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# IRIDOID GLYCOSIDES FROM *THUNBERGIA GRANDIFLORA*

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**Key Word Index**—*Thunbergia grandiflora*; Acanthaceae; iridoid glycosides; unedoside; isounedoside; grandifloric acid; alatoside; isolation.

**Abstract**—The novel iridoid glycosides, isounedoside and grandifloric acid, were isolated from *Thunbergia grandiflora*. Grandifloric acid contains C-10 as a carboxylic acid group, the presence of which was predicted by recent iridoid biosynthesis studies carried out within *T. alata*. Isounedoside contains a rare 6,7-epoxide functional group. A revision in some of the NMR spectral assignments for the known iridoid glycoside alatoside was also made.

## INTRODUCTION

*Thunbergia* is an Old World tropical plant genus of some 100 species, with a number of ornamental and medicinal taxa. Several interesting iridoid glycosides have been reported from *T. alata*, *T. grandiflora*, *T. fragrans* and *T. mysorensis* [1–3] and the biosynthesis of some of these was recently studied in *T. alata* [4]. Plants of the Acanthaceae have sometimes been found to contain bioactive compounds [5, 6]. As part of a screening programme of various Acanthaceae genera, we included extracts of *T. grandiflora*. Since a considerably larger amount of plant extract was available than previously used [2], we reinvestigated the glycoside content of this species.

## RESULTS AND DISCUSSION

Stilbericoside (**1**) was the only isolate previously described [2] from *T. grandiflora*. We did not encounter **1** in our extract, but did find thunaloside (**2**) and alatoside (**3**), both components of *T. alata* [3, 4]. We also found two other iridoid glycosides, both novel substances.

The first new compound (**4**) showed 14 resonances in its $^{13}C$ NMR spectrum, and was assigned the formula $C_{14}H_{20}O_9$ based on an $M_r$ of 332 established from the negative ion electrospray (ES) mass spectrum. These data, as well as a DEPT $^{13}C$ NMR spectrum, indicated that **4** was isomeric with unedoside (**6**) and had the same number of methine, methylene and quaternary carbons as those expected for **6** [7, 8]. Unedoside was originally assigned structure **5** [7], but determination of the $^1H$ NMR spectrum on a new isolate and reinterpretation of the data suggested the epimeric structure **6** [8]. Our $^1H$ NMR analysis on a standard unedoside sample (H. Rimpler) and on a sample isolated here confirmed the detailed earlier work [8]. A HMQC spectrum of **6** allowed assignment of the carbon resonances (Table 1; previously not reported). The carbon resonance chemical shifts for our isolated unedoside isomer (**4**) differed from those of **6** in several relatively small respects, but there were more significant differences in the $^1H$ NMR spectrum. Compound **6** had, for example, a doublet ($J = 9.9$) at $\delta$ 4.79 for H-1, a doublet of doublets ($J = 9.9$ and 7.5 Hz) at $\delta$ 2.48 for H-9 and a *dddd* group ($J = 8.1$, 7.5, 4.8 and 1.8 Hz) at 2.03 for H-5. Compound **4**, in contrast, showed H-1 as a broad singlet at $\delta$ 5.35 and complex groupings at $\delta$ 2.86 and 1.80. The $^1H$–$^1H$ COSY NMR spectrum showed that

Table 1. $^{13}C$ NMR spectral data for iridoid glycosides ($D_2O$; C-6′ at $\delta$ 61.5 as internal standard)

| C | 3 | 4 | 5 | 6 |
|---|---|---|---|---|
| 1 | 95.0 | 97.0 | 93.9 | 96.3 |
| 3 | 140.4 | 141.8 | 140.6 | 139.0 |
| 4 | 105.2 | 104.2 | 101.6 | 109.0 |
| 5 | 40.2 | 37.2 | 31.7 | 33.0 |
| 6 | 76.7 | 79.2 | 59.3 | 29.5 |
| 7 | 42.1 | 59.4 | 59.4 | 32.1 |
| 8 | 73.1 | 56.6 | 73.5 | 46.2 |
| 9 | 48.4 | 42.8 | 42.3 | 48.9 |
| 10 | | | 185.6 | |
| 1′ | 99.3 | 100.2 | 98.9 | 99.3 |
| 2′ | 73.7 | 73.7 | 73.4 | 73.5 |
| 3′ | 76.9 | 76.7 | 76.5 | 76.4 |
| 4′ | 70.5 | 70.4 | 70.4 | 70.5 |
| 5′ | 77.3 | 77.0 | 77.0 | 77.1 |
| 6′ | 61.5 | 61.5 | 61.5 | 61.5 |

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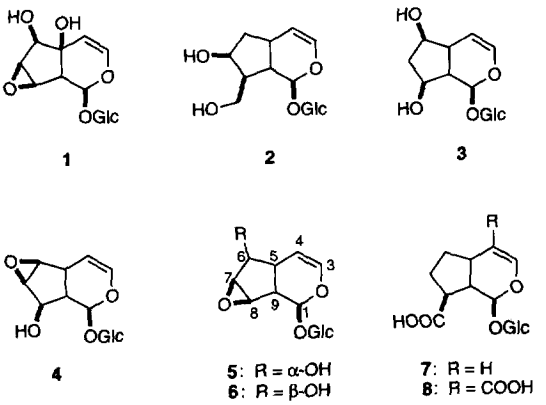
the H-1 resonance was coupled with the $\delta$ 1.80 resonance, which could then be assigned to H-9, while that for H-5 was the resonance at $\delta$ 2.84. This is a reversal of the chemical shift positions for these two protons as compared to the unedoside protons and is similar to the situation observed for 6$\beta$,7$\beta$-epoxyplendoside [9]. A doublet of doublets ($J$ = 8.1 and 1.5 Hz) at $\delta$ 4.06 was comparable to the H-6 resonance in **6**, but if the epoxide was at C-6, C-7 in **4**, this $\delta$ 4.06 resonance would correspond to H-8, not H-6, in the isomeric isolate, **4**. That this was indeed the case, was shown by a large NOE enhancement in H-1 at $\delta$ 5.35 when the $\delta$ 4.06 resonance was irradiated. This would only occur if H-8 and H-1 were on the same side. There was also a large enhancement in a resonance at $\delta$ 3.52, which could therefore be assigned to H-7. The enhancement would require that H-7 and H-8 be on the same side, and hence the epoxide must be in the $\beta$-orientation as in **4**, which we have designated isounedoside. It has been suggested [9] that $^{13}$C resonances at $\delta$ 59.0 and 58.2 are typical of 6,7-epoxides and the resonances for **4** ($\delta$ 59.3 and 59.4) are consistent with this. The HMQC experiment on (**6**) allowed us to assign the relative positions of the C-7 and C-8 resonances, where that for the C-8 epoxy carbon is at $\delta$ 56.6.

A second unknown (**7**) showed 15 resonances in its $^{13}$C NMR spectrum (Table 1) and, with a $M_r$ of 346 established from the ES mass spectrum, could be assigned the formula $C_{15}H_{22}O_9$. The spectra had several characteristics of those for an iridoid glucoside. The $\delta$ 108.3 and 140.3 $^{13}$C resonances for C-3 and C-4, along with the $\delta$ 4.78 and 6.20 $^1$H resonances, showed that C-4 was unsubstituted. The presence of seven $^1$H resonances in the upfield $\delta$ 1.45–2.70 region indicated a lack of hydroxyl groups or unsaturation in the cyclopentano ring. The $\delta$ 184.5 resonance and the molecular formula, which needed two oxygens in addition to those from the glucose, pointed to the presence of a carboxylic acid function, which was placed at C-8 for biosynthetic reasons. $^1$H NMR decoupling experiments and a HETCOR spectrum definitively established the assignments for C-1, C-9, C-8 and their attached protons. Finally, a NOE experiment showed enhancements between H-1 and H-8, which proved the stereochemistry at C-8. During the course of this work, **7**, which we have named grandifloric acid, was also isolated from *T. alata* and its structure proved by decarboxylation of forsythide (**8**) (Jensen, S. R., personal communication).

Column chromatography (on both regular and C-18 reverse phase silica gel) yielded (**3**) apparently complexed with $\alpha$- and $\beta$-glucose in a 1:1:1 ratio [10]. The contaminating glucose anomers were finally removed from **3** by chromatography through charcoal. The $^1$H and $^{13}$C NMR spectra for **3** were closely similar to those reported [3], but analysis of the spectra suggested revision of several assignments. The DEPT $^{13}$C NMR spectrum showed that a 42.0 resonance (which had been assigned [3] to C-5) was due to a




methylene carbon and not a methine. Hence, this must be the resonance for C-7. A HMBC spectrum showed three-bond H to C couplings between an H-7 resonance ($\delta$ 1.59) and carbon resonances at $\delta$ 73.1 and 76.7, which must be those for C-8 and C-7 (or C-7 and C-8). These could be distinguished by the HMQC spectrum, which showed the 4.2 resonance for H-8 was coupled with the $\delta$ 73.1 carbon resonance, which must therefore be that for C-8 (leaving the 76.7 resonance for C-6). The HMQC spectrum also showed that the H-9 and H-7 resonances ($\delta$ 2.46 and 2.68) were correlated to $^{13}$C resonances at $\delta$ 48.4 and 40.2, respectively. All $^{13}$C NMR assignments are given in Table 1.

The isolated iridoids lack C-11 and several lack both C-10 and C-11. The first biosynthesis studies on iridoids which lack C-10 were recently carried out using *T. alata* [4] and the loss of C-10 was attributed to its oxidation to a carboxylic acid function followed by subsequent decarboxylation.

Compound **7** is the first C-10 carboxyl iridoid reported from *Thunbergia* and its finding is consistent with the biosynthetic proposal [4]. The presence of such a functional group is quite rare among isolated iridoid glycosides as is a 6,7-epoxy group [11, 12]. The very low concentration of these iridoids in *T. grandiflora* undoubtedly accounts for their absence in the previous report on this species [2].

## EXPERIMENTAL

*Plant material. Thunbergia grandiflora* (Rottl.) Roxb., 16.5 years old, was collected during the flowering stage on 15 November 1992 from the El Zoharia garden in Cairo, Egypt. It was identified by El-Orman taxonomists and by Prof. Dr Nabeil El Hadedi, Department of Botany, Faculty of Science, Cairo University, Giza, Egypt, and a voucher was deposited in the herbarium of the Department of Pharmacognosy, Faculty of Pharmacy, Al-Azhar University, Nasr City, Cairo.

*Extraction and isolation.* Air-dried leaves and flowers (700 g) were extracted with *n*-hexane–EtOAc (3:2), then with MeOH. The MeOH was evapd *in vacuo* to yield 64 g residue, which was partitioned

between $H_2O$ and $Et_2O$. The $H_2O$ was evapd *in vacuo* to leave 58.5 g of a residue, which was treated with MeOH. The MeOH-soluble part was evapd *in vacuo*, to give 41 g crude iridoid and/or glycoside residue. This residue was purified by dissolving in $H_2O$ and VLC using C-18 silica gel with $H_2O$–MeOH elution (21 frs, 0–100% MeOH). The MeOH content was increased 5% in each 100-ml fr.

Fr. 1 (100% $H_2O$; 14.6 g): 15 mg **6** and 15 mg **4** after rechromatography on C-18 silica gel ($H_2O$–MeOH gradient) and then HPLC ($H_2O$–MeOH, 49:1).

Fr. 2 (5% MeOH; 4.55 g): 40 mg **4** after rechromatography on C-18 silica gel ($H_2O$–MeOH gradient) and normal phase silica gel ($CHCl_3$–MeOH gradient); 55 mg **7** after four column purifications on C-18 silica gel ($H_2O$–MeOH gradient); 40 mg **3** after three column purifications on C-18 silica gel ($H_2O$–MeOH gradient) and three on normal phase silica gel ($CHCl_3$–MeOH gradient). For final purification of **3**, it was necessary to remove complexed glucose by charcoal chromatography.

Fr. 3 (10% MeOH; 3.5 g): 30 mg **2**, after rechromatography on normal phase silica gel ($CHCl_3$–MeOH gradient); 25 mg **7** and 20 mg **3**.

*Isounedoside* (**4**). $[\alpha]_D^{20}$ − 52° (MeOH; *c* 0.35); EI-MS, *m/z* 331 $[M-H]^-$, $C_{14}H_{20}O_9$; $^1H$ NMR ($D_2O$): $\delta$ 5.35 (H-1, *d*, $J$ = 1.2 Hz), 6.14 (H-3, *dd*, $J$ = 1.8, 6.3 Hz), 4.72 (H-4, *dd*, $J$ = 1.2, 6.3 Hz), 1.78 (H-9, *ddd*, $J$ = 1.5, 8.1, 8.7 Hz), 4.06 (H-8, *dd*, $J$ = 1.5, 8.1 Hz), 3.52 (H-7, *dd*, $J$ = 2.1, 1.5 Hz), 3.48 (H-6, *d*, $J$ = 2.1 Hz), 2.86 (H-5, *ddd*, $J$ = 1.8, 6.3, 8.7 Hz), 4.58 (H-1′, *d*, $J$ = 11.7 Hz), 3.12 (H-2′, *dd*, $J$ = 7.9, 9.2 Hz), 3.19–3.41 (H-3′, H-4′, H-5′, *m*), 3.54–3.78 (H-6′, *m*).

*Grandifloric acid* (**7**). $[\alpha]_D^{20}$ − 84° (MeOH; *c* 1.06); EIMS, *m/z* 345 $[M-H]^-$, 369 $[M+Na]^+$, $C_{15}H_{22}O_9$; $^1H$ NMR ($D_2O$): $\delta$ 5.11 (H-1, *d*, $J$ = 3.2 Hz), 6.05 (H-3, *m*), 4.70 (H-4, *m*), 2.57 (H-5, *m*), 1.84 (H-6, *m*), 1.53 (H-6, *m*), 1.84 (H-7, *m*), 1.33 (H-7, *m*), 2.25 (H-8, *m*), 2.43 (H-9, *m*), 4.61 (H-1′, *d*, $J$ = 7.8 Hz), 3.16–3.36 (H-2′, H-3′, H-4′, H-5′, *m*) 3.90 (H-6′, *dd*, $J$ = 13.8, 9.8 Hz), 3.73 (H-6′, *dd*, $J$ = 13.8, 3.3 Hz).

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