6-DEOXYLAMIOSIDE, A NEW IRIDOID GLUCOSIDE FROM *LAMIUM AMPLEXICAULE*[1]

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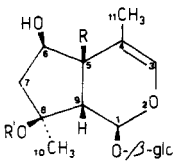
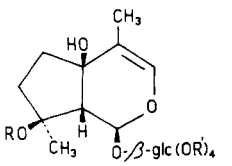
ABSTRACT.—The structure and configuration of 6-deoxylamioside (**5**) has been assigned to a new iridoid glucoside isolated from *Lamium amplexicaule* L. (Labiatae). Conclusive chemical evidence has been achieved by transforming the ipolamiide (**2**) into 6-deoxylamioside tetraacetate (**6**) by successive reduction, acetylation and allylic hydrogenolysis.

From *Lamium amplexicaule* L. (Labiatae), in addition to the well known iridoid glucosides lamiide, lamiol (**1**) (1), ipolamiide (**2**) (2), asperuloside, lamioside (**3**) (1), ipolamiidoside and 5-deoxylamioside (**4**) (3), we isolated a new iridoid glucoside, the 6-deoxylamioside (**5**). Its Rf value is the highest among those of the lamium iridoid glucosides.



1 R=OH R'=H
3 R=OH R'=Ac
4 R=H R'=Ac
12 R=R'=H



5 R=Ac R'=H
6 R=R'=Ac
13 R=R'=H

The isolation of **5** was made difficult in that it was present in only a minute amount and showed a chromatographic behaviour very similar to that of **4** and ipolamiidoside. The final separation was achieved by hplc on Bondapak C_{18} in methanol-water (1:1). Compound **5** is amorphous with the molecular formula $C_{18}H_{28}O_{10}$, identical to that of 5-deoxylamioside (**4**).

Its ir spectrum showed absorptions at 1720 and 1640 cm^{-1} indicating the probable presence of a saturated carbonyl function as well as that of a double bond. The absence of conjugated iridoidic enol-ether function was confirmed by the uv spectrum which did not show any absorption band above 210 nm.

The ^{1}H-nmr spectrum of **5** (D_2O) (see table 1) confirmed an iridoidic structure and showed a close relationship with that of lamioside (**3**). In fact in both spectra

TABLE 1. ^{1}H-nmr chemical shifts assignments (D_2O)[a].

Compounds	3	5
Proton		
1H–C(1).....	5.96, d, $J_{1,9}$=0.8	5.91, d, $J_{1,9}$=1.0
1H–C(3).....	6.17, q, $J_{3,11}$=1.3	6.23, q, $J_{3,11}$=1.5
H–C(6).....	4.07, m (1H)	1.8–2.2 (2H)
2H–C(7).....	1.9–2.2, m	1.8–2.2
2H–C(9).....	2.81, d, $J_{9,1}$=0.8	2.72, d, $J_{9,1}$=1.0
3H–C(10).....	1.42 s	1.49, s
3H–C(11).....	1.58, d, $J_{11,3}$=1.3	1.64, d, $J_{11,3}$=1.5
acetyl.....	2.03, s	2.1, s

[a]Chemical shifts are expressed in ppm and J in Hz. Internal standard HDO (4.70 from TMS). s=singlet, d=doublet, q=quartet, m=multiplet, s* (see data in Experimental)=broad singlet.

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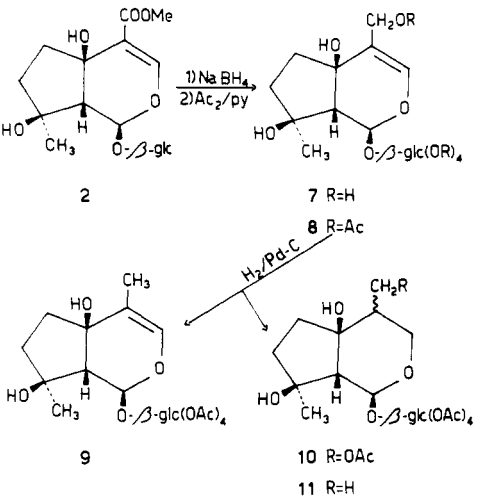
of **3** and **5** the signals of H–1, H–3, H–9, acetyl group, CH_3–4 and CH_3–8 corresponded to each other in chemical shifts or in coupling constant values. The only differences were: i) the absence in **5** of the multiplet of H–6 which appeared at 4.07 ppm in that of **3**; ii) the presence in the spectrum of **5** of a complex signal pattern at ~2.0 ppm (partially obscured by the O-acetyl singlet) corresponding to four protons; in the spectrum of **3** it appeared that the complex signals pattern ~2.0 ppm (partially superimposed to the acetyl singlet) corresponded to only two protons.

The acetylation of **5** in mild conditions gave a tetraacetate (**6**) that still showed hydroxyl bands in the ir spectrum. The ^{1}H-nmr spectrum of **6**, compared with that of **5**, showed deshieldings only for the protons geminal with the glucosylic hydroxyl functions.

All the above data indicated that **5** had the structure and configuration of 6-deoxylamioside.

To get conclusive chemical proof, we decided to convert ipolamiide (**2**) to **6**. Thus, we transformed the $COOCH_3$ group at C–4 of **2** into $-CH_2OH$, by $NaBH_4$ reduction, to get ipolamiidol (**7**). This latter by acetylation under mild conditions gave the pentaacetate (**8**) which by H_2/Pd-C afforded **9**. This was transformed



subsequently to **6** by further acetylation. It should be noted that the use of Pd-C (4) as a catalyst instead of Pd-$BaSO_4$ employed in a similar transformation (3), was necessary to obtain **9** in sufficient amount. By this reaction we obtained also **10** and **11**, the first showing only the hydrogenation of the double bond $\Delta^{3,4}$ and the other both hydrogenolysis and hydrogenation.

Having at our disposal the ^{13}C-nmr data of ipolamiide (**2**) (5), lamiol (**1**) (5) and 5-deoxylamiol (**12**) (3), we prepared the 6-deoxylamiol (**13**) by alkaline hydrolysis of **6** to investigate the effects of the different hydroxylation pattern on the carbons of cyclopentane ring.

The comparison between the ^{13}C-nmr spectra of **1** and **12** (table 2) showed that the C–6 carbon of **12** resonates at lower field than that of **1**. A comparison between the spectra of **1** and **13** showed a slight deshielding of the C–5 of **13** in respect to that of **1**. Therefore, when there is a vic *cis* diol function on C–5/C–6 the steric *cis* interaction between the two hydroxyl groups exerts a shielding effect larger than the β inductive deshielding effect. In the above spectra the other carbons of the cyclopentane ring showed chemical shift values consistent with the known shielding and deshielding effects (5,6,7). The shielding effect found for C–8 in **1** in respect to **12** and **13** is larger than that expected for an additional γ effect and can be related to the presence in **1** of two 1–3 *cis* diaxial interactions (6).

TABLE 2. ^{13}C-nmr chemical shifts assignments (D_2O)[a].

Compounds	13	1[c]	12[b]	2[c]
Carbon n°				
1	93.5	93.1	93.7	94.5
3	135.1	136.1	133.5	152.8
4	115.4	114.6	114.1	114.0
5	73.8	72.6	43.3	71.4
6	35.2	73.9	74.8	38.0
7	39.1	46.8	49.4	39.4
8	79.6	75.8	78.6	78.9
9	60.4	58.9	51.0	60.6
10	23.4	23.8	23.9	22.8
11	11.9	11.9	15.6	169.1
OCH_3				52.5
1'	98.6	98.7	98.7	99.2
2'	73.4	73.3	73.5	73.3
3'	76.3	76.2	76.5	76.2
4'	70.5	70.5	70.5	70.5
5'	77.1	77.0	77.0	77.1
6'	61.5	61.5	61.6	61.6

[a]The standard used was dioxane (67.4 ppm from TMS). Chemical shifts in ppm±0.1. Values with the same superscript in the vertical column are interchangeable.
[b]See ref. [3].
[c]See ref. [5].

A comparison of the ^{13}C-nmr spectrum of **13** with that of **2** (see table 2) shows evidence that the $COOCH_3$ group at C–4 deshields C–3 ($\Delta\delta = 17.6$) and C–6 ($\Delta\delta = 2.8$) while the CH_3 group at C–4 deshields the C–5 ($\Delta\delta = 2.4$) and C–4 ($\Delta\delta = 1.4$).

The deshielding effect on C–5 by the CH_3–4 was previously found in the comparison of the spectra of **12**, shanzhiside methyl ester (which differs from **12** only in the presence of a $COOCH_3$ group at C–4) and ajugol (which is the 11-nor-derivative of **12**) (3).

In conclusion, we wish to point out that *Lamium amplexicaule* contains numerous iridoid glucosides (eight compounds) all showing at C–4 a substituent which is found to be only a CH_3 or a COOR group.

EXPERIMENTAL[2]

ISOLATION OF THE IRIDOIDIC FRACTION.—*Lamium amplexicaule* L. (Labiatae) was collected in May while it was in flower in the neighborhood of Rome. The fresh aerial part of the plant (10 kg) was extracted twice with 90% ethanol (15 liters each) at room temperature for seven days. Paper chromatography with the solvent n-butanol-acetic acid-water (63:10:27) showed seven spots with Rf 0.65 (**5**), 0.60 (**4** and ipolamiidoside), 0.46 (**3**), 0.40 (**2**), 0.36 (asperuloside), 0.30 (**1**) and 0.25 (lamiide). The collected ethanolic extracts were concentrated to an aqueous suspension which was then extracted six times with diethyl ether (500 ml each). The suspension was further concentrated and treated with decolorizing charcoal (0.5 Kg). The resulting suspension was stratified on a gooch funnel (i.d. 18 cm). Monosaccharides were eluted with water (10 liters); disaccharides were eluted with 5% and 10% ethanol (5 liters each); **1** and lamiide were eluted with 30% ethanol (5 liters, Fraction A); asperuloside, **2** and **3** with 50% ethanol (6 liters, Fraction B); and **4**, **5** and ipolamiidoside, contaminated by small quantities of **3** and **2**, with 80% ethanol (6 liters, Fraction C). Fraction C (0.8 g) chromatographed on cellulose (80 g) in n-butanol saturated with water, afforded the following fractions: i) **5**, **4** and ipolamiidoside (0.1 g); ii) **3** (0.2 g); iii) **2** (0.2 g). Fraction i) by hplc on a semipreparative μBondapak C_{18} column (Waters, ø ¼ inc.) in water-methanol (1:1), speed flow 2 ml/min, gave **5** (15 mg) as an amorphous powder, ir (KBr), νmax: 1720 and 1640 cm^{-1}.

TETRAACETATE **6**: Compound **5** (10 mg) was treated with Ac_2O/py (2:1, 0.3 ml) for 1 hr at room temperature. Methanol (1 ml) was added, and the solution was allowed to stand for 20

[2]COLUMN CHROMATOGRAPHY: Si gel 70–230 mesh (Merck) and cellulose CF 11 (Whatman). Tlc: Si gel 60 F_{254} and cellulose (Merck) plates. Pc: Schleicher & Schüll n. 2043 b Mgl paper. Spray reagents: 2N H_2SO_4, vanillin (vanillin 2g, conc. HCl 4 ml, MeOH 100 ml) and resorcin (resorcin 5 g, conc. H_2SO_4 4 ml, EtOH 100 ml). ^{1}H-nmr: Perkin-Elmer R-32 and Jeol C-60; ^{13}C-nmr: Varian CFT-20; ir, uv, or: Perkin-Elmer 357, 137, 141. Hplc: Waters 6000A equipped with uv detector Perkin-Elmer LC 55 B. Melting points were uncorrected (Kofler).
Volatile materials were evaporated under reduced pressure.

with n-butanol saturated with water gave in the first fractions unreacted **2** (70 mg) and subsequently **7** (90 mg) as an amorphous powder which gave a ^{1}H-nmr (D_2O): 6.30 (1H, s*, H-3), 5.60 (1H, s*, H-1), 4.10 (2H, s*, 2H-11), 2.32 (1H, s*, H-9), 1.5–2.3 (4H, 2H-6 and 2H-7), 1.15 (3H, s, 3H-10) ppm.

IPOLAMIIDOL PENTAACETATE **8**: Compound **7** (90 mg) was treated with Ac_2O (1 ml) and pyridine (0.5 ml) for 1 hr at room temperature and then worked up as described for **6**; the residue, when chromatographed on Si gel (9 g) in ether-ethyl acetate (8:2), afforded pure **8** (90 mg), ^{1}H-nmr ($CDCl_3$): 6.30 (1H, s*, H-3), 5.50 (1H, d, $J_{1,9}$=1.5 Hz, H-1), 4.62 (2H, m, 2H-11), 2.50 (1H, d, $J_{9,1}$=1.5 Hz, H-9), 1.26 (3H, s, 3H-10) ppm.

HYDROGENOLYSIS OF **8**; COMPOUNDS **9**, **10** AND **11**.—Compound **8** (90 mg), dissolved in ethanol (10 ml), was added to Pd/C 10% (10 mg) suspended in ethanol (5 ml) and treated with H_2 for 6 min. The reaction was stopped by bubbling in CO_2. The suspension was filtered off in a gooch funnel and the ethanolic solution was evaporated. The residue, chromatographed on Si gel in ether-ethyl acetate (8:2), gave in the first fractions **11** (20 mg), followed successively by the expected tetraacetyl-6-deoxylamiol **9** (35 mg) and finally **10** (15 mg).

Compound **9** crystallized from ethanol. It crystallized as needles, mp 200–201°; ^{1}H-nmr ($CDCl_3$): 5.90 (1H, q, $J_{3,11}$=0.8 Hz, H-3), 5.48 (1H, d, $J_{1,9}$=1.5 Hz, H-1), 2.50 (1H, d, $J_{9,1}$=1.5 Hz, H-9), 1.7–2.2 (4H, 2H-6, 2H-7 partially under acetyl signals), 1.62 (3H, d, $J_{11,3}$= 0.8 Hz, 3H-11), 1.25 (3H, s, 3H-10) ppm.

Compound **10**: ^{1}H-nmr ($CDCl_3$): 2.52 (1H, H-9), 1.22 (3H, s, 3H-10) ppm.

Compound **11**: ^{1}H-nmr ($CDCl_3$): 2.62 (1H, s, H-9), 1.25 (3H, s, 3H-10), 1.06 (3H, d, $J_{11,4}$= 7.5 Hz, 3H-11) ppm.

ACETYLATION OF **9** TO GIVE COMPOUND **6**.—Compound **9** (35 mg) was treated with Ac_2O (0.4 ml) and pyridine (0.2 ml) for 40 hrs at 40°. The reaction, worked up as above, gave a residue which when chromatographed on Si gel in ether-benzene (8:2), afforded a compound (35 mg) which was identical to **6** (^{1}H-nmr, ir superimposable).

DEACETYLATION OF **9** TO GIVE COMPOUND **13**.—Compound **9** (50 mg), dissolved in methanol (3 ml), was treated with 2N NaOH (2 ml) for 4 hrs at room temp. Carbon dioxide was bubbled into the solution until it reached pH≈7. The methanol was evaporated, and the resulting solution was diluted with water and treated with decolorizing charcoal (0.5 g). The resulting suspension was stratified on a gooch funnel, washed with water and eluted with methanol. The residue, chromatographed on Si gel with chloroform-methanol (7:3), gave pure **13** (27 mg) as a colorless amorphous powder, ^{1}H-nmr (D_2O): 6.05 (1H, q, $J_{3,11}$=1.5 Hz, H-3), 5.75 (1H, d, $J_{1,9}$=1.0 Hz, H-1), 2.37 (1H, d, $J_{9,1}$=1.0 Hz, H-9), 1.7–2.1 (4H, 2H-6, 2H-7), 1.58 (3H, d, $J_{11,3}$=1.5 Hz, 3H-11), 1.20 (3H, s, 3H-10) ppm; $[\alpha]^{25}D = -104.5°$ (MeOH, c 2.7).

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