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ISOLATION OF 6-EPIMONOMELITTOSIDE FROM *TECOMA HEPTAPHYLLA* AND ITS CONVERSION INTO MONOMELITTOSIDE*

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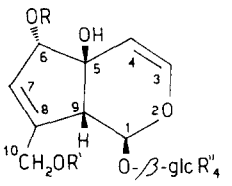
Key Word Index—*Tecoma heptaphylla*; Bignoniaceae; iridoid glucoside, 6-epimonomelittoside, ^{13}C NMR.

Abstract—The isolation of a new iridoid glucoside, 6-epimonomelittoside, from *Tecoma heptaphylla* is reported. The structure and configuration were established by analysis of spectroscopic data and chemical conversion into monomelittoside.

INTRODUCTION

We have previously described the analysis of the glucosidic fraction of *Tecoma chrysantha* [1], a tree common in north and north-east Brazil, where it is known by the trivial name of 'ipê amarelo' From this plant we isolated for the first time the 6-α-hydroxy epimer of aucubin together with its two ester derivatives [2, 3]. Although aucubin is one of the most common iridoid glucosides and several of its derivatives have been isolated [4], natural 6-α-hydroxy iridoids are rather rare [4] and we decided to examine another plant of the *Tecoma* genus for their presence. We chose *Tecoma heptaphylla* (Vell.) Mart, a plant of widespread distribution in Brazil, where it is known by the trivial name of 'ipê roxo' [5], whose leaves contain a glucosidic fraction (0.9–1.0%). The most polar component of this fraction, **1**, was found to be the C-6 epimer of monomelittoside (5-β-hydroxyaucubin) (**2**) [6]. It is noteworthy that aucubin and monomelittoside (**2**) have until now only been found in plants typical of the temperate zone [4, 6–8]



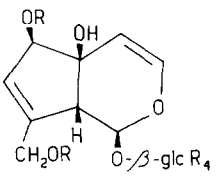
1 R=R'=R''=H

4 R=R'=R''=Ac

5 R=H R'=R''=Ac

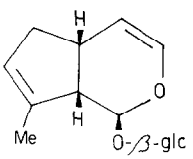
6 R=R''=Ac R'=H

7 R=tosyl R'=R''=Ac



2 R=H

8 R=Ac



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*Part 6 in the series "Iridoids in Equatorial and Tropical Flora" For Part 5 see ref [3]

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RESULTS AND DISCUSSION

Compound **1**, $C_{15}H_{22}O_{10}$, is a colourless amorphous powder and exhibits a green-brown reaction with vanillin reagent. It is transparent in the near UV, while its IR spectrum shows a band at 1650 cm^{-1}, attributable to the conjugated iridoid enol–ether system. Acid hydrolysis of **1**, in boiling 1 M sulphuric acid, gave glucose (1 mol), together with insoluble black products arising from the decomposition of the aglycone.

The ^{1}H and ^{13}C NMR spectra of **1** (Table 1) confirm the iridoid structure and show that the aglycone moiety has a monomelittoside-type structure As the ^{1}H and ^{13}C NMR spectra of **1** and **2** (Table 1), as well as their physical properties (see Experimental), are different these iridoids must differ in the configuration of one or more chiral centres.

Treatment of **1** with lithium–ammonia gave 6,10-bisdeoxyaucubin (**3**) thus demonstrating that **1** and **2** have the same configurations at C-1 and C-9 as well as of the glucosidic linkage. The configuration at C-5 is biogenetically determined Careful analysis of the ^{1}H and ^{13}C NMR data of **1** and **2** suggests a difference in configuration at C-6. In fact, in iridoids having an α-hydroxy group as against a β-hydroxy group at C-6, C-1 is always deshielded, whereas H-1 appears correspondingly deshielded [9]. The absolute value of chemical shift differences between C-3 and C-4 in **1** compared to those in **2** are in good agreement with an α configuration of the 6-hydroxy group according to the criterion proposed by Damtoft *et al.* [10] and as a consequence of an additional 1,3-diaxial interaction between the hydroxyl group and the dihydropyranic ring. Chemical shift differences between **1** and **2** for C-5 and C-6 are very similar to those observed in the dihydrohastatosides [9] and in the couple antirrhinoside–procumbide [10], when allowance is made for the additional interactions due to the presence of the epoxide function. In these examples, C-5 and C-6 always appear shielded in 6-β-hydroxy pairs as a consequence of the interaction between the two hydroxyl groups in a *cis* relationship. In fact, the vicinal diol interaction over-rides the *cis–trans* interaction between the 6-hydroxy group and the side chain at C-5 [10]. Finally, a further diagnostic feature comes out from the H-6 chemical shift value: in all the known 6-β-hydroxy

Table 1 NMR data of compounds **1** and **2**

H No	**1***† (D_2O)	**2**[6]*† (D_2O)	C No	**1*** (CD_3OD)	**2**[7]* (CD_3OD)
1	5 51 *d*	5 70 *d*	1	95.2	93.6
	$J_{1,9}$ = 4 7	$J_{1,9}$ = 2 5	3	142 8	142 4
3	6 45 *d*	6 37 *d*	4	105 7	108 4
	$J_{3,4}$ = 6 5	$J_{3,4}$ = 6.4	5	78 3	72 8
4	5 12 *d*	5 06 *dd*	6	83 9	80 5
	$J_{4,3}$ = 6 5	$J_{4,1}$ = 1.2, $J_{4,3}$ = 6 4	7	129 2	127 7
6	4 57 *m*	4 25 *m*	8	145 4	148 3
7	5 72 *br s*	5 87 *m*	9	55 1	53.6
9	3 00 *m*	3 22 *m*	10	60 9	60 8
10(2H)	4 24 *br s*	4 25 *m*	1′	99 6	99 4
			2′	74 7	74 4
			3′	78 3‡	78 2‡
			4′	71 7	71 6
			5′	77.7‡	77 4‡
			6′	62 8	62 6

*Values in δ-values (ppm) downfield from TMS
†J values in Hz
‡In the same column these assignments may be interchanged

epimers–α-hydroxy pairs, H-6 is shielded *ca* δ 0.3 In agreement with this trend, we observed in the couple **1**/**2** a $\Delta\delta$ value for H-6 of 0.32

To verify the spectroscopic suggestions, we transformed **1** into **2** by inversion of the configuration at C-6 Compound **1** was converted into the crystalline hexa-acetyl derivative, **4**, where the tertiary hydroxyl group at C-5 remained unaffected In the ^1H and ^{13}C NMR spectra of **4**, in comparison with those of **1**, the esterification shifts clearly proved the acylation of a primary and a secondary alcoholic function, present in **1** Compound **4** was hydrolysed in acidic medium (0 1 M sulphuric acid–dioxane, 1:1) affording the 6-deacetyl derivative, **5**, together with the 10-deacetyl derivative, **6** Compound **5** was esterified with tosyl chloride affording the monotosylate, **7**, which gave the hexa-acetate, **8**, by refluxing with tetraethyl ammonium acetate in acetone This compound was finally hydrolysed to obtain an iridoid which was identical to monomelittoside, **2** This transformation definitively demonstrated the structure and configuration of **1** as 6-epimonomelittoside. The chemical proof of the structure of **1** confirmed the validity of our spectroscopic approach in the elucidation of the structures of these natural compounds. Further studies are in progress to test whether the production of 6-hydroxy epimers of aucubin and its related compounds is peculiar of *Tecoma* plants or if a similar biogenetic trend can be found in other tropical genera

EXPERIMENTAL

PC Schleicher and Scull 2043 Mgl, TLC: Si gel F_{254} (Merck) and cellulose (Merck) plates Spray reagents 1 M H_2SO_4, vanillin (2 g vanillin, 4 ml conc HCl, 100 ml MeOH), benzidine (0 5 g benzidine, 20 ml HOAc, 80 ml EtOH) and resorcin (5 g resorcin, 4 ml conc H_2SO_4, 300 ml EtOH) ^1H and ^{13}C NMR spectra XL 100 FT NMR spectrometer. Evaporation of volatile material was performed under red pres

Isolation of the iridoidic fraction Tecoma heptaphylla was collected in June 1981 near Maceiò, Alagoas (Brazil) Voucher specimens of the plant were identified in the Universidade Federal de Alagoas, Maceiò (Brazil). Fresh aerial parts of the plant (0 5 kg) were extracted at room temp with 90% EtOH (3 × 2 l) until negative to the vanillin test PC in *n*-BuOH–HOAc–H_2O (63 10 27) showed the presence of a polar iridoid (**1**), which gave a green-brown reaction with vanillin and had R_f 0 10 The EtOH extract was concd to an aq suspension which was stirred with decolorizing charcoal (0 4 kg, negative vanillin test of the aq suspension) The resulting suspension was stratified on a Gooch funnel (10 cm diameter), monosaccharides were eluted with H_2O (5 l), disaccharides with 5 and 10% EtOH (1 l each), compound **1** with 30% EtOH (4 l)

6-*Epimonomelittoside* (**1**). The 30% EtOH fraction (2 3 g) was chromatographed on Si gel (100 g) in *n*-BuOH satd with H_2O, affording crude **1** (0 5 g) which was purified by HPLC on a semi-prep μ-Bondapak C_{18} column (30 × 0 5 cm) eluted with MeOH–H_2O (7 3), flow rate 3 0 ml/min Compound **1** (0 35 g) was obtained as a colourless amorphous powder $[\alpha]_D^{20}$ −47° (MeOH, *c* 1 0), IR ν_{max}^{KBr} cm^{-1}. 3350, 2900, 1650, 1370, 1050, 1020, 1010 (Found C, 49 58, H, 6 30 Calcd for $C_{15}H_{22}O_{10}$ C, 49 72, H, 6 12%)

6,10-*Bisdeoxyaucubin* (**3**) Compound **1** (100 mg) was dissolved in EtOH (1 ml) and keeping the apparatus at −40°, liquid NH_3 (100 ml) was added Over 4 hr Li (500 mg) was added in small portions until a blue colour persisted, then excess Li was decomposed by EtOH and the NH_3 left to evaporate overnight The residue was dissolved in H_2O (50 ml) and extracted with EtOAc (5 × 50 ml) The organic soln was evaporated and the residue chromatographed on Si gel in $CHCl_3$–MeOH (8 2) to give **3** (35 mg) Direct comparison with an authentic sample of 6,10-bisdeoxyaucubin established the identity (^1H NMR and IR spectra superimposable)

Hexa-O-acetyl-6-epimonomelittoside (**4**) Compound **1** (60 mg) was treated with pyridine (1 ml) and Ac_2O (2 ml) for 2 hr at room temp After addition of MeOH (5 ml) the soln was left for 20 min then evaporated to give crude **4** (100 mg) which, by chromatography on Si gel in C_6H_6–Et_2O (4:6), afforded pure **4** Crystals from EtOH (needles), mp 156–157° IR $\nu_{max}^{CHCl_3}$ cm^{-1}. 2490, 1740, 1650, 1380, 1240, 1030, ^1H NMR ($CDCl_3$). δ 6 20 (H-3, *d*, $J_{3,4}$ = 6 5 Hz), 5 70 (H-7, *m*), 5 60 (H-1, *d*, $J_{1,9}$ = 4 7 Hz), 5 53 (H-6,

m), 4 9–5.3 (H-4), 4 62 (2H-10, *br s*), 3.18 (H-9, *m*); ^{13}C NMR ($CDCl_3$)· δ 91 7 (C-1), 139 6 (C-3), 105.3 (C-4), 75.1 (C-5), 84.2 (C-6), 128.6 (C-7), 137 7 (C-8), 53.1 (C-9), 60 7 (C-10), 96 1 (C-1′), 71.2 (C-2′), 72.2 (C-3′), 68.3 (C-4′), 72.0 (C-5′), 61 6 (C-6′); ^{13}C NMR ($CDCl_3$–CD_3OD): δ 96.5 (C-1), 142.7 (C-3), 105.6 (C-4), 76.9 (C-5), 84.4 (C-6), 127.6 (C-7), 142 1 (C-8), 54.9 (C-9), 62 2 (C-10), 97.4 (C-1′), 71.9 (C-2′), 73.0 (C-3′), 69.2 (C-4′), 72.6 (C-5′), 62.4 (C-6′).

Penta-O-acetyl derivatives (**5** *and* **6**). Compound **4** (300 mg) was dissolved in dioxane–0.1 M H_2SO_4 (1·1) (10 ml) and left for 24 hr at 40°. The soln was neutralized with pyridine, diluted with H_2O and extracted with EtOAc. The residue obtained from the organic phase was chromatographed on Si gel in C_6H_6–EtOAc (3 2) to give unreacted **4** (200 mg), **5** (25 mg) and **6** (20 mg) Compound **5**: IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3550, 2930, 1740, 1640; ^{1}H NMR ($CDCl_3$). δ 6.30 (H-3, *d*, $J_{3,4}$ = 6 5 Hz), 4 65 (2H-10, *br s*), 3 30 (H-9, *m*). Compound **6**: IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3580, 2940, 1740, 1640; ^{1}H NMR ($CDCl_3$)· δ 6.25 (H-3, *d*, $J_{3,4}$ = 6.5 Hz), 4.40 (H-6, *m*), 4 20 (2H-10, *br s*), 3.20 (H-9, *m*)

Tosyl derivative, **7**. Compound **5** (20 mg) was dissolved in pyridine (0 3 ml) and treated with 0.4 ml of a 10% soln of tosyl chloride in pyridine After 4 hr the soln was evaporated and the residue chromatographed on Si gel in C_6H_6–Et_2O (3:2) to give pure **7** (25 mg) as a colourless powder

Epimerization of **7** *to give* **2**. A soln of **7** (25 mg) and tetraethyl ammonium acetate (100 mg) in Me_2CO (5 ml) was heated under reflux for 24 hr and then the solvent removed. The residue was chromatographed on Si gel in Et_2O giving pure **8** (15 mg) whose physical data were identical to those reported for acetylmonomelittoside [6] Compound **8** was dissolved in MeOH–2 M NaOH (1:1) and left overnight at room temp. The soln was neutralized with CO_2, MeOH was removed by evaporation, then charcoal (100 mg) was added until a negative vanillin test was obtained. The suspension was stratified on a Gooch funnel, washed with H_2O until the washings gave a negative salt test, then eluted with MeOH. The residue, obtained by evaporation of MeOH, was chromatographed on Si gel, in *n*-BuOH satd with H_2O, affording a pure compound which was identical to an authentic sample of monomelittoside, **2**

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