CHEMISTRY LETTERS, pp. 1201-1202, 1974. Published by the Chemical Society of Japan

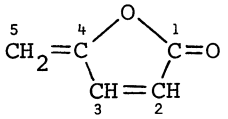
# THE GENUINE PRECURSOR FOR THE BIOSYNTHESIS OF PROTOANEMONIN IN *RANUNCULUS GLABER*

Takayuki SUGA, Toshifumi HIRATA, Tomoko HORIKAWA, and Noriko WAKI
Department of Chemistry, Faculty of Science, Hiroshima University
Higashisenda-machi, Hiroshima 730

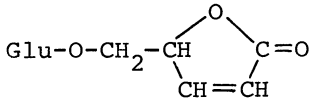
Protoanemonin (I) was biosynthesized from radioactive tracers, such as sodium [5-$^{14}$C]α-ketoglutarate, sodium [1-$^{14}$C]acetate, sodium [2,3-$^{14}$C]succinate, and [2-$^{14}$C]glucose, in *Ranunculus glaber* Makino. Its labeling patterns have demonstrated that the genuine precursor of the lactone I is α-ketoglutarate.

Recently we proposed that protoanemonin (I), having the antibiotic activity and the property of blistering the skin, is biosynthesized by the condensation of glycerate and malonate.[1] However, more detailed examinations using $^{14}$C-labeled tracers, such as sodium [5-$^{14}$C]α-ketoglutarate, sodium [1-$^{14}$C]acetate, sodium [2,3-$^{14}$C]succinate, and D-[2-$^{14}$C]glucose, have demonstrated that the genuine precursor of I is α-ketoglutarate and not glycerate and malonate. We report here evidence for the biosynthetic pathway of protoanemonin (I).

Incorporations of the tracers were carried out on terminal branches of *Ranunculus glaber* Makino (Kitsunenobotan in Japanese) in budding season.[2] It was observed that all the tracers were incorporated into the lactone I, as shown in Table 1. This fact indicated that all the tracers are the biosynthetic precursors of I in a wide sense. However, the labeling patterns (Table 2)[2] in the lactone I have suggested that all these compounds are not the genuine precursors of I. In the case of sodium [1-$^{14}$C]acetate, a substantial amount of the total radioactivity in I was located in C-1 and with [2-$^{14}$C]malonate, as documented earlier,[1] about 60 per cent of the radioactivity was detected in the C-2 carbon atom. These facts demonstrated that the C-1 and C-2 of the lactone I are generated from the $C_2$-unit of acetate, while the C-3~C-5 moiety originate from a different source. On the basis of incorporation of sodium [2,3-$^{14}$C]succinate and degradations of radioactive protoanemonin biosynthesized from the tracer, it was established that succinate forms the potential source for C-3~C-5 moiety. Further it was observed that after the uptake of sodium [5-$^{14}$C]α-ketoglutarate, almost all of the total radioactivity in I was located in the C-1 position. These results unambiguously established that the



I



II

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

| Exp. No. | Precursors* (mCi) | Feeding time (day) | Specific radioactivity of I (dpm/mmole) | Incorporation (%) |
|---|---|---|---|---|
| 1 | SA; 0.1 | 2 | $3.34\times10^4$ | 0.063 |
| 2 | SS; 0.05 | 2 | $1.05\times10^5$ | 0.61 |
| 3 | SK; 0.025 | 1 | $1.20\times10^5$ | 0.21 |
| 4 | SK; 0.025 | 2 | $2.19\times10^5$ | 0.35 |
| 5 | GL; 0.05 | 2 | $3.95\times10^4$ | 0.16 |

* SA, SS, SK, and GL denote sodium [1-$^{14}$C]acetate, sodium [2,3-$^{14}$C]succinate, sodium [5-$^{14}$C]α-ketoglutarate, and D-[2-$^{14}$C]glucose, respectively.

TABLE 2. DISTRIBUTION OF RADIOACTIVITY IN PROTOANEMONIN (I) AFTER THE UPTAKE OF THE $^{14}$C-LABELED TRACERS

| Carbons originated from I | Distribution (%)* | | | |
|---|---|---|---|---|
| | Exp. 1 | Exp. 2 | Exp. 3 | Exp. 5 |
| C-1 | 91.0 | 7.1 | 94.2 | 34.3 |
| C-2 | 3.1 | 7.4 | | 5.9 |
| C-3 | | 34.1 | | 29.7 |
| C-4 | 5.9 | 30.2 | 5.8 | 16.5 |
| C-5 | | 21.2 | | 13.6 |

* "Exp. No." corresponds to the number in Table 1.

genuine precursor of the lactone I is α-ketoglutarate which is generated from acetate and succinate by the tricarboxylic acid cycle (TCA cycle). Moreover, the labeling pattern in I biosynthesized from [2-$^{14}$C]glucose also demonstrate the direct participation of α-ketoglutarate in the formation of I *via* the TCA cycle through the glycolysis and the pentose phosphate cycle. These data lead us to propose that the biosynthesis of protoanemonin (I), which is present as ranunclin (II) in the biological system,[3,4] involves the lactonization of the 5-carboxyl and the carbonyl group (which, probably, is in the enol form) of α-ketoglutarate.

## References and Note

1) T. Suga and T. Hirata, Chem. Lett., 1973, 637.
2) Feeding experiments and degradation of labeled protoanemonin (I) were carried out by the same procedures as described in our previous paper.[1]
3) R. Hill and R. V. Heyningen, Biochem. J., 49, 332 (1951).
4) R. Tschesche, K. Welmer, G. Wulff, and G. Snatzke, Chem. Ber., 105, 290 (1972).

(Received August 26, 1974)