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NEW BIS-IRIDOIDS FROM *DIPSACUS LACINIATUS*

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ABSTRACT.—In addition to the known mono- and bis-iridoid glucosides loganin [**10**], sweroside [**6**], cantleyoside [**9**], and sylvestrosides III [**7**] and IV [**8**], *Dipsacus laciniatus* has provided six new bis-iridoid glucosides, laciniatosides I–VI. These derivatives contain an acidic unit (secologanic acid, swerosidic acid, or 7-deoxyloganic acid) and an alcoholic unit (various loganin-like alcohols) linked by an ester bond. Laciniatosides I [**1**], II [**2**], III [**3**], IV [**4**], and VI [**11**], as well as sylvestroside IV, were characterized by chemical transformations and ^{1}H- and ^{13}C-nmr spectroscopy. NOe measurements were used to determine the steric position of the hydrogens and substituents. Conformational analysis of the separated loganin-type subunit of the new compounds was performed using molecular mechanics calculations. The calculated structures were related to solution structures based on direct comparison of measured and calculated vicinal proton-proton coupling constants.

The first bis-iridoid compound, cantleyoside [**9**], containing a secoiridoid unit and an iridoid unit, was found in *Cantleya corniculata* (1). Five other bis-iridoids related to cantleyoside, namely sylvestrosides I–IV from *Dipsacus sylvestris* (2), and laciniatoside V [**5**] from *Dipsacus laciniatus* L. (Dipsacaceae) (3), were isolated previously. Recently, we have isolated five new bis-iridoids, laciniatosides I–IV [**1–4**] and laciniatoside VI [**11**], from *D. laciniatus*. Here we report the isolation and the structure determination of laciniatosides I–IV and laciniatoside VI as well as the elucidation of the stereostructure of sylvestroside IV [**8**] which was not given by Jensen *et al.* (2).

RESULTS AND DISCUSSION

The above-ground parts of *D. laciniatus* L. were extracted and gave, after purification, a glycosidic fraction. Countercurrent distribution and cc were used to separate laciniatosides I [**1**], II [**2**],l III [**3**], IV [**4**], and V [**5**], sweroside [**6**], and sylvestrosides III [**7**] and IV [**8**] from this fraction. Likewise, cantleyoside [**9**], loganin [**10**], sylvestroside IV, sweroside, and laciniatoside VI [**11**] were isolated from the roots of the same plant.

On the basis of ^{1}H- and ^{13}C-nmr data, a bis-iridoid structure was postulated for laciniatoside I [**1**]. Its ^{13}C-nmr spectrum (Table 1) also contained all the signals corresponding to secologanic acid and reported previously for unit A of sylvestrosides III [**7**] and IV [**8**] (2). Two sets of signals for unit B, with slightly different intensity ratios, were observed in the nmr spectra of **1**, recorded in several solvents; thus compound **1** occurs in solution as a mixture of two isomers. The nmr spectra gave no evidence for unsaturation in unit B.

Acetylation of laciniatoside I [**1**] gave a pentaacetyl derivative suggesting a hydroxyl group in unit "B." The signal of the C-4 atom of the pentaacetylated derivative showed an upfield shift of 3.6 ppm when compared to that of the same atom in laciniatoside I. This shift is in agreement with the presence of the hydroxyl group at C-3 position. The structure of laciniatoside I [**1**] (Scheme 1) was proposed on the basis of the above-mentioned data, and it was further confirmed by chemical reactions and spectroscopic study of the reaction products.

Reduction of laciniatoside I [**1**] by $NaBH_4$ in MeOH followed by acidic intramo-

TABLE 1. ^{13}C-nmr Chemical Shifts of Compounds Studied.[a]

Compound		Carbon											
		C-1	C-2	C-3	C-4	C-5	C-6	C-7	C-8	C-9	C-10	C-11	Me
1 Major	A	96.9	—	153.0	110.1	27.4	44.9	201.7	134.8	44.9	120.4	167.0	—
	B	64.2	—	96.6	53.4	39.2	38.0	77.7	38.5	43.8	12.5	174.3	52.0
	Glu	99.8	74.3	77.8	71.4	77.8	62.8						
1 Minor	B	57.5	—	91.2	49.3	31.9	39.8	77.3	38.2	43.4	12.5	172.5	51.9
1 (Ac)$_5$	A	97.5[b]	—	152.3	110.8	26.3	43.6	201.0	134.1	44.6	120.9	166.7	
	B	64.9	—	94.4	49.8	39.7	38.0	78.5	37.8	44.0	12.7	173.0	52.3
	Glu	97.3[b]	71.7	72.9	69.4	73.3	62.6						
2	A	97.1	—	153.2	110.2	27.4	44.8	201.6	134.9	45.0	120.4	167.0	
	B	68.9	—	173.5	35.0	33.7	39.7	79.2	41.4	43.4	13.4		
	Glu	99.9	74.4	77.9	71.5	77.9	62.9						
3	A	97.6	—	152.0	112.8	34.9	33.7[c]	33.1[c]	36.1	48.8	20.7	167.3	
	B	70.0	—	169.6[b]	52.2	37.4	39.0	79.0	41.8	42.9	13.5	169.9[b]	52.8
	Glu	100.0	74.5	77.9	71.6	77.9	62.9						
4	A	97.3	—	153.2	111.3	30.7	33.8	60.5	135.9	44.8	118.9	167.5	
	B	70.0	—	169.6[b]	52.1	37.3	38.9	79.3	41.9	42.8	13.5	169.9[b]	52.8
	Glu	99.9	74.4	77.9	71.4	77.8	62.8						
7	A	96.9	—	153.0	109.9	27.1	44.8	201.7	134.7	44.8	120.4	166.9	
	B	96.6	—	153.0	111.4	33.3	40.2	77.2	41.1	47.9	14.3	168.2	51.4
	Glu	99.7	74.4	77.5	71.4	77.5	62.6						
8	A	97.1	—	153.5	110.1	27.5	44.9	201.6	134.9	45.0	120.4	166.9	
	B	70.0	—	169.9[b]	52.3	37.9	38.9	79.4	41.9	42.8	13.5	169.6[b]	52.9
	Glu	100.0	74.4	77.9	71.5	77.9	62.9						
10	A	97.6	—	151.8	113.9	30.7	41.3	74.9	41.0	45.8	12.9	170.6	52.6
	Glu	99.5	73.6	76.6	70.5	77.2	61.6						
11	A	195.6	—	91.4	51.5	32.1[b]	32.1	99.6	154.2	143.2	15.0	172.8	54.9
	B	97.3	—	151.7	112.0	32.2[b]	40.3	77.8	40.3	46.5	13.7	167.8	51.4
	Glu	100.0	74.5	77.8	71.5	77.8	62.8						
11 (Ac)$_5$	A	195.4	—	89.6	48.0	32.0	32.9	99.9	154.3	142.5	14.9	171.6	55.5
	B	97.2	—	150.5	113.8	31.5	39.8	78.5	39.8	46.4	13.0	167.4	51.4
	Glu	96.6	71.6	73.1	69.2	72.6	62.4						
14	A	97.4	—	139.0	115.7	26.3	29.8	59.8	134.1	42.8	120.4	61.4	
	Glu	99.6	73.6	76.6	70.5	77.2	61.62						
15		64.3	—	63.5[b]	49.7	37.7	39.1	74.7	43.7[c]	43.3[c]	15.2	63.7[b]	
17 Major		64.4	—	96.5	53.6	39.2	40.4	74.2	39.6	43.0	12.3	174.5	51.8
17 Minor		57.8	—	91.2	49.8	31.7	42.5	73.7	39.2	42.4	12.4	172.5	51.7
19		69.4	—	169.3[b]	52.3	36.3	41.2	75.7	41.2[c]	42.4[c]	12.7	168.8[b]	52.9
21	A	98.1	—	152.2	113.3	33.6	32.1	33.2	35.7	48.6	20.1	171.2	52.6
	Glu	99.7	73.6	76.6	70.5	77.2	61.6						

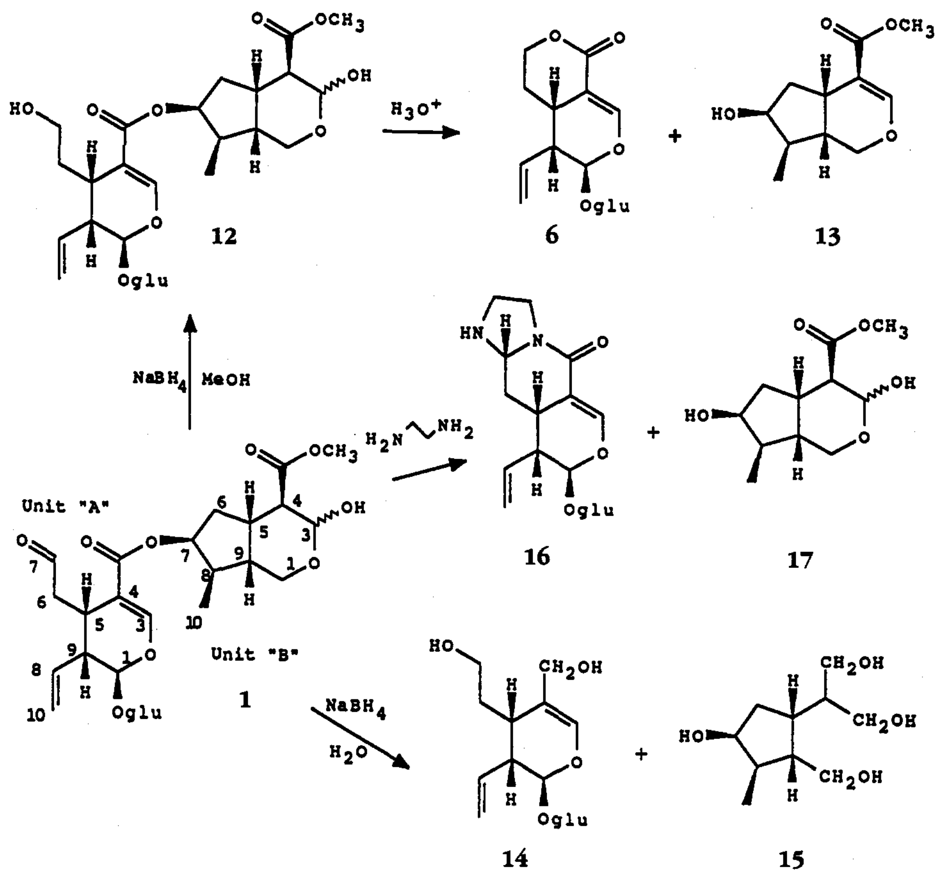
[a]Chemical shifts in ppm versus internal TMS; all spectra were recorded in Me_2CO-d_6 except the spectra of compound **19**, which was measured in $CDCl_3$, and compounds **10**, **14**, and **21**, which were measured in D_2O.

[b,c]Assignment may be interchanged.

lecular transesterification and dehydration yielded sweroside [**6**] and 1-deoxyloganin aglycone [**13**]. The singlet at 7.62 ppm in the ^{1}H-nmr spectrum of the latter compound and the ir absorption bands at 1700 cm^{-1} ($\nu_{C=O}$) and at 1640 cm^{-1} ($\nu_{C=C}$) gave evidence for an olefinic bond conjugated with the carbonyl at C-4. The dehydrated product **13** could be derived from the unit B of **1** containing a hemiacetalic hydroxyl group at C-3. Reduction of compound **1** in aqueous solution provided two products, **14** from unit A and **15** from unit B. Compound **15** was also obtained from loganin aglycone [**18**] (Scheme 3) under the same conditions. This fact proved that the configurations at C-5, C-7, C-8, and C-9 of unit B in compound **1** are the same as in loganin.

Aminolysis of laciniatoside I [**1**] with ethylenediamine resulted in formation of two products. The products were the tricyclic compound **16** from unit A and bicyclic compound **17** from unit B. Compound **16** was also obtained from secologanin under the same conditions, further supporting the structure of unit A of laciniatoside I.

The structure and the conformation of compound **17** were a subject of detailed nmr studies. Optimal spectral dispersion with minimal signal overlapping was obtained in



SCHEME 1

a 1:1 mixture of $CDCl_3$ and C_6D_6. In this solvent mixture, the intensity ratio of two sets of signals was 77:23. The unambiguous assignment of the ^{1}H- and ^{13}C-nmr spectra of this isomeric mixture was based on a 2D heteronuclear chemical shift correlation measurement. The characteristic vicinal coupling constants of the ^{1}H-nmr spectrum of both isomers were determined and are summarized in Table 2. The $J_{3,4}$ and $J_{4,5}$ values suggested that the methoxycarbonyl group was β-equatorial in both isomers, while the position of the hydroxy group was α-equatorial in the major form and β-axial in the minor one. Molecular mechanics calculations were used to study this conformation. Structure I has been found at the lowest energy state for the major isomer and structure II for the minor one (Figure 1). The dihedral angles of the hydrogens of the tetrahydropyran ring were determined for these two structures. From these values the coupling constants were calculated using modified Karplus equations (4) (Table 2). The good agreement between measured and calculated coupling constants proved that in solution the dominant conformations of the isomers are as indicated above. The ^{13}C-nmr results (Table 1) were in agreement with these structures, because, compared to the major isomer, the minor isomer showed significant upfield shift of C-1 and C-5, due to the γ-steric effect of the axial hydroxy group.

The ^{1}H- and ^{13}C-nmr spectra of laciniatoside II suggested a bis-iridoid structure. In the ^{13}C spectrum (Table 1), a set of signals with the same chemical shifts as in unit A of laciniatoside I suggested secologanic acid as one of the building blocks, together with nine more carbon signals which could be attributed to unit B. There were no peaks due

TABLE 2. ^{1}H-nmr Chemical Shifts and Coupling Constants of Isomers of Compounds **17**^{a}.

Proton	Major isomer	Minor isomer
H_{α}-1	3.75 dd	3.43 dd
H_{β}-1	3.68 dd	4.12 dd
H-3	4.75 d	5.29 d
H-4	2.16 dd	2.29 dd
H-5	2.41 dtd	2.77 dtd
H-7	3.99 td	3.95 td
H_3-10	0.86 d	0.90 d
Coupling constant		
$J_{1\alpha,9}$	3.3 [2.5]	2.1 [2.5]
$J_{1\beta,9}$	4.1 [5.0]	3.9 [5.2]
$J_{1\alpha,1\beta}$	12.3	11.9
$J_{3,4}$	8.4 [9.6]	3.4 [2.8]
$J_{4,5}$	11.9 [11.4]	11.8 [11.7]

^{a}δTMS=0.00 ppm; J [Hz]; measured in $CDCl_3$+C_6D_6 at 250 MHz; calculated coupling constants in brackets.

to methoxycarbonyl group or a double bond among these signals. The peak at 172 ppm was assigned to a lactone carbonyl carbon. From these data, structure **2** was postulated for laciniatoside II (Scheme 2). This was confirmed by chemical reactions and spectroscopic studies of the reaction products. The reaction of **2** with ethylenediamine provided two compounds, **16** from unit A and **20** from unit B (Schemes 1 and 2). The presence of the tricyclic compound **16** among the reaction products indicated that unit A of laciniatoside II is identical with unit A of laciniatoside I. Compound **20** was subjected to detailed ^{1}H-nmr studies. The chemical shifts and coupling constants are summarized in Table 3. Assignment of the diastereotopic hydrogens of the methylene groups was based on nOe measurement (the results are listed in Table 4). The data are in full agreement with the proposed structure of compound **20**. The conformation of this compound was also studied by molecular mechanics calculations. The lowest energy was calculated for a structure (shown in Figure 2) in which the six-membered ring is in $^{1}B^{4}$ conformation (boat with C-1 and C-4 above the plane of the other four atoms of the ring) and the five-membered ring is in E^{7} (envelope with C-7 above the plane of the other four atoms of the ring). ^{3}J Coupling constants were calculated from the dihedral angles of the vicinal hydrogens of this structure, using the modified Karplus equation (4). The calculated and the measured coupling constants are listed in Table 3. The good agreement proves that the same dominant conformation of compound **20** exists in

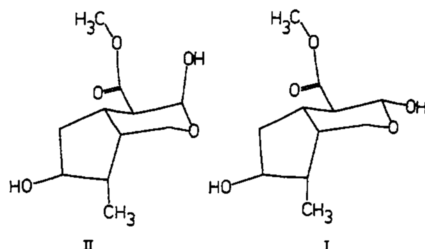
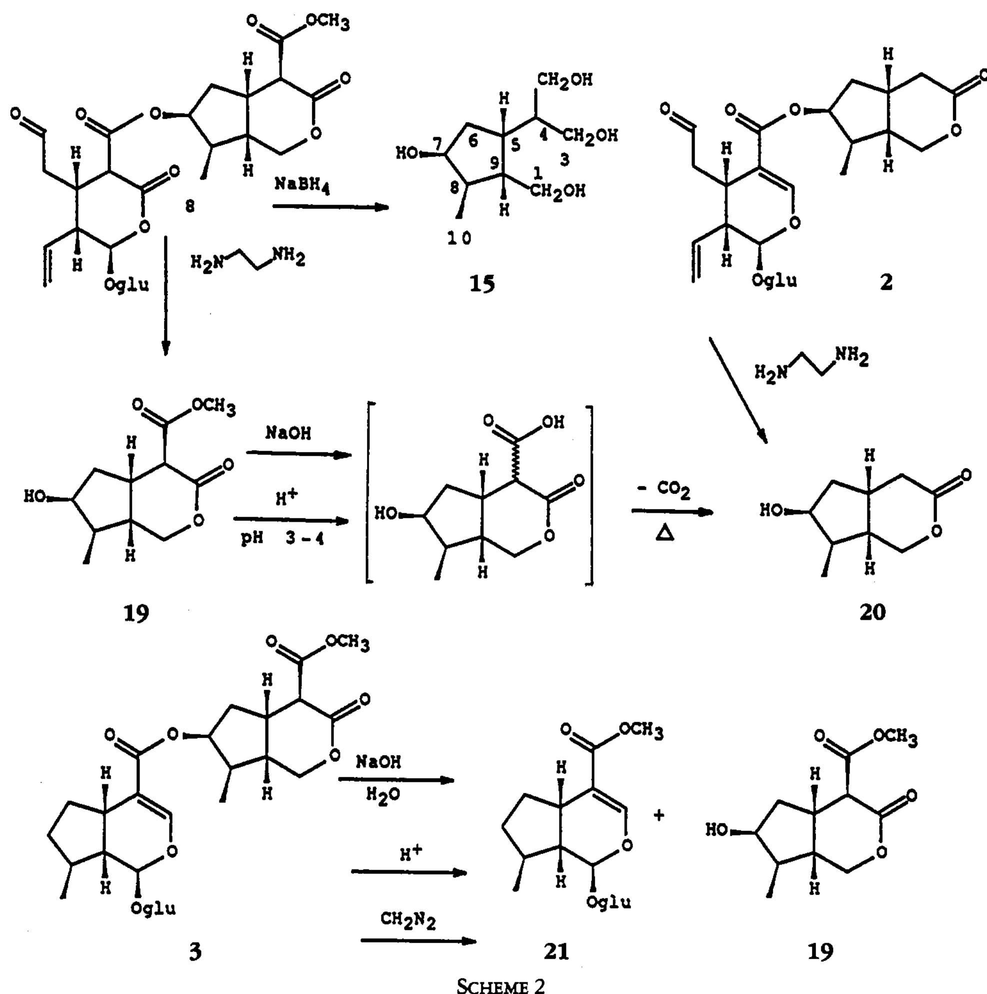


FIGURE 1. The minimal energy structures of the two equilibrium isomers of compound **17** obtained by molecular mechanics calculations.



SCHEME 2

solution too. The significant nOe interaction measured between $H_β$-1 and $H_β$-4 (Table 4) also agrees with the $^1B^4$ conformation of the lactone ring. In a study using various spectroscopic techniques, X-ray crystallography, and molecular mechanics calculations (5), the same boat conformation was found to be dominant in each of the 4 isomers of an analogous iridolactone. The boat conformation is usually considered to have higher energy than the chair form. However, in the lowest energy conformation of these compounds, O-2, C-3, and C-4 of the lactone ring are necessarily planar. The cis ring juncton gives another planar constraint on the site opposite to the lactone ring, and these two planes form the boat conformation.

We first reported the structure of laciniatoside II in 1984 (6). In 1985, Murai *et al.* (7) reported isolation of a compound having the same structure from *Abelia grandiflora* and named it abeloside B. We conclude that the name laciniatoside II has priority.

The ^{1}H- and ^{13}C-nmr spectra of laciniatosides III [**3**] and IV [**4**] were recorded, and a 2D ^{13}C-^{1}H heteronuclear chemcial shift correlation measurement of compound **3** was used for unambiguous assignment of signals (Table 1). The ^{13}C-nmr spectra of both compounds contained signals corresponding to values reported for unit B of sylvestroside IV [**8**] (2). The rest of the signals in the spectra of laciniatosides III and IV corresponded to chemical shifts reported for deoxyloganin [**21**] (8) and for unit A of laciniatoside V

TABLE 3. ^{1}H-nmr Chemical Shifts and Coupling Constants of Compounds **19** and **20**.[a]

Proton	Compound **19**	Compound **20**
H_α-1	4.18	4.15
H_β-1	4.39	4.32
H_α-4	3.61	2.38
H_β-4	—	2.65
H-5	3.10	2.95
H_α-6	1.47	1.42
H_β-6	2.05	2.06
H-7	4.11	4.13
H-8	1.82	1.93
H-9	2.26	2.16
H_3-10	1.06	1.08
Coupling constant		
$J_{1\alpha,1\beta}$	11.5	11.7
$J_{1\alpha,9}$	9.3 [11.6]	3.4 [1.3]
$J_{1\beta,9}$	5.7 [5.0]	4.0 [2.5]
$J_{4\alpha,4\beta}$	—	15.0
$J_{4\alpha,5}$	9.5 [11.6]	3.9 [1.0]
$J_{5,6\alpha}$	9.8 [10.3]	10.3 [9.0]
$J_{5,6\beta}$	7.7 [8.4]	8.3 [9.4]
$J_{5,9}$	11.1 [10.3]	10.3 [10.2]
$J_{6\alpha,6\beta}$	13.2	13.8
$J_{6\alpha,7}$	3.6 [4.7]	3.5 [5.0]
$J_{6\beta,7}$	1.2 [1.1]	1.0 [1.0]
$J_{7,8}$	3.5 [4.3]	3.8 [4.0]
$J_{8,9}$	9.5 [9.8]	10.1 [10.6]

[a]Compound **19** measured in Me_2CO-d_6 at 250 MHz; compound **20** measured in $CDCl_3$ at 250 MHz; chemical shifts in ppm referred to internal TMS; coupling constants in Hz; calculated coupling constants in brackets; α and β denote stereochemical position of relevant hydrogens.

[**5**] (3), respectively. Structures **3** and **4** were assumed, and these structures were further supported by chemical reactions and spectroscopic investigations of the reaction products. The ester bond connecting the two units of laciniatoside III [**3**] was cleaved by basic hydrolysis in aqueous solution. After acidification to pH 3–4, a mixture of two carboxylic acids was isolated and then treated with CH_2N_2. Cc of the reaction mixture gave deoxyloganin [**21**] and compound **19** (Scheme 2). Reduction of sylvestroside III [**7**] and sylvestroside IV [**8**] by $NaBH_4$ in MeOH led to laciniatosides V [**5**] and IV [**4**], respectively (Scheme 3). Intramolecular transesterification of **4** yielded sweroside [**6**] and compound **19**. Similar treatment of **5** gave sweroside [**6**] and loganin aglucone [**18**].

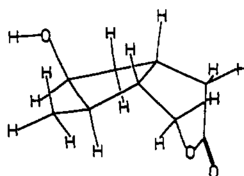
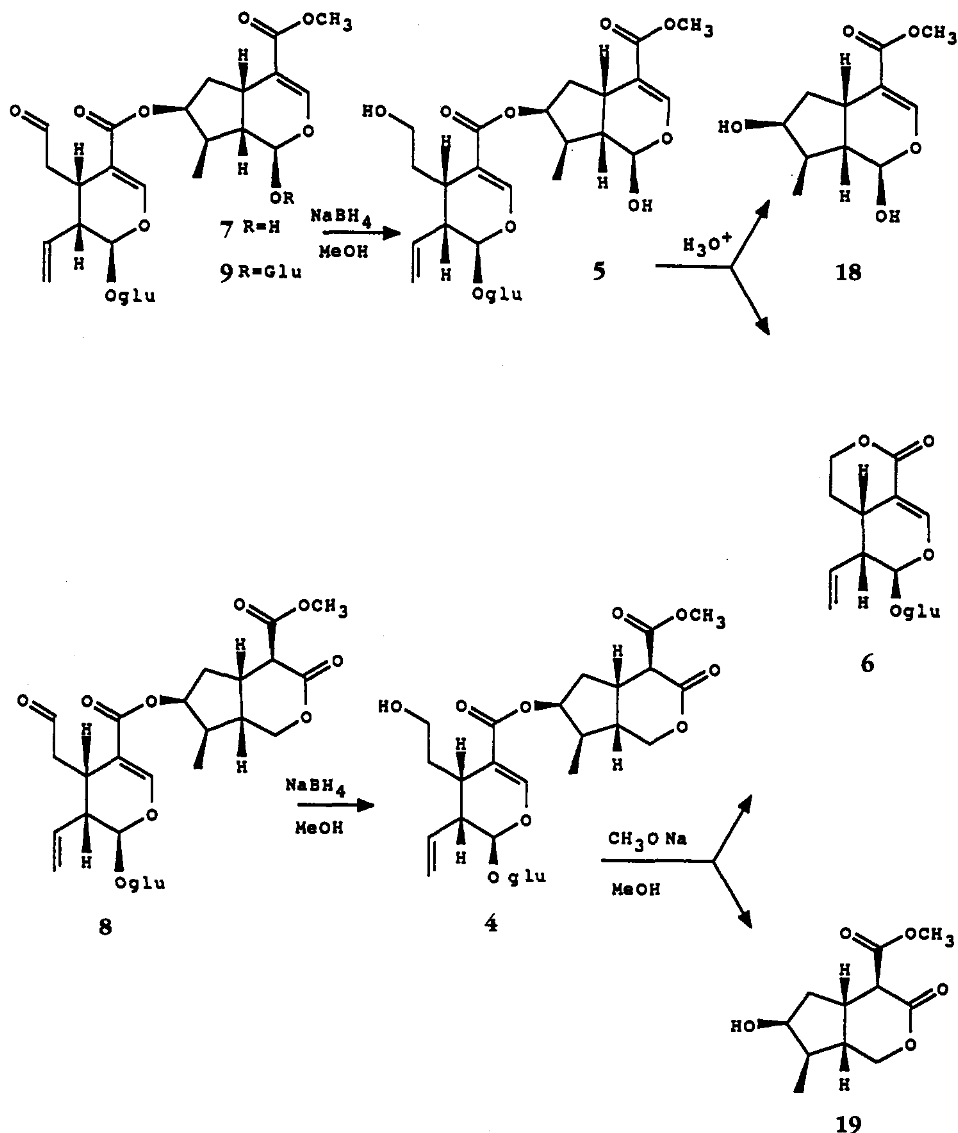


FIGURE 2. The minimal energy structure of compound **20** obtained by molecular mechanics calculations.



SCHEME 3

As the reactions presented above proved that compound **19** was derived from unit B of the three bis-iridoids laciniatoside III [**3**], laciniatoside IV [**4**], and sylvestroside IV [**8**], detailed chemical and spectroscopic studies were performed to elucidate its stereostructure. The reaction of **8** with $NaBH_4$ in aqueous solution cleaved the ester bond between units A and B and simultaneously reduced unit B to compound **15** (Scheme 2). As this compound was obtained from **18** under the same reaction conditions, the configurations of the stereocenters C-5, C-7, C-8, and C-9 of compound **19** and loganin **10** are identical. Compound **19** was hydrolyzed and decarboxylated in mild acidic solution to give compound **20**, the unit B of laciniatoside II. The facile decarboxylation indicated the presence of a β-dicarboxylic acid structure. Full assignment of the ^{1}H-nmr spectrum of compound **19** is reported in Table 3. Unambiguous identification of the diastereotopic protons of the H_2-1 and H_2-6 methylene groups and the steric position of H-4 were obtained from 1D and 2D nOe measurements (Table 4).

TABLE 4. Results of nOe Measurements on Compounds **19** and **20**.[a]

Irradiated at	NOe intensity enhancements [%]	
	19	**20**
H_α-1	H_β-1, 15.3; H-4, 5.4; H_α-6, 1; H-8, 3	
H_β-1	H_α-1, 14.9; H-4, −0.8; H-9, 1.8	H_α-1, 7; H_α-4, −0.1; H_β-4, 2.6; H-8, 0.6; H-9, 5.0
H_α-4	H_α-1, 2.5; H-5, 0.5; H_α-6, 2.5	H_α-1, 0.6; H_β-4, 11.8; H-5, 3.0
H_β-4		H_α-1, −0.4; H_β-1, 2.2; H_α-4, 11.8; H-5, 3.0
H-5	H_α-4, 0.4; H_α-6, −0.4; H_β-6, 2.6; H-9, 5.2	H_α-4, 2.0; H_β-4, 2.5; H_β-6, 3.1; H-9, 3.3
H_α-6	H_α-4, 5.8; H_β-6, 21.4; H-7, 5.7; H-8, 4.5	H_α-4, 2.0; H_β-6, 15.5; H-7, 3.1; H-8, 1.3; H_3-10, −0.3
H_β-6	H-5, 4.0; H_α-6, 17.9, H-7, 1.5; H_3-10, 0.3; OH, 1.5	
H-7	H_α-4, 1.0: H_α-6, 2.0; H-8, 2.5; OH, 3.4	
H-8	H_α-1, 4.5; H_α-4, 1.0; H_α-6, 4.5; H-7, 4.0; H_3-10, 7.0	
H-9	H_α-1, 0.3; H_β-1, 2.8; H-5, 4.8; H_3-10, 1.1	
H_3-10	H_α-1, 2.0; H_β-1, 2.0; H-7, 2.0; H-8, 11.5; H-9, 11.5; OH, 2.3	

[a]Compound **19** was measured in Me_2CO-d_6; compound **20** was measured in $CDCl_3$.

The conformation of this compound was studied by molecular mechanics calculations. In the conformer of lowest energy, the five-membered ring is in the E^7 conformation, while the lactone ring takes up a $_1B_4$ conformation which is necessary for the equatorial position of the 4β-methoxycarbonyl substituent (Figure 3). However, the other boat form ($^1B^4$) was found to be the most stable in compound **20**. Vicinal proton-proton coupling constants were calculated again for this structure, and the good agreement with measured values (Table 3) proved that this was also the dominant conformation in solution. The significant nOe interaction betwen H_α-1 and H-4 is a further proof of this structure in solution. The small difference (<1 ppm) between the chemical shifts of C-9 in unit B of compounds **2** and **3** also supports the equatorial position of the 4-methoxycarbonyl substituent in laciniatoside III.

The reduction of laciniatoside VI [**11**] with $NaBH_4$ in H_2O gave two products (Scheme 4). One was identical to loganin [**10**], and the other proved to be a tetraol, **22**. This suggested that **11** is a bis-iridoid glucoside having only one glucose unit. The ^1H- and ^{13}C-nmr spectra supported this assumption. The chemical shifts of signals corresponding to a loganin unit esterified at the 7-OH group were in close agreement with those in similar compounds (2,3). The remaining signals were characteristic of a

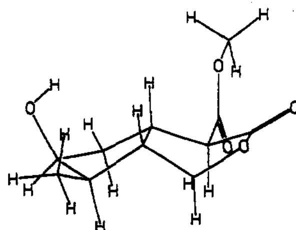
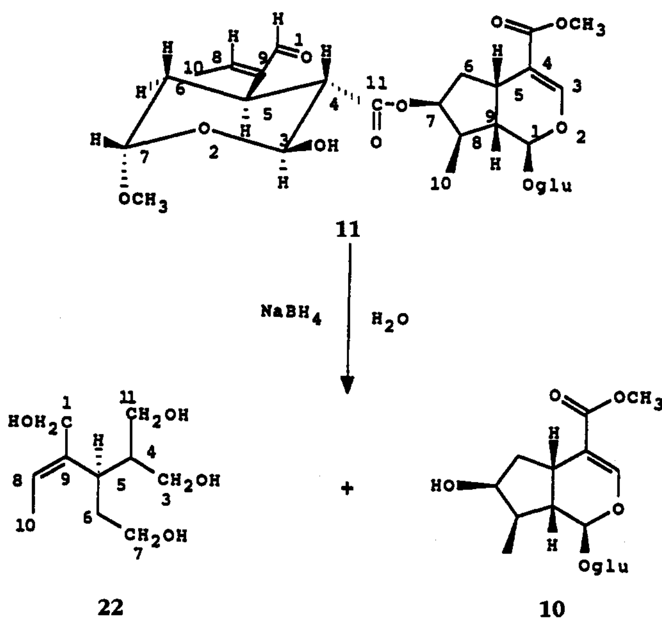


FIGURE 3. The minimal energy structure of compound **19** obtained by molecular mechanics calculations.



SCHEME 4

secologanin derivative having some special structural features: (a) there is no C-3–C-4 double bond, but a hydroxy substituent at C-3 in this unit; (b) the C-8–C-10 double bond of secologanin is rearranged to the C -8–C-9 position as indicated by the quartet at 6.9 ppm in the ^{1}H-nmr spectrum; (c) the formyl proton at 9.4 ppm showed no coupling with vicinal hydrogens. All of these observations can be explained by rearrangement of the secologanin unit via ring opening at the C-1–O-2 bond followed by ring closure between C-7 and O-2.

Total assignment of chemical shifts and coupling constants of both compounds was achieved by means of homonuclear and heteronuclear shift correlation spectra. The ^{1}H-nmr chemical shifts and coupling constants are given in Table 5, and the ^{13}C-nmr chemical shifts are listed in Table 1. A structural problem was the position of the methyl group, which could be attached either to the 7-oxygen or the 3-oxygen. In Me_2CO-d_6, the signal of H-3 was a doublet of doublets. However, adding one drop of D_2O to the solution resulted in its simplification into one doublet; therefore, one of the couplings came from the geminal OH group. Consequently, the methyl group is attached to the 7-oxygen. The ^{1}H-nmr coupling constants of unit A made possible assignment of configuration to the substituents and the conformation of the tetrahydropyran ring. The large values of the $J_{3,4}$, $J_{4,5}$, and $J_{5,6\alpha}$ indicated that the substituents on C-3, C-4, and C-5 were all equatorial. The 7-OMe substituent, however, was axial as indicated by the small value of $J_{6\alpha,7}$. The absolute configuration is based on the assumption that this unit is derived from secologanin without inversion of configuration of the C-5 atom.

^{1}H-nmr nOe measurements were used to solve the stereochemical problem of geometry around the C-8–C-9 double bond. Irradiation of H-1 caused a 26% nOe intensity enhancement of the H-8 signal, and reciprocal irradiation of H-8 induced a 30% intensity enhancement of the H-1 signal. These data clearly indicate that the configuration is *E*. The overall structure of laciniatoside VI [**11**] is shown in Scheme 4.

TABLE 5. ^{1}H-nmr Chemical Shifts and Coupling Constants of Laciniatoside VI [**11**] and its Pentaacetate.[a]

Proton	Compound					
	11			**11** (Ac)$_5$		
	A	B	Glu	A	B	Glu
H-1	9.42	5.21	4.68	9.43	5.23	5.08
H-2				3.13		4.92
H-3	5.17	7.37	3.2–3.6	6.12	7.33	5.30
OR-3	5.69		3.2–3.6	2.0		ca 5.0
H-4	ca 3.4	2.89	3.2–3.6	3.35		4.03
H-5	ca 3.3	1.58	3.86	3.5	2.84	4.30
Hα-6	1.46	ca 2.1	3.65	1.53	1.67	4.14
Hβ-6	2.25	5.04		2.28	ca 5.0	
H-7	4.84	ca 2.1		4.9	ca 2.0	
OMe-7	3.35			3.49		
H-8	6.86	ca 2.1			ca 2.0	
H-9		ca 2.1			ca 2.0	
H_3-10	2.01	1.02			1.02	
Coupling constant						
$^3J_{1,2}$	—	—	8.0	—	—	8.2
$^3J_{1,9}$	—	5.4	—	—	3.7	—
$^4J_{1,5}$	2.0		—	1.8		—
$^3J_{2,3}$		—	—	—	—	9.7
$^3J_{3,4}$	8.4	—		9.1	—	9.7
$^4J_{3,5}$		1			1.3	
$^3J_{4,5}$		—		11.9	—	10.1
$^3J_{5,6\alpha}$	4.0	8.6		4.0	6.9	4.6
$^3J_{5,6\beta}$	13.2	8		12.8	7	2.4
$^3J_{5,9}$	—	8.9	—	—	7	—
$^2J_{6\alpha,6\beta}$	13.2	14.4		13.4	14.8	12.4
$^3J_{6\alpha,7}$	1.0	4.7	—	1.2	4.8	—
$^3J_{6\beta,7}$	3.5	1	—	3.6		—
$^3J_{7,8}$	—	5	—	—		—
$^3J_{8,9}$	—	8.9	—	—		—
$^3J_{8,10}$	7.2	6.9	—	7.0		—
$^3J_{3,OH}$	7.5	—		—	—	

[a]In deuteroacetone at 300 MHz. α,β denote the stereochemical position of the relevant hydrogens.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Nmr spectra were recorded on a Bruker AM-300 spectrometer at 300 MHz (^{1}H) and 75 MHz (^{13}C), on a Bruker AC-250 spectrometer at 250 MHz (^{1}H) and 62 MHz (^{13}C), on a Jeol PS-100 spectrometer at 100 MHz (^{1}H), or on a Bruker WP-80 spectrometer, at 80 MHz (^{1}H) and 20.1 MHz (^{13}C). Internal TMS was used for chemical shift reference. The homonuclear shift correlation (COSY) spectra were measured with the standard microprogram of the Bruker DISNMR software. A 45° mixing pulse, sine-squared apodization in both dimensions, and matrix symmetrization were employed. 2DC nOe spectra were measured with the standard Bruker (NOESY) microprogram. Heteronuclear shift correlation spectra were also measured using standard software, employing polarization transfer from ^{1}H to ^{13}C and polarization transfer and rephasing delays of 3.6 msec and 2.2 msec, respectively. Negative exponential apodization was used in both domains. Molecular mechanics calculations were performed on a 386 PC using the program Alchemy II. The same lowest energy structures were obtained starting the calculations from several conformations. Molar rotations for new compounds were measured at 546 nm (Hg), for known compounds at 583 nm (Na) on a Carl Zeiss Polamat A instrument and on a Carl Zeiss polarimeter, respectively.

ISOLATION OF GLUCOSIDES.—*D. laciniatus* was collected at Szentendre Island, Hungary, in July 1986 and identified at the Botanical Institute of Eötvös Loránd University, Budapest. Voucher specimens (no.

582) are deposited in the Herbarium of the Research Institute of Medicinal Plants, Budakalász, Hungary (Pilis Gene Reservation collection). Fresh aerial parts of second-year *D. laciniatus* (4800 g) were chopped into small pieces and extracted with aqueous EtOH (70%, 14 liters). The extract was concentrated in vacuo to an aqueous suspension (4.0 liters), shaken with Al_2O_3 (Woelm Neutral, 480 g) for 30 min, and filtered. The solution was extracted with Et_2O (3×400 ml), concentrated in vacuo to 400 ml, diluted by slow addition of Me_2CO under vigorous shaking (4 liters), and let stand in a refrigerator for 24 h. The viscous lower phase was separated and the upper phase evaporated to dryness. The residue (105 g) was divided into fractions A, B, C, D, E by counter current distribution (ccd) in a two-phase system, CH_2Cl_2-MeOH-H_2O (14:15:7). The apparatus has 165 tubes with 100-ml phase volumes for the organic stationary and aqueous mobile phases. The number of transfers was 365. Organic phases of tubes 146–165 and eluted aqueous fractions 153–190 were combined and afforded residue E (1.6 g) after evaporation in vacuo. Likewise, eluted fractions 22–33, 34–45, 69–92, and 93–122 gave residues A (10.6 g), B (11.0 g), C (11.2 g), and D (6.3 g), respectively.

ISOLATION OF SYLVESTROSIDE III [7], LACINIATOSIDE I [1], AND SWEROSIDE [6].—Fraction A was chromatographed on Si gel (400 g) with CH_2Cl_2-MeOH-H_2O (320:50:5). Fractions 30–37 (each 90 ml) were combined and afforded, after evaporation in vacuo, a mixture of **7**, **1**, and **6** (1.5 g) which were separated by repeated cc on Si gel (200 g) with EtOAc-iPrOH-H_2O (200:18:9) (each fraction 40 ml). Fractions 22–27 gave sylvestroside III (0.40 g), and fractions 30–37 gave laciniatoside I (0.90 g).

Fractions 38–55 (0.23 g) were combined, evaporated, and rechromatographed on Si gel (24 g) with EtOAc-iPrOH-H_2O (20:5:2) (each fraction 4 ml). Fractions 18–22 afforded sweroside [**6**] (0.18 g), identical to an authentic specimen (^{1}H nmr). Sylvestroside III [**7**]: $[\alpha]^{22}$D −86° (c=0.5%, MeOH); uv (MeOH) 227 nm (log ϵ=4.26); ir (KBr) 3600–3000, 1705, 1695, 1630 cm^{-1}; ^{1}H nmr (Me_2CO-d_6, 300 MHz) 9.72 (t, $J_{6,7}$=1.6, H-A7), 7.56 (d, $J_{3,5}$=1.5, H-A3), 7.40 (d, $J_{3,5}$=1.5 H-B3), 6.47 (d, $J_{1,OH}$=6.4, B1-OH), 5.63 (ddd, $J_{8,9}$=9.2, $J_{8,10E}$=10.3, $J_{8,10Z}$=17.3, H-A8), 5.49 (d, $J_{1,9}$=4.8, H-A1), 5.28 (ddd, $J_{10E,10Z}$=1.8, $J_{9,10Z}$=0.7, H_Z-A10), 5.22 (dd, H_E-A10), 5.21 (td, $J_{6\alpha 7}$=5.1, $J_{7,8}$=5.1, $J_{6\beta,7}$=1.5, H-B7), 5.00 (t, $J_{1,9}$=6.4, H-B1), 4.73 (d, $J_{1',2'}$=7.8, H-1), 4.3–4.7 (m, -2′, -3′, -4′, -6′-OH), 3.88 (dd, $J_{6'a,6'b}$=12.0, H_a-6′), 3.66 (s, OMe), 3.7–3.25 (m, H-B5, H-2′, -3′, -4′, -5′, -H_b-6′), 2.79 (ddd, $J_{6a,6b}$=17.5, $J_{5,6a}$=6.0, H_a-A6), 2.75 (ddd, $J_{8,9}$=9.2, $J_{5,9}$=6.0, H-A9), 2.48 (ddd, $J_{5,6b}$=7.5, H_b-A6), 2.30 (ddd, $J_{6\alpha,6\beta}$=14.1, $J_{5,6\beta}$=7.3, H_β-B6), 2.14 (qd, $J_{8,9}$=7.1, $J_{8,10}$=7.1, H-B8, 1.91 (ddd, $J_{5,9}$=8.5, H-B9), 1.65 (ddd, H_α-B6), 1.08 (d, H_3-B10). Found C 53.02, H 6.30; $C_{27}H_{36}O_{14}$·1.5H_2O requires C 53.04, H 6.43%. Laciniatoside I [**1**]: $[\alpha]^{24}{}_{546}$ −53° (c=1.40%, MeOH); uv (EtOH) 237.5 nm (log ϵ=4.04); ir (KBr) 3650–3100, 1720, 1695, 1630 cm^{-1}; ^{1}H nmr (D_2O, 100 MHz) 9.67 (t, $J_{6,7}$=1.0, H-A7), 7.62 (d, $J_{3,5}$=1.0, H-A3), 6.0–5.1 (m, H-A8, H_2-A10, H-A1, H-B7), 4.96 (d, $J_{3,4}$=8.0, H-B3), 4.90 (d, $J_{1,9}$=7.0, H-A1), 4.15–3.9 (m, H_2-B1), 3.85 (s, OMe), 0.97 (d, $J_{8,10}$=6.0, H_3-B10). Found C 55.16, H 6.62; $C_{27}H_{38}O_{14}$ requires C 55.32, H 6.55%.

ISOLATION OF SYLVESTROSIDE IV [**8**], LACINIATOSIDE II [**2**], AND LACINIATOSIDE IV [**4**].—Fraction C was chromatographed on Si gel (800 g) with CH_2Cl_2-MeOH-H_2O (320:50:5). Fractions 10–12 (each 300 ml) were combined and evaporated in vacuo to afford sylvestroside IV [**8**] (5 g): $[\alpha]^{22}$D − 58° (MeOH, c=0.5%); uv (MeOH) 236 nm (log ϵ=3.99); ir (KBr) 3600–3000, 1750, 1730, 1700, 1630 cm^{-1}; ^{1}H nmr (Me_2CO-d_6, 100 MHz) 9.72 (t, $J_{6,7}$=1.0, H-A7), 7.60 (d, $J_{3,5}$=1.5, H-A3), 5.46 (d, $J_{1,9}$=5.0, H-A1), 4.7 (d, $J_{1,2}$=8.0, H-1′), 4.37 (m, H_2-B1), 3.70 (d, $J_{4,5}$=9.3, H-B4), 2.35 (m, H-B9), 1.04 (d, $J_{8,10}$=7.0, H_3-B10), 3.72 (s, OMe). Found C 55.39, H 6.32; $C_{27}H_{36}O_{14}$ requires C 55.48, H 6.31%. Fractions 13–15 were concentrated to afford residue C1 (2.0 g). Likewise, fractions 16–19 and 20–27 gave, after concentration in vacuo, residues C2 (0.67 g) and C3 (0.53 g), respectively. Residue C1 was chromatographed on Si gel (220 g) with CH_2Cl_2-MeOH-H_2O (320:50:5). Fractions 10–14 (each 100 ml) were combined, evaporated, and rechromatographed on Si gel (100 g) with EtOAc-iPrOH-H_2O (80:10:5). Fractions 13–14 (each 70 ml) were combined and evaporated in vacuo to afford 260 mg amorphous, solid, colorless laciniatoside II [**2**]: $[\alpha]^{25}{}_{546}$−57° (c=0.98%, MeOH); uv (MeOH) 236 nm (log ϵ=3.99); ir (KBr) 3600–3000, 1720, 1690, 1620 cm^{-1}; ^{1}H nmr (D_2O, 100 MHz) 9.80 (t, $J_{6,7}$=1.5, H-A7), 7.76 (d, $J_{3,5}$=1.5, H-A3), 5.67 (d, $J_{1,9}$=6.0, H-A1), 5.8–5.1 (H-B7, H-A8, H_2-A10), 4.79 (d, $J_{1,2}$=7.5, H-1′), 4.6–4.1 (H_2-B1), 0.96 (d, $J_{8,10}$=6.0, H_3-B10). Found C 57.03, H 6.52; $C_{25}H_{34}O_{12}$ requires C 57.10, H 6.52 %.

Residue C2 was chromatographed on Si gel (100 g) with CH_2Cl_2-MeOH-H_2O (320:50:5). Fractions 32–35 (each 20 ml) were combined, evaporated, and rechromatographed on Si gel (50 g) with the same mobile phase. Fractions 14–18 (each 15 ml) were combined, evaporated, and chromatographed again on Si gel (25 g) with EtOAc-iPrOH-H_2O (80:10:5). Likewise, fractions 25–29 (each 5 ml) afforded a colorless solid foam (0.12 g), laciniatoside IV [**4**]: $[\alpha]^{26}{}_{546}$ −101° (c=0.43%, MeOH); uv (EtOH) 237 nm (log ϵ=4.00); ir (KBr) 3650–3100, 1752, 1702, 1630 cm^{-1}: ^{1}H nmr (Me_2CO-d_6, 250 MHz) 7.57 (s, H-A3), 5.80 (m, H-A8), 5.56 (d, $J_{1,9}$=5.5, H-A1), 4.75 (d, $J_{1',2'}$=7.8, H-1′), 3.75 (s, OMe), 3.70 (d, $J_{4,5}$=9.3, H-B4), 2.87 (m, H-A5), 1.05 (d, $J_{8,10}$=6.8, H_3-B10). Found C 55.22, H 6.63; $C_{27}H_{38}O_{14}$ requires C 55.32, H 6.55%.

ISOLATION OF LACINIATOSIDE III [**3**].—Fraction E was chromatographed on Si gel (180 g) with CH_2Cl_2-

MeOH-H_2O (320:50:6). Fractions 24–29 (each 18 ml) were combined, evaporated, and chromatographed again on Si gel (100 g) with EtOAc-iPrOH-H_2O (200:18:9). Fractions 18–22 (each 20 ml) were combined and evaporated in vacuo to afford a colorless solid foam (0.41 g), laciniatoside III [**3**]: $[\alpha]^{26}_{546}$ −67° (c=0.68%, MeOH); uv (EtOH) 238 nm (log ε=4.23); ir (KBr) 3650–3100, 1748, 1700, 1630 cm^{-1}; ^{1}H nmr (Me_2CO-d_6, 250 MHz) 7.46 (d, $J_{3,5}$=1.3, H-A3), 5.23 (td, $J_{6\alpha,7}$=3.9, $J_{7,8}$=3.9, $J_{6\beta,7}$=1, H-B7), 5.19 (d, $J_{1,9}$=5.5, H-A1), 4.71 (d, $J_{1',2'}$=7.8, H-1′), 4.45 (dd, $J_{1\alpha,1\beta}$=11.5, $J_{1\beta,9}$=5.9, H_β-B1), 4.25 (dd, $J_{1\alpha,9}$=9.3, H_α-B1), 3.87 (m, H_a-6′), 3.72 (s, OMe), 3.70 (d, $J_{4,5}$=9.5, H-B4), 3.60 (m, H_b-6′), 3.5–3.2 (m, H-2′, -3′, -4′, -5′), 3.07 (qd, $J_{5,6\alpha}$=10, $J_{5,9}$=10, $J_{5,6\beta}$=8, H-B5), 2.88 (m, H-A5), 2.42 (qd, $J_{8,9}$=10, H-B9), 2.3–1.1 (m, H_2-A6, H_2-B6, H_2-A7, H-A8, H-B8, H-A9), 1.07 (d, $J_{8,10}$=6.5, H_3-A10), 1.04 (d, $J_{8,10}$=6.7, H_3-B10). Found C 57.12, H 6.69; $C_{27}H_{38}O_{13}$ requires C 56.84, H 6.73%.

ISOLATION OF LOGANIN [**10**], CANTLEYOSIDE [**9**], AND LACINIATOSIDE VI [**11**].—Fresh roots of first-year *D. laciniatus* (1200 g) were treated in the same manner as the aerial parts to give a mixture of glycosides (30 g) which was chromatographed on Si gel (500 g) with CH_2Cl_2-MeOH-H_2O (320:70:8; after fraction 30, 32:9:1). Fractions 20–25 (each 16 ml) were combined and evaporated in vacuo to afford loganin [**10**] (1.7 g), identical to an authentic specimen (^{1}H nmr).

The residue of combined and evaporated fractions 36–48 was cantleyoside [**9**] (4.4 g): $[\alpha]^{22}$D −93° (c=0.7%, MeOH); uv (MeOH) 235 nm (log ε=4.31); ir (KBr) 3600–3100, 1705, 1650 cm^{-1}; ^{1}H nmr (D_2O, 100 MHz) 9.73 (t, $J_{6,7}$=1.5, H-A7), 7.65 (s, H-B3), 7.51 (s, H-A3), 3.74 (s, OMe) 0.9 (d, $J_{8,10}$=6.5 H_3-B10). The residue of evaporated fractions 13 and 14 was rechromatographed on Si gel (200 g) with CH_2Cl_2-MeOH-H_2O (320:60:6). Fractions 24–26 (each 30 ml) were combined and evaporated in vacuo, than rechromatographed on Si gel (100 g) with isopropylether-MeOH-H_2O (100:40:11). The residue of evaporated fractions 45–49 (each 10 ml) was laciniatoside VI [**11**] (0.35 g): $[\alpha]^{27}_{546}$ −11° (c=0.2%, MeOH); uv (EtOH) 231 nm (log ε=4.31); ir (KBr) 3650–3100, 1730, 1700, 1640 cm^{-1}.

LACINIATOSIDE I PENTAACETATE.—Laciniatoside I [**1**] (200 mg) dissolved in dry pyridine (2.0 ml) was treated with Ac_2O (1.0 ml) for 2 h at room temperature. After adding MeOH (5 ml), the solution was let stand for 15 min, then evaporated to give a residue which was chromatographed on Si gel (30 g) in Et_2O-C_6H_6 (1:1). After evaporation of the solvents, laciniatoside I pentaacetate (180 mg) was isolated: $[\alpha]^{25}_{546}$ −54° (c=0.67%, $CHCl_3$); uv (EtOH) 235.5 nm (log ε=4.08); ^{1}H nmr ($CDCl_3$, 80 MHz) 8.72 (t, $J_{6,7}$=1.3, H-A7), 7.38 (d, $J_{3,5}$=2.0, H-A3), 5.89 (d, $J_{3,4}$=8.8, H-B3), 3.70 (s, OMe), 2.10, 2.05, 2.02, 2.00, 1.92 (s, OAc), 0.95 (d, $J_{8,10}$=7.0, H_3-B10).

REDUCTION OF LACINIATOSIDE I WITH $NaBH_4$ IN MeOH.—Laciniatoside I [**1**] (300 mg, 0.0005 M) was treated with $NaBH_4$ (12 mg, 0.0003 M) in MeOH (10 ml) for 10 min at room temperature. The solution, after acidification with HOAc to pH 6–7, evaporation of solvent, and purification on Si gel (20 g) in CH_2Cl_2-MeOH-H_2O (320:70:8), gave pure **12** as a colorless foam (250 mg): $[\alpha]^{26}_{546}$ −69° (c=0.85%, MeOH); uv (EtOH) 237.5 nm (log ε=4.20); ir (KBr) 3700–3100, 1740, 1705, 1632 cm^{-1}; ^{1}H nmr (CD_3OD, 100 MHz) 7.59 (s, H-A3), 5.65 (d, $J_{1,9}$=6.0, H-A1), 4.83 (d, $J_{3,4}$=8.0, H-B3), 4.78 (d, $J_{1,2}$=7.5, H-1′), 0.97 (d, $J_{8,10}$=7.0, H_3-B10).

1-DEOXYLOGANIN AGLYCONE [**13**] AND SWEROSIDE [**6**] FROM **12**.—Compound **12** (300 mg, 0.005 M) and Amberlite IR-120 resin (300 mg) in H_2O (25 ml) were refluxed with stirring for 720 min. After filtration, the solution was evaporated to give a residue which was chromatographed on Si gel (15 g) with CH_2Cl_2-MeOH-H_2O (300:28:3; after fraction 23, 320:80:9). Fractions 13–15 (each 5 ml) were combined and evaporated to afford pure **13** (71 mg). The residue of combined and evaporated fractions 30–36 was pure sweroside [**6**], identical to an authentic specimen (^{1}H nmr). 1-Deoxyloganin aglycone [**13**]: colorless, amorphous oil; $[\alpha]^{26}_{546}$ +147° (c=1.08%, $CHCl_3$); uv (EtOH) 239.5 nm (log ε=3.92); ir (KBr) 3700–3100, 1705, 1630 cm^{-1}; ^{1}H nmr ($CDCl_3$, 100 MHz) 7.60 (s, H-3), 4.18 (m, H-7), 3.75 (s, OMe), 3.14 (m, H-5), 1.12 (d, $J_{8,10}$=7.0, H_3-10).

REDUCTION OF LACINIATOSIDE I [**1**] WITH $NaBH_4$ IN H_2O.—Laciniatoside I [**1**] (300 mg, 0.0005 M) was treated with $NaBH_4$ (500 mg, 0.0115 M) in H_2O (10 ml) for 24 h at room temperature. The cooled solution was neutralized with cold 2 M HCl, charcoal (3 g) was added, and the resulting suspension was stratified on a Gooch funnel. The charcoal layer was washed with H_2O until a negative salt test was obtained, then eluted with MeOH (300 ml). After evaporation of the solvent, a crude mixture of **14** and **15** (240 mg) was obtained, which was chromatographed on Si gel (20 g) in 2-butanone saturated with H_2O. Fractions 12–16 (each 10 ml) were combined and evaporated to afford pure **15** (48 mg) as colorless powder crystallized from MeOH. Compound **15**: mp 101–103°; $[\alpha]^{26}_{546}$ +2.2° (c=0.38%, MeOH); ^{1}H nmr (D_2O, 100 MHz) 4.09 (m, H-7), 3.1–3.75 (m, H_2-1, -3, -11), 1.3–2.25 (m, H-4, -5, H_2-6, H-8, -9), 0.84 (d, $J_{8,10}$=7.0, H_3-10). The residue of combined and evaporated fractions 38–52 was pure **14** (163 mg), crystallized from MeOH. Compound **14**: mp 119–22°; $[\alpha]^{26}_{546}$ +72° (c=0.68%, MeOH); ^{1}H nmr (D_2O) 6.36 (s, H-3), 5.85–

5.1 (m, H-1, -8, H_2-10), 4.22 (d, $J_{11a,11b}$=12, H_a-11), 3.91 (d, H_b-11), 4.0–3.2 (m, H_2-7), 3.0–2.55 (m, H-5, -9), 2.2–1.25 (m, H_2-6).

REDUCTION OF SECOLOGANIN WITH $NaBH_4$ IN H_2O.—Secologanin (470 mg, 0.0012 M) was treated with $NaBH_4$ (460 mg, 0.012 M) in H_2O (20 ml) for 16 h at room temperature. The cooled solution, after neutralization with cold 2 M HCl and treatment with charcoal as described above, gave crude **14** which was chromatographed on Si gel (25 g) in CH_2Cl_2-MeOH-H_2O (320:80:9). The residue of combined and evaporated fractions 14–23 (each 15 ml), after crystallization from MeOH, gave pure **14** (200 mg), which was identical with compound **14** prepared from laciniatoside I [**1**].

REDUCTION OF LOGANIN AGLYCONE [**18**] WITH $NaBH_4$ IN H_2O.—Loganin aglycone [**8**] (90 mg, 0.0004 M) was treated with $NaBH_4$ (100 mg, 0.0023 M) in H_2O (5 ml) for 24 h at room temperature. The cooled solution, after neutralization with cold 2 M HCl and treatment with charcoal as described above, gave crude **15** which was chromatographed on Si gel (8 g) in CH_2Cl_2-MeOH-H_2O (320:60:7). The residue of combined and evaporated fractions 12–21 (each 5 ml) was pure **15** (54 mg, crystallized from MeOH) which was identical with compound **15** prepared from laciniatoside I [**1**].

AMINOLYSIS OF LACINIATOSIDE I [**1**] WITH ETHYLENE DIAMINE.—Compound **1** (300 mg, 0.0005 M) and ethylene diamine (0.040 ml, 0.0006 M) were refluxed in 8 ml anhydrous MeOH for 10 h. the residue was chromatographed on Si gel (16 g) in CH_2Cl_2-MeOH-H_2O (320:50:5; after fraction 10, 320:80:9). Fractions 17–21 (each 5 ml) were combined and evaporated to afford **17** (56 mg) as colorless amorphous powder: $[\alpha]^{26}_{546}$ +80° (*c*=0.41%, $CHCl_3$); ir (KBr) 3700–3100, 1738 cm^{-1}. The residue of combined and evaporated fractions 38–46 was a colorless, amorphous powder, compound **16** (160 mg): $[\alpha]^{26}_{546}$ −270° (*c*=1.18%, MeOH); uv (EtOH) 237.5 nm (log ϵ=4.21); ir (KBr) 3700–3000, 1660, 1650 cm^{-1}; ^{1}H-nmr (CD_3OD, 100 MHz) 7.13 (s, H-3), 5.58 (d, $J_{1,9}$=5, H-1), 4.23 (dd, $J_{6\alpha,7}$=10.5, $J_{6\beta,7}$=4.0, H-7), 2.74 (m, H-5), 2.19 (m, H-9), 1.7–1.1 (m, H_2-6).

AMINOLYSIS OF SECOLOGANIN WITH ETHYLENE DIAMINE.—Secologanin (230 mg, 0.0006 M) and ethylene diamine (0.040 ml) were refluxed in 10 ml anhydrous MeOH for 30 min. The residue was chromatographed on Si gel (15 g) in CH_2Cl_2- MeOH-H_2O (320:110:16). Fractions 24–29 (each 4 ml) were combined and evaporated to afford **16** (190 mg) as colorless, amorphous powder, which was identical with compound **16** prepared from laciniatoside I [**1**].

AMINOLYSIS OF LACINIATOSIDE II [**2**] WITH ETHYLENE DIAMINE.—Compound **2** (87 mg. 0.00013 M) and ethylene diamine (0.010 ml, 0.00015 M) were refluxed in anhydrous MeOH (5 ml) for 14 h. The residue was chromatographed on Si gel (5 g) in CH_2Cl_2-MeOH-H_2O (320:30:3, after fraction 10, 320:110:16). Fraction 5 (each 3 ml) was evaporated to afford **20** (15 mg), crystallized from MeOH: mp 95–97°; $[\alpha]^{26}_{546}$ +159° (*c*=0.5%, $CHCl_3$); ir (KBr) 3650–3100, 1743 cm^{-1}. The residue of combined and evaporated fractions 13 and 14 was a colorless, amorphous powder (40 mg) which was identical with compound **16** prepared from secologanin.

AMINOLYSIS OF SYLVESTROSIDE IV [**8**] WITH ETHYLENE DIAMINE.—Compound **8** (360 mg, 0.0006 M) and ethylenediamine (0.040 ml, 0.0006 M) were refluxed in 14 ml anhydrous MeOH (14 ml) for 10 h. The residue was chromatographed on Si gel (25 g) in CH_2Cl_2-MeOH-H_2O (320:30:3; after fraction 19, 320:110:16). Fractions 10–13 (each 4 ml) were combined and evaporated, affording **19** (86 mg), crystallized from EtOH: mp 144–145°; $[\alpha]^{27}_{546}$ +86° (*c*=0.83%, $CHCl_3$); ir (KBr) 3450, 1750, 1735 cm^{-1}. The residue from fractions 40–48 was a colorless, amorphous powder (200 mg) which was identical with compound **16** prepared from secologanin.

PREPARATION OF COMPOUND **20** FROM UNIT B OF SYLVESTROSIDE IV.—Compound **19** (98 mg, 0.00043 M) was treated with NaOH (45 mg, 0.0011 M) in a mixture of THF (3 ml) and H_2O (3 ml) for 18 h at room temperature. The solution was stirred with Amberlite IR-120 to adjust the pH to 4–5. After filtration the solution was refluxed for 5 h. The residue was chromatographed on Si gel (8 g) in CH_2Cl_2-MeOH-H_2O (200:10:1). Fractions 20–25 (each 2 ml) were combined and evaporated to afford 50 mg colorless powder, crystallized from MeOH, identical with compound **20** prepared from laciniatoside II [**2**] (see above).

REDUCTION OF SYLVESTROSIDE IV [**8**] WITH $NaBH_4$ IN H_2O.—Compound **8** (300 mg, 0.005 M) was treated with $NaBH_4$ (500 mg, 0.0115 M) in H_2O (10 ml) for 24 h at room temperature. Treatment as described above for reduction of laciniatoside I [**1**] gave two pure, colorless, amorphous glucosides (38 mg and 147 mg), which were identical with compounds **14** and **15**, respectively.

PREPARATION OF DEOXYLOGANIN [**21**] AND COMPOUND **19** FROM LACINIATOSIDE III [**3**].—Compound **3** (70 mg, 0.00012 M) was treated with NaOH (120 mg, 0.003 M) in H_2O (6 ml) for 64 h at room temperature, and the solution was stirred with Amberlite IR-120 to adjust the pH to 4–5. After filtration, the solution was evaporated to give a residue which was dissolved in MeOH (5 ml), cooled, and treated with

CH_2N_2 in Et_2O. The residue of the evaporated solution was chromatographed on Si gel (8 g) in CH_2Cl_2-MeOH-H_2O (320:50:5). Combined fractions 14–16 (each 3 ml) afforded, after evaporation and crystallization from EtOH, 8 mg of **19**, identical to that prepared previously from sylvestroside IV [**8**] (physical data, see above). The residue of combined and evaporated fractions 23–29 was a colorless powder which was identical with deoxyloganine [**21**] (from MeOH). Compound **21** mp 156.5–158°; $[\alpha]^{25}D$ −90° (0.23%, EtOH); uv (EtOH) (236 nm) (log ϵ=4.03); ir (KBr) 3700–3100, 1710, 1640 cm^{-1}; ^1H nmr (CD_3OD, 100 MHz) 7.4 (d, $J_{3,5}$=1.4, H-3), 5.6 (d, $J_{1,9}$=4.5, H-1), 1.1 (d, $J_{8,10}$=6.0, H_3-10).

PREPARATION OF LACINIATOSIDE V [**5**] FROM SYLVESTROSIDE III [**7**].—Compound **7** (500 mg, 0.0008 M) was treated with $NaBH_4$ (19 mg, 0.0005 M) in MeOH (10 ml) for 10 min at room temperature. The solution was acidified with HOAc at pH 6–7, evaporated, and chromatographed on Si gel (50 g) in CH_2Cl_2-MeOH-H_2O (320:50:5; after fraction 22, 320:80:9). Fractions 25–31 (each 13 ml) were combined and evaporated to afford pure **5** (30 mg) as colorless amorphous foam.

PREPARATION OF LACINIATOSIDE IV [**4**] FROM SYLVESTROSIDE IV [**8**].—Compound **8** (200 mg, 0.00033 M) was treated with $NaBH_4$ (7 mg, 0.00016 M) in MeOH (8 ml) for 10 min at room temperature. The solution was acidified with HOAc at pH 6–7, evaporated, and chromatographed on Si gel (15 g) in CH_2Cl_2-MeOH-H_2O (320:50:5). Fractions 13–18 (each 4 ml) were combined and evaporated affording pure **4** (160 mg).

PREPARATION OF LOGANINE AGLYCON [**18**] AND SWEROSIDE [**6**] FROM LACINIATOSIDE V [**5**].—Compound **5** (300 mg, 0.005 M) and Aberlite IR-120 (300 mg) in H_2O (30 ml) were stirred for 450 min at 100°. After filtering, the solution was evaporated to give a residue which was chromatographed on Si gel (15 g) in CH_2Cl_2-MeOH-H_2O (150:14:15; after fraction 16, 320:80:9). Fractions 13–16 (each 6 ml) were combined and evaporated, affording pure **18** (57 mg) as a colorless, amorphous foam. Loganin aglycone [**18**]: $[\alpha]^{26}_{546}$ +2.3° (c=1.4%, $CHCl_3$); ^1H nmr ($CDCl_3$, 100 MHz) 7.44 (s, H-3), 4.96 (d, $J_{1,9}$=6.0, H-1), 4.15 (m, H-7), 3.74 (s, OMe), 3.16 (m, H-5), 1.14 (d, $J_{8,10}$=7.5, H_3-10). The residue of combined and evaporated fractions 23–29 was pure sweroside [**6**] (170 mg).

PREPARATION OF COMPOUND **19** AND SWEROSIDE [**6**] FROM LACINIATOSIDE IV [**4**].—Compound **4** (150 mg, 0.00025 M) was treated with NaOMe (4.5 mg, 0.0008 M) in anhydrous MeOH (15 ml) for 20 h at room temperature. The solution was neutralized with HOAc (0.008 ml) and evaporated to give a crude mixture of compounds which was purified by chromatography on Si gel in EtOAc-iPrOH-H_2O (10:2:1). Fractions 4–7 (each 5 ml) were combined and evaporated affording **19** as a colorless powder (84 mg). The residue of combined and evaporated fractions 19–24 was an amorphous powder (47 mg) identical with sweroside [**6**].

LACINIATOSIDE VI PENTAACETATE.—Laciniatoside VI [**11**] (50 mg) dissolved in dry pyridine (1 ml) was treated with AC_2O (0.5 ml) for 16 h at room temperature. After adding MeOH (4 ml), the solution was left to stand at room temperature for 15 min, then evaporated to a residue which was chromatographed on Si gel (10 g) in C_6H_6-EtOAc (3:2), yielding pure laciniatoside VI pentaacetate: $[\alpha]^{26}_{546}$ −35° (c=0.68%, $CHCl_3$); uv (EtOH) 228 nm (log ϵ=4.34); ir (KBr) 1735, 1700, 1640 cm^{-1};

REDUCTION OF LACINIATOSIDE VI [**11**], WITH $NaBH_4$ IN H_2O.—Compound **11** (140 mg, 0.0002 M) was treated with $NaBH_4$ (200 mg, 0.005 M) in H_2O (10 ml) for 24 h at room temperature, than the pH of the solution adjusted with Amberlite IR-120 to 6–7. After filtration, the solution was evaporated to give a residue which was chromatographed on Si gel (15 g) in CH_2Cl_2-MeOH-H_2O (320:60:7). Fractions 8–19 (each 5 ml) were combined and evaporated to afford compound **23** (30 mg) as a colorless amorphous foam: $[\alpha]^{27}_{546}$ 0° ($CHCl_3$, c=0.22%); ^1H nmr (CD_3OD, 100 MHz) 5.78 (q, $J_{8,10}$=6.0, H-8), 4.2–3.3 (m, H_2-1, -3, -7, -11), 2.83 (m, H-5), 1.71 (m, H_3-10, H-4, H_2-6). The residue of combined and evaporated fractions 20–25 was pure loganin [**10**].

LITERATURE CITED

1. T. Sevenet, C. Thal, and P. Potier, *Tetrahedron*, **27**, 663 (1971).
2. S.R. Jensen, S.E. Lyse-Jensen, and B.J. Nielsen, *Phytochemistry*, **18**, 273 (1979).
3. B. Podányi, R.S. Reid, Á. Kocsis, and L. Szabó, *J. Nat. Prod.*, **52**, 135 (1989).
4. C.A.G. Haasnoot, F.A.A.M. de Leeuw, and C. Altona, *Tetrahedron*, **36**, 2783 (1980).
5. F. Bellesia, U.M. Pagnoni, R. Trave, G.D. Andretti, G. Bocelli, and P. Sgarabotto, *J. C hem. Soc., Perkin Trans 2*, 1341 (1979).
6. Á. Kocsis, Z. Pál, B. Podányi, L. Szabó, and P. Tétényi, *IUPAC Int. Symp Chem. Nat. Prod., 14th*, Poznan (1984).
7. F. Murai, M. Tagawa, S. Matsuda, T. Kikuchi, S. Uesato, and H. Inouye, *Phytochemistry*, **24**, 2329 (1985).
8. S. Damtoft, S.R. Jensen, and B.J. Nielsen, *Phytochemistry*, **20**, 2717 (1981).

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