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Two octanordammarane triterpenes from *Commiphora kua*

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Abstract

The resin of *Commiphora kwo* yielded two new octanordammarane triterpenes namely 15α-hydroxymansumbinone and 28-acetoxy-15α-hydroxymansumbinone, along with the four known compounds, mansumbinone, mansumbinol, (16*S*, 20*R*)-dihydroxydammar-24-en-3-one and T-cadinol. These structures were elucidated by spectroscopic techniques, including 1D and 2D NMR spectroscopy, and X-ray analysis.

Keywords: *Commiphora kua*; Burseraceae; 15α-Hydroxymansumbinone; 28-Acetoxy-15α-hydroxymansumbinone; Mansumbinone; Mansumbinol

1. Introduction

Commiphora kua (J.F. Royle) Vollesen var. *gowlello* (Sprague) J.B. Gillett (Burseraceae) is a 3–5 m tall tree found mainly in Kenya, Somalia, Ethiopia and Arabia (Vollesen, 1989). The plant is known by different local names such as Lolowi (Turkana, Kenya) and Rahan-Reb or Warab-Reb (Somali). It produces wood that is used to make household utensils (cups and pots), furniture (stools) and tools (hammers). During rainy seasons, its trunk is cut and sucked to quench thirst. In dry seasons, the tree produces a brown resin, which is used as incense.

Previous phytochemical investigations of the resin of *C. kua* (Provan and Waterman, 1986) resulted in the isolation of mansumbinone (**1**), mansumbinol (**2**), and (l6*S*, 20*R*)-dihydroxydammar-24-en-3-one (**3**). In the first report on this plant, it was identified as *C. incisa* but later on this was corrected to *C. kua* by the same workers (Provan et al., 1992). Duwiejua et al. (1993) showed mansumbinone to possess significant anti-inflammatory activity.

Mansumbinane or octanordammarane is the general name given to unique compounds derived from dammarane triterpenes and characterized by lack of the usual C_8 side chain present at C-17. In this paper, we describe the isolation and structure elucidation of two new C-15 hydroxylated mansumbinanes, and four known compounds from the resin of *C. kua*.

2. Results and discussion

TLC analysis of the petrol extract of the resin of *C. kua* indicated the presence of at least six compounds when sprayed with vanillin–H_2SO_4 reagent. The extract was subjected to flash CC on silica gel followed by crystallization affording the four known compounds: mansumbinone (**1**), mansumbinol (**2**), (16*S*, 20*R*)-dihydroxydammar-24-en-3-one (**3**), T-cadinol (**6**), and the two novel octanordammarane triterpenes **4** and **5**. The known compounds were identified by comparison of their spectral data with those available in the literature.

HREIMS of compound **4**, which was isolated as translucent crystal, revealed the molecular formula $C_{22}H_{34}O_2$. The fragment $[C_{14}H_{21}O]^+$ (**7**) indicated a tetracyclic 3-oxotriterpene which shows typical fragmentation in ring C (Provan et al., 1992). Furthermore compound **4** was optically active, and its IR spectrum exhibited absorptions indicating the presence of hydroxyl (3480 cm^{-1}) and carbonyl (1702 cm^{-1}) groups. The presence of a 3-oxotriterpene moiety was evident from the ^1H NMR spectrum which exhibited signals for the H-1 protons at 1.43 ppm (*m*) and 1.90 ppm (*ddd*) and for the H-2 protons

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at 2.42 (*ddd*) and 2.48 ppm (*ddd*). Cross-peaks in the H–H COSY spectrum arose as a result of vicinal coupling between the olefinic protons H-16 and H-17, W-bond spin–spin long-range couplings between H-l5β and H-l3β, and the allylic coupling of H-15β with H-17.

A comparison (cf Table 1) of the ^{13}C NMR spectrum of **4** with that of mansumbinone (**1**) revealed a down-field shift of 3.8 ppm for the C-16 olefinic carbon in **4** (130.1–133.9 ppm), an up-field shift of 7.4 ppm for the Me-30 of **4** (17.1–9.7 ppm), and the replacement of the C(15) signal of **1** at 39.9 ppm by an oxymethine carbon signal at 79.1 ppm. The down-field shift of the olefinic carbon signal at C-16 might be due to the proximity of the hydroxyl group. To the best of our knowledge, this is the first example of C-15 oxidation in the dammarane group of triterpenoids.

HMQC and COLOC connectivities (Fig. 1) confirmed the structure and allowed the assignment of all the ^{13}C NMR signals except for C- 16 and C-17 which might be interchangeable. Furthermore, the relative stereochemistry of **4** at C-15 was deduced from the NOESY spectrum which revealed cross-peaks for H-15/Me-18/H-16/H-17 and H-13/Me-18 (Fig. 2), thus confirming that the structure of compound **4** is 15α–hydroxymansumbinone.

Crystalline **5**, $C_{24}H_{36}O_4$ (HREIMS), was found to be optically active, and showed UV absorption at λ_{max}^{EtOH} 207 nm. The IR spectrum exhibited absorptions indicative of hydroxyl (3429 cm^{-1}), ester carbonyl (1743 cm^{-1}) and ketonic carbonyl (1698 cm^{-1}) groups. Its ^{1}H NMR spectrum was similar to that of **4** but displayed the following differences: the C(28) methyl singlet of **4** was replaced by two protons constituting an AB-system at 4.02 and 4.05 ppm, suggesting a –CH_2O– group, and a methyl singlet at 2.02 ppm (CH_3CO).

The ^{13}C NMR of **5** supported the presence of an acetyl group, an oxymethine carbon and an oxymethylene group by displaying resonances at 170.8 (C), 79.0 (CH), and 67.7 (CH_2), respectively (Table 1). HMQC, HMBC and COLOC connectivities (Fig. 1) were used to assign its ^{13}C signals. Based on these data, compound **5** was assigned the structure 28-acetoxy-l5α-hydroxymansumbinone. This structure was also confirmed by X-ray diffraction analysis. As shown in Fig. 3, ring A manifested two very flexible conformations with high thermal motions. Apart from that there are no major differences between the two independent conformations in the X-ray structure. Crystallographic data of **5** have been deposited at Cambridge Crystallographic Data Centre, UK.

Table 1
^{1}H and ^{13}C NMR spectral data[a] of compounds **1**, **4** and **5**[b]

Position	**1** δ_C	**1** δ_H (multi., *J*, Hz)	**4** δ_C	**4** δ_H (multi., *J*, Hz)	**5** δ_C	**5** δ_H (multi., *J*, Hz)
1	39.9	1.45 (*m*)	40.0	1.43 (*m*)	38.6	1.42 (*m*)
		1.90 (*ddd*, 12.8, 7.7, 4.8)		1.90 (*ddd*, 13.1, 8.1, 4.4)		1.96 (*ddd*, 13.1, 8.1, 3.7)
2	34.2	2.44 (*ddd*, 15.4, 7.9, 4.8)	34.1	2.42 (*ddd*, 14.6, 7.7, 4.4)	35.1	2.44 (*ddd*, 13.7, 7.3, 3.7)
		2.47 (*ddd*, 15.4, 9.8, 7.7)		2.48 (*ddd*, 14.6, 8.1, 5.7)		2.49 (*ddd*, 15.2, 8.1, 6.3)
3	218.2		218.0		215.0	
4	47.5		47.5		50.3	
5	55.5	1.40 (*m*)	55.3	1.41 (*m*)	48.7	1.68 (*m*)
6	19.8	1.48 (*m*)	19.6	1.50 (*m*)	19.6	1.34 (*m*), 1.53 (*m*)
7	34.8	1.33 (*m*), 1.60 (*m*)	35.7	1.54 (*m*)	35.5	1.52 (*m*)
8	39.8		40.4		40.3	
9	50.3	1.54 (*m*)	50.5	1.51 (*m*)	50.3	1.54 (*m*)
10	37.1		37.1		36.8	
11	22.4	1.60 (*m*)	22.3	1.59 (*m*)	22.2	1.34 (*m*), 1.57 (*m*)
12	23.9	1.72 (*m*)	24.4	1.71 (*m*)	24.3	1.46 (*m*), 1.72 (*m*)
13	47.8	2.73 (*m*)	46.2	2.64 (*m*)	46.2	2.64 (*m*)
14	53.0		58.7		58.7	
15	39.9	1.70 (*m*), 2.35 (*m*)	79.1	4.91 (*m*)	79.0	4.91 (*m*)
16	130.1 b	5.65 (*m*)	133.9 c	5.51 (*m*)	133.9 d	5.52 (*m*)
17	134.1 b	5.57 (*m*)	134.6 c	5.65 (*m*)	134.6 d	5.67 (*m*)
18	17.9	1.04 (*s*)	17.9	1.12 (*s*)	18.0	1.14 (*s*)
19	15.9	0.94 (*s*)	16.1	0.94 (*s*)	15.9	0.97 (*s*)
28	26.8	1.07 (*s*)	26.8	1.06 (*s*)	67.7	4.02 (*AB*, 11.0)
						4.05 (*AB*, 11.0)
29	21.1	1.03 (*s*)	21.0	1.03 (*s*)	17.3	1.01 (*s*)
30	17.1	0.99 (*d*,1.1)	9.7	1.01 (*s*)	9.7	1.03 (*s*)
$\underline{CH_3}CO$					21.0	2.02 (*s*)
$CH_3\underline{CO}$					170.8	

[a] ^{1}H: 400 MHz; ^{13}C: 100 MHz; solvent and internal reference: $CDCl_3$; δ values are in ppm and are referenced against residual $CHCl_3$ (δ_H7.25) and $CDCl_3$ (δ_C 77.1) signals.

[b] Signals with the same letters in the same column may be interchangeable.

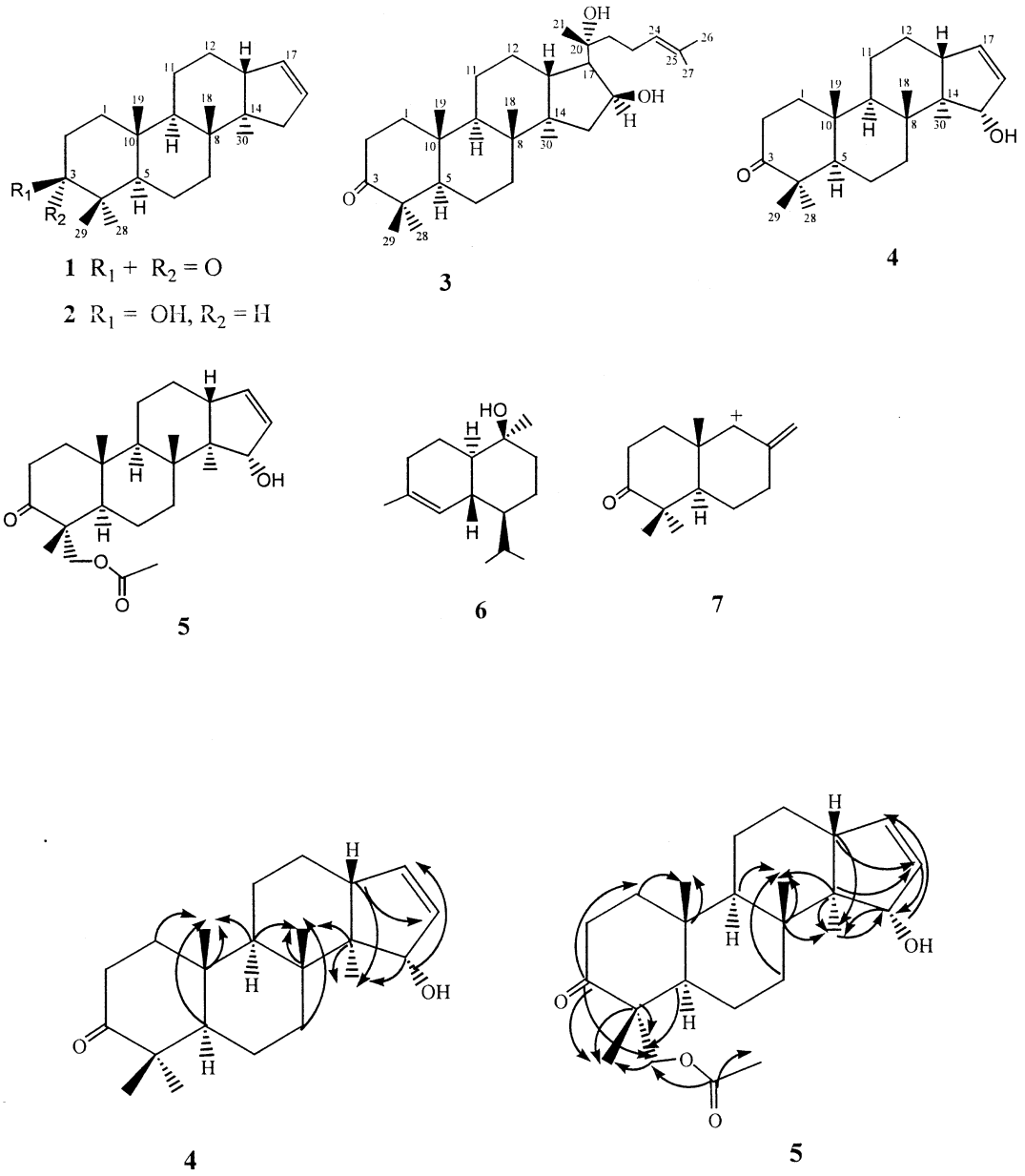

Fig. 1. Selected C/H correlations obtained by COLOC spectra of **4** and **5**. Arrows point from carbon to proton.

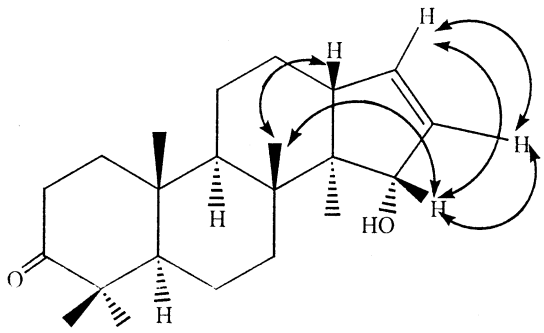

Fig. 2. Relative stereochemistry of **4**. The arrows denote NOESY correlations.

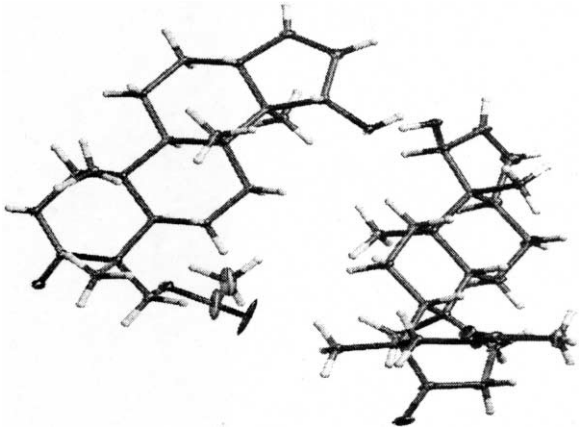

Fig. 3. Molecular structure of **5** showing two conformations as determined by X-ray analysis.

The precursor of the mansumbinanes has been suggested to be a dammarane triterpene such as **3** (Provan and Waterman, 1986) in which fission of the C-17/C-20 bond is achieved via loss of C-20 or the C-16 hydroxy group with concomitant formation of the double bond. The presence of **4** and **5** in *C. kua* may shed light on the biogenesis and metabolism of mansumbinanes.

3. Experimental

3.1. General

Melting points are uncorrected. TLC was performed on pre-coated plates (Silica gel 60 F_{254}, Merck) using *n*-hexane/EtOAc (7:3) 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 tablets. UV spectra were recorded on a Shimadzu UV/vis spectrophotometer. ^{1}H and ^{13}C NMR were recorded on a Jeol JNM-EX400 instrument at 400 and 100 MHz, respectively using $CDCl_3$ as solvent. EIMS data were generated on a VG Quattro quadrupole mass spectrometer operated at 70 eV.

3.2. Plant material

Resin was collected from *C. kua* trees found in Natorbe Hill, north Kenya in May 1998. The plant was identified by Ms Pat Curry (Saltlick, Kenya) and Dr. Kaj Vollesen (Kew Botanic Gardens, UK). Voucher specimens have been deposited at the National Herbarium (number: 072789), Addis Ababa University, Ethiopia and at the East African Herbarium, Nairobi, Kenya (number: Pat Curry-AD5).

3.3. Extraction and isolation

Ground resin (150 g) was extracted with petrol and concentrated to yield 35 g of extract. Part of the extract (25 g) was subjected to CC over silica gel (270 g) eluting with *n*-hexane containing increasing amounts of EtOAc. Fractions eluted with 2–10% EtOAc were rechromatographed and afforded compounds **1** (105 mg), **2** (15 mg) and **6** (97 mg). The fraction eluted with 30% EtOAc was further purified by CC to yield compound **4** (110 mg). Similarly, the fraction eluted with 40% EtOAc furnished compounds **3** (150 mg) and **5** (210 mg).

3.4. Mansumbinone (*1*)

Crystals from *n*-hexane/CH_2Cl_2 (9:1), mp 118–120 °C (lit. 122–123 °C, Provan and Waterman, 1986); R_f = 0.86. $[\alpha]_D^{22}$ +16° ($CHC1_3$, *c* 1.0) [lit. +17° ($CHCl_3$, *c* 0.60), Provan and Waterman, 1986]. ^{1}H NMR, MS and IR spectra were in good agreement with those previously reported (Provan and Waterman, 1986). ^{1}H and ^{13}C NMR data are presented in Table 1.

3.5. Mansumbinol (*2*)

Amorphous, R_f = 0.70. $[\alpha]_D^{22}$ −7° ($CHC1_3$, *c* 0.5) [lit. −23° ($CHC1_3$, *c* 0.18) Provan and Waterman, 1986]. ^{1}H and ^{13}C NMR, MS and IR spectral data were in good agreement with those reported (Provan and Waterman, 1986).

3.6. 16S, 20R-Dihydroxydammar-24-en-3-one (*3*)

Crystals from *n*-hexane/CH_2Cl_2 (3:2), mp 174–176 °C, (lit. 181–183 °C, Provan and Waterman, 1986); R_f = 0.46. $[\alpha]_D^{22}$ +68° ($CHC1_3$, *c* 1.0) [lit. +55° ($CHC1_3$, *c* 0.85), Provan and Waterman, 1986]. ^{1}H NMR, ^{13}C NMR, MS and IR spectral data were consistent with those reported in the literature (Provan and Waterman, 1986).

3.7. 15a-Hydroxymansumbinone (*4*)

Colorless crystals from *n*-hexane/CH_2C1_2 (4:l), mp 149–150 °C, R_f = 0.50. $[\alpha]_D^{22}$ +18°($CHC1_3$, c 0.8). UV λ_{max}^{EtOH} nm (log ε): 206 (3.46). IR ν_{max}^{KBr} cm^{-1}: 3480, 3050, 2946, 2869, 1702, 1452, 1385, 1243, 1026, 984. HREIMS: *m*/*z* 330.2557 $[M]^+$ ($C_{22}H_{34}O_2$ requires *m*/*z* 330.2559), 205.1591 ($C_{14}H_{21}O$ requires *m*/*z* 205.1592). EIMS *m*/*z* (rel. int.): 330 $[M]^+$ (49), 315 (16), 297 (10), 220 (13), 205 (38), 187 (10), 163 (13), 135 (16), 121 (22), 110 (100), 109 (44), 95 (69), 81 (41), 67 (29), 55 (40). ^{1}H and ^{13}C NMR: Table 1.

3.8. 28-Acetoxy-15α-hydroxymansumbinone (*5*)

Colorless crystals from *n*-hexane/CH_2C1_2 (1:2), mp 135–137 °C, R_f = 0.28. $[\alpha]_D^{22}$ + 25° ($CHC1_3$, *c* 1.0). UV λ_{max}^{EtOH} nm (log ε): 207 (3.52). IR ν_{max}^{KBr} cm^{-1}: 3429, 3036, 2947, 2869, 1743, 1698, 1443, 1414, 1374, 1242, 1036, 978, 778. HREIMS: *m*/*z* 388.2614 ($C_{24}H_{36}0_4$ requires *m*/*z* 388.2614). EIMS, *m*/*z* (rel. int): 388 $[M]^+$ (39), 373 (5), 328 (7), 313 (16), 279 (99), 219 (21), 205 (16), 203 (30), 175 (24), 161 (26), 135 (32), 121 (39), 110 (80), 109 (55), 95 (100), 81 (56), 79 (35), 55 (49). ^{1}H and ^{13}C NMR: Table 1.

3.9. T-Cadinol (*6*)

Amorphous, R_f = 0.78. $[\alpha]_D^{22}$ −2° ($CHCI_3$, *c* 1.0) [lit−4.7° ($CHCl_3$, *c* 4.4), Cheng et al., 1967, +3.4°($CHC1_3$, *c* 1.2), Borg-Karlson et al., 1981]. The ^{1}H NMR, ^{13}C NMR and MS data were in agreement with those reported in the literature (Borg-Karlson et al., 1981; Claeson et al., 1990).

3.10. X-ray analysis of *5*

A colorless crystal of dimensions 0.6×0.4×0.4 mm was mounted on a Nonius CAD-4 diffractometer with

graphite-monochromated MoK_α ($\lambda = 0.71073$ Å) radiation. The lattice parameters and orientation matrix were determined by a least-squares fit of 25 reflections with $\theta > 1.43$–$24.99°$. Compound **5**, $C_{24}H_{36}O_4$, crystallizes in the monoclinic space group $P2_1/n$, with $a = 12.238(11)$, $b = 12.226(10)$, $c = 14.382(11)$ Å, $\beta = 97.33(7)$, $V = 2134(3)$ Å^3, $Z = 4$ and with a calculated density of 1.209 Mg m^{-3}. The intensity data were collected at 20 °C using a $\omega 2$-θ scanning procedure to a 2θ limit of 50°. A total of 3949 reflections (independent) were collected. Neither extinction nor absorption corrections were applied to the data. The absorption coefficient was 0.080 mm^{-1}. The crystal structure was solved using direct methods and refined using a full-matrix least-squares procedure. The positions of the H-atoms were all located from difference maps.

All programs used in the solution (SHELXS-97, Sheldrick, 1997a), refinement (SHELXL-97/2, Sheidrick, 1997b) and display (ORTEP III, Burnett and Johnson, 1996) of the structures are included in the OSCAIL program package (McArdle, 1993).

Atomic coordinates, bond lengths and angles, and thermal parameters may be obtained from the Cambridge Crystallographic Data Centre on quoting the depository number CCDC 162142.

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