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OBSERVATIONS ON THE BIOSYNTHESIS OF 24-METHYLCHOLESTEROL AND 24-ETHYLCHOLESTEROL BY *ZEA MAYS*

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Key Word Index—*Zea mays*; Gramineae; sterol biosynthesis; 24-methylcholesterol; 24-ethylcholesterol; sitosterol; cyclolaudenol.

Abstract—Examination of the sterols of *Zea mays* shoots has established that the 24-ethylcholesterol is predominately the 24α-epimer, sitosterol, but the 24-methylcholesterol is a mixture of the 24α- and 24β-epimers. After incubation of *Z. mays* shoots with [2-^{14}C, (4*R*)4-$^{3}H_1$]mevalonic acid the sitosterol had a ^{3}H: ^{14}C atomic ratio of 2.09:5 which is consistent with previous results indicating that a $\Delta^{24(25)}$-sterol is implicated in its biosynthesis. By contrast, the 24α- and 24β-methylcholesterol mixture had a higher ^{3}H: ^{14}C atomic ratio of 2.82:5. This can be explained by the operation of two routes for the elaboration of the 24-methylcholesterol side chain. One may proceed via $\Delta^{24(28)}$- and $\Delta^{24(25)}$-sterols to produce the 24α-methylcholesterol with a ^{3}H: ^{14}C atomic ratio of 2:5. The other route may involve reduction of either a $\Delta^{24(28)}$-, a Δ^{23}- or a Δ^{25}-sterol intermediate to give the 24β-methylcholesterol with a ^{3}H: ^{14}C atomic ratio of 3:5. The proportion of these two labelled compounds in the mixture then determines the observed ^{3}H: ^{14}C atomic ratio (2.82:5). Some evidence for the formation of a Δ^{25}-compound, cyclolaudenol, by *Z. mays* shoots was provided by incorporation studies employing either [2-^{14}C]mevalonic acid or [Me-^{14}C]methionine as the sterol precursor.

INTRODUCTION

The most commonly reported [1–3] sterols in vascular plants are sitosterol (**1a**), stigmasterol (**2a**) and campesterol (**3a**). These sterols have the 24α-configuration† and it was originally assumed that 24α-alkyl sterols were found only in higher plants whilst 24β-alkyl sterols, such as clionasterol (**4a**), dihydrobrassicasterol (**5a**) and ergosterol, were restricted to algae and fungi. This view was modified following the identification of Δ^{25}-24β-ethyl sterols, such as clerosterol (**6a**) and 22-dehydroclerosterol (**7a**) in higher plants belonging to the Cucurbitaceae and Verbenaceae [4–7] and, more recently, other 24β-ethyl sterols have been characterized in seeds of Cucurbitaceae species [8, 9]. By contrast, the 24α-methyl sterol, epibrassicasterol has now been identified as the major sterol in some algae [10–12].

The 24α- and 24β-epimers of various sterols cannot be separated by the preparative chromatographic procedures routinely employed for sterol isolation although recently their separation has been reported using capillary column GC [13, 14]. The 24α- and 24β-epimers can also be distinguished by ^{1}H NMR and ^{13}C NMR spectroscopy and, moreover, the proportions of the two isomers present in a mixture of the two can be approximately estimated by this method [15–20].

The analysis of the sterols from several higher plants by ^{1}H NMR spectroscopy has established that the 24-ethyl sterol consists predominantly of the 24α-epimer but the 24-methyl sterol is often a mixture of the 24α- and 24β-forms [17, 21]. Thus, earlier reports of campesterol (**3a**) in higher plants may warrant reinvestigation using ^{1}H NMR or GC techniques to establish the proportions of the 24α- and 24β-methyl compounds.

The co-occurrence of campesterol (**3a**) and dihydrobrassicasterol (**5a**) in some plants poses questions regarding their biosynthetic origins. Investigations on sitosterol (**1a**) production suggested [22, 23] that a precursor, 24-ethylidine sterol (**8**), is isomerized to a $\Delta^{24(25)}$-sterol (**9**) prior to reduction to yield the 24α-ethyl sterol (**1**). Further evidence for the involvement of this pathway has proved elusive and labelling studies with barley [24] have produced only a very low incorporation of mevalonic acid into stigmasta-5,24-dien-3β-ol (**9a**) and shown poor conversion of **9a** into sitosterol (**1a**). However, the isolation of sterol **9a** and other $\Delta^{24(25)}$-sterols from plants of the Solanaceae family [25, 26] does provide support for this biosynthetic route to the 24α-ethyl sterols (**1** and **2**).

It has been suggested [27] that the 24α-methyl sterol **3** is produced by a similar route requiring isomerization of a 24-methylene intermediate (**10**) to the 24-methyl-$\Delta^{24(25)}$-sterol (**11**), which is then reduced stereospecifically to sterol **3**. The isolation of ergosta-5,24-dien-3β-ol (**11a**) from *Withania somnifera* [28] provides some support for such a route.

The 24β-methyl compound, brassicasterol (**12a**), was first isolated from rapeseed oil [29] and we suggested [1, 3] that a possible precursor might be cyclolaudenol (**13b**) which also has the 24β-methyl configuration [30]. Evidence was reported by McKean and Nes [27] which supports the involvement of a 25-methylene sterol (**13**) in the production of the 24β-methyl sterol (**5**) and the intermediacy of a $\Delta^{24(25)}$-sterol (**11**) in the formation of

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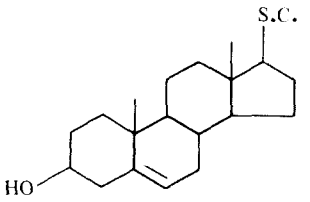
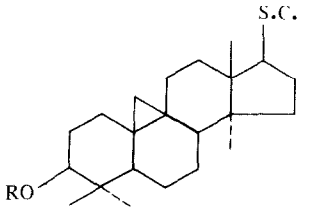
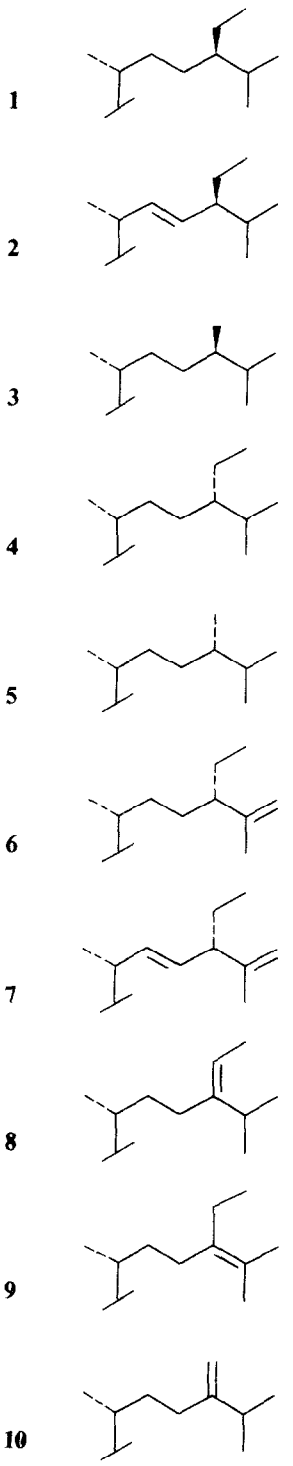
†We have elected in this paper to use the 24α- and 24β-convention to assign the configurations of 24-alkyl sterols rather than the 24*R*- and 24*S*-nomenclature.



a



b R = H
c R = Ac



the 24α-alkyl sterol in *Pinus pinea* seedlings. Recently, Benveniste and co-workers [31, 32] have reported the isolation of Δ^{23}-sterols (**14**) from *Zea mays* coleoptiles and suggested that they may be the precursors of the 24β-methyl sterol. We now present results which are pertinent to an understanding of the mechanism of formation of the 24β-methyl sterol in *Z. mays* seedlings.

RESULTS AND DISCUSSION

The sterols isolated from *Z. mays* shoots were acetylated and separated by chromatography on silver nitrate–alumina to yield the acetates of stigmasterol (**2a**) and the saturated side chain mixture which contained 24-ethylcholesteryl acetate (70% by GC) and 24-meth-

ylcholesteryl acetate (30%). The latter mixture was saponified and the sterols separated by chromatography on hydrophobic Sephadex [33] into the 24-ethylcholesterol (**1** and **4**) and 24-methylcholesterol (**3** and **5**) fractions. The 24-methyl-cholesterol fraction was further purified by reacetylation and prep. TLC on silver nitrate–silica gel TLC. The 220 MHz ^{1}H NMR spectrum of the maize 24-ethylcholesterol was identical [16] to that of authentic sitosterol (**1a**) thus demonstrating that it consisted predominantly of the 24α-isomer, sitosterol (**1a**). Similarly, the ^{1}H NMR spectrum of the stigmasteryl acetate indicated the 24α-ethyl configuration for this compound [16]. However, the ^{1}H NMR spectrum of the 24-methylcholesteryl acetate from *Z. mays* clearly showed it to be a mixture of the 24α- and 24β-isomers [16, 17]. From the intensities of the doublets for the C-21, C-27 and C-28 methyl protons it was estimated that the mixture contained 20–30% of the 24α-epimer (**3a**) and 70–80% of the 24β-epimer (**5a**). Scheid *et al.* [32] reported that coleoptiles of *Z. mays* contain a mixture of the 24α- and 24β-epimers of 24-methylcholesterol (**3a** and **5a**) in equal amounts.

Evidence for the involvement of a $\Delta^{24(25)}$-sterol in 24-ethyl sterol biosynthesis has been provided by the use of [2-^{14}C, (4*R*)4-$^3H_1$]mevalonic acid [22, 23, 34]. As shown in Scheme 1, the 24-ethylidene sterol intermediate isofucosterol (**8a**) produced from this precursor has a tritium atom located at C-25 and a ^{3}H: ^{14}C atomic ratio of 3:5. By contrast, the sitosterol (**1a**) produced from it has a ^{3}H: ^{14}C atomic ratio of 2:5. The replacement of the C-25 tritium atom of **8a** by a proton has been explained by the intermediate production of the $\Delta^{24(25)}$-sterol by isomerization of the 24-ethylidene sterol. A similar conclusion was indicated by the results obtained from the incorporation of [24-$^3H_1$]lanosterol into the sterols of *P. pinea* [27].

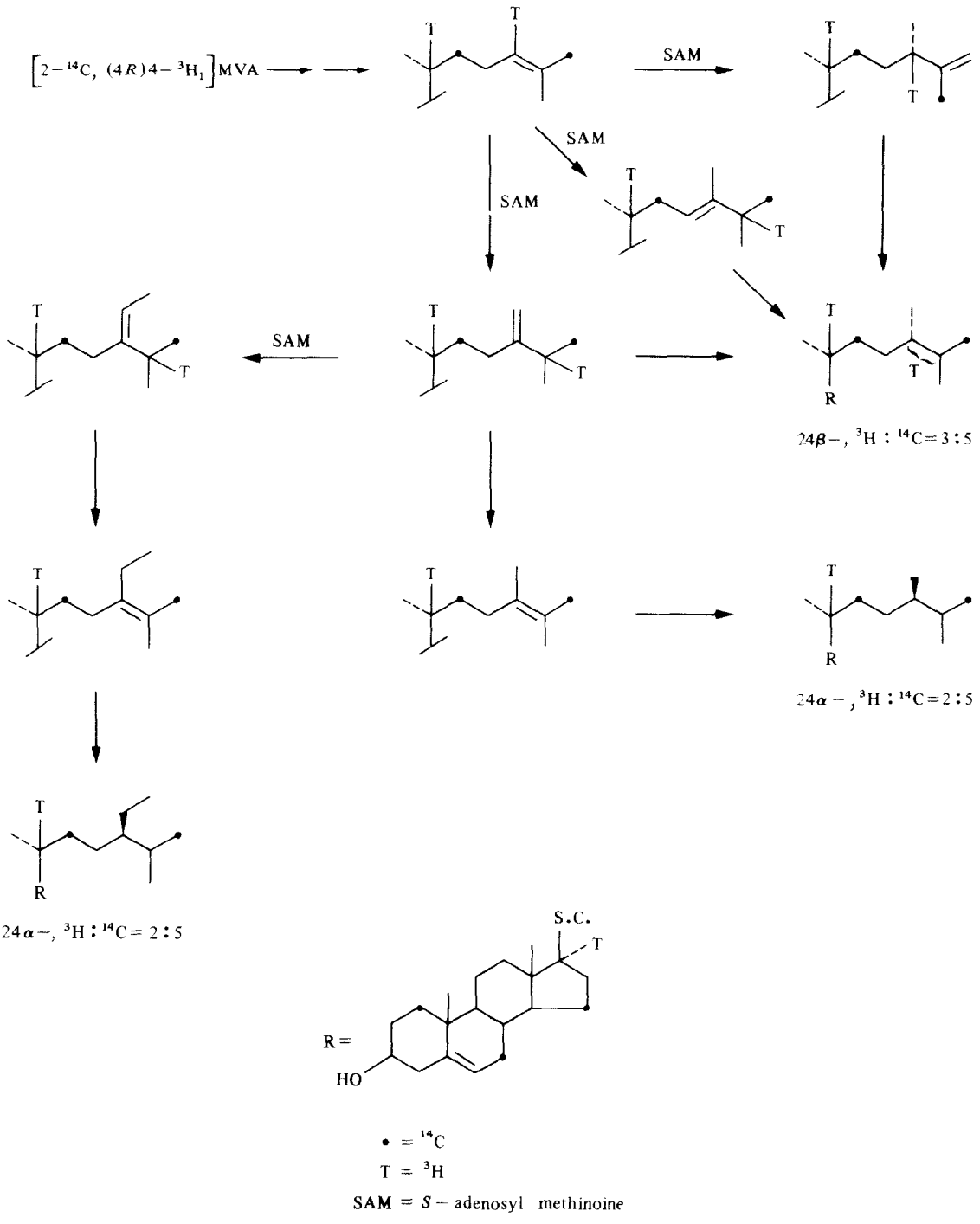
In several of our earlier studies on the incorporation of [2-^{14}C,(4*R*),4-$^3H_1$]MVA into plant sterols, we observed the ^{3}H: ^{14}C atomic ratio of the sitosterol (**1a**) containing fraction could not be lowered to the anticipated theoretical value of 2.0:5 but often stayed persistently in the range 2.2–2.3:5 despite attempted purification. However, the 24-methylcholesterol present in the plant sterol mixtures also accompanied the sitosterol (**1a**) through the purification procedures employed. Therefore, it occurred to us that the anomalous ^{3}H: ^{14}C atomic ratios might be due to the 24-methylcholesterol component having a higher tritium content than the sitosterol. Indeed, this would be predicted if the 24β-methylcholesterol (**5a**) is produced via a 25-methylene intermediate, such as cyclolaudenol (**13b**) as indicated in Scheme 1.

To test this possibility, *Z. mays* shoots were allowed to imbibe [2-^{14}C,(4*R*)4-$^3H_1$]MVA and the labelled sterols produced were extracted and separated as described in the Experimental (Table 1). The 4-demethyl sterol mixture had a ^{3}H: ^{14}C atomic ratio of 3.07:5. Acetylation followed by prep. TLC on silver nitrate–silica gel gave a mixture of the acetates of sitosterol (**1a**) and 24-methylcholesterol (**3a** and **5a**) which had a ^{3}H: ^{14}C atomic ratio of 2.3:5. The observed fall in the ^{3}H: ^{14}C ratio can be ascribed to the removal of isofucosterol (**8a**, theoretical ^{3}H: ^{14}C atomic ratio 3:5) which is a major labelled constituent of the 4-demethyl sterol mixture. Saponification of the steryl acetates followed by chromatography on Lipidex 5000 separated **1a** from the 24-methylcholesterol (**3a** and **5a**) (Table 1).

The purified 24-methylcholesterol fraction had a ^{3}H: ^{14}C atomic ratio of 2.82:5. This observed ratio can be rationalized on the basis of the proportions of 24α-methyl and 24β-methyl epimers shown to be present in *Z. mays* (see above) and their proposed routes of production (Scheme 1). The 24α-methylcholesterol (**3a**) comprises *ca* 20–30% of the mixture and would have a ^{3}H: ^{14}C ratio of 2:5 if produced via a $\Delta^{24(25)}$-sterol, whereas the 24β-methylcholesterol (**5a**) accounts for 70–80% of the mixture and would have a ^{3}H: ^{14}C ratio of 3:5 if its biosynthesis involves a Δ^{25}-sterol intermediate [1, 27] or a Δ^{23}-sterol precursor as recently suggested by Benveniste [32]. Also, direct stereospecific reduction of a 24-methylene sterol (**10**) to 24β-methyl sterol (**5**) (Scheme 1), as observed in fungi [3, 35], would give a ^{3}H: ^{14}C ratio of 3:5. Thus, the predicted ^{3}H: ^{14}C atomic ratio for the 24-methylcholesterol (**3a** and **5a**) was in the range 2.70–2.80:5, which is in agreement with the observed value of 2.82:5. Also, the proportions of sitosterol (*ca* 70% by GC) to 24-methylcholesterol (*ca* 30%) suggest that the ^{3}H: ^{14}C ratio for the initial mixture of the two should be around 2.25–2.30:5, which compares with the experimental value of 2.31:5.

The sitosterol had a ^{3}H: ^{14}C atomic ratio of 2.09:5 compared with the theoretical value of 2:5 if it is assumed to consist solely of the 24α-epimer derived via a $\Delta^{24(25)}$-sterol intermediate. The ^{1}H NMR method for determining the proportions of 24α- and 24β-ethyl sterols in a mixture is not capable of detecting small amounts of either epimer. It is, therefore, possible that the sitosterol fraction of *Z. mays* does in fact contain up to 5–10% of the 24β-ethyl epimer (**4a**) having a ^{3}H: ^{14}C atomic ratio of 3:5. This may possibly arise by a pathway involving a 25-methylene intermediate (**6**) which would retain a tritium at C-24 upon reduction to yield **4** and, indeed, we have detected a very small amount of clerosterol (**6a**) by GC/MS analysis of the *Z. mays* sterols [36].

While the above results confirm that alternative mechanisms are responsible for the production of the 24α- and 24β-methyl sterols of maize they do not differentiate between the various routes which require involvement of $\Delta^{24(28)}$ (**10**), Δ^{25} (**13**) or Δ^{23} (**14**) sterol intermediates (Scheme 1). Therefore, we performed preliminary experiments to investigate the labelling of one potential precursor, cyclolaudenol (**13b**) by incubating *Z. mays* shoots with [2-^{14}C]MVA (see Experimental for details). The labelled 4,4-dimethyl sterol fraction was mixed with appropriate carrier material, acetylated, and separated by prep. silver nitrate–silica gel TLC into the fraction containing cycloartenyl acetate (**15c**) and that containing 24-methylenecycloartanyl acetate (**10c**) and cyclolaudenyl acetate (**13c**) which were not adequately separated by the TLC system used. To establish if the cyclolaudenyl acetate (**13c**) was labelled, the mixture of **10c** and **13c** (4.37 $\times 10^4$ dpm) was subjected to osmium tetroxide oxidation as described previously [37] and the resulting diols separated. Radioactivity accompanied both the 3β-acetoxy-24-methylcycloartan-24,28-diol (**16c**, 2.07 $\times 10^4$ dpm) was subjected to osmium tetroxide oxidation as described previously [37] and the resulting diols separated. Radioactivity accompanied both the 3β-acetoxy-24-methylcycloartan-24,28-diol (**16c**, 2.07 compounds 24-oxocycloartanyl acetate (**18c**, 1.80 $\times 10^4$ dpm) and 24-methyl-25-oxo-26-norcycloartanyl acetate (**19c**, 3.70 $\times 10^3$ dpm), respectively. These results, also confirmed in a second experiment, indicate that both 24-methylenecycloartanol (**10b**) and cyclolaudenol (**13b**) were produced thus providing limited support for the



Scheme 1. Possible routes for the production of the 24-methyl and 24-ethyl sterols of *Zea mays* to show the side chain labelling patterns to be expected following incorporation of [2-^{14}C,(4*R*)4-^{3}H$_1$]mevalonic acid.

suggestion that a Δ^{25}-sterol is produced and may be involved in 24β-methyl sterol elaboration.

To obtain further evidence for the formation of cycloladenol (**13b**) *Z. mays* shoots were also incubated with [Me-^{14}C]methionine which will label the alkyl group introduced at C-24 into the sterols **10b** and **13b** [38]. Incorporation of radioactivity into the 4,4-dimethyl sterols was low but, nevertheless, radioactivity was shown to accompany both **16c** (3200 dpm) and **17c** (340 dpm) derived from **10b** and **13b**, respectively. Cleavage of the diol **16c** resulted in essentially all the radioactivity being lost from **18c** as expected because **16c** will be labelled at C-28. By contrast, although the labelling of **17c** was very low, the radioactivity was largely retained in **19c** as predicted if the labelled parent compound was cyclolaudenol (**13b**). These experiments were performed before the suggestion

Table 1. Incorporation of [2-^{14}C,(4*R*)4-$^{3}H_1$]mevalonic acid into the sterols of *Z. mays* shoots

Sterol	Observed ^{3}H:^{14}C ratio	Corrected ^{3}H:^{14}C atomic ratio*	Theoretical ^{3}H:^{14}C atomic ratio
Cycloartenyl acetate	10.70	6:6	6:6
4-Desmethyl sterol mixture	6.58	3.07:5	—
Sitosteryl acetate plus 24-methylcholesteryl acetate	4.94	2.31:5	—
Sitosterol	4.47	2.09:5	2:5
24-Methylcholesterol	6.04	2.82:5	2:5 for 24α-epimer. 3:5 for 24β-epimer

*The ^{3}H:^{14}C atomic ratios were calculated from the experimental ^{3}H:^{14}C value of 10.70 obtained for cycloartenyl acetate.

by Benveniste and co-workers [31, 32] that Δ^{23}-sterols may be implicated in 24β-methyl sterol production and so no information on the labelling of Δ^{23}-sterols was obtained in the present study.

EXPERIMENTAL

General methods. TLC, GC, MS and radioactivity counting methods were as described previously [23, 24, 37].

Isolation of Zea mays *sterols.* Seeds of *Z. mays* were soaked overnight in H_2O and then placed in trays of moist vermiculite and allowed to germinate for 5 days in the dark followed by 13 days with alternating 14 hr light and 10 hr dark periods. The shoots (715 g wet wt) were harvested and saponified by reflux with 10% KOH in 85% aq. EtOH. The recovered non-saponifiable lipid (1.02 g) was chromatographed on alumina, Brockmann Grade III, eluted with Et_2O-petrol mixtures to give a fraction containing the 4-demethyl sterols which were acetylated (pyridine–Ac_2O). The steryl acetates (205 mg) were chromatographed on a 20 g column of 10% $AgNO_3$–alumina eluted with Et_2O–petrol mixtures to give fractions containing 24-methylcholesteryl plus sitosteryl acetate (112 mg) and stigmasteryl acetate (7.1 mg). The 24-methylcholesteryl plus sitosteryl acetate fraction was hydrolysed (10% KOH in 85% aq. EtOH) and the 24-methylcholesterol–sitosterol mixture (72 mg) applied to a 1.5 × 75 cm column of Lipidex 5000 (Packard Instruments). The column was held at 10° and eluted with hexane–MeOH (1:19) and 5–7 ml fractions collected. GC analysis (3% OV-17) established that sitosterol was eluted in fractions 131–143. These were combined and the sitosterol (20 mg) purified by TLC on silica gel developed with $CHCl_3$ prior to MS and ^{1}H NMR examination. GC analysis showed that fractions 110–113 contained 24-methylcholesterol but fractions 115–123 contained 24-methylcholesterol that was still contaminated by a small amount of sitosterol. The latter fractions were, therefore, combined and rechromatographed on a smaller (1.0 × 35 cm) column of Lipidex 5000. Fractions (6 ml) were again monitored by GC and those containing 24-methylcholesterol were combined with fractions 110–113 from the first Lipidex column. Acetylation (pyridine–Ac_2O) and TLC on first $AgNO_3$–silica gel developed with pure $CHCl_3$ and then silica gel developed with Et_2O–petrol (1:49) gave 24-methylcholesteryl acetate (4.1 mg) which was subjected to MS and 220 MHz ^{1}H NMR examination.

Sitosterol. MS *m/z*: 414 $[M]^+$; ^{1}H NMR ($CDCl_3$): δ0.679 (3H, *s*, H-18), 0.811 (3H, *d*, J = 6.8 Hz, H-27), 0.833 (3H, *d*, J = 6.8 Hz, H-26), 0.843 (3H, *t*, J = 7.2 Hz, H-29), 0.918 (3H, *d*, J = 6.5 Hz, H-21), 1.004 (3H, *s*, H-19).

Stigmasteryl acetate. MS *m/z*: 394 $[M-60]^+$; ^{1}H NMR ($CDCl_3$): δ0.693 (3H, *s*, H-18), 0.791 (3H, *d*, J = 6.8 Hz, H-27), 0.800 (3H, *t*, J = 7.2 Hz, H-29), 0.843 (3H, *d*, J = 6.8 Hz, H-26), 1.018 (3H, *d*, J = 6.5 Hz, H-21), 1.018 (3H, *s*, H-19).

24-Methylcholesteryl acetate. MS *m/z*: 382 $[M-60]^+$; ^{1}H NMR ($CDCl_3$): δ0.668 (3H, *s*, H-18), 0.770 (3H, *d*, J = 6.8 Hz, H-28), 0.780 and 0.798 (3H, *d*, J = 6.8 Hz H-27), 0.848 (3H, *d*, J = 6.8 Hz, H-26), 0.909 and 0.916 (3H, *d*, J = 6.5 Hz, H-21), 1.018 (3H, *s*, H-19).

Incubation of maize shoots with [2-^{14}C, (4R)4-$^{3}H_1$]*mevalonic acid.* Maize seeds were soaked in H_2O and then germinated on moist filter papers in the light. After 8 days the shoots (10.45 g) were excised, chopped into strips and incubated for 6 hr with 1.0 ml [2-^{14}C, (4*R*)4-$^{3}H_1$]mevalonic acid (10 μCi ^{14}C, 100 μCi ^{3}H). The non-saponifiable lipid (21 mg) was extracted in the usual manner, squalene (2 mg) and cycloartenol (2 mg) were added and the mixture then separated by prep. TLC (silica gel developed with $CHCl_3$–EtOH, 49:1) to give the fractions containing squalene (4.7×10^4 dpm ^{14}C), 4,4-dimethylsterols (2.97×10^5 dpm ^{14}C) and 4-demethylsterols (4.51×10^5 dpm ^{14}C). A mixture of carrier cycloartenol and 24-methylenecycloartanol (8 mg) was added to the labelled 4,4-dimethyl sterols and the mixture acetylated (Ac_2O–pyridine) and separated by prep. TLC on 10% $AgNO_3$–silica gel developed with pure $CHCl_3$ to yield cycloartenyl acetate (2 mg; 7.76×10^3 dpm ^{14}C) and 24-methylenecycloartanyl acetate (7 mg; 8.95×10^4 dpm ^{14}C). To the 4-demethyl sterols was added sitosterol plus 24-methylcholesterol (4 mg) and the mixture acetylated (pyridine–Ac_2O). The steryl acetates (4 mg; 4.10×10^5 dpm ^{14}C) were separated by prep. TLC on 10% $AgNO_3$–silica gel developed with pure $CHCl_3$ to yield a mixture of sitosteryl plus 24-methylcholesteryl acetates (3 mg; 8.97×10^4 dpm ^{14}C) and impure stigmasteryl acetate (1.4×10^4 dpm ^{14}C). To the sitosteryl acetate plus 24-methylcholesteryl acetate mixture a further 9 mg of carrier was added and the mixture saponified with ethanolic KOH. The recovered sitosterol plus 24-methylcholesterol (13.8 mg; 8.77×10^4 dpm ^{14}C) was mixed with a further 8 mg of carrier and then separated by chromatography on a column (1.0 × 35 cm) of Lipidex 5000 eluted with hexane–MeOH (1:19) and collecting 7–8 ml fractions. Fractions were monitored by GC and those containing pure components bulked to give 24-methylcholesterol and sitosterol for ^{3}H: ^{14}C ratio determinations.

Incorporation of [2-^{14}C]*mevalonic acid into the* 4,4-*dimethyl sterols by* Z. mays *shoots.* Maize seeds were soaked overnight in H_2O, allowed to germinate in trays of moist vermiculite in the dark for 2 days and then transferred to the light. After 10 days the shoots were excised and 10–15 shoots were allowed to absorb 1 ml H_2O containing 10 μCi [2-^{14}C]mevalonic acid, more H_2O was added and the shoots left for 24 hr. The non-saponifiable

lipid (4.4×10^6 dpm) was extracted in the usual manner and separated by TLC on silica gel ($CHCl_3$–EtOH, 49:1) to give the squalene (2.4×10^4 dpm), 4,4-dimethyl sterol (5.42×10^5 dpm) and 4-demethyl sterol (9.80×10^5 dpm) fractions. The 4,4-dimethylsterol fraction was acetylated (pyridine–Ac_2O) and cycloartenyl acetate (**15c**, 2.5 mg) and 24-methylenecycloartanyl acetate (**10c** 2.5 mg) added prior to their separation by prep. TLC on 10% $AgNO_3$–silica gel developed with pure $CHCl_3$. The recovered cycloartenyl acetate (**15c**) contained 2.94×10^5 dpm. To the radioactive material co-chromatographing with 24-methylenecycloartanyl acetate (**10c**, 4.37×10^4 dpm) was added 41 mg of 24-methylenecycloartanyl acetate (**10c**) and cyclolaudenyl acetate (**13c**). To this mixture was added 30 mg OsO_4 in 1.5 ml pyridine [37] and the soln was kept at room temp. overnight before addition of 125 mg sodium metabisulphite in 0.75 ml H_2O. The mixture was stirred for 2 hr, diluted with H_2O and extracted ($\times 4$) with Et_2O. The extract was washed with H_2O, dried and evaporated to give 45 mg of product. This was separated by prep. TLC [37] on silica gel ($CHCl_3$–MeOH, 23:2) to give 3β-acetoxy-24-methylcycloartan-24,28-diol (**16c**, 27 mg, 2.07×10^4 dpm) and 3β-acetoxy-24-methylcycloartan-25,26-diol (**17c**, 7 mg, 5.53×10^3 dpm). These compounds were characterized by GC and MS. Compound **16c**: MS *m/z* 516 $[M]^+$, 498, 483, 456, 438, 423, 413, 357, 334, 297. Compound **17c** had a similar MS but *m/z* 413 was of lower rel. int. Compound **16c** was dissolved in 3 ml 1,4-dioxan and 30 mg $NaIO_4$ and 1.5 ml H_2O added. The mixture was stirred overnight, diluted with H_2O, extracted ($\times 4$) with Et_2O and the extract washed and taken to dryness. The product was purified by prep. TLC on silica gel ($CHCl_3$–MeOH, 97:3) and crystallized (MeOH) to yield 24-oxocycloartanyl acetate (**18c**, 1.80×10^4 dpm); MS *m/z*: 484 $[M]^+$, 469, 424, 409, 381, 355, 302, 297. Compound **17c** (5.7 mg) was treated in a similar manner to give 24-methyl-25-oxo-26-norcycloartanyl acetate (**19c**, 3.5 mg, 3.70×10^3 dpm); MS *m/z*: 484 $[M]^+$, 469, 474, 409, 381, 355, 302, 297. A second incubation with [2-^{14}C]MVA gave similar results.

Incorporation of [Me-^{14}C]*methionine into the* 4,4-*dimethyl sterols of* Z. mays *shoots.* [Me-^{14}C]Methionine (12.5 μCi) was incubated with *Z. mays* shoots for 24 hr as described above for the [2-^{14}C]MVA experiments and the 4,4-dimethyl sterols (9.9×10^3 dpm) and 4-demethyl sterols (2.1×10^5 dpm) isolated by prep. TLC. Addition of carrier 24-methylenecycloartanol and cyclolaudenol to the 4,4-dimethyl sterol fraction and acetylation and OsO_4 oxidation as above gave **16c** (3200 dpm) and **17c** (340 dpm) which were treated with $NaIO_4$ to yield **18c** (60 dpm) and **19c** (260 dpm), respectively. Two other similar incubations of *Z. mays* shoots with [Me-^{14}C]methionine and degradation of the 4,4-dimethyl sterols gave essentially similar results.

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