A GERANIOL DERIVATIVE - AN INTERMEDIATE IN THE BIOSYNTHESIS OF SQUALENE BY A RAT LIVER ENZYME SYSTEM*

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Six water-soluble, collidine-extractable, acid-labile terpenoid compounds are formed from 2-C^{14}-mevalonic acid by a soluble rat liver enzyme system (Witting and Porter, 1959). The sequence of appearance of these compounds with time was reported previously, and the terpenoid moiety of the third member of this series, in time of appearance, was identified as farnesol (Witting and Porter, 1959). Recent experiments have corroborated the suggestion made in the same report that the terpenoid moiety of the first compound of this group to be synthesized is geraniol. This compound was identified by formation of the 3, 5-dinitrobenzoate and the 4(4'-nitrophenylazo) benzoate and crystallization of each derivative to constant specific radioactivity.

The water-soluble, acid-labile terpenoid derivatives are extractable from the incubation mixture with collidine, and the terpenoid moieties are extractable with petroleum ether from either the incubation mixture or the collidine extract after addition of aqueous acid to pH 1-2. One of the terpenoid moieties thus obtained has the same R_f value (0.90) as authentic geraniol when chromatographed in the kerosene-85% acetic acid system of Kaufmann and Nitsche (1954). The terpenoid derivative which is cleaved to give a C^{14}-

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labeled terpene alcohol corresponding to geraniol in the above chromatographic system reaches a maximum concentration in 10-30 minutes of incubation and then rapidly disappears.

Carrier geraniol was added to the incubation mixture after 10 or 20 minutes of incubation in the experiments in which derivatives of geraniol were prepared. The pH of the incubation mixture was then adjusted to 1-2, and the C^{14}-labeled terpenoid compounds were extracted with petroleum ether. The 3, 5-dinitrobenzoate and 4(4'-nitrophenylazo) benzoate were prepared and crystallized to constant specific radioactivity in separate experiments, Table I. A pure geranyl 3, 5-dinitrobenzoate which melted at the recorded value (Shriner and Fuson, 1948, 63°C) was readily obtained. Geranyl 4 (4'-nitrophenylazo) benzo-

TABLE I

CRYSTALLIZATION OF GERANIOL DERIVATIVES TO CONSTANT SPECIFIC RADIOACTIVITY

	4(4'-Nitrophenylazo) benzoate[1,2]			3,5-Dinitrobenzoate	
Crystallization	Total c/min	Crystals[3] c/min/mg	Supernate c/min/mg	Total c/min	Crystals[4] c/min/mg
1	5050	312	—	5000	38.3
2	1420	308	315	3080	32.5
3	510	308	—	1670	23.7
4				1260	24.1
5				915	23.3
6				689	23.7

1 The derivative was chromatographed twice on alumina prior to crystallization.

2 The weight of sample was determined spectrophotometrically at 332 mμ (Hecker 1955).

3 Samples were counted as infinitely thin films with a thin end-window gas-flow Geiger-Muller counter (Nuclear-Chicago, Model D 47).

4 Samples were counted in a Packard Tri-Carb liquid scintillation spectrometer (Model 314). Quenching was determined through counts of internal standards.

ate was found to melt at 103-4°C when the regenerated geraniol was 95% pure, as determined by gas chromatography. Purification of geraniol with $CaCl_2$ permitted formation of a derivative with the melting point of 107-109°C reported by Hecker (1955). The geraniol derivative was cleaved with mild alkali, and the regenerated geraniol was isolated by gas chromatography. Geraniol gave a single peak with a retention time of 0.54 relative to methyl myristate on a Craig polyester column at 180°C. Radioactivity was obtained in the geraniol, as expected, but recovery of the geraniol was not quantitative.

The present report is the first description of the synthesis of a geraniol derivative in an animal tissue. This finding and the results reported in previous papers (Witting and Porter, 1959; Lynen, Agranoff, Eggerer, Henning and Moslein, 1959) suggest that the biosynthesis of squalene involves the same reactions in yeast and in rat liver. However, the report of Olgivie (1959) of the occurence of 4-carboxy-farnesol and the remaining four intermediates in our system suggest that the condensation of isopentenyl pyrophosphate with dimethyallyl pyrophosphate to form a geranyl derivative and then a farnesyl derivative, followed by a condensation of the latter to squalene (Lynen, et al., 1959) may be an overly simplified concept of the reactions involved in the biosynthesis of squalene.

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