TRITERPENOIDS FROM *HUMATA PECTINATA*[1]

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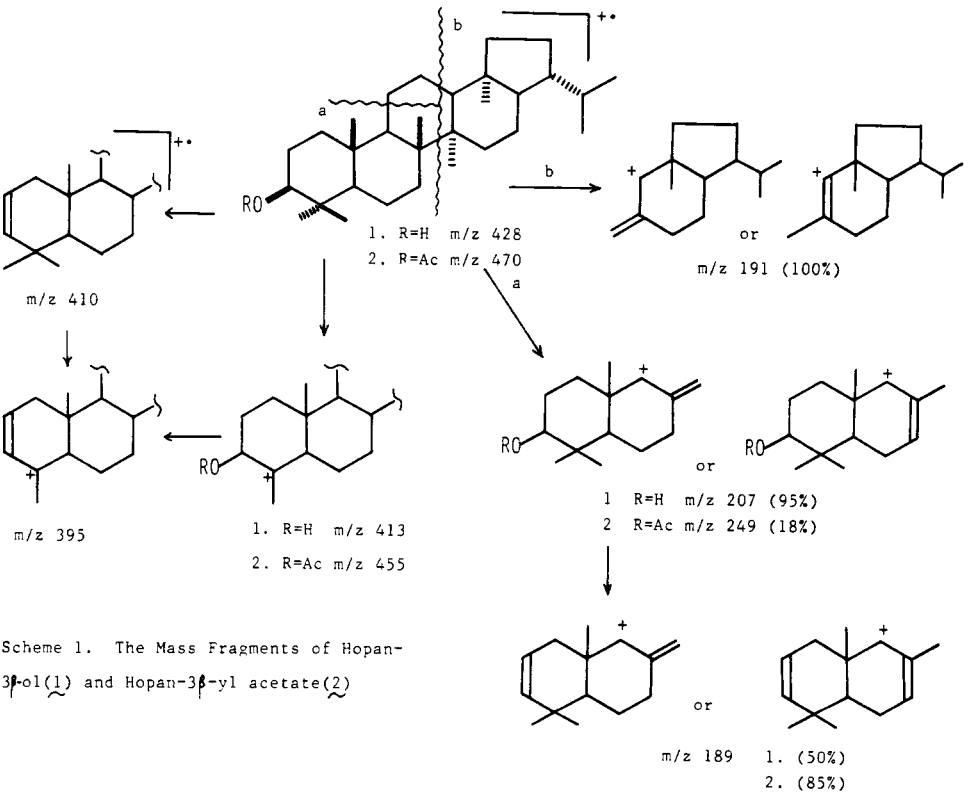
ABSTRACT.—From the alcohol extract of the whole plant of *Humata pectinata*, two new triterpenoids were isolated along with the known compounds neohop-13(18)-ene (**4**), neohop-12-ene (**5**), hop-21-ene (**6**), hop-22(29)-ene (**7**), and diplopterol (**8**). On the basis of spectral and chemical evidences, it was shown that the two new triterpenes have the structures corresponding to hopan-3β-ol (**1**) and hopan-3β-yl acetate (**2**).

Humata pectinata (Davalliaceae) (1) has been used as a folk medicine in South Taiwan for treating rheumatism, but the constituents of this plant have not been previously investigated. The ethanol extract of the whole plant (*H. pectinata*) was treated by the procedures described in the experimental section. Two new triterpenoids, together with five known triterpenoids, were obtained as crystals. This paper describes the first isolation of triterpenoids from this plant and the structure elucidation of two new substances.



Scheme 1. The Mass Fragments of Hopan-3β-ol(1) and Hopan-3β-yl acetate(2)

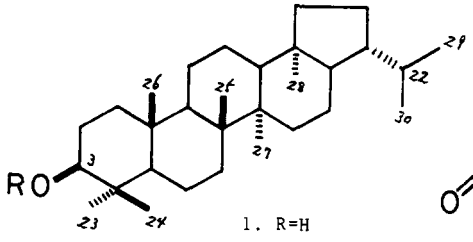
[1]Part XIV in the series of "Constituents of Formosan Folk Medicine". For Part XIII see T-S. Wu and H. Furukawa, *J. Natural Products*, in press preceding paper.

RESULTS AND DISCUSSION

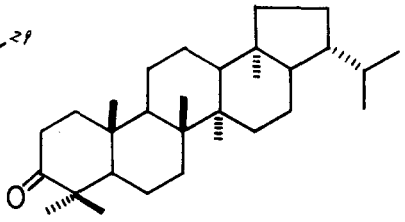
Hopan-3β-ol (**1**), colorless needles, $C_{30}H_{52}O$, $[\alpha]D+37.4°$ ($CHCl_3$), mp 236–238°, showed a positive reaction with Liebermann-Burchard reagent. The ir band at 3600 cm^{-1} and ^1H-nmr signal at δ 3.32, which disappeared in D_2O, indicated the presence of a hydroxyl group. In addition, the ^1H-nmr spectrum of **1** showed a one-proton quartet centered at δ 3.76 with splitting of 10 and 4.5 Hz. This signal is characteristic of an H–atom attached to the carbon bearing a hydroxyl group (2). Furthermore, the splitting pattern of this signal also substantiated the equatorial orientation of the C–3 hydroxyl group. The presence of an isopropyl group and six methyl groups attached on quaternary carbons was shown by the signals of two three-proton doublets at δ 0.77 and 0.82 (each $J=7$Hz), and singlets at δ 0.83(9H), 0.91(3H) and 0.97(6H), respectively. The mass spectrum of **1** showed a characteristic fragmentation pattern with the hopane-type triterpenoids (3,4) (scheme 1).

These spectral data suggested the structure of hopan-3β-ol (formula **1**) for this compound. Further proof of this structure was obtained by identity with the hydrogenation product of moreterol (**9**) by direct comparisons (ir, ^1H-nmr and mixed mp).

Hopan-3β-yl acetate (**2**), colorless needles, $C_{32}H_{54}O_2$, $[\alpha]D+26.7°$ ($CHCl_3$), mp 324–326°. The ir spectrum of **2** showed a band at 1713 cm^{-1} which indicated the presence of an acetyl group, and no absorption band due to a hydroxyl group was present. In the ^1H-nmr spectrum of **2**, a three-proton singlet at δ 2.04, and a proton signal at δ 4.50(1H,m) could be assigned to the protons of the $-CH-OCOCH_3$ moiety. In addition to these signals, isopropyl methyl signals appeared at δ 0.76 and 0.81 (each $J=7$Hz), and six quart. methyl signals at δ 0.86(12H)



1. R=H
2. R=Ac



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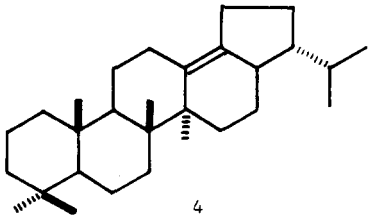

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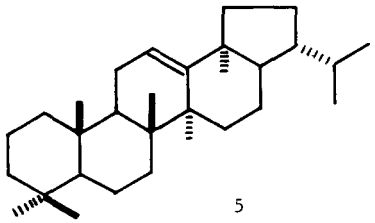

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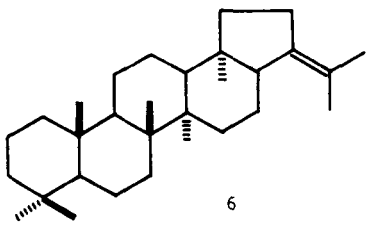

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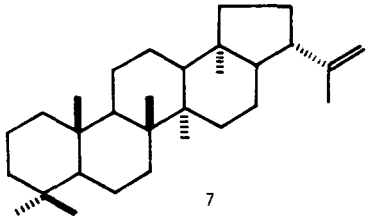

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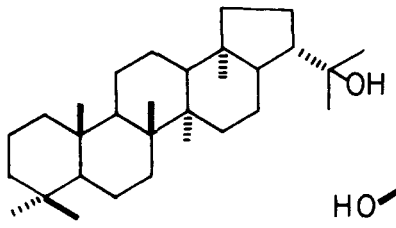

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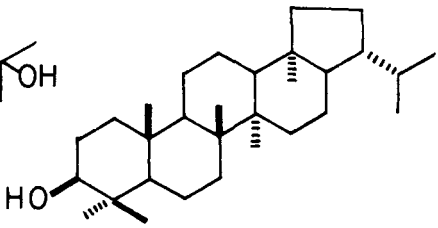

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and 0.97(6H). In the mass spectrum of **2**, the fragmentation pattern was similar to that of **1** (scheme 1). The fragments at *m/z* 395, 191, and 189 were the same as in **1**. On the other hand, the fragments at *m/z* 428(M^+), 413, and 207 in **1** were observed to shift to *m/z* 470(M^+), 455, 249 in **2**, respectively. On the basis of these spectral data, the structure of this compound should be represented by the formula **2** corresponding to hopan-3β-yl acetate. This was confirmed by direct comparisons with the acetylation product of **1** (^{1}H-nmr, ir, mass and mixed mp).

Five additional triterpenoids isolated from the same plant were characterized as neohop-13(18)-ene (**4**) (5), neohop-12-ene (**5**) (5), hop-21-ene (**6**) (5), hop-22(29)-ene (**7**) (5), and diplopterol (**8**) (5) by comparisons with the ^{1}H-nmr and ir spectra of authentic samples supplied by Professor H. Agata (Showa College of Pharmaceutical Sciences).

EXPERIMENTAL[2]

PLANT MATERIAL.—*Humata pectinata* (J. Sm.) Desv. was collected on Orchild island, Taiwan, and verified by Professor C.-S. Kuoh; the specimen is deposited in the Herbarium of Chia-Nan Junior College of Pharmacy, Tainan, Taiwan, Republic of China.

EXTRACTION AND SEPARATION.—The ethanolic extract of the whole herb of *Humata pectinata* (1 kg) was partitioned into chloroform and water. The chloroform layer was evaporated to dryness and subjected to silica gel column chromatography by successive elution with hexane, hexane-benzene (1:1), and benzene. The fractions of hexane-benzene (1:1) and benzene afforded crystalline hopan-3β-yl acetate (**2**) (0.32 g), diplopterol (**8**) (0.03 g), and hopan-3β-ol (**2**) (0.08 g). The hexane fraction was rechromatographed on 10% $AgNO_3$ impregnated silica gel, and the column was eluted with hexane, hexane-benzene (9:1), and hexane-benzene (8:2); neohop-13 (18)-ene (**4**) (1.35 g), hop-21-ene (**6**) (0.04 g), neohop-12-ene (**5**) (0.21 g) and hop-22(29)-ene (**7**) (0.02 g) were obtained.

NEOHOP-13(18)-ENE (**4**).—Colorless needles from ether, mp 192–194°. Liebermann-Burchard test showed a positive reaction. νmax cm^{-1}: 2930, 2850, 1455, 1365, and 1377; ^{1}H-nmr δ: 0.79(6H, s, 24, 28–CH_3), 0.83(3H, s, 26–CH_3), 0.87(6H, s, 23, 25–CH_3), 0.88(3H, d, J=7Hz, 29–CH_3), 0.93(3H, d, J=7Hz, 30–CH_3), and 1.10(3H, s, 27–CH_3), ms *m/z*: 410(M^+), 218(94%), 191(100%), and 189(31%).

HOP-21-ENE (**6**).—Colorless needles from acetone, mp 196–198°, $[\alpha]_D$+22.8° (c=0.18, $CHCl_3$). Liebermann-Burchard test showed a positive reaction. νmax cm^{-1}: 2930, 2850, 1455, 1380, and 1365; ^{1}H-nmr δ: 0.59(3H, s, 28–CH_3), 0.80(3H, s, 24–CH_3), 0.82(3H, s, 26–CH_3), 0.85(3H, s, 23–CH_3), 0.97(6H, s, 25, 27–CH_3), 1.57(3H, s, 30–CH_3), and 1.73(3H, s, 29–CH_3); ms, *m/z*: 410(M^+), 395, 367, 341(100%), 238, 191, and 189.

NEOHOP-12-ENE (**5**).—Colorless plates from ether, mp 208–210°. Liebermann-Burchard test showed a positive reaction. νmax cm^{-1}: 2930, 2850, 1650, 1455, 1375, and 1360; ^{1}H-nmr δ: 0.74(3H, s, 25–CH_3), 0.76(3H, s, 28–CH_3), 0.82(3H, s, 24–CH_3), 0.87(3H, s, 23–CH_3), 0.88(3H, s, 26–CH_3), 0.85(3H, d, J=7Hz, 30–CH_3), 0.94(3H, d, J=7Hz, 29–CH_3), 1.14(3H, s, 27–CH_3), and 5.08(1H, m, 12–H); ms, *m/z*: 410(M^+), 218(100%), 203, 191, 189, 177, and 175.

HOP-22(29)-ENE (**7**).—Colorless plates from ether, mp 208–210°. Liebermann-Burchard test showed a positive reaction. νmax cm^{-1}: 2930, 2850, 1630, 1455, 1380, and 1370; ^{1}H-nmr δ: 0.74(3H, s, 28–CH_3), 0.80 (3H, s, 24–CH_3), 0.84(3H, s, 26–CH_3), 0.86(3H, s, 23–CH_3), 0.95 (3H, s, 25–CH_3), 0.97(3H, s, 27–CH_3), 1.75(3H, s, 30–CH_3), and 4.80(2H, s, 29–CH_2); ms *m/z*: 410(M^+), 395, 367, 354, 343, 301, 299, 191(100%), and 189(94%).

DIPLOPTEROL (**8**).—Colorless needles from acetone, mp 245–248°, $[\alpha]_D$+37.5° (c=0.8, $CHCl_3$). Liebermann-Burchard test showed a positive reaction. νmax cm^{-1}: 3590, 2930,

[2]Mps are uncorrected. ^{1}H-nmr (100 MHz) were recorded in $CDCl_3$. Chemical shifts are given in ppm (δ) with TMS as internal standard. Mass spectra were taken with a direct inlet system. Ir spectra were recorded in chloroform solution.

2850, 1455, 1385, and 1370; ^{1}H-nmr δ: 0.78(3H, s, 28-CH_3), 0.81(3H, s, 24-CH_3), 0.84(3H, s, 26-CH_3), 0.86(3H, s, 23-CH_3), 0.99(6H, s, 25, 27-CH_3), 1.20 (3H, s, 29-CH_3), and 1.23(3H, s, 30-CH_3); ms m/z: 428(M^+), 410, 395, 370, 191(100%), and 189.

HOPAN-3β-OL (**1**).—Colorless needles from acetone, mp 236–238°, $[\alpha]_D$+37.4° (c=0.68, $CHCl_3$). Liebermann-Burchard test showed a positive reaction. νmax cm^{-1}: 3600, 2930, 2850, 1455, 1385, 1372, and 1005; ^{1}H-nmr δ: 0.77(3H, s, J=7Hz, 29-CH_3), 0.82 (3H, d, J=7Hz, 30-CH_3), 0.83(9H, s, 24, 26, 28-CH_3), 0.91(3H, s, 23-CH_3), 0.97(6H, s, 25, 27-CH_3), 3.32(1H, br. s, OH), and 3.76(1H, q, J=4.5 & 10Hz, 3-H); ms, m/z: 428(M^+), 413, 410, 395, 369, 256, 207(95%), 191(100%), and 189(50%). Anal. Calc. for $C_{30}H_{52}O \cdot \frac{1}{2}H_2O$ C, 82.31; H, 12.20. Found C, 82.70; H, 12.61.

HOPAN-3β-YL ACETATE (**2**).—Colorless needles from ether, mp 324–326°, $[\alpha]_D$+26.7° (c=0.55, $CHCl_3$). Liebermann-Burchard test showed a positive reaction. νmax cm^{-1}: 2930, 2850, 1713, 1460, 1365, 1380, and 1250; ^{1}H-nmr δ: 0.76(3H, d, J=7Hz, 29-CH_3), 0.81(3H, d, J=7Hz, 30-CH_3), 0.86(12H, s, 23, 24, 26, 28-CH_3), 0.97(6H, s, 25, 27-CH_3), 2.04(3H, s, $OCOCH_3$), and 4.52(1H, m, 3-H); ms, m/z: 470(M^+), 455, 410, 395, 369, 354, 341, 299, 249, 191(100%0, and 189(85%). Anal. Calc. for $C_{32}H_{54}O_2$ C, 81.64; H, 11.56. Found C, 81.36; H, 11.92.

HYDROLYSIS OF HOPAN-3β-YL ACETATE (**2**).—Hopan-3β-yl acetate (0.1 g) was dissolved in 40 ml of methanol containing 2.0 g NaOH and was stirred at room temp. for 5 hrs., and then neutralized; the solvent was evaporated *in vacuo*. The residue was treated with water and chloroform. Colorless needles were obtained and recrystalized from acetone, mp 237–238°. This product was identified with **1** by comparison of ^{1}H-nmr, ir, and mass spectra, and mixed mp.

ACETYLATION OF HOPAN-3β-OL (**1**).—A mixture of the hopan-3β-ol (**1**) (30 mg), acetic anhydride (0.5 ml) and pyridine (0.5 ml) was left to stand overnight at room temperature. Usual work-up of the reaction mixture gave a crystalline residue which was recrystallized from ether; the colorless needles obtained, mp 318–320°, were identified with **2** by comparison of the ^{1}H-nmr, ir, and mass spectra and mixed mp.

OXIDATION OF HOPAN-3β-OL (**1**).—A mixture of hopan-3β-ol (**1**) (20 mg), CrO_3-pyridine complex (50 mg) and methylene chloride (10 ml) was stirred for 15 hrs. at room temperature. The reaction mixture was subjected to silica gel column chromatography and eluted with chloroform, the crystalline residue was recrystallized from acetone, mp 178–180°, $C_{30}H_{50}O$; νmax cm^{-1}: 1716, 1450, 1380, and 1365; ^{1}H-nmr δ: 0.72, 0.81, 0.84, 0.86, 0.99, and 1.26(24H, 6s, CH_3); ms, m/z: 426(M^+), 411, 384, 369, 273, 231, 206, 205, 191(100%), and 190.

CATALYTIC HYDROGENATION OF MORETENOL (**9**).—A solution of **9** (10 mg) in ethanol (20 ml) was shaken under H_2 gas in the presence of PtO_2 (30 mg) for 4 hrs. at room temperature. The solution was filtered, and evaporated. The residue, when crystallized from acetone, gave colorless needles, mp 236–237°. The ir, ^{1}H-nmr and mass spectra were superimposable with **1**.

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The authors thank Dr. W. H. Hui (University of Hong Kong) for the authentic sample of moretenol and Professor H. Agata (Showa College of Pharmaceutical Sciences) for the ir, ^{1}H-nmr spectra of neohop-13(18)-ene, hop-21-ene, neohop-12-ene, hop-22(29)-ene, and diplopterol. We are also indebted to Miss T. Sakai in the Analytical Center of our University for elemental analyses.

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