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NEW DITERPENOIDS FROM *CROTON ARGYROPHYLLOIDES**

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Key Word Index—*Croton argyrophylloides*; Euphorbiaceae; new diterpenoids; ^{1}H and ^{13}C NMR.

Abstract—Two new diterpenoids, *ent*-Kaur-16-en-15-oxo-18-oic acid and 3, 12-dioxo-15,16-epoxy-4-hydroxycleroda-13 (16),14-diene, were isolated together with other known compounds from *Croton argyrophylloides*. Structural determinations were made by spectrometric data and chemical reactions.

INTRODUCTION

In the continuation of our chemical investigation of *Croton argyrophylloides*, (Euphorbiaceae), a small shrub very common in the hinterland of the semi-arid regions of Brasil. We have isolated further constitutents from this species. Previous work on the trunk wood has yeilded the tetracyclic diterpenes **1** and **1a** [1]. The present paper deals with the identification of two kauren acids (**2** and **3**) and one clerodene (**7**) diterpene.

RESULTS AND DISCUSSION

The spectral properties of **2**, $C_{20}H_{30}O_2$ ($[M]^+$ *m/z* 302) showed it to be kaur-16-en-18-oic acid which has been isolated previously [2]. Further confirmation of its identity was obtained by comparison of its ^{13}C NMR spectrum with other related diterpenoids (Table 1).

The new diterpene **3**, analysed for $C_{20}H_{28}O_3$ by both mass spectral ($[M]^+$ *m/z* 316) and ^{13}C NMR (Table 1) methods and gave an ^{1}H NMR spectrum typical of a kauren skeleton with two tertiary methyl groups [δ 1.16 (*s*) and 1.12 (*s*): Me-4 and Me-10] and an exocyclic double bond (δ 5.96 (*br*), H-17a; 5.26 (*br*), H-17b] in conjugation with a carbonyl group. The IR spectrum exhibited absorption bands at 3500–2500 and 1685 (COOH), 1640 and 890 (C=CH_2) and 1718 cm^{-1} (C=O in 16-en-15-one system in kauren skeleton). Furthermore, it was possible to assign unambiguously the α,β-unsaturated carbonyl system in **3** by 1-pyrazoline (**3c**) formation using the well-known reaction of diazomethane with a conjugated carbonyl system [1].

The assignment of an equatorial-position for the carboxyl group at C-4 was deduced from the chemical shifts of C-4 and C-5 (δ 47.6 and 49.3, respectively). One axial carboxyl function at C-4 is indicated by ^{13}C NMR absorptions (e.g. **5**) at δ 43.7 (*s*, C-4) and δ 56.0 (*d*, C-5) [3]. This comparative analysis showed the expected shielding γ-effect on the C-5 by the oxygenated substituent at C-4 of epimer **3**. The assignments of the remaining signals in the ^{13}C NMR spectrum of **3** were made on the basis of the observed multiplicities (SFORD or APT), empirical shift rules [4] and comparison with data from model compounds **5** and **6** (Table 1). The stereochemistry of the carboxyl functions at C-4 of **3** was also revealed by ^{1}H NMR absorptions of the methyl groups Me-4 and Me-10 (**3**: δ 1.16 and 1.13, $\Delta\delta$ = 0.03; **5**: δ 1.27 and 1.03, $\Delta\delta$ = 0.24). On the other hand, different results were observed in the comparison between the chemicals shifts of

Table 1. ^{13}C NMR chemical shifts data of diterpenoids **2**, **3** and **5**, **6** [3]

C	**2**	**6**	**3**	**5**
1	39.9	40.7	38.7	39.9
2	18.0	19.1	18.0	18.8
3	37.0	37.8	36.8	37.6
4	47.6	43.8	47.6	43.7
5	50.0	57.1	49.3	56.0
6	23.3	21.8	21.7	20.0
7	40.7	41.3	33.0	32.3
8	44.4	44.2	52.7	52.5
9	56.2	55.1	52.4	51.6
10	39.8	39.7	39.5	40.3
11	18.0	18.4	17.8	18.4
12	33.3	33.1	32.3	33.6
13	44.0	43.9	38.1	38.1
14	39.5	39.7	36.6	36.5
15	49.1	49.0	210.4	210.7
16	155.3	155.9	149.3	149.5
17	103.2	103.0	114.8	114.5
18	185.0	29.0	185.0	28.9
19	17.8	184.6	17.5	184.4
20	16.1	15.6	16.1	15.6

The spectra of **2** and **3** were obtained at 25.2 MHz in the Fourier transform mode in $CDCl_3$ solns. The δ values are in ppm downfield from TMS.

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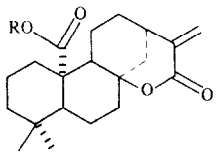
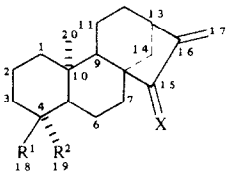
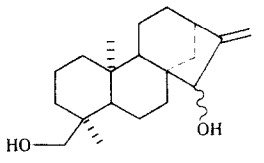


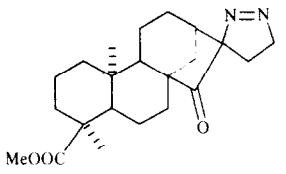
1 R = H
1a R = Me



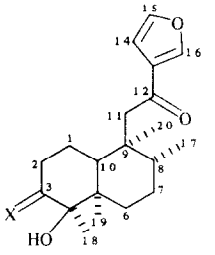
2 X = H,H; R^1 = COOH; R^2 = Me
2a X = H,H; R^1 = COOMe; R^2 = Me
3 X = O; R^1 = COOH; R^2 = Me
3a X = O; R^1 = COOMe; R^2 = Me
4 X = H,OH; R^1 = Me; R^2 = CH_2OH
5 X = O; R^1 = Me; R^2 = COOH
6 X = H,H; R^1 = Me; R^2 = COOH



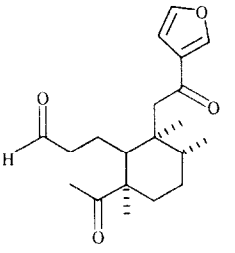
3b



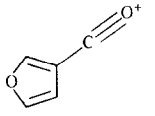
3c



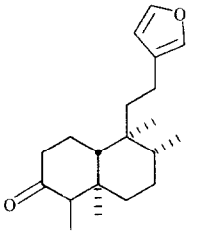
7 X = O
7a X = H,OH
7b X = H,OAc



7c



8 *m/z* 95 (67 %)



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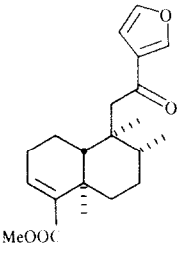

10

these methyl groups of **3b** (δ 1.12 and 0.74, $\Delta\delta = 0.38$), derivative prepared by lithium aluminium hydride reduction of **3a**, and **4** (δ 1.05 and 0.99, $\Delta\delta = 0.06$), also useful for the stereochemistry assignment at C-4 in other terpenoids.

The new clerodane diterpenoid **7** has a molecular formula of $C_{20}H_{28}O_4$, deduced by both mass spectral ($[M]^+$ *m/z* 332) and ^{13}C NMR (Table 2) methods. Its IR spectrum revealed the presence of a hydroxyl group (3420 cm^{-1}) and two carbonyl functions [1680 and 1645 (α,β-unsaturated) cm^{-1}]. The ^1H NMR spectrum showed signals for a secondary methyl group (δ 0.90, *d*, *J* = 7 Hz), a β-substituted furan ring (δ 8.05, H-16; 7.47 H-15; 6.80, H-14) in conjugation with a carbonyl function (ν_{co} 1645 cm^{-1}; δ_{co} 195.0) placed at the C-12 position and three tertiary methyl groups (δ 1.36, *s*, 3H; 0.86 *s*, 3H; 0.82, *s*, 3H). The peak at *m/z* 95 (**8**, 67%) in the mass spectrum was also used to assign the conjugated system involving the furan ring and the carbonyl function at C-12.

These data and the ^{13}C NMR spectra of **7** (Table 2) provided strong support for the structure and stereochemistry proposed. The assignments were based on the application of the usual shift parameters, comparison with literature data [5–10] and observed multiplicities of signals (Table 2). The assignment of an axial-position for the hydroxyl group at C-4 was deduced by chemical shifts of C-2, C-6, and C-10 (δ 36.1, 31.1 and 42.5, respectively) when compared with those of model compounds **9** [δ 39.4 (C-2), 41.5 (C-6) and 49.0 (C-10)] and **10** (δ 35.8 (C-6) and 47.6 (C-10)] [10]. These chemical shifts demonstrated the expected shielding γ-effect of the hydroxyl group at C-4 of **7**. The presence of hydroxyl group at C-4 of **7** was confirmed by chemical reactions. The natural diterpene **7** was treated with sodium borohydride (MeOH, 5°, 15 min) to give **7a**, which on acetylation with Ac_2O/pyridine yielded the monoacetate **7b**. On treatment with $NaIO_4$ (H_2O/EtOH, 25°, 48 hr), **7a** gave **7c**.

The structure and stereochemistry proposed for this

Table 2. ^{13}C NMR chemical shift data of clerodane diterpenoids **7**, and **9**, **10** [10]

C	7	9	10*
1	23.7	23.2	
2	36.1	39.4	
3	215.0	216.6	
4	81.5	58.2	
5	45.2	41.8	
6	31.1	41.5	35.8
7	26.8	27.4	27.7
8	37.4	36.6	38.4
9	42.0	39.4	42.4
10	42.5	49.0	47.6
11	47.0	38.6	47.6
12	195.0	18.1	193.8
13	129.7	125.4	130.4
14	108.7	110.9	109.1
15	144.4	138.5	144.2
16	146.8	142.3	146.8
17	14.9	14.4	
18	21.8	6.8	
19	16.5	15.8	
20	17.8	18.3	

The spectrum of **7** was obtained at 25.2 MHz in the Fourier transform mode in $CDCl_3$ solns. The δ values are in ppm downfield from TMS.

*Only the chemical shift data useful to comparative analysis are described. The spectrum of **10** was recorded in benzene-d_6 solns.

clerodane diterpenoid **7** was confirmed by X-ray diffraction.*

EXPERIMENTAL

Mps: uncorr. NMR (^1H 60 MHz and 90 MHz; ^{13}C, 25,2 MHz) were recorded in $CDCl_3$ soln with TMS as int standard. MS were measured by direct inlet with 70 eV ionization.

Isolation of constituents. Root bark (2 kg) of *C. argyrophylloides* Muell was extd with hexane at room temp. Solvent was removed under vacuum to yield 40.5 g of residue. Treatment of this residue (30 g) with hexane produced a crystalline material ($\mathbf{H}_1 = 25$ g). The filtered hexane soln was concd, affording a yellowish mass ($\mathbf{H}_2 = 5$ g). $\mathbf{H}_2$ was chromatographed on a silica gel column and eluted with hexane–$CHCl_3$ (1:1) yielding **2** (0.54 g) after crystallization from hexane. $\mathbf{H}_1$ (12 g) was chromatographed on a silica gel column and eluted with hexane–$CHCl_3$ (1:9) and $CHCl_3$–MeOH (3:2). Fractions 29 and 37 were recrystallized from hexane–MeOH, affording **7** (8 g) and **3** (0.4 g), respectively.

The root bark, after extraction with hexane (see above), was extracted with EtOH at room temp. Evapn of solvent produced 360 g of residue. The presence of **3** and **7** in this ext was detected by TLC. These two natural diterpenes (**3** and **7**) were also observed (TLC) in the hexane and EtOH exts of the stem and its bark.

ent-*Kaur*-16-*en*-18-*oic acid* (*kaurenic acid*, 2). Mp 163–164° (hexane–MeOH). Me ester (**2a**). MP 118–120° (hexane–MeOH) (lit. [2] 120–121°).

ent-*Kaur*-16-*en*-15-*oxo*-18-*oic acid* (**3**). Mp 202–204° (hexane–MeOH). $[\alpha]_D^{25}$ 73° (*c*, 2.21 in $CHCl_3$). EIMS *m/z* (rel. int.): 316 ($[M]^+$, 24), 315 (100), 282 (48), 270 (70), 255 (52), 227 (14), 199 (15), 148 (67), 122 (34), 121 (78), 105 (67), IR ν_{max}^{KBr} cm^{-1}: 3500–2500, 1718, 1685, 1640, 890. ^1H NMR (60 MHz, $CDCl_3$): δ 5.90 (*br*, H-17), 5.25 (*br*, H-17), 1.16 (*s*, Me-10), 1.13 (*s*, Me-4). ^{13}C NMR (25.2 MHz, $CDCl_3$): see Table 1. *Me ester* (**3a**). CH_2N_2 treatment of **3** (60 mg) in the usual manner (30 min) yielded **3a**, mp 138–141° hexane–MeOH). EIMS *m/z* (rel. int): 330 ($[M]^+$, 8), 270 (18), 255 (11), 167 (22), 149 (100), 121 (50). IR ν_{max}^{KBr} cm^{-1}: 1718, 1640, 1240. ^1H NMR (60 MHz, $CDCl_3$): δ 5.96 (*br*, H-17), 5.26 (*br*, H-17), 3.65 (*s*, CO_2Me), 1.17 (*s*, Me-4 and Me-10). *Pyrazoline* **3c**. CH_2N_2 treatment of **3** (250 mg) in the usual manner (12 hr) yielded **3c** (220 mg). EIMS *m/z* (rel. int): 344 (M-N_2, 3), 274 (7), 243 (7), 215 (8), 167 (9), 149 (35), 121 (41). IR ν_{max}^{KBr} cm^{-1}: 1715, 1700, 1380, 1240. ^1H NMR (60 MHz, $CDCl_3$): δ 4.60 (*t*, $J = 7$ Hz, CH_2-17), 3.65 (*s*, OMe), 1.18 (*s*, Me-4 and Me-10).

ent-*Kaur*-16-*en*-15, 18-*diol* (**3b**). The Me ester of kaur-16-en-15-ol-18-oic acid (100 mg in 15 ml Et_2O), prepared by treatment of **3** (590 mg) in MeOH with $NaBH_4$ (250 mg) at 5° (40 min) and then methylation with CH_2N_2, was reduced with $LiAlH_4$ (50 mg) in 20 ml Et_2O. The mixt. was kept at 0° for 1 hr and usual work-up gave diol **3b**, mp 118–120° (hexane–MeOH). EIMS *m/z* (rel. int.): 304 ($[M]^+$, 7), 219(23), 273 (77), 255 (48), 173 (25), 135 (42), 123 (69), 95 (80), 81 (100). IR ν_{max}^{KBr} cm^{-1}: 3240, 1660, 890. ^1H NMR (60 MHz, $CDCl_3$): δ 4.98 (*br*, H-17), 4.79 (*br*, H-17), 3.80 (*br*, H-15), 3.10 (*d*, $J = 10$ Hz, H-18), 3.40 (*d*, $J = 10$ Hz, H-18), 1.12 (*s*, Me-10), 0.74 (*s*, Me-4).

3,12-*Dioxo*-15,16-*epoxy*-4-*hydroxycleroda*-13(16),14-*diene*-(**7**). Mp 122–124° (hexane–MeOH). $[\alpha]_D^{25}$ +41 (*c*, 1.07 in $CHCl_3$). EIMS *m/z* (rel. int.) 332 ($[M]^+$, 3), 222 (11), 204 (10), 195 (5), 179 (17), 161 (26), 136 (100), 135 (18), 134 (16), 121 (27), 110 (12), 107 (14), 95 (67). IR ν_{max}^{KBr} cm^{-1}: 3420, 1680, 1645, 1540, 1535, 1470, 1140, 860. ^1H NMR (90 MHz, $CDCl_3$): δ 8.05 (*br*, H-16), 7.46 (*t*, $J = 2$ Hz, H-15), 6.80 (*d*, $J = 2$ Hz, H-14), 3.65 (*br*, OH), 2.78 (*s*, CH_2-11), 1.50 (*s*, Me-4), 0.90 (*d*, $J = 7$ Hz, Me-8), 0.86 (*s*, Me-5 and Me-10). ^{13}C NMR (25.2 MHz, $CDCl_3$): see Table 2.

3,4-*Dihydroxy*-15,16-*epoxy*-12-*oxocleroda*-13(16),14-*diene* (**7a**). **7** (260 mg) in MeOH (26 ml) was allowed to react with $NaBH_4$ (60 mg) for 16 min at 5°. After addition of H_2O and HCl (5%), the mixt. was extracted with EtOAc. The EtOAc soln was dried and evapd under vacuum giving **7a** (213 mg), mp 112–114° after crystallization from hexane–MeOH. EIMS *m/z* (rel. int.): 334 ($[M]^+$, 2) 224(9), 206(24), 149(17), 136(64), 121(32), 95(100),. IR ν_{max}^{KBr} cm^{-1}: 3350, 1660, 1550, 1500, 1150. ^1H NMR (60 MHz, $CDCl_3$): δ 8.00 (*br*, H-16), 7.37 (*t*, $J = 2$ Hz, H-15), 6.73 (*d*, $J = 2$ Hz, H-14), 3.50 (*m*, H-3), 2.70 (*s*, CH_2-11), 1.16 (*s*, Me-4), 1.12 (*s*, Me-5), 0.87 (*d*, $J = 7$ Hz, Me-8), 0.84 (*s*, Me-10). *Monoacetate* (**7b**). Mp 121–123° (hexane–MeOH). EIMS *m/z* (rel. int.): 376 ($[M]^+$,5), 358 (2), 334 (2), 316 (4), 298 (4), 266 (6), 206 (31), 173 (28), 121 (26), 95 (100). ^1H NMR (60 MHz, $CDCl_3$): δ 8.00 (*br*, H-16), 7.37 (*br*, H-15), 6.75 (*br*, H-14), 4.80 (*br*, H-3), 2.73 (*s*, CH_2-11), 2.10 (*s*, Me-C=O), 1.20 (*s*, Me-4), 1.16 (*s*, Me-5), 0.90 (*d*, $J = 7$ Hz, Me-8), 0.87 (*s*, Me-10).

Compound **7c**. A soln of **7a** (100 mg) in EtOH (30 ml) was treated with $NaIO_4$, (120 mg) for 48 hr at room temp. After addition of H_2O, the ppt. was filtered and chromatographed on a silica gel column and eluted with $CHCl_3$, affording **7c** (35 mg). Mp 110–113° (hexane–MeOH). ^1H NMR (60 MHz, $CDCl_3$):

*Personal communication of Dra. Yvone P. Mascarenhas (Instituto de Física e Química de São Carlos, Universidade de São Paulo, 13560-São Carlos, São Paulo, Brasil). For publication, in a specialized periodical, it is necessary to refine the data and to use the ORTEP program to obtain a better molecular drawing.

δ 9.60 (s, CHO), 8.0 (*br*, H-16), 7.37 (*br*, H-15), 6.75 (*br*, H-14), 2.75 (*s*, CH_2-11), 2.20 (*s*, Me–C=O), 1.20 (*s*, Me-4), 1.16 (*s*, Me-5), 0.87 (*s*, Me-10), 0.80 (*d*, $J = 7$ Hz, CH_3-8).

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