On The Biosynthesis Of Lichen Substances
Part 2. The Pulvic Acid Derivative Vulpinic Acid

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Lichen substances belonging to the pulvic acid group are the coloring matter of peculiar constitution containing conjugated double bonds. Their occurence is restricted to lichens where they are widely distributed and conspicuous. The structures of eight such compounds have been established (Shibata, 1963). Several speculative schemes have been suggested for the biosynthetic mechanism leading to the formation of this class of pigments, however, none has been confirmed experimentally. The single procedure for the in vivo study of the biosynthesis of lichen metabolites used in elucidating the biogenesis of the depside gyrophoric acid (Mosbach, 1964) has provided a practical opportunity to test these theories. There are essentially two major pathways which can lead to the formation of a pulvic acid derivative such as vulpinic acid (II): (a.) a direct condensation of two branched C_6C_3 units such as 2-C-phenylglyceraldehyde (Mittal, Seshadri, 1957), (b.) oxidative ringopening of a terphenylquinone such as polyporic acid (I) (Asahina, Shibata, 1954, Thomson, 1957). To the author, the latter alternative seemed more attractive because of the observed joint occurence of polyporic acid together with pulvic acid derivatives in some Sticta lichens (Murray, 1952) as well as the ready conversion of a terphenylquinone into pulvic acid dilactone by oxidation (Frank, Clark, Coker, 1950). Recent biogenetic studies on the terphenylquinone volucrisporin (2, 5 - (m-hydroxyphenyl) - 1, 4 - benzoquinone) suggest condensation of two molecules of an unbranched phenylpropanoid intermediate leading to the formation of this pigment (Read, Vining, Haskins, 1962). An efficient utilization of C14-labelled phenylalanine and phenyllactic acid was observed in these experiments.

In the tracer studies to be described on the lichen metabolite vulpinic acid, more than 5 % of the administered radio-

activity from C14-carboxyl labelled DL-phenylalanine was found to be incorporated into this product. In the course of degradation (Figure 2), the total radioactivity of vulpinic acid was determined. Alkaline hydrolysis yielded oxalic as well as phenylacetic acid. By a Schmidt degradation of the phenylacetate, the radioactivity of the carboxyl group could be determined.

The obtained values of the radioactivities listed in Table 1 established that 50 % of the total radioactivity of vulpinic acid was located in the oxalic acid formed in the degradation. The remaining 50 % of radioactivity was found to be present in the carboxyl groups of phenylacetic acid (2 moles of phenylacetic acid from 1 mole of vulpinic acid). In order to determine the radioactivity of a specific carbon atom from one of the four labelled positions, the vulpinic acid was reduced to methyl dihydrocornicularate (III) with concomitant liberation of carbon dioxide. The methyl dihydrocornicularate was subsequently oxidized to carbon dioxide. It was found that 75 % of the total labelling remained in the reduction product, whereas the evolved carbon dioxide contained 25 %. These results suggest that the four labelled carbon atoms carry equal radioactivities.

Table I

DL-3-Phenyl(alanine-1-C14) derived vulpinic acid

compound	cpm/mmole x 10^{-4}
vulpinic acid	129.2
phenylacetic acid	32.5
carboxyl group der. from phenylacetic acid	32.8
barium oxalate	63.8
oxalic acid	64.0
methyl dihydrocornicularate	96.3
carbon dioxide obtained upon reduction of vulpinic acid	33.0

As to the two alternative schemes of the biosynthesis of vulpinic acid outlined in Figure 1, the suggested formation from two C_6C_3 branched units (alt. B) appears less likely in light of the

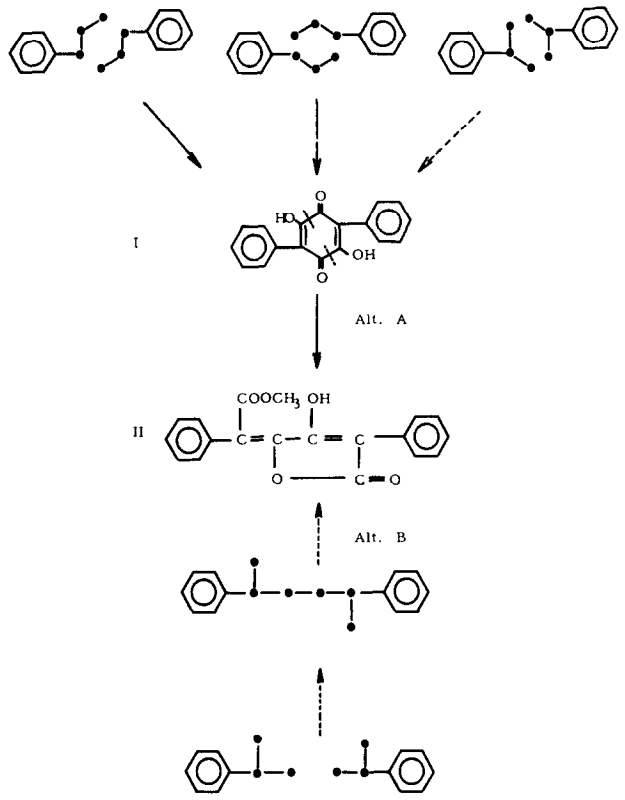

Fig. 1

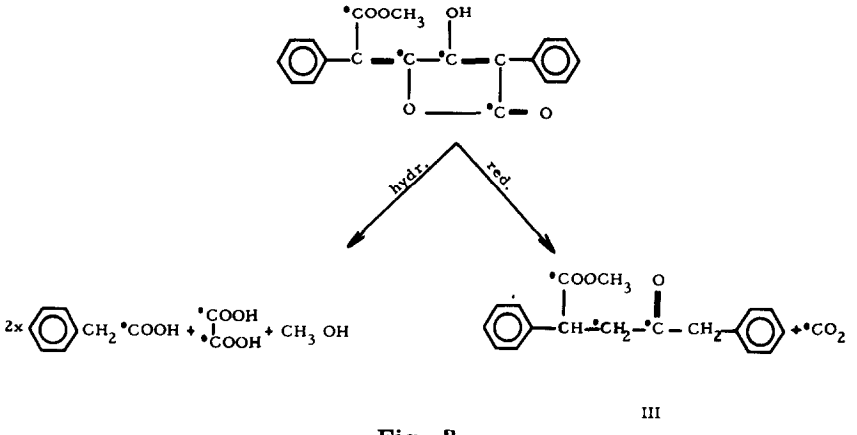

Fig. 2

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results obtained. The labelling pattern found necessiates condensation of two symmetrical C_6C_3 branched chain units. The administered unbranched phenylpropanoid compound would thus have to undergo rearrangement of the chain leading to a forked C_6C_3

unit prior to condensation. It is known from the biosynthesis of tropic acid (Louden, Leete, 1962) that such a biosynthetic mechanism can possibly take place. However, in the case of vulpinic acid, the hypothetical branched C_6C_3 unit would have to undergo further transformations leading to a symmetrical intermediate permitting randomization of the radioactivity. A compound satisfying these conditions would be phenylmalondialdehyde which however is not known to occur in nature. On the other hand, oxidative ringopening of polyporic acid or any related terphenylquinone (alt. A) would directly lead to the formation of the basic structure of vulpinic acid. Subsequent lactonization and methylation would then give rise to the vulpinic acid.

Experimental.

Culture conditions: 2.5 g of freshly collected lichen thallus of Evernia vulpina were transferred to a 250 ml Erlenmayer flask containing 50 ml of sterilized Czapek-Dox medium. After addition of 50 μC of DL-3-phenyl(alanine-1-C14) with a specific activity of 20 mC/mM the lichen was placed on a shaker (250 rpm, 1 inch stroke) at 27°. The incubation proceeded in a illuminated room for 5 days.

Isolation: The lichen thallus was filtered off, washed several times with distilled water and air-dried. After extraction with warm chloroform the solvent was evaporated leaving 110 mg of a yellow residue. Chromatography on Whatman 1 paper of a representative sample of the isolated material was carried out in the following system (Wachtmeister, 1955):n-butanol:ethanol:H_2O, 5:1:4. Scanning of the paper in a strip-counter showed one major radioactive peak: R_f 0.65 = vulpinic acid. After addition of 800 mg of unlabelled vulpinic acid as a carrier, the vulpinic acid was recrystallized several times from ethanol, yield 600 mg, m.p. 148°.

Degradation: 5 mg of the radioactive vulpinic acid were submitted to wetcombustion by the van Slyke-Folch method and the resulting carbon dioxide trapped as barium carbonate. In the course of the degradation the total radioactivities of the following compounds were determined: barium oxalate, oxalic acid, phenylacetic acid and methyl dihydrocorniculate. 500 mg of vulpinic acid were hydrolysed with 10 g of barium hydroxide in 25 ml of water for 2 hours (Koller, Pfeiffer, 1933). The barium oxalate formed on hydrolysis was filtered off from the hot solution and washed

with dilute acetic acid prior to isolation, yield 293 mg. 250 mg of the oxalate were suspended in water and upon acidification with hydrochloric acid extracted with ether. After evaporation of the solvent the formed oxalic acid was sublimed, yield 85 mg, m.p.101°. The filtrate obtained from the hydrolysis with baryta was acidified and extracted with ether. After evaporation of the ether the remaining residue was dissolved in a minimum amount of water and filtered. Upon addition of conc. hydrochloric acid to the filtrate, crystalline phenylacetic acid precipitated out, yield 119 mg, m.p. 77°. 100 mg of the acid were degraded by a Schmidt reaction and the carboxyl group isolated as barium carbonate. Reduction of 250 mg of vulpinic acid with 250 mg of zinc powder in 3 ml of glacial acetic acid yielded 25 mg of the methylester of dihydrocornicularic acid, m.p. 67° (Asano, Kameda, 1935). After completed reaction the carbon dioxide evolved during the course of reduction was flushed through the closed system with nitrogen and collected for radioactive analysis.

Radioactive analysis: In the degradation series measurements were performed in a liquid scintillation counter with the samples as barium carbonate suspended in a gel of Aerosil in a toluene solution of 2,5-diphenyloxazol.

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References

Asahina, Y. and Shibata, S. "Chemistry of Lichen Substances", 206 (1954).
Asano, M. and Kameda, Y. Ber. 68, 1567 (1935).
Frank, R. L., Clark, G. R. and Coker, J. N. J. Am. Chem. Soc. 72, 1824 (1950).
Koller, G. and Pfeiffer, G. Monatsh 62, 160 (1933).
Louden, M. L. and Leete, E. J. Am. Chem. Soc. 84, 4507 (1962).
Mittal, O. P. and Seshadri, T. R., Current Sci. 26, 4 (1957).
Mosbach, K. Acta Chem. Scand. 18, 329 (1964).
Murray, J. J. Chem. Soc., 1345 (1952).
Read, G., Vining, L. C. and Haskins, R. H. Can. J. Chem. 40, 2357 (1962).
Shibata, S. "Modern Methods of Plant Analysis", 6, 154 (1963).
Thomson, R. H. "Naturally Occurring Quinones Butterworths Scientific Publications, London", 7 (1957).
Wachtmeister, C. A. "Papierchromatographie in der Botanik Springer Verlag", 7 (1955).