Microbial Degradation of Methyl Dehydroabietate

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RECENT reports on degradation of dehydroabietic acid by bacteria[1] (*Flavobacterium resinovorus*) and of methyl dehydroabietate by fungi[2] (*Corticium sasakii*) show oxidation at C-3 to form the ketone or β-alcohol, respectively. In the case of the free acid, decarboxylation was observed in addition to oxidation and the question of whether this was a spontaneous or an enzymic process was raised.

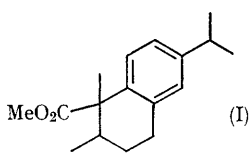
We have been studying the degradation of methyl dehydroabietate by *Arthrobacter sp.* isolated from lodgepole pine. Several acidic degradation products which have been isolated and characterized as their methyl esters may be of interest in the elucidation of the metabolic pathway or pathways involved.

The method employed for the growth and isolation is as follows: a mixture of sterile salt medium and methyl dehydroabietate was inoculated with bacteria and vigorously aerated at 24—26° for 15—20 days. Cells were removed by centrifugation and the supernatant liquid was basified and extracted with methylene chloride to remove neutral material (mainly methyl dehydroabietate). Acidification, extraction with methylene chloride, and removal of solvent yielded a mixture of acids (*ca.* 2% yield). The mixture was treated with diazomethane and the resulting esters were separated by g.l.c. (20% SE 30, 290°). Three components were collected as single peaks and at least eight other components were detected.

One of the acids obtained (characterized as the methyl ester) was dehydroabietic acid. The need for bacteria for the hydrolysis of starting material was demonstrated in a separate experiment in which no bacteria were present; all other conditions of the degradation and isolation procedure were reproduced. Although it is possible that hydrolysis of the ester is the first step of the main path, growth of the bacteria on dehydroabietic acid itself did not proceed at a rate comparable to that observed with the methyl ester.

The same method also yielded methyl 3-oxodehydroabietate. Its structure was formulated from detailed analysis of the mass spectral fragmentation pattern and on the basis of i.r. Confirmation was afforded by comparison of its i.r. spectrum with that of an authentic sample.[2] Although a few differences in peak intensities were noted, the two spectra were virtually identical. The occurrence of 3-oxodehydroabietic acid as a degradation product is of interest because it could be an intermediate between methyl 3-β-hydroxydehydroabietate isolated by Brannon, *et al.*[2] and the decarboxylated 3-oxo-derivative of dehydroabietic acid isolated by Biellmann, *et al.*[1] Also, it would appear that the decarboxylation which Biellmann encountered was probably enzymic since the keto-acid survived our similar isolation procedure.

A third acid obtained had been further degraded, as the corresponding methyl ester had *M* 260 (mass spectrum). The i.r. spectrum indicates that both the aromatic ring (6·21 μ, 6·67 μ, and 6·89 μ) and the isopropyl group (7·30 μ) are intact and a non-conjugated carbonyl group is present (5·73 μ). The fragmentation pattern is consistent with the presence of ring B, the base peak occurring at 159 and suggesting a (dimethylnaphthalene—H) fragment. Further, the presence of fragments at $M - 27$, $M - 41$, and $M - 55$ indicate that the structural unit $\cdot CH{-}CH_2{-}CH_2{-}CH_2$ or $CH_2{-}CH{-}CH_2{-}CH_2$ is present. We therefore assign structure (I) to this product.



(I)

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[1] J. F. Biellmann, R. Wennig, P. Daste, and M. Raynaud, *Chem. Comm.*, 1968, 168.
[2] D. R. Brannon, H. Boaz, J. Mabe, and D. Horton, *Chem. Comm.*, 1968, 681.