Carbohydrate Research, 34 (1974) 99–114

IDENTIFICATION OF 2-*O*-(INDOLE-3-ACETYL)-D-GLUCOPYRANOSE, 4-*O*-(INDOLE-3-ACETYL)-D-GLUCOPYRANOSE AND 6-*O*-(INDOLE-3-ACETYL)-D-GLUCOPYRANOSE FROM KERNELS OF *Zea mays* BY GAS–LIQUID CHROMATOGRAPHY–MASS SPECTROMETRY*

AXEL EHMANN

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

(Received October 3rd, 1973; accepted in revised form, December 24th, 1973)

ABSTRACT

New esters of indole-3-acetic acid and D-glucose have been isolated from mature sweet-corn kernels of *Zea mays*. The esters were resolved by t.l.c. into two fractions having R_F values distinct from that of authentic 1-*O*-(indole-3-acetyl)-β-D-glucopyranose. Analysis of the trimethylsilyl ethers of the two fractions by combined gas–liquid chromatography–mass spectrometry (g.l.c.–m.s.) showed that the esters have a free carbonyl group. Labeling of the carbonyl carbon atom with an *O*-methyloxime group, and analysis of the *O*-trimethylsilyl *O*-methyloxime derivatives by g.l.c.–m.s. permitted the new compounds to be identified as a mixture of 2-*O*-(indole-3-acetyl)-D-glucopyranose, 4-*O*-(indole-3-acetyl)-D-glucopyranose, and 6-*O*-(indole-3-acetyl)-D-glucopyranose.

INTRODUCTION

Several esters of indole-3-acetic acid (**1**) and a sugar have been isolated from plant materials. Zenk[1] first isolated a D-glucose ester of **1** from pea epicotyls and leaves of *Colchicum neapolitanum* after incubating the tissues and labeled **1**. This ester showed characteristics typical of an ester linkage at C-1 of D-glucose and Zenk[1,2] assigned the structure 1-*O*-(indole-3-acetyl)-β-D-glucopyranose to it. Following the incubation of wheat coleoptiles with labeled **1**, Klämbt[3] isolated an ester of **1** and D-glucose which he tentatively identified as 1-*O*-(indole-3-acetyl)-β-D-glucopyranose. A compound isolated from *Avena* coleoptiles by Keglević and Pokorny[4], behaved on t.l.c. as authentic 1-*O*-(indole-3-acetyl)-β-D-glucopyranose, but further characterization is needed before a definitive assignment can be made. Ueda and Bandurski[5] have isolated two unidentified esters of **1**, designated HRF-1 (**2**) and HRF-2 (**3**), in trace amounts from mature kernels of sweet corn. The present report describes the

*This work was supported, in part, by the National Science Foundation (GB-18353X). Journal Article No. 6530 from the Michigan Agricultural Experiment Station.

isolation, purification and identification of these esters, by t.l.c., g.l.c., and combined g.l.c.–m.s. analysis, as three isomers of *O*-(indole-3-acetyl)-D-glucose.

RESULTS AND DISCUSSION

The extraction of 10 kg of dry sweet-corn kernels of *Zea mays* L. (cultivar Stowell's Evergreen Hybrid) yielded 420 μg (as **1**) of **2** and 480 μg (as **1**) of **3**. This concentration (about 100 μg/kg dry weight) is low compared to the cyclitol esters of **1** (about 65 mg/kg dry weight) previously identified[5]. Following purification on "low capacity" styrene–divinylbenzene copolymer resin[6] (Fig. 1-A), Sephadex LH-20 (Fig. 1-B), and separation of the two esters of **1** into **2** and **3** by preparative t.l.c., it became apparent that **2** and **3** undergo interconversion, presumably by acyl migration[7–15]. As is seen in Fig. 1-C, rechromatography of **2** (band 3: central region of **2**) or **3** (band 2: central region of **3**) always yielded a small amount of the other isomer, **2** from **3**, or **3** from **2**.

The ammonolysis products of **2** and **3** were identified by t.l.c. as **1**, indole-3-acetamide (**4**), and D-glucose, and by g.l.c. and combined g.l.c.–m.s. as **1**, **4**, and α+β-D-glucopyranose. By using authentic 1-*O*-(indole-3-acetyl)-β-D-glucopyranose as a working standard the stoichiometry of (**1**+**4**)/glucose was found to be 0.98/1.00 for **2** and 0.97/1.00 for **3**. Compounds **2** and **3** were thus identified as two isomeric *O*-(indole-3-acetyl)-D-glucoses. It can be seen from Fig. 1-C that the R_F values of **2** and **3** are different from that of authentic 1-*O*-(indole-3-acetyl)-β-D-glucopyranose, indicating that **1** in **2** and **3** is esterified to glucose at positions other than O-1.

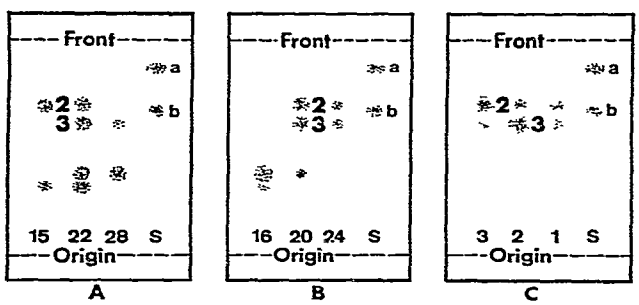


Fig. 1. Thin-layer chromatograms of fractions containing **2** and **3**: (A) chromatography on partially sulfonated styrene–divinylbenzene copolymer resin, fractions 15, 22, and 28; (B) chromatography on Sephadex LH-20, fractions 16, 20 and 24; and (C) preparative t.l.c. of the pooled fractions (16–24) eluted from Sephadex LH-20 (see Experimental section). Conditions for t.l.c. and visualization of the indole compounds were described before[6]. The relative R_F values are based on the R_F value of indole-3-acetic acid (*a*). The relative R_F values for **2** and **3** reported by Ueda and Bandurski[5] were 0.81 and 0.71, compared with 0.80 and 0.71 reported here.

Gas–liquid chromatography. — G.l.c. of the trimethylsilyl (Me_3Si) ethers of a mixture of **2** and **3** (tube no. 22 from the Sephadex LH-20 column; Fig. 1-B) gave five peaks, the relative retention-times of which are shown in Table 1. Besides the liquid

phases listed in Table 1 (OV-1, 2%, and OV-17, 3%), other liquid phases (SE-30, 3%; OV-210, 3%; and SP-2401, 5%) were tried, but none of them improved the resolution of the overlapping peaks. Subsequent g.l.c. of the Me_3Si ethers of **2** and **3** (band no. 3 and band no. 2; Fig. 1-C) showed that **2** gives rise to peaks 2 and 4 (main peaks), and peaks 1, 3, and 5 (minor peaks). Compound **3** on the other hand gives peaks 1, 3, and 5 (main peaks) and peaks 2 and 4 (minor peaks). A correlation of the Ehrlich-positive spots of **2** and **3** on t.l.c. with the peak areas observed by g.l.c. of the Me_3Si ethers of **2** and **3** is shown in Table 1. Compounds **2** and **3** may therefore be assigned to peaks 2 and 4, and peaks 1, 3, and 5, respectively. By contrast, g.l.c. of the Me_3Si ether of 1-*O*-(indole-3-acetyl)-*β*-D-glucopyranose gave one peak having a retention time distinct from that of peaks 1 to 5. This suggests that **2** and **3** are esters of **1** and D-glucose with the ester linkage at positions other than O-1, assuming that the hexose is in the pyranose form.

Mass spectrometry. — Combined g.l.c.–m.s. analysis of the *O*-Me_3Si derivatives of **2** and **3** showed that peaks 2 and 4 and peaks 1, 3, and 5, respectively, had almost identical mass spectra. The mass spectra of peaks 2 (spectrum I) and 3 (spectrum II), and the *O*-Me_3Si derivative of 1-*O*-(indole-3-acetyl)-*β* D-glucopyranose (spectrum III)

TABLE I

THE CONTRIBUTION OF COMPOUNDS **2**[a] AND **3**[a] TO THE EHRLICH-POSITIVE SPOTS ON T.L.C., THE FIVE PEAKS OF THE Me_3Si DERIVATIVES OF A MIXTURE OF **2** AND **3**, AND TO THEIR MeON-*O*-Me_3Si DERIVATIVES[b]

Mixture of **2** *and* **3**	R_F[e] *or* R_T *liquid phase*		*Preparative t.l.c. sample number* 2:2	3:3
			% peak area	*% peak area*
T.l.c.[c]: **2**	0.81		20	90
3	0.71		80	10
G.l.c.[d]: Me_3Si derivatives	OV-17, 3% (1.8 m, 227°)	OV-1, 2% (1.2 m, 220°)		
Peak 1	0.68	0.70	17.3	3.4
Peak 2	0.73	0.75	8.6	38.7
Peak 3	0.96	0.98	41.2	10.1
Peak 4	1.01	1.03	13.7	47.8
Peak 5	1.09	1.14	19.2	
1-*O*-(indole-3-acetyl)-*β*-D-glucopyranose MeON-*O*-Me_3Si derivatives	1.00[f]	1.00[g]		
Peak 1	0.62		21.3	49.2
Peak 2	0.77		6.4	26.8
Peak 3	0.90		72.3	24.0

[a]Band eluted from the **2** and **3** regions (see Fig. 1). [b]The imino group of the indole nucleus is substituted with a Me_3Si group. [c]Percent spot intensity was estimated visually from reference spots of known amounts of indole-3-acetic acid. [d]Percent peak area was determined as described before[6]. [e]R_F values from Fig. 1. [f]Retention time 12.6 min. [g]Retention time 9.65 min.

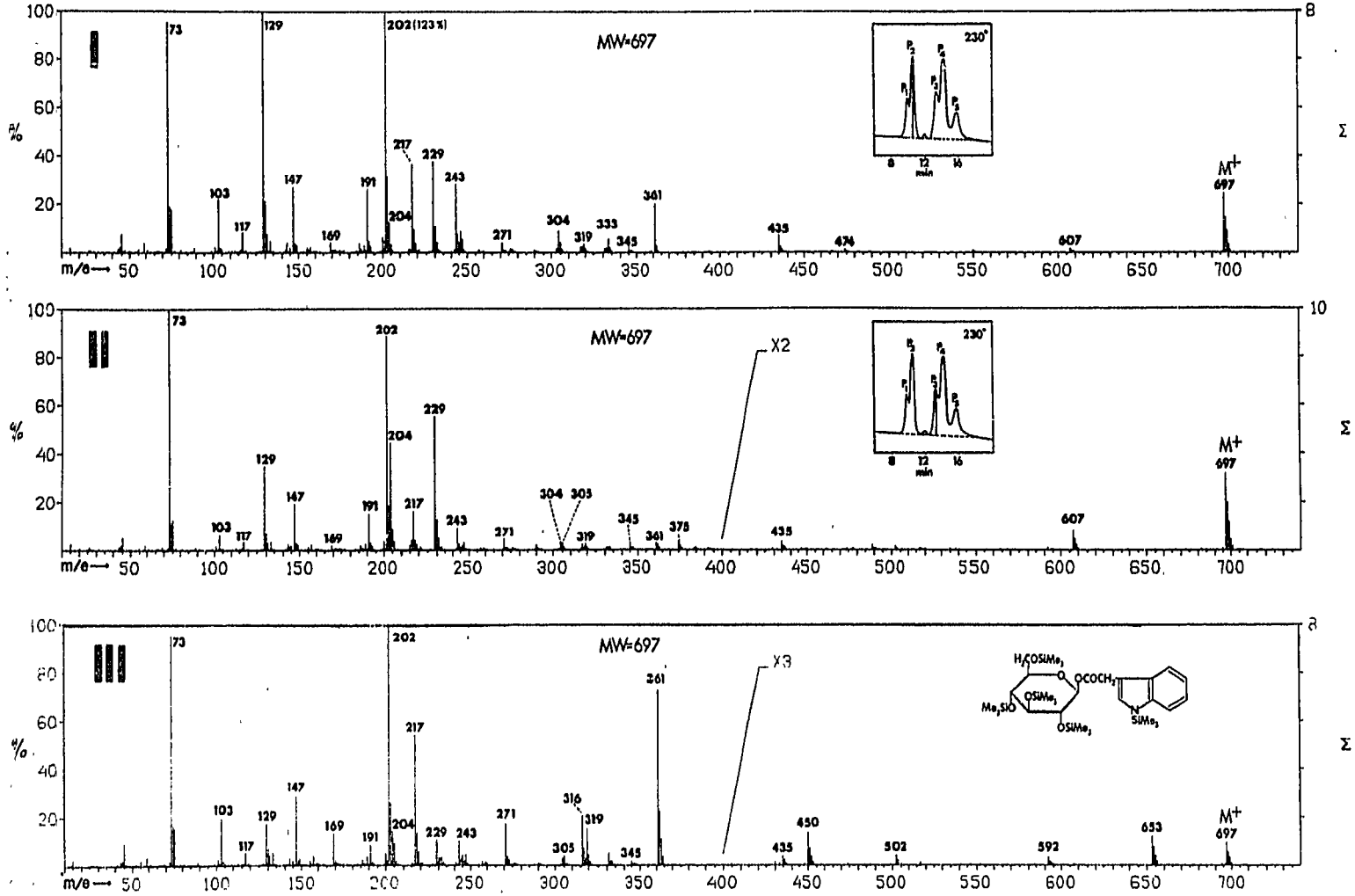
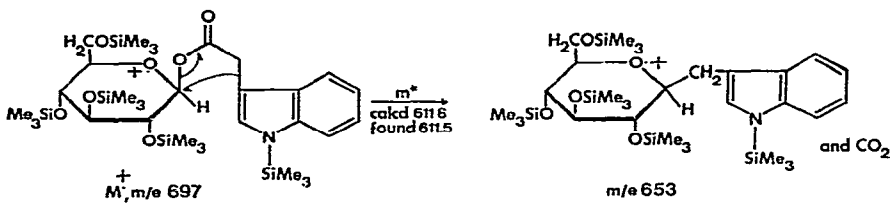


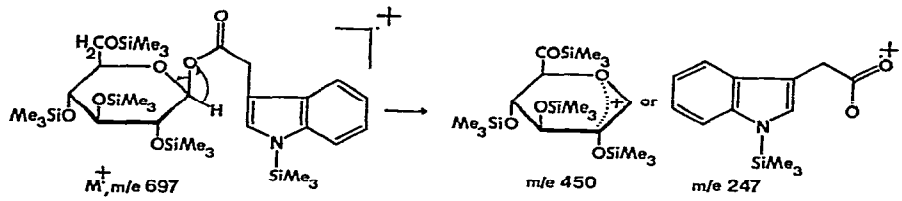
Fig. 2. Mass spectra of peaks 2 (spectrum I) and 3 (spectrum II) of the *O*-Me_3Si derivatives of a mixture of **2** and **3**. Spectrum III is that of the derivative of authentic 1-*O*-(indole-3-acetyl)-*β*-D-glucopyranose. The spectra were recorded isothermally at 230° as described in the experimental section. The inserts show the gas–liquid chromatograms, and the shaded area under each peak represents the contribution of the peak to the individual spectra obtained.

are presented in Fig. 2. The ions *m/e* 653 and 450 in spectrum III are characteristic of the ester linkage in 1-*O*-(indole-3-acetyl)-*β*-D-glucopyranose. The ion *m/e* 653 is formed by the expulsion of CO_2 from the odd-electron molecular ion. This transition is supported by a metastable peak observed at *m/e* 611.5.



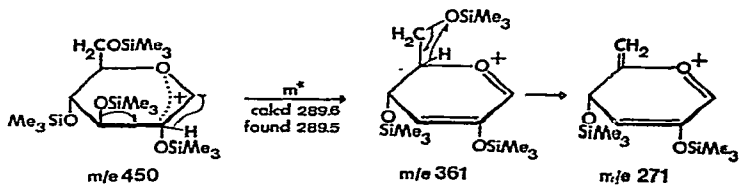
Scheme 1.

The ion *m/e* 450 (ref. 16), rather than the *m/e* 451 observed in tetra-*O*-trimethylsilyl hexopyranosides[17,18], results from the transfer of a hydrogen atom to the leaving group prior to cleavage at the ester linkage. The spectrum of the *O*-Me_3Si-d_9 derivative of 1-*O*-(indole-3-acetyl) *β*-D-glucopyranose (unpublished results) showed that the hydrogen atom comes from the carbon skeleton. Retention of charge on the glucose ring results in the ion *m/e* 450, and charge retention on the leaving group gives rise to *m/e* 247.



Scheme 2.

The ion *m/e* 450 fragments further to *m/e* 361, an ion of high relative intensity, through the loss of a trimethylsilyloxy radical. A metastable peak at *m/e* 289.5 supports this transition. The ion *m/e* 361 loses another Me_3SiOH molecule to give rise to *m/e* 271.



Scheme 3.

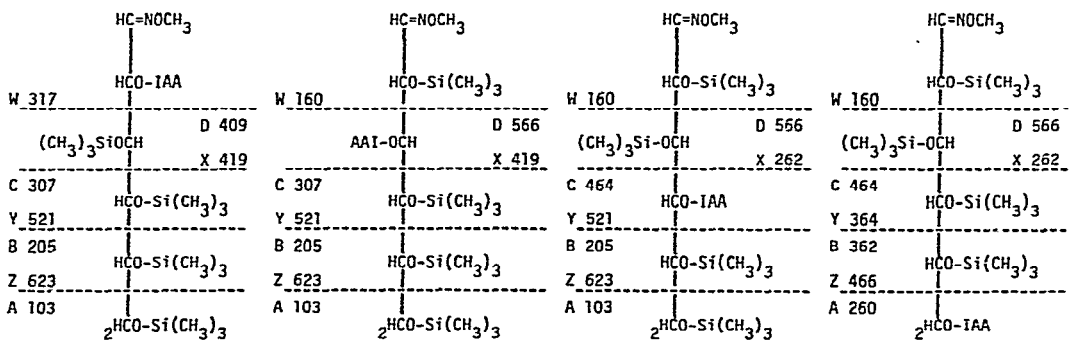
The lack of the ions *m/e* 653, 450 and the metastable peak at *m/e* 289.5 in the mass spectra I and II confirms that **2** and **3** have a free carbonyl group. It is not, however, possible to establish the location of the ester linkage, because of the absence of diagnostic ion-fragments.

The mass spectra of Me_3Si ethers of pyranoses[17] are characterized by an ion of high relative-intensity at *m/e* 204, whereas the furanoses show a higher-intensity ion at *m/e* 217. The ratio of *m/e* 204/217 is therefore considered characteristic of ring size[17,19,20]. However, the mass spectrum of the *O*-Me_3Si derivative of 1-*O*-(indole-3-acetyl)-β-D-glucopyranose[4] shows a *m/e* 204/217 ratio typical of a furanose ring. The presence of the indole-3-acetyl group on C-1 (and also on other positions of the ring) must suppress the formation of *m/e* 204 in favor of *m/e* 217. This suppression of *m/e* 204 has also been observed in the mass spectrum of the *O*-Me_3Si derivative of D-galactosyl phosphate[16].

It is therefore not possible to deduce the ring size or linkage position from the mass spectra I and II, but as the compounds **2** and **3** have a free carbonyl group, they can be converted into their *O*-methyloxime (MeON) *O*-Me_3Si derivatives.

Gas–liquid chromatography–mass spectrometry of the MeON-O-Me$_3$Si derivatives of **2** *and* **3**. — Laine and Sweeley[21,22] have demonstrated the usefulness of MeON-*O*-Me_3Si derivatives of sugars in locating substituents on the carbon skeleton. Preliminary experiments with 1-*O*-(indole-3-acetyl)-β-D-glucopyranose, which cannot react with methoxylamine HCl, showed that the ester linkage is unaffected during the reaction. Subsequent trimethylsilylation with *N,O*-bis(trimethylsilyl)trifluoroacetamide yielded 2,3,4,6-tetra-*O*-trimethylsilyl-1-*O*-[*N*-(trimethylsilyl)indole-3-acetyl]-β-D-glucopyranose quantitatively.

The MeON-*O*-Me_3Si derivatives of **2** and **3** gave only three peaks on g.l.c., with complete resolution of the individual peaks. The relative retention-times for these peaks are listed in Table 1. A very small shoulder (less than 20% contribution to the total peak area) accompanied each peak at the downslope. Laine and Sweeley[22] have shown that the *syn* and *anti*- forms of *O*-methyloximes give similar mass spectra. No attempt was therefore made to separate these two forms. Since the *O*-methyloxime function labels the carbonyl carbon atom, the interpretation of the spectra of the



Form. 1.

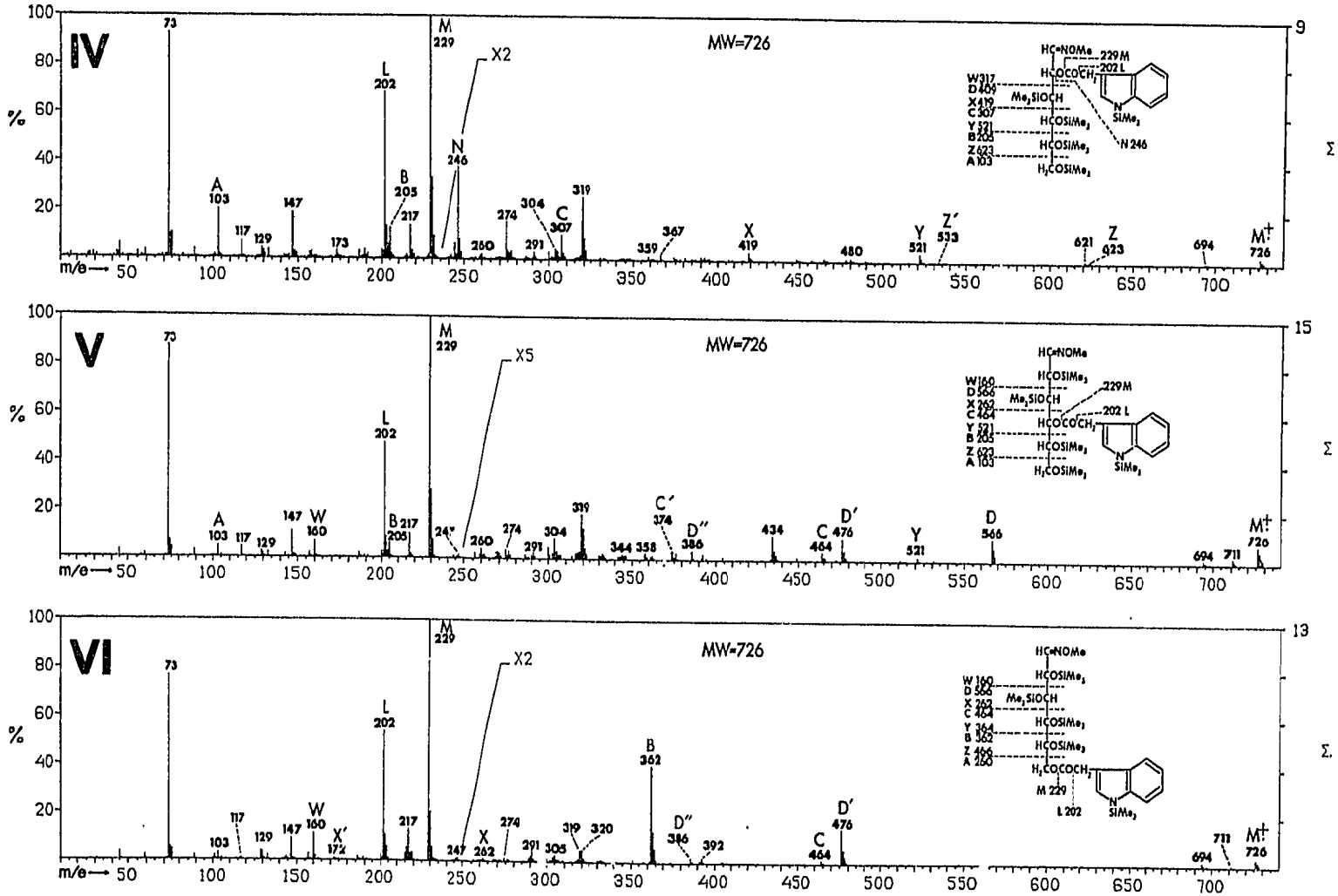
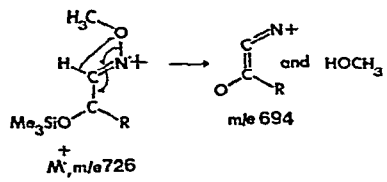


Fig. 3. Mass spectra of the second (spectrum IV), first (spectrum V), and third (spectrum VI) peak of the MeON-*O*-Me_3Si-derivatives of a mixture of **2** and **3**, trimethylsilylated with *N,O*-bis(trimethylsilyl)trifluoroacetamide. The inserts show the ion series resulting from simple homolytic cleavage of the carbon skeleton. Conditions are the same as in Fig. 2.

acyclic MeON-*O*-Me_3Si derivatives of **2** and **3** should allow positive determination of the point of esterification.

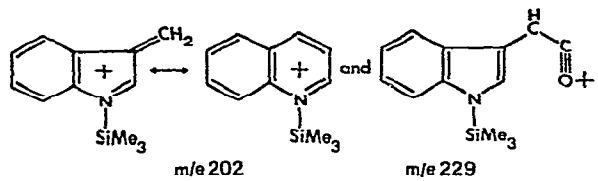
It may be assumed that **2** and **3** both have the pyranose ring, and thus the four acyclic structures of Form. I are possible, with **1** as the ester group at either position 2, 3, 4, or 6.

The mass spectra of the three peaks observed for the MeON-*O*-Me_3Si derivatives of **2** and **3** are shown in Fig. 3 (spectra IV, V and VI). All of the spectra show an ion at *m/e* 726, which corresponds to the molecular ion. This allows the conclusion that **2** and **3** constitute three isomeric esters of **1** and D-glucose. Initial fragmentation results in the loss of $\cdot CH_3$ to give the ion at *m/e* 711. The ion at *m/e* 694 differs from $M^{\ddagger}$ by 32 daltons, and may be explained by the following McLafferty rearrangement of the odd-electron molecular ion, which subsequently leads to the loss of the neutral $HOCH_3$ molecule.



Scheme 4.

The ions *m/e* 202 (L) and 229 (M) are characteristic of *N*-(trimethylsilyl)indole-3-acetyl esters (see Fig. 2) and are due to cleavage of the *N*-(trimethylsilyl)indole-3-methylene and *N*-(trimethylsilyl)indole-3-acetyl group from the carbon skeleton with charge retention on the leaving group.

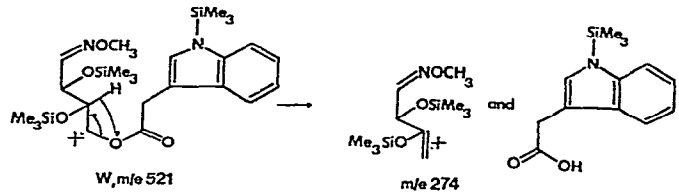


Scheme 5.

In addition, a number of ions, *m/e* 73, 117, 129, 147, 204, 127, 247, 291, 305, and 319, are common ions of Me_3Si ethers of carbohydrates[17,22].

Mass spectrum of peak 1 (Spectrum V). — The ion W at *m/e* 160 arises by homolytic C-2–C-3 cleavage with charge retention on W, and implies that C-2 is not substituted[22]. This is confirmed by the presence of the corresponding ion D at *m/e* 566 and its homologues D′ (D-Me_3SiOH) and D″ (D′-Me_3SiOH). Peaks found at *m/e* 103 (A) and 205 (B) identify the substitution on C-4. This is confirmed by the presence of ion C, *m/e* 464 and its homologue C′ at *m/e* 374, which arises by homolytic

C-3–C-4 cleavage. The ion Y at *m/e* 521 supports this conclusion. A weak ion is found at *m/e* 274, derived from ion Y by loss of *N*-(trimethylsilyl)indole-3-acetic acid.



Scheme 6.

Similarly, the ion *m/e* 376 could be derived from the ion Z, and *m/e* 623 (not present) by the loss of the same group. Two ions, *m/e* 434 and its homologue *m/e* 344, could arise from the successive loss of two $\cdot CH_3$ radicals from the ion C. Indeed an ion of low intensity at *m/e* 449 supports this fragmentation pathway.

Mass spectrum of peak 2 (spectrum IV). — The spectrum is characterized by the absence of *m/e* 160, but the ions *m/e* 103 (A), *m/e* 205 (B), and *m/e* 207 (C) are present, providing evidence that the substitution is on C-2. The ions *m/e* 419 (X), *m/e* 521 (Y), and *m/e* 623 (Z) confirm this conclusion. The ion-pair W and D is missing, suggesting that the homolytic C-2–C-3 cleavage is suppressed in favor of the cleavage between C-2 and the *N*-(trimethylsilyl)indole-3-acetyl group. This results in the rather intense ion N at *m/e* 246. The corresponding ion at *m/e* 480 with charge retention on the carbon skeleton confirms this fragmentation. The ion *m/e* 173 is probably derived from ion X by loss of the *N*-(trimethylsilyl)indole-3-acetyl group.

Mass spectrum of peak 3 (spectrum VI). — The presence of the ion series W (*m/e* 160), X (*m/e* 262) and the corresponding ions D (*m/e* 476), C (*m/e* 464), and B (*m/e* 362) provide conclusive evidence that the substitution is on C-6. By analogy to D (*m/e* 409) in spectrum VII, the ion A (*m/e* 260) is suppressed in favor of the preferred C-4–C-5 cleavage. To support this reasoning, the MeON-*O*-Me_3Si derivative of the model compound D-galacturonic acid was studied. Its spectrum is shown in Fig. 4 (Spectrum VII). As expected, the substituted C-6 completely suppresses the homolytic C-5–C-6 cleavage in favor of C-4–C-5 cleavage, resulting in the ions B at *m/e* 248 and Y at *m/e* 364.

The mass spectra of the MeON-*O*-Me_3Si derivatives of **2** and **3** were obtained by trimethylsilylating the MeON-derivatives with *N,O*-bis(trimethylsilyl)trifluoroacetamide, resulting in the quantitative substitution of the imino group of the indole nucleus by a Me_3Si group. It was known from other experiments that *N*-(trimethylsilyl)imidazole at 25° will not react with primary or secondary amines, and it should be possible to obtain the MeON-*O*-Me_3Si derivatives of **2** and **3** retaining a free imino group. All indole-3-acetyl-containing ion fragments would thus be identified in the mass spectra by a down-shift of 72 daltons, thereby providing additional evidence as to the location of the ester linkage. Trimethylsilylation of the MeON-derivatives of **2**

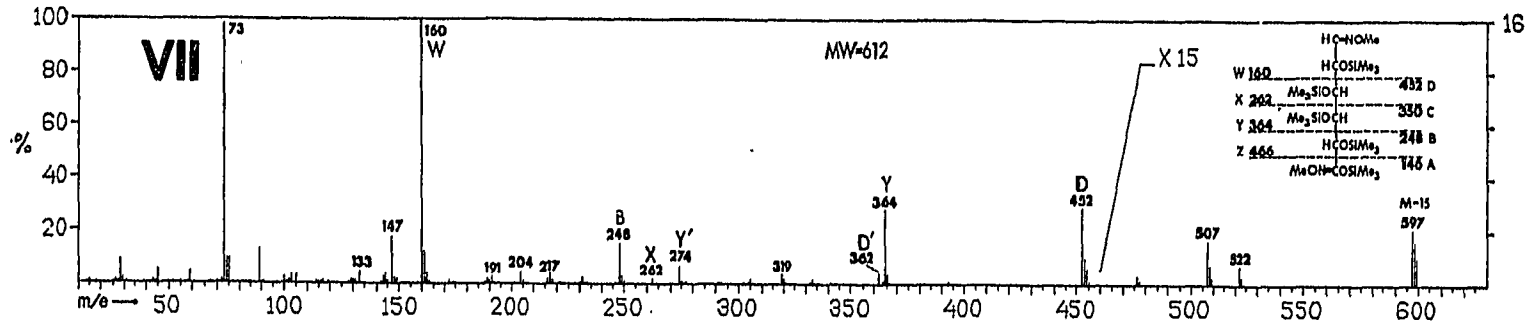


Fig. 4. The mass spectrum of the MeON-*O*-Me_3Si derivative of D-galacturonic acid (spectrum VII). Conditions as described in Fig. 2.

and **3** with *N*-(trimethylsilyl)imidazole at 80° resulted in three doublet peaks on g.l.c., the second peak of each doublet corresponding to the imino-group substituted analogues. This result indicated that about one half of the derivatives had a substituted imino group.

Utilizing the mass spectrometer–computer data-acquisition system for repetitive scans[23], the mass chromatograms shown in Fig. 5 were produced. The bottom panels show the total ion-intensity versus the mass spectrum scan-number of a mixture of imine-substituted and imine-non-substituted MeON-*O*-Me_3Si derivatives of **2** and **3** (solid line). The total ion-intensity versus mass spectrum scan-number of the three

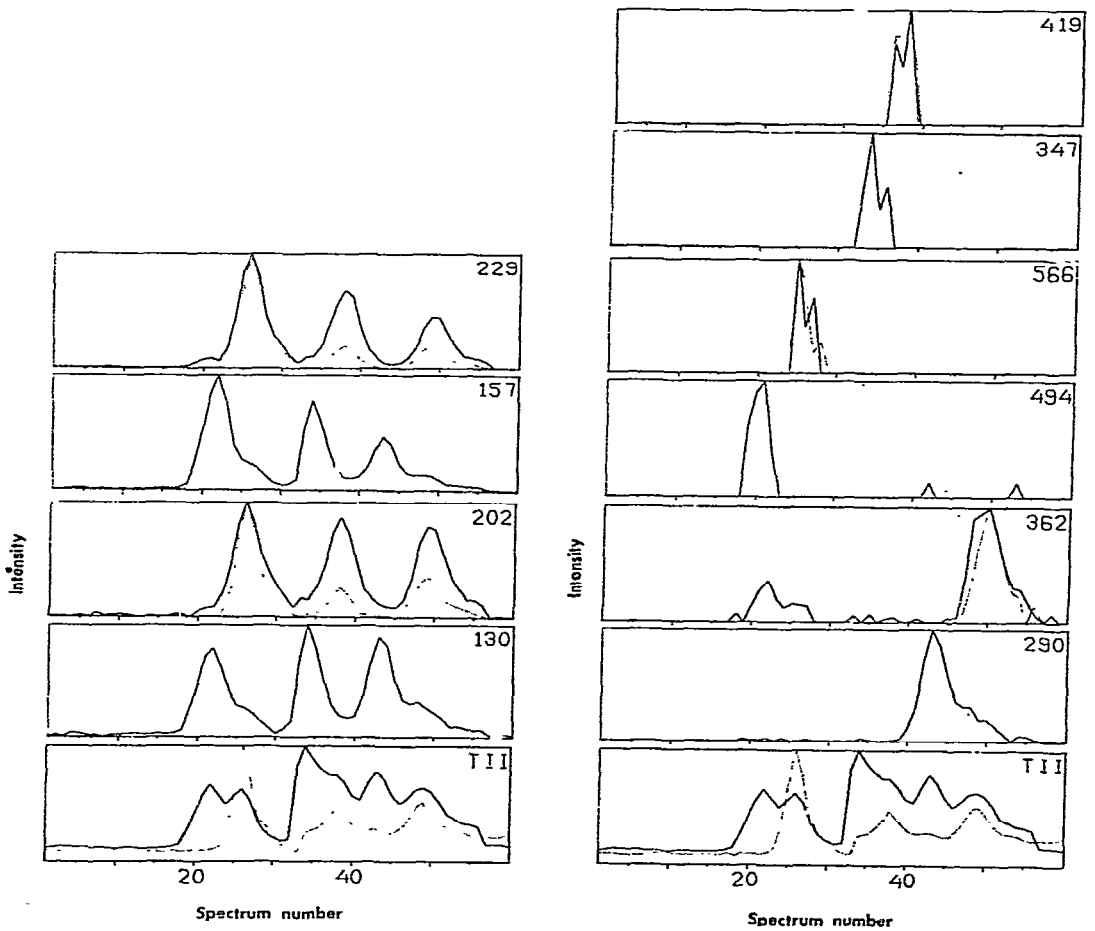


Fig. 5. Mass chromatograms for the intensities of the ion pairs *m/e* 130 and 202, *m/e* 157 and 229, *m/e* 290 and 362, *m/e* 494 and 566, and *m/e* 347 and 419, compared with the total ion-intensity of the same scans (bottom panels). The mass chromatograms were obtained from the repetitive scans of a mixture of MeON-*O*-Me_3Si-derivatives of **2** and **3** having both a free Me_3Si-substituted imino-group (solid lines), and the same mixture containing no free imino group (broken lines). Conditions are as described in Fig. 2 and the Experimental section.

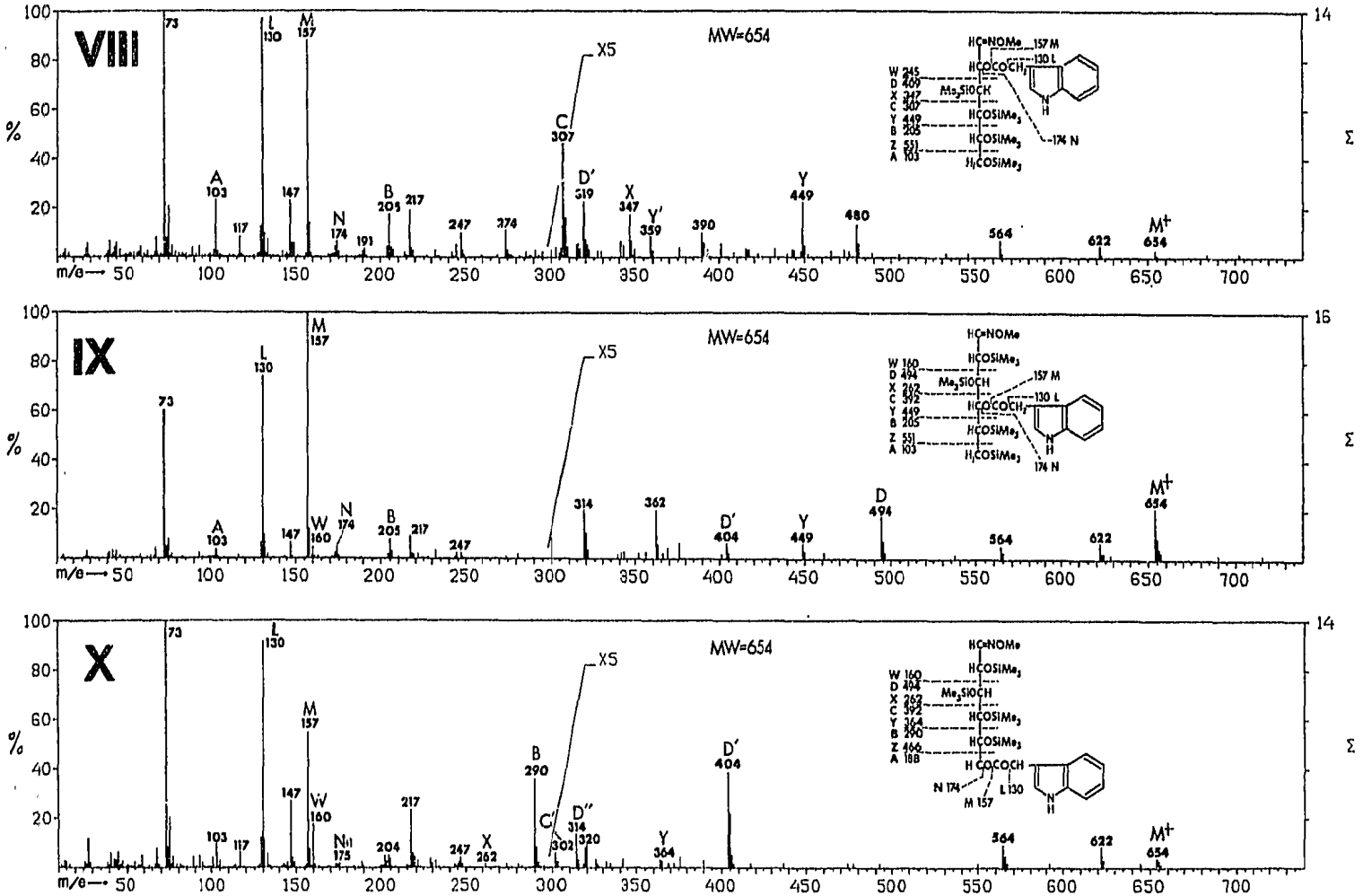


Fig. 6. Mass spectra of the second (spectrum VIII), first (spectrum IX), and third (spectrum X) peak of the MeON-*O*-Me_3Si derivatives of a mixture of **2** and **3** trimethylsilylated with *N*-(trimethylsilyl)imidazole. Conditions as in Fig. 2.

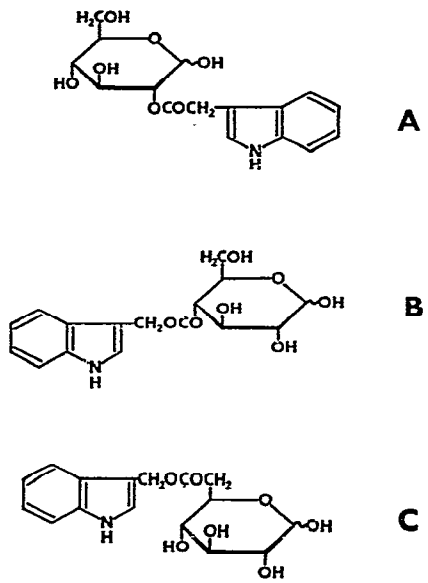


Fig. 7. The structures of 2-*O*-(indole-3-acetyl)-D-glucopyranose (A), 4-*O*-(indole-3-acetyl)-D-glucopyranose (B), and 6-*O*-(indole-3-acetyl)-D-glucopyranose (C).

corresponding peaks of the imino-substituted derivatives is shown by the broken line. The panels above show the mass chromatograms for the intensities of the ion pairs *m/e* 130 and 202, *m/e* 157 and 229, *m/e* 290 and 362, *m/e* 494 and 566, and *m/e* 347 and 419 versus mass spectrum scan-number. These mass chromatograms clearly demonstrate that the first peak of each doublet has the free imino group. As can be seen from the mass chromatograms of the total ion-intensities, there is poor resolution of the doublet peaks. However, by computer subtraction of the second peak in each doublet from the first peak, the three spectra of the MeON-*O*-Me_3Si derivatives of **2** and **3** containing a non-substituted imino-group were obtained. The spectra are shown in Fig. 6 (spectra VIII, IX and X). The difference of 72 daltons between the ions containing a free imino-group (Fig. 6) and a substituted imino-group (Fig. 3) confirm the location of the ester linkage on C-2, 4 (**2**), and 6 (**3**) for peaks 2, 1, and 3. The structures (Fig. 7) of the three isomeric *O*-(indole-3-acetyl)-D-glucopyranoses isolated from kernels of *Zea mays* have thus been established.

EXPERIMENTAL

Extraction and purification. — The extraction of 10 kg of ground sweet-corn kernels of *Zea mays* L. (cultivar, Stowells Evergreen hybrid) was conducted as described before[6]. The butanol phase was taken to dryness under diminished pressure at 45°. Water (40 ml) was added to the residue, and the water-insoluble fraction was removed by filtration. The filtrate was lyophilized and the residue redissolved in

8.0 ml of 1:1 ethanol–water. The sample was chromatographed on "low capacity"[6] styrene–divinylbenzene copolymer resin (column i.d. 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. Aliquots (10–50 μl) of the fractions were monitored by t.l.c.[6] (Fig. 1-A), and the Ehrlich-positive regions of **2** and **3** pooled (tubes 15–28). The pooled sample was dried and redissolved in 4.0 ml of 1:1 ethanol–water and rechromatographed on Sephadex LH-20 (column i.d. 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. (Fig. 1-B). The fractions containing **2** and **3** (tubes 20–24) were pooled, dried, and redissolved in 0.2 ml of 1:1 ethanol–water. Compounds **2** and **3** were separated by preparative t.l.c. on precoated silica gel plates[5,6] into sample no. 1 (**2**+**3**), sample no. 2 (**3**) and sample no. 3 (**2**). Small aliquots were rechromatographed on t.l.c. (Fig. 1-C), and it was apparent that acyl migration had occurred during the elution of the compounds from the silica gel with 1:1 ethanol–water. No attempt was made to further purify **2** and **3**.

Ammonolysis. — Small samples of nos. **2** and **3** (containing 150 μg of **1**) were hydrolyzed in 14% ammonium hydroxide for 15 min at 65° in sealed ignition tubes. The reaction mixture was then dissolved in 1:1 ethanol–water and the solution was evaporated. This operation was repeated several times and the residue was then dissolved in 100 μl of 1:1 ethanol–water. The reaction products were then analyzed by t.l.c., g.l.c., and combined g.l.c.–m.s. Authentic 1-*O*-(indole-3-acetyl)-β-D-glucopyranose served as an internal standard for the stoichiometric determinations of **2** and **3**, which was performed by quantitative g.l.c. as described before[24]. Free **1** and the α and β anomers of D-glucose were also used as internal standards. The amount of **2** and **3** present was determined by g.l.c. by measuring quantitatively compounds **1** and **4** liberated during ammonolysis. The amounts of **2** and **3** isolated from 10 kg of corn kernels were 420 μg and 480 μg (as **1**), respectively.

Preparation of O-methyloximes. — The conversion of **2** and **3** into their corresponding *O*-methyloximes was conducted according to Laine and Sweeley[21,22], with slight modifications. Samples containing 50 μg of **1** were dried in 1.0-ml screw top, Teflon-lined vials, and 50 μl of dry pyridine containing 100 μg of methoxylamine hydrochloride (Supelco, Inc. Bellefonte, Penn. 16823, U.S.A.) was added, and the mixture was allowed to react for 2 h at 80°. *N,O*-Bis(trimethylsilyl)trifluoroacetamide (100 μl) or 100 μl of *N*-(trimethylsilyl)imidazole (Regis Chemical Co., Chicago, Ill. 60610, U.S.A.) was added to the reaction mixture, which was heated for an additional 15 min at 80°. To facilitate sample handling for repeated g.l.c. and g.l.c.–m.s. the Teflon liner was replaced by a Flurorcone™ septum (Pierce Chemical Co., P.O. Box 117, Rockford, Ill. 61105, U.S.A.), which allowed the direct and repeated withdrawal of aliquots of the reaction mixture. Samples stored in this way were stable for at least one month at 4°. The *O*-methyloximes of D-galacturonic acid were also prepared as just described.

Gas–liquid chromatography. — Trimethylsilylation of the samples with *N,O*-bis(trimethylsilyl)trifluoroacetamide or *N*-(trimethylsilyl)imidazole was performed

exactly as described before[6]. The derivatized samples were analyzed on an F & M Model 402 gas chromatograph equipped with flame-ionization detectors. Nitrogen was used as carrier gas at a flow rate of 60 ml/min. Two columns were used, a 1.8 m × 3.0-mm i.d. U-shaped glass column packed with OV-17 (3%) on Gas-chrom Q (100–120 mesh), and a 1.2 m × 3.0-mm i.d. U-shaped column packed with OV-1 (2%) on Gas-chrom Z (100–120 mesh) (Applied Science Lab Inc., State College, Penn. 16801, U.S.A.).

Mass spectrometry. — Combined g.l.c.–m.s. was performed on an LKB-9000 mass spectrometer with a 1.8 m × 3.0-mm i.d. glass column packed with SE-30 (1%) on Supelcoport (100–120 mesh), (Supelco Inc., Bellefonte, Penn. 16823, U.S.A.) and helium as carrier gas at a flow rate of 25 ml/min. The ionizing energy was 70 eV, the flash-heater temperature 250°, the molecular-separator temperature 250°, and the ion-source temperature 290°. The mass spectra and mass chromatograms were recorded with an on-line data-acquisition and processing program[23].

ACKNOWLEDGMENTS

I am grateful to Dr. C. C. Sweeley for his help in the interpretation of the mass-spectral data, Dr. R. S. Bandurski for his continued interest and advice, and Dr. D. Keglević for the generous gift of 1-*O*-(indole-3-acetyl)-β-D-glucopyranose. I thank Mr. Jack Harten for technical assistance in operating the mass spectrometer, and Mr. Norman Young who developed the mass-spectrometry computer system for the acquisition of data from repetitive scans and production of mass chromatograms.

REFERENCES

1 M. H. Zenk, *Nature*, 191 (1961) 493.
2 M. H. Zenk, *Colloq. Int. Cent. Nat. Rech. Sci. Paris*, 123 (1964) 241.
3 H. D. Klämbt, *Planta*, 55 (1961) 618.
4 D. Keglević and M. Pokorny, *Biochem. J.*, 114 (1969) 827.
5 M. Ueda and R. S. Bandurski, *Plant Physiol.*, 44 (1969) 1175.
6 A. Ehmann and R. S. Bandurski, *J. Chromatogr.*, 72 (1972) 61.
7 D. Keglević, *Carbohyd. Res.*, 20 (1971) 293.
8 E. Fischer, M. Bergmann, and A. Rabe, *Ber.*, 53 (1920) 2362.
9 H. Ohle, *Ber.*, 57 (1924) 403.
10 B. Helferich and W. Klein, *Ann.*, 450 (1926) 219.
11 P. Brigl and H. Grümmer, *Ann.*, 495 (1932) 67.
12 R. M. Mann, W. D. Maclay, and C. S. Hudson, *J. Amer. Chem. Soc.*, 61 (1939) 2432.
13 T. N. Cawley and R. Letters, *Carbohyd. Res.*, 19 (1971) 373.
14 C. Labarca, P. B. Nicholls, and R. S. Bandurski, *Biochem. Biophys. Res. Commun.*, 20 (1966) 641.
15 P. B. Nicholls, *Planta*, 72 (1967) 258.
16 D. J. Harvey and M. G. Horning, *J. Chromatogr.*, 76 (1973) 51.
17 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.
18 K. A. Karlsson, I. Pascher, B. E. Samuelsson, and G. O. Steen, *Chem. Phys. Lipids*, 9 (1972) 230.
19 S. M. Kim and C. C. Sweeley, *Carbohyd. Res.*, 5 (1967) 373.
20 M. Zinbo and W. R. Sherman, *J. Amer. Chem. Soc.*, 92 (1970) 2105.

21 R. A. LAINE AND C. C. SWEELEY, *Anal. Biochem.*, 43 (1971) 533.
22 R. A. LAINE AND C. C. SWEELEY, *Carbohyd. Res.*, 27 (1973) 199.
23 C. C. SWEELEY, in *Introduction to Lipid Chemistry*, R. M. BURTON, Ed., Bio-Science International, Webster Groves, Inc. in press.
24 M. UEDA, A. EHMANN, AND R. S. BANDURSKI, *Plant Physiol.*, 46 (1970) 715.