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IRIDOID GLUCOSIDES IN *PLANTAGO ALPINA* AND *P. ALTISSIMA*

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Key Word Index—*Plantago alpina*; *P. altissima*; Plantaginaceae; iridoid glucosides; 10-*O*-acetylgeniposidic acid; alpinoside; desacetylhookerioside.

Abstract—Two species of *Plantago*, namely *P. Alpina* and *P. altissima* were investigated. From the former, nine iridoid glucosides and verbascoside were isolated. Together with the known iridoids gardoside, geniposidic acid, 8-epi-loganic acid, mussaenosidic acid, aucubin, monomelittoside and melittoside, two new glucosides were found: 10-*O*-acetylgeniposidic acid and alpinoside, another compound with a 10-*O*-acetyl group. From *P. altissima* verbascoside and isoverbascoside were isolated together with the known iridoids gardoside, 8-epi-loganic acid, catalpol, aucubin, and hookerioside as well as the new compound desacetylhookerioside.

INTRODUCTION

Plantago alpina and *P. altissima* have previously both been investigated for iridoids and the compounds aucubin (**5**) and melittoside (**6**) were found in the former [1], while deacetyl-asperuloside, aucubin, catalpol (**14**) and globularin were reported from the latter [1, 2]. The caffeic acid ester verbascoside (**10**) has also been reported from *P. altissima* [3]. However, as part of a continuing taxonomic study of the genus *Plantago* we have reinvestigated these two species in detail.

RESULTS AND DISCUSSION

The crude aqueous extract of *P. alpina* was separated by reverse-phase chromatography. Seven known and two new iridoid glucosides were found. The known compounds were gardoside (**1**), geniposidic acid (**2**), 8-epiloganic acid (**3**), mussaenosidic acid (**4**), aucubin (**5**), melittoside (**6**) and monomelittoside (**7**), identified by comparison of NMR spectra with known samples. The new iridoids were compounds **8** and **9**. In addition, verbascoside (**10**) and isoverbascoside (**11**) were isolated.

The ^{1}H NMR spectrum of compound **8** showed a singlet at δ 7.12 suggesting H-3 in an iridoid glucoside and proved that H-4 was absent while C-11 was present as an acid or ester functionality. A characteristic broad signal at δ 5.89 also indicated a double bond in the five-membered ring. Finally, a three proton singlet at δ 2.05 suggested the presence of an acetyl moiety. These facts suggested a compound derived from geniposide (**12**). A comparison with the ^{1}H NMR spectrum of geniposidic acid (**2**) showed great similarity, except that the signals from the 10-CH_2 group were shifted downfield to δ 4.7 in compound **8** (partly covered by the HDO-peak) as compared with δ 4.2 in compound **2**. Taken together with the presence of the acetyl group, this suggested that C-10 was acetylated and that compound **8** was 10-*O*-acetylgeniposidic acid. The FAB-mass spectrum of compound **8** gave a M_r of 416 which is expected for a compound with the suggested structure and composition $C_{18}H_{24}O_{11}$. However, the ^{13}C NMR spectrum of compound **8** showed only 17 visible peaks (Table 1). Of these, six could be assigned to β-glucopyranosyl moiety, leaving 11 peaks for the aglucone part. Comparison of the ^{13}C NMR data of compounds **8** and **2** [4] confirmed this as C-10 and C-7 showed downfield shifts of 3.1 and 3.7 ppm, respectively, and C-8 was shifted upfield by 5.1 ppm. The differences seen for the signals of C-3 and C-4 as well as the missing carbonyl peak in compound **8** were ascribed to the lack of esterification of the C-11 carboxyl group in compound **8**, giving rise to a differing degree of protonation of the carboxyl group.

The FAB-mass spectrum of alpinoside (**9**) indicated the same M_r (416) as compound **8** and thus presumably the same molecular composition. The ^{1}H NMR spectrum contained a broad singlet at δ 7.05 arising from H-3. This, together with the lack of a methyl ester signal suggested that compound **9** also contained a carboxylic acid group. A singlet at δ 6.20 was assigned to H-1. The unusual downfield position and multiplicity of this proton indicated that H-9 was missing and that C-9 was doubly bonded to C-8 as seen in the compound hookerioside (**13**), where H-1 is seen as a singlet at

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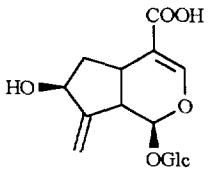

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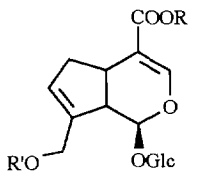
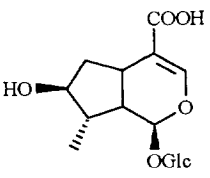


2 R=H, R′=H
8 R=H, R′=Ac
12 R=Me, R′=H



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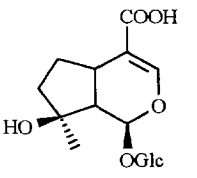

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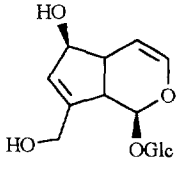

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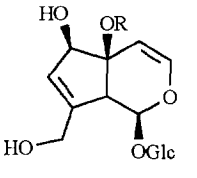

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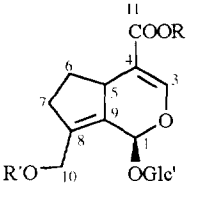
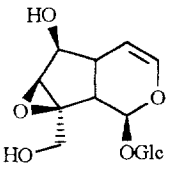
7 R=H



9 R=H, R′=Ac
13 R=Glc″, R′=Ac
15 R=Glc″, R′=H



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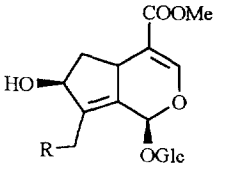
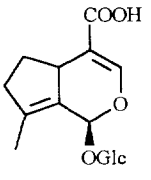


16 R=H
18 R=OH



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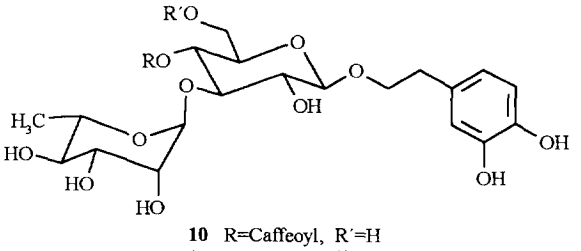

10 R=Caffeoyl, R′=H
11 R=H, R′=Caffeoyl

δ 6.40 [5]. Furthermore, a signal at δ 2.08 showed the presence of an acetyl group, a feature also present in **13**. When comparing the ^{13}C NMR spectrum of alpinoside with that of compound **13** (Table 1) a striking resemblance was seen. The main difference was that the signals arising from the 11-glucosyl moiety in hookerioside were missing from the spectrum of compound **9**. The further differences in the signals of C-3 and C-4, along with the lack of C-11 in the spectrum of compound **9** were again readily explained by partial ionization of the carboxyl group in the compound. We thus conclude that the structure of alpinoside (**9**) is that shown in the structure.

By chromatography of the crude extract of *P. altissima*, five known and one new iridoid glucosides were found together with verbascoside (**10**). The known compounds were gardoside (**1**), 8-epi-loganic acid (**3**), aucubin (**5**), hookerioside (**13**) and catalpol (**14**), identified by their NMR spectra. The new iridoid was **15**.

The ^{13}C NMR spectrum of compound **15** showed 22 peaks of which 12 could be assigned to two glucosyl moieties as in compound **13** and many of the remaining signals were also very similar, except those assigned to C-8, C-9 and C-10. Together with the fact that no acetyl group was present in compound **15**, this suggested that **15** was desacetylhookerioside. When comparing compounds **13** and **15**, the latter showed upfield shifts for C-10 and C-9 (3.2 and 2.4 ppm, respectively) and a downfield shift for C-8 (4.6 ppm). This is what would be expected given the difference in structure with a 10-*O*-acetyl group in **13** and none in **15**. The ^{1}H NMR spectra were also very similar, except for the singlet at δ 2.14 from the acetyl group in hookerioside (**13**) which was not seen in the spectrum of compound **15**. Furthermore, the shift for the 10-oxy-methylene protons which in compound **13** were seen at δ 4.77 while they were located at δ 4.29 and δ 4.19 in the spectrum of compound **15**, was also consistent with the difference in structure. Finally, acetylation of com-

Table 1. ^{13}C NMR data (50 MHz) of new compounds (**8**, **9** and **15**) and model compounds (**2** and **13**) in D_2O

C	2*	8	9	13	15
1	97.8	97.3	91.5	92.5	92.5
3	153.0	151.8	146.8	154.0	154.0
4	112.8	114.3	119.3	113.1	113.1
5	35.2	34.9	39.2	38.1	37.9
6	38.9	39.0	31.4	31.4	31.5
7	129.6	133.3	34.6	34.9	35.9
8	142.4	137.2	137.5	139.0	143.9
9	46.6	47.1	133.6	131.9	129.5
10	60.5	63.8	61.5	61.4	58.2
11	171.8	Not obs.	Not obs.	168.0	168.0
MeCO		175.0	174.7	175.0	
MeCO		21.2	21.1	21.2	
1′	99.7	99.5	99.8	99.2	99.2
2′	73.6	73.6	73.5	73.6	73.5
3′	76.5	76.5	76.4	76.5	76.4
4′	70.3	70.3	70.3	70.4	70.3
5′	76.9	77.0	77.1	77.1	77.1
6′	61.6	61.5	61.5	61.5	61.5
1″				94.7	94.6
2″				72.9	72.8
3″				76.5	76.4
4″				70.1	70.0
5″				77.7	77.6
6″				61.3	61.6

*Data from [4]: some signals were reassigned.

pound **15** yielded a nonacetate which proved identical with the peracetate of hookerioside (**13a**) in all respects. We therefore conclude that the new compound is desacetylhookerioside.

The presence of an 8,9-double bond in iridoids is not common. Except for compounds **9** and **15** in the present work, only four iridoids with this structural feature have been published to date, mainly from the genus *Plantago*. The compounds are majoroside (**16**) from *P. major* [6], anagalloside (**17**) from *Veronica anagallis-aquatica* var. *anagalloides* [7], 10-hydroxymajoroside (**18**) from *P. cornuti* [8] and hookerioside (**13**) from *P. hookeriana* [5].

EXPERIMENTAL

General. The plant material was grown at the experimental field of The Botanical Garden, The University of Copenhagen at Tåstrup, Sjælland. Vouchers (Rahn 699, *P. alpina*) and Rahn, 688, *P. altissima*) have been deposited at the Botanical Museum, University of Copenhagen. Prep. chromatography was performed on Merck Lobar C_{18} RP columns (size B or C) eluting with H_2O–MeOH mixtures as specified in each case. The HDO-peak (δ 4.75) was used as int. standard in ^1H NMR. In the ^{13}C NMR spectra, the C-6′ peak was set to δ 61.5 [9]. Microanalytical analysis was performed by Leo Microanalytical Laboratory, Ballerup, Denmark, FAB-MS: Jed JMS-AX505W.

Work-up of Plantago alpina. Frozen aerial parts (210 g) were homogenized twice with EtOH. The concd extracts were partitioned between Et_2O–H_2O and the aq. phase was concd to yield the crude extract (8.4 g). An aliquot (2.8 g) of the crude extract was applied to a chromatography column and eluted with H_2O–MeOH (25:1 to 1:1). This yielded gardoside (**1**, 130 mg, 0.2%), geniposidic acid (**2**, 15 mg, 0.02%) a 3:2 mixt. of 8-epiloganic acid and mussaenosidic acid (**3** and **4**, 80 mg, 0.06 and 0.05%, respectively), aucubin (**5**, 270 mg, 0.4%), monomelittoside (**6**, 40 mg, 0.06%), mellitoside (**7**, 65 mg, 0.09%), 10-*O*-acetyl-geniposidic acid (**8**, 77 mg, 0.1%), alpinoside (**9**, 36 mg, 0.05%), verbascoside (**10**, 719 mg, 1%) and a 2:3 mixt. of **10** and isoverbascoside (**11**, 50 mg). To the remaining part of the crude extract (5.6 g) was added sat. eq. $NaHCO_3$ (15 ml) and the mixture was chromatographed as above. Elution with H_2O gave first a fr. with no retention followed by the salt of **8** (120 mg) and that of **9** (66 mg).

10-O-*Acetyl-geniposidic acid* (**8**). The combined frs containing **8** were dissolved in 10% aq. AcOH and rechromatographed on a B-column. Elution with H_2O–MeOH (4:1) followed by treatment with activated C yielded pure **8** (77 mg) as a foam. $[\alpha]_D^{20} = +7°$ (c = 0.6, MeOH); ^1H NMR (200 MHz, D_2O); δ 7.12 (*s*, H-3), 5.89 (*m*, *H*-7), 5.28 (*d*, J = 5 Hz, H-1), 4.75 (*m*, partly obscured by solvent peak, 10-CH_2), 2.9 (*m*, H-9),

2.7 and 2.1 (ea. *m*, H_2-6), 2.05 (*s*, $C\underline{H}_3CO$–); ^{13}C NMR: Table 1. $C_{18}H_{24}O_{11}$ (416); FAB-MS (glycerol): $[M+H]^-$ at *m/z* 417; $[M-H]^-$ at *m/z* 415. (Found: C, 50.6; H, 6.1. $C_{18}H_{24}O_{11} \cdot \frac{1}{2}H_2O$ requires: C, 50.8; H, 5.9%)

Alpinoside (**9**). The frs containing **9** were combined and treated as above. This yielded pure **9** (55 mg) as a foam. $[\alpha]_D^{20} = -54°$ (*c* = 0.6, MeOH); ^1H NMR (200 MHz, D_2O): δ 7.05 (*s*, H-3), 6.20 (*s*, H-1), 4.83 (*d*, *J* = 8 Hz, H-1′), 4.75 (*m*, partly obscured by solvent peak, 10-CH_2), 2.5 (*m*, 3H, 2 × H-7 and H-6_A), 2.08 ($C\underline{H}_3CO$–), 1.45 (*m*, H-6_B); ^{13}C NMR: Table 1. $C_{18}H_{24}O_{11}$ (416); FAB-MS (glycerol): $[M+H]^+$ at *m/z* 417; $[M-H]^-$ at *m/z* 415. (Found: C, 47.7; H, 6.1. $C_{18}H_{24}O_{11} \cdot 2H_2O$ requires: C, 47.8; H, 6.2%)

Work-up of Plantago altissima. Frozen aerial parts (190 g) were worked up as above to yield a crude extract (10.4 g). This was applied to a chromatography column. Elution with H_2O gave first fr. A (0.198 g), followed by catalpol (**14**, 518 mg) and elution with MeOH yielded fr. B. Rechromatography of fr. A (H_2O–MeOH; H_2O to 10:1) gave gardoside (**1**, 25 mg, 0.01%), 8-epi-loganic acid (**3**, 10 mg, 0.005%) together with some unidentified iridoid acids. Fr. B was re-chromatographed (H_2O–MeOH; 25:1 to 1:1) to give additional catalpol (44 mg; total 0.3%), aucubin (**5**, 300 mg; 0.16%), a fr. containing mainly desacetylhookerioside (**15**, 74 mg; 0.04%), hookerioside (**13**, 85 mg; 0.05%) and verbascoside (**10**, 371 mg; 0.2%). *Desacetylhookerioside* (**15**). A pure sample was obtained as a foam after rechromatography and treatment with activated C. $[\alpha]_D^{20} = -38°$ (*c* = 0.6, MeOH); ^1H NMR (200 MHz; D_2O): δ 7.63 (*d*, *J* = 1 Hz, H-3), 6.32 (*s*, H-1), 5.61 (*d*, *J* = 8 Hz, H-1″), 4.83 (*d*, *J* = 8 Hz, H-1′), 4.29 (*d*, *J* = 12 Hz, H-10A), 4.19 (*d*, *J* = 12 Hz, H-10_B), 2.5 (multiple signals, 3H, 2 × H-7, H-6_A), 1.50 (*m*, H-6_B); ^{13}C NMR: Table 1. $C_{22}H_{32}O_{15}$ (536); FAB-MS (bis(2-hydroxyethyl)disulphide): $[M+Na]^+$ at *m/z* 559; $[M-H]^-$ at *m/z* 535. (Found: C, 46.1; H, 6.5. $C_{22}H_{32}O_{15} \cdot 2H_2O$ requires: C, 46.2; H; 6.3)

Hookerioside octaacetate (**13a**). Desacetylhookerioside (**15**, 30 mg) was acetylated in Ac_2O-pyridine (1:2, 2 hr at room temp.). The produce **13a** was crystallized from EtOH; mp 194° (reported: 195–196° [5]). $[\alpha]_D^{20} = -43°$ ($CHCl_3$; *c* = 0.6). (Reported: −44° [5]). NMR data also similar to those reported [5].

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