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Short Report

New Tetra-acetylated Oligosaccharide Diterpene from *Cupania vernalis*

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O diterpeno glicosídico inédito, denominado vernanolídeo, possuindo o esqueleto geranil-geraniol, foi isolado do caule de *Cupania vernalis*, uma espécie da família Sapindaceae, conhecida popularmente como "arco de peneira". A estrutura do diterpeno foi determinada por técnicas espectrométricas, incluindo RMN mono e bidimensionais.

A new glucosyl diterpene possessing a geranyl-geraniol skeleton and named vernanolide was isolated from a Sapindaceae plant species, *Cupania vernalis*, popularly named "arco de peneira". The structure of the isolated compound was determined by ^{1}H, ^{13}C NMR, MS, IR and 2D NMR experiments.

Keywords: Sapindaceae, *Cupania vernalis*, diterpene glucoside

Introduction

Plant species of Sapindaceae are known for their traditional medicinal uses as diuretic, stimulant, expectorant, natural surfactant, sedative, vermifuge and against stomach-ache and dermatitis in many parts of the world [1-4]. In Brazil, *Paullinia cupana*, popularly named "guaraná" is the most representative plant species of this family. This plant is well known for its stimulant properties and a soft drink prepared from its fruits is an important article of Brazilian commerce. The chemical investigation of this family has led to the isolation of saponins[4-6], diterpenes[7-9], flavonoids[10], among other secondary metabolites[11-14]. Several saponins and acyclic sesquiterpene and diterpene oligoglycosides have been isolated as main secondary metabolites of several Sapindaceae species used in traditional oriental medicine[6, 15].

Cupania vernalis Camb., known as "camboatã", "arco de pipa" or "arco de peneira", is a common small tree growing from Minas Gerais to Rio Grande do Sul, mainly in the Cerrado and gallery forest. This species was investigated because the $CHCl_3$ crude extract from its bark proved to be active against all mutant strains of *Saccharomyces cerevisiae* Rad+, Rad 52Y and RS 321, which suggests the presence of antifungal constituents. This report describes the isolation and structure elucidation of a new acyclic diterpene oligosaccharide (**1**), besides the mixtures of the known sterols sitosterol, stigmasterol, 3-*β*-*D*-glucosylsitosterol, 3-*β*-*D*-glucosylstigmasterol and the coumarin scopoletin, this latter responsible for the moderate activity against the mutant strains of *S. cerevisiae* detected in the crude extract.

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Experimental

General

Infrared spectra were measured on a Perkin-Elmer spectrophotometer. ^{1}H NMR spectra were recorded at 200 or 300 MHz (Bruker AC 200 and AM 500 WB spectrometer, respectively); TMS was used as internal standard. ^{13}C NMR spectra were recorded at 75 MHz. Mass spectra were recorded at 70 eV on a Platform II spectrometer. Optical rotation measurements were conducted on a Polamat polarimeter using a quartz cuvete (length 10 cm). Silica gel PF_{254} and silica gel (230-400 mesh) were used for separation procedures. All solvents used were of analytical or HPLC grade.

Plant material

Cupania vernalis was collected in Campininha Farm Ecological Reserve, situated in Mogi-Guaçú, São Paulo

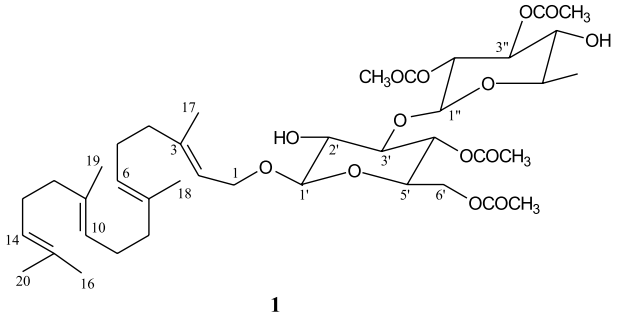
State. Voucher specimens (Young 04) are deposited in the Herbarium of the Botanic Garden, São Paulo.

Extraction and isolation

Air-dried and powdered trunk bark (1.0 kg) was repeatedly extracted with MeOH to afford MeOH crude extract (80.0 g). Part of this extract (40.0 g) was dissolved in aqueous MeOH (80%) and then submitted to liquid/liquid partition with hexane, which gave a hexane fraction (1.96 g) after concentration. The aqueous MeOH solution was concentrated to 60% and then extracted with $CHCl_3$ and *n*-BuOH affording the $CHCl_3$ (3.70 g) and *n*-BuOH (5.40 g) fractions, respectively, after concentration. The $CHCl_3$ fraction was submitted to gel filtration with gradient elution on Sephadex LH-20, according to methodology previously described[16]. Fifteen sub-fractions were obtained and combined to give 10 fractions, after comparison by TLC analysis. Flash chromatography was carried out with the first combined fractions 1-5, which gave a mixture of sitosterol/stigmasterol and 3-*β*-*D*-O-glucosylsitosterol/3-*β*-*D*-O-stigmasterol. Combined fractions 7-10 (1.70 g) were further separated by silica gel column chromatography ($CHCl_3$/MeOH gradient) and subsequent prep. TLC (5% MeOH/$CHCl_3$), affording the bioactive coumarin scopoletin (**2**) (10 mg) and the new diterpene **1** (15.5 mg).

Results and Discussion

Compound **1** was obtained as a colorless powder from the stems of *C. vernalis*, collected at Campininha Farm, Mogi-Guaçú, São Paulo State. The ES-MS data showed the molecular ion at *m/z* 789 $[M+Na]^+$ and four major fragments at *m/z* 541, 525, 295 and 92. In addition, ^1H and ^{13}C NMR analyses indicated the presence of four acetyl groups besides a disaccharide moiety and suggested the molecular formula $C_{40}H_{62}O_{14}$ for this compound. Analysis of the IR spectral absorptions at 3450, 1720 and 1650 cm^{-1} suggested the presence of hydroxyl, ester carbonyl and double bond functions, respectively. The ^1H NMR spectrum of **1** (Table 1) showed a signal at δ 4.35 (2H, d, *J* 5.2 Hz) for hydrogens of a $-CH_2O-$ system probably linked to an olefinic carbon and to a sugar moiety. In addition, this spectrum showed two signals at δ 5.10 (3H, br t, *J* 5.0 Hz) and δ 5.40 (1H, br t, *J* 5.2 Hz) for four hydrogens, each on an olefinic carbon linked to a methylene group; and three singlets at δ 1.59 (9H, s), 1.68 (3H, s), 1.69 (3H, s), assigned to five methyl groups on sp^2 carbons. These data suggested an open chain terpene derived partial structure for compound **1**. The presence of four additional singlets at δ 1.99, 2.03, 2.05 and 2.09 for methyl groups corroborated the acetyl moieties in the proposed structure. The observation of several signals at δ 3.20-4.28 for hydroxymethine and hydroxymethylene hydrogen atoms and at δ 5.18 (1H, d, *J* 7.4 Hz) and 5.30 (1H, br s) for anomeric hydrogen atoms, besides one doublet at δ 1.14 (3H, d, *J* 6.5 Hz) for one methyl group on an sp^3 carbon, suggested the presence of a glucose and a rhamnose unit linked to the terpene moiety. Inspection of ^{13}C NMR spectra (Table 1) indicated a great similarity with diterpene geranylgeraniol [6]. The comparison of all carbon values of **1** with those of geranylgeraniol led us to establish the aglycone skeleton of diterpene **1**. Furthermore, two signals for anomeric carbons at δ 97.8 and 100.1, eight signals for hydroxymethine carbons at δ 77.8, 77.0, 69.8, 69.4, 73.5, 70.9, 70.1, 66.3, signals for one hydroxymethylene carbon at δ 64.7 and one methyl group at δ 17.0, besides signals at δ 171.6, 170.7, 170.4, 169.8 for four carbonyl groups and at δ 21.1, 20.9, 20.8, 20.7 for four methyl groups suggested the presence of a tetra-acetylglucosyl-rhamnopyranosyl moiety attached at C-1 of diterpene **1**. These spectral features were in agreement with data obtained from the ES-MS spectrum, which showed major ions at *m/z* 789, 541, 277, 295 and 92 assigned to $[M+Na]^+$, [789-diacetylrhamnosyl+Na]$^+$, [541-diacetylglucosyl+Na]$^+$, [geranylgeraniol-H_2O+Na]$^+$ and [dimethylallyl+Na]$^+$, respectively. The sequential loss of rhamnose and then glucose moieties confirms the presence of a disaccharide chain linked to the aglycone geranylgeraniol with rhamnose being the terminal sugar. The exact sequence of the disaccharide and its linkage to the aglycone, as well as the positions of each acetyl group on the sugar units, were solved by extensive use of 2D NMR spectroscopic techniques aided by the analysis of chemical shifts described in the literature[6, 15]. The COSY ^1H-^1H spectrum of **1** enabled quite extensive hydrogen couplings to be verified, as the strong correlation of signals at δ 5.40 and δ 4.35, assigned to H-2 and H-1, respectively. This spectrum also evidenced the correlation between signals at δ 2.05 and 1.98, which were assigned to methylene hydrogens at



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C-4, C-8, C-12 and C-5, C-9, C-13, respectively, after analysis of additional data from the HETCOR spectrum. Additionally, these data confirmed the positions of each methyl group on the diterpene unit and the methyl signal at δ 1.14 corresponding to C-6" (δ 17.0) of the rhamnosyl unit. Based on such spectral evidence, compound **1** was shown to contain four acetyl groups and a β-*D*-glucopyranosyl-α-*L*-rhamnopyranoside linked at C-1 of the aglycone geranylgeraniol. The positions of the acetyl units at each sugar moiety, the detailed sugar arrangement and the linkages between the sugar and the aglycon were determined from HMBC experiments (Figure 1) with the same conclusions regarding the sugar sequence drawn from the NOESY experiment (Table 1). The signal at δ 63.4 attributed to C-1 showed HMBC correlation to the signal at δ 5.40, attributed to the olefinic hydrogen H-2, and to the anomeric hydrogen at δ 5.18, and confirmed that the glucosyl unit is linked to the diterpene skeleton. The HMBC correlation between the broad singlet at δ 5.30, attributed to the anomeric hydrogen of the terminal rhamnosyl unit and the carbon signals at δ 77.8 (C- 3'), 69.8 (C- 2") and 73.5 (C-5"), respectively, established a (1→3) linkage between the two sugar units. Further correlations between the hydrogen signal at δ 4.28 (H-6') to carbon signals at

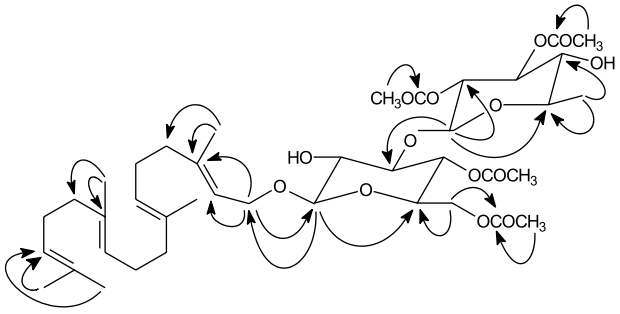

Figure 1. Selected HMBC correlations for compound **1**.

Table 1. ^{1}H and ^{13}C NMR spectral data for diterpene **1** (500 and 125 MHz, respectively, $CDCl_3$)

Position	δ^{13}C	δ^{1}H (mult., *J* (Hz))	COSY	NOESY
1	63.4	4.35 (d, 5.2)	2	2, 1'
2	119.3	5.40 (br t, 5.2)	1, 17	
3	141.2			
4	39.6	2.05 (m)	5, 17	17
5	26.3	1.98 (m)	4, 6	18
6	123.5	5.10 (br t, 5.0)	5, 18	
7	135.4			
8	39.6	2.05 (m)	9	
9	26.5	1.98 (m)	8, 10	
10	124.0	5.10 (br t, 5.0)	9	9
11	135.4			
12	39.6	2.05 (m)	13	
13	26.5	1.98 (m)	12, 14	
14	124.0	5.10 (br t, 5.0)	13, 20	20
15	131.1			
16	17.6	1.59 (s)	15	20
17	16.3	1.69 (s)		4
18	15.9	1.59 (s)		6, 8
19	15.9	1.59 (s)		12
20	25.6	1.68 (s)		14, 16
1'	97.8	5.18 (d, 7.4)	2'	3'
2'	77.0	3.20 (br t, 11.0)		4',C$\underline{H}_3$COC-2"
3'	77.8	4.22 (br t, 11.0)	2', 4'	5',1'
4'	69.4	4.14 (br t, 11.0)	3', 5'	2', 6', 1"
5'	66.3	3.68 (m)	4', 6'	1', 3'
6'	64.7	4.28 (dd, 11.4, 5.5)	5'	4', C$\underline{H}_3$COC-6'
1"	100.1	5.30 (br s)	2"	4', 5"
2"	69.8	3.80 (br d, 9.8)	1", 3"	4", C$\underline{H}_3$COC-3"
3"	70.1	3.68 (m)	2", 4"	
4"	70.9	3.70 (br t, 9.5)	3", 5"	6"
5"	73.5	3.70 (dq, 9.5, 6.5)	4", 6"	1"
6"	17.0	1.14 (d, 6.5)	5"	4"
C$\underline{H}_3$C$\underline{C}$O-O-C-6'	169.8/20.9	1.99 (s)		
C$\underline{H}_3$C$\underline{C}$O-O-C-4'	171.6/21.1	2.05 (s)		
C$\underline{H}_3$C$\underline{C}$O-O-C-2"	170.4/20.7	2.03 (s)		
C$\underline{H}_3$C$\underline{C}$O-O-C-3"	170.7/20.8	2.09 (s)		

δ 66.3 (C-5'), 64.7 (C-6') and 169.8 (CH_3COO-C-6') led to the definition of the acetyl position at the sugar moiety in the diterpene molecule. The NOESY experiments also showed significant interactions among hydrogens and permitted us to assign the relative stereochemistry on the basis of coupling constants (Table 1) and corroborated the proposed structure for compound **1**.

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