The Biosynthesis of (+)-Protolichesterinic Acid

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WE report the first biosynthetical work on the aliphatic lichen acids. Protolichesterinic acid (I), obtained from *Cetraria islandica*[1] and *Cladonia papillaria*,[2] is of interest for its antibiotic properties[3] as well as its structural relationships to the fatty-acid cycle and the tricarboxylic acid cycle.[4] Our studies indicate that (I) represents a very minor metabolic pathway of *C. islandica* and is not produced by the isolated mycobionts of either lichen in submerged culture. Although sixteen of the carbon atoms in (I) are derived from straightforward head-to-tail linkage of acetate units, our experiments indicate that C-1, C-2, and C-5 of (I) are not derived from oxalacetate as was previously postulated[4] but probably from a C_3-unit.

The degradation of (I) to (IIa), and (IIa) to (III) has been previously described.[5,6] The keto-acid (III) was decarboxylated by copper chromite in refluxing quinoline to yield carbon dioxide, collected as barium carbonate, and a mixture of saturated and unsaturated ketones which was not separable by usual t.l.c. and v.p.c. methods, but which could be converted into (IV) by hydrogenation using palladium catalyst. N.m.r. studies indicated that the mixture consisted of (IV), (Va), and (Vb). The ketone (IV) was then readily purified by v.p.c.

A second series of degradations on (IIa) involved oxidation with potassium permanganate under carefully controlled conditions, whereby a mixture of acetic acid and homologous fatty acids (consisting chiefly of myristic, tridecanoic, and dodecanoic acids) was obtained, as well as a small amount of (III). The mixture of homologous fatty acids was separated by v.p.c. of the corresponding methyl esters.

A large number of feeding experiments were done on *C. islandica*, *Cl. papillaria*, and their isolated mycobionts.† In no case was it possible to obtain incorporation of radioactive sodium-[1-^{14}C]acetate under normal conditions of growth, either for the whole lichen or for the isolated mycobiont. Since (I) occurs only in small amount (*ca.* 0·1%) in the whole lichen in its natural state, it seems reasonably certain that (I) represents only a very minor metabolic route from acetate, at least under the conditions used, including feeding experiments on dry lichen (using very small amounts of feeding solution), agar slants (with direct injection of labelled acetate into the body of the lichen fungus), stationary cultures, and shake cultures.

We have spent considerable effort in attempts to determine if isolated *C. islandica* and *Cl. papillaria* mycobionts can produce (I); however, all attempts have failed in submerged culture, and the high density of mycobiont cells has thus far ruled out ordinary stationary culture techniques. [Detection of (I) as (IIb) by v.p.c. techniques was sensitive to as little as 1—2 μg. of material.] Studies with partially submerged and slant cultures are still under investigation, as aeration seems to be a key factor in production of (I).

Glucose solution and sodium [1-^{14}C]acetate were hydroponically administered to whole *C. islandica* lichen‡ for one week, and (I) was extracted into the ether-soluble acidic fraction followed by t.l.c. at all stages with scintillation-counting of all t.l.c. fractions. After the first t.l.c. on silica gel plates using benzene–methanol–acetic acid,[7] the radioactivity and weight profiles of the plates were

† Provided by Vernon Ahmadjian, Clark University, Worcester, Mass.
‡ Provided by Dr. Johan Santesson, University of Uppsala, Sweden.

essentially superimposable. As a cross-check a sample of (IIb) was brought to constant activity by multiple v.p.c. The incorporation of activity was in the range 0·01—0·08% for a number of experiments.

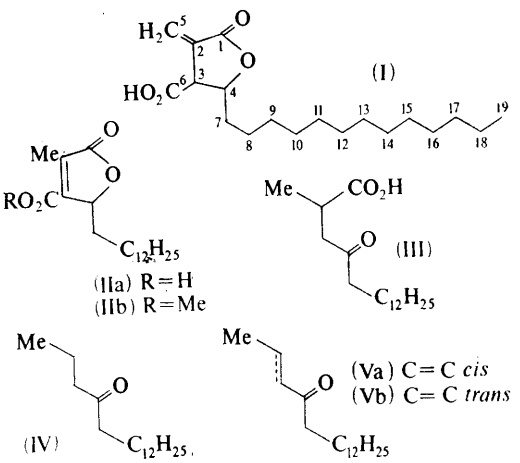
Results of our partial degradations to date on sodium [1-^{14}C]acetate derived (I) are summarized in the Table [which refers to formula (I)].

TABLE

Carbon atoms	Estimated as	% of Total radioactivity
C-1	CO_2	2·8
C-2	CO_2	1·8
C-5	CO_2	1·6
C-6	CO_2	11·7
C-4, C-7—19	$C_{13}H_{27}CO_2Me$	80·2
C-7—19	$C_{12}H_{25}CO_2Me$	66·6
C-8—19	$C_{11}H_{23}CO_2Me$	66·6
C-1—5, C-7—19	(III)	84·9
C-2, C-5	CH_3CO_2Na	3·6
C-2—5, C-7—19	(IV)	80·5
C-1—19	(IIa)	100·0

The results are consistent with the formation of (I) by the condensation of a fatty acid derivative corresponding to C-6,3,4,7—19 with a C_3 or C_4 fragment derived from an alternative pathway of acetate metabolism. The equality of incorporation of radioactivity into C-2 and C-5 originally suggested a symmetrical intermediate such as succinate, which could condense by conversion to oxalacetate followed by loss of one carbon; however, the relatively high degree of incorporation into C-2 and C-5 would not be expected from [1-^{14}C]acetate as this would in effect involve the conversion of [1-^{14}C]acetate into [2,3-$^{14}C_2$]succinate, a biologically unlikely process unless reactions other than the tricarboxylic acid cycle are playing a significant role in the metabolism. Biosynthetic studies on carolic[8] and glauconic[9] acids with [1-^{14}C]acetate showed no evidence for such conversion.

To investigate the possibility of a C_4 fragment giving rise to C-1, C-2, and C-5 of (I) we have carried out a feeding experiment under identical conditions to the acetate work above but with [1,4-$^{14}C_2$]succinic acid as tracer. One would expect [1,4-^{14}C]succinic acid to be converted into [1,4-$^{14}C_2$]oxalacetic acid *via* the tricarboxylic acid cycle; furthermore, if results were comparable to those for carolic acid biosynthesis,[8] where we have a C_4 fragment plus a polyacetate chain, one should observe essentially no breakdown of succinic acid to acetate units. In our experiment, (I) obtained from [1,4-$^{14}C_2$]succinic acid was almost inactive. This suggests that (I) may not be formed from oxalacetate as was previously thought, but arises instead from pyruvate or one of its C_3 precursors from the glycolytic (Embden–Meyerhof) pathway. The role of these C_3 precursors is currently under investigation.



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