New *cis*-Clerodane Diterpenoids from *Croton schiedeanus*

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The acid fraction of extracts from the aerial part of *Croton schiedeanus* afforded six *cis*-clerodane type diterpenoids. Two of them (1 and 4) are new natural compounds. Structural elucidation was achieved on the basis of their spectral data.

Key words *Croton schiedeanus*; Euphorbiaceae; diterpenoid; *cis*-clerodane

The genus *Croton* (family Euphorbiaceae) comprises about 800 species of trees, shrubs and herbs distributed in tropical and subtropical regions, and is one of the richest sources of diterpenes with a clerodane skeleton.[1,2] Clerodanes are distributed in higher plants, with an enormous stereochemical and functional variability. *Croton schiedeanus* SCHLECHT grows widely in the centre and the north of South America. In Colombia it is popularly known as "almizclillo" and used in traditional medicine for the treatment of stomachache and hypertension.[3,4] Previously, we studied the neutral fraction of this species and reported the isolation and identification of four *neo*-clerodanes.[5] In continuation of our studies on the diterpenoids from this plant, we have now investigated the acid fraction. In this paper we report the isolation and structure elucidation of six other clerodanes, two of them (**1**, **4**) being new compounds. In addition, two known flavonoids, ayanin[6,7] and quercetin-3,7-dimethyl ether[8] were also obtained from this fraction. Structural elucidation was achieved by spectroscopic experiments and by comparison with other closely related compounds.

The aerial part of *C. schiedeanus* was extracted with EtOH and was partitioned between $CHCl_3$ and H_2O. The residue of $CHCl_3$ extract was extracted with aqueous 4% NaOH yielding an acidic and a neutral part.

The acid fraction purification, using a combination of chromatography on silica gel and Sephadex LH-20, has led to the isolation of six *cis*-clerodane type diterpenoids (**1**—**6**) (Fig. 1).

The known compounds **2**, **3**, **5**, **6** were determined to be (−)-12,16-dihydroxy-*cis*-cleroda-3,13-dien-15-oic acid-15,16-olide (**2**),[9] floridolide A (**3**),[10,11] haplopappic acid (**5**),[12] and (+)-15-hydroxy-*cis*-cleroda-3,13-dien-18-oic-acid (**6**)[11] by comparison of physical data with literature values and from spectroscopic evidence.

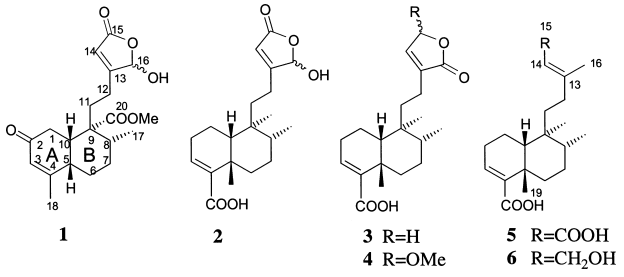

Fig. 1. Structures of Compounds Isolated from *C. schiedeanus*.

Compound **1** was isolated as colourless gum. Its molecular formula was deduced to be $C_{20}H_{26}O_6$ on the basis of the pseudomolecular ion at m/z=363.1812 $[M+H]^+$ by high-resolution mass spectrometry (HR-FAB-MS). The IR spectrum exhibited bands of α,β-unsaturated γ-lactone (1760 cm^{-1}), ester group (1727 cm^{-1}) α,β-unsaturated carbonyl (1649 cm^{-1}) and hydroxyl group (3308 cm^{-1}). The ^{1}H- and ^{13}C-NMR spectra were very close to those of the 19-*nor* clerodane *cis*-cajucarin B,[13] except for the absence of the signals associated with the furan ring. The singlet at δ_H 6.00 was assigned to an acetalic proton, since a correlation of this signal with that of a methine carbon atom at δ_C 98.8 was observed in the heteronuclear multiple quantum coherence (HMQC). The ^{13}C-NMR spectrum showed signals due to conjugated carbonyl group (δ_C 170.6), and a double bond (δ_C 165.8, 117.6). These facts suggested the replacement of the side-chain furanic ring by a hydroxylated α,β-unsaturated γ-lactone moiety. The ^{13}C-NMR spectrum of **1** also showed upfield signals at δ_C 37.3 (C-1), 21.0 (C-6), 27.4 (C-7), 31.8 (C-8) and 38.5 (C-10), indicative of a *cis*-A/B ring junction.[13] The assignment was supported by the analysis of the ^{1}H–^{1}H correlation (COSY), HMQC and the heteronuclear multiple bond correlation (HMBC) (Table 1, Fig. 2). Thus, the structure of (−)-methyl 16-hydroxy-19-*nor*-2-oxo-*cis*-cleroda-3,13-dien-15,16-olide-20-oate, was proposed for compound **1**.

The molecular formula of compound **4**, $C_{21}H_{30}O_5$, was determined by HR-FAB-MS (m/z 363.2135) $[M+H]^+$. The ^{1}H- and ^{13}C-NMR spectra were very close to those of compound **3**, and an additional methoxyl signal was visible at δ_H 3.56. The A/B ring *cis* fusion was proposed on the basis of the low field ^{13}C-NMR signal of the bridgehead methyl carbon (C-19, δ_C 33.2). According to Manabe and Nishino the chemical shift of C-19 shows a 10 ppm downfield shift compared with that of *trans*-clerodanes.[14] The two-dimensional nuclear Overhauser effect spectroscopy (NOESY) confirmed the *cis*-configuration of the decalin moiety, since a correlation between H-19 and H-10 was observed. On the basis of ^{1}H–^{1}H COSY, HMQC and HMBC (Table 1), all proton and carbon signals were unambiguously assigned. Thus, the structure of compound **4** was established as (+)-15-methoxyfloridolide A.

Although in previous studies we have reported the antihypertensive and vaxorelaxant activities of the ethanolic extract

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Table 1. ^{13}C-, ^{1}H-NMR and HMBC Data of Compounds Spectral Data for Compounds **1** and **4**

Position	**1**			**4**		
	^{13}C-NMR	^{1}H-NMR	HMBC	^{13}C-NMR	^{1}H-NMR	HMBC
1	37.3	2.60 dd (10.1; 2, 6)	2, 10	16.8	1.82 m; 2.01 m	2, 3, 5, 9, 10
2	199.0	—	—	24.3		
3	126.0	5.86 s	5, 18	142.2	6.80 br s	
4	168.2	—	—	137.5	—	—
5	38.9	2.28 m		40.2	—	—
6	21.0	1.75 m		35.3	1.10 m; 2.70 m	5, 10
7	27.4	1.67 m; 1.90 m		28.5	1.20 m; 1.40 m	
8	31.8	2.20 m		37.8	1.50 m	
9	52.0	—	—	36.3	—	—
10	38.5	2.40 m		45.2	1.48 m	2, 4, 5, 9, 17, 19
11	33.7	2.05 m		36.6		
12	22.6	2.09 m		18.8	2.21 m	13, 14, 16
13	165.8	—	—	139.2	—	—
14	117.6	5.83 s	15, 16	141.1	6.76 br s	
15	170.6	—	—	102.4	5.72 br s	
16	98.8	6.08 br s		171.4	—	—
17	18.2	1.13 d (7.2)	7, 8, 9	15.8	0.77 d (2.4)	7, 12
18	22.8	2.00 s	3, 4, 5	172.9	—	—
19	—	—	—	33.2	1.23 s	4, 6, 10
20	174.6	—	—	18.0	0.80 s	5, 6, 8, 10
OMe	51.6	3.68 s	20	56.9	3.56 s	

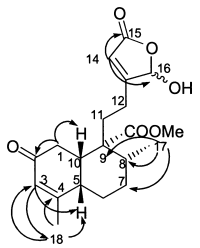

Fig. 2. ^{1}H–^{13}C Long-Range Correlation by HMBC of Compound **1**

from *Croton schiedeanus*,[15] the diterpenoids **1**—**6** did not show any significant vasodilator effect in an *in vitro* screening in rat aortic ring.

Experimental

General Procedures ^{1}H- and ^{13}C-NMR were recorded on Bruker AC 200 and Bruker 400 DRX instruments. Chemical shifts (δ) are given in ppm and tetramethylsilane was used as internal standard. The IR spectra were recorded in dichloromethane film in a Nicolet (Impact 410) spectrometer. Sephadex LH-20 (Fluka, 25—100 μm) and silica gel 60 (Merck, 230—400 mesh) were used for flash chromatography; precoated silica gel plates (Merck, Kieselgel 60 F254, 0.25 mm) were used for TLC analysis. For MS analyses, a VG-TS250 apparatus (70 eV) was used. Optical rotations were measured on a Perkin-Elmer 241 polarimeter.

Plant Material The aerial part of *Croton schiedeanus* was collected from the region of Tocaima, Cundinamarca, Colombia, in April 2002. Its identity was confirmed by the botanist Dr. José Luis Fernández and a voucher specimen has been deposited under No. 432164 in the Herbarium of Natural Sciences Institute of National University of Colombia.

Extraction and Isolation The dried and triturated aerial part of *C. schiedeanus* (8 kg) was soaked in 96% EtOH (35 l) at room temperature for three days. The EtOH was removed *in vacuo* to yield a dark residue (120 g), which was partitioned between $CHCl_3$ and H_2O to give $CHCl_3$ soluble fraction (90 g). The residue of $CHCl_3$ extract was fractionated with aqueous 4% NaOH yielding two parts: an acid part (12 g) and a neutral part (55 g). The acid part was successively rechromatographed on Sephadex LH-20 in hexane–CH_2Cl_2–MeOH 2 : 2 : 1, and silica gel using 0—100% EtOAc–hexane to afford ayanin (1.5 g), quercetin 3,7-dimethyl ether (0.5 g), **1** (200 mg), **2** (280 mg), **3** (90 mg), **4** (45 mg), **5** (30 mg) and **6** (75 mg).

(−)-Methyl 16-Hydroxy-19-*nor*-2-oxo-*cis*-cleroda-3,13-dien-15,16-olide-20-oate (**1**): $[\alpha]_D$ −8.1° (c=0.3, $CHCl_3$). IR ($CHCl_3$) cm^{-1}: 3308, 1760, 1727, 1649, 1450, 1380, 874. ^{1}H-NMR (400 MHz, $CDCl_3$) δ and ^{13}C-NMR (100 MHz, $CDCl_3$) δ given in: Table 1. HR-FAB-MS: 363.1812 $[M+H]^+$ (Calcd for $C_{20}H_{27}O_6$, 363.1808).

(+)-15-Methoxyfloridolide A (**4**): $[\alpha]_D$ +50.6° (c=1.35, $CHCl_3$). IR ($CHCl_3$) cm^{-1}: 2960—3360, 1768, 1679, 1446, 1365, 936. ^{1}H-NMR (400 MHz, $CDCl_3$) δ and ^{13}C-NMR (100 MHz, $CDCl_3$) δ given in Table 1. HR-FAB-MS: 363.2135 $[M+H]^+$ (Calcd for $C_{21}H_{31}O_5$, 363.2172).

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