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NOVEL BIOSYNTHESIS OF D-PINITOL IN *SIMMONDSIA CHINENSIS*

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Abstract—Chase experiments with $^{14}CO_2$ and feeding experiments with labelled inositols showed that D-pinitol in leaves of *Simmondsia chinensis* arises via epimerization of D-ononitol This finding represents an alternative pathway, since D-pinitol is formed in gymnosperms and other plants by epimerization of sequoyitol

INTRODUCTION

D-Pinitol (5-*O*-methyl-D-*chiro*-inositol) is one of the most abundant cyclitols in plants Practically all gymnosperms and many other plant families sometimes contain considerable concentrations of this cyclitol in leaves or wood The pathway by which D-pinitol is formed has been elucidated independently in gymnosperms, Leguminosae and Asclepiadaceae [1] The reaction sequence was found to proceed by methylation of *myo*-inositol and subsequent epimerization of the resulting sequoyitol to yield D-pinitol During the present phytochemical studies on *Simmondsia chinensis* (jojoba), we found that the leaves of this economically important desert shrub contain D-pinitol, but no traces even of the mandatory precursor sequoyitol The following report describes the occurrence of a novel biosynthetic pathway via D-ononitol for the formation of D-pinitol

RESULTS AND DISCUSSION

The carbohydrate and cyclitol metabolism was investigated on a long-term basis in leaves of *Simmondsia chinensis* using a pulse–chase experiment with $^{14}CO_2$, which was assimilated via photosynthesis, the chase period followed by exposure to natural $^{12}CO_2$ for 2 months In the course of this kinetic study it was found that *myo*-inositol and ononitol were turned over consecutively, while pinitol clearly accumulated This sequence of synthesis, turnover and accumulation, obviously reflects a biogenetic relationship, in a similar mode the biosynthesis of D-1-*O*-methyl-*muco*-inositol had been elucidated in gymnosperms [2] However, in the present study the expected precursor of pinitol, namely sequoyitol, could not be detected Instead, ononitol showed the required kinetic behaviour suitable for a precursor of pinitol

As depicted in Fig 1, it is feasible from a theoretical standpoint to convert ononitol to pinitol via inversion of the same hydroxyl group that would have to be inverted if the pathway proceeded via sequoyitol Since pinitol and ononitol can occur in D- or L-configuration, the more abundant pinitol was isolated from *S chinensis* leaves in order to elucidate its stereochemical nature The crystallized pinitol showed an optical activity of $[\alpha]_D^{20} + 63°$, which is in accord with D-pinitol In order to validate the biogenetic relationship between the two methylated inositols, ononitol should also occur in the D-form Supported by the stereochemical feasibility and the results from the $^{14}CO_2$ pulse–chase experiment, two feeding experiments with labelled cyclitols were carried out to confirm the proposed formation of D-pinitol via D-ononitol *myo*-[U-^{14}C]Inositol was infiltrated via the transpiration stream into a young leaf of *S chinensis* Samples taken daily were analysed for metabolic products The data are compiled in Table 1 Accordingly, *myo*-inositol was converted first to D-ononitol, which subsequently was turned over into D-pinitol The low rates of D-*chiro*-inositol which arose are generally considered to be a consequence of demethylation of D-pinitol Since a young leaf was employed in this experiment, about one-third of the recovered label was found in pectic compounds [3]

The direct relationship between D-ononitol and D-pinitol was finally shown when D-[U-^{14}C]ononitol was likewise fed to a young leaf More than two-thirds of the radioactivity converted was recovered in D-pinitol The

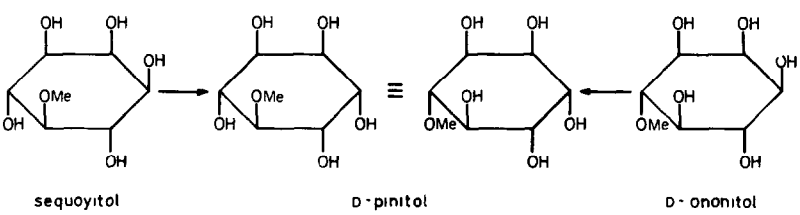

Fig 1 Alternative pathways of D-pinitol formation

Table 1 Metabolites of two feeding experiments with a young leaf of *Simmondsia chinensis* using labelled precursors

Compound fed	*myo*-Inositol				D-Ononitol	
Feeding time (hr)	24	48	72	120	12	36
Metabolites						
myo-Inositol	26 0	13 0	6 1	4 0	2 0	1 5
D-Ononitol	18 0	13 0	6 9	4 3	13 2	7 0
D-Pinitol	20 7	32 0	45 0	57 2	66 2	73 3
D-*chiro*-Inositol	2 0	5 1	3 8	1 5	7 2	8 2
Galacturonic acid	5 1	2 9	0	0	0	0
Pectic compounds	28 0	33 0	38 0	33 0	11 2	9 5

Data given in % of total radioactivity recovered from the leaf sample Compounds fed were uniformly labelled with ^{14}C

remaining label rests in degradation products like *myo*-inositol or D-*chiro*-inositol or in polymers like pectic compounds

From these data it is quite clear than in addition to the well-established sequence sequoyitol → D-pinitol, an alternative pathway via D-ononitol exists Interestingly, the biosynthesis of D-pinitol via sequoyitol has also been shown to operate in *Ononis spinosa* [1] This leguminous plant, however, not only contains considerable amounts of D-ononitol, but also gave its name to this cyclitol, thus, one might be tempted to speculate that both pathways occur together in the same plant

EXPERIMENTAL

PC, radioautography and high-voltage electrophoresis for compound identification and analysis were carried out as previously described [4]

Chase expt An intact branch of a potted plant of a 2-year-old *Simmondsia chinensis* was sealed into a transparent plastic bag of *ca* 100 ml vol Gaseous $^{14}CO_2$ (100 μCi) was injected with a syringe into the bag and the plant was exposed to natural sunlight of *ca* 50 klx for 4 hr Subsequently, the bag was removed and the plant allowed to carry on with photosynthesis under natural $^{12}CO_2$ Leaf samples were taken at intervals of 3 days for a period of 2 months

Feeding expt A short shoot section with a young leaf was placed in a small test-tube containing the aq soln of either 10 μCi *myo*-[U-^{14}C]inositol or 2 μCi D-[U-^{14}C]ononitol When the soln had been consumed, distilled H_2O was refilled Leaf sections were cut out at intervals and analysed for radioactive products

Isolation of D-*pinitol* Fresh leaves of *Simmondsia chinensis* (3 kg) were extracted with hot H_2O The conc extract was hydrolysed with 2 M HCl at 100° for 3 hr The ppt was filtered and the clear soln subjected to osazone and phenylhydrazone formation After filtration the soln was deionized and filtered through activated carbon The clear filtrate was concd and chromatographed on cellulose powder according to ref [5] D-Pinitol crystallized from 96% EtOH Yield 10 2 g, mp 184–186°, $[\alpha]_D^{20}$ +63° (H_2O, *c* 5)

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