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IRIDOID GLUCOSIDES FROM *CLERODENDRUM INCISUM*

ELISABETH STENZEL, HORST RIMPLER* and DIETER HUNKLER†

Institut für Pharmazeutische Biologie, Schänzlestr. 1, D-7800 Freiburg, West Germany; †Institut für Organische Chemie und Biochemie, Albertstr. 21, D-7800 Freiburg, West Germany

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Key Word Index—*Clerodendrum incisum*; Verbenaceae; iridoid glucosides; 8-*O*-foliamenthoyleuphroside; 2′-*O*,8-*O*-difoliamenthoyleuphroside.

Abstract—8-*O*-Foliamenthoyleuphroside and 2′-*O*,8-*O*-difoliamenthoyleuphroside, new foliamenthic acid esters of euphroside, and the known iridoid euphroside have been isolated from the leaves and twigs of *Clerodendrum incisum*. From the roots of the same plant the known iridoid plantarenaloside has been isolated. The taxonomic significance of these findings is discussed.

INTRODUCTION

Several attempts [1–6] to classify the large genus *Clerodendrum* (Verbenaceae) into subgenera or sections have so far not led to a natural classification of the whole genus. In continuation of our chemotaxonomic studies on the distribution of iridoids within the genus *Clerodendrum* [6], we now wish to describe the isolation and identification of four iridoids from different parts of *C. incisum*, a member of the hitherto unexamined section *Konocalyx*.

RESULTS AND DISCUSSION

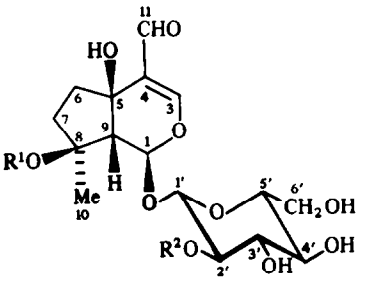
Column chromatography of alcoholic extracts from different parts of *C. incisum* on silica gel and XAD 7 resin followed by HPLC separations yielded four iridoid glucosides. From the leaves and twigs two new esteriridoids (**1**, **2**) as well as the known iridoid euphroside (**3**) [7] were isolated. From the roots we isolated another known iridoid glucoside, plantarenaloside (**4**) [8]. Glucosides **3** and **4** were identified by comparison with authentic samples. The structures of **1** and **2** were determined mainly by spectroscopic methods.

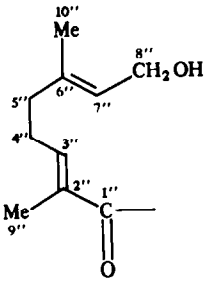
The IR spectrum of compound **1** showed the typical absorption of the enol ether system of iridoids at 1627 cm^{-1} [9], of an aldehyde function at 1676 cm^{-1} and of an ester function at 1688 cm^{-1}.

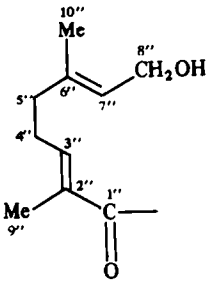
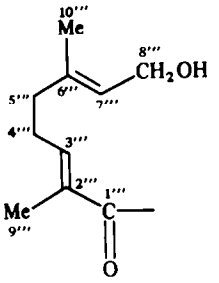
The UV maximum (229 nm) revealed the presence of a conjugated carboxyl group [10] and a shoulder at 245 nm was attributable to an enol ether system conjugated with a carbonyl function [9]. Methanolysis of **1** yielded foliamenthic acid methyl ester (**5**, 8-hydroxy-2,6-dimethyl-2(*E*),6(*E*)-octadienoic acid methyl ester), which was identified by GC/MS comparison with an authentic sample of **5** prepared from nemoroside [11]. The iridoid moiety was destroyed under these conditions, but Hakamori methylation [12] of **1** gave a compound which showed a $[M]^+$ at *m/z* 488 and the typical fragments expected for 5,8-dimethoxy-8-methyl-3-methylene-4-methoxymethinyl-1-(2′,3′,4′,6′-tetra-*O*-methyl-β-D-glucopyranosyloxy)-1,3,4,4a,5,6,7,7a-decahydrocyclopenta[c]pyran (**6**), the main methylation product of euphroside [13]. The presence of a euphroside moiety was further confirmed by ^1H and ^{13}C NMR data (Tables 1 and 2). The ^1H NMR signals for the iridoid moiety of **1** and of **3** differed only in the chemical shifts for H-1 and H-9. These signals were shifted downfield by 0.32 and 0.24 ppm in the spectrum of **1**. These differences are to be expected if the acyl moiety is linked to C-8 of euphroside [9]. In the ^{13}C NMR spectra (Table 2) also, most of the signals for the iridoid moieties of **1** and **3** had very similar chemical shifts. Significant differences were the large downfield shift (+9.24 ppm) of the C-8 signal and smaller upfield shifts for C-7, C-9 and C-10 (−1.09, −2.29 and −1.95 ppm) in **1** which are typical for α- and β-effects [14, 15] caused by acylation of the tertiary 8-hydroxyl group. Additionally, selective hetero-^1H, ^{13}C-NOE difference NMR spectra [16] established the position of the acyl group, as irradiation of the C-10 protons increased the intensities of the signals for C-2″ and for C-8. The configuration at C-8 was confirmed by NOE experiments; irradiation of the signal of H_3-10 increased the intensity of H-1, but the H-9 signal intensity remained unchanged. The C(10)H_3 group must therefore be in the α-position. The 2(*E*),6(*E*) configuration of the acyl moiety was also confirmed by NOE experiments on **1**; irradiation of the H_3-9″ signal increased the intensity of H_2-4″ and irradiation of the H_3-10″ signal increased the intensity of H_2-8″. Thus compound **1** is 8-*O*-foliamenthoyleuphroside.

The UV spectrum of compound **2** was nearly identical to that of **1**. Its IR spectrum was also similar to that of **1**, but it showed an additional peak at 1700 cm^{-1} for another ester function. Partial methanolysis of **2** yielded **1** and **5**. Compound **2** must therefore be a difoliamenthoyleuphroside. All the signals in the ^1H NMR spectrum of **2** for the iridoid and for one acyl moiety were in good agreement with those of **1**. However, there was a second set of signals for another foliamenthoyl moiety (Table 1). The ^{13}C NMR spectrum of **2** (Table 2) also showed two sets of 10 signals each for the foliamenthoyl moieties. The occurrence of fragment **7** (*m/z* 310) in the mass spectrum

*To whom correspondence should be addressed.



1 R^1 =  , R^2 = H

2 R^1 =  , R^2 = 

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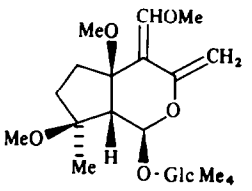

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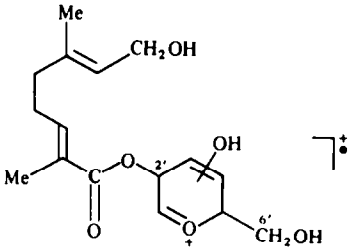

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Table 1. ^{1}H NMR spectral data of compounds **1**, **2**, **3** and **5**

	1*	2*	3*	5† [11]
H-1	6.32 *br s*	6.37 *br s*	6.00 *br s*	
H-3	7.69 *s*	7.58 *s*	7.63 *s*	
H_2-6	1.61–2.42 *m*	1.58–2.43 *m*	1.59–2.26 *m*	
H_2-7				
H-9	2.82 *br s*	2.82 *br s*	2.58 *br s*	
H_3-10	1.48 *br s*	1.47 *br s*	1.29 *br s*	
H-11	9.30 *s*	9.16 *s*	9.34 *s*	
H-1′	4.90 *d*	5.18 *d*	4.90 *d*	
H-2′	3.34 *t*	4.84 *t*	3.35 *t*	
	$J_{1',2'} = 7.5$	$J_{1',2'} = 7.5$	$J_{1',2'} = 7.5$	
H-3′				
H-4′	3.42–3.61 *m*	3.54–3.63 *m*	3.47–3.66 *m*	
H-5′				
H_2-6′	3.79–4.02 *m*	3.90–4.10 *m*	3.82–4.00 *m*	
	$J_{6'A,6'B} = 12$	$J_{6'A,6'B} = 12$	$J_{6'A,6'B} = 12$	
	$J_{6'A,5'} = 1.5$	$J_{6'A,5'} = 1.5$	$J_{6'A,5'} = 1.5$	
	$J_{6'B,5'} = 6.0$	$J_{6'B,5'} = 6.0$	$J_{6'B,5'} = 6.0$	
H-3″	6.85 *br t*	6.87 *br t*		6.71 *qt*
	$J_{3'',4''} = 7.5$	$J_{3'',4''} = 7.5$		$J_{3'',4''} = 7.3$
				$J_{3'',9''} = 1.4$
H_2-4″	2.42 *m*	2.43 *m*		2.28 *br q*
	$J_{4'',5''} = 8.0$	$J_{4'',5''} = 8.0$		$J_{4'',5''} = 7.8$
H_2-5″	2.18 *m*	2.24 *m*		2.14 *br t*
H-7″	5.48 *br t*	5.48 *br t*		5.41 *qt*
	$J_{7'',8''} = 7.2$	$J_{7'',8''} = 7.2$		$J_{7'',8''} = 6.9$
				$J_{7'',10''} = 1.3$
H_2-8″	4.12 *d*	4.14 *d*		4.14 *d*
H_3-9″	1.84 *d*	1.82 *d*		1.81
	$J_{3'',9''} = 1.5$	$J_{3'',9''} = 1.5$		
H_3-10″	1.72 *d*	1.74 *d*		1.67
	$J_{7'',10''} = 1.5$	$J_{7'',10''} = 1.5$		
-OMe				3.71
H-3‴		6.96 *br t*		
		$J_{3''',4'''} = 7.5$		
H_2-4‴		2.43 *m*		
		$J_{4''',5'''} = 8.0$		
H_2-5‴		2.24 *m*		
H-7‴		5.56 *br t*		
		$J_{7''',8'''} = 7.2$		
H_2-8‴		4.19 *d*		
H_3-9‴		1.85 *d*		
		$J_{3''',9'''} = 1.5$		
H_3-10‴		1.77 *d*		
		$J_{7''',10'''} = 1.5$		

*250.0 MHz, solvent D_2O, capillary D4-TSPNa as external standard with 0.03 ppm deviation to internal D4-TSPNa standard.

†Solvent $CDCl_3$.

Chemical shift values as δ; coupling constants in Hz.

of **2** showed that the second acyl group is linked to the glucose moiety. The ^{1}H NMR spectrum of **2** indicated that the second ester group is linked to the 2′-oxygen, since the signal due to H-2′ was shifted downfield by *ca* 1.5 ppm as compared with **1**, whereas the other glucose signals remained essentially unchanged. The ^{13}C NMR spectrum of **2** (Table 2) confirmed this structure. The C-2′ signal was deshielded by 0.54 ppm as compared with **1** while the signals for the β-carbons C-1′ and C-3′ were shifted upfield by 2.63 and 1.25 ppm. The other glucose signals agreed with those of **1**. Compound **2** is therefore 2′-*O*,8-*O*-difoliamenthoyleuphroside.

Compounds **1** and **2** represent a comparatively rare type of glucosidic iridoid derived from the junction of two monoterpenic units. Esters of foliamenthic acid with iridoids and secoiridoids have so far been detected in species belonging to the Menyanthaceae (*Menyanthes trifoliata*: foliamenthin [10, 17]), Bignoniaceae (*Tecoma chrysantha*: amareloside [18]) and Scrophulariaceae (*Penstemon nemorosus*: nemoroside and nemorososide

Table 2. ^{13}C NMR spectral data of compounds **1**, **2**, **3** and **5**

C	1*	2†	3‡ [19]	5§ [11]
1	96.03	95.38	95.5	
3	165.81	159.54	164.3	
4	122.62	122.40	124.7	
5	72.01	72.00	71.0	
6	34.80	33.16	36.8	
7	38.21	37.85	39.3	
8	88.24	86.47	79.0	
9	58.21	57.84	60.5	
10	21.15	21.14	23.1	
11	193.97	188.79	194.5	
1′	99.52	96.89	99.4	
2′	73.05	73.59	73.2	
3′	77.07	75.82	76.1	
4′	70.31	70.25	70.4	
5′	75.99	74.16	77.1	
6′	61.50	61.50	61.5	
1″	170.24	168.18		168.6
2″	128.95	128.03		127.9
3″	144.10	143.46		141.6
4″	27.34	25.98		26.9
5″	38.21	37.40		38.1
6″	139.86	137.01		138.3
7″	123.83	124.67		124.3
8″	58.59	58.74		59.3
9″	12.47	11.84		12.3
10″	16.15	15.56		16.2
–OMe				51.6
1‴		167.50		
2‴		126.79		
3‴		141.85		
4‴		25.87		
5‴		37.30		
6‴		136.84		
7‴		124.60		
8‴		58.68		
9‴		11.84		
10‴		15.49		

*In D_2O (20.15 MHz).
†In $CDCl_3$ (62.83 MHz).
‡In D_2O (22.6 MHz).
§In $CDCl_3$ (100.5 MHz).
All chemical shifts were aligned to C-6′ = 61.50 ppm.

[11]). All the iridoids isolated from *C. incisum* bear a formyl group at C-4. Within the genus *Clerodendrum*, this type of iridoid has so far been found only in the section *Cyclonema* [6]. Since *C. incisum* is a typical representative of the section *Konocalyx*, our results indicate a closer relationship between the sections *Konocalyx* and *Cyclonema* which up to now had not been recognized. This relationship is also indicated by our numerical taxonomic studies on morphological characters of representative *Clerodendrum* species which will be published elsewhere.

EXPERIMENTAL

Plant material. C. incisum Vent. was cultivated in a greenhouse at Freiburg. The leaves, twigs and roots were collected from flowering plants and lyophilized immediately. A voucher specimen (012-010) has been deposited at the herbarium of the Institut für Pharmazeutische Biologie, Freiburg.

Analytical methods. CC: Celite 545, silica gel 60 (Merck), Amberlite XAD 7 (Serva). HPLC: μ-Bondapack C_{18} column (250 mm × 9.4 mm i.d.), **1** was eluted with 50% MeOH, **2** with 70% MeOH, **3** with 20% MeOH, **4** with 30% MeOH, flow rate 2 ml/min. TLC: silica gel 60, CH_2Cl_2–MeOH–H_2O (90:10:1 and 70:30:3). Spray reagent: vanillin (3%) and H_2SO_4 (1%) in 100 ml EtOH followed by heating at 110° for 5 min. GC/MS: **6**: OV-17 (3% on Chromosorb W) column (1.2 m × 2 mm i.d.), injector temp. 280°, column 270°, source 250°, He 40 ml/min, EIMS 70 eV. **5**: FFAP (10% on Gas Chrom Q) column (2 m × 2 mm i.d.), inj. temp. 230°, column 200°, 2°/min up to 240°, source 220°, He 35 ml/min, EIMS 70 eV. ^1H NMR: 250 MHz, 22°, decoupling and NOE expts, 2D NMR spectra (COSY 90). ^{13}C NMR: 20 MHz, 37° and 63 MHz, 25°, spinecho expts, bb, gated, selective decoupled and hetero NOE spectra. When D_2O was used as solvent, most of the ^1H NMR spectra were acquired by selectively inverting the HDO signal by a 180° decoupler pulse and applying a 90° acquisition pulse after an appropriate delay, optimized for near-zero intensity of HDO. Optical rotations were measured at 21° using a 1 dm cell for **1** and 1 cm ORD short cell for **2**.

Isolation. Twigs and leaves (70 g) were extracted by refluxing for 30 min successively with 96%, 80% and 70% EtOH. The combined EtOH extracts were evaporated *in vacuo* and chromatographed on a Celite column. Elution with *n*-hexane–CH_2Cl_2 (1:1) afforded the lipophilic fraction; subsequent elution with CH_2Cl_2–MeOH (1:1) yielded the hydrophilic fraction containing the iridoids. By chromatography of the hydrophilic fraction on silica gel with CH_2Cl_2–MeOH–H_2O (70:30:3) we obtained three iridoid-containing fractions: A, B and C. Fraction A purified only by HPLC yielded 70 mg **2**. Fraction B was rechromatographed on silica gel with CH_2Cl_2–MeOH–H_2O (17:3:0.3). Further purification by CC on XAD 7 with MeOH (40%) followed by HPLC afforded 180 mg **1**. Fraction C yielded 30 mg **3** after CC on XAD 7 with MeOH (10%) followed by HPLC. Roots (10 g) were extracted in a similar fashion to twigs and leaves except that the combined EtOH extracts were evaporated *in vacuo* and then chromatographed once on silica gel with CH_2Cl_2–MeOH–H_2O (70:30:3). The iridoid-containing fractions were rechromatographed on XAD 7 with MeOH (10%) and finally purified by HPLC yielding 6 mg **4**.

8-O-*Foliamenthoyleuphroside* (**1**). $[\alpha]_D^{21}$ −59.15° (*c* 2.0; MeOH). EIMS, solid probe (source 140°), 30 eV, −1.4 kV, *m/z* (rel. int.): 296 (0.36), 284 (0.22), 268 (0.27), 256 (0.58), 213 (0.77), 197 (0.68), 196 (0.77), 185 (1.31), 179 (2.17), 178 (2.76), 166 (6.30), 160 (10.02), 121 (28.61).

2′-O,8-O-*Difoliamenthoyleuphroside* (**2**). $[\alpha]_D^{21}$ −17.5° (*c* 2.0; MeOH). EIMS, solid probe (source 140°), 30 eV, −1.4 kV, *m/z* (rel. int.): 310 (0.31), 296 (0.41), 284 (0.31), 268 (0.52), 256 (0.83), 244 (0.73), 213 (1.15), 196 (0.52), 195 (0.62), 185 (1.67), 178 (1.25), 166 (11.22), 160 (15.32), 121 (77.75).

Identification of known compounds. The known iridoids **3** and **4** were identified by comparison with authentic samples (IR, UV, MS, HPLC, TLC).

Methylation. The method of Hakamori [12] with NaH–DMSO–MeI was used with some modifications [13]. **1** (20 mg) gave 6 mg **6**.

5,8-*Dimethoxy*-8-*methyl*-3-*methylene*-4-*methoxymethinyl*-1-(2′,3′,4′,6′-*tetra*-O-*methyl*-β-D-*glucopyranosyloxy*)-1,3,4,4a,5,6,7,7a-*decahydro-cyclopenta*[*c*]*pyran* (**6**). GC/MS *m/z* (rel. int.): 488 (0.33), 269 (0.67), 253 (1.86), 249 (6.94), 237 (2.37), 221 (3.22), 218 (11.01), 189 (11.69), 187 (48.81), 174 (3.38), 167 (1.69), 155 (19.32), 145 (8.98), 127 (19.49), 115 (10.00), 111 (73.05), 101 (70.84), 89 (56.77), 75 (41.01).

Methanolysis. **1** or **2** (25 mg) was dissolved in 0.5 ml MeOH

and 1.0 ml 0.05 M NaOMe was added. The soln was stirred for 1 hr at room temp. (**2**), or at 60° (**1**). The reaction was stopped by the addition of 1.5 ml Amberlyst 15. After evaporation of solvent and addition of H_2O, **5** was extracted with Et_2O and identified by GC/MS. The aq. soln was lyophilized and then purified by HPLC. From **2**, 13 mg **1** was isolated and identified by ^1H NMR.

Foliamenthic acid methyl ester (**5**). GC/MS *m/z* (rel. int.): 198 (1.14), 180 (15.26), 165 (5.34), 148 (27.86), 133 (10.30), 121 (61.83), 105 (46.18), 93 (100.00), 91 (55.34), 59 (26.33).

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