Phytochemistry 1974 Vol 13 pp 243 to 253 Pergamon Press. Printed in England

STRUCTURE OF INDOLE-3-ACETIC ACID MYOINOSITOL ESTERS AND PENTAMETHYL-MYOINOSITOLS

MINORU UEDA* and ROBERT S BANDURSKI

Department of Botany and Plant Pathology, Michigan State University, East Lansing, MI 48823, U S A

(*Received* 31 *May* 1973 *Accepted* 16 *July* 1973)

Key Word Index—*Zea mays* Gramineae corn, indole-3-acetic acid esters myoinositol, myoinositol glycosides, MS trimethylsilyl ethers pentamethyl-myoinositols myoinositol arabinoside myoinositol galactoside

Abstract—GC–MS properties of three isomeric esters of indole-3-acetic acid and myoinositol three esters of indole-3-acetic acid and myoinositol arabinoside and three esters of indole-3-acetic acid and myoinositol galactoside are presented MS fragmentation patterns for the four possible pentamethyl myoinositols are also shown These data indicated that the arabinose, and galactose of the glycosides were in the pyranose form and that C-1 of the sugar was linked to the 5 hydroxyl of myoinositol Homologies in fragmentation patterns for the esters and the glycoside esters together with knowledge of the properties of 2-*O*-indole-3-acetyl-myoinositol permitted identification of one of the arabinosides as 5-*O*-L-arabinopyranosyl-2-*O*-indole-3-acetyl-myoinositol and one of the galactosides as 5-*O*-D-galactopyranosyl-2-*O*-indole-3-acetyl-myoinositol The remaining two GLC peaks observed for the arabinoside were then, most likely, the two mixtures of diastereoisomers 1 D- and 1 L-5-*O*-L-arabinopyranosyl-1-*O*-indole-3-acetyl myoinositol and 1 D- and 1 L-5-*O*-L-arabinopyranosyl-4-*O*-indole-3-acetyl-myoinositol The remaining two GLC peaks observed for the galactoside would then be the 1 D- and 1 L-5-*O*-D-galactopyranosyl-1-*O*-indole-3-acetyl-myoinositol and 1 D- and 1 L-5-*O*-D-galactopyranosyl-4-*O*-indoleacetyl-myoinositol

INTRODUCTION

PREVIOUS publications from this laboratory have described the isolation and characterization of esters of indole-3-acetic acid (IAA)† and myoinositol, myoinositol arabinoside and myoinositol galactoside [2–6] The purpose of the present report is to present additional GLC and MS properties of the esters isolated from *Zea mays*, to provide data on the linkage of the sugar to myoinositol, and, to provide MS characterizations of the pentamethyl-myoinositols

* This work was performed while on leave from the Sumitomo Chemical Company, Ltd Japan

† *Abbreviations used* IAA (or ROH for figs and tables)—indole-3-acetic acid, RO^-—indole-3-acetate, R^-—the indole-3-acetyl radical, TMS (or T for figs and tables)—trimethylsilyl, TMSOH (or TOH for figs and tables)—trimethylsilanol, M—the molecular ion, G—the sugar moiety of the glycosides, or GOH if the departing group retains a bridge oxygen plus hydrogen I—the inositol moiety

[1] The cyclitol nomenclature used follows IUPAC Tentative Rules (1968) *European J Biochem* **5**, 1 There are 6 possible indole-3-acetyl myoinositols and using this nomenclature they are designated as the axial-2-*O*-ester and the 5 equatorial esters comprised of the 5-*O*-ester and the two enantiomorphic pairs 1 D and 1 L and 4 D and 4 L The GLC peaks observed are most likely attributable to the 2-*O* ester, the 5-*O* ester and the two pairs of enantiomorphs 1 D and 1 L and 4 D and 4 L

[2] LABARCA, C, NICHOLLS, P B and BANDURSKI, R S (1966) *Biochem Biophys Res Commun* **20**, 641

[3] NICHOLLS, P B (1967) *Planta* **72**, 258

[4] UEDA, M and BANDURSKI, R S (1969) *Plant Physiol Suppl* **44**, 27

[5] UEDA, M AND BANDURSKI, R S (1969) *Plant Physiol* **44**, 1175

[6] ULDA, M, EHMANN, A and BANDURSKI R S (1970) *Plant Physiol* **46**, 715

RESULTS AND DISCUSSION

Isomerism of the IAA-inositols and IAA-inositol glycosides

The structures of the 2-*O*-esters of IAA-myoinositol and the IAA-myoinositol glycosides are shown in Fig 1 That one of the isomeric[1] IAA-inositols, isolated from *Zea mays* was the 2-*O*-(axial) ester was suggested by its slower mobility on paper and TLC chromatograms,[2,3] its higher mobility on Sephadex[5] and Dowex columns,[7] and its lower retention times on silicone gum GLC columns[6] Later the axial structure was established by comparison with authentic synthetic material[3] and by NMR comparison of the chemical shift observed for the equatorial proton of the naturally occurring IAA-inositol and synthetic 2-*O*-acetyl-myoinositol[8] The MS fragmentation patterns for the 2-*O*-ester and for two of the three, resolvable, equatorial esters are presented below The third resolvable equatorial ester has recently been observed[7] Based upon ease of formation from the 2-*O*-ester the most abundant equatorial ester may be the mixture of enantiomers DL-1-*O*-indoleacetyl myoinositol The remaining peaks described here and elsewhere[7] would then be 5-*O*-indoleacetyl myoinositol and the mixture of enantiomers DL-4-*O*-indoleacetyl myoinositol

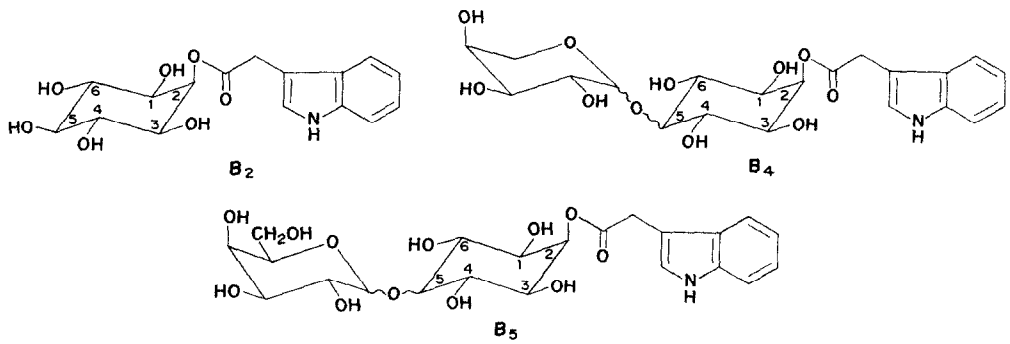

FIG 1 INDOLE-3-ACETIC ACID ESTERS OF MYOINOSITOL

The esters shown are 2-*O*-indole-3-acetyl-myoinositol B_2 5-*O*-L-arabinopyranosyl-2-*O*-indole-3-acetyl-myoinositol B_4, 5-*O*-D-galactopyranosyl-2-*O*-indole-3-acetyl-myoinositol B_5 The figure is adapted from Ehmann and Bandurski[7]

The IAA-myoinositol arabinoside and IAA-myoinositol galactoside of Fig 1 are also shown as 2-*O* esters although NMR characterization and comparison with authentic synthetic IAA-inositol glycoside has not yet been accomplished In view of the low concentrations in which these compounds occur such characterization is presently impossible Moreover, the structures cannot be established by hydrolysis of the glycoside to IAA-inositol since, owing to acyl migration, a mixture of axial and equatorial IAA-inositols results following glycosidase catalyzed removal of the sugar from the glycoside[2] Quantitatively, hydrolysis of the equatorial IAA-inositol arabinoside[6] does lead to the formation of mainly equatorial IAA-inositols[2] and this may provide a means for characterization If the IAA-inositol glycosides behave analogously to the IAA inositols with respect to MS fragmentation patterns then it is possible to identify the axial ester of the glycosides, as is shown below GLC retention times and chromatographic properties, to be published elsewhere support the conclusions from MS fragmentation patterns The

[7] EHMANN A and BANDURSKI R S (1972) *J Chromatog* **72**, 61
[8] NICHOLLS P B ONG B L and TAIT M F (1971) *Phytochemistry* **10**, 2297

remaining two esters observed would then be the DL-1-*O* pair and the DL-4-*O* pair since the 5-*O*-position is occupied by the sugar Thus for IAA-inositol-arabinoside and IAA-inositolgalactoside, 3 isomers are possible (the axial 2-*O* and the two mixtures of diastereoisomers, DL-1-*O*- and DL-4-*O*) and the expected 3 isomers are observed

MS of trimethysilyl-IAA-myoinositols

The three isomeric IAA-inositols studied here gave almost the same ion species, varying only in their relative abundance, as is shown in Table 1 Peak 3 lacks some ions owing to the smaller quantity of this isomer available relative to peaks 1 and 2 but is very similar to peak 2

TABLE 1 RELATIVE ABUNDANCE OF IONS IN THE 70 eV MS OF TRIMETHYSILYL ETHERS OF THE IAA-MYOINOSITOLS*

	Isomers			Ion species	
m/e	Peak-1	Peak-2	Peak-3	Origin	Ref
73	100	100	100	TMS	9
103	8 5	7 5	6 4	I and ROH	9, 11
129	13 0	13 3	9 2	I and ROH	9, 11, Fig 2
130	85 7	54 5	45 2	ROH	11
133	5 6	5 4	7 1	I	9, 10
147	36 3	31 7	30 6	I	9
157	85 8	89 8	106	ROH	9, Fig 2
169	1 8	7 6	1 8		
189	1 6	2 6	3 1	I	9
191	17 8	19 1	13 7	I	9
204	11 1	11 5	7 3	I	9
217	23 7	27 2	22 0	I	9
265	3 0	3 8	–	I	9
271	1 9	2 1	–	I	9
291	2 0	2 5	1 8	I	9
293	2 5	3 4	2 2		
305	7 2	9 8	6 0	I	9
318	20 7	28 1	15 0	I	10
343	5 8	4 6	2 9	I	10
361	0 6	0 9	–		10
390	3 0	2 6	1 3	M–307	Fig 2
403	2 0	0 9	0 9	M–294	Fig 2
417	1 3	2 2	2 0	I	10
433	4 2	4 6	2 8	I	10
502	0 6	0 5	–	M–(Me + 2TOH)	
507	1 8	2 5	1 8	M–(Me + ROH)	
523	0 5	–	–	M–RO	
607	–	6 7	0 7	M–TOH	
682	0 1	0 1	–	M–Me	
697	4 7	5 1	2 0	M	

* Ions originating from inositol, **(I)**, which are analogous to those derived from sugars or indoleacetic acid, ROH have usually been previously described[9 12] M designates the molecular ion TMS is $(Me)_3Si$ TOH is $(Me)_3SiOH$ Ion abundance is normalized to *m/e* 73 = 100%

A striking feature of the MS is the relatively high abundance of the molecular ion as compared to the intensity of the molecular ion of TMS ethers of simple sugars[9,10] and

[9] KOCHETKOV N K and CHIZHOV O S (1966) *Adv Carbohydr Chem* **21**, 39
[10] DEJONGH, D C, RADFORD, T, HRIBAR, J D, HANESSIAN, S, BIEBER, M DAWSON, G and SWEELEY, C C (1969) *J Am Chem Soc* **91**, 7

the cyclitols [11] possibly owing to a stabilizing effect of the IAA moiety The occurrence of ions can be explained by the following reactions (1) Elimination of Me , TMSOH RO or ROH from M (2) Ring cleavage of the inositol moiety of M (3) Degradation of the inositol moiety starting from *m/e* 523 (4) Degradation of the IAA moiety starting from *m/e* 157

The difference in fragmentation patterns among the isomers may be summarized as follows (1) Peak 1 lacks the ion at *m/e* 607 and has instead a peak at *m/e* 523 The ion at *m/e* 523 can be explained by the preferential elimination of IAA Peak 1 is IAA-2-*O*-myoinositol and this preferential elimination of an axial substituent is expected (2) By contrast, peaks 2 and 3 lose a TMSOH more easily than ROH and produce an ion at *m/e* 607 Peaks 2 and 3 are equatorial esters but complete structures can not be assigned Peak 2 may be DL-1-*O*-indoleacetyl-myoinositol since it is most abundant and might be expected to be formed by cis acyl migration (3) The ratio of ion intensities at *m/e* 157 compared to *m/e* 130 is peak 1 < 2 < 3 These ratios, see below, are useful in establishing homologies

The fragmentation path and possible structures of ions which have not previously been described are shown in Fig 2

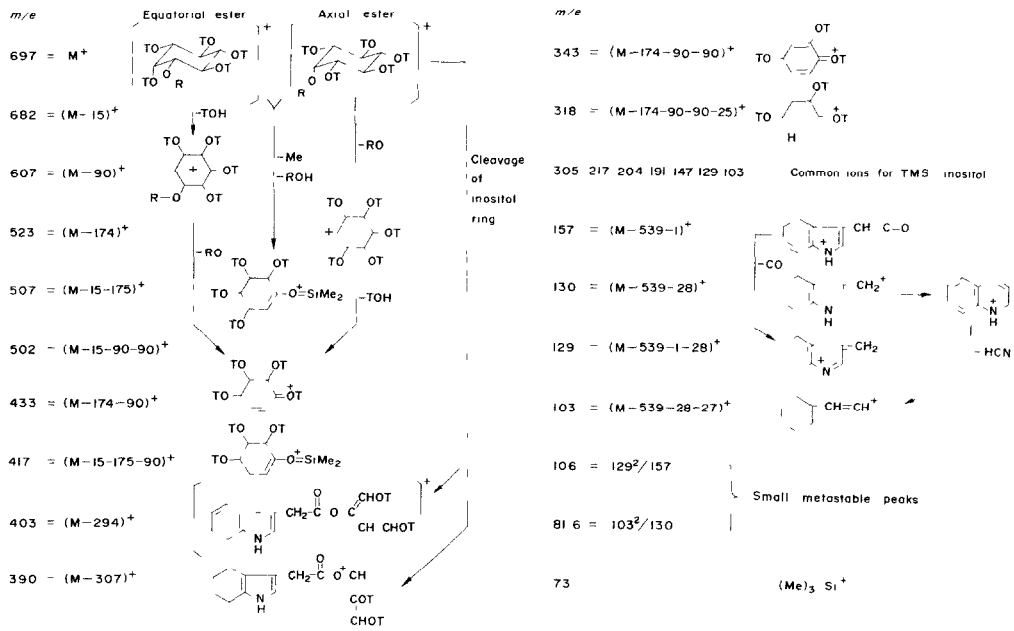

FIG 2 POSSIBLE FRAGMENTATION OF IAA-INOSITOL ESTERS

Fragmentation pathways are shown for the trimethylsilyl ethers of an equatorial DL-1-*O*-ester, and an axial 2-*O*-ester The molecular ion M^+ is at mass 697 since the indolylic nitrogen is not silylated

MS of TMS-myoinositol glycosides

The mass spectra of the TMS-myoinositol glycosides obtained from the IAA-myoinositol glycosides were examined and compared with two known inositol glycosides (Table 2)

[11] SHERMAN W R EILERS N C and GOODWIN S L (1970) *Organ Mass Spec* 3, 829

TABLE 2 RELATIVE ABUNDANCE OF IONS IN THE 70 eV MS OF TRIMETHYLSILYL ETHERS OF THE MYOINOSITOL GLYCOSIDES*

	Glycosides						
	inos-arab	inos-gal	galac-tinol	inos-glu		Ion species	
m/e	G-1	G-2	G-3	G-4	Origin	Type	Ref
73	100	100	100	100	P & H		9
103	11 1	14 4	32 1	16 4	P & H		9
115	2 6	0 6	0 6	0 7	P	K_1	
117	3 5	3 6	3 3	5 1	P & H		9
129	14 9	20 4	18 2	18 0	P & H		9
131	3 0	3 0	3 3	3 7	P & H	H_2	
133	5 4	4 7	4 4	4 8	P & H		9, 10
141	0 7	0 5	0 5	0 7	P & H	C_3	
147	35 8	40 0	37 0	42 2	P & H		9
157	2 8	1 7	2 1	2 4			
161	2 9	1 1	0 9	1 0			
169	1 7	3 2	5 2	3 6	P	A_3	
189	3 4	3 8	3 6	4 1	P & H		9
191	42 1	30 0	37 0	35 9	P & H		9
204	120	226	173	127	P & H		9
217	54 3	50 0	56 2	39 9	P & H		9
221	9 8	8 2	8 2	9 6	P & H		9, 10
230	4 5	4 4	5 3	5 8			
231	2 9	3 2	3 2	4 6	P & H	C_2	
243	2 2	4 4	5 5	5 5	P & H		9
253	+	+	0 2	0 7			
257	0 3	0 7	0 6	1 2	H	E_3	
259	12 4	0 6	0 8	0 9	P	A_2	
265	3 1	4 0	3 3	2 8	P & H		9
271	0 9	2 5	4 0	5 5	H	A_3	
291	2 2	3 0	3 3	4 3	P & H		9, 10
293	7 3	4 3	4 5	5 2	P & H		10
305	9 5	9 0	14 1	14 9	P & H		9
318	6 3	4 1	4 9	5 4	P & H		10
319	9 2	6 7	7 3	7 4	P & H		9
331	0 9	2 3	2 3	2 4			
341	1 1	1 2	1 2	2 0			
343	2 7	2 5	4 0	3 3	P & H		10
349	2 5	0 3	0 2	0 2	P	A_1	
361	0 3	9 3	17 5	26 6	H	A_2	
369	1 4	1 1	1 1	1 2			
377	0 2	–	0 2	0 6			
433	9 5	5 2	11 4	14 9	P & H		10
451	–	0 4	0 8	2 6	H	A_1	
479	–	0 1	–	0 4	H	*m/e* 569–TOH	
507	0 9	1 2	0 8	1 3	P & H	M–Me–GOH	
523	0 5	0 3	1 2	0 8	P & H	M–GO	
529	–	–	–	1 1			
539	–	0 3	0 6	1 5	P & H	M–G	
569	–	0 4	0 5	0 5	H	M–421	Fig 3a
594	0 3	–	–	–	P	M–294	Fig 3b
603	–	–	–	0 5			
641	1 1	0 7	5 9	5 3	P & H	J_1	
654	–	0 1	0 3	2 6	P & H	H_1	
683	–	2 2	0 3	1 2	H	M–307	Fig 3c
755	1 5	0 5	0 1	0 4	P & H	B_2	
783	0 2	–	–	–	P	M–Me–TOH	
795	–	0 2	–	–	H	M–Me–2TOH	
843	–	0 1	0 2	0 6	P & H	B_1–Me	

TABLE 2- *continued*

	Glycosides						
	inos-arab	inos-gal	galac-tinol	inos-glu		Ion species	
m/e	G-1	G-2	G-3	G-4	Origin	Type	Ref
885	–	0 5	0 1	0 5	H	M-Me TOH	
888	0 2	–	–	–	P	M	
975	–	–	+	0 5	H	M Me	
990	–	0 1	0 1	+	H	M	

* The glycosides are inositol arabinoside (inos-arab) or inositol galactoside (inos-gal) derived by alkaline hydrolysis of the IAA-esters [5] Galactinol was a commercial sample and inositol glucoside (inos-glu) was from plant phytoglucolipid [13] The origin of the peaks are classified as hexoside (H) or pentoside (P) The type of structure is according to the classification of Kochetkov and Chizhov[8] and reference is made to previously described fragments or to Fig 3 for new fragments G designates a departing sugar moiety and GO the sugar plus glycosyl bridge oxygen Ion abundance is expressed relative to *m/e* 73 = 100 The – sign means the ion was not detectable and the + signifies the ion was just detectable

The origin of the fragments are as follows (1) Elimination of Me–, TMSOH G–, GOH or IO– from M (2) Ring cleavage of either inositol or the sugar moiety For most of the ions produced by the latter reaction the types of ions conform to those described by Kochetkov and Chizhov [9] Analysis of the fragmentation patterns (259 and 349 for the pentoside and 361, 451 and 539 for the hexoside) obtained for the TMS-derivatives and comparison with those described for the methylated disaccharides showed that the inositol is bound to C_1 of the sugars That the reducing group of the sugar was not free was also shown by the failure of the glycosides to form a methoxime derivative under conditions where known compounds formed a derivative [13] (3) Degradation of the inositol moiety starting at *m/e* 523 (4) Degradation of the sugar moiety starting at *m/e* 349 (pentoside) or *m/e* 451 (hexoside) Some structures, not previously proposed are shown in Fig 3

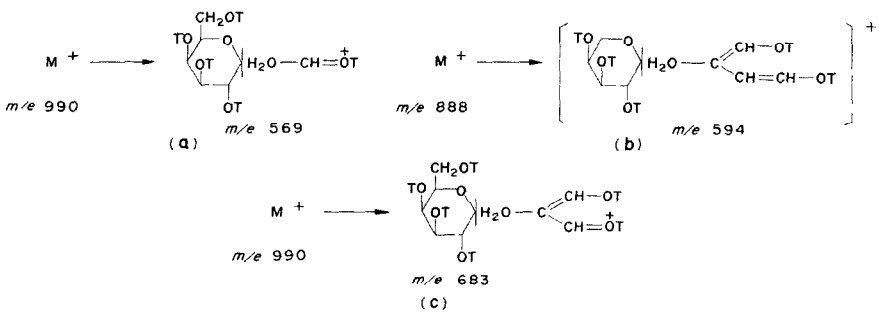

FIG 3 TRIMETHYLSILYL ETHERS OF MYOINOSITOL GLYCOSIDES

Characteristic heavy ions in the 70 eV MS of trimethylsilyl ethers of myoinositol glycosides (a) and (b) Fragment ions derived from a myoinositol hexoside by elimination of 307 and 421 respectively from the molecular ion (c) Fragment ion derived from a myoinositol pentoside by elimination of 294 from the molecular ion

[12] WILLIAMS C M, PROTER A H and GREER M (1969) *Mass Spectrometry of Biologically Important Aromatic Acids* Univ of Florida Gainesville Florida
[13] FEHMANN A unpublished

The MS of the inositol hexoside derived from the IAA ester (G-2) is different from galactinol, 1 L-1-*O*-γ-D-galactopyranosyl-myoinositol,[14] (G-3) or inositol substituted with glucose on the 6 position from phytoglycolipid (G-4).[15] A careful study of these spectra might be sufficient to determine the structure of the inositol glycoside without permethylation

TABLE 3 RELATIVE ABUNDANCE OF IONS IN THE 70 eV MS OF TRIMETHYLSILYL ETHERS OF IAA-MYOINOSITOL GLYCOSIDES*

	Glycosides			Ion species	
m/e	Peak-8	Peak-9	Origin	Type	Ref
73	100	100	P & H		9
103	10 1	9 9	P & H		9,11
129	11 1	11 9	P & H		11
130	37 8	23 6	P & H, ROH		11
133	7 5	7 7	P & H		9, 10
147	35 8	39 8	P & H		9
157	38 8	29 2	P & H ROH		9
169	2 8	2 3	P	A_3	
189	3 5	3 4	P & H		9
191	15 6	12 0	P & H		9
204	44 3	52 8	P & H		9
217	30 2	21 2	P & H		9
221	15 6	21 2	P & H		10
259	23 2	2 3	P	A_2	
265	1 6	2 4	P & H		9
271	3 7	3 2	H	A_3	
291	1 5	0 8	P & H		9
293	–	0 6	P & H		10
305	2 8	2 5	P & H		9
318	3 5	2 6	P & H		10
343	4 9	2 8	P & H		10
349	4 6	0 7	P	A_1	
361	+	8 4	H	A_2	
390	+	–	P & H		10
417	+	–	P & H		10
433	2 0	1 8	P & H		10
479	0 2	–	P & H	*m/e* 653–RO	
563	0 4	0 3	P & H	*m/e* 653–TOH	
607	0 4	0 3	P & H	*m/e* 697–TOH	
609	+	0 2			
653	0 6	0 9	P & H		Fig 4a
682	+	0 1	P & H	*m/e* 697–Me	
697	2 1	1 9	P & H	IAA-Inositols	
739	+	+	P & H		Fig 4b
868	0 2	–	P	M–Me–TOH	
880	–	0 07	H	M–Me–2TOH	
883	+	–	P	M–TOH	
970	–	0 2	H	M–Me–TOH	
973	3 8	–	P	M	
985	–	0 03	H	M–TOH	
1060	-	0 05	H	M–Me	
1075	–	1 4	H	M	

* Abbreviations as for Table 2

[14] KABAT, E A, MACDONALD, D L, BALLOU, C E and FISCHER, H O L (1953) *J Am Chem Soc* **75**, 4507
[15] CARTER, H E, STROBACH, D R and HAWTHORNE, J N (1969) *Biochemistry* **8**, 383

The presence of ions of the (*B*) series for both the pentosides and hexosides strongly suggests that they are pyranose derivatives Characteristic ions of the *E* series are found in the MS of the hexosides Although E_1 and E_2 are not clear owing to the effect of neighboring isotopic peaks in the MS of TMS compounds (*m/e* 887 and 707 respectively) permethylated galactose obtained by methanolysis of inositol galactoside produced the whole series of *E* ions The K_1 type ion, *m/e* 115, found in the spectra of the pentosides, also supports the pyranose structure of arabinose in our compounds Permethylated arabinose gave a pattern characteristic of arabinopyranoside

TABLE 4 RELATIVE ABUNDANCE OF HEAVY IONS IN THE 70eV MS OF TRIMETHYLSILYL ETHERS OF IAA-MYOINOSITOL GLYCOSIDES*

	Glycosides							Ion species
m/e	Peak-5	Peak-6	Peak-8	Peak-4	Peak-7	Peak-9	Origin	Structure
563	16	88	17	7	27	15	P & H	*m/e* 653 TOH
607	43	42	17	31	18	18	P & H	*m/e* 697 TOH
609	22	70	+	14	18	13		
653	33	92	31	19	85	46	P & H	Fig 4a
682	4 4	11	+	3 1	13	4 0	P & H	*m/e* 697 Me
697	100	100	100	100	100	100	P & H	IAA-Inositols
739	35	21	+	2 4	+	+	P & H	Fig 4b
778	6 2	–	–	–	–	–	P	M Me-2TOH
799	–	7 3	–	0 3	+	+	P	M-RO
868	5 3	6 1	11	–	–	–	P	M-Me-TOH
880	–	–	–	0 6	+	3 6	H	M Me-2TOH
883	7 1	4 9	+	+	–	–	P	M TOH
901	–	(6 1)	–	0 4	–	–	H	M-RO
958	+	+	–	+	–	–	P	M Me
970	–	–	–	1 5	9 2	9 5	H	M-Me-TOH
973	28	190	183	(3 6)	–	–	P	M
985	–	–	–	0 4	+	1 8	H	M TOH
1060	–	–	–	+	6 2	2 6	H	M Me
1075	–	–	–	2 0	–	72	H	M

* Abbreviation as for Table 2 except that *m/e* 697 = 100

MS of the TMS-IAA-myoinositol glycosides

In Table 3 typical MS of an IAA-myoinositol pentoside (peak 8) and an IAA-myoinositol hexoside (peak 9) are given Some of the characteristic ion species found in the MS of the TMS-IAA-myoinositols are also observed in the spectra of the TMS-myoinositol glycosides and it is noteworthy that the molecular ion is abundant despite the high MW of these compounds A more detailed presentation of the heavy ions is given in Table 4 From these data it may be concluded that peaks 5 6 and 8 are pentosides and peaks 4, 7 and 9 are hexosides [16] This confirms earlier studies of hydrolysis products [6] The fragmentation patterns can be summarized as follows (1) Elimination of Me, TMSOH or ROH from M (2) Elimination of the sugar moiety and transfer of TMS^+ from the sugar to C-5 of inositol to form TMS-IAA-myoinositol, *m/e* 697 (3) Ring cleavage of the sugar moiety This cleavage is different from that of TMS-myoinositol glycosides as shown in Fig 4 (4) Degradation of the IAA moiety (5) Degradation of the TMS-IAA-myoinositol (6) Degradation of the inositol moiety (7) Degradation of the sugar moiety

[16] The peak number designation used here and earlier[6] is arbitrary and does not necessarily represent the order of emergence from a GLC column The complexity of the mixture together with the large difference in relative amounts of the esters in any single preparation has necessitated special techniques to be published later [13]

Reactions (2) and (3) show that IAA is bound to inositol and not to the sugar moiety This conclusion confirms that reached earlier[2] based on the observation that enzymatic hydrolysis of IAA-inositol arabinoside led to the production of IAA-inositols

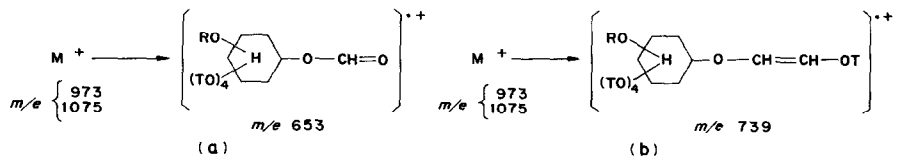

FIG 4 TRIMETHYLSILYL ETHERS OF IAA-MYOINOSITOL GLYCOSIDES

Characteristic fragment ions in the 70 eV MS of trimethylsilyl ethers of IAA-myoinositol glycosides Both ions (a) and (b) retain the indolyl group during fragmentation of the glycosyl moiety of both the pentoside and the hexoside

Examination of the mass spectra reveals that peaks 1, 4 and 6 belong to a homologous group since all yield the ion M–RO Since peak 1 is 2-*O*-indoleacetyl-myoinositol it may be concluded that peak 4 is the 2-*O*-IAA-inositol-galactoside and peak 6 is the 2-*O*-IAA-inositol-pentoside If the high intensity of the ion M–TOH is used as a guide, peak 5 can be grouped with peak 2 Peak 7 probably also belongs to this group because M–Me–TMSOH is abundant Peaks 8 and 9 have low ratios of M–TOH to M When the value of the ratio of *m/e* 157/130 are employed as indicators[6] similarities in structure of the pairs, peak 1 and 4, 2 and 7, 3 and 9 are seen As data are lacking for peak 6 no similar series can be made for the pentosides When the relative abundance of the molecular ions are taken into account, peak 5 is homologous with 7 and 8 with 9 There is no substantial difference between peaks 2 and 3 Collecting all this information, it is most likely that peaks 2, 5 and 7 belong to the B_1 type ester where IAA is bound to an equatorial hydroxyl, and is possibly the diastereoisomeric DL-1-*O* pair Peaks 3, 8 and 9 would then be the DL-4-*O*-myoinositol esters Except for peaks 8 and 9, the other four peaks give ions at *m/e* 625 which might be the molecular ion of tetra-TMS-IAA-myoinositol or loss of TMS–H Peaks 4, 6 and 7 give ions at *m/e* 1002 and 900 The mass number of hepta-TMS-IAA-myoinositol-hexoside and hexa-TMS-IAA-myoinositol pentoside are 1003 and 901 respectively Since the ions of the type M–T have never been found in the MS of completely silylated IAA-myoinositols or inositol glycosides, *m/e* 1002 and 900 should not be derived by the reactions M_{1075}–T or M_{973}–T but rather by M_{1003}–H or M_{901}–H Although varying in amount peaks 4 to 7 gave ions of the type M_{1003}–H–Me, M_{901}–H–Me, M_{1003}–TOH Peaks 4 and 7 gave a small amount of ion current at *m/e* 930 and 915 These should have been derived from hexa-TMS-IAA-myoinositol hexosides whose mass number is 931 These ion peaks were not strong and were thus omitted from Table 4 Two figures of Table 4 are shown in parenthesis since they are of doubtful origin The *m/e* 901 of P-6 could be due to contamination with hexa-TMS-IAA-myoinositol pentoside (or loss of $(Me)_3$ Si less one H from 973) and *m/e* 973 of P-4 might be contamination from neighboring peaks although extensive rearrangement from the large M^+ is possible

MS of pentamethyl-myoinositols

In order to determine the position of the sugars on inositol the methanolysed permethylated inositol glycosides were studied (Table 5) Some possible fragment ions are as shown in Fig 5

TABLE 5 RELATIVE ABUNDANCE OF IONS IN THE 70 eV MS OF PENTAMETHYL-MYOINOSITOLS*

	Pentamethyl-myoinositols and their sources								
m/e	1 2 3 4 5-Me-inos Glucosyl-inos	1 2 4 5 6-Me-inos 1 4 5 6-Me-inos	Galac-tinol	1 3 4 5 6-Me-inos 1 3 4 6-Me-inos	1 4 5 6-Me-inos	1 3 4 6-Me-inos	1 2 3 4 6-Me-inos Prep 6	Prep 7	Ion species structure reference
88	98 5	86 2	69 5	109	75 0	48 0	67 5	72 2	8
101	300	300	300	300	300	300	300	300	8
114	15 8	12 8	11 1	14 8	15 7	7 4	8 8	10 3	Fig 5a
115	6 0	5 1	3 9	4 1	4 5	6 5	4 6	5 2	Fig 5b
117	20 1	19 8	17 2	17 4	16 4	7 7	9 2	10 8	Fig 5c
127	13 5	8 9	7 8	11 8	14 1	3 9	5 5	6 5	
129	6 4	8 3	7 0	4 7	5 2	4 5	6 3	6 2	
130	24 7	10 2	12 8	22 9	22 7	11 9	12 2	13 9	Fig 5d e
131	22 0	20 7	20 5	21 2	24 4	11 9	10 4	14 9	Fig 5f g
143	7 3	4 5	3 6	6 2	5 9	1 2	3 0	1 9	
144	22 9	31 9	27 8	21 2	23 4	6 8	6 7	6 5	Fig 5h
145	4 4	(26 2)	4 4	6 0	6 6	2 6	3 4	3 2	Fig 5i
147	0 9	2 9	3 3	1 7	2 8	0 9	0 8	0 6	
154	1 1	1 3	1 4	0 9	1 2	0 4	0 5	0 2	*m e* 186–MeOH
155	1 1	2 6	1 4	1 1	1 2	0 6	1 7	1 2	*m e* 187–MeOH
156	0 9	0 6	0 8	0 9	0 9	0 4	+	0 3	*m e* 187 MeO
157	2 3	3 5	2 8	2 4	2 3	1 3	1 5	1 8	
158	4 6	(5 4)	1 9	4 9	5 9	0 8	1 3	0 8	
159	2 3	1 0	0 6	0 6	0 7	0 3	1 3	0 6	
186	3 4	0 6	0 6	3 0	3 3	0 3	–	0 3	*m/e* 218–MeOH
187	1 1	1 0	0 8	0 6	0 7	0 4	+	0 8	*m/e* 219 MeOH
201	0 2	0 6	0 6	1 3	1 6	0 3	+	0 3	*m/e* 219 H_2O
218	0 2	8 9	11 7	1 5	1 2	6 8	4 6	5 2	M MeOH
219	0 2	1 3	1 4	0 2	+	0 7	1 3	1 5	M–MeO
249	?	0 3	0 3	0 4	0 2	+	?	+	M–H
250	2 1	1 0	0 8	1 7	2 1	0 1	+	0 2	M

* Abbreviations as for Table 2 Abundancies are normalized to *m e* 101 = 300

From the MS, it is evident that inositol glycosides derived from IAA-myoinositol glycosides produce 1,2 3,4,6-pentamethyl myoinositol, and thus the sugars in the origin1 compounds must have been linked to the 5-*O* of inositol The isomer having a free axial OH produce more ions at *m/e* 201 by elimination of OH or H_2O The isomer with a free OH at the 5-position (para to the axial OMe) is comparatively unstable Some of these data were presented briefly in a prior paper [4 6]

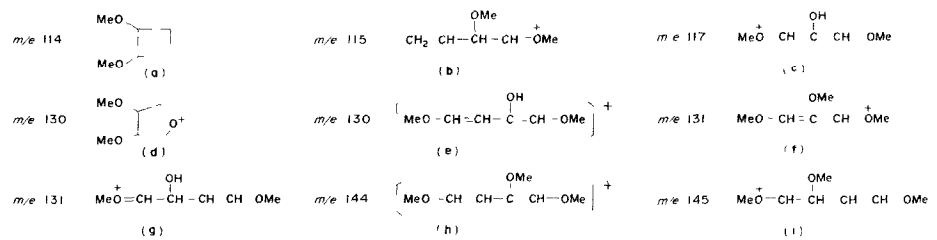

FIG 5 PENTAMETHYL-MYOINOSITOLS
Characteristic fragment ions in the 70 eV MS of pentamethyl-myoinositols The relative abundancy of these ions as given in Table 5 serves to differentiate 1 2 3,4 6 pentamethyl-myoinositol from the other pentamethyl-myoinositols

EXPERIMENTAL

Preparation of the indole-3-acetic acid myoinositol esters The esters used in the present study were isolated as previously described [5] A more convenient procedure adapted to larger scale preparations and yielding identical results has been published [6] The preparation of the trimethylsilyl ethers for GC–MS analysis was as previously described[5] as is the permethylation of the inositol glycosides with methyl iodide in dimethyl formamide [5]

GC–MS analysis GLC analysis were made with an F and M model 402 equipped with an FID with N_2 carrier gas flow rate 60 ml/min The column used for the esters was a 2% OV-1, 3 2 mm × 183 cm on Gas-Chrom Z at 245° for the IAA-inositols, 220° for TMS-myoinositol glycosides, and 270° for the IAA-inositol glycosides MS was with an LKB-9000 analyzer unit coupled to a 183 cm OV-1 column on Gas-Chrom Z with the column operating at 15°above the temps indicated above The separator was at 250° and the source at 290° He gas was used as carrier with a flow rate of 25 ml/min Scan rates were about 80 mu/sec Mass identification was established with perfluorokerosene and the LKB mass marker In tabulating the spectra (a) the contributions from isotopes were not corrected for, (b) the clusters of peaks due to isotopes were represented by the smallest mass numbers, (c) in the light ion region, where many peaks occur, only intense peaks are reported, and (d) background peaks are subtracted from the spectra

Acknowledgements—We gratefully acknowledge the support of the National Science Foundation Grant GB 18353-X Journal Article 6358 from the Michigan Agricultural Experiment Station We are indebted to Professor C C Sweeley and Mr Jack Harten of this university for the use of the mass spectrometer and for advice Professor Laurens Anderson of the University of Wisconsin helped with nomenclature and Professors C E Ballou of the University of California and H E Carter of the University of Illinois kindly supplied tetramethylinositols and glucosylinositol Mr Axel Ehmann provided unpublished data on methoxime derivatives, assistance and advice