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SHORT COMMUNICATION

FORMATION OF L-QUEBRACHITOL FROM D-BORNESITOL IN LEAVES OF *ACER PSEUDOPLATANUS*

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Abstract—D-Bornesitol and L-quebrachitol have been found in the leaves of *Acer pseudoplatanus* L. The results of incorporation studies using labeled *myo*-inositol-^{14}C, L-inositol-^{14}C and D-bornesitol-^{14}C indicate that L-quebrachitol is produced by epimerization of D-bornesitol. In *Artemisia vulgaris*, however, the precursor of L-quebrachitol is L-inositol.

INTRODUCTION

L-QUEBRACHITOL (2-*O*-methyl-L-inositol) was isolated in 1889 from the bark of *Aspidosperma quebracho-blanco* by Tanret.[1] Its presence in leaves of *Acer pseudo-platanus* and *Acer platanoides* was shown by Plouvier[2] and Stinson *et al.*[3] found L-quebrachitol in maple syrup. Previous investigations of the biosynthesis of L-quebrachitol have shown good incorporation from L-inositol in *Artemisia vulgaris*.[4] It was concluded, therefore, that, in the conversion of *myo*-inositol to L-quebrachitol, epimerization is the first step which is followed by methylation at position 2. A second possible biosynthetic pathway, mentioned by Scholda,[4] is the methylation of *myo*-inositol followed by epimerization of the resulting 1-*O*-methyl-*myo*-inositol (D-bornesitol). Until now, this second pathway has not been shown to occur in any plant. The results described in this communication, however, show that in the leaves of *Acer pseudo-platanus* the synthesis of L-quebrachitol occurs in fact via the second pathway.

RESULTS

Identification of D-*Bornesitol in* Acer pseudo-platanus

When leaves of *Acer pseudo-platanus* were allowed to fix $^{14}CO_2$ for several hours, the paper chromatograms of the extract showed an unknown radioactive spot. Its R_f values were similar to D-bornesitol. However, this methyl inositol is not known to occur in *Acer* and its identity was determined by co-chromatography in 4 solvent systems with an authentic sample. In addition, the demethylated product was shown to be identical with *myo*-inositol by co-chromatography. Configuration of the bornesitol was determined by comparative feeding experiments described in the next section. As shown in Table 2, D-bornesitol from *Myosotis* and the bornesitol from *Acer* were converted to L-quebrachitol and an unknown compound to the same extent, while L-bornesitol remained unchanged. This demonstrated that the bornesitol from *Acer* is of the D-configuration.

[1] C. TANRET, *Compt. Rend.* **109**, 908 (1889).
[2] V. PLOUVIER, *Compt. Rend.* **224**, 1842 (1947).
[3] E. E. STINSON, C. J. DOOLEY, J. M. PURCELL and J. S. ARD, *Agric. Food. Chem.* **15**, 394 (1968).
[4] R. SCHOLDA, G. BILLEK and O. HOFFMANN-OSTENHOF, *Monatsh. Chem.* **95**, 541 (1964).

Kinetics of Labeling of Cyclitols in Acer pseudo-platanus

Young attached leaves of *Acer pseudo-platanus* were allowed to photosynthesize in an atmosphere of 0·1% $^{14}CO_2$ and air for 4 and 8 hr in a plastic bag out of doors. After removal of the bag, the leaves were allowed to continue to grow under normal conditions for 5–90 days, parts of the leaves being removed and analysed as shown in Table 1. After 4 hr, *myo*-inositol and D-bornesitol are labeled, while L-quebrachitol contained measurable

TABLE 1. DISTRIBUTION OF ACTIVITY AMONG THE INOSITOLS ISOLATED FROM THE LEAVES OF *Acer pseudo-platanus*

Exposure time	*myo*-inositol		D-bornesitol		L-quebrachitol	
	%*	dpm/10 mg Fr. wt.	%	dpm/10 mg Fr. wt.	%	dpm/10 mg Fr. wt.
4 hr $^{14}CO_2$	0·7	43 486	0·6	37 273	0	0
8 hr $^{14}CO_2$	1·7	216 613	3·0	382 259	1·8	229 355
8 hr $^{14}CO_2$ + 5 days $^{12}CO_2$	1·1	61 313	0	0	9·7	540 677
8 hr $^{14}CO_2$ + 10 days $^{12}CO_2$	0·7	20 350	0	0	11·0	319 794
8 hr $^{14}CO_2$ + 90 days $^{12}CO_2$	0	0	0	0	2·3	16 241

* % of ^{14}C in the soluble fraction.

radioactivity only after 8 hours. In the further course of the chase experiment, the radioactivity in L-quebrachitol increased up to 5 days and then decreased. This indicates that this cyclitol is either translocated or further metabolized. L-inositol which was expected to occur as an intermediate of L-quebrachitol biosynthesis according to the experiments with *Artemisia*, was not detected in our experiments with *Acer*. Instead D-bornesitol behaved like an intermediate.

Feeding Experiments with Acer *Leaves*

In order to further elucidate the biosynthetic pathway of L-quebrachitol, we fed various possible precursors to *Acer* leaves. The results obtained are summarized in Table 2. Feeding of *myo*-inositol-^{14}C yielded radioactive D-bornesitol, L-quebrachitol and an unknown compound which is most likely a derivative of D-bornesitol. (No final data in this compound, X, are yet available.) Again D-bornesitol behaves like an intermediate, while L-quebrachitol and compound X behave like end products. L-Inositol was not labeled.

D-Bornesitol from *Myosotis vulgaris* as well as bornesitol from *Acer* are readily converted to L-quebrachitol and to a much lesser extent to compound X. In contrast, however, L-bornesitol from *Vinca minor* remained completely unchanged. L-inositol was converted to L-quebrachitol in significant amounts, but to a much less extent than was D-bornesitol. No compound X was formed from L-inositol.

These data show that D-bornesitol is an intermediate in the biosynthesis of L-quebrachitol in *Acer*. Although L-inositol is slowly converted to quebrachitol when applied to the leaves, it is not labeled during photosynthesis in $^{14}CO_2$ or during feeding of labeled *myo*-inositol. This indicates that D-bornesitol is the actual intermediate in normal conditions.

TABLE 2. DISTRIBUTION OF RADIOACTIVITY AMONG THE VARIOUS CYCLITOLS IN *Acer pseudo-platanus* AFTER APPLICATION OF *myo*-INOSITOL, D-BORNESITOL, L-BORNESITOL AND L-INOSITOL

Compound applied	Compound isolated	Incorporation of radioactivity 21 hr		44 hr	
		dpm	%*	dpm	%
U-^{14}C-*myo*-inositol	D-bornesitol	6480	8·7	1350	1·8
	L-quebrachitol	6910	9·3	44 840	61·0
	Compound X	1350	1·8	7700	10·8
U-^{14}C-bornesitol from *Acer*	L-quebrachitol	28 650	19·1	151 114	76·1
	Compound X	1380	0·7	10 280	5·2
U-^{14}C-D-bornesitol from *Myosotis*	L-quebrachitol	27 600	22·8	95 170	79·0
	Compound X	6860	5·7	8220	6·8
U-^{14}C-L-bornesitol from *Vinca*	L-quebrachitol	0	0	0	0
	Compound X	0	0	0	0
U-^{14}C-L-inositol	L-quebrachitol	1270	0·7	7920	4·7
	Compound X	0	0	0	0

* % of precursor applied.

Feeding Experiments with Leaves of Artemisia vulgaris

Since Scholda *et al.*[4] had only applied L-inositol and not D-bornesitol to leaves of *Artemisia*, experiments were also carried out with this plant. When leaves of *Artemisia vulgaris* were allowed to photosynthesize in $^{14}CO_2$ for several hours, labeled L-inositol as well as *myo*-inositol and L-quebrachitol were found, but no radioactive D-bornesitol. The same is true when *myo*-inositol-U-^{14}C is fed as shown in Table 3. In contrast to leaves of *Acer*, D-bornesitol is not converted to L-quebrachitol, while feeding of L-inositol results in high yields of L-quebrachitol. No compound X could be detected.

TABLE 3. RADIOACTIVITY IN THE ISOLATED L-INOSITOL AND L-QUEBRACHITOL IN *Artemisia vulgaris* AFTER APPLICATION OF *myo*-INOSITOL, L-INOSITOL AND D-BORNESITOL

Compound applied	Compound isolated	Incorporation of radioactivity 16 hr		60 hr	
		dpm	%*	dpm	%
U-^{14}C-*myo*-inositol	L-inositol	1210	3·0	1720	4·8
	L-quebrachitol	650	1·6	2460	6·9
U-^{14}C-L-inositol	L-quebrachitol	6780	43·0	12 050	57·2
U-^{14}C-D-bornesitol	L-quebrachitol	0	0	0	0

* % of precursor applied.

DISCUSSION

The data show clearly that two different pathways of biosynthesis of L-quebrachitol exist. While in *Artemisia*, epimerization of *myo*-inositol preceeds the methylation of L-inositol, methylation of *myo*-inositol to D-bornesitol followed by the epimerization of the

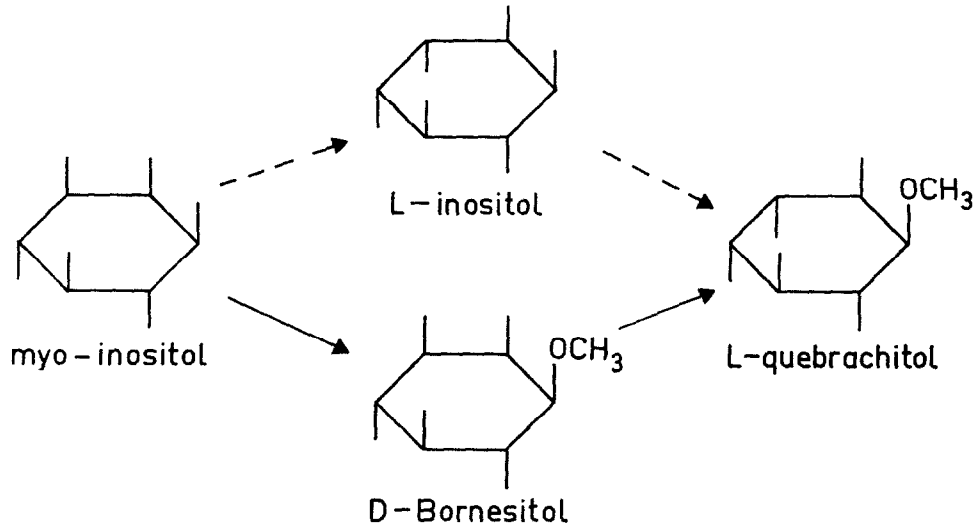

FIG. 1. THE BIOGENETIC RELATIONSHIP OF THE CYCLITOLS IN *Acer pseudo-platanus* ——→ AND *Artemisia vulgaris* – – –→.

latter is the pathway in *Acer* (Fig. 1). Although the *Acer* leaves are able to methylate L-inositol to some extent, they are obviously unable to epimerize *myo*-inositol to L-inositol, since no labeled L-inositol could be found either after photosynthesis in $^{14}CO_2$ or after feeding of *myo*-inositol. Therefore it seems likely that only the pathway via D-bornesitol occurs in *Acer* leaves. The failure to epimerize labeled D-bornesitol by leaves of *Artemisia* as well as the absence of labeled D-bornesitol after photosynthesis in $^{14}CO_2$ demonstrates that the biosynthesis of L-quebrachitol proceeds exclusively via L-inositol in *Artemisia*.

The biosynthesis of L-quebrachitol is a further example of the fact that secondary plant products may be synthesized by different pathways in different taxa.[5] It supports the warning of Mothes[6] that the occurrence of the same secondary plant product in different taxa of the plant kingdom cannot be taken as evidence for a close relationship, as long as the identity of the biosynthetic pathway has not been proven.

EXPERIMENTAL

The ^{14}C-uniform labeled cyclitols, *myo*-inositol, D-bornesitol and L-quebrachitol, were isolated by two dimensional paper chromatography from leaves of *Acer pseudo-platanus* and *Myosotis arvensis* after photosynthesis in $^{14}CO_2$. U-^{14}C-L-inositol was obtained by demethylation of U-^{14}C-L-quebrachitol with HI (30 min at 100°).

The radioactive inositols were applied to leaves by immersing the severed petiols of *Acer pseudo-platanus* and *Artemisia vulgaris* in solutions containing the precursor substances. The leaf samples were extracted with acetone and water and the concentrated extract was chromatographed on filter paper (Whatman No. 1). The following solvent systems were used for paper chromatography: *Iso*PrOH–HOAc–H_2O (75:10:15); EtOAc–*n*-BuOH–HOAc–H_2O (3:4:2·5:4); BuOH–H_2O (15:1) and Propionic acid–H_2O (352:448) (Equal volumes mixed immediately before use).

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[5] R. DORAND and M. H. ZENK, *Tetrahedron Letters* **32**, 3009 (1971).
[6] K. MOTHES, *Naturwissenschaften* **21**, 571 (1968).

Key Word Index—Acer pseudoplantanus; Aceraceae; cyclitols; biosynthesis; D-bornesitol; L-quebrachitol.