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PHENYLPROPANOID GLYCOSIDES, A FURANONE GLUCOSIDE AND GENIPOSIDIC ACID FROM MEMBERS OF THE RUBIACEAE*

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Key Word Index—*Psydrax livida*; *Oxyanthus speciosus*; *Canthium gilfillanii*; Rubiaceae; phenylpropanoid glycosides; psydroside; furanone glucoside; psydrin; geniposidic acid.

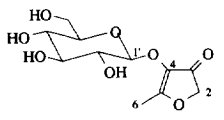
Abstract—The new 5-methyl-4-hydroxy-3(2*H*)-furanone glucoside (psydrin) and the new (5-*O*-*E*-caffeoyl-β-D-apio-D-furanosyl-(1 → 6)-β-D-glucopyranosyl benzoic acid ester (psydroside) were isolated from the leaves of *Psydrax livida*. The new 2-(2-hydroxy)-ethanol-β-D-glucopyranoside was found in the leaves of *Oxyanthus speciosus* subsp. *gerrardii* and geniposidic acid in the leaves of *Canthium gilfillanii*. All species belong to the Rubiaceae.

INTRODUCTION

During our work on cyanogenic glycosides of *Canthium*, *Psydrax* and *Oxyanthus* species of the Rubiaceae [1], we detected four compounds which gave outstanding colour reactions in the TLC systems used. Their structural elucidation will be reported here.

RESULTS AND DISCUSSION

Psydrin (**1**, 5-methyl-4-hydroxy-3(2*H*)-furanone) was obtained from the methanol extract of the leaves of *P. livida* (Hiern) Bridson by DCCC chromatography. The compound showed a red-brownish false positive spot in the TLC sandwich test [2] for cyanogenic glycosides and a strong absorption on the plate at 254 nm UV detection. Acidic hydrolysis resulted in glucose (TLC, GC). The DCI-MS indicated a M_r of 276; upon standing in CD_3OD, $[M]^+$ of 277 and 278 were observed indicating an exchangeable methylene group. The ^1H NMR spectrum in DMSO-d_6 (Table 1) showed, besides the H2–H6 glucose protons, the anomeric proton at 4.68 ppm indicating the β-configuration from its coupling constant of 7.8 Hz. Only two additional resonances were observed; one singlet at 2.22 ppm indicated an isolated Me attached to an olefinic carbon, and another singlet at 4.65 ppm pointed to an isolated methylene group. The latter disappeared in CD_3OD indicating the exchange of the methylene protons with deuterium via an enolic structure already described for such systems [3]. The ^{13}C NMR spectrum exhibited 11 carbon resonances, of which the aglycone carbons were assigned as indicated in Table 1. Strong cross peaks between H-1′ and C-4, and H-6 and C-5 in the 2D HMBC spectrum indicated the substitution of the glucosyloxy residue at C-4 and the methyl group at C-5 of the aglycone; further structural evidence was obtained from cross peaks between H-2 and



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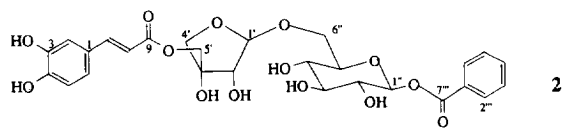

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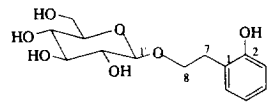

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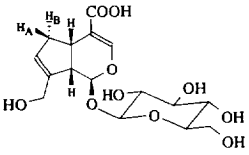

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*In honour of Prof. F.-C. Czygan, Würzburg, on the occasion of his 60th birthday.

Table 1. ^{1}H and ^{13}C NMR data of **1**

	Methanol-d_4 ^{1}H NMR	^{13}C NMR	DMSO-d_6 ^{1}H NMR	^{13}C NMR
2	-	—	4.65	73.6
2	—	198.0	—	194.6
4		136.5	—	133.9
5		186.1	—	181.7
6	2.35 (*s*)	14.2	2.22 (*s*)	13.9
1′	4.77 (*d*, 7.8)	104.6	4.68 (*d*, 7.9)	102.3
2′	3.34 (*dd*, 7.8, 9.1)	74.8	3.06	73.3
3′	3.43 (*dd*, 9.1, 9.1)	77.7[a]	3.16	76.2[a]
4′	3.38 (*dd*, 9.1, 9.1)	71.2	3.09	69.8
5′	3.31 (*ddd*, 2.5, 5.5, 9.1)	78.3[a]	3.08 (*m*)	77.1[a]
6′	3.87 (A, *dd*, 2.0, 11.9)	62.5	3.63 (A, *dd*, 2.1, 11.6)	60.9
	3.72 (B, *dd*, 5.5, 10.4)		3.43 (*B*, *dd*, 5.4, 11.7)	

[a]Assignments are interchangeable.

C-3 as well as C-5, and H-6 and C-4; no cross peaks were observed for C-2 and C-6. The ^{1}H and ^{13}C NMR data are in good agreement with 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone glucoside isolated previously from strawberries [3], the aglycone of which, furaneol, is an aroma constituent of strawberry and pineapple [4]. Furaneol differs, however, from **1** by one methyl moiety at C-2. It seems likely that both compounds stem from carbohydrate metabolism, the aglycone of **1** from pentose and furaneol from hexose metabolism.

(5-*O*-*E*-Caffeoyl)-β-D-apio-D-furanosyl-(1 → 6)-β-D-glucopyranosyl benzoic acid ester (**2**, psydroside) was isolated from the ethyl acetate extract of the leaves of *P. livida* by using radial accelerated TLC and MLCC techniques as described in the Experimental. Its R_f was very similar to that of the cyanogenic glycoside oxyanthin benzoate [1] isolated from the same source. Upon acidic hydrolysis apiose, glucose, caffeic acid and benzoic acid were detected by TLC. The FAB-MS yielded a $[M - H]^-$ of 557 indicating a M_r of 578. The ^{1}H and ^{13}C NMR data are presented in Table 2. They show striking similarities with the caffeoylapiose partial structure of the cyanogenic compound xeranthin [5], the apiosylglucose partial structure of the cyanogenic glycoside oxyanthin [1] and with benzoylglucose [6]. The β-configuration (C-1/C-2 *trans* OH) of the apiose is assigned from the ^{13}C resonance [7] of C-1′, and the C-2/C-3 *cis* relative configuration by the equivalence of the C-5′ methylene group protons [8]. Thus the apiose moiety is identical, with respect to its spectroscopic properties, to that assigned in oxyanthin [1] of the same plant, and thus has most probably the β-D-apio-D-furanose conformation. Clear cross peaks in the 2D HMBC between H-5′ and C-9, H-1′ and C-6″ and H-1″ and C-7‴ revealed the connectivities between the different moieties of the molecule as shown for **2**. Compounds with the apiosylglucose unit as the glucosidic moiety are not rare and occur in the flavonoids, phenols, lignans, coumarins, monoterpenes, xanthones, phenylpropanes [9] and cyanogenic glycosides [1]. Forsythoside B [10], leucosceptoside B [11] and phenylpropanoid glycosides isolated from *Pedicularis striata* [12] contain the caffeic acid, glucose and apiose units but not in the same sequence.

Table 2. ^{1}H and ^{13}C NMR data of **2** in MeOH-d_4

		^{1}H NMR	^{13}C NMR
Caffeic acid	1	—	128.0
	2	7.08 (*d*, 2.0)	115.5
	3	—	147.1
	4	—	149.9
	5	6.82 (*d*, 8.0)	116.7
	6	6.98 (*dd*, 2.1, 8.5)	123.3
	7	7.62 (*d*, 15.6)	147.6
	8	6.30 (*d*, 15.6)	115.0
	9	-	169.3
Apiose	1′	5.06 (*d*, 2.0)	110.8
	2′	4.00 (*d*, 2.1)	78.6[a]
	3′	—	79.1
	4′	A 4.09 (*d*, 9.8)	75.2
		B 3.87 (*d*, 9.7)	
	5′	A 4.30 (*m*)	67.6
		B 4.29	
Glucose	1″	5.76 (*d*, 7.1)	96.4
	2″	3.53 (*m*)	74.1
	3″	3.61 (*m*)	78.5[a]
	4″	3.47 (*m*)	71.4
	5″	3.70 (*m*)	78.1[a]
	6″	A 4.06 (*dd*, 1.7, 11.2)	68.4
		B 3.72 (*dd*, 5.8, 11.2)	
Benzoic acid	1‴	—	131.0
	2,6‴	8.10 (*m*)	131.2
	3,5‴	7.43–7.50 (*m*)	129.8
	4‴	7.62 (*m*)	134.9
	7‴	—	167.0

[a]Assignments are interchangeable.

Table 3. ^{1}H and ^{13}C NMR data of **3** in MeOH-d_4

	^{1}H NMR	^{13}C NMR
1	—	126.1
2	—	156.5
3	6.74 (*dd*, 8.0, 1.3)	116.1
4	7.01 (*ddd*, 7.7, 7.7, 1.8)	128.5
5	6.74 (*ddd*, 7.5, 7.5, 1.2)	120.6
6	7.12 (*dd*, 7.7, 1.8)	131.8
7	2.94 (*dd*, 7.5, 7.3)	31.9
8	A 4.06 (*ddd*, 7.4, 7.4, 9.9)	70.8
	B 3.76 (*ddd*, 7.4, 7.4, 9.9)	
1′	4.32 (*d*, 7.6)	104.5
2′	3.19 (*dd*, 7.6, 9.1)	75.1
3′		78.1[a]
4′	3.24–3.42 9 (*m*)	71.6
5′		77.9[a]
6′	A 3.86 (*dd*, 11.9, 1.9)	62.8
	B 3.67 (*dd*, 11.9, 5.1)	

[a]Assignments are interchangeable.

To our knowledge the diacyl glycoside **2** is a new natural compound.

The acetone extract of the leaves of *Oxyanthus speciosus* subsp. *gerrardii* (Sond.) Bridson showed a red-brownish zone on TLC with anisaldehyde/H_2SO_4 and a blue one with dichloroquinone chloroimide; the latter indicating a free phenol. The substance was purified by column chromatography on silica gel and Sephadex LH 20, and **3** was obtained after lyophilization as an amorphous powder. Acidic hydrolysis of **3** yielded glucose (TLC). The ^{1}H NMR (Table 3) showed the anomeric glucose proton at 4.32 ppm (7.6 Hz, β-configuration). A triplet was visible at 2.94 ppm of a methylene group indicating the presence of a $-CH_2-CH_2-$ grouping. The *o*-disubstituted aromatic ring system showed a characteristic pattern at 6.7–7.2 ppm. The ^{13}C NMR (Table 3), with 14 carbon resonances, corroborated these data. Irradiation at H-7 (2.94 ppm) caused NOE effects on H-8 and H-6. The data fit well with those reported for 2-phenylethanol-β-D-glucoside [13], 2-(4-hydroxyphenyl)-ethanol-β-D-glucoside [14] and 3,4-dihydroxyphenylethanol glycosides [12] and allow **3** to be assigned as 2-(2-hydroxyphenyl)ethanol-β-D-glucopyranoside. To our knowledge this is a new natural product.

Geniposidic acid (**4**) was isolated from the leaves of *Canthium gilfillanii* (N.E.Br.) O.B. Miller by methanol extraction. The substance showed a blackish zone in the TLC sandwich test for cyanogenic glycosides [2]. The extract was finally purified by DCCC. Compound **4** exhibited a $[M + NH_4]^+$ of 392 indicating a M_r of 374. After acidic hydrolysis, glucose was detected (TLC, GC). The ^{1}H and ^{13}C NMR data, including the HMBC spectrum of **4**, were consistent with the proposed structure and with data reported for genoposidic acid in [15–17]. The $[\alpha]_D^{22}$ of + 7.5° differed from that of + 19.3 reported in ref. [16] due to some impurities. Compound **4** is fairly widespread within the Rubiaceae and has been reported for *Genipa* [18], *Gardenia* [15], *Ixora* [16] and *Wendlandia* [17] species.

EXPERIMENTAL

General. NMR spectra were recorded either on a Varian Gemini 200 (200.0, 50.3 MHz), a Bruker AM-300 (300.1 MHz) or a Bruker AM-600 (600.1, 150.9 MHz). UV spectra were recorded on a Shimadzu UV-260 and $[\alpha]$ values on a Perkin Elmer 241.

Plant material. Air dried leaves of *Psydrax livida* were collected in 1989/90 in the Pretoria National Botanic Garden; comparison with authentic specimens in the herbaria in Pretoria, Durban and Kew Gardens confirmed their identity; a voucher is deposited in the herbarium of the Institute in Münster under PBMS 20. Air-dried leaves of *Oxyanthus speciosus* subsp. *gerardii* were collected in the Pretoria Garden and the Durban area; a voucher is deposited under PBMS 48. Air-dried leaves of *Canthium gilfillanii* stem from collections in Pretoria which were gathered in 1987/88; their identity was confirmed by comparison with an authentic specimen in the herbarium of the Pretoria Botanical Garden; vouchers are deposited under PBMS 17.

Extraction and isolation. Psydrin (**1**). The MeOH extract of the defatted (petrol) leaves of *P. livida* was dried and dissolved in the upper phase of the solvent system $CHCl_3$/MeOH/H_2O/iPrOH (5:6:4:1) and chromatographed in a Büchi 670 DCCC in the ascending mode using 296 columns (I.D. 2.7 mm). Fractions between 166 and 250 ml contained **1**; these were combined, dried and dissolved in the lower phase of the above system and chromatographed in the same system but in the descending mode. Fractions between 2070 and 2200 ml contained **1**; they were combined, dried and chromatographed on Sephadex LH 20 (27 × 2 cm) with EtOH/H_2O 1:1; elution of **1** occurred between 45 and 50 ml. Dried leaves (100 g) gave 29 mg of **1**; λ_{max} 274 nm (logε 3.65); $[\alpha]_D^{20}$ − 66.2° (MeOH; *c* 0.12).

Psydroside (**2**). The EtOAc extract of the defatted leaves of *P. livida* was chromatographed by centrifugally accelerated radial TLC (Chromatotron; Al_2O_3, 2 mm) first with 100 ml *n*-hexane/EtOAc 1:4 followed by EtOAc. Compound **2** eluted after *ca* 350 ml together with oxyanthin benzoate [1]. The dried fraction was used for MLCC (multilayer coil separator P.C. Inc. Potomac, MD, U.S.A.; 1.6 mm i.d. coil with a total volume of 325 ml; 700 rpm) with the upper phase of the system EtOAc/H_2O/*n*-hexane/iPrOH 140:80:10:8 as the mobile phase. Compound **2** eluted between 328 and 336 ml. Final purification was obtained on a Sephadex LH 20 column as above; **2** eluted between 37.5 and 45 ml. Leaves (100 g) yielded 115 mg of **2**; λ_{max} 230 nm (log ε 4.28), 325 nm (4.15); $[\alpha]_D^{25}$ − 45° (MeOH; *c* 0.5).

2-(2-Hydroxyphenyl)ethanol-β-D-glucoside (**3**). The Me_2CO extract of the dry leaves of *O. speciosus* subsp. *gerrardii* was taken to dryness and chromatographed on silica gel (60 × 5 cm) with EtOAc/MeOH/H_2O (79:11:10). Fractions between 1428 and 1533 contained **3**; they were combined, dried and were twice chromatog-

raphed on Sephadex LH20 as above; **3** eluted between 57 and 63 ml. Leaves (100 g) gave *ca* 90 mg of **3**. λ_{max} 217 nm ($\log\varepsilon = 3.18$); $[\alpha]_D^{20} - 19°$ (MeOH; $c = 0.5$).

Geniposidic acid (**4**). The dried leaves of *C. gilfillanii* were extracted with MeOH and the dried extract was chromatographed in the DCCC apparatus as described for **1** using the same solvent system in the ascending mode. Fractions 175–262 ml contained **4**; these were dried and chromatographed again in the same system for final purification; this time the 180–200 ml contained **4**. Leaves (100 g) yielded *ca* 200 mg of **4**. The compound was not quite pure as indicated by its comparatively low $[\alpha]_D^{24}$ of 7.5° (MeOH; *c* 0.5) and its log of 3.11 at λ_{max} of 235 nm ($\log\varepsilon$ 3.64 at 237 nm in ref. [16]).

TLC systems. Silica gel plates (Merck 5735). Solvent systems. A: $EtOAc/Me_2CO/CH_2Cl_2/H_2O/MeOH$ 40:30:12:8:10. B: $EtOAc/H_2O/MeOH$ 79:10:11. C: $CS_2/MeCN/H_2O$ 5:90:5. R_f values: **1**: 0.43 (A), 0.24 (B); **2**: 0.69 (A), 0.49 (B); **3**: 0.66 (A), 0.47 (B); **4**: 0.29 (A), 0.12 (B); benzoic acid: 0.85 (A); glucose: 0.08 (C); apiose: 0.31 (C); caffeic acid: 0.54 (C). Detection was carried out mostly with anisaldehyde/H_2SO_4 reagent.

Hydrolysis. Performed with 1 N HCl by refluxing; in some cases the substance was hydrolysed according to ref. [19] directly on the TLC plate followed by development of the plate.

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