

## Studies on the Chemistry of Lichens

## 17\*. The Structure of Umbilicin

BENGT LINDBERG and BÖRJE WICKBERG

*Organisk-kemiska Institutionen och Träkemiska Institutionen, Kungl. Tekniska Högskolan, Stockholm, Sweden*

A kinetic study of the lead tetraacetate oxidation of the D-arabinitol- $\beta$ -D-galactofuranoside, umbilicin, and some model substances, and of their tritylation products, showed that the arabinitol residue was substituted in the 2- or 4-position. A choice between these alternatives could be made by comparing the electrophoretic mobilities of umbilicin and suitable model substances in germanate buffer and sulphonated phenyl boronic acid buffer; this showed that umbilicin is 2-O- $\beta$ -D-galactofuranosyl-D-arabinitol.

An arabinitol galactoside, umbilicin, was isolated from the lichen *Umbilicaria pustulata*<sup>1</sup> and was shown to occur in several other lichens of the order Gymnocarpene<sup>2</sup>. It was shown to be a D-arabinitol- $\beta$ -D-galactofuranoside<sup>3</sup>, the position of substitution in the D-arabinitol residue being undetermined. In the present paper studies directed at the determination of the full structure are reported.

When umbilicin is treated either with periodate or lead tetraacetate, a considerable "overoxidation" is observed, due to the intermediate formation of a malonaldehyde structure. The same effect is observed when ethyl- $\beta$ -D-galactofuranoside is oxidised with these agents<sup>3,4</sup>. This "overoxidation" was also severe when these furanosides were oxidised with lead tetraacetate in the presence of potassium acetate<sup>5</sup>, and neither in the consumption of oxidant nor the formation of formaldehyde *versus* time curves could any marked discontinuities be observed. However, when the oxidation was performed in chloroform-acetic acid-pyridine, umbilicin and ethyl- $\beta$ -D-galactofuranoside rapidly consumed about 5 and 2 moles of oxidant, respectively, and subsequently suffered a slower "overoxidation" (Fig. 1). Formic acid, under the same conditions, rapidly consumed one mole of oxidant. The very rapid glycol cleavage and the enhancement of the formic acid oxidation in the presence of pyridine have been observed previously (*cf.* Ref.<sup>6</sup>).

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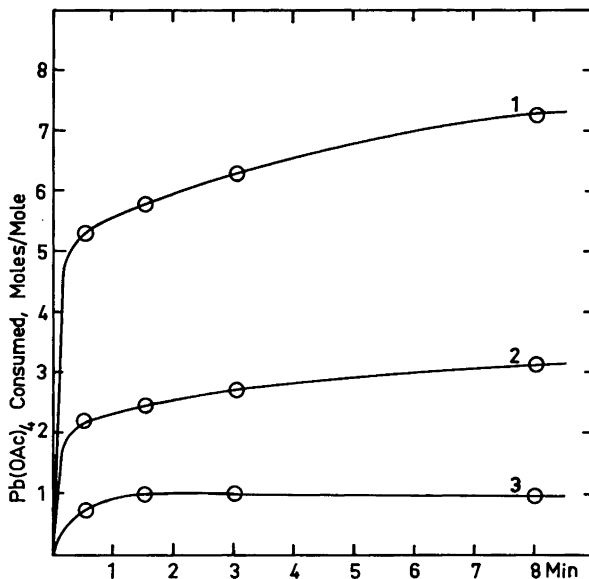


Fig. 1. Rates of consumption of lead tetraacetate by umbilicin (1), ethyl- $\beta$ -D-galactofuranoside (2) and formic acid (3).

Assuming that 5 moles of oxidant were used up for normal glycol cleavage of umbilicin and for oxidation of the formic acid formed, 2 of these should have been used up by the galactofuranose residue, leaving 3 moles for the oxidation of the arabinitol residue. This indicates a substitution of position 2 or 4 in the latter, with 2 moles of the oxidising agent being consumed by glycol cleavage and one by the formic acid formed. An arabinitol residue substituted in the 1- or 5-position would consume 5 moles of lead tetraacetate and an arabinitol residue substituted in the 3-position would consume only 2 moles.

Further support for this structure was obtained when D-arabinitol, umbilicin and ethyl- $\beta$ -D-galactofuranoside were treated with trityl chloride in pyridine and the reaction mixtures subjected to lead tetraacetate oxidation (Fig. 2). For arabinitol the consumption of oxidant rapidly approached the value of 3 moles, in agreement with the assumption that tritylation was restricted to the primary groups and was complete for these and that the ether groups were not hydrolysed during the oxidation. For the two galactofuranosides more than the theoretical amounts of oxidant was consumed but the curves showed marked discontinuities at about one mole for the ethyl- $\beta$ -D-galactofuranoside and 2 moles for umbilicin. Further, during the first 60 min. umbilicin consumed about one mole more lead tetraacetate than ethyl- $\beta$ -D-galactofuranoside. This difference is also in agreement with a substitution in position 2 or 4 in the arabinitol residue in umbilicin, since a substitution in position 1 or 5 would require a difference of 3 moles and, in position 3, no difference.

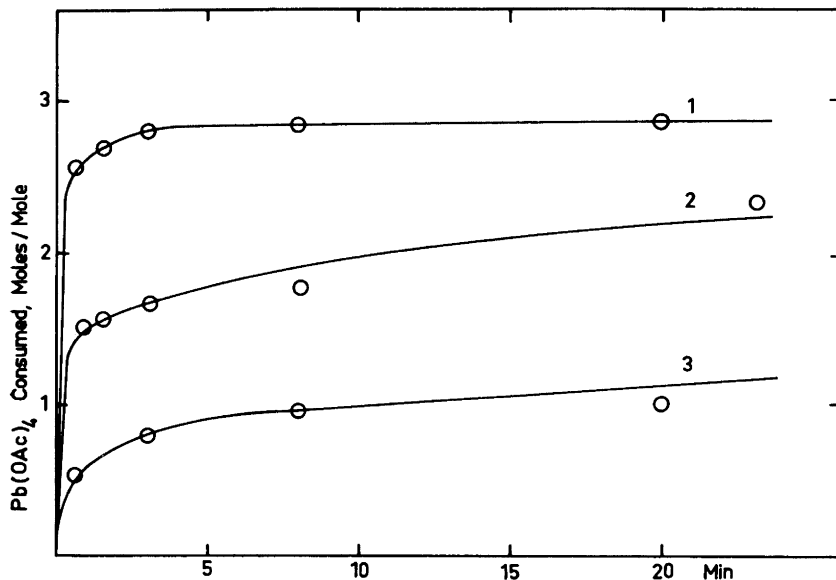
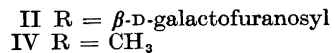
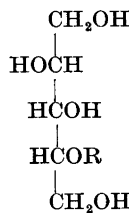
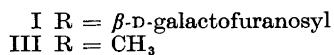
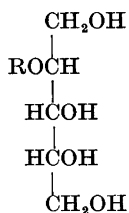


Fig. 2. Rates of consumption of lead tetraacetate by tritylated D-arabinitol (1), umbilicin (2) and ethyl- $\beta$ -D-galactofuranoside (3).

These results thus clearly indicate that umbilicin is either 2- or 4- $O$ - $\beta$ -D-galactofuranosyl-D-arabinitol (I or II) but do not distinguish between these alternatives. One of these (I) contains a *cis*- $\alpha$ -diol (*erythro*-configuration),



the other (II) a *trans*- $\alpha$ -diol grouping (*threo*-configuration). It is known that the latter has a much greater tendency than the former to form 5-membered rings, *e.g.* cyclic acetals and ketals<sup>7</sup> or borate complexes<sup>8</sup>. The contribution from non-cyclic  $\alpha$ -*trans*-diol structures to the electrophoretic mobility of carbohydrates is especially pronounced in germanate buffer at pH 10<sup>9</sup> and sulfonated phenyl boronic acid at pH 6.5<sup>10</sup>. The paper electrophoretic mobilities of umbilicin and some model substances in these buffers were therefore deter-

Table 1.  $M_A$ -values \* of umbilicin and some model substances.

Substance	$M_A$	
	In germanate buffer	In sulphonated phenylboronic acid buffer
Umbilicin	0.3	0.2
Ethyl- $\beta$ -D-galactofuranoside	0.1	0.1
D-Arabinitol	1	1
2-O-Methyl-D-arabinitol	0.2	0.1
4-O-Methyl-D-arabinitol	0.8	0.8

\*  $M_A$  = electrophoretic mobility, relative to that of arabinitol.

mined (Table 1). Ethyl- $\beta$ -D-galactofuranoside and 2-O-methyl-D-arabinitol \* (III) have low mobilities, but 4-O-methyl-D-arabinitol \* (IV) has a high mobility, close to that of D-arabinitol. Umbilicin has a low mobility, in agreement with the expected mobility of 2-O- $\beta$ -D-galactofuranosyl-D-arabinitol (I) but not with that of the 4-O-substituted isomer (II).

#### EXPERIMENTAL

*Oxidation experiments.* The oxidations were carried out at 0°. A 0.1 M solution (4 ml) of lead tetraacetate in chloroform containing 10 % v/v of acetic acid was added with stirring to a solution of the substance (0.025 mmole) in pyridine (1 ml). At intervals samples (0.5 ml) were withdrawn and immediately transferred to a flask containing sodium iodide (0.15 g) in acetic acid (1 ml) and saturated aqueous sodium acetate (2 ml). Liberated iodine was titrated with sodium thiosulphate (0.05 N) using starch indicator.

The trityl compounds were prepared by adding anhydrous calcium sulphate (50 mg) and trityl chloride (0.2 mmole) to a solution of the sample (0.05 mmole of D-arabinitol or ethyl- $\beta$ -D-galactofuranoside, 0.025 mmole of umbilicin) in pyridine (1 ml). After standing at 60° for 12 h and then being cooled to 0°, the reaction mixtures were oxidised as described above. Blanks were run parallel to these experiments and were found not to change significantly within 20 min. oxidation time.

*Paper electrophoresis* was performed in 0.05 M germanate buffer<sup>9</sup> at pH 10 and 40° and in 0.05 M sulphonated phenyl boronic acid buffer<sup>10</sup> at pH 6.5 and 40°. The spots were developed with the periodate-benzidine<sup>13</sup> reagent.

*2-O-Methyl-D-arabinitol.* 3-O-Methyl-D-glucose was oxidised with lead tetraacetate, the reaction mixture worked up as described by Charlson and Perlin<sup>11</sup> and the syrupy 2-O-methyl-D-arabinose reduced with Raney-nickel<sup>12</sup> in boiling 80 % aqueous ethanol. The resulting 2-O-methyl-D-arabinitol contained traces of impurities, as demonstrated by paper chromatography and paper electrophoreses, but was not further characterised.

*4-O-Methyl-D-arabinitol* was prepared in the same manner from 3-O-methyl-D-galactose. It also contained traces of impurities and was characterised by paper chromatography and paper electrophoresis only.

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\* These previously unknown compounds were prepared by lead tetraacetate oxidation of 3-O-methyl-D-glucose and 3-O-methyl-D-galactose, respectively, according to Charlson and Perlin<sup>11</sup>, followed by reduction of the resulting 2-O-methyl-D-arabinose and 2-O-methyl-D-lyxose with Raney-nickel<sup>12</sup>.

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