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BIOSYNTHESIS OF USNIC ACID IN LICHENS

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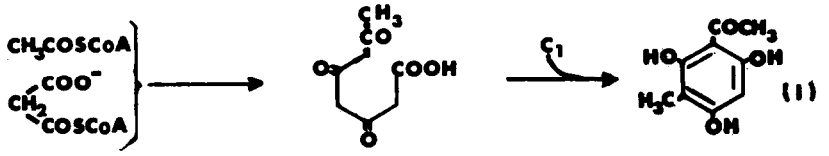
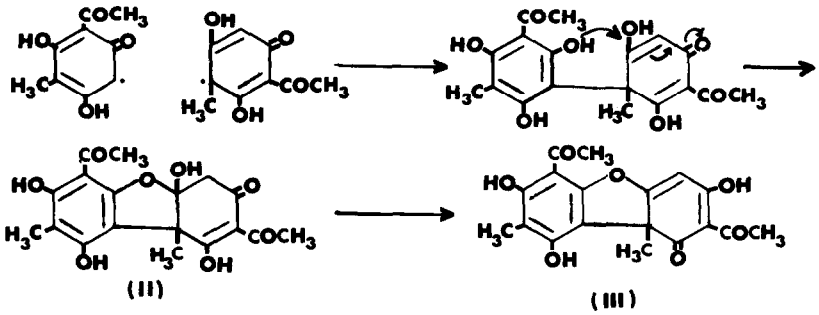
Usnic acid , a yellow colouring matter which is widely distributed in lichens, particularly in Usnea , Cladonia, Evernia and Parmelia , had long been investigated by several workers, and the structure (III) (1,2) was unequivocally confirmed by the elegant synthesis achieved by Barton et al. (3).

The present paper deals with the biosynthesis of usnic acid in living lichens. The biogenesis of usnic acid by the condensation of 2 molecules of methylphloroacetophenone (I) had originally been suggested by Schöpf and Ross (1), and was later demonstrated chemically by Barton et al. (3) in their synthesis of (±) usnic acid.

The methylphloroacetophenone skeleton would biogenetically be derived by the acetate-malonate condensation accompanying C_1-fragment incorporation.





(II) (III)

The lichens which have been employed in the present study are Usnea longissima Ach., U. diffracta Wain., Evernia mesomorpha Müll. Arg., Parmelia caperata (L.) Ach., Cladonia mitis Sandst., and Cl. alpestris (L.)Rabh. The isotopically labelled precursors, sodium acetate-1^{14}C, -2^{14}C, sodium formate-^{14}C, diethyl malonate-2^{14}C, phloroacetophenone(CO^{14}CH$_3$), phloroacetophenone-T, methylphloroacetophenone(CO^{14}CH$_3$), methylphloroacetophenone-T, were administered respectively by the following methods to the fresh lichens immediately after collection in the field:

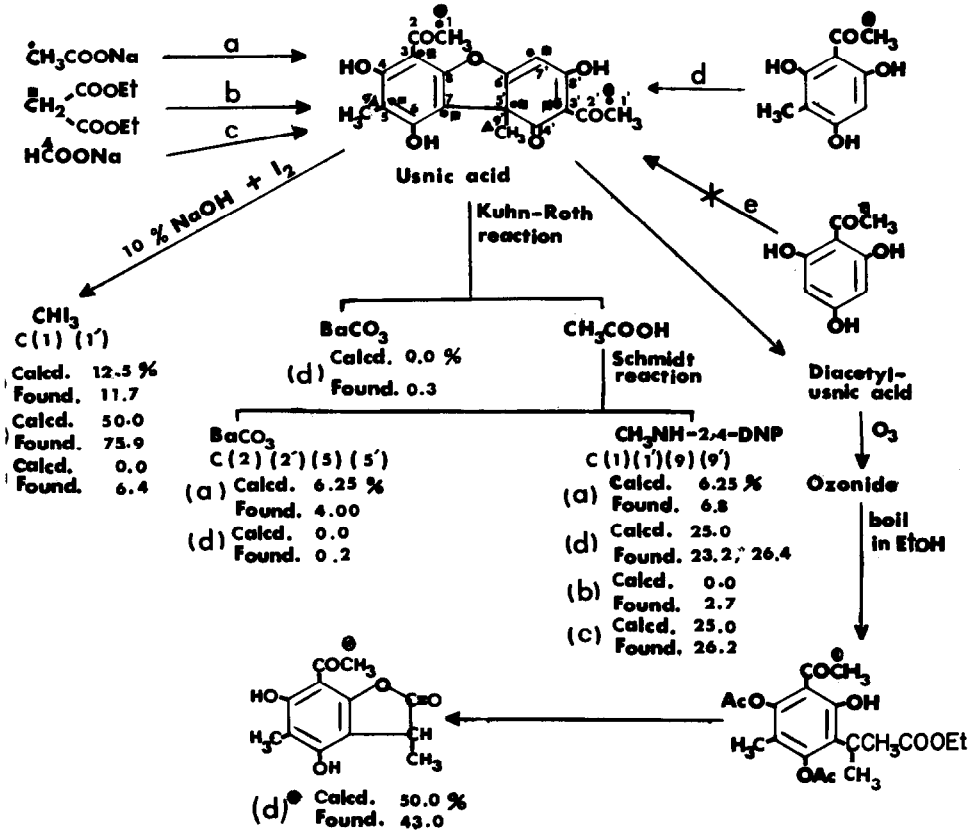
i) Incubation by shaking the fresh lichens in sterilized Czapek-Dox medium added with the solution of labelled precursors using 500 ml. flask, at 25° for 3-4 days under the illumination of an ordinary electric lamp (200 w / 100 volt) ii) Immersion of the labelled precursors by dropping the solution in 10 % aq.ethanol on the surface of the fresh part of lichen thalli.

After certain metabolic period, the lichen was extracted with benzene to isolate usnic acid, which was recrystallized from the same solvent. The radioactivities were measured by the Packard Tricarb scintillation counter.

The incorporation ratios of ^{14}C in usnic acid in 24 experiments

were 0.001-1.11% depending on the lichen species and the experimental conditions. The labelled usnic acid isolated was degraded with sodium hypoiodite, by the Kuhn-Roth oxidation followed by the Schmidt reaction, or by the ozonisation of the acetate.

The degradation reactions of the labelled usnic acid and the incorporations of ^{14}C in the fragments are illustrated in the following chart:



The figures in the chart indicate the calculated and observed activities of the carbon fragments on the basis of the specific activity(per mmole) of usnic acid as 100%)

The tritium labelled methylphloroacetophenone and phloroacetophenone were prepared by the Wilzbach method, recrystallized twice from aqueous methanol. The radioactive solution was administered to Cladonia mitis and Parmelia caperata by shaking in the Czapek-Dox medium. Tritium was incorporated efficiently in usnic acid (0.022-0.018 %) from tritium labelled methylphloroacetophenone, whereas it was not introduced from tritium labelled phloroacetophenone. The corresponding results were obtained when methylphloroacetophenone($CO^{14}CH_3$) (0.07-0.28 %) and phloroacetophenone($CO^{14}CH_3$) (0 %) were administered respectively to the lichens both by shaking and immersion methods.

Accordingly, it has been proved that usnic acid is biosynthesized in lichens by the oxidative coupling of two methylphloroacetophenone moieties whose methyl group is introduced into the molecule prior to the completion of aromatic ring closure.

As for the introduction of methyl group, this result is in accord with the findings in the C_1-fragment incorporation in the course of biosynthesis of atranorin (4).

The enzymatic studies of usnic acid biosynthesis are now in progress.

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