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CLERODANE DITERPENES OF *CROTON HOVARUM*

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Key Word Index—*Croton hovarum*; Euphorbiaceae; diterpene; clerodane; 3,12-dioxo-15,16-epoxy-cleroda-13(16),14-dien-9-al; 3α,4β-dihydroxy-15,16-epoxy-19-nor-12-oxo-cleroda-5(10), 13(16),14-triene.

Abstract—A clerodane- and a nor-clerodane-type furano-diterpene were obtained from the methanolic extract of the leaves of *Croton hovarum*. Structural determinations which were made from spectroscopic data led to 3,12-dioxo-15,16-epoxy-cleroda-13(16),14-dien-9-al and 3α,4β-dihydroxy-15,16-epoxy-19-nor-12-oxo-cleroda-5(10),13(16),14-triene. Furthermore, Vitexin was isolated.

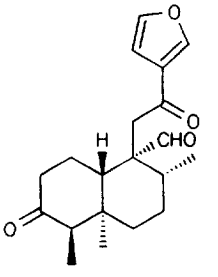
INTRODUCTION

Croton spp. are well-known as toxic plants. Various species are used in Africa as sources of poison for hunting and fishing [1]. *Croton hovarum* is a toxic tree, endemic to Madagascar [2]. In a previous paper [3] we reported the isolation of two clerodane-type diterpenes of the bark, one of them was an aldehyde. In this paper we describe isolation and structural elucidation of two new diterpenes, **1** and **2**, from the leaves.

RESULTS AND DISCUSSION

Chromatographic separation on silica gel of the ethanolic extract of the leaves yielded two crystalline compounds. The IR spectrum of **1** showed absorption at 3136, 1514 and 873 cm^{-1} suggesting the presence of a furan ring system. A peak at 1658 cm^{-1} revealed an α,β-unsaturated carbonyl group and three peaks at 1714, 2726 and 2891 cm^{-1} were assigned to an aldehyde function. There was no signal corresponding to a hydroxyl group. The mass spectrum of **1** supported the β-substituted furan ring by the presence of a strong peak at *m/z* 95 ($C_5H_3O_2$), arising from a furanyl-carbonyl group. The molecular formula of $C_{20}H_{26}O_4$ was deduced from the high-resolution mass spectrum.

The ^1H NMR spectrum of **1** showed three signals at δ 8.09, 7.46 and 6.77 due to a β-substituted furan ring. A singlet at δ 0.70 and two doublets at δ 0.93 and 1.03 demonstrated the presence of three methyl groups, one of them located at a quarternary carbon. The presence of an aldehyde group was significant, and was proved by a signal at δ_H 10.03 in the ^1H NMR spectrum and at δ_C 205.1 in the ^{13}C NMR spectrum. Two keto groups showed signals at δ 193.7 and 211.7. Homo- and heteronuclear COSY experiments led to the assignment of the peaks in the ^1H NMR and ^{13}C NMR spectra. The ^{13}C NMR signal for the methylene group in position C-11 was shifted to a higher field (δ 38.5), compared with the signal in similar compounds bearing a methyl group at C-9, due to the β-position



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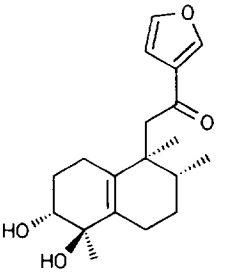

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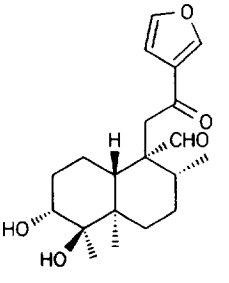

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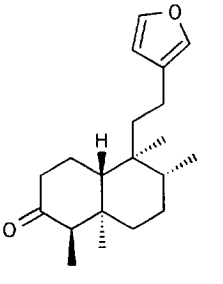

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Table 1. ^{13}C NMR chemical shift data of compounds **1** (in $CDCl_3$), **2** (in $CDCl_3/CD_3OD$), **3** [3] and **4** [4]

C-	1	3	4	2
1	23.9	17.6	23.2	21.2
2	38.0	29.7	39.4	29.5
3	211.7	75.1	216.6	75.4
4	56.7	75.6	58.2	77.3
5	41.4	41.2	41.8	127.5
6	41.0	31.5	41.5	30.8
7	27.4	26.5	27.4	28.4
8	35.3	36.3	36.6	34.3
9	55.3	54.7	39.4	44.1
10	49.3	44.5	49.0	138.8
11	38.5	40.8	38.6	41.4
12	193.7	194.4	18.1	194.0
13	129.0	128.9	125.4	131.2
14	108.5	108.3	110.9	108.5
15	144.5	144.3	138.5	144.2
16	147.2	147.4	142.3	147.4
17	17.2	17.5	14.4	20.2
18	7.1	20.7	6.8	22.9
19	15.4	17.9	15.8	-
20	205.1	206.3	18.3	19.0

to the aldehyde [3]. Thus, compound **1** was determined to be 3,12-dioxo-15,16-epoxy-cleroda-13(16),14-dien-9-al. The ^{13}C NMR data (Table 1) agreed with those of reference compounds **3** [3] and **4** [4].

The IR spectrum of **2** showed only one carbonyl absorption, 1650 cm^{-1}, for an α,β-unsaturated ketone. Again, three peaks at 3139, 1513 and 873 cm^{-1} pointed to the presence of a furan ring system. Further, the spectrum showed hydroxyl absorptions at 3482 and 3462 cm^{-1}. From the ^1H NMR spectrum it was seen that **2** contained three methyl groups, two of them located at quarternary carbons and one adjacent to a CH. The ^{13}C NMR spectrum only showed 19 peaks and suggested that this substance contained one keto group, three double bonds, a tertiary and a secondary hydroxyl function, as well as three methyl groups. Assignment of the peaks has been done by homo- and heteronuclear COSY experiments.

The molecular formula of $C_{19}H_{26}O_4$ was established by both high-resolution mass spectral and ^{13}C NMR methods. The mass spectrum of **2** showed a molecular ion at *m/z* 318 and a peak at *m/z* 275 $[M\text{-}CH_3\text{-}CH{=}CH_2\text{-}H]^+$ formed by a retro Diels–Alder reaction in the B-ring. This proved that the double bond must be located in position 5(10). The base peak at *m/z* 95 again arose from a furanyl-carbonyl group. All this data revealed the structure of **2** as 3α,4β-dihydroxy-15,16-epoxy-19-nor-12-oxo-cleroda-5(10), 13(16),14-triene.

In addition to **1** and **2**, vitexin was isolated from the leaves of *C. hovarum*. Spectroscopic data were in agreement with the literature [5].

EXPERIMENTAL

General. Plant material was collected in October 1991 near Ankazobe (125 km north-west from Antananarivo), Madagascar. NMR spectra (^1H 300 MHz; ^{13}C 75 MHz) recorded in $CDCl_3$ (**1**) and $CDCl_3$–CD_3OD (**2**) soln with TMS as int. standard. MS were measured by direct inlet with 70 eV ionisation. IR: in KBr.

Extraction and isolation. The powdered leaves of *C. hovarum* were extracted 3× with 80% EtOH in H_2O at room temp. for 48 hr, each. After filtration and evapn of the solvent, the residue was partitioned between $CHCl_3$ and H_2O. From the H_2O phase vitexin could be obtained after extraction ×3 with *n*-BuOH and chromatograpy on silica gel. The $CHCl_3$-phase was evapd and partitioned between hexane and MeOH–H_2O (10:9:1). The MeOH extract was conc. under red. pres. and the residue was chromatographed on a silica gel column and eluated with petrol–EtOAc (gradient from pure petrol to pure EtOAc). By further chromatography on silica gel with $CHCl_3$–hexane (6:4) (for **1**) and petrol-EtOAc (1:1) followed by rechromatography on silica gel with hexane–Me_2CO (4:1) (for **2**) compounds **1** and **2** were obtained.

3,12-*Dioxo*-15,16-*epoxy*-*cleroda*-13(16),14-*dien*-9-*al* (**1**). Mp 134–135° (MeOH). IR ν_{max}^{KBr} cm^{-1}: 3136, 2969, 2945, 2891, 2726, 1714, 1658, 1563, 1514, 1454, 1399, 1374, 1282, 1156, 873, 604. EIMS *m/z* (rel. int.): 330 (5) ([M^+] measured 330.18309 $C_{20}H_{26}O_4$ required 330.18311), 312 (4), 284 (5), 220 (12), 135 (10), 119 (21), 110 (100), 95 (87), 84 (27). ^1H NMR (300 MHz, $CDCl_3$): 10.03 (1H, *s*, H-20), 8.09 (1H, *s*, H-16), 7.46 (1H, *s, br*, H-15), 6.77 (1H, *s, br*, H-14), 3.35 (1H, *d*, J = 17.5 Hz, 1/2 H_2-11), 2.92 (1H, *d*, J = 17.5 Hz, 1/2 H_2-11), 2.73–2.81 (1H, *dd*, J = 13/3 Hz, H-10), 2.17–2.41 (4H, *m*, H-4 + H_2-6 + H-8), 2.03–2.13 (2H, *m*, H_2-1), 1.78–1.87 (1H, *m*, 1/2 H_2-2), 1.64–1.78 (2H, *m*, H_2-7), 1.45–1.64 (1H, *m*, 1/2 H_2-2), 1.03 (3H, *d*, J = 7.1 Hz, H_3-17), 0.93 (3H, *d*, J = 6.7 Hz, H_3-18), 0.70 (3H, *s*, H_3-19). ^{13}C NMR (75 MHz, $CDCl_3$): see Table 1.

3α,4β-*Dihydroxy*-15,16-*epoxy*-19-*nor*-12-*oxo*-*cleroda*-5(10), 13(16), 14-*triene* (**2**). Mp 163–164° (MeOH-$CHCl_3$). IR ν_{max}^{KBr} cm^{-1}: 3482, 3462, 3139, 2979, 2874, 1650, 1559, 1513, 1455, 1289, 1161, 1080, 1002, 965, 873, 832, 603. EIMS *m/z* (rel. int.): 318 (13) ([M^+] measured 318.18318 $C_{19}H_{26}O_4$ required 318.18311), 299 (5), 275 (4), 208 (31), 147 (13), 135 (32), 134 (22), 122 (36), 107 (24), 95 (100). ^1H NMR (300 MHz, $CDCl_3/CD_3OD$): 8.19 (1H, *s*, H-16), 7.48 (1H, *s, br*, H-15), 6.79 (1H, *s, br*, H-14), 3.84 (1H, *d*, J = 17.5 Hz, 1/2 H_2-11), 3.67 (1H, *d*, J = 17.5 Hz, 1/2 H_2-11), 3.60 (1H, *m*, H-3), 2.27–2.42 (1H, *m*, 1/2 H_2-1), 1.98–2.10 (3H, *m*, H-8 + 1/2 H_2-6 + 1/2 H_2-1), 1.83–1.98 (1H, *m*, 1/2 H_2-2), 1.67–1.77 (2H, *m*, H_2-7), 1.57–1.67 (1H, *m*, 1/2 H_2-2), 1.36–1.48 (H, *m*, 1/2 H_2-6), 1.37 (3H, *s*, H_3-18), 1.26 (3H, *s*, H_3-20), 0.97 (3H, *d*, J = 7.0 Hz, H_3-17). ^{13}C NMR (75 MHz, $CDCl_3$): see Table 1.

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