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Sterol Composition and Ecdysteroid Content of Eggs of the Root-knot Nematodes *Meloidogyne incognita* and *M. arenaria*

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Abstract: Free and esterified sterols of eggs of the root-knot nematodes *Meloidogyne incognita* races 2 and 3 and *M. arenaria* race 1 were isolated and identified by gas-liquid chromatography–mass spectrometry. The major sterols of eggs of each race were 24-ethylcholesterol (33.4–38.8% of total sterol), 24-ethylcholestanol (18.3–25.3%), 24-methylcholesterol (8.6–11.7%), 24-methylcholestanol (7.7–12.5%), and cholesterol (4.6–11.6%). Consequently, the major metabolic transformation performed by *Meloidogyne* females or eggs upon host sterols appeared to be saturation of the sterol nucleus. The free and esterified sterols of the same race did not differ appreciably, except for a slight enrichment of the steryl esters in cholesterol. Although the sterol composition of *Meloidogyne* eggs differed from that of other life stages of other genera of plant-parasitic nematodes, the three *Meloidogyne* races could not be distinguished from each other by their egg sterols. Ecdysteroids, compounds with hormonal function in insects, were not detected by radioimmunoassay in the *Meloidogyne* eggs either as free ecdysteroids or as polar conjugates.

Key words: ecdysteroid, mass spectrometry, *Meloidogyne arenaria, M. incognita,* root-knot nematode, steroid, sterol.

Unlike plants and vertebrate animals, nematodes possess a sterol nutritional requirement because of the inability of nematodes to biosynthesize sterols de novo (1,5). The importance of this metabolic difference between parasitic nematodes and their hosts has been emphasized by the discovery that several compounds that inhibit growth and development of various free-living, animal-parasitic, and plant-parasitic nematodes disrupt the conversion of plant sterols to nematode sterols in the free-living nematode *Caenorhabditis elegans* (7,21,23).

The difficulty in culturing plant-parasitic nematodes axenically has impeded the direct examination of their sterol metabolism. In the few phytoparasitic species in which sterol composition has been determined, the nematodes have contained substantially larger proportions of cholesterol or other typical animal sterols than their hosts (3,9,30,37). Consequently, speculation is that plant-parasitic nematodes remove the methyl or ethyl substituents at C-24 in the side chains of typical plant sterols (3,9,37). Additionally, the occurrence of stanols (defined as sterols without nuclear double bonds) is another conspicuous aspect of the sterol composition of a few phytoparasitic nematodes, such as *Globodera tabacum solanacearum* (30), and, to a lesser extent, *Heterodera zeae* (3). It is possible that these two cyst nematodes selectively concentrate minute quantities of naturally occurring plant stanols, but the fact that the free-living nematode *Panagrellus redivivus* produces substantial quantities of cholestanol (8) indicates that some plant-parasitic nematodes may be capable of a similar reduction of the Δ^5-bond typical of most plant and vertebrate sterols.

Despite the great agronomic importance of the root-knot nematodes, their sterols have not been characterized. Also, comparison of the sterols of two species within the same genus of nematodes has not been reported. The purpose of this investigation was to isolate and identify the sterols of eggs of *Meloidogyne incognita* and *M. arenaria* in order to further characterize phy-

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tosterol dealkylation and saturation in plant-parasitic nematodes. Additionally, because ecdysteroids (compounds with hormonal function in insects) have been identified in some nematodes (32) and because insect eggs are often unusually rich sources of ecdysteroids (11,40), we attempted to isolate and identify these compounds in *Meloidogyne* eggs.

Materials and Methods

Culture methods: M. incognita race 2 (North Carolina State University population #E1135) and *M. arenaria* race 1 (NCSU #352) were greenhouse-propagated on eggplant (*Solanum melongena* 'Black Beauty'). *M. incognita* race 3 (25) was cultured similarly on chili pepper (*Capsicum frutescens* 'Anaheim'). Eggs were released from egg masses, concentrated, rinsed, and immediately lyophilized (26).

Lipid extraction: Lyophilized eggs (duplicate samples, 154–1,905 mg dry weight, for each race) were extracted serially with two 25-ml portions of methanol : water 3:1 (v:v), methanol, and chloroform : methanol 2:1. The extract was evaporated to dryness and partitioned between equal volumes (15 ml) of countersaturated hexane and 70% aqueous methanol, with five transfers of upper phase across three centrifuge tubes containing lower phase. This partition separated the crude extract into a hexane layer containing the majority of the lipids (including phospholipids, triglycerides, hydrocarbons, aliphatic alcohols, free sterols, and steryl esters) and an aqueous methanolic layer containing highly polar lipids, including free ecdysteroids, ecdysteroid conjugates, and other polar material (40).

Sterol isolation: The combined hexane layers were evaporated to dryness and applied to a silica column, and chromatographic fractions containing steryl esters and free sterols were obtained and saponified (3). The saponification products were then fractionated on a 5-g column (10.5-mm i.d.) of 60–100 mesh Florisil (J. T. Baker, Phillipsburg, New Jersey) previously deactivated with 7% water. Compounds were eluted with 50 ml hexane, 50 ml 1% diethyl ether in hexane, 50 and 100 ml 5% ether, 50 ml 20% ether, and 50 ml ether. All column chromatographic separations were monitored by thin-layer chromatography (TLC) on high performance silica gel 60 chromatoplates (E. Merck, Darmstadt, Fed. Rep. Germany) developed with a solvent system of hexane : diethyl ether : acetic acid 70:30:1.

Sterol identification: Purified sterols were tentatively identified and quantified by gas-liquid chromatography (GLC) in a Varian model 3700 gas chromatograph equipped with a flame ionization detector and connected to a Shimadzu model C-R1B recording integrator. Detection of nanogram quantities of sterols was facilitated by fitting the chromatograph with an on-column injector (J&W Scientific, Folsom, California) connected to a 14-m × 0.25-mm J&W DB-1 fused silica capillary column (0.25-μm film); the column temperature was held at 100 C for 1 minute and increased at a rate of 15 C per minute until 240 C was reached. In addition, GLC was performed isothermally at 245 C on a 2-m × 2-mm i.d. glass column containing 2% OV-17 liquid phase. Relative retention times (RRT) were calculated with respect to cholesterol for isothermal GLC. To facilitate comparison with our previous investigations where GLC was performed isothermally on a DB-1 capillary column with split injection, during temperature programming we arbitrarily set the RRT of cholesterol and sitosterol (24α-ethylcholesterol) at 1.00 and 1.60 and calculated the RRT of peaks from biological samples by interpolation or extrapolation.

Subsequent to tentative GLC identification, sterols were converted to steryl acetate derivatives (3) and then purified on 5-g columns of deactivated Florisil by elution with 50-ml portions of 0, 1, 6, and 100% diethyl ether in hexane. The steryl acetates emerged in the 6% ether fraction and were analyzed by GLC similarly to the free sterols, except column temperatures were 5 C higher. Tentative identifications of the steryl acetates were confirmed by capillary

GLC–mass spectrometry (GC–MS) (3). The steryl acetates were then fractionated according to number and location of double bonds by chromatography on silica columns impregnated with $AgNO_3$ (3) and analyzed by GLC and argentation TLC (7).

Synthesis of 24-methylenecholestanyl acetate and isofucostanyl acetate: During GC–MS, two steryl acetates derived from nematodes possessed fragmentation patterns consistent with acetates of 24-methylenecholestanol and isofucostanol. Consequently, authentic reference compounds were synthesized as follows. First, 3β-acetoxycholanyl chloride was prepared by acetylation of 3β-hydroxy-Δ^5-cholenic acid (Calbiochem, San Diego, California), hydrogenation of the resulting acetate over a palladium catalyst, and subsequent conversion to the acid chloride with a slight excess of thionyl chloride in benzene containing a catalytic amount of N,N-dimethylformamide. Subsequently, 24-ketocholestanyl acetate was produced via reflux of the acid chloride in benzene containing isopropylmagnesium bromide and $CdCl_2$ (34) and purified on silica columns by elution with 16% ether in hexane. For synthesis of 24-methylenecholestanyl acetate, 0.75 mmol methyltriphenylphosphonium iodide in 5 ml of tetrahydrofuran was first deprotonated with 0.5 mmol 1.6 N butyllithium in hexane for 1 hour at 22 C, and then 0.25 mmol of 24-ketocholestanyl acetate in tetrahydrofuran was added (36). After reaction for 90 minutes and partition between ether and water, the product, 24-methylenecholestanol, was reacetylated and purified by silica column chromatography and recrystallized from methanol, melting point 121.0–121.5 C; literature mp 124 C (35,36). Isofucostanyl acetate was synthesized by an analogous procedure, except deprotonated ethyltriphenylphosphonium bromide was used. As in a similar synthesis of isofucosteryl acetate, the reaction with 24-ketocholestanyl acetate was slow, requiring reflux for 23 hours (12).

Ecdysteroid analysis: The aqueous methanol phase from the original hexane–70% methanol partition was repartitioned across equal volumes (12 ml) of countersaturated *n*-butanol and water, with five transfers across three centrifuge tubes (40). This and subsequent ecdysteroid fractionations were monitored by TLC on high performance silica gel 60 F254 chromatoplates (E. Merck) developed with a solvent system of chloroform : 95% ethanol 7:3. The butanol layer, containing putative free ecdysteroids, was evaporated to dryness and applied in chloroform to a Florisil SEP–PAK cartridge (Waters, Milford, Massachusetts) previously conditioned with 5 ml chloroform. Compounds were eluted from the cartridge with 5 ml chloroform, 5 ml chloroform : ethanol 19:1, 5 ml chloroform : ethanol 9:1, 10 ml chloroform : ethanol 6:4, and 5 ml methanol. Previous experimentation showed that free ecdysteroids emerge in the 40% ethanol fraction. This fraction was subsequently applied in 2 ml methanol : water 1:9 to a C_{18} SEP–PAK previously conditioned with 5-ml portions of methanol, water, methanol, water, and methanol : water 1:9. Compounds were eluted with 5 ml methanol :water 3:7, 6 ml methanol : water 6:4, and 5 ml methanol (43). Ecdysteroids, which emerge in the 60% methanol fraction, were quantified by radioimmunoassay (RIA) (13), as were all Florisil and C_{18} SEP–PAK fractions.

The aqueous layer from the butanol–water partition, which contained putative polar conjugates of ecdysteroids as well as other polar material, was dissolved in 6 ml of a solution containing 0.1 M NaCl, 0.1 M sodium acetate buffer (pH 5.0), 3.5 mg β-glucuronidase from *Helix pomatia* (Sigma, St. Louis, Missouri), 3.5 mg β-glucuronidase from *Patella vulgata* (Sigma), and 1.0 mg β-glucosidase from almond (Sigma) (40). After incubation at 35 C for 40 hours, 6 ml water and 12 ml butanol were added. Free ecdysteroids liberated from the polar conjugates were isolated by butanol–water solvent partition and C_{18} SEP–PAK chromatography and quantified by RIA.

In one instance, a compound that behaved nearly identically to ecdysone during TLC was characterized by chemical ionization–mass spectrometry (CI–MS)

TABLE 1. Lipid and sterol content of eggs of *Meloidogyne incognita* and *M. arenaria*.

	M. incognita race 2	*M. incognita* race 3	*M. arenaria*
Percentage of dry weight as lipid	53.0	53.6	58.1
Percentage of lipid as sterol	0.0202	0.0196	0.0165
Percentage of dry weight as sterol	0.0107	0.0105	0.0096
Percentage of total sterol as esterified sterol	9.9	8.5	16.9

with ammonia, methane, and isobutane as reagent gases (24).

RESULTS

Approximately one-half of the dry weight of the eggs of each species consisted of lipid (Table 1), with lipid defined as the material in the hexane and butanol layers of the solvent partitions. Examination by TLC indicated that the egg lipids were primarily triglycerides. Following column chromatographic purification of saponification products, TLC indicated that the second 5% ether fraction contained 4α-methylsterols and 4,4-dimethylsterols; 4-desmethylsterols were in the 20% ether fraction. Quantification of the purified sterols by GLC revealed that sterols comprised approximately 0.01% of the dry weight of the eggs of each race, with a small proportion of the sterols being esterified (Table 1).

The sterols identified in the eggs were identical to authentic reference compounds by GLC RRT (Table 2) of both the free sterols and steryl acetate derivatives, as well as by GC–MS and argentation chromatography of the steryl acetates. The RRT of the free sterols vs. cholesterol were identical to the RRT of the steryl acetates vs. cholesteryl acetate, except for the seven 4α-methylsterols or 4,4-dimethylsterols, which possessed characteristic decreases in RRT after acetylation (31).

We lacked authentic reference material for the following compounds: cycloartanol, 24-methylenecycloartanol, 24-ethylcholest-22-enol, 24Z-ethylidenelathosterol, 4α,24-dimethylcholestanol, 4α-methyl-24-ethylcholestanol, 24-methylenecholestanol, isofucostanol, 4α-methyl-24-methylenelathosterol, and 4α-methyl-24Z-ethylidenelathosterol. The first four of these compounds were identical during GLC, GC–MS, and argentation chromatography to the corresponding compounds we isolated previously from *H. zeae* or *Zea mays* (3). Similarly, 4α,24-dimethylcholestanol and 4α-methyl-24-ethylcholestanol were identical to the same compounds previously isolated by us from *C. elegans* (6,22). Because MS data were previously obtained from the free sterols, we herein provide similar data obtained from the steryl acetates: 4α,24-dimethylcholestanol (mass/charge m/z [relative intensity] 458 [molecular ion M^+, 18], 443 [M-CH_3, 3], 398 [M-CH_3COOH, 48], 383 [M-CH_3COOH-CH_3, 31], 369 [M-CH_3COOH-C_2H_5, 7], 290 [M-C_9H_{19} side chain-C_3H_5, 32], 289 [M-C_9H_{19}-C_3H_6, 18], 271 [M-C_9H_{19}-CH_3COOH, 6], 244 [M-C_9H_{19}-CH_3COOH-C_2H_3, 23], 230 [M-$C_9H_{19}CH_3COOH$-C_3H_5,-57], 229 [M-C_9H_{19}-CH_3COOH-C_3H_6, 100], and 215 [M-C_9H_{19}-CH_3COOH-C_4H_8, 22]); 4α-methyl-24-ethylcholestanol (m/z 472 [16], 457 [3], 412 [47], 397 [24], 383 [7], 290 [32], 289 [19], 271 [7], 244 [21], 230 [56], 229 [100], and 215 [23]). Isofucostanol and 24-methylenecholestanol from *M. incognita* and *M. arenaria* were identical to our newly synthesized authentic standards by GLC (Table 2) and argentation chromatography. The mass spectra of their acetates were similar to literature spectra (35,36,39), were identical to spectra of the newly synthesized reference compounds, and included several ions resulting from allylic cleavage of the C-22 bond accompanied by hydrogen transfer: 24-methylenecholestanyl acetate (m/z 442 [M^+, 1], 427 [M-CH_3, 1], 358 [M-C_6H_{12}, 46], 343 [M-C_6H_{12}-CH_3, 11], 315 [M-C_9H_{17} side chain-2H, 21], 298 [M-CH_3COOH-C_6H_{12}, 2], 283 [M-CH_3COOH-C_6H_{12}-CH_3, 4],

TABLE 2. Gas-liquid chromatographic relative retention times of sterols from eggs of *Meloidogyne incognita* and *M. arenaria*, expressed relative to cholesterol.

Sterol	DB-1	OV-17
Cholesterol	1.00	1.00
Cholestanol	1.02	1.02
Lathosterol	1.12	1.19
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
Fucosterol	1.60	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-Ethyllathosterol	1.81	1.96
24Z-Ethylidenelathosterol	1.89	2.20
4α,24-Dimethylcholestanol	1.58	1.52
	(1.53)†	(1.48)
4α-Methyl-24-methylenelathosterol	1.75	1.86
	(1.68)	(1.80)
4α-Methyl-24-ethylcholestanol	2.10	1.87
	(1.91)	(1.82)
4α-Methyl-24Z-ethylidenelathosterol	2.26	2.52
	(2.12)	(2.44)
Cycloartanol	1.71	1.67
	(1.60)	(1.53)
Cycloartenol	1.86	2.05
	(1.73)	(1.85)
24-Methylenecycloartanol	2.14	2.28
	(1.99)	(2.06)

GLC was performed isothermally on a packed glass column (2 m × 2-mm i.d.) containing 2% OV-17 stationary phase or on a temperature programmed DB-1 fused silica capillary column (14 m × 0.25-mm i.d., 0.25 μm film).

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; cycloartanol, 4,4,14α-trimethyl-9β,19-cyclo-5α-cholestan-3β-ol; cycloartenol, 4,4,14α-trimethyl-9β,19-cyclo-5α-cholest-24-en-3β-ol.

† Values in parentheses are RRT of steryl acetate derivatives relative to cholesteryl acetate.

255[M-CH_3COOH-C_9H_{17}-2H, 11], 215 [M-CH_3COOH-C_9H_{17}-C_3H_6, 23], and 55 [C_4H_7, 100]); isofucostanyl acetate (m/z 456 [2], 358 [83], 343 [15], 315 [7], 298 [9], 283 [10], 255 [8], 215 [24], and 55 [100]).

The GLC RRT of 4α-methyl-24-methylenelathosterol and 4α-methyl-24Z-ethylidenelathosterol from *Meloidogyne* (Table 2) were consistent with literature values (16,31). The mass spectra of the acetylated compounds were similar to literature spectra (33,42) and included base peaks at m/z 327, 14 units higher than the characteristic base peaks of 4-desmethylsterols with $\Delta^{7,24(28)}$-bond systems isolated by us from *C. elegans* and *H. zeae* (3,7). Fragments at m/z 370 (relative intensity of 24% and 40%, respectively) reflected allylic cleavage at C-22 due to a $\Delta^{24(28)}$-bond, and weak molecular ions occurred at m/z 454 and 468, respectively.

The major egg sterols were cholesterol, 24-methylcholesterol, 24-methylcholestanol, 24-ethylcholesterol, and 24-ethylcholestanol (Table 3). The three races were similar in the composition of their free or esterified sterols. Within each race, the steryl esters contained greater relative proportions of cholesterol and several 4α-methylsterols and 4,4-dimethylsterols than the free sterols.

During attempted quantification of free ecdysteroids, none of the Florisil or C_{18} SEP–PAK fractions from extracts of eggs of any of the races displayed immunoreactivity towards the antiserum employed in our ecdysteroid RIA, except for slight activity (930 pg per gram dry weight) in the methanol eluate of the C_{18} cartridge during fractionation of extracts from *M. arenaria*. Similarly, ecdysteroids were not detected in any of the C_{18} SEP–PAK fractions following enzymic hydrolysis of the potential ecdysteroid conjugate fractions, i.e., the aqueous layers of the butanol–water partitions from extracts of eggs of any race. An interesting compound that migrated nearly identically to ecdysone was observed during TLC. It comprised 0.1% of the dry weight of the eggs of each race. Unlike ecdysone, it occurred in the hexane layer of the hexane–70% methanol partition, it was not UV-absorbent during TLC, and a 100-μg sample was not immunoreactive during RIA. Because CI–MS indicated that its apparent molecular weight was 811, it was not characterized further.

DISCUSSION

The high lipid content in eggs of *M. incognita* and *M. arenaria* agrees with previously reported values in the same species (20). Compared with the two other Het-

TABLE 3. Relative percentages of free and esterified sterols in eggs of *Meloidogyne incognita* and *M. arenaria*.

Sterol	*M. incognita* race 2		*M. incognita* race 3		*M. arenaria*	
	Free	Esterified	Free	Esterified	Free	Esterified
Cholesterol	4.7	14.0	3.8	12.9	10.7	15.8
Cholestanol	2.4	4.0	2.6	3.1	3.9	3.3
Lathosterol	0.2	0.5	0.1	0.2	0.3	0.3
24-Methylenecholesterol	0.2	0.1	0.1	*	0.4	0.4
24-Methylenecholestanol	0.3	0.3	0.3	0.3	0.6	0.5
24-Methylcholesterol	8.7	7.2	8.9	6.4	11.4	13.2
24-Methylcholestanol	7.9	5.7	12.9	8.3	8.6	8.6
24-Ethylcholest-5,22-dienol	4.3	1.9	2.1	1.2	3.0	1.5
24-Ethylcholest-22-enol	2.0	1.5	2.3	0.5	2.0	1.0
Fucosterol	0.1	0.1	0.1	0.2	0.2	*
24-Ethylcholesterol	39.6	31.5	33.6	31.7	35.6	31.9
24-Ethylcholestanol	24.2	21.1	25.2	26.3	18.9	15.6
Isofucosterol	2.8	5.1	4.7	4.7	2.2	2.2
Isofucostanol	0.8	2.5	1.3	1.4	1.0	0.8
24-Ethyllathosterol	0.3	0.4	0.6	0.6	0.3	0.5
24Z-Ethylidenelathosterol	0.2	0.2	0.1	0.1	0.2	0.2
4α,24-Dimethylcholestanol	0.2	*	0.3	0.5	0.1	0.1
4α-Methyl-24-methylenelathosterol	0.2	1.9	0.1	0.3	0.1	1.7
4α-Methyl-24-ethylcholestanol	0.4	0.2	0.5	0.8	0.1	0.2
4α-Methyl-24Z-ethylidenelathosterol	0.2	0.4	0.2	0.2	0.1	0.6
Cycloartanol	0.1	0.3	*	*	0.1	0.4
Cycloartenol	0.1	0.9	0.1	0.1	0.1	0.8
24-Methylenecycloartanol	0.1	0.2	0.1	0.2	0.1	0.4

* Not detected.

eroderoidea previously examined, the sterol content of *Meloidogyne* eggs (0.01% of nematode dry weight) is similar to that of cysts of *Globodera tabacum solanacearum* (0.01%) but less than that of cysts of *Heterodera zeae* (0.05%) (3,30). As in *H. zeae* and *C. elegans* (3,7), most of the sterol from *Meloidogyne* eggs is unesterified.

The principal *Meloidogyne* egg sterols are 24-ethylcholesterol, 24-ethylcholestanol, 24-methycholesterol, 24-methylcholestanol, and cholesterol. The major sterols of vascular plants are usually 24-alkyl derivatives of cholesterol, especially 24-ethylcholesterol, 24-methylcholesterol, and 24-ethylcholesta-5,22-dienol; small quantities of many other sterols, including cholesterol, are frequently present (29). A literature search revealed no direct studies of the sterol composition of eggplant or chili pepper roots. The seed sterols of eggplant and *Capsicum annuum* include 24-ethylcholesterol (49–69%), 24-methylcholesterol (9–20%), 24-ethylcholesta-5,22-dienol (8–11%), isofucosterol (1–11%), and cholesterol (3–10%) (17). Given this somewhat high concentration of cholesterol, as well as other investigations in which sterols from roots of other Solanaceae (tomato and potato) contained as much as 10% cholesterol (41), we cannot conclude if *Meloidogyne* cholesterol is obtained directly by adult females from the host roots or is produced by the adult or eggs from plant 24-alkylsterols via a dealkylation pathway similar to that in free-living nematodes (4). In many plant-feeding insects, which, like nematodes, cannot biosynthesize sterols de novo, C-24 dealkylation pathways are common, but some species possess selective uptake mechanisms for cholesterol (38).

Because plant tissue seldom contains greater than trace quantities of stanols and because approximately one-third of the *Meloidogyne* egg sterols do not contain a nuclear double bond, the major metabolic transformation of sterols in *Meloidogyne* appears to be Δ^5-bond reduction. The free-living nematodes *C. elegans, Turbatrix aceti,* and *Panagrellus redivivus* (8) and the insect associate *Steinernema feltiae* (28) reduce the Δ^5-bonds of various 24-desalkylsterols, such

as cholesterol or 7-dehydrocholesterol. Interestingly, *C. elegans, T. aceti,* and *P. redivivus* produce only traces of 24-ethylcholestanol from 24-ethylcholesterol, unlike *Meloidogyne,* which produces 24-ethylcholestanol in abundance.

One of the most unusual aspects of sterol metabolism in free-living nematodes is the ability of certain free-living species to attach a 4α-methyl group to the sterol nucleus, a transformation thus far unique to nematodes (6,8). The occurrence of 4α-methylsterols in *Meloidogyne* eggs is the first record of their detection in parasitic nematodes. However, the small relative proportions of these compounds, our failure to detect any 4α-methyl-24-desalkylsterols, and the occurrence of 4α-methyl-24-methylenelathosterol and 4α-methyl-24-ethylidenelathosterol in seeds of eggplant and *C. annuum* (18) indicate that *Meloidogyne* did not produce these compounds by nuclear methylation. Similarly, the three 4,4-dimethylsterols—cycloartanol, cycloartenol, and 24-methylenecycloartanol—are common constituents of vascular plants and are undoubtedly not synthesized by *Meloidogyne.*

The sterol compositions of eggs of the three *Meloidogyne* races are qualitatively identical and quantitatively very similar. Consequently, the chemotaxonomic value of nematode sterols at the species or race level must be regarded as poor, at least in the root-knot nematodes. Compared to sterols of cysts of *G. tabacum solanacearum* and *H. zeae, Meloidogyne* egg sterols contain approximately the same relative proportion of cholesterol but are intermediate in the relative percentage of stanols. *H. zeae* sterol contains 10% stanols (3); *Meloidogyne,* 34–44%; and *G. tabacum solanacearum,* 71% (30). Stanols do not occur in *Ditylenchus dipsaci* (9) and comprise only 4% of the total sterol of *Rotylenchulus reniformis* (37). The reason for the wide divergence of stanol abundance among different genera of phytoparasitic nematodes remains unknown; free-living rhabditids possess a similar, curious variation in stanol abundance among genera (8).

Within each race, the free and esterified sterols differed only slightly and to a much lesser extent than the free and esterified sterol of *C. elegans* or *H. zeae* (3,7,22). As in *H. zeae,* the steryl esters were richer in cholesterol than the free sterol fraction. The role of steryl esters in nematodes has not been determined experimentally. In other organisms, free sterols are the major steroidal membrane components, whereas steryl esters are utilized for sterol transport and storage (19).

Ecdysteroids are a family of structurally related polyhydroxy Δ^7-6-ketosteroids that hormonally control molting and other processes in insects (15). Although a few nematodes have been reported to contain ecdysteroids, neither a hormonal role for ecdysteroids nor their biosynthesis from a sterol precursor in nematodes has yet been established (4,32). Quantification by RIA has indicated nearly a 4,000-fold difference in the levels of ecdysteroids in various nematodes: *Ascaris suum* reproductive tissue contained 255 pg free ecdysteroids per gram fresh weight (14); *Dirofilaria immitis* adults, 1.9–3.9 ng free ecdysteroids and 3.9 ng conjugated ecdysteroids per gram fresh weight (27); *Haemonchus contortus* juveniles, 200–213 ng free ecdysteroids per gram fresh weight (10); mixed stages of *P. redivivus,* 2.4–271 ng free ecdysteroids per gram fresh weight (10); mixed stages of *Aphelenchus avenae,* 115–996 ng free ecdysteroids per gram fresh weight (10); and adults and third-stage juveniles of *Nippostrongylus brasiliensis,* 41–151 ng free ecdysteroids and 43–150 ng conjugated ecdysteroids per gram dry weight (2). Consequently, our inability to detect either free or conjugated ecdysteroids in *Meloidogyne* eggs is of considerable interest. Ecdysteroids may occur in *Meloidogyne* eggs at a level below our limit of detection (150 pg), or they could be of such structure as to be poorly immunoreactive with the antiserum used in our investigation. (For example, ecdysone has 7.4 times the immunoreactivity of 26-hydroxyecdysone in our RIA.) On the contrary, given that some insect eggs are very rich sources of ecdysteroids

(e.g., up to 22 μg and 72 μg per gram fresh weight of eggs of *Manduca sexta* and *Schistocerca gregaria*) (11,40), one might expect relatively high concentrations of ecdysteroids to occur in nematode eggs, especially if nematode and insect ecdysteroids have similar functions. Further investigation of nematode ecdysteroid biochemistry is necessary to determine if these compounds have specific physiologic roles in nematodes.

Although the significance of ecdysteroids in nematodes remains unclear, the nutritional requirement for sterol by nematodes demonstrates the importance of sterols in the nematode life cycle. The lack of de novo sterol biosynthesis in nematodes remains one of the few major biochemical differences between parasitic nematodes and their plant or vertebrate hosts. The fact that *Meloidogyne* females or eggs saturate a large percentage of their dietary sterols indicates that the stanols produced may have a key structural role in nematode membranes or some other undetermined physiological function. Appropriate in vitro experiments with radiolabeled sterols could determine some of these specific roles.

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