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SESQUITERPENES FROM *CARISSA EDULIS**

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Key Word Index—*Carissa edulis*; Apocynaceae; carissone; cryptomeridiol; β-eudesmol; new hydroxylated carissones; germacrenone.

Abstract—The methanolic extract from the root of *Carissa edulis* contains about 5% sesquiterpenes. Besides carissone, cryptomeridiol and β-eudesmol, three hitherto unknown sesquiterpenes of the eudesmane-type and a novel germacrane derivate have been isolated.

INTRODUCTION

Carissa edulis is widely used in West African folk medicine [1, 2]. In a previous paper we reported on the lignans and some of the other compounds present in this plant [3]. In continuation of this work we have investigated the sesquiterpene fraction of the methanolic root extract. This resulted in the isolation of seven sesquiterpenes: carissone (**1**), which had already been isolated from other *Carissa* species [4–6], was found to be the main sesquiterpene; as minor components, we isolated cryptomeridiol (**2**) [7–9] and β-eudesmol (**3**) [10] along with the hitherto unknown hydroxylated carissones **4** to **6** and the novel germacrane type sesquiterpene **7**.

RESULTS AND DISCUSSION

The methanolic extract of the root of *C. edulis* was treated as described in an earlier paper [3]. Repeated chromatography of the petrol and of the tetrachloromethane soluble fraction of this extract on silica gel and Sephadex LH 20 afforded the sesquiterpenes **1–7**.

Compounds **1–3** were identified on the basis of their physicochemical data as carissone (**1**), cryptomeridiol (**2**) and β-eudesmol (**3**).

The structures of **4–7** were deduced from their ^{1}H, ^{13}C NMR and MS.

The sesquiterpenes **4–6** all showed IR and UV spectra very similar to those of carissone (**1**). The NMR and mass spectra indicated the presence of hydroxylated carissones: careful studies of the ^{1}H NMR spectra of **4–6** and **1** (Table 1) led to the assignment of a 6α-hydroxy group in **4**, a 6β-hydroxy group in **5** and a 2α-hydroxy group in **6**. The configurations followed from the characteristic coupling constants and the observed influence of the hydroxy groups on neighbouring protons. Thus H-8 in the spectrum of **5** appeared at lower field than in the spectrum of **4**, because of the influence of the syn-axial hydroxy group at C-6. The observed deshielding of H-7 in **4** and of H_{eq}-1 in **6** was in good agreement with the equatorial positions of the hydroxy groups at C-6 (in **4**) and at C-2 (in **6**) respectively.

Further evidence for structures **4** and **5** came from oxidation experiments: when treated with pyridiniumchlorochromate both compounds yielded the same diketone **8**, which exhibited the typical electron spectrum of a 2-butene-1,4-dione [11].

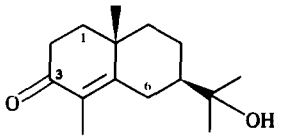
Additional proof was provided by ^{13}C NMR studies of **1**, **5**, and **6** (Table 2).

The signals of **1** were assigned by SFORD experiments and by comparison with the spectral data published for **9** [12] and **10** [13]. Correlation with the spectrum of **1** allowed the assignment of all ^{13}C-resonances of **5** and **6**.

Sesquiterpene **7** belonged to another structural group; its mass spectrum [M^+ at m/z 238 ($C_{15}H_{26}O_2$)] exhibited a significant fragment at m/z 59 (C_3H_7O) suggesting the presence of a hydroxyisopropyl group. Study of the ^{1}H NMR spectrum (Table 3) led to partial structure **11**.

From the ^{13}C NMR signals, **11** had to contain four methyl, five methylene, and two methine groups and in addition one quarternary carbon atom; therefore, **11** could be completed to **7**. Stereochemistry at the double bond followed from the chemical shift of the allylic methyl

*Part 17 in the series "Constituents of West African Medicinal Plants". For Part 16 see, Addae-Mensah, I., Waibel, R. and Achenbach, H. (1985) *Liebigs Ann. Chem.* 1284.



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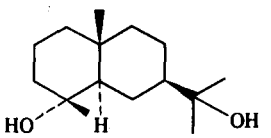

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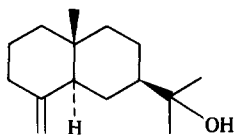

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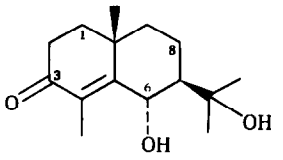

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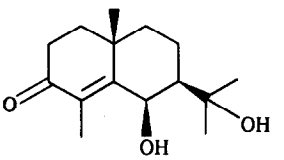

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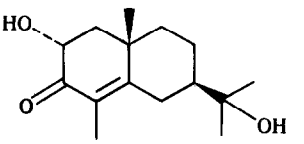

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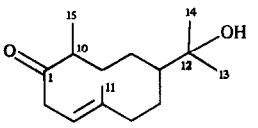

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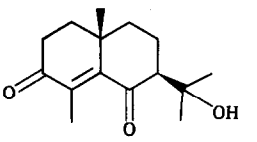

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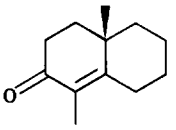

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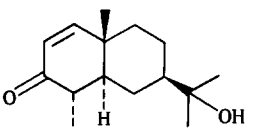

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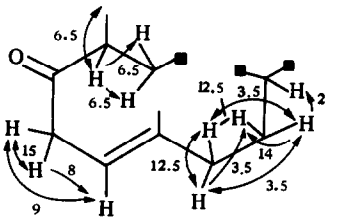

11

group in the ^{13}C NMR, which was smaller than 18 ppm [14].

Eudesmane-type sesquiterpenes seem to be typical constituents of the genus *Carissa*; carissone, the main sesquiterpene of *C. edulis*, has also been found in *C. lanceolata*, *C. carandas* and *C. congesta* [4–6]. By contrast only one plant not belonging to the genus *Carissa* is known to contain **1** [15].

Table 1. ^1H NMR data of **1**, **4**, **5** and **6**

Proton	1	4	5	6
H_{ax}-2	2.53 (*ddd*, $J_1 = 16$–17, $J_2 = 11$, $J_3 = 7.5$)	2.50 (*ddd*, $J_1 = 16$–17, $J_2 = 13$, $J_3 = 5$–6)	2.65 (*ddd*, $J_1 = 17$, $J_2 = 14$, $J_3 = 6$)	4.28 (*dd* (*br*), $J_1 = 14$, $J_2 = 5.5$)
H_{eq}-2	2.39 (*ddd*, $J_1 = 16$–17, $J_2 \cong J_3 \cong 4$)	2.38 (*ddd*, $J_1 = 16$–17, $J_2 = 5$, $J_3 = 4$)	2.44 (*ddd*, $J_1 = 17$, $J_2 = 5$, $J_3 = 3$)	—
H_{ax}-6	1.92 (*ddq*, $J_1 \cong J_2 \cong 13$, $J_3 = 1$)	4.76 (*dq*, $J_1 = 11$, $J_2 = 1$)	—	1.91 (*ddq*, $J_1 \cong J_2 \cong 13$–14, $J_3 = 1.5$)
H_{eq}-6	2.87 (*ddd*, $J_1 = 13$, $J_2 \cong J_3 \cong 3$)	—	5.30 (*d* (*br*), J = 3)	2.84 (*ddd*, $J_1 = 13$–14, $J_2 = J_3 \cong 3$)
H-11	1.79 (*d*, $J = 1$)	2.09 (*d*, $J = 1$)	1.89 (*s*)	1.82 (*d*, $J = 1.5$)
H-13	1.27* (*s*)	1.35* (*s*)	1.46* (*s*)	1.30* (*s*)
H-14	1.26* (*s*)	1.29* (*s*)	1.42* (*s*)	1.24* (*s*)
H-15	1.21* (*s*)	1.25* (*s*)	1.30* (*s*)	1.22* (*s*)
H_{eq}-1	†	†	†	2.14 (*dd*, $J_1 = 12.5$, $J_2 = 5.5$)
H-7	†	1.94 (*ddd*, $J_1 \cong J_2 \cong 10$–11, $J_3 = 5$)	†	†
H_{ax}-8	†	†	2.10 (*dddd*, $J_1 \cong J_2 \cong J_3 \cong 13$, $J_4 = 3$)	†

*Assignments interchangeable.

†Not observable because of overlapping.

Table 2. ^{13}C NMR data of 1, 5 and 6

C	1	5	6
1	37.41	39.07	45.66
2	33.82	34.11	68.88
3	199.02	200.34	200.39
4	128.90	131.31	125.93
5	162.62	158.94	164.21
6	28.80	68.57	28.86
7	49.70	49.73	49.81
8	22.55	16.13	22.49
9	42.01	41.57	42.72
10	35.93	35.00	37.02
11	10.88	10.38	11.02
12	72.42	73.40	72.36
13	26.81*	28.75*	26.70*
14	27.54*	28.94*	27.71*
15	22.63	24.60	22.91

*Assignments interchangeable.

The occurence of 7 together with a variety of eudesmanes, points to the role of germacranes as biogenetic precursors of the eudesmanes [16].

EXPERIMENTAL

General procedures. If not stated otherwise, all general procedures and instruments were as described in [17]. NMR: $CDCl_3$ with TMS used as internal standard; MS: 70 eV; TLC: 0.25 mm silica gel N/UV_{254} (Macherey-Nagel), petrol–EtOAc (7:3), detection by UV at 254 nm and by anisaldehyde reagent No. 15 according to Stahl [18].

Plant material. Roots of *Carissa edulis* Vahl were collected near Legon, Ghana, in March 1979 and identified by Mr. A. A. Enti. A herbarium specimen is deposited in our collection under No. 79/1.

Extraction procedure. Powdered dried roots (2.5 kg) were extracted with 15 l MeOH in a Soxhlet apparatus. On evaporation this extract yielded 155 g residue, which was redissolved in 3 l MeOH–H_2O (1:1) and then treated successively with 8 × 250 ml petrol (upon evaporation: 35 g extract A), 8 × 250 ml CCl_4 (8 g extract B), 20 × 250 ml Et_2O (11 g extract C) and 20 × 500 ml EtOAc (13 g extract D).

Extract A was separated over 300 g silica gel (column operated at *ca* 30 psi) into eight fractions (A1–A8) using cyclohexane with increasing amounts of EtOAc as eluent. Further separation of A4 over 450 g silica gel (petrol–Me_2CO, 9:1) gave five fractions (A4.1–A4.5). *β-Eudesmol* (3). Purification of A4.3 over 60 g silica gel with $CHCl_3$–MeOH (49:1) as eluent and subsequent purification of A4.3.4 over 60 g silica gel (C_6H_6–EtOAc, 9:1) afforded 15 mg colourless needles [mp 80.5, $[\alpha]_D^{20}$ +36.2° ($CHCl_3$; *c* 1.5)]

Table 3. ^1H NMR data of 7

Proton	δ	Multiplicity	Multiplicity on irradiation at: 5.54ppm	2.22ppm	2.90ppm	1.00ppm
H_A	3.11	*dd* $J_1=15, J_2=9$	*d* $J=15$	*	†	*
H_B	2.96	*dd* $J_1=15, J_2=8$	*d* $J=15$	*	†	*
H_C	5.54	*dd* $J_1\sim J_2\sim 8.5$	↳	*	†	*
H_D	2.22	*ddd* $J_1=12.5$ $J_2\sim J_3\sim 3.5$	*	↳	*	*
H_E	2.01	*ddd* $J_1\sim J_2=12.5$ $J_3=3.5$	*	*dd* $J_1=12.5$ $J_2=3.5$	*	*
H_F	1.73	*dddd* $J_1=14$ $J_2\sim J_3\sim 3.5$ $J_4=2$	*	*ddd* $J_1=14$ $J_2=3$ $J_4=2$	*	*
H_G	2.90	*ddq* $J_1\sim J_2\sim J_3\sim 6.5$	*	*	↳	*dd* $J_1\sim J_2\sim 6.5$
CH_3-11	1.63	*s*	*s*	*s*	*s*	*s*
CH_3-13	1.23	*s*	*s*	*s*	*s*	*s*
CH_3-14	1.19	*s*	*s*	*s*	*s*	*s*
CH_3-15	1.00	*d* $J=6.5$	*	*	*s*	↳

*No change.
†Signal disturbed by irradiation.

physicochemical data identical with those published for **3** [10, 19].

Extract B was separated over 300 g silica gel (column operated at *ca* 30 psi) into seven fractions (B1–B7) using cyclohexane with increasing amounts of EtOAc as eluent. *Germacrenone* (**7**). Chromatography of B2 over 220 g Sephadex LH 20 with MeOH–$CHCl_3$ (7:3) gave two fractions. Purification of B 2.1 over 8 g silica gel (C_6H_{12}–EtOAc, 4:1) afforded 18 mg colourless oil $[\alpha]_D^{20}$ + 173.2° ($CHCl_3$; *c* 1.1); MS *m/z* (int. ≥ 10%): 238.19328 ($C_{15}H_{26}O_2$) (12) $[M]^+$, 195.13851 ($C_{12}H_{19}O_2$) (41), 177 (10), 137 (28) [137.09664 ($C_9H_{13}O$), 137.13303 ($C_{10}H_{17}$)], 123 (15), 121 (19), 111 (15), 109.10173 (C_8H_{13}) (30), 108 (10), 107 (22), 97 (64), 96 (47), 95 (31), 94 (10), 93 (19), 84 (22), 83 (37), 82 (41), 81 (53), 79(15), 74 (18), 71 (12), 70 (19), 69 (74), 68.06260 (C_5H_8) (100), 67 (57), 59.04969 (C_3H_7O) (73), 55 (52), 53 (10), 43 (45), 41 (38); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3450, 1700; ^1H NMR: Table 3; ^{13}C NMR: 211.1 (*s*), 116.4 (*d*), 141.2 (*t*), 74.6 (*s*), 46.1 (*d*), 42.8 (*t*), 41.7 (*d*), 41.0 (*t*), 32.0 (*t*), 29.9 (*t*), 28.9 (*t*), 27.5 (*q*), 27.3 (*q*), 16.9 (*q*), 16.5 (*q*). *Carissone* (**1**). Purification of B5 over 450 g silica gel (C_6H_{12}–EtOAc, 3:2) yielded 2.9 g colourless crystals [mp 78–79, $[\alpha]_D^{20}$ + 132 ($CHCl_3$; *c* 1.05)] physicochemical data identical with literature data [4–6]; MS *m/z* (unless ion at highest observable mass int. ≥ 10%): 221 (4) $[M-Me]^+$, 219 (16), 218.16706 ($C_{15}H_{22}O$) (100), 203 (25), 175 (16) [175.11228 ($C_{12}H_{15}O$), 175.14866 ($C_{13}H_{19}$)], 163.11228 ($C_{11}H_{15}O$) (28), 161 (13), 147 (15), 146 (10), 91 (11), 59.0469 (C_3H_7O) (81), 43 (19), 41 (17).

Chromatography of B6 over 150 g silica gel with $CHCl_3$–MeOH (49:1) gave ten fractions (B6.1–B6.10). Rechromatography of B6.9 over 60 g silica gel (petrol–EtOAc, 3:2) yielded fractions B6.9.1 and B6.9.2. *6β-Carissanol* (**5**). On evaporation B6.9.1 afforded 15 mg colourless plates; mp 115–118; $[\alpha]_D^{20}$ + 10 ($CHCl_3$, *c* 0.3); MS *m/z* (unless M^+ int. ≥ 15%): 252 (0.1) $[M]^+$, 234.16198 ($C_{15}H_{22}O_2$) (32), 207 (16), 177 (43), 176 (72), 164 (21), 163 (19), 162 (23), 161 (41), 152 (17), 149 (25), 148 (27), 147 (28), 137 (20), 135 (24), 134 (35), 133 (32), 124 (26), 123 (32), 121 (24), 119 (29), 109 (32), 108 (18), 107 (33), 105 (32), 97 (17), 95 (38), 93 (36), 91 (28), 83 (23), 82 (20), 81 (47), 79 (27), 75 (18), 71 (16), 69 (29), 67 (29), 59 (100), 57 (16), 55 (38), 43 (87), 41 (37); UV λ_{max}^{EtOH} nm (log ε): 245 (4.15); IR ν_{max}^{KBr} cm^{-1}: 3450, 1645, 1605; ^1H NMR: Table 1; ^{13}C NMR: Table 2. *Oxidation of* **5**. 4 mg **5** dissolved in 2 ml CH_2Cl_2 were oxidized with 20 mg pyridiniumchlorochromate as described in [20]. Chromatographic purification afforded 2.5 mg **8** as colourless oil; $[\alpha]_D^{20}$ + 70 ($CHCl_3$, *c* 0.25); UV λ_{max}^{EtOH} nm (log ε): 255 (4.08); IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3550, 1620, 1610; ^1H NMR: δ 3.69 ($1H_{ex}$, *s*, –OH), 2.65–2.38 (3H, *m*), 2.23–1.75 (6H, *m*), 1.74 (3H, *s*, Me), 1.31 (6H, *s*, 2 × Me), 1.17 (3H, *s*, Me); MS *m/z* (unless M^+ int. ≥ 10%): 250 (4) $[M]^+$, 232 (16), 193 (23), 192 (32), 177 (16), 165 (10), 164 (68), 150 (11), 149 (41), 137 (10), 136 (16), 135 (12), 121 (11), 93 (12), 91 (15), 79 (17), 77 (16), 69 (11), 67 (13), 65 (10), 59 (100), 57 (10), 55 (48), 53 (17), 43 (97), 41 (64).

Fraction B6.9.2 was separated over 4 g silica gel into fractions B6.9.2.1 and B6.9.2.2. using C_6H_{12}–EtOAc (7:3) as eluent. *6α-Carissanol* (**4**). B6.9.2.1 on evaporation afforded 6 mg colourless oil: $[\alpha]_D^{20}$ + 66.4 ($CHCl_3$, *c* 0.25); MS *m/z* (unless M^+ int. ≥ 15%): 252 (0.5) $[M]^+$, 234.16198 ($C_{15}H_{22}O_2$, 39), 206.13067 ($C_{12}H_{18}O_2$, 42), 191 (37), 177 (51), 176 (23), 164 (21), 163 (20), 161 (29), 149 (29), 148 (17), 137 (19), 136 (16), 135 (28), 134 (21), 133 (23), 124.08881 ($C_8H_{12}O$, 92), 123 (65), 122 (20), 121 (23), 119 (25), 110 (22), 109 (36), 107 (21), 105 (20), 95 (41), 93 (26), 91 (29), 83 (56), 82 (40), 81 (28), 79 (27), 77 (18), 69 (40), 67 (35), 59 (81), 57 (18), 55 (64), 53 (17), 44 (16), 43 (100), 41 (63); UV λ_{max}^{EtOH} nm (log ε): 245 (4.09); IR ν_{max}^{KBr} cm^{-1}: 3450, 1645, 1605; ^1H NMR: Table 1. *Oxidation of* **4**. 1.5 mg **4** were oxidized like **5**. The product was identical in all respects with **8**. *α-Carissanol* (**6**). B6.9.2.2 on evaporation yielded 11 mg colourless oil; $[\alpha]_D^{20}$ + 124 ($CHCl_3$, *c* 0.07); MS *m/z* (unless M^+ int. ≥ 15%): 252 (0.1) $[M]^+$, 234.16198 ($C_{15}H_{22}O_2$) (58), 191 (21) 190.13576 ($C_{13}H_{18}O$) (91), 175 (16), 165 (50), 163 (38), 147 (33), 135 (15), 123 (16), 91 (18), 59 (100), 55 (16), 43 (43), 41 (31); UV λ_{max}^{EtOH} nm (log ε): 245 (4.09); IR ν_{max}^{KBr} cm^{-1}: 3225, 1640, 1605; ^1H NMR: Table 1; ^{13}C NMR: Table 2.

Cryptomeridiol (**2**). Chromatography of fraction B7 over 150 g silica gel with $CHCl_3$–MeOH (49:1) as eluent afforded eight fractions (B7.1–B7.8). Purification of B7.8 over 5 g silica gel (petrol–EtOAc, 3:2) gave 20 mg **2** as colourless needles [mp 134–136°, $[\alpha]_D^{20}$ – 27 ($CHCl_3$, *c* 2.0] physicochemical data identical with [7–9].

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