70 (*d*, J = 2.7 Hz, H-1), 5.41 (*dd*, J = 2.5 and 2.5 Hz, H-10a), 5.40 (*dd*, J = 2.5 and 2.5 Hz, H-10b), 4.55 (*ddddd*, J = 9.6, 7.1, 2.5, 2.5 and 2.3 Hz, H-7), 3.24 (*dddd*, J = 7.2, 2.7, 2.5 and 2.5 Hz, H-9), 3.09 (*dddd*, J = 7.2, 6.8, 2.2 and 1.5 Hz, H-5), 2.33 (*ddd*, J = 12.8, 6.9 and 2.2 Hz, H-6a), 1.86 (*ddd*, J = 12.8, 9.4 and 6.8 Hz, H-6b); β-glucopyranosyl moiety: δ 4.90 (*d*, J = 8.1 Hz, H-1′), 4.00 (*dd*, J = 12.4 and 2.1 Hz, H-6′a), 3.80 (*dd*, J = 12.4 and 5.8 Hz, H-6′b), 3.69 (*t*-like, J = *ca*. 9.5 Hz, H-3′), 3.55 (*ddd*, J = 10.0, 5.8 and 2.1 Hz, H-5′), 3.50 (*dd*, J = 10.0 and 9.1 Hz, H-4′), 3.48 (*dd*, J = 9.6 and 8.1 Hz, H-2′); apiofuranosyl moiety: δ 5.39 (*d*, J = 2.4 Hz, H-1″), 4.04 (*d*, J = 2.4 Hz, H-2″), 3.97 (*d*, J = 10.2 Hz, H-4″a), 3.87 (*d*, J = 10.2 Hz, H-4″b), 3.66 (2H, *s*, H-5″): ^{13}C NMR: Table 1.

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