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THE BIOSYNTHESIS OF PROTOANEMONIN IN RANUNCULUS GLABER MAKINO

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Sodium [2-^{14}C]malonate and D-[U-^{14}C]glucose were incorporated into protoanemonin (I) in Ranunculus glaber Makino in 0.60 and 0.09% yield respectively at the highest value. Its labeling pattern suggests that the lactone I is biosynthesized by the condensation of glycerate and malonate in a terrestrial part of the plant.

Many of the plants belonging to Ranunculaceae have the antibiotic activity and the property of blistering the skin. Such physiological activities have been reported to be due to protoanemonin (I).1-5) Tissues of Ranunculus species are said to liberate enzymically the protoanemonin from ranunculin (II) in the plant upon crushing the tissues.6) Tschesche7) reported recently glycosides of analogous C_5 compounds present in extracts of Ranunculus species. Although the biosynthetic pathway of such a simple lactone nucleus is of considerable interest, it has not been reported yet. We now have tested the labeling pattern in protoanemonin (I) biosynthesized from ^{14}C-labeled precursors, sodium [2-^{14}C]malonate and D-[U-^{14}C]-glucose, by Ranunculus glaber Makino (Kitsunenobotan in Japanese) in order to clarify the biosynthetic pathway of the lactone I.

Feeding experiments were carried out on terminal branches (ca. 20 cm long) of the young plant in April. A phosphate buffered solution (pH 7.38) of each of sodium [2-^{14}C]malonate and D-[U-^{14}C]glucose was fed through a cut-stem into the plant (ca. 100-150 g). The leaves and stems were then subjected to steam-distillation to obtain the lactone I, which was then converted into *n*-valeric acid by hydrogenation with Adam's platinum oxide.3) After the valeric acid was derived to the silver salt, the salt was purified to constant specific activity upon recrystallization.

TABLE 1. INCORPORATION OF RADIOACTIVE TRACERS INTO PROTOANEMONIN (I) IN RANUNCULUS SPECIES

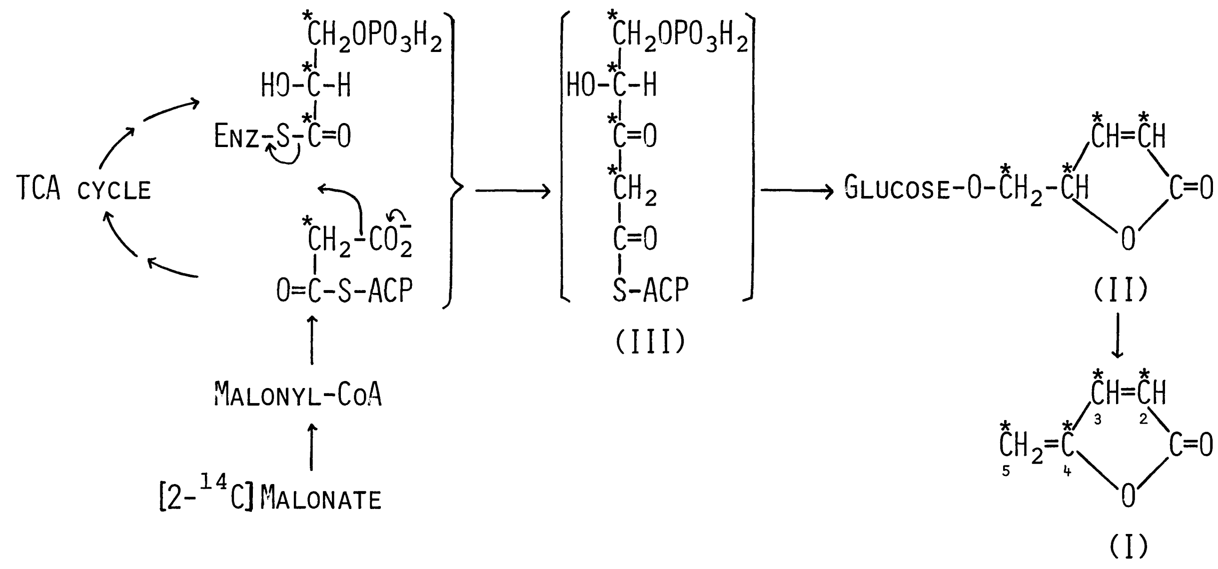
Exp. No.	Precursors§ (mCi)	Feeding time (day)		Specific radioactivity of I (dpm/mM)	Incorporation (%)
1	SM; 0.015	1	In budding season	7.10×10^4	0.37
2	SM; 0.015	2		1.05×10^5	0.60
3	SM; 0.01	1	In seeding season	2.22×10^4	0.09
4	SM; 0.01	2		1.80×10^4	0.09
5	SM; 0.01	3		1.24×10^4	0.06
6	SM; 0.01	4		1.92×10^4	0.10
7	GL; 0.015	1	In flowering season	2.04×10^4	0.06
8	GL; 0.015	2		2.61×10^4	0.09
9	GL; 0.015	3		2.60×10^4	0.09

§SM and GL denote sodium [2-^{14}C]malonate and D-[U-^{14}C]glucose respectively.

TABLE 2. DISTRIBUTION OF RADIOACTIVITY IN PROTOANEMONIN (I) AFTER UPTAKE OF SODIUM [2-^{14}C]MALONATE

Carbons originated from I	Specific radioactivity (dpm/mM)	Distribution (%)
C-1 ∿ C-5	1.05×10^5	100
C-1	3.78×10^3	3.6
C-2	6.38×10^4	60.8
C-3	1.26×10^4	12.0
C-4	1.28×10^4	12.2
C-5	1.20×10^4	11.4

Both malonate and glucose were found to be incorporated into the lactone I, as is shown in Table 1. This fact indicates that both malonate and glucose are the biosynthetic precursor of I. In addition, the incorporation experiments of



* = Radioactive carbon

Scheme 1. Biosynthetic pathway of protoanemonin (I)

malonate show that the lactone I is best biosynthesized in the budding season.

The Schmidt reaction of the labeled silver valerate yielded carbon dioxide and *n*-butylamine, which was further converted to butyric acid by potassium permanganate oxidation. Repetition of the procedure with the acid having carbon atoms less one atom gave successively carbon dioxide originated from each carbon atom of I. The carbon dioxide produced were absorbed in a barium hydroxide solution to be converted into barium carbonate. On the other hand, the Kuhn-Roth oxidation of the valerate yielded acetic acid which involves C-4 and C-5 of the lactone I. The acetic acid was further cleaved into carbon dioxide and methylamine by the Schmidt reaction. The methylamine was converted into barium carbonate by Van Slyke-Folch oxidation. The radioactivity of these barium carbonate was detected on planchets at an infinite thickness under an Aloka LBC-1 2π-gas flow low back counter, as is shown in Table 2.

About 60 per cent of the total radioactivities was detected in carbon dioxide originated from C-2 of protoanemonin (I) after the uptake of sodium [2-^{14}C]malonate. On the other hand, the distribution of the radioactivity on each carbon atom of C-3, C-4, and C-5 was nearly equal. These facts suggest that the C-1 and C-2 moiety of the lactone I is incorporated directly from malonate, while the C-3$\sim$C-5 moiety is derived from such a pathway as explained below. Some molecules of radioactive malonate are introduced to the tricarboxylic acid cycle via acetate, which certainly is acetyl-CoA produced probably by the decarboxylation of malonyl-CoA with malonyl-CoA decarboxylase, to be converted into glycerate via oxalacetate. This makes the tracer at C-2 of malonate distribute uniformly among three carbon atoms of the glycerate. The incorporation experiments now imply that the biosynthesis of protoanemonin (I), which actually is ranunclin (II) in biological systems, involves the condensation of glycerate with malonate with the loss of carbon dioxide and subsequently the lactonization accompanied with the reduction and the dehydration, as is shown in the scheme. The condensation occurs presumably in such way that 3-phosphoglycerate is first bound to the enzyme (Enz.-S-) to turn out to be more reactive and then the reactive glycerate reacts with malonate activated by acyl carrier protein (ACP-S-) as in the biosynthesis of fatty acids. The participation of glycerate in the biosynthetic pathway of I was further supported by the incorporation of [U-^{14}]glucose into I, since D-glucose is well known to be degraded to glycerate by glycolysis. Thus, the pathway proposed in this paper is a unique example in the biosynthesis of naturally occurring C_5 compounds.

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