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IRIDOIDS IN *THUNBERGIA* SPECIES

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Abstract—Four species of *Thunbergia* were all found to contain the iridoid glucoside stilbericoside. In addition to this compound, one species also contained 6-*epi*-stilbericoside while another one had thunbergioside, both compounds being described here for the first time. Thunbergioside was originally described as the product of water addition to stilbericoside, but in the present paper it has been shown to be the chlorohydrin of the latter compound.

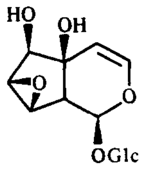
INTRODUCTION

In a recent paper on the chemotaxonomy of the Acanthaceae [1], we reported the presence of iridoid glucosides in the subfamily Thunbergioideae and the four species of *Thunbergia* investigated were all found to contain such compounds. Here we describe the experimental details.

The incorrect structure **4**, originally assigned to thunbergioside by us [1], was regrettably based solely on NMR spectral data, while awaiting more material of *T. fragrans*. However, because the plant cultivated in The Botanical Garden of Copenhagen has died in the meantime, and this species could not be located in other botanical gardens, we decided to finish the work with the small amount initially available. Data supporting another structure (**3**) are presented here.

RESULTS AND DISCUSSION

Two of the species investigated, namely *Thunbergia grandiflora* (Rottl.) Roxb. and *T. mysorensis* (Wight) T. Anders. contained the iridoid glucoside stilbericoside (**1**), a compound so far only encountered in *Stilbe ericoides* L. [2] and in *Retzia capensis* Thunb. [3]. The compound was identified by comparison with the reported [2] ^{1}H NMR spectrum and by the pentaacetate.



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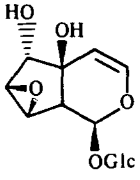

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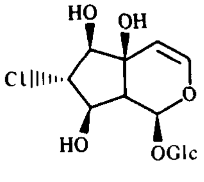

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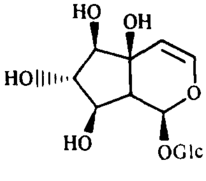

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When examining the aqueous extract of *T. alata* (black-eyed Susan), we were able to isolate two iridoid glucosides, the minor one again being stilbericoside (**1**). The elemental analysis of the major constituent (**2**) showed it to be an isomer of **1** and the NMR spectra of the two compounds were very similar. Thus the ^{13}C NMR spectrum contained the same number of signals with major differences only for C-3 to C-7, indicating that **2** was the 6-epimer of **1**. The C-5 signal for **1** (δ73.4) compared with that of **2** (δ79.8) was highly indicative of the *cis*-disposition of the 5- and 6-hydroxy groups in the former and of the *trans*-disposition in the latter. A similar behaviour is seen for the pair 6β- and 6α-dihydrohastatoside [4]. Further proof was found in the difference between the C-3 and the C-4 shift values in the two compounds, namely δ35.7 for **1** and 40.6 for **2**, showing **1** to be the 6β-epimer and **2** the 6α-epimer [4]. The ^{1}H NMR spectrum confirmed this as the coupling constant $J_{6,7}$ was 1.7 Hz and <0.5 Hz in the spectra of **1** and **2**, respectively, showing the protons at C-6 and C-7 to be *cis* and *trans*, respectively [5]. Finally, the coupling constant $J_{8,9}$ was found to be <0.5 Hz in both compounds, proving the position of the oxirane ring to be on the β-face of the molecule in both compounds. Assuming the usual absolute configuration at C-1, C-5 and C-9 the structure of **2** was that depicted in Fig. 1, namely 6-*epi*-stilbericoside.

From *Thunbergia fragrans* Roxb., **1** was the major iridoid found. Additionally, a second iridoid (**3**), which we have named thunbergioside, could be isolated in a modest amount only. Due to the small amount of plant material at hand we initially obtained only 11 mg of **3**. In the ^{13}C NMR spectrum of thunbergioside, signals from 14 carbon atoms could be seen, indicating a close relationship with **1** and **2**. Eleven signals could comfortably be assigned to the carbons of the β-glucopyranosyl moiety and the dihydropyrane ring, leaving three signals at δ81.5, 74.0 and 67.4 to be allotted to C-6, C-7 and C-8, all apparently carrying electronegative substituents. The ^{1}H NMR spectrum was in good agreement with this conclusion. The coupling pattern allowed assignment of all signals (see Experimental) showing those for H-6, H-7 and H-8 to be situated at low field, namely at δ3.82, 3.97 and 3.84, respectively. Furthermore, the coupling constants $J_{6,7}$, $J_{7,8}$ and $J_{8,9}$ were all larger than 8.5 Hz indicating a pairwise *trans*-relationship between consecutive protons. Initially (see below), the low field shifts in the NMR spectra were thought to show hydroxy substitution at C-6, C-7 and C-8. However, since acetylation under mild conditions provided a hexaacetate (**3a**) and not the expected heptaacetate, an alternative had to be found. Chloro-substitution at C-7 was a likely alternative, as other chlorohydrins of iridoid epoxides are known, i.e. linarioside [6], eustoside [7] and 7-chlorodeutziol [8], and **3** would thus be the chlorohydrin of **1**. The NMR spectra of **3a** was in agreement with this. Thus, in the ^{1}H NMR spectrum the H-6 and H-8 signals were shifted downfield 1.1 and 1.2 ppm, respectively, while H-7 was shifted only 0.2 ppm, when compared to **3**. In the ^{13}C NMR spectrum acetylation caused the C-6 and C-8 signals to shift slightly downfield while C-7 shifted 6 ppm upfield, similar to the effects seen for 7-chlorodeutziol [8]. In order to finally clarify the positions of the substituents ^{1}H NMR NOE experiments were performed on **3**. Irradiation of the H-1 signal gave enhancements of the H-8 and H-9 signals (each 6%). Likewise, irradiation of the H-9 signal gave large enhancements of the H-1 and H-7 signals (9 and 14%, respectively) with a smaller effect at H-8 (4%). Finally, irradiation of the H-4 signal gave enhancements of the H-3 and H-6 signals (6 and 3%, respectively). This is best explained by the structure given as **3**. The presence of a chlorine atom in **3a** was finally confirmed by chemical ionization mass spectroscopy using ammonia as reagent gas. The spectrum exhibited weak peaks at *m/z* 654 and 656 with an intensity ratio of 2:1, corresponding to $[M+NH_4]^+$ ions.

In an effort to prepare either **3** or **4** from stilbericoside (**1**), the latter compound or its pentaacetate (**1a**) was treated with strong aqueous base [9] or concentrated hydrochloric acid in ethyl acetate [7], respectively, in order to open the epoxide ring. Despite the reported [5, 9] facile openings of some iridoid epoxide rings no reaction was detected with neither **1** nor **1a** in the present work.

EXPERIMENTAL

Microanalyses were performed by LEO Microanalytical Laboratory, Ballerup, Denmark. Mps are corrected. The plant material was grown in greenhouses in The Botanical Garden, The University of Copenhagen. Voucher nos are given in ref. [1]. Prep. chromatography was performed on Merck Lobar reversed phase columns and eluted with H_2O–MeOH mixtures specified in each case.

Thunbergia alata. Fresh foliage (100 g) was homogenized with EtOH and the concd extract partitioned in Et_2O–H_2O. The aq. phase was passed through alumina (100 g) which was washed with H_2O (250 ml). Evapn followed by trituration with MeOH (2 × 5 ml) and passage through activated C provided a white foam (705 mg). Chromatography (RP-8, size C; 22 ml/min) eluting with H_2O gave first a polar fraction which was discarded followed by trigonellin (5 mg, identified by ^{1}H NMR [1]). Elution with H_2O–MeOH (10:1) gave a fraction (183 mg) consisting of two iridoids. Rechromatography (RP-18; size B; 11 ml/min) eluting with (25:1) provided first 6-*epi*-stilbericoside (**2**, 133 mg, 0.13%), isolated as a foam $[\alpha]_D^{23}$ −67° (EtOH; *c* 0.5): ^{1}H NMR (500 MHz, D_2O): δ6.59 (*d*, $J = 6.2$ Hz, H-3), 5.25 (*d*, $J = 9.6$ Hz, H-1), 4.96 (*d*, $J = 6.2$ Hz, H-4), 4.85 (*d*, $J = 8.1$ Hz, H-1′), 4.08 (*s*, H-6), 3.88 (*d*, $J = 1.7$ Hz, H-8), 3.69 (*d*, $J = 1.7$ Hz, H-7), 2.48 (*d*, $J = 9.6$ Hz, H-9). ^{13}C NMR (125 MHz, D_2O): δ144.9 (C-3); 104.3 (C-4); 97.1 (C-1); 79.8 (C-5); 77.1 (C-6); 59.3 (C-8*); 58.8 (C-7*); 49.9 (C-9); 100.1, 73.6, 76.7, 70.4, 77.2 and 61.5 (C-1′ to C-6′). (Found: C, 45.94; H, 6.34. $C_{14}H_{20}O_{10}$, H_2O requires: C, 45.90; H, 6.05%). The next fraction contained stilbericoside (**1**, 40 mg, 0.04%) as a foam, characterized by ^{1}H NMR (500 MHz, D_2O): δ6.48 (*d*, $J = 6.2$ Hz, H-3), 5.33 (*d*, $J = 8.2$ Hz, H-1), 5.04 (*d*, $J = 6.2$ Hz, H-4), 4.83 (*d*, $J = 8.0$ Hz, H-1′), 4.23 (*d*, $J = 1.7$ Hz, H-6), 3.79 (*d*, $J = 2.5$ Hz, H-8), 3.75 (*m*, H-7), 2.58 (*d*, $J = 8.2$ Hz, H-9) virtually as reported [2]. ^{13}C NMR (125 MHz, D_2O): δ143.1 (C-3); 107.4 (C-4); 96.5 (C-1); 77.9 (C-6); 73.4 (C-5); 59.4 (C-7*); 56.3 (C-8*); 49.7 (C-9); 99.9, 73.5, 76.6, 70.4, 77.2 and 61.5 (C-1′ to C-6′).

6-*epi*-Stilbericoside pentaacetate (**2a**) was prepared by acetylation (pyridine–Ac_2O; 2:1; 2 hr at room temp.). Crystd from EtOH, mp 156–157°; $[\alpha]_D^{23}$ −87° ($CHCl_3$; *c* 0.7): ^{1}H NMR (250 MHz, $CDCl_3$): δ6.38 (*d*, $J = 6.3$ Hz, H-3), 5.11 (*d*, $J = 8.5$ Hz, H-1), 4.90 (*d*, $J = 8.0$ Hz, H-1′), 4.85 (*d*, $J = 6.3$ Hz, H-4), 3.69 (*d*, $J = 2.4$ Hz, H-7), 3.57 (*d*, $J = 2.4$ Hz, H-8), 2.43 (*d*, $J = 8.5$ Hz, H-9). ^{13}C NMR (63 MHz; D_2O): δ142.9 (C-3); 102.4 (C-4); 94.3 (C-1); 77.3 (C-5); 77.2 (C-6); 57.2 (C-8*); 56.5 (C-7*); 49.7 (C-9); 96.9, 70.6, 71.9, 68.1, 72.1, 61.5 (C-1′ to C-6′). (Found: C, 51.74; H, 5.52. $C_{24}H_{30}O_{15}$ requires: C, 51.61; H, 5.41%).

Stilbericoside pentaacetate (**1a**) was prepared as above. By crystallization from EtOH a product was obtained with a broad mp range and recrystallizations did not improve this. However, after seeding with an authentic sample, we obtained a preparation with mp 137–138° (reported [2]: 138–140°); $[\alpha]_D^{20}$ −101° ($CHCl_3$; *c* 0.6); ^{1}H NMR (250 MHz, $CDCl_3$): δ6.32 (*d*, $J = 6.1$ Hz, H-3), 5.09 (*d*, $J = 8.0$ Hz, H-1), 4.99 (*d*, $J = 1.6$ Hz, H-6), 4.96 (*d*, $J = 6.1$ Hz, H-4), 3.70 (*dd*, $J = 2.7$ and 1.6 Hz, H-7), 3.64 (*d*, $J = 2.7$ Hz, H-7), 2.91 (*br s*, 5-OH), 2.56 (*d*, $J = 7.8$ Hz, H-9), virtually as reported [2]. ^{13}C NMR (63 MHz, $CDCl_3$): δ141.9 (C-3); 106.8 (C-4); 94.5 (C-1); 78.1 (C-6); 72.6 (C-5); 56.5 (C-7*); 55.2 (C-8*); 49.1 (C-9); 96.6, 70.7, 72.2, 68.2, 72.2 and 61.5 (C-1′ to C-6′).

Thunbergia fragrans. Fresh foliage (16 g) was worked-up as above to give a foam (540 mg). Chromatography on a RP-8 column (size B; 25:1 and 10:1; 11 ml/min) gave after the most polar fraction stilbericoside (**1**; 144 mg, 0.9%) followed by thunbergioside (**3**; 11 mg, 0.07%) as a syrup. ^{1}H NMR (500 MHz, D_2O): δ6.40 (*d*, $J = 6.5$ Hz, H-3), 5.54 (*d*, $J = 3.5$ Hz, H-1), 5.19 (*dd*, $J = 6.5$ and 0.5 Hz, H-4), 4.78 (*d*, $J = 8.0$ Hz, H-1′), 3.97 (*t*, $J = 8.5$ Hz, H-7), 3.84 (*t*, $J = 9$ Hz, H-8), 3.82 (*d*, $J = 8.5$ Hz, H-6), 2.35 (*ddd*, $J = 9$, 3.5 and 0.5 Hz, H-9). ^{13}C NMR (125 MHz, D_2O): δ141.4 (C-3); 109.0 (C-4); 94.4 (C-1); 81.5 (C-6); 74.0 (C-8); 68.3 (C-5); 67.4 (C-7); 53.8 (C-9); 99.3, 73.3, 76.2, 70.4, 77.0 and 61.5 (C-1′ to C-6′).

Thunbergioside hexaacetate (**3a**) was prepared by acetylation as above followed by prep. TLC (Et_2O). The product (11 mg) was isolated as a syrup. Due to the small amount, it was solely characterized by NMR and MS. ^{1}H NMR (250 MHz, $CDCl_3$): δ6.32 (*d*, $J = 6.4$ Hz, H-3), 5.47 (*d*, $J = 1.9$ Hz, H-1), 5.25 (*dd*, $J = 6.3$ and 1.7, H-4), 5.03 (*dd*, $J = 10.0$ and 7.5 Hz, H-8), 4.92 (*d*, $J = 5.2$ Hz, H-6), 4.15 (*dd*, $J = 7.5$ and 5.2 Hz, H-7), 2.66 (*dt*, $J = 10.0$ and 1.8 Hz, H-9), 2.01–2.17 (6 × OAc). ^{13}C NMR (125 MHz, $CDCl_3$): δ140.5 (C-3); 108.6 (C-4); 91.9 (C-1); 81.9 (C-6); 75.6 (C-8); 66.7 (C-5); 61.4 (C-7); 51.5 (C-9); 96.2, 71.1, 71.8, 68.1, 72.1 and 61.5 (C-1′ to C-6′). CIMS (NH_3, probe) 70 eV, *m/z* (rel. int.): 656.6 $[M+NH_4]^+$ (0.07), 654.6 $[M+NH_4]^+$ (0.14), 135.1 (100).

NOE (500 MHz, D_2O) on **3**: Irr. at δ5.54 (H-1) gave enhancements at 3.84 (H-8; 6%) and 2.35 (H-9; 6%). Irr. at δ5.19 (H-4) gave enhancements at 6.40 (H-3; 6%) and 3.82 (H-6; 3%).

Irr. at δ2.35 (H-9) gave enhancements at 5.54 (H-1; *ca* 9%), 3.97 (H-7; *ca* 14%) and 3.84 (H-8; *ca* 4%).

Thunbergia grandiflora. Fresh foliage (136 g) was worked-up as above resulting in 920 mg of a foam. Chromatography on a RP-8 column (size B; 25:1; 11 ml/min) gave first a polar fraction with no retention, followed by stilbericoside (**1**; 186 mg, 0.14%).

Thunbergia mysorensis. Fresh leaves (7 g) provided 110 mg of extract. Chromatography as above gave stilbericoside (**1**; *ca* 3 mg, 0.05%).

Treatment of stilbericoside (**1**) *with base.* Compound **1** (50 mg) dissolved in satd $Ba(OH)_2$ soln (5 ml) or in 1 M NaOH (5 ml) showed no reaction after 24 hr. The compound was recovered almost quantitatively by chromatography.

Treatment of stilbericoside hexaacetate (**1a**) with acid. To **1a** (60 mg) in EtOAc (10 ml) was added concd HCl (4 drops) under stirring for 10 min. After extraction with satd $NaHCO_3$, **1a** was recovered unchanged according to ^1H NMR.

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