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## Chemical Studies on Lichens

### 5.\* Separation and Identification of the Antipodes of Usnic Acid by Thin Layer Chromatography

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A thin layer chromatographic method to investigate different lichens as to their content of (+) or (-) usnic acid has been worked out. It was not possible to resolve the acid chromatographically by using methanol containing brucine, the base earlier used for the resolution of the racemic acid.<sup>1</sup> The same negative result was also obtained when two solutions, each containing brucine and (+) or (-) usnic acid, were used. Each solution yielded one spot and the two spots had identical  $R_F$ -values. It was then found that a chromatographically resolvable brucine ( $\pm$ )-usnate was not formed just by mixing the two compounds. For the formation of such a salt or complex heating was necessary, a fact indicating that some kind of reaction must first take place between brucine and usnic acid. When the heat-treated solution was applied on the chromatographic plate and developed with methanol, two spots were

obtained. One spot travelled with the methanol front and was identified as brucine (-)-usnate. The other one had a lower  $R_F$ -value and was a mixture of brucine and brucine (+)-usnate. If the solvent front was allowed to travel more than 4 cm, the brucine (+)-usnate was slowly eluted from the brucine spot and the difference in the  $R_F$ -values of the usnates became too small for their identification. Evidently their chromatographical resolution is due to the fact that only brucine (+)-usnate is forming some kind of complex with brucine resulting in a decrease in its  $R_F$ -value. The nature of this complex is still obscure.

A useful reagent for usnic acid as well as for brucine-usnate is an aqueous solution of titanium trichloride. It forms a gray-green complex with usnic acid and develops a yellow-green colour on chromatographic plates. This reagent was also used to detect usnic acid in lichens with the "filter paper" method.<sup>2</sup>

*Experimental.* The thin layer chromatography was carried out on Eastman "Chromagram sheets" type K 301 R 2, cut down to a height of 6.7 cm. The plates were activated at 100° for 30 min and stored over silica gel. The spots were applied 1.0 cm above the lower edge and the solvent was allowed to travel to a height of 4 cm. (-)-Usnic acid was isolated from *Cladonia alpestris* and (+)-usnic acid from *Cladonia silvatica*.

The following solutions were used:

- (-)-Usnic acid in acetone (saturated) (A)
- (+)-Usnic acid in acetone (saturated) (B)
- Brucine in methanol (0.1 g/ml) (C)

Spots of brucine-usnate were detected either by their dark colour in UV-light (365 nm) or by the yellow-green colour produced by spraying the plates with an aqueous solution of titanium trichloride (10 %).

No resolution was obtained when A or B were chromatographed with C as solvent. Each solution yielded one spot and the two spots had identical  $R_F$ -values. The same result was obtained with either a mixture of A (0.1 ml) and C (0.2 ml) or of B (0.1 ml) and C (0.2 ml), using C or methanol as solvents. Simultaneously, the two mixtures (A + C and B + C) were warmed on the steam-bath until half of the solvent had evaporated. On cooling some of the brucine (+)-usnate was precipitated (from B + C) and the solution was filtered. No precipitate of brucine (-)-usnate was formed. When the solutions were chroma-

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tographed with methanol as solvent, brucine (-)-usnate travelled with the methanol front about 3 cm and brucine (+)-usnate together with brucine about 2 cm. These two solutions were then used as references.

A mixture of A (0.1 ml), B (0.1 ml), and C (0.4 ml) was warmed on the steam bath until half of the solvent had evaporated and some brucine (+)-usnate had been precipitated. The filtrate was chromatographed in methanol with brucine (+)-usnate and brucine (-)-usnate as markers. Two spots were obtained, one at the methanol front identified as brucine (-)-usnate and the other, with the lower  $R_F$ -value, as brucine (+)-usnate.

The "filter paper" method was performed according to Santesson.<sup>2</sup> The lichen specimen pressed down on a filter paper was treated dropwise with chloroform. Each drop of chloroform was allowed to evaporate leaving the extracted substances in a ring round the lichen fragment. When tested with an aqueous solution of titanium trichloride (8%), a green colour was formed if usnic acid was present.

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## A Rapid and Sensitive Method for the Measurement of Biological Oxidation of an Aromatic Hydrocarbon Catalyzed by Liver Microsomes

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In a previous communication<sup>1</sup> we have described the radiation-induced formation of polar oxidation products from

aromatic hydrocarbons used for the measurements of low doses of ionizing radiations. In these experiments an aqueous solution of tritium-labelled naphthalene was irradiated and the activity of the polar compounds formed was measured in a window-less proportional counter.

In higher animals naphthalene is mainly converted to dihydronaphthalene-1,2-diol and naphthols which are excreted as conjugates of glucuronic and sulphuric acid.<sup>2</sup> In view of the high degree of accuracy by which small amounts of radioactive compounds may be measured, it would be of interest if the "naphthalene-dosimeter" could be adapted and modified for biological use. Preliminary experiments with rat liver microsomes confirmed previous observations<sup>3,4</sup> that naphthalene in fact, is oxidized *in vitro* in the presence of oxygen and NADPH by this subcellular fraction and that the formation of radioactive oxidation products could readily be determined.<sup>5</sup> The experimental procedures used for extraction in the "naphthalene-dosimeter" were, however, disturbed by the presence of proteins, salts etc., resulting in high background values and lack of reproducibility. Incidentally, during the development of the "naphthalene-dosimeter" it was found that most of the activity vanished completely from the water phase when aqueous solutions of labelled naphthalene were stored in vials made of polyethene.<sup>6</sup> Polyethene was found to function as a very rapid extraction medium for aromatic hydrocarbons in water solutions. Based on these findings it has been possible to work out a simple and rapid method for the separation of the parent hydrocarbon from its oxidation products which, thus, could be utilized for the following convenient modification of the "naphthalene-dosimeter" for the estimation of hydroxylation activity in biological systems.

*Experimental.* Naphthalene-1-T\* (specific activity 17 C/mole, Radiochemical Centre, Amersham) was dissolved in distilled water at 20°C (magnetic stirring for 12 h) and stored at 5°C. Activities were measured in a liquid scintillation counter. As seen from Table 1, shaking for 20 min followed by transfer into a new vial gives satisfactory results. The naph-

\* Naphthalene-1-T is no longer produced by the Radiochemical Centre, Amersham, but <sup>14</sup>C labelled naphthalene or methyl-naphthalene available from several sources is equally suitable.