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DAMMARANE TRITERPENES FROM THE RESINS OF COMMIPHORA *CONFUSA*

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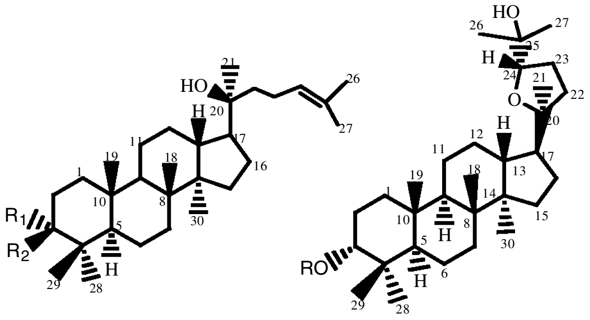
ABSTRACT. The resin of *Commiphora confusa* afforded two new dammarane triterpenes, (3*R*,20*S*)-3,20-dihydroxydammar-24-ene and (3*R*,20*S*)-3-acetoxy-20-hydroxydammar-24-ene along with the known triterpenes, cabraleadiol 3-acetate and α-amyrin.

KEY WORDS: *Commiphora confusa*, Dammarane triterpenes, (3*R*,20*S*)-3,20-Dihydroxydammar-24-ene, (3*R*,20*S*)-3-Acetoxy-20-hydroxydammar-24-ene, Triterpenes, Cabraleadiol 3-acetate, α-Amyrin

INTRODUCTION

Commiphora confusa Vollesen (Burseraceae) is a tree about four meters high, which is known to occur in Ethiopia, Kenya and Tanzania [1]. It is also to some extent planted in villages to serve as hedge or fence. Its leaves are browsed by livestock. The plant produces three types of gums: white chewable, white non-chewable and brown or black chewable gums. In northern Kenya, the resin is sold in markets as incense and is also used in folk medicine to treat snakebite.

The isolation and structural elucidation of two new dammarane triterpenes, (3*R*,20*S*)-3,20-dihydroxydammar-24-ene (**1**), (3*R*,20*S*)-3-acetoxy-20-hydroxydammar-24-ene (**3**) along with the known compounds cabraleadiol 3-acetate (**4**) and α-amyrin constitute the subject of this report. To the best of our knowledge, this is the first chemical study on the resin of *C. confusa*. Dammarane triterpenes have previously been reported as constituents of *C. dalzielii* [2] and *C. kua* [3].



1: R_1 = OH, R_2 = H
2: R_1 = H, R_2 = OH
3: R_1 = OAc, R_2 = H

4: R = Ac
5: R = H

RESULTS AND DISCUSSION

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Extraction of the resin of *C. confusa* with EtOAc yielded copious amounts (60%) of a yellowish oil which after column chromatography furnished four compounds with R_f values of 0.14, 0.19, 0.28 and 0.36 (*n*-hexane/EtOAc, 9:1). α-Amyrin (R_f 0.19) was readily identified by comparison of its data with previous reports [4, 5].

EIMS of compound **1** (R_f 0.14) revealed a molecular ion peak at *m/z* 444 with fragments resulting from loss of one and two molecules of water, respectively (*m/z* 426 and 408) indicating two hydroxyl groups. The presence of hydroxyl group(s) was supported by IR absorption at 3450 cm^{-1}. The mass and NMR spectra indicated the molecular formula $C_{30}H_{52}O_2$. The ^{13}C- and ^{1}H-NMR spectra of **1** were identical to a compound derived by Yukiko *et al.* [6] through reduction of the corresponding 3-ketone using sodium borohydride and lithium tri-*sec*-butyl borohydridie [6]. The chemical shifts of the methine carbon, C-5 (δ 50.5), and the quaternary carbon, C-14 (δ 49.8), were assigned on the basis of DEPT and HMBC experiments and comparison with literature report [6]. Hence, this compound is identified as (3*R*,20*S*)-3,20-dihydroxydammar-24-ene (**1**), a compound not previously reported as a natural product. However, the C-3 epimer of **1** (3*S*,20*S*) is known as constituents of the frankincense of *Boswellia freerana* (Burseraceae) [7].

Although the molecular ion of compound **3** (R_f 0.36) at *m/z* 486 in the EIMS is weak (1%), the HRMS of the $[M-H_2O]^+$ fragment and NMR data including DEPT experiments supported the molecular formula $C_{32}H_{54}O_3$. The ^{1}H-NMR spectrum showed one-proton triplets at δ 4.59 and 5.09, indicating oxymethine proton and olefinic proton, respectively. A three-proton singlet at δ 2.06 and IR absorption at 1720 cm^{-1} revealed the presence of an acetyl group. IR absorption at 3500 cm^{-1} and the ^{13}C-NMR resonance at δ 75.8 disclosed the presence of a tertiary hydroxyl group. The ^{13}C-NMR (Table 1) and DEPT spectra indicated nine methyl, ten methylene, six methine and seven quaternary carbons, which were further characterized as a carbonyl carbon at δ 171.2, two oxygen-bearing carbons at δ 78.7 and 75.8, and two olefinic carbons at δ 125.1 and 131.9, respectively. It has been pointed out that in 3β- and 3α-oxygenated dammarane triterpenes the C-5 methine carbons appear at *ca.* δ 55 and δ 50, respectively [8, 9]. In the case of compound **3**, the appearance of the signal for C-5 at δ 50.7 clearly suggested 3α-orientation of the acetoxy group. This 3α-configuration is further supported by the fact that the resonance for C-3 appeared at δ 78.7 rather than at δ *ca.* 81 which is expected for the corresponding 3β-isomer [10]. Furthermore, irradiation of the H-3 signal at δ 4.59 showed NOE response of the 29-Me resonance and *vice versa*. The double bond was placed between C-24 and C-25 as judged from the mass spectrum which displayed a major fragment at *m/z* 69, $[C_5H_9]^+$, which is ascribed to a methylbutenyl fragment from the C-17 side chain. As shown in Figure 1, COLOC of **3** revealed connectivities of H-3/C-1,C-5; Me-19/C-1,C-9,C-10; Me-21/C-20,C-22; Me-26/C-24,C-25; Me-27/C-24,C-25. Based on the above data compound **3** was characterized as (3*R*,20*S*)-3-acetoxy-20-hydroxydammar-24-ene.

The MS and NMR spectral characteristics of compound **4** (R_f 0.28) indicated that it belongs to a triterpene of the dammarane group. The EIMS displayed a base peak at *m/z* 143, which is ascribed to a tetrahydrofurylisopropanol ion. The IR spectrum revealed hydroxyl and ester carbonyl absorptions at 3450 cm^{-1} and 1725 cm^{-1}, respectively. The ^{1}H- and ^{13}C-NMR data were virtually identical to those reported for cabraleadiol 3-acetate (**4**) [11, 12], a compound previously isolated from *Commiphora dalzielii* [2].

Hydrolysis of **4** yielded cabraleadiol (**5**), which was previously reported from *Cabralea polytricha* (Meliaceae) [13] and *C. dalzielii* [2]. Based on NMR data, Aalbersberg and Singh [14] reported the configurations of the two stereocenters in the side chain of **4** as (20*S*,24*R*) while Hisham *et al.* [11] proposed (20*S*,24*S*). However recently using X-ray diffraction analysis, the structure of compound **4** was determined as (*3R,20S*,24*S*)-3-acetoxy-20,24-epoxy-25-hydroxydammarane [12]. The occurrence of dammarane triterpenes in both Meliaceae and Burseraceae indicates a close chemotaxonomic relationship of the two families [2].

Table 1. ^{13}C-NMR spectral data of compounds **1-5** (100 MHz, $CDCl_3$).

C N0.	**1**	**1** lit. [6]	**2** lit. [6]	**3**	**4**	**5**
1	33.7	33.7	39.1	34.6	34.4	34.1
2	24.8	25.5	27.4	23.2	23.0	25.8
3	76.4	76.4	79.0	78.7	78.5	76.7
4	37.7	37.7	39.0	37.5	36.9	38.0
5	50.5	49.6	55.9	50.7	50.9	50.2
6	18.3	18.3	18.3	18.4	18.2	18.6
7	35.2	35.2	35.3	35.4	35.2	35.2
8	40.7	40.6	40.4	40.8	40.7	41.0
9	50.5	50.5	50.7	51.1	50.7	51.0
10	37.3	37.4	37.1	37.1	37.3	37.7
11	21.5	21.5	21.6	21.7	21.8	22.1
12	25.4	24.9	24.8	25.1	27.1	27.4
13	42.3	42.3	42.3	42.5	42.9	43.2
14	49.8	50.6	50.3	50.7	50.3	50.5
15	31.2	31.2	31.2	31.5	31.6	31.8
16	27.6	27.7	27.6	27.9	26.0	26.2
17	49.6	49.9	49.9	50.2	49.9	49.9
18	16.6	16.6	16.5	15.8	15.6	15.9
19	16.1	16.1	16.2	16.3	16.2	16.5
20	75.5	75.5	75.4	75.8	86.7	86.7
21	25.4	25.5	25.4	25.7	27.3	27.5
22	40.6	40.7	40.5	40.9	34.8	35.7
23	22.6	22.7	22.6	22.9	26.5	26.7
24	124.8	124.8	124.7	125.1	86.5	86.9
25	131.6	131.7	131.6	131.9	70.4	70.6
26	25.8	25.8	25.7	26.1	27.9	28.2
27	17.8	17.8	17.7	18.1	24.2	24.5
28	28.4	28.4	28.0	28.2	28.0	28.7
29	22.2	22.2	15.4	22.1	21.6	22.5
30	15.6	15.6	15.5	16.9	16.7	16.9
$CH_3\underline{C}O$	-	-	-	171.2	171.0	-
$\underline{CH_3}CO$	-	-	-	21.7	21.8	-

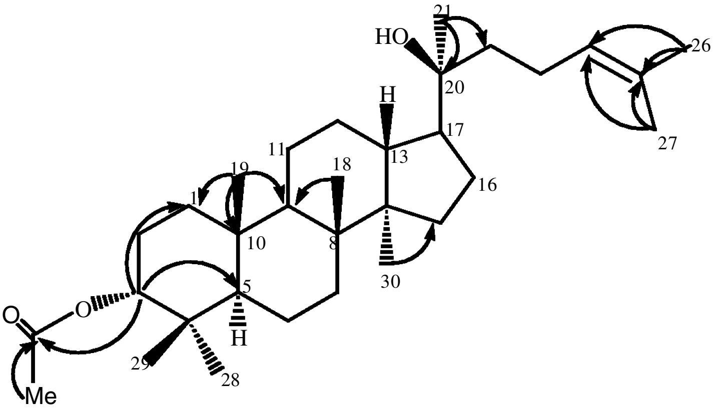

Figure 1. Selected COLOC of **3**. Arrows point from H to C.

EXPERIMENTAL

General. Melting points are uncorrected. TLC was performed on precoated plates (Silica gel 60 F_{254}, Merck) using *n*-hexane/EtOAc (9:1) as eluent and with vanillin-H_2SO_4 as detecting reagent. CC was performed on silica gel. IR spectra were measured on a Perkin Elmer 1600 using KBr. ^{1}H- and ^{13}C-NMR were recorded on a JEOL JNM-EX400 instrument at 400 MHz and 100 MHz, respectively, using $CDCl_3$ as solvent and internal reference. EIMS data were obtained on VG Quattro quadrupole mass spectrometer operated at 70 eV.

Plant material. The plant material was collected from Noloworu Hill, N. Kenya in May 1998. The plant is known by vernacular names EKIGNATE (Turakana), LCHANI-NGIRO (Samburu) and SILATACHO (Boran) and was identified by Ms Pat Curry and Dr. Kaj Vollesen (Kew Botanic Gardens). A voucher specimen has been deposited at the National Herbarium, Addis Ababa University, Addis Ababa, Ethiopia (Voucher Number: 072798) and at the East African Herbarium, Nairobi, Kenya (Voucher Number, Pat Curry PC-AD1).

Extraction and isolation. The powdered black gum (100 g) of *C. confusa* was extracted with EtOAc for 24 h at room temp. The solvent was removed *in vacuo* to give a yellowish oil (60 g, 60%). Part of this oil (30 g) was chromatographed on silica gel (290 g) using increasing amounts of EtOAc in *n*-hexane as eluents. Fractions of 150 mL were collected. The fraction (2.1 g) eluted with EtOAc/*n*-hexane (3:7) was rechromatographed with EtOAc/*n*-hexane (1:9) furnishing 67 mg of compound **1**. The fraction (4.2 g) eluted with EtOAc/*n*-hexane (1:9) was rechromatographed repeatedly to yield compound **3** (98 mg), compound **4** (420 mg) and α-amyrin (8 mg).

(3*R*,20*S*)-*3,20-dihydroxydammar-24-ene* (**1**). White amorphous solid, mp 65-67 ^{0}C $[\alpha]^{22}_D$ + 20^o ($CHCl_3$, *c* 0.25); R_f 0.14; UV λ^{EtOH}_{max} nm (log ε): 206 (3.35); IR ν^{KBr}_{max} cm^{-1}: 3450, 3010, 2940, 2860, 1450, 1370, 1240, 1190, 1040, 750; ^{1}H NMR: δ 5.10 (1H, *t*, *J* = 6.9 Hz, H-24), 3.38 (1H, *t*, *J* = 3.0 Hz, H-3), 1.67, 1.61, 1.13, 0.94, 0.92, 0.87, 0.84, 0.82 (3H each, *s*, 8 x Me); ^{13}C NMR: Table 1; EIMS *m/z* (rel. int.): 444 $[M]^+$ (2), 426 (40), 408 (12), 383 (13), 357 (11), 317 (8.0), 273

(18.0), 207 (37), 189 (40), 149 (42), 143 (58), 135 (46), 121 (37), 109 (74), 95 (59), 81 (50), 69 (81), 55 (57), 43 (100).

(3*R*,20*S*)-*3-Acetoxy-20-hydroxydammar-24-e*ne (**3**). Colourless oil, $[\alpha]_D^{22}$ + 8° ($CHCl_3$, *c* 0.25); R_f 0.36; UV λ_{max}^{EtOH} nm (log ε): 203 (3.38); IR ν_{max}^{KBr} cm^{-1}: 3500, 3010, 2940, 2860, 1720, 1450, 1370, 1240, 1180, 1030, 750; ^1H NMR: δ 5.09 (1H, *t*, *J* = 7.0 Hz, H-24), 4.59 (1H, *t*, *J* = 3.0 Hz, H-3), 2.06, 1.66, 1.59, 1.12, 0.94, 0.89, 0.86, 0.84, 0.81 (3H each, *s*, 9 x Me); ^{13}C NMR: Table 1; EIMS *m/z* (rel. int.): 486 $[M]^+$ (1), 468 (50), 408 (7), 343 (6), 300 (12), 218 (9), 189 (36), 141 (36), 121 (29), 109 (70), 69 (73), 43 (100); HRMS: *m/z* 468.3981 $[M-H_2O]^+$ ($C_{32}H_{52}O_2$ requires *m/z* 468.3967), 408.3730 $[M-H_2O-AcOH]^+$ ($C_{30}H_{48}$ requires *m/z* 408.3756).

(3R,20S,24S)-3-acetoxy-20,24-epoxy-25-hydroxydammarane or cabraleadiol 3-acetate (**4**). White powder, mp 147-149 °C (lit. 155 °C [2], 148-149 °C [13]); R_f 0.28; $[\alpha]_D^{22}$ + 14.8° ($CHCl_3$, *c* 0.25) (lit. + 10° [2], + 12° [13]); IR ν_{max}^{KBr} cm^{-1}: 3450, 2950, 2880, 1725, 1450, 1370, 1240, 1180, 1050, 880; ^1H NMR: δ 4.61 (1H, *t*, *J* = 2.6 Hz, H-3), 3.65 (1H, *dd*, *J* = 10.0, 4.6 Hz, H-24), 2.08, 1.18, 1.14, 1.10, 0.97, 0.91, 0.87, 0.86, 0.82 (3H each, *s*, 9 x Me); ^{13}C NMR: Table 1; EIMS *m/z* (rel. int.): 487 $[M-Me]^+$ (0.9), 443 (3), 427 (2), 383 (25), 218 (6), 203 (10), 191 (16), 175 (7), 143 (100), 125 (13), 121(8), 95 (8), 85 (6), 59 (5), 43 (11).

Hydrolysis of compound **4**. 6% Methanolic KOH (20 mL) was added to 150 mg of compound **4** and refluxed for 12 h. The mixture was diluted with water (50 mL) and the product was extracted with $CHCl_3$. Removal of the solvent followed by CC and crystallization (*n*-hexane-EtOAc, 2:8) gave 130 mg colourless crystals (**5**). Mp 172-174 °C (lit. 175 °C [2]). R_f 0.14; IR ν_{max}^{KBr} cm^{-1}: 3440, 2960, 2830, 1450, 1380, 1300, 1150, 1050, 990; ^1H-NMR: δ 3.39 (1H, *t*, *J* = 2.6 Hz, H-3), 3.64 (1H, *dd*, *J* = 10.0, 5.3 Hz, H-24), 1.19, 1.14, 1.11, 0.97, 0.94, 0.89, 0.86, 0.84 (3H each, *s*, 8 x Me); ^{13}C NMR: Table 1; EIMS *m/z* (rel. int.): 424 $[M-2H_2O]^+$ (7), 384 (7), 383 (24), 203 (6), 191 (11), 189 (13), 175 (7), 143 (100), 125 (51), 121 (15), 107 (15), 93 (13), 81 (16), 71 (12), 59 (10), 43 (31).

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REFERENCES

1. Vollesen, K. in Vol. 3, *Flora of Ethiopia*, Hedberg, I.; Edwards, S., (Eds); National Herbarium, Science Faculty, Addis Ababa University: Addis Ababa; **1989**; p 442.
2. Waterman, P.G.; Ampofo, S. *Phytochemistry* **1985**, 24, 2925.
3. Provan, G.J.; Waterman, P.G. *Phytochemistry* **1986**, 25, 917.
4. Lau-Cam, C.A. *Phytochemistry* **1973**, 12, 475.
5. Menezes, F.S.; Borsatto, Â.S.; Pereira, N.A.; Matos, F.J.A.; Kaplan, M.A.C. *Phytochemistry* **1998**, 48, 323.

6. Yukiko, I.; Yoshioki, H.; Atsushi, K.; Seiji, O. *Bull. For. and For. Prod. Res. Inst.* **1996**, 371, 43.
7. Fattorusso, E.; Santacroce, C.; Xaasan, C.F. *Phytochemistry* **1985**, 24, 1035.
8. Asakawa, J.; Kasai, R.; Yamasaki, K.; Tanaka, O. *Tetrahedron* **1977**, 33, 1935.
9. Grover, S.H.; Stothers, J.B. *Can. J. Chem.* **1974**, 52, 870.
10. Rouf, A.S.S.; Ozaki, Y.; Rashid, M.A.; Rui, J. *Phytochemistry* **2001**, 56, 815.
11. Hisham, A.; Bai, M.D.A.; Fujimoto, Y.; Hara, N.; Shimada, H. *Magn. Reson. Chem.* **1996**, 34, 146.
12. Qiu, S-X.; Hung, N.V.; Xuan, L.T.; Gu, J-Q.; Lobkovsky, E.; Khanh, T.C.; Soejarto, D.D.; Clardy, J.; Pezzuto, J.M.; Dong, Y.; Tri, M.V.; Huong, L.M.; Fong, H.H.S. *Phytochemistry* **2001**, 56, 775.
13. Cascon, S.C.; Brown, K.S. *Tetrahedron* **1972**, 28, 315.
14. Aalbersberg, W.; Singh, Y. *Phytochemistry* **1991**, 30, 921.