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CLERODANE DITERPENES FROM THE BARK OF *CASEARIA TREMULA*

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Key Word Index—*Casearia tremula*; Flacourtiaceae; *rel*-18(*S*),19(*R*)-diacetoxy-18,19-epoxy-6(*R*)-methoxy-2(*S*)-2(ξ)-methylbutanoyloxy) 5(*R*),8(*S*),9(*S*), 10(*R*)-cleroda-3,13(16),14-triene; *rel*-18(*S*),19(*R*)-diacetoxy-18,19-epoxy-2(*S*)-(2ξ-methylbutanoyloxy)-5(*R*),8(*S*),9(*S*),10(*R*)-cleroda-3,13(16),14-triene; *rel*-18(*S*),19(*R*)-diacetoxy-18,19-epoxy-2(*R*)-hexanoyloxy-5(*R*),8(*S*),9(*S*), 10(*R*)-cleroda-3,13(16),14-triene; *rel*-18(*S*),19(*R*)-diacetoxy-18,19-epoxy-6(*R*)-hydroxy-2(*S*)-octanoyloxy-5(*R*),8(*S*),9(*S*) 10(*R*)-cleroda-3,13(16),14-triene; *rel*-18(*S*),19(*R*)-diacetoxy-18,19-epoxy-6(*R*)-hydroxy-2(*S*)-undecanoyloxy-5(*R*), 8(*S*),9(*S*),10(*R*)-cleroda-3,13(16),14-triene; *rel*-18(*S*),19(*R*)-diacetoxy-18,19-epoxy-6(*R*)-hydroxy-2(*S*)-(2ξ-methylbutanoyloxy)-5(*R*),8(*S*),9(*S*), 10(*R*)-cleroda3,13(16),14-triene; *rel*-18(*S*),19(*R*)-diacetoxy-18, 19-epoxy-6(*R*)-hydroxy-2(*S*)-(3ξ-hydroxyoctanoyloxy)-5(*R*),8(*S*),9(*S*), 10(*R*)-cleroda-3, 13(16),14-triene; HMBC NMR Spectroscopy.

Abstract—Six novel clerodane diterpenes have been isolated from the bark of *Casearia tremula*. They were characterized by extensive use of Heteronuclear Multiple Bond Coherence NMR spectroscopy as *rel*-18(*S*),19(*R*)-diacetoxy-18, 19-epoxy-6(*R*)-methoxy-2(*S*)-(2ξ-methylbutanoyloxy) 5(*R*),8(*S*),9(*S*),10(*R*)-cleroda-3,13(16),14-triene, *rel*-18(*S*),19(*R*)-diacetoxy-18,19-epoxy-2(*S*)-(2ξ-methylbutanoyloxy)-5(*R*),8(*S*),9(*S*), 10(*R*)-cleroda-3,13(16),14-triene, *rel*-18(*S*),19(*R*)-diacetoxy-18,19-epoxy-2(*R*)-hexanoyloxy-5(*R*),8(*S*),9(*S*), 10(*R*)-cleroda-3,13(16),14-triene, *rel*-18(*S*),19(*R*)-diacetoxy-18,19-epoxy-6(*R*)-hydroxy-2(*S*)-octanoyloxy-5(*R*),8(*S*),9(*S*),10(*R*)-cleroda-3,13(16),14-triene and *rel*-18(*S*),19(*R*)-diacetoxy-18,19-epoxy-6(*R*)-hydroxy-2(*S*)-undecanoyloxy-5(*R*),8(*S*),9(*S*), 10(*R*)-cleroda-3,13(16),14-triene, *rel*-18(*S*), 19(*R*)-diacetoxy-18,19-epoxy-6(*R*)-hydroxy 2(*S*)-(2ξ-methylbutanoyloxy)-5(*R*),8(*S*),9(*S*),10(*R*)-cleroda-3,13(16),14-triene and *rel*-18(*S*),19(*R*)-diacetoxy-18,19-epoxy-6(*R*)-hydroxy-2(*S*)-(3ξ-hydroxyoctanoyloxy)-5(*R*),8(*S*),9(*S*), 10(*R*)-cleroda-3,13(16),14-triene.

INTRODUCTION

In a series of studies on the chemistry of the Flacourtiaceae we have recently reported on friedelane triterpenes and cyclohexenone glycosides from *Phyllobotryon spathulatum* [1], *Poliothrysis sinensis* [2] and *Xylosma flexuosum* [3].

Casearia tremula Grisebach. is a tree found in the Guanacaste region of Costa Rica (D. H. Janzen, personal communication). The genus *Casearia* has been reported to produce coumarins [4], aryltetralin lignans [6] and a series of highly oxygenated clerodane diterpenes [5–8]. The presence of these interesting metabolites in this genus prompted us to study *C. tremula*, from which we have isolated six novel clerodane diterpenes. Elucidation of the structure of these compounds was achieved by one- and two-dimensional NMR, with particular emphasis on Heteronuclear Multiple Bond Coherence (HMBC) spectroscopy [9].

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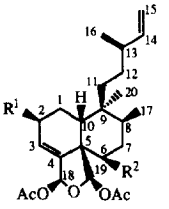
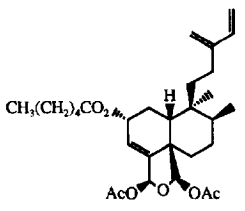
RESULTS AND DISCUSSION

By VLC [10] and circular preparative TLC six compounds (**1**–**6**) were isolated from the combined petrol and chloroform extracts of the bark of *C. tremula*. The ^{1}H and ^{13}C NMR (Tables 1 and 2) and EI mass spectra data of these compounds were very similar to those of one of the diterpenes isolated by Khan and coworkers [11] from *C. corymbosa* (e.g., **7**).

The IR spectrum of **1** revealed bands for the occurrence of three carbonyls (1757, 1731, 1679 cm^{-1}) and two exo-methylenes (3080, 3060 cm^{-1}). The EI mass spectrum of **1** solved for $[M]^+$ $C_{30}H_{44}O_8$, and the molecular ion underwent facile loss of two molecules of acetic acid and a $C_5H_{10}O_2$ fragment. The ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) revealed the presence of one methoxy, one 2-methylbutanoyloxy and two acetoxy substituents. Removal of these elements from the molecular formula left 20 carbons and suggested a diterpene. Signals in the ^{1}H NMR spectrum for an olefin (δ 5.90, *dd*, J = 1.6, 4.2 Hz), two doubly deshielded acetal-acetoxy methines (δ 6.36, *s*, 6.58, *t*, J = 1.6 Hz), a broad singlet (δ 5.37), an axial oxymethine proton (δ 3.27, *dd*, J = 3.8, 12.0 Hz)





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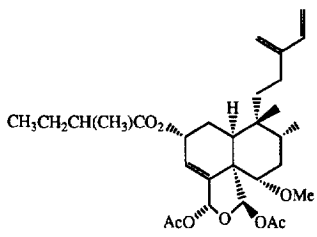
	R^1	R^2
1	2(β)-methylbutanoyloxy	methoxy
2	2(β)-methylbutanoyloxy	H
4	*n*-octanoyloxy and *n*-undecanoyloxy	hydroxy
5	2(β)-methylbutanoyloxy	hydroxy
6	3(β)-hydroxyoctanoyloxy	hydroxy



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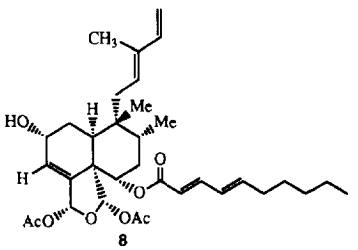

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and a CH_2–CH_2–(C=CH_2)–CH=CH_2 moiety were similar to those reported for the C-9 side chain in the clerodane diterpenes from *C. corymbosa* [6] (e.g. **7**).

Further signals, comparable to those of **7**, included a methyl doublet (δ0.89, J = 6.8 Hz) and a methyl singlet (δ0.86). In the HMBC spectrum (Table 3) a methyl triplet (δ0.91, J = 7.4 Hz) (associated with the side chain), exhibited ^2J and ^3J H–C couplings to one methylene carbon and one methine carbon. Both of these carbons were coupled to a methyl doublet (δ1.12, J = 7.0 Hz) which showed a further coupling to an ester carbonyl carbon (δ_C 175.9). The side chain was therefore confirmed as a 2-methylbutanoate ester.

The HMBC study (Table 3) confirmed the placement of the six carbon side chain at position C-9 and oxygenation at positions C-2, C-6 and the C-18/C-19 acetal. The deshielded acetal oxymethines exhibited ^3J couplings to the carbonyls of their attached acetates, placing the acetoxy groups at C-18 and C-19. The C-2 oxymethine resonance was deshielded (δ5.37), which was indicative of esterification at this position and so the 2-methylbutanoate moiety could be placed here. The methoxy must therefore be placed at position C-6.

The final problem remaining was to assign the stereochemistry at the eight chiral centres of **1**. The C-2 oxymethine resonance was a broad singlet with no large couplings and is therefore assigned to the equatorial (*rel*-α) conformation which requires that the ester be axial (*rel*-β). The H-6 oxymethine resonance (δ3.27) was a double doublet with a large *trans* di-axial coupling (J = 12.0, 3.8 Hz) and therefore axial (*rel*-α), which requires that the methoxy is equatorial and *rel*-β.

The presence of a *cis*-A/B ring junction was inferred by the deshielded C-20 methyl resonance (δ_C 25.5) [8]. This requires the substituent at position C-5 to be *rel*-β. Methyl-17 was assigned as *rel*-β (equatorial) on the basis of an NOE interaction with H_2-11. Methyl-20 was also assigned an equatorial (*rel*-α) conformation on the basis of NOE interactions with H-10 and H-1_{eq} (Fig. 1). The acetal acetoxy methines of C-18 and C-19 are assigned an *rel*-α conformation on the basis of NOESY interactions between H-18 and H-19, between H-18 and the methoxyl (Fig. 1) and between H-19 and H-7_{ax} and both protons of H_2-11.

Based on the spectral evidence above compound **1** is assigned as the novel clerodane diterpene *rel*-18(*S*), 19(*R*)-diacetoxy-18,19-epoxy-6(*R*)-methoxy-2(*S*)-(2ξ-methylbutanoyloxy) 5(*R*),8(*S*),9(*S*), 10(*R*)-cleroda-3,13(16),14-triene. The (*rel*)-β configuration has been chosen as the $[\alpha]_D$ of **1** is of the opposite sign to that of **7**. The orientation of the C-18 and C-19 positions of **1** is identical to that found in the diterpene pitumbin (**8**) isolated from *C. pitumba* [8].

The ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) of **2** were very similar to those of **1**. GC–mass spectrometry in the positive-ion chemical ionization mode suggested a molecular ion of *m/z* 502.30, which solved for $C_{29}H_{42}O_7$, which is CH_2O short of (**1**). Further principle ions in the GC–mass spectrum included *m/z* 400.30 $[M - C_4H_9CO_2H]^+$ and *m/z* 340 $[M - C_4H_9CO_2H - AcOH]^+$. The ^{1}H and ^{13}C NMR data (Tables 1 and 2) showed chemical shift values very similar to those of **1** but the methoxy was absent and position C-6 was not oxygenated. H-2 was a deshielded broad singlet (δ5.34) and so the 2-methylbutanoate ester moiety must again be axial and placed at C-2.

Relative stereochemistry was identical to that of **1** with the same characteristic NOESY interactions. The stereochemistry of the two acetoxy groups differed from those of the compounds isolated by Khan *et al.* [6], for example **7**, and was identical to the corymbotins isolated from *C. corymbosa* by Chen and Wiemer [12]. On the above evidence **2** is assigned as the novel clerodane diterpene *rel*-18(*S*),19(*R*)-diacetoxy-18,19-epoxy-2(*S*)-(2ξ-methylbutanoyloxy)-5(*R*),8(*S*),9(*S*),10(*R*)-cleroda-3,13(16),14-triene. A similar diterpene with the same ester moiety has been isolated from *C. corymbosa* [6], but with a hydroxy in position C-6 and with opposite relative stereochemistry at positions C-2 and C-19.

The third compound, **3**, was the least polar of the diterpenes obtained in this study. Gas chromatography–mass spectrometry of **3** in the CI mode gave a molecular ion of *m/z* 516 which solved for $C_{30}H_{44}O_7$.

Table 1. ^{1}H NMR data for diterpenes **1** to **6**

H	1	2	3	4	5	6
1	1.88 *m*	1.90 *m*	1.75 *m*, 2.06 *m*	1.88 *m*	1.90 *m*	1.95 *m*
2	5.37 *bs*	5.34 *bs*	5.50 *bt* (7.8)	5.67 *bs*	5.40 *bs*	5.48 *bs*
3	5.90 *dd* (4.2, 1.6)	5.87 *dd* (4.6, 1.4)	5.69 *d* (6.0)	6.20 *d* (4.0)	5.96 *d* (4.1)	5.93 *d* (3.0)
6	3.27 *dd* (3.8, 12.0)	1.71 *m*, 1.53 *m*	1.70 *m*	3.83 *dd* (7.1, 10.6)	3.78 *bt* (8.4)	3.75 *bt* (7.0)
7	1.45 *m*, 1.80 *m*	1.45 *m*	1.45 *m*	1.68 *m*	1.70 *m*	1.68 *m*
8	1.65 *m*	1.60 *m*	1.68 *bd* (13.2)	1.70 *m*	1.75 *m*	1.75 *m*
10	2.29 *bt* (9.6)	2.20 *dd* (11.9, 5.7)	2.13 *bd* (13.5)	2.50 *bt* (7.4)	2.31 *dd* (10.8, 6.1)	2.22 *dd* (13.4, 4.0)
11	1.16 *dd* (2.3, 12.4) 1.41 *m*	1.25 *m*, 1.46 *m*	1.29 *m*	1.50 *m*	1.25 *m*, 1.48 *m*	1.25 *m*, 1.45 *m*
12	2.05 *m*	2.07 *m*	2.05 *m*	2.24 *m*	2.06 *m*	2.07 *m*
14	6.37 *dd* (10.8, 17,4)	6.40 *dd* (17.6, 10.8)	6.36 *dd* (17.6, 10.9)	6.45 *dd* (10.9, 17.6)	6.42 *dd* (17.7, 11.0)	6.40 *dd* (17.6, 11.0)
15	4.96 *bd* (10.5) 5.10 *d* (17.4)	4.99 *d* (10.8) 5.15 *d* (17.6)	4.97 *d* (9.8) 5.16 *d* (17.6)	4.98 *d* (10.9) 5.21 *d* (17.6)	4.95 *d* (10.3) 5.14 *bd* (17.7)	5.01 *d* (10.6) 5.10 *d* (17.6)
16	4.87 *s*, 4.98 *s*	4.91 *s*, 5.01 *s*	4.86 *s*, 4.96 *s*	5.14 *s*, 5.23 *s*	4.93 *s*, 5.01 *s*	4.92 *s*, 5.01 *s*
17	0.89 *d* (6.8)	0.85 *d* (6.7)	0.82 *d* (6.8)	0.82 *d* (6.6)	0.90 *d* (6.0)	0.89 *d* (6.5)
18	6.58 *t* (1.6)	6.65 *bd* (1.4)	6.58 *s*	7.24 *bs*	6.69 *bs*	6.68 *t* (1.6)
19	6.36 *s*	6.27 *bs*	6.20 *s*	6.84 *bs*	6.46 *bs*	6.46 *s*
20	0.86 *s*	0.91 *s*	0.91 *s*	0.81 *s*	0.89 *s*	0.91 *s*
Ac	1.80 *s*, 2.00 *s*	1.86 *s*, 2.03 *s*	1.84 *s*, 2.09 *s*	1.80 *s*, 1.77 *s*	1.85 *s*, 2.03 *s*	1.88 *s*, 2.05 *s*
1′	—	—	—	—	—	—
2′	2.40 *m*	2.42 *m*	2.26 *t* (7.4)	—	2.42 *m*	2.46 *dd* (16.1, 8.8) 2.55 *dd* (16.1, 3.4)
3′	1.51 *m*, 1.64 *m*	1.66 *m*	1.65 *m*	—	1.63 *m*	4.00 *bs*
4′	0.91 *t* (7.4)	0.94 *t* (7.4)	1.68 *m*	—	0.94 *t* (7.4)	1.53 *m*
5′	1.12 *d* (7.0)	1.15 *d* (7.0)	1.25 *m*	—	1.15 *d* (7.0)	1.25 *m*
6′	—	—	0.87 *t* (7.4)	—	—	1.22 *m*
7′	—	—	—	—	—	1.25 *m*
8′	—	—	—	—	—	0.85 *t* (7.0)
CH_3	—	—	—	0.97 *t* (6.8)	—	—
CH_3	—	—	—	0.93 *t* (7.2)	—	—
2′–10′	—	—	—	1.25—1.40 *m*	—	—
MeO	3.24 *s*	—	—	—	—	—

Table 2. ^{13}C NMR data for the diterpenes **1** to **6**

C	1	2	3	4*	5	6
1	26.9	26.2	25.9	27.7	26.9	27.0
2	66.1	66.4	71.0	67.1	66.3	67.0
3	121.4	120.5	123.1	122.0	121.8	121.4
4	145.1	145.4	145.1	146.8	145.7	146.1
5	53.1	49.4	49.3	54.6	53.9	53.8
6	81.9	29.4	30.7	73.3	73.0	73.0
7	31.1	27.3	27.6	37.1	37.0	37.0
8	36.9	37.3	37.3	37.6	37.6	37.4
9	37.4	37.3	37.9	37.9	37.5	37.5
10	36.4	34.3	38.6	37.4	36.5	36.7
11	27.8	28.2	27.8	28.7	28.0	28.2
12	23.8	23.7	23.6	24.7	23.9	24.0
13	146.1	147.3	145.5	146.0	145.2	145.4
14	140.4	140.5	140.3	141.3	140.6	140.5
15	112.1	112.1	112.4	112.5	112.2	112.6
16	115.5	115.4	115.0	116.4	115.6	115.5
17	15.8	15.8	15.5	16.1	15.8	14.3
18	96.0	94.5	93.7	96.8	95.7	95.8
19	98.3	99.6	98.9	99.3	98.1	98.2
20	25.5	26.1	26.1	25.7	25.5	25.4
AcC=O	169.8, 170.2	169.9, 170.2	169.7, 169.5	169.8, 170.4	169.8, 170.2	169.9, 170.3
Ac-Me	20.5, 21.5	21.2, 21.3	21.3, 21.2	21.2, 22.0	21.3, 21.5	21.1, 21.3
1′	175.9 *s*	175.9 *s*	173.2 *s*	173.3 *s*	176.0 *s*	172.4 *s*
2′	41.1 *d*	41.3 *d*	34.8 *t*	—	41.3 *d*	42.0 *t*
3′	26.9 *t*	27.1 *t*	24.6 *t*	—	27.1 *t*	68.4 *d*
4′	11.6 *q*	11.7 *q*	31.3 *t*	—	11.7 *q*	36.8 *t*
5′	16.6 *q*	11.7 *q*	22.3 *t*	–	16.7 *q*	29.7 *t*
6′	—	—	13.9 *q*	—	—	32.0 *t*
7′	—	—	—	—	—	22.8 *t*
8′	—	—	—	—	—	14.2 *q*
CH_3	—	—	—	1.47 *q*, 14.6 *q*	—	—
MeO	57.5 *q*	—	—	—	—	—

*Ester carbons for **4** found at 34.9, 32.6, 32.4, 30.4, 30.2, 30.1, 29.7, 29.6, 29.6, 25.7, 23.4 and 23.3. All spectra run in $CDCl_3$ except **4** in C_6D_6.

Table 3. HMBC data for compound **1**

H	^2J	^3J
H-3	—	26.9, 53.1, 96.0
H-6	53.1	57.5
H-10	26.9, 53.1	36.9, 66.1, 81.9
H-14	112.1, 146.1	23.8, 115.5
H_2-15	140.4	146.1
H_2-16	146.1	23.8, 140.4
Me-17	36.9	31.1, 37.4
H-18	—	98.3, 170.2
H-19	53.1	81.9, 96.0, 169.8
Me-20	37.4	27.8, 36.4, 36.9
MeO	—	81.9
AcO-18	—	170.2
AcO-19	—	169.8

Electron-impact mass spectrometry indicated the loss of acetic acid $[M - AcOH]^+$ and of a hexanoate ester $[M - C_6H_{11}O_2]^+$. The ^1H and ^{13}C NMR spectra (Tables 1 and 2) were very similar to those of **1** and **2**. In the HMBC spectrum a methyl triplet (δ0.87, J = 7.4 Hz) exhibited ^2J and ^3J H–C couplings to two methylene carbons. The protons of a deshielded methylene triplet (δ2.26) also coupled to one of these methylene carbons via ^3J coupling and to another methylene carbon and a carbonyl carbon via ^2J coupling (δ_C173.5), thus supporting the presence of a hexanoate ester.

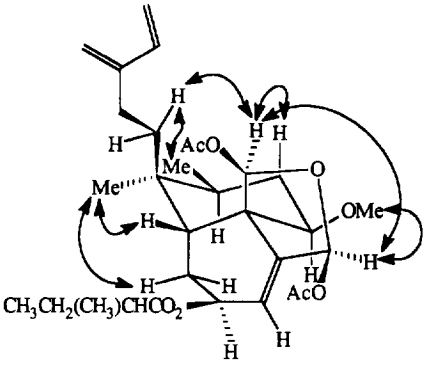

Fig. 1. NOESY interactions for compound **1**.

HMBC analysis once more confirmed the 2-substituted clerodan-18,19-acetal nucleus. The C-2 oxymethine resonance was deshielded, which was indicative of esterification at this position and the *n*-hexanoyl moiety must be placed here. Direct H–C connectivities were established by an HCCOBI experiment. The relative stereochemistry of the hexanoate ester at position C-2 was assigned as α owing to the pseudoaxial-axial coupling between the C-2 oxymethine proton and the axial proton of the C-1 methylene (7.8 Hz). This was confirmed by a NOESY interaction between H-2 and H-10. Assignment of stereochemistry at C-5, C-8, C-9, C-10, C-18 and C-19 was carried out on the basis of correlations in the NOESY experiment and all of these positions were of the same relative stereochemistry as **1** and **2**. On the above evidence **3** is assigned as the novel diterpene *rel*-18(*S*),19(*R*)-diacetoxy-18,19-epoxy-2(*R*)-hexanoyloxy-5(*R*),8(*S*),9(*S*),10(*R*)-cleroda-3,13(16),14-triene.

The GC–mass spectrum of **4** showed two major ions in the chromatogram for $[M]^+$ at *m/z* 560 ($C_{32}H_{48}O_8$) and $[M]^+$ at *m/z* 602 ($C_{35}H_{54}O_8$). The high-resolution electron impact mass spectrum gave fragments at (*m/z* 416) for $[M - C_7H_{15}CO_2H]^+$ and $[M - C_{10}H_{21}CO_2H]^+$ which corresponded to the loss of octanoic acid from $C_{32}H_{48}O_8$ and undecanoic acid from $C_{35}H_{54}O_8$. Further fragmentation included successive loss of two acetic acid molecules to give the parent diterpene. There were marked similarities in the ^1H and ^{13}C NMR spectra (Tables 1 and 2) of **4** with those of **1**–**3**. The presence of a β-hydroxy at position C-6 was inferred by a proton at δ3.83, which exhibited typical axial–axial coupling (J = 16 Hz) to H-7ax. In both compounds an ester substituent was attached to position C-2 which, in the proton domain, was deshielded when compared with position C-6. The ester at C-2 was assigned the axial (*rel*-β) orientation because of the broad nature of the C-2 oxymethine proton, its distinct lack of axial–axial coupling and the lack of any 1,3-diaxial NOESY interaction between H-2 and H-10. Stereochemistry was otherwise identical to the preceding diterpenes. Therefore, **4** is a mixture of two diterpenes with *n*-alkanoyl ester substituents of different chain lengths at position C-2 which could be assigned as *rel*-18(*S*),19(*R*)-diacetoxy-18,19-epoxy-6(*R*)-hydroxy-2(*S*)-octanoyloxy-5(*R*),8(*S*),9(*S*)10(*R*)-cleroda-3,13(16),14-triene and *rel*-18(*S*),19(*R*)-diacetoxy-18,19-epoxy-6(*R*)-hydroxy-2(*S*)-undecanoyloxy-5(*R*),8(*S*),9(*S*),10(*R*)-cleroda-3,13(16),14-triene.

GC–mass spectrometry of compound **5** in the chemical ionisation mode gave a molecular ion of *m/z* 518, which solved for $C_{29}H_{42}O_8$. Major fragments in the EI mass spectrum suggested loss of acetic acid $[M - 60]^+$ and of a C_5 ester substituent ($[M - C_4H_9CO_2H]^+$). The ^1H and ^{13}C NMR spectra (Tables 1 and 2) were very similar to those of **1** except that the methoxy was absent and from the molecular formula, its place must be taken by a hydroxyl group. Stereochemistry was identical to **1**. Compound **5** is therefore assigned as the novel isozuelanin *rel*-18(*S*),19(*R*)-diacetoxy-18,19-epoxy-6(*R*)-hydroxy-2(*S*)-(2ξ-methylbutanoyloxy)-5(*R*),8(*S*),9(*S*),10(*R*)-cleroda-3,13(16),14-triene.

The ^1H NMR spectrum of the final compound, **6**; was very similar to that of **5** differing only in the C-2 esterifying group. This ester was a hydroxyoctanoic acid (oxymethine: δ_H4.00 *bs*, δ_C68.4). In the EI mass spectrum this was confirmed by fragments at *m/z* 143 $[C_8H_{15}O_2]^+$ and at *m/z* 125 $[C_8H_{13}O]^+$ which indicated that the octanoate ester readily lost the elements of water. The oxymethine proton in the octanoate ester exhibited a ^3J coupling to the ester carbonyl (δ_C172.4) and a ^2J coupling to the α-methylene carbon, indicating that the hydroxyl should be placed at the C-3 position and that the ester substituent is 3-hydroxy-octanoate. This was confirmed by a COSY-45 experiment which showed that the oxymethine proton coupled to two methylenes.

Compound **6** is therefore assigned as the novel diterpene *rel*-18(*S*),19(*R*)-diacetoxy-18,19-epoxy-6(*R*)-hydroxy-2(*S*)-(3ξ-hydroxyoctanoyloxy)-5(*R*),8(*S*),9(*S*),10(*R*)-cleroda-3,13(16),14-triene.

EXPERIMENTAL

NMR spectra of compounds (**1**–**6**) were recorded in $CDCl_3$. Spectra run using a Bruker AMX-400 spectrometer. The HMBC experiment was set up using the Bruker pulse program inv4lplrnd set for d6 (1/2J) approximately equal to 7 Hz. Direct correlation was established using a variant of H–C COSY (HCCOBI) and the experiment gave information for direct H–C connectivity with an optimum value of J_{CH} in the order of 135 Hz. The NOESY experiment used a mixing time (d8) of 0.8 s.

Plant material. Collected from the Santa Rosa National Park in the Guanacaste Region of north Costa Rica in May 1992. A voucher specimen has been deposited at the Instituto Nacional de Biodiversidad at Heredia, Costa Rica.

Extraction and isolation of compounds. The ground bark (1000 g) was extracted in a Soxhlet with petrol (bp 60–80°). On concentration a residue of 40.5 g of oil was obtained. 10 g of this extract was fractionated by VLC over silica gel. Subsequent CPTLC of the fraction eluted with 18% EtOAc in petrol (silica gel; solvent, toluene:EtOAc, 95:5) and then further VLC (silica gel; solvent, toluene:EtOAc, 85:15) yielded **1** (445 mg). CPTLC of the fraction eluted with 30% EtOAc in petrol (silica gel; solvent, toluene:EtOAc, 90:10) led to the isolation of **2** (100 mg). MPTLC (×2) of VLC fraction eluted with 35% EtOAc in petrol (silica gel; solvent, toluene–EtOAc–AcOH, 88:10:2) gave **3** (125 mg). VLC fraction eluted with 40% EtOAc was further purified by MPTLC (×2) (silica gel; solvent, toluene–EtOAc, 92:8) to give **4** (65 mg). CPTLC of fraction eluted with 50% EtOAc in petrol (silica gel; solvent, toluene–EtOAc, 85:15) led to the isolation of **5** (95 mg). Finally, fraction eluted with 70% EtOAc in petrol was subjected to further VLC (silica gel; solvent, petrol–EtOAc, 66:34) and then MPTLC (×5) (silica gel; solvent, petrol–EtOAc, 8:2) to yield **6** (130 mg).

rel-18(S),19(R)-*diacetoxy*-18,19-*epoxy*-6(R)-*methoxy*-2(S)-(2ξ-*methylbutanoyloxy*)-5(R),8(S),9(S), 10(R)-*cleroda*-3,13(16),14-*triene* (**1**). Pale yellow oil. $[\alpha]_D$ −84

($CHCl_3$, *c* 0.11); Found: $[M]^+$ at *m/z* 532.3042, $C_{30}H_{44}O_8$ requires 532.3036; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) Tables 1 and 2; IR ν_{max}^{KBr} cm^{-1}: 3080, 3060, 2965, 2937, 2877, 1762, 1734, 1598, 1227, 1068, 961, 852, 602, 478, 385; EIMS *m/z* (rel. int): $[M]^+$ 532 (2), 489 (11), 472 (28), 430 (71), 413 (19), 371 (30), 328 (45), 296 (20), 161 (31), 85 (100).

rel-18(S),19(R)-*diacetoxy*-18,19-*epoxy*-2(S)-(2ξ-*methylbutanoyloxy*)-5(R),8(S),9(S),10(R)-*cleroda*-3,13(16),14-*triene* (**2**). Gum. $[\alpha]_D$ −6 ($CHCl_3$, *c* 0.14); Found: $[M + H]^+$ at *m/z* 503, $C_{29}H_{43}O_7$ requires 503; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) Tables 1 and 2; IR ν_{max}^{KBr} cm^{-1}: 2968, 2939, 2878, 1757, 1730, 1692, 1618, 1462, 1375, 1229, 1114, 1067, 1005, 958, 933, 888, 601, 406; GC–MS *m/z* (rel. int.): 502 (100), 444 (2), 400 (2), 399 (7), 358 (2), 356 (3), 343 (1), 342 (68), 289 (2), 203 (2), 161 (2), 102 (3), 101 (63).

rel-18(S),19(R)-*diacetoxy*-18,19-*epoxy*-2(R)-*hexanoyloxy*-5(R),8(S),9(S),10(R)-*cleroda*-3,13(16),14-*triene* (**3**). Pale yellow oil. $[\alpha]_D$ 0 ($CHCl_3$, *c* 0.14); (GC–MS (CI)): Found $[M + H]^+$ 517, $C_{30}H_{45}O_7$ requires 517. ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) Tables 1 and 2; IR ν_{max}^{KBr} cm^{-1}: 2967, 2933, 2879, 1757, 1731, 1679, 1641, 1597, 1375, 1230, 1179, 1100, 1024, 948, 544; EIMS *m/z* (rel. int.): 414 (12), 359 (15), 346 (15), 316 (53), 298 (88), 280 (19), 203 (13), 187 (100), 173 (23), 159 (35).

rel-18(S),19(R)-*diacetoxy*-18,19-*epoxy*-6(R)-*hydroxy*-2(S)-*octanoyloxy*-5(R),8(S),9(S) 10(R)-*cleroda*-3,13(16),14-*triene and rel*-18(S),19(R)-*diacetoxy*-18,19-*epoxy*-6(R)-*hydroxy*-2(S)-*undecanoyloxy*-5(R),8(S),9(S) 10(R)-*cleroda*-3,13(16),14-*triene* (**4**). Gum. $[\alpha]_D$ 0° ($CHCl_3$, *c* 0.16); Found: 560 and 602, $C_{32}H_{48}O_8$ and $C_{35}H_{54}O_8$ require 560 and 602. ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) Tables 1 and 2; IR ν_{max}^{KBr} cm^{-1}: 3300–3694 broad OH, 2961, 2928, 2733, 1756, 1735, 1639, 1375, 1239, 1169, 1070, 1051, 896, 601, 404; GC–MS *m/z* (rel. int.): $[M]^+$ 602 (3), 588 (2), 560 $[M]^+$ (1), 528 (13), 458 (5), 372 (4), 358 (6), 356 (14), 314 (34), 286 (26), 171 (100).

rel-18(S),19(R)-*diacetoxy*-18,19-*epoxy*-6(R)-*hydroxy*-2(S)-(2ξ-*methylbutanoyloxy*)-5(R),8(S),9(S), 10(R)-*cleroda*-3,13(16),14-*triene* (**5**). Pale yellow oil. $[\alpha]_D$ +13° ($CHCl_3$, *c* = 0.24); GCMS (NCI) Found: $[M - H]^-$ 517, $C_{29}H_{41}O_8$ requires 517; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) Tables 1 and 2; IR ν_{max}^{KBr} cm^{-1}: 3300–3695 broad OH, 3008, 2970, 2938, 2879, 1757, 1726, 1639, 1463, 1374, 1227, 1151, 1082, 1053, 979, 945, 601; EIMS *m/z* (rel. int.): $[M]^+$ 518 (1), 416 (12), 374 (9), 314 (26), 268 (21), 215 (24), 199 (18), 187 (100), 177 (18), 135 (95).

rel-18(S),19(R)-*diacetoxy*-18,19-*epoxy*-6(R)-*hydroxy*-2(S)-(3ξ-*hydroxyoctanoyloxy*)-5(R),8(S),9(S), 10(R)-*cleroda*-3,13(16),14-*triene* (**6**). Pale yellow oil. $[\alpha]_D$ +3° ($CHCl_3$, *c* = 0.33); Found: $[M - C_8H_{16}O_2]^+$ 416.2299, $C_{24}H_{32}O_7$ requires 416.2352; ^1H NMR (400 MHz, $CDCl_3$) and ^{13}C NMR (100 MHz, $CDCl_3$) Tables 1 and 2; IR ν_{max}^{KBr} cm^{-1}: 3236–3549 broad OH, 2960, 2927, 2877, 1753, 1639, 1618, 1375, 1230, 1172, 1002, 976, 896, 756; EIMS *m/z* (rel. int.): 555 (2), 501 (4), 416 (12), 374 (8), 356 (40), 314 (40), 239 (13), 187 (100), 136 (29).

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REFERENCES

1. Gibbons, S., Gray, A. I., Hockless, D. C. R., Lavaud, C., Nuzillard, J., Massiot, G., Waterman, P. G. and White, A. H. (1993) *Phytochemistry* **34**, 273.
2. Gibbons, S., Gray, A. I. and Waterman, P. G. (1993) *Nat. Prod. Letters* **3**, 59.
3. Gibbons, S., Gray, A. I., Waterman, P. G., Hockless, C. R., Skelton, B. W. and White, A. H. (1995) *J. Nat. Prod.* **58**, 554.
4. Talapatra, S. K., Ganguly, N. C. and Talapatra, B. (1983) *J. Nat. Prod.* **46**, 401.
5. Shaari, K. (1994) Ph.D. Thesis, Strathclyde University, Glasgow.
6. Khan, M. R., Gray, A. I., Sadler, I. H. and Waterman, P. G. (1990) *Phytochemistry* **29**, 11 3595.
7. Itokawa, H., Totsuka, N., Takeya, K., Watanabe, K. and Obata, E. (1988) *Chem. Pharm Bull.* **36**(4), 1585.
8. Guittet, E., Stoven, V., Lallemand, J.-Y., Ramiandrasoa, F., Kunesch, G. and Moretti, C. (1988) *Tetrahedron* **44**, 2893.
9. Bax, Ad and Summers, M. F. (1986) *J. Am. Chem. Soc.*, **108**, 2093.
10. Pelletier, S. W., Chokshi, H. P. and Desai, H. K. (1986) *J. Nat. Prod.* **49**, 893.
11. Khan, M. R., Gray, A. I., Reed, D. R., Sadler, I. H., Waterman, P. G. (1990) *Phytochemistry* **29**, 5 1609.
12. Chen, T. and Wiemer, D. F. (1991) *J. Nat. Prod.* **54**(6), 1612.