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NOVEL *BIS*-IRIDOID GLUCOSIDES FROM *DIPSACUS SYLVESTRIS*

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Key Word Index—*Dipsacus sylvestris*; Dipsacaceae; *bis*-iridoid glucosides; sylvestrosides I–IV; cantleyoside; methyl glucoside; structural determination.

Abstract—In addition to the known iridoid glucosides loganin, sweroside and cantleyoside, *Dipsacus sylvestris* has provided 4 novel *bis*-iridoid glucosides named sylvestrosides I–IV, composed of swerosidic acid, secologanic acid, loganin and loganin aglucone. Sylvestroside I, II and III have been fully characterized by chemical conversions and by ^{13}C NMR spectroscopy. A probable formula for sylvestroside IV is presented.

INTRODUCTION

Dipsacus sylvestris Huds. has been investigated chemically several times (see Hegnauer [1] for a complete account). Of particular interest is the observation of Tammes [2] that leaves of several species within the family Dipsacaceae (among these some *Dipsacus* species) exhibit a blue colour on gentle heating in a moist atmosphere. Three species of *Scaevola* investigated also showed this behaviour. The blue colouring matter was named dipsacotin and later [1] suggested as being of iridoid origin. From seeds of a number of Dipsacaceae (incl. two *Dipsacus* species) Lys [3] isolated the bitter glucoside, cephalarioside [mp 208–210°; $[\alpha]_D^{20}$ −104° (MeOH); λ_{max}^{EtOH} 240 nm (log ε 4)], for which he determined the formula $C_{16}H_{24}O_{10}$ and pointed out the similarities to loganin.

In a preliminary investigation of *D. sylvestris* [4], we isolated as the acetate, a *bis*-iridoid glucoside which we presumed to be an ester of secologanic acid with loganin aglucone. We now report our findings in detail.

RESULTS AND DISCUSSION

Two amorphous *bis*-iridoid glucosides, named sylvestroside I (SI) (**1**) and sylvestroside II (SII) (**2**), have been isolated from seeds of *Dipsacus sylvestris*. The structures have been determined in the following way: acetylation of SI or SII gives the crystalline nonaacetate (**3**). That SII is a monoacetate of SI follows from its PMR spectrum and from the fact that SII yields SI upon methanolysis. In the same reaction loganin (**4**) and sweroside (**6**) are produced, defining the stereochemistry of the components of **1** and **2** supposedly combined through an ester bond in SI and SII. The structural problem was solved by comparing the ^{13}C NMR spectra of **1**, **2** and **3** with those of secologanin (**21**), secologanin acetate (**22**), loganin (**4**), loganin acetate (**5**) and sweroside (**6**). From the spectrum of **3** a complete set of signals, the 'b' part, corresponding to loganin acetate (dev. <0.2 ppm) can be picked out. The 'a' part of the spectrum of **3** corresponds partly to that of **22**, the differences being accounted for by the different functionalities at C-7: an aldehyde function in **22** and a primary acetate in **3**. In both **1** and **2** the oxygen at C-7 of the loganin unit is evidently esterified, as the chemical shifts of this carbon atom (78.8 and 78.6 ppm, respectively), coincide better with the corresponding shift in **5** (77.1 ppm) than with that in **4** (74.9 ppm). This shows that in **1**, swerosidic acid is esterified to the oxygen function at C-7 in loganin. In **2**, C-7a is shifted 3.5 ppm downfield relative to **1**, while C-6a and C-5a are shifted upfield (2.1 and 1.1 ppm, respectively), proving the position of the acetoxy group at C-7a.

D. sylvestris is a biennial, the first year forming a rosette with a taproot; the two parts of the plant have been investigated separately. The roots afford loganin and sweroside in minor quantities, together with three *bis*-iridoid glucosides, the main component being cantleyoside (**8**), first found in *Cantleya corniculata* (Icacinaceae) [6] and characterized as the octaacetate **9**. Reduction of **8** with sodium borohydride in methanol gives sylvestroside I (**1**), thereby interrelating these compounds.

The two minor *bis*-iridoid glucosides, named sylvestroside III (SIII) and sylvestroside IV (SIV), are assigned structures **10** and **15**, respectively. Upon acetylation, SIII gives a pentaacetate (**11**), and therefore contains only one molecule of glucose. Comparison with the PMR spectra of secologanin and loganin indicates that the aglucone parts of these compounds are present in **10**. In the spectrum of the pentaacetate **11**, a doublet (6.1 ppm, J = 2.5 Hz) is assigned to the proton of an acetylated hemiacetalic centre. The ^{13}C NMR spectrum contains a set of signals almost identical to those assigned to the secologanin (a) moiety of cantleyoside (**8**). The other set of signals, corresponding to the loganin (b) moiety of (**8**), shows a larger deviation, particularly with respect to the absorptions assigned to the dihydropyrane ring. When comparing the ^{13}C NMR spectrum of SIII pentaacetate (**11**) with those of loganin aglucone diacetate (**13**) and secologanin tetraacetate (**22**) a complete match is obtained, except for a deviation of *ca* 0.5 ppm from the signals of the latter. Sylvestroside III is thus composed of secologanic acid esterified to the OH-group at C-7 of loganin aglucone.

Sylvestroside IV could not be purified completely.

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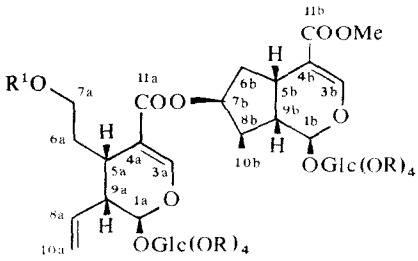

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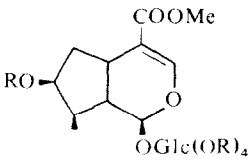

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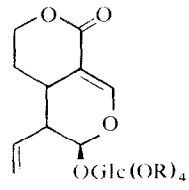

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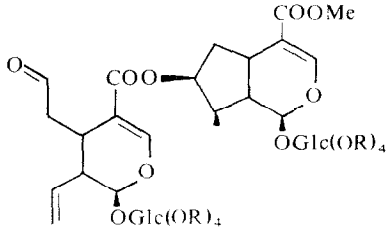

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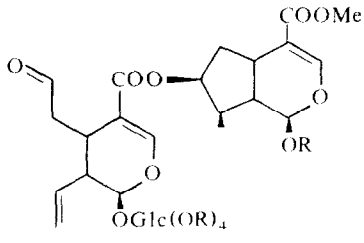

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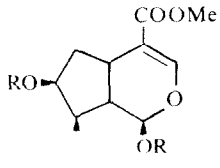

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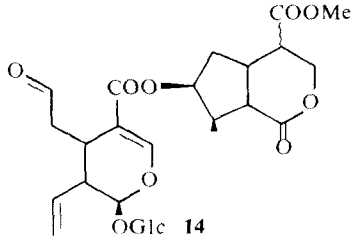

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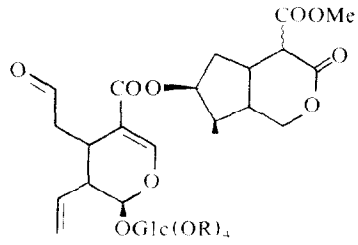

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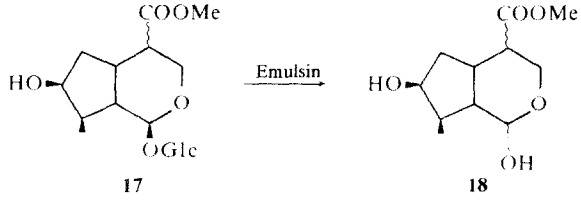

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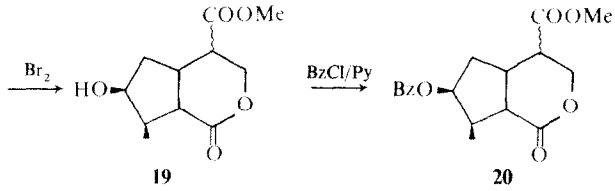

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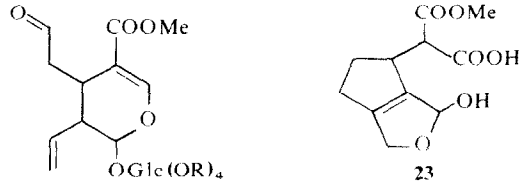

21 R = H
22 R = Ac

23

Since a tetraacetate is produced on acetylation only one glucose is present in the molecule. In the ^{13}C NMR spectrum of the tetraacetate (**16**) one set (a) of signals corresponds closely to that of secologanin acetate (**22**), with a deviation of *ca* 0.6 ppm. Seven ester carbonyl signals are seen in the spectrum of **16**: 5 of these can be accounted for in the secologanin moiety (a), leaving two ester groups in the 'b'-unit of the molecule. The remaining signals correspond well with the presence of an intact cyclopentane ring having the same substitution pattern as loganin. The abnormal high-field shift of the C-10b absorption in particular (also found in the other loganin derivatives (12–14 ppm)) suggests the same *cis*-relationship between this methyl group and the oxygen at C-7 in all of these compounds [10]. The biosynthetically probable structures **14** and **15** have been considered for sylvestroside IV. For comparison, **20**, the 'b'-unit of **14**, was synthesized from loganin (**4**). Hydrogenation [7] of the double bond of **4** gave **17**, which was cleaved with emulsin to **18**. Oxidation with bromine [8, 9] of the cyclic hemiacetal gave **19**. Benzoylation yielded **20**, possessing the correct functionality at C-7 as a model compound. The ^{13}C NMR spectra of **20** and of the 'b' part of SIV tetraacetate did not show satisfactory correspondence. A signal at 51.8 ppm present in the latter, which could be

assigned to either C-9 in structure **14** or to C-4 in structure **15**, however, was absent in the spectrum of **20** (and **19**), thus eliminating structure **14** for SIV.

That the correct structure is **15**, biosynthetically derivable from loganin aglucone, is anticipated from the structures of the accompanying compounds and from the evidence (^{13}C NMR) for the presence of the C_{10}-moiety carrying the appropriate functionalities. Genipinic acid (**23**) [11], an iridoid with a structure reminiscent of the 'b'-unit of **15** behaves in a way similar to that of SIV. Thus, in solution, **23** easily isomerizes at C-4, as demonstrated by an extra methyl signal in the PMR spectrum. A strongly UV-absorbing chromophore (274 nm) is formed by addition of alcoholic alkali to a solution of **23**, interpreted as the formation of an enolate ion. SIV shows the same phenomenon: a PMR spectrum of the tetraacetate, run immediately after dissolution in $CDCl_3$, indicates a 9:1 equilibrium mixture. In the UV spectrum of **16** there is a single maximum (233 nm), shifted to 272 nm upon addition of sodium methoxide. This evidence, together with the biosynthetic considerations, makes **15** a probable structure for sylvestroside IV.

Leaves from the rosette as well as leaves and stems from flowering plants have also been examined. Here sylvestroside III is the major glycosidic constituent, accompanied by loganin, methyl glucoside, and some minor unidentified iridoids.

bis-Iridoids have been described from *Cantleya* (Icacinaceae) [6] and from *Fraxinus* (Oleaceae) [12], in the latter case consisting of two molecules of oleoside linked through a molecule of salidroside. We have found cantleyoside and the sylvestrosides in a number of species of Dipsacaceae and Caprifoliaceae. They are also present in the two species of *Scaevola* (Goodeniaceae) that we have investigated. This is in agreement with the observations of Tammes [2] concerning the distribution of dipsacotin.

Cephalarioside, the crystalline glucoside isolated by Lys [3] from seeds of *Cephalaria syriaca*, is apparently a monomer and not identical with any of the sylvestrosides.

EXPERIMENTAL

General procedures were as earlier described [5, 13]. Microanalyses were performed at Bernhardt, Microanalytisches Laboratorium, W. Germany. Seeds of *D. sylvestris* were collected near Faxe, Denmark, in September 1974 and plants grown from these (voucher nos IOK-81/74, -47/76, deposited at The Botanical Museum, Copenhagen (C)) were used in the present study.

Seeds. Two-year-old, viable seeds (50 g, collected Sept. 1974, 2 km south of Faxe, Sjælland) were homogenized with EtOH (2 × 500 ml) to give 5 g H_2O solubles. Treatment as described earlier [5, 13] gave a mixture of crude glycosides (3.74 g). An aliquot (900 mg) was chromatographed by prep-TLC ($CHCl_3$–MeOH: 3:1) yielding 2 fractions. The slower moving band consisted of sylvestroside I (**1**, 94 mg, 0.8%), a colourless syrup. The syrup was adsorbed on activated C/celite, washed with H_2O, eluted with MeOH and then thoroughly dried to give the pure glucoside: $[\alpha]_D^{21}$ −106° (EtOH, *c* 0.4); λ_{max}^{EtOH} nm (log ε): 236 (4.32); PMR: (D_2O, DSS): δ 7.58 (s, H-3a), 7.47 (s, H-3b), 3.76 (*s*, OCH_3), 1.06 (*br d*, 10b-CH_3). (Found: C, 51.69; H, 6.55. $C_{33}H_{48}O_{19}$, H_2O requires: C, 51.65; H, 6.57%). The faster moving band, sylvestroside II (**2**), 540 mg, 3.6%, was treated as above to give a colourless foam: $[\alpha]_D^{20}$ −99° (MeOH, *c* 1.5) λ_{max}^{EtOH} nm (log ε): 238 (4.38); PMR (D_2O, DSS): δ 7.58 (s, H-3a), 7.47 (s, H-3b), 4.1 (*m*, 7a-CH_2), 3.76 (*s*, OCH_3), 2.08 (*s*, OAc), 1.11 (*br d*, 10b-CH_3). (Found: C, 52.98; H, 6.41. $C_{35}H_{50}O_{20}$ requires: C, 53.16; H, 6.37%).

Sylvestroside I nonaacetate (**3**). This could be prepared by acetylation of either **1** or **2**. Recrystallization from EtOH afforded the pure compound, mp 154–155°; $[\alpha]_D^{20}$ −85° ($CHCl_3$, *c* 0.4); λ_{max}^{MeOH} nm (log ε): 235 (4.39); PMR ($CDCl_3$, TMS): δ 7.33 (*d*, *J* = 1 Hz, H-3a), 7.31 (*s*, H-3b), 3.71 (*s*, OCH_3), 2.11–1.97 (2,1,2,2,1 and 1 × OAc), 1.09 (*d*, *J* = 6.5 Hz, 10b-CH_3). (Found: C, 54.17; H, 5.86. $C_{51}H_{66}O_{28}$ requires: C, 54.35; H, 5.90%).

Methanolysis of **2**. To **2** (200 mg) in MeOH (5 ml) was added a soln of Na (5 mg) in MeOH (5 ml) with stirring. After 10 min the mixture was neutralized with IR-120. Chromatography (Si gel: $CHCl_3$–MeOH, 3:1) gave 3 fractions (*ca* 70 mg each): (i) a mixture of loganin (**4**) and sweroside (**6**), (ii) unreacted **2** and (iii) **1**. The 3 fractions were mixed and acetylated. Chromatography (Si gel: Et_2O) again provided 3 fractions. The fastest moving band consisted of loganin pentaacetate (**5**, 66 mg), crystallized from EtOH, mp 141°, identical to an authentical specimen (PMR, mmp). The next band provided sweroside tetraacetate (**7**, 56 mg), mp 167°, identified as above. The slowest moving band gave **3** (120 mg), mp 154°.

Roots of 1st year plants. Fresh roots (150 g) were treated in the same manner as the seeds to give a mixture of glycosides (1550 mg). Chromatography as above provided 5 fractions. The slowest moving band (641 mg, 0.4%) consisted of cantleyoside (**8**), a colourless foam; $[\alpha]_D^{22}$ −93° (MeOH, *c* 0.6); λ_{max}^{MeOH} nm (log ε): 235 (4.31); PMR (D_2O, DSS): δ 9.73 (*br s*, 7a-CHO), 7.65 (*br s*, H-3a), 7.58 (*s*, low intensity signal from hydrated form of **8**; H-3a), 7.51 (*br s*, H-3b), 3.74 (*s*, OCH_3), 1.09 (*m*, 10b-CH_3; *m* due to higher order couplings). (Found: C, 51.15; H, 6.20. $C_{33}H_{46}O_{19}$, 1.5 H_2O requires: C, 51.20; H, 6.38%). The next bands provided loganin (**4**, 120 mg, 0.08%) and sweroside (**6**, 36 mg, 0.03%) which were identified as the acetates **5** and **7**, respectively. The fourth fraction contained sylvestroside III (**10**, 150 mg, 0.1%) contaminated by some minor constituents, see below. The fastest running band afforded almost pure sylvestroside IV (**15**, 230 mg, 0.16%) as a foam. Rechromatography on Si gel with EtOAc–EtOH–C_6H_6 (4:1:1) or BuOH–MeOH–H_2O (18:1:2) and on Sephadex G-15 (75% aq. MeOH) did not improve the purity of this constituent as demonstrated by PMR (Me absorptions at 3.79 and 3.73 ppm, *ca* 9:1) $[\alpha]_D^{20}$ −57° (MeOH, *c* 0.4); λ_{max}^{MeOH}, nm (log ε): 236 (3.99); PMR (Me_2CO-d_6, TMS): δ 9.79 (*t*, *J* = 1 Hz, 7a-CHO), 7.65 (*d*, *J* = 1.5 Hz, H-3a), 5.52 (*d*, *J* = 5 Hz, H-1a), 4.76 (*d*, *J* = 7 Hz, H-1a'), 4.42 (2 H, AB part of ABX system, 1b-CH_2; X at 2.4 ppm), 3.79 (*s*, OCH_3), 1.11 (*d*, *J* = 7 Hz, 10b-CH_3). Also low integr. signals from isomer. (Found: C, 55.30; H, 6.38. $C_{27}H_{36}O_{14}$ requires: C, 55.48; H, 6.21%).

Sylvestroside IV tetraacetate (**16**). This was prepared by acetylation of **15**. It was crystallized from EtOH, mp 137–139°; $[\alpha]_D^{22}$ −60° ($CHCl_3$, *c* 0.4); λ_{max}^{MeOH} nm (log ε): 233 (3.97). Addition of NaOMe soln shifted the maximum to 272 nm with strong intensity. PMR ($CDCl_3$, TMS): δ 9.75 (*br t*, 7a-CHO), 7.42 (*d*, *J* = 1.5 Hz, H-3a), 3.84 (*s*, OCH_3), 3.44 (*d*, *J* = 7 Hz, H-4b), *ca* 2 (4 × OAc), 1.07 (*d*, *J* = 7 Hz, 10b-CH_3) also low integr. signals from minor isomer. (Found: C, 55.62; H, 6.04. $C_{35}H_{44}O_{18}$ requires: C, 55.85; H, 5.89%).

Cantleyoside octaacetate (**9**). (**8**) was acetylated and the product crystallized from EtOH: mp 146–148°; $[\alpha]_D^{22}$ −89° ($CHCl_3$, *c* 0.4); λ_{max}^{MeOH} nm (log ε): 233 (4.24); PMR ($CDCl_3$, TMS): δ 9.76 (*br t*, *J* = 1 Hz, 7a-CHO), 7.42 (*d*, *J* = 1.5 Hz, H-3a), 7.35 (*d*, *J* = 1 Hz, H-3b), 3.73 (*s*, OCH_3), 2.2–1.9 (8 × OAc), 1.07 (*d*, *J* = 7 Hz, 10b-CH_3), in agreement with reported values [6]. (Found: C, 54.51; H, 5.93. Calc. for $C_{49}H_{62}O_{27}$: C, 54.34; H, 5.77%).

Reduction of cantleyoside. **8** (300 mg) in MeOH (10 ml) was treated with $NaBH_4$ (24 mg) for 10 min and provided, after neutralization with IR-120, crude **1** (295 mg). Chromatography (Si gel; $CHCl_3$–MeOH, 2.5:1) gave pure **1** (250 mg), identical PMR, TLC) to the sample from the seeds.

Leaves of 1st year plants. Frozen leaves (330 g) were treated in the same manner as the seeds to give a mixture of glycosides

(3.2 g). An aliquot (625 mg) was chromatographed as above. The slowest band (not detected by UV) contained methyl β-D-glucoside (100 mg, 0.15%); mp and mmp of tetraacetate 101°. The next band contained loganin (62 mg, 0.1%), identified as above. An unidentified fraction (35 mg) was followed by the fastest moving band, sylverstoside III (**10**, 275 mg, 0.4%), not quite pure. A larger amount (*ca* 1.2 g), obtained by pooling from several separations, was subjected to column chromatography on Si gel using EtOAc–EtOH–C_6H_6 (4:1:1), BuOH–MeOH–H_2O (95:1:5) and $CHCl_3$–MeOH (4:1), each time collecting the fastest moving fraction. 300 mg of pure **10** was obtained as a foam, $[\alpha]_D^{20}$ −85° (MeOH, *c* 0.4); λ_{max}^{MeOH} nm (log ε): 237 (4.25; PMR Me_2CO-d_6, TMS); δ 9.73 (*br t*, *J* = 1 Hz, 7a-CHO), 7.56 (*d*, *J* = 1.5 Hz, H-3a), 7.42 (*d*, *J* = 1 Hz, H-3b), 4.70 (*d*, *J* = 7 Hz, H-1a'), 3.64 (*s*, OCH_3), 1.07 (*d*, *J* = 7 Hz, 10b-CH_3). (Found: C, 53.09; H, 6.05. $C_{27}H_{36}O_{14}$, 1.5 H_2O requires: C, 53.03; H, 6.43%).

Sylvestroside III pentaacetate (**11**) was obtained as a foam by acetylation of **10**: $[\alpha]_D^{21}$ −81 ($CHCl_3$, *c* 0.5); λ_{max}^{MeOH} nm (log ε): 238 (4.28); PMR ($CDCl_3$, TMS): δ 9.76 (*t*, *J* = 1 Hz, 7a-CHO), 7.43 (*d*, *J* = 1.5 Hz, H-3a), 7.39 (*d*, *J* = 1 Hz, H-3b), 6.10 (*d*, *J* = 2.5 Hz, H-1b), 3.75 (*s*, OCH_3), 2.14–1.96 (5 × OAc), 1.13 (*d*, *J* = 6 Hz, 10b-CH_3). (Found: C, 55.71; H, 5.80. $C_{37}H_{46}O_{19}$ requires: C, 55.92; H, 5.83%).

2nd year plants. Leaves and stems of flowering plants provided a mixture of glucosides almost identical to that from the rosettes.

Loganin aglucone (**12**) was prepared by treatment of **4** (400 mg)

Table 1. ^{13}C NMR data* of sylvestrosides I–IV, some derivatives and model compounds

Compound (solvent) no.	Carbon unit	C_1	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	C=O	OMe	C_1'	C_2'	C_3'	C_4'	C_5'	C_6'
21																		
(D_2O)	a	97.6 *d*(174)	154.0 *d*(194)	109.6 *s*	27.5 *d*(134x)	44.6 *t*	206.8 *d*(177)	133.8 *d*(156)	44.6 *d*(132x)	121.6 *t*(161)	169.8 *s*	52.6 *q*(147)	99.6 (161)	73.5	76.6	70.5	77.2	61.6
22																		
($CDCl_3$)	a	95.4	150.7	109.1	25.1	43.5†	199.7	131.8	43.1†	120.6	166.1	51.1	95.4	70.4	72.0	67.9	72.0	61.4
6																		
(D_2O)	a	98.4 *d*(184x)	154.2 *d*(195)	105.6 *s*	27.3 *d*(136x)	24.8 *t*(128x)	70.4 *t*(153x)	132.2 *d*(161)	42.5 *d*(134)	121.5 *t*(160)	170.2 *s*		99.2 (158x)	73.5	76.4	70.4	72.1	61.6
4																		
(D_2O)	b	97.6 *d*(172)	151.8 *d*(192)	113.9 *s*	30.7 *d*(135)	41.3 *t*(129)	74.9 *d*(148)	41.0 *d*(123)	45.8 *d*(134)	12.9 *q*(124)	170.6 *s*	52.6 *q*(147)	99.5 (161)	73.6	76.6	70.5	77.2	61.6
5																		
($CDCl_3$)	b	94.8 *d*(173)	149.1 *d*(193)	113.7 *s*	29.9 *d*(138)	38.9 *d*(127x)	77.1 *d*(156)	38.9 *d*(127x)	45.6 *d*(132)	12.5 *q*(126)	167.1 *s*	51.2 *q*(147)	96.0 (163)	70.8	72.3	68.4	72.6	61.8
12																		
(D_2O)	b	96.3	153.4	112.2	32.1	41.5	74.8	41.5	46.8	13.7	170.9	52.6						
13																		
($CDCl_3$)	b	91.0 *d*(174)	150.2 *d*(195)	112.6 *s*	30.6 (139)	39.3 *t*(133)	76.9 *d*(155)	39.3 *d*(126x)	45.2 *d*(136x)	12.6 *q*(127)	167.0 *s*	51.2 *q*(147)						
1 (D_2O)	a	98.3	153.6	111.5	30.7	33.2	60.8	134.7	44.5	120.4	169.5		99.6‡	73.5	76.6	70.5	77.2	61.6
	b	97.4	152.2	113.2	31.3	40.2	78.8	39.3	46.4	13.3	170.5	52.7						
2 (D_2O)	a	98.0	153.7	111.2	29.6	31.1	64.3	134.6	44.4	120.6	168.9		99.6†	73.5	76.6	70.4	77.2	61.6
	b	97.4	152.2	113.0	31.4	40.1	78.6	39.5	46.4	13.5	170.1	52.6						
3	a	96.0 *d*	150.7 *d*(194)	111.0 *s*	27.6 *d*(135)	27.6 *t*(127x)	62.1 *t*(148x)	132.8 *d*(157)	43.0 *d*(132)	120.3 *t*(159)	165.9 *s*		96.0‡ *d*	70.6	72.2	68.3	72.5	61.7
($CDCl_3$)	b	94.6 *d*(173)	149.0 *d*(194)	113.7 *s*	29.9 *d*(138x)	39.1 *t*(131)	77.0 *d*(148x)	39.1 *d*(126x)	45.7 *d*(134)	12.6 *q*(125)	167.0 *s*	51.3 *q*(147)						
8																		
(D_2O)	a	97.7	154.2	109.8	28.2	44.5†	206.7	133.9	45.1†	121.6	168.9		99.6‡	73.5	76.6	70.5	77.2	61.6
	b	97.4	152.2	113.1	31.3	40.1	78.8	39.3	46.3	13.2	170.3	52.7						
10																		
(Me_2CO-d_6)	a	96.9 *d*(175x)	153.0 *d*(192)	109.9 *s*	27.3 *d*	44.8 *t*	201.7 *d*(177)	134.7 *d*(159)	44.8 *d*	120.4 *t*(161x)	166.9 *s*		99.7 (162)	74.2	77.5	71.2	77.5	62.6
	b	96.4 *d*(175x)	153.0 *d*(192)	111.4 *s*	33.1 *d*	40.2 *d*(133x)	77.5 *d*	41.1 *d*(128x)	47.9 *d*(135x)	14.3 *q*(126)	168.2 *s*	51.4 *q*(147)						
10 (D_2O)	a	97.6	153.8	109.7	28.1	45.0†	206.2	133.9	44.5†	121.7	168.5		99.6	73.4	76.5	70.3	77.1	61.6
	b	96.0	153.8	111.3	32.7	39.9	78.4	40.9	47.3	14.2	170.3	52.6						
11 ($CDCl_3$)	a	95.9 *d*	151.1 *d*(194)	109.6 *s*	25.4 *d*(138)	43.7 *t*(126)	200.3 *d*(174)	132.4 *d*(159)	43.3 *d*(131)	121.1 *t*(159)	166.1 *s*		95.9 *d*	70.6	72.3	68.2	72.3	61.7
	b	90.7 *d*(175)	150.2 *d*(195)	112.4 *s*	30.3 *d*(134x)	39.1 *d*(133)	76.9 *d*(157)	39.4 *d*(127x)	45.2 *d*(134x)	12.9 *q*(126)	167.3 *s*	51.4 *q*(147)						
15 (Me_2CO-d_6)	a	96.9	153.3	109.8	27.2	44.9	201.4	134.7	44.9	120.2	166.8		99.7	73.4	76.5	70.3	77.1	61.6
	b	69.9	169.8†	52.2	37.2†	41.8†	79.2	38.7†	42.7†	13.4	169.5†	52.8						
16 ($CDCl_3$)	a	96.1 *d*	151.4 *d*(194)	109.6 *s*	25.6 *d*(138x)	43.8 *t*	200.2 *d*(175)	132.4 *d*(158)	43.5 *d*	121.0 *t*(159)	165.8 *s*		96.1 *d*	70.8	72.3	68.3	72.5	61.8
	b	68.7 *t*(145x)	168.5† *s*	51.8 *d*(135)	36.2† *d*(140x)	41.2† *t*	78.3 *d*(158x)	38.5† *d*	42.0† *d*	13.2 *q*(127)	168.3† *s*	53.0 *q*(149)						
17 (D_2O)	b	95.2 *d*(171)	59.7 *t*(150)	44.4† *d*(133)	34.5 *d*(138)	39.9 *t*(130)	73.8 *d*(147x)	39.1† *d*(127)	46.3† *d*(132)	12.3 *q*(126)	176.0 *s*	53.3 *q*(148)	98.1 *d*(162)	73.8	76.7	70.5	77.1	61.6
19 ($CDCl_3$)	b	—	67.2	45.9†	37.2	41.0	74.8	43.9	46.1†	14.0	—	52.4						
20 ($CDCl_3$)	b	173.2 *s*	67.2 *t*(152)	46.0† *d*(130)	37.5 *d*(136)	39.0 *t*(131)	78.0 *d*(156)	42.7 *d*(126)	46.8† *d*(130)	14.4 *q*(128)	171.3 *s*	52.5 *q*(147)						

*The spectra were recorded as earlier described. Standards used were TMS and dioxane (in D_2O). Chemical shifts in ppm +0.1. Coupling constants in Hz ±2 (if the value is followed by an 'x': ‡5 Hz).

†Interchangeable in the same horizontal column.

‡The signals arising from the sugar moieties are of double intensity.

in H_2O (20 ml) with emulsin (200 mg) for 20 hr. The reaction mixture was passed through activated C and celite (2 + 2 g). The cake was washed with H_2O (100 ml) and then eluted with MeOH (100 ml) to give **12** (242 mg) as an oil, which was characterized only by its ^{13}C (see Table 1) and PMR spectra ($CDCl_3$, TMS): δ 7.44 (*s*, H-3), 4.96 (2H, *m*, H-1 and OH), 4.15 (*m*, H-7), 3.74 (*s*, OCH_3), 3.16 (*br q*, $J = ca$ 8 Hz, H-5), 2.58 (*dd*, $J_{5,6\beta} = 7.5$ Hz, $J_{6\alpha,6\beta} = 13$ Hz, H-6β), 1.95 (2H, *m*, H-9 and H-8), 1.57 (*m*, $J_{5,6\alpha} = 8.5$ Hz, $J_{6\alpha,6\beta} = 14$ Hz, $J_{6\alpha,7} = 4$ Hz, H-6α), 1.14 (3H, *d*, $J_{8,10} = 7.5$ Hz, 10-CH_3).

Dihydrologanin (**17**) was prepared by dissolving loganin (**4**, 500 mg) in HOAc (10 ml), adding PtO_2 (100 mg) and hydrogenating for 2.5 hr until 1 mol of H_2 had been absorbed. Crystallization of the product gave **17** (300 mg), mp 187–188°, containing *ca* 10% of **4**. The crystals and mother liquors were dissolved in H_2O (15 ml) and emulsin (200 mg) was added. After 24 hr the mixture was passed through activated C and celite, (2 + 2 g). The cake was washed with H_2O and eluted with MeOH (200 ml). The extract was concd *in vacuo* to give a syrup (375 mg). Crystallization (EtOH) afforded pure **17**, (175 mg), mp 160–162° (reported: 160–162° [7]).

Preparation of lactone **19**. **17** (150 mg) was dissolved in H_2O (5 ml), emulsin (150 mg) added and the mixture stirred for 7 days when TLC showed that hydrolysis was complete. The mixture was filtered and extracted with CH_2Cl_2 (5 × 20 ml) and Et_2O (2 × 40 ml) to give 75 mg of an oil. A PMR spectrum showed the signals expected for dihydrologanin aglucone (**18**). The compound was not further characterized. The oil was dissolved in H_2O (1 ml) and $BaCO_3$ (100 mg) was added. Under stirring, Br_2 (21 µl) was added till the colour remained for more than 1 min. The mixture was diluted with Me_2CO (10 ml), filtered and concd to an oil, which was extracted with $CHCl_3$ (2 × 30 ml). Concn gave crude **19** (67 mg); crystals from Et_2O, mp 98–98.5°; $[\alpha]_D^{22}$ +17° ($CHCl_3$, *c* 0.3); PMR spectrum ($CDCl_3$, TMS): δ 4.42 (*m*, AB part of ABX system, 3-CH_2), 4.18 (*m*, H-7), 3.76 (*s*, OCH_3), 1.27 (*d*, $J = 7$ Hz, 10-CH_3). (Found: C, 57.86; H, 7.00. $C_{11}H_{16}O_5$ requires: C, 57.88; H, 7.07%).

Benzoylation (BzCl/Py) of **19** provided the lactone benzoate **20** mp 114–115°, $[\alpha]_D^{20}$ + 36° ($CHCl_3$, *c* 0.3); PMR spectrum ($CDCl_3$, TMS): δ 8.1 and 7.6 (2H and 3H, arom.), 5.50 (*t*, $J =$ 3.5 Hz, H-7), 4.48 (*m*, AB part of ABX system, 3-CH_2), 3.76 (*s*, OCH_3), 1.31 (*d*, $J = 7$Hz, 10-CH_3). (Found: C, 64.87; H, 6.11. $C_{18}H_{20}O_6$ requires: C, 65.05: H, 6.07%).

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