Sterol content of the Myxomycetes *Physarum polycephalum* and *P. flavicomum*

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Abstract The sterol content of two Myxomycetes, *Physarum polycephalum* and *P. flavicomum* has been examined. The sterols of the two species are apparently identical, the two major sterols in each being poriferasterol and 22-dihydroporiferasterol. Three minor sterols are probably Δ^5-ergostenol, ergostanol, and poriferastanol. The triterpenoids of the two species differ in that, though lanosterol was identified in both, 22-dihydrolanosterol was indicated only in *P. flavicomum*. The occurrence of lanosterol together with a typical mixture of plant sterols is somewhat unusual.

Supplementary key words slime molds · poriferasterol · 22-dihydroporiferasterol · Δ^5-ergostenol · lanosterol · labeled methionine · sterol biosynthesis

The Myxomycetes or acellular slime molds are unusual organisms that cannot be readily classified as plants or animals. In the vegetative phase the species examined here form a free-living, multinucleate, acellular, mobile mass of protoplasm called the plasmodium. Apart from the question of classification, studies on the chemical composition of the plasmodium are significant because nuclear division in the acellular mass is highly synchronous and the organisms are widely used in research on the cell cycle (for a comprehensive view of the Myxomycetes see (1)).

Sterols have been isolated from the plasmodium of the Myxomycete *Physarum polycephalum* by several authors (2–4). One of the more recent reports (5), in addition to claiming identification of a typical phytosterol mixture of campestanol, campesterol, stigmastanol, stigmasterol, and sitosterol, together with smaller amounts of cholesterol and brassicasterol, also noted the presence of lanosterol, 24-methylenedihydrolanosterol, and possibly 4α-ethylcholestenol in the triterpenoids of the lipid fraction (3).

The occurrence of plant sterols, which contrasts with the presence of typically protozoal unsaturated fatty acids in *P. polycephalum* (6), emphasizes the problems involved in the classification of the Myxomycetes. In addition the occurrence of lanosterol, the usual first cyclic intermediate in the biosynthesis of animal sterols, rather than cycloartenol, the normal first cyclic intermediate in the biosynthesis of plant sterols, is of interest.

In this report we have reexamined the sterol and triterpenoid content of *P. polycephalum* and also report findings for a second Myxomycete, *P. flavicomum*.

MATERIALS

Cultures of *P. polycephalum* and *P. flavicomum* were kindly donated by Dr. C. J. Alexopoulos of the Department of Botany, University of Texas at Austin.

Samples of unlabeled amino acids were purchased from Nutritional Biochemicals Inc. (Cleveland, Ohio), and the [methyl-$^2H_3$]iodide was from Mercke, Sharp and Dohme (Montreal, Quebec).

Stigmasterol (Nutritional Biochemicals Inc.) was purified by several recrystallizations from ether–ethanol. "β-Sitosterol" (Nutritional Biochemicals Inc.) was separated by GLC to give pure samples of campesterol and sitosterol; similarly "lanosterol" gave pure samples of lanosterol and 24-dihydrolanosterol. A sample of poriferasterol from Dr. L. J. Goad, University of Liverpool, is also acknowledged.

Abbreviations: TLC, thin-layer chromatography; GLC, gas–liquid chromatography; NMR, nuclear magnetic resonance. Trivial names are used for the sterols and triterpenoids in accordance with the following: campestanol, (24R)-24-methyl-5α-cholestan-3β-ol; campesterol, (24R)-24-methylcholest-5-en-3β-ol; stigmastanol, (24R)-24-ethyl-5α-cholestan-3β-ol; sitosterol, (24R)-ethylcholest-5-en-3β-ol; stigmasterol, (24S)-24-ethylcholesta-5,22-dien-3β-ol; ergostanol, (24S)-24-methyl-5α-cholestan-3β-ol; Δ^5-ergostanol, (24S)-24-methylcholest-5-en-3β-ol; poriferastanol, (24S)-24-ethyl-5α-cholestan-3β-ol; 22-dihydroporiferasterol, (24S)-24-ethylcholest-5-en-3β-ol; poriferasterol, (24R)-24-ethylcholesta-5,22-dien-3β-ol; brassicasterol, (24R)-24-methylcholesta-5,22-dien-3β-ol; ergosterol, (24S)-24-methylcholesta-5,7,22-trien-3β-ol; cycloartenol, 9,19-cyclo-9β-lanost-24-en-3β-ol.

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METHODS

Optical rotations were determined at the sodium D line on a Perkin-Elmer 141 polarimeter (Perkin-Elmer Corp., Norwalk, Conn.). Infrared absorptions were recorded in carbon tetrachloride solution on a Perkin-Elmer 237B spectrophotometer. A Hitachi Perkin-Elmer RMU-6E mass spectrometer was used to record the mass spectra. Samples were introduced as solids with an inlet temperature of 200°C. Spectra were recorded with an ionization voltage of 70eV, which was reduced to 20eV to determine the identity of molecular ions. Proton magnetic resonance spectra were determined in $CDCl_3$ on a Varian HA 100 spectrometer (Varian Assoc., Palo Alto, Cal.) with tetramethylsilane as an internal reference. Melting points, recorded on a Fisher-Johns block, are uncorrected.

Gas–liquid chromatography (GLC)

GLC separation and analysis of the sterols and triterpenoids were effected on either a Perkin-Elmer PE 881 gas chromatograph fitted with a flame ionization detector and a 4.5 × 6.6 mm OD glass column with helium as the carrier gas at a flow rate of 40 ml/min, or on a Varian Aerograph 1520 with a thermal conductivity detector and a 3.75 m stainless steel column (OD 6.6 mm) again using helium as the carrier gas at a flow rate of 65 ml/min. Both columns were operated with a column temperature of 270°C and injector and detector temperatures of 300°C.

The columns were packed with acid-washed, silanized Chromosorb W (100–120 mesh) that was coated with one of the following liquid phases: 3% OV 17, 4% OV 101, 4% OV 25, or 3% SE 30. The OV 101 column was used for all preparative separations; the other three columns were used solely for analytical work. The lipids were collected from the gas chromatograph in narrow U-tubes cooled in dry ice.

Similar retention times on at least three different columns and an increase in peak height on co-injection were used as criteria in establishing that two lipids were either identical or closely related structurally.

Quantitative determinations were made by measuring peak areas.

Culture of *P. polycephalum* and *P. flavicomum*

When received, a portion of each culture was transferred onto agar plates that had been prepared using a partially defined medium (7). The plasmodium was allowed to grow in the dark at 25°C for several days, when the tip of the migrating plasmodium was excised and transferred to a second plate. This procedure coupled with the use of antibiotics (penicillin G-procaine and streptomycin sulfate) was repeated until the culture was free from bacterial contamination.

Shake flask culture

Pieces of each Myxomycete plasmodium were separately transferred into a partially defined liquid medium (7). In a typical run six 3-l Erlenmeyer flasks, each containing 600 ml of medium, were inoculated by plasmodium from an agar plate or from a previous shake flask culture. The flasks were shaken in the dark at 25°C for 4 days before the plasmodium was harvested by centrifugation after the plasmodium had partially settled.

Culture conditions in the presence of added methionine or [methyl-$^2H_3$]methionine

The Myxomycetes were cultured in 250-ml flasks, each containing 50 ml of the usual liquid medium, to which was added 40 mg of additional methionine or [methyl-$^2H_3$]methionine. In each case growth was normal and the plasmodium was harvested after 4 days.

Culture conditions for growth in a defined amino acid medium

The normal protein source in the medium (casein hydrolysate) was replaced by the following mixture: DL-methionine, 250 mg/l; glycine, 450 mg/l; L-arginine hydrochloride, 600 mg/l; DL-alanine, 1.2 g/l; and ammonium sulfate, 250 mg/l. In other experiments [methyl-$^2H_3$]methionine replaced the unlabeled methionine. Growth on this medium was slow, particularly in the case of *P. polycephalum*, and up to 2 weeks was required to obtain a satisfactory quantity of plasmodium.

Extraction of the triterpenoids and sterols from the Myxomycete plasmodia

The plasmodium was homogenized with three times its volume of acetone in a Waring Blendor for 30 sec and the aqueous extract was filtered under suction. The residue was reextracted with acetone, and the combined extracts were concentrated at 35°C until the smell of acetone could no longer be detected. The resulting solution was extracted four times with an equal volume of petroleum ether (bp 35–60°C); the petroleum ether layers were combined, concentrated, and dried over anhydrous sodium sulfate. The solution was evaporated to dryness under reduced pressure. The residue was dissolved in warm ethanol and an equal volume of

aqueous NaOH (20 g in 100 ml H_2O) was added. After standing overnight, the solution was diluted with water, and the unsaponifiable lipid was extracted into petroleum ether; this latter solution was evaporated to leave a slightly yellow semicrystalline solid.

In later experiments the plasmodium was freeze-dried, crushed, and extracted in a Soxhlet apparatus with dry ether. This method did not extract any pigment from the plasmodium, and it became the preferred method.

Column chromatography of the unsaponifiable lipid component

In a typical separation the lipid (203 mg) was chromatographed on a 15 × 2 cm column of neutral alumina (Woelm, activity 3) first with benzene (150 ml) and then with 5% ether in benzene (200 ml). The benzene eluate yielded mainly triterpenoids and the benzene–ether solution the sterols. Each fraction was rechromatographed to complete the separation.

Separation of the sterol components

The mixture of sterols was first separated by TLC on silica gel G layers impregnated with silver nitrate (30% w/w) using chloroform as the developing solvent. Visualization of the sterols was achieved by placing a template over the plate and spraying two narrow bands with 50% sulfuric acid. After heating at 110°C for 15 min a deep blue color showed the presence of two separate bands containing the saturated (R_f 0.34) and the unsaturated sterols (R_f 0.25). (For typical yields see Table 3.)

Separation of the mixture of unsaturated sterols was achieved either by GLC on the OV 101 column or, alternatively, by effecting partial separation with additional TLC. Thus poriferasterol was separated from the other unsaturated sterol components using the thin-layer system previously described. The rear third of the broad unsaturated sterol band was scraped from the plate. Elution and GLC of the sterols from this sample showed significant enrichment of poriferasterol. Repetition of this procedure gave a sample that was virtually homogeneous (as detected on a GLC analytical column).

Each sterol, whether separated by GLC or TLC, was crystallized from ether–ethanol until only a single component could be detected by GLC.

Acetylation of the sterols

Acetylation of the mixtures of sterols, and of individual purified sterols, was performed using the same procedure. In a typical experiment the sterol (50 mg) was dissolved in anhydrous pyridine (5 ml) and acetic anhydride (1.10 ml) was added. The mixture was allowed to stand at room temperature overnight; deionized water (10 ml) was added and the sterol acetates were extracted into ether. This solution was evaporated to dryness, and the residue was chromatographed on a column of neutral alumina (activity 3; 200 mg); the sterol acetates were eluted by petroleum ether. Crystallization of the acetates from ether–ethanol yielded white plates (ca. 40 mg).

Ozonolysis of the sterols

The mixture of sterols from *P. flavicomum* and an authentic sample of stigmasterol were treated in the same way; only the former is described here.

The mixture of sterols (150 mg) was dissolved in a warm mixture of methyl acetate (10 ml) and methanol (10 ml) which was then cooled in an ice bath, when small white crystals formed. The crystals dissolved as a stream of ozone, generated by a Welsbach ozonizer, was slowly bubbled through the cool solution. The state of completion of the reaction was determined by TLC on silica gel G plates using 10% acetone in petroleum ether as the developing solvent. In this system the R_f of the unchanged sterol was 0.60 while that of the product was 0.10.

Reduction of the ozonide

On completion of the ozonolysis the excess ozone was blown off and 5% palladium on charcoal (20 mg) was added to the solution. The mixture was hydrogenated at room temperature and atmospheric pressure until no more gas was taken up. The catalyst was filtered off, the product was extracted into ether, and the solution was dried over molecular sieves.

Preparation of 2-ethyl-3-methylbutanal-2,4-dinitrophenyl-hydrazone

The dried ethereal solution above was fractionally distilled, 2-ethyl-3-methylbutanal distilling at about 135°C. This product was immediately added to a warmed solution of 2,4-dinitrophenylhydrazine in ethanol–phosphoric acid (8) and after 15 min the yellow crystals were filtered off and chromatographed on neutral alumina thin layers using benzene as the eluting solvent. In this system the R_f of the product was 1.0, which separated it from unchanged dinitrophenylhydrazine (R_f 0.2). The product crystallized from 95% ethanol as orange needles (20 mg); mp 119–119.5°C; $[\alpha]_D^{23}$ + 10.5° (benzene; 2 mg/ml).

A similar product was prepared from an authentic sample of stigmasterol; mp 120°C; $[\alpha]_D^{25}$ −6.3° (benzene; 2 mg/ml).

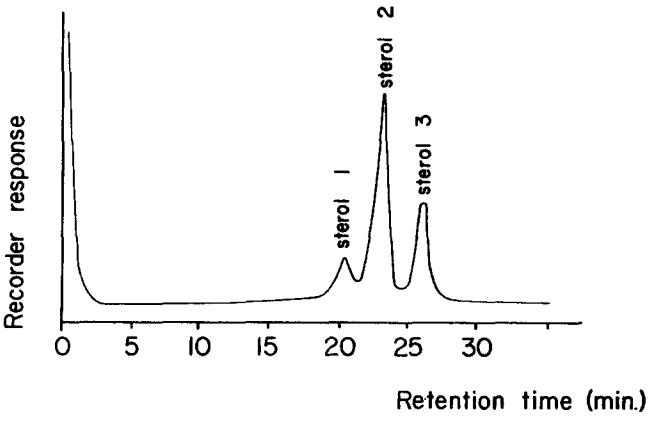

Fig. 1. The GLC profile of the mixture of unsaturated sterols extracted from *Physarum flavicomum*. GLC on 4% OV 101 supported on acid-washed, silanized Chromosorb W: 4.5 × 7 mm OD glass column; helium carrier gas at 40 cm^3/min; column temperature 270°C. Sterol 1, a 24-methylcholest-5-en-3β-ol (probably Δ^5-ergostenol); sterol 2, poriferasterol; sterol 3, 22-dihydroporiferasterol.

Preparation of methionine and [methyl-$^2H_3$]-methionine

DL-Methionine (mp 240–245°C (decomp.)) was prepared from DL-homocystine essentially by the method of du Vigneaud, Dyer, and Harmon (9); similarly [methyl-$^2H_3$]methionine (mp 245–260°C (decomp.)) was prepared by substituting [$^2H_3$]methyl iodide for methyl iodide in this preparation. Comparison of mass spectra with published data (10) confirmed the identity of both compounds.

RESULTS

The mass spectrum of the isolated mixture of sterols from both *P. polycephalum* and *P. flavicomum* showed molecular ions at *m/e* 400, 402, 412, 414, and 416 suggesting the presence of a mixture of phytosterols. However no prominent ion was observed at either *m/e* 386 or 398 indicating that cholesterol and brassicasterol, which were identified by Lenfant, Lecompte, and Garrugia (5) in their strain of *P. polycephalum*, were not present in either organism.

TLC on silver nitrate-impregnated layers readily separated the saturated sterols from the unsaturated sterols and GLC and mass spectrometry indicated that, except for the relative amounts of the different components, these fractions were apparently identical in the two species. For this reason the sterols of the two species are discussed together and note is made wherever any difference in procedure was used or a difference in properties was noted.

Saturated sterols in *P. polycephalum* and *P. flavicomum*

The mass spectrum of the mixture of saturated sterols (stanols) showed only two molecular ions at *m/e* 402 and 416 with obvious fragmentation ions at *m/e* 401 (416 − CH_3), 398 (416 − H_2O), 387 (402 − CH_3) and 384 (402 − H_2O). After acetylation the mixture gave molecular ions at *m/e* 444 and 458.

GLC analysis revealed the presence of two components, the first had chromatographic properties identical with campestanol and the second with stigmastanol. No attempt was made to separate larger amounts of the individual stanols.

Unsaturated sterols in *P. polycephalum* and *P. flavicomum*

Mass spectrometry showed molecular ions at *m/e* 400, 412, and 414 while the acetylated mixture gave no molecular ions but, instead, abundant ions at *m/e* 382, 394, and 396, (corresponding to the loss of acetic acid from the three expected molecular ions) dominated the spectra.

GLC of the unsaturated sterol mixture showed three components (**Fig. 1**: sterols 1, 2, and 3 in order of elution) that were identical with campesterol, stigmasterol, and sitosterol, respectively, in their GLC properties.

This mixture of sterols was separated into its three components by GLC and, in addition, sterol 2 was separated from sterols 1 and 3 by TLC on silver nitrate-impregnated silica gel plates.

As the separation from sterol 2 was rather unsatisfactory, sterol 1 was never obtained in sufficient amounts for complete characterization. However, on several columns sterol 1 had a retention time identical with that of campesterol and its mass spectrum showed a single molecular ion at *m/e* 400, with fragmentation ions similar to those reported for campesterol (11).

Although these results show the expected similarity between sterol 1 and campesterol, neither the GLC nor the mass spectral data can distinguish between

TABLE 1. Relative amounts of the three unsaturated sterols in the mixture of unsaponifiable lipids extracted from the Myxomycetes *Physerum flavicomum* and *P. polycephalum*

Sterol	*P. flavicomum*	*P. polycephalum*
	% of total unsaturated sterols[a]	
Sterol 1 (a 24-methylcholest-5-en-3β-ol)	10	15
Sterol 2 (poriferasterol)	57	39
Sterol 3 (22-dihydroporiferasterol)	33	45

[a] Average values from three determinations.

TABLE 2. Melting points of the diunsaturated sterol (sterol 2) isolated from *Physarum flavicomum* and *P. polycephalum*, and of some derivatives

Sterol or Derivative	Sterol 2 (Poriferasterol), ex. *P. flavicomum*	Sterol 2 (Poriferasterol), ex. *P. polycephalum*	Literature Values[a]	
			Porifer-asterol	Stigmas-terol
		°C		
Sterol	156	154–155	156	170
3β-Acetoxy sterol	147	146.5–147	146.5–147	144–144.6
Tetrahydro-3β-acetoxy sterol	139–140	139–140	140–141	130–131

[a] Reference 20.

the C-24 epimers of 20-methylcholest-5-en-3β-ol (campesterol and Δ^5-ergostenol) and thus complete characterization of sterol 1 was not possible using these methods.

Sterol 2, the most abundant sterol in *P. flavicomum* (**Table 1**), gave a molecular ion at *m/e* 412 and fragmentation ions at *m/e* 397 ($M - CH_3$) and 394 ($M - H_2O$) together with numerous other ions observed in the mass spectrum of stigmasterol (11), e.g., an obvious ion at *m/e* 300, which is characteristic of Δ^{22}-sterols (12). The mass spectrum of the acetate was extremely similar to that recorded for stigmasteryl acetate and showed no molecular ion, though an abundant ion at *m/e* 394 was most likely formed by the loss of acetic acid from the expected molecular ion (11).

GLC analysis on several columns revealed the expected similarity with stigmasterol; similarly the acetate closely resembled stigmasteryl acetate. However, the melting point of the sterol and of some derivatives (**Table 2**) suggested that the sterol was not stigmasterol but rather the 24-ethyl epimer, poriferasterol.

Further confirmation of this assignment was made by comparison of the 100 MHz NMR spectrum of sterol 2 from *P. polycephalum* with the spectra of authentic poriferasterol and stigmasterol. Thus, poriferasterol and sterol 2 showed a broad singlet at 83 Hz (to low field of tetramethylsilane) while stigmasterol has a clearly resolved doublet resonance at the corresponding position (cf. 13).

Sterol 3 and its acetate had mass spectra identical to those of sitosterol and its acetate (11) and GLC properties virtually identical with those of sitosterol and its acetate. Again, however, the melting points of the sterol (139.5–140°C) and of its acetate (140–141°C) showed that the compound was not sitosterol but the 24-ethyl epimer, 22-dihydroporiferasterol.

Further confirmation of the stereochemical assignment of the 24-ethyl side chain in poriferasterol was achieved by ozonolysis of the mixture of sterols isolated from *P. flavicomum*. The ozonide formed from this mixture was worked up reductively, and the 2-ethyl-3-methylbutanal, produced from the sterol side chain by fission of the Δ^{22}-bond of the diunsaturated component (sterol 2) of the mixture, was converted into its 2,4-dinitrophenylhydrazone. This compound has a rotation opposite to that of a similar product prepared from stigmasterol, confirming that the major sterol in the mixture was poriferasterol (4).

Triterpenoid components

The GLC profile on an OV 101 column of the small triterpenoid fraction from *P. flavicomum* (4 mg from 125 g. wet) showed two major and several minor components. The major peaks corresponded with those of lanosterol and 24-dihydrolanosterol, while comparison of retention times and coinjection with authentic cycloartenol indicated that this substance was absent from the slime mold triterpenoid fraction. GLC of the mixed acetates and comparison with authentic specimens confirmed the presence of lanosterol and 24-dihydrolanosterol in approximately equal amounts. Further, mass spectrometry of the mixed triterpenoids supported the assignments, with molecular ions at *m/e* 426 and 428. The relative intensities of fragment ions at *m/e* 413, 411, 395, 393 were consistent in abundance with those expected from a mixture of lanosterol and 24-dihydrolanosterol. Similar experiments with the

TABLE 3. Typical yields of the various lipid components at different stages in the isolation of the sterols and triterpenoids from the plasmodia of the Myxomycetes *Physarum flavicomum* and *P. polycephalum*

	P. flavicomum[a]	*P. polycephalum*[a]
Wet wt. plasmodium	125 g	144 g
Nonsaponifiable lipid	101 mg	125 g
From alumina column		
(a) triterpenoids	4 mg	6 mg
(b) sterols	93 mg	108 mg
TLC on silica gel–$AgNO_3$		
(a) stanols	7 mg	28 mg
(b) unsaturated sterols	77 mg	71 mg

[a] All yields are from a total of 4 l of culture medium.

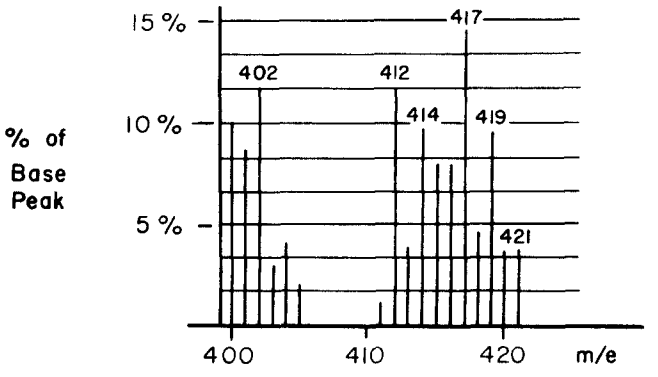

Fig. 2. High mass region of the spectrum of the sterols extracted from *Physarum polycephalum* following its culture in the semi-defined medium (7) containing [methyl-$^2H_3$]methionine.

triterpenoid fraction from *P. polycephalum* (6 mg from 144 g) showed that lanosterol was the only major triterpenoid present with apparently no 24-dihydrolanosterol or cycloartenol and very small amounts of other substances. The mass spectra of the free triterpenoids and their acetates from both organisms showed no significant ion at *m/e* 286, which is characteristic of cycloartenol and its acetate (14).

Biosynthesis of the C-24 alkyl group

When cultured in small batches in the usual medium but with the addition of [methyl-$^2H_3$]methionine, both species grew normally. The extracted sterols from the plasmodia of these organisms gave a very complex mixture of ions in the region *m/e* 400–412 (**Fig. 2**), indicating the incorporation of deuterium atoms into the sterol molecules; the molecular ions of the triterpenoids were unaffected by this treatment.

In an attempt to simplify interpretation of the mass spectra, the Myxomycetes were grown in a medium where the protein source was replaced by a defined mixture of amino acids including [methyl-$^2H_3$]-methionine, since it has been established that methionine serves as the major source of the two C_1- units used in the biosynthesis of the 24-alkyl groups. However, growth in this medium was extremely slow and the mass spectra of the mixtures of sterols showed no better incorporation of deuterium than when labeled methionine was simply added to the usual medium.

DISCUSSION

This work confirms the previous reports (5) of the presence of a typical mixture of plant sterols in some Myxomycete species. However, we differ in the assignment of the stereochemistry of the alkyl group of the sterols at C-24. Although neither report attempts to define the stereochemistry of every component sterol, Lenfant et al. (5) state, as a result of the ozonolysis of the mixture of sterols, that in *P. polycephalum* the major sterol is stigmasterol.[2] However, it is clear that the equivalent sterol in both Myxomycete species examined here is poriferasterol.

Such a difference in results must be closely examined. Lenfant et al (5) reported a yield of 258 mg of 2-ethyl-3-methylbutanoic acid derived from the ozonolysis of 328 mg of a mixture of sterols, which is far in excess of the maximum possible yield. Furthermore, the reported circular dichroism curve of this compound, on which the stereochemical assignment is made, had a maximum at 288 nm. Such a maximum cannot be due to the presence of a carboxylic acid, for which any Cotton effect can relate only to a weak n-π^* transition of the carboxyl group at about 215 nm (15). These observations do not therefore constitute evidence for the stereochemistry at C-24 of the sterols.

The work reported here defines the stereochemistry of the C-24 alkyl group of only those sterol components that could be isolated as pure compounds, though it appears most likely that all the sterols have the same configuration at the C-24 asymmetric center. Thus poriferasterol and 22-dihydroporiferasterol have been shown to be the major sterols of both organisms, while the minor sterols are probably poriferastanol, Δ^5-ergostenol, and ergostanol.

Biosynthesis of the phytosterols

Several different routes operate in different organisms for the C-24 alkylation of the sterol side chain. In the presence of [methyl-$^2H_3$]methionine, either two (16) or three (17) deuterium atoms may be incorporated into the side chain of the methyl sterols and either four (12) or five (17) deuterium atoms into the 24-ethyl group of the ethyl sterols.

In both *P. polycephalum* and *P. flavicomum* the mass spectrum of the sterols synthesized in the presence of labeled methionine was complex and not easily interpreted (Fig. 2); however the highest mass ion at *m/e* 421 showed that five deuterium atoms were incorporated into the 24-ethyl-cholestanol component (M = 416), similarly other ions at *m/e* 417 and 419 were largely attributable to the addition of five mass units to the molecular ions at 412 and 414.

[2] It should be noted that the optical rotations of the two 2-ethyl-3-methylbutanal-2,4-dinitrophenylhydrazones derived from stigmasterol and from the naturally occurring mixture of sterols in *P. flavicomum* were of opposite sign but unequal magnitude. This difference probably arises because of the low concentrations used in these determinations.

It is not possible to draw definite conclusions regarding the incorporation of deuterium atoms into the 24-methyl sterols from the mass spectrum of the mixture of sterols, as the region *m/e* 400–404 was complicated by ions formed by the loss of H_2O and CH_3 from the various isotopic forms of the 24-ethyl sterols. However the mass spectrum of the acetylated mixture, which showed molecular ions corresponding to the stanol acetates only (11), gave ions from 458 to 463, confirming the uptake of up to five deuterium atoms into the 24-ethylcholestanol and at *m/e* 444–446, demonstrating a maximum incorporation of two labeled atoms into the 24-methylcholestanol.

The location of the incorporated deuterium atoms in the 24-ethyl sterols was readily shown to be in the side chain for, though there was a complex mixture of ions between *m/e* 394 and 404, the ions at *m/e* 273 (M − side chain), 271 (M − side chain − 2H), 255 (M − side chain − H_2O), and 213 (M − side chain − H_2O − 47) (11), which are characteristic of all the sterols present, were unaccompanied by isotope peaks with intensities corresponding to deuterium enrichment. In addition the characteristic *m/e* 369 (M − isopropyl) ion of poriferasterol was accompanied by several major isotope peaks, thereby confirming that the side chain isopropyl group was not derived from the labeled methionine and indicating that the deuterium atoms are almost certainly in the 24-ethyl group.

Significance of the presence of lanosterol

Lanosterol has previously been claimed as accompanying plant sterols in a few species (see (18) for review) but later work has demonstrated that the claims were incorrect, insufficiently documented or, in the case of *Euphorbia*, that the lanosterol was found only in the latex and was apparently an 'end product' rather than an intermediate in the sterol biosynthesis that occurs in the plant tissue (19). Our failure to demonstrate the presence of cycloartenol, the generally accepted first cyclic intermediate in the biosynthesis of plant sterols, does not totally exclude the existence of the usual phytosterol synthetic route in the slime molds. Similarly, the presence of lansterol in both species does not necessarily implicate that compound in a possibly novel pathway to the 24-alkyl sterols.

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