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Article

Antifungal Iridoids from the Stems of *Tocoyena formosa*

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In honor of Professor Otto R. Gottlieb's Seventy-Fifth Birthday

Um novo iridóide, com atividade antifúngica, foi isolado dos caules de *Tocoyena formosa*, juntamente com outros três iridóides (apodantosídeo, galiosídeo e sua correspondente aglicona), uma saponina triterpênica, ácido 3-O-β-D-glicopiranosil-28-0-β-D-glicopiranosil-quinóvico e o flavonol glicosilado 3-O-β-D-ramnosil-3-O-metilquercetina, de estruturas conhecidas. A substância nova foi caracterizada através de dados espectroscópicos como sendo ferulato de 11-O-*trans*-teucreína.

A new antifungal iridoid compound, together with three active known iridoids, galioside, galioside aglucone, and apodanthoside, as well as 3-O-β-D-glucopyranosyl-28-O-β-D-glucopyranosyl quinovic acid and quercetin-3-O-methyl-3-O-β-D-rhamnopyranoside, were isolated from the stems of *Tocoyena formosa*. This new compound was characterized by spectral data as 11-O-*trans*-feruloylteucrein.

Keywords: Tocoyena formosa, *Rubiaceae, triterpene saponin, flavonol glycoside, antifungal iridoids*

Introduction

Several tropical Brazilian Rubiaceae species from the rain forests and *cerrado*, produce phytoalexins in response to fungal inoculation[1]. Among the 15 assayed species, plants of the *Tocoyena formosa* collected in Mogi-Guaçú, in the state of São Paulo, showed positive phytoalexin response during the entire year except during winter[2]. A positive response is not necessarily associated with the appearance of phytoalexins, but could be caused by inhibitins. After TLC bioassays the extracts obtained from the leaves and stems of this species revealed several different fungitoxic zones. Recently we reported the isolation and structural determination of four iridoids from the leaves of *T. formosa*. Two of them, α- and β- gardiol, showed inhibitory activity against fungal growth[3]. We now describe the isolation and characterization of four iridoids, **3-6**, from the stems of *T. Formosa,* with antifungal properties, as well as the inactive, known compounds **1-2**. The new iridoid **6** and the known iridoids **3-5** showed activity against the growth of *Cladosporium cladosporioides* (Fres.) de Vries (Dematiaceae). This is a common phyllocladane fungus and a contaminant of many species of seeds[4]. Some strains of *C. cladosporioides* appear to be weakly pathogenic[5]. In this paper we report the isolation and structural elucidation of this new iridoid.

Experimental

IR spectra were obtained on a Perkin-Elmer Model 1600 spectrometer, ^{1}H-NMR (200 and 400 MHz) and ^{13}C-NMR (50 and 100.57 Mhz) spectra on a Brucker and a Varian Unity 400, respectively, at 25°; EI-MS and HRMS: MS Laboratory, Department of Chemistry, Virginia Polytechnic Institute and State University, Virginia, USA.

Plant material

Stems of *Tocoyena formosa* were collected from preserved areas of a Brazilian *cerrado* in the biological reserve of the Mogi-Guaçú Ecological and Experimental Station, São Paulo, Brazil. A voucher specimen (# SP 178799) was deposited in the Maria Eneida Fidalgo Herbarium of the Instituto de Botânica de São Paulo.

Extraction and isolation of the constituents

Dried and powdered stems (2.0 kg) of *T. formosa* were successively extracted in soxhlet with hexane and EtOH. The hexane extract (310 mg) was fractioned by silica gel column chromatography (6.0 g) yielding sitosterol (10 mg), β-amirin (30 mg), and fatty material. The crude ethanolic extract (200 g) was solubilized in EtOH:H_2O (8:2) and partitioned into hexane (30 g), $CHCl_3$ (22 g) and n-BuOH (45.5 g). The n-BuOH soluble fraction (250 mg) was submitted to CC on silica gel (10 g) and eluted with CH_2Cl_2 with increasing amounts of MeOH. After TLC, some fractions were combined and submitted to prep. TLC developed with CH_2Cl_2:AcOEt (4:2) and $CHCl_3$:MeOH:H_2O (68:38:10) gave **1** (13.5 mg), **2** (14.0 mg), **3** (6.2 mg, colorless gum **4** (10.5 mg, mp 113-115°, [lit. 110-115°]), **5** (15.0 mg, colorless gum) and **6** (20.1 mg, colorless gum).

Bioassay

Fungitoxic zones were detected using a spore suspension of *C. cladosporioides*[12] on TLC silica gel plates developed with $CHCl_3$:MeOH (7:3) and $CHCl_3$:MeOH:H_2O (68:38:10).

11-O-*trans*feruloylteucrein **6**. Colorless gum: $[\alpha]_D$ -65.5 (c = 0.1, MeOH); IR: ν_{Max}^{KBr} (cm^{-1}): 3294, 1680, 1605,

Table 1. ^1H- and ^{13}C-NMR chemical shifts δ and coupling constants (Hz) of 11-O-*trans*-feruloylteucrein **6** and teucrein **7**[13].

atom	**6**		**7**	
	^1H	$^{13}C^a$	$^1H^b$	^{13}C
1	5.02 (1H d, 4.5)	90.1 d	5.87 (1H d, 4.3)	91.5 d
3	6.35 (1H, s)	140.7 d	6.30 (1H, s)	140.6 d
4	-	113.0 s	-	112.8 s
5	2.66 (1H, m)	48.0 d	2.66 (1H, m)	48.0 d
6	1.56 (1H-6a, m), 2.31 (1H-6b, m)	33.0 t	-	32.9 t
7	1.73 (H-7, m)	30.2 t	-	30.0 t
8	1.78 (H-8, m)	35.8 d	-	35.0 d
9	2.66 (1H, dd, J = 4.5; 9.8)	35.0 d	-	35.0 d
10	1.07 (3H, d, J = 6.0)	20.0 q	1.07 (3H, d, J = 5.5)	20.8 q
11	4.70 (1H, d, J = 12.0) 4.66 (1H, d, J = 12.0)	66.9 t	4.24 (1H, d, J = 11.9) 4.66 (1H, d, J = 11.9)	63.9 t
1'	-	127.5 s	-	-
2'	7.36 (1H, d, J = 2.0)	116.5 d	-	-
3'	-	146.0 s	-	-
4'	-	149.1 s	-	-
5'	7.20 (1H, d, J = 8.5)	115.3 d	-	-
6'	7.42 (1H, dd, J = 8.5; 2.0)	123.9 d	-	-
7'	7.63 (1H, d, J = 16.0)	148.0 d	-	-
8'	6.40 (1H, d, J = 16.0)	114.5 d	-	-
9'	-	168.9 s	-	-
CH_3CO	-	-	2.10; 2.04 (6H, s)	21.1q
CH_3CO	-	-	-	169.8s

The ^1H-NMR spectrum was recorded in $(CD_3)_2CO$ at 200 and 400 MHz. The ^{13}C-NMR spectrum was recorded in $(CD_3)_2CO$ at 50 and 100 MHz.

a. Multiplicities were based on DEPT-135 experiments, assignments were supported by HETCOR and ^1H-^1H COSY-45 experiments.

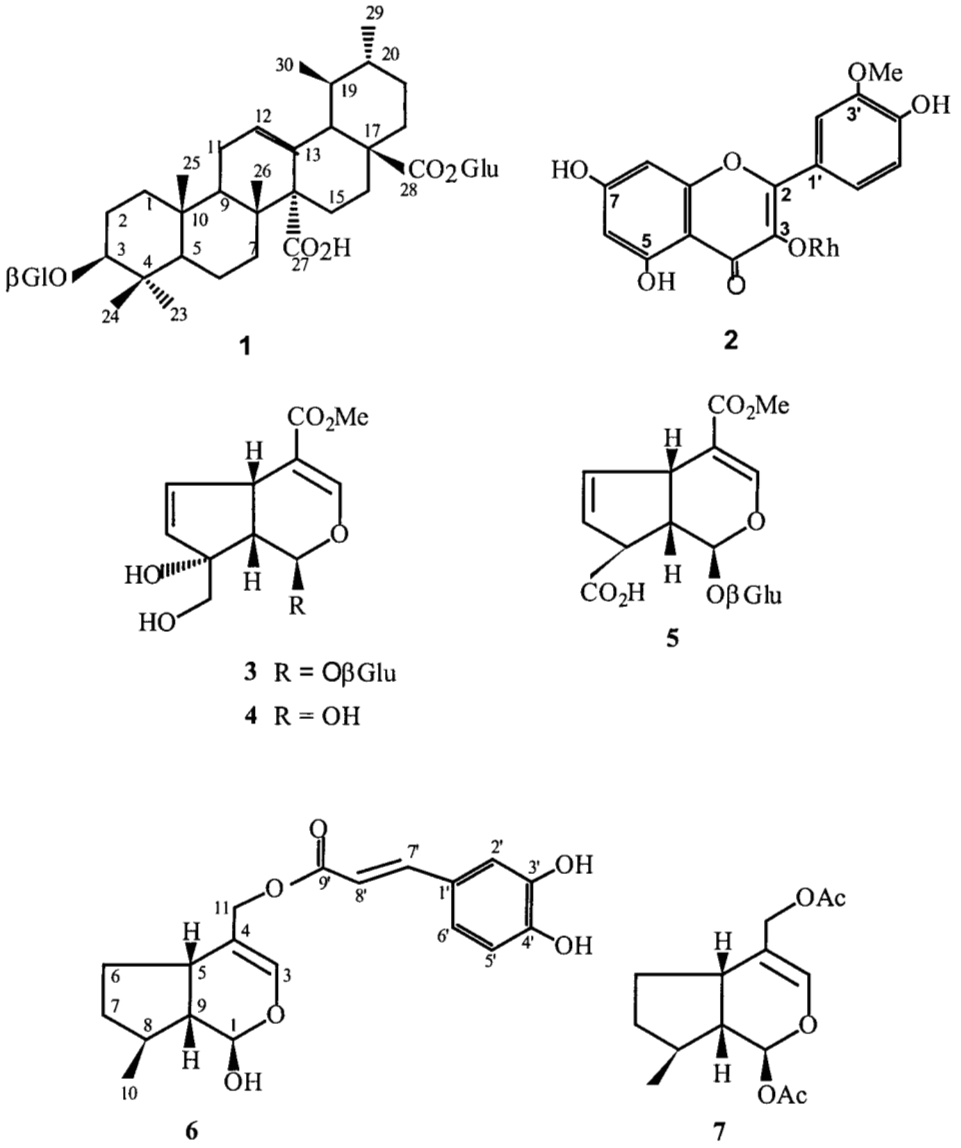
b. Only these chemical shifts were reported.

1516, 1438, 1200, 1025, 986, 825; ^{1}H-NMR [200, 400 MHz, $(CD_3)_2CO$]: (Table 1); ^{13}C-NMR [50, 100 MHz, $(CD_3)_2CO$]: (Table 1); EIMS (70 eV) m/z (%): 336 ($[M]^+$, 10), 167 (90), 163 (100), 149 (26), 139 (16), 135 (58), 109 (28).

Results and Discussion

Column chromatography of the EtOH extract from stems of *T. formosa* on silica gel resulted in six compounds **1-6**, among which the iridoid **6** is being reported for the first time. The other five compounds**1-5**, are known compounds and were identified as the triterpene saponin (3-O-β-D-glucopyranosyl-28-O-β-D-glucopyranosyl quinovic acid) **1**[6], the flavonol glycoside (quercetin-3-O-β-D-methyl-3-rhamnopyranoside) **2**[7], and the iridoids galioside **3**[8,9], galioside aglicone **4**, and apodanthoside **5**[10,11].

The iridoid **6** showed structural features very related with teucrein, which was isolated as an acetyl derivative from *Teucrium marum* (Labiateae)[13]. The molecular formula of **6** was determined as $C_{19}H_{22}O_6$ from its HRMS (m/z 346.1305; calc. 346.1314). The EI-mass fragments at m/z 163 $[C_9H_7)O_3]^+$, 135 $[C_8H_7O_2]^+$ and 109 $[C_6H_5O_2]^+$ indicated the presence of a feruloyl unity in the side chain of **6**. The IR spectrum showed diagnostic absorption bands occurring at 3294 (νOH), 1680 (νC = O α,β-unsaturated ester), 1630 (νC = C), and 1605 and 1438 cm^{-1} (νC = C, aromatic unity). The presence of a *trans*-feruloyl moiety was confirmed by ^{1}H- and ^{13}C-NMR resonance spectral data (Table 1). Analysis of the ^{1}H- and ^{13}C-NMR spectral data of **6**, including DEPT 135 and HETCOR experiments also indicated that this compound bears an iridoidal skeleton, which was identified as teucrein **7**, by comparison of



Scheme 1.

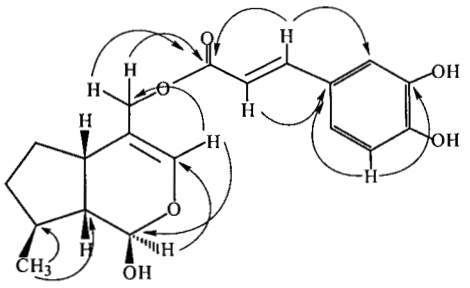


Figure 1. Some selected HMBC correlations of iridoid **6**.

the ^{1}H- and ^{13}C-NMR chemical shifts with those reported in the literature[13]. In the ^{1}H-NMR spectrum of **6** relative to **7**, H-1 (δ 5.00) was shielded by 0.87 ppm, while in the ^{13}C-NMR spectrum C-1 (δ 90.1) it was shielded by 1.4 ppm. The ^{1}H-NMR spectrum also showed resonances at δ 4.70 d and 4.66 d (δ 66.9), attributable to hydroxymethylene H-11, in agreement with structure **7**. This observation indicated that C-11 should be the site of esterification and that in C-1 the hydroxyl group should be free. An HMBC ^{1}H- ^{13}C-NMR (Fig. 1) correlation confirmed not only the substitution of *trans*feruloyl unity at C-11, but also confirmed that the hydroxyl group at C-1 is free. The structure of **6** was therefore established as the novel 11-O-*trans*-feruloylteucrein.

The presence of aglicone iridoids with antifungal activity suggests that they can play a defensive role against fungal attack. However an additional study must be undertaken in order to prove whether these compounds are phytoalexins or inhibitins.

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