



Fig. 1. A. Hydrocarbon from *Cladonia deformis*. B. Taraxerene.

taraxerene and a mixture of the two all melted at 238–239°, thereby confirming the identity. A further confirmation of identity was obtained by X-ray powder diagrams of the two substances (Fig. 1) and Weissenberg diagrams. The measurements were very kindly carried out by Mr. O. Borgen (Theoretical Chemistry Department).

It is a curious fact of experience that when proceeding from monoterpenoids through triterpenoids hydrocarbons become progressively more rare, and as far as this author is aware no cyclic triterpene has hitherto been reported to occur in nature*. The only indication that such a one might be found in lichens are reports by Hesse⁵ and Zopf⁶ that *Nephroma arcticum* (L.) Forss., *N. lusitanicum* Schaer. and *N. laevigatum* Ach. contain nephrine, m. p. 168°, which Hesse⁵ considered to be a diterpene crystallising with one molecule of water. If this may be construed as evidence for the presence of a terpene in lichens, the reported m. p. would probably indicate a triterpene rather than a diterpene. When considering this problem, it should be taken into account that it may be questionable whether the analytical technique at that time permitted any greater accuracy on the scale that Hesse had to work (ca. 40 mg) when analysing his nephrine. In the writer's opinion it seems more probable that an oxygenated compound may have been present.

So far 5 triterpenoids are known to occur in lichens: ursolic acid⁷, zeorin⁸, leucotylin⁹, friedelin and epifriedelinol² and now taraxerene. Of these only ursolic acid and taraxerene have fairly well established constitutions, the former being related to α -amyrin¹⁰, the latter a stereoisomer of germanicene, with the C/D ring junction *cis* instead of *trans*⁴, i. e. it belongs in the β -amyrin series.

* The triterpene squalene is of the alifatic type.

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The Biosynthesis of Riboflavin in *Eremothecium ashbyii*

LEIV KLUNGSOYR

Biochemical Laboratory, University Clinic
of Bergen, Norway

The hypothesis of MacLaren¹ that a substance with purine structure is utilized for the synthesis of riboflavin by *E. ashbyii*, is supported by recent work by Goodwin and Pendlington² and by Klungsöyr³.

Previous studies have shown that the carboxyl group of acetic acid is incorporated into the position 2 of the isoxaloxazine part of the riboflavin molecule². This observation was taken as indirect evidence of the utilization of a purine intermediate

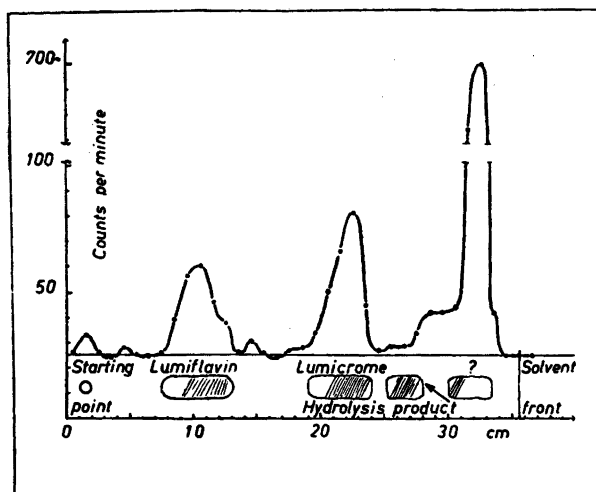


Fig. 1. C^{14} distribution curve and spots visible in ultraviolet light. Counted along the intact paper. The spots represent degradation products of riboflavin, formed by *E. ashbyii* incubated in a phosphate buffer containing C^{14} formate.

in riboflavin synthesis. The conclusion was based on the assumption that a formyl-compound may be formed from the carboxyl group of acetic acid in *E. ashbyii*⁴. If this assumption is valid C^{14} formic acid should label the riboflavin molecule in the same position as does $1-C^{14}$ acetic acid. Experiments were therefore made to study the incorporation of formic acid into riboflavin.

Eremothecium ashbyii was collected from a liquid medium, washed, and resuspended in phosphate buffer. To 20 ml suspension 40 microCurie C^{14} sodium formate was added, and the suspension was shaken at 28° C for 48 hours. At the end of the incubation period the riboflavin was degraded, and the degradation products were isolated by extraction with chloroform and separated by paper chromatography. The positions of the spots were marked off in ultraviolet light, and the C^{14} distribution curve was determined by counting along the paper, one cm at a time. (The details of the technique have been described previously³). The results are presented in Fig. 1.

Fig. 1 shows that some C^{14} activity is present in lumiflavin and in lumicrome, but none in the hydrolysis product of

lumiflavin. Positions 1(N), 2(C) and 3(N) are split off from lumiflavin by hydrolysis, and C^{14} from formic acid is therefore present in position 2 in lumiflavin.

The findings that C^{14} from $1-C^{14}$ acetic acid and from C^{14} formic acid are both incorporated into position 2 of riboflavin show that the carboxyl group of acetic acid may be converted into a formyl-compound by *E. ashbyii*, in accordance with the scheme suggested by Krebs *et al.*⁴

The C^{14} sodium-formate was purchased from The Radiochemical Centre, Amersham, England, and the $1-C^{14}$ acetic acid from Nyegaard & Co. A/S, Oslo. (The acetic acid was prepared by the reaction between carbon dioxide and methyl-magnesium-bromide and should be free from formic acid.)

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