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IRIDOID GLUCOSIDES IN *AVICENNIA OFFICINALIS*

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Key Word Index—*Avicennia officinalis;* Verbenaceae; iridoids; avicennioside; 7-cinnamoyl-8-epiloganic acid; geniposidic acid; 2'-cinnamoyl-mussaenosidic acid.

Abstract—The new iridoids avicennioside and 7-cinnamoyl-8-epiloganic acid, as well as the known compounds geniposidic acid and 2'-cinnamoyl-mussaenosidic acid, have been isolated from the leaves of *Avicennia officinalis*. In the plant the acids are accumulated as salts. The taxonomic significance of these findings is discussed.

INTRODUCTION

The genus *Avicennia* has been classified differently by several authors. There are different opinions concerning (i) the systematic rank of *Avicennia* as a monotypic family Avicenniaceae [1–6], as a subfamily Avicennioideae [7, 8], or as a tribe Avicenniae [9, 10]; (ii) the phylogenetic relationship of *Avicennia* to the Verbenaceae [11, 12], Santalales [13], Celastrales [14], Dipterocarpaceae and Ancistrocladaceae [1]; and (iii) the subdivision of the genus into sections and the definition of the species. Schauer [9] and Moldenke [1] divide the genus *Avicennia* into two sections. Moldenke [1], who recognizes 11 different *Avicennia* species, includes *A. marina* in the section *Upata* and *A. officinalis* in the section *Donatia*. Other authors such as Lam [15] and Schimper [16], however, recognize only three *Avicennia* species and unite *A. marina* with *A. officinalis*. Since the distribution of iridoids can be a useful character at the generic level, we examined the occurrence of iridoids in some *Avicennia* species. Previously we described the iridoids isolated from *A. marina* (Forsk.) Vierh. [17]. We have examined now a second species, *A. officinalis* L.

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RESULTS

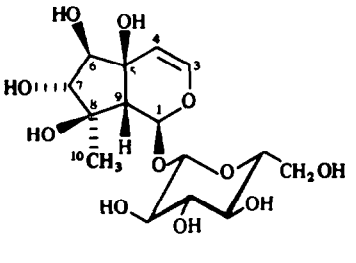
Hydrophobic adsorption chromatography of an alcoholic extract of the leaves from *A. officinalis* followed by LPLC and HPLC separations on RP material afforded four iridoid glycosides.

Compound **1** is a C_9-iridoid glucoside: UV (203 nm, $\log \varepsilon = 3.8$), IR (1650 cm^{-1}) and ^1H NMR data for H-3 (δ6.32, *d*) and H-4 (5.11, *dd*, $J_{3,4} = 6.5$ Hz, $J_{4,9} = 1.5$ Hz) indicated the presence of a 4-unsubstituted enol ether system of iridoids. The ^1H NMR spectrum also showed typical signals for one β-glucopyranosyl moiety. The assignments of the sugar protons were confirmed by a two-dimensional (2D) NMR spectrum (COSY 45). The aglycone part of the one-dimensional (1D) spectrum showed an AB system for two protons adjacent to hydroxyl groups, which could be assigned to H-6 and H-7 by the COSY 45 spectrum and NOE difference spectra (see Table 1). The 2D NMR showed small ($J < 1$ Hz) but significant *W*-type long-range couplings between H_3-10 and H-7 as well as H-9, which were not resolved in the 1D spectra. These couplings allowed the assignment of the signal at δ4.03 to H-7 and they indicated a *trans*-relationship between Me-10 and H-7 as well as H-9. The signal at 3.63 showed a positive NOE to H-4 and could therefore be assigned to H-6. The coupling constant $J_{6,7} = 10$ Hz indicated a *trans*-diaxial arrangement of H-6 and H-7, which was confirmed by the lack of any NOE

Table 1. Significant ^1H NMR spectral data (250.1 MHz/TMS) and NOEs of compounds **1**, **1a** and **1b**

	1*			**1a***			**1b†**		
		Irrad.	NOE‡		Irrad.	NOE‡		Irrad.	NOE‡
H-1	5.64	+	H-9, H-10, H-1'	5.47	–		5.81	+	H-9
H-3	6.32	+	H-4, H-10	6.35	–		6.32	–	
H-4	5.11	+	H-3, H-6	5.12	+	H-3, H-6	5.04	+	H-3, H-6
H-6	3.63	+	H-4, H-10	4.16	+	H-4, H-10	4.17	+	H-4
H-7	4.03	+	H-9	4.08	+	H-9	4.90	–	
H-9	2.41	+	H-7, H-1	2.47	+	H-7, H-1	3.26	+	H-1, H-7
H-10	1.15	+	H-1, H-3, H-6	1.10	+	H-1, H-3, H-6	—	–	
H-1'	4.64	–		4.68	–		4.84	–	

*In $CD_3CN + 5\%$ D_2O.
†In $CDCl_3$.
‡Positive NOE on the H-atoms listed.



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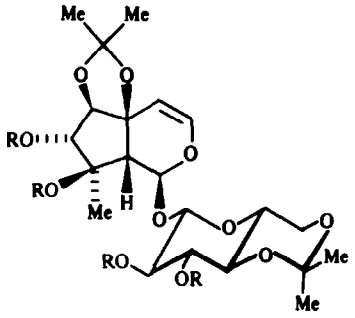
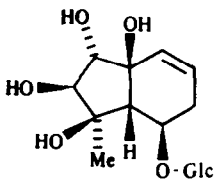


1a R = H
1b R = Ac



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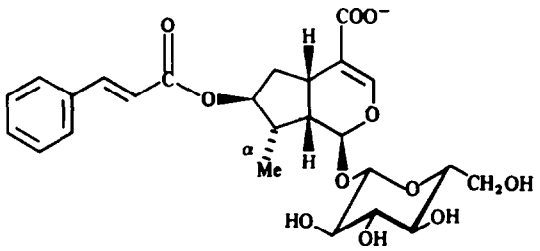

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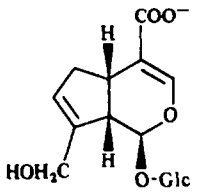

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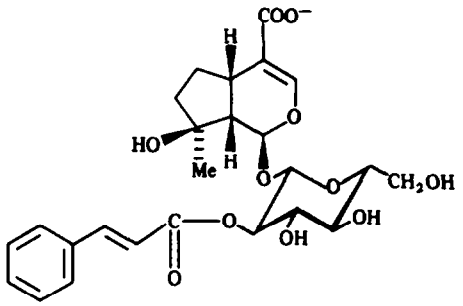

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between them. These arguments together with the observations that there were no signals for H-5 and H-8 and that the ^{13}C NMR shifts for C-8 and C-9 (Table 2) were very similar to other 8β-hydroxy-8α-methyl-iridoids [18–20] led to structure **1** for this compound.

Structure **1** has already been claimed for 7α-hydroxyharpagide (**2**), a compound prepared semisynthetically from anthirrhinoside [21, 22]. However, the published ^{13}C NMR data of that compound [19], especially the chemical shifts for C-6 and C-7, differed markedly from the data of our compound, which are substantiated by APT and selective decoupling experiments. Since we were unable to obtain an authentic sample of compound **2**, we looked for further evidence to prove structure **1** and to exclude structure **3** for our compound. The ^1H NMR spectrum of the diacetonide **1a** showed a significant downfield shift of 0.53 ppm for H-6 whereas H-7 remained unchanged. Acetylation of **1a** gave a tetraacetate (**1b**). In the ^1H NMR spectrum of this compound, H-1, H-9, and H-7 were shifted downfield by 0.33, 0.79 and 0.81 ppm, respectively, whereas the chemical shifts of H-4 and H-6 remained unchanged. These results are compatible only with one isopropylidene group being linked to O-5 and O-6 and, therefore, with a *cis*-relationship of OH-5 and OH-6 in **1**. Moreover, the acetylation shifts for H-1 and H-9 corresponded well with the shifts observed for other 8β-hydroxy-8α-methyliridoids [23], again confirming the configuration of **1** at C-8.

NOE difference spectra of **1**, **1a** and **1b** (Table 1) showed positive NOEs between H-6 and H-4, H-6 and H_3-10, H-7 and H-9, thus proving that H-6 and Me-10 are on the concave α-side and H-7 is on the convex β-side of the iridoid moiety. Thus we have no doubt that **1** is the correct structure of our compound, for which we suggest the name avicennioside in order to avoid confusion with compound **2**. Although the base-catalysed ring opening of 7,8-epoxyiridoid glucosides has been studied extensively [22], and although a wide range of polyhydroxylated iridoid glucosides with structures similar to **1** are known, it seems to be difficult to determine the configuration of **2**

Table 2. ^{13}C NMR spectral data of compounds **1**, **2**, **4** and 8-epiloganin

Carbon No.	1* (D_2O)	2† [21] (D_2O)	4‡ (DMSO-d_6)	8-Epiloganin [24]* (D_2O)
1	93.12	93.18	93.31	96.5
3	141.31	141.13	(—)§	152.2
4	110.20	110.65	(—)	114.0
5	66.63	65.34	31.32	29.4
6	81.33	83.81	38.02	39.6
7	72.92	80.09	81.14	79.0
8	75.98	76.08	41.28‖	43.5
9	58.44	57.53	40.86‖	41.8
10	19.26	17.86	13.81	14.0
11	—	—	(—)	170.7
1′	99.96	99.91	98.09	99.1
2′	74.55	74.39	73.22	73.5
3′	77.42	77.45	76.78	76.6
4′	71.75	71.78	70.19	70.5
5′	78.25	78.23	77.15	77.1
6′	62.80	62.80	61.29	61.6

trans-Cinnamoyl moiety of **2**: 118.35 (C-α), 128.23 (C-3″/C-5″), 128.79 (C-2″/C-6″), 130.29 (C-4″), 133.90 (C-1″), 144.20 (C-β), 165.94 (C=O).

*Capillary d_4-TSPNa/D_2O as external standard.

†Adjusted to $\delta_{C\text{-}6'} = 62.80$ ppm.

‡TMS as internal standard.

§(—), not observed.

‖Interchangeable.

with certainty by prediction of the reaction mechanism of the epoxide opening and the comparison of ^{13}C NMR data only.

The IR spectrum of compound **4** showed typical absorptions of the enol ether system of iridoids at 1635 cm^{-1}, an ester function at 1700 cm^{-1} and a carboxylate group at 1540 and 1400 cm^{-1}. Analysis by atomic absorption spectroscopy indicated that the counterion of the carboxylate was 70% sodium. Since we could not detect significant amounts of other metal ions, we suppose that the compound contained about 30% of the free acid. In the ^{1}H NMR spectrum of **4**, the signals for H-7 and H-1′ were covered by the HDO signal of the solvent, which could be suppressed by an inversion recovery technique with optimized recovery delay. With this method we obtained a completely resolved spectrum of **4**, with only H-3 being of reduced signal intensity, typical for protons with no potent neighbours for dipolar relaxation. Especially in DMSO-d_6 the width at half height of the H-3 signal was markedly broadened due to the slow exchange process carboxylate→carbonic acid. For the same reason, we assume that the signals for C-3, C-4 and C-11 were not observable in the ^{13}C NMR spectrum of **4**.

The ^{1}H NMR and ^{13}C NMR spectra (Table 2) were similar to those of 7-*O*-(5-phenyl-2,4-pentadienoyl)-8-epiloganin [17], but showed typical signals of a *trans*-cinnamoyl moiety. Compared with 8-epiloganin [24], the signal for H-7 was shifted downfield by *ca* 1 ppm in the ^{1}H NMR spectrum of **4**. The ^{13}C NMR signal for C-7 was shifted downfield by 2.14 ppm whereas the signals for C-6 and C-8 were shifted upfield by 1.58 and 2.22 ppm, confirming that the ester group is linked to C-7. Methylation of the acid form of **4** with diazomethane followed by methanolysis yielded 8-epiloganin. After permethylation of **4** with methyl iodide in dimethyl sulphoxide we obtained penta-*O*-methyl-8-epiloganin, which was identified by GC/MS [25, 26]. Compound **4** is therefore 7-*O*-*trans*-cinnamoyl-8-epiloganin.

The mass spectra of the TMSi derivatives of compounds **5** and **6** were identical with those of geniposidic acid and 2′-cinnamoyl-mussaenosidic acid, which we have previously isolated from *A. officinalis* [17]. The IR spectra, however, showed the typical absorption for carboxylate groups at 1540 and 1400 cm^{-1}. The main counterion, as determined by atomic absorption spectroscopy, was sodium. Geniposidic acid-Na (**5**) was identified by spectral analysis. The data were identical with those of the authentic sample [27]. The ^{1}H NMR spectrum of **5** also showed only a weak signal for H-3. As with **4**, this is probably due to the lack of nearby protons for dipolar relaxation; by applying large pulse angles (e.g. 90°) the signal intensity of H-3 can be significantly reduced. 2′-Cinnamoyl-mussaenosidic acid-Na (**6**) was methylated with diazomethane. The reaction product proved to be identical with 2′-cinnamoyl-mussaenoside.

DISCUSSION

Avicennia officinalis and *A. marina* accumulate iridoid glucosides of the geniposide and lamiide types. In addition, *A. officinalis* contains avicennioside, an iridoid of the harpagide type. All these iridoids, which are characterized by an α-orientated methyl group at C-8 or a 7,8-double bond and a hydroxymethyl group at C-8, are probably derived from the same biogenetic precursors. They are formed via 8-epideoxyloganic acid [28–30], whereas iridoids with a β-orientated methyl group at C-8 are biosynthesized via deoxyloganic acid [31–33]. Iridoids of the lamiide and harpagide types are known from the subfamilies Viticoideae [34–36] and Verbenoideae

[37, 38] of the Verbenaceae and from other families of the Lamiiflorae [39]. Geniposidic acid has been isolated from *Aloysia triphylla* (Verbenaceae) [27] and from some species of the Rubiaceae (Gentianiflorae) [40–42]. Thus the iridoid types in *A. marina* and *A. officinalis* show no closer relationship to any of the subfamilies of the Verbenaceae than to other families of the Lamiiflorae. Morphologically, *Avicennia* differs from the typical verbenaceous plants in its incomplete four-celled ovary with a free central placenta, the orthotropous ovules, fruits with endosperm, a viviparous embryo, wood anatomy and pollen morphology [5, 6, 8, 43–45]. Our results combined with these morphological characters are more consistent with a relatively independent position of *Avicennia* amongst the Lamiales as a family or a subfamily [1, 11, 12] than with other opinions.

The iridoids of *A. marina* and *A. officinalis* are similar but they seem to be well distinguished. In *A. marina* only iridoid acids have been found, some of which are esterified with cinnamic or 5-phenyl-2,4-pentadienoic acid. From *A. officinalis* we exclusively obtained the salts of iridoid acids and their cinnamic acid esters. The C_9-iridoid avicennioside has been found only in *A. officinalis*. These results favour the recognition of two different species, as suggested by Moldenke [1].

EXPERIMENTAL

Plant material. Air-dried leaves of *A. officinalis* were obtained from wild plants growing in Sri Lanka. A voucher specimen (005-002) has been deposited at the herbarium of the Institut für Pharmazeutische Biologie, Freiburg.

Analytical methods. CC: Amberlite XAD 7 and XAD 2 (Serva), H_2O–MeOH (7:3)/(1:9); LPLC: LiChroprep-RP 8, size C (Merck), MeOH (10–60%), flow rate 120 ml/hr; HPLC: μ-Bondapak C_{18}, 9.4 mm × 250 mm, MeOH (5–45%), flow rate 3 ml/min; TLC: silica gel 60, CH_2Cl_2–MeOH–H_2O (70:30:3–90:10:1). Spray reagent for iridoids: vanillin (3%) and H_2SO_4 (1%) in 100 ml EtOH followed by heating at 110° for 5–10 min. GC/MS: geniposidic acid-Na and 2′-cinnamoyl-mussaenosidic acid-Na were 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 column 250°/260°, He 40 ml/min, EIMS 30 eV; hepta-*O*-methyl-8-epiloganin: OV-17 (3%) column, 1.2 m × 2 mm i.d., temp. of column 250°, He 38 ml/min, EIMS 30 eV.

Isolation procedure. The plant material (1 kg) was extracted by refluxing for 30 min once 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 a lipophilic fraction, which was discarded. Subsequent elution with CH_2Cl_2–MeOH (1:1) yielded a hydrophilic fraction, which was separated by CC on XAD-7 into three iridoid-containing fractions. The first fraction was rechromatographed on silica gel with CH_2Cl_2–MeOH–H_2O (70:30:3). Purification of the separated iridoids by LPLC and HPLC yielded 100 mg avicennioside and 70 mg geniposidic acid-Na. Rechromatography of the other two fractions on XAD-2 followed by LPLC and HPLC yielded 50 mg 2′-cinnamoyl-mussaenosidic acid-Na and 100 mg 7-cinnamoyl-8-epiloganic acid-Na.

Avicennioside (**1**). ^{1}H NMR (250.1 MHz, CD_3CN/D_2O): δ1.15 (3H, *s*, H-10), 2.41 (1H, *br s*, H-9), 3.24 (*dd*, $J_{2'/3'}$ = 9, $J_{1'/2'}$ = 8 Hz, H-2′), 3.22 (*dd*, $J_{4'/5'}$ = 9, $J_{3'/4'}$ = 8 Hz, H-4′) 3.39 (*ddd*, $J_{5'/6'a}$ = 2, $J_{5'/6'b}$ = 5.5 Hz, H-5′), 3.42 (*dd*, H-3′), 3.63 (1H, *d*, $J_{6/7}$ = 10 Hz, H-6), 3.65 (1H, *dd*, $J_{6'a/6'b}$ = 13 Hz, H-6′b), 3.9 (1H, *dd*, H-6′a), 4.03 (1H, *d*, H-7), 4.64 (1H, *d*, H-1′), 5.11 (1H, *dd*, $J_{3/4}$ = 6.5, $J_{4/9}$ = 1.5 Hz, H-4), 5.64 (1H, *br s*, H-1), 6.32 (1H, *d*, H-3).

5,6-4′,6′-Bis-O-isopropylidene-avicenmioside (**1a**). Compound **1** (36 mg) was treated with Me_2CO and $H_3[P(Mo_3O_{10})_4]$ as described in ref. [46]. RP-HPLC of the residue with 50% MeOH yielded 4 mg **1a**. ^{1}H NMR (250.1 MHz, CD_3CN/D_2O): δ6.35 (1H, *d*, $J_{3,4}$ = 6.8 Hz, H-3), 5.47 (1H, *s*, H-1), 5.12 (1H, *dd*, $J_{4/9}$ = 1.5 Hz, H-4), 4.68 (1H, *d*, $J_{1'/2'}$ = 8 Hz, H-1′), 4.16 (1H, *d*, $J_{6/7}$ = 7.5 Hz, H-6), 4.08 (1H, *d*, H-7), 3.85 (*dd*, $J_{6'ax/6'eq}$ = 10.5, $J_{5'/6'eq}$ = 5.5 Hz, H-6′eq), 3.73 (*t*, $J_{5'/6'ax}$ = 10.5 Hz, H-6′ax), 3.55 (*t*, $J_{3'/4'}$ = 9.3, $J_{4'/5'}$ = 9.3 Hz, H-4′), 3.43 (*t*, $J_{2'/3'}$ = 9.3 Hz, H-3′), 3.27 (*ddd*, H-5′), 3.20 (*dd*, H-2′), 2.47 (1H, *br s*, H-9), 1.43, 1.41, 1.33, 1.30 (12H, 4*s*, isopropyl), 1.1 (3H, *s*, H-10).

Tetraacetate of **1a** (**1b**). Compound **1a** (4 mg) treated with Ac_2O (0.8 ml) in dry pyridine (0.8 ml) for 48 hr at room temp. and worked up as usual yielded 0.4 mg crude **1b**. ^{1}H NMR (250.1 MHz, D_2O): δ7.71 (1H, *d*, $J_{\alpha/\beta}$ = 16 Hz, H-β), 7.58 (2H, *d*, $J_{4/9}$ < 1.4 Hz, H-1), 5.13 (*dd*, $J_{2'/3'}$ = $J_{3'/4'}$ = 9 Hz, H-3′), 5.04 (*dd*, H-4), 4.96 (*dd*, $J_{1'/2'}$ = 7.8 Hz, H-2′), 4.89 (*d*, $J_{6/7}$ = 6.3 Hz, H-7), 4.84 (*d*, H-1′), 4.17 (*d*, H-6), 4.00 (*dd*, $J_{5'/6'eq}$ = 5.5, $J_{6'ax/6'eq}$ = 10.5 Hz, H-6′eq), 3.81 (*t*, $J_{5'/6'ax}$ = 10.5 Hz, H-6′ax), 3.75 (*t*, $J_{4'/5'}$ = 9 Hz, H-4′), 3.38 (*ddd*, H-5′), 3.26 (*br s*, H-9).

7-Cinnamoyl-8-epiloganic acid-Na (**4**). $[\alpha]_D^{25}$ −71.93° (H_2O; *c* 0.75); UV λ_{max}^{MeOH} (log ε) nm: 278 (4.03), 236 (sh), 222 (3.93), 217 (3.98), 205 (3.91). IR ν^{KBr} cm^{-1}: 1700, 1635, 1535, 1400. ^{1}H NMR (250.1 MHz, D_2O): δ7.71 (1H, *d*, $J_{\alpha/\beta}$ = 16 Hz, H-β), 7.58 (2H, *m*, H-2″, H-6″), 7.43 (3H, *m*, H-3″, H-4″, H-5″), 7.02 (1H, *d*, $J_{3/5}$ < 1.5 Hz, H-3), 6.54 (1H, *d*, H-α), 5.52 (1H, *d*, $J_{1/9}$ = 2.3 Hz, H-1), 4.91 (1H, *m*, H-7), 4.72 (*d*, $J_{1'/2'}$ = 8 Hz, H-1′), 3.90 (1H, *dd*, $J_{6'a/6'b}$ = 13, $J_{6'b/5'}$ = 1.5 Hz, H-6′b), 3.69 (1H, *dd*, $J_{6'a/5'}$ = 5.3 Hz, H-6′a), 3.46 (*t*, $J_{3'/4'}$ = $J_{2'/3'}$ = 9 Hz, H-3′), 3.44 (*ddd*, $J_{4'/5'}$ = 9 Hz, H-5′), 3.36 (*t*, H-4′), 3.25 (*dd*, H-2′), 3.08 (1H, *dddd*, $J_{5/9}$ = $J_{5/6\alpha}$ = 9, $J_{5/6\beta}$ = 5.3 Hz, H-5), 2.76 (1H, *ddd*, $J_{8/9}$ = 9 Hz, H-9), 2.47 (1H, *m*, $J_{7/8}$ = 3.8 Hz, H-8), 2.24 (1H, *ddd*, $J_{6\alpha/6\beta}$ = 15, $J_{6\alpha/7}$ < 3 Hz, H-6α), 1.93 (1H, *ddd*, $J_{6\beta/7}$ = 5 Hz, H-6β), 1.00 (3H, *d*, $J_{8/10}$ = 7.5 Hz, H-10).

7-Cinnamoyl-8-epiloganin (**4a**). Obtained from **4** by methylation with CH_2N_2. EIMS (solid probe, source 270°) 30 eV, *m/z* (rel. int.): 91 (100), 103 (73.76), 127 (24.62), 131 (52.42), 139 (11.2), 145 (38.05), 149 (19.11), 150 (17.57), 178 (10.42), 179 (16.78), 182 (13.85), 193 (10.05), 210 (11.89), 211 (4.11), 228 (3.54), 341 (0.31), 358 (0.14).

Penta-O-methyl-8-epiloganin (**4b**). Obtained from **4** by methylation with MeI in DMSO, as described by Hakomori [25] and Franke [26]. GC/MS *m/z* (rel. int.): 458 (0.11), 429 (0.11), 428 (0.07), 396 (0.07), 382 (0.07), 351 (0.29), 313 (0.11), 285 (0.59), 281 (0.65), 256 (0.44), 240 (0.40), 225 (2.54), 224 (1.84), 219 (5.16), 193 (11.29), 187 (99.99), 161 (2.14), 155 (19.11), 145 (6.78), 139 (2.17), 133 (1.77), 127 (13.17), 115 (6.42), 111 (71.43), 101 (43.21).

Atomic absorption spectroscopy. The salts of geniposidic acid (**5**) and 2′-cinnamoyl-mussaenosidic acid (**6**) were measured by AAS. They contained 3.6% and 2.43% Na, corresponding to 70 and 57%, respectively, of the theoretical value.

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