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EFFECT OF GIBBERELLIC ACID ON STEROL PRODUCTION IN *CORYLUS AVELLANA* SEEDS

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Key Word Index—*Corylus avellana*; Betulaceae; hazel; gibberellic acid; germination; sterols; [2-^{14}C]MVA.

Abstract—Gibberellic acid-induced germination of hazel seeds was accompanied by little change in the sterol content of the cotyledons. Dormant and germinating cotyledons rapidly incorporated [2-^{14}C]MVA into squalene which was slowly converted to sterols. Gibberellin treatment induced an increase in the incorporation of [2-^{14}C]MVA into cotyledon esterified sterols. An increase in free sterols occurred in the germinating embryonic axes, with increased relative amounts of stigmasterol and campesterol in the free 4-desmethylsterols. Germination was accompanied by increased incorporation of [2-^{14}C]MVA into free and esterified sterols in the embryonic axes.

INTRODUCTION

HAZEL seeds (*Corylus avellana* L.) require either prechilling or gibberellic acid pretreatment to induce germination.[1] With such experimental material it is possible to divorce the biochemical events due to germination proper from those due to hydration.[2,3] Gibberellic acid (GA) treatment enhances the activity of enzymes responsible for mevalonate activation and decarboxylation.[4] Increased mevalonic acid (MVA) activation probably represents (providing endogenous substrate is available) increased terpenoid synthesis. Triterpenoid and sterol analyses have been reported for seeds of pea,[5] maize,[6] *Calendula*[7] and species of the Cruciferae.[8] Biosynthetic investigations with whole pea seeds have been reported by Baisted's group[9–13] and Bennett *et al.*[14] have reported a time course for sterol

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synthesis in *Happlopappus* in the post germination stage. The present report deals with the effects of GA treatment on sterol metabolism in the isolatable organs of hazel seeds.

RESULTS

GA treatment resulted in germination of the seeds, the radicle emergence being 37% at 6 days and 100% at 9 days. The water treated seeds did not germinate. The fresh weight of the water treated cotyledons increased over the period of treatment from 20 to 32 g/15 pairs of cotyledons. GA treatment resulted in a further increase in fresh weight, the weight of 15 pairs of 12-day-old GA treated cotyledons being 52 g. The dry weights of the H_2O and GA treated cotyledons did not change.

GA treatment also resulted in increases in the fresh and dry weights of the embryonic axes. The fresh weight increased from 0·8467 to 109·02 g/250 axes and the dry weight from 0·1587 to 5·19 g/250 axes.

Sterol analysis

Cotyledons. The levels of fast and slow Liebermann–Burchard reacting free and esterified sterols in the cotyledon tissue of germinating seeds showed little change (Table 1). Esterified sterols were present at concentrations too low for accurate analysis. Free sterols, however, were fractionated by TLC (see Experimental) into 4-methylsterols (a group which chromatographed with 4,4-dimethylsterols, 4α-methylsterols and amyrins) and 4-desmethylsterols. The relative amounts of 4-methylsterols and 4-desmethylsterols remained constant with germination (Table 2).

TABLE 1. THE STEROL CONTENT OF COTYLEDON TISSUE FROM GA AND H_2O TREATED SEEDS

Sample	Free sterols*		Esterified sterols*		Total*
	Fast reacting†	Slow reacting†	Fast reacting†	Slow reacting†	
Dry seeds	0·274	13·694	0·066	0·657	14·691
3 days H_2O	0·236	12·603	0·075	0·499	13·413
3 days GA	0·210	12·041	0·067	0·448	12·766
9 days H_2O	0·356	12·828	0·043	0·499	13·726
9 days GA	0·336	13·760	0·040	0·470	14·606

* mg/15 Pair cotyledons.
† Liebermann–Burchard slow and fast reacting sterols.

GLC on OV17 of the cotyledon 4-methylsterols gave eight peaks. The identities of the 4-methylsterols were not established, but the major component co-chromatographed with β-amyrin. GLC of the 4-desmethylsterols demonstrated the presence of sitosterol, stigmasterol, campesterol and traces of cholesterol. A slight decrease in sitosterol and corresponding increases in campesterol and stigmasterol occurred in both H_2O and GA treated cotyledons (Table 3).

Embryonic axes. Germination of the embryonic axes was accompanied by large increases in the total sterol content (Table 4). The increase was accounted for by increases in fast and slow Liebermann–Burchard reacting free sterols with only slight increases in esterified sterols (Table 4). The levels of esterified sterols in the 12-day-old GA treated plumules, radicales + hypocotyls, and cotyledonary petioles were too low for further analyses. The relative amounts of 4-methylsterols and 4-desmethylsterols in the free and esterified

sterols are given in Table 5. An increase in the relative amount of 4-desmethylsterols occurred in the free sterol fractions and of 4-methylsterols in the esterified sterol fractions of the axes from GA and water treated seeds.

TABLE 2. THE RELATIVE AMOUNTS OF DIFFERENT STEROLS IN THE FREE STEROL FRACTION OF COTYLEDON TISSUE FROM H_2O AND GA TREATED SEEDS

Sample	4-Methylsterols*	4-Desmethylsterols*
Dry seeds	35	65
3 days H_2O	24	76
3 days GA	29	71
9 days H_2O	21	79
9 days GA	24	76

* Expressed as a percentage of total free sterol.
Amounts of sterols were determined by GLC using 5α-cholestane as an internal standard.

The esterified 4-methylsterols were further fractionated by GLC into 10 peaks. Eight of these corresponded to the component peaks of the free 4-methylsterol fractions of the cotyledons. The free 4-methylsterols of the embryonic axes gave nine components by GLC which corresponded to nine of the component peaks of the embryonic axis esterified 4-methylsterol fractions. Further identification was not carried out although the quantitatively major component of the 4-methylsterol fractions again co-chromatographed with β-amyrin.

TABLE 3. THE RELATIVE AMOUNTS OF STEROLS IN THE FREE 4-DESMETHYLSTEROL FRACTION OF COTYLEDON TISSUE FROM GA AND H_2O TREATED SEEDS

Sample	Campesterol*	Stigmasterol*	Sitosterol*
Dry seeds	2·88	0·49	96·63
3 days H_2O	4·84	0·93	94·23
3 days GA	4·23	1·42	94·35
9 days H_2O	6·75	1·45	91·80
9 days GA	4·99	1·21	93·80

* Expressed as a percentage of total free 4-desmethylsterols.

The free 4-desmethylsterol fraction of the embryonic axes contained campesterol, stigmastrol, sitosterol and traces of cholesterol. The relative amounts of campesterol and stigmaserol increased with germination (Table 6). The esterified 4-desmethylsterol fractions contained largely campesterol and sitosterol with only trace amounts of stigmasterol and cholesterol. The relative amounts of sterols in the esterified 4-desmethylfraction showed little change with germination (Table 6).

Radioisotope experiments

Whole seeds. [2-^{14}C]MVA was administered to the seeds during imbibition. The seeds were then divided into two groups and each treated with either GA solution or water. The dry weights of the H_2O and GA treated cotyledons were constant, but the fresh weights increased. The fresh weight of the water treated cotyledons increased from 30 to 35 g/20

pairs of cotyledons and of the GA treated cotyledons from 29 to 41 g/20 pairs of cotyledons. At least 30% of the radioactivity was recovered in the cotyledon lipid fractions. Only small quantities of label were recovered from the embryonic axes.

TABLE 4. THE STEROL CONTENT OF EMBRYONIC AXES OF GA AND H_2O TREATED SEEDS

Sample	Free sterols* Fast reacting†	Free sterols* Slow reacting†	Esterified sterols* Fast reacting†	Esterified sterols* Slow reacting†	Total*
Dry seeds	0·085	0·690	0·190	0·680	1·645
3 days H_2O	0·045	0·760	0·155	0·400	1·360
3 days GA	0·030	0·700	0·150	0·710	1·590
6 days H_2O	0·050	0·550	0·085	0·760	1·445
6 days GA	0·065	1·140	0·160	0·700	2·065
9 days H_2O	0·045	0·670	0·150	0·820	1·685
9 days GA	0·195	3·650	0·235	0·800	4·880
12 days H_2O	0·050	0·760	0·140	0·640	1·590
12 days GA					
Whole axes	0·979	15·090	0·264	0·813	17·146
Plumules	0·527	8·283	0·075	0·301	9·186
Radicles† Hypocotyls	0·256	4·669	0·068	0·301	5·294
Cotyledonary petioles	0·196	2·138	0·121	0·211	2·666

* mg/250 axes.
† Liebermann–Burchard slow and fast reacting sterols.

Cotyledon lipids were fractionated on alumina (see Experimental) to give hydrocarbons, free sterols and sterols derived from esters. TLC of the hydrocarbon fraction gave only one radioactive product which co-chromatographed with authentic squalene. Radioactivity in squalene, total sterols and free and esterified sterols is expressed as a percentage

TABLE 5. THE RELATIVE AMOUNTS OF DIFFERENT STEROLS IN THE FREE AND ESTERIFIED STEROL FRACTIONS OF AXES FROM H_2O AND GA TREATED SEEDS

Sample	Free sterols 4-Methyl-sterols*	Free sterols 4-Desmethyl-sterols*	Esterified sterols 4-Methyl-sterols†	Esterified sterols 4-Desmethyl-sterols†
Dry seeds	26	74	14	86
3 days H_2O	32	68	15	85
3 days GA	20	80	12	88
9 days H_2O	12	88	24	76
9 days GA	8	92	36	64
12 days H_2O	16	84	22	78
12 days GA				
Whole axes	10	90	levels too low for analysis	
Plumules	11	89		
Radicles + hypocotyls	11	89		
Cotyledonary petioles	9	91		

* Expressed as a percentage of total free sterols.
† Expressed as a percentage of total esterified sterols.

of the total radioactivity recovered in triterpenoids (Table 7). The percentage of radioactivity recovered in total sterols increased with H_2O or GA treatment, but was greater in the H_2O than the GA treated tissue at 12 hr, 2 days and 4 days. Gibberellic acid treatment, however, stimulated the incorporation of radioactivity into the esterified sterol fraction.

TABLE 6. THE RELATIVE AMOUNTS OF STEROLS IN THE FREE AND ESTERIFIED 4-DESMETHYL-STEROL FRACTIONS OF AXES FROM GA AND H_2O TREATED SEEDS

Sample	Free sterols			Esterified sterols		
	Campesterol*	Stigmasterol*	Sitosterol*	Campesterol†	Stigmasterol†	Sitosterol†
Dry seed	6·95	1·71	91·34	5·64	Tr.	94·36
3 days H_2O	7·04	1·39	91·57	4·12	Tr.	95·88
3 days GA	6·75	1·85	91·40	4·31	Tr.	95·69
9 days H_2O	7·87	1·76	90·37	5·70	Tr.	94·30
9 days GA	19·50	7·11	73·39	4·37	Tr.	95·63
12 days H_2O	8·19	2·08	89·73	4·15	Tr.	95·85
12 days GA						
Whole axes (average)	15·91	9·71	74·38	levels too low for analysis		
Plumules	14·90	9·25	75·85			
Radicles + hypocotyls	18·52	10·61	70·87			
Cotyledonary petioles	14·31	9·28	76·41			

* Expressed as a percentage of total free 4-desmethylsterols.
† Expressed as a percentage of total esterified 4-desmethylsterols.

Free sterols and sterols derived from esters were fractionated by TLC (see Experimental) into groups corresponding to 4,4-dimethylsterols + amyrins, 4α-methylsterols and 4-desmethylsterols. The radioactivity recovered in the free 4,4-dimethylsterols, 4α-methylsterols and 4-desmethylsterols is expressed as a percentage of the radioactivity recovered in total free sterols (Table 8). Similar changes were found in the cotyledons of both GA and H_2O treated seeds. The percentage incorporation into 4-desmethylsterols was high but declined to less than half the initial value within 96 hr whilst the percentage incorporation into 4-methylsterols increased over the same period. Radioactivity in the esterified 4,4-dimethyl-, 4α-methyl-, and 4-desmethylsterols is expressed as a percentage of the radioactivity revered in the total esterified sterol fraction (Table 8). Most radioactivity was found in the esterified 4,4-dimethylsterols in cotyledons of GA and H_2O treated seeds.

TABLE 7. RECOVERY OF RADIOACTIVITY FROM [2-^{14}C]MVA IN TERPENOID FRACTIONS OF COTYLEDONS FROM SEEDS TREATED WITH EITHER GA OR H_2O

Sample	Total sterols*	Free sterols*	Esterified sterols*	Squalene*
12 hr H_2O	36·52	26·95	9·57	63·48
12 hr GA	16·35	11·86	4·49	83·65
2 day H_2O	44·35	32·69	11·66	55·65
2 day GA	36·82	16·46	20·36	63·18
4 day H_2O	58·16	43·98	14·18	41·84
4 day GA	47·23	29·11	18·12	52·77
10 day H_2O	71·90	61·77	10·13	28·10
10 day GA	72·35	48·03	24·32	27·65

* Expressed as a percentage of the total radioactivity recovered in triterpenoids.

Embryonic axes. Embryonic axis tips were incubated with [2-^{14}C]MVA for 24 hr before extraction (see Experimental). The radicle emergence of the GA treated seeds was 26% at 6 days and 100% at 11 days. The fresh weight of the germinating embryonic axes increased from 0·1619 to 3·16 g/50 axes and the dry weight from 0·0219 to 0·1720 g/50 axes. The H_2O treated seeds did not germinate and the fresh and dry weights of the embryonic axes remained unchanged.

TABLE 8. RECOVERY OF RADIOACTIVITY FROM [2-^{14}C]MVA IN THE DIFFERENT STEROLS OF THE FREE AND ESTERIFIED STEROL FRACTIONS OF COTYLEDONS FROM SEEDS TREATED WITH EITHER GA OR H_2O

Sample	Free sterol fractions* 4,4-Dimethylsterols	4α-Methylsterols	4-Desmethylsterols
12 hr H_2O	9·70	9·70	80·60
12 hr GA	12·83	19·42	67·75
1 day H_2O	12·65	21·57	65·78
1 day GA	30·20	25·30	44·50
4 day H_2O	47·71	26·64	25·65
4 day GA	44·77	25·75	29·48
10 day H_2O	45·14	19·96	34·90
10 day GA	36·78	19·14	44·08
	Esterified sterol fractions†		
12 hr H_2O	37·24	31·06	31·70
12 hr GA	46·10	23·45	30·45
1 day H_2O	50·67	21·67	27·66
1 day GA	44·56	23·85	31·59
4 day H_2O	55·50	25·81	18·69
4 day GA	39·63	27·78	32·59
10 day H_2O	48·21	25·54	26·25
10 day GA	54·94	22·73	22·33

* Expressed as a percentage of radioactivity found in total free sterols.
† Expressed as a percentage of radioactivity found in total esterified sterols.

Extraction and purification of terpenoids was as described for cotyledon tissue. TLC of the hydrocarbon fraction gave only one radioactive product and this co-chromatographed with authentic squalene. Germination of the embryonic axes was accompanied by an increase in the percentage recovery of radioactivity in free and esterified sterols whereas that in squalene decreased from 62 to 2% (Table 9). Only slight increase in percentage incorporation into free and esterified sterols occurred in the embryonic axes of H_2O treated seeds. The results indicate that germination of the axes was accompanied by increased incorporation of squalene into total sterols.

TABLE 9. RECOVERY OF RADIOACTIVITY FROM [2-^{14}C]MVA IN TERPENOID FRACTIONS OF EMBRYONIC AXES FROM SEEDS TREATED WITH EITHER GA OR H_2O

Sample	Total sterols*	Free sterols*	Esterified sterols*	Squalene*
24 hr imbibed	36·67	20·58	17·09	62·33
3 day H_2O	34·50	18·76	15·74	65·50
3 day GA	45·55	22·99	22·56	54·45
6 day H_2O	35·05	12·86	22·19	64·95
6 day GA	65·88	36·26	29·62	34·12
11 day H_2O	47·35	28·14	19·21	52·65
11 day GA	97·87	59·54	38·33	2·13

* Expressed as a percentage of the total radioactivity recovered in triterpenoids.

Radioactivity recovered in free and esterified 4,4-dimethylsterols, 4α-methylsterols and 4-desmethylsterols is given in Table 10. The percentage radioactivity in free 4,4-dimethylsterols was initially high whilst that in free 4α-methylsterols and free 4-desmethylsterols was low in axes of H_2O and GA treated seeds. Germination produced an increased incorporation of [2-^{14}C]MVA into free 4-desmethylsterols with a decrease into free 4,4-dimethylsterols and free 4α-methylsterols. Slight changes in incorporation of radioactivity occurred in the esterified sterol fractions of H_2O and GA treated axes.

TABLE 10. RECOVERY OF RADIOACTIVITY FROM [2-^{14}C]MVA IN THE DIFFERENT STEROLS OF THE FREE AND ESTERIFIED STEROL FRACTIONS OF THE EMBRYONIC AXES FROM SEEDS TREATED WITH EITHER GA OR H_2O

Sample	Free sterol fractions* 4,4-Dimethylsterols	4α-Methylsterols	4-Desmethylsterols
24 hr imbibed	62·2	27·9	9·9
3 day H_2O	62·8	23·6	13·6
3 day GA	65·4	23·7	10·9
6 day H_2O	48·6	25·8	25·6
6 day GA	28·2	26·7	45·1
11 day H_2O	35·7	21·4	42·9
11 day GA	17·8	8·6	73·6
	Esterified sterol fractions†		
24 hr imbibed	54·2	21·8	24·0
3 day H_2O	49·6	31·0	19·4
3 day GA	59·3	23·5	17·2
6 day H_2O	43·0	30·1	26·9
6 day GA	46·0	34·0	20·0
11 day H_2O	43·2	31·6	25·2
11 day GA	39·2	32·2	28·6

* Expressed as a percentage of radioactivity found in total free sterols.
† Expressed as a percentage of radioactivity found in total esterified sterols.

DISCUSSION

The constant total sterol content in germinating cotyledons contrasts with the decreases reported for germinating cotyledons of pea[5] and bean.[15] The sterol ester content of hazel cotyledons was low compared to results reported for maize scutella tissue.[6] Although increases in the relative and total amounts of fast Liebermann–Buchard reacting esters are reported for maize[6] and *Calendula*[7] little change was observed in hazel cotyledon tissue. The constant composition of the 4-desmethylsterol fractions in hazel cotyledons agrees with the relative amounts of 4-desmethylsterols in the free sterol fractions of germinating maize scutella[6] and the total sterol fractions in germinating pea seeds.[5]

The rapid incorporation of [2-^{14}C]MVA into squalene in H_2O and GA treated cotyledons suggests that the enzymes catalysing the conversion of MVA to squalene were present in non-limiting amounts. Similar results have been reported for pea seeds[9,10,13] and the suggestion made that isoprenoid biosynthesis is regulated by the supply of MVA. Baisted *et al.* have reported that ^{14}C-squalene is incorporated solely into β-amyrin in the initial stages of pea seed germination and only later is it incorporated into sitosterol. They suggest that this represents a recapitulation of the evolutionary chemical history of the seed.[9] Certainly the results reported here suggest, if such an argument is viable, that a similar evolutionary sequence is not evident in hazel seeds.

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The pattern of incorporation of [2-^{14}C]MVA into cotyledon free 4,4-dimethylsterols, 4α-methylsterols, and 4-desmethylsterols can be explained on the basis of substrate availability and changes in the relative activities of enzymes catalysing the cyclization of squalene and the conversion of cyclic products to 4-desmethylsterols.

The increase is total sterol content of the germinating embryonic axes agrees with reports for maize,[6] pea,[5] bean[15] and *Calendula*.[7] The increased sterol levels were accounted for by an increase in free sterols. The decreasing relative amounts of 4-methylsterols in the free sterol fractions may be due to the synthesis of large quantities of 4-desmethylsterols in axes from GA treated seeds and the conversion of 4-methylsterol precursors to 4-desmethylsterols in the axes of H_2O treated seeds. The increases in relative levels of campesterol and stigmasterol in the free 4-desmethylsterol fractions of germinating hazel axes are comparable to results reported for pea axes.[5] Kemp *et al.*[6] report increases in stigmasterol in free and esterified sterol fractions of roots but not shoots of germinating maize seeds.

Grunwald has shown that free sterols are active in controlling alcohol-induced permeability in beet discs[16] and barley roots.[17] The changes in the composition of the free 4-desmethylsterol fraction of the embryonic axes of germinating hazel seeds may be related to changes in the permeability of constituent membranes. Gibberellic acid treatment may affect specific membrane permeability and activity by inducing sterol changes. Phospholipids, an important class of functional and structural membrane lipid are also affected by GA treatment of hazel seeds.[18,3]

[2-^{14}C]Mevalonate was rapidly incorporated into squalene in the axes of H_2O and GA treated seeds. Germination was accompanied by a massive increase in label in the free 4-desmethylsterols. The increased synthesis of free 4-methylsterols and free 4-desmethylsterols may indicate a structural role in newly synthesized membrane material.

The significance of the high relative incorporation of [2-^{14}C]MVA into 4,4-dimethylsterols of the esterified fractions of cotyledons and axes of H_2O and GA treated seeds is difficult to assess. The occurrence of biosynthetically important 4α-methylsterols and 4,4-dimethylsterols in a predominantly or partly esterified form has been reported in other tissues.[19–24] The function of sterol esters is still unknown, although it has been suggested that they are involved in intracellular transport mechanisms.[6] The occurrence of, and rapid incorporation of radioactive precursors into esterified forms of biosynthetically important sterols suggests that the biosynthesis of 4-desmethylsterols proceeds via esters. However, this suggestion is incompatible with evidence indicating the formation of a 3-ketone as an essential step in the conversion of cycloartenol to phytosterols[25] and lanosterol to cholesterol in animals.[26]

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EXPERIMENTAL

Chemicals and plant material. Most lipid standards were commercial samples. Campesterol was obtained as an impurity in sitosterol supplied by R. N. Emanuel Ltd. Dr. L. J. Goad provided samples of cycloartenol and cycloeucalenol; lophenol was a gift from the MRC steroid collection. All solvents were redistilled prior to use. Light petroleum (b.p. 40–60°) was used throughout. Fruits of *Corylus avellana*. L. (Kent Cob Nuts) were purchased from R. Gould, Mereworth, Kent.

Sterol analyses. After deshelling and sterilizing, the seeds were imbibed and placed in Petri dishes containing 20 ml 3×10^{-4} M GA or H_2O. Samples (15 pairs of cotyledons or 250 embryonic axes) were harvested at regular intervals. Tissue was extracted in acetone and the residue exhaustively re-extracted in hot acetone in a Soxhlet for 6 hr. The bulked extracts were reduced in vol., diluted with aq 0·7% (w/v) NaCl and the lipids extracted in Et_2O by phase separation. The Et_2O extracts were dried and reduced to dryness. Samples for saponification were redissolved in absolute EtOH, 60% (w/v) aq. KOH added (1 ml/9 EtOH), and boiled under reflux for 60 min. The non-saponifiable material was extracted in Et_2O and reduced to dryness under vacuum. The lipid residues were redissolved in a small vol. petrol. and fractionated on columns of Brockman III alumina[6,27] (Woelm, acid. 10 g alumina/g lipid). Elution was as follows (1 vol. = 10 ml solvent/g alumina):

Fraction 1. 1 vol. petrol: hydrocarbons, squalene.

Fraction 2. 1 vol. 2% Et_2O in petrol; 1 vol. 4% Et_2O in petrol: sterol esters.

Fraction 3. 2 vol. 40% Et_2O in petrol: sterols.

In analytical experiments fractions 1–3 were collected separately and fraction 2 saponified to release free sterols. However, in the isotope experiments fractions 1 and 2 were bulked, saponified and the non-saponifiable material applied to a second column of grade 111 alumina and eluted as follows:

Fraction 1. 2 vol. petrol: hydrocarbons, squalene.

Fraction 2. 2 vol. 40% Et_2O in petrol: sterols derived from esters.

TLC. (1) *Hydrocarbons*: chromatographed on kieselgel G thin layers impregnated with rhodamine 6G. in either: (a) Hexane.[27] R_f standard squalene 0·78; or (b) Petrol.–C_6H_6 (3:2).[28] R_f squalene 0·68. (2) *Sterol esters*: chromatographed on kieselgel G thin layers in C_6H_6–hexane (2:3).[23] R_f for standard squalene and cholesterol palmitate were 0·76 and 0·45 respectively. (3) *Sterols*: Free 4,4-dimethylsterols, 4α-methylsterols and 4-desmethylsterols were separated on rhodamine 6G impregnated layers of kieselgel G in either: (a) $CHCl_3$;[6] (b) EtOAc–C_6H_6 (1:5);[27] or (c) Heptane–EtOAc (22:3).[11] 4,4-Dimethylsterols and 4α-methylsterols were incompletely resolved by one migration in any solvent system. However four migrations particularly in solvent system (b) resulted in the separation of three groups which correspond to standard lanosterol, cycloeucalenol and sitosterol.

GLC. Sterols were separated on a 1·5 m × 8 mm o.d. silanized glass column of 3% OV17 on 80–100 mesh gas chrom Q at 250° in Pye 104 gas chromatograph. Argon at 60 ml/min was used as carrier. 4-desmethylsterols were separated as free sterols with 5α-cholestane as internal standard. 4-Methylsterols were separated as trimethylsilyl ethers[29] (TMS) with 5α-cholestane as internal standard. Liebermann–Burchard Reaction was carried out by the method outlined by Kemp *et al.*[6] Calibration curves were prepared with ergosterol (fast reacting) and sitosterol (slow reacting).

Radioisotope experiments. Whole seeds. Seeds were deshelled, the distal ends removed, and the seeds stood on the cut surfaces in Petri dishes containing a total of 16 ml D,L [2-^{14}C]MVA (8 μmol, 80 μCi) in 0·04 M phosphate buffer, pH 8·0. The soln was taken up by the seeds within 6 hr. The seeds were washed in dist. H_2O and placed in 9 cm Petri dishes containing 10 ml of either H_2O or 3×10^{-4} M GA with 50 μmol cold D,L-MVA lactone. Samples of 20 seeds were extracted and assayed for radioactive terpenoids at regular intervals after imbibition. Wound callus at the cut surface was removed before analysis. *Embryonic axes.* Seed tips were removed (50 per treatment), incubated in 3·75 μCi D,L [2-^{14}C]MVA (0·375 μmol) for 24 hr and the embryonic axes dissected out and extracted. Seed tips were used in the isotope feeding stage since isolated axes could not be maintained in a healthy condition for the time of incubation necessary for sufficient label to enter sterols and sterol esters. Radioactivity was determined on a Nuclear Chicago Mark 1 model 6860 liquid scintillation counter. The scintillant was 5 g PPO and 0·3 g POPOP in 1l. toluene.

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