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## SHORT COMMUNICATION

# CHEMICAL CONSTITUENTS OF LICHENS: APHTHOSIN, A HOMOLOGUE OF PELTIGERIN

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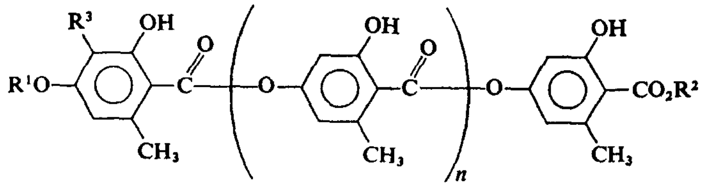
**Abstract**—Aphthosin, the first example of a tetradepside, has been isolated from *Peltigera aphthosa* (L.) Willd. Its structure was determined from spectroscopic data and hydrolysis to everninic acid, orsellinic acid and methyl orsellinate.

Until now, the largest depsides known have been the tridepsides, all of which are simple derivatives of gyrophoric acid (Ia), with the exception of hiasic acid (II) which contains an additional phenolic hydroxyl group.[1] We have now isolated what we believe to be the first example of a tetradepside which we have called aphthosin (III).



$n = 1$ (Ia) $R^1 = R^2 = R^3 = H$
(Ib) $R^1 = R^2 = CH_3, R^3 = H$
(II) $R^1 = R^2 = H, R^3 = OH$
$n = 2$ (III) $R^1 = R^2 = CH_3, R^3 = H$

Extraction of *Peltigera aphthosa* (L.) Willd. with hexane followed by benzene yielded a relatively insoluble high melting compound. This compound, aphthosin, was virtually insoluble in most simple organic solvents, but was sparingly soluble in pyridine, dimethylsulfoxide, nitrobenzene and anisole. All attempts to recover aphthosin from these latter solvents lead to extensive decomposition. As yet we have not been able to obtain aphthosin other than as a fine gray powder.

The i.r. spectrum of aphthosin (KBr) was virtually superimposable on that of tenuiorin (Ib),[2] which we have also isolated from the same source; however, the strikingly different solubilities ruled out the possibility that we had another form of this compound. It was obvious that we were dealing with a higher homologue of tenuiorin, but standard methods for estimating the molecular weight were negated by its insolubility.

Tenuiorin is quantitatively hydrolyzed in concentrated sulfuric acid to yield three

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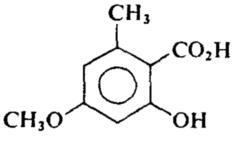
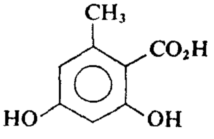
[1] C. F. Culberson, *Chemical and Botanical Guide to Lichen Products*, p. 120, The University of North Carolina Press, Chapel Hill (1969).

[2] S. Huneck and R. Tümmler, *Ann. Chem.* 128 (1965).

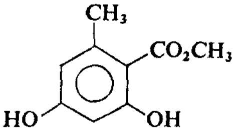
components, everninic acid (IV), orsellinic acid (V), and methyl orsellinate (VI).[2] A similar treatment of aphthosin yielded the same three products and no others. These were identified by TLC vs. standards in two solvent systems[3] (Table 1) and by isolation of the individual components according to Huneck.[2] Prolonged hydrolysis in both cases led to a decrease and eventually the total disappearance of methyl orsellinate with a corresponding increase in orsellinic acid.



(IV)



(V)



(VI)

TABLE 1

| Compound | $R_f$* |  |
|---|---|---|
|  | *A*† | *B*‡ |
| Orsellinic acid | 0·36 | 0·20 |
| Everninic acid | 0·53 | 0·63 |
| Methyl orsellinate | 0·62 | 0·46 |
| Tenuiorin hydrolysate | 0·37, 0·55, 0·61 | 0·22, 0·47, 0·64 |
| Aphthosin hydrolysate | 0·36, 0·54, 0·60 | 0·22, 0·46, 0·64 |

* Average of two runs on silica gel G; spots were developed with $I_2$ vapour.

† Benzene–dioxane–HOAc (90:25:4).

‡ Toluene–HOAc (17:3).

The number of orsellinic acid units in aphthosin was finally evaluated by quantitative NMR studies on the hydrolysis products. Although a weak NMR spectrum of aphthosin was available in dilute pyridine solution it was not possible to integrate this spectrum. A

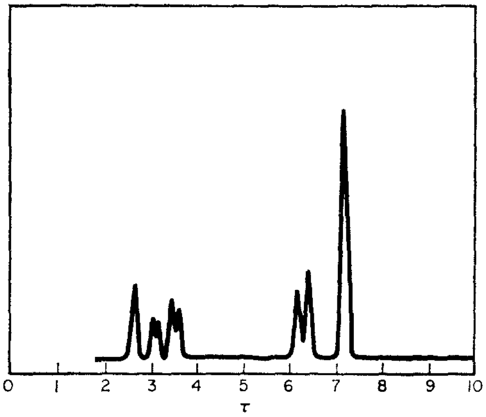


FIG. 1. NMR SPECTRUM OF SULFURIC ACID HYDROLYSATE OF APHTHOSIN IN PYRIDINE-$d_5$.

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[3] C. F. CULBERSON, personal communication.

short-time hydrolysis (10–15 min) was rapidly worked up by pouring into ice-water and extracting with ether. The ether was then removed and the residue dissolved in deuteropyridine for NMR analysis. A comparison of the total area under the peak for the aromatic methyls to the area under the methoxyl peaks (methyl ether plus methyl ester) gives us the number of orsellinic acid units in aphthosin (see Fig. 1). A similar analysis was run on tenuiorin for control. It was obvious in both cases that a small amount of hydrolysis of the methyl orsellinate had taken place by the decrease of the methoxyl peak at $\tau$ 6·19. When this was taken into account the number of aromatic methyl groups in aphthosin was found to be equivalent to four. Thus aphthosin is a tetramer of orsellinic acid.

It is interesting to speculate that other tetramers also exist and possibly even higher polymers, but because of their insolubility they have either been ignored or possibly not even extracted from the lichens themselves.

## EXPERIMENTAL

Analyses were performed by Mrs. S. Swaddle of the Department of Chemistry, University of Calgary, Canada. NMR spectra were recorded on a Varian Model A60 NMR spectrometer.

### *Extraction of Aphthosin from* Peltigera aphthosa

1500 g of thallus material from *Peltigera aphthosa* were extracted 24 hr in a Soxhlet extractor with hexane. Cooling of this extract led to the precipitation of *ca.* 10 g of tenuiorin. The extracted residue was then further extracted with benzene for 24 hr. On cooling the benzene extract a solid precipitated (*ca.* 7 g). This solid could not be redissolved in hot benzene, alcohol, acetone, acetic acid or $CHCl_3$. It was washed extensively with hot acetone, and the residue melted with decomposition at 300°. This residue was used for subsequent experiments. The i.r. spectrum (KBr) showed strong peaks at 3420 (broad, OH), 1670 (hydrogen bonded aromatic carbonyl), 3070, 1610, 1580 (aromatic) and 1250 $cm^{-1}$ (ester). The NMR spectrum (pyridine-$d_5$) showed peaks at $\tau$ 6·2, 6.25 ($OCH_3$); 7·19, 7·35, 7·52 (aromatic $CH_3$); all singlets. (Found: C, 62.77; H, 4.98. Calc. for $C_{34}H_{30}O_{13}$: C, 63·15; H, 4·64 %.)

### *Hydrolysis of Aphthosin*

Aphthosin (150 mg) was stirred at room temp. with 10 ml conc. $H_2SO_4$ until solution occurred (about 10–15 min). The solution was poured onto 50 g of crushed ice and immediately extracted three times with ether. The ether was dried over $MgSO_4$, removed *in vacuo*, and the residue taken up in pyridine-$d_5$ for NMR analysis. Pertinent peaks in the NMR appeared at $\tau$ 6·19, 6·29 ($OCH_3$) and 7·18 (aromatic $CH_3$) with a ratio of methoxyl to methyl of 1:2.

If the hydrolysis solution is allowed to stand for longer times the peak at $\tau$ 6·19 decreases and eventually vanishes due to hydrolysis of the methyl ester.

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