(+)-PINPOLLITOL: A DI-*O*-METHYL D-(+)-*CHIRO*-INOSITOL FROM *PINUS RADIATA*

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Abstract—(+)-Pinpollitol, a new cyclitol recently isolated from the pollen of *Pinus radiata*, was found in the needles of this species. (+)-Pinpollitol was found to be a di-*O*-methyl ether of D-(+)-*chiro*-inositol, and tentative isomeric structures have been proposed for the cyclitol. (+)-Pinpollitol is the first di-*O*-methyl inositol to be found in a gymnosperm and is one of only three di-*O*-methyl inositols yet found in nature.

INTRODUCTION

The cyclitols D-pinitol and a new di-*O*-methyl inositol named (+)-pinpollitol, were recently found in *Pinus radiata* pollen water diffusate [1]. These cyclitols had previously been detected [1] in a purified *P. radiata* pollen extract fraction called Aq2 exhibiting growth regulating properties [2]. We now wish to report the isolation and partial structure identification of (+)-pinpollitol from *P. radiata* needles.

Although at least 10 naturally occurring mono-*O*-methyl inositols are known [3, 4], dambonitol and liriodendritol were the only di-*O*-methyl inositols hitherto found in nature [3, 5]. The *O*-methyl inositols are widely distributed in higher plants, and it appears that their distribution is of taxonomic value in a number of cases [3, 6–12]. (+)-Pinpollitol is the first di-*O*-methyl inositol to be found in a gymnosperm, and the possible chemotaxonomic significance of its presence in the Pinaceae is briefly discussed.

RESULTS AND DISCUSSION

(+)-Pinpollitol was isolated as a hygroscopic glass from both pollen and needles of *P. radiata*, and formed a non-crystalline tetraacetate. Such non-crystallinity is known for other cyclitols e.g. D- 1-*O*-methyl-*muco*-inositol and its pentaacetate [11, 13]. However, (+)-pinpollitol is readily characterized by its chromatographic properties and its optical rotation.

(+)-Pinpollitol is a di-*O*-methyl inositol. Thus, it is a neutral H_2O-soluble compound which was recovered unchanged following the action of aq. boiling $Ba(OH)_2$ and subsequent deionization, a process which efficiently removes reducing sugars from aqueous solution [14, 15]. The MS of (+)-pinpollitol showed a molecular ion at m/e 208, and high resolution MS established the molecular formula as $C_8H_{16}O_6$. It also formed a TMS ether which was shown to be a tetrakis-TMS ether by high resolution MS. (+)-Pinpollitol could be demethylated with HI to give D-(+)-*chiro*-inositol, thus establishing its identity as a di-*O*-methyl ether of D-(+)-*chiro*-inositol. The NMR of (+)-pinpollitol in D_2O showed signals at τ6·40 and 6·60, attributable to the methyl protons of two non-equivalent methoxyl groups. That (+)-pinpollitol was different from both dambonitol and liriodendritol was clearly shown by direct MS comparison with these compounds (of the free compounds and also their TMS derivatives). As well, the TMS ether derivative of (+)-pinpollitol shows a significantly lower retention time (R_G 0·68, relative to the TMS ether derivative of α-D-glucose as R_G 1·00) on GLC

analysis on SE-30, compared to the TMS ethers of dambonitol (R_G 1·06) and liriodendritol (R_G 0·87).

There are nine possible isomeric di-O-methyl ethers of D-(+)-*chiro*-inositol. However, three of these have equivalent methyl groups owing to the two-fold axis of symmetry in *chiro*-inositol, and since the NMR of (+)-pinpollitol clearly indicated two non-equivalent methyl groups (see above), this leaves six possible structures for (+)-pinpollitol. The NMR (in CDCl₃) of (+)-pinpollitol tetraacetate, prepared by acetylation of (+)-pinpollitol with Ac₂O and NaOAc showed signals at τ6·52 and 6·57 due to the non-equivalent methyl groups of the two methoxyls, and signals at τ7·85 (3H), 7·93 (6H) and 7·97 (3H) attributable to the methyl protons of the acetyl groups. According to a published correlation of chemical shifts of acetyl-methyl protons with the conformational disposition of the acetyl groups of a large number of acetylated carbohydrates [3, 16], these chemical shift values for (+)-pinpollitol tetraacetate allow tentative assignment of the acetyl groups. The signal at τ7·85 appears to represent an axial acetyl group, and that at τ7·97 an equatorial acetyl group. The signal at τ7·93 just falls within the region for equatorial groups, according to the correlation, and a little outside the region for axial groups. These results indicate that (+)-pinpollitol tetraacetate would have only one axial acetyl group, and this would require that one of the methoxyl groups was also axial. The assignment is supported by chemical shift values from published NMR spectra of D-*chiro*-inositol hexaacetate, and quebrachitol pentaacetate in CDCl₃ [16, 17]. Further support was obtained from the NMR spectrum of D-pinitol pentaacetate in CDCl₃, which showed two axial acetyl methyl signals at τ7·85 and three equatorial acetyl methyl signals at τ7·92, 7·97 and 8·00.

The above data limit the number of possible structures of (+)-pinpollitol, and if it is assumed that (+)-pinpollitol arises biosynthetically by methylation of D-pinitol, then (+)-pinpollitol must have either structure (1) or (2). Further chemical

evidence is required to decide between these possibilities.

From the systematic investigations carried out on the O-methyl inositols it appears that the di-O-methyl inositols dambonitol and liriodendritol are much more restricted in their occurrence than the known mono-O-methyl inositols [3, 6]. It is possible that in the same way (+)-pinpollitol is restricted in its distribution too; a preliminary examination has revealed its presence in other members of the Pinaceae.
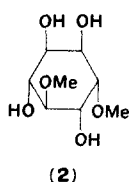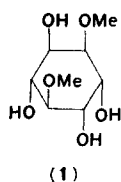
It is known that members of the Pinaceae differ from all other conifers in the distribution of an O-methyl inositol [11]. Thus, in a recent careful survey, Dittrich et al. [11] showed the widespread presence in the gymnosperms of D-1-O-methyl-*muco*-inositol, which was, however, conspicuously absent from members of the Pinaceae. From the results of labelling experiments, Dittrich and Kandler [18] outlined the biosynthetic sequence leading via D-pinitol to D-1-O-methyl-*muco*-inositol. These investigators proposed the possibility that through evolution, members of the Pinaceae had lost the enzyme required to cause conversion (an epimerization) of *d*-pinitol to the *muco*-inositol methyl ether.

These observations are of interest in considering the occurrence of (+)-pinpollitol in a *Pinus* species, and the relationship of (+)-pinpollitol to D-pinitol — the cyclitols are both methyl ethers of D-*chiro*-inositol, and it is thus possible that (+)-pinpollitol arises from methylation of the very widely distributed D-pinitol. In this connection, dambonitol (a *myo*-inositol di-methyl ether) has been shown [19] to be formed biosynthetically via methylation of D-bornesitol (a *myo*-inositol mono-methyl ether), and a methylating enzyme has actually been isolated [20] from pea seedlings, which methylates bornesitol to dambonitol.

(1)   (2)

EXPERIMENTAL

Extraction of needles. Needles, dried at 100° overnight, were ground to a powder, vigorously stirred with cold H₂O (100 g

powdered needles/1·5 l. H_2O), and filtered through Celite 545. The resulting aq. extract was reduced in vol., sat. with an. K_2CO_3 and filtered. The filtrate was then shaken with an equal vol. of 95% EtOH, and the resulting aq. and ethanolic layers separated. The aq. layer was extracted a further 2× with 95% EtOH. Each EtOH layer obtained was filtered and evaporated to a thick syrup. The syrup was dissolved in H_2O and treated with small amounts of Dowex 50W H⁺ ion-exchange resin until the pH was neutral. The neutral solution was filtered from the resin, evaporated to dryness, and the residue sublimed at 140° under *vacuo* (0·1 mm Hg). The sublimate was partitioned between H_2O and EtOAc, and the aq. layer evaporated under *vacuo* and taken to dryness (0·1 mm Hg) to yield (+)-pinpollitol as a colourless hygroscopic glass. This material rapidly absorbed moisture from the air until an equilibrium was established; the syrup had $[\alpha]_D^{20}$ +31° (c 0·44, H_2O); PC: R_f 0·56; MS: M⁺ 208 [found: 208·0952, required for $C_8H_{16}O_6$: 208·0946]; NMR:(D_2O), τ6·40 (3H, *s*) and 6·60 (3H, *s*). (+)-Pinpollitol could also be obtained from crude extracts by repeat preparative PC on Whatman 3MM paper followed by vacuum sublimation.

(+)-*Pinpollitol tetraacetate*, formed by refluxing (+)-pinpollitol (dried at room temp. under vac., 0·1 mm Hg) with Ac_2O–NaOAc was obtained as a non-crystalline gum. MS: M⁺ 376 [found: 376·1379, required for $C_{14}H_{22}O_9$: 376·1367]. NMR: ($CDCl_3$), τ6·52 (3H, *s*), 6·57 (3H, *s*), 7·85 (3H, *s*), 7·93 (6H, *s*), 7·97 (3H, *s*). (+)-Pinpollitol formed a tetra-kis TMS ether; MS: (M⁺–Me) 481 [found: 481·2288, required for C_6H_6(O-Me)$_2$(OSiMe$_3$)$_4$–Me: 481·2292].

Demethylation of (+)-*pinpollitol*, was achieved by refluxing the cyclitol with constant boiling HI for 70 min. Workup of the product yielded a crystalline solid shown to be identical with authentic D-(+)-*chiro*-inositol, by polarimetry, MS, and GLC and GC–MS of its TMS ether derivative.

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