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IRIDOIDS FROM ENDEMIC SARDINIAN *LINARIA* SPECIES*

ARMANDODORIANO BIANCO, MARCELLA GUISO, MARIACELESTE MARTINO, MARCELLO NICOLETTI,† MAURO SERAFINI,† LAMBERTO TOMASSINI,† LUIGI MOSSA‡ and FERRUCCIO POLI‡

Dipartimento di Chimica, Centro CNR di Studio per la Chimica delle so Stanze Organichenaturali, Università 'La Sapienza', P. le A. Moro 5, I 00185 Rome, Italy; †Dipartimento di Biologia Vegetale, Università 'La Sapienza', P. le A. Moro 5, I 00185 Rome, Italy; ‡Istituto e Orto Botanico, Università di Cagliari, Via Frà Ignazio de Laconi, Cagliari, Italy

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Key Word Index—*Linaria* sp.; Scrophulariaceae; iridoid; bisiridoid; monoterpene; arcusangeloside; antirrhinoside; linarioside; antirrhide; linaride; 5-glucosyl-antirrhinoside; 5-deoxyantirrhinoside.

Abstract—*Linaria arcusangeli* contains a new bisiridoid, named arcusangeloside, besides the known antirrhinoside, linarioside, antirrhide, linaride and 5-glucosylantirrhinoside. The new bisiridoid is formed from a non-glycosidic cyclopentanoid which esterified the iridoid glucoside 5-deoxyantirrhinoside, also isolated as a free compound from the plant extract. *L. flava* subsp. *sardoa* contains the new bisiridoid, 5-deoxyantirrhinoside, antirrhinoside, linarioside and antirrhide.

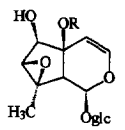
INTRODUCTION

The flora of Sardinia is characterized by a high percentage of endemic plants. However, in recent years several endemic species have been subjected to a severe restriction of their distribution and are at present in serious risk of extinction. Therefore, we have started a programme to examine the phytochemical composition of the endemic Sardinian species. In this paper, we report on the glycosidic components of the recently described *Linaria arcusangeli* Atzei et Camarda [2] and of *Linaria flava* subsp. *sardoa* Arrigoni [3]. Both species represent interesting cases of endemism, being at present restricted to areas of few square kilometres. The first plant is located *ca* 40 km northeast of Cagliari, whereas the second, once present in several areas, is now to our knowledge restricted to a single area of the east coast 45 km from Cagliari.

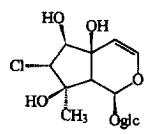
The genus *Linaria* contains several species considered as medicinal plants based on their healing (*L. vulgaris* Mill.), diuretic and laxative (*L. japonica* Miq.), diuretic, tonic and antiscorbutic (*L. cymbalaria* Mill. = *Cymbalaria muralis* P. Gaertn., B. Meyer et Scherb.) properties [4–6]. Chemically, they are characterized by flavonoid and iridoid constituents [7].

RESULTS AND DISCUSSION

The iridoid fraction of *L. arcusangeli* yielded antirrhinoside (**2**), as the main component, linarioside (**3**), antirrhide, (**4**), linaride (**5**), 5-glucosylantirrhinoside (**6**) and the two new compounds **1** (arcusangeloside), in significant yield, and **7**, in smaller amounts. Compound **7** (5-deoxyantirrhinoside) has already been obtained by deacetylation of genistifolin, an iridoid from *L. genistifolia* Mill, but it was only partly characterized. Its presence was hypothesized on the basis of TLC data [8].



2 R=H
6 R=Oglc



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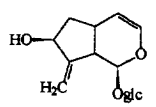

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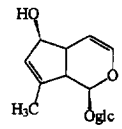

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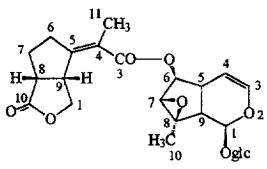
Linaria flava subsp. *sardoa* showed a very similar iridoid pattern: **1** and **7**, together with **2**, **4** and **3**.

Compound **1** presented a complicated ^{1}H NMR spectrum consistent with a bisiridoid structure. Part of the spectrum (part b) could be assigned to a 5-deoxyantirrhinoside unit, where the chemical shift value (δ



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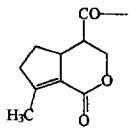


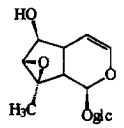
8 Alternative structure for part a

deduced by the ^{13}C NMR data. On considering the spectrum in D_2O the signal at δ 48.9 must be assigned both to C-9 of the iridoid glucoside unit (part b) and to a methine of part a, as evident by the $^{13}C-^{1}H$ HETCOR spectrum and by the ^{13}C NMR spectrum in CD_3OD.

The ^{1}H NMR spectrum of part a (D_2O) showed an ABX system, whose AB part (δ 4.57, *dd*, and 4.48, *dd*) was assigned to an oxymethylene which was part of a lactone system (carbonyl chemical shift value at δ 175.7). The X part was a methine at δ 3.15, which was further coupled with another methine at δ 3.43, which was coupled in turn (as evident in the $^{1}H-^{1}H$ COSY) with a methylene (δ 2.17 and 2.40). The last group was linked to a second methylene (δ 2.56 and 2.40), which did not present further couplings apart from a long-range one with a methyl group, whose NMR data accounted for the presence of a totally substituted double bond conjugated with a carbonyl group. Therefore, part a was a δ-lactone ring fused with a cyclopentane having a double bond which was involved in the bridge linkage with part b, as reported in structure **1**. The assignment was confirmed by the $^{13}C-^{1}H$ HETCOR, $^{1}H-^{1}H$ COSY and $^{1}H-^{1}H$ COSY-LR spectra, which, together with NOE-difference experiments, allowed us to assign the configurations of all chiral centres, while the configuration of the methyl group was assigned on the basis of its long-range coupling with the methylene protons.

The alternative structure **8** for the non-glycosidic iridoid moiety of (**1**) (part a), described by Otsuka [9] for iridolinarin C, a bisiridoid isolated from *L. japonica*, and also consistent with the sequence $=COOCH_2CH\ CH\ CH_2CH_2=$, must be discarded on the basis of the COLOC NMR experiments: in particular, the long-range couplings H-11/C(3), H-11/C(5) and H-9/C(5) are not in accordance with the $^{13}C-^{1}H$ long-range correlations reported by Otsuka [9] for the non-glycosidic iridoid moiety of iridolinarin C.

Alkaline hydrolysis of arcusangeloside afforded two separate iridoid units. The glucosidic one was, as previously hypothesized, 5-deoxyantirrhinoside (**7**), and the NMR data in comparison with those of **1** showed the expected upfield shifts of H-6 and C(6), demonstrating that the secondary hydroxyl is esterified by the non-glucosidic moiety. The non-glucosidic unit, under the hydrolytic conditions used, underwent opening of the lactone ring, which remained open even after acidification, thus making necessary its conversion into biogenetically related to the dialdehydic skeleton of iridoid precursors by internal oxyreductive opening of the dihydropyrane ring and formation of the δ-lactone by a reaction between the carbonyl at C-10 and the primary alcoholic function at C-1. On the basis of this conjecture the usual numeration of the iridoid skeleton was adopted also for part a of structure **1**.



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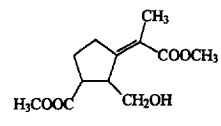

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The structures of the iridoids isolated from *L. arcusangeli* and *L. flava* are well in accordance with previous isolations from *Linaria* spp. [9–12], which are mainly characterized by a C-10 as a methyl group, an epoxide function at C-7/C-8 and the absence of C-11, as well as with the occurrence of 6-acyl derivatives of antirrhinoside as in *L. genistifolia* [8], *L. Japonica* [9] and *L. clementei* Haensel [12].

EXPERIMENTAL

Materials and methods. TLC: silica gel SiF_{254} (Merck) and RP-8 F_{254} (Merck); Spray reagents: 1 M H_2SO_4, vanillin (3 g vanillin, 4 ml HCl, 100 ml MeOH); NMR: Bruker AM 500; $^{13}C-^{1}H$ coupling constants for COLOC NMR experiments: 5.0, 6.2, 7.5 and 10.0 Hz; MS: Kratos-80. $[\alpha]_D$: Jasco DIP-370, IR.

Plant material. Linaria arcusangeli Atzei et Camarda (whole plant, 400 g) and *L. flava* (Poiret) Desf. subsp. *sardoa* (Sommier) Arrigoni (whole plant, 200 g) were collected in Sardinia (see above) in April 1994. Voucher specimens were identified by Dr M. Ballero, Istituto di Botanica, Università di Cagliari, where they are deposited.

Extraction and separation. Each species was exhaustively extracted with EtOH at room temp. and the extract evapd to an aq. suspension. Charcoal (50 g) was added until a negative vanillin test was obtained and the resulting suspension stratified on a Gooch funnel. Elution with aq. 10% EtOH removed salts and sugar, whereas 30, 60 and 90% EtOH eluted iridoid-containing frs. The 90% EtOH fr. was subjected to CC on silica gel (15 g) in $CHCl_3$–MeOH (17:3) to give partially pure iridoid frs. For *L. arcusangeli*, the less polar fr. was purified using the same conditions to afford pure 1 (140 mg), as an amorphous powder.

Arcusangeloside (**1**). $[\alpha]_D^{20} = -153$ (MeOH, c = 0.2); IR (KBr), ν_{max} cm^{-1}: 3500, 3000, 1730, 1600, 1400, 1360, 1250; UV (MeOH), λ_{max} = 248 nm, log ε = 2.32; ^{1}H NMR (D_2O): part a, δ: 4.57 (1 H, *dd*, J = 12.2, 2.0 Hz, H-1a), 4.48 (1 H, *dd*, J = 12.2, 3.7 Hz,

2.40 (1 H, partially overlapped with H-5 of part b, H-6b), 2.17 (1 H, *m*, H-7a), 2.10 (3 H, *bt*, $J = 2.0$ Hz), 1.57 (1 H, *m*, H-7b); part b: 6.39 (1 H, *dd*, $J = 6.2$, 1.6 Hz, H-3), 5.08 (1 H, *d*, $J = 10.0$ Hz, H-1), 4.96 (1 H, *dd*, $J = 8.5$, 1.5 Hz, H-6), 4.89 (1 H, *dd*, $J = 6.2$, 4.3 Hz, H-4), 3.60 (1 H, *bd*, $J = 1.5$ Hz, H-7), 2.46 (1 H, *dd*, $J = 10.0$, 7.5 Hz, H-9), 2.38 (1 H, partially overlapped with H-6b of part a, H-5), 1.50 (3 H, *s*, 3H-10); glucose unit: 4.82 (1 H, *d*, $J = 9.2$ Hz, H-1), 3.85 (1 H, *dd*, $J = 13.2$, 2.0 Hz, H-6a), 3.67 (1 H, *dd*, $J = 13.2$, 4.3 Hz, H-6b), 3.45 (1 H, *t*, $J = 9.2$ Hz, H-3), 3.40 (1 H, *m*, H-5), 3.37 (1 H, *t*, $J = 9.2$ Hz, H-4), 3.32 (1 H, *t*, $J = 9.2$ Hz, H-2); ^{13}C NMR (D_2O): part a, δ: 175.7, C(10); 169.4, C(4); 167.0, C(3); 124.5, C(5); 72.7, C(1); 48.9, C(8); 45.1, C(9); 40.9, C(6); 29.3, C(7); 18.9, C(11); part b: 143.7, C(3); 104.7, C(4); 97.3, C(1); 83.6, C(6); 67.3, C(8); 65.3, C(7); 48.9, C(9); 38.3, C(5); 19.4, C(10); glucose unit: 101.1, C(1); 78.9, C(5); 78.5, C(3); 75.5, C(2); 72.2, C(4); 63.4, C(6). ^{13}C NMR (CD_3OD): part a, δ 172.7, C(10); 163.3, C(3); 162.1, C(4); 123.8, C(5); 71.9, C(1); 45.9, C(8); 44.8, C(9); 39.2, C(6); 28.1, C(7); 18.0, C(11); part b: 142.5 C(3); 102.4, C(4); 95.4, C(1); 82.6, C(6); 64.1, C(8); 63.1, C(7); 45.7, C(9); 36.8, C(5); 16.6, C(10); glucose unit: 99.8, C(1); 78.6, C(3); 77.9, C(5); 74.9, C(2); 70.8, C(4); 62.7, C(6); FAB-MS *m/z*: 537 $[M + Na]^+$, 553 $[M + K]^+$.

The other frs of the 90% elution were further purified on RP-8 silica gel F_{254} in H_2O–MeOH (7:3) affording **4** (15 mg) and **5** (10 mg). The 60% fr. (1.6 g) was subjected to CC on silica gel in $CHCl_3$–MeOH (4:1) and successively on RP-8 silica gel F_{254} in H_2O–MeOH (7:3) affording **1** (50 mg), **2** (200 mg), **3** (55 mg), **4** (30 mg), **5** (25 mg) and **7** (10 mg), whose NMR and physical data proved identical with those of the product obtained by alkaline hydrolysis of **1**. The 30% fr. (6 g) was subjected to CC on silica gel in $CHCl_3$–MeOH (3:2), affording **2** (1 g), **3** (100 mg) and **6** (50 mg). All known compounds were identified by comparison with authentic samples. The sepn of *L. flava* subsp. *sardoa* extract in the same conditions gave **1** (80 mg), **2** (500 mg), **3** (60 mg), **4** (30 mg) and **7** (10 mg).

Alkaline hydrolysis of **1**. Compound **1** was dissolved in satd $Ba(OH)_2$ soln (3 ml) and the mixt. allowed to stand for 2 hr at room temp. The soln was then neutralized by bubbling with CO_2 and, after filtration to remove the $Ba(CO_3)_2$ residue, the H_2O was removed *in vacuo*. The organic residue was dissolved in MeOH (5 ml), acidified by bubbling with CO_2, and then CH_2N_2 was added. The MeOH was evapd, H_2O added and the soln extracted several times with EtOAc. The aq. phase contained **7** and the organic one **9**.

Compound **7**. Amorphous powder, $[\alpha]_D^{20} = -48$ (MeOH, $c = 0.2$). IR (KBr), ν_{max} cm^{-1}: 3500, 1640, 1250; ^{1}H NMR (D_2O) δ: 6.30 (1 H, *dd*, $J = 6.2$, 1.6 Hz, H-3), 5.07 (1 H, *dd*, $J = 6.2$, 4.3 Hz, H-4), 4.99 (1 H, *d*, $J = 10.0$ Hz, H-1), 4.80 (1 H, *d*, $J = 8.0$ Hz, H-1′), 3.99 (1 H, *dd*, $J = 7.0$, 1.5 Hz, H-6), 3.82 (1 H, *dd*, $J = 13.2$, 4.3 Hz, H-6′a), 3.66 (1 H, *dd*, $J = 13.2$, 2.0 Hz, H-6′b), 3.44 (1 H, *bd*, $J = 1.5$ Hz, H-7), 2.40 (1 H, *dd*, $J = 9.5$, 7.5 Hz, H-9), 2.21 (1 H, *m*, H-5), 1.49 (3 H, *s*, 3H-10); ^{13}C NMR (D_2O): δ 145.2, C(3); 104.2, C(4); 95.8, C(1); 78.9, C(6); 66.0, C(7); 64.9, C(8); 45.5, C(9); 38.4, C(5); 17.7, C(10); glucose unit: 99.3, C(1); 77.0, C(3); 76.6, C(5); 74.0, C(2); 70.4, C(4); 61.5, C(6); FAB-MS *m/z*: 369 $[M + Na]^+$, 385 $[M + K]^+$.

Compound **9**. Amorphous powder, $[\alpha]_D^{20} = -6.0$ (MeOH, $c = 0.1$). IR (KBr), ν_{max} cm^{-1}: 3500, 3035, 3000, 1750, 1725, 1638, 1600, 1372, 1350, 1250, 1043; UV (MeOH), $\lambda_{max} = 238$ nm $\log \varepsilon = 3.53$; ^{1}H NMR ($CDCl_3$) δ: 3.78 (1 H, *dd*, $J = 11.2$, 8.5 Hz, H-1a), 3.75 (3 H, *s*, OCH_3), 3.70 (3 H, *s*, OCH_3), 3.55 (2 H, *m*, H-1b and H-9), 3.08 (1 H, *m*, H-8), 2.49 (1 H, *dt*, $J = 17.5$, 8.0 Hz, H-6a), 2.40 (1 H, *bs*, OH), 2.32 (1 H, *ddd*, $J = 17.5$, 9.5, 3.0 Hz, H-6b), 2.08 (3 H, *bs*, 3H-11), 1.90 (1 H, *m*, H-7), 1.68 (3 H, *ddt*, $J = 13.6$, 8.0, 3.4 Hz, H-7b); ^{13}C NMR ($CDCl_3$): δ 175.2, C(10); 166.0, C(3); 158.3, C(4); 127.3, C(5); 60.0, C(1); 49.5, C(9); 45.6, C(6); 25.1, C(7); 16.6, C(11); FAB-MS *m/z*: 243 $[M + H]^+$.

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