2. Ladroside (= 6′-Caffeoyl-mussaenoside), a New Iridoid Glucoside from *Veronica officinalis* L. *(Scrophulariaceae)* and the Elucidation of the Absolute Configuration at C(8) of Mussaenoside[1])

by **Fatma Ü. Afifi-Yazar**[2]) and **Otto Sticher**

Eidgenössische Technische Hochschule, Pharmazeutisches Institut, ETH-Zentrum, CH-8092 Zürich

and **Shinichi Uesato, Kimiko Nagajima** and **Hiroyuki Inouye**

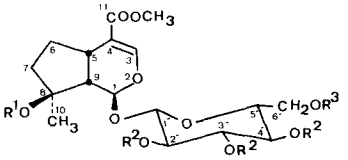
Faculty of Pharmaceutical Sciences, Kyoto University, Sakyo-ku, Kyoto, Japan

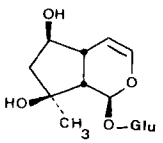
(2.X.80)

Summary

A new iridoid glucoside, named ladroside, together with mussaenoside (**1**) [2], has been isolated from *Veronica officinalis* L. The structure of ladroside (**4**) and the identity of mussaenoside have been established by spectral analysis. Additionally, the absolute configuration at C(8) carrying the tertiary hydroxyl group has been established by chemical evidence.

Introduction. – Continuing our work on iridoid glucosides from *Veronica officinalis* L. [3–5] we have now isolated mussaenoside (**1**) and a new iridoid ester, named ladroside (**4**)[3]). This paper describes the full spectral and chemical results which led to structure **4** for ladroside and established the absolute configuration at C(8) in mussaenoside (**1**) as well as in ladroside (**4**).





1 mussaenoside
$R^1 = R^2 = R^3 = H$

2 mussaenoside tetraacetate
$R^1 = H$, $R^2 = R^3 = Ac$

3 mussaenoside pentaacetate
$R^1 = R^2 = R^3 = Ac$

4 ladroside
$R^1 = R^2 = H$, R^3 = caffeoyl

5 ladroside pentaacetate
$R^1 = H$, $R^2 = Ac$, R^3 = diacetylcaffeoyl

6 ladroside hexaacetate
$R^1 = R^2 = Ac$, R^3 = diacetylcaffeoyl

7 ajugol

[1]) Presented by *O. St.* and *F. Ü. A.-Y.* at the 25. Jubiläumsvortragstagung der Gesellschaft für Arzneipflanzenforschung, Zürich, Switzerland, September 12–16, 1977; s. [1a].

[2]) Part of the thesis of *F. Ü. A.-Y.* [1b].

[3]) The name ladroside is deduced from the French expression 'herbe aux ladres' for the crude drug herba veronicae.

0018-019X/81/1/0016-09$01.00/0

Results and discussion. – The crude water-soluble part of *Veronica officinalis* L. was fractionated into five fractions (1–5) by counter-current distribution (CCD.) using EtOAc/EtOH/H_2O 403:220:390. Further chromatography of the fraction 3 followed by semipreparative scale purification (reversed phase HPLC.) afforded mussaenoside (**1**) characterized by spectral analysis, comparison with an authentic sample [2], and its acetates **2** and **3** (s. below).

The fractions 4 and 5 were further subjected to CCD. using H_2O/MeOH/EtOH/$CHCl_3$ 2:1:1:3. Repeated chromatography of the appropriate fractions over silica gel followed by preparative liquid chromatography using MeOH/H_2O 2:3 gave pure ladroside (**4**). Hydrolysis of **4** yielded mussaenoside (**1**) and caffeic acid (= 3,4-dihydroxycinnamic acid) which were identified by TLC., HPLC. and spectra (s. exper. part).

Ladroside is a yellow amorphous powder, $[\alpha]_D^{20} = -68.9°$ ($c = 0.72$, CH_3OH) with a molecular formula $C_{26}H_{32}O_{13}$. The UV. spectrum of ladroside exhibits absorption typical for iridoid glucosides with a C(4) carbomethoxy group (λ_{max} at 236 nm). The IR. spectrum (KBr) of **4** shows prominent bands at 3400 (OH), 1698 (COO), 1642 (C=C, iridoid), 1635 (C=C, caffeic acid), 1610, 1520 and 1448 cm^{-1} (aromatic ring). The ^{1}H-NMR. (100 MHz, CD_3OD) of **4** suggests an iridoid structure. The singlet of H–C(3) (observed at 7.37 ppm) is highly deshielded by the presence of a carbomethoxy group at C(4) (singlet at 3.62 ppm). Besides the signals due to 3 aromatic (at 6.78–7.02 ppm) and 2 olefinic protons (at 7.53 and 6.22 ppm, *AB*-system, $J = 16.0$ Hz, arising from the *trans*-caffeoyl moiety) a doublet is observed at 5.19 ppm, assignable to H–C(1). The assignments of the protons at 3.02–3.50 (H–C(5)) and 2.09 ppm (H–C(9)) were confirmed by appropriate decoupling experiments. A three-proton singlet at 1.28 ppm is attributed to the methyl group at C(8), deshielded by the tertiary OH-group.

A comparison of the ^{1}H-NMR. of **4** with that of **1** indicates, that the esterification with caffeic acid has taken place at HO–C(6′) of the glucose moiety since the signals due to the protons at C(6′) are shifted downfield (in **4**), and all other glucose signals are almost unchanged.

Further confirmation of the proposed structure came from the ^{13}C-NMR. spectrum (25.2 MHz) of **4** which is, apart from the signals due to the caffeoyl part, very similar to the one of mussaenoside (**1**), so that the ester linkage can't be on the iridoid moiety (s. *Table* in the exper. part). The chemical shifts of the four glucose C-atoms (C(1′), C(2′), C(3′), C(4′)) being the same in both compounds, the linkage of caffeic acid can only be at HO–C(6′). The signal for C(6′) appears in **1** at 62.55 ppm whereas in **4** this signal is shifted downfield by 1.48 ppm and the signal of the C-atom in β-position, C(5′), upfield by 2.19 ppm. In fact, this downfield shift of the signal of the α-C-atom and the upfield shift of the signal of the β-C-atoms upon acylation is well documented [6].

Acetylation of **4** under mild conditions gave after column chromatography only a crystalline ladroside pentaacetate (**5**) ($C_{36}H_{42}O_{18}$, m.p. 191.1–193.3°, $[\alpha]_D^{20} = -45.4°$ ($c = 0.83$, $CHCl_3$)), still showing hydroxyl absorption in the IR. spectrum (KBr, 3540 cm^{-1}). The ^{1}H-NMR. of **5** ($CDCl_3$, 100 MHz), with the exception

of the signals of the caffeoyl moiety, is nearly identical with the spectrum of mussaenoside tetraacetate (**2**). It shows only three acetoxy groups bound to saturated C-atoms at 1.92–2.14 ppm – a further proof for the position of the caffeoyl residue on the glucose moiety –, while the signals of the two acetoxy groups bound to aromatic C-atoms appear with the signals of one H–C(6) and H–C(9) at 2.18–2.32 ppm. The expected signal for the unacetylated hydroxyl group is visible as singlet at 1.84 ppm and disappears after shaking with D_2O. The chemical shift of the methyl group at C(8) remains nearly unchanged at 1.29 ppm in agreement with the fact that the tertiary hydroxyl group is not easy to acetylate. The three proton singlet at 3.69 ppm can be assigned to the carbomethoxy group. The same absorption for the two protons at C(6′) in **4** and **5** supports again the structure for ladroside.

In addition to these data the definitive structure for ladroside has been confirmed by the fragmentations in the MS. The MS. of ladroside pentaacetate (**5**) shows, besides the molecular ion at *m/z* 762, the fragments resulting after the cleavage of the O–C(1′) bond (*m/z* = 211, 193, 161, 139, 133, 105 and 81), the fragments arising from the glucose part esterified with caffeic acid (*m/z* = 535), and the fragments resulting from the diacetylated caffeoyl moiety (*m/z* = 264, 247, 205, 163, 135, 103 and 77).

Prolonged acetylation (48 h) of ladroside pentaacetate (**5**) with pyridine/acetic anhydride at 40° provided after column chromatography on silica gel ladroside hexaacetate (**6**), which was recrystallized from EtOH to give white crystals, $C_{38}H_{44}O_{19}$, m.p. 106–108°, $[\alpha]_D^{20} = -12.5°$ ($c = 0.62$, MeOH). In the IR. spectrum (KBr) of **6** no hydroxy group absorption appears, but significant bands are observed at 1640 (C=C), 1710 (C=O, ester) and 1760 cm^{-1} (C=O, acetyl). The UV. spectrum (EtOH) shows λ_{max} at 217 and 273 nm.

Elucidation of the configuration at C(8), carrying the tertiary hydroxyl group. (a) *By ^{13}C-NMR. spectral analysis.* The shielding caused by a quarternary C(8)-hydroxyl group to the C-atom in β-position, C(9), is a diagnostic constant for the determination of the C(8) configuration of such compounds [6] [7], the shift of $H_3C(10)$ is also very informative for that purpose [6]. Thus, comparison of the C(9) and C(10) chemical shifts of **1**, **4** and ajugol (**7**) unequivocally suggests an axial hydroxyl and an equatorial methyl group at C(8).

(b) *By IR.-spectral analysis.* Comparison of the IR. spectra in the range of 4000–2500 cm^{-1} of mussaenoside tetraacetate (**2**) and 8-epi-mussaenoside tetraacetate (**8**) shows an extra-absorption at 3605 cm^{-1} for **2** but not for **8**. We attribute this absorption to an intramolecular H-bonding between the C(8)-hydroxyl group and the anomeric proton at C(1′) of the glucose moiety. This intramolecular H-bonding is only possible when C(8) carries an axial hydroxyl group. It might be mentioned that this is the first time that the relative configuration of C(8)–OH epimeric iridoids has been determined by IR. spectroscopy.

(c) *By chemical correlation.* In order to establish the absolute structure of mussaenoside (**1**) as well as of ladroside (**4**) we correlated **1** chemically with 10-deoxygeniposide tetraacetate (**9**) whose absolute structure is known [8] [9]. Treatment of **9** with *m*-chloroperbenzoic acid gave the β,β-epoxide **10** and the α,α-epoxide **11** in 30.8 and 7.7% yield, respectively [9]. A methanolic solution

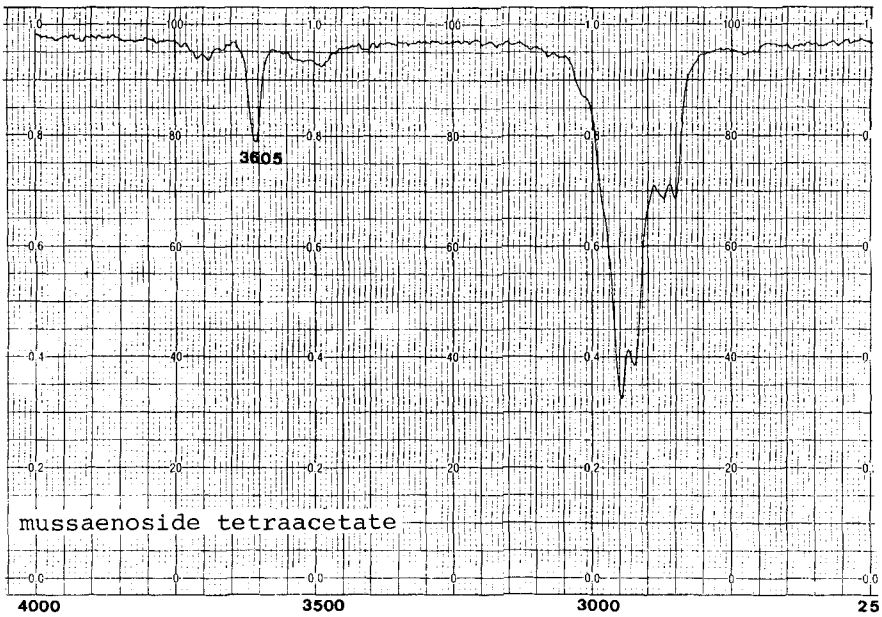


Fig. 1. *IR. spectrum of mussaenoside tetraacetate* (**2**) in the range of 4000–2500 cm^{-1} (*Perkin-Elmer* IR. spectrophotometer model 580)

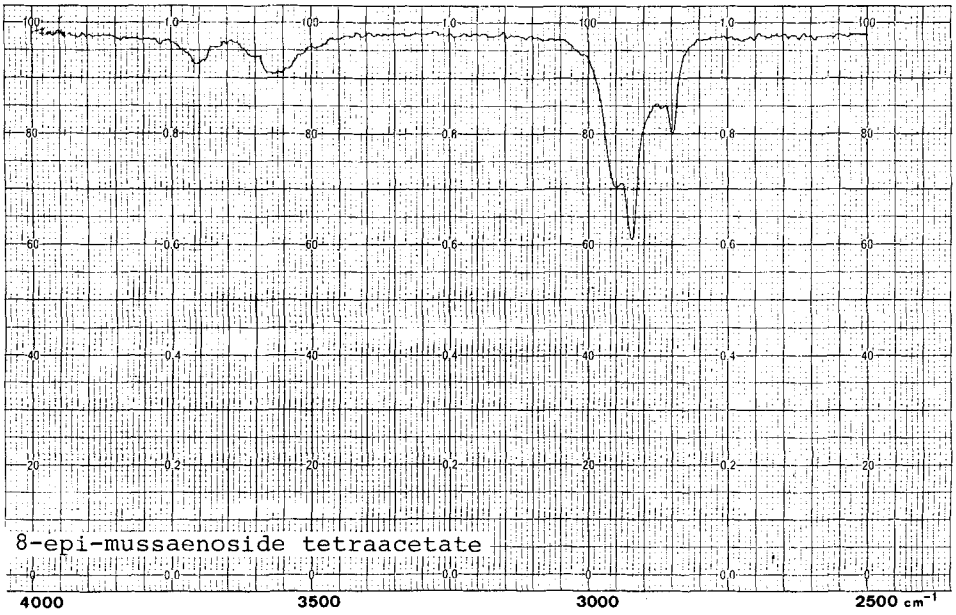
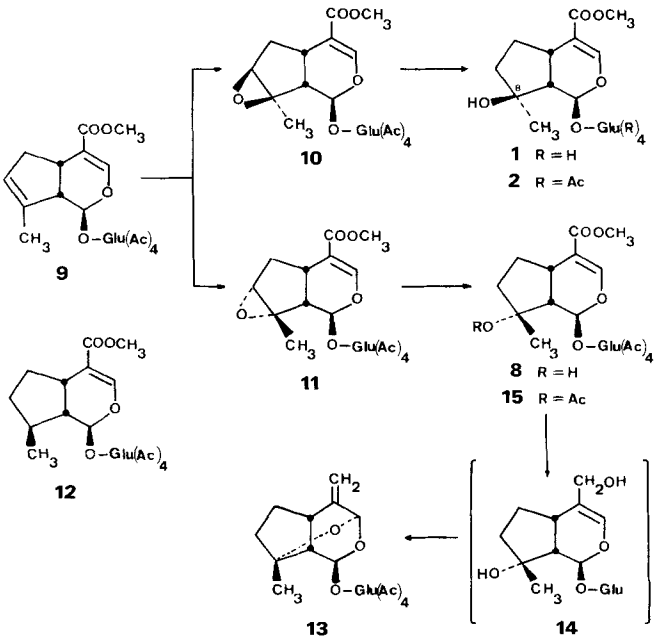


Fig. 2. *IR. spectrum of 8-epi-mussaenoside tetraacetate* (**8**) in the range of 4000–2500 cm^{-1} (*Perkin-Elmer* IR. spectrophotometer model 580)

of **10** was hydrogenated over Pd/C in the presence of aqueous $HClO_4$ solution yielding the 8β-alcohol **2** [10], the physical data of which were in accordance with those of mussaenoside tetraacetate (**2**) [2] except the melting point (8β-alcohol **2**: m.p. 86–87° [10]; mussaenoside tetraacetate (**2**): m.p. 124–126° [2]).

Thus, we prepared the 8β-alcohol **2** again by a somewhat modified method. A solution of the β,β-epoxide **10** in AcOH was hydrogenated over Pd/C giving the 8β-alcohol **2** and deoxyloganin tetraacetate (**12**), the former being contaminated with a small amount of unknown by-products. The crude 8β-alcohol **2** after a conventional workup with Ac_2O and pyridine was purified by preparative TLC. in two different solvent systems (benzene/AcOEt 6:4, ether) followed by recrystallization from ether. The 8β-alcohol **2** thus obtained showed the m.p. 134–136°, whereas mussaenoside tetraacetate (**2**) freshly recrystallized from ether showed the m.p. of 126–127°. On admixture of both substances the m.p. was 130–131°.

On the other hand, the α,α-epoxide **11** was converted to the ether **13** *via* the 8α-alcohol **8** which was not identical with mussaenoside tetraacetate (**2**) as described in the experimental part.



On the basis of the above mentioned data the structure of ladroside (**4**) is established as 6′-caffeoyl-mussaenoside and the absolute configuration at C(8) carrying an axial OH group is (*S*).

We could not detect the presence of loganin in *V. officinalis* as reported earlier by *Grayer-Barkmeijer* [11]. However, the colour reactions and TLC. behaviour of loganin and mussaenoside are almost identical; this might have misled the author in identifying the compound. Consequently, the presence of mussaenoside instead of loganin in other *Veronica* species could be expected.

Financial support of this work by the *Swiss National Science Foundation* is gratefully acknowledged. The authors wish to thank Prof. *H. Gerlach* (University of Bayreuth, FRG) and Dr. *R.K. Chaudhuri* (ETH Zürich) for helpful discussions. Thanks are also due to Dr. *Y. Kuroda* and Mrs. *R. Omoto* (Kyoto University) for measurement of ^{1}H-NMR. spectra and to the members of the Microanalytical Centre (Kyoto University) for microanalyses.

Experimental Part

General remarks. The IR. spectra of **2** and **8** were measured on a *Perkin-Elmer* IR. spectrophotometer model 580 in CCl_4 *(Uvasol, Merck)* using a NaCl-cell (thickness 1 mm). Other general procedures used by the ETH group were described in previous papers [3–5]. Only the methods and apparatus used by the Kyoto group (elucidation of the absolute configuration at C(8) of mussaenoside (**1**) are described in the following. Melting points are uncorrected. Optical rotations were measured with a *Hitachi* automatic digital polarimeter PM-201. IR. spectra were recorded on a *Hitachi* model 215 grating infrared spectrophotometer. UV. spectra were measured on a *Hitachi* ESP-3 spectrophotometer. ^{1}H-NMR. spectra were taken on a *Varian* A-60 or a *Varian* HA-100 spectrometer in $CDCl_3$ with tetramethylsilane as the internal standard. Silica gel G *(Merck)* was employed for TLC. Spots were visualized by exposure to I_2 vapor or spraying with a mixture of anisaldehyde (0.5 ml), conc. H_2SO_4 solution (0.5 ml), AcOH (a few drops) and 95% EtOH (9 ml) followed by heating. Silica gel 60 PF_{254} *(Merck)* was used for prep. TLC., where, unless otherwise specified, plates (20×20 cm, 1 mm coating) were employed. Bands were visualized by I_2 exposure or UV. light. Active charcoal *(Wako)* was used for column chromatography.

Isolation of Mussaenoside. Fraction 3 of the first *Craig* distribution [3] was chromatographed on silica gel (CH_2Cl_2/MeOH/H_2O 4:1:0.1, $CHCl_3$/EtOH 2:1, CH_2Cl_2/MeOH 9:1) and *Sephadex* (acetone). After semipreparative HPLC. (MeOH/H_2O 1:3) pure **1** was obtained as a white powder, $[\alpha]_D^{20} = -106.5°$ ($c = 0.57$, CH_3OH). – UV. (CH_3OH): 236 (3.84). – IR. (KBr): 3410, 1695, 1645. – ^{1}H-NMR. (CD_3OD): 7.39 (*s*, 1 H, H–C(3)); 5.43 (*d*, $J = 4.4$, 1 H, H–C(1)); 4.66 (*d*, $J = 8.8$, 1 H, H–C(1′)); 3.95 (*m*, 2 H, 2 H–C(6′)); 3.66 (*s*, 3 H, CH_3O); 3.00–3.44 (*m*, 1 H, H–C(5), together with the signals of the glucose protons); 2.14–2.38 (*m*, 1 H, H–C(6)); 2.24 (*d*×*d*, $J = 8.0$ and 4.4, 1 H, H–C(9)); 1.46–1.82 (*m*, 3 H, H–C(6) and 2 H–C(7)); 1.31 (*s*, 3 H, CH_3)). – ^{13}C-NMR.: s. *Table*. – $C_{17}H_{26}O_{16}$.

Mussaenoside tetraacetate (**2**). Acetylation of **1** with acetic anhydride/pyridine at room temp. gave **2**, m.p. 119.3–122.3°, $[\alpha]_D^{20} = -86.7°$ ($c = 0.97$, $CHCl_3$). – UV. (EtOH): 205 (3.41), 233 (3.48). – IR. (KBr): 3480, 1755, 1710, 1645. – ^{1}H-NMR. ($CDCl_3$): 7.40 (*d*, $J = 1.0$, 1 H, H–C(3)); 5.34 (*d*, $J = 3.0$, 1 H, H–C(1)); 4.84–5.30 (4 H, H–C(1′), H–C(2′), H–C(3′), H–C(4′)); 4.24 (*m*, 2 H, 2 H–C(6′)); 3.70 (*s*, 3 H, CH_3O); 3.68–3.84 (*m*, 1 H, H–C(5′)); 3.09 (*m*, 1 H, H–C(5)); 2.30 (*d*×*d*, $J = 9.0$ and 3.0, 1 H, H–C(9)); 2.21–2.40 (*m*, 1 H, H–C(6)); 1.88–2.14 (4 CH_3COO, HO–C(8)); 1.54–1.81 (*m*, 3 H, H–C(6) and 2 H–C(7)); 1.32 (*s*, 3 H, CH_3). – ^{13}C-NMR.: s. *Table*. – MS.: 558 (M^+), 43 (64.0), 53 (1.9), 69 (2.7), 73 (2.2), 77 (1.0), 81 (8.0), 85 (4.0), 97 (8.0), 103 (5.6), 109 (62.0), 115 (11.2), 127 (24.0), 135 (8.0), 139 (24.0), 145 (8.8), 152 (9.0), 157 (3.1), 161 (6.0), 169 (100.0), 179 (40.0), 187 (4.0), 193 (8.0), 211 (30.0), 229 (6.0), 245 (0.9), 271 (22.0), 289 (1.1), 331 (85.0), 379 (0.9), 509 (7.2), 541 (4.8), 558 (0.4). – $C_{25}H_{34}O_{14}$.

Mussaenoside pentaacetate (**3**). Compound **2** was further acetylated with acetic anhydride/4-dimethylaminopyridine for 30 min. and after usual chromatographic purification white crystals of **3** were obtained, m.p. 135.8–138°, $[\alpha]_D^{20} = -64.9°$ ($c = 0.52$, $CHCl_3$). – UV. (EtOH): 232 (3.84). – IR. (KBr): no OH!, 1755, 1730, 1645. – ^{1}H-NMR. ($CDCl_3$): 7.33 (*d*, $J = 1.0$, 1 H, H–C(3)); 5.65 (*d*, $J = 2.0$, 1 H, H–C(1)); 4.74–5.22 (*m*, 4 H, H–C(1′), H–C(2′), H–C(3′), H–C(4′)); 4.20 (*m*, 2 H, 2 H–C(6′)); 3.65 (*s*, 3 H, CH_3O); 3.64–3.80 (*m*, 1 H, H–C(5′)); 2.99 (*m*, 1 H, H–C(5)); 2.62 (*d*×*d*, $J = 9.0$ and 2.0, 1 H, H–C(9)); 1.64–2.10 (5 CH_3COO, 2 H–C(6), 2 H–C(7)); 1.48 (*s*, 3 H, CH_3). – MS.: 600 (M^+, no peak), 43 (100.0), 55 (17.9), 57 (25.0), 69 (15.2), 81 (13.4), 85 (8.9), 91 (2.7), 97 (10.7), 103 (6.3), 105 (3.6), 109 (50.0), 115 (10.7), 127 (17.9), 135 (3.6), 139 (12.5), 145 (8.9), 149 (6.0), 157 (2.8), 161 (4.7), 169 (100.0), 179 (9.0), 187 (3.0), 193 (35.7), 211 (7.0), 229 (3.5), 271 (8.4), 331 (35.7), 347 (4.0), 360 (1.5), 368 (0.8), 388 (3.0), 420 (1.5), 437 (1.0), 497 (1.5), 509 (2.2), 541 (1.8). – $C_{27}H_{36}O_{15}$.

Table. *^{13}C-NMR. Chemical Data*

C-Atom	1	2	4	5
C(1)	95.05	93.86	95.57	93.94
C(3)	151.78	149.64	152.01	149.55
C(4)	112.98	113.12	112.67	113.09
C(5)	31.49	30.25	32.88	30.28
C(6)	30.39	29.29	30.62	29.40
C(7)	40.44	40.69	39.63	40.77
C(8)	80.22	79.08	81.03	79.04
C(9)	51.67	51.05	51.58	51.08
C(10)	24.43	24.14	24.97	24.20
C(11)	169.11	169.12	169.26	169.34
C(1′)	99.39	95.69	99.43	95.77
C(2′)	74.25	70.74	74.47	70.86
C(3′)	77.75	72.56	77.44	72.66
C(4′)	71.22	68.34	71.41	68.61
C(5′)	77.46	72.05	75.27	72.12
C(6′)	62.55	61.70	64.03	62.11
C(1″)			127.34	132.94
C(2″)			115.11	123.94
C(3″)			146.32	142.40
C(4″)			149.19	142.52
C(5″)			116.41	122.97
C(6″)			123.01	126.50
CH_3O	51.67	51.17	51.76	51.26
C(α)			147.12	143.73
C(β)			114.62	118.40
CO		167.23–170.61	168.87–169.26	165.88–170.05
CH_3COO		20.17–20.66		20.32–20.68

Conversion of the α,α-epoxide **11** *into 8-epi-mussaenoside tetraacetate* (**8**). $LiBH_4$ (600.0 mg) was added to an iced solution of **11** (306.4 mg) in dry THF (15 ml). The mixture was stirred at room temp. for 30 min and then heated under reflux for 30 min. After cooling, 10% methanolic AcOH was added dropwise to decompose the excess reagent and to adjust the resulting solution to pH 6. The solvent was removed *in vacuo*, and an aq. solution of the residue was transferred to a charcoal (3 g) column, eluted with H_2O (200 ml) and then with MeOH (200 ml). After concentration of the MeOH eluate, the residue (196.1 mg) was subjected to prep. TLC. (2 plates, 2 developments) with $CHCl_3$/MeOH 8:2 as eluent. The major band was extracted with $CHCl_3$/MeOH 9:1. The residue (109.2 mg) of the extract was acetylated with 2 ml of pyridine/Ac_2O 1:1 in the usual manner. The product, after purification by prep. TLC. (1 plate) with ether as eluent, was recrystallized from EtOH yielding the 8α-alcohol **8** (86.4 mg) as colorless needles, m.p. 144–145°, $[\alpha]_D^{29} = -70.73°$ ($c = 0.21$, $CHCl_3$). – UV. (MeOH): 237.3 (4.01). – IR. (KBr): 3480, 2925, 1740, 1680, 1640, 1370, 1220. – ^{1}H-NMR. ($CDCl_3$): 1.41 (*s*, 3 H, CH_3); 1.97–2.08 (4 CH_3COO); 3.71 (*s*, 3 H, CH_3O); 7.38 (*d*, $J = 1.2$, H–C(3)).

$C_{25}H_{34}O_{14}$ Calc. C 53.76 H 6.14% Found C 53.55 H 5.99%

Conversion of **8** *into the ether* **13** via *the 11-alcohol* **14**. To a stirred suspension of $LiAlH_4$ (323.4 mg) in dry THF (22 ml) precooled to −20 to −15° was added dry MeOH (0.85 ml) during 20 min keeping the temp. below −15°. Having stirred the suspension for further 30 min below −15°, a solution of **8** (165.0 mg) in dry THF (8 ml) was added dropwise during 5 min. The mixture was stirred for further 1.5 h below −15°. Excess reagent was decomposed by slow addition of AcOEt.

The inorganic material, precipitated by the successive addition of satd. aq. Na_2SO_4 solution (20 ml), was filtered off and then digested with H_2O (20 ml) to dissolve the glucoside. The H_2O layer was extracted with BuOH (4 times 25 ml) and the combined extracts were washed with satd. aq. NaCl solution. The filtrate and BuOH layer were combined and concentrated *in vacuo* to give a residue containing **14** and inorganic salts. The residue was dissolved in MeOH/AcOH 7:3 and heated at 55–60° for 45 min. After concentration of the mixture, an aq. solution of the residue was applied to a charcoal (3 g) column, eluted with H_2O (200 ml) and then with EtOH (200 ml). The EtOH eluate was concentrated *in vacuo* and the residue submitted to prep. TLC. (2 plates, 2 developments) with $CHCl_3$/MeOH 4:1 as eluent. Of the three major bands, the middle one was extracted with $CHCl_3$/MeOH 9:1, the extract evaporated and the residue (42.1 mg) acetylated with 2 ml of Ac_2O/pyridine 1:1 in the usual manner. The product, after purification by prep. TLC. (1 plate, ether), was recrystallized from EtOH giving **13** (26.0 mg) as colorless needles, m.p. 150–151°, $[\alpha]_D^{24} = -17.95°$ ($c = 0.39$, $CHCl_3$). – IR. (KBr): 2930, 1740, 1370, 1230, 1040. – ^{1}H-NMR. ($CDCl_3$): 1.40 (*s*, 3 H, CH_3); 1.97–2.08 (4 CH_3COO); 5.47 (*d*, $J = 3.0$, 1 H, H–C(1)).

$C_{24}H_{32}O_{12}$ Calc. C 56.25 H 6.29% Found C 56.13 H 6.44%

Conversion of **8** *into 8-epi-mussaenoside pentaacetate* (**15**). To a solution of **8** (24.0 mg) in Ac_2O (2 ml) was added BF_3-etherate (2 drops), the mixture was allowed to stand at room temp. for 2 min, poured into iced H_2O and extracted with $CHCl_3$. The $CHCl_3$ layer was washed with 5% aq. $NaHCO_3$ solution and then with H_2O, dried over $MgSO_4$ and concentrated. The residue was subjected to prep. TLC. (1 plate, 10×20 cm, coating 0.75 mm) with ether as eluent. Of the two major bands, the less mobile one was extracted with $CHCl_3$/MeOH 9:1. Removal of the solvent gave **15** (16.0 mg) as a white powder[4]). – ^{1}H-NMR. ($CDCl_3$): 1.65 (*s*, 3 H, CH_3); 1.90–2.09 (5 CH_3COO); 2.66 (*d*×*d*, $J = 2.0$ and 9.0, 1 H, H–C(9)); 3.70 (*s*, 3 H, CH_3O); 5.52 (*d*, $J = 2.0$, 1 H, H–C(1)); 7.35 (*d*, $J = 1.0$, 1 H, H–C(3)).

Isolation of Ladroside (**4**). Fractions 4 and 5 of the first *Craig* distribution were combined and submitted to a second *Craig* distribution with H_2O/MeOH/EtOH/$CHCl_3$ 2:1:1:3. Four fractions were collected. Fraction 3 was chromatographed on silica gel with $CHCl_3$/EtOH 2:1 (2 times) and CH_2Cl_2/MeOH/H_2O 4:1:0.1. Prep. liquid chromatography (MeOH/H_2O 2:3; *Waters* Prep. LC/System 500 using a Prep Pak-500/C_{18} reversed phase column 30 cm × 5.7 cm), gave pure ladroside (**4**) as a yellow amorphous powder, $[\alpha]_D^{20} = -68.9°$ ($c = 0.72$, CH_3OH). – UV. (CH_3OH): 221 (4.07), 236 (4.09), 328 (3.99). – IR. (KBr): 3400, 1642, 1635, 1610, 1520, 1445. – ^{1}H-NMR. (CD_3OD): 7.53 (*d*, $J = 16$, 1 H, H–C(*α*)); 7.37 (*s*, 1 H, H–C(3)); 6.78–7.02 (3 arom. H); 6.22 (*d*, $J = 16$, 1 H, H–C(*β*)); 5.19 (*d*, $J = 6$, 1 H, H–C(1)); 4.38–4.48 (*m*, 2 H, 2 H–C(6′)); 3.62 (*s*, 3 H, CH_3O); 3.02–3.50 (*m*, 1 H, H–C(5)); 2.09 (*d*×*d*, $J = 8$ and 6, 1 H, H–C(9)); 2.02–2.30 (*m*, 1 H, H–C(6)); 1.52–1.72 (*m*, 3 H, H–C(6) and 2 H–C(7)); 1.28 (*s*, 3 H, CH_3). – ^{13}C-NMR.: s. *Table*. – $C_{26}H_{32}O_{13}$.

Ladroside pentaacetate (**5**). Acetylation of **4** with acetic anhydride/pyridine for 2 h at room temp. gave crude **5**. Chromatography (silica gel, $CHCl_3$/C_6H_6/MeOH 3:1:0.1) followed by crystallization from EtOH gave pure crystalline **5**, m.p. 191.1–193.3°, $[\alpha]_D^{20} = -45.5°$ ($c = 0.83$, $CHCl_3$). – UV. (CH_3OH): 224 (4.09), 280 (4.08). – IR. (KBr): 3450 (OH), 1755, 1720, 1648, 1632. – ^{1}H-NMR. ($CDCl_3$): 7.64 (*d*, $J = 16$, 1 H, H–C(*α*)); 7.14–7.48 (3 arom. H, H–C(3)); 6.40 (*d*, $J = 16$, 1 H, H–C(*β*)); 5.32 (*d*, $J = 3.4$, 1 H, H–C(1)); 4.80–5.38 (*m*, H–C(1′), H–C(2′), H–C(3′), H–C(4′)); 4.35 (*m*, 2 H, 2 H–C(6′)); 3.62–3.92 (*m*, 1 H, H–C(5′)); 3.69 (*s*, 3 H, CH_3O); 3.08 (*m*, 1 H, H–C(5)); 2.18–2.32 (*m*, 2 H, H–C(6), H–C(9)); underneath 2 CH_3COO–C(arom.)); 1.92–2.14 (3 CH_3COO–C(aliph.)); 1.84 (HO); 1.56–1.86 (*m*, 3 H, H–C(6) and 2 H–C(7)); 1.29 (*s*, 3 H, CH_3). – ^{13}C-NMR.: s. *Table*. – MS.: 762 (M^+), 43 (87.5), 53 (2.8), 60 (4.8), 77 (5.0), 81 (4.8), 85 (2.3), 91 (3.2), 97 (4.7), 103 (3.1), 105 (3.8), 109 (3.0), 115 (3.4), 127 (10.0), 133 (4.4), 135 (4.0), 139 (6.4), 145 (2.8), 152 (2.1), 161 (6.0), 163 (12.5), 169 (100.0), 179 (6.5), 187 (2.9), 193 (14.0), 205 (10.0), 211 (6.0), 229 (8.0), 247 (2.5), 271 (3.8), 289 (6.2), 331 (1.3), 373 (1.9), 397 (0.9), 435 (0.9), 467 (1.9), 493 (3.5), 535 (7.5), 541 (2.0), 585 (0.8), 628 (1.0), 660 (0.6), 762 (0.3). – $C_{36}H_{42}O_{18}$.

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[4]) In this reaction **8** contaminated with the 8-epimer **1** was employed, and hence the product **15** was not pure. However, the ^{1}H-NMR. clearly shows the signals of H–C(1) and H–C(9) of **15**, suggesting that the tertiary hydroxy group in **8** has the *α*-configuration on the basis of the reported assignment [12].

Ladroside hexaacetate (**6**). A further treatment of **5** with acetic anhydride/pyridine for 48 h at 40° gave after chromatography on silica gel with $CHCl_3/C_6H_6/CH_3OH$ 3:1:0.1 and recrystallization in EtOH abs. pure **6**, m.p. 106–108°, $[\alpha]_D^{20} = -12.5°$ ($c = 0.62$, $CHCl_3$). – UV. (EtOH): 217 (4.57), 273 (4.59). – IR. (KBr): no OH absorption, 1760, 1710, 1640. – MS.: 804 (M^+, no peak), 43 (100.0), 55 (6.1), 60 (3.4), 69 (4.8), 77 (6.8), 81 (6.5), 89 (6.1), 97 (5.9), 103 (4.8), 105 (6.1), 109 (22.0), 115 (3.5), 127 (10.9), 136 (26.7), 139 (5.0), 145 (3.7), 149 (2.7), 160 (7.2), 162 (16.2), 163 (13.3), 169 (31.4), 180 (28.6), 189 (7.8), 192 (10.6), 193 (16.2), 205 (15.2), 210 (2.7), 222 (5.4), 229 (8.9), 239 (1.4), 247 (14.3), 264 (2.0), 271 (1.6), 289 (10.9), 331 (7.5), 339 (0.9), 348 (0.9), 373 (1.8), 450 (1.2), 477 (20.9), 493 (33.3), 523 (4.8), 535 (60.9), 549 (10.9), 551 (12.2), 563 (2.7), 577 (38.1), 602 (14.3), 660 (0.8), 702 (1.8), 744 (2.3). – $C_{38}H_{44}O_{19}$.

Hydrolysis of Ladroside (**4**). A solution of **4** in methanolic 0.1N NaOH was kept overnight at room temp., then neutralized with 0.1N HCl and chromatographed on silica gel with EtOAc/EtOH/H_2O 100:16.5:13.5. Pure caffeic acid (TLC., UV.) and crude mussaenoside (**1**) were obtained. For identification (IR., UV., ^{1}H-NMR., TLC.) **1** was purified by semipreparative HPLC. and lyophilized.

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