DIRECT EVIDENCE FOR BIOSYNTHETIC RELATIONSHIPS AMONG HYDROCARBONS, SECONDARY ALCOHOLS AND KETONES IN BRASSICA OLERACEA

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Summary: n-Nonacosane-U-^{3}H (C_{29} hydrocarbon) fed to young broccoli (Brassica oleracea) leaves was converted into labeled nonacosanol and nonacosan-15-one. Exogenous nonacosan-15-ol-U-^{3}H was converted into labeled nonacosan-15-one in the same tissue. The structure of the ketone was determined by chemical degradation and analysis of products. These results show the following biosynthetic relationships: hydrocarbon → secondary alcohol → ketone.

Hydrocarbons are widely distributed in nature. Frequently ketones and/or secondary alcohols, with the functional groups in the middle of the carbon chain, occur together with the corresponding hydrocarbons. This observation has led to the proposal of a head-to-head condensation hypothesis for the biosynthesis of these very long carbon chains (1). Experimental evidence obtained in the recent years disproved the classical head-to-head condensation hypothesis and suggested an elongation decarboxylation hypothesis (2). However, it was not clear how this hypothesis could account for the synthesis of the oxygenated derivatives. Indirect evidence suggested that the oxygen is introduced into a preformed chain of carbon atoms (3). Recently certain double labeling experiments suggested that the probable sequence of reactions is hydrocarbon → secondary alcohols → ketone (4). The hydroxylation of the carbon chain was assumed to be either at C_{30} fatty acid level or at the paraffin level. This paper describes experimental

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results which show that exogenous n-nonacosane is converted into n-nonacosanol and nonacosan-15-one whereas n-nonacosan-15-ol is converted into n-nonacosan-15-one in Brassica oleracea leaves.

Experimental

Substrates: n-nonacosane isolated from cabbage leaves and n-nonacosan-15-ol synthesized as described before (5) were exposed to 6 curies of ^{3}H gas at New England Nuclear Corporation, Boston, Massachusetts. The tritiated materials were purified by repeated thin-layer chromatography (5) to yield radiochemically and chemically homogeneous n-nonacosane (sp. activity 0.83 curie/m mole) and nonacosan-15-ol (sp. activity about 10 curie/m mole). The tritiated substrates were dispersed in water with the aid of Tween-20 as described before (6) and then the solutions were sonicated with a Biosonik III for 5 minutes at maximum power. Appropriate amounts of these solutions were incubated with chopped young broccoli leaves (6) at 30° for varying periods in a gyratory waterbath shaker. Only freshly purified (by TLC) substrates were used. In control experiments boiled tissues were incubated with the radioactive solutions for the same amount of time. At the end of the incubation period the surface lipids were extracted by stirring the reaction mixture in 100 ml of chloroform - methanol (2:1) for 30 seconds. The lipids were recovered from this solution by the method of Folch et. al (7) and thin-layer chromatographed on silica gel G with benzene as the solvent. The radioactivity on the chromatogram was determined by a scanner and with a liquid scintillation counter (6).

Ketone fractions were isolated and purified by repeated thin-layer chromatography from 12 grams of tissue that was incubated for 6 hrs. with nonacosane-U-^{3}H (396 x 10^{6} cpm), and from an identical experiment with nonacosan-15-ol-U-^{3}H (540 x 10^{6} cpm). The chemically and radiochemically homogeneous ketone fraction (7 x 10^{6} cpm and 1.0 x 10^{6} cpm from the secondary alcohol and hydrocarbon experiments respectively) was mixed with synthetic nonacosan-13-one (3). Oxime prepared from the ketone was subjected to

Beckmann rearrangement and the resulting substituted amide was hydrolysed (5). The fatty acid and amine fractions were separated by thin-layer chromatography with hexane: ethyl ether: formic acid (40:10:1). Methyl esters of the fatty acid fraction were subjected to gas liquid chromatography as described under the figures.

Results and Discussion

The radioactivity distribution in figure 1 shows that nonacosane-U-^{3}H was converted by intact young broccoli leaves into secondary alcohol and ketone fractions. The ketone fraction contained much more radioactivity than in the secondary alcohol. Similar conversions were observed when chopped leaves were incubated with the labeled hydrocarbon. If this conversion is representative of the natural synthesis of ketone the carbonyl

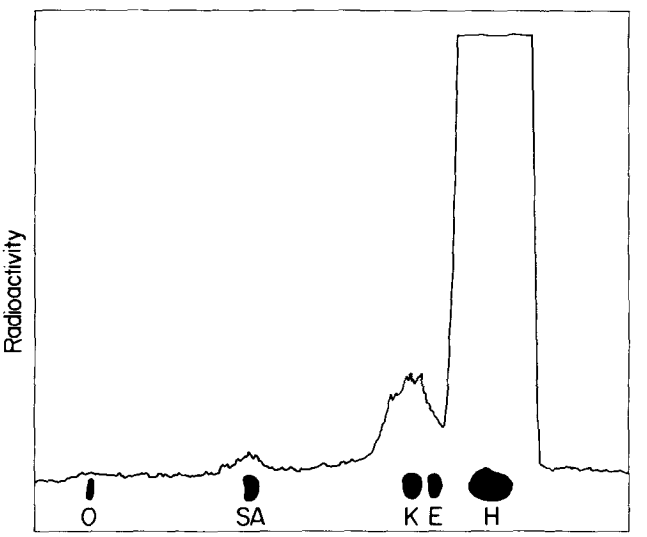

Figure 1: Thin-layer chromatogram of the surface lipids isolated from an intact leaf which had metabolized for 24 hours nonacosane-U-^{3}H solution painted on it. Chromatography on silica gel G with Benzene as the solvent. O-origin, SA-secondary alcohol, K-ketone, E-wax ester, H-hydrocarbon; the other components are not shown.

group in the radioactive ketone derived from nonacosane-U-^{3}H should be at C-15. When the oxime of the ketone was subjected to Beckmann rearrangement followed by hydrolysis the acid fraction and amine fraction contained

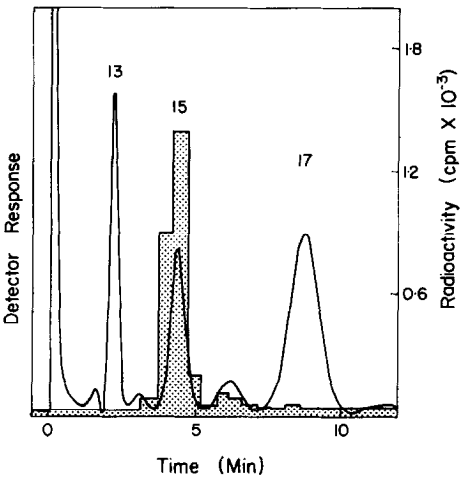

Figure 2: Gas-chromatogram of the fatty acid methyl esters derived from the degradation of the ketone produced from nonacosane-U-^{3}H. The C_{13} and C_{17} acids are from the 10 mg synthetic nonacosan-13-one added to the radioactive ketone before degradation. Chromatography on 6 ft. 0.25" O.D. coiled copper column packed with 12% diethylene glycol succinate on 90-100 mesh gas chrom Q at 150°C and 90 ml Argon carrier gas flow. Column effluent split into the hydrogen flame and sample collector which was washed with counting solution (5) and assayed for ^{3}H in a liquid scintillation counter.

approximately equal amounts of ^{3}H. The radioactivity distribution among the fatty acids (fig. 2) clearly showed that essentially all the ^{3}H was in the C_{15} acid proving that the radioactive ketone was indeed nonacosan-15-one.

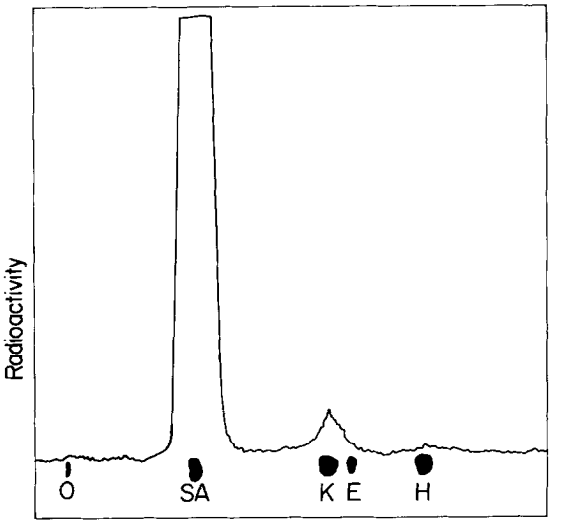

Figure 3: Thin-layer chromatogram of the surface lipids isolated from an intact leaf which had metabolized for 24 hours nonacosan-15-ol-U-^{3}H solution painted on it. Conditions as in figure 1.

Apparently nonacosane is hydroxylated specifically at C-15 position and subsequently oxidized to the ketone. In support of such a hypothesis is the observation that exogenous nonacosan-15-ol was converted into ketone by broccoli leaves (fig. 3). Control experiment with boiled leaves showed no products. This labeled ketone was subjected to the same degradation reactions mentioned above and the radioactivity distribution in the acid fraction is shown in fig. 4. Clearly the radioactive ketone produced from nonacosan-15-ol by broccoli leaves was nonacosan-15-one. Thus broccoli leaves can oxidize nonacosane into nonacosan-15-ol which in turn is oxidized to nonacosan-15-one.

These results provide direct evidence for the conclusions derived from earlier double labeling experiments (4). From such experimental results it

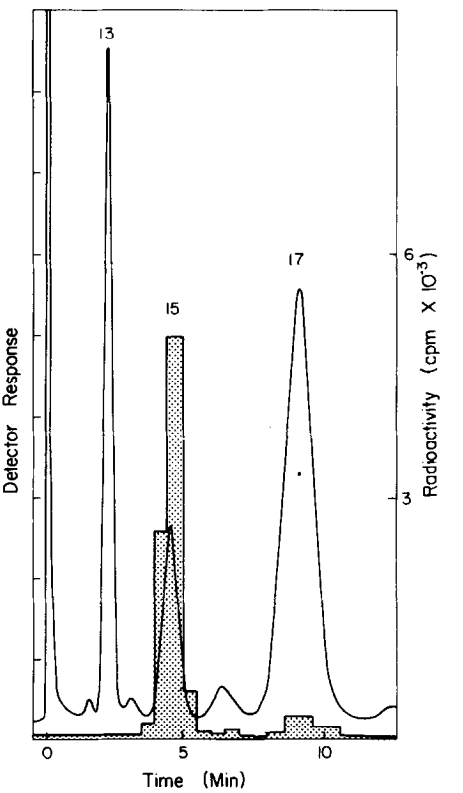

Figure 4: Gas-chromatogram of the fatty acid methyl esters derived from the degradation of the ketone produced from nonacosan-15-ol-U-^{3}H. Conditions as in figure 2 except that 20 mg nonacosan-13-one was used in this case.

was concluded that pea leaves contain n-hentriacontane and hentriacontan-16-ol but not the corresponding ketone because they lack the enzyme that oxidizes the secondary alcohol. In agreement with this hypothesis nonacosane-U-^{3}H was converted into labeled secondary alcohol but not into ketone by young pea leaves. Furthermore exogenous nonacosan-15-ol-U-^{3}H was not converted into ketone by young pea leaves.

Many if not most organisms that contain hydrocarbons also contain corresponding secondary alcohols and/or ketones (2) suggesting biosynthetic relationships among them. According to the classical head-to-head condensation mechanism for hydrocarbon synthesis, the ketones and secondary alcohols are precursors of hydrocarbons. However it has been established that the carbonyl group of the ketone does not arise from the carboxyl group of the precursor fatty acid (3, 4) and thus the biosynthetic relationships among the hydrocarbons and secondary alcohols and ketones were obscure. Results discussed in this communication provide for the first time direct experimental evidence that hydrocarbons are most probably hydroxylated at a specific carbon atom in the chain and then the resulting secondary alcohol is oxidized to the ketone.

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