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SHORT COMMUNICATION

# BIOSYNTHESIS OF D-1-*O*-METHYL-*muco*INOSITOL IN GYMNOSPERMS

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**Abstract**—Chase experiments with $^{14}CO_2$ and application of labelled precursors show that D-pinitol is epimerized to D-1-*O*-methyl-*muco*inositol in needles of gymnosperms.

## INTRODUCTION

THE BIOSYNTHETIC interconversion of inositols and the biosynthesis of *O*-methyl inositols have been under investigation for some time.[1] It has been shown that epimerization of *myo*inositol is a key reaction by which other inositols and their derivatives arise. Inositol methyl ethers are synthesized by methylation of the parent inositol with *S*-adenosyl-methionine after initial epimerization of *myo*inositol.[2] The formation of D-pinitol seems to constitute up to now the only known exception to this general pathway insofar as this cyclitol arises by epimerization of an already methylated inositol, sequoyitol.[3] The data in this paper demonstrate that D-pinitol can be epimerized to give D-1-*O*-methyl-*muco*inositol. The latter has recently been shown to occur in many gymnosperms and some angiosperms.[4]

## RESULTS AND DISCUSSION

The investigation of the biosynthesis of methyl-*muco*inositol was preferentially carried out by two types of experiments: first by chase experiments using a pulse label of $^{14}CO_2$ followed by exposure to $^{12}CO_2$ and second, by applying labeled precursors.

### *Chase Experiments with* $^{14}CO_2$

The plants used were *Juniperus communis* and *Taxus baccata.* Young shoots o these plants were allowed to assimilate $^{14}CO_2$ for 6 hr and were exposed thereafter to the $^{12}CO_2$ of normal air. During a period of 115 days, samples of needles were taken and assayed for the distribution of label. The results obtained are given in Figs. 1 and 2.

In *Juniperus communis* (Fig. 1), *myo*inositol is turned over very rapidly, as indicated by the sharp decrease of its radioactivity during chase conditions. Although some *myo*inositol may be converted to lipids or to galacturonic acid derivatives, the data suggest that much of it is methylated to sequoyitol. Figure 1 shows that the radioactivity in *myo*inositol decreases from the beginning of the chase, while that in sequoyitol reaches a maximum at 5 days. Thereafter the label appears in D-pinitol, showing a maximum at 14 days. In contrast to *myo*inositol as primary precursor and sequoyitol and pinitol as obvious intermediates,

[1] H. KINDL and O. HOFFMANN-OSTENHOF, *Progress Chem. Org. Nat. Prod.* **24**, 149 (1966).
[2] I. WAGNER, H. HOFMANN and O. HOFFMANN-OSTENHOF, *Hoppe-Seyler's Z. Physiol. Chem.* **350**, 1460 (1969).
[3] R. SCHOLDA, G. BILLEK and O. HOFFMANN-OSTENHOF, *Monatsh. Chem.* **95**, 1311 (1964).
[4] P. DITTRICH, M. GIETL and O. KANDLER, *Phytochem.* **11**, 245 (1972).

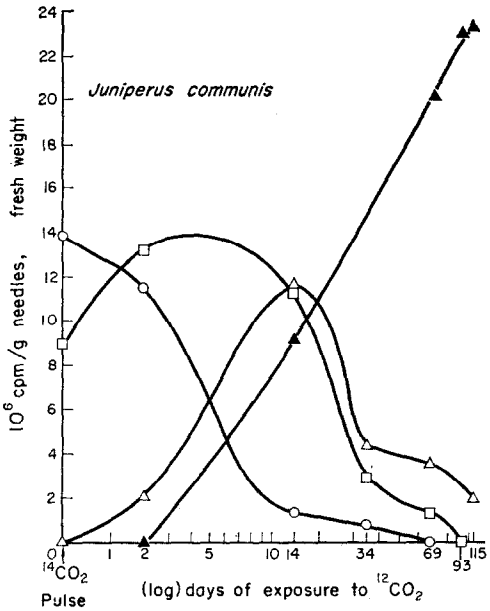


FIG. 1. CHASE EXPERIMENT WITH *Juniperus communis*, PULSE LABELED WITH $^{14}CO_2$ ON JUNE 4.

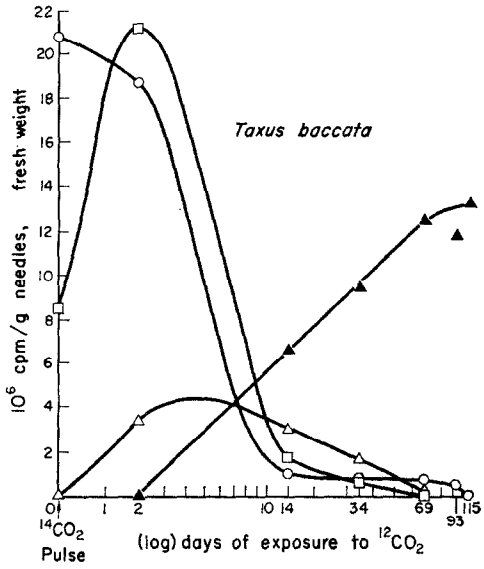


FIG. 2. CHASE EXPERIMENT WITH *Taxus baccata*, PULSE LABELED WITH $^{14}CO_2$ ON JUNE 2.

○, *myo-inositol;* □, sequoyitol; △, D-pinitol; ▲, D-1-*O*-methyl-*muco*inositol.

D-1-*O*-methyl-*muco*inositol is the end product of two subsequent epimerizations as it accumulates during the course of our observations (June–Sept.).

The data obtained from the experiment with *Taxus baccata* lead to the same conclusions (Fig. 2). The radioactivity in D-1-*O*-methyl*muco*inositol also increases as that in sequoyitol and D-pinitol decreases. In contrast to the experiment with *Juniperus*, D-pinitol accumulates much less radioactivity, probably due to a smaller pool size of this cyclitol in *Taxus*. Whereas the pool of pinitol in *Juniperus* contains about 100 μmol/g fr. wt, it is about 30 μmol/g in the needles of *Taxus*, thus favoring a quicker turnover of radioactivity.

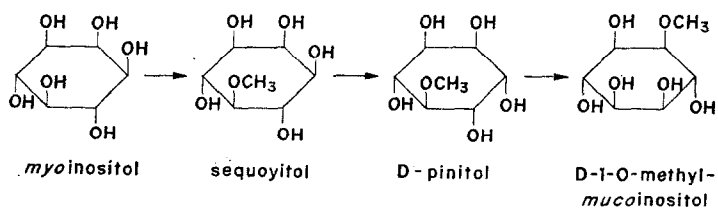


FIG. 3. PROPOSED ROUTE FOR THE FORMATION OF D-1-*O*-METHYL *muco*INOSITOL.

From purely structural considerations, it might instead be expected that D-1-*O*-methyl-*muco*inositol arises by methylation of *mucoinositol* or by epimerization of L-quebrachitol or D-1-*O*-methyl-D-inositol. In course of these chase-experiments none of these compounds, however, could be detected. Their absence, together with the data reported, point to a close relationship of D-pinitol and D-1-*O*-methyl-*muco*inositol as indicated in Fig. 3.

*Feeding Experiments*

Two young shoots of *Taxus* (5 May) were fed $^{14}$C-*myo*inositol and $^{14}$C-D-pinitol respectively, for a period of 6 days. Thereafter they were extracted and analysed for radioactive metabolic compounds.

Table 1 shows the distribution of $^{14}$C after feeding the labeled precursors. The application of *myo*inositol gives rise to the whole series of inositols present in the needles of *Taxus*. The main part of the metabolized radioactivity is found in D-1-*O*-methyl-*muco*inositol, while the other inositols contain less $^{14}$C in accord with their position in the pathway. Thus the role of D-1-*O*-methyl-*muco*inositol as an accumulating product of the metabolism of inositol-methylethers is apparent, supporting our interpretation of the chase experiment above.

TABLE 1. DISTRIBUTION OF RADIOACTIVITY IN METABOLIC PRODUCTS AFTER APPLICATION OF U-$^{14}$C-*myo*INOSITOL AND U-$^{14}$C-D-PINITOL TO *Taxus baccata*

| Precursor | %* of radioactivity found in | | | | | | |
|---|---|---|---|---|---|---|---|
| | D-1-*O*-Methyl-*muco*inositol | D-Pinitol | Sequoyitol | D-Inositol | Sucrose | Citric acid | Malic acid |
| *myo*inositol† | 42·4 | 18·0 | 11·9 | 1·5 | 5·3 | 4·5 | 0·6 |
| D-Pinitol‡ | 98·4 | 0 | 0 | 0·9 | 0 | 0·7 | 0 |

* Because of the uncertainty about the actual amount of fed precursor which penetrates to site of enzymatic interconversion, percentages were calculated on the basis of total activity found in all radioactive compounds except the fed precursor.

† *myo*Inositol taken up 4 100 000; converted 2 480 000 dpm.

‡ D-Pinitol taken up 9 130 000; converted 3 304 000 dpm.

The presence of other compounds labeled to a minor extent, like sucrose and some acids related to the Tricarboxylic Acid Cycle, is certainly caused by the catabolism of *myo*inositol. The variety of metabolic products however is drastically reduced when D-pinitol is applied as precursor (Table 1). D-1-*O*-Methyl-*muco*inositol contains nearly all the radioactivity metabolized, while citric acid and D-inositol are present in very small amounts. The latter has been previously shown to be a demethylation product of D-pinitol.[1] The appearance of radioactive citric acid cannot yet be explained.

This experiment provides the final proof that D-pinitol is actually the precursor of D-1-*O*-methyo-*muco*inositol. The already known sequence of biosynthesis from *myo*inositol to D-pinitol via sequoyitol can now be extended to D-1-*O*-methyl-*muco*inositol as demonstrated in Fig. 3.

## EXPERIMENTAL

The plant material was obtained from the Botanical Garden, Munich. Paper chromatography and extraction procedures were as described previously.[4]

*Chase experiment.* An intact twig of *Juniperus communis* or of *Taxus baccata* on a tree growing out of doors was enclosed in an air-tight plastic bag (vol. 100 ml), along with a beaker containing 500 $\mu$CiBa$^{14}CO_3$. By injection of phosphoric acid through a thin tube into the beaker, $^{14}CO_2$ was liberated. The light intensity during $^{14}CO_2$ assimilation was in the range of 50–70 000 lx. After 6 hr the bag was removed and samples of 6 needles, taken at random, were collected over a period of 115 days.

*Feeding experiment.* Young shoots of *Taxus baccata* (fr. wt about 40 mg) were placed in a small test tube containing an aqueous solution of the labeled inositols. When the solution had been consumed,

distilled water was added. After 6 days the shoots were washed with water, extracted and assayed for radioactive products.

*Origin of radioactive inositols.* $^{14}$C-*myo*-inositol and $^{14}$C-D-pinitol were obtained from *Trifolium incarnatum* after two days of photosynthesis in $^{14}CO_2$ followed by a $^{12}CO_2$ chase period of 6 days. The inositols were isolated by repeated one-dimensional paper chromatography.[4] Prior to chromatography, interfering sugars were converted to their osazones or hydrazones by usual procedures.

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*Key Word Index—Juniperus communis Cupressaceae; Taxus baccata;* Texaceae; biosynthesis; D-1-*O*-methyl- *muco*inositol, D-pinitol epimerization.