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Sterol Composition of the Corn Root Lesion Nematode, *Pratylenchus agilis*, and Corn Root Cultures

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ABSTRACT: Sterols from mixed stages of the corn root lesion nematode, *Pratylenchus agilis*, and uninfected corn root cultures were analyzed by gas chromatography-mass spectrometry. Twenty-eight sterols were identified in *P. agilis*, including 9 not previously detected in nematodes. The major sterols were 24-ethylcholest-22-enol, 24-ethylcholesta-5,22-dienol, 24-methylcholestanol, 24-ethylcholestanol, isofucostanol, and 24-ethylcholesterol. The principal corn root sterols were 24-ethylcholesta-5,22-dienol, 24-methylcholesterol, 24-ethylcholesterol, isofucosterol, and cycloartenol. Therefore, the major metabolic transformation of sterols by *P. agilis* was saturation of the sterol nucleus. In addition, very small amounts of 4α-methylsterols were biosynthesized by *P. agilis*. The 4-methylation pathway is unique to nematodes and was previously demonstrated only in free-living Rhabditida. The sterol composition of *P. agilis* is remarkably different from that of other nematodes in its very small relative percentage of cholesterol (0.4% of the total nematode sterol).

KEY WORDS: corn, lesion nematode, lipid, maize, nematode, *Pratylenchus agilis*, sterol, *Zea mays*.

The development of new control strategies for parasitic nematodes could exploit biochemical differences between them and their vertebrate or plant hosts. One of the few such areas is steroid metabolism; unlike higher animals and plants, nematodes possess a nutritional requirement for sterol as a result of their lack of de novo sterol biosynthesis (for review: Chitwood et al., 1986). Sterols have 2 major functions; they are integral components of cell membranes as well as metabolic precursors of steroid hormones (Nes and McKean, 1977). Interest in nematode sterol biochemistry has intensified in recent years because of the discovery that several azasteroids and aliphatic amines strongly disrupt growth and development and also inhibit sterol metabolism in nematodes (Chitwood, 1987).

Because free-living nematodes are easily propagated in artificial media, they have been utilized in most investigations involving metabolism of radiolabeled dietary sterols by nematodes (Chitwood et al., 1986). The difficulty in in vitro culture of parasitic nematodes has restricted investigation of sterol metabolism in these organisms to comparison of sterol compositions of host and parasite. As expected, cholesterol is the major sterol of mammalian parasites (Cole and Krusberg, 1967b; Castro and Fairbairn, 1969; Barrett et al., 1970; Fulk and Shorb, 1971; Chung et al., 1986).

A significant biochemical difference between higher animals and plants is that plant sterols usually contain alkyl (i.e., methyl or ethyl) substituents at C-24 of the sterol side chain (Nes and McKean, 1977). Comparison of the sterols of plant-parasitic nematodes and their hosts has indicated that plant parasites are similar to free-living nematodes in that both groups can remove the C-24 alkyl groups of plant sterols (Chitwood, 1987). Unequivocal proof in the case of plant parasites, however, would involve experiments with radiolabeled plant sterols in an in vitro system. Because large quantities of the corn root lesion nematode, *Pratylenchus agilis* Thorne and Malek, 1968, can be readily obtained from corn root explant cultures, we examined the sterols of this plant parasite and its host in order to determine if it was a suitable candidate for radiotracer studies of dealkylation of plant sterols by phytoparasitic nematodes.

Materials and Methods

Seeds of *Zea mays* cv. Iochief were surface sterilized by immersion in distilled water for 2 hr and then 1.3% sodium hypochlorite containing 0.1% (v:v) Tween 80 for 25 min. The sterilized seeds were germinated for 4 days on 1.5% water agar in culture dishes, and then 2.5-cm root pieces were transferred to culture dishes containing Gamborg's B5 medium formulated with 1.5% agar and 2.0% sucrose, and lacking cytokinins or auxins (Huettel, 1990). The agar had been extracted previously with methanol and chloroform : methanol 2:1 (v:v, twice each) to reduce the high amounts of endogenous cholesterol in commercial agar (Nes, 1987) to an acceptable level (20.6 ng of cholesterol per gram dry weight as determined by subsequently described

Table 1. Sterol content of *Pratylenchus agilis* and root cultures of *Z. mays*.

	% dry weight		% sterol esterified
	Lipid	Sterol	
P. agilis	14.6	0.03	26.8
Z. mays	3.9	0.83	—*

* Not determined.

methods). One-week-old root cultures were inoculated with agar plugs from similar cultures containing sterile *P. agilis*. Nematodes from 8-week-old cultures maintained at 25°C were harvested with modified Baermann funnels, floated on 30% sucrose, rinsed 5 times with distilled water, frozen immediately, and lyophilized.

Lipids were extracted from lyophilized nematodes (2.0–2.2 g dry wt) by homogenization in a Ten-Broeck tissue grinder 3 times with chloroform : methanol 2:1 and were purified by partition against 0.85% NaCl (Folch et al., 1957). Neutral lipids were separated from polar lipids and were fractionated into various lipid classes by column chromatography on silica (Chitwood et al., 1985). The lipid fractions containing steryl esters and free sterols were saponified separately in methanolic KOH (Chitwood et al., 1985); the liberated 4-desmethylsterols and 4-methylsterols were purified by Florisil column chromatography (Chitwood et al., 1987c).

Roots from uninfected cultures were gently removed with forceps, frozen, and lyophilized. Extraction of roots (0.3–0.5 g dry wt) was identical to that of nematodes, except a Virtis homogenizer was used. The root lipids were saponified directly without prior fractionation on a silica column. The 4-methylsterols and 4-desmethylsterols were isolated as described above. As a precautionary measure, any potentially tightly bound sterols were removed from the extraction residue by saponification of the residue and analyzed identically to the root extracts.

Sterols were analyzed by gas-liquid chromatography (GLC) on a DB-1 fused silica capillary column and an OV-17 packed glass column as described previously (Chitwood et al., 1987c). Following preliminary analysis, the sterols were acetylated, the steryl acetates were purified on Florisil columns and analyzed by GLC, and then tentative identifications were confirmed by gas chromatography-mass spectrometry (GC-MS, Chitwood et al., 1987c). The steryl acetates were also fractionated according to degree and position of unsaturation by chromatography on columns of silica impregnated with $AgNO_3$ and analyzed by GLC (Chitwood et al., 1987c).

Results are the means of two replicates.

Results

Gravimetric analysis indicated that lipid constituted approximately 15% of the dry weight of *P. agilis*; subsequent quantification of sterols by GLC revealed that sterol comprised only a very small proportion of the lipid extract (Table 1).

Table 2. Gas-liquid chromatographic relative retention times (RRT's) of sterols from *Pratylenchus agilis* and *Zea mays*, expressed relative to cholesterol. GLC was performed on a temperature-programmed DB-1 fused silica capillary column (14 m × 0.25-mm i.d., 0.25-μm film) and on an isothermally operated packed glass column containing 2.0% OV-17 stationary phase.

Sterol	DB-1	OV-17
Cholesterol	1.00	1.00
Cholestanol	1.02	1.02
24-Methylcholesta-5,22-dienol	1.11	1.14
Lathosterol	1.12	1.19
24-Methylcholest-22-enol	1.14	1.17
24-Methylcholesta-5,23-dienol	1.25	1.38
24-Methylenecholesterol	1.26	1.37
24-Methylenecholestanol	1.30	1.39
24-Methylcholesterol	1.30	1.33
24-Methylcholestanol	1.33	1.35
24-Ethylcholesta-5,22E-dienol	1.40	1.45
24-Ethylcholest-22E-enol	1.43	1.47
24-Methylcholesta-5,24(25)-dienol	1.47	1.67
24-Ethylcholesta-5,23-dienol	1.53	1.67
24-Ethylcholesta-7,22-dienol	1.57	1.70
Fucosterol	1.59	1.76
24-Ethylcholesterol	1.60	1.67
24-Ethylcholestanol	1.63	1.69
Isofucosterol	1.66	1.85
Isofucostanol	1.69	1.88
24-Ethylcholesta-5,24(25)-dienol	1.77	1.98
24-Ethyllathosterol	1.81	1.96
24-Ethylcholest-24(25)-enol	1.81	2.04
24Z-Ethylidenelathosterol	1.89	2.20
Obtusifoliol	1.52	1.56
	(1.49)*	(1.50)
4α,24-Dimethylcholestanol	1.58	1.52
	(1.53)	(1.48)
4α-Methyl-24-ethylcholest-22-enol	1.71	1.66
	(1.67)	(1.61)
4α-Methyl-24-ethylcholestanol	2.10	1.87
	(1.91)	(1.87)
4α-Methylisofucostanol	2.07	2.11
	(1.95)	(2.06)
4α-Methyl-24Z-ethylidenelathosterol	2.26	2.52
	(2.12)	(2.44)
Cycloartanol	1.71	1.67
	(1.60)	(1.53)
Cycloeucalenol	1.74	1.88
	(1.69)	(1.78)
Cycloartenol	1.86	2.05
	(1.73)	(1.85)
24-Methylenecycloartanol	2.14	2.28
	(1.99)	(2.06)

* Values in parentheses are RRT's of steryl acetates relative to cholesteryl acetate. Trivial and systematic names: cholesterol, cholest-5-en-3β-ol; cholestanol, 5α-cholestan-3β-ol; lathosterol, 5α-cholest-7-en-3β-ol; fucosterol, 24E-ethylidenecholest-5-en-3β-ol; isofucosterol, 24Z-ethylidenecholest-5-en-3β-ol; isofucostanol, 24Z-ethylidene-5α-cholestan-3β-ol; obtusifoliol, 4α,14-dimethyl-24-methylene-5α-cholest-8-en-3β-ol; cycloartanol, 4,4,14-trimethyl-9β,19-cyclo-5α-cholestan-3β-ol; cycloeucalenol, 4α,14-dimethyl-24-methylene-9β,19-cyclo-5α-cholestan-3β-ol; cycloartenol, 4,4,14-trimethyl-9β,19-cyclo-5α-cholest-24-en-3β-ol.

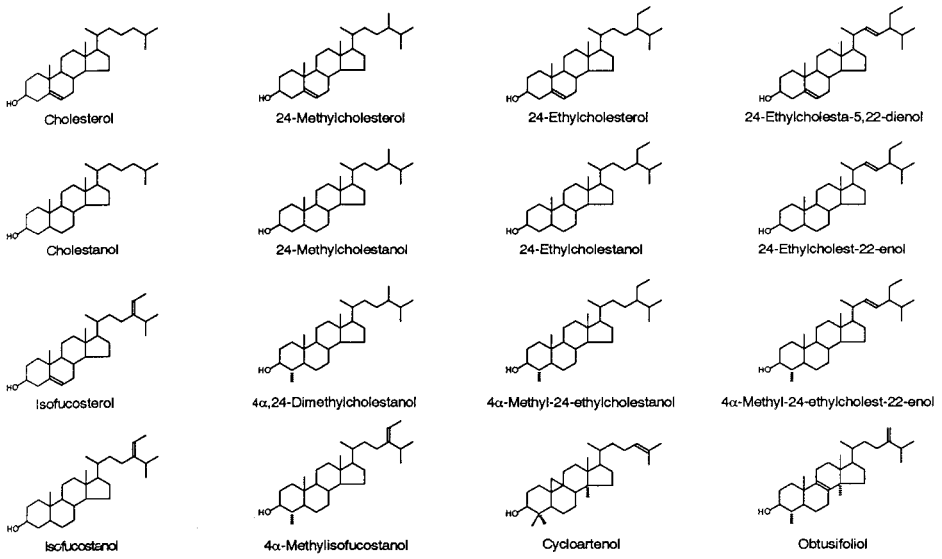

Figure 1. Some representative sterols from *Pratylenchus agilis* or *Zea mays*.

Most of the nematode sterol was unesterified. Total sterols were much more abundant in *Z. mays*. Saponification of the root extraction residue yielded only an additional 0.1% sterol.

All sterols from *P. agilis* and *Z. mays* (Fig. 1) were identical to authentic standards (when available) by GLC relative retention times (RRT's, Table 2) of both the free sterols and steryl acetate derivatives, argentation column chromatographic behavior, and GC-MS. Authentic 24-methylcholesta-5,24(25)-dienol was prepared by isomerization of 24-methylenecholesterol by iodine (Salt et al., 1986). Its GLC RRT's were similar to literature values (Itoh et al., 1982) and the mass spectrum of the steryl acetate (mass/charge m/z [structure and relative intensity]: 380 [molecular ion M^+-CH_3COOH, 27], 365 [M-CH_3COOH-CH_3, 5], 296 [M-CH_3COOH-C_6H_{11}-H, 53], 281 [296-CH_3, 17], 253 [M-CH_3COOH-C_9H_{17} side chain-2H, 19], 213 [M-CH_3COOH-side chain-C_3H_6, 17], and 83 [C_6H_{11}, 100]) was nearly identical to that of 24-methylenecholesterol but contained an abundant side chain fragment at m/z 83 produced by allylic cleavage at C-22 due to the $\Delta^{24(25)}$-bond. We lacked authentic standards for several compounds, including 24-ethylcholest-22E-enol, 24Z-ethylidenelathosterol, 4α,24-dimethylcholestanol, 4α-methyl-24-ethylcholestanol, 4α-methyl-24Z-ethylidenelathosterol, cycloartanol, and 24-methylenecycloartanol. These were indistinguishable by GLC and GC-MS from the same compounds previously identified by us in *Heterodera zeae* and *Meloidogyne incognita* (Chitwood et al., 1985, 1987c).

Other sterols for which we lacked authentic standards were 24-methylcholest-22-enol, 24-ethylcholesta-5,23-dienol, 24-ethylcholesta-5,24(25)-dienol, 24-ethylcholest-24(25)-dienol, obtusifoliol, 4α-methyl-24-ethylcholest-22-enol, 4α-methylisofucostanol, and cycloeucalenol. These were identified as follows.

As is typical for other sterols with saturated ring systems (Patterson, 1971), the GLC RRT's for 24-methylcholest-22-enol were characteristically slightly greater than those of the corresponding sterol containing a Δ^5-bond, i.e., 24-methylcholesta-5,22-dienol. The mass spectrum of the steryl acetate (442 [M^+, 18], 344 [M-C_7H_{13}-H, 29], 329 [344-CH_3, 3], 315 [M-C_9H_{17} side chain-2H, 36], 257 [M-CH_3COOH-side chain, 57], 215 [M-CH_3COOH-side chain-C_3H_6, 14], and 55 [C_4H_7, 100]) was very similar to that of 24-ethylcholest-22-enyl acetate (Chitwood et al., 1985), but all ions containing an intact side chain were 14 mass units smaller. The GLC RRT's of 24-ethylcholesta-5,23-dienol were similar to literature values (Itoh et al., 1982); the mass

spectrum of the acetate (394 [M-CH_3COOH, 100], 379 [M-CH_3COOH-CH_3, 7], 283 [M-CH_3COOH-C_8H_{15}, 16], 253 [M-CH_3COOH-$C_{10}H_{19}$ side chain-2H, 22], and 213 [M-CH_3COOH-side chain-C_3H_6, 11]) included a diagnostic loss of C_8H_{15} resulting from allylic cleavage of the C-20(22) bond. The GLC RRT's for 24-ethylcholesta-5,24(25)-dienol were similar to literature values (Itoh et al., 1982). The mass spectrum of the steryl acetate (394 [M-CH_3COOH, 32], 379 [M-CH_3COOH-CH_3, 6], 296 [M-CH_3COOH-C_7H_{13}-H, 100], 281 [296-CH_3, 23], 253 [M-CH_3COOH-$C_{10}H_{19}$ side chain-2H, 18], and 213 [M-CH_3COOH-side chain-C_3H_6, 9]) was similar to a literature spectrum (Kim et al., 1988), contained a more abundant side chain fragment at m/z 97 (analogous to the m/z 83 fragment in the spectrum of 24-methylcholesta-5,24(25)-dienyl acetate), but was otherwise indistinguishable from the spectra of fucosteryl and isofucosteryl acetates. The RRT's of 24-ethylcholest-24(25)-enol were slightly greater than RRT's of 24-ethylcholesta-5,24(25)-dienol. The mass spectrum of the $\Delta^{24(25)}$-acetate was indistinguishable from that of its $\Delta^{24(28)}$-isomer, i.e., authentic isofucostanyl acetate (Chitwood et al., 1987c).

The fact that the RRT's of the newly identified 4α-methylsterols were greater as free sterols than as the acetate derivatives is indicative of the presence of a 4α-methyl substituent (Patterson, 1971). The RRT's of obtusifoliyl acetate (Table 2) and its mass spectrum (468 [M^+, 28], 453 [M-CH_3, 64], 393 [M-CH_3COOH-CH_3, 22], 369 [M-CH_3-C_6H_{11}-H, 9], 343 [M-C_9H_{17} side chain, 4], 309 [M-CH_3COOH-CH_3-C_6H_{11}-H, 11], 287 [M-side chain-CH_3-D ring, 19], 227 [287-CH_3COOH, 14], and 55 [C_4H_7, 100]) were similar to literature values (Itoh et al., 1978, 1982; Staphylakis and Gegiou, 1985; Rahier et al., 1989). The mass spectrum included ions produced by loss of a C_6H_{11} fragment resulting from allylic cleavage of the C-22 bond. The mass spectrum of 4α-methyl-24-ethylcholest-22-enyl acetate (470 [M^+, 28], 455 [M-CH_3, 2], 427 [M-C_3H_7, 2], 367 [M-CH_3COOH-C_3H_7, 17], 358 [M-C_8H_{15}-H, 24], 343 [M-C_8H_{15}-H-CH_3, 4], 329 [M-$C_{10}H_{19}$ side chain-2H, 23], 271 [M-CH_3COOH-side chain, 33], 229 [M-CH_3COOH-side chain-C_3H_6, 8], and 55 [C_4H_7, 100]) included ions produced by the side chain fragmentations characteristic of Δ^{22}-sterols (Lenfant et al., 1967; Hutchins et al., 1970) and nuclear ions (229, 271) characteristic of 4α-methylsterols. The mass spectrum of 4α-methylisofucostanyl acetate (470 [M^+, 4], 455 [M-CH_3, 1], 372 [M-C_7H_{13}-H, 100], 357 [M-C_7H_{13}-H-CH_3, 12], 329 [M-$C_{10}H_{19}$ side chain-2H, 6], 312 [M-CH_3COOH-C_7H_{13}-H, 4], 269 [M-CH_3COOH-side chain-2H, 3], 230 [M-CH_3COOH-sidechain-C_3H_5, 27], and 229 [M-CH_3COOH-side chain-C_3H_6, 18]) was similar to a literature spectrum (Piretti and Viviani, 1989) and virtually identical to that of authentic isofucostanyl acetate (Chitwood et al., 1987b), but all ions containing an intact steroid nucleus were 14 mass units higher. The GLC RRT's of cycloeucalenyl acetate (Table 2) and its mass spectrum (468 [M^+, 4], 453 [M-CH_3, 7], 408 [M-CH_3COOH, 62], 393 [M-CH_3COOH-CH_3, 37], 300 [M-CH_3COOH-A ring, 6], 283 [M-CH_3COOH-C_9H_{17} side chain, 8], 281 [M-CH_3COOH-side chain-2H, 7], and 55 [C_4H_7, 100]) agreed with literature values (Itoh et al., 1978, 1982; Staphylakis and Gegiou, 1985; Rahier et al., 1989). The mass spectrum included the loss of the A ring characteristic of 9β,19-cyclopropyl sterols (Rahier and Benveniste, 1989).

The major sterols of corn root cultures were 24-ethylcholesta-5,22E-dienol, 24-methylcholesterol, and 24-ethylcholesterol (Table 3). In addition to these 3 sterols, *P. agilis* contained 24-ethylcholest-22E-enol as its major sterol and substantial quantities of 24-methylcholestanol, 24-ethylcholestanol, and isofucostanol. The free and esterified sterol fractions of *P. agilis* were qualitatively similar but quantitatively different; the steryl esters contained a much greater proportion of cycloartenol and 24-methylenecycloartenol.

Discussion

The lipid content of *P. agilis* (15%) was less than that found by Krusberg (1967) in *Pratylenchus penetrans* (25%) propagated on alfalfa callus. The small percentage of dry weight of *P. agilis* as sterol (0.03%) is within the range reported in other phytoparasitic nematodes: *Globodera tabacum solanacearum* (0.01%), *Meloidogyne incognita* (0.01%), *M. arenaria* (0.01%), *Rotylenchulus reniformis,* (0.02%), *Heterodera zeae* (0.05%), and *Ditylenchus dipsaci* (0.06%) (Cole and Krusberg, 1967a; Svoboda and Rebois, 1977; Orcutt et al., 1978; Chitwood et al., 1985, 1987c). These values are lower than the range of 0.2%–0.6% reported in the animal-par-

Table 3. Relative percentages of sterols in root cultures of *Zea mays* and in free sterol and steryl ester fractions from *Pratylenchus agilis*.

	P. agilis		
	Free sterols	Steryl esters	*Z. mays*
Cholesterol	0.3	0.7	0.2
Cholestanol	0.4	0.4	Trace*
24-Methylcholesta-5,22-dienol	0.1	Trace	0.3
Lathosterol	0.1	Trace	Trace
24-Methylcholest-22E-enol	0.1	Trace	—†
24-Methylcholesta-5,23-dienol	—	—	0.1
24-Methylenecholesterol	0.2	0.1	1.2
24-Methylenecholestanol	0.4	0.2	0.1
24-Methylcholesterol	2.3	4.7	14.2
24-Methylcholestanol	11.0	4.8	0.5
24-Ethylcholesta-5,22E-dienol	24.6	21.9	60.3
24-Ethylcholest-22E-enol	34.2	18.4	1.4
24-Methylcholesta-5,24(25)-dienol	—	—	0.1
24-Ethylcholesta-5,23-dienol	—	—	0.2
24-Ethylcholesta-7,22E-dienol	0.4	0.1	0.3
Fucosterol	—	—	0.1
24-Ethylcholesterol	4.5	4.8	9.0
24-Ethylcholestanol	10.2	11.9	0.6
Isofucosterol	1.8	2.1	4.3
Isofucostanol	5.2	5.1	0.1
24-Ethylcholesta-5,24(25)-dienol	Trace	—	0.2
24-Ethyllathosterol	0.3	0.9	0.1
24-Ethylcholest-24(25)-enol	0.1	—	—
24Z-Ethylidenelathosterol	0.8	3.0	0.2
Obtusifoliol	0.1	0.2	0.1
4α,24-Dimethylcholestanol	0.6	0.3	—
4α-Methyl-24-ethylcholest-22E-enol	0.4	0.4	—
4α-Methyl-24-ethylcholestanol	0.6	0.2	—
4α-Methylisofucostanol	0.2	0.4	—
4α-Methyl-24Z-ethylidenelathosterol	0.1	Trace	0.1
Cycloartanol	—	—	0.3
Cycloeucalenol	0.1	—	0.4
Cycloartenol	0.7	16.1	3.9
24-Methylenecycloartanol	0.2	3.3	1.7

* Less than 0.05%.
† None detected.

asitic nematodes *Ascaris suum, Nippostrongylus brasiliensis, Strongyloides ratti,* and *Trichostrongylus colubriformis* (Cole and Krusberg, 1967b; Barrett, 1968, 1969; Tarr and Fairbairn, 1973; Chung et al., 1986). A small fraction of the sterols in *P. agilis* are esterified, as in *H. zeae, Meloidogyne* spp., and the free-living nematodes *Caenorhabditis elegans* and *Panagrellus redivivus* (Chitwood et al., 1984, 1985, 1987c; Salt et al., 1989). The composition of the free and esterified sterol fractions from *P. agilis* were qualitatively identical but quantitatively different. Most notably, the steryl esters were rich in cycloartenol and 24-methylenecycloartanol, 2 important intermediates in de novo sterol biosynthesis in higher plants (Nes and McKean, 1977). These two 4,4,14-trimethylsterols are probably not utilized by nematodes, as a related 4,4,14-trimethylsterol, lanosterol, does not support growth and reproduction in *C. elegans* or the insect-parasitic nematode, *Steinernema feltiae* (Chitwood et al., 1987a; Ritter, 1988). Perhaps *P. agilis* and other nematodes isolate nonutilizable sterols by sequestering them as steryl esters.

The major sterols of *P. agilis* are 24-ethylcholest-22-enol, 24-ethylcholesta-5,22-dienol, 24-methylcholestanol, 24-ethylcholestanol, isofucostanol, and 24-ethylcholesterol. The principal sterols of corn root cultures are 24-ethylcholesta-5,22-dienol, 24-methylcholesterol, 24-ethylcholesterol, and isofucosterol. Our results support other reports of 24-ethylcholesta-5,22-dienol

(stigmasterol) as the predominant sterol of *Z. mays* roots and 24-ethylcholesterol (sitosterol) and 24-methylcholesterol as other abundant sterols (Rohmer et al., 1972; Bladocha and Benveniste, 1983). The most striking difference between *P. agilis* and its host is in the percentage of stanols (i.e., sterols without double bonds in the ring system). Less than 3% of the corn root sterols were stanols, whereas approximately two-thirds of the sterols from *P. agilis* were stanols. Although selective uptake of the small quantities of plant stanols cannot be excluded, it seems likely that *P. agilis* can hydrogenate the Δ^5-bonds of corn sterols to produce a variety of stanols. Two free-living nematodes, *Turbatrix aceti* and *P. redivivus,* reduce radiolabeled dietary cholesterol to form cholestanol (Chitwood et al., 1987b), and comparison of sterols of hosts and parasite indicate that *S. feltiae, G. tabacum solanacearum, H. zeae,* and *Meloidogyne* spp. also saturate Δ^5-bonds (Chitwood et al., 1985, 1987b; Morrison and Ritter, 1986; Orcutt et al., 1978).

One interesting aspect of sterol metabolism in free-living Rhabditida is their production of significant quantities of 4α-methylsterols (Chitwood et al., 1984, 1987b). This 4-methylation pathway does not occur in any other class of organisms. Interestingly, we identified very small amounts of several 4α-methylstanols in *P. agilis*: 4α-methyl-24-ethylcholest-22-enol, 4α,24-dimethylcholestanol, 4α-methyl-24-ethylcholestanol, and 4α-methylisofucostanol. Their small relative percentage (less than 2% of the total *P. agilis* sterol) makes any conclusion about their biosynthetic origin speculative. However, their endogenous biosynthesis by *P. agilis* is suggested by the facts that we were unable to isolate them from corn roots and that they are the 4-methyl derivatives of the 4 major products of sterol biosynthesis in *P. agilis,* i.e., the 4 abundant stanols.

Many compounds that inhibit sterol metabolism and growth and development in free-living nematodes inhibit the C-24 dealkylation pathway (Chitwood, 1987). Because free-living nematodes remove the C-24 methyl and ethyl groups of plant sterols and because the sterols of all other plant-parasitic nematodes examined consist of 5%–50% cholesterol, plant-parasitic nematodes are thought similarly to dealkylate phytosterols (Chitwood, 1987). *P. agilis,* however, is distinguished from other nematodes by the small (0.4%) relative percentage of cholesterol and may lack the ability to dealkylate phytosterols. The following sterols previously undetected in nematodes were identified in *P. agilis* in this investigation: 24-methylcholest-22-enol, 24-methylcholesta-5,24(25)-dienol, 24-ethylcholesta-5,23-dienol, 24-ethylcholesta-5,24(25)-dienol, 24-ethylcholest-24(25)-enol, 4α-methyl-24-ethylcholest-22-enol, 4α-methylisofucostanol, obtusifoliol, and cycloeucalenol. We are unaware of identification in *Z. mays* of the following: 24-methylenecholestanol, 24-methylcholesta-5,24(25)-dienol, 24-ethylcholesta-5,23-dienol, and 24-ethylcholesta-5,24(25)-dienol. Sterols with $\Delta^{24(25)}$- or Δ^{23}-bonds have been implicated in the biosynthesis of the major sterols of corn (Zakelj and Goad, 1983), although few of these have been identified previously in *Z. mays.*

The sterol composition of *P. agilis* reflects the diversity of sterol metabolism in nematodes. There is no evidence for phytosterol dealkylation in *P. agilis,* in contrast to other plant-parasitic and free-living nematodes. Instead, *P. agilis* saturates the nucleus of its dietary sterols; the specific value to nematodes of stanol production is presently unknown. Further examination of sterol metabolism in taxonomically distant orders could reveal even greater diversity in sterol biochemistry between nematodes and their hosts.

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Literature Cited

Barrett, J. 1968. Lipids of the infective and parasitic stages of some nematodes. Nature 218:1267–1268.

———. 1969. The effect of ageing on the metabolism of the infective larvae of *Strongyloides ratti* Sandground, 1925. Parasitology 59:3–17.

———, **G. D. Cain, and D. Fairbairn.** 1970. Sterols in *Ascaris lumbricoides* (Nematoda), *Macracanthorhynchus hirudinaceus* and *Moniliformis dubius* (Acanthocephala), and *Echinostoma revolutum* (Trematoda). Journal of Parasitology 56:1004–1008.

Bladocha, M., and P. Benveniste. 1983. Manipulation by tridemorph, a systemic fungicide, of the sterol composition of maize leaves and roots. Plant Physiology 71:756–762.

Castro, G. A., and D. Fairbairn. 1969. Carbohydrates and lipids in *Trichinella spiralis* larvae and their utilization in vitro. Journal of Parasitology 55:51–58.

Chitwood, D. J. 1987. Inhibition of steroid or hormone metabolism or action in nematodes. Pages 122–130 *in* J. A. Veech and D. W. Dickson, eds. Vistas on Nematology. Society of Nematologists, Hyattsville, Maryland.

———, **P. A. Hutzell, and W. R. Lusby.** 1985. Sterol composition of the corn cyst nematode, *Heterodera zeae,* and corn roots. Journal of Nematology 17:64–68.

———, **R. Lozano, and W. R. Lusby.** 1986. Recent developments in nematode sterol biochemistry. Journal of Nematology 18:9–17.

———, ———, ———, **M. J. Thompson, and J. A. Svoboda.** 1987a. Metabolism and function of sterols in nematodes. Pages 200–217 *in* G. Fuller and W. D. Nes, eds. Ecology and Metabolism of Plant Lipids. ACS Symposium Series 325. American Chemical Society, Washington, D.C.

———, **W. R. Lusby, R. Lozano, M. J. Thompson, and J. A. Svoboda.** 1984. Sterol metabolism in the nematode *Caenorhabditis elegans.* Lipids 19:500–506.

———, ———, **and T. A. Salt.** 1987b. Sterol metabolism in the nematodes *Panagrellus redivivus, Turbatrix aceti* and *Caenorhabditis elegans.* Comparative Biochemistry and Physiology 86B:103–107.

———, **M. A. McClure, M. F. Feldlaufer, W. R. Lusby, and J. E. Oliver.** 1987c. Sterol composition and ecdysteroid content of eggs of the root-knot nematodes *Meloidogyne incognita* and *M. arenaria.* Journal of Nematology 19:352–360.

Chung, W.-L., E. J. Parish, and L. W. Bone. 1986. Sex steroid content and metabolism in *Trichostrongylus colubriformis* (Nematoda). Journal of Parasitology 72:326–329.

Cole, R. J., and L. R. Krusberg. 1967a. Sterol composition of the nematodes *Ditylenchus triformis* and *Ditylenchus dipsaci,* and host tissues. Experimental Parasitology 21:232–239.

———, **and** ———. 1967b. Sterols in *Ascaris lumbricoides.* Comparative Biochemistry and Physiology 21:109–114.

Folch, J., M. Lees, and G. H. Sloane Stanley. 1957. A simple method for the isolation and purification of total lipides from animal tissues. Journal of Biological Chemistry 226:497–509.

Fulk, W. K., and M. S. Shorb. 1971. Lipids of male and female *Ascaridia galli* at different ages. Journal of Parasitology 57:840–844.

Huettel, R. N. 1990. Monoxenic culturing of plant parasitic nematodes using carrot discs, callus tissues, and root-explants. Pages 163–172 *in* B. M. Zuckerman, W. F. Mai, and L. R. Krusberg, eds. Plant Nematology Laboratory Manual, revised ed. University of Massachusetts Agricultural Experiment Station, Amherst.

Hutchins, R. F. N., M. J. Thompson, and J. A. Svoboda. 1970. The synthesis and the mass and nuclear magnetic resonance spectra of side chain isomers of cholesta-5,22-dien-3β-ol and cholesta-5,22,24-trien-3β-ol. Steroids 15:113–120.

Itoh, T., T. Ishii, T. Tamura, and T. Matsumoto. 1978. Four new and other 4α-methylsterols in the seeds of Solanaceae. Phytochemistry 17:971–977.

———, **H. Tani, K. Fukushima, T. Tamura, and T. Matsumoto.** 1982. Structure-retention relationship of sterols and triterpene alcohols in gas chromatography on a glass capillary column. Journal of Chromatography 234:65–76.

Kim, S. K., T. Akihisa, T. Tamura, T. Matsumoto, T. Yokota, and N. Takahashi. 1988. 24-Methylene-25-methylcholesterol in *Phaseolus vulgaris* seed: structural relation to brassinosteroids. Phytochemistry 27:629–631.

Krusberg, L. R. 1967. Analyses of total lipids and fatty acids of plant-parasitic nematodes and host tissues. Comparative Biochemistry and Physiology 21:83–90.

Lenfant, M., E. Zissman, and E. Lederer. 1967. Biosynthesis of the ethyl side chain of stigmasterol derivatives by the slime mould *Dictyostelium discoideum.* Tetrahedron Letters 1967:1049–1052.

Morrison, A. H., and K. S. Ritter. 1986. Effects of host insect sterols on the development and sterol composition of *Steinernema feltiae.* Molecular and Biochemical Parasitology 19:135–142.

Nes, W. D. 1987. Biosynthesis and requirement for sterols in the growth and reproduction of Oomycetes. Pages 304–328 *in* G. Fuller and W. D. Nes, eds. Ecology and Metabolism of Plant Lipids. ACS Symposium Series 325. American Chemical Society, Washington, D.C.

Nes, W. R., and M. L. McKean. 1977. Biochemistry of Steroids and Other Isopentenoids. University Park Press, Baltimore.

Orcutt, D. M., J. A. Fox, and C. A. Jake. 1978. The sterol, fatty acid, and hydrocarbon composition of *Globodera solanacearum.* Journal of Nematology 10:264–269.

Patterson, G. W. 1971. Relation between structure and retention time of sterols in gas chromatography. Analytical Chemistry 43:1165–1170.

Piretti, M. V., and R. Viviani. 1989. Presence of 4α-methylsterols in the bivalve mollusc *Scapharca inaequivalvis* (Bruguiere): their significance and metabolism. Comparative Biochemistry and Physiology 93B:753–756.

Rahier, A., and P. Benveniste. 1989. Mass spectral identification of phytosterols. Pages 223–250 *in* W. D. Nes and E. J. Parish, eds. Analysis of Sterols and Other Biologically Significant Steroids. Academic Press, San Diego.

———, **M. Taton, and P. Benveniste.** 1989. Cycloeucalenol-obtusifoliol isomerase: structural requirements for transformation or binding of substrates and inhibitors. European Journal of Biochemistry 181:615–626.

Ritter, K. S. 1988. *Steinernema feltiae* (=*Neoaplectana carpocapsae*): effect of sterols and hypolipidemic agents on development. Experimental Parasitology 67:257–267.

Rohmer, M., G. Ourisson, and R. D. Brandt. 1972. Hydrosoluble complexes of sterols, sterol esters and their precursors from *Zea mays* L. European Journal of Biochemistry 31:172–179.

Salt, T. A., D. J. Chitwood, and W. R. Lusby. 1989. Sterol metabolism in the nematode *Panagrellus redivivus.* Lipids 24:325–328.

———, **R. Lozano, W. R. Lusby, D. J. Chitwood, and M. J. Thompson.** 1986. 24-Methyl-23-dehydro-

cholesterol: a new sterol intermediate in C-24 demethylation from the nematodes *Panagrellus redivivus* and *Caenorhabditis elegans*? Steroids 48:451–460.

Staphylakis, K., and D. Gegiou. 1985. Free, esterified, and glucosidic sterols in cocoa butter. Lipids 20:723–728.

Svoboda, J. A., and R. V. Rebois. 1977. Sterol composition of *Rotylenchulus reniformis* and its host plant, cotton. Journal of Nematology 9:286. (Abstr.)

Tarr, G. E., and D. Fairbairn. 1973. Glycerides, waxes and sterols in ovaries of *Ascaris lumbricoides* (Nematoda). Lipids 8:303–310.

Zakelj, M., and L. J. Goad. 1983. Observations on the biosynthesis of 24-methylcholesterol and 24-ethylcholesterol by *Zea mays*. Phytochemistry 22: 1931–1936.