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TWO DITERPENE ALCOHOLS FROM *CROTON SUBLYRATUS*

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Key Word Index—*Croton sublyratus*; Euphorbiaceae; structural determination; anti reserpine ulcer; labdane; kaurane.

Abstract—The isolation and structural elucidation of two diterpene alcohols from *Croton sublyratus* are described. These compounds are *ent*-3α-hydroxy-13-epimanool and *ent*-16β,17-dihydroxykaurane.

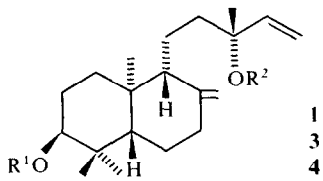
INTRODUCTION

In the course of our search for constituents of plant origin with antipeptic ulcer activity, we have isolated 18-hydroxygeranylgeraniol [1] and plaunol A, B, C, D and E [2, 3] as principles with anti reserpine-induced and anti-Shay ulcer activity, respectively, from the Thai medicinal plant named Plau-noi, identified with stems of *Croton sublyratus* Kurz (Euphorbiaceae). Further investigation led to the isolation of two new diterpene alcohols (**1** and **2**) and we now report their characterization.

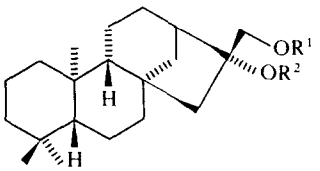
RESULTS AND DISCUSSION

Systematic fractionation of an acetone extract of the plant led to the isolation of two crystalline diterpene alcohols (**1** and **2**) and a mixture of dihydroxygeranylgeraniols by extensive silica gel chromatography.

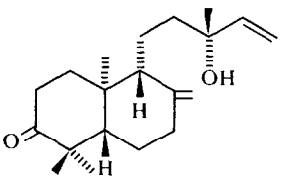
Diterpene alcohol **1** was recrystallized from acetone as colourless crystals, mp 86–87°, $[\alpha]_D^{22}$ − 30.4° ($CHCl_3$, *c* 1.0), MS *m/e*: 288 ($M^+ - H_2O$). Its molecular formula was determined as $C_{20}H_{34}O_2$ by elemental analysis and MS. The IR spectrum had absorption bands at 3430 (—OH), 1645, 1410, 995, 915 (—CH=CH_2), 890 (>C=CH_2) and 1385, 1215, 1198 cm^{-1} (>CMe_2). The ^1H NMR spectrum indicated the presence of four tertiary methyl groups (δ: 0.68, 0.79, 0.90 and 1.20). ABC-type signals at δ 4.90, 5.12 and 5.85 were characteristic of the vinyl group, and broad singlets at 4.49 and 4.75 were assigned as an end methylene group. The presence of a



1 $R^1 = H$; $R^2 = H$
3 $R^1 = Ac$; $R^2 = H$
4 $R^1 = Ac$; $R^2 = Ac$



2 $R^1 = H$; $R^2 = H$
8 $R^1 = Ac$; $R^2 = H$
9 $R^1 = Ac$; $R^2 = Ac$



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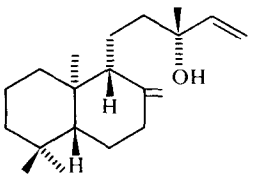

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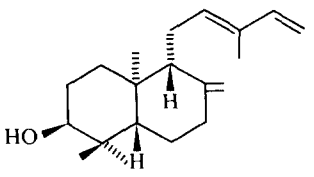

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primary and a tertiary hydroxyl group was indicated by the fact that when **1** was treated with Ac_2O–pyridine at room temperature it gave the monoacetate (**3**) [^{1}H NMR δ: 4.57 (H, *brs*) 2.03 (3H, *s*), IR ν: 3460 cm^{-1} (—OH)] and when **1** was heated with Ac_2O–NaOAc at 130° for 3.5 hr it gave the diacetate (**4**) [^{1}H NMR δ: 1.95 (3H, *s*), 2.01 (3H, *s*)]. In the ^{1}H NMR spectrum of **4** the singlet at 1.20 of **1** shifted to 1.49, which indicated that **1** had the partial formula —C(Me)(OH)—CH=CH_2. These results and spectral data provided clear indications that **1** was a manool [4] (or 13-epimanool [5]) derivative possessing an additional secondary hydroxyl group. To determine the absolute structure of **1**, the following chemical conversions were carried out. First, **1** was treated with CrO_3–pyridine to give the ketone (**5**), mp 95.8°, MS *m/e* 304 (M^+), which gave the deoxy compound (**6**) of **1** by Huang-Minlon reduction. All physical and spectral properties of **6** accorded with those of *ent*-13-epimanool [6]. Secondly, the monoacetate (**3**) was treated with $POCl_3$–pyridine at −5°, followed by deacetylation, to give an anhydro mixture, which was chromatographed over Si gel and $AgNO_3$–Al_2O_3 (15:85) [7] to give **7**. Compound **7** was identical with 3α-hydroxy-12,13*E*-biformen [8] in all respects except for the direction of optical rotation. The optical rotation of **7** and 3α-hydroxy-12,13*E*-biformen were −1.0° and +4°, respectively, **7** being the enantiomer of the latter. These chemical correlations indicated the absolute structure of compound **1** to be *ent*-3α-hydroxy-13-epimanool.

Diterpene alcohol **2** was recrystallized from acetone as colourless prisms, mp 186–188°, $[\alpha]_D^{16}$ − 36.2° ($CHCl_3$, *c* 0.93), MS *m/e* 306 (M^+). The IR spectrum of **2** had absorption bands at 3380 (—OH), 1385, 1370 cm^{-1} ($\diagdown\!\!\diagup CMe_2$) and no absorption band characteristic of an olefine structure. Its ^{1}H NMR spectrum had signals at δ 0.79, 0.84 and 0.98 assigned as tertiary methyl groups. On treatment with Ac_2O pyridine at room temperature overnight and with Ac_2O at 130° for 4 hr, **2** [^{1}H NMR δ: 4.12, 4.02 (AB, J = 12 Hz, H-17)] gave the monoacetate (**8**) [IR ν: 3460 cm^{-1} (—OH), ^{1}H NMR δ: 4.22 (2H, *s*, H-17), 2.31 (3H, *s*)] and the diacetate (**9**) [^{1}H NMR δ: 4.77, 4.35 (AB, J = 12 Hz, H-17), 2.08 (3H, *s*), 2.00 (3H, *s*)], respectively. These results and spectral properties characterized diterpene alcohol **2** as *ent*-16β,17-dihydroxykaurane derived from (−)-kaurane [9] or sugeroside [10]. Comparison of the spectral and physical data of **2** and **8** with those of *ent*-16β,17-dihydroxykaurane and its monoacetate showed them to be identical.

EXPERIMENTAL

^{1}H NMR spectra were run with TMS as an int. reference. Analytical GLC was carried out with a glass column (1.0 m × 3 mm) packed with 2% OV-225 on 80–100 mesh Chromosorb G, injection and detection temp.: 250°; column temp.: 205°, carrier gas: He at 60 ml/min.

Extraction and isolation. Crushed stems (81.5 kg) of *Croton sublyratus* were extracted 3× with Me_2CO under reflux. After evapn of the solvent, the residue was fractionated [1] to give diterpene alcohols **1** (1.47 g) and **2** (1.53 g) after Si gel chromatography (C_6H_6–EtOAc).

Diterpene alcohol **1**. Mp 86–87°, $[\alpha]_D^{22}$ − 30.4° ($CHCl_3$, *c* 1.0); MS (75 eV) *m/e* (rel. int.): 288 (M^+ − H_2O, 17), 273 (14), 270 (11), 255 (21), 175 (21), 152 (22), 135 (100), 107 (34), 93 (38); IR ν_{max}^{nujol} cm^{-1}: 3430, 1645, 1410, 1215, 1198, 1110, 995, 915, 890; ^{1}H NMR (CCl_4): δ 5.85, 4.90, 5.12 (3H, ABC, J_{AC} = 17, J_{BC} = 10, J_{AB} = 2 Hz), 4.75 (1H, *brs*), 4.49 (1H, *brs*), 3.31 (1H, *brs*), 2.27 (1H, *brs*), 2.2 − 1.3 (15H, *m*), 1.20 (3H, *s*), 0.90 (3H, *s*), 0.79 (3H, *s*), 0.68 (3H, *s*). [Found: C, 78.44; H, 11.21. $C_{20}H_{34}O_2$ requires: C, 78.39; H, 11.18%.]

Diterpene alcohol **2**. Mp 186–188°, $[\alpha]_D^{16}$ − 36.2° ($CHCl_3$, *c* 0.93); MS (75 eV) *m/e* (rel. int.) 306 (M^+, 1), 288 (5), 276 (36), 275 (100), 257 (23), 232 (9), 137 (15), 123 (20), 95 (17), 81 (18), 69 (16); IR ν_{max}^{KBr} cm^{-1}: 3380, 2930, 2870, 2840, 1480, 1465, 1450, 1435, 1385, 1370, 1065, 1040, 1025, 1025, 993, 880; ^{1}H NMR (Py-d_5): δ 5.00 (2H, *brs*), 4.12, 4.02 (AB, J = 12 Hz), 2.43 (1H, *brs*), 2.1–1.1 (20H, *m*), 0.98 (3H, *s*), 0.84 (3H, *s*), 0.79 (3H, *s*). [Found: C, 76.02; H, 11.14. $C_{20}H_{34}O_2 \cdot \frac{1}{2}H_2O$ requires [9, 10]: C, 76.14; H, 11.18%.] [lit. [9]: mp 189–190°, [10]: mp 187.5–188.5°, $[\alpha]_D^{16}$ − 36.5° ($CHCl_3$, *c* 0.91)].

Acetylation of **1**. (1) Acetylation of 80 mg **1** with Ac_2O–pyridine for 2 days gave 48.3 mg the monoacetate (**3**) and 15.1 mg unreacted **1**. **3**: mp 65.9–67°; MS (75 eV) *m/e* (rel. int.): 330 (M^+ − H_2O, 11), 288 (3), 270 (59), 255 (70), 188 (30), 176 (38), 135 (100), 119 (57), 107 (59), 93 (67); IR ν_{max}^{nujol} cm^{-1}: 3460, 3070, 1740, 1730, 1710, 1635, 1415, 1245, 1185, 1120, 1045, 988, 925, 895; ^{1}H NMR (CCl_4): δ 5.86, 5.09, 4.92 (3H, ABC, J_{AC} = 17, J_{BC} = 10, J_{AB} = 2 Hz), 4.78 (1H, *brs*), 4.57 (1H, *brs*), 4.54 (1H, *brs*), 2.03 (3H, *s*), 1.21 (3H, *s*), 2.6 − 2.1, 2.0 − 1.3, 1.2 − 1.0 (15H, *m*), 0.90 (3H, *s*), 0.86 (3H, *s*), 0.70 (3H, *s*). [Found: C, 75.69; H, 10.46. $C_{22}H_{36}O_3$ requires: C, 75.82; H, 10.41%.]

(2) A mixture of 33 mg **1** and Ac_2O (1.5 ml)–AcONa (30 mg) was refluxed for 3.5 hr. Usual work-up gave 22 mg the diacetate (**4**) as a colourless oil, MS (75 eV) *m/e* (rel. int.): 390 (M^+, 0.4), 330 (37), 315 (12), 270 (76), 255 (62), 202 (23), 175 (35), 135 (100), 134 (65), 133 (49), 92 (12); IR ν_{max}^{liquid} cm^{-1}: 3060, 2920, 1730, 1635, 1445, 1370, 1245, 1180, 1040, 1015; ^{1}H NMR (CCl_4): δ 5.84, 4.99, 4.98 (3H, ABC, J_{AC} = 18, J_{BC} = 10, J_{AB} = 2 Hz), 4.73 (1H, *brs*), 4.52 (1H, *brs*), 4.42 (1H, *brs*), 2.01 (3H, *s*), 1.95 (3H, *s*), 1.49 (3H, *s*), 0.90 (3H, *s*), 0.86 (3H, *s*), 0.70 (3H, *s*), 2.7 − 2.1, 1.85 − 1.6, 1.45 − 0.95 (14H, *m*).

Oxidation of **1**. A mixture of 122 mg CrO_3, 1.2 ml pyridine and 0.12 ml H_2O was added to 70 mg **1**. The reaction mixture was allowed to stand overnight, and usual work-up gave 49 mg of the desired compound **5**, mp 95.8°; MS (75 eV) *m/e* (rel. int.): 304 (M^+, 5), 286 (60), 271 (51), 258 (47), 243 (16), 201 (32), 135 (51), 133 (49), 123 (64), 107 (66), 93 (82), 71 (100); IR ν_{max}^{nujol} cm^{-1}: 3450, 3080, 1710, 1705, 1695, 1640, 1203, 1000, 910, 897; ^{1}H NMR (CCl_4): δ 5.87, 5.14, 4.98 (3H, ABC, J_{AC} = 18, J_{BC} = 10, J_{AB} = 2 Hz), 4.87 (1H, *brs*), 4.62 (1H, *brs*), 2.7 − 1.3 (15H, *m*), 1.23 (3H, *s*), 1.06 (3H, *s*), 1.00 (3H, *s*), 0.88 (3H, *s*). [Found: C, 78.40; H, 10.52. $C_{20}H_{32}O_2$ requires: C, 78.90; H, 10.59%.]

Huang-Minlon reduction of **5**. A mixture of 134 mg **5**, 0.2 ml 90% hydrazine-hydrate, 3 ml diethyleneglycol and 180 mg KOH was heated at 150–160° for 1 hr and at 240–250° for 1.5 hr under a N_2 atm., followed by usual work-up, to give 113 mg **6** as a colourless oil, $[\alpha]_D^{22}$ − 44.9° ($CHCl_3$, *c* 2.3) [Lit. [6]: $[\alpha]_D$ − 46° ($CHCl_3$, *c* 1.0)], spectral data were identical with those of *ent*-13-epimanool [6].

Dehydration and deacetylation of monoacetate (**3**). To a soln of 470 mg **3** in 8 ml pyridine was added 2.8 ml $POCl_3$ in 4 ml pyridine. The reaction mixture was allowed to stand overnight at −5°. Usual work-up [7] gave 84.3 mg a colourless oil [MS (75 eV) *m/e* 330 (M^+)], which was hydrolyzed to yield three components detected on TLC (R_f: 0.83, 0.73 and 0.64, solvent system: C_6H_6–EtOAc, 1:1). The compound showing R_f 0.73 was separated by Si gel chromatography, and found to have three components on GLC. This olefinic mixture was chromatographed over $AgNO_3$–Al_2O_3 (15:85) eluting successively with Et_2O–hexane (9:1), Et_2O–MeOH (9:1) and Et_2O–MeOH (4:1). Elution with Et_2O–MeOH (4:1) gave desired **7** (colourless oil),

showing a single peak on GLC, $[\alpha]_D^{22} - 1.0°$ ($CHCl_3$, *c* 0.7), [lit. [8], enantiomer: $[\alpha]_D^{24} + 4°$ ($CHCl_3$, *c* 29.6)]. Spectral data were identified with those of 3α-hydroxy-12,13*E*-biformen [8].

Acetylation of **2**. (1) Diterpene alcohol **2** (50 mg) was treated with Ac_2O–pyridine to afford 40 mg monacetate (**8**), mp 151–152° [lit. [9]: 153.5–154°]. Spectral data were identical with those of *ent*-16β,17-dihydroxykaurane-17-acetate [9]. (2) A mixture of 20 mg **2** and 2 ml Ac_2O was refluxed for 3 hr to give 20 mg diacetate (**9**), mp 135–135.5°. MS (70 eV) *m/e* (rel. int.): 330 (M^+ − HOAc, 47), 315 (14), 288 (14), 270 (100), 255 (44), 165 (29), 123 (37), 109 (24), 91 (35), 81 (39); IR ν_{max}^{KBr} cm^{-1}: 2930, 1745, 1723, 1480, 1465, 1450, 1385, 1370, 1255, 1215, 1165, 1105, 1033, 1023, 935; ^1H NMR ($CDCl_3$): δ 4.94, 4.46 (2H, AB, *J* = 12 Hz), 2.49 (1H, *m*), 2.08 (3H, *s*), 2.00 (3H, *s*), 2.2 – 1.0 (20H, *m*), 1.02 (3H, *s*), 0.86 (3H, *s*), 0.81 (3H, *s*).

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REFERENCES

1. Ogiso, A., Kitazawa, E., Kurabayashi, M., Sato, A., Takahashi, S., Noguchi, H., Kuwano, H., Kobayashi, S. and Mishima, H. (1978) *Chem. Pharm. Bull Jpn* **26,** 3117.
2. Kitazawa, E., Ogiso, A., Sato, A., Kurabayashi, M., Kuwano, H., Hata, T. and Tamura, C. (1979) *Tetrahedron Letters* 1117.
3. Kitazawa, E., Sato, A., Takahashi, S., Kuwano, H. and Ogiso, A. (1980) *Chem. Pharm. Bull. Jpn* **28,** 227.
4. Brandt, C. W. and Thomas, B. R. (1952) *Nature (London)* 1018.
5. Rowe, J. W. and Scroggins, J. H. (1964) *J. Org. Chem.* **29,** 1554.
6. Hugel, G., Oehlschlager, A. C. and Ourisson, G. (1966) *Tetrahedron Supp.* 8, Part 1, 203.
7. Carman, R. M. and Dennis, N. (1967) *Aust. J. Chem.* **20,** 157.
8. Bohlmann, F. and Czerson, H. (1979) *Phytochemistry* **18,** 115.
9. Hanson, J. R. (1963) *J. Chem. Soc.* 5061.
10. Ichikawa, N., Ochi, M. and Kubota, H. (1973) *J. Chem. Soc. Jpn. Chem. Ind. Chem.* 785.