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Pernambucone, a new tropone derivative from *Croton argyroglossum*

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Received April 7, 2007, accepted January 23, 2009

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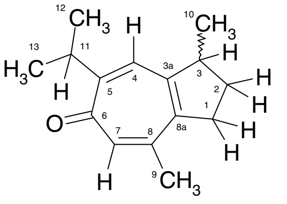
Pharmazie 64: 350–351 (2009)
doi: 10.1691/ph.2009.7592

Two tropone derivatives, orobanone (**1**), previously isolated from *Orobanche rapum-genistae*, and the new natural product pernambucone (3,8-dimethyl-5-isopropyl-2,3-dihydro-1*H*-azulene-1,6-dione, **2**), were isolated from the sterm bark of *Croton argyroglossum*. The structures were elucidated from spectroscopic data.

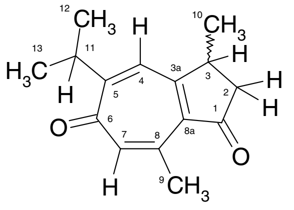
The genus *Croton* (Euphorbiaceae) consists of about 1300 species of trees, shrubs and herbs, growing in tropical and subtropical regions (Webster 1993, 1994a, b; Salatino et al. 2007). *Croton* species are distributed throughout Brazil, in the State of Pernambuco 35 species have been identified. In Brazilian folk medicine numerous *Croton* preparations are used as stimulating tonic, febrifuge, diuretic and purgative agents, anti-inflammatory, anti-hypertensive, analgesic agents and for the treatment of gastric ulcera and cancer (Hartwell 1969; Farnsworth et al. 1969; Kupchan et al. 1976; Bettolo and Scarpati 1979; Guerrero et al. 2001; Randau et al. 2002; Salatino et al. 2007). The secondary metabolites isolated from this genus are mainly alkaloids and terpenoids (essential oils and diterpenoids) (Farnsworth et al. 1969; Seigler 1994; Eisenreich et al. 2003; Bracher et al. 2004; Salatino et al. 2007). Some time ago we isolated some new nitrogen-free natural products, the phenanthrene crotoflavol from *Croton flavens* (Eisenreich et al. 2001), and the monocyclic tropone crototropone from *Croton zehntneri* (Bracher et al. 2008).

Croton argyroglossum Baill., commonly known as "Marmeleiro" and "Sacatinga", is a shrub growing in northeastern Brazil. Until now no investigations on the chemical composition of *C. argyroglossum* have been published.

In continuation of our research on secondary metabolites from *Croton* species, we describe here the isolation and structural elucidation of two tropone derivatives from the stem bark of *C. argyroglossum*. The first of them, was identified as the known tropone orobanone (**1**) (Fruchier et al. 1981). In addition, we found a second tropone derivative **2**, named pernambucone.



1



2

Separation of the defatted MeOH extract of the bark of *Croton argyroglossum* by column chromatography on silica, followed by preparative TLC gave two oily products.

Analysis of the HR-MS data gave the molecular formulas $C_{15}H_{20}O$ and $C_{15}H_{18}O_2$ for the two compounds, thus suggesting that both compounds might be sesquiterpenes. The UV maxima at 236/249 nm and 327/297 nm are in good accordance with values published for tropone derivatives (Hosoya et al. 1962). Based on these preliminary informa-

Table 1: ^{1}H-NMR and ^{13}C-NMR data of orobanone (1) and pernambucone (2)

	^{1}H NMR		^{13}C NMR	
	1	**2**	**1**	**2**
1	2.82 (m) 2.72 (m)	–	33.52	205.40
2	2.14 (dddd, 6.0/8.3/8.9/12.8 Hz) 1.52 (dddd, 6.0/6.0/8.8/12.8 Hz)	2.17 (dd, 2.3/19.0 Hz) 2.76 (dd, 7.3/19.0 Hz)	29.71	42.90
3	3.12 (m)	3.15 (ddq, 2.3/7.0/7.3 Hz)	43.88	35.33
3a	–	–	148.79*	168.48
4	7.03 (s)	7.09 (s)	128.78	127.82
5	–	–	157.20	163.26
6	–	–	184.40	185.25
7	6.93 (br s)	6.80 (br s)	137.87	137.98
8	–	–	143.68*	142.25
8a	–	–	144.59	135.43
9	2.15 (d, 1.0 Hz))	2.47 (d, 1.0 Hz)	24.04*	22.53
10	1.17 (d, 7.0 Hz))	1.29 (d, 7.0 Hz)	19.45*	20.60
11	3.40 (sept, 6.9 Hz)	3.40 (sept, 6.8 Hz)	28.83	29.75
12/13	1.11 (d, 6.9 Hz) 1.09 (d, 6.9 Hz)	1.17 (d, 6.8 Hz) 1.13 (d, 6.8 Hz)	21.55 21.54	21.38 21.42

* correlations corrected on the basis of HMBC and HMQC experiments

Table 2: HMBC data of compounds 1 and 2

	HMBC	
	1	**2**
C-1	H-2a/b	H-2a/b
C-2	H-1a/b, H-10	H-10
C-3	H-4, H 2a/b, H1a/b, H-10	H-4, H-2a/b, H-10
C-3a	H-3, H-2a/b, H-1a/b, H-10	H-3, H-2a/b, H-10
C-4	H-11	H-11
C-5	H-4, H-7, H-11, H-12/13	H-4, H-7, H-11, H-12/13
C-6	H-4, H-11	H-4, H-11
C-7	H-9	H-9
C-8	H-9	H-9
C-8a	H-4, H-7, H-3, H-1a/b	H-4, H-7, H-3, H-9
C-9	H-7	H-7
C-10	H-3, H-2a/b	H-3, H-2a/b
C-11	H-4, H-12/13	H-4, H-12/13
C-12/C-13	H-11, H-13/H-12	H-11, H-13/H-12

tions, and the NMR data of compound **1**, a literature search revealed that **1** is identical to the tropone orobanone (Figure 1), previously isolated from *Orobanche rapum-genistae* (Orobranchaceae) (Fruchier et al. 1981). The NMR data were in good accordance with those published before, but some ^{13}C correlations could be revised on the basis of our HMBC and HMQC experiments (Table 1).

Detailed analysis of 2D ^{1}H and ^{13}C NMR spectra of the second product, including DEPT, COSY, HMQC and HMBC experiments, confirmed that this natural product is closely related to orobanone (**1**), and allowed to determine the locations of the substituents.

In contrast to **1**, the second metabolite pernambucone (**2**) has two carbonyl groups, as can be seen from carbonyl absorptions in the IR spectrum at 1710 and 1604 cm^{-1}, and ^{13}C absorptions at 185.25 (tropone) and 205.40 ppm. Taking into account the differences in the molecular compositions from the HR-MS data (see above), and the NMR data (five quaternary carbons, four secondary carbons, two methylene groups, and four methyl groups for **1**; six quaternary carbons, four secondary carbons, only one methylene group, and four methyl groups for **2**) it was evident, that **2** should be an analogue of orobanone (**1**) in which one methylene group is replaced by a carbonyl group.

The HMBC experiments clearly revealed that the additional carbonyl group must be located at position 1. The most important informations were that the methyl carbon C-10 still shows a HMBC coupling with the protons at C-2, and in the ^{1}H NMR spectrum H-3 gives a complex coupling (ddq) which can only be explained by the fact that there is still a neighboring methylene group at 2-position. The complete set of HMBC data, which allows unambiguous correlation of all NMR resonances, is presented in Table 2.

The absolute configurations of both **1** and **2** could not yet be determined.

Experimental

1. General

Flash column chromatography (FCC) was performed on Kieselgel 60 (Merck), preparative TLC (PTLC) on silica gel 60F-254 plates (0.5 mm) (Merck). NMR spectra: JEOL GSX-400 and GSX-500 spectrometer, solvent: $CDCl_3$, with TMS as internal standard. Mass spectra (EI, 70 eV): Hewlett Packard 5989A. HR-MS: Finnigan MAT 95 Q spectrometer. IR spectra: Jasco FT-IR 410. UV spectra: Jasco V-530 spectrometer. Optical rotations: Perkin Elmer 241 Polarimeter. HPLC: Merck Lachrom L 7100 pump, L 7455 UV/VIS diode array detector, LiChrospher 100 RP-18 column.

2. Plant material

The stem bark of *Croton argyroglossum* Baill. was collected in Buíque, Pernambuco, Brazil, in November 2002. Botanical identification was confirmed by Dr. Grady Webster and a voucher specimen has been deposited under number 39941 in the Herbarium Vasconcelos Sobrinho (PEUFR) of University Federal Rural de Pernambuco.

3. Extraction and isolation

The dried powdered plant material (1000 g) was exhaustively extracted with MeOH (4 l) at room temperature for 24 h. The extract was evaporated in vacuo to obtain a brown residue (40.7 g). Part of the residue (18.9 g) was thoroughly defatted with hexane (300 ml), and then extracted with EtOAc (200 ml). The EtOAc extract (10 g) was fractionated by FCC on silica gel by elution with hexane and EtOAc (increasing polarity). The fraction eluted with hexane : EtOAc (9 : 1) gave a crude mixture of compounds (483 mg). Further purification of this fraction was performed by PTLC with hexane : EtOAc (4 : 1) to afford, after extraction of the corresponding zone with dichloromethane, 273 mg of a mixture of two compounds. These were separated by a second PTLC with dichloromethane : MeOH (39 : 1) to give orobanone (**1**) (135 mg), and pernambucone (**2**) (22 mg), both as viscous, pale yellow oils.

4. Isolated natural products

4.1. Orobanone (3,8-dimethyl-5-isopropyl-1,2-dihydro-1H-azulen-6-one) (**1**)

$[\alpha]_D^{20}$: −5.21° (c = 0.15, MeOH); UV (EtOH): λ_{max} (log ε) nm: 236 (4.02); 327 (3.53); 397 (1.90); IR (NaCl) ν_{max} cm^{-1}: 2963, 2931, 2872, 2359, 1708, 1111; HR-MS m/z: 216.1514 (calculated for $C_{15}H_{20}O$ 216.1514); purity: 96% (HPLC); NMR data: see Tables 1 and 2.

4.2. Pernambucone (3,8-dimethyl-5-isopropyl-2,3-dihydro-1H-azulene-1,6-dione) (**2**)

$[\alpha]_D^{20}$ = +14° (c = 0.05, MeOH); UV (MeOH): λ_{max} (log ε) nm: 207 (3.97), 249 (4.18), 297 (3.64), 395 (3.20); IR (NaCl) ν_{max} cm^{-1}: 2964, 2957, 1738, 1710, 1604, 1373, 1242, 1045, 731; HR-MS m/z: 230.1311 (calculated for $C_{15}H_{18}O_2$ 230.1307); purity: 95% (HPLC); NMR data: see Tables 1 and 2.

Acknowledgements: This project was supported by a grant from Deutscher Akademischer Austauschdienst for K.P.R. The authors are grateful to Dr. Grady Webster, University of Davis, California for the botanical identification.

References

Bracher F, Eisenreich WJ, Mühlbacher J, Dreyer M, Bringmann G (2004) Saludimerines A and B, novel-type dimeric alkaloids with stereogenic centers and configurationally semistable biaryl axes. J Org Chem 69: 8602–8608.

Bracher F, Randau KP, Lerche H (2008) Crototropone, a new tropone derivative from Croton zehntneri. Fitoterapia 79: 236-237.

Eisenreich WJ, Bracher F (2001) Crotoflavol, a new phenanthrene from *Croton flavens*. Nat Prod Lett 15: 147–150.

Eisenreich WJ, Höfner G, Bracher F (2003) Alkaloids from *Croton flavens* L. and their affinities to GABA-receptors. Nat Prod Res 17: 437–440.

Farnsworth NR, Blomster RN, Messmer WM (1969) Phytochemical and biological review of the genus *Croton*. Lloydia 32: 1–28.

Fruchier A, Rascol JP, Andary C, Privat GA (1981) A tropone derivative from *Orobanche rapum-genistae*. Phytochemistry 20: 777–779.

Guerrero MF, Carrón R, Martín ML, San Román L, Reguero MT (2001) Antihypertensive and vasorelaxant effects of aqueous extract from *Croton schiedeanus* Schlecht in rats. J Ethnopharmacol 75: 33–36.

Hartwell JL (1969) Plants used against cancer. Lloydia 32: 153–205.

Hosoya H, Tanaka J, Nagakura S (1962) Ultra-violet absorption spectra of tropone, troponium ion, tropolone and 2,4,6-octatrienal. Tetrahedron 18: 859–874.

Kupchan SM, Branfman UAR, Dailey Jr. RG, Yu Fei B. (1976) Antileukemic principles isolated from Euphorbiaceae plants. Science 191: 571–572.

Marini Bettolo R, Scarpati ML (1979) Alkaloids of *Croton draconoides*. Phytochemistry 18: 520.

Randau KP, Xavier HS, Dimech GS, Wanderley AG (2002) Preliminary evaluation of pharmacological activity (antispasmodic and antiulcerogenic) of raw aqueous of *Croton rhamnifolius* H.B.K. and *Croton rhamnifolioides* Pax & Hoffm. (Euphorbiaceae). Lecta 20: 61–68.

Salatino A, Salatino MLF, Negri G (2007) Traditional uses, chemistry and pharmacology of Croton species (Euphorbiaceae). J Braz Chem Soc 18: 11–33.

Seigler DS (1994) Phytochemistry and systematics of the Euphorbiaceae. Annals Missouri Botanical Garden 81: 380–401.

Webster GL (1993) A provisional synopsis of the section of the genus *Croton* (Euphorbiaceae). Taxon 42: 793–823

Webster GL (1994a) Classification of the Euphorbiaceae. Annals Missouri Botanical Garden 81: 3–32.

Webster GL (1994b) Synopsis of the genera and suprageneric taxa of Euphorbiaceae. Annals Missouri Botanical Garden 81: 33–144.