THEVERIDOSIDE, AN IRIDOID GLUCOSIDE FROM *Thevetia gaumeri*

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ABSTRACT

The identification of theveridoside (**1**), an iridoid glucoside isolated from the roots of *Thevetia gaumeri* is reported. The structure of **1** was established from its spectroscopic data and those of its peracetylated derivative **1a**.

Key Word Index. *Thevetia gaumeri*, Apocynaceae, iridoid glucoside, theveridoside.

RESUMEN

Se reporta la identificación de teveridósido (**1**), un iridoide glucosilado aislado de la raíz de *Thevetia gaumeri*. La estructura de **1** se estableció con base en la interpretación de sus datos espectroscópicos y los de su derivado peracetilado **1a**.

INTRODUCTION

In the Yucatan Peninsula there exist more than 800 plants used in the practice of traditional medicine. However, the flora of this region has not been significantly explored for its production of biologically active metabolites and the number of phytochemical studies carried out on Yucatecan plant species is limited (Sousa and Cabrera, 1983). One of the plants most commonly used in Yucatecan traditional medicine is *Thevetia gaumeri* Hemsley, commonly known as "k´aanloo", "sakits", or "campanita" (Argueta Villamar *et al.*, 1994). The latex or resin of the plant is widely used as an antimalarial and to heal wounds; it is also reported to soothe itching or mange, and to be of use to treat ulcers, cutaneous eruptions, hemorrhoids, snake bites, tumors, and edemas in general (Pulido Salas and Serralta Peraza, 1993; Mendieta and Del Almo, 1981). Previous investigations of some *Thevetia* spp. report the isolation of cardenolides (Abe *et al.*, 1994), flavonols (Abe *et al.*, 1995b), iridoids (Abe *et al.*, 1995a), and polyhydroxydinormonoterpenoids (Abe *et al.*, 1996).

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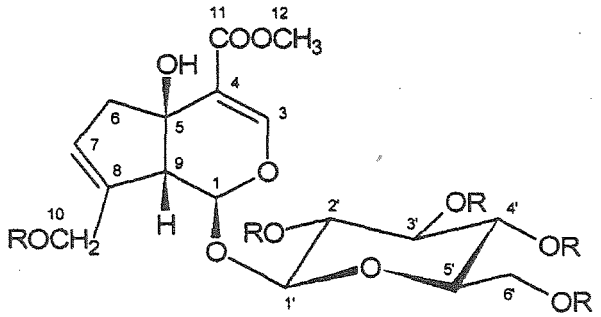

1 R = H
1a R = Ac

As part of a project directed towards the phytochemical investigation of some of the most popular Yucatecan medicinal plants, we wish to report herein the isolation and identification of the previously reported iridoid glucoside theveridoside (**1**) from the root extract of *T. gaumeri* (Sticher and Schmid, 1969; Sticher, 1970).

RESULTS AND DISCUSSION

An aqueous suspension of the methanolic extract of the roots of *T. gaumeri* was successively partitioned between hexane, CH_2Cl_2, EtOAc, and *n*-BuOH. The high polarity fraction (*n*-BuOH) showed a major component on TLC, which was obtained in pure form after purification by VLC. The IR spectrum of the isolated metabolite showed bands at 3400, 1700, and 1625 cm^{-1}, indicating the presence of hydroxyl, carbonyl, and alkene functionalities in the molecule. Its ^1H NMR spectrum (Table 1) showed, among other signals, one singlet at δ 7.49, characteristic of a proton at the β position of an α,β-unsaturated carbonyl system; and a broad singlet at δ 5.69, assigned to the vinylic proton of a trisubstituted double bond. A COSY experiment showed the latter signal as being coupled to the protons of a methylene group appearing as a doublet at δ 2.83. The presence of a natural methyl ester in the structure of the purified product was suggested by signals at δ 3.72 and δ 168.2 in its ^1H- and ^{13}C NMR spectra, corresponding to a methoxyl group and a carbonyl ester carbon, respectively. These results, together with the low frequency observed for the carbonyl absorption band in the IR of the isolated metabolite, confirmed the presence of an α,β-unsaturated ester in the structure. The two doublets at δ 5.54 and δ 4.61 in the ^1H NMR spectrum of the metabolite were assigned to the methine protons of two acetal groups. The presence of acetals in the structure was confirmed by two signals at δ 97.0 and δ 100.0 in the ^{13}C NMR spectrum. Furthermore, the acetal signal at δ 4.61 and a group of signals between δ 3.21 and 3.38 in the ^1H NMR spectrum, strongly indicated the presence of a carbohydrate unit in the molecule. In addition to the signals assigned to the methylene protons of the primary alcohol of the sugar moiety, the ^1H NMR spectrum showed two doublets at δ 4.22 and δ 4.11 indicating the presence of a second hydroxylated methylene in the aglycone moiety. To confirm unequivocally the presence of a sugar moiety in the structure of the purified metabolite, as well as to improve its solubility in organic solvents, the natural product was acetylated in the usual manner. The IR spectrum of the acetylated derivative showed the expected new carbonyl carbon band at 1750 cm^{-1}, corresponding to the carbonyl group of the various acetate units, but still showed an absorption band at 3530 cm^{-1}, indicating the presence of a remaining tertiary hydroxyl group in the molecule. Three oxymethine and two oxymethylene protons of the sugar moiety showed the expected downfield chemical shift of 0.5-2.0 ppm in the ^1H NMR spectrum of the acetylated derivative (Table 1). The all-*trans*-diaxial coupling observed between the acetal and oxymethine protons, including that next to the primary alcohol, in the ^1H NMR spectrum of the acetylated derivative, allowed the identification of the sugar moiety as a glucose

Table 1. ^{1}H NMR and ^{13}C NMR Spectral Data of Theveridoside (**1**) and Theveridoside pentaacetate (**1a**).[a]

	1[b]		**1a**[c]	
	^{1}H	^{13}C	^{1}H	^{13}C
1	5.54 d (J = 5.5 Hz)	97.0	5.41 d (J = 5.7 Hz)	95.2
2	—	—	—	—
3	7.49 s	154.0	7.40 s	151.4
4	—	114.5	—	113.8
5	—	76.4	—	74.9
6	2.83 *br* d (J = 16.8 Hz)	46.9	2.87 *br* d (J = 18.6 Hz)	45.5
7	5.69 *br* s	126.7	5.77 *br* s	129.9
8	—	141.9	—	134.6
9	3.03 d (J = 5.5 Hz)	56.7	3.18 d (J = 5.7 Hz)	54.9
10a	4.22 d (J = 14.3 Hz)	61.0	4.67 d (J = 13.4 Hz)	61.5
10b	4.11 d (J = 14.3 Hz)		4.60 d (J = 13.4 Hz)	
11	—	168.2	—	169.3
12	3.72 s	51.7	3.76 s	51.5
1′	4.62 d (J = 8.0 Hz)	100.0	4.61 d (J = 8.0 Hz)	96.5
2′	3.21 dd (J = 9.2, 8.0 Hz)	74.6	5.01 dd (J = 9.6, 8.0 Hz)	70.7
3′	3.38 t (J = 9.2 Hz)	77.6	5.25 t (J = 9.6 Hz)	72.0[d]
4′	3.29 m	71.5	5.09 t (J = 9.7 Hz)	68.1
5′	3.29 m	78.4	3.72 ddd (J = 9.7, 4.6, 2.5 Hz)	72.1[d]
6′a	3.87 dd (J = 11.9, 1.8 Hz)	62.7	4.26 dd (J = 11.7, 4.6 Hz)	61.5
6′b	3.64 dd (J = 11.9, 5.6 Hz)		4.17 dd (J = 11.7, 2.5 Hz)	
OCOMe	—	—	1.97 – 2.10 (5 Me)	20.4 – 20.8 (5 Me)
OCOMe	—	—	—	166.7 – 170.6 (5C)

[a] (^{1}H NMR at 300 MHz, ^{13}C NMR at 75 MHz, TMS as internal standard). [b] (CD_3CD). [c] ($CDCl_3$). [d] Exchangeable values.

unit. Two additional oxymethylene signals, also showing a downfield chemical shift (δ 4.67 and δ 4.60) in the ^{1}H NMR spectrum of the acetylated derivative, were readily assigned to the primary alcohol functionality present in the aglycone moiety. The purified metabolite was identified as theveridoside (**1**), an iridoid glucoside originally isolated from *Thevetia peruviana*, and its peracetylated derivative (**1a**) by comparing the spectroscopic data discussed above with those reported in the literature (Sticher and Schmid, 1969). To date, no biological activity has been reported for **1**. However, a number of interesting reports exist on the pharmacological properties of iridoid glucosides; currently **1** is being evaluated on a number of bioassays, the results of which will be published in due course.

EXPERIMENTAL

The IR spectra were determined using a Perkin-Elmer 683 spectrophotometer. The ^{1}H-(300 MHz) and ^{13}C NMR (75 MHz) spectra were recorded in a JEOL Eclipse 300 MHz spectrometer, using either $CDCl_3$ or CD_3OD as solvents and TMS as internal standard. The VLC chromatography (Pelletier *et al.*, 1986) was run using silica gel 60 GF_{254} for TLC from Merck.

Plant Material. Roots of *T. gaumeri* were collected at Km 7 of Merida-Sierra Papacal road, Yucatan, Mexico in June 1996. A voucher specimen (*No. 1992*) was deposited in the herbarium of the Unidad de Recursos Naturales of the Centro de Investigación Científica de Yucatán, Mexico.

Extraction and Isolation. Dried and powdered roots (724.0 g) were extracted with

MeOH in a soxhlet apparatus. Evaporation of the solvent under reduced pressure yielded the MeOH extract (106.0 g), which was suspended in a H_2O-MeOH (9:1) mixture and successively partitioned between hexane, CH_2Cl_2, EtOAc, and *n*-BuOH. The *n*-BuOH fraction (10.0 g) was purified by means of a VLC column, eluting with CH_2Cl_2-MeOH mixtures of increasing polarity, to produce 10 major fractions. Fraction TPR-3D (2.3 g) was shown to contain pure theveridoside (**1**).

Theveridoside (**1**): Yellow oil. IR (film) ν_{max} 3400 (OH), 1700, 1625 (C=C-COOR) cm-1. ^{1}H- (300 MHz, CD_3OD) and ^{13}C NMR (75 MHz, CD_3OD): see Table 1.

Theveridoside pentaacetate (**1a**): Yellow oil. IR (film) υ_{max} 3530, 1750, 1620 cm-1. ^{1}H- (300 MHz, $CDCl_3$) and ^{13}C NMR (75 MHz, $CDCl_3$): see Table 1.

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