Pergamon

PII: S0031-9422(97)00696-1

Phytochemistry, Vol. 47, No. 6, pp. 1067–1072, 1998

Printed in Great Britain
0031-9422/98 $19.00+0.00

2′-, 4′-, AND 6′-*O*-SUBSTITUTED 1,5,9-EPIDEOXYLOGANIC ACIDS FROM *NEPETA GRANDIFLORA*

TAMÁS NAGY, ÁKOS KOCSIS,* MIKLÓS MORVAI, LÁSZLÓ F. SZABÓ, BENJAMIN PODÁNYI,† ANDRÁS GERGELY‡ and GYULA JERKOVICH§

Institute of Organic Chemistry, Semmelweis Medical University, Högyes E. u. 7, H-1092 Budapest, Hungary; ‡ Institute of Pharmaceutical Chemistry, Semmelweis Medical University, Högyes E. u. 7, H-1092 Budapest, Hungary; § Institute for Drug Research, P.O. Box 82, H-1325 Budapest, Hungary

(*Received in revised form* 27 *June* 1997)

Key Word Index—*Nepeta grandiflora*; Lamiaceae; iridoid glucosides; conformational analysis; *O*-substituted-1,5,9-epi-deoxyloganic acid.

Abstract—In addition to the known 1,5,9-epideoxyloganic acid and 1,5,9-epideoxyloganin, *Nepeta grandiflora* has provided four new iridoid glucosides: 6′-*O*-acetoacetyl-, 2′-*O*-methyl-, 4′-*O*-methyl-, and 6′-*O*-methyl-1,5,9-epideoxyloganic acid. Their structures were proved by ^{1}H and ^{13}C-NMR studies. The conformation of the molecules was studied by molecular modelling and ^{1}H NMR coupling constant analysis. The absolute configurations were supported by CD spectroscopy.

INTRODUCTION

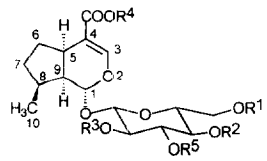
1,5,9-Epideoxyloganic acid (**1**) was the first iridoid isolated with opposite chirality at stereocentres C-1, C-5 and C-9 to those of common iridoids [1]. Recently, we have observed four new iridoids with this chirality (**3**–**6**) in *Nepeta grandiflora* (*Lamiaceae*). Here we report on the isolation and the structure elucidation of these compound and their derivatives (**7**–**10**) (Scheme 1). The absolute configuration of 1,5,9-epideoxyloganic acid is based on the fact that the aglucone methyl ester was shown to be the enantiomer of the aglucone of 8-epideoxyloganin of known stereochemistry [1]. This was established by measuring the specific rotations at several wavelengths and also the CD-curves were of the same magnitude but opposite sign. In the present work, we report the study of the conformational states of **1** using molecular modelling and NMR coupling constants.

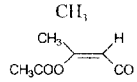
RESULTS AND DISCUSSION

The above-ground parts of *N. grandiflora* were extracted and gave, after an initial purification, a glycosidic fraction. Repeated silica gel column chromatography was used to separate compound **1**, **2** and **3** (Scheme 1). The ^{1}H and ^{13}C NMR data of compounds **1** and **2** were identical with the literature spectra of 1,5,9-epideoxyloganic acid, as well as of 1,5,9-epideoxyloganin, respectively [1]. The chemical shift assignment of the sugar carbons of compound **1** and **2** was based on detailed 2D NMR studies of strictosidine [2]. On comparing the ^{1}H and ^{13}C (Table 1) NMR spectra of compound **3** recorded in deuteromethanol with the data for compound **1**, we observed chemical shift differences only for the H-5′, H-6′, C-5′ and C-6′ signals. Therefore, compound **3** had to be a derivative of 1,5,9-epideoxyloganic acid substituted at C-6′. In the ^{1}H NMR spectrum of compound **3** recorded in pyridine two additional signals, a singlet at δ 3.72 with two proton intensity and singlet at δ 2.24 with three proton intensity, appeared compared to the spectrum of compound **1**. In the ^{13}C NMR spectrum of compound **3** four additional peaks, two signals due to carbonyls at δ 200.9 and 167.8, a methylene carbon at δ 50.2 and a methyl carbon at δ 29.9 appeared compared to the spectrum of **1**. These signals were in agreement with an acetoacetyl group. Acetylation of the methylester of **3** gave unexpectedly a tetraacetate (**7**) instead of a triacetate. Furthermore, in the ^{13}C NMR spectrum of **7**, the expected signals of the acetoacetyl moiety were replaced by signals of a methyl group, a carboxyl group, a tertiary olefinic carbon, and a very low field quaternary carbon. These signals, together with the presence of the additional acetyl group suggested that structure **7** was formed by acetylation of the enol form of the acetoacetyl group. In order to settle the stereochemistry of the newly formed double bond, a NOE experiment was perfor-

* Author to whom correspondence should be addressed.

† Permanent address: Chinoin Pharmaceutical and Chemical Works Ltd., Tó utca 1–5, H-1045, Budapest, Hungary.



	R^1	R^2	R^3	R^4	R^5
1	H	H	H	H	H
2	H	H	H	CH_3	H
3	CH_3COCH_2CO	H	H	H	H
4	H	H	CH_3	H	H
5	H	CH_3	H	H	H
6	CH_3	H	H	H	H
7		CH_3CO	CH_3CO	CH_3	CH_3CO
8	H	H	CH_3	CH_3	H
9	H	CH_3	H	CH_3	H
10	CH_3	H	H	CH_3	H

Scheme 1. Formulas of isolated iridoids.

Table 1. ^{13}C NMR spectral data for compounds **1–3** and **7–10**

C	**1**	**2**	**2**	**3**	**3**	**7**	**8**	**9**	**10**
solvent	MeOD	MeOD	D_2O	MeOD	Pyridine-d_5	$CDCl_3$	MeOD	MeOD	MeOD
-1	100.7	100.7	101.0	100.7	99.8	99.7[c]	100.6	100.7	100.7
-3	153.2	153.2	152.7	153.3	151.9	151.0	153.2	153.1	153.3
-4	113.3	113.2	113.1	113.5	113.3	112.1	113.3	113.3	113.2
-5	34.0	34.0	33.1	34.0	33.8	33.6[a]	34.0	34.0	34.0
-6	32.3	32.3	31.7	32.4	31.8	32.2[a]	32.3	32.3	32.4
-7	33.6	33.7	33.0	33.8	33.0	31.2[a]	33.8	33.6	33.8
-8	37.1	37.2	36.3	37.2	36.3	36.2	37.0	37.1	37.1
-9	44.3	44.3	43.4	44.2	43.5	43.0	44.6	44.3	44.3
-10	16.8	16.7	16.6	16.7	16.5	16.3	16.6	16.7	16.7
COO	170.9	169.6	170.5	171.0	169.5	170.4[e]	169.4	169.5	169.5
-1′	103.8	104.0	103.1	103.6	103.8	99.2[c]	103.7	103.9	103.8
-2′	75.2	75.3	74.0	75.5	75.4	71.5[d]	84.99	75.3	75.1
-3′	78.0	78.1	76.5	77.9	78.3	73.1[d]	77.8	78.1	78.1
-4′	71.1	71.2	70.1	71.4	71.3	68.6	71.2	80.3	71.4
-5′	78.2	78.4	77.1	75.0	75.1	72.3[d]	78.2	77.4	77.4
-6′	62.5	62.5	61.4	65.3	65.4	62.2	62.5	62.0	73.0
$COOCH_3$	-	51.6	52.5	—	—	51.1	51.6	51.6	51.6
-1″				168.9	167.8	169.4[e]			
-2″					50.2	109.3			
-3″				203.4	200.9	169.2[e]			
-4″				30.2	29.9	18.3[f]			
AC(COO)				—	—	168.0[e]			
Ac(COO)						167.5[e]			
Ac(COO)					—	165.5[e]			
Ac(COO)				—	—	165.0[e]			
OCH_3				—	—		61.2	60.8	59 76
$Ac(CH_3)$				—	—	21.2-20.6[f]			

[a,b,c,d,e,f]: tentative assignment is also possible.

med. This showed a large interaction between the olefinic hydrogen and the methyl group and thus proved the presence of a Z-isomer. The CI and the FAB mass spectrum of compound **7** displayed the $[M + H]^+$ ion at *m/z* 627. No molecular ion was observed in the EI mass spectrum, and the largest ion was observed at *m/z* 415, which is formed from the glucose derivative after cleavage of the glycosidic bond. The elemental composition of the relevant ions together with the quasi-molecular ions of the CI and FAB mass spectra further proved the structure of compound **7** and its precursor compound **3**. The methylene protons of the

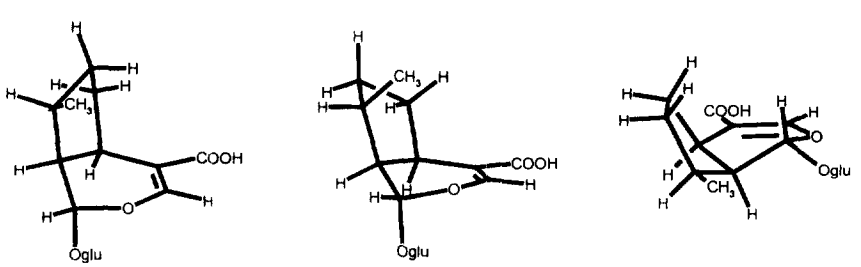
6′-*O*-acetocetyl group exchanged with deuterium when the spectrum of compound **3** was recorded in deuteromethanol; therefore, their signal was not observed and neither was the methylene carbon signal in the ^{13}C NMR spectrum.

In the ^{13}C NMR spectrum of the crude glycosidic fraction, beside the intensive peaks of compound **1**, some small, characteristic signals were observed. We proved by the DEPT spectrum editing technique that a few signals between δ 55 and 60 were due to methyl groups. This chemical shift range corresponds to methyl ethers. Furthermore, signals of methine groups were identified in the δ 80–85 chemical shift range. These may correspond to carbons of substituted glucose units. These data indicated the presence of further minor compounds in the glycosidic fraction, however in acidic form they could not be separated from compound **1** and from each other. Therefore, the whole acidic part of the glycosidic fraction was esterificated with diazomethane, and then repeated column chromatography was applied to separate the components. Five compounds were isolated. The main product was 1,5,9-epideoxyloganin (**2**), which was present as a minor compound in the glycosidic fraction; however, it was also formed from the main compound (**1**) in this fraction in the reaction with diazomethane.

In the ^{1}H and ^{13}C NMR spectra of the three further isolated compounds of the esterificated glycosidic fraction (compound **8**, **9** and **10**), the chemical shifts of the aglycone unit agreed with the data of 1,5,9-epideoxyloganin (**2**). On the other hand, characteristic differences in the chemical shift of the glucose unit and one additional peak due to a methoxy group was observed for each compound. Therefore, compounds **8–10** are methylated derivatives of 1,5,9-epideoxyloganin. Selective O-alkylation on a sugar is known to give a large (ca. 10 ppm) downfield shift of the corresponding ^{13}C NMR signal, while the neighbouring carbon signals show only minor changes [3]. The data in Table 1 shows that only C-2′ in compound **8**, only C-4′ in compound **9** and only C-6′ in compound **10** have a downfield shift of about 10 ppm compared to the data for compound **2**. Therefore, compounds **8**, **9** and **10** are 2′-O-methyl-, 4′-O-methyl-, and 6′-O-methyl-1,5,9-epideoxyloganin, respectively. Long range carbon–proton couplings in compounds **8** and **9** were identified by 1D selective INEPT experiments. The data for compound **8** supported the chemical shift assignment of Table 1. The data for compound **9** gave an independent proof for the C-4′ position of the methoxy group by the detection of the spin–spin interaction of the methoxy carbon with the H-4′ proton. The structure elucidation of compounds **8–10** and the ^{13}C spectrum of the crude glycosidic fraction described above, suggested that besides 6′-*O*-acetoacetyl-1,5,9-epideoxyloganic acid (**3**) *N. grandiflora* contains three further minor iridoids: 2′-*O*-methyl-, 4′-*O*-methyl-, and 6′-*O*-methyl-1,5,9-epideoxyloganic acid (compounds **4–6**).

Conformational analysis

The values of the vicinal proton–proton coupling constants of compound **2** (Table 2) indicated that the aglycone cannot be characterised by one dominant conformation. To obtain real representatives of all conformational arrangements of compound **2**, the two possible half-chair forms ($^{9}H_{1}$ and $^{1}H_{9}$ where the atomic number in sub- and superscript means the atoms below and above the plain of the further atoms of the ring) of the dihydropyrane ring were attached to 10 envelope conformations (E) of the cyclopentane ring by a molecular modelling program [4]. MM+ energy minimisation (geometry optimisation) of the obtained 20 structure gave three stable conformers: **A** E_{6} $^{9}H_{1}$, **B** ^{8}E $^{9}H_{1}$; **C** ^{9}E $^{1}H_{9}$. (Scheme 2.) Their vicinal proton–proton coupling constants were calculated from the corresponding dihedral angles by Altona's Karplus equation [5]. Assuming that the sample is a mixture of the three energetically stable conformers their proportions can be determined. The populations of these conformers (**A** 50%, **B** 40%, **C** 10%) were obtained by linear regression fitting of the measured and calculated coupling constants (Table 2). Since the coupling constant values of the aglycone unit are identical within experimental error for all compounds, the same conformational equilibrium is characteristic for all of them.



A **B** **C**

Scheme 2. The three stabile conformations of the aglycone unit.

Table 2. Measured and calculated coupling constant in the aglycone of compound **1–3** and **7–10**

	A		B		C			
	$E_6\ ^9H_1$		$^8E\ ^9H_1$		$^9E\ ^1H_9$			
	ϕ	J_A (Hz)	ϕ	J_B (Hz)	ϕ	J_C (Hz)	J_E (Hz)	J_M (Hz)
$J_{1,9}$	−64.0°	2.0	−70.2	1.5	−171.2°	9.1	2.5	3.8
$J_{5,6\alpha}$	44.6°	5.3	−13.1	9.9	−32.0°	7.7	7.4	8.4
$J_{5,6\beta}$	−76.5°	1.4	−133.2°	6.4	−151.2°	9.7	4.2	5.0
$J_{5,9}$	−30.5°	8.2	−24.1	9.1	41.6°	6.5	8.4	8.6
$J_{6\alpha,7\alpha}$	−168.2°	12.8	−82.7°	0.4	−119.5°	3.8	6.9	7.5
$J_{6\alpha,7\beta}$	−48.0°	5.3	37.5	7.4	−0.2°	11.9	6.8	8.2
$J_{6\beta,7\alpha}$	−49.2°	5.1	35.2°	8.0	−0.1°	11.9	6.9	4.9
$J_{6\beta,7\beta}$	71.0°	1.4	155.5°	11.1	119.2°	3.6	5.5	7.7
$J_{7\alpha,8}$	154.0°	10.4	78.4	1.2	152.4°	10.1	6.7	7.4
$J_{7\beta,8}$	32.9°	7.3	−41.9	8.9	33.4°	7.3	7.9	8.0
$J_{8,9}$	0.1°	10.7	40.1°	6.4	−43.7°	5.5	8.5	8.7
Populations	**0.5**		**0.4**			**0.1**		

ϕ, dihedral angle.

J_A, J_B, J_C are the calculated coupling constants for A, B and C conformers by Altona's Karplus equation.

$J_E = (0.5 * J_A + 0.4 * J_B + 0.1 * J_C)/3$.

J_M, measured coupling constant.

The assignment of the diastereotopic methylene hydrogens is based on the NOE interactions detected in the NOESY spectrum of compound **2**.

The absolute configuration of isolated compounds

L.-F. Tietze *et al.* have already examined the CD spectroscopic characteristics of iridoids [6]. According to their results, positive Cotton effect for 1*R*,5*R*,9*S* and negative Cotton effect for 1*S*,5*S*,9*R* loganin-like iridoids with axial glucosiloxy group is expected. We obtained a positive Cotton effect for compounds **2**, **3**, **8**, **9**, **10** and the NMR data proved that $^9H_1$ (Scheme 2) is the dominant conformation of the six membered ring. Therefore, the CD and NMR data were in accordance with the absolute configuration of 1*R*,5*R*,9*S* for these compounds, just as in the case of compound **1** [1].

EXPERIMENTAL

General experimental procedures

NMR: Bruker AC-400 instrument. DEPT, selective INEPT, NOE difference, and NOESY spectra were recorded using the standard microprograms of the DISNMR software. CD: JASCO J-720 dichrograph, spectra registered between 200–300 nm, cell length: 2 cm; MS: Finnigan MAT 8430 mass spectrometer; EI: 70 eV; CI: isobutane as the reagent gas; FAB: Xe FAB gun, in *m*-nitrobenzyl alcohol matrix.

Isolation of iridoids

N grandiflora was collected and identified in the garden of the Institute of Etology and Botany of the Hungarian Academy of Sciences, Vácrátót, in July, 1992. A voucher specimen has been deposited (No. L827) in the Herbarium of Institute of Etology and Botany of the Hungarian Academy of Sciences, Vácrátót.

Fresh aerial parts (6000 g) were chopped into small pieces and extracted with aq EtOH (70%, 18 litres). The extract was concentrated *in vacuo* to an aq suspension (1,5 litre), which was treated with charcoal-celite (2:1) (450 g). The resulting suspension was stratified on a Gooch funnel (25 cm diameter) containing a layer of charcoal-celite (2:1) (150 g).

Monosacharides were eluted with H_2O (8 litres) and H_2O-EtOH (19:1) (4 litres), disacharides with H_2O-EtOH (9:1) (4 litres), fraction I with H_2O-EtOH (17:3) (4 litres), fraction II with H_2O-EtOH (7:3) (4 litres) and fraction III (49 g) with H_2O-EtOH (1:1) (12 litres). Fraction III was chromatographed on silica gel (800 g) with EtOAc-EtOH-H_2O (40:6:3, after fraction 31, 10:4:2). Fractions 14–38 (each 250 ml) were combined and evaporated *in vacuo* to afford a purified iridoid fraction (20.0 g)

Isolation of 1,5,9-*epideoxyloganic acid* (**1**) 1,5,9-*epideoxyloganin* (**2**) *and* 6′-O-*acetoacetyl*-1,5,9-*epideoxyloganic acid* (**3**).

The purified iridoid fraction (5 g) was chromatographed on silica gel (300 g) with CH_2Cl_2-MeOH-H_2O (32:7:0.8; after fraction 47, 32:13:1.5). Fractions 51–62 (each 18 ml) gave **1** (1,4 g); $[\alpha]_D$ and IR, UV, ^1H NMR and ^{13}C NMR spectra superimposable on those of 1,5,9-epideoxyloganic acid [1].

The residue of the combined and evaporated fractions 47–50 after rechromatography on silica gel (15 g) afforded **2** (70 mg); $[\alpha]_D$ and IR, UV, ^{1}H NMR and ^{13}C NMR spectra superimposable on those of 1,5,9-epideoxyloganin [1].

The residue of the combined and evaporated fractions 28–46 was purified by flash chromatography on silica gel (70 g, 0.015–0.04 mm) with CH_2Cl_2-MeOH-H_2O (32:5:0.6). Fractions 13–25 were combined and evaporated *in vacuo* then rechromatographed in the same manner to afford pure 6′-O-acetoacetyl-1,5,9-epideoxyloganic acid (**3**) (40 mg), $[\alpha]_D = +88.28°$ (c = 0.069, EtOH), CD (EtOH) λ_{max}: 231, mol. ellipticit.: 10.901. IR (KBr): ν_{max} (cm^{-1}): 3600-2500, 1743, 1706, 1684, 1636; UV (EtOH) 236 nm, ($\log \varepsilon = 3.95$); ^{1}H NMR (MeOD, 400 MHz): δ 1.06 (*d*, Me-10), 1.28 (*dq*, H-7β), 1.61 (*ddt*, H-6β), 1.77 (*dtd*, H-7α), 2.00 (*dtd*, 6α), 2.25 (*s*, CH_3), 2.26 (*m*, H-8), 2.42 (*td*, H-9), 2.90 (*td*, H-5), 3.22 (*m*, H-2′), 3.45-3.30 (*m*, H-4′-H-3′), 3.52 (*m*, H-5′), 4.21 (*dd*, H-6′β), 4.50 (*dd*, H-6′α), 4.57 (*d*, H-1′), 5.26 (*d*, H-1), 7.37 (*s*, H-3), coupling constants see Table 2; ^{13}C NMR: Table 1.

Isolation of 6′-O-*methyl*-1,5,9-*epideoxyloganin* (**10**), 1,5,9-*epidexoyloganin* (**2**), 2′-O-*methyl*-1,5,9-*epideoxyloganin* (**8**) *and* 4′-O-1,5,9-*epideoxyloganin* (**9**)

10 g of purified iridoid fraction was dissolved in MeOH (200 ml), cooled, and treated with CH_2N_2 in Et_2O.

The residue of the evaporated solution was chromatographed on silica gel (700 g) in MeOH-$CHCl_3$ (1:10). Fractions 24–25 (each 150 ml) gave **10** as colourless powder (67 mg), $[\alpha]_D = +95.35°$ (c = 0.17, EtOH), CD (EtOH) 231, mol. ellipticit.: 10.665. IR (KBr): ν_{max} (cm^{-1}): 3600-3300, 1698, 1635; UV (EtOH) 237 nm ($\log \varepsilon = 3.95$); C% = 55.3 (calc 55.7) H% = 7.5 (calc 7.3); CIMS: [M + H] = *m/z* 389; ^{1}H NMR (MeOD, 400 MHz): δ 1.06 (*d*, Me-10), 1.28 (*dq*, H-7β), 1.58 (*ddt*, H-6β), 1.77 (*dtd*, H-7α), 1.99 (*dtd*, H-6α), 2.28 (*m*, H-8), 2.43 (*td*, H-9), 2.92 (*td*, H-5), 3.21 (*dd*, H-2′), 3.27 (*t*, H-4′), 3.32 (*t*, H-3′), 3.38 (*ddd*, H-5′), 3.39 (*s*, OMe), 3.55 (*dd*, H-6′α), 3.69 (*s*, COOMe), 3.70 (*dd*, H-6′β), 4.54 (*d*, H-1′), 5.26 (*d*, H-1), 7.37 (*s*, H-3), (pyridine-d5): δ 1.18 (*d*, Me-10), 1.20–2.20 (7-Hα, 7-Hβ, H-6α, 6-Hβ, H-8), 2.24 (*s*, CH_3), 2.53 (*m*, H-9), 3.28 (*m*, H-5), 3.90–4.30 (*m*, H-2′–H-5′), 4.76 (*dd*, H-6′b), 5.04 (*dd*, H-6′a), 5.22 (*d*, H-1′), 5.68 (*d*, H-1), 7.98 (*s*, H-3), coupling constants see Table 2; ^{13}C NMR: Table 1.

The residue of combined fractions 30–47 afforded, after evaporation and crystallisation from EtOH, 2.8 g of **2**, mp, $[\alpha]^D$ and IR, UV ^{1}H NMR and ^{13}C NMR spectra superimposable on those of 1,5,9-epideoxyloganin [1]. Fractions 19–23 were evaporated to afford residue A (0.84 g), a mixture of three compounds with R_f values 0.31, 0.28 and 0.24 (silica gel TLC, MeOH-$CHCl_3$, 1:15).

Residue A was flash chromatographed on silica gel (80 g, 0.015–0.04 mm) with MeOH-$CHCl_3$ (1:15). Fractions 20–22 were concentrated to afford residue A1. Likewise, fractions 23–33 and 34–39 gave, after concentration *in vacuo*, residues A2 and A3, respectively. Residues A2 was chromatographed four times in the same manner to give some more residue A1 and residue A3, as well as pure residue A2, (80 mg) $R_f = 0.28$, (silica gel, MeOH-$CHCl_3$, 1:15). On the basis of the NMR spectra, the main component of residue A2 is the methyl ester of compound **3**.

6′-O-*acetoacetyl*-1,5,9-*epideoxyloganin*

IR (KBr): ν_{max} (cm^{-1}): 3600-3300, 1740, 1690, 1635; ^{1}H NMR (MeOD, 400 MHz): δ 1.06 (*d*, Me-10), 1.28 (*m*, 7-Hβ), 1.57 (*m*, 6-Hβ) 1.76 (*m*, 7-Hα), 1.98 (*m*, H-6α), 2.25 (*s*, CH_3), 2.28 (*m*, H-8), 2.42 (*m*, H-9), 2.91 (*m*, H-5), 3.20 (*m*, H-2′), 3.45-3.20 (*m*, H-3′, H-4′), 3.50 (*m*, H-5′), 3.69 (*s*, OMe), 4.21 (*dd*, H-6′b), 4.49 (*dd*, H-6′a), 4.56 (*d*, H-1′), 5.27 (*d*, H-1), 7.35 (*s*, H-3); ^{13}C-NMR (MeOD, 100 MHz): δ 16.6 (C-10), 30.2 (C-4″), 32.3 (C-6), 33.7 (C-7), 33.9 (C-5), 37.1 (C-8), 44.1 (C-9), 51.6 ($COOCH_3$), 65.1 (C-6′), 71.4 (C-4′), 75.0 (C-5′), 75.5 (C-2′), 77.9 (C-3′), 100.7 (C-1), 103.7 (C-1′), 113.4 (C-4), 153.3 (C-3), 168.8 (C-1″), 169.5 (COO).

Unified residue A1 (150 mg) was rechromatographed four times on silica gel (80 g) with MeOH-$CHCl_3$ (1:15), then finally with EtOH-$CHCl_3$ (1:10) to give pure 4′-*O*-methyl-1,5,9-epideoxyloganin (**9**) (36 mg), $R_f = 0.31$, $[\alpha]_D = +82.09°$ (c = 0.06, EtOH), CD (EtOH): 233, mol. ellipticit.: 9.791. IR (KBr): ν_{max} (cm^{-1}): 3600-3300, 1700, 1634; UV (EtOH) 237 nm ($\log \varepsilon = 3.95$); CIMS: [M + H] = 389; ^{1}H NMR (MeOD, 400 MHz): δ 1.06 (*d*, Me-10), 1.28 (*dq*, 7-Hβ), 1.57 (*ddt*, 6-Hβ), 1.76 (*dtd*, 7-Hα), 1.99 (*dtd*, H-6α), 2.28 (*m*, H-8), 2.40 (*td*, H-9), 2.90 (*td*, H-5), 3.12 (*t*, H-4′), 3.22 (*dd*, H-2′), 3.27 (*ddd*, H-5′), 3.45 (*t*, H-3′), 3.55 (*s*, OMe), 3.69 (*s*, COOMe), 3.66 (*dd*, H-6′b), 3.80 (*dd*, H-6′a), 4.54 (*d*, H-1′), 5.30 (*d*, H-1), 7.39 (*s*, H-3); ^{13}C NMR: Table 2; INEPT: selectively excited hydrogen (carbon showing coupling with the excited proton): H-1′ (C-1, C-3′, C-5′), H-3′ (C-1′, C-2′, C-2′, C-4′), H-4′ (C-3′, C-5′, C-6′, OCH_3), Ha-6′ (C-4′, C-5′), OCH_3 (C-4′).

Unified residue A3 (110 mg) was rechromatographed three times on silica gel (80 g) with MeOH-$CHCl_3$ (1:15) to give pure 2′-*O*-methyl-1,5,9-epideoxyloganin (**8**), (27 mg) $R_f = 0.24$ $[\alpha]_D = +64.6°$ (c = 0.05, EtOH), CD (EtOH): 230, mol. ellipticit.: 8.905. IR (KBr): ν_{max} (cm^{-1}): 3600-3300, 1698, 1634, UV (EtOH) 237 nm ($\log \varepsilon = 3.95$); CIMS: [M + H] = *m/z* 389; ^{1}H NMR (MeOD, 400 MHz): δ 1.05 (*d*, Me-10), 1.28 (*dq*, 7-Hβ), 1.60 (*ddt*, 6-Hβ), 1.77 (*dtd*, 7-Hα), 2.00 (*dtd*, H-6α), 2.30 (*m*, H-8), 2.39 (*td*, H-9), 2.90 (*td*, H-5), 2.90 (*dd*, H-2′), 3.4-3.3 (*m*, H-3′, H-4′), 3.26 (*m*, H-5′), 3.56 (*s*, OMe), 3.67 (*dd*, H-6′b), 3.69 (*s*, COOMe), 3.83 (*dd*, H-6′a), 4.60 (*d*, H-1′), 5.31 (*d*, H-1), 7.40 (*s*, H-3); ^{13}C NMR Table; selective INEPT: selectively excited hydrogen (carbon showing coupling with the excited proton) H-1′ (C-1), H-6′ (C-4′), OCH_3 (C-2′).

2′,3′,4′-Tri-O-acetyl-6′-O-([Z]-3-acetoxy-2-butenoyl)-1,5,9-epideoxy-loganin (**7**) *from residue A2*

Residue A2 (30 mg dissolved in dry pyridine (0.5 ml) was treated with Ac_2O (0.25 ml) for 16 h at room temp. After adding MeOH (2 ml), the sol. was left to stand at room temp. for 15 min, then evaporated to give a residue which was chromatographed on silica gel (6 g) in Me_2CO-CH_2Cl_2 (1:17). Fractions 19–24 (each 1.5 ml) were combined and evaporated affording **7** (6 mg); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 1.01 (*d*, Me-10), 1.27 (*m*, 7-Hβ), 1.52 (*m*, 6-Hβ), 1.79 (*m*, 7-Hα), 2.00, 2.03, 2.04, 2.18 (*s*, Ac), 2.08 (*m*, H-6α), 2.20 (*m*, H-8), H-9), 2.35 (*s*, CH_3), 2.90 (*m*, H-5), 3.70 (*s*, COOMe), 3.78 (*m*, H-5′), 4.25 (*dd*, H_2-6′a), 4.83 (*d*, H-1′), 5.10 (*d*, H-1), 5.25-5.00 (*m*, H-2′, H-3′, H-4′), 7.32 (*s*, H-3); ^{13}C NMR: Table 2; EI MS *m/z* (rel. int): 415 (35), 373 (16), 331 (10), 289 (10), 253 (10), 229 (8), 211 (10), 195 (14), 169 (24), 127 (100), 85 (40), 43 (40). Accurate mass measurements: *m/z* 415.1187 (calculated for $C_{18}H_{23}O_{11}$: 415.1240), 373.1105 (calculated for ($C_{16}H_{21}O_{10}$: 373.1135). 253.0708 (calculated for $C_{12}H_{13}O_6$: 253.0712), 195.1011 (calculated for $C_{11}H_{15}O_3$: 195.1021).

REFERENCES

1. Murai, F., Tagawa, M., Damtoft, S., Jensen, S. R. and Nielsen, B. J., *Chem. Pharm. Bull.*, 1984, **32**, 2809.
2. Patthy, Á., Károlyházy, L., Szabó, L. F., Podányi, B., *J. Nat. Prod.*, 1997, **60**, 69.
3. Utamura, T., Kuromatsu, K., Suwa, K., Koizumi, K., Shingu, T., *Chem. Pharm. Bull.*, 1986, **34**, 2341.
4. Hyperchem for Windows, Hypercube Inc., 1994 HC40-00-02-01.
5. Haasnoot, C. A. G., de Leeuw, F. A. A. M. and Altona, C., *Tetrahedron*, 1980, **36**, 2783.
6. Tietze, L. F., Niemeyer, U., Marx, P., Glüsenkamp, K.H. and Shwenen, L., *Tetrahedron*, 1980, **36**, 735.