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CLERODANE DITERPENES OF *CROTON URURUCANA*

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Key Word Index—*Croton ururucana*; Euphorbiaceae; clerodanes; sonderianin; 15,16-epoxy-3,13(16)-clerodatriene-2-one; 12-*epi*-methyl-barbascoate.

Abstract—Sonderianin and two novel furoclerodane diterpenes with a *trans* A/B ring junction were obtained from the barks of *Croton ururucana*. Their structures were elucidated by detailed NMR investigation, and the relative configurations established by NOE experiments. ^{13}C NMR values for the carbonyls of sonderianin were corrected. Furthermore, two catechins were isolated.

INTRODUCTION

Croton ururucana Baill. is a tree growing in Brazil from Bahia to Rio Grande do Sul and Mato Grosso States. The wood is highly appreciate for cheap furniture, building and naval constructions, and the wood for tanning [1]. The blood red latex is locally known as "Sangre de aqua" or "Sangre de drago" (Dragon's blood), and used in popular medicine for wound healing and ulcer of animals.

The vernacular name Dragon's blood is attributed to the sap of many South American Euphorbiaceae, mainly *Croton* species [2–4]. From the phytochemical viewpoint, the genus *Croton* is a rich source of diterpenoids, mainly with a clerodane skeleton [5].

In this paper, we describe the structures of two new clerodanes (**1**, **2**) isolated from the stem barks of *C. ururucana* together with sonderianin (**3**) and other known compounds.

RESULTS AND DISCUSSION

In addition to acetylaleuritolic acid [6] and a mixture of sterols (sitosterol, stigmasterol and campestrol), the chloroform soluble portion of the methanol extract of the stem barks yielded three diterpenes.

Compound **1** was obtained as a colourless laevorotatory oil, whose molecular formula $C_{20}H_{28}O_2$ was deduced by the mass spectrum and NMR data. Its IR spectrum suggested an α,β-unsaturated carbonyl (1670 cm^{-1}) and a furyl (1505 and 870 cm^{-1}). The ^{13}C NMR spectrum revealed twenty carbon signals including characteristic signals due to a β-substituted furan ring and an α,β-unsaturated carbonyl. Moreover, the ^1H NMR spectrum exhibited two singlets and two doublets for four methyl groups. The unambiguous ^1H and ^{13}C assignments of compound **1** are reported in Table 1 as a result of HETCOR, Long Range HETCOR and selective INEPT experiments. In particular, in the Long Range HETCOR spectrum the C-1 showed a cross peak with H-10, and C-2 with H-1. The assignations of the signals for the methylenes in position C-6, C-7 and C-11 came from the selective irradiation (INEPT) of the signals of the neighbouring methyl groups (Me-19, Me-17 and Me-20, respectively). The last methylene (C-12) was assigned by comparison with the data of other clerodanes [7].

The *trans*-A/B ring junction was supported [8] by the ^{13}C NMR chemical shifts of Me-19 (δ 18.3) and Me-20 (δ 19.7), and the relative configuration of the methyls by difference NOE experiments. In particular, on irradiation of the Me-19 signal (δ 1.13) a NOE effect was observed on the Me-20 at δ 0.82 and vice versa; however, the irradiation had no effect on the Me-17 doublet. Thus, the compound was assigned the structure depicted in **1**.

The second compound (**2**) had spectral mass ($[M]^+$ at m/z 358) and NMR data consistent with the molecular formula $C_{21}H_{25}O_5$, indicating nine double-bond equivalents in the molecule. The detailed ^1H and ^{13}C parameters are reported in Table 2. In addition, the IR spectrum showed the presence of two carbonyls. Analysis of the data suggested the presence of a β-substituted furan ring, an α,β-unsaturated methyl ester, a δ-lactone, two methyl groups located at quaternary carbons, five methylenes, three methines and two quaternary carbons. These findings account for six double-bond equivalents and make necessary three further cycles in the compound. Cumulatively, the

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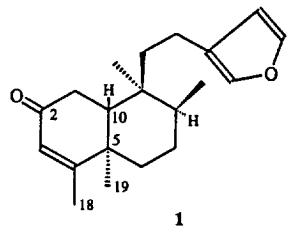

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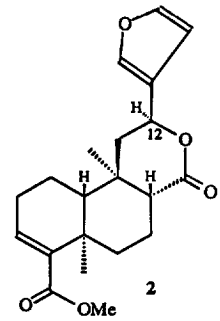

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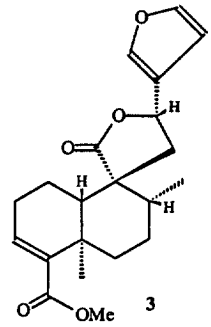

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Table 1. NMR data* of compound **1**

Position	δ_C	δ_H	Long range C-H connectivities	Long range H-C connectivities
1	34.8	2.40 *d* (9.4)	H-10	C-2, C-5, C-10
2	200.3		H-1	
3	125.4	5.73 *q* (1.4)	Me-18	C-1, C-5, C-18
4	172.6		Me-18, Me-19	
5	39.8			
6	35.5	†	Me-19	
7	26.8	†		
8	35.9	†		
9	38.7		Me-17	
10	45.6	1.96 *t* (9.4)	Me-19, Me-20	
11	37.9	†	Me-17	
12	17.9	2.30–2.10 *m*		
13	124.9			
14	110.9	6.23 *dd* (1.0; 1.6)		C-13, C-16, C-15
15	138.4	7.18 *dd* (1.6; 1.0)		C-13, C-16, C-14
16	142.8	7.33 *t* (1.6)		C-14, C-15, C-13
17	15.7	0.86 *d* (6.1)		C-7, C-9, C-8
18	18.9	1.89 *d* (1.4)		C-3, C-5, C-4
19	18.3	1.13 *s*		C-4, C-6, C-10, C-5
20	17.7	0.82 *s*		C-8, C-10, C-11, C-9

* In $CDCl_3$. ^{13}C at 75 MHz, ^1H at 300 MHz. Coupling constants (in Hz) in parentheses.
† 1.60–1.50 overlapped multiplets.

Table 2. NMR data* of compound **2**

Position	δ_C	δ_H	Long range H-C connectivities
1	17.4	1.65 *m*	
2	26.8	2.35 *m*	
3	137.1	6.63 *m*	C-1, C-5, C-18
4	141.2		
5	36.7		
6	34.8	2.55 *m*	
		1.17 *dd* (13.0; 3.3)	
7	18.3	1.75 *m*	
8	51.2	2.16 *m*	
9	37.0		
10	52.5	1.28 *dd* (13.0; 6.0)	
11	44.0	2.40 *d* (6.0)	
		1.60 *m*	
12	71.8	5.53 *dd* (11.0; 6.0)	C-13
13	125.9		
14	108.5	6.42 *br s*	C-16, C-13, C-15
15	143.8	7.42 *t* (1.7)	
16	139.3	7.45 *br s*	
17	172.4		
18	167.3		
19	20.9	1.33 *s*	C-4, C-10, C-5
20	14.5	1.10 *s*	C-8, C-11, C-9
OMe	51.3	3.70 *s*	C-18

* See footnote to Table 1.

data fit well with structure **2** without regard to the stereochemistry. The gross structure **2** was previously assigned to methyl barbascoate isolated from *Croton californicus*, whose structure was determined by X-ray analysis. However, the comparison of the available physical and ^{1}H NMR data [9] revealed that the two compounds were not identical. In particular, in the ^{1}H NMR spectrum methyl barbascoate exhibited the C-12 lactone proton as a triplet ($J = 9$ Hz) at δ 5.3, suggesting a different stereochemistry at that chiral centre. In the difference NOE experiments on **2**, the irradiation of H-12 (δ 5.53) enhanced the signal at δ 1.10 (Me-20); furthermore, on irradiation of Me-20 the NOE effect was observed on both H-12 and Me-19 (δ 1.33). Therefore, compound **2** is 12-*epi*-methyl-barbascoate.

A third diterpene was isolated from the extract. The NMR data were identical to those of sonderianin (**3**) [10], except for the ^{13}C NMR values for C-19 and C-20 (δ 167.5 and 176.7 vs 165.4 and 173.0, respectively). Even if the physical constants were also similar, the difference was initially attributed to an unlikely different stereochemistry. The problem was solved once an authentic sample of sonderianin from *C. sonderianus* was available. In our hands, the specimen exhibited the carbonyl signals at δ 167.5 and 176.7. The identity of the two compounds was confirmed by an undepressed mixed melting point.

Finally, (+)catechin, (+)gallocatechin and sitosterol glucoside were isolated from the acetate soluble portion of the extract (see Experimental).

EXPERIMENTAL

Plant material

Croton ururucana Baillon was collected (May 1994) in Dourados (Mato Grosso do Sul, Brazil) and identified by Dr C. A. Conceição. A voucher specimen is deposited in the Depto. de Biologia (Universidade Federal de Mato Grosso do Sul) under the cifher 5009.

Extraction and separation

The powdered stem barks (3.5 kg) were extracted with cold MeOH ($\times 3$) to give a reddish-brown residue (320 g). Part (32 g) of the new extract was applied to silica gel and eluted with hexane, CH_2Cl_2, EtOAc and MeOH, successively. Repeated column chromatography (silica gel; hexane with increasing amount of EtOAc) of the CH_2Cl_2 portion (2.5 g) gave **1** (120 mg), acetylaleuritolic acid (250 mg), a mixt. (150 mg) of sitosterol, stigmasterol and campesterol, **2** (60 mg) and **3** (60 mg).

Flash chromatography on silica gel (EtOAc) of the EtOAc soluble portion (4 g) yielded (+)catechin (420 mg), (+)gallocatechin (50 mg) and sitosterol glucoside (130 mg).

15,16-*Epoxy*-3,13(16)-*clerodatriene*-2-*one* (**1**). Oil, $C_{20}H_{28}O_2$. $[\alpha]^{20} = -30$ ($c = 1.1$, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1670 (CO), 1505, 870. EI MS, *m/z* (rel. int.): 300 $[M]^+$ (40), 285 (47), 206 (22), 191 (36), 136 (37), 135 (40), 124 (37), 121 (100). NMR data in Table 1.

12-epi-*methyl-barbascoate* (**2**). Mp 115–7°, $[\alpha]^{20} = -53$, ($c = 0.2$, $CHCl_3$). IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1720, 1708 (CO). EI MS, *m/z* (rel. int.): 358 $[M]^+$ (33), 327 (23), 299 (28), 281 (24), 232 (23), 205 (100). NMR data in Table 2.

Identification of the known compounds

Acetylaleuritolic acid [6], sitosterol, stigmasterol, campesterol, sitosterol glucoside, (+)catechin and (+)gallocatechin were identified by NMR data, $[\alpha]$, mmp and co-TLC with authentic specimens previously obtained in our laboratories. Sonderianin (**3**) mp 136–8°, $[\alpha]^{20} = -51$ (lit. [10]; mp 134–7°, $[\alpha]^{20} = -55.2$). IR, MS, NMR as in Ref. [10] except ^{13}C NMR (75 MHz, $CDCl_3$): δ 176.7 (C-20), 167.5 (C-19) (lit. [10] δ 173.0 and 165.4, respectively). The identification was confirmed by co-TLC and mmp with an authentic sample of sonderianin from *Croton sonderianus*.

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REFERENCES

1. Correa, M. P., *Dicionário das Plantas Uteis do Brasil e das Exóticas Cultivadas*, Vol. VI, Impresa Nacional, Río de Janeiro, 1969, p. 360.
2. Hartwell, J. L., *Lloydia*, 1969, **32**, 158.
3. Bettolo, R. Marini and Scarpati, M. L., *Phytochemistry*, 1979, **18**, 520.
4. Cai, Y., Chen, Z. P. and Phillipson, J. D., *Phytochemistry*, 1963, **32**, 755.
5. Merritt, A. T. and Ley, S. V., *Natural Product Reports*, 1992, **9**, 243.
6. McLean, S., Perpick-Dumont, M. and Reynolds, W. F., *Canadian Journal of Chemistry*, 1987, **65**, 2519.
7. Atta-Ur-Rahman and Ahmad, V. U., *^{13}C of Natural Products*, Vol. 2, Plenum Press, New York and London, 1989.
8. Manabe, S. and Nishino, C., *Tetrahedron*, 1986, **42**, 3461.
9. Wilson, S. R., Neubert, L. A., Huffman, J. C., *Journal of the American Chemical Society*, 1976, **98**, 3669.
10. Craveiro, A. A., Silveira, E. R., Braz Filho, R. and Mascarenhas, I. P., *Phytochemistry*, 1981, **20**, 852.