Phytochemistry, Vol. 24, No. 6, pp. 1245–1248, 1985.
Printed in Great Britain.

0031–9422/85 $3.00 + 0.00

IRIDOID GLUCOSIDES IN *AVICENNIA MARINA*

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(*Received* 16 *August* 1984)

Key Word Index—*Avicennia marina*; Verbenaceae; iridoids; geniposidic acid; mussaenosidic acid; 2′-cinnamoyl-mussaenosidic acid; 10-*O*-(5-phenyl-2,4-pentadienoyl)-geniposidic acid; 7-*O*-(5-phenyl-2,4-pentadienoyl)-8-epiloganic acid.

Abstract—2′-Cinnamoyl-mussaenoside, 10-*O*-(5-phenyl-2,4-pentadienoyl)-geniposide, 7-*O*-(5-phenyl-2,4-pentadienoyl)-8-epiloganin, and the known iridoids geniposide and mussaenoside have been isolated from a methylated extract of the leaves of *Avicennia marina*. Evidence is presented that the iridoids occur as the free acids in the plant. The taxonomic significance of these findings is discussed.

INTRODUCTION

The systematic position of the genus *Avicennia* is uncertain [1–4]. In the course of our chemotaxonomic investigations of the Verbenaceae, we therefore examined the occurrence of iridoid glycosides in *Avicennia marina* (Forsk.) vierh. as an additional character. The hydrophilic chemical constituents in *Avicennia* have received little attention. Thus, except for the flavonoid velutin [5], mainly lipophilic compounds, such as triterpenes, steroids, fatty acids and hydrocarbons have been reported [6–9].

RESULTS AND DISCUSSION

A preliminary screening by TLC revealed, that the crude extract of the leaves of *A. marina* contained several iridoid glycosides, the majority of which were iridoid acids. Of these we isolated free geniposidic acid (**1**) and 2′-cinnamoyl-mussaenosidic acid (**2**).

Because the separations proved to be difficult, the crude extract was methylated with diazomethane and the iridoids isolated as their methyl esters.

In order to prove that all the iridoids detectable by TLC occured as free acids, we compared the original and the methylated extracts by two-dimensional TLC. After methylation all iridoid spots were shifted to higher R_f-values in the first dimension (CH_2Cl_2–MeOH–H_2O). No methylated iridoids were detectable in the original extract. We therefore assumed that all the iridoids, of which larger amounts were isolated after methylation, were accumulated as free acids in the plant. Column chromatography on silica gel followed by XAD-7 resin and subsequent HPLC separations afforded five iridoid glycosides, two of which were identified as geniposide (**3**) [10] and mussaenoside (**4**) [11] by spectral analysis and by comparison with authentic samples. The other three compounds were new esters of mussaenoside, geniposide and 8-epiloganin respectively.

The methanolysis of **5** yielded cinnamic acid methylester and mussaenoside. The ^{1}H NMR spectrum of **5** indicated that the ester group was linked to the 2′-oxygen of the glucose moiety, since the signal due to H-2′ was shifted downfield by about 1.4 ppm as compared with non acylated iridoid glucosides [12]; the other glucose signals were almost unchanged.

The proposed structure was also in accordance with the data of the ^{13}C NMR spectrum (Table 1). All signals of the iridoid part of **5** were very similar to those of mussaenoside (**4**). The C-2′ signal at δ75.89 in the spectrum of **5** was deshielded by 1.33 ppm as compared with **4**, whilst the signals for the carbons in β-positions C-1′ and C-3′ were shifted upfield by 2.15 and 3.27 ppm. The linkage of the cinnamic acid therefore had to be at the 2′-oxygen. This structure was confirmed by MS data. In addition to all the fragments resulting from the aglycone part (m/z = 228, 211, 210, 139), we obtained a fragment at m/z 293 (**8**) arising from the glucose part esterified with cinnamic acid.

Compound **6** showed a UV absorption maximum at 308 nm (log ε = 4.47; MeOH), due to an aromatic system conjugated with a diene. Methanolysis of **6** yielded methyl-5-phenyl-2,4-pentadienoate and geniposide, which were identified by comparison with authentic samples. 5-Phenyl-2,4-pentadienoic acid rarely occurs as a natural product. It has been found as a triterpenoid ester in *Marsdenia pringlei* and termed juarezic acid [13].

The ^{1}H NMR data of **6** indicated, that the acyl group was linked to the 10-oxygen. The signals at δ5.23 and 4.64 (d, J = 15 Hz) due to the AB-system of the C-10 protons were shifted downfield by 1.07 and 0.48 ppm as compared with **3**. The structure was confirmed by MS, which showed an ion at m/z 365 (**9**), arising from the geniposide oxonium-ion esterified with 5-phenyl-2,4-pentadienoic acid.

Compound **7** after methanolysis also gave methyl-5-phenyl-2,4-pentadienoate. The iridoid moiety was proved to be 8-epiloganin. Thus its IR spectrum was identical with that of an authentic sample of 8-epiloganin, whereas it was clearly different from the spectrum of loganin. By comparing the ^{13}C NMR data of **7** with those of the model compounds **11** [14] and **12** [15], the best fit was seen with the 8-epiloganin derivative **12**, while differences

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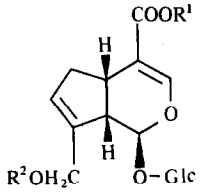
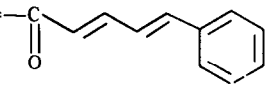
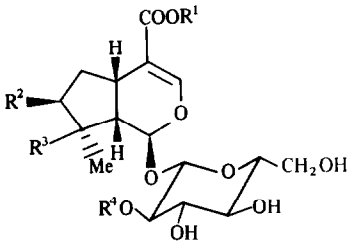


1 $R^1 = R^2 = H$

3 $R^1 = Me$, $R^2 = H$

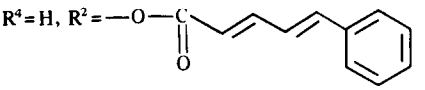
6 $R^1 = Me$, $R^2 =$ 



2 $R^1 = R^2 = H$, $R^3 = OH$, $R^4 = t$-cinnamoyl–

4 $R^1 = Me$, $R^2 = R^4 = H$, $R^3 = OH$

5 $R^1 = Me$, $R^2 = H$, $R^3 = OH$, $R^4 = t$-cinnamoyl–

7 $R^1 = Me$, $R^3 = R^4 = H$, $R^2 =$ 

of up to 4 ppm for the signals of C-7, C-8 and C-9 were observed for the corresponding loganin derivative **11**. The similarity of the chemical shift values for **7** and **12** also showed that the 5-phenyl-2,4-pentadienoyl moiety was attached to the 7-oxygen. The ^1H NMR chemical shifts and coupling constants were also in agreement with structure **7** assuming that the ^7V conformation of the cyclopentane ring with quasi axial orientation of the acyloxy group at C-7 was predominant.

The iridoids accumulated in *A. marina* leaves are similar to those found in other verbenaceous plants [16–18], although 5-phenyl-2,4-pentadienoic acid has not been detected in Lamiales so far. Our results, therefore, are in agreement with a fairly close relationship between the genus *Avicennia* and the Verbenaceae as suggested by Junell [1], Takhtajan [19] and Thorne [20]. They are neither compatible with the suggestion of Croizat, as cited by Moldenke [4], to regard *Avicennia* as having arisen from the Dipterocarpaceae or Ancistrocladaceae nor with the suggestions of van Tieghem [21] and Dahlgren [3] to place *Avicennia* near Santalales, or in Celastrales respectively. To our knowledge in none of these orders or families as defined by Dahlgren are iridoids accumulated. Interestingly, in Dahlgrens revised classification of the Angiosperms [22] *Avicennia* is not mentioned in the synopsis of the orders and families.

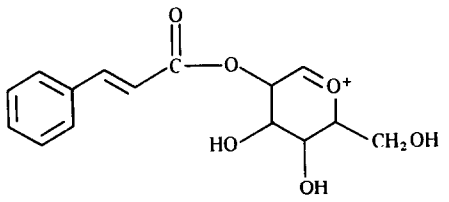
EXPERIMENTAL

Plant material. Air-dried leaves of *A. marina* were obtained from wild growing plants in Ceylon. A voucher specimen

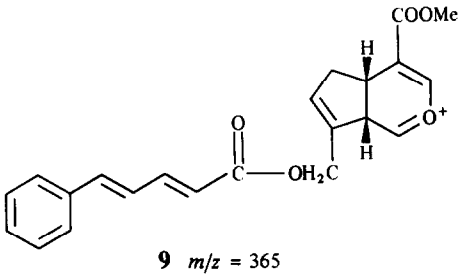
Table 1. ^{13}C NMR data (20.15 MHz) of compounds **4**, **5**, **7**, **11** and **12**

C	**4** (CD_3OD)	**5** (CD_3OD)	**7** ($CDCl_3$–CD_3OD)	**11** ($CDCl_3$)	**12** ($CDCl_3$)
1	95.41	94.8	95.18	91.0	100.7
3	152.01	151.2	151.11	150.2	151.3
4	113.43	114.18	112.70	112.6	111.6
5	32.02	31.03	30.51	30.6	31.3
6	30.74	30.09	37.96	39.3	38.3
7	40.77	41.66	80.96	76.9	81.0
8	80.49	79.54	42.03*	39.3	42.1
9	52.02	51.31	42.14*	45.2	42.1
10	24.65	24.22	13.73	12.6	14.0
11	169.36	168.67	167.80	167.0	170.9
1′	99.86	97.52	98.68		
2′	74.75	75.89	73.43		
3′	78.36	74.90	76.50		
4′	71.76	71.77	70.34		
5′	78.03	78.58	77.60		
6′	62.97	62.78	62.05		
–OMe	52.36	52.58	—	51.2	51.2
α		118.7	121.15		
β		146.30	145.20		
γ			126.22		
δ			141.02		
1″		135.86	136.15		
2″		130.04	128.94		
3″		129.44	127.36		
4″		131.54	129.25		
5″		129.44	127.36		
6″		130.04	128.94		

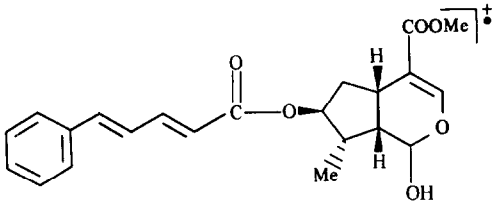
*Interchangeable.



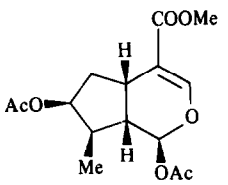
8 *m/z* = 293



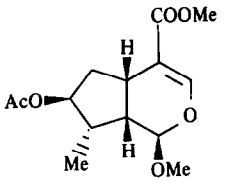
9 *m/z* = 365



10 *m/z* = 384



11



12

(005–001) is deposited in The Herbarium of the Institut für Pharmazeutische Biologie, Freiburg.

Analytical methods. CC: silica gel (Merck), CH_2Cl_2–MeOH–H_2O (90:10:1)/(70:30:3); Servachrom XAD 7 (Serva), MeOH (40–60%); Sephadex G15 (Pharmacia), H_2O; TLC: silica gel 60, CH_2Cl_2–MeOH–H_2O (70:30:3)/(80:20:2); 2D-TLC: silica gel 60, CH_2Cl_2–MeOH–H_2O (70:30:3), *n*-BuOH–MeOH–H_2O (4:1:5). Spray reagent for iridoids: Vanillin (3%) and H_2SO_4 (1%) in 100 ml EtOH followed by heating at 110° for 5–10 min. GLC/MS: 2′-Cinnamoyl-mussaenosidic acid (**2**) was trimethylsilylated with TMSI-S (Serva) for 2 hr at room temp. OV-101 (1.5%) column (1.2 m × 2 mm i.d.), temp. of injector 290°, column 270°, source 270°, He 30 ml/min; EIMS 70 eV. Methyl-5-phenyl-2,4-pentadienoate and methyl-*t*-cinnamate were analysed by a FFAP (10%) column (2 m × 2 mm i.d.), temp. of injector 230°, column 200°, 2°/min up to 240°, source 220°, He 30 ml/min; EIMS/70 eV; HPLC: μ-Bondapak-phenyl (9.5 mm × 250 mm), MeOH (45%); μ-Bondapak C_{18} (9.4 mm × 250 mm), MeOH (30–65%), MeOH–MeCN–H_2O (22.5:18.5:59); silica gel 60 (20 mm × 600 mm) CH_2Cl_2–MeOH–H_2O (85:15:1.5). The flow rate varied from 2.0 to 4.0 ml/min.

Isolation procedure. The plant material (960 g) was extracted by refluxing for 30 min with 96% EtOH and twice with 70% EtOH. The combined extracts were evaporated *in vacuo* and chromatographed on a Celite column. Elution with *n*-hexane–CH_2Cl_2 (1:1) afforded the lipophilic fractions, elution with CH_2Cl_2–MeOH (1:1) the hydrophilic fractions. The hydrophilic fractions were rechromatographed on XAD 7 (MeOH 10–50%) and then methylated with CH_2N_2. After further CC on silica gel and XAD 7 it was necessary to purify all compounds by HPLC.

Identification of known compounds. The known iridoids geniposide (60 mg), mussaenoside (110 mg) and 8-epiloganin (obtained by methanolysis of **7**) were identified by comparison with authentic samples (TLC, HPLC, MS, IR).

Methanolysis. To 5 mg substance dissolved in 0.1 ml MeOH 0.2 ml 0.05 M Na–methylate was added. The mixture was stirred for 1 hr at room temp. after which the reaction was stopped by the addition of 0.3 ml Amberlyst 15. After evaporation of the solvent and addition of H_2O the methyl-ester was extracted with Et_2O and identified by GC/MS. R_t values and mass spectra were identical with samples prepared by methylation of 5-phenyl-2,4-pentadienoic acid (Aldrich Comp.) and cinnamic acid (Roth). The iridoids were isolated from the aqueous phase, purified by HPLC and identified as described before.

2′-Cinnamoyl-mussaenosidic acid (**2**; 22 mg). UV $\lambda_{max}^{H_2O}$ nm (log ε): 282 (4.2); 223 (4.1); 218 (4.1); 204 (4.1); IR ν_{max}^{KBr} cm^{-1}: 2500–2800, 1700, 1680, 1630; MS of penta-(trimethylsilyl)-2′-cinnamoyl-mussaenosidic acid, *m/z* (rel. int.): 509 (2.8), 419 (0.68), 430 (0.05), 361 (0.22), 340 (0.27), 341 (0.35), 325 (0.22), 312 (0.35), 271 (1.59), 251 (5.12), 217 (4.56), 197 (1.9), 161 (7.88), 131 (100), 103 (17.97).

2′-Cinnamoyl-mussaenoside (**5**; 40 mg): UV λ_{max}^{MeOH} nm (log ε): 278 (4.0), 238 (3.6), 222 (4.0), 217 (4.1), 205 (3.9); IR ν_{max}^{KBr} cm^{-1}: 1710, 1639; EIMS (solid probe, 390°, source 230°), 70 eV, *m/z* (rel. int.): 358 (0.24), 293 (2.77), 275 (1.28), 228 (1.18), 211 (3.78), 210 (2.62), 131 (100), 103 (44.12); ^{1}H NMR (250.1 MHz). 1. C_5D_5N: δ7.91 (*d*, H-β), 6.71 (1H, *d*, $J_{\alpha/\beta}$ = 15 Hz, H-α), 6.3 (1H, *s*, H-3), 6.01 (1H, $J_{1/9}$ = 3 Hz, H-1), 5.76 (1H, *t*, $J_{1'/2'}$ = 8 Hz, H-2′), 5.52 (1H, *d*, H-1′), 4.56–4.00 (5H, *m*, sugar protons), 3.47 (1H, *ddd*, $J_{5/9}$ = 10 Hz, $J_{5/6b}$ = 4 Hz, $J_{5/6a}$ = 9 Hz, H-5), 3.24 (3H, *s*, –OMe), 2.84 (1H, *dd*, H-9), 2.4 (1H, *m*, H-6a), 1.9 (1H, *ddd*, $J_{7a/7b}$ = 12 Hz, $J_{7a/6b}$ = 8 Hz, $J_{7a/6a}$ = 7 Hz, H-7a), 1.8–1.5 (2H, *m*, H-6b, H-7b), 1.55 (3H, *s*, H-10); 2. $CDCl_3/D_2O$: δ7.45 (2H, *m*, H-6″, H-2″), 7.3 (*m*, H-3″, H-4″, H-5″).

10-O-(5-Phenyl-2,4-pentadienoyl)-geniposide (**6**; 30 mg). UV λ_{max}^{MeOH} nm (log ε): 308 (4.4), 232 (4.3), 219 (sh), 203 (4.2); IR ν_{max}^{KBr} cm^{-1}: 1705, 1622, 1440; EIMS (solid probe 380°, source 250°) 30 eV, *m/z* (rel. int.): 365 (0.22), 351 (0.11), 226 (3.96), 209 (23.1), 208 (21.23), 174 (29.92), 157 (41.69), 129 (100), 91 (25.7); ^{1}H NMR (250.1 MHz, $CDCl_3$): δ7.55–6.8 (*m*, H-β, H-3, aromatic protons, H-γ, H-δ), 6.00 (1H, *d*, $J_{\alpha/\beta}$ = 15 Hz, H-α); 5.73 (1H, *s* (*br*), H-7), 5.23 (1H, *d*, $J_{10a/10b}$ = 15 Hz, H-10a), 4.88 (1H, *d*, $J_{1/9}$ = 8 Hz, H-1), 4.71 (1H, *d*, $J_{1'/2'}$ = 7 Hz, H-1′), 4.64 (1H, *d*, H-10b), 3.66 (3H, *s*, –OMe), 3.3–3.9 (sugar protons), 3.22 (1H, *ddd*, H-5), 2.87 (1H, *dd*, $J_{6a/6b}$ = 17 Hz, $J_{5/6a}$ = 7 Hz, H-6a), 2.65 (1H, *t*, $J_{5/9}$ = 7 Hz, H-9), 2.05 (1H, *dd*, $J_{5/6b}$ = 7 Hz, H-6b).

7-O-(5-Phenyl-2,4-pentadienoyl)-8-epiloganin (**7**; 11 mg). UV λ_{max}^{MeOH} nm (log ε): 308 (4.3), 232 (4.2), 219 (sh), 205 (3.9); IR ν_{max}^{KBr} cm^{-1}: 1702, 1623, 1434; EIMS (solid probe 380°, source 270°), 30 eV, *m/z* (rel. int.): 384 (2.96), 367 (0.25), 353 (0.25), 228 (7.56), 211 (15.69), 210 (36.94), 157 (61.28), 129 (100); ^{1}H NMR (250.1 MHz, $CDCl_3/C_5D_5N$): δ7.3–6.9 (9H, *m*, H-3, H-β, aromatic protons, H-γ, H-δ), 5.99 (1H, *d*, $J_{\alpha/\beta}$ = 15 Hz, H-α), 5.4 (1H, *d*, $J_{1/9}$ = 4 Hz, H-1), 4.99 (1H, *ddd*, $J_{7\alpha/6\beta}$ = 6 Hz, $J_{7\alpha/8}$ = 4 Hz,

$J_{7\alpha/6\alpha}$ = 3 Hz, H-7α), 4.8 (1H, *d*, $J_{1'/2'}$ = 8 Hz, H-1'), 4.05 (1H, *dd*, $J_{6'a/6'b}$ = 12 Hz, $J_{6'a/5'}$ = 4 Hz, H-6'a), 3.95 (1H, *dd*, $J_{6'b/5'}$ = 5 Hz, H-6'b), 3.8 (3H, *s*, –OMe), 3.4–3.8 (4H, *m*, H-2', H-3', H-4', H-5'), 3.09 (1H, *ddd*, $J_{5/9}$ = 9 Hz, $J_{5/6\alpha}$ = 9 Hz, $J_{5/6\beta}$ = 7 Hz, H-5), 2.65 (1H, *ddd*, $J_{8/9}$ = 7 Hz, H-9), 2.4 (1H, *m*, H-8), 2.3 (1H, *ddd*, $J_{6\alpha/6\beta}$ = 15 Hz, $J_{6\alpha/7\alpha}$ = 3 Hz, H-6α), 1.96 (1H, *ddd*, $J_{6\beta/7\alpha}$ = 6 Hz, H-6β), 1.05 (3H, *d*, $J_{8/10}$ = 7 Hz, H-10).

Acknowledgements—We wish to thank Dr. A. H. M. Jayasuriya, Peradeniya, for collecting and identifying the plant material, Dr. D. Hunkler, Institut für Organische Chemie und Biochemie, and Mr. Brecht, Institut für Pharmazeutische Chemie, for the NMR-spectra and Mrs. M. Weber for the GC/MS analysis. We are grateful to Professor O. Sticher, ETH Zürich (mussaenoside), Professor H. Inouye, Kyoto University (geniposide) and Professor A. Bianco (loganin, 8-epiloganin) for providing us with authentic samples.

REFERENCES

1. Junell, S. (1934) *Symb. Bot. Upsal.* **4**, 1.
2. Melchior, H. (ed.) (1964) *Syllabus der Pflanzenfamilien.* Berlin.
3. Dahlgren, R. (1975) *Bot. Notiser* **128**, 119.
4. Moldenke, H. N. (1960) *Phytologia* **7**, 123, 179, 259.
5. Majumdar, S. G., Ghosh, P. and Thakur, S. (1981) *Indian J. Chem.* **20B**, 632.
6. Majumdar, S. G. and Patra, G. (1979) *J. Indian Chem. Soc.* **56**, 111.
7. Subramanian, S. S. and Vedantham, T. N. C. (1974) *Indian J. Pharm.* **36**, 105.
8. Wannigama, G. P., Volkman, J. K., Gillan, F. T., Nichols, P. D. and Johns, R. B. (1981) *Phytochemistry* **20**, 659.
9. Hogg, R. W. and Gillan, F. (1984) *Phytochemistry* **23**, 93.
10. Inouye, H. and Saito, S. (1969) *Tetrahedron Letters* 2347.
11. Takeda, Y., Nishimura, H. and Inouye, H. (1977) *Phytochemistry* **16**, 1401.
12. Tietze, L. F., Niemeyer, U., Marx, P. and Glüsenkamp, K. H., (1980) *Tetrahedron* **36**, 1231.
13. Dominguez, X. A., Marroquin, J., Olguin, L. M., Morales, F. and Valdez, V. (1974) *Phytochemistry* **13**, 2617.
14. Jensen, S. R., Lyse-Petersen, S. E. and Nielsen, B. J. (1979) *Phytochemistry* **18**, 273.
15. Bianco, A. and Passacantilli, P. (1981) *Phytochemistry* **20**, 1873.
16. Sehgal, C. K., Taneja, S. C., Dhar, K. L. and Atal, C. K. (1982) *Phytochemistry* **21**, 363.
17. Sehgal, C. K., Taneja, S. C., Dhar, K. L. and Atal, C. K. (1983) *Phytochemistry* **22**, 1036.
18. Dutta, K. P., Chowdhury, S. U., Chakravarty, K. A., Achari, B. and Pakrashi, S. C. (1983) *Tetrahedron* **39**, 3067.
19. Takhtajan, A. (1973) *Evolution und Ausbreitung der Blütenpflanzen.* Stuttgart.
20. Thorne, R. F. (1983) *Nord. J. Bot.* **3**, 85.
21. Van Tieghem, M. P. (1898) *J. Bot.* **12**, 345.
22. Dahlgren, R., Jensen, S. R. and Nielsen, B. J. (1981) in *Phytochemistry and Angiosperm Phylogeny* (Young, D. A. and Siegler, S., eds.), p. 149. Praeger, New York.