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IRIDOIDS IN *PHYSOSTEGIA VIRGINIANA*

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Key Word Index—*Physostegia virginiana*; Lamiaceae; iridoid glucosides; physoside; virginioside.

Abstract—Two varieties of *Physostegia virginiana* were investigated for iridoid glucosides. The previously reported glucoside deoxyloganin (bisdeoxydihydromonotropein) could not be detected in either variety. Two new iridoid glucosides, named physoside and virginioside, were isolated and the structures elucidated mainly by NMR spectroscopy. Additionally, the known compounds myoporoside, antirrhinoside, 5-*O*-glucosyl-antirrhinoside, galiridoside, 8-*O*-acetyl-myoporoside, 8-*O*-acetyl-harpagide, ajugoside and 8-epiloganic acid were isolated and identified by NMR.

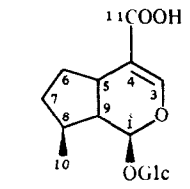
INTRODUCTION

The iridoid glucoside deoxyloganic acid (bisdeoxydihydromonotropein, **1**) was isolated from *Physostegia virginiana* (L.) Benth. by Rimpler and von Lehmann [1]. Furthermore, Kooiman [2] isolated three compounds which were characterized only by R_fs and specific optical rotations. Iridoid glucosides are widespread in certain subfamilies of the Lamiaceae [2–5], but except for the abnormal compounds found in *Nepeta cataria* [6–8], the glucosides so far reported from the family are presumably derived from *epi*-deoxyloganic acid (**2**) by transformations of the cyclopentane ring followed by decarboxylation [9]. We have now reinvestigated *P. virginiana* in order to, if possible, resolve the apparent discrepancy and here we report the results.

RESULTS AND DISCUSSION

Two varieties of *Physostegia virginiana* Benth. were available, namely *P. virginiana* var. *speciosa* (Sweet) A. Gray and *P. virginiana* var. *virginiana*, i.e. apparently those used in the earlier work [1]. The aqueous extract from *P. virginiana* var. *speciosa* provided the known iridoid glucosides myoporoside (**3**), antirrhinoside (**4**), 5-*O*-β-glucosyl-antirrhinoside (**5**), galiridoside (**6**), acetylmyoporoside (**7**), acetylharpagide (**8**) and ajugoside (**9**) as well as two previously unknown compounds which we have named physoside (**10**) and virginioside (**11**).

The ^{13}C NMR spectrum of physoside (**10**) exhibited 15 signals of which six could be assigned to a β-glucopyranosyl moiety. Additionally, three signals could be assigned to C-1, C-3 and C-4 of an iridoid glucoside decarboxylated at C-4, as in the above compounds. The ^{1}H NMR data and the remaining ^{13}C NMR signals were consistent with a structure such as **10**, assuming the usual stereochemistry at C-1, C-5 and C-9, but leaving the configuration at C-6, C-7 and C-8 open. NOE experiments showed that when the signal of the 10-methyl group was irradiated the largest effects were seen for H-7 and H-1 (5–7%) together with a smaller effect for H-9 (2%). Similarly, irradiation of H-9 gave the largest effects



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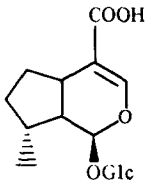

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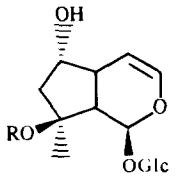

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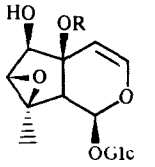
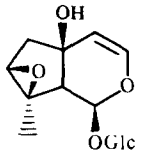
7 R = Ac



4 R = H
5 R = Glc



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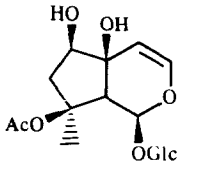

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at H-1 and H-5 (6–8%) with a smaller one for H-6 (3%) and a minimal one for H-7 (0.2%). These results showed the β-configuration of H-6 and H-9, and that H-7 and the 10-methyl group were placed on the α-face of the molecule, thus proving the structure **10** for physoside. In agreement with the structure shown, acetylation under non-forcing conditions gave a hexaacetate (**10a**) consistent with a tertiary hydroxy group at C-8.

The structure of virginioside was similarly proved to be **11**. Thus the ^{13}C NMR signals from a β-glucopyranosyl moiety and those from C-1, C-3 and C-4 could be

assigned as above. Of the remaining five signals, the one at δ51.9 could be assigned to C-9 in a molecule substituted with hydroxy groups at C-5 (δ64.1) and C-8 (δ77.1). The last two signals at δ61.3 and 62.1 indicated the presence of an oxirane ring at C-6 and C-7. The ^{1}H NMR spectrum was consistent with this conclusion. Also two doublets (J = 2.9 Hz each) at δ3.51 and 3.41 assigned to H-6 and H-7, respectively, showed the presence of an oxirane ring at this position. Acetylation under non-forcing conditions provided a tetraacetate (**11a**) in agreement with the gross structure **11**. Assuming the usual stereochemistry at C-1, C-5 and C-9, the stereochemistry of the remaining centres were proved by ^{1}H NOE experiments as above (see Experimental). Irradiation at the 10-Me signal showed the largest enhancements for H-1 and H-7 (3–4%) with minor ones for H-3 and H-4. Furthermore, irradiation at the C-9 signal showed the largest enhancement for H-1 (4%) and minimal enhancements for H-6 and H-7 (0.4%), proving the β-disposition of the oxirane ring oxygen and the 8-hydroxy group as shown for **11** in Fig. 1. The presence of oxirane rings in iridoid glucosides is common and it is usually situated at the 7,8-position [10]. To our knowledge, only a single iridoid with a 6,7-oxirane ring has so far been published [11].

Physoside (**10**), myoporoside (**3**) and galiridoside (**6**) were similarly isolated from the other variety, *P. virginiana* var. *virginiana*. In order to isolate iridoid acids, the usual work-up procedure was modified to circumvent difficulties owing to the degree of dissociation during reversed phase chromatography. Sodium hydrogen carbonate was added first to ensure complete conversion to the salt form and thus very little retention during the first chromatography step. Secondly, excess acetic acid was added to the polar fraction from the first step, this time causing protonation and thus retention on the column. The fractions were monitored at 235 nm in order to detect the UV absorption from the iridoid acids. A number of small UV-positive fractions were found, but according to ^{1}H NMR only one contained an iridoid, namely 8-*epi*-loganic acid (**12**). Neither deoxyloganic (**1**) nor 8-*epi*-deoxyloganic acid (**2**) could be detected.

The iridoids found in the two varieties of *P. virginiana* tested in the present work showed, on the one hand, that these two varieties are also different with regard to iridoid glucosides. On the other hand, the spectrum of iridoids present in the two varieties is so similar, that they may easily belong to the same species. Additionally, the type of compounds found in the present work are in harmony with the systematic position of *Physostegia* among the iridoid containing genera of Lamiaceae.

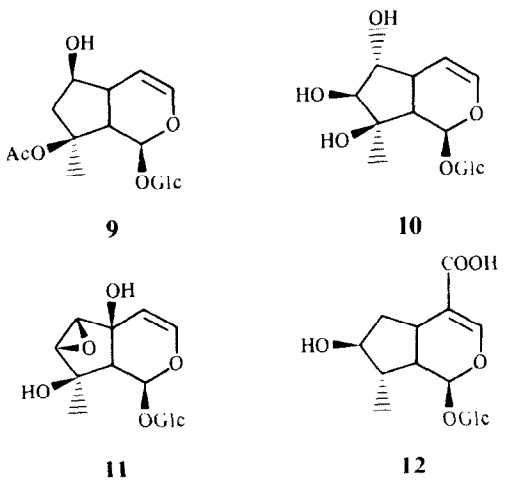

Fig. 1.

In the 1968 edition of 'The New Britton and Brown Illustrated Flora of the Northeastern United States and Adjacent Canada' [12] *Physostegia* was included in the genus *Dracocephalum*. However, recent classification [13, 14] of Lamiaceae split the family into two main taxa, placing *Physostegia and Dracocephalum* in each of these. Furthermore, Kooiman [2] in his investigation of a large number of genera and species within the family found that iridoids were present only in one of the above main taxa (including *Physostegia*), and verified that *Dracocephalum* (three species) apparently did not contain iridoids. The above incorporation of *Physostegia* into *Dracocephalum* thus seems unjustified.

EXPERIMENTAL

Microanalyses were performed at LEO Microanalytical Laboratory, Ballerup, Denmark. Mps: corrected. Preparative chromatography was performed on Merck Lobar reversed phase columns eluting with H_2O–MeOH mixtures, monitored at 206 and/or 254 nm. For ^{1}H NMR spectra the standards used were the HDO-peak (δ4.75 in D_2O) or TMS. Plant material was obtained from The Botanical Garden, The University of Copenhagen. Vouchers (see nos below) have been deposited at the Herbarium of the Botanical Museum, Copenhagen and were verified by Dr K. Rahn.

Physostegia virginiana var. *speciosa* (voucher no IOK 1–89). Frozen plants (180 g) harvested in Oct. were homogenized twice with EtOH and the extract taken to dryness. The residue was partitioned in Et_2O–H_2O whereupon the aq. fraction was passed through a column of Al_2O_3 (300 g) followed by H_2O (600 ml) and the combined eluates concd. The resulting dark syrup was dissolved in MeOH (40 ml) and filtered through activated charcoal (8 g) to give a colourless product (4.1 g). Application to a C-8 column (size C) eluting with H_2O–MeOH (10:1) to (1:1) gave first a polar fraction consisting (NMR) mainly of carbohydrates etc. The second fraction (10:1) was pure physoside (**10**; 31 mg, 0.02%), crystallized from EtOH, mp 222°; $[\alpha]_D^{23}$ −232° (H_2O; *c* 0.5); ^{1}H NMR (250 MHz; D_2O): δ6.29 (*dd*, J = 6.5 and 1.9 Hz, H-3), 5.57 (*d*, J = 1.9 Hz, H-1), 5.02 (*br dd*, J = 6.5 and 2.1 Hz, H-4), 4.72 (*d*, J = 8 Hz, H-1′), 4.08 (*dd*, J = 9.5 and 7.0 Hz, H-6), 3.53 (*d*, J = 9.5 Hz, H-7), 2.78 (*m*, H-5), 2.36 (*br d*, J = 9 Hz, H-9), 1.27 (3H, *s*, 10-Me); ^{13}C NMR (63 MHz, D_2O): δ140.3 (C-3), 102.6 (C-4), 93.4 (C-1), 80.6 (C-7*), 75.5 (C-6*), 74.9 (C-8), 47.6 (C-9), 30.3 (C-5), 23.2 (C-10), 98.7, 73.5, 76.4, 70.4, 77.0 and 61.5 (C-1′ through C-6′). (Found: C, 49.33; H, 6.75. $C_{15}H_{24}O_{10}$ requires: C, 49.45; H, 6.64). The third fraction (5:1) was myoporoside [15, 16] (**3**; 93 mg, 0.05%) characterized by ^{1}H NMR (250 MHz, D_2O): δ6.34 (*dd*, J = 6.5 and 2.0 Hz, H-3), 5.5 (*d*, J = 2.4 Hz, H-1), 4.96 (*br dd*, J = 6.5 and 2.2 Hz, H-4), 4.73 (*d*, H-1′), 4.46 (*dt*, J = 10.3 and 6.5 Hz, H-6), 2.90 (*m*, H-5), 2.30 (*br d*, J = 8.0 Hz, H-9), 1.94 (*br dd*, J = 13.4 and 6.5 Hz, H-7β), 1.76 (*dd*, J = 13.4 and 10.4 Hz, H-7α), 1.33 (3H, *s*, 10-Me); ^{13}C NMR (63 MHz, D_2O): δ141.0 (C-3), 101.7 (C-4), 93.6 (C-1), 77.7 (C-8), 72.0 (C-6), 50.8 (C-9), 47.3 (C-7), 36.1 (C-5), 25.9 (C-10), 98.8, 73.5, 76.4, 70.4, 77.0 and 61.5 (C-1′ through C-6′). The fourth fraction (**A**; 101 mg; 5:1) consisted of a mixture of **4**, **5** and **11** (see below). The fifth fraction (4:1) was galiridoside (**6**; 43 mg, 0.02%), compared with an authentic sample [17]. The last fraction (**B**; 387 mg; 1:1) contained **7**–**9** (see below).

Rechromatography of fraction **A** on a C-18 column (5:1) provided first 5-*O*-glucosyl-antirrhinoside (**5**; 18 mg, 0.01%) characterized by ^{1}H NMR (90 MHz, D_2O): δ6.63 (*d*, J = 6.0 Hz, H-3), 5.43 (*d*, J = 7.5 Hz, H-1), 5.10 (*d*, J = 6.0 Hz, H-4), 4.31 (*d*, J = 1.5 Hz, H-6), 3.58 (*d*, J = 1.5 Hz, H-7), 1.51 (3H, *s*, 10-Me), identical to that reported [15]. ^{13}C NMR (63 MHz, D_2O): δ145.1 (C-3), 106.3 (C-4), 95.9 (C-1), 80.4 (C-5), 78.2 (C-6), 65.8 (C-7), 64.4 (C-8), 50.3 (C-9), 17.5 (C-10), 99.3, 98.9, 77.1, 76.6, 76.5, 76.5, 73.8, 73.7, 70.4, 70.2, 61.5 and 61.4 (two β-glucopyranosyl moieties). Then antirrhinoside (**4**; 22 mg, 0.01%) [17] was eluted, followed by virginioside (**11**; 16 mg, 0.01%), isolated as an amorphous powder $[\alpha]_D^{22}$ −170° (EtOH; *c* 0.5); ^{1}H NMR (250 MHz, D_2O): δ6.39 (*d*, J = 6.6 Hz, H-3), 5.68 (*br s*, H-1), 5.09 (*dd*, J = 6.5 and 1.6 Hz, H-4), 4.70 (*d*, J = 8 Hz, H-1′), 3.51 (*d*, J = 2.9 Hz, H-6), 3.41 (*d*, J = 2.9 Hz, H-7), 2.16 (*m*, H-9), 1.18 (3H, *s*, 10-Me). ^{13}C NMR (63 MHz, D_2O): δ143.4 (C-3), 102.6 (C-4), 93.3 (C-1), 77.3 (C-8), 69.3 (C-5), 62.3 (C-7*), 61.7 (C-6*), 52.1 (C-9), 20.2 (C-10), 99.5, 73.4, 76.3, 70.6, 77.2 and 61.5 (C-1′ through C-6′). (Found: C, 45.16; H, 6.28. $C_{15}H_{22}O_{10}\cdot 2H_2O$ requires: C, 45.22; H, 6.57).

Rechromatography of fraction **B** as above (2.5:1) gave first 8-*O*-acetylmyoporoside (**7**; 15 mg, 0.01%), the NMR spectra being virtually identical with those reported [cf. 16, 19], except for the different standard used. The second fraction contained 8-*O*-acetylharpagide (**8**; 54 mg, 0.03%) identified by the NMR spectra [20, 21]. The last fraction consisted of ajugoside (**9**; 21 mg, 0.01%), identified similarly [16, 20].

Physoside hexaacetate (**10a**) was prepared by acetylation (pyridine–Ac_2O, 2:1; 2 hr at room temp.). Crystd from EtOH; mp 134–135°; $[\alpha]_D^{20}$ −164° ($CHCl_3$; *c* 0.4); ^{1}H NMR (250 MHz, $CDCl_3$): δ6.25 (*dd*, J = 6.5 and 2.0 Hz, H-3), 5.40 (*d*, J = 2.0 Hz, H-1), 5.31 (*dd*, J = 7.3 and 8.2 Hz, H-6), 5.03 (*d*, J = 8.2 Hz, H-7), 4.84 (*br dd*, J = 6.5 and 2.4 Hz, H-4), 2.97 (*m*, H-5), 2.59 (*br d*, J = 9 Hz, H-9), 2.00–2.13 (*s*'s, 6 × OAc), 1.33 (3H, *s*, 10-Me); ^{13}C NMR (63 MHz, $CDCl_3$): δ139.8 (C-3), 100.9 (C-4), 91.4 (C-1), 79.4 (C-7*), 75.1 (C-6*), 74.1 (C-8), 46.5 (C-9), 28.7 (C-5), 23.1 (C-10), 95.2, 70.4, 71.7, 68.0, 72.3 and 61.5 (C-1′ through C-6′). (Found: C, 52.58; H, 5.98. $C_{27}H_{36}O_{16}$ requires: C, 52.59; H, 5.89).

Virginioside tetraacetate (**11a**) was prepared as above. Crystd from EtOH; mp 203–204°; $[\alpha]_D^{21}$ −127° ($CHCl_3$; *c* 0.9); ^{1}H NMR (250 MHz, $CDCl_3$): δ6.24 (*d*, J = 6.3 Hz, H-3), 5.62 (*d*, J = 1 Hz, H-1), 5.07 (*dd*, J = 6.3 and 1.6 Hz, H-4), 3.42 (*d*, J = 2.6 Hz, H-6), 3.25 (*d*, J = 2.7 Hz, H-7); 2.28 (*t*-like, J = *ca* 1 Hz, H-9), 2.00–2.10 (*s*'s, 4 × OAc), 1.22 (3H, *s*, 10-Me); ^{13}C NMR (63 MHz, $CDCl_3$): δ140.6 (C-3), 104.9 (C-4), 92.8 (C-1), 76.5 (C-8), 68.2 (C-5), 60.9 (C-7*), 60.4 (C-6*), 53.4 (C-9), 19.4 (C-10), 95.9, 71.1, 71.7, 68.1, 72.0 and 61.5 (C-1′ through C-6′). (Found: C, 51.95; H, 5.92. $C_{23}H_{30}O_{14}$ requires: C, 52.02; H, 5.70).

Myoporoside hexaacetate (**3a**) was obtained by forced acetylation of **3** [pyridine–Ac_2O, 2:1; 4 d after addn of 4-(dimethylamino)-pyridine]. Crystd from EtOH; mp 173° (reported [15] mp 174–175°); $[\alpha]_D^{22}$ −151° ($CHCl_3$; *c* 1.7); ^{1}H NMR (250 MHz, $CDCl_3$): δ6.27 (*dd*, J = 6.5 and 2.0 Hz, H-3), 5.76 (*d*, J = 1.8 Hz, H-1), 5.22 (*m*, H-6), 4.83 (*br d*, J = 6.5 Hz, H-4), 2.94 (*t*-like, J = 8.5 Hz, H-5), 2.61 (*br d*, J = 7.5, H-9), 2.42 (*dd*, J = 13.8 and 6.9 Hz, H-7β), 1.98–2.11 (*s*′ *s*, 6 × OAc), 1.62 (*dd*, J = 13.8 and 10.9 Hz, H-7α), 1.55 (3H, *s*, 10-Me). ^{13}C NMR (63 MHz, $CDCl_3$): δ140.1 (C-3), 100.1 (C-4), 92.1 (C-1), 84.6 (C-8), 71.6 (C-6), 46.7 (C-9), 43.4 (C-7), 33.3 (C-5), 21.7 (C-10), 95.3, 70.3, 72.3, 68.1, 72.5 and 61.5 (C-1′ through C-6′).

NOE (500 MHz, D_2O) on **10**: Irr. at δ2.36 (H-9); effect at: 5.56 (6%, H-1), 4.08 (3%, H-6), 3.53 (0.8%, H-7), 2.78 (8%, H-5). Irr. at δ1.27 (H-10), effect at: 5.56 (5%, H-1), 3.53 (7%, H-7), 2.36 (2%, H-9).

NOE (500 MHz, D_2O) on **11**: Irr. at δ2.16 (H-9), effect at: 5.56 (4%, H-1), 3.51 and 3.41 (0.4% each, H-6 and H-7). Irr. at δ1.28 (10-Me), effect at: 6.39 (1%, H-3), 5.68 (3%, H-1), 5.09 (0.3%, H-4), 3.41 (4%, H-7).

Physostegia virginiana var. *virginiana* (voucher no IOK 2–89). Work-up of 205 g frozen plant as above gave **10** (131 mg, 0.06%), **3** (143 mg, 0.07%) and **6** (20 mg, 0.01%). In an additional work-up of 200 g fresh plant harvested in July, satd $NaHCO_3$ soln (10 ml) was added to the crude extract. After concn and partitioning between Et_2O–H_2O, the aq. extract gave a syrup (9.7 g), which was dissolved in H_2O (10 ml) and MeOH (100 ml) added. The resulting suspension was filtered through activated charcoal and taken to dryness (4.6 g). Half of this was applied to a C-18 (size C) column eluting with (10:1) and the most polar fraction collected (1.5 g). Rechromatography (C-18, size B, 10:1 to 1:1) and monitoring at 254 nm gave only a single iridoid fraction (9 mg, 0.01%), mainly consisting of 8-epiloganic acid (**12**) solely characterized by NMR. The ^{1}H and ^{13}C NMR spectra were superimposable with spectra of authentic 8-epiloganin [22], except for the methyl ester signal of the latter.

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