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THE STRUCTURE OF PHLEBIC ACID B, A CONSTITUENT OF THE LICHEN *PELTIGERA APHTHOSA*, AND THE OCCURRENCE OF 15α-ACETOXY- AND 7β-ACETOXY-22-HYDROXYHOPANE IN *P. DOLICHORRHIZA*

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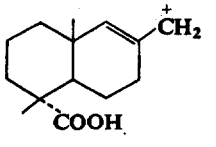
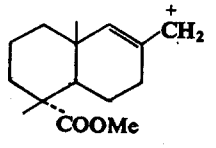
Abstract—The lichen *Peltigera aphthosa* (L.) Willd. yielded phlebic acid B, the structure of which was established as 22-hydroxyhopan-23-oic acid. 15α-Acetoxy and 7β-acetoxy-22-hydroxyhopane were isolated from *P. dolichorrhiza* (Nyl.) Nyl.

IN THE previous paper[1] we reported that *Peltigera aphthosa* (L.) Willd. contains 15α-acetoxy-22-hydroxyhopane (dustanin monoacetate) and phlebic acid A along with zeorin, tenuiorin and some other unknown minor constituents. Phlebic acid A was identified as 28-acetoxy-22-hydroxyhopan-23-oic acid (I). Phlebic acid B (II), $C_{30}H_{50}O_3$, m.p. 258–260°, $[\alpha]_D$ +52·9°, which has a higher R_f than phlebic acid A, has now been isolated from the petroleum ether extracts of *P. aphthosa*. It gives a positive Liebermann–Burchard reaction and a negative tetranitromethane reaction. It shows i.r. maxima at 3480 (OH), 1715 (COOH) and 1705 (sh) cm^{-1} and mass spectral peaks at *m/e* 458 (M^+) and 440 (M^+-H_2O). The methyl ester (III) of phlebic acid B, m.p. 231–232°, possesses only one hydroxyl which is a tertiary one since it is not acetylated with acetic anhydride and pyridine and the NMR spectrum reveals the absence of proton at the carbon bearing the hydroxyl.

The above results suggest that phlebic acid B, $C_{29}H_{48}$ (OH)(COOH), is a hopane-type pentacyclic triterpene similar to phlebic acid A. This was proved by the following reactions. The methyl ester (III) of phlebic acid B was reduced with $LiAlH_3$ to a diol (IV), m.p. 240–243°; the aldehyde (V), the main product of oxidation of the diol with chromic acid, was reduced by the Huang–Minlon method to yield the hydroxyhopane (VI), identified by mixed m.p. and by i.r. and TLC comparison.



m/e 221



m/e 235

The mass spectra of phlebic acid B (II) and its methyl ester (III) show a peak at *m/e* 221 and 235, respectively, indicating the carboxyl or the carbomethoxyl group is located in the

[1] R. TAKAHASHI, O. TANAKA and S. SHIBATA, *Phytochem.* **8**, 2345 (1969).

A-ring.[2] The monoacetate of the diol (IV) shows a pair of doublet signals of methylene at δ 3·65 and 3·89 (1H each, $J = 11$ c/s),[3] the aldehyde (V) shows the aldehyde proton signal at δ 9·21 (s, 1H),[4] and the methyl ester of phlebic acid B (II) has an i.r. absorption of C—O—C at 1235 cm^{-1}.[5] All these facts reveal the equatorial orientation of the carboxyl group in phlebic acid B. This was also supported by the high field shift, 0·11 and 0·05 ppm of two methyl signals of phlebic acid B methyl ester (III) in comparison with those of phlebic acid B (II) (see Table 1).[6] Consequently, phlebic acid B is formulated as 22-hydroxyhopan-23-oic acid (II).

TABLE 1

	4βMe	10βMe	Other Me groups
I	1·37	0·90	0·90, 0·98 (2Me), 1·32, 1·38
II	1·26	0·85	0·92, 0·98 (2Me), 1·33, 1·38
	0·11	0·05	

Determined in C_5D_5N (δ ppm).

Zeorin and tenuiorin have already been reported in *P. dolichorrhiza* (Nyl.) Nyl.; the present study reveals that another two triterpenes are also present. One of these compounds, previously named dolichorrhizin,[7] m.p. 200°, has now been identified as 15α-acetoxy-22-hydroxyhopane (VIII), which is contained in *P. aphthosa* (L.) Willd., by mixed fusion, TLC and i.r. spectral comparison. The second constituent, $C_{32}H_{50}O_3$, m.p. 247–249°, $[\alpha]_D$ +20°, is identical with 7β-acetoxy-22-hydroxyhopane (IX), which was isolated by Corbett *et al.*[8] from *Sticta billardierii* Del., by mixed fusion, TLC and i.r. spectral comparison with the authentic sample. An unknown compound X_3, m.p. 183–184°, was isolated from both *P. aphthosa* and *P. dolichorrhiza.*

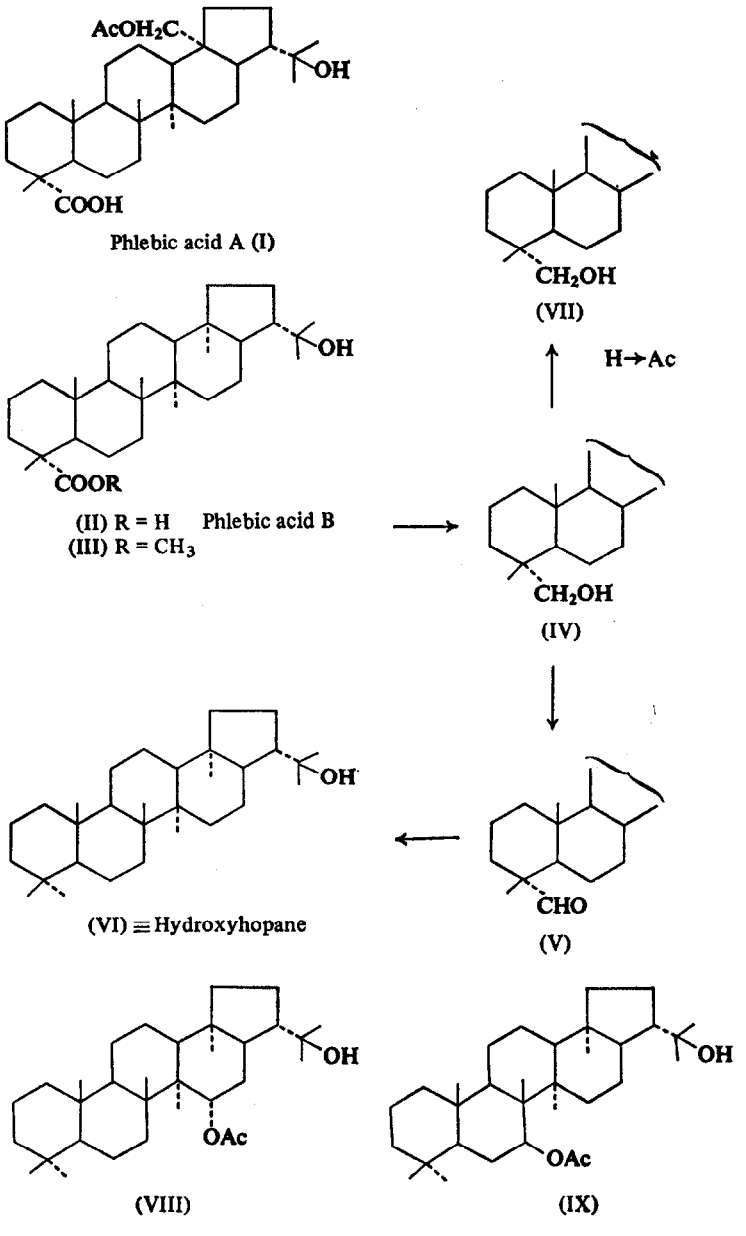
The constituents of these two species, as present known, are listed in Table 2.

TABLE 2. CONSTITUENTS OF *Peltigera species*

	*P. aphthosa**	*P. dolichorrhiza*†
Phlebic acid A	+	–
Phlebic acid B	+	trace
Compound X_3	+	+
Zeorin	+	+
15α-Acetoxy-22-hydroxyhopane	+	+
7β-Acetoxy-22-hydroxyhopane	–	+
Tenuiorin	+	+

* Belongs to section Phlebia of the genus.
† Belongs to section Eprostea of the genus.

[2] H. BUDZIKIEWICZ, C. DJERASSI and D. H. WILLIAMS, *Structure Elucidation of Natural Products by Mass Spectrometry*, Vol. II, Holden-Day, San Francisco (1964).
[3] A. GAUDEMER, J. POLONSKY and E. WENKERT, *Bull. Soc. Chim. France* 407 (1964).
[4] M. FÊTIZON, G. MARAU and N. MARAU, *Bull. Soc. Chim. France* 3295 (1968).
[5] S. BORY and M. FÊTIZON, *Bull. Soc. Chim. France* 570 (1964).
[6] C. R. NARAYANAN and N. K. VENKATASUBRAMANIAN, *Tetrahedron Letters* 3639 (1965).
[7] S. KUROKAWA, Y. JUNZENJI, S. SHIBATA and H-C. CHIANG, *Bull. Nat. Sci. Museum* **8**, 101 (1966).
[8] R. E. CORBETT and H. YOUNG, *J. Chem. Soc.* **18**, 1556 (1966).



EXPERIMENTAL

M.ps were taken on a Kofler hot-stage apparatus and are uncorrected, and NMR spectra were determined on 100 Mc.

Phlebic Acid B (II)

The total fraction which contained phlebic acid B, as isolated previously,[1] was chromatographed again on a column of silica gel using benzene–$CHCl_3$ (1:2) as eluting solvent, and crystallization from $CHCl_3$–MeOH gave phlebic acid B (0·5 g), m.p. 258–260°, colourless crystals, $[\alpha]_D^{23°}$ +52·9 (C = 0·59, in pyridine), ν_{max}^{KBr} 3480, 1715, 1705 cm^{-1}, and *m/e* 458 (M^+), 440 (M^+-H_2O), 415, 387, 361, 261, 221, 207, 189. (Found: C, 78·27; H, 10·85. $C_{30}H_{50}O_3$ required: C, 78·55; H, 10·99%.)

Phlebic Acid B Methyl Ester (III)

Treatment of II with CH_2N_2 gave III, m.p. 231–233°, colourless crystals (from $CHCl_3$–MeOH) $[\alpha]_D^{23}$ +54·3 (C = 0·40, in $CHCl_3$), $\nu_{max}^{CS_2}$ 3400 (br), 1730, 1725 (sh), 1240 cm^{-1}, δ 0·78, 0·86, 0·96, 0·99, 1·15, 1·19, 1·22 (1Me each), 3·67 ($COOCH_3$) ppm (in $CDCl_3$) and *m/e* 472 (M^+), 454 (M^+-H_2O), 439, 412, 386, 235 (base peak), 207, 189. (Found: C, 78·72; H, 10·96. $C_{31}H_{52}O_3$ required: C, 78·76; H, 11·09%.)

$LiAlH_4$ Reduction of III

Phlebic acid B methyl ester (III) (0·2 g) in dry ether (60 ml) was heated under reflux with $LiAlH_4$ (0·7 g) for 5·5 hr. After working up in the usual way, the product was crystallized from $CHCl_3$–MeOH to give diol (IV), m.p. 240–243°, colourless crystals, $[\alpha]_D^{23}$ +41·3 (C = 0·75, in pyridine), ν_{max}^{KBr} 3300, no carbonyl, and δ 0·95 (1Me), 1·00 (2Me), 1·03, 1·09, 1·45, 1·50 (1Me each), 3·395, 3·765 (a pair of doublets, 1 H each, J = 11 c/s) (in C_5D_5N). (Found: C, 81·37; H, 11·61. $C_{30}H_{52}O_2$ required: C, 81·02; H, 11·79%.)

Chromic Acid Oxidation of IV

A solution of IV (0·11 g) in pyridine (6·5 ml) was combined with CrO_3 (0·15 g) in pyridine (5 ml), and the mixture was stirred at room temperature for 5·5 hr. The reaction mixture was poured into water and extracted with ether. The crude products were submitted to chromatography on silica gel using benzene–$CHCl_3$ (5:1) as eluting solvent, and the fraction (0·040 g) was obtained, which contained mainly V, δ 0·78, 0·88 (1Me each), 0·97 (2Me), 1·04, 1·81, 1·21 (1Me each), 9·21 (1H s) ppm (in $CDCl_3$), and $\nu_{max}^{CHCl_3}$ 3300 (br), 1725 cm^{-1}.

Huang–Minlon Reduction of V

A mixture of the above-mentioned fraction (0·035 g) in EtOH (7 ml), diethyleneglycol (10 ml) and 80% hydrazine hydrate (1 ml) was heated for 0·5 hr at 130°. Then KOH (0·5 g) was added to the solution, which was further heated for an additional 0·5 hr at 130°. After removing the EtOH and H_2O, the temperature was allowed to rise to 220°, and refluxing was continued for 2·5 hr. The cooled solution was diluted with water, neutralized with 1 N HCl and extracted with ether. The colourless solid was crystallized from acetone to give VI. Identity of VI with authentic sample was established by m.p., TLC and i.r. spectral comparison.

Acetylation of Diol (IV)

Diol (IV) (0·1 g) in pyridine (3 ml) and Ac_2O (1·5 ml) was allowed to stand overnight at room temperature. Crystallization from acetone gave the monoacetate (VII), m.p. 197·5–198·5, colourless crystals, $[\alpha]_D^{23}$ +45·6 (C = 0·46 in $CHCl_3$), $\nu_{max}^{CCl_4}$ 1740, 1240 cm^{-1}, and δ 0·77, 0·83, 0·86 (1Me each), 0·97 (2Me), 1·18, 1·21 (1Me each), 2·06 (OAc), 3·65, 3·89 (a pair of doublets, 1 H each J = 11 c/s). (Found: C, 78·97; H, 11·31. $C_{32}H_{54}O_3$ required: C, 78·96; H, 11·18%.)

The Extraction of Peltigera dolichorrhiza

The lichen was collected in the foot of Mt. Fuji in June 1967. Air-dried lichen (1700 g) was ground into fine powder and extracted (Soxhlet) with hexane for 2 days. The extract (26 g) was chromatographed on a column of silica gel (330 g). When the proportion of $CHCl_3$ to benzene was increased, the following were successively eluted: tenuiorin, 7β-acetoxy-22-hydroxyhopane (*ca.* 2 g), dolichorrhizin (*ca.* 0·6 g) and zeorin.

7β-Acetoxy-22-hydroxyhopane, m.p. 247–248° (from $CHCl_3$–acetone), colourless crystals, $[\alpha]_D$ +20·0 (C = 0·32 in $CHCl_3$), ν_{max}^{KBr} 3530, 1725 (sh), 1710, 1270 cm^{-1}, and δ 0·77, 0·79, 0·84, 0·88, 1·04, 1·10, 1·22 (1Me each), 1·97 (OAc), 5·08 (1H, q). (Found: C, 79·14; H, 11·27. $C_{33}H_{54}O_3$ required: C, 79·0; H, 11·2%.)

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