Microbial Degradation of Diterpenic Acids

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ONE of us has isolated from the soil of a *Pinus maritima* forest a new strain of micro-organism (*Flavobacterium resinovorum*) which is able to use the nonvolatile portion of oleoresin from pine trees and other origins as the sole source of carbon.[1] This kind of micro-organism may be one of the agents responsible for biodegradation of natural products.

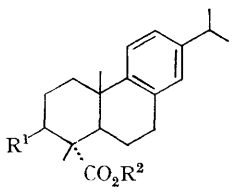
In a previous paper we showed that acids with an abietane skeleton and with unsaturation in the c ring could be metabolized. Moreover, it appeared that an acid function at C-4 is not a requirement for degradation.[2] Here we describe the structure of a metabolite obtained from dehydroabietic acid (I). We have chosen this acid because of its relatively high stability towards oxygen.

The medium was prepared as follows: the substrate was dissolved in ethanol and sterilized by filtration, then added to an inorganic solution[3] sterilized by heating in an autoclave. After inoculation with *Flavobacterium resinovorum*, incubation was carried out on a reciprocating shaker at 30° for 20 to 40 hr. Then the organic material was extracted with ether and chloroform, the solvent removed, and the residue then treated with diazomethane. Besides recovering the starting material, traces of other products were found. For example, by successive chromatography over silica gel, one degradation product was obtained pure, but in low yield [0·06% to the consumed (I)].

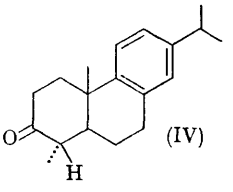
Its structure was formulated as (IV) on the basis of spectroscopic arguments.† The synthesis of (IV) was accomplished by treating (I) with lead tetra-acetate according to Huffman's procedure.[4] We did not obtain as reported the olefin (Va) in high yield, but instead a mixture of three olefins, (Va) (40%), (Vb) 20%, and (Vc) 20%, which could be separated by chromatography on silica gel impregnated by silver nitrate. The hydroboration of olefin (Vb) and oxidation gave a

† To be published in the complete paper.

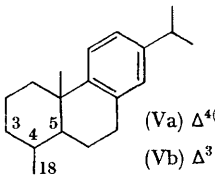
mixture of two alcohols. The major was oxidized by sodium hydrogen chromate in acetic acid to the epimer in C-4 of ketone (IV). Treatment with sodium hydroxide in ethanol gave the product (IV). The metabolite is identical with the ketone (IV) by comparison of physical properties (i.r., n.m.r., circular dichroism).



(I) $R^1=R^2=H$
(II) $R^1=H$, $R^2=Me$
(III) $R^1=OH(\alpha \text{ or } \beta)$, $R^2=H$



(IV)



(Va) $\Delta^{4(18)}$
(Vb) Δ^3
(Vc) Δ^4

It is highly probable that the biodegradation of (I) to (IV) takes the following pathway: enzymic hydroxylation at C-3 to form the alcohol (III) (α or β at C-3), followed by its oxidation to the ketone which then undergoes decarboxylation either spontaneously or enzymically. No compounds corresponding to (III) were isolated.

Hydroxylation at C-3 in (I) is somewhat surprising. Biodegradation was expected to take place on the aromatic ring or at least on the benzylic position, as usually the resulting metabolites no longer contain the aromatic ring. It may well be that (IV) is not on the main path. Structural elucidation of the other metabolites formed is in progress.

The present results resemble the hydroxylation of lanostadien to lanosterol by yeast,[5] and of androst-5-en-7-one to 3-hydroxyandrost-5-en-7-one.[6]

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