

## Preliminary Studies on the Biosynthesis of the Terpenoids in *Artemisia monogyna*

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The present work was carried out as a preliminary investigation on the biosynthesis<sup>1)</sup> of santonin and the essential oils by *Artemisia monogyna*.

[2-C<sup>14</sup>] Mevalonic acid<sup>2)-5)</sup> and 5-phosphomevalonic acid<sup>6)7)</sup> were used as precursors of the terpenoid biosynthesis in the plant. Terpenoids were separated into several fractions by using various solvents and radioactivity incorporated into each fraction was determined by using a gas flow counter.

### Materials and Methods

#### *Labeled compounds and other chemicals.*

The DL-[2-C<sup>14</sup>] mevalonic acid used was obtained from the Radiochemical Centre, Amersham (England). Radioactive L-5-phosphomevalonic acid was prepared from DL-[2-C<sup>14</sup>] mevalonic acid by enzymic preparation of *Staphylococcus aureus*<sup>8)</sup>.

Petroleum benzin used as the solvent was refined by washing with concentrated H<sub>2</sub>SO<sub>4</sub>, mixed solution of concentrated H<sub>2</sub>SO<sub>4</sub> and concentrated HNO<sub>3</sub>, 2% solution of NaOH, and water in succession.  $\alpha$ -Santonin used as a carrier was supplied by the Nippon Shinyaku Company Ltd. Veronalacetate,  $\beta$ -indoleacetate, and other reagents were commercial goods.

#### *Administration of labeled compounds.*

*Artemisia monogyna* (*Mibuyomogi*) used in this experiment were *Yamashina No. 3* and a common species.

The plants were cut to the approximately equal sizes (about 20 cm. length), and were dipped into an aqueous solution containing a labeled compound and other chemicals as shown in Table 1. After a few days cultivation, the plants were harvested and were dealt with by the following chemical treatment. The details of dip method carried out in this study are given in Table 2.

#### *Separation of radioactive products.*

The plants were cut to pieces and ground with a mortar. To this homogenate 25mg. of  $\alpha$ -santonin was added as a carrier, and 1 ml. of ethanol was also added to stop enzymatic reactions. After boiling for 10 minutes under reflux the mixture was extracted with petroleum benzin, chloroform, and distilled water. The each extract was washed, dried, and the radioactivity was measured as infinitely thin samples in a  $2\pi$  gas flow counter. From the chloroform solution crude santonin was obtained by the usual chemical method as calcium santoninate. The essential oil was separated into a petroleum benzin fraction.

### Results and Discussion

The results obtained using DL-[2-C<sup>14</sup>] mevalonic acid and L-[2-C<sup>14</sup>] phosphomevalonic acid are shown in Table 2.

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Table 1

Exp.	No.	Labeled compound ( $\times 10^3$ c.p.m.)		Added chemicals ( $\mu$ moles)				
		DL-MVA	L-P-MVA	Mn <sup>++</sup>	ATP	Phosphate buffer	Veronal acetate	$\beta$ -Indole acetate
I	1	20	—	2	5	10	—	—
	2	20	—	2	—	10	—	—
	3	20	—	—	5	10	—	—
	4	20	—	—	—	10	—	—
II	1	20	—	2	5	10	—	—
	2	20	—	2	—	10	—	—
	3	20	—	—	5	10	—	—
	4	20	—	—	—	10	—	—
III	1	20	—	2	—	10	—	—
	2	20	—	2	—	10	—	—
	3	20	—	2	—	10	—	—
IV	1	40	—	—	—	—	—	—
	2	40	—	—	—	10	—	—
	3	40	—	—	—	—	10	—
	4	40	—	2	—	—	10	—
	5	40	—	2	5	—	10	—
	6	—	4	—	—	—	—	—
V	1	200	—	—	—	—	—	—
VI	1	—	20	—	—	—	—	—
VII	1	200	—	—	—	—	—	—
	2	200	—	10	—	—	—	—
	3	100	—	—	—	—	—	0.01
	4	100	—	—	—	—	—	1.00
VIII	1	100	—	—	—	—	—	—

The incorporation of C<sup>14</sup> into santonin and essential oil fractions were observed. The rate of incorporation into these fractions were varied with the growth phase of the plant.

For incorporation into santonin, administration of a large amount of mevalonic acid and phosphomevalonic acid were advantageous. The effects of manganese ion and ATP were not so distinct under these cultivation. While, in the dark the incorporation of mevalonic acid into santonin was scarcely observed. This fact suggests that the biosynthesis of santonin may be occurred joining with photosynthesis by uncertain ways.

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Table 2

Exp.	Date	Dip method			C <sup>14</sup> incorporation (c.p.m.)				Crude santonin
		Species	Weather and Lighting	No.	Petroleum benzin	Chloroform	Water	Total	
I	Jul. 12	common	fine	1	1,900	700	2,400	5,000	
				2	600	500	500	1,600	
				3	300	500	500	1,300	
				4	900	700	400	2,000	
II	Jul. 14	common	fine	1	300	2,000	6,000	8,300	250
				2	2,300	3,200	6,600	12,100	10
				3	1,600	2,100	4,700	8,400	225
				4	2,200	1,600	3,100	6,900	200
III	Jul. 22	Yamashina No. 3	fine	1	400	2,400	4,000	6,800	200
				2	100	2,100	6,460	8,660	225
				3	300	1,900	2,260	4,460	300
IV	Jul. 25	Yamashina No. 3	cloudy	1	5,300	8,100	15,700	29,100	275
				2	300	3,400	8,300	12,000	350
				3	700	4,400	12,350	17,450	50
				4	1,100	4,100	9,600	14,800	50
				5	200	2,200	3,900	6,300	100
				6	300	600	500	1,400	250
V	Aug. 5	Yamashina No. 3	fine	1	23,350	16,800	64,200	104,350	1,700
VI	Aug. 14	common	a fluorescent light	1	5,110	2,710	2,720	10,540	420
VII	Aug. 18	Yamashina No. 3	a fluorescent light	1	20,450	13,100	48,800	82,350	216
				2	20,850	15,550	57,900	94,300	110
				3	12,100	7,900	32,350	52,350	10
				4	8,150	4,900	28,950	42,000	140
VIII	Sep. 12	common	in the dark	1	24,500	5,600	35,625	65,725	0