Triterpenes of *Commiphora holtziana* oleo-gum resin

Lawrence Onyango Arot Manguro, Sylvia Awino Opiyo, Eberhardt Herdtweck, and Peter Lemmen

Abstract: Chemical analysis of the acetone extract of *Commiphora holtziana* gum resin has led to the isolation of triterpenes characterized as methyl 3-oxo-1α,19α,28-trihydroxyurs-12-en-24-oate (**1**), methyl 3β-acetyl-2α,11α,19α,28-tetrahydroxyurs-12-en-24-oate (**2**), methyl 3β,11α-diacetyl-1α,2α,28-trihydroxyurs-12-ene-24-oate (**3**), and 3β,28-diacetyl-1α,2α,25-trihydroxydammar-23-ene (**4**). The known compounds isolated from the same extract included cabraleadiol monoacetate (**5**), mansumbinol (**6**), 3β-acetylamyrin (**7**), 3α-acetylboswellic acid (**8**), 2-methoxy-8,12-epoxygermacra-1(10),7,11-trien-6-one (**9**), 2-methoxy-5-acetylfuranogermacra-1(10),7,11-trien-6-one (**10**), furadienone (**11**), 2-methoxy-5-acetyl-4-furanogermacra-1(10)*Z*-en-6-one (**12**), α-amyrin (**13**), sistosterol (**14**) and stigmasterol 3-*O*-acetate (**15**). Structural elucidation was carried out using spectroscopic and physical methods as well as by comparison with the literature data.

Key words: Commiphora holtziana, Burseraceae, triterpenes, gum-oleo-resin exudate.

Résumé : L'analyse chimique des produits extraits à l'acétone à partir de la gomme résine *Commiphora hostziana* a permis d'extraire des triterpènes caractérisés comme le 3-oxo-1α,19α,28-trihydroxyurs-12-én-24-oate de méthyle (**1**), le 3β-acétyl-2α,11α,19α,28-tétrahydroxyurs-12-én-24-oate de méthyle (**2**), le 3β,11α-diacétyl-1α,2α,28-trihydroxyurs-12-én-24-oate de méthyle (**3**) et le 3β,28-diacétyl-1α,2α,25-trihydroxydamma-23-ène (**4**). On a aussi isolé les produits connus suivants: monoacétate de cabraléadiol (**5**), mansumbinol (**6**), 3β-amyrine (**7**), acide 3-α-acétylboswellique (**8**), 2-méthoxy-8,12-époxy-germacra-1(10),7,11-trién-6-one (**9**), 2-méthoxy-5-acétylfuranogermacra-1(10),7,11-trién-6-one (**10**), furadiénone (**11**), 2-méthoxy-5-acétyl-4-furanogermacra-1(10)*Z*-én-6-one (**12**), α-amyrine (**13**), sistostérol (**14**) et 3-*O*-acétate du stigmastérol (**15**). L'élucidation des structures a été réalisée à l'aide de méthodes physiques et spectroscopiques ainsi que par comparaison avec des données tirées de la littérature.

Mots-clés : Commiphora holtziana, Burseraceae, triterpènes, exsudate de gomme-oléo-résine.

[Traduit par la Rédaction]

Introduction

The genus *Commiphora* (*Burseraceae*) is widely distributed in the tropics and subtropics, particularly in Africa, Asia, and Australia.[1] Over 46 *Commiphora* species are acclimatized in the northeastern province of Kenya, and a good number of them are characterized by oleo-resin exudates used in medicine, food, and perfumery industries.[2] *Commiphora holtziana* (syn. *Commiphora erythraea*) is one such species growing in the wild and produces resin, an article of commerce.[3] The plant is used in the indigenous system of medicine as a remedy for microbial infections, anti-inflammatory, and as an acaricide.[4,5] Previous chemical analyses of the plant resin exudate yielded 2-methoxy-8,12-epoxygermacra-1(10),7,11-trien-6-one, 2-methoxy-5-acetylfuranogermacra-1(10),7,11-trien-6-one, and furadienone.[5,6] In the present study, steam-distilled residue of *C. holtziana* resin exudate was examined chemically leading to the isolation and identification of triterpenes of ursane and dammarane types.

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Experimental part

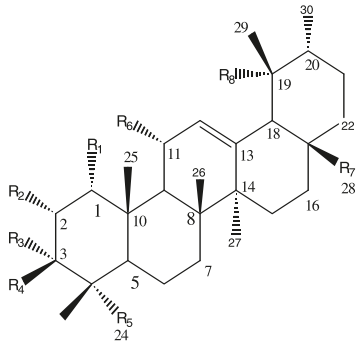
General experimental procedure

IR data were recorded on a PerkinsElmer FTIR 600 series. NMR data were measured in $CDCl_3$ and $CDCl_3$ + DMSO-d_6 on a Bruker DRX-400 NMR spectrometer operating at 400 and 100 MHz, respectively. MS data were obtained on a MAT 8200A instrument of Varian (Bremen). Preparative HPLC was performed on an instrument of JASCO labor und daten technik GmBH (RP-18, 250 × 20 mm, 7.4 μm JASCO Kromasil 100).

Oleo-gum resin material

Oleo-gum resins were collected from *C. holtziana* trees in Samburu district of Kenya by Mr. Norman Gachathi in March 2002 and were kept in a deep freezer in sealed polyethylene bags. Voucher specimens were confirmed after comparison with authentic herbarium samples at the Kenya Forestry Research Institute, Muguga, Kenya.

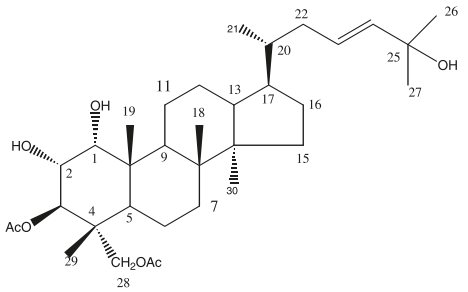
Chart 1.



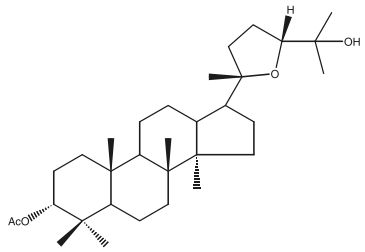
1: $R_1 = R_8 = OH$, $R_2 = R_6 = H$, $R_3 = R_4 = O$, $R_5 = CO_2Me$, $R_7 = CH_2OH$

2: $R_1 = R_3 = H$, $R_2 = R_6 = R_8 = OH$, $R_4 = OAc$, $R_5 = CO_2Me$, $R_7 = CH_2OH$

3: $R_1 = R_2 = OH$, $R_3 = R_8 = H$, $R_4 = R_6 = OAc$, $R_5 = CO_2Me$, $R_7 = CH_2OH$



4



5

Extraction and isolation

Steam-distilled residues of the resin (~1 kg) were extracted at room temperature with acetone (2.5 L) for 5 days. The extract was filtered and concentrated to give a brownish gummy material (350 g). Approximately 245 g was dissolved in a small amount of acetone and then adsorbed onto silica gel. Removal of acetone under reduced pressure yielded a free-flowing mass that was chromatographed over silica gel (medium pressure, pressure ~ 1 bar); eluted with *n*-hexane/EtOAc mixtures with increasing concentration of the more polar solvent, EtOAc, followed by EtOAc/MeOH mixtures (9:1, 4:1, 7:3, and 2:1), and elution concluded with MeOH, affording 250 fractions (100 mL each) whose compositions were monitored by TLC (eluent: *n*-hexane/EtOAc (9:1, 4:1, 2:1) and EtOAc/MeOH (9:1, 4:1). Those showing similar TLC profiles were combined, resulting into four pools (I–IV). Pool I (fractions 1–105, 35 g), an oily resinous material was further purified by medium-pressure chromatography to afford 2-methoxy-8,12-epoxygermacra-1(10), 7,11-trien-6-one (**9,** 78 mg), furadienone (**11,** 105 mg), 2-methoxy-5-acetylfuranogermacra-1(10),7,11-trien-6-one (**10,** 62 mg), and 2-methoxy-5-acetyl-4-furanogermacra-1(10)*Z*-en-6-one (**12,** 85 mg).[6–8] Fractions 111–170 constituted pool II (25 g) and was found to contain four major spots, which were resolved into α-amyrin (**13**, 200 mg), sistosterol (**14**, 104 mg), stigmasterol 3-*O*-acetate (**15**, 84 mg), and mansumbinol (**6,** 65 mg), using *n*-hexane/ethyl acetate (4:1, 3:2).[9–11] Pool III (fractions 171–205, 10 g) upon repeated fractionation using *n*-hexane/ethyl acetate (3:2, 1:2), collecting 20 mL each, allowed the isolation of 3β-acetylamyrin (**7,** 150 mg), 3α-acetylboswellic acid (**8,** 97 mg),[12,13] methyl-3β,11α-diacetyl-1α,2α,28trihydroxyurs-12-ene-24-oate (**3**, 35 mg), and cabraleadiol monoacetate (**5**, 95 mg).[14] Pool IV (21 g) was a gummy material, and its constituents were resolved into individual compounds using preparative HPLC (eluent: acetonitrile/H_2O, 35:65), mobile flow rate 10 mL min^{-1}, injecting 10 μL each time. The process afforded methyl 3-oxo-1α,19α, 28-trihydroxyurs-12-en-24-oate (**1**, 65 mg), methyl-3β-acetyl-2α,11α,19α,28-tetrahydroxyurs-12-en-24-oate (**2**, 87 mg), and 3β, 24-diacetyl-1α,2α,25-trihydroxydammar-23-ene (**4**, 75 mg).

Compound 1

Colorless amorphous powder, mp > 250 °C. $[\alpha]_D^{25}$ +13 (MeOH, *c* 0.05). UV (CH_3CN) λ_{max} (nm): 202 (log ε 3.4). IR ν_{max} (KBr) (cm^{-1}): 3430–3300 (OH), 2950, 2863, 1719 (CO_2Me), 1706 (C=O), 1646 (C=C), 1450, 1380, 1260, 1190, 1020, 960. ^1H NMR ($CDCl_3$ + DMSO-d_6, 400 MHz): see Table 1. ^{13}C NMR ($CDCl_3$ + DMSO-d_6, 100 MHz): data are in Table 2. EIMS *m/z* (%): 516 $[M]^+$ (1), 498 $[M - H_2O]^+$ (3), 480 $[M - 2H_2O]^+$ (7), 448 (2), 440 (3), 266 $[C_{15}H_{22}O_4]^+$ (10), 250 $[C_{16}H_{26}O_2]^+$ (3), 248 (15), 238 (100), 232 (1), 231 (45), 219 (17), 207 (2), 203 (11), 201 (21), 189 (75), 173 (27), 119 (55), 95 (34), 56 (48). HR-EIMS *m/z*: 516.3589 $[M]^+$, (calcd. for $C_{31}H_{48}O_6$: 516.3473).

Compound 2

Colorless amorphous powder, mp > 250 °C. $[\alpha]_D^{25}$ +73 (MeOH, *c* 0.2). UV λ_{max} (nm): 206 (log ε 4.0). IR ν_{max} (KBr) (cm^{-1}): 3420 (OH), 2950, 2856, 1732 (ester), 1721 (CO_2Me), 1640 (C=C), 1450, 1376, 1245, 1221, 1130, 980. ^1H NMR ($CDCl_3$ + DMSO-d_6, 400 MHz): see Table 1. ^{13}C NMR ($CDCl_3$ + DMSO-d_6, 100 MHz): see Table 2. EIMS *m/z* (%): 540 $[M - 2H_2O]^+$ (6), 522 (2), 481 (3), 310 $[C_{17}H_{26}O_5]^+$ (1), 292 (4), 266 $[C_{16}H_{26}O_3]^+$ (5), 250 (7), 249 $[C_{15}H_{21}O_4]^+$ (21), 248 $[C_{16}H_{24}O_2]^+$ (4), 235 $[C_{15}H_{23}O_2]^+$ (12), 231 $[C_{16}H_{23}O]^+$ (100), 213 (8), 218 $[C_{15}H_{22}O]^+$ (10), 203 (2), 189 (21). HR-ESIMS *m/z*: 576.3834 $[M + Na]^+$, (calcd. for $C_{33}H_{52}O_8Na$: 576.3827).

Compound 3

Colorless amorphous powder, mp > 250 °C. $[\alpha]_D^{25}$ +7.9 ($CHCl_3$, *c* 0.5). UV λ_{max} (nm): 198 (log ε 4.1). IR ν_{max} (KBr) (cm^{-1}): 3540 (OH), 2945, 2870, 1736 (ester), 1725 (CO_2CH_3), 1635 (C=C), 1460, 1382, 1250, 1120, 1040, 950. ^1H NMR ($CDCl_3$, 400 MHz): see Table 1. ^{13}C NMR ($CDCl_3$, 100 MHz): see Table 2. EIMS *m/z* (%): 618 $[M]^+$ (3), 600 $[M - H_2O]^+$ (1), 587 (4), 582 (2), 569 (6), 558 $[M - CH_3COOH]^+$ (11), 551 (7), 527 (9), 498 $[M - 2CH_3COOH]^+$ (6), 491 (11), 467 (2), 441 (1), 431 (5), 410 (1), 408 (3), 326

Table 1. ^{1}H NMR spectra of compounds **1**–**5**.

C	**1**	**2**	**3**	**4**	**5**
1	3.60 t (10.0, 4.5)	1.96 dd (12.0, 4.6), 0.94 dd (12.0, 9.0)	3.75 d (3.6)	3.60 d (4.5)	1.94 m, 1.12 m
2	1.84 m, 1.54 m	3.65 ddd (12.4 10.0, 3.2)	3.50 dd (10.2, 3.4)	3.77 dd (11.0, 3.3)	1.66 m, 1.59 m
3	—	4.50 d (11.6)	4.40 d (12.0)	4.65 d (12.4)	4.56 d (4.6)
4	—	—	—	—	—
5	1.02 m	1.00 m	0.98 m	0.92 m	0.84 m
6	1.68 m, 1.43 m	1.63 m, 1.40 m	1.62 m, 1.38 m	1.65 m, 1.35 m	1.54 m, 1.43 m
7	1.44 m, 1.24 m	1.56 m, 1.27 m	1.54 m, 1.28 m	1.46 m, 1.20 m	1.52 m, 1.25 m
8	—	—	—	—	—
9	1.66 dd (12.7, 6.6)	1.98 dd (10.3, 4.0)	1.95 d (9.3)	1.36 m	1.33 m
10	—	—	—	—	—
11	2.00 m, 1.92 m	3.70 dd (10.2, 3.6)	4.23 dd (8.8, 3.4)	1.47 m, 1.28 m	1.48 m, 1.16 m
12	5.26 br s	5.21 br s	5.25 br s	1.74 m, 1.16 m	1.63 m, 1.22 m
13	—	—	—	1.60 m	1.62 m
14	—	—	—	—	—
15	1.74 m, 1.16 m	1.60 m, 1.13 m	1.86 m, 1.01 m	1.46 m, 1.10 m	1.45 m, 0.96 m
16	1.92 m, 1.58 m	2.01 m, 1.62 m	1.96 m, 0.96 m	1.56 m, 1.42 m	1.69 m, 1.30 m
17	—	—	—	1.75 m	1.86 m
18	2.36 s	2.38 s	1.75 d (12.5)	0.94 s	0.95 s
19	—	—	1.53 m	0.86 s	0.88 s
20	1.20 m	1.17 m	1.46 m	1.30 m	—
21	1.58 m, 1.18 m	1.35 m, 1.17 m	1.51 m, 1.29 m	0.95 d (6.5)	1.02 s
22	1.86 m, 1.61 m	1.70 m, 1.51 m	1.59 m, 1.23 m	1.44 m, 1.06 m	1.90 m, 1.66 m
23	1.16 s	1.15 s	1.13	5.64 m	1.84 m, 1.76 m
24	—	—	—	5.58 m	3.45 dd (10.6, 5.3)
25	0.98 s	0.99 s	0.94 s	—	—
26	1.10 s	1.18 s	1.15 s	1.36 s	1.32 s
27	1.22 s	1.20 s	1.17 s	1.36 s	1.32 s
28	4.00 d (10.6), 3.80 d (10.6)	4.20 (11.0), 3.90 d (11.0)	4.10 d (11.1), 3.86 d (11.1)	0.95 s	0.88 s
29	1.30 s	1.33 s	0.88 d (6.8)	1.04 s	0.82 s
30	0.89 d (6.6)	0.95 d (6.7)	0.87 d (6.6)	0.84 s	0.89 s
CO_2*Me*	3.54 s	3.46 s	3.50 s	—	—
OCO*Me*	—	1.98 s	2.00 s, 2.03 s	1.99 s, 2.02 s	2.01 s

Table 2. ^{13}C NMR of compounds **1**–**5**.

C	1	2	3	4	5
1	73.4	46.2	71.5	74.3	38.8
2	44.1	67.6	74.0	72.0	24.7
3	216.0	78.2	80.1	80.0	81.0
4	54.2	55.0	54.0	42.2	37.6
5	48.4	48.8	48.3	43.5	54.7
6	20.8	18.1	20.6	20.3	18.3
7	33.2	32.4	33.1	36.2	34.8
8	39.4	39.8	40.4	40.8	41.4
9	38.4	48.2	46.7	46.8	51.1
10	38.9	38.6	40.6	37.4	37.4
11	23.8	71.4	79.5	25.7	22.0
12	127.3	126.0	125.4	33.0	27.0
13	140.0	141.6	139.0	42.6	43.2
14	42.4	43.6	42.3	52.4	50.0
15	27.2	28.8	28.1	36.2	32.4
16	23.7	26.0	23.4	27.9	25.8
17	46.1	47.4	45.9	54.2	49.7
18	52.6	54.0	37.8	15.8	16.0
19	72.8	72.4	39.9	16.3	16.6
20	43.0	42.6	37.8	35.7	86.6
21	26.9	27.2	31.8	18.8	24.4
22	36.9	38.4	38.5	36.2	35.5
23	14.1	16.1	14.4	126.3	26.8
24	181.4	179.7	180.8	141.4	86.2
25	16.0	15.0	14.2	79.9	73.0
26	16.8	16.9	17.0	25.3	26.6
27	26.3	25.0	26.5	25.1	27.0
28	66.6	65.7	67.5	26.4	16.6
29	28.7	26.7	26.3	19.7	28.0
30	16.9	17.0	16.8	16.1	17.3
CO_2*Me*	53.0	54.5	54.0		
O*C*OMe		171.0	170.1, 169.5	170.3, 169.8	171.1
OCO*Me*		23.0	23.8, 24.1	24.1, 23.5	22.8

$[C_{17}H_{26}O_6]^+$ (5), 298 (1), 292 $[C_{18}H_{28}O_3]^+$ (11), 266 $[C_{15}H_{22}O_4]^+$ (15), 261 $[C_{17}H_{25}O_2]^+$ (10), 249 $[C_{15}H_{21}O_3]^+$ (20), 234 (3), 232 $[C_{16}H_{24}O]^+$ (12), 231 (1), 201 $[C_{15}H_{21}]^+$ (100), 198 (41), 174 (23), 95 (51), 69 (70). HR-EIMS *m/z*: 618.2354 $[M]^+$, (calcd. for $C_{35}H_{54}O_9$: 618.2337).

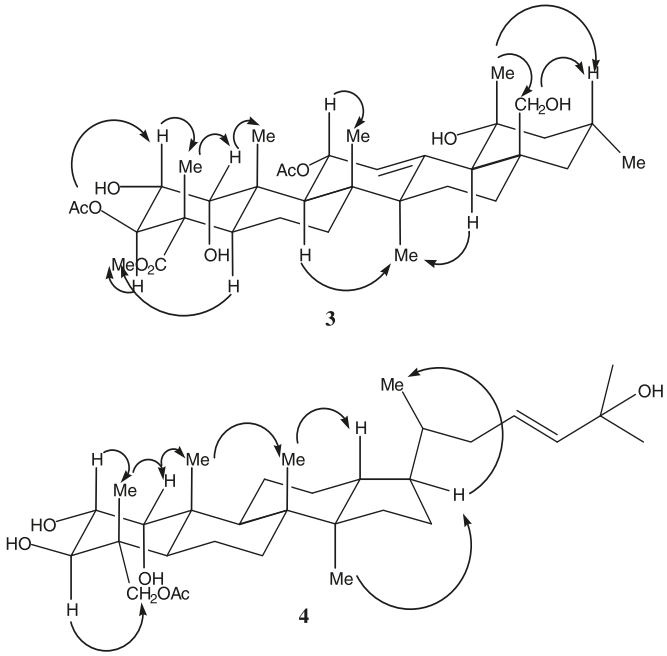
Compound 4

Colorless amorphous powder, mp 248–250 °C. $[\alpha]_D^{25}$ 116 ($CHCl_3$, *c* 0.02). UV λ_{max} (nm): 228 (log ε 4.1). IR ν_{max} (KBr) (cm^{-1}): 3500 (OH), 1736 (ester), 1644 (C=C). ^1H NMR ($CDCl_3$, 400 MHz): see Table 1. ^{13}C NMR ($CDCl_3$, 100 MHz): see Table 2. EIMS *m/z* (%): 558 $[M - H_2O]^+$ (4), 540 $[M - 2H_2O]^+$ (13), 516 $[M - HOAc]^+$ (4), 435 (22), 420 (3), 373 (31), 250 (42), 189 (100), 109 (75). HR-ESIMS *m/z:* 599.2495 $[M + Na]^+$, (calcd. for $C_{34}H_{56}O_7{}^+Na$: 599.2431).

Compound 5

Colorless crystals, mp151–153 °C. $[\alpha]_D^{25}$ + 61° ($CHCl_3$, *c* 0.1). IR ν_{max} (KBr) (cm^{-1}): 3440 (OH), 2952, 2860, 1734 (ester), 1450, 1380, 1255, 1145, 1040, 1020, 970. ^1H NMR ($CDCl_3$, 400MHz): see Table 1. ^{13}C NMR ($CDCl_3$, 100 MHz): see Table 2. CIMS *m/z* (%): 502 $[M]^+$ (9), 442 $[M - HOAc]^+$ (12), 424 $[M - H_2O - HOAc]^+$ (9), 292 (11), 249 (15), 248 (20), 213 (10), 218 (36), 201 (100).

Fig. 1. Key NOESY correlations for compounds **3** and **4**.

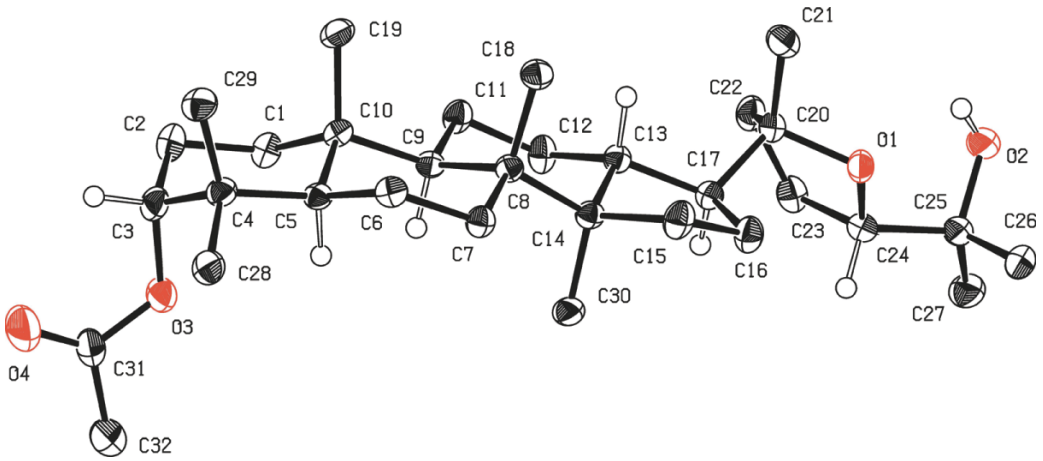


Results and discussion

Fractionation of the acetone extract of *C. holtziana* yielded compounds (**1**–**4**) together with eleven known ones (**5–15**) identified on the basis of spectroscopic and physical methods.

Compound **1**, isolated as colorless amorphous powder afforded positive Liebermann–Burchard test, suggesting the presence of a triterpene or sterol skeleton. Its molecular-ion peak at *m/z* 516.3589 (calcd. *m/z* 516.3473) by accurate mass measurement corresponds to the formula $C_{31}H_{48}O_6$, confirmed by ^{13}C NMR and DEPT spectra, which exhibited 31 carbon atoms resolved into 9 methylenes, 7 methyls, 6 methines, and 9 quaternary. The ^1H NMR spectrum (Table 1) indicated five tertiary methyls (δ 1.30, 1.22, 1.10, 1.06, 0.98), a methoxy carbonyl (δ 3.54), a secondary methyl group (δ 1.16, d, J = 6.6 Hz, CH_3-30), an oxymethine (δ 3.60, t, J = 10.0, 4.5 Hz, H-1), a terminal hydroxymethylene (δ 4.00, d, J = 10.6 Hz, H-28b; δ 3.80, d, J = 10.6 Hz, H-28a), and a trisubstituted double bond (δ 5.26, d, J = 4.0 Hz, H-12). In combination with the characteristic EIMS fragmentation ions at *m/z* 266 $[C_{15}H_{22}O_4]^+$ and 250 $[C_{16}H_{26}O_2]^+$ attributed to retro-Diels–Alder cleavage between C-9 and C-11, and between C-8 and C-14 bonds, it suggested that compound **1** is either an ursane- or oleanane-type triterpene containing a keto, two hydroxyls, a methyl carbonyl, and a terminal hydroxymethylene functional group. On the basis of the cleavage, it was suggested that the oxo, one hydroxyl, and the methoxy carbonyl were present in the rings A/B, whereas the terminal hydroxymethylene and another hydroxyl group were positioned in the rings C/D part of the molecule.[15–19] The ^1H NMR singlet peak at δ 2.36 is a characteristic signal for the H-18 of an ursane-type with 19-O-substitution.

Fig. 2. ORTEP-style plot of compound **5** showing the atom-numbering scheme and the solid-state conformation. Thermal ellipsoids are drawn at the 50% probability level.



Together with the ^{13}C NMR olefinic carbon signals at δ 127.3 (C-12) and δ 140.0 (C-13) and an oxygen bearing quaternary carbon at δ 72.8 (C-19), it confirmed the 19α-hydroxyurs-12-ene skeleton for the compound.[17,20] The positions of the substituents and their stereochemistry were established by 2D experiments, whereby the key proton resonances were assigned by gHSQC correlations. The long-range correlation (gHMBC) showed significant cross peaks between H-1 (δ 3.60) and C-3 (δ 216.0), between H-18 (δ 2.36) and C-19 (δ 72.8) and C-28 (δ 66.6), between H-5 (δ 1.02) and C-24 (δ 181.4), and between H-18 and C-20 (δ 43.0). The position of a hydroxyl-bearing methine proton at δ 3.60 (H-1) was confirmed to be at C-1, as its carbon resonance (δ 73.4) showed a gHMBC correlation with Me-25, a fact further corroborated by the ^{13}C NMR shielded resonances for C-5 (δ 48.4) and C-9 (δ 38.4), respectively.[21] The stereochemistry of the hydroxyl group at C-1 was determined as α and axial on the basis of NOESY cross peaks between H-1 and Me-23 and also between H-1 and Me-25. This was further supported by the coupling $J_{H\text{-}1/H\text{-}2}$ (cis) = 4.5 Hz consistent with the 1α-hydroxy-substituted ring A, which is in agreement with literature report for rings A/B part of 1α-hydroxy-3-oxoolean-12-en-oic acid.[21] Thus, based on the results, compound **1** was concluded to be methyl-3-oxo-1α,19α,28-trihydroxyurs-12-en-24-oate.

Compound **2** was isolated as colorless powder from CH_2Cl_2/MeOH (9:1). According to EIMS, ^{13}C NMR, and DEPT, its composition is $C_{33}H_{52}O_8$, consistent with nine degrees of unsaturation. Data from ^1H and ^{13}C NMR (Tables 1 and 2) indicated a secondary metabolite with a structure similar to that of compound **1**. The major structural difference is the additional acetyl group (δ 1.98) and two oxygenated methines (δ 4.50, d, J = 11.6 Hz, H-3; δ 3.70, dd, J = 10.2, 3.6 Hz, H-11). Both the ^1H and ^{13}C NMR depicted data typical of a triterpene of ursane skeleton containing a double bond between C-12 and C-13.[17,22,23] Support for this conclusion was further provided by the mass spectrum. Retro-Diels–Alder cleavage, characteristic of Δ^{12}-pentacyclic triterpenes, leads to m/z 310 $[C_{17}H_{26}O_5]^+$ and 250 $[C_{16}H_{26}O_2]^+$. The fragments at m/z 249 $[C_{15}H_{21}O_4]^+$, resulting from m/z 310, and those at m/z 232 $[C_{16}H_{24}O]^+$ and 201 $[C_{16}H_{21}]^+$, resulting from m/z 250, indicated the presence of hydroxyl, acetyl, and a methoxy carbonyl group in rings A/B, whereas another hydroxyl, an oxymethylene and an oxygenated tertiary methyl group were in rings D/E part of the molecule.[24] This was further corroborated by the singlet proton signal at δ 2.38 (H-18), typical of 19α-hydroxyurs-12-ene skeleton, which correlated with C-28 (δ 65.7), C-12 (δ 126.0), and C-20 (δ 42.6) in the HMBC spectrum. On the other hand, in the ^1H–^1H homonuclear chemical correlation spectrum, the comparatively low field oxymethine proton at δ 4.50 correlated with one proton and hence was assigned to C-3, further evidenced by gHSQC correlation between H-3 (δ 4.50) and C-3 (δ 78.2). The large coupling constant (J = 11.6 Hz) allowed the assignment of β and equatorial orientation of the acetyl group. Similarly, the positions of two hydroxyl-bearing methine protons at δ 3.65 (ddd, J = 12.4, 10.0, 3.2 Hz, H-2) and δ 3.70 (d, J = 10.2, 3.6 Hz, H-11) and their disposition as both α was suggested by the coupling constants, which agreed well with 2α, 11α-dihydroxy substitution on rings A/C,[25,26] respectively, and were confirmed by gHMBC cross peaks between H-2 (δ 3.65) and C-4 (δ 55.0), and between H-11 (δ 3.70) and C-13 (δ 141.6). Spin-decoupled and NOESY experiments allowed the complete assignment of the stereochemistry of compound **2**. Thus, on the basis of accrued data, **2** was concluded to be methyl 3β-acetyl-2α,11α,19α,28- tetrahydroxyurs-12-en-24-oate.

Compound **3** was isolated as colorless powder from CH_2Cl_2–MeOH (9:1). It afforded an IR spectrum indicating the presence of hydroxyl, ester, methoxylated carbonyl group, and a double bond (3540, 1734, 1725, 1635 cm^{-1}). Its ^1H NMR spectrum showed a vinyl proton (δ 5.25, br s, H-12), an oxymethine proton (δ 4.40, d, J = 12.0 Hz, H-3), an allylic methine proton attached to an oxygen functionality (δ 4.23, d, J = 8.8, 3.4 Hz, H-11), a terminal hydroxymethylene (δ 4.10, d, J = 11.1 Hz) and δ 3.86 (d, J = 11.1 Hz, CH_2-28), two hydroxy methine protons (δ 3.50, dd, J = 10.2, 3.4 Hz, H-2; δ 3.75, d, J = 3.6 Hz, H-1), two acetyl groups (δ 2.03, 2.00, both singlets), four tertiary methyls (δ 1.17, δ 1.15, δ 1.13, δ 0.94), and two secondary methyl groups (δ 0.88, d, J = 6.8 Hz, Me-29; δ 0.87, d, J = 6.6 Hz, Me-30). The compound exhibited an HR-EIMS molecular ion peak at m/z 618.2354, compatible with the formula $C_{35}H_{54}O_9$. Other significant peaks at m/z 326 $[C_{17}H_{26}O_6]^+$

and 234 $[C_{16}H_{26}O]^+$ were in accord with the retro-Diels–Alder cleavage, typical of C-12 unsaturated pentacyclic triterpenes.[27] From the fragmentation pattern, it was evident that rings A/B contained two hydroxyls, an acetyl, and a methoxy carbonyl group, a fact that was evidenced by the daughter ions at *m/z* 266 $[C_{15}H_{22}O_4]^+$ and 249 $[C_{15}H_{21}O_3]^+$ from the ions *m/z* 326 $[C_{17}H_{26}O_6]^+$. Similarly, fragments at *m/z* 261 $[C_{17}H_{25}O_2]^+$, 232 $[C_{16}H_{24}O]^+$, and 201 $[C_{15}H_{21}]^+$ signified the presence of another acetoxy and an hydroxymethylene group in the rings D/E. The foregoing conclusions were confirmed by gHSQC and gHMBC experiments; in particular, the gHMBC correlations observed between H-18 (δ 1.75) and C-28 (δ 67.5), between H-11 (δ 4.23) and C-13 (δ 139.0), and between H-5 (δ 0.98) and C-24 (δ 180.8) and C-3 (δ 80.1). The relative stereochemistry of substituents was assigned by NOESY experiments (Fig. 1). Therefore, on the basis of spectroscopic data, as well as comparison with known compounds, **3** was structurally concluded to be methyl 3β,11α-diacetyl-1α,2α,28-trihydroxyurs-12-en-24-oate.

Compound **4** was obtained as colorless powder, mp 248–250 °C after crystallization from a CH_2Cl_2/MeOH. Its molecular formula, $C_{34}H_{56}O_7$, was deduced from the HR-ESIMS molecular ions at *m/z* 599.2495 $[M + Na]^+$ (calcd. 599.2431) and by quantification of methyls, methylenes, methines, and quaternary carbon atoms revealed in the ^{13}C NMR and DEPT spectra. The IR spectrum displayed significant bands at 3500, 1736, and 1644 cm^{-1}, signifying the presence of OH, ester, and a carbon–carbon double bond, respectively. The EIMS fragment peaks at *m/z* 558 $[M - H_2O]^+$, 540 $[M - 2H_2O]^+$, and 516 $[M - HOAc]^+$ are characteristic features of the dammarane skeleton with two acetoxy and two hydroxyl groups in the rings A/B.[20,28] The ^1H NMR spectrum corroborated the findings by showing nine signals for methyl groups, two of them acetoxy, a fact further supported by two ester carbonyl peaks at δ 170.3 and δ 169.8 with corresponding methyls at δ 24.1 and δ 23.5, respectively. A singlet at δ 1.36, typical of a hydroxyl isopropyl moiety, and signals of an isolated double bond at δ 5.64 and δ 5.58 evidenced the presence of a $–CH(CH_3)–CH_2–CH=C(CH_3)_2–OH$ system in the C-17 side chain.[12] This was confirmed by ^1H-detected gradient heteronuclear single quantum coherence (gHSQC) experiments where the two peaks at δ 126.3 (C-23) and δ 141.4 (C-24) represented the isolated double bond while the other peak at δ 79.9 corresponded to hydroxy isopropyl moiety. Further insight into the structure of **4** was provided by close examination of both the mass spectrum and the ^1H NMR data, whereby the EIMS fragments in combination with ^1H NMR peaks at δ 4.65 (d, J = 12.4 Hz, H-3), δ 3.77 (dd, J = 11.0, 3.3 Hz, H-2), and δ 3.60 (d, J= 4.5 Hz, H-1) confirmed the presence of an acetoxy and three oxymethines linked in a $CH_{eq}(O-H_{ax})–CH_{eq}(OH_{ax})–CH_{ax}(OAc_{eq})–CCH_3(CH_2OAc)$ system requiring the two oxymethine protons to be at C-1 and C-2 both as α, while the acetoxy to be at C-3 as β[20,28] and was consistent with gHSQC, gHMBC, and NOESY correlations (Fig. 1). On this basis, the structure of compound **4** was concluded as 3β,28-diacetyl-1α,2α,25-trihydroxydammar-23-ene.

Compound **5** was isolated as colorless crystals from *n*-hexane/EtOAc (2:1). Its HR-EIMS showed a peak at *m/z* 502.4074 $[M]^+$, corresponding to the formula $C_{32}H_{54}O_4$. The ^1H and ^{13}C NMR spectral data revealed a close relationship, if not identity, with cabraleadiol monoacetate.[14] However, HMQC and HMBC experiments lead to minor but possibly significant differences from the reported ^1H NMR spectrum data, as concluded from HETCOR experiments. Thus, the structure of **5** was validated by an X-ray crystallographic study (Fig. 2) to be cabraleadiol monoacetate.[29]

Supplementary data

Supplementary data for this article are available on the journal Web site (canjchem.nrc.ca) or may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Ottawa, ON K1A 0R6, Canada. DUD 3974. For more information on obtaining material, refer to cisti-icist.nrc-cnrc.gc.ca/cms/unpub_e.shtml. CCDC 727028 contains the X-ray data in CIF format for this manuscript. These data can be obtained, free of charge, via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax +44 1223 336033; or deposit@ccdc.cam.ac.uk).

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