Notes

8-*epi*-Muralioside, an Iridoid Glucoside from *Linaria arcusangeli*†

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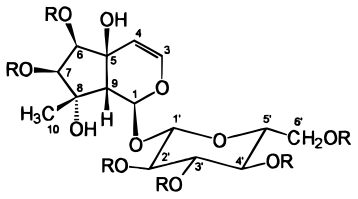
A new polyhydroxylated iridoid glucoside, **1**, was isolated from *Linaria arcusangeli*. The structure of **1** could arise from a regioselective acid-catalyzed-like opening of the epoxide ring of antirrhinoside, the main iridoid constituent of the plant. Compound **1** is the 8-epimer of muralioside, previously isolated from *Cymbalaria muralis*.

In a program aimed toward the phytochemical investigation of endemic Sardinian species, we have examined *Linaria arcusangeli* Atzei et Camarda,[1] an interesting case of endemism located only in a restricted area about 40 km northeast of Cagliari. The plant is rich in iridoids,[2] including antirrhinoside and antirrhide, which are chemotaxonomic markers of the Scrophularioideae-Antirrhineae tribe of the family of Scrophulariaceae, to which the genus *Linaria* belongs.[3,4] An examination of the most polar glycosidic constituents resulted in the isolation of macfadienoside and a new iridoid glucoside, **1**.



1 R = H

2 R = Ac

From an examination of its ^{1}H-NMR spectrum, compound **1** was assigned as a polyhydroxylated iridoid glucoside. Hydroxyl groups could be located at several positions, namely, C-5 owing to the signal observed for H-4 (5.70 ppm), which appeared as a doublet of doublets ($J_{3,4}$ = 6.5 Hz and $J_{4,9}$ = 0.5 Hz); C-6 and C-7 because of the signals of two doublets at 4.68 and 4.10 ppm ($J_{6,7}$ = 4.2 Hz); and C-8 from the singlet occurring at 1.68 ppm, assigned to H_3-10. To confirm the hydroxyl pattern, reaction of **1** with Ac_2O/pyridine under mild conditions gave the hexaacetyl derivative, **2**, whose NMR analysis demonstrated the presence of two secondary esterified hydroxyl groups at C-6 and C-7 and two free tertiary hydroxyl groups at C-5 and C-8.

Concerning the stereochemistry of the hydroxyls present in the aglucone part of **1**, the value of $J_{6,7}$ (4.2 Hz) is indicative of a *cis* relative configuration between H-6 and H-7, since in the *trans* configuration the coupling constant value would lie in a range between 9.0 and 9.7 Hz. A β configuration could be assigned to OH-5 by analogy with that of other known iridoid glycosides, in contrast to the recent isolation of a C-5, C-9 *trans* iridoid.[5] In any case, the configurations at C-5, C-6, and C-9 in **1** could be confirmed by the presence of an evident NOE effect between H-4 and H-6. Other stereogenic centers were assigned for **1** on the basis of NOE experiments: H-9 and H_3-10 exhibited a strong NOE effect, as did H-6 and H-7. Owing to these observations and the absence of a NOE effect between H_3-10 and H-7, a β configuration could be assigned to OH-6, OH-7, and H_3-10. Furthermore, the chemical shift value of the last group appears to be very different from that observed at 1.34 ppm in muralioside, an iridoid glucoside epimeric to **1** at C-8, recently isolated from *Cymbalaria muralis*.[6] Similar data for H_3-10 have been reported for other polyhydroxylated iridoids with the same configuration of muralioside at C-8, as exemplified by physoside (1.27 ppm)[7] and lamiide (1.08 ppm).[8] A deshielded value for the β configuration of H_3-10 *versus* an α configuration is in accordance with previous data (e.g., in the pair plantarenaloside/stansioside).[9] Interestingly, in the ^{13}C-NMR spectrum of **1** the differences for the C-10 signal among these iridoids are not so evident. Therefore, on the basis of the interpretation of the above-reported data, the structure of 8-*epi*-muralioside can be assigned to **1**.

Although C-8 iridoid epimers are known when substituted with CH_3/H or with CH_2OH/OH,[10] and several iridoids exhibit an α-CH_3/β-OH C-8 substitution,[11] to our knowledge compound **1** is the second case of glycosidic iridoid having β-CH_3/α-OH C-8 substitution.[12]

From a biogenetic point of view, the structure **1** could arise from a regioselective acid catalyzed-like opening of the epoxide ring of antirrhinoside, the main iridoid constituent of the plant.

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Experimental Section

General Experimental Procedures. Optical rotations were measured on a JASCO DIP-370 instrument. IR spectra were obtained on a Shimatzu IR-470 instrument. NMR spectra were recorded with a Bruker AM 500 instrument (HDO at 4.72 ppm as reference peak for ^{1}H-NMR spectra in D_2O and C-6′ at 61.5 ppm for ^{13}C-NMR spectra in D_2O). MS were obtained with a Kratos-80 instrument.

TLC was performed on Si gel SiF_{254} (Merck), and plates were visualized using as spray reagents 2 N H_2SO_4 or vanillin (3 g of vanillin, 4 mL of HCl, 100 mL of MeOH).

Plant Material. *L. arcusangeli* Atzei et Camarda (whole plant, 400 g) was collected in Sardinia at about 40 km northeast of Cagliari in April 1994. Voucher specimens were identified by Dr. M. Ballero at the Istituto di Botanica, Università di Cagliari, where they are deposited.

Extraction and Isolation. The whole plant was exhaustively extracted with EtOH at room temperature and the extract evaporated to generate an aqueous suspension. Charcoal (50 g) was added until a negative vanillin test was obtained and the resulting suspension filtered through a Gooch funnel. Elution with water−10% EtOH removed salts and sugars, whereas 30, 60, and 90% EtOH eluted iridoid-containing fractions. The 30% EtOH fraction (6 g) was chromatographed on Si gel in $CHCl_3$−CH_3OH (6:4), affording, besides antirrhinoside (1 g) and linarioside (100 mg), a crude more polar fraction (100 mg) that contained a mixture of at least two different iridoids. Further purification on Si gel in *n*-BuOH−CH_3OH−H_2O (7:1:3) allowed the separation of macfadienoside (50 mg) and 8-*epi*-muralioside (**1**) (15 mg). All known compounds were identified by comparison with authentic samples.

8-*epi*-Muralioside (1): white amorphous powder; $[\alpha]^{20}_D$ −50° (*c* 0.2, MeOH); IR (KBr) ν_{max} 3500, 1645, 1250 cm^{-1}; ^{1}H-NMR (D_2O) δ 6.31 (1H, d, J = 6.5 Hz, H-3), 5.70 (1H, d, J = 1.0 Hz, H-1), 5.11 (1H, dd, J = 6.5, 0.5 Hz, H-4), 4.68 (1H, d, J = 8.0 Hz, H-1′), 4.10 (1H, d, J = 4.2 Hz, H-6), 3.96 (1H, d, J = 4.2 Hz, H-7), 3.74 (1H, dd, J = 12.5, 2.2 Hz, H-6′a), 3.62 (1H, dd, J = 12.5, 4.6 Hz, H-6′b), 3.40 (1H, t, J = 9.2 Hz, H-4′), 3.39 (1H, ddd, J = 9.2, 4.6, 2.2 Hz, H-5′), 3.32 (1H, t, J = 9.2 Hz, H-3′), 3.21 (1H, dd, J = 9.2, 8.0 Hz, H-2′), 2.68 (1H, sharp m, H-9), 1.68 (3H, s, H_3-10); ^{13}C-NMR (D_2O) δ 141.1 (C-3), 107.7 (C-4), 98.7 (C-1′), 93.4 (C-1), 81.0 (C-8), 77.2 (C-3′)*, 77.1 (C-5′)*, 76.1 (C-6), 73.6 (C-4′), 73.3 (C-7), 70.4 (C-2′), 68.5 (C-5), 61.5 (C-6′), 56.7 (C-9), 27.5 (C-10), assignments bearing an asterisk may be interchanged; FABMS *m*/*z* 403 [M + Na]$^+$, 419 [M + K]$^+$.

8-*epi*-Muralioside Hexaacetate (2). Compound **1** (10 mg) was dissolved in pyridine (0.1 mL) and allowed to stand in Ac_2O (0.2 mL) for 2 h at room temperature. Then, MeOH was added, the volatile material evaporated *in vacuo*, and the resulting residue, chromatographically pure, directly submitted to NMR analysis: ^{1}H-NMR ($CDCl_3$) δ 6.29 (1H, d, J = 6.5 Hz, H-3), 5.61 (1H, d, J = 1.5 Hz, H-1), 5.40 (1H, d, J = 4.2 Hz, H-6), 5.24 (1H, t, J = 9.2 Hz, H-3′), 5.19 (1H, dd, J = 6.5, 0.5 Hz, H-4), 5.18 (1H, d, J = 4.2 Hz, H-7), 5.02 (1H, t, J = 9.2 Hz, H-4′), 4.93 (1H, dd, J = 9.2, 8.0 Hz, H-2′), 4.80 (1H, d, J = 8.0 Hz, H-1′), 4.29 (1H, dd, J = 12.5, 4.6 Hz, H-6′b), 4.11 (1H, dd, J = 12.5, 2.2 Hz, H-6′a), 3.71 (1H, ddd, J = 9.2, 4.6, 2.2 Hz, H-5′), 2.87 (1H, sharp m, H-9), 2.09, 2.08, 2.07, 2.00, 1.98 (18H, 5 s, 6 × Ac), 1.78 (3H, s, H_3-10); ^{13}C-NMR ($CDCl_3$) δ 170.9, 170.6, 169.9, 168.8 ($COCH_3$), 141.1 (C-3), 107.1 (C-4), 95.8 (C-1′), 92.4 (C-1), 79.1 (C-8), 75.7 (C-6), 72.1 (C-3′)*, 71.8 (C-5′)*, 71.1 (C-4′), 69.7 (C-7), 68.1 (C-2′), 66.8 (C-5), 61.6 (C-6′), 56.4 (C-9), 21.1, 20.7, 20.5 ($COCH_3$), 29.6, (C-10), assignments bearing an asterisk may be interchanged.

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