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6β-HYDROXYIPOLAMIIDE, AN IRIDOID GLUCOSIDE FROM *STACHYTARPHETA MUTABILIS*

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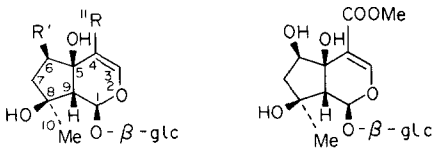
Key Word Index—*Stachytarpheta mutabilis*, Verbenaceae, iridoid glucosides, 6β-hydroxyipolamiide

Abstract—A new iridoid glucoside has been isolated from *Stachytarpheta mutabilis* and assigned the structure and configuration of 6β-hydroxyipolamiide on the basis of ^{1}H NMR and ^{13}C NMR evidence. The conversion of this compound into penta-acetyllamiol proved the above assignment.

INTRODUCTION

Stachytarpheta mutabilis Jacq. Vehl (Verbenaceae), a shrub growing in tropical America and Africa, is used in the traditional medicine of East Africa to cure headache, dysentery and eye infections [1]. In the Caribbean area *Stachytarpheta* species are considered as medicinal plants in traditional medicine due to their antifever and antidysenteric properties.

In the *Stachytarpheta* genus the presence of iridoid glucosides, mainly ipolamiide (**1**) [2], has been established [3, 4]. However **1** was isolated only recently from *S. mutabilis* [5]. A chromatographic examination of the ethanolic extract of this plant revealed the presence of ipolamiide (**1**), the most abundant iridoid component, and of one other compound with a probable iridoid structure We now demonstrate the structure of this compound to be 6β-hydroxyipolamiide (**2**)



1 R = COOMe, R' = H
4 R = Me, R' = OH

2

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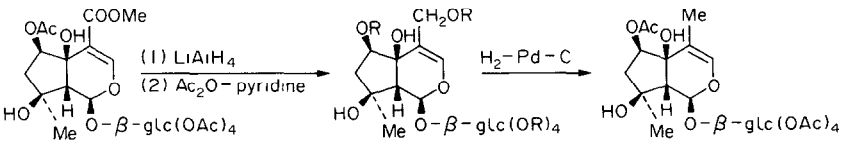
RESULTS AND DISCUSSION

Compound **2** was crystalline with mp 192–193°, $[\alpha]_D = -161°$ and molecular formula $C_{17}H_{26}O_{12}$ Its UV spectrum showed an intense absorption band at 231 nm (log ε, 4.0) and the IR spectrum revealed bands at 1710 and 1645 cm^{-1}. These data show the presence of a conjugated carbonyl function Acid hydrolysis of **2** gave glucose and tars arising from extensive decomposition of the aglycone. Acetylation of **2** under mild conditions afforded the penta-acetate (**3**) which still showed hydroxy bands in the IR spectrum Therefore, in the aglycone moiety of **2** there must be one hydroxyl group which is readily acetylated and one which is difficult to acetylate.

The ^{1}H NMR spectrum of **2** (see Experimental) revealed an iridoid structure and showed the presence of a carbomethoxy group at C-4, as supported by the chemical shift value of H-3, and of a methyl geminal with a hydroxyl function at C-8. The signals of H-3 and H-9, both singlets, excluded the presence of a proton at C-5 The presence of an ABX system suggested that a methylene group adjacent to a secondary alcoholic function must be present in the cyclopentane ring Finally, the doublet at δ 4.80, $J = 7.0$ Hz, was due to the glucose β-anomeric proton.

On the basis of the above data, **2** appeared to have the structure of hydroxyipolamiide, but it was different from the lamiide (7β-hydroxyipolamiide) which was the only known isomer [2] The decoupled and SFORD ^{13}C NMR spectra of **2** (Table 1) confirmed the suspected structure and the comparison with model iridoids allowed



3

5 R = H
6 R = Ac

7

Table 1 ^{13}C NMR spectral data of compounds **1**, **2** and **4** (20 MHz, D_2O, dioxane as internal standard)

Carbon No	1*	2	4*
1	94.5 *d*	94.2 *d*	93.1 *d*
3	152.8 *d*	154.5 *d*	136.1 *d*
4	114.0 *s*	112.8 *s*	114.6 *s*
5	71.4 *s*	70.4† *s*	72.6 *s*
6	38.0 *t*	74.5 *d*	73.9 *d*
7	39.4 *t*	47.0 *t*	46.8 *t*
8	78.9 *s*	74.7 *s*	75.8 *s*
9	60.7 *d*	59.1 *d*	58.9 *d*
10	22.8 *q*	23.6 *q*	23.8 *q*
11	169.1 *s*	169.0 *s*	11.9 *q*
OMe	52.5 *q*	52.7 *q*	—
1′	99.2 *d*	99.3 *d*	98.7 *d*
2′	73.3 *d*	73.3 *d*	73.3 *d*
3′	76.2 *d*‡	76.1 *d*‡	76.2 *d*‡
4′	70.5 *d*	70.4 *d*	70.5 *d*
5′	77.2 *d*‡	77.2 *d*‡	77.0 *d*‡
6′	61.6 *t*	61.5 *t*	61.5 *t*

*See ref [6].

†The C-5 signal appears as a singlet in SFORD in the middle of the C-4′ doublet

‡These values are interchangeable within each column

the identification of **2** as 6β-hydroxyipolamiide In fact the chemical shift value of the C-10 methyl (δ 23.6) was very similar to those of compounds like harpagide (δ 24.7) [6], lamiol (**4**) (δ 23.8) and shanzishide methyl ester (δ 24.2) [6] all having a β-hydroxyl function at C-6, a methylene group at C-7 and a β-hydroxyl and an α-methyl at C-8 Comparison of the ^{13}C NMR spectra of **2**, ipolamiide (**1**) and lamiol (**4**) (Table 1) shows that the signal of C-8 is more deshielded in **1** than in either **2** or **4** owing to the lack of the γ-effect of the C-6 hydroxyl and that of C-5 appears slightly more deshielded in **1** than in **2** as found for other iridoid glucosides having at C-5/C-6 a β-*cis*-diol function With lamiol (**4**) C-5 resonates at δ 72.6 while in 5-deoxylamiol it is at 74.8 [7] and in 6-deoxylamiol at 73.8 [8] This behaviour is in accord with a *cis*-diaxial interaction between the two hydroxyl functions [9]

The structure of 6β-hydroxyipolamiide proposed for **2** was also in accordance with the chemical shift value, in the ^1H NMR spectrum of **2**, of the X part of the ABX system, in that this signal appeared deshielded owing to the presence of the C-11 carbomethoxy group A comparison of the ^1H NMR spectra of **2** and **4** [10] showed for the H-6 of **2** a deshielding of δ 0.25; this value is very close to that found in the related compounds, shanzishide methyl ester [11] and 5-deoxylamiol [7], which are the 5-deoxy derivatives of **2** and **4**, respectively

To obtain chemical evidence that **2** had the structure and configuration of 6β-hydroxyipolamiide, we treated **3** with lithium aluminium hydride to prepare 6β-hydroxyipolamiidol (**5**) This latter compound was transformed by acetylation under mild conditions into its hexa-acetate (**6**) which, by allylic hydrogenolysis with H_2/Pd–C, afforded a penta-acetate found to be identical to penta-acetyllamiol (**7**) [10]

EXPERIMENTAL

CC Si gel 70–230 mesh (Merck), cellulose CF_{11} (Whatman), TLC Si gel 60 F_{254} and cellulose pre-coated plates (Merck), PC Schleicher and Schull No 2043 b Mgl paper, Spray reagents 2 N H_2SO_4, vanillin (2 g vanillin, 4 ml conc HCl, 100 ml MeOH), removal of all volatile materials was performed under red pres

Isolation of iridoids S mutabilis was collected in Guataparo near Valencia (Estado Carabobo, Venezuela) Voucher specimens of this plant were identified by Dr Victor M Badillo, at the Universidad Central de Venezuela, Maracay, and deposited in the herbarium (labelled Carlo De Luca No 10)

Dried plant material (1 kg) was roughly chopped and extracted with EtOH (6 × 0.5 l) at room temp by percolation PC with *n*-BuOH–HOAc–H_2O (63 10 27) showed at least four spots R_f 0.51 unknown *X*, 0.37 **1**, 0.25 **2** and 0.18 unknown **Y** The ethanolic extracts were concd to a viscous fluid, then distributed between H_2O and EtOAc and the aq layer further extracted (twice) with EtOAc The resulting aq soln was concd and treated with decolorizing charcoal (300 g) The suspension was stratified on a Gooch funnel (14 cm i d) Monosaccharides were eluted with H_2O (10 l), di- and oligosaccharides with 5% EtOH (11), 10% EtOH (11), **1**, **2** and unknown **Y** with 30% EtOH (5 l) and 50% EtOH (2 l) (fraction A), small amounts of **1**, other compounds and unknown *X* with 70% EtOH (2 l) (fraction B) Fraction A (8 g) was chromatographed on Si gel in *n*-BuOH satd with H_2O to give **1** (3 g), and **2** (0.5 g) still contaminated with **1** This latter was rechromatographed as above to give **2** (0.35 g) which was twice crystallized from MeOH–Me_2CO, mp 192–193°, $[\alpha]_D^{25}$ −161° (MeOH, *c* 0.2), UV λ_{max}^{MeOH} nm (log ε) 231 (4.0), IR ν_{max}^{KBr} cm^{-1} 1710, 1645, 1395, 1310, 1080, ^1H NMR (90 MHz, D_2O) δ 7.60 (1H, *s*, H-3), 5.86 (1H, *s*, H-1), 4.15 (1H, *pt*, H-6, X part of ABX system) 3.77 (3H, *s*, COOMe-11) 2.63 (1H, *s*, H-9), 2.16 and 1.90 (2H, AB part, $J_{7\alpha,7\beta}$ = 13.5, $J_{7\alpha,6}$ = 8.0, $J_{7\beta,6}$ = 6.4 Hz, 2H-7), 1.18 (3H, *s*, 3H-10) (Found C, 48.20, H, 6.32 $C_{17}H_{26}O_{12}$ requires C, 48.34, H, 6.20%)

Penta-O-acetyl derivative (**3**) *of* **2** Compound **2** (50 mg) was treated with dry pyridine (0.4 ml) and Ac_2O (0.8 ml) for 1 hr at room temp After addition of MeOH (3 ml) the soln was left for 30 min, then evaporated to give crude **3** which, when chromatographed on Si gel in Et_2O–EtOAc (6 4), gave pure **3** (55 mg) Needles from MeOH, mp 202.5–203.5°, IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3650, 3500, 1760, 1645, 1445, 1380, 1310, 1260, 1130, 1075, 1045, 960, 920, 875, ^1H NMR (90 MHz, $CDCl_3$) δ 7.40 (1H, *s*, H-3), 5.63 (1H, *d*, $J_{1,9}$ = 3.0 Hz, H-1), 5.49 (1H, *pt*, H-6, X part of ABX), 3.75 (3H, *s*, COOMe-11), 2.71 (1H *d*, $J_{9,1}$ = 3.0 Hz, H-9), 1.29 (3H *s*, 3H-10)

Reduction of **3** *to yield 7β-hydroxyipolamiidol* (**5**) $LiAlH_4$ (45 mg) suspended in dry THF (3 ml) was added dropwise to a stirred soln of **3** (60 mg) in dry THF (5 ml) After 1 hr at room temp the suspension was cooled and MeOH was added dropwise to destroy the excess $LiAlH_4$ The resulting suspension was diluted with H_2O (10 ml) and CO_2 was bubbled through the soln until pH *ca* 8 Finally it was concd, further treated with CO_2 (pH *ca* 7) and centrifuged The residue was washed (× 5) with H_2O until it gave a negative reaction with the vanillin reagent The collected aq solns were treated with decolorizing charcoal (0.5 g) and the suspension was stratified on a Gooch funnel (2 cm i d), washed with H_2O (200 ml) and eluted with MeOH (50 ml). The MeOH soln was evaporated to give a residue (30 mg) which, after chromatography on cellulose developed with *n*-BuOH satd with H_2O gave pure **5** (20 mg) as an amorphous powder ^1H NMR (60 MHz, D_2O) 6.25 (1H, *br s*, H-3), 5.51 (1H, *s*, H-1), 4.10 (2H-11), 2.42 (1H, *s*, H-9), 1.6–2.2 (2H-7), 1.10 (3H, *s*, 3H-10)

Acetylation of **5** *to give the hexa-acetate* (**6**) Compound **5** (20 mg) was acetylated and worked-up as described for **3** to give

pure **6** (22 mg) ^{1}H NMR (90 MHz, $CDCl_3$) 6.43 (1H, *br s*, H-3), 5.64 (1H, *br s*, H-1), 4 64 (2H, *br s*, 2H-11), 2 65 (1H, *br s*, H-9), 1 22 (3H, *s*, 3H-10)

Hydrogenolysis of **6** *to yield penta-acetyllamiol* (**7**) Compound **6** (22 mg) dissolved in 95% EtOH (5 ml) was added to 10% Pd–C (10 mg) previously suspended in 95% EtOH (3 ml) and satd with H_2 After 5 min the catalyst was removed by filtration and the soln gave a residue which, after chromatography on Si gel in Et_2O–EtOAc (8 2) gave a compound (6 mg) shown to be identical to penta-acetyllamiol (**7**) (mp and mmp identical, IR and ^{1}H NMR spectra superimposable)

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