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Diterpenoids from the roots of *Croton macrostachys*

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Abstract

Three novel diterpenoids have been isolated from the roots of *Croton macrostachys*. The structure and stereochemistry of the compounds have been unambiguously settled as neoclerodan-5,10-en-19,6β;20,12-diolide, 3α,19-dihydroxytrachylobane, and 3α,18,19-trihydroxytrachylobane from detailed spectroscopic evidence.

Keywords: *Croton macrostachys*; Euphorbiaceae; Diterpenoid; Neoclerodan-5,10-en-19,6β;20,12-diolide; 3α,19-Dihydroxytrachylobane; 3α,18,19-Trihydroxytrachylobane

1. Introduction

Croton macrostachys A. Rich is a medium-sized deciduous tree of East Africa particularly widespread between 1100 and 2500 m in Kilimanjaro, Meru, Iringa, and Mbeya regions. In Tanzania its roots are used as antidiabetic (Mbuya et al., 1994). During the course of our program aimed to identify new natural pharmacologically active compounds, we have recently investigated the terpenoid contact of the title plant. We herein report the structure of three new diterpenoids isolated from the root of *C. macrostachys* as **4**, **5** and **6**. Triterpenoid 3β-acetoxy taraxer-14-en-28-oic acid (**1**), diterpenoids trachyloban-18-oic acid (**2**) and trachyloban-19-oic acid (**3**) were also isolated during this investigation.

2. Results and discussion

The roots of *C. macrostachys* were extracted with ethanol. The dried extract was carefully chromatographed on silica gel using a slow gradient of ethy1 acetate in petroleum ether. Six compounds were isolated: three (**1–3**) were known compounds and were rapidly identified as 3β-acetoxy taraxer-14-en-28-oic acid (**1**), trachyloban-19-oic acid (**2**) trachyloban-18-oic acid (**3**) by comparing their chemical and spectral data with literature values (Bohlmann et al., 1979; Mahato et al., 1988; Leong and Harrison, 1997).

The mass spectrum of **4** displayed a molecular peak at m/z 343 (MH^+) establishing molecular formula: $C_{20}H_{22}O_5$. Compound **4** exhibited also in its IR spectrum a particularly broad and intense band at 1747 cm^{-1} (COO) and another band at 875 cm^{-1}, suggesting a furan moiety. The 75 MHz ^{13}C NMR of **4** displayed 20 resonances confirming the mass data and was highly informative for it contained signals for three methine groups at δ 144.2 (C-16), 139.0 (C-15), and 107.9 (C-14) characteristic of furoclerodanes. The fourth furan carbon was indeed observed at δ 125.5 as a singlet. Other peaks of interest were four deshielded quaternary signals; two at δ 176.5 and 176.4 that had

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each to belong to a lactone group in order to be consistent with the molecular formula and two others at δ 141.1 and 133.5 evidencing an additional unsaturation. Remaining peaks were due to two CH_3, five CH_2, three CH, and two C (see Table 1). The 300 MHz ^1H NMR spectrum of **4** displayed, in addition to the furan signals [δ 7.4 (2H), 6.32 (*br. s.*, 1H)], two methyl peaks: singlet at δ 1.31 (*s*) and doublet at δ 0.89 ($J =$ 6.6 Hz). Furthermore, two downfield doublet of doublets, each integrating for one proton, were observed at δ 5.5 ($J = 9$, 5.6 Hz) and 4.9 ($J = 3.7$, 1.4 Hz) corresponding to two hydroxylated CHs. On the basis of these results, a planar structure could be proposed for **4**. The C-12 configuration was deduced from careful comparison of the ^{13}C NMR data with related compounds. Criteria to differentiate the C-12 series have been proposed; the main ones are the observations of a strong NOE between H-17 and H-12, exclusively in the (*R*) series and the chemical shift of C-8 and C-10 (Fayos et al., 1984). In our case, no NOE could be observed between H-17 and H-12. However, considering that a lack of NOE is hardly a proof of structure, we used the ^{13} C chemical shifts as an alternative and additional proof. The chemical shifts of C-8 and C-10 have been shown to be the most sensitive to a C-12 change of configuration at C-12 (Fayos et al., 1984). In our case, since C-10 is a part of a double bond, we could not use it for comparison purposes. However, C-8 was suitable for this purpose; its chemical shift (35.6 ppm) unequivocally established the configuration of **4**. The configuration shown for C-8 and C-9 was proposed on biogenetic grounds and confirmed by comparing their chemical shifts and those of the neighbouring atoms with those of the known compounds (Gacs-Baitz et al., 1982; Fayos et al., 1984). Finally, the γ-lactone bridging the A and B rings was placed on the β face of the molecule, on the following basis. On biogenetic grounds (Gacs-Baitz et al., 1982) CH_3-17 was pseudo-equatorial and the similar pseudo-equatorial orientation observed for H-6 ($J_{H6-H7,7'} = 3.7$, 1.4 Hz), implied that the lactone was above the plane of the molecule. Consequently, compound **4** is neoclerodan-5,10-en-19,6β;20,12-diolide; to the best of our knowledge its structure has not been reported previously.

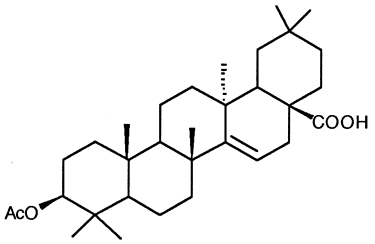
Table 1
^{13}C NMR data for compounds **4**–**6** in $CDCl_3$

Carbon	**4**	**5**[a]	**6**[a]
1	26.8	36.9	36.8
2	20.7	26.6	26.1
3	28.3	80.1	75.3
4	46.1	40.2	40.2
5	141.1	55.3	52.8
6	75.3	19.7	19.6
7	25.9	38.8	38.5
8	35.6	41.8	45.3
9	49.3	52.8	49.0
10	133.5	37.4	37.3
11	40.0	19.7	19.9
12	72.3	20.2	20.2
13	125.5	23.8	23.9
14	107.9	33.0	33.0
15	139.0	49.9	49.9
16	144.2	23.8	22.2
17	15.3	20.2	20.2
18	15.5	22.1	65.3
19	176.4[b]	64.1	62.4
20	176.5[b]	14.9	14.7

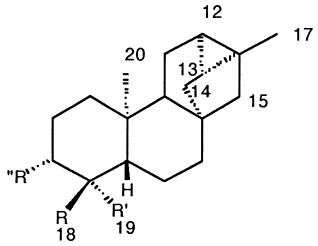
[a] Recorded in $CDCl_3$, CD_3OD, 4:1.
[b] Interchangeable values.

The mass spectrum of compound **5** displayed a pseudo-molecular peak at m/z 305 (MH^+) which, consequently, suggested the molecular formula $C_{20}H_{32}O_2$. Its IR spectrum showed a hydroxy1 frequency at 3333 cm^{-1}. The ^{13}C NMR spectrum displayed only two signals above 60 ppm [δ 80.2 (CH) and 64.1 (CH_2)]. The trachylobane nature of **5** was deduced after observation of the characteristic cyclopropane carbons [δ 20.2 (C-12) and 23.8 (C-13 and C-16)]. Substitution of this skeleton at position 3 and 19 was deduced on comparison with closely related natural or synthetic compounds (Hugel et al., 1965; St Pyrek, 1970; Achmatowicz et al., 1971; Bohlmann et al., 1979; Leong and Harrison, 1997; Arnone et al., 1979; Cory et al., 1980). Since CH_3-17 and CH_3-20 could be identified in the NMR spectrum of **5**, an hydroxy1 group had to be located at either C-18 or C-19. The choice between these two locations was realised only after determination of the configuration of C-3. As H-3 appeared as a *dd* ($J = 11$, 4.7 Hz), it was axial. The cross peak observed in the long range COSY spectrum between H-3 and one proton of the CH_2OH group indicated that this latter group had also to be in an axial position to provide the necessary geometry required for this kind of ^4J coupling. Consequently, the last hydroxy1 group was placed on C-19 and compound **5** is 3α,19-dihydroxytrachylobane.

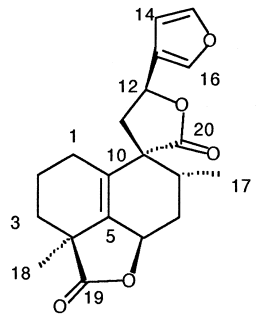
The mass spectrum of compound **6** displayed a quasi-molecular peak at m/z 321 (MH^+) indicating the molecular formula $C_{20}H_{32}O_3$. The ^{13}C NMR spectrum of **6** was closely similar to that of **5**, the only major difference being the presence of an extra deshielded carbon (δ 65.3, CH_2) and the lack of the signal corresponding to C-18. Consequently, **6** was identified as 3α,18,19-trihydoxytrachylobane. Although, the structure of some 18,19-disubstituted trachylobanes has been reported recently (Leong and Harrison, 1997) the natural occurrence of 3,18,19-trisubstituted trachylobanes, if not unprecedented (Harrison and Asakawa, 1989), seems quite rare.



1



2: R= CH_3, R'= COOH, R"= H
3: R= COOH, R'= CH_3, R"= H
5: R= CH_3, R'= CH_2OH, R"= OH
6: R= CH_2OH, R'= CH_2OH, R"= OH



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3. Experimental

3.1. General procedures

Melting points are uncorrected. Optical rotations were determined on a Perkin Elmer polarimeter, IR spectra were measured as a film on a NaCl pellet using a FTIR-1600 Perkin Elmer spectrometer. NMR spectra were obtained at 300 MHz (^{1}H frequency) on a Bruker instrument. Mass spectra were obtained on a Nermag R10-10 spectrometer (direct insertion) using NH_3 as ionising gas. Column chromatography was performed using Kieselgel 60 (70–230 mesh, EM science-Merck). TLC were performed on aluminium backed kieselgel 60 GF254 plates (Merck) developed with petroleum ether:ethyl acetate (4:1 or 3:2) and visualised by spraying vanillin sulphuric acid and heating for 10 min at 110°C.

3.2. Plant material

The roots of *C. macrostachys* were collected at Handeni District, Tanga region by MJM and identified by E.B. Mhoro. Voucher specimen #MJ-53 is deposited at the herbarium of the Institute of Traditional Medicine, Dar-es-Salaam, Tanzania.

3.3. Extraction and isolation

Air-dried roots of *C. macrostachys* (500 g) were extracted with 80% aqueous ethanol. The filtrate was concentrated in vacuo to afford 140 g of residue from which 5 g were chromatographed on silica gel using petroleum ether and increasing volumes of ethy1 acetate. Six compounds: 3β-acetoxy taraxer-14-en-28-oic acid (12 mg), trachyloban-18-oic (15 mg), trachyloban-19-oic (50 mg), neoclerodan-5,10-en-19,6β;20,12-diolide (10 mg), 3α,19-dihydroxytrachylobane (22 mg), and 3α,18,19-trihydroxytrachylobane (25 mg) were isolated.

3.4. Compound *4*: neoclerodan-5,10-en-19,6β;20,12-diolide

Mp 139–140°C; $[\alpha]_D^{18}$ +50° ($c = 1.9$, $CHCl_3$); CIMS *m/z* (rel. int.) 343 (100) MH^+, 299 (10); IR ν_{max} cm^{-1} 3146, 2939, 1747, 1503, 875, 730; ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.89 (3H, *d*, $J = 6.6$ Hz, CH_3-17), 1.31 (3H, *s*, CH_3-18), 2.33 (1H, *dd*, $J = 14$, 5.6 Hz, H-11), 2.79 (1H, *dd*, $J = 14$, 9 Hz, H-11), 4.9 (1H, *dd*, $J = 3.7$, 1.4 Hz, H-6), 5.5 (1H, *dd*, $J = 9$, 5.6 Hz, H-12), 6.32 (1H, *br. s.*, H-14). 7.38 (2H, *m*, H-15 and H-16); ^{13}C NMR (75 MHz, $CDCl_3$) see Table 1.

3.5. Compound *5*: 3α,19-dihydroxytrachylobane

Mp 149–150°C; $[\alpha]_D^{18}$ −35° ($c = 4$, $CHCl_3$–MeOH, 1:1); CIMS *m/z* (rel. int.) 305 (100) MH^+, 287 (100), 269 (7); IR ν_{max} cm^{-1} 3333, 2929, 1442, 1021, 738; ^{1}H NMR (300 MHz, $CDCl_3$–CD_3OD 4:1) δ 0.5 (1H, *m*, H-12), 0.80 (3H, *s*, CH_3-20), 1.05 (3H, *s*, CH_3-17), 1.12 (3H, *s*, CH_3-18), 3.14 (1H, *d*, $J = 11$ Hz, H-19), 3.21 (1H, *dd*, $J = 11$, 5 Hz, H-3), 4.03 (1H, *d*, $J = 11$ Hz, H-19); ^{13}C NMR (75 MHz, $CDCl_3$) see Table 1.

3.6. Compound *6*: 3α,18,19-trihydroxytrachylobane

Mp 180–181°C; $[\alpha]_D^{18}$ −39° ($c = 3$, $CHCl_3$–MeOH, 1:1); CIMS *m/z* (rel. int.) 321 (100) MH^+, 303 (12), 285 (7); IR ν_{max} cm^{-1} 3300, 2918, 1436, 1032; ^{1}H NMR (300 MHz, $CDCl_3$–CD_3OD, 4:1) δ 0.37 (1H, *m*, H-12), 0.70 (3H, *s*, CH_3-20), 0.89 (3H, *s*, CH_3-17), 3.31 (1H, *d*, $J = 7.8$ Hz, H-19), 3.41 (1H, *d*, $J = 11.8$ Hz, H-18), 3.42 (1H, *dd*, $J = 11$, 5 Hz, H-3), 3.81 (1H, *d*, $J = 7.8$ Hz, H-19), 4.00 (1H, *d*, $J = 11.8$ Hz, H-18); ^{13}C NMR (75 MHz, $CDCl_3$) see Table 1.

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