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Article

Clerodane Diterpenes from Bark of *Croton urucurana* Baillon

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O novo diterpeno clerodano 3-oxo-12-epibarbascoato de metila foi isolado das cascas do caule de *Croton urucurana*, juntamente com o diterpeno conhecido como 12-epibarbascoato de metila. As estruturas destes compostos foram elucidadas através de técnicas espectroscópicas e por comparação com dados da literatura. A obtenção de cristais possibilitou a análise cristalográfica da difração de raios X dos diterpenos, confirmando assim as estruturas propostas.

The new clerodane diterpene methyl 3-oxo-12-epibarbascoate was isolated from the stem barks of *Croton urucurana* together with the known diterpene methyl 12-epibarbascoate. The structures of these compounds were elucidated by spectroscopic techniques and comparison with the literature data. The obtainment of crystals allowed the crystallographic analysis of X-ray diffraction of diterpenes, thus confirming the proposed structures.

Keywords: *Croton urucurana*, X-ray structure, stereochemistry, clerodane diterpene

Introduction

Croton is a large genus of Euphorbiaceae, comprising around 1,300 species of trees, shrubs and herbs distributed in tropical and subtropical regions of both hemispheres.[1,2] Some species of this genus produce a blood-red latex, which gave to the genus the vernacular name of Dragon's Blood.[3] Among a great variety of shikimic and acetate pathway metabolites, diterpenoids with various skeletons are well represented in this genus, which is one of the richest sources of clerodanes.[2] The *nor*-clerodane diterpene *trans*-dehydrocrotonin was isolated from the bark of *C. cajucara* Benth.[4] This compound was also isolated from the aerial part of *C. schiedeanus*, besides two new neo-clerodane type furan diterpenoids and *cis*-dehydrocrotonin.[5] Diterpene crotonin-derived clerodanes were also found in *C. macrobothrys*.[6] Two new clerodane diterpenes, crotobrasilin A and crotobrasilin B, were isolated from leaves and stems of *C. brasiliensis*.[7] In addition to clerodane diterpenes, a variety of diterpene types can be found in the *Croton* genus, such as cembrane diterpenoids in *C. gratissimus*,[8] casbane in *C. nepetaefolius*,[9] abietane and *ent*-kaurene derivatives in *C. argyrophylloides*[10] and *C. kongensis*,[11] diterpenes derived of the tigliane and daphnane types in *C. steenkampianus*,[12] and trachylobane derivatives in *C. zambesicus.*[13]

There are very few reports available concerning the phytochemical compounds of *C. urucurana* Baill., a tree commonly found in the region of Dourados City, Mato Grosso do Sul State, Brazil. This plant is widely used in traditional medicine to treat wound infection and to accelerate wound healing, and also to treat rheumatism, cancer and other illnesses.[14] In our previous phytochemical studies on the bark of *C. urucurana*, three clerodane diterpenes were found and identified as methyl 12-epibarbascoate (**1**), sonderianin (**2**) and 15,16-epoxyclerodan-3,13(16),14(15)-trien-2-one (**3**), along with acetyl aleuritolic acid, stigmasterol, campesterol, β-sitosterol, β-sitosterol-3-O-glucoside, catechin and gallocatechin.[15] In the essential oil of the stem bark borneol, bornyl acetate, 1-isopropyl-7-methyl-4-methylene-1,3,4,5,6,8-hexahydro-2*H*-naphthalen-4α-ol sesquicineole and γ-gurjunene epoxide were identified as the main components.[16] The polysaccharide

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fucoarabinogalactan has been isolated as the main constituent of the gum exudate of this species.[17] In terms of biological activity, the methanolic extract of the bark has been reported to be antimicrobial[14] and analgesic,[18] and the aqueous extract is apparently a promising antidote for the effects of *Bothrops jararaca* venom.[19] In the case of the blood-red latex, antifungal,[17] visceral nociception[20] and antidiarrhoeal activities[21] have been observed. Insecticidal activity has also been verified for semipurified fractions of the stem bark extract of *C. urucurana.*[22]

As a continuation of our investigation on the chemical constituents of the stem barks of *C. urucurana*, this work describes the structures of two clerodane diterpenes, the previously reported diterpene methyl 12-epibarbascoate (**1**) and the new diterpene methyl 3-oxo-12-epibarbascoate (**4**). The structures of these compounds were elucidated by spectroscopic techniques and comparison with the literature. Furthermore, the obtainment of crystals allowed the crystallographic analysis of X-ray diffraction of methyl 12-epibarbascoate and 3-oxo-12-epibarbascoate, thus confirming the proposed structures.

Experimental

General experimental procedures

Melting points were determined with a Microquímica APF-302 apparatus, and are uncorrected. IR spectral data were acquired on a Perkin-Elmer FT 16PC instrument with KBr disks. The optical rotation was evaluated on a Polartronic E polarimeter (Schmidt Haensch). ^{1}H (400 MHz) and ^{13}C nuclear magnetic resonance (NMR) (100 MHz) spectra were recorded with a Varian spectrometer, using $CDCl_3$ as the solvent and TMS (tetramethylsilane) as the internal standard. Gas chromatographic analyses were obtained on a Shimadzu GC 14B with a flame ionization detector (FID) and a CBP20 fused silica capillary column. The initial column temperature was 100 ºC and the final temperature was 290 ºC, with heating at 10 ºC min^{-1}. The injector and detector temperature were 235 ºC and the carrier gas used was N_2. Column chromatography was performed using silica gel (230-400 mesh, Merck). Thin layer chromatography (TLC) was performed on a pre-coated silica gel type 60 plate (Merck) and spots were located by spraying with sulfuric anisaldehyde followed by heating. ESI-Q-TOFMS (electrospray ionization quadrupole time-of-flight mass spectrometry) measurements were performed with a micrOTOF Q-II (Bruker Daltonics) mass spectrometer equipped with an automatic syringe pump from KD Scientific for sample injection. The ESI-QTOF mass spectrometer was running at 4.5 kV at a desolvation temperature of 180 ºC. The mass spectrometer was operating in the positive ion mode. The standard ESI source was used to generate the ions. Samples were injected using a constant flow (3 μL min^{-1}). The solvent was an acetonitrile/methanol mixture. The ESI-Q-TOF MS instrument was calibrated in the range *m/z* 50-3000 using an internal calibration standard (low concentration tuning mix solution) which was supplied from Agilent Technologies. Data were processed via Bruker Data Analysis software version 4.0.

X-ray crystallographic analysis

The isolated diterpenes were submitted to crystallization, and both **1** and **4** afforded crystals suitable for X-ray diffraction determination. A crystal of each sample was selected from representative crystalline samples. The intensity data for these compounds were collected with an Enraf-Nonius CAD4 diffractometer, at room temperature, with graphite-monochromated Mo K_α radiation. The unit cell parameters were determined based on the setting angles of 25 centered reflections. All data were corrected for Lorentz and polarization effects. The structure was solved by direct methods and refined by full-matrix least-squares methods using SIR97[23] and SHELXL97[24] programs, respectively. All non-hydrogen atoms were refined anisotropically. H atoms attached to C atoms were placed at their idealized positions, with C–H distances and U_{eq} values taken from the default settings of the refinement program. Due to the absence of an efficient anomalous scattering source, the absolute configuration of the structures could not be determined. The obtainment of ORTEP plots and the cif (crystallographic information file) validation procedure were performed using PLATON software.[25] Further data obtained from the crystallographic analysis of compounds **1** and **4** are summarized in Table 1. Crystallographic data for the structures in this work were deposited in the Cambridge Crystallographic Data Centre as supplementary publication numbers CCDC 837380 and CCDC 837381, respectively.

Plant material

The stem barks of *C. urucurana* Baill. were collected along the Dourados highway (km 21) in a town called Itahum (Mato Grosso do Sul State, Brazil) in October 2006. The plant material was identified by Ubirazilda Resende, retired Botanist of the Universidade Federal do Mato Grosso do Sul Herbarium, and a voucher specimen was deposited in the CGMS Herbarium under the registered number 16860.

Table 1. Crystal data and structure refinement of compounds **1** and **4**

	1	**4**
Empirical formula	$C_{21}H_{26}O_5$	$C_{21}H_{26}O_6$
Formula weight	358.42	374.42
Temperature / K	293(2)	293(2)
Wavelength / Å	0.71069	0.71069
Crystal system	orthorhombic	monoclinic
Space group	$P2_12_12_1$	$P2_1$
Unit cell dimensions		
a / Å	7.536(1)	9.1547(8)
b / Å	11.043(2)	11.160(2)
c / Å	22.576(3)	9.2186(4)
β / degree		99.109(5)
Volume / Å^3	1878.8(5)	929.9(2)
Z	4	2
Density (cal.) / (mg m^{-3})	1.267	1.337
μ / mm^{-1}	0.089	0.097
F(000)	768	400
Crystal size / mm^3	0.50 × 0.33 × 0.13	0.50 × 0.26 × 0.20
Theta range / degree	1.80 to 25.97	2.24 to 26.97
Index ranges	$-9 \le h \le 3, -13 \le k \le 3, -27 \le l \le 0$	$-11 \le h \le 11, -14 \le k \le 5, -11 \le l \le 5$
Reflections collected	3925	5104
Independent reflections	2129 [R(int) = 0.0247]	2141 [R(int) = 0.0201]
Refinement method	Full-matrix least-squares on F^2	Full-matrix least-squares on F^2
Data / restraints / parameters	2129 / 0 / 239	2141 / 1 / 247
Goodness-of-fit on F	1.033	1.023
Final R indices [I > 2sigma(I)]	R1 = 0.0409, wR2 = 0.0997	R1 = 0.0348, wR2 = 0.0893
R indices (all data)	R1 = 0.0806, wR2 = 0.1156	R1 = 0.0498, wR2 = 0.0967
Extinction coefficient	0.004(1)	
Largest diff. peak and hole / (e Å^{-3})	0.153 and –0.197	0.191 and –0.153

Extraction and isolation

The powdered stem barks of *C. urucurana* (1.2 kg) were extracted sequentially with enough volume to cover the plant material of *n*-hexane, EtOAc and EtOH (three times for seven days each) at room temperature. The resulting extracts were filtered and concentrated under reduced pressure at 50 ºC to give the *n*-hexane (37 g), EtOAc (17 g) EtOH (66 g), respectively. Part of the EtOAc extract (7 g) was submitted to chromatographic fractionation on a silica gel column eluted with *n*-hexane and increasing amounts of EtOAc (0-100%) to give 20 fractions (125 mL each). Fractions **8** and **9** (75:25 *n*-hexane-EtOAc), isolated initially in the form of a yellow oil, were combined and after successive recrystallization in CH_2Cl_2 yielded 80.0 mg of **1** in the form of crystals. Finally, fractions **15** and **16** (60:40 *n*-hexane-EtOAc) were combined and purified by recrystallization in CH_2Cl_2 to yield 22.0 mg of **4**, also in crystal form.

Methyl 12-epibarbascoate (**1**)

Colorless crystals (CH_2Cl_2); mp 115-117 ºC;[15] TLC Rf 0.7 (70:30 *n*-hexane-EtOAc); IR (KBr) ν_{max}/cm^{-1} 3128, 2951, 2845, 1713, 1495, 1444, 1388, 1361, 1314, 1246, 1140, 1070, 1022, 954, 909, 871, 799, 766, 724, 688, 598; ^{1}H NMR ($CDCl_3$) δ 7.42 (s, H-16), 7.40 (t, *J* 1.8 Hz, H-15), 6.61 (t, *J* 3.3 Hz, H-14), 6.40 (br s, H-3), 5.51 (dd, *J* 5.9 and 11.4 Hz, H-12), 3.68 (s, O-Me), 2.53 (dt, *J* 3.3 Hz, H-2), 2.37 (d, *J* 5.9 Hz, H-11), 2.33 (m, H-8), 2.17 (m, H-7), 1.73 (d, *J* 3.7 Hz, H-1), 1.66 (m, H-6), 1.63 (m, H-10), 1.31 (s, H-19), 1.08 (s, H-20). For ^{13}C NMR spectroscopic data see Table 2.

Table 2. NMR data for compounds **1** and **4**

Position	**1**		**4**	
	δ_C / ppm[a]	δ_C / ppm[a]	δ_H / ppm (J[b] / Hz)	^2J, ^3J ^{1}H-^{13}C correlated
1	17.6	22.4	2.00 m	
2	27.0	40.6	2.54 dt (*J* 3.3)	
3	137.4	204.7	–	
4	141.4	69.6	3.22 s	C-3, C-5, C-19
5	37.0	37.3	–	
6	35.0	37.7	1.90 m 1.34 m	
7	18.6	18.4	2.10 dd (*J* 12.5; 3.3)	
8	51.5	51.6	2.23 d (*J* 3.3)	
9	37.3	41.5	–	
10	51.5	54.2	1.60 m	
11	44.3	44.6	2.36 dd (*J* 13.2; 5.9) 1.75 d (*J* 3.7)	
12	72.1	71.9	5.49 dd (*J* 11.4; 5.9)	C-13
13	126.2	125.8	–	
14	108.7	108.6	6.41 br s	C-13, C-15, C-16
15	144.0	144.1	7.41 br s	
16	139.6	139.6	7.44 br s	
17	172.7	171.9	–	
18	167.5	168.3	–	
19	21.2	15.4	1.25 s	C-4, C-5, C-6, C-10
20	14.8	15.1	1.11 s	C-8, C-10, C-11
OMe	52.7	51.9	3.68 s	C-18

[a]$CDCl_3$, assignments confirmed by DEPT; [b]assignments confirmed by ^{1}H-^{1}H COSY.

Methyl 3-oxo-12-epibarbascoate (**4**)

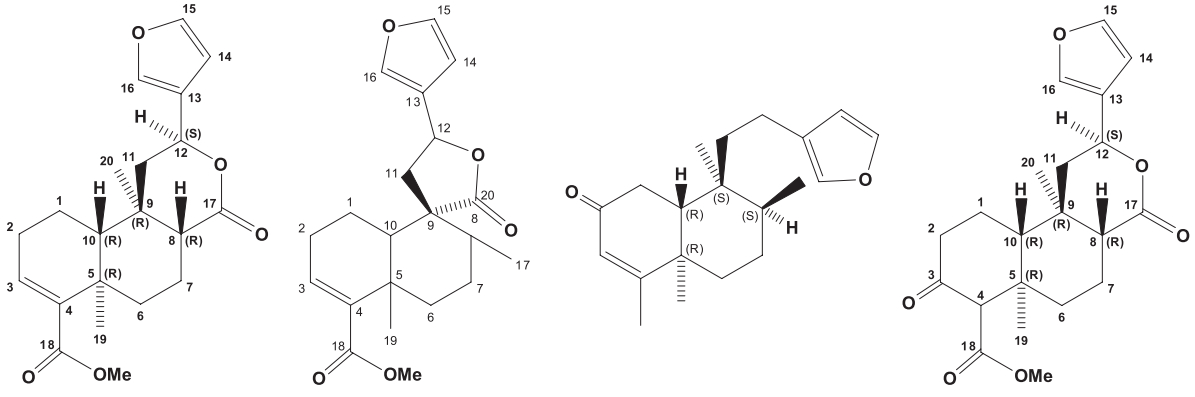
Colorless crystals (CH_2Cl_2); $[\alpha]_D^{20}$ +37.6 (acetone, *c* 7.60 × 10^{-4} g mL^{-1}); mp 156 ºC; TLC Rf 0.3 (70:30 *n*-hexane-EtOAc); HRMS (ESI$^+$) *m/z* calculated for $C_{21}H_{26}O_6$ $[M + Na]^+$ 397.1621; found 397.1622; IR (KBr) ν_{max}/cm^{-1} 3151, 2952, 1736, 1705, 1504, 1452, 1345, 1265,1225, 1159, 1069, 1018, 870, 788, 749, 701, 653, 602, 522. For ^{1}H and ^{13}C NMR spectroscopic data see Table 2.

Results and Discussion

Following our investigation of the EtOAc extract of *C. urucurana* stem barks, herein the isolation and structural elucidation of a new clerodane diterpene, methyl 3-oxo-12-epibarbascoate (**4**), together with the known compound methyl 12-epibarbascoate (**1**), is reported.

The compound **1** was isolated initially in the form of a yellow oil that after successive recrystallization in CH_2Cl_2 yielded 80.0 mg of crystals with mp 117-120 ºC and TLC Rf 0.7, at 70:30 *n*-hexane-EtOAC. Structure of this compound **1** (Figure 1) was confirmed by comparison of the NMR data with those reported in the literature.[15] The obtainment of single crystals through recrystallization in CH_2Cl_2, allowed the crystallographic analysis of **1** by X-ray diffraction (Figure 2, Table 1), an observation that has not been previously reported in the literature.

Compound **4**, Figure 1, was isolated as colorless crystals (mp 156 ºC, TLC Rf 0.3, with 70:30 *n*-hexane-EtOAc) and identified through spectroscopic methods, and confirmed by X-ray crystallography (Figure 2). Complete assignments of ^{1}H and ^{13}C signals were achieved (Table 2) through detailed NMR spectral analysis including ^{1}H-^{1}H COSY (correlation spectroscopy), HMQC (heteronuclear single quantum coherence) and HMBC (heteronuclear multiple-bond correlation spectroscopy).



1 **2** **3** **4**

Figure 1. Diterpenes isolated from *Croton urucurana*.

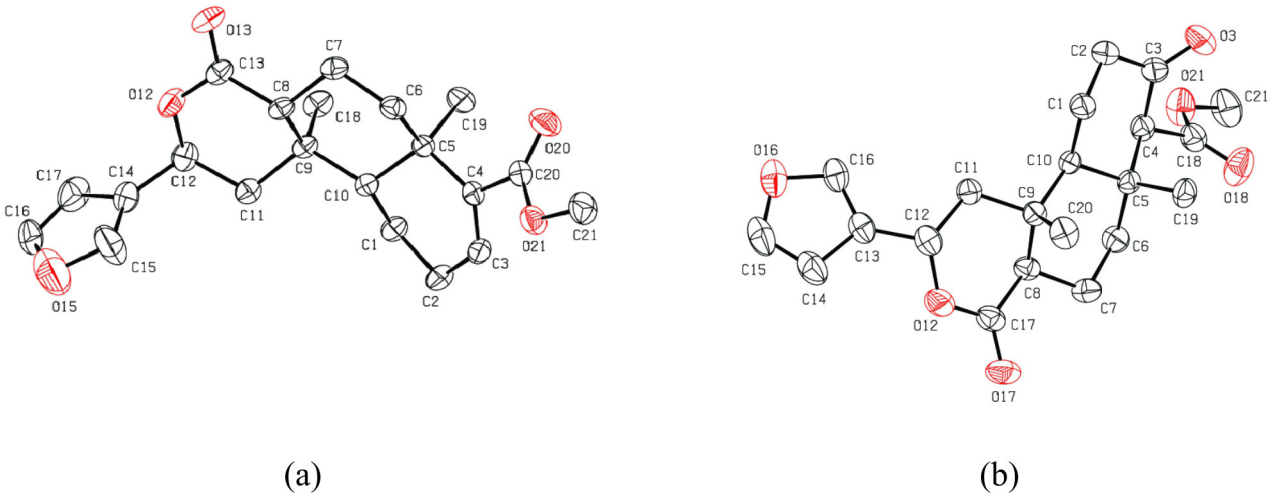


Figure 2. Perspective view of the X-ray structures of compounds **1** (a) and **4** (b).

The ^{1}H and ^{13}C NMR results were very similar to those observed for methyl 12-epibarbascoate (**1**), with a lack of a resonance for the vinyl hydrogen H-3 (δ 6.61).[18] The ^{13}C NMR spectrum showed an additional carbonyl C-3 signal (δ 204.7) for the cyclohexanone moiety and a CH (δ_C 69.6; δ_H 3.22, C-4) alpha to two carbonyl groups. Gas chromatographic analysis confirmed the purity of the compound through the observation of a single peak with a retention time of 25.1 min.

With regard to the infrared spectroscopy, there are broad absorption bands for esters and lactone carbonyl at ν 1736 cm^{-1}, and an exocyclic ketone carbonyl at ν 1705 cm^{-1}. In the ^{1}H NMR spectrum, two signals at δ 7.44 (H-16) and δ 7.41 (H-15) were observed indicating the presence of two hydrogens each attached to a sp^2 carbon of the aromatic ring or neighbors to an electronegative atom such as oxygen, for example. These signals, along with the signal at δ 6.41 (H-14) and in comparison with the ^{1}H NMR spectrum of the known compound methyl 12-epibarbascoate (**1**) suggested the presence of a furan ring. Several other signals between δ 1.0 and 2.0 for CH, CH_2 and CH_3 groups, as well as a double doublet at δ 5.49 (H-12) related to a CH (C-12) endocyclic connected to oxygen and a neighbor CH_2 (C-11) were observed. By observing the correlation between the signal of this hydrogen with the signal of the CH_2 hydrogens at δ 2.36 and δ 1.75 in the ^{1}H-^{1}H COSY spectrum, it was confirmed the existence of the C12-C11 (CH-CH_2) system. In the ^{13}C NMR spectrum, 21 carbons in the spectral region between δ 15.1 and 204.7 were observed, Table 2. This spectrum confirms the existence of the furan ring due to the presence of signals at δ 139.6, 144.1 and 108.6, besides the signal at δ 125.8 corresponding to a quaternary carbon. In addition to these signals, absorptions related to another three quaternary carbons appear, probably corresponding to ester carboxyl (δ 168.3 and 171.9) and ketone carbonyl (δ 204.7). The ^{13}C NMR DEPT (distortionless enhancement by polarization transfer) spectrum indicated signals for three methyl, five methylene and seven methyne carbons. Analyzing these data and comparing them with reports in the literature,[15] many consistencies were noted in the absorption signals. These data can be attributed to a clerodane-type diterpene structure, consisting of a system known as 5-(furyl)-δ-valerolactone.[26] Through the HMQC bidimensional spectrum analysis, it was possible to assign of all chemical shifts of the hydrogens and carbons, Table 2. This information together with the ^{1}H NMR spectrum allowed the identification of the hydrogens and the calculation of their coupling constants (J), however, the structural elucidation of the molecule was only possible through X-ray diffractometry.

To the best of our knowledge, the new clerodane diterpene reported herein has not been described before in the literature and thus the diterpene isolated from the *C. urucurana* can be considered as previously unpublished.

Conclusions

This study reports the isolation and identification of two clerodane diterpenes **1** and **4** from the stem barks of *C. urucurana*, of which the compound **4**, to the best of our knowledge, has not been previously reported in the literature. The structures of the isolated diterpenes were established by spectroscopic data and the novel clerodane diterpene methyl 3-oxo-12-epibarbascoate (**4**) had its structure confirmed by X-ray crystallography, together with that of compound **1**, which, although a known compound, did not have its structural configuration identified by X-ray crystallographic analysis.

Supplementary Information

Supplementary information associated with this article (Figures S1-S13 and Tables S1-S3) is available free of charge at http://jbcs.sbq.org.br as a PDF file.

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