Phytochemistry, 1969, Vol. 8, pp. 2345 to 2349. Pergamon Press. Printed in England

OCCURRENCE OF 15α-ACETOXY-22-HYDROXYHOPANE AND PHLEBIC ACID A IN THE LICHEN, *PELTIGERA APHTHOSA*

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(*Received* 29 *May* 1969)

Abstract—15α-Acetoxy-22-hydroxyhopane (Dustanin monoacetate) (I) and phlebic acid A were isolated from a lichen *Peltigera aphthosa* (L.) Willd., and the structure of phlebic acid A was established to be 28-acetoxy-22-hydroxyhopan-23-oic acid (II).

UNTIL recently, zeorin and peltigerin were the only known constituents of lichens of *Peltigera* spp.[1] Huneck and Tümmler[2] reported that peltigerin was identical with tenuiorin[3] which had been isolated earlier from *Lobaria linita* (Ach.) Rabh. var. *tenuior* (Hne) Asah, and *L. sachalinensis* Asah. A recent chemotaxonomic TLC survey[4] showed the occurrence of some new constituents in various *Peltigera* species and their correlation with the plant classification.

The present paper concerns with some new triterpenoids of *Peltigera aphthosa* (L.) Willd. A triterpenoid, $C_{32}H_{54}O_3$, m.p. 203–205°, $[\alpha]_D$ +22·1°, which was isolated from the readily soluble portion of petroleum ether extracts of the lichen, revealed the presence of eight tertiary C-methyl groups by NMR spectrum (in $CDCl_3$) [δ 0·80 (3 Me), 0·85, 1·04, 1·10, 1·15, 1·19 ppm (1 Me each)] and an acetoxyl group by i.r. absorption (in $CHCl_3$) at 1720 cm^{-1} and NMR spectrum at δ 2·00 ppm (1 Me). This substance proved to be identical with 15α-acetoxy-22-hydroxyhopane (dustanin monoacetate)[5] (I) by m.p., TLC and i.r. spectral comparison with the authentic sample.

From the sparingly soluble portion of the above extracts, a new triterpene, named phlebic acid A (formerly named phlebin A)[4] (II), $C_{32}H_{52}O_5$, m.p. 243–245°, $[\alpha]_D$ +70·2°, was isolated, which gave positive Liebermann–Burchard reaction and negative tetranitromethane reaction. The i.r. absorption (in KBr) at 1717 cm^{-1} revealed a carboxyl group and the band at 1742 cm^{-1} an alcoholic acetate grouping. This is also confirmed by the mass spectrum giving peaks at *m/e* 516 (M^+), 498 (M^+-H_2O), 443 ($M^+-CH_2OCOCH_3$), and 425 ($M^+-H_2O-CH_2OCOCH_3$). The methyl ester (III) of phlebic acid A, m.p. 191–192·5°, showed in the NMR spectrum the presence of six tertiary C-methyl groups [δ 0·84, 0·96, 1·08, 1·15, 1·20 and 1·27 ppm (1 Me each)], $C\underline{H}_2OCOC\underline{H}_3$ grouping [δ 2·05 ppm (1Me), 4·26, 4·57 ppm (a pair of doublets, 1 H each, $J=12$ cps)] and $COOC\underline{H}_3$ [δ 3·66 ppm (1 Me)].

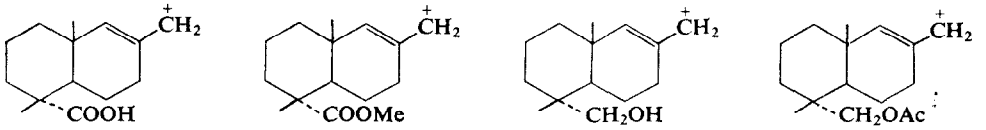
The free hydroxyl in phlebic acid A must be tertiary, since the presence of a proton attached to the carbon atom bearing hydroxyl was not observed in the NMR spectrum, and

[1] W. ZOPF, *Ann. Chem.* **364**, 273 (1909).
[2] S. HUNECK and R. TÜMMLER, *Ann. Chem.* **685**, 128 (1965).
[3] S. SHIBATA and H-C. CHIANG, *Bull. Nat. Inst. Sci. India*, No. 31, 151 (1965).
[4] S. KUROLAWA, Y. JUNZENJI, S. SHIBATA and H-C. CHIANG, *Bull. Nat. Sci. Museum* **8**, 101 (1966).
[5] R. E. CORBETT and H. YOUNG, *J. Chem. Soc.* **18**, 1564 (1966); Y. TSUDA and K. ISOBE, *Tetrahedron Letters* 3337 (1965).

the hydroxyl could not be acetylated with acetic anhydride and pyridine. From the above results and from the co-occurrence of the hopane-type triterpenoids, zeorin and 15α-acetoxy-22-hydroxyhopane, in the same lichen, it is reasonable to assume that phlebic acid A, $C_{28}H_{45}(OH)(CH_2OAc)(COOH)$, is also a hopane-type pentacyclic triterpene.

Triterpenes having no functional groups in A and B rings give a peak, *m/e* 191, in the mass spectra caused by the cleavage of C-ring,[6] whereas phlebic acid A, its methyl ester, the corresponding triol (IV) prepared by $LiAlH_4$ reduction from the methyl ester, and the triol diacetate (X) show a peak at *m/e* 221, 235, 207 and 249, respectively.[6] This can be explained by the presence of a carboxyl at 4α, 4β or 10β position in A-ring of phlebic acid A.

According to the above mass spectral result, the primary alcoholic acetate grouping cannot be located in the A and B rings, so that it must be situated alternatively at 14α or 18α. The latter is most probable, since the NMR signal of angular methyl at 18α of hopane ring system appears in higher field as shown by 22-hydroxyhopane at δ 0·78 ppm, and no such a signal is observed in the NMR spectrum of phlebic acid A. According to Ageta,[7] in hopane-type triterpenoids there is no significant shift of NMR signal of 14α methyl when 18α methyl is replaced by an acetoxymethylene group. This also agrees with the disposition of the acetoxymethylene group and tertiary methyl in phlebic acid A at 18α and 14α, respectively.



m/e 221 *m/e* 235 *m/e* 207 *m/e* 249

CHART 1.

The triol (IV) was oxidized with chromic acid in pyridine under mild conditions. Chromatography of the reaction mixture followed by fractional recrystallization afforded a diol-aldehyde (VI), m.p. 249–250·5°, i.r. (in $CHCl_3$): 1725 (CHO) and 3410 cm^{-1} (OH). NMR (in C_5D_5N): δ 9·27 ppm (1 H, s, CHO) and 3·73, 4·18 ppm (a pair of doublets, 1 H each, $J=11·5$ cps, —CH_2OH). On Huang-Minlon reduction, VI yielded a diol which was proved to be identical with 22,28-dihydroxyhopane (VII) prepared by Ageta *et al.*[8] from adipedatol (XI), a constituent of a fern, *Adiantum pedatum*. It should be noted that 22,28-dihydroxyisohopane, the 21-αH isomer of VII, can easily be distinguished from VII by TLC.[8]

In the chromic acid oxidation of IV, a hemiacetal compound (V) (i.r.: no carbonyl absorption, NMR (in C_5D_5N): δ 5·67 ppm (1 H, s, —O—CH(OH)—O—)) was formed along with VI. Although V was not obtained completely pure, the NMR signals (in C_5D_5N) of V at δ 3·28 and 3·63 ppm (a pair of doublets, 1 H each, $J=10·5$ cps) could be assigned as a methylene of the carbinol group attached to C_4 in comparison with the NMR spectra of IV and VIII.

The 4α equatorial and 4β axial acetoxymethylene can be distinguished by the chemical shift in NMR spectra, since the former gives δ 3·67–3·86 ppm and the latter δ 4·09–4·31 ppm.[9] The acetate (X) of triol (IV) gave an AB type methylene signal at δ 3·63, 3·89 ppm (a pair of

[6] H. BUDZIKIEWICZ, C. DJERASSI and D. H. WILLIAMS, *Structure Elucidation of Natural Products by Mass Spectrometry*, Vol. II, Holden-Day, New York.
[7] H. AGETA and K. IWATA, *Tetrahedron Letters* 6069 (1966).
[8] H. AGETA and K. SHIOJIMA, *Chem. Commun.* 1372 (1968).
[9] A. GAUDEMER, J. POLONSKY and E. WENKERT, *Bull. Soc. Chim. France* 407 (1964).

TABLE 1.

	4β Me	10β Me	Other Me groups			
II	1·41	0·90	1·00	1·08	1·35	1·47
III	1·25	0·83	0·97	1·07	1·34	1·47
Δ	0·16	0·07				

Determined in C_5H_5N (60 Mc; δ ppm).

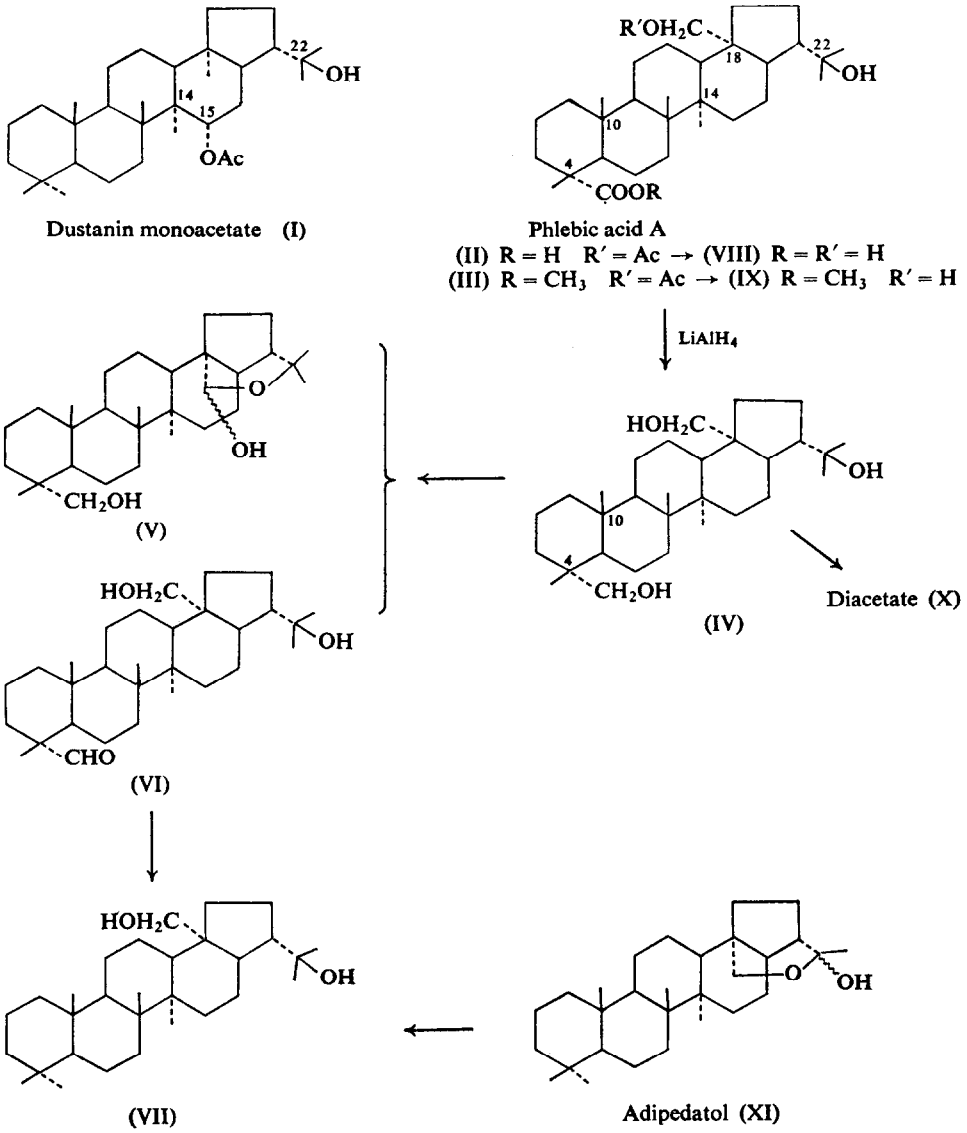


CHART 2.

doublets, 1 H each, $J = 11$ cps) showing an equatorial orientation. The equatorial orientation (α) of the carbomethoxyl group at position 4 in the original methyl ester (III) was also indicated by the strong i.r. absorption at 1235 cm^{-1} for C—O—C stretching vibration.[10]

According to Narayanan *et al.*[11] the carboxyl anion at $C_4\beta$ (axial) shows a 1,3 diaxial deshielding effect to the C_{10} methyl, causing a down-field shift (0·28 ppm) in comparison with the methyl ester when the NMR spectra is measured in pyridine. No such remarkable effect of the $C_4\alpha$ (equatorial) carboxyl anion on the C_{10} methyl is observed with the free acid; there is only a slight down-field shift of 0·07 ppm. The C_4 methyl suffers the deshielding effect in both cases to show a down-field shift about 0·17 ppm.

Phlebic acid A shows the 0·07 ppm and 0·16 ppm down-field shifted NMR signals (in C_5H_5N) of methyl groups at C_{10} and C_4, respectively, in comparison with those of its methyl ester. (See Table 1). Thus the orientation of carboxyl at C_4 of phlebic acid A is deduced to be equatorial (α), and the structure is finally formulated as 28-acetoxy-22-hydroxyhopan-23-oic acid (II).

The genus *Peltigera* is divided into two sub-genera Phlebia and Emprostea. The latter consists of three groups, *P. malacea* group, *P. polydactyla* group and *P. canina* group. The lichens in *P. canina* group contain neither tenuiorin nor triterpenoids. Phlebic acid A is found only in Section Phlebia with one exception, *P. nigripunctata*, which is morphologically very similar to *P. aphthosa* and *P. variorosa*.

EXPERIMENTAL

Extraction of the Lichen

The lichen was collected in the Mt. Fuji area in June 1967. Air-dried lichen (1140 g) was ground into fine powder and extracted (Soxhlet) with petroleum ether for 2 days. The precipitates (3·6 g) which resulted during the extraction were chromatographed on a column of silica gel (65 g). When the proportion of $CHCl_3$ to benzene increased, tenuiorin [benzene–$CHCl_3$ (5:1)]; zeorin (benzene–$CHCl_3$ (1:1)) and phlebic acid A (*ca.* 1 g) [benzene–$CHCl_3$ (1:2)] were eluted. Evaporation of the supernatant gave a dark-green semi-solid residue (11·5 g) which was subjected to chromatography in the same way as above to give tenuiorin, zeorin, 15α-acetoxy-22-hydroxyhopane and phlebic acid A.

15α-*Acetoxy-22-hydroxyhopane* (*I*). m.p.[12] 203–205° (from acetone–MeOH), colourless crystals, $[\alpha]_D$ + 22·1 (C = 0·58 in $CHCl_3$), ν_{max}^{KBr} 3490 (OH), 1730 (OAc), 1260 (OAc) cm^{-1}; and δ 0·80 (3 Me), 0·85, 1·04, 1·10, 1·15, 1·19 (1 Me each), 2·00 (OAc), 5·09 (1 H, m) ppm (60 Mc in $CDCl_3$). (Found: C, 79·13; H, 11·17. $C_{32}H_{54}O_3$ required: C, 78·96; H, 11·18 per cent.)

Phlebic acid A (*II*). m.p. 243–245° (from MeOH–H_2O), colourless crystals, $[\alpha]_D$ + 70·2 (C = 0·79 in $CHCl_3$), ν_{max}^{KBr} 3470 (OH), 1742 (OAc), 1717, 1703 (COOH), 1265 (OAc) cm^{-1}, 0·84, 0·95, 1·09, 1·13 (1 Me each), 1·24 (2 Me), 2·02 (OAc), 4·25, 4·42 (a pair of doublets, 1 H each, $J = 12$ cps) ppm (100 Mc in $CDCl_3$), and *m/e* 516 (M^+), 498 ($M^+ - H_2O$), 456 ($M^+ - AcOH$), 443 ($M^+ - CH_2OCOCH_3$), 438 ($M^+ - H_2O - AcOH$), 425 ($M^+ - H_2O - CH_2OCOCH_3$), 221, 203, 189, 59. (Found: C, 74·57; H, 9·93. $C_{32}H_{52}O_5$ required: C, 74·37; H, 10·14 per cent.)

Phlebic Acid A Methyl Ester (*III*)

Treatment of II with CH_2N_2 gave III, m.p. 191–192·5° (from MeOH), colorless crystals, $\nu_{max}^{CCl_4}$ 3640, 3530, 1745 (sh), 1740, 1245 cm^{-1}, and δ 0·84, 0·96, 1·08, 1·15, 1·20, 1·27 (1 Me each), 2·05 (OAc), 3·66 (OCH_3), 4·26, 4·57 (a pair of doublets, 1 H each, $J = 12$ cps) ppm (60 Mc in $CDCl_3$). (Found: C, 74·76; H, 10·17. $C_{33}H_{54}O_5$ required: C, 74·67; H, 10·26 per cent).

$LiAlH_4$ Reduction of III

Phlebic acid A methyl ester (III) (0·1 g) in dry ether (30 ml) was heated under reflux with $LiAlH_4$ (0·5 g) for 6 hr. After working up in the usual way the product was crystallized from acetone to give triol (IV), m.p. 276–277·5°, colorless crystals, i.r.: no carbonyl, and δ 0·88 (1 Me), 0·94 (1 Me), 1·05 (2 Me), 1·32 (1 Me), 1·54 (1 Me), 3·37, 3·74 (a pair of doublets, 1 H each, $J = 11$ cps), 3·78, 4·26 ppm (a pair of doublets, 1 H each, $J = 12$ cps) (60 Mc in C_5D_5N). (Found: C, 78·43; H, 11·15. $C_{30}H_{50}O_3$ required: C, 78·20; H, 11·38 per cent.)

[10] S. BORY and M. FÊTIZON, *Bull. Soc. Chim. Fr.* 570 (1964).
[11] C. R. NARAYANAN and N. K. VENKATASUBRAMANIAN, *Tetrahedron Letters* 3639 (1965).
[12] M.ps were taken on Kofler hot-stage apparatus and are uncorrected.

Chromic Acid Oxidation of IV

A solution of IV (0·2 g) in pyridine (4·5 ml) was combined with CrO_3 (0·25 g) in pyridine (6 ml) and the mixture was stirred under ice cooling for 1 hr. The reaction mixture was poured into water and extracted with ether. The crude products were submitted to chromatography on silica gel using benzene–acetone (30:1) as eluting solvent.

The fraction obtained after less polar substances were eluted was recrystallized from acetone–MeOH mixture to separate VI, colourless crystals, m.p. 249–250·5°, sparingly soluble in MeOH, $\nu_{max}^{CHCl_3}$ 3410, 1727 cm^{-1} and δ 0·75, 0·91, 1·01, 1·06, 1·25, 1·47 (1 Me each), 3·73, 4·18 (a pair of doublets, 1 H each, J=11·5 cps), 9·27 (1 H, s) ppm (100 Mc in C_5D_5N). On the basis of the NMR (δ 0·86, 0·92, 1·07, 1·17, 1·29, 1·34 (1 Me each), 3·28, 3·63 (a pair of doublets, 1 H each, J=10·5 cps), 5·67 (1 H, s) ppm (100 Mc in C_5D_5N)) and i.r. ($\nu_{max}^{CCl_4}$ 3660, 3620, 3380, 1180, 1095 cm^{-1}), the subsequent fraction as well as the mother liquor of recrystallization of VI, contained mainly V, being more soluble in MeOH, though its further purification has not been made as yet. Further elution with benzene–acetone (5:1) afforded unchanged triol (IV).

Huang-Minlon Reduction of VII

A mixture of VII (0·013 g) in EtOH (2 ml), diethyleneglycol (3 ml) and 80% hydrazine hydrate was heated for 0·5 hr at 130°. Then KOH (0·2 g) was added to the solution, which was further heated for additional 0·5 hr at 130°. After the condenser was replaced to a downward cooler ethanol and water was removed, the temperature being allowed to rise to 220°, and then refluxing was continued for 2·5 hr longer. The cooled solution was diluted with water, neutralized with 1 N HCl and extracted with ether. The colourless solid was crystallized from methanol to give VII. Identity of VII with the authentic sample[8] was established by comparison of the i.r. spectrum and by TLC.

Acetylation of Triol (IV)

Triol (IV) (0·05 g) in pyridine (3 ml) and Ac_2O (1·5 ml) was allowed to stand overnight at room temperature After working up in the usual way, crystallization from methanol gave the diacetate (X), m.p. 218·5–220°, colourless crystals, δ 0·81, 0·84, 0·96, 1·05, 1·18, 1·26 (1 Me each), 2·01, 2·03 (1 OAc each), 3·63, 3·89 (a pair of doublets, 1 H each, J=11 cps), 4·29, 4·52 (a pair of doublets, 1 H each, J=12 cps) ppm (100 Mc in $CDCl_3$).

Deacetylation of Phlebic Acid A (II)

Phlebic acid A (II) (0·2 g) was refluxed with 5% ethanolic KOH (30 ml) for 1 hr; after the concentration of the solution and the neutralization with 1 N HCl, the resulted precipitate was extracted with ether. The product was recrystallized from methanol to give VIII, as colourless crystals, m.p. 245–247°, δ 0·89, 1·01, 1·06, 1·27, 1·37, 1·47 (1 Me each), 3·72, 4·17 (a pair of doublets, 1 H each, J=12 cps) ppm (100 Mc in C_5D_5N). On methylation with CH_2N_2 in ether VIII gave a methyl ester (IX), m.p. 265–270°, i.r. $\nu_{max}^{CS_2}$ 1725, 1235 ($COOCH_3$) cm^{-1}.

Acknowledgements—We are grateful to Dr. S. Kurokawa, National Science Museum, Tokyo, for his kind advice in lichenology and Dr. H. Ageta, Showa College of Pharmacy, for showing unpublished experimental data and providing samples. We are also indebted to Dr. R. E. Corbett, University of Otago, New Zealand, and Dr. Y. Tsuda, Showa College of Pharmacy, for supplying samples.