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OBSERVATIONS ON THE BIOSYNTHESIS AND METABOLISM OF β-SITOSTEROL, β-AMYRIN AND RELATED METHYL STEROLS

R. AEXEL, S. EVANS, M. KELLEY and H. J. NICHOLAS

Institute of Medical Education and Research, St. Louis, Missouri, and Department of Biochemistry, St. Louis University School of Medicine, St. Louis, Missouri

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Abstract—The incorporation of mevalonic acid-2-^{14}C into cut flower stalks of *Taraxacum officinale* L., whose principal sterol and triterpene respectively in the dried form is β-sitosterol and β-amyrin, was studied at intervals of 0·5 hr to 2 weeks. A trace of product possibly identical with lanosterol was indicated by gas chromatography. However, it was only weakly radioactive at all intervals studied, and it was concluded that lanosterol was not an obligatory intermediate in the biosynthesis of β-sitosterol under the conditions of these particular experiments. Several compounds which may be transient 4α-methyl sterols and therefore possible precursors of β-sitosterol were indicated by gas chromatography, but none could be identified with the methyl sterols or triterpenes available as reference compounds. The ^{14}C-content of β-sitosterol slowly increased to a relatively constant value after 8 hr, thereafter remaining stable. The ^{14}C-content of β-amyrin, on the other hand, rose slowly to a maximum in about 20 hr and then declined. The data suggest a moderate rate of turnover of β-amyrin.

INTRODUCTION

FOR a study of the biosynthesis and metabolism of the widely distributed triterpene β-amyrin and the more ubiquitous sterol β-sitosterol, flower stalks of *Taraxacum officinale* L. (dandelion) were selected. The roots of this plant have been reported to contain a complex mixture of triterpenes and sterols,[1] but in our laboratory the flower stalks were found to contain predominantly β-sitosterol and β-amyrin. In some preliminary studies in which mevalonic acid-2-^{14}C was presented to cut stems of flowering dandelion, the β-amyrin-^{14}C isolated indicated a definite degree of turnover, while the isolated β-sitosterol-^{14}C remained quite stable over the period studied. Since observations of this type may one day provide a clue as to the physiological role of sterols and triterpenes in higher plants, the work has been repeated and investigated in more detail. During the course of these investigations several important observations from other laboratories were published. Bennett and Heftmann[2] demonstrated the direct conversion of squalene-^{14}C to β-sitosterol-^{14}C in *Pharbitis nil* seedlings, and Capstack *et al.*[3] showed the direct conversion of squalene-^{14}C to β-amyrin-^{14}C in cell-free extracts of *P. sativum*. Of particular interest are the observations of Benveniste *et al.*,[4] who have indicated that the triterpene cycloartenol rather than lanosterol may be a critical intermediate in the formation of higher plant sterols in tissue culture. The work herein described was completed with these observations in mind.*

* We are indebted to Dr. W. W. Reid of the British Tobacco Company (Australia) Ltd., for calling our attention to the work of Professor Ourisson, and for informative comments on his own studies with tobacco tissue, and to Professor Ourisson for providing his manuscript to us before publication.

[1] S. BURROWS and J. C. E. SIMPSON, *J. Chem. Soc.* 2042 (1939).
[2] R. D. BENNETT and E. HEFTMANN, *Phytochem.* **4**, 475 (1966).
[3] E. CAPSTACK, JR., N. ROSIN, G. A. BLONDIN and W. R. NES, *J. Biol. Chem.* **240**, 3285 (1965).
[4] P. BENVENISTE, L. HIRTH and G. OURISSON, *Phytochem.* **5**, 45 (1966).

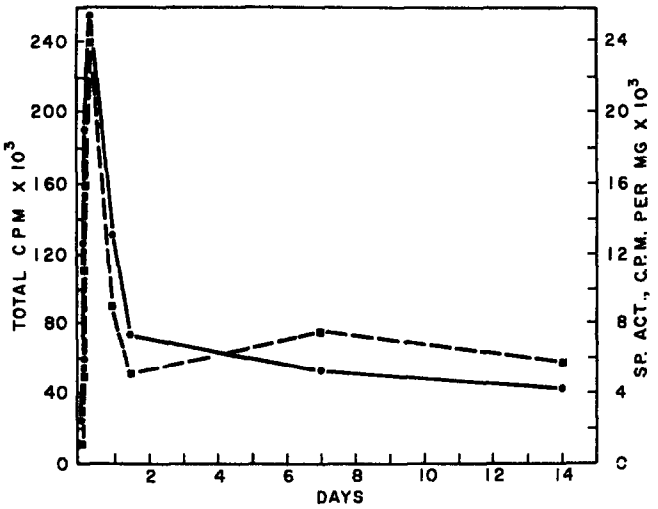

FIG. 1. INCORPORATION OF 10 μc OF MEVALONIC ACID-2-^{14}C INTO β-AMYRIN OF *T. officinale* AT VARYING TIME INTERVALS. THE SOLID LINE (LEFT ORDINATE) REPRESENTS THE TOTAL ^{14}C IN EACH β-AMYRIN FRACTION, THE DOTTED LINE (RIGHT ORDINATE) THE SPECIFIC ACTIVITY OF EACH CORRESPONDING β-AMYRIN-^{14}C SAMPLE.

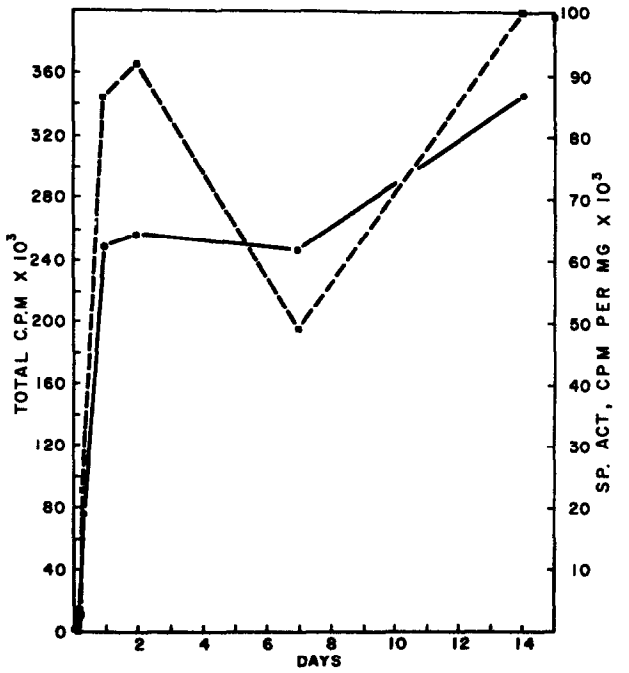

FIG. 2. INCORPORATION OF 10 μc OF MEVALONIC ACID-2-^{14}C INTO β-SITOSTEROL OF *T. officinale* AT VARYING TIME INTERVALS. THE SOLID LINE (LEFT ORDINATE) REPRESENTS THE TOTAL ^{14}C IN EACH β-SITOSTEROL FRACTION, THE DOTTED LINE (RIGHT ORDINATE) THE SPECIFIC ACTIVITY OF EACH CORRESPONDING β-SITOSTEROL-^{14}C SAMPLE.

RESULTS AND DISCUSSION

In a preliminary experiment 10 μc of mevalonic acid-2-^{14}C was presented to each of several groups of cut dandelion flower stalks under carefully controlled conditions. β-Amyrin and β-sitosterol were separated and isolated from non-saponifiable extracts by chromatography on alumina; the results shown in Figs. 1 and 2 were obtained. The values

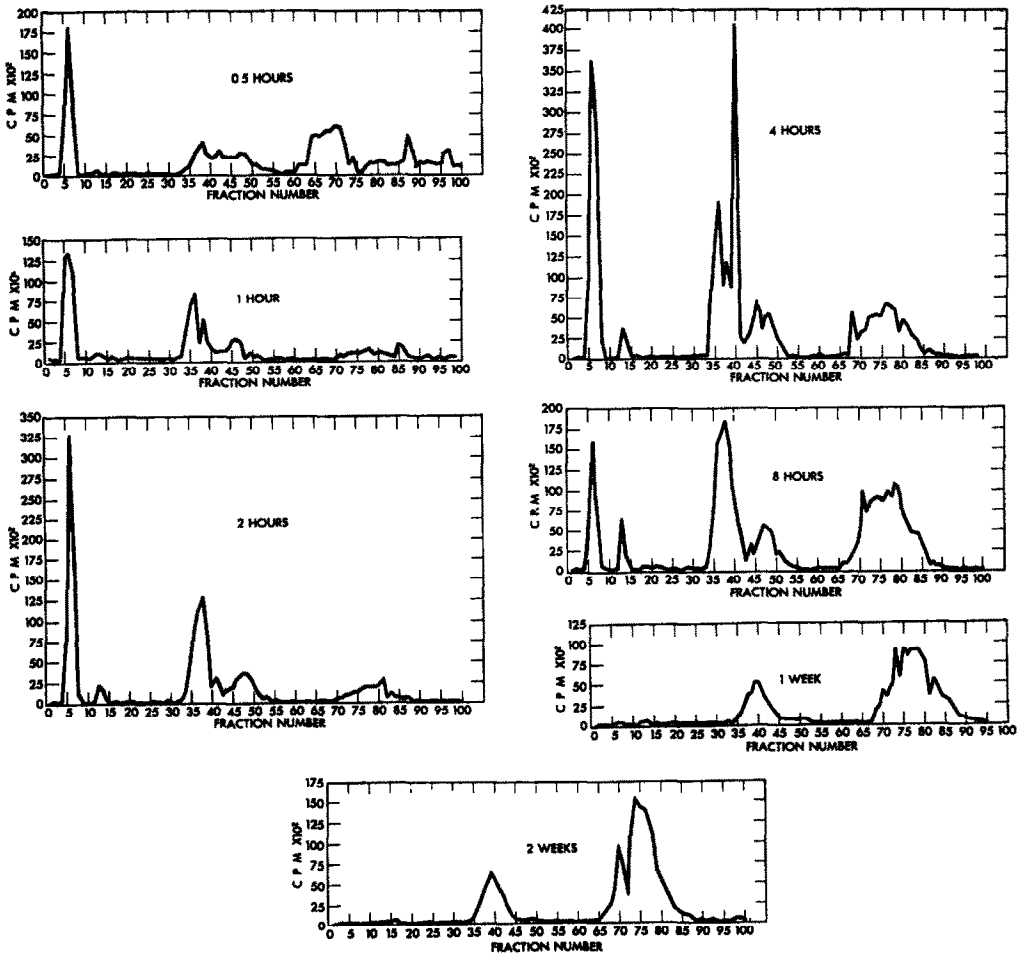

FIG. 3. CHROMATOGRAPHY OF NON-SAPONIFIABLE FRACTIONS OF *T. officinale* FLOWERING STEMS WHICH HAD RECEIVED 10 μc OF MEVALONIC ACID-2-^{14}C FOR VARIOUS TIME INTERVALS. FRACTIONS 2–10 CONSISTED OF 20 ML OF PETROLEUM ETHER; FRACTIONS 11–100 CONSISTED OF 20 ML OF BENZENE.

for the β-amyrin curve were obtained at the various intervals by co-crystallization to constant radioactivity of β-amyrin-^{14}C fractions with a fixed amount of non-radioactive β-amyrin. A definite drop in specific activity of β-amyrin-^{14}C is noted after the 24-hr period. In contrast, the β-sitosterol-^{14}C specific activity (Fig. 2) rose to a reasonably steady value after 24 hr and remained stable over the 2-week period investigated. However, at the time no method was available to effectively separate β-amyrin-^{14}C from lanosterol-^{14}C suspected to be present,

nor any way to quantitate their amount, a problem which could have obscured the evaluation of the β-amyrin data. For this reason the work was repeated when a gas chromatograph became available; the latter instrument has proved to be the only effective way to satisfactorily separate the two C_{30} triterpenes. Since "turnover" of β-amyrin seemed to be indicated by the preliminary work, in the present manuscript only the earliest time intervals and two advanced periods were investigated. Non-saponifiable fractions from batches of cut dandelion flower stalks incubated with 10 μc of mevalonic acid-2-^{14}C for various time intervals were chromatographed on alumina. A plot of fraction number versus ^{14}C content for the various time intervals is shown in Fig. 3. Based on previous observations[5] the product in Fractions 4–8 is largely squalene-^{14}C. An orange-colored band was consistently found in Fractions 13 and 14. It is presumably at least partly carotenoid, but was not further examined. It is interesting that the ^{14}C-content of these fractions rises and falls as does that of squalene over the intervals studied.

β-Amyrin and Lanosterol

Fractions 32–40 (Fig. 3) contained β-amyrin-^{14}C and presumably lanosterol, if present. For each respective time period these fractions were combined. The weights, total ^{14}C-content and percentage ^{14}C in these fractions is shown in Table 1. β-Amyrin and lanosterol

TABLE 1. INCORPORATION OF MEVALONIC ACID-2-^{14}C INTO CRUDE β-AMYRIN-LANOSTEROL FRACTIONS OF *T. officinale*

Incubation period	Yield (mg)	Total ^{14}C incorporation, c.p.m. × 10^5	Percentage ^{14}C incorporation*
0·5 hr	11·0	1·50	1·9
1·0 hr	12·0	2·41	3·1
2·0 hr	15·0	18·22	23·6
4·0 hr	21·0	10·57	13·7
8·0 hr	55·0	9·78	12·7
1 week	25·0	3·15	4·0
2 weeks	34·0	3·72	4·8

* Based on 10 μc of administered DL-mevalonic acid-2-^{14}C, and assuming only one isomer was utilized in the biosynthesis.

can be satisfactorily separated by gas chromatography. When 50 μc quantities of the respective combined fractions were subjected to gas chromatography, the results shown in Fig. 4 were found. It will be noted that the mass of β-amyrin is fairly constant over the entire period studied. Radioactivity corresponding to the individual time periods from 0·5 hr to 1 week and their analogous mass peaks are also shown in Fig. 4. The radioactive content of the β-amyrin peak area is detectable within 1 hr and up to 8 hr, and is practically negligible at the longer intervals.* That the radioactivity in this peak area is due primarily to β-amyrin-^{14}C rather than lanosterol-^{14}C is also evident from the data given in Table 2. Co-crystallization of weighed amounts of each of the fractions with the unlabeled lanosterol (see also Capstack

* The radioactive monitor peaks shown obviously cannot distinguish between the ^{14}C-contribution from Peaks 3 and 4 (β-amyrin) with retention times so nearly identical. The limited sensitivity of the monitor system in the present studies has also limited interpretation of the identity of peaks in the methyl sterol area. (See next section.)

[5] H. J. NICHOLAS, *J. Biol. Chem.* **237**, 1485 (1962).

et al.[3]) produced lanosterol, low in ^{14}C and practically constant in radioactivity for all of the intervals studied. Similar treatment of the fractions with unlabeled β-amyrin, however, produced β-amyrin-^{14}C in which the specific activity rose during the periods up to 8 hr, and then declined over the 1- and 2-week intervals, substantiating the data previously obtained in Fig. 1. Since the mass of β-amyrin remained essentially constant during the intervals, these data suggest that the triterpene is undergoing some metabolic change, characteristic of

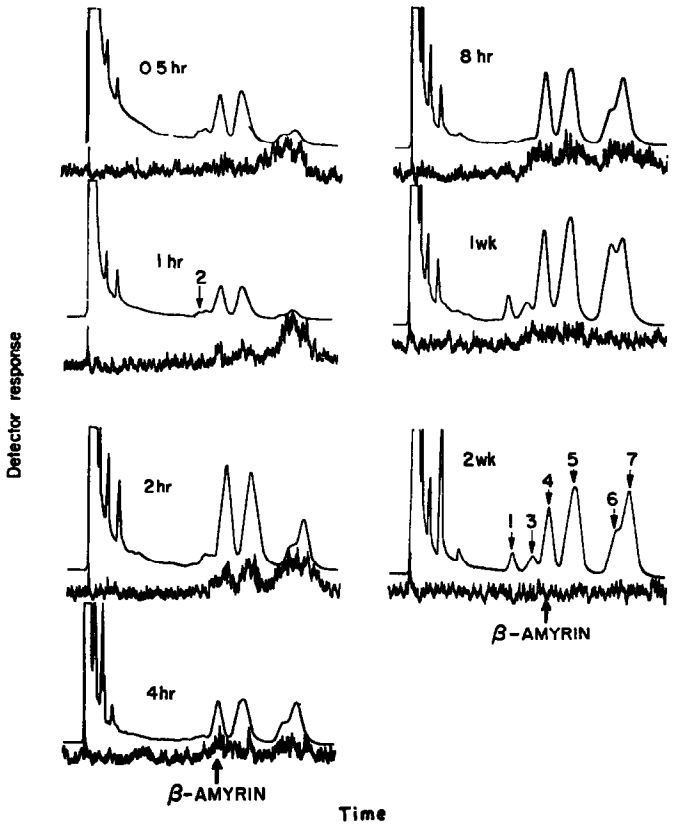

FIG. 4. RELATIONSHIP OF MASS (UPPER CURVES) AND RADIOACTIVITY (LOWER CURVES) TO RETENTION TIME IN GAS–LIQUID CHROMATOGRAPHY. THE SAMPLES CHROMATOGRAPHED WERE THE "β-AMYRIN-^{14}C-LANOSTEROL FRACTIONS" OBTAINED BY CHROMATOGRAPHY ON ALUMINA OF NON-SAPONIFIABLE FRACTIONS OF *T. officinale* WHICH HAD BEEN PRESENTED WITH MEVALONIC ACID-2-^{14}C FOR THE TIME INTERVALS INDICATED.

"turnover". The data also suggest, what is implied by the work of Benveniste *et al.*[4] with tobacco tissue culture studies, that lanosterol is not an obligatory intermediate in the biosynthesis of higher plant sterols, in this case β-sitosterol. Some lanosterol may be present in the *T. officinale* flowering stems, as indicated in Fig. 5, where peak enrichment with authentic lanosterol at the 1-week interval did not produce a detectable change in the peak position or shape assigned to this triterpene. However, to serve as a precursor of β-sitosterol in the present experiments lanosterol would have to be labeled appreciably with ^{14}C at one of the earliest time intervals of the experiment. From the data in Table 2 this is evidently not so.

It seems unlikely that labeled lanosterol would be found at intervals of less than 0·5 hr if this were experimentally possible under the conditions imposed here.

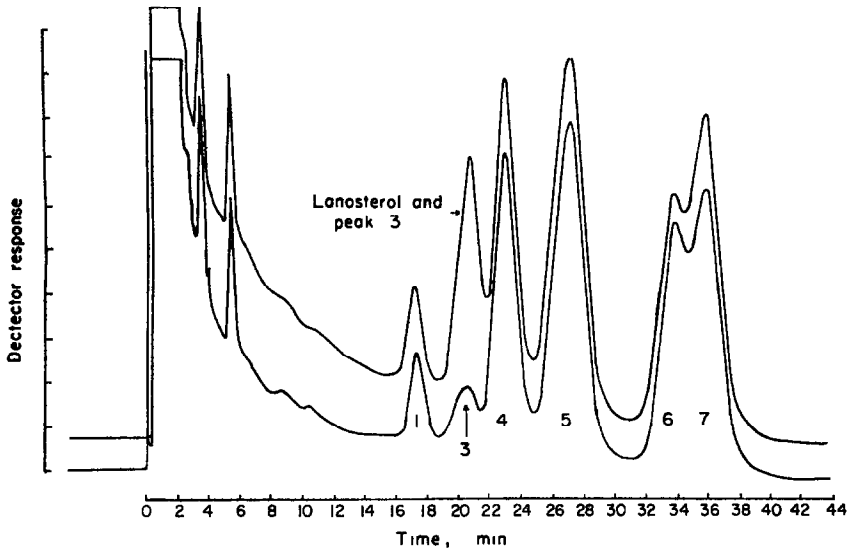

FIG. 5. GAS–LIQUID CHROMATOGRAPHY OF "β-AMYRIN-^{14}C-LANOSTEROL FRACTION" (TABLE 1, FIG. 5; 1-WEEK INTERVAL). LOWER CURVE, FRACTIONS 36–45, UPPER CURVE, FRACTIONS 36–45 PLUS LANOSTEROL. THE COLUMN CONSISTED OF 6 FT OF 1% SILICONE GE XE-60 ON GAS CHROM Q, 100/120 MESH, AND THE CARRIER GAS WAS ARGON. OTHER DETAILS ARE GIVEN IN EXPERIMENTAL SECTION.

TABLE 2. CO-CRYSTALLIZATION OF β-AMYRIN AND LANOSTEROL WITH "β-AMYRIN-^{14}C-LANOSTEROL" FRACTIONS OF *T. officinale* NON-SAPONIFIABLE EXTRACTS, FOLLOWING ADMINISTRATION OF MEVALONIC-2-^{14}C ACID

Time interval following MVA-2-^{14}C administration	Specific activity* after crystallization to constant radioactivity	
	β-Amyrin†	Lanosterol†
0·5 hr	271	37
1·0 hr	288	49
2·0 hr	462	34
4·0 hr	1840	99
8·0 hr	2392	33
1 week	667	35
2 weeks	642	30

* Expressed as c/min per mg.

† Appropriate fractions (Fig. 1 and Table 1) of 0·500 mg each were diluted with 10·00 mg of β-amyrin or lanosterol, then crystallized to constant radioactivity from absolute ethanol or acetone.

The identity of Peaks 1, 2, 5, 6 and 7 (Figs. 4 and 5) is not known, although the retention time of Peak 1 is identical to that of lophenol (4α-methyl-5α-cholesta-7-ene-3β-ol). There was no indication that this product was markedly radioactive at any early time interval; such would be the case if it were a precursor of β-sitosterol. It seems probable that it is identical to Peak 8 (Fig. 6) due to overlapping of fractions taken from the alumina column (Fig. 3).

Peaks 5, 6 and 7 are especially interesting since they increase in mass over the time periods studied; the ^{14}C-content of Peaks 6 and 7 are radioactive at the earliest time interval (Fig. 4), decreasing in ^{14}C-content after 4 hr, and at the final interval of 2 weeks they represent a mass almost equal to that of β-amyrin. The retention time of Peak 5 is identical to that of lupeol, but there was no indication during chemical analyses of the dried flower stalks that this triterpene was present.

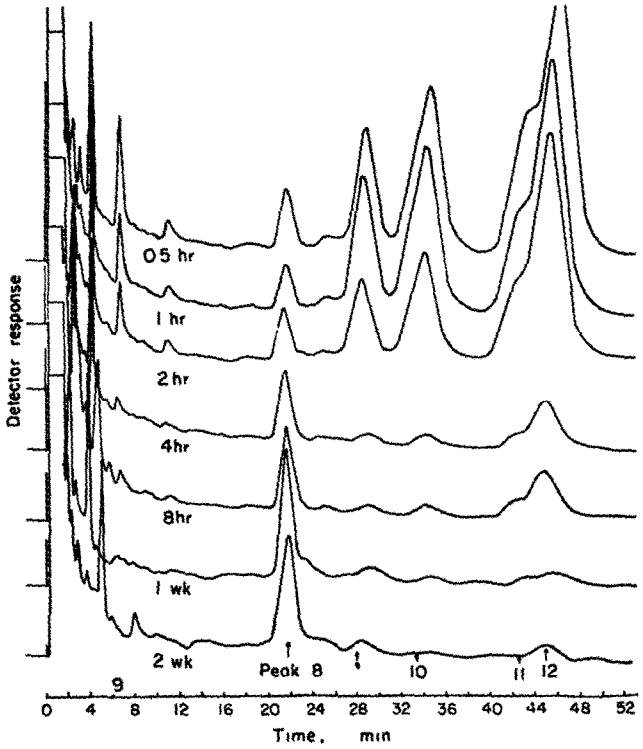

FIG. 6. GAS–LIQUID CHROMATOGRAPHY OF "METHYL STEROL FRACTIONS" (TABLE 4, FIG. 3). EACH CURVE REPRESENTS A FIXED AMOUNT OF MATERIAL FROM *T. officinale* FLOWERING STEMS THAT HAD BEEN INCUBATED WITH MEVALONIC ACID-2-^{14}C FOR THE TIME INTERVAL INDICATED. THE SEPARATIONS WERE ACCOMPLISHED ON A 6 FT GLASS COLUMN (I.D. = 4 mm) CONTAINING 1% SILICONE GE XE-60 ON GAS CHROM Q, 100/120 MESH. OTHER DETAILS ARE GIVEN IN THE EXPERIMENTAL SECTION.

The Methyl Sterols

Fractions 42–50 (Fig. 3) should contain the methyl sterols (see Table 5). The ^{14}C-content and other pertinent data for these fractions at the time intervals selected are shown in Table 3. When these fractions were subjected to gas chromatography, the curves shown in Fig. 6 were obtained. Peaks 8 and 9 may be identical, respectively, to Peaks 1 and 4 (β-amyrin) of Figs. 4 and 5 since their mass shows the same relationship over the time periods studied; some overlapping in sampling may have occurred in the chromatography on alumina (Fig. 3). Peaks 10, 11 and 12 all decrease in mass over the 2-week period, and are therefore *not* identical, despite similar retention times, to Peaks 5, 6 and 7, respectively, in Figs. 4 and 5. The interesting observations of Benveniste *et al.*[4] have suggested the probable sequence

shown in Fig. 7 for the biosynthesis of plant sterols, in which a cycloartenol type of triterpene rather than lanosterol is a critical precursor. This sequence also implies a two-step biosynthesis of the origin of the ethyl group at C-24 in the C_{29} sterols. It will be noted that there is no provision in this sequence for the cactus methyl sterol lophenol of Djerassi *et al.*[6]

TABLE 3. INCORPORATION OF MEVALONIC ACID-2-^{14}C INTO CRUDE 4α-METHYL STEROL FRACTIONS FROM CUT STEMS OF FLOWERING *T. officinale*

Incubation period	Yield (mg)	Total ^{14}C incorporation, c.p.m. × 10^5	Percentage ^{14}C incorporation*
0·5 hr	21·8	2·50	3·2
1·0 hr	19·6	3·48	4·5
2·0 hr	17·1	3·01	3·9
4·0 hr	7·5	3·86	5·0
8·0 hr	21·7	4·70	0·6
1 week	6·5	2·70	0·3
2 weeks	15·9	4·50	0·5

* Based on 10 μc of administered DL-mevalonic acid-2-^{14}C, and assuming only one isomer was utilized in the biosynthesis.

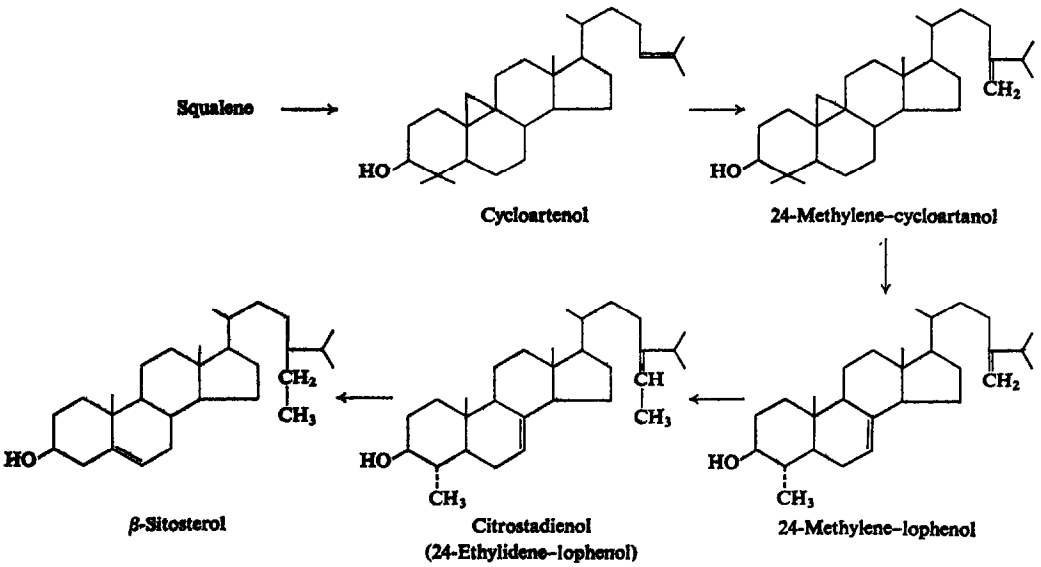

FIG. 7. SUGGESTED SEQUENCE FOR THE BIOSYNTHESIS OF β-SITOSTEROL FROM SQUALENE.

Despite repeated attempts, none of the authentic reference samples available to us, with the possible exception of lophenol, had retention times identical with the peaks shown in Fig. 6. This is indicated more graphically in Fig. 8, where arrows have been inserted showing the retention times experimentally determined. The presence of citrostadienol could not be ascertained because both control samples received were probably contaminated.

[6] C. DJERASSI, G. W. KRAKOWER, A. J. LEMIN, L. H. LIU, J. S. MILLS and R. VILLOTTI, *J. Am. Chem. Soc.* **80**, 6284 (1958).

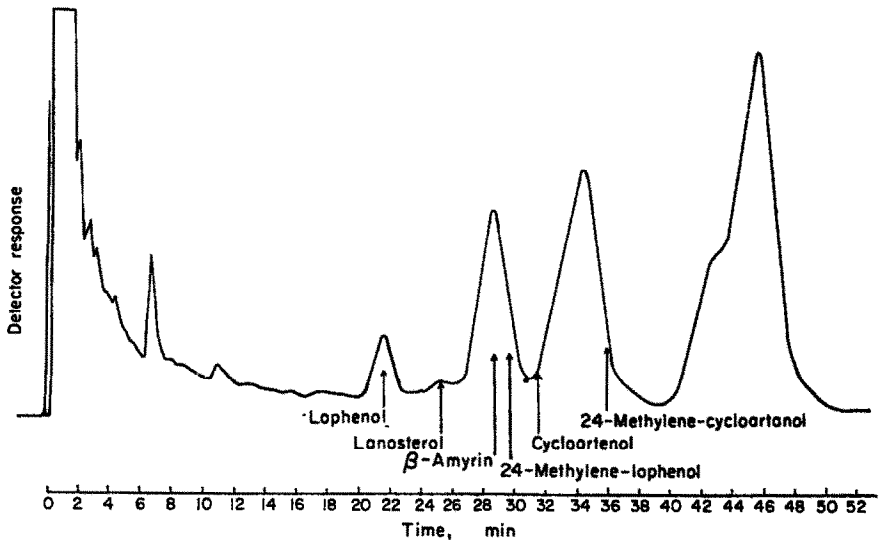

FIG. 8. GAS-LIQUID CHROMATOGRAPHY OF "METHYL STEROL FRACTION", 0·5 HR INTERVAL (TABLE 4, FIG. 6) WITH RETENTION TIMES OF REFERENCE SAMPLES INDICATED.

β-Sitosterol

In agreement with previous observations[5] mevalonic acid-2-^{14}C was slowly incorporated into β-sitosterol, which increased in total counts and specific activity (Fig. 3 and Table 4) over the entire period studied. It would appear that in contrast to β-amyrin, the sterol molecule remains fairly stable in the plant. The time lag in formation of the compound is also consistent with the likelihood that the ethyl group at C-24 is being formed from some presently unknown precursor.

TABLE 4. INCORPORATION OF MEVALONIC ACID-2-^{14}C INTO β-SITOSTEROL-^{14}C FROM CUT FLOWERING STEMS OF *T. officinale*

Time interval following administration of MVA-2-^{14}C	β-Sitosterol-^{14}C*		Total ^{14}C incorporation, c.p.m. × 10
	Weight in mg	Specific activity†	
0·5 hr	19·2	0	0
1·0 hr	20·1	0	0
2·0	20·1	492	0·01
4·0 hr	22·7	2,338	0·047
8·0 hr	18·7	48,600	0·87
1 week	20·6	48,000	0·99
2 weeks	20·2	50,000	1·01

* After chromatography on silicic acid as the *p*-phenylazobenzoyl ester and saponification to the free compound.

† Expressed as c.p.m. per mg.

Efforts are currently directed towards identifying the unknown components of Figs. 4, 5 and 6.

CONCLUSIONS

In agreement with Benveniste *et al.*[4] it must be concluded that lanosterol is not an obligatory intermediate in the biosynthesis of β-sitosterol in the present experiments. A probable trace of lanosterol or a compound closely allied to it was indicated in *T. officinale*, following administration of mevalonic acid-2-^{14}C, but the compound contained at best only a trace of radioactivity at any time interval studied. A time interval of 0·5 hr was the shortest capable of being studied in the system investigated; the absorption of ^{14}C within 5 min, the earliest period investigated by Ourisson and co-workers in tobacco tissue culture[4] would be too limiting for study. It seems unlikely from the data presented that radioactive lanosterol would appear in higher specific activity if it were possible to study shorter incubation periods.

Radioactive β-sitosterol in *T. officinale* was relatively stable when studied over an interval of 2 weeks. In contrast, the radioactive content of β-amyrin in the same plant rose and declined, suggesting turnover. The significance of this observation is not apparent at the moment.

There was an indication of transient products, possibly 4α-methyl sterols, present in *T. officinale* cut stems incubating in aqueous solution. In the present experiments it was not possible to positively identify them with any of the known naturally occurring or synthetic methylated sterols available for experimentation. Further attempts to identify them are in progress.

EXPERIMENTAL

Solvents and Materials

All solvents were A.R. grade, distilled before use. The alumina used was Merck acid-washed.

Radioactive Determinations

Aliquots of fractions in toluene were counted in a liquid scintillation spectrometer having an efficiency of 70%. PPO (2·0 mg) and 0·15 mg of POPOP* per 15 ml of toluene were added before counting.

Other Analyses

Optical rotations were determined in chloroform by Drs. G. Weiler and F. B. Strauss, Microanalytical Laboratory, Oxford, England. Melting points were determined on a Fisher-Johns hot plate.

Thin-layer Chromatography

Silica Gel G (Merck, Germany) plates 0·305 mm thick were utilized, with the solvent system ethyl acetate–benzene (1:3) (ascending) to examine the non-radioactive β-amyrin preparations. The chromatography was performed at room temperature.

Administration of Mevalonic Acid-2-^{14}C

Flowering dandelion stalks obtained locally were cut with a razor near the base of the stalk, then immediately immersed in H_2O. If this is not done, the latex soon makes a seal at the cut surface, preventing the uptake of solutions into the stem. In the laboratory the base

* PPO = 2,5-diphenyloxazole; POPOP = 1,4-bis-2-(5-phenyloxazolyl)-benzene.

of each stalk was blotted for several minutes on absorbent paper. If this is not done, the stems exude H_2O for several minutes rather than absorb it, preventing the rapid uptake of radioactive solutions for short-term studies. Batches of exactly 21·4–21·5 g of stems so treated were placed in a small vessel containing 10 μc of the sodium salt of DL-mevalonic acid-2-^{14}C prepared and administered otherwise as previously described.[5] Examination of the vessels at the conclusion of the absorption period showed that 80% of the counts administered had entered the plant during the 0·5 hr intervals, and 97% at the longer intervals. There was some loss of turgor in the flower stalks maintained in H_2O over the 2-week period.

Preparation of Non-saponifiable Fractions

After the desired absorption period the stems were refluxed for 1 hr in 500 ml of 15% KOH in 50% ethanol. The cooled mixture was filtered through glass wool and the pulp and glass wool were rinsed with the first light petroleum wash. The alkaline fraction was extracted five times with 100 ml of light petroleum (b.p. 30–60°). The combined extracts were washed with H_2O and distilled to dryness, the last traces of solvent being removed under N_2. The residues, weighing from 40 to 50 mg, were chromatographed on alumina.

Chromatography on Alumina

Merck acid-washed alumina (60·0 g) was slurried in light petroleum into a 10 mm diameter column fitted with a coarse fritted glass disc. To enhance uniformity of the chromatography the same glass column was used throughout. The non-saponifiable fractions dissolved in 40 ml of light petroleum were placed on the column and the eluate collected as Fraction 1. Nine fractions of 20 ml of light petroleum were subsequently collected, followed by ninety fractions each of 20 ml benzene, making a total of 100 fractions. The radioactive content of non-saponifiable fractions chromatographed in this manner is shown in Fig. 3.

Chromatography on Alumina of Authentic Reference Samples

Reference samples were very kindly provided by the following individuals: β-amyrin and lophenol—Dr. Carl Djerassi; 24-methylene-lophenol and 24-ethylidene-lophenol—Dr. K. Schreiber; lanosterol-^{14}C—D. J. L. Gaylord; cycloartenol and 24-methylenecycloartanyl acetate—Professor G. Ourisson; campesterol—Dr. A. Kuksis; β-sitosterol was obtained from dried peas[7] and from flowering dandelion stems. On gas chromatography a small percentage of campesterol was indicated but this is not believed to interfere with the interpretation of radioactive data obtained.

A sample of citrostadienol acetate was received from Dr. Mazur with the knowledge that it was at least 6 yr old. It is believed that considerable decomposition had occurred since recovery of the free compound (Table 5) was not consistent with that of the other 4α-methyl sterols. Pure lanosterol was obtained from 3β-acetoxy-24,25-dibromo-Δ^8-lanostene by the method of Bloch and Urech.[8] Squalene was obtained from a commercial source.

When each of these authentic compounds in 6–20 mg quantities was chromatographed on alumina as described, they were recovered in the fractions indicated in Table 5.

[7] D. J. BAISTED, E. CAPSTACK and W. R. NES, *Biochemistry* **1**, 537 (1962).
[8] K. BLOCH and J. URECH, *Biochemical Preparations* (Edited by C. S. VESTLING), Vol. 6, p. 32. John Wiley, New York (1958).

TABLE 5. CHROMATOGRAPHY OF REFERENCE TRITERPENES, STEROLS AND 4α-METHYL STEROLS ON ALUMINA

Compound	Fraction in which recovered*
Squalene	4–8
β-Amyrin and lanosterol	32–40
24-Methylene-lophenol, synthetic (4α-methyl-5α-ergosta-7,24(28)-diene-3β-ol)	42–50
24-Ethylidene-lophenol, from potato leaves (4α-methyl-5α-stigmasta-7,24(28)-diene-3β-ol)	42–50
Lophenol, from a cactus species (4α-methyl-5α-cholesta-7-ene-3β-ol)	42–46
Citrostadienol, from grapefruit peels (4α-methyl-5α-stigmasta-7,24(28)-diene-3β-ol)†	80–83
Cycloartenol	47–55
24-Methylene-cycloartanol	52–59
β-Sitosterol	66–80
Campesterol	77–96

* Chromatography was performed on a glass column containing 60·0 g of Merck acid-washed alumina. Fractions 1–10 consisted of light petroleum, Fractions 11–100, of benzene. See Experimental Section for details.

† According to Schreiber[9], citrostadienol and 24-ethylidene-lophenol differ only in configuration at C-24.

Gas Chromatography

Gas chromatography was performed on a Barber–Colman Model 5000 Gas Chromatograph equipped with a radioactive monitoring system. The radioactive monitor was a Barber–Colman Model 5190.

Isothermal operation was used exclusively in gas chromatography. The separations were made using a 6 ft glass column with an I.D. of 4 mm. The column contained a packing of 1% silicone GE XE-60 on Gas Chrom Q, 100/120 mesh (Applied Science Laboratories, Inc.).

The samples were chromatographed at a column temperature of 231°, detector 235°, flash heater 295°; the carrier gas was argon, with a flow rate of 56 ml/min, inlet pressure 24 psi. Samples were injected in dry benzene solution using a Hamilton microliter syringe equipped with Chaney adapter.

The Barber–Colman radioactive monitoring system uses a proportional counter detection system. The system was equipped with a stream splitter which allowed one part of the effluent to flow to the detector and ten parts to flow to the counter. Before entering the counter the carrier gas (Argon) was diluted with a low concentration (10%) of the quench gas (Propane). The mass of the sample and its radioactivity were simultaneously recorded on separate recorders.

Isolation of β-Amyrin and β-Sitosterol from T. officinale

One kilogram of dried dandelion flowers and stalks were cut into small pieces and extracted exhaustively with hot ethanol. The extract was distilled to dryness *in vacuo* and the residue was saponified with 10% alcoholic KOH by several hours' heating on a steam bath.

[9] K. SCHREIBER and G. OSSKE, *Experientia* **19**, 69 (1963).

After dilution with H_2O the mixture was extracted thoroughly with ethyl ether. The ether was washed with water, dried over anhydrous Na_2SO_4, then distilled, leaving a non-saponifiable residue of 22 g of orange solid. This was boiled briefly in 600-ml portions of light petroleum with each portion filtered through coarse filter paper. The filtrate was poured onto a column containing 200 g of alumina, which was subsequently washed with an additional 200 ml of light petroleum. The combined petroleum eluates were discarded. The column was now washed with 500 ml of 1:1 ethyl ether–acetone. The eluate was freed of solvent and the residue was combined with the insoluble material originally filtered from the light petroleum solution, giving 16·0 g of orange wax. This was dissolved in 80 ml of warm benzene and poured onto a 3·5 cm diameter column containing 300 g of alumina. An additional 100 ml of benzene was passed through, the first total eluate being collected as Fraction 1. Thirty-six additional 100 ml fractions of benzene were collected, followed by a 200 ml fraction of ethyl ether and a 400 ml fraction of 1:1 benzene–ethanol respectively. Evaporation of solvent from Fractions 1–12 yielded material varying from low-melting orange gum to yellow–orange, semi-crystalline solid. Fractions 13–37 all yielded white solid. The ethyl ether and ethanol–benzene fractions yielded yellowish-brown, gummy solid. A small amount of each fraction subjected to the Liebermann–Burchard test indicated that sterol was concentrated in Fractions 15–30.

Isolation of β-Amyrin

Fractions 6–11 were combined (total weight 2·2 g) and acetylated with acetic anydride and anhydrous pyridine under reflux. The cooled mixture was processed in the usual manner, yielding whitish solid. A portion of this (71 mg) was dissolved in 10 ml of 1:1 light petroleum–benzene and poured onto a 1·2 cm dia. column containing 5 g of silicic acid (Mallinckrodt) prepared as a slurry in light petroleum. The first eluate was collected as Fraction 1. Two additional fractions of 10 ml of light petroleum were collected, followed by seven additional fractions (10 ml each) of 5% ethyl ether in light petroleum (v/v). The bulk of the material (69 mg) was recovered in Fraction 6. This was crystallized to constant m.p. from benzene–ethanol, giving scales, m.p. 238–239°; $[\alpha]_D^{21°}$ +90·7.

Saponification of the acetate and crystallization to constant melting point from aqueous ethanol gave β-amyrin, m.p. 201–202°; $[\alpha]_D^{21°}$ +86·0°. On thin-layer chromatography (room temperature) in the solvent system ethyl acetate–benzene (1:3), 20 μg did not separate from an equal quantity of β-amyrin, R_f 0·76, and exhibited only a single spot when sprayed with 20% $SbCl_5$ in $CHCl_3$ (purple color). In the same solvent system taraxerol travels with the solvent front.*

Isolation of β-Sitosterol

Fractions 16–25 were combined (total weight 5·3 g) and acetylated with acetic anhydride and anhydrous pyridine under reflux. Work-up and crystallization of the product from acetone–methanol gave β-sitosteryl acetate, m.p. 127–128°; $[\alpha]_D^{21°}$ −37·2°. Free β-sitosterol (m.p. 140–141°; $[\alpha]_D^{21°}$ −36·4°) and from this β-sitosteryl benzoate (m.p. 146–148°; $[\alpha]_D^{21°}$ −12·4°) were obtained in the usual manner.

* This work was performed before a gas chromatograph was available; thin-layer chromatography may not be capable of separating the triterpenes indicated as being present in *T. officinale* roots by Burrow and Simpson.[1] However, none was detected in the mother liquors from the described preparation of β-amyrin and β-sitosterol by standard crystallization procedures.

Examination of β-Sitosterol-^{14}C Fractions

Fractions 60–80 (Fig. 3) from each respective time period were combined, converted to the *p*-phenylazobenzoyl esters and chromatographed on silicic acid as previously described.[5] Fractions representing β-sitosteryl *p*-phenylazobenzoyl ester were saponified to the free compound, crystallized to constant radioactivity and counted for ^{14}C-activity. The critical data are given in Table 4.

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