Crotofolane- and Casbane-Type Diterpenes from *Croton argyrophyllus*

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Phytochemical analysis of *Croton argyrophyllus* led to the isolation of five new diterpenes named (5*β*,6*β*)-5,6 : 13,16-diepoxycrotofola-4(9),10(18),13,15-tetraen-1-one (**1**), (5*β*,6*β*)-5,6 : 13,16-diepoxy-2-epicrotofola-4(9),10(18),13,15-tetraen-1-one (**2**), (5*β*,6*β*)-5,6 : 13,16-diepoxy-16-hydroxy-2-epicrotofola-4(9),10(18),13,15-tetraen-1-one (**3**), (5*β*,6*β*)-5,6 : 13,16-diepoxy-16-hydroxy-2-epicrotofola-4(9),10(18),13,15-tetraen-1-one (**4**) and (2*E*,5*β*,6*E*,12*E*)-5-hydroxycasba-2,6,12-trien-4-one (**5**), in addition to the known diterpenes crotonepetin and depressin, and acetylaleuritolic acid and spinasterol. The structures of the isolated compounds were established by a combination of spectroscopic methods, including HR-ESI-MS, 2D-NMR, and X-ray crystallography.

Introduction. – The *Croton* genus (Euphorbiaceae) is considered as a prolific source of diterpenoids of several different skeletal types from which the crotofolane- and casbane-types are considered the rarest. Up to now, the occurrence of crotofolane diterpenoids in higher plants is restricted to five species of *Croton* native to Central America, Africa, and Asia, and their presence as potential chemomarkers of the genus has already been discussed [1 – 5]. Although more abundant than crotofolanes, casbanes have been so far only described as a small group of *cis*- or *trans*-fused bicyclic compounds isolated from few species of the same genera within the Euphorbiaceae family. More recently, they were reported to occur also in soft corals of the genus *Sinularia* [6 – 11].

During the course of our continuing study of plants searching for new bioactive diterpenoids, we here present the phytochemical investigation of *Croton argyrophyllus*, a shrub growing predominantly in the northeast of Brazil, where it is popularly known as 'alecrim-de-vaqueiro' [12]. A previous chemical investigation of the stems of *C. argyrophyllus* led to the isolation of casbane diterpenes [13]. The phytochemical analysis was now extended to the roots leading to the isolation and structure elucidation of new crotofolane and casbane diterpenes named (5*β*,6*β*)-5,6 : 13,16-diepoxycrotofola-4(9),10(18),13,15-tetraen-1-one[2]) (**1**), (5*β*,6*β*)-5,6 : 13,16-diepoxy-2-epicrotofola-4(9),10(18),13,15-tetraen-1-one[2]) (**2**), (5*β*,6*β*)-5,6 : 13,16-diepoxy-16-hydroxycrotofola-4(9),10(18),13,15-tetraen-1-one[2]) (**3**), (5*β*,6*β*)-5,6 : 13,16-diepoxy-16-

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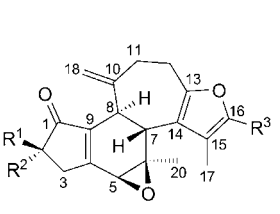
[2]) Trivial or arbitrary atom numbering; for systematic names, see *Exper. Part.*

hydroxy-2-epicrotofola-4(9),10(18),13,15-tetraen-1-one[2]) (**4**), and (2*E*,5*β*,6*E*,12*E*)-5-hydroxycasba-2,6,12-trien-4-one[2]) (**5**) (*Fig. 1*), in addition to crotonepetin [7], depressin [12], acetylaleuritolic acid [14], and spinasterol.



	R^1	R^2	R^3
1	Me	H	H
2	H	Me	H
3	Me	H	OH
4	H	Me	OH

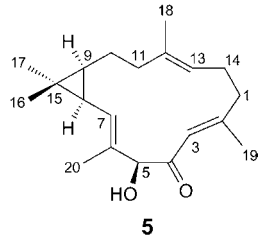


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Fig. 1. *Compounds* **1**–**5**[2])*, isolated from* Croton argyrophyllus

Results and Discussion. – Compound **1** was isolated as optically active small colorless crystals. Its molecular formula was established by HR-ESI-MS (*m*/*z* 311.1621 ($[M+H]^+$, $C_{20}H_{23}O_3^+$). Inspection of the ^{1}H-NMR spectrum (*Table 1*) suggested a diterpene scaffold for **1** by the signals of three Me groups at δ(H) 1.14 (*d*, $J=6.5$ Hz, Me(19)), 1.21 (*s*, Me(20)), and 1.96 (*d*, $J=1.3$ Hz, Me(17)), in addition to the signals due to an exocyclic CH_2 group at δ(H) 4.40 and 4.68 (2*d*, $J=1.3$ Hz, CH_2(18)). Moreover, the deshielded resonance at δ(H) 7.14 (*s*, H–C(16)) was assignable to an olefinic H-atom of an *α*,*β*,*β*′-trisubstituted furan moiety, and the signal at δ(H) 3.23 (*s*, H–C(5)) was related to an epoxymethine H-atom. Combined analysis of the ^{13}C-NMR and DEPT spectra (*Table 1*) showed 20 C-atom resonances corresponding to three Me, three CH_2, one CH_2=, and five CH groups, and eight quaternary C-atoms. A detailed analysis of 2D-NMR experiments allowed the unequivocal assignments of all H- and C-atoms and established connectivities within the molecule. Further evidences of a diterpene-type skeleton containing a furan ring were possible by the observed long-range correlations in the HMBC spectrum, that showed the cross-peaks δ(H) 7.14 (H–C(16)) δ(C) 118.1 (C(14)), 122.5 (C(15)), 151.8 (C(13)), and 9.0 (Me(17)). In addition, the correlations of the CH group at δ(H) 2.39 (H–C(2)) with δ(C) 17.5 (Me(19)), 146.7 (C(9)), 167.5 (C(4)), and 207.4 (C(1)) supported the presence of an *α*,*β*-conjugated cyclopentenone moiety, and the correlations δ(H) 3.23 (H–C(5)) δ(C) 20.0 (Me(20)), 60.6 (C(6)), 146.7 (C(9)), and 167.5 (C(4)), revealed the position of the oxirane ring at C(5) and C(6). The analysis of the HMBC spectrum also led to the location of the exocyclic C=C bond at C(10) due to the observed cross-peaks between both diastereotopic H-atoms at δ(H) 4.40 (H_b–C(18)) and 4.68 (H_a–C(18) with the C-atoms at δ (C) 37.2 (C(11)), 37.9 (C(8)), and 146.2 (C(10)). On the basis of the ten degrees of unsaturation, deduced from the molecular composition $C_{20}H_{22}O_3$, a crotofolane-type structure was suggested for **1**. To determine the relative configurations, compound **1** was submitted to single-crystal X-ray crystallography. This analysis showed that the epoxy moiety between C(5) and C(6), the Me(19) group, and C(7) are

on same face of the molecule, while Me(20) and the methine H–C(8) are on the opposite face (*Fig. 2*). Thus, the above observations led to the deduction of compound **1** as being 5*β*,6*β*-5,6 : 13,16-diepoxycrotofola-4(9),10(18),13,15-tetraen-1-one[2]), a novel irregular diterpenoid.

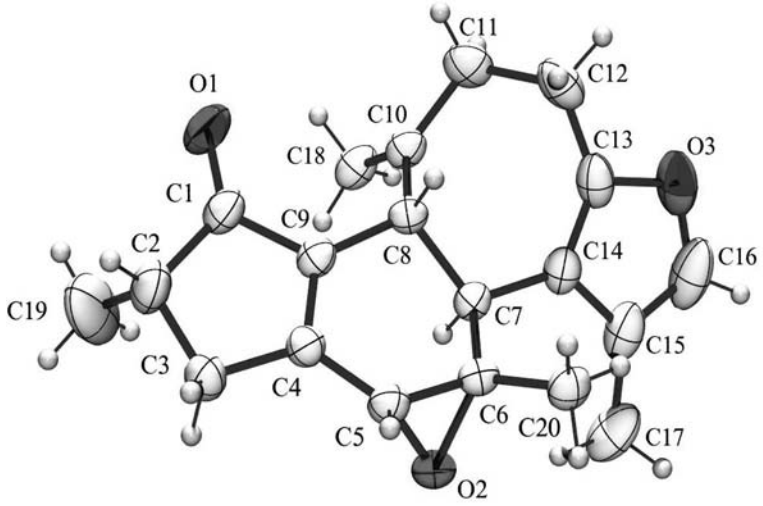

Fig. 2. *ORTEP-3 Drawing showing the relative configuration of* **1**[2])

Compound **2** was isolated as an optically active colorless resin which showed a molecular-ion peak at *m*/*z* 311.1630 ($[M+H]^+$, $C_{20}H_{23}O_3^+$) in the HR-ESI-MS. The ^{1}H-NMR spectrum (*Table 1*) was very similar to that of **1**, the slight differences being the deshielded shift of H–C(2) at δ(H) 2.37 – 2.40 in **1**, compared to δ(H) 2.54 – 2.56 in **2**. Also the ^{13}C-NMR spectrum fo **2** (*Table 1*) closely resembled that of **1**, except by for chemical shift of the C-atom at δ(C) 15.4 (Me(19)), that was shielded in comparison with the same C-atom of **1** at δ(C) 17.5, suggesting that the metabolites **1** and **2** are C(2) epimers. The NOE cross-peaks observed in the NOESY experiment were in agreement with this proposal by the diagnostic correlations H–C(5)/Me(19), H_b–C(3), and H–C(8), besides the correlations Me(19)/Me(20), and H–C(2)/H–C(7). Thus, compound **2** was identified as the new (5*β*,6*β*)-5,6 : 13,16-diepoxy-2-epicrotofola-4(9),10(18),13,15-tetraen-1-one[2]).

Compound **3** was isolated as an optically active yellow resin which showed the molecular-ion peak in the HR-ESI-MS at *m*/*z* 365.1630 ($[M+K]^+$, $C_{20}H_{22}KO_4^+$). A major support for **3** being a crotofolane diterpene came from a comparative analysis of its ^{1}H-NMR data (*Table 1*) with those of **1** and **2**, which revealed the same signals related to the exocyclic C=C bond at δ(H) 4.91 and 4.45 (2*s*, CH_2(18)), the oxymethine group at δ(H) 3.31 (*s*, H–C(5)), and three Me groups at δ(H) 1.17 (*d*, $J = 8.0$ Hz, Me(19)), 1.30 (*s*, Me(20)), and 1.86 (*d*, $J = 1.0$ Hz, Me(17)). However, the signal of H–C(16) at δ(H) 7.14 observed for **1** and **2** was absent in **3**. In addition, the ^{13}C-NMR spectrum of **3** (*Table 1*) presented a deshielded signal attributable to an oxygenated sp^2 C-atom at δ(C) 173.0, instead of the CH(16) group of both **1** and **2**. These findings

Table 1. *^{1}H- and ^{13}C-NMR Data* (500 and 125 MHz) *of Compounds* **1**–**4**[2]). δ in ppm, J in Hz.

Position	**1**[a])		**2**[b])		**3**[a])		**4**[b])	
	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)	δ(H)	δ(C)
C(1)	–	207.4	–	207.1	–	210.9	–	210.57
H–C(2)	2.37–2.40 (*m*)	41.4	2.54–2.56 (*m*)	41.5	2.46–2.48 (*m*)	42.1	2.60–2.62 (*m*)	42.51
CH_2(3)	3.02 (*ddd*, *J* = 3.5, 6.5, 17.5), 2.32 (*ddd*, *J* = 1.7, 3.5, 17.5)	36.8	3.03 (*ddd*, *J* = 3.5, 6.5, 17.5), 2.38 (*ddd*, *J* = 1.7, 3.5, 17.5)	37.2	3.05 (*ddd*, *J* = 3.5, 6.5, 17.5), 2.38 (*ddd*, *J* = 1.7, 3.5, 17.5)	37.4	3.08 (*ddd*, *J* = 3.5, 8.5, 18.5), 2.38 (*td*, *J* = 3.5, 18.5)	37.70
C(4)	–	167.5	–	166.3	–	169.3	–	168.21
H–C(5)	3.23 (*s*)	54.8	3.23 (*s*)	54.8	3.31 (*s*)	55.9	3.31 (*s*)	55.77
C(6)	–	60.6	–	60.7	–	61.2	–	61.19
H–C(7)	2.48 (*dd*, *J* = 3.5, 11.5)	53.1	2.53–2.55 (*m*)	52.9	2.75 (*dd*, *J* = 1.3, 12.0)	54.5	2.76 (*d*, *J* = 11.5)	54.33
H–C(8)	3.63 (*td*, *J* = 1.3, 11.5)	37.9	3.63 (*dt*, *J* = 3.5, 11.5)	38.1	3.59 (*td*, *J* = 3.5, 12.0)	41.3	3.57 (*td*, *J* = 11.5)	41.61
C(9)	–	146.7	–	147.7	–	145.8	–	146.78
C(10)	–	146.2	–	146.5	–	148.5	–	148.70
CH_2(11)	2.69–2.72, 2.55–2.57 (2 *m*)	37.2	2.70–2.73, 2.53–2.56 (*m*)	37.2	2.49–2.52, 2.37–2.398 (2*m*)	36.0	2.49 (*ddd*, *J* = 2.5, 5.0, 13.0), 2.37 (*dt*, *J* = 5.0, 13.0)	36.00
CH_2(12)	3.20–3.22, 2.70–2.73 (2 *m*)	23.1	3.20–3.22, 2.71–2.74 (2*m*)	23.2	2.52–2.56 (*ddd*, *J* = 3.0, 4.5, 13.5), 1.48 (*dt*, *J* = 8.5, 13.5)	42.9	2.63–2.66 (*m*), 1.42 (*dt*, *J* = 5.0, 14.0)	42.89
C(13)	–	151.8	–	151.8	–	161.6	–	161.63
C(14)	–	118.1	–	118.1	–	110.1	–	110.11
C(15)	–	122.5	–	122.5	–	130.9	–	130.85
H–C(16) or C(16)	7.14 (*s*)	137.8	7.14 (*s*)	137.8	–	173.0	–	173.03
Me(17)	1.96 (*d*, *J* = 1.3)	9.0	1.97 (*d*, *J* = 1.3)	9.0	1.86 (*d*, *J* = 1.0)	10.0	1.86 (*s*)	10.08
CH_2(18)	4.68, 4.40 (2*d*, *J* = 1.3)	111.7	4.69, 4.46 (2*d*, *J* = 1.3)	111.7	4.91, 4.45 (2*s*)	113.0	4.91, 4.52 (2*s*,)	113.08
Me(19)	1.14 (*d*, *J* = 6.5)	17.5	1.15 (*d*, *J* = 6.5)	15.4	1.17 (*d*, *J* = 8.0)	17.6	1.18 (*d*, *J* = 7.5)	15.45
Me(20)	1.21 (*s*)	20.0	1.22 (*s*)	20.0	1.30 (*s*)	20.3	1.33 (*s*)	20.38

[a]) In CD_3OD. [b]) In $(CD_3)_2CO$.

revealed a fully substituted furan moiety bearing an additional OH group at C(16) in the case of **3**. Furthermore, the selective NOE NMR experiment provided clues for the proposed relative configuration of **3**. Irradiation of the H-atom at δ(H) 3.31 (H–C(5)) resulted in the selective enhancement of the signals at δ(H) 2.37–2.398 (H_b–C(11)) and 1.30 (Me(20)). On the other hand, the irradiation of the H-atom at δ(H) 3.59 (H–C(8)) showed enhancement of the signals at δ(H) 1.30 (Me(20)), 2.37–2.398 (H_b–C(11)), 2.46–2.48 (H–C(2)), and 4.45 (H_b–C(18)). These informations led to the identification of compound **3** as (5β,6β)-5,6:13,16-diepoxy-16-hydroxycrotofola-4(9),10(18),13,15-tetraen-1-one[2]).

Compound **4** was isolated as an optically active dark brown resin which showed a molecular-ion peak at *m*/*z* 365.1155 ($[M+K]^+$, $C_{20}H_{22}KO_4^+$) in the HR-ESI-MS. Its ^{1}H- and ^{13}C-NMR data (*Table 1*) closely resembled those of **3**. The most remarkable difference in the ^{1}H-NMR spectrum was the deshielding of H–C(2) from δ(H) 2.46–2.48 in **3** to δ(H) 2.60–2.62 in **4**. The chemical-shift value attributable to Me(19) at δ(C) 15.45 was shielded when compared with that of Me(19) of **3** δ(C) 17.6. These observations led to the conclusion that compounds **3** and **4** are indeed C(2) epimers, as observed before in the case of the pair **1** and **2** (*Table 1*). This suggestion was confirmed by selective NOE irradiation of Me(19) group at δ(H) 1.18 that resulted in enhancement of the signals at δ(H) 4.52 (H_b–C(18)) and 2.38 (H_b–C(3)). On the other hand, the irradiation of the H-atom at δ(H) 3.57 (H–C(8)) enhanced the signals at δ(H) 4.52 (H_b–C(18)), 2.38 (H_b–C(3)), and 1.33 (Me(20)), and the irradiation of the H-atom at δ(H) 3.31 (H–C(5)) increased the signal at δ(H) 1.33 (Me(20)). Therefore, the structure of **4** was determined to be (5β,6β)-5,6:13,16-diepoxy-16-hydroxy-2-*epi*-crotofola-4(9),10(18),13,15-tetraen-1-one[2]).

Compound **5** was isolated as an optically active colorless oil. Its molecular formula was deduced by the HR-EI-MS (*m*/*z* 325.2122 ($[M+Na]^+$, $C_{20}H_{30}NaO_2^+$), indicating six degrees of unsaturation. Its ^{1}H-NMR spectrum (*Table 2*) displayed five distinct Me *s* at δ(H) 1.07 (Me(16)), 1.08 (Me(17)), 1.54 (Me(20)), 1.58 (Me(18)), and 2.20 (Me(19)), which were assigned to two Me groups at a quaternary C-atom and three olefinic Me groups, respectively. Moreover, signals of three olefinic H-atoms at δ(H) 5.95 (*s*, H–C(3)), 5.42 (*d*, J = 7.7 Hz, H–C(7)), and 4.92 (*t*, J = 6.8 Hz, H–C(13)), and an oxymethine group at δ(H) 4.50 (*s*, H–C(5)) were observed. Scalar ^{1}H,^{1}H correlations from the COSY experiment allowed the identification of the allylic coupling of the olefinic Me group at δ(H) 1.58 (Me(18)) with the olefinic H-atom at δ(H) 4.92 (H–C(13)), that also coupled with the diastereotopic CH_2 H-atoms at δ(H) 2.23 (H_a–C(14)) and 2.04 (H_b–C(14)), the latter showing cross-peaks with two other diastereotopic H-atoms at δ(H) 2.32 (H_a–C(1)) and 2.04 (H_b–C(1)). Further ^{1}H,^{1}H-COSY cross-peaks were observed at δ(H) 1.29 (H–C(8)) δ(H) 5.42 (H–C(7)) and δ(H) 0.74 (H–C(9)), and δ(H) 0.74 (H–C(9))/δ(H) 1.84 (H_a–C(10)) and 0.81 (H_b–C(10)); the latter diastereotopic CH_2 H-atoms were further coupled with another similar CH_2 group at δ(H) 2.20 (H_a–C(11)) and 1.82 (H_b–C(11)). Chemical shifts and comparative analysis of broad-band-decoupled (BB) and DEPT ^{13}C-NMR spectra (*Table 2*) revealed 20 lines in agreement with the suggested molecular formula of **5**. Among these, three trisubstituted C=C bonds were evident by the presence of six sp^2 C-atom signals, *i.e.*, three methine groups at δ(C) 129.8 (C(7)), 122.5 (C(13)), 120.8 (C(3)) and three quaternary C-atoms at δ(C) 161.6 (C(2)), 137.6 (C(12)), 135.1 (C(6)),

in addition to one C=O group at δ(C) 199.3 (C(4)). The two remaining degrees of unsaturation were ascribed to two rings, and revealed compound **5** as a bicyclic diterpene. The unambiguous assignment of all C- and H-atoms was possible by analysis of the HMQC spectrum (*Table 2*). The typical ^{13}C-NMR chemical shifts of the CH groups at δ(C) 25.6 (C(8)) and 32.2 (C(9)) correlated in the HSQC spectrum with the H-atoms at δ(H) 1.29 (H–C(8)) and 0.74 (H–C(9)), respectively; together with the two geminal Me groups at δ(C) 15.9 (Me(16)) and 28.7 (Me(17)), this established the presence of a disubstituted cyclopropane moiety bearing the geminal Me groups [7]. These informations suggested the structure of a diterpene containing a casbane-type 14-membered macrocyclic skeleton with a fused cyclopropane ring. The *cis* junction of the cyclopropane ring at C(8) and C(9) was deduced from the chemical shifts of the geminal Me groups at δ(C) 15.9 (Me(16)) and 28.7 (Me(17)), that are in accordance with those reported for *cis*-fused cyclopropane moieties of casbane-type diterpenoids [11]. Further evidences of a casbane-type skeleton were provided by the long-range correlations in the HMBC spectrum. The HMBCs δ(H) 4.50 (H–C(5)) δ(C) 199.3 (C(4)), 135.1 (C(6)), 129.8 (C(7)), and 120.8 (C(3)), and δ(H) 5.95 (H–C(3)) δ(C) 199.3 (C(4)), 161.6 (C(2)), 83.9 (C(5)), 42.1 (C(1)), and 19.9 (Me(19)) determined the exact location of the OH and keto groups at C(5) and C(4), respectively. The relative configuration of **5** was established with the aid of molecular models and by the NOESY experiment. The key NOE cross-peaks observed between H–C(3), H–C(7), and H–C(13) indicated that they were oriented in the same direction, and determined (*E*) configuration of the C=C bonds at C(2), C(6), and C(12). In addition, the β-orientation of OH–C(5) was determined by the NOE H–C(5) H–C(3) and H–C(7) (*Fig. 3*). From the foregoing evidence, compound **5** was identified as (2*E*,5β,6*E*,12*E*)-5-hydroxy-casba-2,6,12-trien-4-one[2]). It may be noted that the casbane derivative **5** contains a

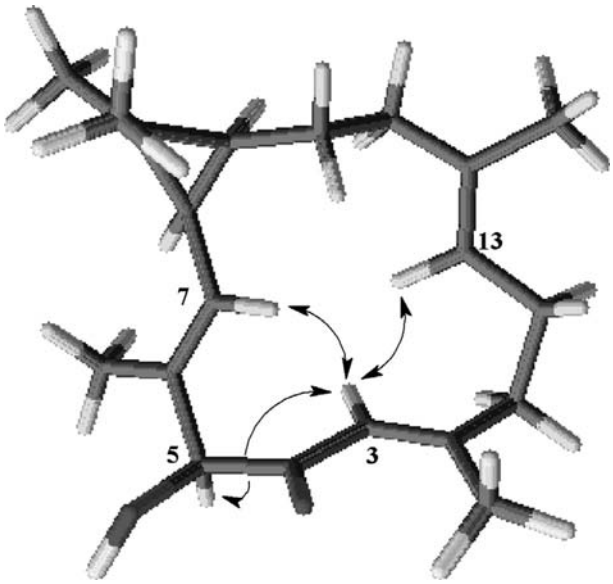

Fig. 3. *Key NOE correlations observed for compound* **5**[2])

Table 2. *^{1}H- and ^{13}C-NMR Data* (500 and 125 MHz, resp.; $CDCl_3$) of *compound* **5**. δ in ppm, J in Hz.

Position	δ(H)	δ(C)	HMBC	
			^2J(C,H)	^3J(C,H)
CH_2(1)	2.32, 2.04 (2*m*)	42.1	–	H–C(3), Me(19)
C(2)	–	161.6	H–C(3), H–C(1), Me(19)	–
H–C(3)	5.95 (*s*)	120.8	–	Me(19), H–C(5)
C(4)	–	199.3	H–C(5), H–C(3)	–
H–C(5)	4.50 (*s*)	83.9	–	H–C(3), Me(20)
C(6)	–	135.1	H–C(5), Me(20)	H–C(8)
H–C(7)	5.42 (*d*, J = 7.7)	129.8	–	H–C(5), Me(20)
H–C(8)	1.29 (*m*)	25.6	H–C(7)	Me(16), Me(17)
H–C(9)	0.74 (*m*)	32.2	H–C(8), H_b–C(10)	CH_2(11), Me(16), Me(17)
CH_2(10)	1.84, 0.81 (2*m*)	25.0	CH_2(11)	–
CH_2(11)	2.20, 1.82 (2*m*)	40.8	–	H–C(13), Me(18)
C(12)	–	137.6	CH_2(11), Me(18)	CH_2(10), CH_2(14)
H–C(13)	4.92 (*t*, J = 6.8)	122.5	–	Me(18), H–C(1)
CH_2(14)	2.23, 2.04 (2*m*)	24.1	CH_2(11)	–
C(15)	–	20.3	Me(16), Me(17)	H–C(7)
Me(16)	1.07 (*s*)	15.9	–	Me(17)
Me(17)	1.08 (*s*)	28.7	–	Me(16)
Me(18)	1.58 (*s*)	15.7	–	H–C(13)
Me(19)	2.20 (*s*)	19.9	–	H–C(3)
Me(20)	1.54 (*s*)	11.4	–	H–C(7), H–C(5)

regular diterpene skeleton from a biogenentic point of view, while compounds **1**–**4** are all endowed with an irregular skeleton.

The isolated diterpenes **1**–**5** had their cytotoxicity tested with five tumor cell lines: two human leukemias (HL-60 and CEM), the human-breast adenocarcinoma (MCF-7), the human-colon adenocarcinoma (HCT-8), and the murine melanoma (B16), but none showed any cytotoxic activity.

Physical and spectroscopic-data comparison of the additionally isolated compounds with those from literature allowed to identify then as crotonepetin [7], depressin [11], acetylaleuritolic acid [14], and spinasterol.

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Experimental Part

General. Column chromatography (CC): *Merck* silica gel *60* (70–230 mesh; *EMD Millipore*) or *Sephadex LH-20* (*Amersham Pharmacia Biotech*, Uppsala, Sweden). TLC: *Merck* aluminium sheets. HPLC: *Shimadzu* chromatographer with a ternary pump (*Shimadzu LC-20AT*) and UV detector (*Shimadzu SPD-M20A*). NMR Spectra: *Bruker-Avance-DRX-500* spectrometer; at 500.13 (^{1}H) and 125.77 MHz (^{13}C); δ in ppm rel. to Me_4Si as internal standard, J in Hz. HR-MS: *UltrOTOF-Q* mass spectrometer (*LC-IP-TOF* model *225-07100-34*, *Shimadzu*); positive ionization mode of the ESI source; in *m*/*z*.

Plant Material. Stems and roots of *Croton argyrophyllus* MULL. were collected in Jacobina County (Bahia State, Northeast of Brazil). Voucher specimens (# 39.434) were deposited with the Herbário

Prisco Bezerra (EAC) and identified by *Edson Paula Nunes*, Departamento de Biologia, Universidade Federal do Ceará, Brazil.

Extraction and Isolation. Stems (2.8 kg) and roots (0.83 kg) of *C. argyrophyllus* were separately pulverized and extracted with hexane at r.t. The solvent was evaporated to give the corresponding extracts. Both residues obtained after hexane extraction were extracted with EtOH. Partition of the EtOH extract from stems (96.9 g) in MeOH/H_2O 1:1 and extraction with hexane, $CHCl_3$, AcOEt, and MeOH, yielded *Fractions A–D*, resp. The hexane *Fr. A* (5.8 g) was submitted to CC (SiO_2, hexane/AcOEt 10:0 → 0:10): *Fractions A1–A7. Fr. A*2 (365.8 mg) was submitted to semi-prep. HPLC (hexane/$CHCl_3$ 9.5:0.5): *depressin* (12.8 mg) and **5** (10.5 mg). The $CHCl_3$ *Fr. B* (21.8 g) was submited to CC (SiO_2, $CHCl_3$/AcOEt 4:1): *crotonepetin* (39.6 mg). Filtration and recrystallization of the hexane extract from roots (7.2 g) in hexane yielded **1** (524.5 mg). Fractionation of the mother liquor by elution with hexane, $CHCl_3$, AcOEt, and MeOH, yielded *Fractions E–H*. The $CHCl_3$ *Fr. E* was submitted to CC (SiO_2, hexane/AcOEt 4:1): *acetylaleuritolic acid* (585.0 mg) and *spinasterol* (5.3 mg). The EtOH extract of the roots (12.2 g) was subjected to CC (*Sephadex LH-20* (MeOH): *Fractions I–O. Fr. M* (1.61 g) was separated by CC (SiO_2, hexane/AcOEt 85:15): **2** (26.5 mg). *Fr. N* (249.0 mg) was separated by HPLC ($CHCl_3$/MeOH 8:2): **3** (23.7 mg) and **4** (16.7 mg).

(5β,6β)-5,6:13,16-Diepoxycrotofola-4(9),10(18),13,15-tetraen-1-one (= rel-*(1a*R,*3*S,*4b*S,*10b*S,*10c*S)-*2,3,4b,5,6,7,10b,10c-Octahydro-3,10,10c-trimethyl-5-methyleneoxireno[6′,7′]indeno[5′,4′:3.4]cyclohepta[1,2*-b*]furan-4(1a*H*)-one*; **1**): Colorless crystals. M.p. 126–129°. $[\alpha]_D^{20} = -70$ ($c = 0.214$, Me_2CO). ^{1}H- and ^{13}C-NMR: *Table 1*. HR-ESI-MS: 311.1621 ($[M+H]^+$, $C_{20}H_{23}O_3^+$; calc. 311.1647).

(5β,6β)-5,6:13,16-Diepoxy-2-epicrotofola-4(9),10(18),13,15-tetraen-1-one (= rel-*(1a*R,*3*R,*4b*S,*10b*S,*10c*S)-*2,3,4b,5,6,7,10b,10c-Octahydro-3,10,10c-trimethyl-5-methyleneoxireno[6′,7′]indeno[5′,4′:3.4]-cyclohepta[1,2*-b*]furan-4(1a*H*)-one*; **2**): Colorless resin. $[\alpha]_D^{20} = +50.3$ ($c = 0.408$, $CHCl_3$). ^{1}H- and ^{13}C-NMR: *Table 1.* HR-ESI-MS: 311.1630 ($[M+H]^+$, $C_{20}H_{23}O_3^+$; calc. 311.1647).

(5β,6β)-5,6:13,16-Diepoxy-16-hydroxycrotofola-4(9),10(18),13,15-tetraen-1-one (= rel-*(1a*R,*3*S,*4b*S,*10b*S,*10c*S)-*2,3,4b,5,6,7,10b,10c-Octahydro-9-hydroxy-3,10,10c-trimethyl-5-methyleneoxireno[6′,7′]indeno[5′,4′:3.4]cyclohepta[1,2*-b*]furan-4(1a*H*)-one*; **3**): Yellow resin. $[\alpha]_D^{20} = -83.4$ ($c = 0.314$, MeOH). ^{1}H- and ^{13}C-NMR: see *Table 1.* HR-ESI-MS: 365.1630 ($[M+K]^+$, $C_{20}H_{22}KO_4^+$; calc. 365.1155).

(5β,6β)-5,6:13,16-Diepoxy-16-hydroxy-2-epicrotofola-4(9),10(18),13,15-tetraen-1-one (= rel-*(1a*R,*3*R,*4b*S,*10b*S,*10c*S)-*2,3,4b,5,6,7,10b,10c-Octahydro-9-hydroxy-3,10,10c-trimethyl-5-methyleneoxireno[6′,7′]indeno[5′,4′:3.4]cyclohepta[1,2*-b*]furan-4(1a*H*)-one*; **4**): Dark brown resin. $[\alpha]_D^{20} = +66.4$ ($c =$ 2.14, MeOH). ^{1}H- and ^{13}C-NMR: *Table 1.* HR-ESI-MS: 365.1155 ($[M+K]^+$, $C_{20}H_{22}KO_4^+$; calc. 365.1155).

*(2*E,*5β,6*E,*12*E*)-5-Hydroxycasba-2,6,12-trien-4-one* (= rel-*(1*R,*2*E,*4*S,*6*E,*10*E,*14*S*)-4-Hydroxy-3,7,11,15,15-pentamethylbicyclo[12.1.0]pentadeca-2,6,10-trien-5-one*; **5**): Colorless oil. $[\alpha]_D^{20} = +13.5$ ($c = 0.15$, $CHCl_3$). ^{1}H- and ^{13}C-NMR: *Table 1.* HR-EI-MS: 325.2122 ($[M+Na]^+$, $C_{20}H_{30}NaO_2^+$; calc. 325.2143).

X-Ray Crystallography of **1**. X-ray data collection was accomplished on an *Enraf-Nonius*-Kappa-CCD area-detector diffractometer. The programs used in the crystallographic study were: data collection, COLLECT [15]; cell refinement, SCALEPACK [16]; data reduction, DENZO and SCALEPACK; structure resolution, SHELXS97 [17]; structure refinement, SHELXL97 [18]; molecular graphics, ORTEP-3 [19]. Software used to prepare material for publication, WinGX-Routine [20]. The structure was solved by direct methods and refined through the interactive blocked-matrix least-squares calculations. The refinement was conducted until all atomic-parameter shifts were smaller than their standard deviations. The positions of H-atoms bonded to C-atom were determined based on stereochemical parameters, and U_{iso} was set to 1.5 (Me) or 1.2 (other) times the value of the equivalent isotropic displacement parameter of the attached atom. In the final difference *Fourier* map, there were no peaks greater than 0.28 Å^{-3}. Crystallographic Data: $C_{20}H_{23}O_3$, M_r 311.4; crystal size 0.37 × 0.31 × 0.28 mm; space group orthorhombic, $P2_12_12_1$; T 295(2) K; $a = 9.9429(4)$ Å, $b = 12.8575(3)$ Å, $c =$ 13.5790(5) Å; $V = 1735.9(3)$ Å^3; $F(000) = 668$; $Z = 4$, $D_x = 1.19$ Mg/m^3; 12179 reflections collected with 3844 independent $R_{int} = 0.032$; data, restraints and parameters, 3081, 0, and 209 resp.; goodness-of-fit on $F^2 = 1.075$; final indices $R_1 = 0.060$, $wR_2 = 0.152$; largest difference peak and hole 0.280 and −0.216 eÅ^{-3},

resp. CCDC-871581 contains the supplementary crystallographic data for this paper. These data can be obtained, free of charge, *via* http://www.ccdc.cam.ac.uk/data_request/cif.

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