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CLERODANE DITERPENES AND OTHER CONSTITUENTS OF *CROTON HOVARUM*

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Key Word Index—*Croton hovarum*; Euphorbiaceae; diterpene; clerodane; 3α,4β-dihydroxy-15,16-epoxy-12-oxo-cleroda-13(16),14-diene; 3α,4β-dihydroxy-15,16-epoxy-12-oxo-cleroda-13(16),14-dien-9-al.

Abstract—Two clerodane-type furano-diterpenes were obtained from the methanolic extract of the bark of *Croton hovarum*. Structural determinations were made by spectroscopic data. One compound is a novel substance, 3α,4β-dihydroxy-15,16-epoxy-12-oxo-cleroda-13(16),14-dien-9-al, while the other is 3α,4β-dihydroxy-15,16-epoxy-12-oxo-cleroda-13(16),14-diene. Furthermore, three well-known triterpenes and 4-hydroxyhygrinic acid were also isolated.

INTRODUCTION

Croton spp. (Euphorbiaceae) 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]. We investigated the constituents of the bark and we isolated two furano-diterpenes, **1** and **2**, belonging to the rare clerodane-type compounds.

RESULTS AND DISCUSSION

The methanolic extract yielded two crystalline compounds. The IR spectra of **1** and **2** were rather similar, showing hydroxyl absorption at ν_{max} 3430/3500 cm^{-1}. Peaks at 3114/3114, 1511/1512 and 872/873 cm^{-1} suggested the presence of a furan ring system. Peaks at 1658/1661 cm^{-1} revealed an α,β-unsaturated carbonyl group. The spectrum of **2** contained additional peaks at 2868 and 1710 cm^{-1} for an aldehyde function.

The ^1H NMR spectrum of **1** showed three signals at δ7.99, 7.40 and 6.75 due to a β-substituted furan ring. Singlets at δ1.20, 1.12 and 0.84 and a doublet at δ0.86 demonstrated the presence of four methyl groups, three of them located at quaternary carbons. A secondary alcoholic group gave rise to a broad singlet at δ3.54. The ^{13}C NMR data (Table 1) agreed with the results above. In addition, a peak at δ195.4 for a quaternary carbon showed that the molecule must contain a keto group.

The ^1H and ^{13}C NMR spectra of **2** were rather similar to those of **1**. The presence of an aldehyde group is significant, and could be proved by peaks at δ9.93 and 206.3. Instead, one of the three quaternary bound methyl groups of **1** was missing in **2**. The mass spectrum of **2** supported the β-substituted furan ring by the presence of the base peak at *m/z* 95 ($C_5H_3O_2$), arising from a furanyl-carbonyl group. The molecular formula of $C_{20}H_{28}O_5$ can be deduced by both high-resolution mass spectral and ^{13}C NMR (Table 1) methods. Both compounds **1** and **2** have a *trans*-fused decaline ring-system, as concluded from the methyl resonance in the ^{13}C NMR spectrum at δ17.2 (**1**) and 17.9 (**2**), respectively, attributed to C-19. Homo- and hetero-nuclear COSY experiments led to the assignment of the peaks in the ^1H and ^{13}C NMR spectra.

Compound **1** was identical to synthetic 3α,4β-dihydroxy-15,16-epoxy-12-oxo-cleroda-13(16),14-diene, obtained from natural 3,12-dioxo-15,16-epoxy-4-hydroxy-cleroda-13(16),14-diene (**3**) by treatment with sodium borohydride [3]. The ^1H NMR spectrum was in accordance with the spectrum described; the ^{13}C NMR data of **1** are not given in the literature [3]. Our ^{13}C NMR data were in good agreement with published values for **3** and chiromodine (**4**) [3, 4] (Table 1). The aldehyde function of **2** must be attached to C-9 because of the low-field shift of the C-9 signal and the high-field shift of the C-11 signal. Thus, **2** was identified as 3α,4β-dihydroxy-15,16-epoxy-12-oxo-cleroda-13(16),14-dien-9-al. In addition to **1** and **2**, friedelin, β-amyrin, 3β-acetoxy-friedoolean-14-en-28-oic acid and 4-hydroxyhygrinic acid were isolated from the bark of *C. hovarum*. Spectroscopic data were in agreement with the literature [5, 6].

EXPERIMENTAL

General. Plant material was collected in October 1991 near Ankazobe (125 km north-west from Antananarivo),

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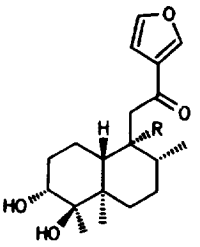
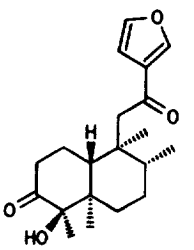


R
1 Me
2 CHO



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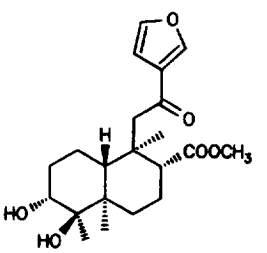

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Table 1. ^{13}C NMR chemical shift data of **1** (in $CDCl_3$), **2** (in $CDCl_3/CD_3OD$), **3** [3] and **4** [4]

C	1	2	3	4
1	17.7	17.6	23.7	18.4
2	30.2	29.7	36.1	31.5
3	76.3	75.1	215.0	76.5
4	76.3	75.6	81.5	76.0
5	42.2	41.2	45.2	42.3
6	32.1	31.5	31.1	32.1
7	26.5	26.5	26.8	21.8
8	37.2	36.3	37.4	50.1
9	41.6	54.7	42.0	40.9
10	41.8	44.5	42.5	42.4
11	47.8	40.8	47.0	49.1
12	195.4	194.4	195.0	194.7
13	129.8	128.9	129.7	130.1
14	108.9	108.3	108.7	109.3
15	144.1	144.3	144.4	144.7
16	146.8	147.4	146.8	148.2
17	16.8	17.5	14.9	175.5
18	21.1	20.7	21.8	22.0
19	17.2	17.9	16.5	17.7
20	17.6	206.3	17.8	19.7

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 ionization. IR: KBr.

Extraction and isolation. The powdered stem bark of *C. hovarum* was extracted 3 × with EtOH at room temp. for 48 hr, each. After filtration and evapn of the solvent, the residue was partitioned between $CHCl_3$ and H_2O. The aq. phase was extracted 3 × with *n*-BuOH; 4-hydroxy-hygrinic acid was isolated from the butanol layer. The chloroform-phase was evapd and partitioned between hexane and MeOH:H_2O (9:1). From the hexane layer friedelin, β-amyrin and 3β-acetoxy-friedoolean-14-en-28-oic acid were obtained. The methanolic extract was concentrated 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 Sephadex LH 20 with MeOH and rechromatography on silica gel with EtOAc:petrol (55:45) compounds **1** and **2** were obtained.

3α,4β-Dihydroxy-15,16-epoxy-12-oxo-cleroda-13(16),14-diene (**1**). Mp 121–122° (hexane–MeOH). IR ν_{max}^{KBr} cm^{-1}: 3431, 3114, 2956, 1658, 1511, 1159, 1093, 1047, 941, 872, 600. ^1H NMR (300 MHz, $CDCl_3$): δ 7.99 (1H, *m*, H-16), 7.40 (1H, *m*, H-15), 6.75 (1H, *m*, H-14), 3.54 (1H, *m*, H-3), 2.62–2.81 (2H, *m*, H_2-11), 2.13–2.18 (1H, *m*, H-10), 1.80–1.93 (2H, *m*, 1/2 H_2-2 + H-8), 1.54–1.71 (4H, *m*, 1/2 H_2-2 + 1/2 H_2-6 + 2 × OH), 1.33–1.49 (5H, *m*, H_2-1 + H_2-7 + 1/2 H_2-6), 1.20 (3H, *s*, H_3-18), 1.12 (3H, *s*, H_3-19), 0.86 (3H, *d*, J = 7 Hz, H_3-17), 0.84 (3H, *s*, H_3-20). ^{13}C NMR (75 MHz, $CDCl_3$): see Table 1.

3α,4β-Dihydroxy-15,16-epoxy-12-oxo-cleroda-13(16),14-dien-9-al (**2**). Mp 139–141° (MeOH). IR ν_{max}^{KBr} cm^{-1}: 3504, 3114, 2956, 2868, 1710, 1661, 1512, 1158, 1108, 1046, 943, 873, 599. EIMS *m/z* (rel. int.): 348 (3) ([M^+] measured 348.1927 $C_{20}H_{28}O_5$ required 348.1937), 330 (2), 317 (5), 239 (16), 221 (18), 179 (16), 177 (13), 175 (19), 173 (15), 159 (13), 149 (11), 147 (15), 135 (15), 133 (17), 121 (18), 110 (27), 95 (100). ^1H NMR (300 MHz, $CDCl_3/CD_3OD$): 9.93 (1H, *s*, H-20), 8.00 (1H, *s*, H-16), 7.34 (1H, *s*, *br*, H-15), 6.63 (1H, *s*, *br*, H-14), 3.38 (1H, *m*, H-3), 2.80–3.19 (2H, *m*, H_2-11), 2.31–2.36 (1H, *m*, H-10), 1.86–2.00 (1H, *m*, H-8), 1.58–1.85 (2H, *m*, 1/2 H_2-7 + 1/2 H_2-2), 1.37–1.56 (6H, *m*, 1/2 H_2-2 + H_2-6 + 1/2 H_2-7 + H_2-1), 1.11 (3H, *s*, H_3-18), 0.94 (3H, *s*, H_3-19), 0.89 (3H, *d*, J = 7 Hz, H_3-17). ^{13}C NMR (75 MHz, $CDCl_3/CD_3OD$): see Table 1.

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