Neoclerodane Diterpenoids from *Croton eluteria*

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Five new neoclerodane diterpenoids, *rel*-(3*R*,4*S*,5*R*,7*R*,8*S*,9*R*,10*S*,12*R*,20*S*)-7-acetoxy-15,16,12,20-diepoxy-3,4-dihydroxy-20-methoxyneocleroda-13(16),14-diene (**1**), *rel*-(3*R*,4*S*,5*R*,7*R*,8*S*,9*R*,10*S*,12*R*,20*S*)-7-acetoxy-15,16,12,20-diepoxy-3,4,20-trihydroxyneocleroda-13(16),14-diene (**2**), *rel*-(3*R*,4*S*,5*S*,6*R*,7*S*,8*S*,9*R*,10*S*,12*R*,20*S*)-6,7-diacetoxy-3,4,15,16,12,20-triepoxy-20-hydroxyneocleroda-13(16),14-diene (**3**), *rel*-(3*R*,4*S*,5*R*,7*R*,8*S*,9*R*,10*S*,12*R*,20*S*)-7-acetoxy-3,4,15,16,12,20-triepoxy-20-hydroxyneocleroda-13(16),14-diene (**4**), and *rel*-(3*R*,4*S*,5*R*,7*R*,8*S*,9*R*,10*S*,12*R*,20*R*)-7,20-diacetoxy-3,4,15,16,12,20-triepoxyneocleroda-13(16),14-diene (**5**), have been isolated from the bark of *Croton eluteria*. The structures of the compounds **1**−**5** (cascarillins E−I) were determined by spectroscopic data interpretation.

Croton eluteria Bennett (Euphorbiaceae) is a tropical shrub or small tree, native to the West Indies and northern South America. Its dried bark, called “cascarilla”, has been used in modern medicine as well as in traditional folk medicine for the treatment of various diseases, including bronchitis, diarrhea, dysentery, fever, and malaria.[1,2] Moreover, due to its diffusiveness and spicy-aromatic odor character, the oil of cascarilla has attracted great interest in the fragrance industry.[3]

In previous papers,[4−6] bark extracts of *C. eluteria* were shown to contain terpenes derivatives such as cascallin, cascarillon, and cascarillin A. The nonpolar constituents of this plant have been investigated, and cascarillins B−D were isolated in our laboratory.[7] This work has been continued and five new neoclerodanes have been isolated, called cascarillins E−I.

The molecular formula, $C_{23}H_{34}O_7$, of compound **1** was established from HRFABMS. The skeleton was deduced from characteristic NMR signals (Tables 1 and 2) and by comparison of spectral data with similar diterpenes also isolated from this plant material.[7] ^{1}H and ^{13}C NMR signals were indicative of a furan ring (C-13: δ_C 129.8; C-14: δ_H 6.44 t, J = 1.2 Hz, δ_C 110.0; C-15: δ_H 7.32 s, δ_C 143.6; C-16: δ_H, 7.32 s, δ_C 139.7), three methyl groups (C-17: δ_H 1.06 d, J = 7.3 Hz; δ_C 15.3; C-18: δ_H 1.22 s, δ_C 22.0; C-19: δ_H 1.19 s; δ_C 19.4), two quaternary carbons (C-5: δ_C 41.4; C-9: δ_C 54.2), and a C−H group (C-10: δ_H 1.9 m, δ_C 45.8). Furthermore, an acetate group (3H, δ_H 2.02 s, δ_C 21.65; 170.8), two hydroxyl groups (δ_C 77.2; 75.9), and an *O*-methyl function (3H, δ_H 3.17 s, δ_C 54.2) were also determined as substituents.[8−10] The remaining oxygen was involved in an *O*-methoxy lactol function as indicated by the signals at δ_C 108.8 (C-20), δ_C 71.6 (C-12) and δ_H 5.29 s (H-20), δ_H 5.00 m (H-12).[11] This assignment was supported by the cross-peak displayed in the HMBC spectrum between the methyl at δ_H 3.17 ppm and the signal assigned to C-20 (δ_C 108.8). The locations of the different substituents were determined by analysis of the HMBC spectrum, whose main correlations are represented in Figure 1. The HMBC cross-peak between the deshielded carbon at δ_C 170.8 ppm and the proton at δ_H 5.01 ppm (H-7) allowed the acetoxy function be placed at C-7. Finally, the quaternary carbon that resonated at δ_C 75.9 ppm (C−OH, tertiary alcohol) exhibited a cross-peak in the HMBC spectrum with protons assigned to H-2α, H-2β, Me-18, and Me-19, and the hydroxymethine signal at δ_C 77.2 ppm (CH−OH, secondary alcohol) with protons H-2β and H-18. Consequently, these hydroxyl groups were located unambiguously at positions C-3 and C-4, respectively. The *trans* A/B ring junction for the decalin was suggested by the ^{13}C NMR signal value of Me-19.[12] Concerning the relative stereochemistry, the α-orientation for the acetoxyl group was proposed on the basis of the *J*-values observed for H-8 in the ^{1}H NMR spectrum of **1**: $J_{8\beta ax-7\beta eq}$ = 2.8 Hz in accordance with an approximate dihedral angle between H-7β and H-8β of 60°. The β-orientation of H-7 was corroborated by NOE correlations of H-7β to H-6β and H-11b located above the plane of the decalin ring (Figure 1). The α-orientation deduced for the acetate group was also confirmed by NOE cross-peaks between $OCOCH_3$ and Me-17α and Me-19α. Similarly, the α-configuration of the hydroxy group at C-3 was deduced from the multiplicity of the equatorial proton H-3 (δ_H 3.53 t, J = 2.4 Hz) and confirmed by NOE correlations between H-3β and H-1β and Me-18β. The 12*R* stereochemistry of compound **1** was established by NOE correlations between Me-17 and H-12. Indeed, this result established that Me-17 and H-12 are on the same side of the plane defined by the 20,12-hemiacetal ring. In the same way, the C-20 stereochemical center was established to be *S*, on the basis of the NOE correlation between *O*-Me and H-14, located on the same side of the plane defined by the hemiacetal ring. Although the absolute stereochemistry of compound **1** has not been ascertained (we were unable to crystallize **1** in a suitable form for X-ray analysis), it is reasonable to assume that both compounds have a neoclerodane absolute configuration, as diterpene derivatives isolated from the title plant whose neoclerodane absolute configurations have been established. These data allowed us to establish the structure of compound **1** as *rel*-(3*R*,4*S*,5*R*,7*R*,8*S*,9*R*,10*S*,12*R*,20*S*)-7-acetoxy-15,16,12,20-diepoxy-3,4-dihydroxy-20-methoxyneocleroda-13(16),14-diene.

Compound **2**, $C_{22}H_{32}O_7$, as determined by HRFABMS, displayed many similarities to **1** in its ^{1}H and ^{13}C NMR spectra. All signals were present, except those corresponding to the *O*-methyl unit, in the deshielded areas of the spectra. Thus, in accordance with literature,[7,13] a hemiacetal function was shown by the characteristic NMR signals at δ_H 5.92 (H-20) and δ_C 100.7 (C-20). An acetoxyl

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Table 1. ^{1}H NMR Spectral Data (400 MHz) of Compounds **1**−**5** (δ in ppm, multiplicities, J in Hz)

proton	**1**[a]	**2**[b]	**3**[a]	**4**[a]	**5**[a]
1α	2.27 dddd (14.6, 13.2, 2.9)	2.47 br dd (12.2, 2.7)	1.90 m	1.73 m	1.69 m
1β	1.70 br dd (14.6, 1.6)	1.73 m	1.76 m		1.59m
2α	1.64 dddd (13.8, 2.4)	1.65 br dd (13.7, 2.7)	1.59 m	1.52 m	1.58 m
2β	1.85 m	1.92 m	2.15 m	2.11 m	2.11 m
3	3.53 t (2.4)	3.50 t (2.7)	2.90 s	2.93 s	2.94 s
6α	1.65 m	1.80 dd (14.8, 2.5)		2.05 dd (14.5, 2.6)	2.05 dd (14.5, 2.8)
6β	2.07 dd (14.6, 4.2)	2.10 dd (14.8, 2.5)	4.95 d (4.2)	1.65 dd (14.5, 4.1)	1.64 dd (14.5, 3.7)
7	5.01 m	5.04 m	5.36 dd (4.2, 3.0)	5.03 m	5.03 ddd (3.0)
8	1.53 qd (7.3, 2.8)	1.58 qd (7.3, 2.8)	1.73 qd (7.2, 3.0)	1.56 qd (7.2, 3.4)	1.60 m
10	1.90 m	1.96 dd (12.2, 1.6)	1.28 br d (10.0)	1.19 br s	1.28 br d (10.3)
11a	2.53 dd (13.3, 6.8)	2.62 dd (13.2, 7.1)	2.57 dd (13.4, 7.0)	2.55 dd (13.3, 7.0)	2.54 dd (13.4, 6.8)
11b	1.82 m	1.92 m	1.88 dd (13.4, 9.0)	1.81 dd (13.3, 9.1)	1.92dd (13.4, 9.0)
12	5.00 m	5.02 m	5.10 dd (9.0, 7.0)	5.05 m	5.11 dd (6.8, 9.0)
14	6.44 t (1.2)	6.63 dd (1.7, 0.7)	6.54 br s	6.50 br s	6.34 dd (1.7, 0.7)
15	7.32 s	7.39 t (1.7)	7.39 d (2.0)	7.35 s	7.33 t (1.7)
16	7.32 s	7.44 t (0.7)	7.40 d (0.3)	7.34 br s	7.27 t (0.7)
Me-17	1.06 d (7.3)	1.11 d (7.3)	1.10 d (7.2)	1.07 d (7.2)	1.10 d (7.3)
Me-18	1.22 s	1.19 s	1.18 s	1.16 s	1.14 s
Me-19	1.19 s	1.35 s	1.39 s	1.21 s	1.00 s
20	5.29 s	5.92 s	5.83 d (3.1)	5.74 s	6.63 s
OCO*CH*$_3$ in C-7	2.02 s	2.04 s	2.12 s	2.04 s	1.94 s
OCH$_3$	3.17 s				
OH in C-20			2.3 d (3.1)		
OCO*CH*$_3$			2.03 s		2.08 s

[a] CDCl$_3$ solution. [b] CD$_3$OD solution.

Table 2. ^{13}C NMR Spectral Data (100 MHz) of Compounds **1**−**5**

carbon	**1**[a]	**2**[b]	**3**[a]	**4**[a]	**5**[a]
C-1	19.3	19.5	18.6	19.0	18.9
C-2	31.5	30.8	29.2	29.4	29.4
C-3	77.2	77.9	63.6	62.8	62.4
C-4	75.9	75.4	64.6	65.3	65.0
C-5	41.4	41.4	42.1	37.1	37.0
C-6	36.7	36.6	75.2	41.2	41.0
C-7	76.1	76.3	74.2	74.1	74.5
C-8	45.5	45.3	44.4	46.1	46.3
C-9	54.1	54.0	54.6	55.0	54.2
C-10	45.8	45.3	50.5	51.3	51.2
C-11	43.3	44.5	43.4	43.3	42.6
C-12	71.6	71.6	72.3	72.2	73.1
C-13	129.9	130.6	129.9	129.9	129.2
C-14	110.0	109.9	109.6	109.7	109.0
C-15	143.6	143.0	143.8	143.6	143.5
C-16	139.7	139.7	139.7	139.7	139.3
C-17	15.3	14.6	14.3	14.7	14.7
C-18	22.0	20.0	13.4	20.3	20.3
C-19	19.4	19.4	22.0	18.0	17.4
C-20	108.1	100.7	100.4	100.6	99.1
OCO*CH*$_3$ (C-7)	21.7	20.3	21.2	21.5	21.5
O*C*OCH$_3$ (C-7)	170.8	171.3	170.5	170.9	170.7
OCH$_3$	54.2				
OCO*CH*$_3$ (C-6/C-20)			21.6		21.9
O*C*OCH$_3$ (C-6/C-20)			170.7		169.9

[a] CDCl$_3$ solution. [b] CD$_3$OD solution.

group was still present as supported by ^{1}H and ^{13}C NMR spectral data (3H, δ_H 2.04, δ_C 20.3; δ_C 171.3) at position C-7 (HMBC correlation between OCOCH$_3$ and C-7) with an α-orientation ($J_{8\beta ax-7\beta eq} = 2.8$ Hz). The hydroxyl groups on C-3 and C-4 (C-3: δ_H 3.50 t, $J = 2.7$ Hz, δ_C 76.3; C-4: δ_C 75.4) were also present, with axial and equatorial orientations, respectively. The structure of **2** was thus assigned as *rel*-(3*R*,4*S*,5*R*,7*R*,8*S*,9*R*,10*S*,12*R*,20*S*)-7-acetoxy-15,16,12,20-diepoxy-3,4,20-trihydroxyneocleroda-13(16),14-diene.

The positive HRFABMS of compound **3** showed peaks at *m*/*z* 471.2006 corresponding to [M + Na$^+$], suggesting the elemental formula $C_{24}H_{32}O_8$. The MS and NMR data indicated that **3** differed from **2** in the substitution of the hydroxyl groups between C-3 and C-4 by an epoxide group and by the presence of an additional acetoxy group. The latter was located at position C-6, as supported by HMBC correlations between the deshielded carbon at δ_C 75.2 ppm and protons H-7, H-10, and Me-19. Strong NOE effects of H-6 and H-8, H-10, and Me-18 indicated a β-orientation of H-6. The substituent was thus located unambiguously in an α-orientation. This assignment was supported by the deshielded position of Me-19. The epoxide group positioned between C-3 and C-4 was deduced from the cross-peaks observed in the HMBC spectrum between C-3 and Me-18 and between C-4 and H-6, H-10, and Me-19. A strong NOE effect of H-6β with Me-18 (in contrast to a smaller NOE effect with Me-19) was indicative of the β-orientation of Me-18 and thus an α-orientation of the epoxide group. These results led to the assignment as *rel*-(3*R*,4*S*,5*S*,6*R*,7*S*,8*S*,9*R*,10*S*,12*R*,20*S*)-6,7-diacetoxy-3,4,15,16,12,20-triepoxy-20-hydroxyneocleroda-13(16),14-diene for compound **3**.

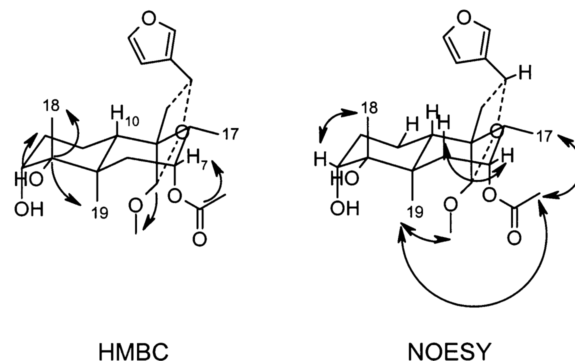
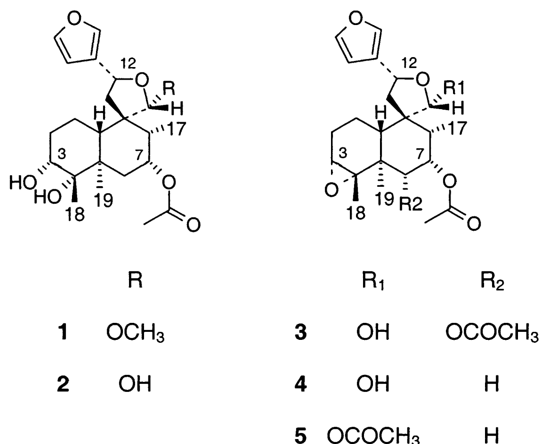


Figure 1. Main HMBC and NOESY correlations of compound **1**.

Compound **4**, determined as $C_{22}H_{30}O_6$ by HRFABMS, exhibited spectral data closely compatible to compound **3**. Comparison of the spectral data of these two compounds indicated that **4** differs from **3** only in the lack of an acetoxy group at position C-6. Thus, the structure of **4** was established as *rel*-(3*R*,4*S*,5*R*,7*R*,8*S*,9*R*,10*S*,12*R*,20*S*)-7-acetoxy-3,4,15,16,12,20-triepoxy-20-hydroxyneocleroda-13-(16),14-diene.

The molecular formula of **5** was determined as $C_{24}H_{32}O_7$ by HRFABMS. The ^{13}C NMR data indicated that **5** was

the acetyl derivative of **4**. A combination of $^1H-^1H$ COSY, HSQC, and HMBC experiments determined unambiguously the position of the additional acetyl group at C-20. Thus, the structure of **5** was established as *rel*-(3*R*,4*S*,5*R*,7*R*,8*S*,9*R*,10*S*,12*R*,20*R*)-7,20-diacetoxy-3,4,15,16,12,20-triepoxyneocleroda-13(16),14-diene.



	R		R₁	R₂
1	OCH_3	**3**	OH	$OCOCH_3$
2	OH	**4**	OH	H
		5	$OCOCH_3$	H

For the sake of simplicity, we have named compounds **1**–**5** as cascarillins E–I, respectively, following the earlier isolation of similar derivatives from the same plant source.[7]

Experimental Section

General Experimental Procedures. Melting points were determined on a Electrothermal instrument and are uncorrected. Optical rotations were measured on a Perkin-Elmer 241 polarimeter with a sodium lamp ($\lambda = 589$ nm) in a 10 cm microcell. UV spectra were obtained with a Perkin-Elmer Lambda 20 UV–vis spectrometer. ^1H NMR (400 MHz) and $J_{mod.}$ ^{13}C NMR (100 MHz) spectra were obtained with CD_3OD or $CDCl_3$ as solvent, on a Bruker ARX-400 spectrometer. Chemical shifts are given in δ (ppm) using the solvent peaks for CD_3OD and $CDCl_3$ at δ_H 4.87, δ_C 48 ppm and δ_H 7.23, δ_C 77 ppm, respectively. Coupling constants are reported in Hz. ESIMS (positive-ion mode), 3.5 kV (MeOH–$CHCl_3$), were measured on a Perkin-Elmer API Sciex 365 mass spectrometer. HRFABMS (positive-ion mode) were recorded on an Autospec 6F mass spectrometer manufactured by Micromass. Silica gel column chromatography was carried out using silica 60 SDS (70–200 μm). Medium-pressure column chromatography (10 bar) was carried out using silica 60 SDS (6–35 μm), with a Büchi 688 pump. Reversed-phase chromatography was performed on a Mega Bond Elut Varian C_{18} cartridge. Preparative HPLC was performed using a C_{18} Varian Dynamax 100 Å (21.4 × 250 mm) column. Fractionations were monitored by TLC (silica gel 60 F-254, Merck) with visualization under UV (254 and 365 nm) and with vanillin-sulfuric acid and Ehrlich reagents.

Plant Material. *Croton eluteria* was collected near Guayaquil on the Pacific coast of Ecuador (summer 1998). Voucher specimens were identified by Professor Isabelle Fourasté and deposited at the herbarium of the Pharmacognosy Department (Faculty of Pharmaceutical Sciences, Toulouse, France) under accession number Casc.09.99.

Extraction and Isolation. The air-dried powdered bark (1 kg) of *C. eluteria* was extracted successively with Me_2CO and MeOH. The Me_2CO extract (70 g) was taken up in $CHCl_3$ and chromatographed on a silica gel column eluted with a $CHCl_3$–MeOH gradient. The chloroform fractions, containing the main furanoid diterpenes (revealed by TLC with Ehrlich reagent), were evaporated to dryness and separated on silica gel (70–200 μm) columns eluted with a toluene–EtOAc gradient. Further purification of the crude diterpenes performed by medium-pressure (10 bar) silica gel chromatography (toluene–EtOAc, 98:2), followed by semipreparative HPLC (MeOH–H_2O, 13:7; 10 mL·min^{-1}, 204 nm), gave pure compounds **4** (10 mg) and **5** (28 mg). Purification by neutral aluminum oxide gel column chromatography with toluene–EtOAc gradient as eluent gave 26 mg of compound **3**. Compound **1** (15 mg) was obtained from the methanolic fraction after partition between $CHCl_3$–H_2O followed by silica gel column chromatography with toluene–EtOAc (98:2), reversed-phase chromatography (MeOH–H_2O, 3:2), and preparative HPLC (MeOH–H_2O, 3:2, 6 mL·min^{-1}, 204 nm).

The MeOH extract was partitioned between $CHCl_3$–H_2O (1:1.3). The combined $CHCl_3$ filtrates were concentrated and then chromatographed by two successive medium-pressure silica gel columns (toluene–EtOAc, 15:5 and 39:11). Separation and purification were carried out with crystallization, to obtain 20 mg of compound **2**.

Cascarillin E (1): colorless small crystals, 15 mg; mp 183–184 °C; $[\alpha]_D$ +19.3° (*c* 1.45, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 200 (3.49) nm; ^1H NMR, see Table 1; ^{13}C NMR, see Table 1; ESIMS $[M + Na]^+$ *m/z* 445.2, $[M + K]^+$ *m/z* 461.2; HR-FABMS (positive mode) $[M + Na]^+$ *m/z* 445.2206 (calcd for $C_{23}H_{34}O_7$ + Na, 445.2202).

Cascarillin F (2): colorless small crystals, 20 mg; mp 204–205 °C; $[\alpha]_D$ +2.2° (*c* 8, MeOH); UV (MeOH) λ_{max} (log ϵ) 209 (3.70) nm; ^1H NMR, see Table 1; ^{13}C NMR, see Table 1; ESIMS $[M + Na]^+$ *m/z* 431.2, $[M + K]^+$ *m/z* 447.2; HR-FABMS (positive mode) $[M + Na]^+$ *m/z* 431.2058 (calcd for $C_{22}H_{32}O_7$ + Na, 431.2046).

Cascarillin G (3): colorless small crystals, 26 mg; $[\alpha]_D$ +10.2° (*c* 14.7, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 207 (3.65) nm; ^1H NMR, see Table 1; ^{13}C NMR, see Table 1; ESIMS $[M + Na]^+$ *m/z* 471.1, $[M + K]^+$ *m/z* 487.1; HR-FABMS (positive mode) $[M + Na]^+$ *m/z* 471.2006 (calcd for $C_{24}H_{32}O_8$ + Na, 471.1995).

Cascarillin H (4): clear yellowish resin, 10 mg; $[\alpha]_D$ −35.7° (*c* 2.8, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 207 (3.72) nm; ^1H NMR, see Table 1; ^{13}C NMR, see Table 1; ESIMS $[M + Na]^+$ *m/z* 413.3, $[M + K]^+$ *m/z* 429.2; HR-FABMS (positive mode) $[M + Na]^+$ *m/z* 413.1933 (calcd for $C_{22}H_{30}O_6$ + Na, 413.1940).

Cascarillin I (5): clear yellowish resin, 28 mg; $[\alpha]_D$ +10.0° (*c* 3, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 207 (3.69) nm; ^1H NMR, see Table 1; ^{13}C NMR, see Table 1; ESIMS $[M + Na]^+$ *m/z* 455.0, $[M + K]^+$ *m/z* 471.0; HR-FABMS (positive mode) $[M + Na]^+$ *m/z* 455.2057 (calcd for $C_{24}H_{32}O_7$ + Na, 455.2046).

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