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Rearranged (4 → 2)-*abeo*-clerodane and clerodane diterpenes from *Aristolochia chamissonis*

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Abstract

From stems of *Aristolochia chamissonis* Duch., five new diterpenes, one rearranged (4 → 2)-*abeo*-clerodane and four *ent*-clerodanes, were isolated. Their structures were determined to be (+)-(4 → 2)-*abeo*-kolavelool-3-oic acid, (−)-13-*epi*-2-oxokolavelool, (−)-2β-hydroxykolavelool, (−)-2β-hydroperoxykolavelool and (+)-13-*epi*-2α-hydroxykolavelool. In addition, lignans, sesquiterpenes, steroids and two known *ent*-clerodane diterpenes were isolated.

Keywords: *Aristolochia chamissonis*; Aristolochiaceae; Diterpene; Clerodane; Rearranged (4 → 2)-*abeo*-clerodane; Lignans; Sesquiterpenes; Sitosterol; Stigmastenone

1. Introduction

In continuation of our investigation of the chemistry of Brazilian *Aristolochia* species, we have examined the constituents of the stems of the *Aristolochia chamissonis* Duch. (Aristolochiaceae). This study led to the characterisation of one rearranged (4 → 2)-*abeo*-clerodane diterpene and six clerodane diterpenes, i.e. (−)-kolavelool (**1**), (−)-3α,4β-dihydroxykolavelool (**2**), (−)-13-*epi*-2-oxokolavelool (13-*epi*-roseostachenone, **3**), (−)-2β-hydroxykolavelool (**4**), (+)-13-*epi*-2α-hydroxykolavelool (13-*epi*-roseostachenol, **5**), (−)-2β-hydroperoxykolavelool (**6**) and (+)-(4 → 2)-*abeo*-kolavelool-3-oic acid (**7**). The last five, showing 13*R* configurations, were hitherto unreported in the literature. From these diterpenes, only kolavelool was previously isolated from the Aristolochiaceae (Lopes & Bolzani, 1988; Leitão, Kaplan & Galeffi, 1992). In addition, (+)-spathulenol, (+)-β-cariophyllene-8,9-epoxy, (−)-cubebin, and (−)-hinokinin were isolated, together with sitosterol and 3β-hydroxystigmast-5-en-7-one. The occurrence of this last compound in the family has been reported only from *A. indica* (Achari, Chakrabarty & Pakrashi, 1981). The structural elucidation of these new compounds is discussed.

2. Results and discussion

From the hexane extract of stems, fractionated by chromatographic column, followed by preparative TLC and/or recrystallization, thirteen compounds were isolated. The known compounds were identified by comparison of their physical and spectroscopic (IR, UV, MS, ^{1}H and ^{13}C NMR) data with those reported in the literature (Lopes & Bolzani, 1988; Wu & Asakawa, 1988; Meragelman, Espinar, Sosa, Uriburu & De La Fuente, 1996; Iwabuchi, Yoshikura & Kamisako, 1989; Barrero, Molina, Oltra, Altarejos, Barragán, Lara et al., 1995; Koul, Taneja, Dhar & Atal, 1983; Lopes, Bolzani & Trevisan, 1988; Honda, Kimura, Sato, Kato & Tominaga, 1994; Nes, Norton & Benson, 1992; Guerriero, D'Ambrosio & Pietra, 1993).

From the spectroscopic data for the diterpene **3**, it was possible to identify its constitution as that of roseostachenone, already isolated from *Stachys rosea* (Fazio, Passannanti, Paternostro & Piozzi, 1992) (Table 1 and Table 2). However, the melting point (154–155°) and $[\alpha]_D$ (−25°) observed for **3** were different from those previously reported of 121° and −7.9°, respectively (Fazio et al., 1992). This led us to postulate that **3** could be the 13-*epi* isomer of (−)-roseostachenone. Previously (−)-roseostachenone had its

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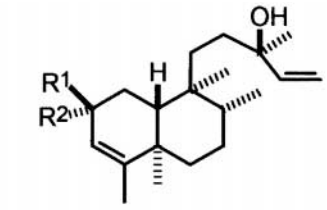
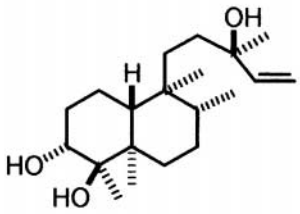


	R1	R2
1	H	H
3	=O	
4	OH	H
5	H	OH
6	OOH	H



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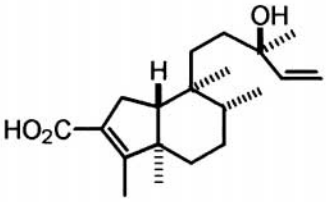

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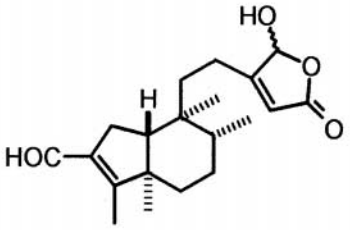

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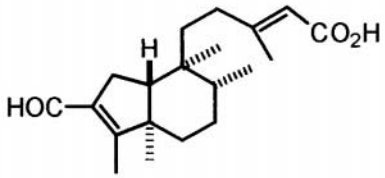

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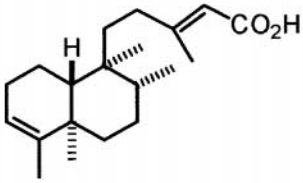

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absolute configuration established by CD and ORD methods as being 5*R*, 8*R*, 9*S*, and 10*R*. Although the configuration of C-13 remains undetermined, it was suggested to be 13*S*, by analogy with others constituents isolated from the same plant (Fazio, Paternostro, Passannanti & Piozzi, 1994). The relative configuration of **3** was established by the X-ray analysis (Fig. 1 and Section 3, Experimental). The CD curve of **3** showed three maxima at 326.0 nm ($\Delta\epsilon$ −28.8), 257.4 nm ($\Delta\epsilon$ +37.8) and 208.8 nm ($\Delta\epsilon$ −94.0). By applying the reversed octant rule (Snatzke, 1965), the stereochemistry of C-5 and C-10 could be deduced as being 5*R*, 10*R*. This is in agreement with the literature data for *ent*-clerodanes (Fazio et al., 1994; Teresa, Urones, Herrero, De LaMano & Benito, 1978). We have named this diterpene (−)-13-*epi*-2-oxokolavelool by analogy to the other compounds previously discussed.

The ^{1}H and ^{13}C NMR spectra of **4** and **5** were very similar to those of **1** (Tables 3 and 4); the main difference was due to a hydroxyl group at C-2. This was evidenced by signals for carbinolic carbons (**4**: δ 65.6, **5**: δ 69.5) and protons (**4**: δ 4.15, **5**: δ 4.18) as well as by the lack of the signals corresponding to one methylene (CH_2-2). The chemical shifts observed for methyl groups at **A**, **B** rings suggested a *trans* clerodane structures as presented for **1**. However, the chemical shifts of CH-2, CH-3, and CH-10 showed significant differences, suggesting that the stereochemistry of the chiral center C-2 should be opposite. It is known that *trans* clerodanes with a β-OH group at C-2 show a coupling constant for the carbinolic proton ($W_{1/2H\text{-}2\alpha}$∼8–10 Hz) smaller than α-OH ($W_{1/2H\text{-}2\beta}$∼17–20 Hz) (Fazio et al., 1994; Khan, Gray, Reed, Sadler & Waterman, 1990). Thus, we could infer a β-OH group in **4** and an α-OH

Table 1
^{1}H NMR spectral data for compounds **2**, **3**, **7** and **8** (200 MHz, $CDCl_3$)

H	**2***	**3***	**7***	**8** (Kijjoa et al., 1990)
1		2.28 *m*		
3	3.56 *t* (2.7)	5.66 *d* (1.2)		9.89 *s*
14	5.90 *dd* (17.4, 10.7)	5.82 *dd* (17.4, 10.7)	5.82 *dd* (17.4, 10.7)	
15	5.18 *dd* (17.4, 1.2)	5.14 *dd* (17.4, 1.3)	5.13 *dd* (17.4, 1.3)	–
	4.96 *dd* (10.7, 1.2)	5.03 *dd* (10.7, 1.3)	5.00 *dd* (10.7, 1.3)	
16	1.24 *s*	1.22 *s*	1.18 *s*	6.04, 5.96 *br s*
17	0.75 *d* (5.9)	0.79 *d* (6.8)	0.71 *d* (5.9)	0.77 *d* (7.0)
18	1.22 *s*	1.83 *d* (1.1)	1.93 *br s*	2.04 *br s*
19	1.10 *s*	1.06 *s*	0.81 *s*	0.93 *s*
20	0.72 *s*	0.76 *s*	0.77 *s*	0.89 *s*

* Assignments were made with the assistance of ^{1}H–^{13}C COSY spectroscopy.

in **5**. This was confirmed by comparison of the ^{13}C NMR of both compounds. The –OH at the pseudoequatorial position deshielded more C-2 of **5** than the pseudoaxial of **4** ($\Delta\delta$ = 3.9). Moreover, when the –OH was pseudoaxial, a γ effect was observed at C-10 ($\Delta\delta$ = 4.8). Thus, **4** was 2β-hydroxykolavelool, the C-2 epimer of **5**. The correlations observed between the protons and carbons of the methyl groups by ^{1}H–^{13}C COSY led us to interchange the chemical shifts of C-18, C-19, and C-20 in relation to those previously reported (Fazio et al., 1994) for **5**. It is interesting to notice that **5** is unstable, being rapidly transformed into **3** under storage, and that the mass spectra of **5** and **3** were very similar. So, **5** was identified as being 2α-hydroxykolavelool, earlier described in the literature as roseostachenol, whose absolute configuration was again proven except at C-13 (Fazio et al., 1994). Based on the transformation of **5** into **3** we established the absolute configuration of **5** as 2*R*, 5*R*, 8*R*, 9*S*, 10*R*, and 13*R*.

Table 2
^{13}C NMR spectral data for compounds **3**, **7** and **8** ($CDCl_3$, 50 MHz)

C	**3***	**7***	**8** (Kijjoa et al., 1990)
1	34.3 *t* [a]	29.4 *t*	29.1 *t*
2	200.5 *s*	125.6 *s*	136.9 *s*
3	127.4 *d*	171.0 *s*	189.1 *d*
4	172.6 *s*	168.7 *s*	169.9 *s*
5	39.7 *s*	50.6 *s*	50.9 *s*
6	35.5 *t*	34.4 *t*	36.6 *t*
7	26.8 *t*	28.3 *t*	26.0 *t*
8	35.8 *d*	37.1 *d*	37.3 *d*
9	38.3 *s*	37.5 *s*	37.9 *s*
10	45.5 *d*	53.9 *d*	53.8 *d*
11	31.1 *t*	33.5 *t*	33.9 *t*
12	34.7 *t* [a]	35.5 *t*	21.7 *t*
13	73.0 *s*	73.0 *s*	171.2 *s*
14	144.8 *d*	144.9 *d*	117.1 *d*, 116.8 *d*
15	111.9 *t*	111.9 *t*	173.0 *s*, 173.1 *s*
16	27.7 *q*	27.8 *q*	99.3 *d*, 98.6 *d*
17	15.6 *q*	15.0 *q*	15.1 *q*
18	18.9 *q* [b]	11.7 *q*	9.7 *q*
19	18.2 *q* [b]	16.9 *q*	17.1 *q*
20	17.9 *q* [b]	18.2 *q*	17.7 *q*

* Multiplicity was established by DEPT pulse sequence.
[a,b,c] Assignments within the same column may be interchanged.

The main difference observed between **4** and **6** by ^{1}H and ^{13}C NMR spectroscopy was due to the presence of a hydroperoxy group at C-2 instead of an hydroxyl group (Tables 3 and 4). The last diterpene was characterised by signals at δ 4.40 (*m*) and at δ 10.19 (*br s*), the latter disappearing upon addition of D_2O, by the deshielded carbinolic carbon ($\delta_{C\text{-}2}$: 79.2), and by γ effect on C-3 ($\delta_{C\text{-}3}$: 116.7) and C-10 ($\delta_{C\text{-}10}$: 40.4). The γ effect on C-10 was analogous to that previously observed for **4**, which also led to the establishment of the β-OOH configuration at C-2. The ESI–MS spectrum of **6** displayed an $[M + Na]^+$ at *m*/*z* 345 (for $C_{20}H_{34}O_3$) along with the fragment ions at *m*/*z* 286 $[M - 2H_2O]^+$ and 205 $[M - H_2O$-side chain$]^+$ which

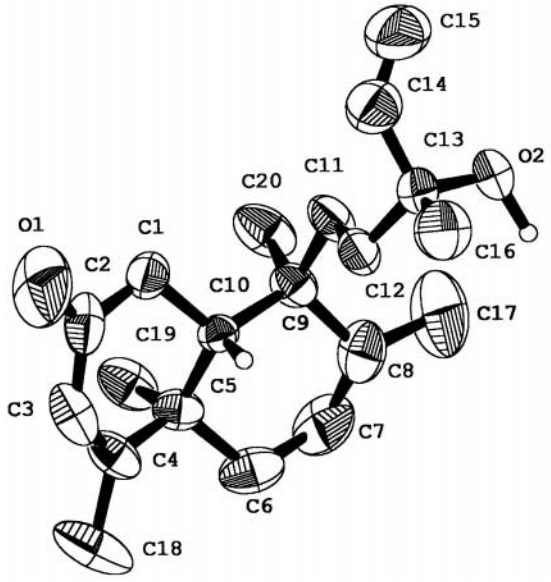

Fig. 1. ZORTEP (Zsolnai & Pritzkow, 1996) drawing of compound **3** and atom labels. Displacement ellipsoids are shown at the 50% probability level.

Table 3
^{1}H NMR spectral data for compounds **1** and **4**–**6** ($CDCl_3$, 200 MHz)

H	1	4	5	6*
1				2.20 *br d* (16.2) 1.45 *dd* (16.2, 3.4)
2		4.15 *m* ($W_{1/2}$ 11)	4.18 *m* ($W_{1/2}$ 20)	4.40 *m* ($W_{1/2}$ 11)
3	5.16 *s*	5.33 *m*	5.19 *m*	5.20 *m*
6				1.35 *m*, 1.63 *m*
7				1.38 *m*
8				1.35 *m*
10				1.70 *m*
11				1.40 *m*, 1.70 *m*
12				1.65 *m*
14	5.84 *dd* (17.2, 10.8)	5.93 *dd* (17.4, 10.7)	5.88 *dd* (17.4, 10.7)	6.01 *dd* (17.3, 10.8)
15	5.07 *dd* (17.2, 1.5) 4.99 *dd* (10.8, 1.5)	5.17 *dd* (17.4, 1.4) 4.98 *dd* (10.7, 1.4)	5.19 *dd* (17.4, 1.4) 5.06 *dd* (10.7, 1.4)	5.22 *dd* (17.3, 1.0) 5.00 *dd* (10.8, 1.0)
16	1.24 *s*	1.22 *s*	1.27 *s*	1.31 *s*
17	0.75 *d* (5.9)	0.79 *d* (6.2)	0.77 *d* (5.9)	0.81 *d* (5.3)
18	1.54 *d* (1.5)	1.62 *t* (1.2)	1.60 *t* (1.5)	1.63 *t* (1.4)
19	0.95 *s*	0.94 *s*	0.97 *s*	0.97 *s*
20	0.68 *s*	0.73 *s*	0.73 *s*	0.78 *s*
OOH				10.19 *br s*

* Assignments were made with the assistance of ^{1}H–^{13}C COSY spectroscopy.

are in agreement with the proposed structure **6** for this new compound. As earlier reported in the literature (Lopes, Bolzani & Trevisan, 1987), this kind of hydroperoxy is not stable, and **6** is easily transformed into its 2-oxo derivative.

Compound **7** exhibited a molecular ion at *m/z* 320, analysing for $C_{20}H_{32}O_3$. This mass spectrum supported the presence of the same side chain observed for **1**–**6** by the presence of the fragment ions at *m/z* 221, 220 and 219 arising from the loss of that side chain. The IR spectrum displayed a characteristic broad band absorption for hydroxyl (3270 cm^{-1}) and a carbonyl stretching band (1692 cm^{-1}) in an α,β-unsaturated carboxylic acid. Comparing the ^{13}C NMR spectrum of **7** with those discussed previously (Tables 2 and 4), we could observe that it showed signals attributable to the side chain; signals for four methyl groups, one of them upfield (δ 11.7); two deshielded quaternary carbons (δ

Table 4
^{13}C NMR spectral data for compounds **1**, **2** and **4**–**6** ($CDCl_3$, 50 MHz)*

C	1	2	4	5	5 (Fazio et al., 1994)†	6
1	18.2 *t*	16.2 *t*	27.3 *t*[a]	28.9 *t*	28.8 *t*	22.0 *t*
2	27.4 *t*	30.3 *t*	65.6 *d*	69.5 *d*	69.3 *d*	79.2 *d*
3	120.4 *d*	76.2 *d*	122.1 *d*	124.4 *d*	124.4 *d*	116.7 *d*
4	144.5 *s*	76.5 *s*	150.1 *s*	147.9 *s*	147.4 *s*	155.0 *s*
5	38.1 *s*[a]	38.3 *s*	38.0 *s*	38.0 *s*	37.6 *s*	37.8 *s*
6	36.8 *t*[b]	32.4 *t*[a]	36.4 *t*	36.5 *t*	36.4 *t*	33.2 *t*[a]
7	26.8 *t*	26.4 *t*	27.2 *t*[a]	27.2 *t*	27.1 *t*	27.1 *t*
8	36.1 *d*	36.0 *d*	36.3 *d*	35.9 *d*	35.8 *d*	36.4 *d*
9	38.3 *s*[a]	41.2 *s*	38.9 *s*	38.6 *s*	38.4 *s*	39.1 *s*
10	46.3 *d*	40.7 *d*	40.4 *d*	45.2 *d*	45.1 *d*	40.4 *d*
11	31.8 *t*	32.2 *t*[a]	31.0 *t*	31.8 *t*	31.7 *t*	30.7 *t*
12	35.3 *t*[b]	35.4 *t*	36.4 *t*	35.2 *t*	35.1 *t*	36.1 *t*[a]
13	73.4 *s*	73.5 *s*	73.2 *s*	73.3 *s*	73.3 *s*	73.9 *s*
14	145.1 *d*	145.0 *d*	146.4 *d*	145.0 *d*	144.9 *d*	146.3 *d*
15	111.8 *t*	111.6 *t*	110.9 *t*	111.9 *t*	111.9 *t*	111.2 *t*
16	27.7 *q*	27.4 *q*	26.3 *q*	27.7 *q*	27.8 *q*	25.0 *q*
17	15.9 *q*	15.9 *q*	15.8 *q*	15.9 *q*	15.9 *q*	15.6 *q*
18	18.0 *q*[c]	21.3 *q*	17.9 *q*	17.7 *q*	19.8 *q*	18.3 *q*
19	19.8 *q*[c]	17.2 *q*	18.3 *q*	19.9 *q*	18.5 *q*	18.1 *q*
20	18.4 *q*[c]	18.5 *q*	18.3 *q*	18.5 *q*	17.7 *q*	18.4 *q*

* Multiplicity was established by DEPT pulse sequence.
[a–c] Assignments within the same column may be interchanged.
† ^{13}C NMR 62.9 MHz.

50.6 and δ 168.7), and one CH (δ 53.9) also deshielded. Moreover, the olefinic CH-3 was not observed. The multiplicity of carbons, established by DEPT experiments, and the correlation observed between the protons and carbons by ^{1}H–^{13}C COSY suggested a rearranged skeleton. The comparison of the NMR data of **1** with those published for **8** (Kijjoa, Pinto, Pinho, Tantisewie & Herz, 1990) (Tables 1 and 2), and for the methyl ester derivative of **9** (Bohlmann, Singh, Singh, Joshi & Jakupovic, 1985), supported the (4 → 2) rearranged structure.

Taking into account the optical rotation signal, the configurations established by NMR, CD and X-ray data, the transformations of **5** and **6** into **3**, as well as the isolation of **1**–**7** from the same plant, we could infer that the diterpenes isolated (**1**–**7**) belong to the same *ent*-clerodane series, with 5*R*, 8*R*, 9*S*, 10*R* and 13*R* configurations. It has been suggested (Bohlmann et al., 1985) that solidagonal acid (**9**) could be formed from kolavenic acid (**10**) via oxidative cleavage followed by aldol condensation, or via a rearrangement of 2-hydroxy-3,4-epoxy kolavenic acid. Due to the co-occurrence of **2** and **7**, the first pathway seemed to fit better to the formation of **7**, since the yielded rearranged aldehyde could be oxidised into the corresponding carboxylic acid.

3. Experimental

3.1. General

The NMR spectra were measured on a Bruker spectrometer, ^{1}H NMR, and ^{1}H–^{1}H COSY spectra were obtained at 200 MHz; ^{13}C NMR and DEPT were taken at 50 MHz; ^{1}H–^{13}C COSY were optimised for J = 7 Hz and 145 Hz. The mass spectra were obtained on an ITD 800 Finnigan MAT (ion trap detector) spectrometer and on a Fisons Platform II by flow injection into the electrospray source. The instrument was operated in the positive ion mode. The mobile phase carrier was a CH_3CN–H_2O (1:1). The carrier was pumped into the source at a flow rate of 10 µl min^{-1}. Data acquisition was obtained by scanning from to 200–450 u/e in 0.8 second scans. The samples were dissolved in MeOH. IR: KBr discs. The UV absorption was measured in a Hewlett Packard 8452A, Diode arrays spectrophotometer. TLC: Silica gel 60 PF_{254}.

3.2. Plant material

The botanical material was identified as *Aristolochia chamissonis* Duch., var. *paulistana* Hoehne, by Dr. Condorcet Aranha and a voucher specimen was deposited at the herbarium of the Instituto Agronômico de Campinas, Campinas, SP, Brazil. The stems (1400 g) of *A. chamissonis*, collected in São Joaquim da Barra, SP, were dried at 45°, ground and extracted exhaustively at room temp. for three days with hexane, Me_2CO and EtOH successively and then conc.

3.3. Isolation

The hexane extract (2.6 g) was fractionated by CC (silica gel, 75 g, hexane–EtOAc–MeOH) affording 9 fractions. The frs. 2, 4, 6 yielded sitosterol (5.5 mg), (−)-cubebin (12.6 mg), and 3β-hydroxystigmast-5-en-7-one (20.4 mg), respectively. The fr. 1 by prep. TLC, toluene–EtOAc (19:1) yielded **1** (33.0 mg), **3** (64.8 mg), **6** (19.1 mg), (+)-spathulenol (33.9 mg) and (+)-β-cariophyllene-8,9-epoxy (4.0 mg). The fr. 3 by prep. TLC, hexane–EtOAc (4:1) 5× eluted, gave (−)-hinokinin (4.0 mg). The fr. 5 and 7 by prep. TLC, toluene–EtOAc (7:3) 3× eluted, yielded **4** (9.0 mg) and **5** (25.8 mg). The fr. 8 by prep. TLC, $CHCl_3$–MeOH (19:1) afforded **7** (5.0 mg) and fr. 9 using the same eluant system, $AgNO_3$–silica gel (1:99), afforded **2** (18.0 mg).

3.3.1. 13-epi-2-oxo-kolavelool (*3*)

(Found: C, 78.8; H, 10.6. $C_{20}H_{32}O_2$ requires: C, 78.9; H, 10.5%). Colourless amorphous solid, mp 158–159° (hexane), lit. (Fazio et al., 1994) 121° (EtOH). $[\alpha]_D^{25}$ −25.0° ($CHCl_3$; *c* 0.10), lit. (Fazio et al., 1994) −7.9° (EtOH; *c* 0.50). CD nm ($\Delta\epsilon$) 326 (−28.8), 257 (+35.8), 208.8 (−94.0) (MeOH; c 0.2). IR ν_{max}^{KBr} cm^{-1}: 3437, 1658, 1453, 1377, 1281, 995, 916. EIMS: Comparable with lit. values (Fazio et al., 1994). ^{1}H NMR: see Table 1, ^{13}C NMR: see Table 2.

3.3.2. X-ray analysis of diterpene *3*

An irregular colourless crystal of 0.15 × 0.30 mm ($C_{20}H_{32}O_2$), obtained by recrystallization from hexane, was mounted at random on an CAD 4 Mach3 Enraf–Nonius diffractometer. Cell dimensions were determined by least-squares fitting of the setting angles of 25 reflection ($10 < \theta < 17°$). Intensity measurements were carried out up to θ = 25.5°, using the $\theta/2\theta$ scan mode and graphite monochromated Mo–Kα radiation. The crystal data are: a = 6.4265(5), b = 13.763(1), c = 21108(3) Å, V = 1867.0(3) Å^3, space group $P2_12_12_1$, Z = 4, d_x = 1.083 g cm^{-3}, 2024 reflections were measured, 1123 with $I \geq 4^*\sigma(I)$ were considered observed. Lorentz and polarization corrections were applied, but not absorption corrections [μ(Mo–Kα) = 0.067 mm^{-1}]. The intensities of three standard reflections varied about 0.5% throughout the experiment. The structure was solved by direct methods (Sheldrick, 1986). An E-map based on the solution with the best combined figure of merit revealed the positions of most of the non-hydrogen atoms. The rest

of the structure was obtained by difference Fourier calculations. The H-atoms were located on stereochemical grounds, except that on the hydroxyl moiety. H atoms were refined with fixed geometry, each riding on a carrier atom, with an isotropic displacement parameter amounting to 1.5 (for methyl H atoms) or 1.2 (for the other H atoms) times the value of the equivalent isotropic displacement parameter of the atom they are attached, non-H atoms were refined anisotropically. Refinements was carried on F^2 (Sheldrick, 1993) for all reflection, until atomic-parameter shifts were smaller than their standard deviations. The final unweighted R factor omitting unobserved reflections was 0.0418 and the goodness of fit S based on F^2 was of 1.048. The atomic scattering factors used were those included in Sheldrick's program (Sheldrick, 1993). In the crystalline state, the molecules are linked through an hydrogen bond: O-2...O-1^i = 2.850(4) Å, HO-2...O-1^i = 1.788(4) Å, O-2–HO-2...O-1^i = 168.2(3)° (symmetry operation: $i = 2 - x$, $0.5 + y$, $1.5 - z$). Atomic coordinates, bond lengths and angles, and thermal parameters have been deposited at the Cambridge Crystallographic Data Center, U.K. (102469).

*3.3.3. (−)-2β-hydroxykolavelool (**4**)*

(Found: C, 78.5; H, 11.0. $C_{20}H_{34}O_2$ requires: C, 78.4; H, 11.1%). Colourless amorphous solid. $[\alpha]_D^{25}$ −38.5° ($CHCl_3$; *c* 0.09). IR ν_{max}^{KBr} cm^{-1}: 3423, 1639, 1458, 1382, 1024, 920. Positive ESI–MS *m/z* (rel. int.): 329 $[M + Na]^+$ (100). EIMS *m/z* (rel. int.): 273 $[M - H_2O - CH_3]^+$ (5), 205 $[M - H_2O$-side chain$]^+$ (100), 189 (41), 123 (57), 105 (43), 95 (35), 81 (34), 69 (44), 55 (51). ^1H NMR: see Table 3, ^{13}C NMR: see Table 4.

*3.3.4. (+)-2α-hydroxykolavelool (13-epi-roseostachenol, **5**)*

(Found: C, 78.5; H, 11.0. $C_{20}H_{34}O_2$ requires: C, 78.4; H, 11.1%). Colourless amorphous solid. $[\alpha]_D^{25}$ +10.0° ($CHCl_3$; *c* 0.10). ^1H NMR: see Table 3, ^{13}C NMR: see Table 4.

*3.3.5. (−)-2β-hydroperoxykolavelool (**6**)*

(Found: C, 74.3; H, 10.6. $C_{20}H_{34}O_3$ requires: C, 74.5; H, 10.6%). Colourless amorphous solid. $[\alpha]_D^{25}$ −20.0° ($CHCl_3$; *c* 0.10). IR ν_{max}^{KBr} cm^{-1}: 3436, 1653, 1457, 1384, 1094, 1022, 918. Positive ESI–MS *m/z* (rel. int.): 345 $[M + Na]^+$ (25), 327 $[M - H_2O + Na]^+$ (100), 286 $[M - 2H_2O]^+$ (80), 205 $[M - H_2O$-side chain$]^+$ (15). EIMS *m/z* (rel. int.): 286 $[M - 2H_2O]^+$ (5), 205 (62), 189 (45), 135 (49), 123 (54), 121 (100), 119 (59), 109 (70), 95 (69), 81 (62), 69 (50), 55 (77). ^1H NMR: see Table 3, ^{13}C NMR: see Table 4.

3.3.6. (+)-(4 → 2)-abeo-kolavelool-3-oic acid (7)

(Found: C, 74.2; H, 10.6. $C_{20}H_{34}O_3$ requires: C, 74.5; H, 10.6%). Colourless amorphous solid. $[\alpha]_D^{25}$ +23.8° ($CHCl_3$; *c* 0.04). IR ν_{max}^{KBr} cm^{-1}: 3428, 3270, 1692, 1547, 1461, 1378, 1249, 1070, 920. Positive ESI–MS *m/z* (rel. int.): 343 $[M + Na]^+$ (100). EIMS *m/z* (rel. int.): 320 $[M]^+$ (<1), 302 $[M - H_2O]^+$ (6), 287 (20), 220 (46), 219 (100), 205 (38), 175 (36), 164 (40), 151 (40), 133 (31), 123 (50), 121 (42), 109 (40), 107 (47), 95 (49), 91 (53), 81 (47), 71 (57), 55 (59). ^1H NMR: see Table 1, ^{13}C NMR: see Table 2.

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