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STUDIES ON THE BIOSYNTHESIS OF PECTINIC ACID METHYL ESTERS^{1,2,3}

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A previous report from this laboratory (4) showed that the methyl group of methionine can be transferred as a unit to form methyl esters of pectinic acid in radish plants. That study has now been extended, in the present work, to examine other compounds which have contributed to the "one carbon" pool in animal and plant metabolism and may also be introduced into pectinic acid methyl esters in the radish plant. Included in the group of methyl precursors tested were glycine, serine, formate and formaldehyde.

MATERIALS AND METHODS

PREPARATION OF RADISH PLANTS: The radish plant, *Raphanus sativus*, var. Comet, was used throughout the present work. Seeds were sprouted on damp cheese cloth and grown in constantly aerated Hoagland nutrient solution (1) following the procedures used by Sato and co-workers (4). When the enlarged hypocotyl and upper root, hereafter referred to as the "radish," had developed to about 1.0 to 1.5 cm diameter, which generally took 5 to 7 weeks depending on the time of the year, the plant was considered to be ready for the feeding experiment.

ADMINISTRATION OF LABELED COMPOUNDS: Glycine-2-C¹⁴, DL-serine-3-C¹⁴, sodium formate-C¹⁴ and formaldehyde-C¹⁴ were fed to separate groups of

radish plants in the nutrient solution. These compounds had been shown to be readily absorbed by various plants through the root system. The plants were placed individually in 100 ml wide-mouth cylinders which were wrapped with black paper to shield their contents from light. On the first day of a 9-day feeding period, each plant received 0.0134 millimoles of radioactive compound having 6.09×10^4 counts per minute. At the end of the 9-day period, the plants were harvested and pectinic acid was isolated from each group of radishes using the method of Kertesz (2).

The incorporation of carbon-14 in the methyl ester group was ascertained by demethylation of pectinic acid as described by Sato and co-workers (4). In the demethylation procedure the methyl esters were hydrolyzed, and the resulting methanol then converted to BaCO₃. Experiments indicated that no scission of S-methyl, N-methyl or methyl ether groups occurred under the conditions of the demethylation procedure. Although no test for purity of the pectinic acid was carried out, the assumption was made that only methyl esters of pectic substances were hydrolyzed and that possible contaminants, such as protein, yielded no methanol in the procedure.

All radioactivities were measured with either the end-window Geiger-Müller tube or an internal flow-tube. The efficiency of the end-window tube as used was 2%, and the other 20%.

RESULTS

The radioactivities of pectinic acid isolated from different groups of plants administered various radio-

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TABLE I
COMPARISON OF VARIOUS COMPOUNDS AS METHOXYL GROUP PRECURSORS FOR PECTINIC ACID

EXP. NO. (NO. OF PLANTS)	COMPOUNDS FED	ACTIVITY OF PECTINIC ACID CPM/100 MG $\times 10^{-3}$	ACTIVITY OF METHYL ESTER CARBON (AS BaCO ₃) FROM 100 MG PECTINIC ACID CPM $\times 10^{-3}$	% ACTIVITY IN METHYL ESTER GROUP OF PECTINIC ACID
1. (40)	Na-formate-C ¹⁴ *	9.57	7.75	82
2. (40)	Na-formate-C ¹⁴	13.2	9.40	75
3. (25)	Glycine-2-C ¹⁴ **	4.55	3.73	82
4. (25)	Glycine-2-C ¹⁴	4.18	3.08	73
5. (25)	Serine-3-C ¹⁴ ***	2.74	1.17	43
6. (23)	Serine-3-C ¹⁴	2.71	0.75	27
7. (12)	Formaldehyde-C ¹⁴ †	9.07	7.57	83
8. (21)	Formaldehyde-C ¹⁴	6.16	5.24	85

* Obtained from Technical Associates, Burbank, California.

** Obtained from Tracerlab, Inc., Boston, Massachusetts.

*** Obtained from California Foundation for Biochemical Research.

† Obtained from Isotopes Specialties Co., California, and from Volk Radiochemical Co., Chicago, Illinois.

active nutrients are presented in table I. Since the molecular weight of pectinic acid is not known, its radioactivity is expressed as counts per minute per 100 mg. The radioactivity of pectinic acid methyl ester carbon, counted as BaCO₃, is also presented in table I. The radioactivity of BaCO₃ is expressed as counts per minute obtained from 100 mg pectinic acid. All counts were corrected for self-absorption of the sample.

A comparison of the extent of incorporation of labelled carbon of the various compounds into pectinic acid reveals that the carbon of both formate and formaldehyde is introduced to a greater extent and the alpha-carbon of glycine and the beta-carbon of serine to a lesser extent. When methionine-methyl-C¹⁴ was administered to a group of radish plants (4) pectinic acid was about as radioactive as it was following administration of glycine-2-C¹⁴. The variation in radioactivity of pectinic acid between duplicate experiments after feeding a single material might have been due to seasonal influences in rate of growth and metabolism.

Except after feeding DL-serine-3-C¹⁴, the majority of the radioactivity of the pectinic acid could be recovered upon demethylation of pectinic acid. No attempt was made to determine the position in the pectinic acid molecule of the radioactive carbon not located in the methoxyl groups.

DISCUSSION

The general metabolic pattern of formation of methyl ester groups of pectinic acid in the radish plant appears to be similar to the formation of the N-methyl group of nicotine and of O-methyl group of lignin in tobacco plants. However, certain specific differences are noted. The carbon of formate was incorporated into methyl ester groups to a much greater extent than into N-methyl or O-methyl groups. Furthermore, the β -carbon of serine was more randomized in entering pectinic acid than in entering nicotine or lignin in tobacco plants. These findings may represent a difference between metabolism in radish

and tobacco plants or, on the other hand, may represent differences in the biosynthesis of methyl ester groups and N-methyl or O-methyl groups.

Bonner and co-workers (3) recently have suggested that indoleacetic acid exerts its effect on plant growth by increasing the rate of transfer of methyl groups from methionine to form pectin methyl esters. Presumably, the esterified pectin allows for expansion of the cell wall as water is taken in by the cell. Since the present study shows that pectinic acid may be methylated by a process other than by transmethylation from methionine, it would be of interest to measure the effect of indoleacetic acid on the rate of methylation of pectinic acid by glycine, serine, formate and formaldehyde.

SUMMARY

The labelled carbons of glycine-2-C¹⁴, serine-3-C¹⁴, formaldehyde-C¹⁴ and formate-C¹⁴ were incorporated in pectinic acid in radish plant metabolism. From 70 to 80 % of the radioactivity of pectinic acid was found in the methyl ester carbon after feeding glycine, formaldehyde and formate, whereas, about 30 to 40 % of the radioactivity was in the methyl ester carbon after feeding serine. When these methyl precursors were fed under similar conditions, formate was incorporated into methyl esters to the greatest extent followed in order by formaldehyde, glycine and serine.

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