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THE PHENOLIC CONSTITUENTS OF *PELTIGERA APHTHOSA**

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Key Word Index—*Peltigera aphthosa*; Peltigeraceae; lichen acids; orsellinate type esters; depsides; tridepsides; aphthosin.

Abstract—In addition to tenuiorin, methyl gyrophorate and methyl evernate have been isolated from *Peltigera aphthosa*. The occurrence of a tetradepside (*aphthosin*) in all the specimens investigated of this species has not been verified.

INTRODUCTION

Peltigera has frequently been the subject of a chemical investigation of its phenolic constituents. Tenuiorin has been isolated on several occasions [1–4] and, in addition, a tetradepside named aphthosin has been described from *P. aphthosa* [4].

In the course of chemotaxonomical surveys of the lichen genera *Pseudocyphellaria* and *Peltigera* by two-directional TLC it was found that the depsides occurring in several species of *Peltigera* resemble some of those found in *Pseudocyphellaria crocata* and related species [5,6]. The present paper deals with the occurrence of tenuiorin, methyl gyrophorate, methyl lecanorate, methyl evernate, methyl orsellinate, 4-*O*-methylgyrophoric acid, gyrophoric acid, and evernic acid [Fig. 1] in *Peltigera aphthosa* and with the search for aphthosin.

RESULTS AND DISCUSSION

In *Peltigera aphthosa* collected in Nova Scotia, Manitoba, the Northwest Territories and British Columbia the following orsellinate derivatives were demonstrated by either isolation or two-directional TLC: tenuiorin, methyl gyrophorate, methyl evernate, methyl lecanorate and methyl orsellinate as well as trace amounts of 4-*O*-methylgyrophoric acid, gyrophoric acid and evernic acid.

Work-up for the isolation of aphthosin according to Bachelor and King [4] resulted in a microcrystalline powder which had a number of characteristics in common with aphthosin, such as the relative insolubility, high-melting behaviour and IR spectrum. An NMR spectrum of this powder gave the same number of methyl- and methoxyl signals although their chemical shifts did not fully agree with those reported for aphthosin (Table 1). On the other hand, TLC and column chromatography revealed this powder to be a mixture of lower-molecular weight compounds. The main constituents were tenuiorin and methyl gyrophorate, the ratio of these compounds being between 10:1 and 5:1 as judged by the size of the reacted spots. Repeated washing of the powder with hot acetone did not apparently change this ratio.

It is possible that Bachelor and King were dealing with a species of *Peltigera* other than *P. aphthosa*, or that their *P. aphthosa* represents a chemical strain not encountered in this study. No indication for the presence of a tetradepside homologous to the tridepside tenuiorin could be found on the two-directional chromatograms [5] obtained from extracts of the lichen collected in Nova Scotia and in a few other localities (see Experimental).

The original evidence [4] for the existence of aphthosin was based essentially on a quantitative NMR analysis of the acid hydrolysis mixture, which revealed a methoxyl- to aromatic methyl

* Lichen Substances Part VI. Issued as NRCC No. 14731.

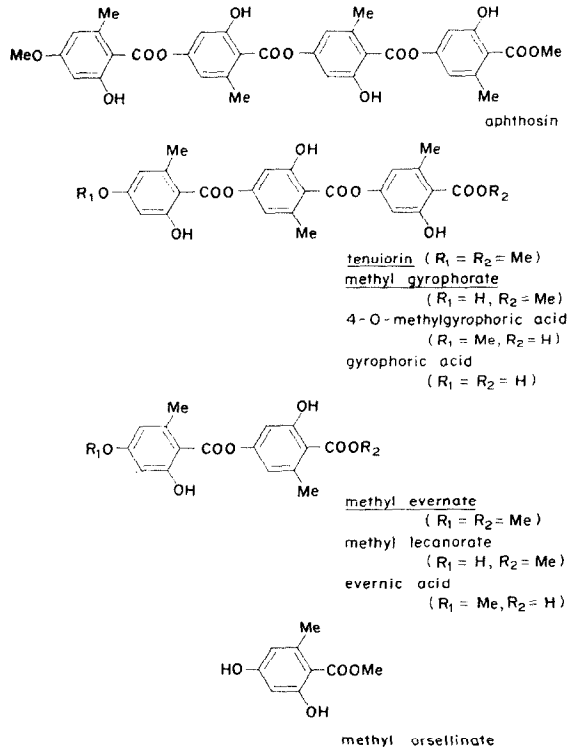

Fig. 1. Orsellinate derivatives reported from or detected in *Peltigera aphthosa*. Compounds underlined were isolated in the course of this study.

ratio of 1:2, the hydrolysis products being the same as those obtained from tenuiorin. Hydrolysis of depsides with conc. sulfuric acid is optimal at temperatures between 0° and −10° [7]. The yields under the conditions employed by Bachelor and King (10–15 min at room temp.) may be low and could seriously affect the ratio of the hydrolysis products.* Neither the homogeneity nor the MW of aphthosin was determined. It appears that more physical and chemical data for the original aphthosin isolate are needed, particularly as no other naturally occurring tetra-aryl depside structure has been reported to date [8].

EXPERIMENTAL

Mass spectra (70 eV) were recorded on a Bell & Howell C.E.C. 21–110 instrument using a direct introduction probe, NMR spectra on a Varian A-60A instrument. Two-directional TLC on Si gel G plates (developed in C_6H_6–dioxane–HOAc 90:25:4 in the first direction and hexane–Et_2O–HCOOH 5:4:1 in the second) and column chromatographic methods were the same as described in a previous paper [5]. For the detection of phenols on TLC plates, a spray of aqueous Fast Bordeaux BD Salt followed by an application of aq Na_2CO_3 was used. From a freshly prepared acetone extract of *Peltigera aphthosa* (30 g) collected in a mountainous area near Baddeck, Cape Breton Island, N.S., during the summer of 1966, tenuiorin, methyl gyrophorate and methyl evernate were isolated in yields of 900, 120 and 30 mg respectively. The same compounds were obtained in yields of 1100, 100 and 35 mg respectively from *P. aphthosa* (30 g) collected on Marvin Island near Chester, N.S., during the fall of 1974. Material from the same source was also worked up for the isolation of aphthosin by following the procedure of Bachelor and King [4] as closely as possible. Air-dry thalli (80 g) were extracted in a soxhlet with *n*-hexane (1800 ml) for 24 hr to remove tenuiorin, and then with benzene (1800 ml). The C_6H_6 soln was concentrated to half of vol and insoluble material filtered off. This was boiled with Me_2CO several times and the remaining insoluble portion, a grayish-white powder representing the aphthosin fraction, was investigated further: it melted at 292–298°

* The authors referred to Huneck and Tümmler [2] in stating that tenuiorin is quantitatively hydrolyzed in conc. sulfuric acid although in the original report no yields for the hydrolysis products (obtained within 15 min at 20°) were given.

Table 1. Comparison of NMR data of depsides from *Peltigera aphthosa*, obtained in pyridine-d_5

Signal from	δ-values				
	Aphthosin*	Mixture†	Methyl gyrophorate	Tenuiorin	Methyl evernate
Arom. Me	2·48	2·42	2·42	2·42	2·43
			2·46‡	2·47‡	
	2·65	2·56	2·55	2·55	
	2·81	2·59 sh	2·62	2·59	2·59
Methoxyls	3·75	3·72		3·70	3·70
			3·76‡	3·77‡	
	3·80	3·86	3·84	3·85	3·84

* Figures calculated from the τ-values reported by Bachelor and King [4]. The location of the fourth aromatic methyl group, the presence of which is implied by the published structure for aphthosin, remains in doubt.

† This is a rather insoluble and high-melting powder which was obtained in an attempt to re-isolate aphthosin, by following the isolation procedure of Bachelor and King [4] as closely as possible; two-directional TLC and column chromatography revealed tenuiorin and methyl gyrophorate as the main constituents of this powder.

‡ These are relatively small signals and are obviously due to chemical breakdown or rearrangement of the compounds in C_5H_5N as they strongly increase with time, at the expense of the signals at δ 2·55 and 3·84–3·85 respectively. The NMR spectra of methyl gyrophorate and tenuiorin in DMSO-d_6 do not show any evidence for these impurities, as all of their functional groups are fully accountable by well integrated proton signals.

(with hardly noticeable softening around 233°, reported 300°) and gave an IR spectrum (ν_{max}^{KBr} 3415 w, 1680, 1613, 1585) similar to the spectrum of tenuiorin as reported for aphthosin; δ(pyridine d_5) 2·00 (impurity, diminished in relation to the other signals after further extraction with acetone), 2·42, 2·56, 2·59 (sh) aromatic methyls, 3·72, 3·86 methoxyls (ratio of aromatic methyls/methoxyls approximately 2/1), 6·53, 6·66 (arom. H, integrating for just 1 proton each), multiplet around 6·93 (arom. H, integrating for 3–4 protons). TLC revealed the presence of tenuiorin and methyl gyrophorate in this powder, and from 100 mg of the powder, 72 mg tenuiorin and 8 mg methyl gyrophorate were subsequently isolated by column chromatography and identified by MP, IR spectra, as well as co-chromatography with authentic compounds in the two-directional TLC system. The powder also contained small amounts of everninic acid (4-*O*-methylorsellinic acid) and of the other orsellinate derivatives already mentioned. The depsides isolated from *P. aphthosa* showed the following characteristics: *Tenuiorin* (4-*O*-methyl methyl gyrophorate): mp 174° and 225–236° d, ν_{max}^{KBr} 1680, 1665 (sh), 1613 cm^{-1}; (DMSO-d_6) 2·27, 2·38, 2·40 (3 arom. Me); 3·76 (1 OMe), 3·82 (1 COOMe), 6·38 (2 arom. H), 6·66 (multiplet of 4 arom. H centered here), 11·33 (3 bonded OH): δ(pyridine d_5) 2·42, 2·55, 2·59 (3 arom. Me, an additional signal at 2·47 was initially small but increased with time), 3·70, 3·85 (2 methoxyls, an additional signal at 3·77 was initially small but increased with time), 6·50 (broad), 6·64 (unresolved multiplet centered here), 6·88 (unresolved multiplet); molecular ion not observed, *m/e* 346, 182, 165, 150, 122.

Methyl gyrophorate. mp 293–297°d or 298–299·5°d respectively, ν_{max}^{KBr} 1674, 1662 (sh), 1612; δ(DMSO-d_6) 2·26 (1 arom. Me), 2·38 (2 arom. Me), 3·82 (1 COOMe), 6·24 (2 arom. H), 6·64, 6·68 (4 arom. H), 10·19 (3 bonded OH); δ(pyridine d_5) 2·42, 2·55, 2·62 (3 arom. Me, an additional signal at 2·36 was initially small but increased with time), 3·84 (1 COOMe, an additional methoxyl signal at 3·76 was initially small but increased with time), 6·58–6·94 (unresolved area of arom. H, including hydroxyls?); molecular ion not observed, *m/e* 332, 182, 151, 150, 122.

Methyl evernate (4-O-*methyl methyl lecanorate*). mp 142–143° (m mp with authentic methyl evernate underpressed); ν_{max}^{KBr} 1658, 1620; δ(DMSO-d_6) 2·25, 2·39 (2 arom. Me), 3·76 (1 OMe), 3·82 (1 COOMe), 6·37 (2 arom. H), 6·60 (2 arom. H), 10·27 (2 OH); δ(pyridine-d_5) 2·43, 2·59 (2 arom. Me), 3·70 (1 OMe), 3·84 (1 COOMe), 6·50 (1 OH?), 6·64 (doublet of 2 arom. H), 6·78 (doublet of 2 arom. H), 7·08 (1 OH?); *m/e* 346 (M+), 182, 165 (100%), 150, 122. Herbarium specimens of *P. aphthosa* examined: 1. Manitoba, Churchill, Warkworth, mile 498 of C.N.R. on hummocks in moist tundra, 14 Aug. 1950, J. W. Thomson 3394 (US). 2. Northwest Territories, Keller L., 64°N, 121°30'W, in damp areas of thick spruce forest, 15 July 1952, J. P. Kellsal L 16, det. Ahti (US). 3. British Columbia, Vancouver Island, Nanaimo River Valley, Rush Creek, on moss-covered rocks at 1500 ft elev., 18 May 1955, A. F. Szczawinski V.I.-556 (US).

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