Carbohydrate Research, 36 (1974) 1–12
© Elsevier Scientific Publishing Company, Amsterdam – Printed in Belgium

THE ISOLATION OF DI-*O*-(INDOLE-3-ACETYL)-*myo*-INOSITOL AND TRI-*O*-(INDOLE-3-ACETYL)-*myo*-INOSITOL FROM MATURE KERNELS OF *Zea mays**

AXEL EHMANN AND ROBERT S. BANDURSKI

Department of Botany and Plant Pathology, Michigan State University, East Lansing, Michigan 48824 (U.S.A.)

(Received October 1st, 1973; accepted in revised form, March 15th, 1974)

ABSTRACT

Di-*O*-(indole-3-acetyl)-*myo*-inositol and tri-*O*-(indole-3-acetyl)-*myo*-inositol have been isolated from kernels of *Zea mays* and identified by gas–liquid chromatographic–mass spectrometric analysis of their trimethylsilyl ethers.

INTRODUCTION

Mature kernels of *Zea mays* contain between 20–100 mg/kg of "bound" indole-3-acetic acid[1–5] (**1**) that is hydrolyzable by dilute alkali. Studies from this laboratory established that "bound" **1** (about 65 mg/kg) consists[6–10] of one-half water-soluble esters of **1** and *myo*-inositol (**2**) or glycosides of **2** and one-half water-insoluble cellulosic-glucan containing esterified **1** (ref. 11). In the present work, we have characterized by t.l.c., g.l.c., and combined g.l.c.–m.s. two new, less polar, esters of **1** present in trace amounts in 1-butanol extracts of the water-soluble fraction: these esters are a di-*O*-(indole-3-acetyl)-*myo*-inositol (**3**) and a tri-*O*-(indole-3-acetyl)-*myo*-inositol (**4**).

RESULTS AND DISCUSSION

Ueda and Bandurski[9] have partitioned with 1-butanol the aqueous condensate of the 1:1 acetone–water extract of mature, sweet-corn kernels. They found trace amounts of two unidentified esters of **1**, which have recently been identified as esters of **1** and D-glucose[12]. Preliminary t.l.c. analysis of the remaining water-insoluble 1-butanol fraction, which was soluble in 1:1 ethanol–water, showed that it contained **1**, the previously identified esters of **1** and **2**, and glycosides of **2**, and minute amounts of a new, unknown indole derivative (**3**). Ammonolysis of **3** yielded **1** and indole-3-acetamide (**5**). Compound **3** is therefore an ester of **1**. The extraction of 10.5 kg of

---

*This work was supported by the National Science Foundation (GB-18353-X and GB-40821-X). Journal Article No. 6532 from the Michigan Agricultural Experiment Station.

mature, sweet-corn kernels yielded 510 μg of **3** (as **1**) and 210 μg (as **1**) of a second unknown indole derivative (**4**). Ammonolysis of **4** showed that it was also an ester of **1**. Compounds **3** and **4** each gave a single spot on t.l.c. (Fig. 1, t.l.c. insert, Samples 1 and 2), but, following storage in 1:1 ethanol–water, both **3** and **4** gave an additional, minor spot on t.l.c. (Fig. 1, t.l.c. insert, Sample 3). These minor components result from acyl migration, as has previously been observed[6,7] with esters of **1** and **2**, glycosides[9] of **2**, and D-glucose[12]. No attempt was made to separate these components, and all experiments were performed with isomeric mixtures of **3** and **4**.

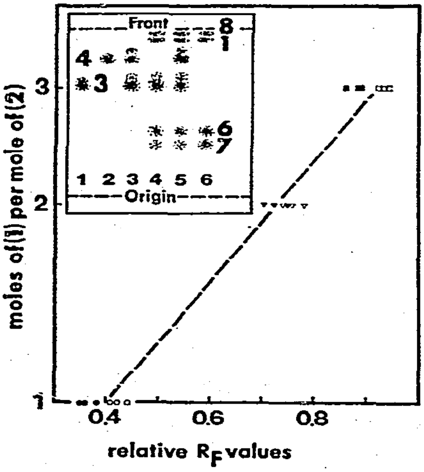


Fig. 1. Plot of three sets of R_F values of **6** (white circles), **7** (black circles), **3** (black triangles) and its isomer (white triangles), and **4** (black squares) and its isomer (white squares), versus the logarithm of moles of **1** per mole of **2**. The insert shows the t.l.c. behavior of **3** (1), **4** (2), the isomers of **3** and **4** (3), and the incomplete hydrolysis products of **3** (4) and **4** (5). Sample 6 is a mixture of standards: 1-DL-*O*-(indole-3-acetyl)-*myo*-inositol (**6**), 2-*O*-(indole-3-acetyl)-*myo*-inositol (**7**), indole-3-acetic acid (**1**) and ethyl indole-3-acetate (**8**). The samples were chromatographed on silica gel plates (Merck, Darmstadt) in 3:5:1:1 butanone–ethyl acetate–ethanol–water and visualized with a modified Ehrlich reagent[14].

Analysis of components and stoichiometry of **3** *and* **4**. — Ammonolysis of **3** and **4** yielded two Ehrlich-positive products that co-chromatographed on t.l.c. with **1** and **5**. Identification was confirmed by g.l.c. and combined g.l.c.–m.s. analysis of the trimethylsilyl (Me_3Si) ethers of the hydrolysis products. Compounds **3** and **4** were, therefore, identified as esters of **1**. Charring of the t.l.c. plates with concentrated sulfuric acid revealed an additional component that co-chromatographed with **2** on t.l.c. and was identified as **2** by g.l.c. and combined g.l.c.–m.s. analysis of the Me_3Si ether. Thus, the hydrolysis products of **3** and **4** were identical with those of previously characterized esters[6,7] of **1** and **2**, but the R_F values by t.l.c. were greater, suggesting that **3** and **4** contained an additional lipophilic moiety. As the only hydrolysis products were **1** and **2**, the additional component must thus have been one, or more, additional moles of **1** per mole of **2**. The R_F values of the mono-, di-, and tri-acyl esters of **1** should vary in a predictable manner[13]. Plotting the R_F values of the two isomers of **1** and **2**, 1-DL-1-*O*-(indole-3-acetyl)-*myo*-inositol (**6**) and 2-*O*-(indole-3-acetyl)-*myo*-inositol (**7**), and **3** and **4**, against the logarithm of the number of moles of **1** per mole of **2** (see Fig. 1) yielded a straight line. This result indicates that **3** and **4** had two and three moles of **1** per mole of **2**, respectively. Incomplete hydrolysis of **3** should yield **1** and **3**, and the ester of **1** and **2**, whereas **4** should yield **1**, **3** and **4**, and the ester of **1** and **2**. Preliminary experiments with a mixture of **6** and **7** in 1:1 ethanol–water showed that 4.6% ammonium hydroxide hydrolyzed about 20% of the ester groups after 3 min at room temperature and 70% after 30 min. Under these hydrolysis conditions, only free **1** and the transesterification product **8** (the ethyl ester of **1**), were formed. Compound **5** was not formed with dilute ammonium hydroxide although it accounted for about 50% of **1** when 28% ammonium hydroxide was used. Hydrolysis of a sample of **3** with 2.15% ammonium hydroxide for 5 min resulted in **1**, its ethyl ester, intact **3**, and a mixture of **6** and **7** (Fig. 1, t.l.c. insert, Sample 4), whereas similar hydrolysis of **4** yielded **1**, the ethyl ester of **1**, **3** and **4**, and **6** and **7** (Fig. 1, t.l.c. insert, Sample 5). This result shows that **4** can be hydrolyzed to **3** by loss of **1** and that both **3** and **4** can by hydrolyzed to yield the esters of **1** and **2**. The stoichiometry of the hydrolysis products of **3** and **4** is given in Table I and shows that **3** and **4** are a di-*O*-(indole-3-acetyl)-*myo*-inositol and a tri-*O*-(indole-3-acetyl)-*myo*-inositol, respectively.

TABLE I

STOICHIOMETRY OF DI-*O*-(INDOLE-3-ACETYL)-*myo*-INOSITOL (**3**) AND TRI-*O*-(INDOLE-3-ACETYL)-*myo*-INOSITOL (**4**)

Structural component	*Tri-ester* (**4**)		*Di-ester* (**3**)	
	Column sample[c]	*T.l.c. sample*[d]	*Column sample*[c]	*T.l.c. sample*[d]
	Mole ratios of indole-3-acetic acid to *myo*-inositol			
myo-Inositol[a]	1.00	1.00	1.00	1.00
Indole-3-acetic acid[b]	3.15	3.06	2.14	2.05
Indole-3-acetic acid[a]	3.04	2.88	1.94	1.97
Moles of indole-3-acetic acid per mole of *myo*-inositol	3.10	2.97	2.04	2.01

[a]Determined by quantitative g.l.c.[15]. [b]Determined spectrophotometrically at 221 and 280.5 nm. [c]This sample was purified by the two column-chromatographic steps described in the text. [d]These samples were prepared by the two column-chromatographic steps described and by further purification on t.l.c.

Gas-liquid chromatography. — The Me_3Si ethers of **3** and **4** were prepared as described previously[14]. G.l.c. analysis of both compounds on several liquid phases (OV-1, 2%; SE-30, 3%; and OV-17, 3%) in a 1.8-m or 1.2-m glass column showed only two peaks, corresponding to mono-substituted **2** (compounds **6** and **7**) indicating thermal instability of the di- and tri-substituted esters of **2**. Use of a 0.4-m column

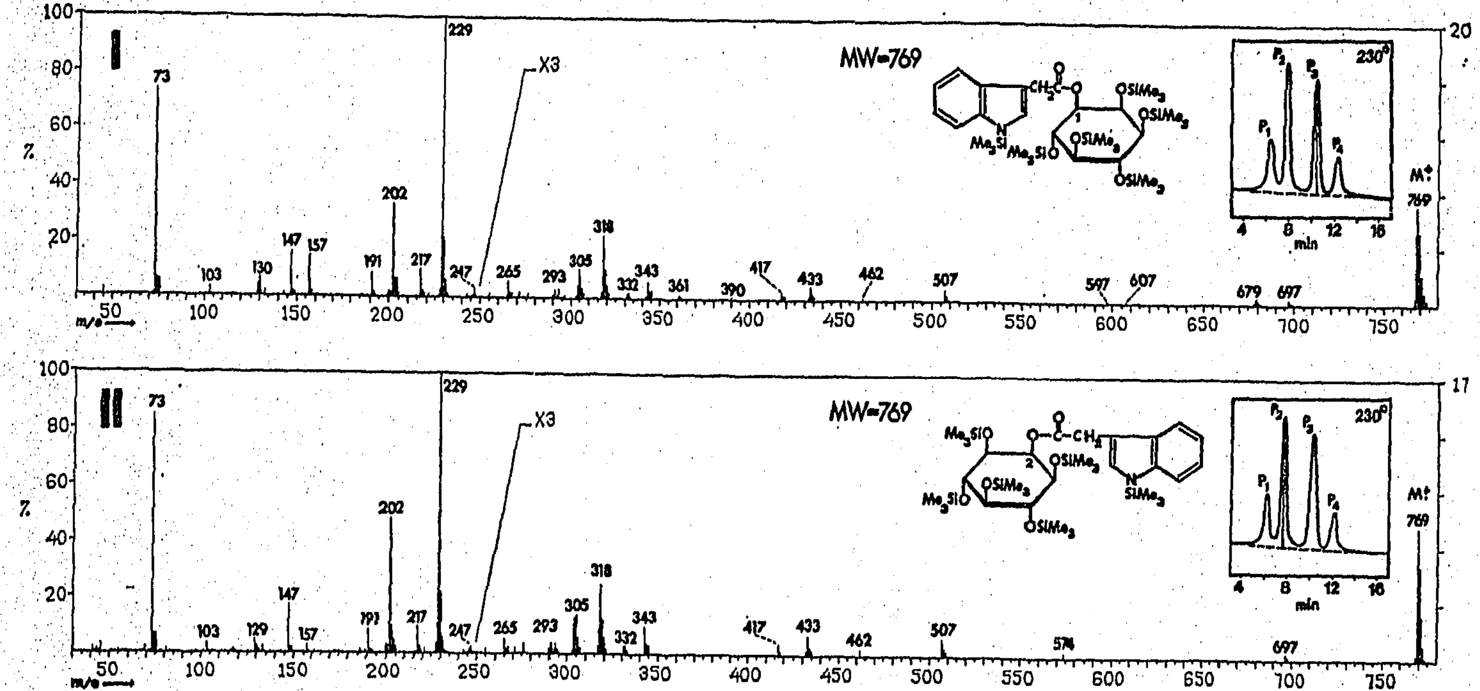


Fig. 2. Mass spectra of the Me_3Si ethers of **6**: 1-DL-1-*O*-(indole-3-acetyl)-*myo*-inositol (Spectrum I) and **7**: 2-*O*-(indole-3-acetyl)-*myo*-inositol (Spectrum II). The spectra were recorded as described in the experimental *section at a column temperature* of 230°. The inserts show the g.l.c. profiles and the shaded area under peaks 2 and 3 represent the contribution of the peaks to the individual spectra obtained.

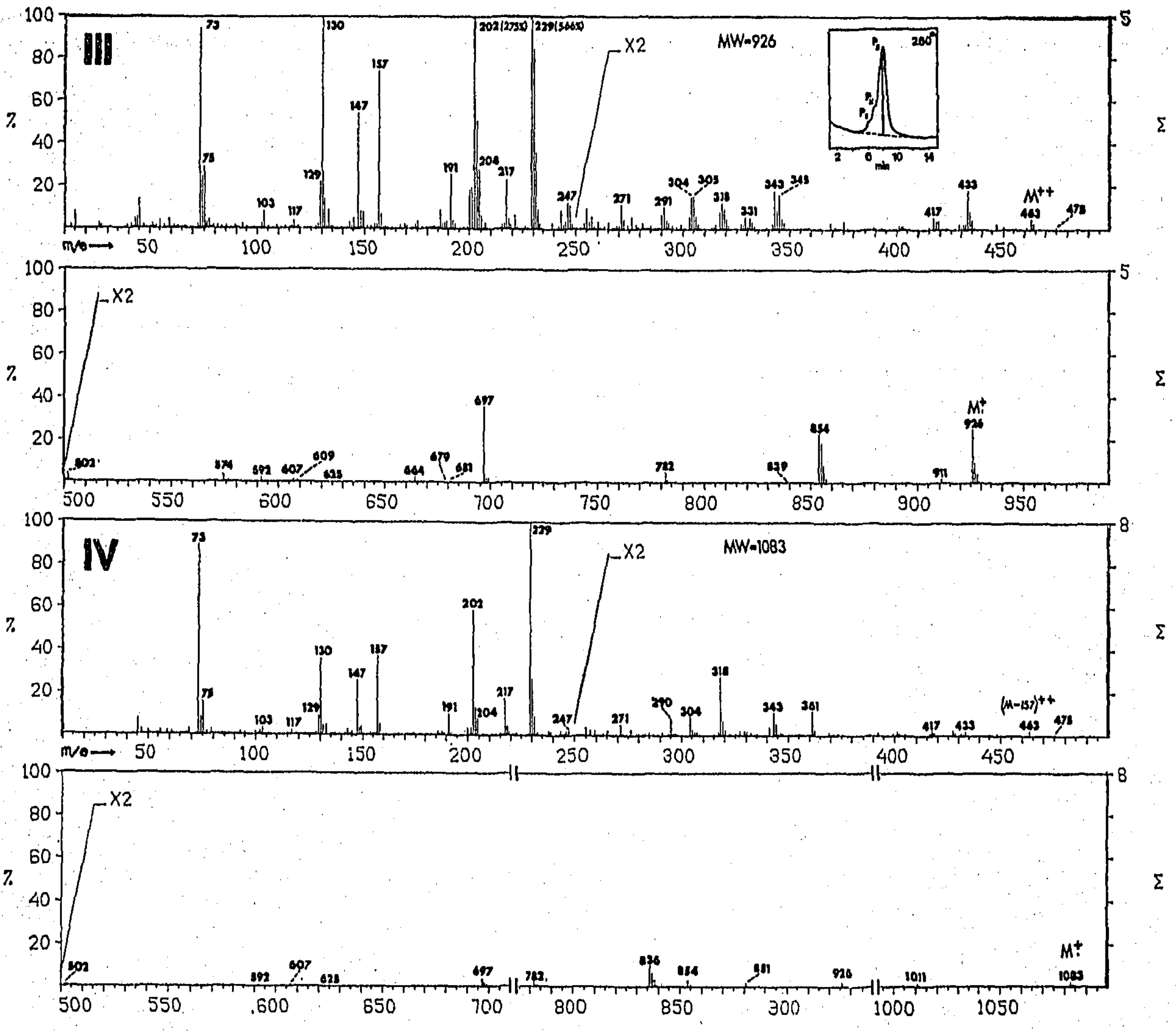


Fig. 3. Mass spectra of peak 3 of the Me_3Si ethers of 3 (Spectrum III) and of the intact Me_3Si ether of 4 (Spectrum IV). Spectrum III was recorded at a column temperature of 260°. Spectrum IV was obtained at a probe temperature of 135°.

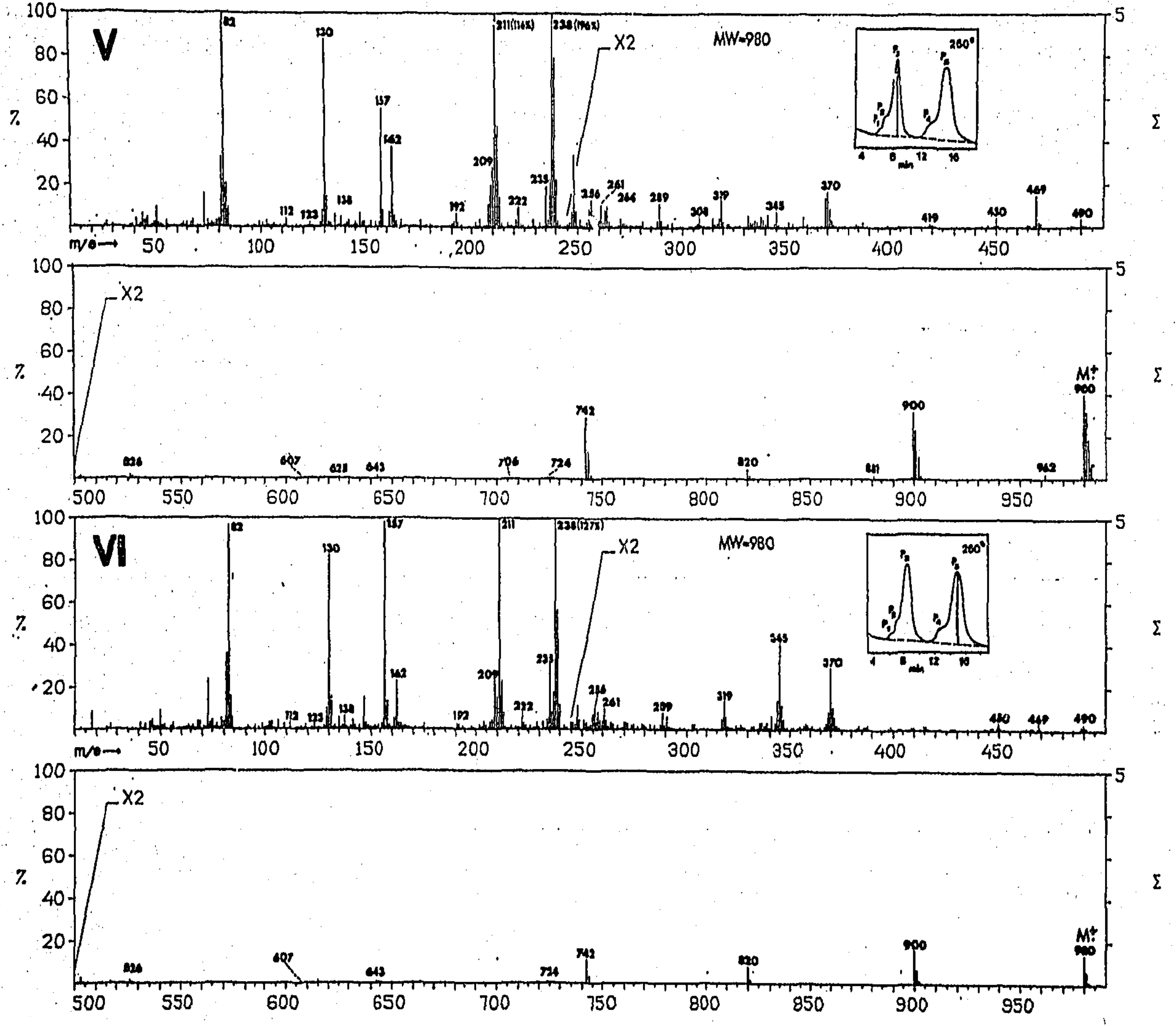
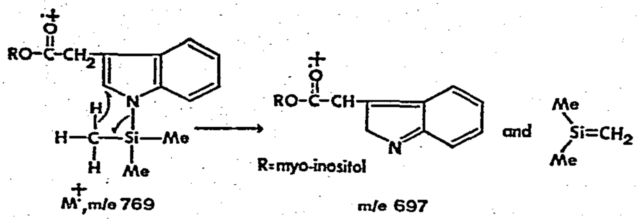


Fig. 4. Mass spectra of peaks 3 and 5 of the thermal decomposition products of the Me_3Si-d_9 ethers of 4 (Spectra V and VI). For conditions see experimental sections.

decreased the temperature for the elution of **7** from 230° to 190°, and the Me_3Si ether of **3** could be chromatographed on this column at 270° yielding three peaks (see Fig. 3, g.l.c. profile in Spectrum III). G.l.c. analysis of the intact Me_3Si derivative of **4** was not possible, as it decomposed to **3** and two isomers of **3** (peaks 4 and 5, Fig. 4, g.l.c. insert in Spectrum VI).

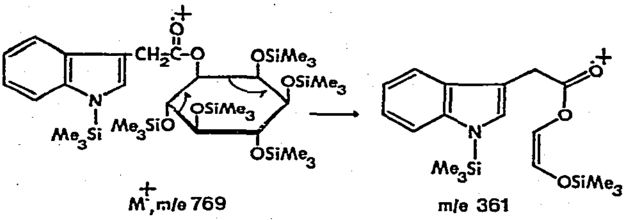
Mass spectrometry. — Combined g.l.c.–m.s. was performed on the Me_3Si-ethers of **3** and **4** to confirm the stoichiometry and to determine, insofar as present methods permit, the positions of substitution of **1** on **2**. Ueda, *et al.*[15] studied the g.l.c. behavior of the esters of **1** and **2**, the parent compounds of **3** and **4**. The esters of **1** and **2** yielded two spots on t.l.c., designated by those authors as B_1 and B_2 and here as **6** and **7** (see Fig. 1, Sample 6), and Nicholls *et al.*[16] showed by n.m.r. spectroscopy that **7** is 2-*O*-(indole-3-acetyl)-*myo*-inositol. As **7** is known to isomerize to **6**, and acyl migration presumably occurs more readily between vicinal groups, it has been concluded that **6** is, most likely, 1-DL-1-*O*-(indole-3-acetyl)-*myo*-inositol. Ueda *et al.*[15] observed three peaks for the Me_3Si ethers of **6** and **7** by g.l.c., and, more recently, Ehmann and Bandurski[14] resolved four of the six possible isomeric esters (two pairs of mirror images and two optically inactive esters). The g.l.c. inserts in Spectrum I of Fig. 2 show these four isomers. Compound **7** prepared by preparative t.l.c. gives rise to peaks 1 and 2. Peak 1, the preponderant component, is therefore the 2-ester, whereas the minor peak 2, is probably the 5-ester. Compound **6** on the other hand, gives rise to peaks 3 and 4; by analogy peak 3 is the 1-DL-1-ester and peak 4 possibly the 1-DL-4-ester. However the substitutional assignments for peaks 2, 3, and 4 have not yet been confirmed. Ueda *et al.*[15], and Ueda and Bandurski[17], studied the Me_3Si ethers of **6** and **7** by combined g.l.c.–m.s. with derivatives where the imino group of the indole ring was not substituted by a Me_3Si-group. They noted that **7** (the 2-ester) fragments preferentially by loss of the acyl group, whereas **6** (the 1-DL-1-ester) fragments by loss of Me_3SiOH. The mass spectra of the fully trimethylsilylated derivatives of the major peaks of **6** and **7** have not previously been published and are shown in Fig. 2 (Spectra I and II). Both spectra were recorded at about equal sample-pressures and under identical conditions to facilitate comparison of the fragmentation patterns. It is apparent that the spectra of both compounds in the mass range from 30 to 350 daltons are almost identical. The ions *m/e* 130, 157, 202, 229, and 247 characteristic for the indole moiety have been described[12], as have the ions arising from fragmentation of the inositol moiety[17].

*Mass spectrum of trimethylsilylated 1-*DL*-1-*O*-(indole-3-acetyl)-*myo*-inositol* (**6**) (*Spectrum I*). — The introduction of a Me_3Si group at the imino group of the indole moiety confers high stability on the molecular ion ($M^{+\cdot}$ at *m/e* 769). This ion ($M^{+\cdot}$) loses 72 daltons to give the ion *m/e* 697, which results from transfer of a hydrogen atom to the indole nucleus from one of the methyl groups of the *N*-Me_3Si group, with subsequent loss of the neutral $\cdot CH_2(CH_3)_2Si$ radical[18]. This step was confirmed by the presence of corresponding ions at *m/e* 823 ($M^{+\cdot}$) and *m/e* 743 ($M^{+\cdot}$ – 80 daltons) in the mass spectra of the *O*-Me_3Si-d_9-derivatives of **6** and **7** (unpublished results). The absence of *m/e* 742, the molecular ion of the *O*-Me_3Si-d_9-derivative having a free



Scheme 1.

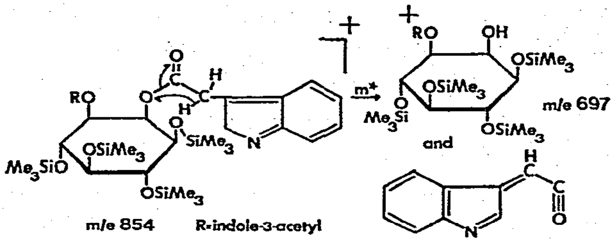
imino group, also confirms that the ion having *m/e* 697 is not due to an incompletely trimethylsilylated contaminant. Loss of Me_3SiOH from M^{+} leads to *m/e* 679. This transition is supported by a metastable peak at *m/e* 600 (calc. 599.5), followed by the elimination of a neutral $\cdot CH_2(CH_3)_2Si$ radical to give rise to *m/e* 607. The ion *m/e* 597 results from the loss of the indole moiety from M^{+}. The ion having *m/e* 361, a characteristic ion of Me_3Si-derivatives of glycosides[19], and esters of **1** and D-glucose[12], is not normally observed in Me_3Si derivatives of cyclitols. Its origin may be explained by ring opening of the inositol moiety followed by the loss of a C_4 unit (408 daltons) with charge retention on the indole moiety.



Scheme 2.

*Mass spectrum of trimethylsilylated 2-O-(indole-3-acetyl)-*myo*-inositol* (**7**) (*Spectrum II*). — Loss of Me_3SiOH from M^{+} is suppressed, but an ion is found at *m/e* 574 that arises from the elimination of two molecules of Me_3SiOH and a $\cdot CH_3$ radical from M^{+}. The ions having *m/e* 679, 607, 597, and 361 are absent, permitting differentiation of **7** and **6**. This difference, together with the difference of retention time by g.l.c., permits the identification of **6** (1-DL-1-ester) and **7** (2-ester).

Mass spectrum of trimethylsilylated **3**. — The mass spectrum of the Me_3Si derivative of **3** is shown in Fig. 3, Spectrum III. It yields three peaks by g.l.c., with peak 3 accounting for more than 80% of the total peak area. The mass spectra of peaks 1 and 2 have also been studied, and are virtually identical with the mass spectrum of peak 3. The ion found at *m/e* 926 corresponds to the molecular ion of a di-*O*-[*N*-(trimethylsilyl)indole-3-acetyl]-*O*-tetra-*O*-trimethylsilyl-*myo*-inositol, confirming the stoichiometry found for **3** of 2 moles of **1** per mole of **2**. The molecular ion loses $\cdot CH_3$ to yield a low-intensity ion at *m/e* 911. The M^{+} ion also loses 72 daltons to yield *m/e* 854, which fragments to *m/e* 697 by the loss of the indole-3-ketene radical.

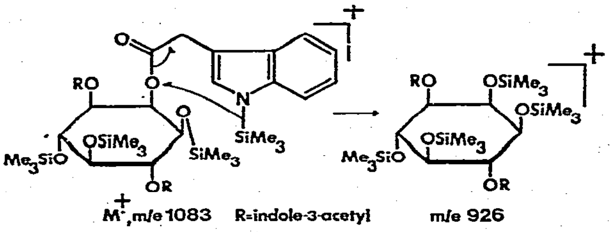


Scheme 3.

This transition is supported by a small metastable ion observed at *m/e* 568.5 (calc. 568.9). Loss of the corresponding ketene (158 a.m.u.) from the *O*-Me_3Si-d_9 analogue confirms this pathway (see Fig. 4, Spectrum V). The $M^{+\cdot}$ ion can also lose an *N*-(trimethylsilyl)indole-3-ketene group to yield *m/e* 697 directly. The metastable peak observed at *m/e* 524.5 (calc. 524.6) supports this transition. The ion *m/e* 911 may lose 72 daltons to give the ion *m/e* 839. The ion *m/e* 782 arises from the loss of two $\cdot CH_2(CH_3)_2Si$ radicals from the molecular ion. The ion of high intensity at *m/e* 697 fragments to *m/e* 625, an ion of low intensity, by the loss of a $\cdot CH_2(CH_3)_2$ Si radical, and to *m/e* 607 and 592 by the successive loss of Me_3SiOH and $\cdot CH_3$. The ion *m/e* 664 corresponds to *m/e* 507 of the mass spectra of **6** and **7** by having one Me_3Si-group replaced by an *N*-(trimethylsilyl)indole-3-acetoxy group. The ion having *m/e* 592 then loses one Me_3SiOH to give *m/e* 502. The ion observed at *m/e* 463 does not relate to any obvious fragmentation pathway, and examination of the oscillographic recording of the mass spectrum shows that *m/e* 463 is accompanied by ions at *m/e* 463.5, 464, and 464.5, affording evidence that *m/e* 463 is the doubly charged analog of the molecular ion (*m/e* 926). The presence of the corresponding ion at *m/e* 490 in the *O*-Me_3Si-d_9 analogue of **3** confirms this assignment (Fig. 4, Spectrum V). The monoacylated parent compounds **6** and **7** do not show a doubly charged molecular-ion, suggesting that, in **3**, charge localization may occur simultaneously on both indole nuclei.

In comparing Spectrum II (**7**) with Spectrum III (**3**) it is apparent that the fragmentations from $M^{+\cdot}$ are identical, and it may be concluded that peak 3 of **3** is the di-*O*-(indole-3-acetyl)-*myo*-inositol that has one of the indole-3-acetyl groups on the 2-position of **2**. This conclusion is supported by the fact that **3** decomposes thermally mainly to **6** upon g.l.c. on 1.2- and 1.8-m columns, by the favored elimination of the axial 2-substituent. The position of the second indole-3-acetyl group is not certain, however. It is very probably at the 1-DL-1- position, as there is no steric hindrance between the acyl groups vicinal to each other, and as no major, monoacylated peaks were found that would correspond to the 5-ester (peak 2) of the 1-DL-4-ester (peak 4) on g.l.c.

Mass spectra of trimethylsilylated **4**. — The composition and stoichiometry of **4** showed three moles of **1** esterified to one mole of **2**. G.l.c. of the Me_3Si ethers of **4** on a 0.4-m column showed five peaks, three of which (peaks 1–3) corresponded in retention time to the three peaks of **3**. Peaks 4 and 5 were not observed with **3**. As mentioned previously, observation indicates that **4** thermally decomposes to **3** and two isomers not previously observed. Combined g.l.c.–m.s. of the Me_3Si ethers of **4** confirmed this assumption. Peaks 1–5 all showed the same molecular ion at *m/e* 926. The mass spectra of the first three peaks were indistinguishable from the mass spectra of the Me_3Si ethers of **3**. The mass spectra of the Me_3Si-d_9 analogue of **4** are shown in Fig. 4; Spectra V and VI. Comparison of these spectra with Spectrum III (**3**) confirms the fragmentation pathways of **3** by the corresponding mass shifts in the spectra. Spectrum V is almost identical with Spectrum III, and it may therefore be concluded that at least two acyl groups in **4** occupy the same position as in **3**, one acyl group at the 2-position and the second one at either the 1-DL-1 or 1-DL-4 position of **2**. Peaks 4 and 5, by virtue of their longer retention-times, are thus probably diacyl isomers of the following types: 1-DL-1,4-, 1-DL-1,6-, 1-DL-2,3-, 4,6-, or 1-DL-1,5-, as there are fifteen theoretical di-*O*-(indole-3-acetyl)-*myo*-inositols, six pairs of mirror images and three symmetrical esters. At the present time it is not possible to assign peak 5 of **4** to any one of these possible substitution modes. Merely by analogy with **6** and **7** it may be inferred that peaks 4 and 5 belong to **6**. By first converting **4** into the Me_3Si ether and subsequent m.s. analysis by direct probe it was possible to obtain the mass spectrum of the intact compound. The spectrum is shown in Fig. 3; Spectrum IV. The ion at *m/e* 1083 corresponds to the Me_3Si ether of the tri-*O*-(indole-3-acetyl)-*myo*-inositol, confirming the assignment of three moles of **1** per mole of **2**. The molecular ion fragments to *m/e* 881 by losing one *N*-(trimethylsilyl)indole-3-methylene radical (202 daltons). The $M^{+\cdot}$ ion also loses one *N*-(trimethylsilyl)indole-3-ketene radical (229 daltons) yielding *m/e* 854 or 836 by the loss of *N*-(trimethylsilyl)indole-3-acetic acid. A weak ion at *m/e* 926 is the result of a rearrangement of the odd-electron molecular-ion, leading to the elimination of an indole-3-acetoxyl radical.



Scheme 4.

The ion having *m/e* 361 is intense in comparison with that in Spectrum I, indicating that cleavage of the inositol ring is more readily achieved in **4** than in **3** or **6**. The remaining ions have been discussed in connection with **3**, **6**, and **7**. It may be assumed that the mass spectrum of **4** is a composite of spectra of at least two or more isomers. Two isomers of **4** have been detected by t.l.c., and thermal degradation on g.l.c. showed evidence of at least five isomers of the twenty theoretically possible esters: 8 pairs of mirror images and four symmetrical esters. By analogy with the major breakdown products of **6** and **3**, it may however be concluded that in **4** the acyl groups predominantly occupy the 1,2,3- and 2,4,6-positions of *myo*-inositol.

EXPERIMENTAL

Extraction and purification. — The extraction of 10.5 kg of ground corn kernels of *Zea mays* L. (cultivar Stowell's Everygreen hybrid) has been described previously[9,14]. The acetone-free, aqueous extract was partitioned with 1-butanol, and the butanol phase dried and redissolved in water. The water-insoluble residue was removed by filtration and dried to yield 2.89 g containing[9,12,14] 4.35% of **1**. This residue was dissolved in 1.5 ml of 1:1 ethanol–water and chromatographed on a partially sulfonated styrene–divinylbenzene copolymer (column inside diameter 9.0 mm, bed volume 38.2 ml, void volume 13.5 ml) with 1:1 ethanol–water as eluent, collecting 2.0-ml fractions. Small aliquots of the fractions (10–50 μl) were monitored by t.l.c.[14] and the fractions containing **3** and **4** (tubes 25–80) were pooled. The pooled sample was dried, redissolved in 1.5 ml of 1:1 ethanol–water and chromatographed on Sephadex LH-20 (column inside diameter 9.0 mm, bed volume 38.5 ml, void volume 8.5 ml) with 1:1 ethanol–water as eluent. The 2.0-ml fractions were again monitored by t.l.c., and the fractions containing **3** and **4** (tubes 40–70) were pooled and dried. The dry weight of the residue was 2.3 mg and the content of **1** was 31.6%. The residue was dissolved in 1.0 ml of 1:1 ethanol–water and chromatographed again on the same Sephadex LH-20 column. The fractions containing mainly **4** (tubes 43–55) and **6** (tubes 56–72) were pooled and further purified by preparative t.l.c. as described previously[12]. Following this step, 510 μg of **3** and 210 μg of **4** were obtained.

Ammonolysis. — Samples of **4** and **6** were hydrolyzed in 14% ammonium hydroxide and the hydrolysis products analyzed by t.l.c., g.l.c., and g.l.c.–m.s.[13,14]. Compound **1** was determined in two ways: spectrophotometrically at 221 and 280.5 nm, and by the peak area on g.l.c.[15]. Compound **2** was determined by the peak area on g.l.c.[15]. Incomplete hydrolysis with 2.15% ammonium hydroxide in 1:1 ethanol–water was conducted exactly as with 14% ammonium hydroxide. After 1, 5, and 30 min, the reaction was stopped by rapidly drying the solution *in vacuo*. The hydrolysis products were assayed by t.l.c. and visualized with Ehrlich reagent and concentrated sulfuric acid[14].

Gas–liquid chromatography. — Trimethylsilylation of **3**, **4**, **6**, and **7** with *N*,*O*-bis(trimethylsilyl)trifluoroacetamide or *N*-(trimethylsilyl-d_9)imidazole was performed as described before[13,14]. The Me_3Si ethers were analyzed on a F and M Model 402 gas chromatograph equipped with flame-ionization detectors, with nitrogen as carrier gas at a flow rate of 60 ml per min. Glass columns of 1.8, 1.2, and 0.4 m × 3.0 mm (inside diameter) were used, packed with either OV-17, 3% on GasChrom Q (100–120 mesh); OV-1, 2% on GasChrom Z (100–120 mesh) (Applied Science Lab. Inc., State College, Pa., 16801, U. S. A.); or SE-30, 1% on Supelcoport (100–120 mesh) (Supelco Inc., Bellefonte, Pa., 16823, U. S. A.).

Mass spectrometry. — Combined g.l.c.–m.s. was performed on an LKB-9000 mass spectrometer with a 0.3 m × 3.0 mm (inside diameter) glass column packed with OV-1, 2% on GasChrom Z (100–120 mesh), and helium as carrier gas at a flow rate of 25 ml/min. The ionizing energy was 70 eV, the flash heater 270°, the molecular separator 260°, and the ion-source temperature 290°. The mass spectra were recorded with an on-line data acquisition and processing program[20]. The mass spectrum of **4** was recorded at a probe temperature of 135°.

ACKNOWLEDGMENTS

Dr. M. Ueda kindly provided the 1-butanol concentrates of the cyclitols used in the early stages of this work. The mass-spectrometry facility made available to us by Professor C. C. Sweeley was supported by a grant from the National Institute of Health (RR-00480). We thank Professor W. Wells for his helpful discussions concerning the thermal instability of the di- and tri-acyl esters and Mr. J. Harten for technical assistance in operating the mass spectrometer.

REFERENCES

1 G. S. AVERY, H. B. CREIGHTON, AND B. SHALUCHA, *Amer. J. Bot.*, 27 (1940) 289.
2 G. S. AVERY, J. BERGER, AND B. SHALUCHA, *Amer. J. Bot.*, 29 (1942) 765.
3 J. BERGER AND G. S. AVERY, *Amer. J. Bot.*, 31 (1944) 199.
4 J. BERGER AND G. S. AVERY, *Amer. J. Bot.*, 31 (1944) 202.
5 A. J. HAAGEN-SMIT, W. D. LEECH, AND W. R. BERGEN, *Amer. J. Bot.*, 29 (1942) 500.
6 C. LABARCA, P. B. NICHOLLS, AND R. S. BANDURSKI, *Biochem. Biophys. Res. Commun.*, 20 (1966) 641.
7 P. B. NICHOLLS, *Planta*, 72 (1967) 258.
8 R. S. BANDURSKI, M. UEDA, AND P. B. NICHOLLS, *Ann. N. Y. Acad. Sci.*, 165 (1969) 655.
9 M. UEDA AND R. S. BANDURSKI, *Plant. Physiol.*, 44 (1969) 1175.
10 M. UEDA AND R. S. BANDURSKI, *Plant Physiol. Suppl.*, 44 (1969) 27.
11 Z. PISKORNIK AND R. S. BANDURSKI, *Plant Physiol.*, 50 (1972) 176.
12 A. EHMANN, *Carbohyd. Res.*, (1974) in press.
13 A. J. P. MARTIN, *Biochem. Soc. Symp.*, 3 (1949) 4.
14 A. EHMANN AND R. S. BANDURSKI, *J. Chromatogr.*, 72 (1972) 61.
15 M. UEDA, A. EHMANN, AND R. S. BANDURSKI, *Plant Physiol.*, 46 (1970) 715.
16 P. B. NICHOLLS, B. L. ONG, AND M. E. TATE, *Phytochem.*, 10 (1971) 2207.
17 M. UEDA AND R. S. BANDURSKI, *Phytochem.*, 13 (1974) 243.
18 A. EHMANN, *Phytochem.*, (1974) in press.
19 D. C. DEJONGH, T. RADFORD, J. D. HRIBAR, S. HANESSIAN, M. BIEBER, G. DAWSON, AND C. C. SWEELEY, *J. Amer. Chem. Soc.*, 91 (1967) 1728.
20 C. C. SWEELEY, in R. M. BURTON (Ed.), *Introduction to Lipid Chemistry*, Bio-Science International, Webster Groves, Inc., 1973, in press.