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DITERPENOIDS FROM *KYLLINGA ERECTA*

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Key Word Index—*Kyllinga erecta*; Cyperaceae; diterpenoids; manoyloxide derivatives; 16-hydroxy-manoyloxide; ambreinolide; norambreinolide or sclareolide.

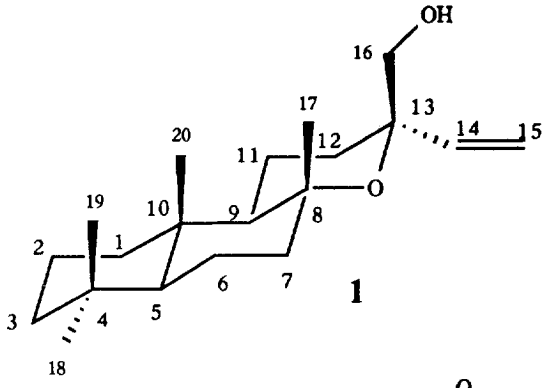
Abstract—16-Hydroxy-manoyloxide, a new derivative of manoyloxide, ambreinolide and norambreinolide have been isolated from the ethyl acetate extract of *Kyllinga erecta* rhizomes.

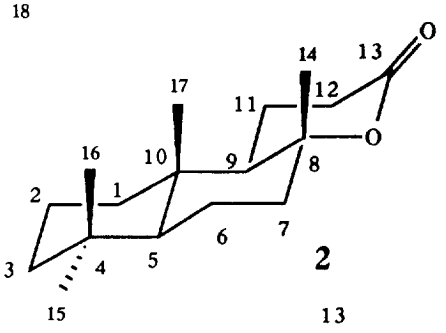
INTRODUCTION

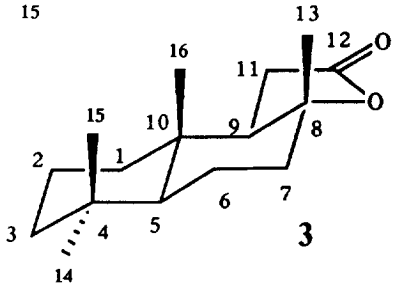
In a continuation of our studies on the diterpenoids from *Kyllinga erecta* S. [1, 2], we have now investigated the ethyl acetate extract of the rhizomes. From this source a new diterpenoid, 16-hydroxy-manoyloxide (**1**) has been isolated and its structure established by chemical and spectroscopic means, including a chemical correlation with manoyloxide. In addition, ambreinolide (**2**), the natural sources of which were limited [3, 4], and norambreinolide (**3**), a product of economic interest, have been also isolated and identified by their spectral characteristics and optical rotation values.

RESULTS AND DISCUSSION

The new diterpenoid (**1**) exhibited IR bands for a hydroxyl group (3350–3450 cm^{-1}) and a vinyl moiety (3050, 1640 cm^{-1}). Its ^1H NMR spectrum (200 MHz) showed signals of four tertiary methyl groups at δ1.28 (Me-17, deshielded by the oxygen atom of the tetrahydropyran ring), 0.86 and 0.80 (2 Me), of a vinyl group attached to a fully substituted sp^3 carbon atom (an ABX system, δ_A5.12, δ_B5.26, δ_X5.81; J_{AB} = 1.5 Hz, J_{AX} = 10.7 Hz, J_{BX} = 17.4 Hz), and of two protons geminal to a primary hydroxyl group (an AB system, δ_A3.34, δ_B3.28; J_{AB} = 11.1 Hz). This ^1H NMR spectrum was different from that of its known ent-16-hydroxy-13-epi-manoyloxide epimer [5] which has antiflammatory properties [6]. With ent-16-hydroxy-13-epi-manoyloxide, the protons of Me-17 are abnormally shielded. This shielding is the result of the reduction of the electronic density of the oxygen of the tetrahydropyran ring, consequent upon a hydrogen bond between this oxygen atom and the hydrogen of the hydroxyl group. The ^{13}C NMR spectral data of C-1 to C-11 and C-17 to C-20 of **1** were identical to those of manoyloxide [1], whereas the carbon resonances of C-12 to C-16 led us to place the hydroxyl group at C-16. The mass spectra of **1** closely matched the spectra of the ent-16-hydroxy-13-epi-manoyloxide epimer [5]. Finally, confirmation of the structure of the alcohol (**1**) was established by chemical correlation with manoylox-







ide through reduction of the methane sulphonate with LAH.

The structures of **2** and **3** were established by comparison of their spectroscopic properties with those reported in the literature [7–12].

EXPERIMENTAL

Plant material. Rhizomes of *K. erecta*, growing wild in Moundou (Southern Chad), were collected in July 1990 and authenticated. A voucher specimen is deposited at the herbarium of the Laboratoire Vétérinaire et Zootechnique de Farcha, N'Djamena, Chad.

General. Mps: uncorr.; IR: neat or $CHCl_3$; ^1H and ^{13}C NMR: $CDCl_3$, δppm, TMS; EI-MS: Hewlett Packard: chromatograph type 5890 equipped with capillary DB1 column 25 m × 0.23 mm (eluent He), mass quadripole detector type 5970 A series mass selective detector (electron impact to 70 eV), 60° (2 mn) and then 10° mn^{-1} until 200°; optical rotations; $CHCl_3$; TLC: aluminium plate precoated with a 0.2 mm layer of silica gel 60 F_{254} (Kieselgel), CH_2Cl_2–EtOAc (9:1); HPLC: Waters Model 510, differential refractometer Waters 410, column Bondapak C18, 19 × 150 mm, MeOH–H_2O (4:1).

Extraction and isolation of the diterpenoids. Air-dried rhizomes (60 g) were powdered and extracted at reflux with EtOAc. The EtOAc extract was concd and the residue (9.6 g) taken up with MeOH and filtered. The mother liquors were evapd (8.4 g) and the residue was subjected to CC on silica gel, eluting with cyclohexane–EtOAc (4:1). Eight frs, F_1–F_8, were collected and concd. Frs F_4 (600 mg), F_5 (250 mg) and F_8 (260 mg) were submitted to prep. HPLC, eluting with MeOH–H_2O (4:1). Fr. F_4 gave 5 frs, F_{41}, F_{42}, F_{43}, F_{44}, and F_{45}, of decreasing polarity. These frs were concd then purified by CC on silica gel. F_{45} (32 mg) gave an unidentified compound. F_{44} (27 mg) and F_{43} (8 mg) gave, respectively, 11α-hydroxymanoyloxide and 1β-hydroxymanoyloxide already isolated from *Kyllinga* [1, 2]. F_{42} (25 mg) gave norambreinoliole (**3**) isolated the first time from *Kyllinga*. F_{41} (26 mg) was a mixt. which was not explored. Fr. F_5 gave three frs, F_{51}, F_{52}, F_{53}. As above, these frs were concd. The main fr., F_{53} (36 mg), was purified by CC on silica gel. It gave the new compound, 16-hydroxymanoyloxide (**1**). Fr. F_8 gave three frs, F_{81}, F_{82} and F_{83}. As above, these were concd. The main fr. F_{82} (30 mg), was purified by CC on silica gel to give ambreinolide (**2**), a natural compound previously isolated in small quantities from tobacco [4].

16-Hydroxymanoyloxide (**1**). (18 mg) R_f 0.36; $[\alpha]_D$ + 13.3° (*c* 0.90); IR ν_{max} cm^{-1}: 3450 3350, 3040, 1640; ^1H NMR (200 MHz): δ5.81 (1H, *dd*, *J* = 17.4, 10.7 Hz, H-14), 5.26 (1H, *dd*, *J* = 17.4, 1.5 Hz, H-15), 5.12 (1H, *dd*, *J* = 10.7, 1.5 Hz, H-15'), 3.34 (1H, *d*, *J* = 11.1 Hz, H-16), 3.28 (1H, *d*, *J* = 11.1 Hz, H-16'), 1.28 (3H, *s*), 0.86 (3H, *s*), 0.80 (6H, *s*); ^{13}C NMR: δ144.0 (C-14), 114.0 (C-15), 76.3 (C-8), 75.6 (C-13), 68.5 (C-16), 56.4 (C-5), 52.8 (C-9), 43.6 (C-7), 42.0 (C-3), 38.9 (C-1), 37.2 (C-10), 33.4 (C-18), 33.2 (C-4), 27.2 (C-12), 25.8 (C-17), 21.5 (C-19), 20.2 (C-6), 18.5 (C-2), 15.1 (C-20), 14.5 (C-11); MS *m/z* (rel. int.): 306 ($[M]^+$ absent), 276 (7), 275 (19), 258 (26), 257 (100), 161 (6), 149 (7), 147 (7), 137 (39), 123 (20), 109 (19), 107 (17), 105 (13), 95 (24), 93 (16), 92 (22), 81 (31), 69 (39), 55 (75), 41 (38). Treatment of **1** (18 mg) with mesyl chloride (0.1 ml) for 24 hr and later reduction with $LiAlH_4$ (20 mg) in dry Et_2O for 6 hr at ambient temp. then 12 hr with reflux, gave manoyloxide identified by its ^1H NMR chracteristics and its optical rotation value [1].

Ambreinolide (**2**). (15 mg) [7–11] R_f 0.52; mp 142°; $[\alpha]_D$ + 28.3° (*c* 0.75); IR ν_{max} cm^{-1}: 1725; ^1H NMR (200 MHz): δ2.77–2.45 (2H, *m*, H-12), 2.18–1.98 (1H, *m*), 1.39 (3H, *d*, *J* = 0.7 Hz), 0.90 (3H, *s*), 0.85 (3H, *d*, *J* = 0.7 Hz), 0.82 (3H, *s*); ^{13}C NMR: δ171.7 (C-13), 83.8 (C-8), 56.0 (C-5), 53.6 (C-9), 41.8 (C-3), 41.2 (C-12), 39.2 (C-1), 37.3 (C-10), 33.3 (C-4), 33.2 (C-15), 29.0 (C-7), 22.9 (C-14), 21.5 (C-16), 19.6 (C-6), 18.4 (C-2), 15.8 (C-11), 15.1 (C-17); MS *m/z* (rel. int.): $[M]^+$ 264 (1), 250 (4), 249 (22), 236 (1), 221 (3), 220 (4), 205 (4), 193 (18), 192 (100), 191 (55), 177 (74), 163 (4), 149 (18), 137 (33), 136 (30), 123 (41), 121 (26), 109 (45), 95 (49), 81 (54), 67 (78), 55 (70), 43 (94), 41 (77).

Norambreinolide. (10 mg) [7, 10–12] R_f 0.44; mp 117°; $[\alpha]_D$ 37.6° (*c* 0.50); IR ν_{max} cm^{-1}: 1755; ^1H NMR: δ2.41 (1H, *dd*, *J* = 16.1, 14.8 Hz, H-11), 2.23 (1H, *dd*, *J* = 16.1, 6.5 Hz, H-11'), 1.33 (3H, *s*), 0.91 (3H, *s*), 0.89 (3H, *s*), 0.84 (3H, *s*); ^{13}C NMR: δ176.9 (C-12), 84.4 (C-8), 59.1 (C-9), 56.7 (C-5), 42.2 (C-3), 39.5 (C-7), 38.7 (C-1), 36.1 (C-10), 33.2 (C-14), 33.1 (C-4), 28.7 (C-11), 21.6 (C-13), 20.9 (C-15), 20.6 (C-6), 18.1 (C-2), 15.1 (C-16); MS *m/z* (rel. int.): 250 (1), 236 (4), 235 (28), 217 (1), 208 (4), 207 (24), 206 (23), 191 (19), 189 (9), 177 (9), 163 (8), 150 (21), 137 (28), 125 (56), 123 (77), 109 (49), 95 (55), 83 (17), 82 (49), 81 (51), 69 (49), 67 (58), 55 (57), 43 (100), 41 (70).

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