Iridoids from *Avicennia marina*

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Three new iridoid glucosides, 10-*O*-[(*E*)-cinnamoyl]-geniposidic acid, 10-*O*-[(*E*)-*p*-coumaroyl]-geniposidic acid, 10-*O*-[(*E*)-caffeoyl]-geniposidic acid and the known iridoid glucoside, 2′-*O*-[(*E*)-cinnamoyl]-mussaenosidic acid have been isolated from *Avicennia marina*. The structures were determined primarily by NMR spectroscopy. The assignment of NMR signals was performed by means of $^1H-^1H$ COSY, HMQC and HMBC experiments.

Introduction

Iridoids are very common in the plant family of Verbenaceae. The genus *Avicennia* belongs to the family Verbenaceae and can be separated into eight species, three in the West Africa/America area and five in the Indo-Pacific/East Africa area (Bousquet-Melou and Fauvel, 1998). Recently, *Avicennia marina* was divided into three subspecies *A. marina* ssp. *australasica*, *A. marina* ssp. *eucalyptifolia* and *A. marina* ssp. *marina* (Everett, 1994). The bark, leaves and fruits of *A. marina* are used in the folk medicine for treatment of skin diseases (Fauvel *et al.*, 1993). Previously, flavonoids (Sharaf *et al.*, 2000), fatty acids, sterols and hydrocarbons (Wannigama *et al.*, 1981, König and Rimpler, 1985) have been isolated from *A. marina*. The iridoids geniposidic acid, 10-*O*-(5-phenyl-2,4-pentadienoyl)-geniposidic acid, mussaenosidic acid, 2′-*O*-[(*E*)-cinnamoyl]-mussaeno-

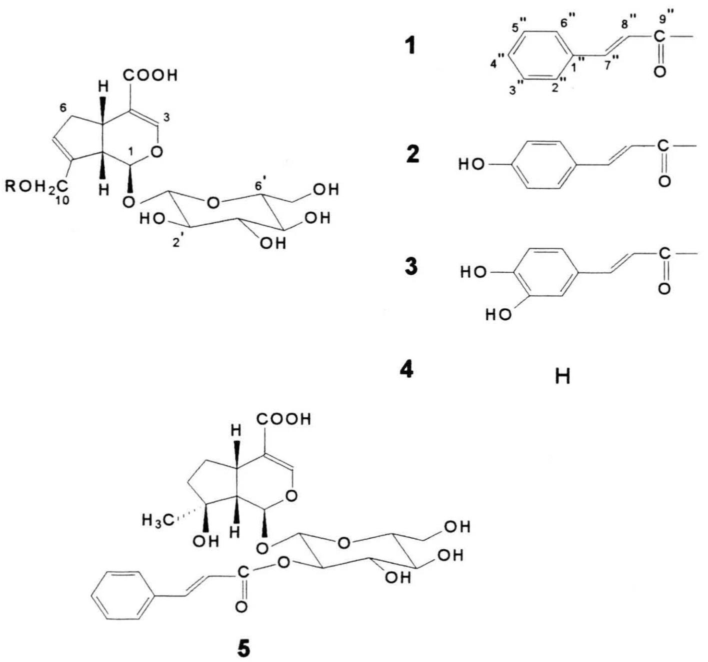

Fig. 1. Iridoids from *Avicennia marina*, 10-*O*-[(*E*)-cinnamoyl]-geniposidic acid (**1**), 10-*O*-[(*E*)-*p*-coumaroyl]-geniposidic acid (**2**), 10-*O*-[(*E*)-caffeoyl]-geniposidic acid (**3**), geniposidic acid (**4**), 2′-*O*-[(*E*)-cinnamoyl]-mussaenosidic acid (**5**).

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sidic acid and 7-*O*-(5-phenyl-2,4-pentadienoyl)-8-epiloganic acid have also been obtained from this plant (König and Rimpler, 1985). In this paper we describe the isolation of three new geniposidic acid esters **1–3** and the known 2′-*O*-[(*E*)-cinnamoyl]-mussaenosidic acid (**5**) from *A. marina* (Forsk.) Vierh.

Results and Discussion

The butanol extract of the whole plants of *A. marina* was obtained as described in the experimental section. The chromatographic separation of the butanol extract was achieved on silica gel columns. The elution was performed with $CHCl_3$, $CHCl_3$–MeOH, $CHCl_3$–MeOH–H_2O with increasing amounts of MeOH and H_2O. Further purification was carried out by MPLC (RP-18 material, silica gel), followed by Sephadex LH-20 column chromatography to give the pure iridoids.

The LSI mass spectra of **1–3** exhibited the $[M-1]^-$ ions at *m/z* 503 (**1**), 519 (**2**) and 535 (**3**) which together with ^1H and ^{13}C NMR data allowed us to propose the molecular formulas $C_{25}H_{28}O_{11}$ (**1**), $C_{25}H_{28}O_{12}$ (**2**) and $C_{25}H_{28}O_{13}$ (**3**). The fragment ions at *m/z* 341 (**1**), 357 (**2**) and 373 (**3**) show the loss of a hexose moiety $[M-1-162]^-$.

The ^1H and ^{13}C NMR spectra of **1–3** revealed the esterification of geniposidic acid (**4**) with (*E*)-cinnamic acid, (*E*)-*p*-coumaric acid and (*E*)-caffeic acid, respectively. The ^1H downfield shifts of the both geniposidic acid signals 2H-10 in **1** (Δδ +0.62, +0.68), **2** (Δδ +0.61, +0.68) and **3** (Δδ +0.60, +0.67) (Table 1) in comparison with **4** (δ 4.26, D_2O) (Cameron *et al.*, 1984) indicated the acylation in position 10 of **4**. This acylation of **1–3** caused also the ^{13}C downfield shifts of the C-10 signal in **1** (Δδ +2.8) and **2**, **3** (Δδ +2.6) and the upfield shifts of the C-8 signal in **1**, **2**, **3** (Δδ −4.6, −4.5, −4.4) compared with **4** (δ 61.13 (C-10), 144.22 (C-8), CD_3OD) (Chaudhuri *et al.*, 1980). The (*E*)-configuration of the 7″,8″- double bond in **1–3** was proved by the coupling constants J = 16.0 Hz (**1**) and J = 15.9 Hz (**2**, **3**). In case of a (*Z*)-configuration a coupling constant J = 12.8 Hz would be observed (Vesper and Seifert, 1994). The ^1H NMR spectra of **1–3** showed the characteristic aromatic proton signals in **1** of cinnamoyl moiety at δ 7.61 (H-2″, H-6″), 7.40 (H-3″, H-4″, H-5″), in **2** of *p*-coumaroyl moiety at δ 7.48, d, J = 8.5 Hz (H-2″, H-6″), 6.81, d, J = 8.5 Hz (H-3″, H-5″) and in **3** of caffeoyl moiety at δ 7.05, d, J = 1.5 Hz (H-2″), 6.78, d, J = 8.1 Hz (H-5″), 6.96, dd, J = 8.1 Hz, J = 1.5 Hz (H-6″).

Geniposidic acid (**4**) shows interesting biological activities. Intraperitoneal administration of **4** to mice led to a dose-dependently decrease of the growth of implanted tumor ascites cells (Hsu *et al.*,

Table 1. ^1H NMR spectral data of the iridoids **1–3** in CD_3OD.

C	1	2	3
1	5.19, d, J = 7.9 Hz	5.19, d, J = 7.8 Hz	5.19, d, J = 7.7 Hz
3	7.53 br.s	7.53 br.s	7.53 br.s
5	3.20 m	3.20 m	3.21 m
6	2.15/2.88	2.15/2.87	2.16/2.88
7	5.90	5.89	5.88
9	2.79 m	2.79 m	2.78 m
10	4.88/4.94, d, J = 14.2 Hz	4.87/4.94, d, J = 14.2 Hz	4.86/4.93, d, J = 14.2 Hz
1′	4.73, d, J = 7.8 Hz	4.74, d, J = 7.8 Hz	4.74, d, J = 7.7 Hz
2′	3.24	3.26	3.24
3′	3.39	3.38	3.39
4′	3.30	3.32	3.32
5′	3.30	3.31	3.30
6′	3.65/3.87	3.67/3.88	3.66/3.88
2″	7.61	7.48, d, J = 8.5 Hz	7.05, d, J = 1.5 Hz
3″	7.40	6.81, d, J = 8.5 Hz	
4″	7.40		
5″	7.40	6.81, d, J = 8.5 Hz	6.78, d, J = 8.1 Hz
6″	7.61	7.48, d, J = 8.5 Hz	6.96, dd, J = 8.1 Hz, J = 1.5 Hz
7″	7.72, d, J = 16.0 Hz	7.65, d, J = 15.9 Hz	7.58, d, J = 15.9 Hz
8″	6.57, d, J = 16.0 Hz	6.38, d, J = 15.9 Hz	6.30, d, J = 15.9 Hz

Table 2. ^{13}C NMR spectral data of the iridoids **1–3** and **5** in CD_3OD.

C	1	2	3	5
1	98.3	98.3	98.4	95.0
3	153.2	153.3	153.3	151.2
4	112.8	112.6	112.7	114.1
5	36.7	36.6	36.6	31.3
6	40.0	39.9	39.9	30.2
7	131.5	131.2	131.2	41.3
8	139.6	139.7	139.8	79.8
9	47.3	47.3	47.4	52.5
10	63.9	63.7	63.7	24.3
11	170.9	170.9	170.8	170.2
1′	100.5	100.5	100.5	97.7
2′	74.8	74.8	74.8	74.8
3′	77.9	77.9	77.9	75.9
4′	71.4	71.4	71.4	71.6
5′	78.4	78.3	78.4	78.5
6′	62.8	62.7	62.8	62.7
1″	135.7	127.1	127.8	135.8
2″	129.3	131.3	115.2	129.4
3″	130.0	116.8	147.2	129.9
4″	131.5	161.3	149.6	131.4
5″	130.0	116.8	116.5	129.9
6″	129.3	131.3	123.0	129.4
7″	146.5	146.8	146.8	146.5
8″	118.7	114.9	114.9	118.6
9″	168.4	169.1	169.1	167.5

1997). The collagen synthesis in false aged rats was stimulated by the administration of **4** (Li *et al.*, 1998). Geniposidic acid inhibited the elongation of coleoptiles of wheat embryos (Cameron *et al.*, 1984) and its methyl ester geniposide the growth of rice and lettuce seedlings (Komai *et al.*, 1990).

Experimental

General

Negative ion MS: MAT 8500 (Finnigan), matrix glycerol. NMR: 500.13 MHz (^1H) and 125.76 MHz (^{13}C), reverse probehead, δ in ppm, solvent CD_3OD, CD_3OD signals were used as int. standard (^1H: 3.30, ^{13}C: 49.0), temp. 290 K, HMQC: phase-sensitive using TPPI (Time Proportional Phase Increment), BIRD (Bilinear Rotation Decoupling) sequence, GARP decoupled, HMBC: using TPPI, delay to achieve long range couplings: 71 msec ($J_{C,H}$ = 14 Hz).

CC: silica gel (0.063–0.2 mm); TLC: silica gel (0.25 mm precoated plates 60 F254, Merck, the spots were sprayed with 10% H_2SO_4 in MeOH.

Isolation

A. marina was collected in 1999 near Hurghada Egypt and identified by Dr. M. Elgebaly from the National Research Centre (NRC) Cairo. A voucher specimen of the plant is deposited at the Herbarium of the NRC, Department of Chemotaxonomy. Dried powder of the whole plant of *A. marina* (3.0 kg) was exhaustively extracted with 80% MeOH (15 l) to give 70 g of crude material after evaporation of the solvent. The residue was successively partitioned between H_2O and petrol, H_2O and $CHCl_3$ and H_2O and *n*-BuOH. The butanolic fraction was evaporated under red. pressure at 45 °C to obtain a crude iridoid mixture (10 g). CC on silica gel eluting with $CHCl_3$–MeOH–H_2O with increasing amounts of MeOH and H_2O afforded two main fractions F_1 (300 mg) and F_2 (200 mg). F_1 was subjected to MPLC (Medium Pressure Liquid Chromatography) using RP-18 material and eluting with MeOH–H_2O 13:7 v/v followed by Sephadex LH-20 chromatography eluting with MeOH–H_2O 17:3 v/v to give **1** (20 mg) and **5** (45 mg). MPLC of F_2 on silica gel eluting with $CHCl_3$–MeOH–H_2O 9:3:0.5 v/v and CC on Sephadex LH-20 eluting with MeOH yielded **2** (12 mg) and **3** (15 mg).

Spectroscopic data

10-*O*-[(*E*)-cinnamoyl]-geniposidic acid (**1**): ($C_{25}H_{28}O_{11}$, *Mr* 504). LSI-MS negative ion mode *m/z* (rel. int.): 503 $[M-H]^-$ (55), 341 $[M-H-Glc]^-$ (3). ^1H NMR and ^{13}C NMR: Tables 1 and 2.

10-*O*-[(*E*)-*p*-coumaroyl]-geniposidic acid (**2**): ($C_{25}H_{28}O_{12}$, *Mr* 520). LSI-MS negative ion mode *m/z* (rel. int.): 519 $[M-H]^-$ (87), 357 $[M-H-Glc]^-$ (6). ^1H NMR and ^{13}C NMR: Tables 1 and 2.

10-*O*-[(*E*)-caffeoyl]-geniposidic acid (**3**): ($C_{25}H_{28}O_{13}$, *Mr* 536). LSI-MS negative ion mode *m/z* (rel. int.): 535 $[M-H]^-$ (**4**), 373 $[M-H-Glc]^-$ (**7**). ^1H NMR and ^{13}C NMR: Tables 1 and 2.

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