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The Sterol Esters of Maize Seedlings

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1. The composition of the sterol ester fraction of the shoot, root, scutellum and endosperm of 10-day-old maize seedlings was investigated. 2. The scutellum and endosperm together contain 80% of the sterol ester of the seedling. 3. β-Sitosteryl linoleate is the major sterol ester of the scutellum and endosperm. 4. β-Sitosteryl and stigmasteryl palmitate, palmitoleate, oleate and linoleate are the major sterol esters of the root. 5. In the shoot phytosterol linoleate is less abundant than phytosterol myristate, palmitate, palmitoleate and oleate. 6. There is a greater proportion of cholesteryl ester in the shoot than in the other tissues of the seedling.

The administration of [2-^{14}C]mevalonate to maize seedlings results in rapid labelling of both free and esterified sterols (Threlfall, Griffiths & Goodwin, 1964; Mercer & Goodwin, 1963; Treharne, Mercer & Goodwin, 1966; Goad, 1967; Kemp, Hammam, Goad & Goodwin, 1968). This was discussed in relation to phytosterol biosynthesis by Kemp *et al.* (1968). Other workers have discussed the esterification of possible phytosterol biosynthetic intermediates (Paasonen, 1964, 1965; Williams, 1966; Hammam, 1966; Bernard & Reid, 1967). In addition, sterol esters have been studied in relation to the neutral lipid content of higher plants (Gilles & Youngs, 1964; Krepinsky, Herout & Šorm, 1958; Weenink, 1962; McKillican & Sims, 1963; Iskhakov & Vereschagin, 1964; Tiwari & Bajpai, 1966; Richards, Sell & Thomas, 1967). However, the detailed composition of the sterol esters of a particular plant species has not been reported. We previously studied the changes in the sterol ester content and composition of maize seedlings during germination (Kemp, Goad & Mercer, 1967). Here we report the detailed analysis of the sterol esters of 10-day-old maize seedlings.

EXPERIMENTAL

Seed germination and lipid extraction. Maize seeds [*Zea mays* var. South African White Horse Tooth, obtained from Gunsons (Seeds) Ltd., London, E.C. 3] were soaked in water for 24hr. and then planted 0·5in. deep in moist vermiculite contained in stainless-steel trays (200 seeds/14in. × 12in. × 2in. tray). They were allowed to germinate for 10 days at 27° under continuous illumination. At the end of this period the length of the shoots was 15–23cm. The plants were harvested and carefully dissected into four morphological units, the shoot, root, scutellum and endosperm, and the lipid was extracted from each as described by Kemp *et al.* (1967).

Column chromatography. The lipid was chromatographed on columns of Brockmann grade III acid-washed alumina (Woelm, anionotropic) which were developed in a stepwise fashion with increasing concentrations of dry peroxide-free diethyl ether in light petroleum (b.p. 40–60°). The first fraction, eluted with light petroleum, contained hydrocarbons and was discarded. The second fraction, eluted with 2% diethyl ether in light petroleum, contained the sterol esters. It was collected and analysed further.

Thin-layer chromatography. The sterol ester fraction was purified by chromatography on 0·25mm. layers of Kieselgel G (E. Merck A.-G., Darmstadt, Germany) impregnated with the dye Rhodamine 6G (Avigan, Goodman & Steinberg, 1963) with benzene–hexane (2:3, v/v) used for development. When the developed plates were examined under u.v. light (Hanovia Chromatolite lamp) the sterol esters and other non-u.v.-absorbing materials were detected as pink zones on a pale-yellow background. The R_F values of a range of sterol esters (β-sitosterol and campesterol esterified with C_{12}–C_{24} even-numbered and C_{17} saturated fatty acids, palmitoleic acid, linoleic acid and α-linolenic acid) were found to be between 0·42 and 0·53 with this system. Accordingly the broad zone chromatographing between these R_F values was eluted with diethyl ether. The resulting mixture of sterol esters was then chromatographed on 0·25mm. layers of Kieselgel G impregnated with 10% (w/w) $AgNO_3$ with benzene–hexane (2:3, v/v) used for development. This system separated the sterol esters according to the degree of unsaturation of the fatty acid moiety. The sterol ester zones were detected by spraying the developed chromatograms with 0·1% (w/v) Rhodamine 6G solution in acetone. Four zones were scraped off such plates and extracted with diethyl ether: zone 1 (R_F 0·45–0·70), zone 2 (R_F 0·23–0·45), zone 3 (R_F 0·07–0·23) and zone 4 (R_F 0·01–0·07), which co-chromatographed with sterol esters of saturated, monounsaturated, diunsaturated

and triunsaturated fatty acids respectively. The sterol esters from each zone were then analysed by gas–liquid chromatography.

Methyl esters of fatty acids were purified by chromatography on 0·25 mm. layers of Kieselgel G impregnated with Rhodamine 6G with benzene–hexane (2:3, v/v) used for development. All esters chromatograph in this system with R_F 0·5.

Free sterols, isolated after saponification of the sterol esters, were separated into 4,4-dimethyl, 4α-methyl and 4-demethyl sterols on 0·25 mm. layers of Kieselgel G impregnated with Rhodamine 6G with chloroform used for development (Kemp *et al.* 1967).

Saponification of sterol esters followed by isolation of the sterols and fatty acids. Sterol esters were refluxed for 1 hr. in 6% (w/v) KOH in aq. 90% (v/v) ethanol. The saponification mixture was then cooled, diluted with 4 vol. of water and extracted four times with diethyl ether to obtain the sterols. The saponification mixture was then acidified to pH 1 with HCl and extracted four times with diethyl ether to obtain the fatty acids. The extracts were then washed thoroughly with water, dried over anhydrous Na_2SO_4 for 30 min. and evaporated to dryness under N_2.

Gas–liquid chromatography. All samples were analysed with a Packard Gas Chromatograph Series 7500 with hydrogen flame detectors.

Unesterified sterols were chromatographed on 3% OV-1 supported on 100–120-mesh silane-treated Gas Chrom Q and 1% QF-1 supported on 80–100-mesh silane-treated Gas Chrom Q, which were packed into coiled glass columns (6 ft. long × 2 mm. internal diam.). The columns were developed at 225° with an argon flow rate of 40 ml./min. Cholestane was chromatographed with each sample and retention times were determined relative to cholestane.

Sterol esters were chromatographed on 3% SE-30 supported on 60–80-mesh silane-treated Chromosorb W packed into coiled glass columns (18 in. long × 2 mm. internal diam.). Dual-column-temperature programming was necessary for satisfactory resolution (Kuksis, 1964); the starting temperature was 250°, rising at a rate of 1°/min. to a final temperature of 275°. The argon flow rate was 112 ml./min. This system separated sterol esters according to the number of carbon atoms in the molecule; the retention times and temperatures of elution of the range of sterol esters mentioned above were determined with authentic samples.

Fatty acid methyl esters were chromatographed on 25% DEGS supported on 60–80-mesh silane-treated Chromosorb W packed into coiled glass columns (6 ft. long × 2 mm. internal diam.). Dual-column-temperature programming was used; the starting temperature was 110°, rising at a rate of 3°/min. to a final temperature of 175°. The argon flow rate was 40 ml./min.

All samples (approx. 1 μg.) were injected on to the column dissolved in 1 μl. of cyclohexane. Peak areas were measured by triangulation (height of peak × width of peak at half the height).

Determination of sterols. 'Fast-reacting' sterols and 'slow-reacting' sterols were determined by the procedure of Moore & Baumann (1952), which is based on the Liebermann–Burchard reaction. Ergosterol and β-sitosterol were used as representative 'fast-reacting' and 'slow-reacting' sterols respectively for the preparation of standard curves. The 4,4-dimethyl sterols were assayed by a modification of the Liebermann–Burchard reaction used by Mercer (1961) for the assay of lanosterol. The sterol was dissolved in 1 ml. of acetic acid, mixed with 2 ml. of 5% (v/v) conc. H_2SO_4 in acetic anhydride and incubated for 40 min. at 25°. The extinction at 460 mμ was then determined. Lanosterol was used for the preparation of a standard curve.

Preparation of sterol esters. The sterol (0·2 m-mole), fatty acid (0·2 m-mole) and toluene-*p*-sulphonic acid (0·015 m-mole) were dissolved in 10 ml. of dry benzene and refluxed for 4 hr. The mixture was then evaporated to dryness under N_2, triturated with 1 ml. of acetone and crystallized from methanol (Bergmann, Ikan & Harel, 1964).

Preparation of fatty acid methyl esters. An ethereal solution of diazomethane, generated from 5 ml. of 20% (w/v) NaOH, 5 ml. of diethyl ether and 10 mg. of nitrosomethylurea, was added to the fatty acid (less than 1 mg.) dissolved in 2 ml. of diethyl ether until a permanent yellow colour was obtained.

RESULTS

A total of 200 10-day-old maize seedlings were dissected into shoots, roots, scutella and endosperms. The lipid was extracted from each type of morphological unit and the sterol ester fractions were isolated by chromatography on alumina. The four sterol ester fractions were purified several times by thin-layer chromatography. Each fraction was then divided into two parts. The first part, comprising three-quarters of the sterol ester fractions (therefore derived from the equivalent of 150 seedlings) was saponified, and the liberated free sterols and fatty acids were analysed. The second part, comprising the remaining one-quarter of the sterol ester fractions, was analysed without prior hydrolysis.

Part 1. The sterols liberated after saponification were separated into 4,4-dimethyl, 4α-methyl and 4-demethyl sterols by thin-layer chromatography, and samples of each type of sterol were assayed colorimetrically. Table 1 gives the total weight of these three types of sterol in each of the four morphological units. The seed residues (scutellum and endosperm) together possessed 81·3% of the total sterol in ester form in the seedling; the scutellum itself possessed 56·3%, whereas the shoot had only 5·35%. More than 87% of the total sterol in ester form in each morphological unit was 4-demethyl sterol; such a high proportion was not unexpected, since the 4,4-dimethyl and 4α-methyl sterols are generally regarded as precursors of the 4-demethyl sterols, which are themselves regarded as the end of the biosynthetic line. The components of each type of sterol were then determined by gas–liquid chromatography. The OV-1 column separated the main 4-demethyl sterols, cholesterol (RRT* 1·90), campesterol (RRT 2·51), stigmasterol (RRT 2·74) and β-sitosterol (RRT 3·16), very well but failed to separate sterols from their corresponding stanols.

* Abbreviation: RRT, retention time relative to cholestane.

Table 1. *Weights and proportions of 4,4-dimethyl, 4α-methyl and 4-demethyl sterols derived from the sterol ester fraction of the shoots, roots, scutella and endosperms of the equivalent of* 150 *10-day-old maize seedlings*

	4,4-Dimethyl sterols		4α-Methyl sterols		4-Demethyl sterols		Total
	Wt. (mg.)	% of total	Wt. (mg.)	% of total	Wt. (mg.)	% of total	wt. (mg.)
Shoots	0·062	8·1	0·028	3·6	0·678	88·3	0·768
Roots	0·102	5·3	0·143	7·4	1·678	87·3	1·923
Scutella	0·082	1·0	0·201	2·5	7·770	96·5	8·053
Endosperms	0·148	4·1	0·307	8·6	3·134	87·3	3·589

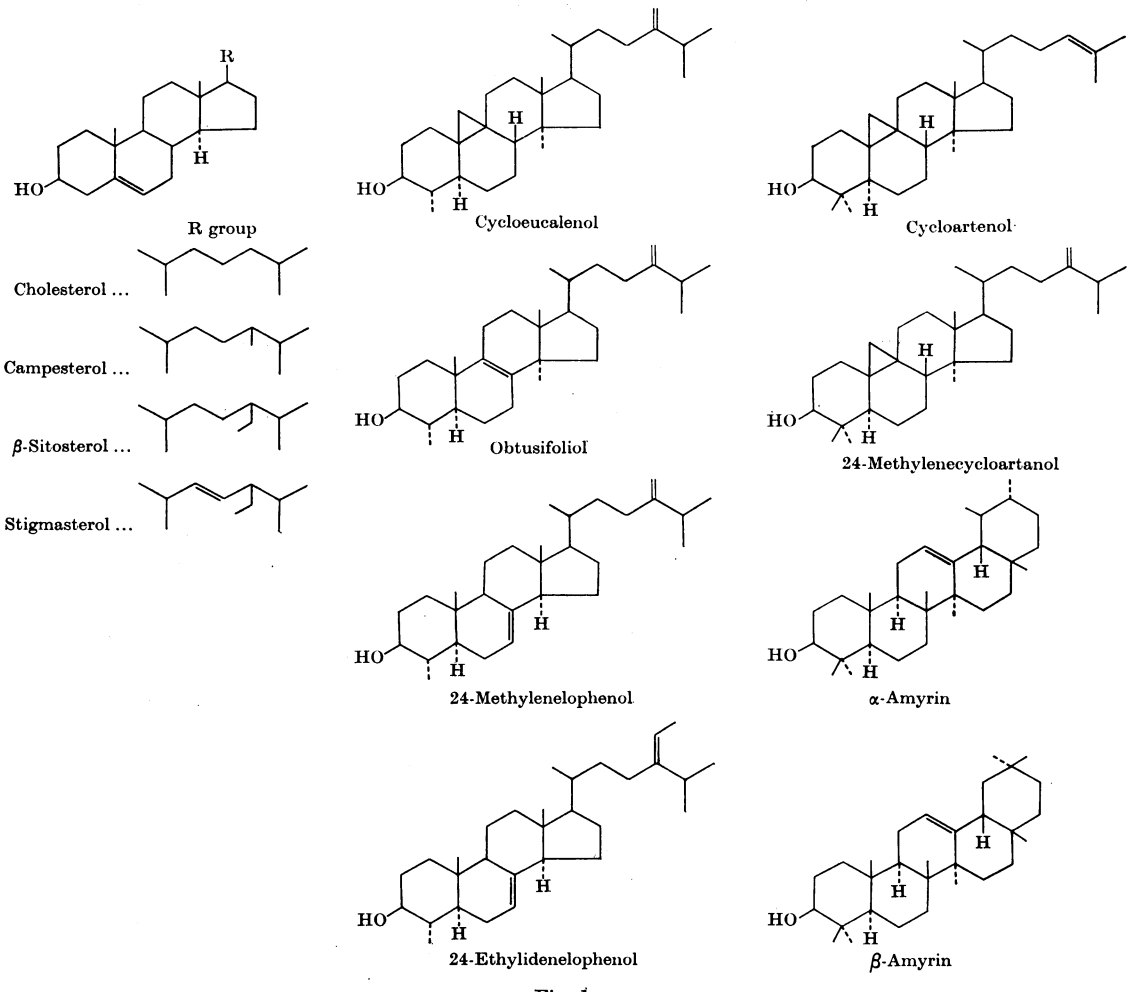

Fig. 1.

The QF-1 column separated sterols from their corresponding stanols but failed to resolve campesterol and stigmasterol (RRT: cholesterol, 2·73; campesterol, 3·57; stigmasterol, 3·57; campestanol, 3·91; stigmastanol, 4·67). Of the four major 4α-methyl sterols, the OV-1 column separated obtusifoliol (RRT 3·06) and 24-ethylidenelophenol (RRT 4·61) from an unresolved mixture of cycloeucalenol and 24-methylenelophenol (RRT 3·49). The QF-1 column, however, separated cycloeucalenol (RRT 5·11) and 24-ethylidenelophenol (RRT 5·62) but failed to resolve obtusifoliol and 24-methylenelophenol (RRT 4·46). The main 4,4-dimethyl sterols were cycloartenol and 24-methylenecycloartanol. These were resolved on the OV-1 column (RRT 3·69 and 4·35 respectively)

Table 2. *Composition of the sterol fraction derived from the 4-demethyl sterol esters of shoot, root, scutellum and endosperm of 10-day-old maize seedlings*

	% of sterols determined after gas–liquid-chromatographic separation on 3% OV-1			
	Cholesterol	Campesterol	Stigmasterol	β-Sitosterol
Shoot	19	16	18	47
Root	4	17	27	52
Scutellum	Trace	18	10	72
Endosperm	Trace	17*	5	78*

	% of sterols determined after gas–liquid-chromatographic separation on 1% QF-1				
	Cholesterol	Campesterol+ stigmasterol	Campestanol	β-Sitosterol	Stigmastanol
Shoot	22	31	—†	47	—†
Root	4	43	—†	53	—†
Scutellum	0·8	27	—†	72	—†
Endosperm	0·9	11	10	45	33

* These values are high because of the presence of the corresponding stanols.
† Not detected.

Table 3. *Composition of the sterol fraction derived from the 4α-methyl sterol esters of the shoot, root, scutellum and endosperm of 10-day-old maize seedlings*

	% of sterols determined after gas–liquid-chromatographic separation on 3% OV-1		
	Obtusifoliol	24-Methylenelophenol+ cycloeucalenol	24-Ethylidene-lophenol
Shoot	16	65	19
Root	34	47	19
Scutellum	28	46	26
Endosperm	32	41	27

	% of sterols determined after gas–liquid-chromatographic separation on 1% QF-1		
	Obtusifoliol+ 24-methylenelophenol	Cycloeucalenol	24-Ethylidene-lophenol
Shoot	67	12	21
Root	55	26	19
Scutellum	56	25	19
Endosperm	57	21	22

	% of sterols calculated from the results of the gas–liquid-chromatographic separations on both 3% OV-1 and 1% QF-1			
	Obtusifoliol	24-Methylene-lophenol	Cycloeucalenol	24-Ethylidene-lophenol
Shoot	16	52	12	20
Root	34	21	26	19
Scutellum	28	24	25	23
Endosperm	32	22	21	25

and were also separated from two other components, which co-chromatographed with α-amyrin (RRT 3·47) and β-amyrin (RRT 3·16) respectively. The QF-1 column failed to resolve the components of the 4,4-dimethyl sterol fraction adequately. The structures of all these sterols are given in Fig. 1.

Tables 2–4 give the compositions of the three types of sterols isolated from the four morphological

Table 4. *Composition of the sterol fraction derived from the 4,4-dimethyl sterol esters of the shoot, root, scutellum and endosperm of 10-day-old maize seedlings*

	% of components determined after gas–liquid-chromatographic separation on 3% OV-1			
	α-Amyrin	β-Amyrin	Cycloartenol	24-Methylene-cycloartanol
Shoot	12	30	34	24
Root	Trace	Trace	71	27
Scutellum	Trace	Trace	53	45
Endosperm	Trace	Trace	34	64

units, determined from peak-area measurements on the gas–liquid-chromatographic traces on OV-1 and QF-1. In all four morphological units the major esterified 4-demethyl sterol was β-sitosterol, accounting for about 50% of the total in the shoot and root and about 75% of the total in the scutellum and endosperm. The percentage of campesterol was fairly constant at 16–18% in all four parts, whereas the percentage of stigmasterol tended to be higher in the shoot and root (18–27%) than in the components of the seed residue (5–10%). The most striking difference, however, was in the percentages of cholesterol; the components of the seed residue had scarcely any, the root had 4% and the shoot had 19%. The endosperm was peculiar in that it possessed considerable amounts of saturated sterols (campestanol, 10%; stigmastanol, 33%), which were resolved from their Δ^5-sterol counterparts on the QF-1 column. Saturated sterols were not found in the 4-demethyl sterols from shoot, root or scutellum.

The major 4α-methyl sterols were found to be obtusifoliol, cycloeucalenol, 24-methylenelophenol and 24-ethylidenelophenol. In spite of the fact that neither the OV-1 column nor the QF-1 column completely resolved all four components, a combination of the chromatogram traces from both columns enabled the percentages of the four components to be estimated. The root, scutellum and endosperm were found to have very similar compositions. The shoot, however, differed in that it had 52% of 24-methylenelophenol, rather more than double the percentage that the other parts of the seedling had, and only half as much obtusifoliol (16%) and cycloeucalenol (12%) as the other parts.

The major 4,4-dimethyl sterols were found to be cycloartenol and 24-methylenecycloartanol. The root, scutellum and endosperm appeared to contain only these 4,4-dimethyl sterols; of these three tissues the root contained the greatest proportion of cycloartenol (71%) and the smallest proportion of 24-methylenecycloartanol (27%). The endosperm reversed the proportions, having the smallest proportion of cycloartenol (34%) and the greatest proportion of 24-methylenecycloartanol (64%); the scutellum had intermediate proportions. The shoot, in contrast with the other parts of the seedling, had two other major components, constituting over 40% of the total 4,4-dimethyl sterol fraction, as well as cycloartenol and 24-methylenecycloartanol. These co-chromatographed with the pentacyclic triterpenes, α- and β-amyrin (Fig. 1) and have been tentatively identified as such.

The fatty acids derived from the sterol esters from the four components of the seedlings were methylated, purified by thin-layer chromatography and then analysed by gas–liquid chromatography. The results are shown in Table 5. In the root, scutellum and endosperm the major fatty acid was linoleic acid, constituting 21%, 50% and 34% of the total respectively in these tissues. Palmitic acid, oleic acid and palmitoleic acid were also abundant in these tissues. The shoot, however, presented a different picture, in that linoleic acid constituted only 6% of the total, being less than myristic acid (8%), palmitic acid (18%), palmitoleic acid (9%) and oleic acid (8%).

Part 2. This was one-quarter of the sterol ester fractions, derived from the equivalent of 50 seedlings. It was separated into four fractions, the sterol esters of saturated, monounsaturated, diunsaturated and triunsaturated fatty acids, by thin-layer chromatography. The four different types of sterol ester were then analysed by gas–liquid chromatography. The system used separated the sterol esters according to the number of carbon atoms in their molecules. It appeared to be unaffected by the degree of unsaturation, since β-sitosteryl stearate, oleate, linoleate and linolenate co-chromatographed; this difficulty was circumvented by the preliminary thin-layer chromatography. Because of the relatively small number of theoretical plates inherent in the short columns used, there was a danger of overloading. Consequently only small amounts (less than 10 μg.) could be analysed, and thus only the major components in the mixture could be detected and estimated. The most satisfactory results were obtained with the sterol esters of the saturated fatty acids, and in the root and shoot it was possible to

Table 5. *Composition of the fatty acid fraction derived from the sterol esters of 10-day-old maize seedlings*

	% of fatty acids determined after gas–liquid chromatographic separation on 25% DEGS			
Fatty acid*	Shoot	Root	Scutellum	Endosperm
12:0	3·2	1·8	0·7	0·5
14:0	7·7	5·4	1·9	3·0
14:1	3·5	3·4	1·7	2·0
15:0	4·7	3·3	1·7	2·5
15:1	2·5	2·6	1·2	1·1
16:0	18·1	13·6	9·6	9·6
16:1	8·9	12·6	5·1	6·5
17:0	2·4	2·5	1·3	2·2
17:1	2·3	2·8	1·0	2·2
18:0	4·5	3·7	2·1	3·3
18:1	8·3	12·8	11·5	8·3
18:2	6·1	20·8	49·8	34·4
18:3	3·2	2·8	2·7	3·5
19:0	2·1	1·9	†	1·9
20:0	3·0	1·6	1·7	1·7
21:0	1·7	0·9	0·7	1·9
22:0	3·8	1·2	1·6	1·6
>22	14·0	6·3	5·7	13·8

* The number before the colon is the number of carbon atoms in the fatty acid; the number after the colon is the number of double bonds present.

† Not detected.

determine the percentages of the components present from the chromatographic traces. For sterol esters of unsaturated fatty acids from all four parts of the seedling it was not possible to determine the percentages because only the major components could be detected. Table 6 gives the results of gas–liquid-chromatographic analysis of the sterol ester fractions of the four parts of the seedlings.

The major components of the sterol esters of di- and tri-unsaturated fatty acids of all four parts of the seedling had carbon numbers of 46 and 47. A sterol ester with 46 carbon atoms can be derived from a C_{27} sterol (e.g. cholesterol) plus a C_{19} fatty fatty acid, a C_{28} sterol (e.g. campesterol) plus a C_{18} fatty acid, or a C_{29} sterol (e.g. stigmasterol or β-sitosterol) plus a C_{17} fatty acid. Since the major diunsaturated and triunsaturated fatty acids in these tissues were linoleic acid and linolenic acid, it follows that the C_{46} diunsaturated sterol ester is likely to have been mainly campesteryl linoleate and the C_{46} triunsaturated sterol ester campesteryl linolenate. Similar reasoning suggests that the C_{47} di- and tri-unsaturated sterol esters were mainly β-sitosteryl and stigmasteryl linoleate and linolenate respectively.

The major components of the sterol esters of monounsaturated fatty acids of shoot had carbon numbers of 43, 45 and 47. Since the main monounsaturated fatty acids of shoot were palmitoleic acid and oleic acid, the main sterol esters were the palmitoleate and oleate of cholesterol, β-sitosterol and stigmasterol. In the root, scutellum and endosperm the major sterol esters of monounsaturated fatty acids had carbon numbers of 46 and 47, and were probably mainly campesteryl and β-sitosteryl oleate, since oleic acid was the major monounsaturated fatty acid in these tissues.

The sterol esters of saturated fatty acids in the shoot had relatively high proportions of components with carbon numbers of 41, 43, 45 and 47. The C_{41} component was probably β-sitosteryl laurate with some cholesteryl myristate. The C_{43} component was probably β-sitosteryl myristate with some cholesteryl palmitate. The C_{45} component was probably β-sitosteryl palmitate with some cholesteryl stearate. The C_{47} component was probably β-sitosteryl stearate with some cholesteryl arachidate. However, all these four components probably also contained a small proportion of campesterol esterified with the corresponding odd-numbered saturated fatty acid. The root had a higher proportion of sterol esters of saturated fatty acids with carbon numbers of 44 and 46 than the shoot, but again had high proportions of those with carbon numbers of 45 and 47. The sterol esters of saturated fatty acids in the scutellum and endosperm had carbon numbers of 44 and 45, and were probably the palmitate esters of

Table 6. *Composition of the sterol ester fraction of the shoot, root, scutellum and endosperm of* 10*-day-old maize seedlings*

The sterol esters were separated into sterol esters of saturated, monounsaturated, diunsaturated and triunsaturated fatty acids by thin-layer chromatography. Each type of sterol ester was then separated according to the number of carbon atoms in the molecule by gas–liquid chromatography. A, B, C and D denote sterol esters of saturated fatty acids, monounsaturated fatty acids, diunsaturated fatty acids and triunsaturated fatty acids respectively. +, ++ and +++ denote the relative proportions of the most abundant sterol esters of that type in the tissue concerned. They are used when the sensitivity of the gas–liquid chromatographic system to overloading prevented the detection of minor components, which were almost certainly present.

	% or estimate of each component															
	Shoot				Root				Scutellum				Endosperm			
Carbon no.	A	B	C	D	A	B	C	D	A	B	C	D	A	B	C	D
39	4·3				2·4											
40					0·7											
41	10·4				2·8			+				+				+
42	0·9				0·7							+				
43	21·6	30·0			2·8											
44	3·3				16·6				+				+			
45	25·2	29·1			29·0				+++							
46	7·3		+	+	18·4	+	+	+		+	+	+	+++	+	+	+
47	18·5	40·9	+++	+++	26·6	+++	+++	++		+++	+++	++	+	+++	+++	++
48	1·8															
49	4·3															
50																
51	2·4															

campesterol, stigmasterol and β-sitosterol plus, in the endosperm, those of the corresponding stanols.

The scutellum was found to contain the greatest amount of sterol ester (8·1mg./150 seedlings), followed by the endosperm (3·6mg./150 seedlings). The root (1·9mg./150 seedlings) and particularly the shoot (0·8mg./150 seedlings) were low in their sterol ester content (Table 1).

DISCUSSION

The seed residue (scutellum and endosperm) contains about 80% of the sterol ester content of 10-day-old maize seedlings, of which β-sitosteryl linoleate is quantitatively the most important. Phytosterol palmitate, palmitoleate and oleate are also present in the seed residue in significant amounts.

The root, unlike the shoot, generally resembles the seed residue in respect of its sterol ester composition.

In the shoot phytosterol linoleate, though present, is not the major sterol ester, being less abundant than phytosterol myristate, palmitate, palmitoleate and oleate.

Apart from these differences it is clear from Table 6 that there is a wide range of fatty acids, both saturated and unsaturated, esterified to sterols in all four parts of the seedling. This suggests that the abundance of particular sterol esters in particular tissues reflects the relative abundances of free sterols and fatty acids in those tissues rather than any specificity in the esterification process. Indeed, analyses of maize oil indicate that the main fatty acids, whether esterified or unesterified, are palmitic acid, oleic acid and linoleic acid (Craig & Murty, 1959; Baker, 1962; French, 1962; Hilditch & Williams, 1964; Rankov, Rankov & Ivanova, 1967; Jellum, 1968). These, together with palmitoleic acid, are the main fatty acids esterified to sterols.

The presence of cholesterol in higher plants is now well established (Jacobsohn & Frey, 1967). The maize shoot contains far more cholesterol in ester form than the other parts of the seedling. In fact, the seed residue contains only trace amounts of cholesteryl ester and the root only 4% cholesteryl ester in contrast with the 19–22% found in the shoot. The presence of cholesteryl ester in plastid-containing tissues (shoot and root) seemed to correlate with the finding that the sterols isolated from the chloroplasts of dwarf-bean (*Phaseolus vulgaris*) leaves contained a high proportion of a sterol co-chromatographing with cholesterol on gas–liquid chromatography (Mercer & Treharne, 1966). However, the idea that cholesterol is confined to the chloroplast has been disproved (Kemp & Mercer, 1968).

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