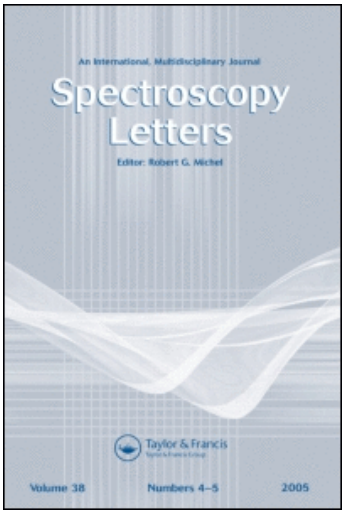

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Isolation and Structural Determination of Xerophytolic Acid, a Novel 3-Geranyl-4-Hydroxybenzoate Derivative from *Xerophyta Plicata*

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ISOLATION AND STRUCTURAL DETERMINATION OF XEROPHYTOLIC ACID, A NOVEL 3-GERANYL-4-HYDROXYBENZOATE DERIVATIVE FROM *XEROPHYTA PLICATA*

Key Words: 3-geranyl-4-hydroxybenzoate, Xerophyta, Velloziaceae, NMR.

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ABSTRACT

A novel 3-geranyl-4-hydroxybenzoic acid derivative, methyl xerophytolate (**1**), was isolated from the crude extract of *Xerophyta*

plicata (*Velloziaceae*) collected in Rio de Janeiro, Brazil. The structure of **1** was elucidated using spectroscopic methods. This is the first time that this type of skeleton is reported in this family.

INTRODUCTION

Higher plant natural products presenting the 3-prenyl-4-hydroxybenzoate skeleton are rarely found in the literature. The most common members of this class of compounds are the intermediates in the ubiquinone biosynthesis, which possess relatively long prenyl chains.[1, 2] The 3-geranyl derivatives, the rarest members of this class of compounds, have been isolated from *Lithospermum erythrorhizon* (Boraginaceae)[3,4] and *Piper aduncum* (Piperaceae).[5]

The Velloziaceae constitute a family of tropical monocotyledons with most of its 250 species occurring in Brazil[6-8]. The chemistry of the genus *Xerophyta* (Velloziaceae) is not well known. The only phytochemical studies on this genus are the taxonomic investigation of cuticular leave wax[9,10] and foliar flavonoids.[11,12] We carried out chemical studies on *Xerophyta plicata* and isolated a novel 3-geranyl-4-hydroxybenzoic acid derivative as its methyl ester, which we call methyl xerophytolate (**1**).

EXPERIMENTAL

Xerophyta plicata was collected in Nova Friburgo (Rio de Janeiro, Brazil) in September 1990. The crude hexane extract of the whole plant was separated in fractions using silica gel column chromatography eluted with hexane-ethyl acetate. Fraction 9 (eluted with hexane:ethyl acetate, 1:1) was treated with an excess of an ethereal solution of diazomethane. Further separation of the resulting mixture on an alumina GF_{254} Merck preparative TLC plate eluted with hexane:ethyl acetate (4:1) afforded compound **1** in 0.001% overall yield.

NMR spectra, in $CCl_4(D_2O)$, were recorded on a BRUKER DRX-300 and VARIAN UNITY-300 spectrometers, using TMS as internal reference. All the ^1H

and ^{13}C assignments were obtained through 1D ^{1}H, ^{13}C, and DEPT, and 2D COSY, HMQC, and HMBC experiments. High resolution impact (EI) and chemical ionization (CI) mass spectra were run on a KRATOS MS-50 apparatus. A PERKIN ELMER 1600-FTIR was used for infrared spectroscopy (neat, NaCl plates).

RESULTS AND DISCUSSION

The high resolution mass spectrum of **1** gave a molecular ion at *m/z* 362.17284, corresponding to $C_{20}H_{26}O_6$. The IR spectrum shows absorptions consistent with the presence of hydroxyl (3451 cm^{-1}), ester (1737 cm^{-1}), α,β-unsaturated ester (1716 cm^{-1}), and an aromatic ring (900 and 832 cm^{-1}). A combined analysis of the ^{1}H, ^{13}C, DEPT, COSY, and HMQC NMR spectra of **1** (see Table 1) led to the identification of six structural fragments shown in Scheme 1.

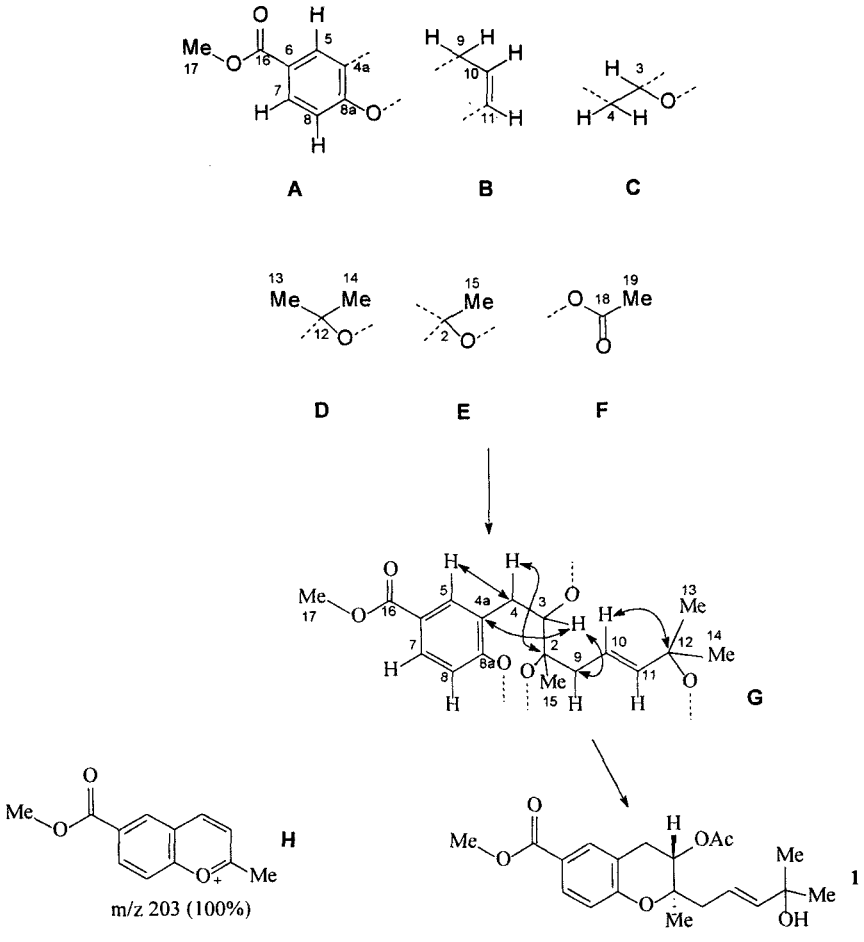
Fragment A contains a 1,3,4-trisubstituted aromatic ring, whose hydrogen signals are two mutually coupled one-hydrogen doublets at δ 7.93 (H7) and δ 6.84 (H8), and a one-hydrogen singlet at δ 7.91 (H5). The HMQC spectrum shows their directly bonded carbons at δ 126.4 (C5), δ 131.0 (C7), and δ 108.5 (C8). The shielding of C8 suggests the vicinity of a carbon bearing an oxygen at C8a. The *meta* relationship between H5 and H7 was assumed considering that C5 is not influenced by the oxygen substituent at C8a. The presence of an α,β-unsaturated ester (C=O stretch 1716 cm^{-1}) in the IR spectrum of **1** suggests that one of the substituents in the aromatic ring is a carbomethoxy group.

Fragment B contains a 1,2-disubstituted carbon-carbon double bond *vicinal* to a methylenic carbon. The methylenic hydrogens H9a and H9b are assigned to δ 2.97 (1H, dd, J= 6.6 and 14.2 Hz) and δ 2.81 (1H, dd, J= 7.0 and 14.3 Hz), respectively. These hydrogens show a *geminal* coupling of 14 Hz and a coupling with a two-hydrogen multiplet at δ 5.75 (H10 and H11). Selective irradiation of the two methylenic hydrogens (H9a and H9b) converted the original multiplet at δ

TABLE 1

^{1}H NMR, ^{13}C NMR, DEPT, COSY and HMQC Data of **1.**

#C	$\delta^{1}H$	$\delta^{13}C$	HMBC (#C)	^{1}H - ^{1}H COSY (#H)
2	-	83.6	9, 4	
3	5.33 (1H, t, 8.7)	85.1	9	4a, 4b
4	3.33 (2H, d, 8.5)	29.8	5	3
4a	-	122.9	4	-
5	7.91 (1H, s)	126.4	7	-
6	-	126.5	8	-
7	7.93 (1H, d, 8.3)	131.0	5	8
8	6.84 (1H, d, 8.3)	108.5	-	7
8a	-	163.4	4, 7	-
9	2.97 (1H, dd, 6.6, 14.3)	37.9	3, 11	10, 11, 4b
	2.81 (1H, dd, 7.0, 14.2)			10, 11, 4a
10	5.75 (2H, m)	142.9	9	9a, 9b
11	5.75 (2H, m)	119.8	9	9a, 9b
12	-	70.0	10, 11	-
13	1.45 (3H, s)	30.0	11	-
14	1.45 (3H, s)	30.0	11	-
15	1.50 (3H, s)	18.5	-	-
16	-	168.6	17	-
17	2.10 (3H, s)	21.7	-	-
18	-	166.4	19	-
19	3.96 (3H, s)	51.3	-	-



Scheme 1

5.75 into an AB system with J_{AB} = 15.0 Hz (*trans*) and resonances of δ 5.73 and δ 5.80 for H10 and H11, respectively. The exact reproduction of the second order coupling pattern for this system was accomplished using WINDAISY 2.0 spin simulation program. Analysis of the HMQC spectrum of **1** shows that the three carbons of fragment B appear at δ 37.9 (C9), δ 142.9 (C10), and δ 119.8 (C11).

Fragment C is also an isolated spin system, which consists of a methylenic carbon at δ 3.33 (H4a and H4b, d, *J*= 8.5 Hz) *vicinal* to a carbinolic methyne at δ 5.33 (H3, t, *J*= 8.5 Hz). The respective carbons for this fragment appear at δ 85.1 (C3) and δ 29.8 (C4). Fragment D is defined by a quaternary carbon (C12) linked to two equivalent methyl groups (C13 and C14) with ^{1}H and ^{13}C NMR resonances at δ 1.45 (6H, s) and δ 30.0, respectively. The ^{13}C NMR spectra of **1** shows the presence of only two sp^3 hybridized quaternary carbons, both bonded to oxygen (δ 70.0 and δ 83.6), one of which corresponds to C12. The other oxygen bearing sp^3 quaternary carbon is part of fragment E (C2), which is bonded to a methyl group (C15) with ^{1}H and ^{13}C NMR resonances at δ 1.50 and δ 18.5, respectively. The last fragment of **1**, fragment F, corresponds to an acetate group with ^{1}H and ^{13}C NMR resonances at δ 2.10 (H19), δ 21.7 (C19) and δ 168.6 (C18).

Analysis of the HMBC spectrum of **1** led to the definition of the fragments and how they are assembled to produce the backbone skeleton of **1**. Correlation of H7 (δ 7.93) with C16 (δ 166.4) indicates that the carbomethoxy group of fragment A is bonded to C6, which leaves C4a as the remaining free position of fragment A. Correlation of H5 (δ 7.91) with C4 (δ 29.8) and H3 (δ 5.33) with C4a (δ 122.9) suggests that C4 of fragment C is bonded to C4a of fragment A. Confirmation of the C4-C4a bond is found in the correlation between H3 (δ 5.33) and C8a (δ 108.5). Another important correlation is observed between H4a/H4b (δ 3.33) and C2 (δ 83.6), which defines the connection between fragment C and fragment E through the C3-C2 bond. Correlation of H3 (δ 5.33) with C9 (δ 37.9) indicates the existence of a bond between C2 and C9, which connects fragment E and fragment B. Since the carbon at δ 83.6 is directly bonded to C3 and C9, it is clear that it corresponds to C2. The other sp^3 oxygenated carbon must be C12 (δ 70.0), which shows correlation with the vinylic hydrogens H10 and H11 (δ 5.73 and δ 5.80, respectively). This is an additional evidence for the connection between fragment B and fragment D, which occurs through the bond between C11 and C12.

According to HREIMS, compound **1** has only 6 oxygen atoms, therefore two of the oxygens shown in the tentative backbone skeleton (partial structure G) have to become one by cyclizing two of the fragments. Considering that the partial structure G has an acetyl group bonded to one of the oxygens of fragments C, D, or E (not shown), the remaining unsaturation equivalent of compound **1** can be easily accounted for by the presence of a heterocyclic ring. This ring could be a 5-, 6- or 10-membered ring depending on the position of the acetate and the hydroxyl groups. The position of the hydroxyl group was determined by measuring the deuterium isotopic effect on the chemical shifts of the neighboring carbons.[13] When a D_2O treated sample of **1** was set into an 5 mm nmr tube having within it a coaxial tube containing a solution with the same concentration of an untreated sample of **1**, only C12 showed a significant chemical shift variation ($\Delta\delta$ = 0.06), indicating that the hydroxyl group is bonded to this carbon. This result eliminates the existence of a 10-membered ring in the structure of **1**. If the heterocyclic ring were a 5-membered ring, a correlation between the hydrogen at C3 and C8a would have been observed in the HMBC spectrum of **1**. Since this correlation is not present, it was assumed that **1** contains a 6-membered heterocyclic ring, formed by the superposition of the oxygen at C8a (from fragment A) with the oxygen at C2 (from fragment E), which places the acetyl group at C3. This is confirmed by the analysis of the high resolution mass spectrum of **1**, which shows a fragment with *m/z* 203 (100%), fragment H, which is compatible with the chromane ring system.[14]

The relative stereochemistry of **1** was determined from a NOESY experiment, which shows a strong correlation between H9 and H3, showing that the acetyl group and the unsaturated side chain are *trans* to each other.

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