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Article

Preparation, X-ray Structural Studies and Plant Growth Regulatory Activity of Methyl 6α,7β-Thiocarbonyldioxyvouacapan-17β-oate

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O ácido 6α,7β-di-hidroxivouacapan-17β-óico (**1**) e o 6α,7β-di-hidroxivouacapan-17β-oato de metila (**2**) são produtos naturais isolados do extrato hexânico de frutos de *Pterodon polygalaeflorus* Benth. Este trabalho descreve a preparação e caracterização por espectroscopias no infravermelho, de ressonância magnética nuclear e por análise elementar do derivado inédito 6α,7β-tiocarbonildioxivouacapan-17β-oato de metila (**5**), a partir do éster **2**. A estrutura do composto **5** foi determinada por difração de raios-X. Na concentração de 100 ppm, este composto inibiu o crescimento radicular de *Sorgum bicolor L.* (-28%) e não mostrou efeitos significantes sobre *Cucumis sativus L.*.

The 6α,7β-dihydroxyvouacapan-17β-oic acid (**1**) and methyl 6α,7β-dihydroxyvouacapan-17β-oate (**2**) are natural products isolated from the hexane extract of *Pterodon polygalaeflorus* Benth fruits. Here we describe the preparation and characterization by infrared and nuclear magnetic resonance spectroscopy, and by elemental analysis of the novel derivative methyl 6α,7β-thiocarbonyldioxyvouacapan-17β-oate (**5**), from the ester **2**. The structure of compound **5** was determined by X-ray diffraction. At the concentration of 100 ppm, this compound inhibited the radicle growth of *Sorgum bicolor L.* (-28%) and showed no significant effect on *Cucumis sativus L.*.

Keywords: Vouacapane derivatives, plant growth regulation, crystal structure determination

Introduction

The 6α,7β-dihydroxyvouacapan-17β-oic acid (**1**) (Scheme 1) and its methyl ester (**2**) (Scheme 2) are natural products isolated from the hexane extract of *Pterodon polygalaeflorus* Benth fruits.[1] These diterpenes have shown a variety of interesting biological activities,[2] being potent analgesic and anti-inflammatory agents. It was recently discovered that compound **1**, at a concentration of 100 ppm, possess an inhibitory effect (41%) on the radicle growth of *Sorgum bicolor L.* and a small stimulatory growth effect (4%) on the radicle of *Cucumis sativus L.*.[3]

Due to the possibility of application of these diterpenes as medicines or as agrochemicals, a series of derivatives for biological evaluation has been synthesized.[4,5] The bis-xanthate **4**, was unexpectedly obtained from the lactone **3** (Scheme 1) upon reaction with NaH, imidazole, CS_2 and MeI.[3]

As compound **4** showed an inhibitory effect on the radicle growth of *Sorgum bicolor L.* (-50%), and a stimulatory effect (+10%) on the radicle growth of *Cucumis sativus L.*,[3] a variation of this methodology[6] was applied directly onto the natural product **2**, as an attempt of preparing larger amounts of the bis-xanthate **4** to further investigate the biological activity detected. Using the conditions showed in the Scheme 2, compound **4** was obtained as a secondary product and a new derivative

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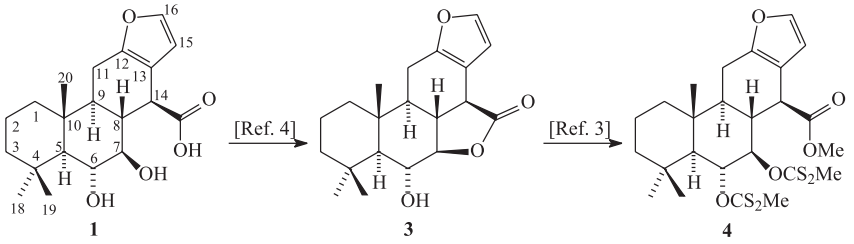


1 3 4

Scheme 1.

containing a five membered ring fused to the vouacapane skeleton was isolated and characterized as the methyl 6α,7β-thiocarbonyldioxyvouacapan-17β-oate (**5**). We undertook crystallization and structure elucidation by X-ray diffraction of compound **5** and the effects of this compound on the radicle growth of *C. sativus L.* and *S. bicolor L.* were determined by the method described by Demuner *et al.*[3] and compared to the results obtained for compounds **1** and **4**.

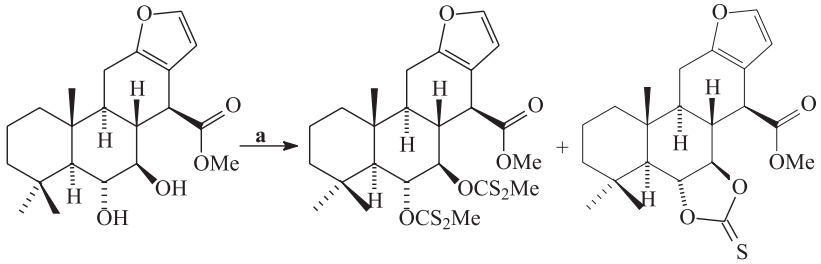
Results and Discussion

The starting material, methyl 6α,7β-dihydroxyvouacapan-17β-oate (**2**) was isolated from *P. polygalaeflorus* Benth fruits, according to the procedure described in the literature.[1] Treatment of the ester **2** with carbon disulphide in the presence of sodium hydride and imidazole in THF at 0 °C, followed by the addition of methyl iodide (Scheme 2), resulted in the formation of compounds **4** and **5** (21 and 54%, respectively). At higher temperatures, the yields decrease and the formation of several other products is observed by TLC.

The structure of compound **4** was confirmed by infrared and NMR spectroscopy and all the data obtained were in accord with the literature.[3] The infrared spectrum of compound **5** showed all the expected signals, specially a sharp band at 1296 cm^{-1} due to the C=S bond stretching. The ^{13}C NMR spectrum of **5** showed six signals with no correlation in the HMQC contour map assigned to carbons 12 and 13 of the furan ring, the quaternary carbons 4 and 10, the ester carbonyl and the thiocarbonyl. The signals of carbons 6 and 7 were unequivocally assigned by the correlations observed in the COSY contour map. The two pseudo-triplets at δ 4.47 and 4.08 (H-6 and H-7) were correlated to each other (J(6,7) 11.6 Hz), and to the doublet at δ 1.49 (H-5) and the multiplet at δ 2.70-2.78 (containing H-8 signal), respectively. All the remaining signals were in accordance with the proposed structure.

An ORTEP-III[7] drawing of the compound **5** is shown in Figure 1. The bond distances and angles are within normally expected ranges. The molecule has a skeletal conformation similar to that of the compound **1**.[8] The junctions between the three six-membered carbon rings in compound **5** are *trans*. The torsion angles C(3)-C(4)-C(5)-C(6), C(2)-C(1)-C(10)-C(9) and C(6)-C(7)-C(8)-C(14) have values of 175.3(3)°, 174.0(3)° and 176.9(3)°, respectively (the expected value is 180°). The C(1)-C(10)-C(9)-C(11) torsion angle is 60.6(4)° (the expected value is 60°). The conformation of the ester group is also similar to the acid group of the compound **1**.[8]

The furan ring of compound **5** is planar. The bond lengths of the furan ring confirm the expected π-bond character. According to Cremer-Pople parameters,[9] the ring fused to furan [q_2 =0.085(1)Å, q_3 = 0.826(1) Å, Q = 0.831(1) Å, θ = 5.843(1) and φ = 3.651(1)°] presents a half-chair conformation similar to the compound **1**[8] and



2 4 (21%) 5 (54%)

a: *i*. NaH, imidazole, THF, CS_2, N_2; *ii*. MeI.

Scheme 2.

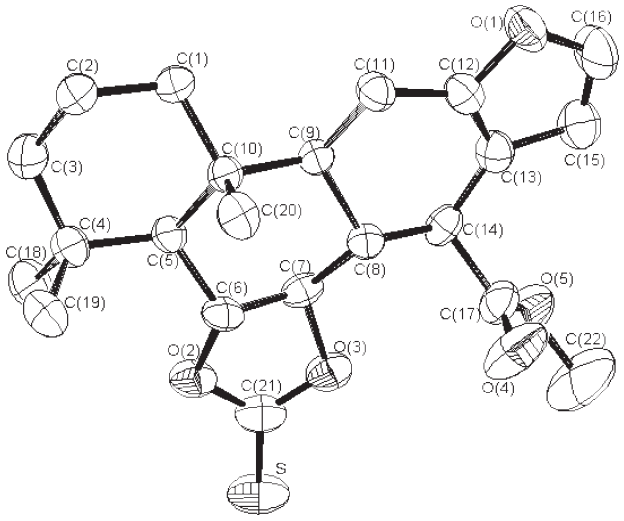


Figure 1. Labeled drawing of the molecular structure of the compound **5** (ORTEP III).

different from the compound **3**[10] in which the conformation is half-boat. The other cyclohexane rings adopt chair conformation like in compounds **1** and **3**. The ring C(6)-O(2)-C(21)-O(3)-C(7) [q_2=0.462(1) Å and φ_2=109.479(1)°] is in an envelope conformation.

Differences were observed in the bond angles O(2)-C(6)-C(5), O(2)-C(6)-C(7), O(3)-C(7)-C(6) and O(3)-C(7)-C(8) of compounds **1** and **5** (Table 1) due to the formation of the fused five-membered ring. Apart from the angle C(7)-C(8)-C(9), no other significant change was observed in the angles within ring C(5)-C(6)-C(7)-C(8)-C(9)-C(10).

Table 1. Comparison of selected bond angles for compounds **1**[8] and **5** obtained by X-ray diffraction

	Compound **1**	Compound **5**
O(2)-C(6)-C(5)	112.9(3)	119.8(3)
O(2)-C(6)-C(7)	107.6(4)	99.0(3)
O(3)-C(7)-C(6)	109.1(3)	101.3(3)
O(3)-C(7)-C(8)	108.3(3)	116.2(3)
C(7)-C(8)-C(9)	110.7(3)	103.9(3)
C(7)-C(6)-C(5)	109.7(3)	110.1(3)
C(8)-C(7)-C(6)	114.8(3)	113.2(3)
C(8)-C(9)-C(10)	113.7(4)	113.4(3)
C(5)-C(10)-C(9)	108.0(4)	109.7(3)

It is interesting to note that the crystal packing of compound **5** is formed by discrete molecules maintained by van der Waals forces, while the crystal packing of compound **1** involves three intermolecular hydrogen bonds which link the molecules in chains.[8]

The effects of compounds **1** and **4** on the radicle growth of *C. sativus L.* and *S. bicolor L.* were previously reported.[3] As compound **5** was obtained in a relatively good yield, being another sulphur derivative of the natural product **1** and presenting a new ring fused to the vouacapane skeleton, we decided to evaluate its influence in plant radicle growth. The same preliminary test conditions previously described[3] were applied to compound **5**. A sample of the natural product **1** was also tested as reference, for the results usually vary according to the seeds lot used. The generality of the results previously published[3] were confirmed, the effect of compound **1** on the radicle growth of *S. bicolor* being much greater than that on *C. sativus*. Compounds **1** and **5** reduced the growth of the *S. bicolor* radicle by 30 and 28%, respectively. No significant effects of compounds **1** and **5** were observed on the radicle growth of *C. sativus* (+7 and -7%, respectively).

In summary, the preparation of the new derivative described here might allow a number of other transformations on the vouacapane natural products **1** and **2** and their biological activities may be further evaluated. The preliminary biological assay described here suggests that the OH groups at C-6 and C-7 might not be essential for the plant growth activity of the vouacapanes, and the small variations in the skeleton conformation due to the new five-membered ring present in compound **5** were not significant to the biological assays results, as compound **5** was approximately as active as **1**. The presence of sulphur atoms as in compounds **4** and **5** did not affect considerably the activity of the vouacapanes.

Experimental

The melting points were determined on a MQRPF - APE - 301 apparatus (Microquímica Ind. e Com. LTDA) without correction. Elemental analyses were taken on a Perkin Elmer 2400 apparatus. Infrared Spectra were recorded on a Perkin Elmer PARAGON 1000 grating spectrometer, scanning from 625 to 4000 cm^{-1}. The samples were prepared as KBr disks. ^1H and ^{13}C NMR spectra were recorded on spectrometers Bruker DRX 400 *AVANCE* (400 MHz for ^1H and 100 MHz for ^{13}C) and Bruker DPX 200 *AVANCE* (200 MHz for ^1H and 50 MHz for ^{13}C). Deuteriochloroform was used as the solvent. Tetramethylsilane was used as the internal standard (δ = 0). Chemical shifts are given in the δ-scale (ppm), coupling constants *J* in Hz. COSY and HMQC experiments were carried out using pulse sequence and program provided by the manufacturer. The methyl 6α,7β-dihydroxyvouacapan-17β-oate (**2**) [R_f 0.34 (hexane-diethyl ether 1:10). mp 201.8-203.5ºC. IR ν_{max}/cm^{-1}: 3537, 3002, 2922, 1727, 1281, 1171, 1021, 727] used as starting material was extracted from the fruits of *Pterodon polygalaeflorus* Benth by the procedure described in the literature.[1] It was further purified by column chromatography (silica gel, hexane-diethyl ether 1:10),

and recrystallized from ethyl acetate and hexane. Alternatively, the ester **2** can be prepared from lactone **3**[4] by reaction with sodium methoxide.

*Preparation of methyl 6α,7β-bis-(dithiocarbonate)-vouacapan-17β-oate (**4**) and methyl 6α,7β-thiocarbonyldioxyvouacapan-17β-oate (**5**)*

To a solution of ester **2** (250 mg, 0.69 mmol) in dry tetrahydrofuran (5 mL) was added a suspension of sodium hydride (70% dispersion in oil, 72 mg, 2.1 mmol) and a solution of imidazole (12 mg) in dry tetrahydrofuran (10 mL) under a nitrogen atmosphere at 0 °C. Carbon disulphide (0.41 mL, 6.8 mmol) was then added and the mixture was stirred at 0 °C for 30 min. Methyl iodide (0.43 mL, 6.7 mmol) was added and the reaction mixture was stirred for a further 2 h. A cold saturated solution of ammonium chloride (10 mL) was added. Extractions with dichloromethane (4 x 20 mL) were performed, the combined organic extracts were washed with a 10% sodium thiosulfate solution (10 mL) and with brine (10 mL), dried over magnesium sulphate and concentrated under reduced pressure. The resulting yellow oil was purified by column chromatography (silica gel, hexane-diethyl ether 3:1), yielding **4** (ref.3; white solid, 81 mg, 0.15 mmol, 21%) and **5** (white solid, 150 mg, 0.37 mmol, 54%).

*Methyl 6α,7β-thiocarbonyldioxyvouacapan-17β-oate (**5**).* R_f 0.33 (hexane-diethyl ether 3:1). mp 247.7-248.8 °C. Anal.Calc. for $C_{22}H_{28}O_5S$: C, 65.3; H, 7.0. Found: C, 65.1; H, 6.9 %. IR ν_{max}/cm^{-1}: 2990, 2950, 2868, 1739, 1350, 1296, 1125, 957, 739. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.26 (1H, d, *J* 1.9 Hz, H-16), 6.20 (1H, d, *J* 1.9 Hz, H-15), 4.47 (1H, *pseudo* t, *J* 11.6 Hz, H-6), 4.08 (1H, *pseudo* t, *J* 11.6 Hz, H-7), 3.83 (3H, s, OCH_3), 3.52 (1H, ddd, *J* 9.1, 2.7 and 1.7, H-14), 2.70-2.78 (2H, m, H-8 and H-11β), 1.10 (3H, s, CH_3-19), 2.52 (1H, ddd, *J* 16.2, 11.6 and 2.7, H-11α), 1.75-1.79 (1H, m, H-1β), 1.47-1.65 (4H, m, H-2α, H-2β, H-3β and H-9), 1.49 (1H, d, *J*(5,6) 11.6, H-5); 1.17-1.28 (1H, m, H-3α), 1.08-1.15 (1H, m, H-1α), 1.13 (3H, s, CH_3-18), 1.11 (3 H, s, CH_3-20). ^{13}C NMR (50 MHz, $CDCl_3$): 192.4 (C=S), 172.7 (C=O), 149.7 (C-12), 141.8 (C-16), 112.5 (C-13), 108.5 (C-15), 91.5 (C-7), 84.8 (C-6), 56.1 (C-5), 52.7 (OCH_3), 48.7 (C-9), 44.6 (C-14), 41.0 (C-3), 40.7 (C-10), 39.1 (C-1), 38.4 (C-8), 34.7 (C-18), 33.2 (C-4), 22.6 (C-19), 21.7 (C-11), 18.3 (C-2), 16.3 (C-20).

Other data obtained: DEPT 135, COSY, HMQC.

*X-ray structure determination of the compound **5***

Colorless crystals of the compound **5** were obtained by slow evaporation at room temperature in ethanol solution. *Crystal data*: $C_{22}H_{28}O_5S$, M_r=404.50, Orthorhombic, $P2_12_12_1$, a=6.980(2), b=14.902(3), c=20.035(2) Å, V=2084.0(8) Å^3, Z=4, D=1.289 g cm^{-3}, F(000)=864, μ=0.185 mm^{-1}. *Data collection*: prismatic single crystal 0.77 x 0.50 x 0.10 mm, Enraf-Nonius CAD-4 diffractometer,[11] T=243(2) K, λ (MoK$_\alpha$)= 0.71073 Å, 3515 intensities (3468 unique), 2.45° < θ < 29.96°.

Structure solution and refinement. Absorption corrections (PSI SCAN),[12] data reduction (program XCAD-4),[13] direct methods, refined on F^2 (program SHELX-97),[14] hydrogen atoms with riding model, wR(F_o^2) = 0.1229, R [$F^2 > 2\sigma(F^2)$] = 0.0588, 258 parameters, Flack parameter = -0.2(2), S=1.050, $\Delta\rho_{max}$=0.238 e/Å^3.

Crystallographic data (excluding structure factors) for the structure of compound **5** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 166447. Copies of the data can be obtained, free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Crambridge Crystallographic Data Centre, 12, Union Road, Cambridge CB2 1EZ, UK; fax: +44 1223 336033; or e-mail: deposit@ccdc.cam.ac.uk).

Biological Assay

The biological assays were carried out at 25 °C under fluorescent light (8 x 40 W) in an incubator for 3 days, in 100 by 15 mm glass Petri dishes lined with one sheet of Whatman No. 1 filter paper and sealed with Parafilm. To each dish were added 2.00 mL of a solution of compounds **1** or **5**, in CH_2Cl_2 (100 ppm). The solvent was evaporated before the addition of 2.00 mL of water and 20 seeds of *C. sativus L.* or *S. bicolor L.*. Four repetitions were prepared for each compound. A control experiment was prepared by the addition of 2.00 mL of solvent only, to four Petri dishes, following the same conditions described. Radicles were measured and the averages compared to the control experiment results.

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