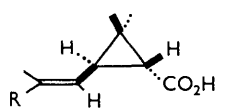
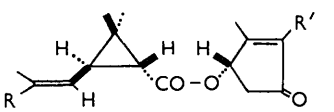
1118. *Biosynthesis of the Pyrethrins. Part II.*[1] *Pyrethric Acid and the Origin of Ester-methyl Groups.*

By P. J. Godin, Helene S. Inglis, M. Snarey, and E. M. Thain.

(+)-Pyrethric acid (I; R = CO_2Me) is formed biogenetically, as is (+)-chrysanthemum-monocarboxylic acid (I; R = Me), from two molecules of mevalonic acid. The stereochemistry of the fusion is partly determined and it is shown that the side-chain carboxyl group is derived from the 2-carbon atom of mevalonic acid. The ester-methyl group of (+)-pyrethric acid is derived from L-methionine.

It has been shown[1] that (+)-chrysanthemum-monocarboxylic acid (I; R = Me), the acid constituent of pyrethrin I (II; R = Me, R′ = CH_2·CH:CH·CH:CH_2) and cinerin I (II; R = Me, R′ = CH_2·CH:CHMe), is derived by condensation of two isoprene units in an unusual manner for which the name " middle-to-tail " fusion is suggested. At the same time it was found that the remaining major insecticidal constituents of *Chrysanthemum cinerariaefolium*, pyrethrin II (II; R = CO_2Me, R′ = CH_2·CH:CH·CH:CH_2) and cinerin II (II; R = CO_2Me, R′ = CH_2·CH:CHMe), which are esters of (+)-pyrethric acid (I; R = CO_2Me), when isolated from ovules fed with [2-^{14}C]mevalonic acid showed a level of incorporation of carbon-14 comparable with that of the other esters above.

(I)   (II)

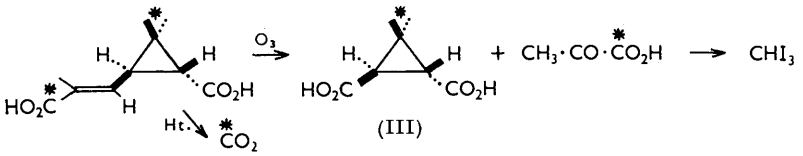
By treating " [^{14}C]pyrethrin II "* with 2,4-dinitrophenylhydrazine in methanolic sulphuric acid under vigorous conditions 4-*O*-methylpyrethrolone 2,4-dinitrophenylhydrazone was isolated which contained none of the initial radioactivity. This confirmed

* Throughout this paper " pyrethrin II " refers to the mixture of pyrethrin II and cinerin II, in which the former predominates, isolated from a mixture of all four constituents by chromatography on silica gel.[1] Similarly " pyrethrin I " is a mixture of pyrethrin I and cinerin I.

[1] Part I, Crowley, Godin, Inglis, Snarey, and Thain, *Biochim. Biophys. Acta* 1962, **60**, 312.

the finding[1] that the pyrethrolone molecule was not derived biogenetically from mevalonate.

This work has now been carried further by hydrolysis of " [^{14}C]pyrethrin II," derived from [2-^{14}C]mevalonic acid, to give (+)-[^{14}C]chrysanthemumdicarboxylic acid (I; R = CO_2H) which contained all the initial radioactivity. The diacid was then further degraded by ozonolysis to give (—)-*trans*-[^{14}C]caronic acid (III) and [^{14}C]pyruvic acid, counted as a mixture of its *cis*- and *trans*-2,4-dinitrophenylhydrazones. The caronic and pyruvic



acids each contained approximately half the initial radioactivity. Finally, treatment of the [^{14}C]pyruvic acid gave inactive iodoform, showing the side-chain terminal *C*-methyl group of chrysanthemumdicarboxylic acid is not derived from the 2-carbon atom of mevalonic acid.

It has been reported[2] that pyrolysis of (+)-chrysanthemumdicarboxylic acid (I; R = CO_2H) gives norchrysanthemum-monocarboxylic acid (I; R = H) in good yield. When this reaction was carried out on the inactive acid, ~1 mol. of carbon dioxide was isolated as barium carbonate and the residue was, as reported, a complex mixture from which a liquid acid (10—20%) was isolated having the spectral properties expected of the nor-acid (I; R = H). Then, after pyrolysis of (+)-[^{14}C]chrysanthemumdicarboxylic acid, it was found that the activity of the derived [^{14}C]carbon dioxide was approximately half that of the original acid. Thus the side-chain carboxyl group, and not the methyl group, is derived from the 2-carbon of mevalonic acid. The carboxyl group is *trans* to the cyclopropane ring, as found by others for the more common " head-to-tail " isoprenoid condensations.[3] This suggests that, in this " middle-to-tail " condensation, the isoprene unit which forms the side-chain is αα-dimethylallyl pyrophosphate. It also appears possible that one explanation for the unusual fusion is that it involves two molecules of this phosphate and not the more normal condensation between one molecule of isopentenyl pyrophosphate and one of αα-dimethylallyl pyrophosphate.

Feeding experiments with L-(Me-^{14}C]methionine were carried out in the same manner, and it was found that approximately twenty times as much radiocarbon was present in " [^{14}C]pyrethrin II " as in " [^{14}C]pyrethrin I " isolated from the treated ovules. The difference in the acids from these two groups of esters is that the methyl-ester grouping of (+)-pyrethric acid (I; R = CO_2Me) has replaced one of the side-chain methyl groups of chrysanthemum-monocarboxylic acid (I; R = Me). Thus it appeared reasonable that the methanol derived from this group is formed biogenetically from the *S*-methyl group of L-methionine. This was confirmed by hydrolysis of " [^{14}C]pyrethrin II " with concentrated alkali and oxidation of the methanol formed to give [^{14}C]formaldehyde which was counted as its dimedone derivative.

The activity of the [^{14}C]formaldehyde confirmed the view that the ester-methyl group in this compound, and presumably in other methyl esters, is derived from L-methionine. Presumably the much lower but still significant activity of the " [^{14}C]pyrethrin I," and a small part of the activity of the " [^{14}C]pyrethrin II," is due to incorporation of the methyl group of L-methionine into the cyclopentenolone part of the pyrethrins.

Experimental

For general experimental details see Part I. In the experiments with " pyrethrins " and their degradation products labelled from [2-^{14}C]mevalonate, samples were counted as

[2] Staudinger and Ruzicka, *Helv. Chim. Acta*, 1924, **7**, 201.
[3] Birch, Kocor, Sheppard, and Winter, *J.*, 1962, 1502 and references cited therein.

infinitely thin films as previously described.[1] The compounds and their degradation products isolated from feeding experiments with L-[^{14}C]methionine were counted in dry and redistilled dioxan containing 0·3% of 2,5-diphenyloxazole, with an Ekco scintillation counter of type N664A. These results were corrected for quenching by using [^{14}C]hexadecane as an internal standard. Gas–liquid chromatography was carried out as described in the literature.[4] Activities are recorded in 10^4 counts per mmole per min.

Incorporation of [2-^{14}C]*Mevalonic Acid into the "Pyrethrins."*—The vacuum-impregnation technique used, and the method of isolation of "pyrethrin I" and "pyrethrin II" from the ovules of *C. cinerariaefolium*, were as previously described.[1]

Hydrolysis of " [^{14}C]*Pyrethrin II."*—"[^{14}C]Pyrethrin II" (250 mg.) was hydrolysed with an excess of aqueous 0·1N-sodium hydroxide in dioxan (titrimetric control). When hydrolysis was complete the acid was isolated as described for the monoacid.[1] The crude diacid was then further purified by chromatography on 1 : 1 charcoal–kieselguhr in chloroform, to give pure (+)-[^{14}C]chrysanthemumdicarboxylic acid (73 mg.), m. p. and mixed m. p. with unlabelled sample 164°. Purity was confirmed by infrared spectra (in CS_2 and $CHCl_3$) and by paper chromatography; the activity was 6·43.

Ozonolysis of the Diacid.—The diacid (60 mg.) from above, in chloroform, was treated with ozone for 6 hr. The chloroform was removed in a stream of oxygen, water (10 ml.) was added, and the solution was warmed at 40° for 1 hr. The residual solution was divided into three equal parts.

(1) A portion, on treatment with an excess of 2N-sodium hydroxide and then with an excess of iodine in potassium iodide, gave iodoform (16 mg.), m. p. 119°, activity 0·089. (Control, 0·074). In these conditions (−)-*trans*-caronic acid gave no iodoform.

(2) A portion was treated with 2,4-dinitrophenylhydrazine in 5% methanolic sulphuric acid. The solution was saturated with sodium chloride, and the precipitated solids were filtered off and purified by chromatography on 1 : 1 bentonite–kieselguhr in 1 : 19 methanol–chloroform, to give a mixture of *cis*- and *trans*-[1-^{14}C]pyruvic acid 2,4-dinitrophenylhydrazones, (6 mg.). This gave two spots, with R_F values identical with those of a preparation from pyruvic acid, on chromatography in light petroleum (b. p. 100—120°) on paper saturated with dimethylformamide. Identity was confirmed by the infrared spectra (mull); the activity was 2·67.

(3) The final portion was extracted with chloroform. The aqueous layer, on concentration, gave (−)-*trans*-[^{14}C]caronic acid (9 mg.), m. p. and mixed m. p. 212—213°, activity 3·75.

Pyrolysis of (+)-[^{14}C]*Chrysanthemumdicarboxylic Acid.*—The diacid (30 mg.) was heated at 250—300° in a stream of nitrogen, and the carbon dioxide evolved was converted into barium carbonate; the activity of this was 2·23. The residue from a large-scale reaction was a complex mixture.

Incorporation of the Methyl Group of L-[Me-^{14}C]*Methionine in the "Pyrethrins."*—Dissected ovules (80 g.) were treated with *L*-[^{14}C]methionine (0·05 mC., 7·8 mg.) and after 24 hr. the "pyrethrins" were isolated and purified, giving "[^{14}C]pyrethrin I" (107·6 mg.), activity 2·3, and "[^{14}C]pyrethrin II" (57 mg.), activity 43·8. The purity of these samples was confirmed by gas–liquid chromatography. Other small-scale experiments gave activity ratios ("pyrethrin II"/"pyrethrin I") between 24 and 18.

Degradation of " [^{14}C]-*Pyrethrin II."* (*Methionine-labelled*).—"[^{14}C]Pyrethrin II" (54 mg.) was refluxed with 0·1N-sodium hydroxide (5 ml.) for 6 hr. The mixture was cooled and just acidified with 4N-sulphuric acid, and sodium dichromate (30 mg.) in 4N-sulphuric acid (3 ml.) was added. The mixture was then distilled quantitatively into dimedone in 50% aqueous ethanol. The [^{14}C]formaldehyde dimedone derivative (7·3 mg.) was filtered off, dried, and counted, m. p. and mixed m. p. 189°, activity 37·6.

We thank the Department of Agriculture, Kenya, and the Pyrethrum Board of Kenya for financial assistance, Mr. T. J. Coomes for the cultivation of plants of *C. cinerariaefolium*, and Miss G. Morgan for assistance in feeding experiments.

The Tropical Products Institute, 56—62 Gray's Inn Road,
London W.C.1. [*Received, May 23rd,* 1963.]

[4] Donegan, Godin, and Thain, *Chem. and Ind.*, 1962, 1420.