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SHORT COMMUNICATION

ASSIGNMENT OF THE ESTER LINKAGE OF 2-*O*-INDOLEACETYL-*MYO*-INOSITOL ISOLATED FROM *ZEA MAYS*

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Abstract—An ester of indole-3-acetic acid and *myo*-inositol was crystallized from an extract of *Zea mays*. A comparison of the ^{1}H NMR spectrum of the ester with synthetic 2-*O*-acetyl-*myo*-inositol supports the 2-*O*-indoleacetyl-*myo*-inositol structure for the natural product.

THE STRUCTURE 2-*O*-indoleacetyl-*myo*-inositol was tentatively assigned to a bound auxin isolated from *Zea mays* kernels on the basis of solubility and chromatographic properties.[1] Further confirmation of the structure is desirable in view of the increasing number of indoleacetyl esters isolated from *Zea mays*.[2,3] We wish to report NMR data of the model compound 2-*O*-acetyl-*myo*-inositol and the major indole acetyl-*myo*-inositol which confirms the earlier assignment of that structure to the natural product. Improved methods of isolation and purification of these compounds are also recorded.

The NMR data for the equatorial proton summarized in Table 1 and compared with other data from the literature, show that acylation of the axial hydroxyl induces the usual downfield shift in the equatorial proton.

The close correspondence in chemical shift observed for the equatorial proton between the synthetic 2-*O*-acetyl-*myo*-inositol and the naturally-occurring indoleacetyl-*myo*-inositol of slower mobility in paper, thin layer chromatographic systems and higher mobility in a Sephadex G-10 column, coupled with published data for 2-*O* acylated and non-acylated *myo*-inositols, strongly supports the axial ester configuration.

EXPERIMENTAL

Isolation and Purification of 2-O-indoleacetyl-myo-inositol

7 kg of Stowells evergreen hybrid *Zea mays* kernels were ground dry and extracted three times with 14 l. of 1:1 v/v acetone H_2O mixture. In each extract the acetone was salted out of the mixture and the aqueous layer washed twice with fresh acetone. All the acetone extracts were concentrated at room temp. under

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TABLE 1. VALUES OF THE CHEMICAL SHIFT (δ) DOWNFIELD FROM AN EXTERNAL TRIMETHYLSILANE STANDARD FOR THE EQUATORIAL PROTON (H_2) ON THE *myo*-INOSOTOL RING, AND THE COUPLING CONSTANT (J) FOR THE OBSERVED TRIPLET IN HERTZ (Hz)

Substance	Solvent	H_2	JHz	Ref.
Myo-inositol	D_2O	4·07	2·2	
2-*O*-acetyl-*myo*-inositol	D_2O	5 40	2·0	
Indoleacetyl-*myo*-inositol	D_2O	5·40	2·0	
Indoleacetyl-*myo*-inositol	D_2O/CD_3COCD_3(v/v)	5·54	3·0	
1-*O*-benzyl-*myo*-inositol	D_2O	4 24	2·5	
5-*O*-methyl-*myo*-inositol	D_2O	4·01	2·6	4
1,3,4,5,6-penta-*O*-acetyl-2-*O*-benzyl-*myo*-inositol	$CDCl_3$	4·12	2·5	5
1,2,4,5,6-penta-*O*-acetyl-3-*O*-benzyl*myo*inositol	$CDCl_3$	5·80	2·7	5
4,5,6,tri-*O*-acetyl-1,2,3-*O*-tribenzyl-*myo*-inositol	$CDCl_3$	3·95	2·0	5
2,4,5,6-tetra-*O*-acetyl-1-3-di-*O*-benzyl-*myo*-inositol	$CDCl_3$	5·81	2·75	5

reduced pressure and the resulting saline syrup (350 ml) was made acid by adding 35 ml 0 1 N glycine-H_2SO_4 buffer pH 2·5. The acidified extract was washed 3 × 350 ml EtOAc and finally 6 × 350 ml *n*-BuOH. The BuOH washings were centrifuged to remove suspended H_2O, combined and concentrated at 50° under reduced pressure. Results for alkali labile indoleacetic acid (IAA) conjugates determined by the modified Salkowski technique[1] were as follows:— 12·6 mg IAA in EtOAc washings, 54 mg IAA in BuOH washings and 6·4 mg in aqueous residue after BuOH extraction. The EtOAc and BuOH fractions were combined, lyophilized and taken up in H_2O loaded onto Sephadex G-10 (92·5 × 2·5 cm column) and eluted with H_2O (10 ml fractions collected). Fractions 38–65 which contained compounds B_3 and B_4 were combined as described previously[2] and 6·25 mg IAA was found; fractions 67–87 were combined, lyophilized and 26·6 mg IAA was found in 168 mg of lyophilized material; fractions 88–108 were combined, lyophilized and 30·8 mg IAA was found in 141 mg of lyophilized material. Total recovery of IAA-conjugates was 96%.

Chromatographic analysis, using paper and TLC silica gel systems, of the fractions obtained from the G-10 Sephadex column, showed that fractions 67–87 contained only one indoleacetyl ester with mobility corresponding to the material described previously as B_2[2] and tentatively given the structure 2-*O*-indoleacetyl-*myo*-inositol.[1] The indoleacetyl ester found in fractions 88–108 was found to have a faster mobility in the same systems and was comparable to that described for B_1.[2]

A portion of the lyophilized material from fractions 67–87, containing 17 5 mg alkali labile IAA, was re-chromatographed on Sephadex G-10 with H_2O as the eluant and appropriate fractions lyophilized, taken up in 1 ml of 90% acetone, to which 4 ml of dry acetone was added slowly The solution was stood at 0° for 2 hr and centrifuged. The supernatant was concentrated till it was a dark syrup and 0·3 ml H_2O added and as soon as crystallization had started the mixture was chilled to 0° and left in a refrigerator overnight, filtered and washed with ice cold H_2O and dried (yield 12·4 mg of 2-*O*-indoleacetyl-*myo*-inositol, i e. 6·44 mg alkali-labile indoleacetic acid; recovery 37%). This sample was used for NMR spectroscopy.

Synthesis of 2-O-acetyl-myo-inositol

The starting point of this compound was *myo*inositol which was converted into 2-*O*-acetyl-1,3,4,5,6 penta-*O*-benzyl-*myo*-inositol by the route described.[6] The 5·0 g of the 2-*O*-acetyl-1,3,4,5,6 penta-*O*-benzyl-*myo*-inositol was hydrogenated in glacial HOAc over a reduced PdCl/C catalyst. At the end of the hydrogenolysis of the benzyl ethers, the catalyst was centrifuged, washed with water and re-centrifuged. The combined supernatants were evaporated *in vacuo* and the residue taken up in MeOH from which 1 21 g of crystalline 2-*O*-acetyl-*myo*-inositol was obtained by crystallization; m.p. 175–180° (yield 73%). For analysis a sample was recrystallized three times from methanol; m.p. 175–180°. (Found C, 42·9; H, 6·4; Calc for $C_8H_{14}O_7$ C, 43·2; H, 6·3%.)

Sample of ± 1-*O*-benzyl*myo*-inositol used for NMR spectroscopy was prepared by method described by Angyal *et al.*[6]

NMR Spectroscopy

The ^1H NMR spectra were obtained on a Varian 60 MHz NMR spectrometer at room temp; the solvent used was D_2O, and a 1/1 by volume mixture of CD_3COCD_3 and D_2O. The 2-*O*-indoleacetyl-*myo*-inositol

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was prepared for NMR spectroscopy by dissolving in D_2O and evaporating off the D_2O twice to remove the readily exchangeable hydrogen on the hydroxyls of the *myo*-inositol.

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