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BIOSYNTHESIS OF PROTOANEMONIN IN *RANUNCULUS GLABER*. THE STEREOCHEMISTRY OF THE HYDROGEN ELIMINATION IN THE FORMATION OF THE DOUBLE BOND OF PROTOANEMONIN

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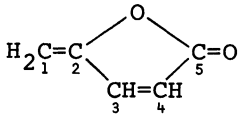
In order to determine the stereochemistry of the hydrogen elimination in the formation of the Δ^3-double bond of protoanemonin (I) by *Ranunculus glaber* Makino, [(3*R*)-3-$^3H_1$;3,4-^{14}C]- and [3-$^3H_2$;3,4-^{14}C]-α-ketoglutaric acids were administered to the plants. The $^3H/^{14}C$ ratios in I biosynthesized from these doubly labeled acids demonstrated that the formation of the Δ^3-double bond occurs with the stereospecific loss of the *pro*-3*S*-hydrogen atom of α-ketoglutaric acid.

Many of the plants belonging to the Ranunculaceae family have the antibiotic activities and the properties of burning and blistering the skin.[1~3] We investigated the biosynthetic pathway of protoanemonin (I) in *Ranunculus glaber* Makino (Japanese name: Kitsune-no-botan) by administering a variety of ^{14}C-labeled compounds to the plants[4,5] and documented that the genuine precursor for the biosynthesis of I is α-ketoglutaric acid (IIa).[5] It is, however, unsolved that the formation of the Δ^3-double bond in the biosynthesis of I from the precursor IIa occurs whether with the stereospecific loss of one of the C-3 hydrogen atoms of IIa or with the random loss of one of the hydrogen atoms.[6] We have tested the stereochemistry of the hydrogen elimination by determining the $^3H/^{14}C$ ratios in I biosynthesized from [(3*R*)-3-$^3H_1$;3,4-^{14}C]- and [3-$^3H_2$;3,4-^{14}C]-α-ketoglutaric acids by the plants, and here wish to report the results.

By reference to the methods in literatures 7 and 8, [3-$^3H_2$]α-ketoglutaric acid (IIb) (31 mg, 12.5 μCi) was prepared by hydrogen exchange of sodium α-ketoglutarate (50 mg) with tritiated water (0.16 ml, 160 μCi) and [(3*R*)-3-$^3H_1$]α-ketoglutaric acid (IIc) (5.9 mg, 1.1 μCi) was prepared by incubation of IIb (7.5 mg, 3.0 μCi) with isocitrate dehydrogenase. On the other hand, [3,4-^{14}C]α-ketoglutaric acid (IId) (0.019 mg, 3.0 μCi) was prepared by condensation of [2,3-^{14}C]dimethyl succinate (0.26 mg, 50 μCi) with diethyl oxalate (2.0 mg) following the literature method.[9] The appropriate doubly labeled precursors, [(3*R*)-3-$^3H_1$;3,4-^{14}C]- and [3-$^3H_2$;3,4-^{14}C]-α-



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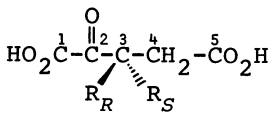

IIa: $R_R=R_S=H$
IIb: $R_R=R_S=^3H$
IIc: $R_R=^3H$, $R_S=H$

TABLE 1. INCORPORATION OF [(3*R*)-3-$^{3}H_1$;3,4-^{14}C]- and [3-$^{3}H_2$;3,4-^{14}C]-α-KETOGLUTARIC ACIDS, (IIe) and (IIf), INTO PROTOANEMONIN (I) AND THE $^{3}H/^{14}C$ RATIOS IN THE ACIDS USED AND PROTOANEMONIN BIOSYNTHESIZED

Exp. No.	$^{3}H,^{14}C$-labeled α-ketoglutaric acids			Protoanemonin (I)		
	The acids	$^{3}H/^{14}C$ Ratio	$^{3}H:^{14}C$ (Normalized)[a]	$^{3}H/^{14}C$ Ratio	$^{3}H:^{14}C$ (Normalized)[b]	Incorp. of IIe and IIf[c]
1	IIe	1.96±0.01	1:1	1.89±0.07	0.96:1	0.61
2	IIe	6.21±0.31	1:1	6.01±0.66	0.97:1	0.47
3	IIf	13.9±0.22	2:1	6.18±0.51	0.89:1	0.52
4	IIf	12.8±0.98	2:1	6.56±0.45	1.03:1	0.73

a) This is the normalized ratio as related to the number of ^{3}H-label at C-3 of α-ketoglutaric acid.
b) The normalized $^{3}H:^{14}C$ ratio was obtained by dividing the $^{3}H/^{14}C$ ratio of I by the $^{3}H/^{14}C$ ratio of α-ketoglutaric acid and multiplying the answer by the number of ^{3}H-label at C-3 of the acid.
c) The incorporations are calculated with respect to only ^{14}C.

ketoglutaric acids, (IIe) and (IIf), were prepared by mixing the above-described, singly labeled α-ketoglutaric acids to give the $^{3}H/^{14}C$ ratios as shown in Table 1.

A phosphate buffered solution (pH 7.0) of each of the doubly labeled precursors, (IIe) and (IIf), was fed through a cut-stem to the terminal branches of the plants (20 cm in length and 70 g in weight) in the flowering season. After uptake of the precursor, the terminal branches were further maintained in the phosphate buffer for 12 hr. and then subjected to steam-distillation to give protoanemonin (I). This lactone (I) on hydrogenation with PtO_2 was converted[10] to *n*-valeric acid, which was then derived to the *p*-bromophenacyl derivative. The phenacyl derivative was purified to a constant specific activity on repeated recrystallization to determine the radioactivities. The $^{3}H/^{14}C$ ratios determined from the radioactivities and the incorporations of the precursors are shown in Table 1. The $^{3}H/^{14}C$ ratio of I biosynthesized from IIf resulted in a half of the $^{3}H/^{14}C$ ratio in [3-$^{3}H_2$;3,4-^{14}C]α-ketoglutaric acid (IIf), whereas the $^{3}H/^{14}C$ ratio in I was retained when [(3*R*)-3-$^{3}H_1$;3,4-^{14}C]α-ketoglutaric acid (IIe) was administered. This clearly demonstrates that the hydrogen elimination that takes place during the formation of the Δ^{3}-double bond of protoanemonin (I) occurs with the stereospecific loss of the *pro-S*-hydrogen atom (R_S) and the stereospecific retention of the *pro-R*-hydrogen atom (R_R) at C-3 of α-ketoglutaric acid (IIa).

References and Note

1) Y. Asahina, Yakugaku Zasshi, No. 396, 81 (1915).
2) R. Hill and R. V. Heyningen, Biochem. J., 49, 332 (1951) and references cited therein.
3) R. Tschesche, K. Welmer, G. Wulff, and G. Snatzke, Chem. Ber., 105, 290 (1972).
4) T. Suga and T. Hirata, Chem. Lett., 637 (1973).
5) T. Suga, T. Hirata, T. Horikawa, and N. Waki, ibid., 1201 (1974).
6) In this report, for convenience, the carbon atoms of protoanemonin (I) are numbered as shown in structural formula I as related with α-ketoglutaric acid (IIa).
7) Z. B. Rose, J. Biol. Chem., 235, 928 (1960).
8) A. C. Stoolmiller and R. H. Abeles, ibid., 241, 5764 (1966).
9) L. Friedman and E. Kosower, Org. Syn., 26, 42 (1946).
10) F. B. Kipping, J. Chem. Soc., 1145 (1935).

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