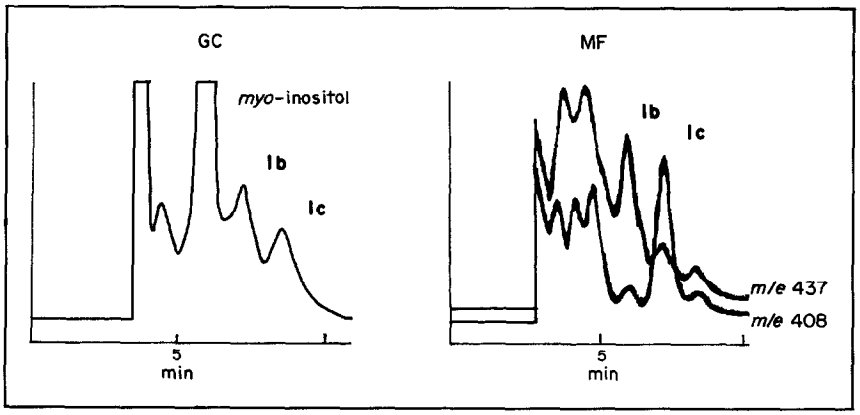

Fig. 1. Gas chromatogram and mass fragmentogram of the trimethylsilylated extract of *C. japonica*.

Co-existence of sedoheptulose and *manno*-heptulose was also proved by MF using the *m/e* 539 ion which is strongly exhibited in the mass spectra of these 2-heptuloses.

The PC data (*n*-BuOH–C_6H_5N–H_2O, 6:4:3; coloured with orcinol–Cl_3CCOOH), obtained after removing fermentable sugars from larger amounts of the extracts of *Coriaria* species by treatment with baker's yeast, were in accord with the above findings.

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D-BORNESITOL ACETATE IN *ACER PSEUDOPLATANUS*

NORBERT SCHILLING, WALTER KUFFER and PETER DITTRICH
Botanisches Institut der Universität, Menzinger Straße 67, 8000 München 19, BRD, W. Germany

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INTRODUCTION

A number of esters of organic acids with *myo*-inositol have been detected in higher plants, such as IAA esters in seeds [1] the acetic acid ester in some Campanulaceae [2] and an ester of *p*-coumaric acid in gymnosperms [3]. During biosynthetic studies [4] leaves of *Acer pseudoplatanus* were found to convert labelled D-bornesitol to L-quebrachitol, and a further unknown compound, which released D-bornesitol on alkaline hydrolysis [5]. Our purpose in this report is to describe the identification of this compound as D-bornesitol acetate in leaves of *A. pseudoplatanus*.

RESULTS AND DISCUSSION

Since the unknown compound was labelled only in the D-bornesitol moiety of the molecule after feeding D-bornesitol [^{14}C] to *Acer* leaves, a different approach towards the elucidation of its structure had to be taken. To obtain the unknown derivative of D-bornesitol uniformly labelled, *Acer* leaves were subjected to photo-

synthesis in $^{14}CO_2$. As indicated in Table 1, label was detected in all major inositols and the derivative of D-bornesitol. After PC isolation, alkaline hydrolysis and rechromatography of the unknown compound, only D-bornesitol, as in the previous experiments, was found to be labelled. Thus it was concluded that the missing moiety, which had to be labelled because of its origin by photosynthesis, was volatile. The chromatographic behaviour of the intact compound (R_f 0.55 in solvent b) suggested that the substituting molecule was small and of an aliphatic nature, comparable to a Me gorup. *O*-Me groups, however, are preferentially eliminated by HI at 100°, whereas our compound was already hydrolyzed by mild alkaline conditions. Thus the presence of an ester bond appeared to be very likely, which is in good agreement with the volatile nature of the substituting moiety. To elucidate the nature of the presumed aliphatic acid, the compound was subjected to alkaline hydrolysis and the resulting products were chromatographed on a column of Si gel designed for the separation of acids. It was found that one ^{14}C-labelled compound co-chromatographed with acetic acid. The only other ^{14}C-product which could be detected was that eluted from the column by H_2O; PC confirmed its identity as D-bornesitol. Thus, the substituting moiety of D-bornesitol was identified as acetic acid, and consequently the novel compound in *A. pseudoplatanus* is an acetic acid ester of D-bornesitol.

In order to obtain further information about the binding site of the acetate group, the ester was subjected to electrophoresis in borate buffer (pH 9.8) [6]. While D-bornesitol exhibited a migration value relative to that of glucose (M_G) of 0.11, the D-bornesitol ester did not show a significantly different value (0.10). Although the ester was sensitive to alkaline conditions, no hydrolysis was observed during electrophoresis, since the radioactivity applied to the paper at the beginning was the same as at the end of the electrophoretic run. From the apparently unchanged complex formation with borate it is concluded that the OH groups at C-1 and C-5 are not substituted [6–8]. If we further assume that according to Kindl and Hoffmann-Ostenhoff [9], the substituents of *myo*-inositol in nature do not occur in axial positions, the OH group at C-2 should also be free. Consequently, it is tentatively suggested that D-bornesitol is esterified with acetic acid via the OH groups at C-4 or C-6. The exact localisation of the binding site, however, will depend on the isolation of larger amounts of D-bornesitol acetate.

Table 1. Distribution of radioactivity among the inositols isolated from the leaves of *Acer pseudoplatanus* after photosynthesis in $^{14}CO_2$

Isolated inositol	%*	cpm/g fr. wt
myo-inositol	0.77	295000
D-bornesitol	1.18	442000
L-quebrachitol	0.55	201000
D-bornesitol-acetate	0.16	59800

* % of total soluble ^{14}C.

EXPERIMENTAL

Five *Acer pseudoplatanus* plants (15 cm high) were exposed to $^{14}CO_2$ (3 mCi, 58 mCi/mmol) for 2 days in 12 hr light (30000 lx) and 12 hr dark. Subsequently, leaves were harvested and extracted 2 × with 70% boiling EtOH. The conc extract was subjected to 2-D PC (Whatman No 3, descending). The following solvents were used: (a) *n*-BuOH–propionic acid–H_2O (7.5:3.5:5.5) and (b) *n*-BuOH–Pyr–HOAc–H_2O (6:4·0.3:3). Radioactivity on the chromatograms was located by autoradiography and determined quantitatively with a CH_4–Ar gas flow counter. D-bornesitol acetate [^{14}C] was eluted and re-chromatographed on paper with Me_2CO–H_2O (4:1). Alkaline hydrolysis (15 min, 15% NH_4OH, 100°) of D-bornesitol acetate yielded D-bornesitol. The demethylation of D-bornesitol was carried out with HI (30 min, 100°). High voltage paper electrophoresis was performed on Whatman No. 1 paper with 0.05 M $Na_2B_4O_7$, according to ref. [6]. For the identification of the HOAc moiety from D-bornesitol acetate, the procedure according to ref. [10] was used. Si gel (12 g) was thoroughly mixed with 7 ml of a 0.025% aq. methyl orange soln and taken up in 80 ml of H_2O satd $CHCl_3$. The suspension was transferred into a glass column (1 × 20 cm) and firmly packed with a glass rod. D-bornesitol acetate (0.2 ml) was hydrolysed with 0.1 ml of 0.1 N KOH for 15 min at 100°, and the soln was supplemented with 0.04 ml HOAc, 0.04 ml HCO_2H and 0.02 ml $HClO_4$. This soln was transferred to the top of the column which was then developed with H_2O satd $CHCl_3$, to which *n*-BuOH was added in successive graduations of 5%. The radioactivity of the emerging fractions was determined with a liquid scintillation counter, and the acidity by titration with 0.1 N NaOH. After HOAc and HCO_2H had appeared in the fractions, the column was eluted with H_2O. The subsequently emerging fractions containing ^{14}C were concentrated, re-chromatographed on paper and identified as D-bornesitol.

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