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Synthetic Lethal Interactions Involving Loss of the Yeast *ERG24*-the Sterol C-14 Reductase Gene.

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Abstract

ERG2 and *ERG24* are yeast sterol biosynthetic genes and are targets of the morpholine antifungals. *ERG2* and *ERG24* encode the C-8 sterol isomerase and the C-14 reductase, respectively. *ERG2* is considered a non-essential gene but the viability of *ERG24* is dependent upon genetic background, type of medium, and CaCl_2 concentration. We demonstrate that *erg2* and *erg24* mutants are viable in the deletion consortium background but are lethal when combined into the same haploid strain. The *erg2erg24* double mutant can be suppressed by mutations in the sphingolipid gene *ELO3* but not *ELO2*. However, suppression occurs on rich but not on synthetic complete medium. We also demonstrate that the suppressed *elo3erg2erg24* does not show a sterol composition markedly different from *erg24*. Further genetic analysis indicates that *erg24* when combined with mutations in *erg6* or *erg28* are synthetically lethal but when combined with mutations in *erg3* are weakly viable. These results suggest that novel sterol intermediates likely contribute to the synthetic lethality observed in this investigation.

Introduction

The synthesis of lanosterol is the first committed step in yeast sterol biosynthesis. The yeast *ERG24* gene encodes the C-14 reductase required to complete C-14 demethylation of lanosterol. This gene was cloned and disrupted by several investigators who demonstrated that the C-14 reductase enzyme is essential for viability (1,2). Originally, *erg24* mutants were found to be lethal in wildtype genetic backgrounds but viable in genetic backgrounds containing mutations in *elo2/fen1* (1,2). Mutations in the *ELO2/FEN1* (3) gene result in resistance to the morpholine, fenpropimorph (1,2). The morpholines inhibit both the C-8 isomerase and the C-14 reductase encoded by *ERG2* and *ERG24*, respectively (4,5) possibly indicating a common target site on the two enzymes or an uncharacterized protein-protein interaction between them. However, resistance to fenpropimorph due to a mutation in *FEN1* results in tolerance to the

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Abbreviations:

CSM

synthetic complete media

GC

gas chromatography

PCR

polymerase chain reaction

YPAD

yeast extract, peptone, adenine, dextrose

accumulation of the sterol intermediate, ergosta-8,14-dienol. Lorenz and Parks were able to construct a deletion mutation in the *ERG24* gene that was viable only in a *fen1* background (1). Similarly, Ladeveze et al. also demonstrated that *ERG24* mutants were viable in a *fen1* background (6). Subsequent work by Baudry et al. demonstrated that *erg24* mutants were also suppressed by mutations in *elo3/sur4* (7). *ELO2/FEN1* and *ELO3/SUR4* encode enzymes that are involved in sphingolipid synthesis and are components of a fatty acid elongation system that elongates C16/C18 to C24/C26 (3). Further analyses indicated that *erg24* mutants could be grown in some genetic backgrounds (wildtype for *ELO2* and *ELO3*) on synthetic complete or rich medium containing Ca^{+2} or Mg^{+2} (8,9). Thus the replacement of ergosterol by ergosta-8,14-dienol is not lethal in itself but requires an adequate level of these ions. Even though both the C-8 isomerase and C-14 reductase enzymatic reactions are sensitive to the morpholine antifungals, overexpression of the non-essential gene, *ERG2* does not lead to fenpropimorph resistance whereas overexpression of the essential gene, *ERG24* does (10). While *erg2* mutants have been generally isolated as non-essential in various genetic backgrounds, a single *erg2* mutant isolate in a FL100 wildtype genetic background was demonstrated to be lethal but suppressible by mutations in either the *ELO2* or *ELO3* gene (11,3).

Previously, we demonstrated that *ERG24* is an essential gene in a wildtype Y294 genetic background (10) but the *erg24* mutant strain produced by the deletion consortium project using the S288C wildtype genetic background clearly indicates that viable *erg24* mutants can occur in genetic backgrounds not containing the *ELO2* or *ELO3* mutations. In this study we demonstrate the various synthetic lethal interactions that can occur with an *erg24* strain derived from the deletion consortium genetic (S288C) background.

MATERIALS AND METHODS

Strains, media, and growth conditions.

Yeast strains used in this study are derived from W303; SCY325 strain (*MATa, ade2-1, his3-11,15, leu2-3,112, trp1-1, ura3-1*) and SCY328 (*MATa, ade2-1, his3-11,15, leu2-3,112, trp1-1, ura3-1*) are wildtype laboratory strains and were used to generate all ergosterol mutant strains other than *erg24*. The *erg24* strain was obtained from the deletion consortium (12) and the construction of other ergosterol deletion strains *erg2*, *erg3*, *erg4*, *erg5*, *erg6*, and *erg28* were as published previously (13). The deletion mutant strains *elo2* and *elo3* were generated by homologous recombination with PCR products (14) from plasmid containing selectable marker genes (15) and oligonucleotides containing gene specific sequences. The *elo2* and *elo3* strains derived in the W303 background were isolated as *elo2::TRP1* and *elo3::TRP1* disruptions using the forward and reverse primers (ELO2fwd: 5'-ATGAATTCACCTCGTTACTCAATATGCTGCTCCGTTGTTTCGAGCGTTATCCCCAAC TTCATTGGCGGGTGTCGGGGCTGGC-3' and ELO2rev: 5'-TAGGAACGTTTTCAAGTCAACGTTAACATACTCATTAACCTTTGCGGGAACAC CGCCGTTTGCCGATTTTCGGCCTATTG-3' for *elo3::TRP1* and ELO3fwd: 5'-AGCTTACTTCTAGTTTATTTATTCGGCTTTTTTCCGTTTGTTCGAAACATAAAC AGTCGGCGGGTGTCGGGGCTGGC-3' and ELO3rev: 5'-TAAGCAGCAGCAGCCTGAGTACCATAACAAGTACCCTTGTGTTGGTAAAATACCG TCCAAGTTGCCGATTTTCGGCCTATTG-3' for *elo3::TRP1*). Verification of the deletion was performed by PCR analysis using checking primers. Gene specific oligonucleotides were obtained from Invitrogen. Disruptions containing the kanamycin (G418) resistance marker were obtained from deletion consortium or isolated as segregants from different crosses. Yeast strains were grown in either YPAD (1% yeast extract, 2% bacto-peptone, 2% glucose, and .012% adenine sulfate) or in synthetic complete media (CSM) containing 0.67% yeast nitrogen base (Difco) supplemented with appropriate amino acids. Additional uracil, (.004%), leucine

(.02%), histidine (.004%), and tryptophan (.01%) were supplemented when required. CSM lacking specific nutrients was used for selection of various marker genes. Ergosterol, when used, was added to autoclaved media at 20 µg/ml to rescue synthetically lethal double mutant strains. For anaerobic growth, anaerobic jars containing the GasPaks system (BBL Microbiology System) were used. Sporulation media has been described previously (14). All solid media for growth of yeast contained 2% Bacto Agar (Difco).

Genetic methods

Mating, sporulation, and genetic dissections to obtain double and triple mutants were as described previously (14). Crosses and asci dissections of the four-spore meiotic products were done on YPAD solid media supplemented with 20 µg/ml ergosterol in a Tween 80/ethanol (1:1v/v) solution and grown anaerobically. Briefly, diploids grown on YPAD medium were transferred to a 1% potassium acetate medium to induce sporulation. After a 1 hr treatment with glucylase (Perkin Elmer Life science), the four spored-asci were dissected under a microscope using a micromanipulator. All meiotic segregants were analyzed by replica plating on YPAD and CSM plates with and without ergosterol for synthetic lethal screening and the genotype confirmed by genetic markers.

Sterol analysis

Sterols were isolated as described previously (16). Briefly, cells were grown for 20-24 hrs in YPAD liquid or solid media containing 20 µg/ml of cholesterol. Cells were pelleted, washed twice with Igepal (1%), and twice with distilled H₂O, saponified with alcoholic KOH and extracted in *n*-heptane. Sterols were preliminarily analyzed by gas chromatography (GC) with a Hewlett-Packard HP5890 series II chromatograph equipped with the HP CHEMSTATION software package. The DB-5 capillary column (15 m × 0.25 mm × 0.25 µm film thickness) with nitrogen as carrier gas (30 cm/sec) was programmed from 195° to 300°C (3 min at 195° C and then an increase at 5.5°C/min to 300°C and then held for 10 min). All injections were run in the splitless mode with an inlet temperature of 280°C. In order to identify novel sterols, gas-chromatography mass-spectrometry (GC-MS) analyses was also performed using a HP5890 GC coupled to a HP5972 mass selective detector. The GC separations were done on a fused silica column (DB-5 15 m × 0.32 mm × 0.25 m film thickness), programmed from 40° C to 300°C (40°C for 1 min, 30°C/min to 300°C and then held for 4 min). Helium was the carrier gas with a linear velocity of 50 cm/sec in the splitless mode. Mass spectra were generated in the electron impact mode at electron energy of 70 eV and an ion source temperature of 150° C. The instrument was programmed to scan from 40 to 700 amu at 1-s intervals.

RESULTS

Synthetic lethality of *erg2erg24*

The observations that both *erg2* and *erg24* mutants could be suppressed by *elo2* or *elo3* mutations and the further observation that *erg24* was lethal in some genetic backgrounds but not in others led us to ask whether the *erg2erg24* double mutant strain was viable. The *erg2erg24* double mutant was obtained by mating viable *erg2* and *erg24* haploid strains to produce diploid heterozygotes; diploids were sporulated, and the four-spored asci were dissected on rich medium containing ergosterol (or cholesterol) and grown anaerobically. From a total of 100 tetrads, 83 *erg2erg24* double mutants were obtained all of which could grow anaerobically with sterol supplement but failed to grow aerobically without sterol supplement (table 1). In fig. 1, wildtype, *erg2*, *erg24*, and *erg2erg24* strains were plated on both aerobic YPAD rich medium and synthetic complete medium (CSM). On YPAD medium, *erg24* strains grew but growth was diminished at lower inocula; on YPAD medium, *erg2erg24* growth was completely absent. However, on CSM medium both *erg2* and *erg24* strains grew normally but the *erg2erg24* strain demonstrated minimal growth indicating that it is not synthetically lethal

on this medium. In the presence of ergosterol (which requires anaerobic growth conditions for sterol uptake) all four strains grew equally well. On YPAD or CSM media supplemented with 10 or 20 mM CaCl_2 , the growth responses were the same as without calcium. Results with 20mM CaCl_2 (not shown) were exactly the same as for 10mM CaCl_2 (fig. 1).

Suppression of *erg2erg24*

Mutations in *ELO2* and *ELO3* are known suppressors of *erg2* or *erg24* mutants (6,7). We generated *elo2erg2erg24* and *elo3erg2erg24* triple mutant strains to determine whether either or both suppressed lethality of the *erg2erg24* strain. Fig. 2 demonstrates that only *elo3* suppressed *erg2erg24* lethality on YPAD medium as indicated by growth on this medium without ergosterol. However, on CSM medium neither *elo2* or *elo3* was found to be an efficient suppressor as there was only small residual growth on this medium without ergosterol. Again, CaCl_2 supplementation in YPAD or CSM media did not rescue the synthetic lethality of *erg2erg24*.

Sterol analysis of *erg2erg24* and *erg2erg24elo3*

We proceeded to determine the sterol composition of each of these strains to assess whether a sterol composition difference could be attributable to the suppressed *erg2erg24elo3* strain. Table 2 indicates the sterol composition of the wildtype, *elo3*, *erg2*, *erg24*, *erg2erg24*, *erg2elo3*, *erg24elo3*, and the suppressed *erg2erg24elo3* strains grown in YPAD media under aerobic conditions. Identification of sterols was based on molecular ions and key fragment ions as well as comparison to standard sterol spectra and relative retention times (Table 3). The *erg2erg24* strain was grown anaerobically in the presence of cholesterol. There was essentially no difference in sterol profiles between the wildtype and *elo3* strains. The *erg2erg24* strain accumulated ergosta-8,14-diene sterols as well as squalene, lanosterol, and the cholesterol which was provided in the growth medium. Sterol profiles from *erg24* and *erg2erg24elo3* strains were also similar in that 94% and 99% of the sterols accumulated in these two strains were ergosta-8,14, or cholesta-8,14 sterols, respectively, suggesting that the major effect of *elo3* suppression did not involve an alteration of sterols. For the most part, *erg2elo3* and *erg24elo3* reflected the sterol compositions of *erg2* and *erg24*, respectively.

Synthetic lethality of *erg24* in combination with other ergosterol biosynthetic mutants.

It was of interest to ascertain whether other ergosterol mutations such as *erg3*, *erg4*, *erg5*, *erg6*, and *erg28* would be synthetically lethal in combination with the *erg24* mutation. *ERG3* encodes the C-5 desaturase, *ERG4* encodes the C-24 reductase, *ERG5* encodes the C-22 desaturase, *ERG6* encodes the C-24 transmethylase; mutations in these genes are viable (fig. 3B). *ERG28* encodes a scaffold protein required for complete C-4 demethylation and it, too, is non-essential for growth. We crossed all single mutants with the *erg24* strain to generate the haploid double mutants. All tetrads were dissected and grown anaerobically with ergosterol and then grown aerobically without ergosterol to assess viability (fig.3). Genetic analyses (table 1) and spot-plating (fig.3) on YPAD medium demonstrated that the double mutant *erg4erg24* and *erg5erg24* were viable but the *erg6erg24* and *erg28erg24* double mutants were not. However, the *erg3erg24* double mutants was only weakly viable. The addition of CaCl_2 had a growth enhancing effect on *erg5erg24* on YPAD medium but not on CSM medium; on CSM medium, CaCl_2 also had a growth enhancing effect on *erg6erg24* and *erg28erg24*. Finally, *elo3* failed to suppress *erg6erg24* or *erg28erg24* on YPAD or CSM media (data not shown). The observation that *elo3* did not suppress *erg6erg24* is expected as Eisenkolb et al. (17) demonstrated that the double mutant *elo3erg6* is synthetically lethal.

DISCUSSION

The genes in the latter part of the yeast ergosterol pathway comprised of *ERG6*, *ERG2*, *ERG3*, *ERG4*, and *ERG5* are considered non-essential because the sterols produced by mutations of these genes function nearly as well as ergosterol and meet the biophysical and biochemical requirements of a sterol molecule. However, mutations in *ERG24* are lethal in some genetic backgrounds but not others.

The results presented here extend initial observations that the viability of *erg24* is dependent on factors such as type of media, metal ion concentrations, and strain background. Studies in Parks' lab (8,9) indicated that *erg24* could not grow on a rich media (YPAD) but could grow on a synthetic complete media due to a greater abundance of Ca^{+2} in the latter. Addition of Ca^{+2} to rich medium restored growth. Our studies confirm some of these findings but differ in that the deletion consortium *erg24* strain is viable on YPAD medium but growth is not enhanced with the addition of Ca^{+2} (fig.1). However growth is enhanced on CSM medium. The combination of *erg2* and *erg24*, however, resulted in lethality on YPAD medium that was not rescued by Ca^{+2} supplementation. Growth of the *erg2erg24* strain was marginal on synthetic complete medium. Suppression of this lethality was obtained by introducing a third mutation, *elo3*. The *elo3* mutation was previously found to individually suppress lethal isolates of *erg2* and *erg24*. However, while *elo3* was able to restore growth of the *erg2erg24* strain on YPAD medium, it failed to restore growth on CSM medium (fig. 2). Surprisingly *elo2* failed to suppress the lethality of *erg2erg24* on any media, even media containing Ca^{+2} supplementation.

We next asked the question whether other ergosterol mutants were lethal in combination with *erg24* and the results indicated that *erg6erg24* and *erg28erg24* were also inviable on rich YPAD medium and this lack of growth was not suppressed by Ca^{+2} supplementation. The double mutant *erg3erg24* grew poorly and only *erg4erg24* and to a lesser extent *erg5erg24* was clearly growing on YPAD. However, on synthetic complete medium, we observed slightly better growth in the presence of Ca^{+2} (fig.3). All double mutants grew at least to some degree in synthetic complete medium if Ca^{+2} was added to the medium.

Crowley et al. (9) demonstrated that the concentration of Ca^{+2} was greater in a synthetic complete than on rich YPD medium and that increased Ca^{+2} resulted in resistance to fenpropimorph suggesting that Ca^{+2} enhances membrane integrity in strains in which ergosterol is replaced by ergosta-8,14 sterol intermediates. It is not obvious why *erg2erg24* mutants are more compromised than *erg24* alone since *erg24* mutants are epistatic to *erg2* and thus accumulate only C-8 sterols. Thus, in an *erg24* strain there is no evidence of Erg2p activity as all accumulated sterols are C-8-sterols. Further work will determine whether there is a regulatory sterol defect that must be taken into consideration. However, in considering the synthetically lethality of *erg6erg24* and *erg28erg24* strains, each individual mutation should contribute to an altered sterol profile and thus may help to explain why the double mutants in these cases are lethal.

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Fig.1.

Synthetically lethality of the *erg2erg24* double mutant strain. Cells were serially diluted and 10⁵ to 10² cells were sequentially spotted onto YPAD (upper panels) or CSM (lower panels) media with or without ergosterol or with 10 mM CaCl₂ to compare the phenotype of strains. The strains tested were wild type, *erg2*, *erg24*, and *erg2erg24*. Plates containing ergosterol were incubated anaerobically and those without ergosterol aerobically for 3 days at 30°C. The *erg2erg24* double mutant strain was able to grow only in presence of ergosterol.

Fig.2.

The mutation *elo3* but not the *elo2* mutation suppresses the *erg2erg24* double mutant. The growth response of strains *elo2*, *erg2erg24elo2*, *elo3*, and *erg2erg24elo3* was compared by spotting serial dilutions of 10⁵ to 10² cells of each strain onto YPAD (upper panels) or CSM (lower panels) media with or without ergosterol or with 10 mM CaCl₂. Plates containing ergosterol were incubated anaerobically and those without ergosterol aerobically for 3 days at 30°C. The *erg2erg24elo3* triple mutant strain was able to grow on YPAD but not on CSM media without ergosterol or with 10 mM CaCl₂. The *erg2erg24elo2* triple mutant strain was unable to grow on YPAD and very poorly on CSM media.

Fig.3.

An *erg24* mutation is synthetically lethal with *erg6* or *erg28* but not with *erg3*, *erg4*, or *erg5*. (A) Growth of the *erg24* mutant in combination with additional mutations in the ergosterol biosynthetic pathway-*erg3*, *erg4*, *erg5*, *erg6*, and *erg28*. Cells from the double mutants were serially spotted onto YPAD (upper panels) or CSM (lower panels) media with or without ergosterol or with 10 mM CaCl₂. Plates containing ergosterol were incubated anaerobically and those without ergosterol aerobically for 3 days at 30°C. The *erg6erg24* and *erg28erg24* mutant strains did not grow on YPAD medium without ergosterol; the *erg3erg24* and *erg5erg24* mutant strains grew poorly; and the *erg4erg24* mutant strain grew well. All strains grew poorly or very poorly on CSM media without ergosterol or with 10 mM CaCl₂. (B) *erg3*, *erg4*, *erg5*, *erg6*, and *erg28* single mutant strains were viable on both YPAD and CSM media without ergosterol.

Table 1Genetic analysis to determine synthetic lethality of *erg24* double mutants in YPAD medium.

Mating	Tetrads Disected	Viable Double Mutants ^a	Inviabile Double Mutants ^a
<i>erg2 x erg24</i>	100	0	83
<i>erg3 x erg24</i>	20	17 ^b	0
<i>erg4 x erg24</i>	14	14	0
<i>erg5 x erg24</i>	22	18 ^b	0
<i>erg6 x erg24</i>	13	0	13
<i>erg28 x erg24</i>	21	0	16

^a double mutants are *erg24* in combination with either *erg2,erg3,erg4,erg5,erg6,or erg28*^b poorly viable

Table 2
Sterol analyses of *erg2erg24*, *erg2erg24elo3*, and related strains

Sterol	Percent Sterol							
	Wild type	<i>elo3</i>	<i>erg2</i>	<i>erg24</i>	<i>erg2 erg24^a</i>	<i>erg2 elo3</i>	<i>erg24 elo3</i>	<i>erg2 erg24 elo3</i>
Squalene	0.27	0.98	-	5.58	28.04	-	0.82	0.78
Cholesterol	-	-	-	-	47.84	-	-	-
Ergosta-5,8,22,24 (28) tetraen-3-ol	-	-	1.88	-	-	-	-	-
Zymosterol	18.73	18.46	1.30	-	-	-	-	-
Ergosterol	61.30	56.98	-	-	-	-	-	-
Ergosta-7,22 dien-3-ol	2.43	2.53	-	-	-	-	-	-
Ergosta-5,7,22,24 (28) tetraen-3-ol	1.59	1.97	-	-	-	-	-	-
Ergosta- 8,14,24 (28) trien-3-ol	-	-	-	25.20	-	-	40.50	26.67
Ergosta-5,8,22-trien-3-ol	-	-	31.31	-	-	38.67	-	-
Ergosta-8,14 dien-ol	-	-	-	56.44	14.00	-	37.51	63.40
Ergosta-8-en-3-ol	-	-	11.21	-	-	17.05	-	-
Fecosterol	6.93	7.67	51.55	-	-	37.31	-	-
Ergosta-8,14-dien-3-one	-	-	-	1.85	-	-	-	-
Episterol	6.12	6.75	-	-	-	-	-	-
Lanosterol	0.51	2.54	0.61	-	8.19 ^b	-	9.20 ^b	-
4,4 Dimethyl Cholesta-8,14,22-trien-3-ol	-	-	-	10.53	-	-	-	3.61
4,4-Dimethyl Zymosterol	0.78	0.40	1.11	-	-	-	-	-
Ergosta-8,22-dien-3-ol	-	-	-	-	-	3.75	-	-
Ergosta-dien-3-one	-	-	-	-	-	-	0.45	-
Ergosta-trien-3-one	-	-	-	-	-	-	0.31	-
Ergosta-5,8,14,22-tetraen-3-ol +Cholesta-8,14,22-trien-3-ol	-	-	-	-	-	-	9.71	5.06

^a grown anaerobically in the presence of cholesterol

^b consists of lanosterol and 4,4-dimethylcholesta-8,14,22-trien-3-ol

Table 3
GC/MS data for *erg2erg24*, *erg2erg24elo3*, and related strains

Sterol ^a	m/z (relative ion intensity)
Squalene	410 [M] (80), 367 (12), 341 (15), 136 (85), 123 (72), 121 (84), 109 (62), 107 (52), 95 (81), 93 (60), 81 (99), 69 (100)
Cholesterol	386 [M] (100), 371 (40), 368 (67), 353 (42), 301 (91), 275 (84), 255 (40), 231 (33), 213 (57), 145 (56)
Ergosta-5,8,22,24 (28)-tetraen-3-ol	394 [M] (29), 379 (5), 376 (18), 361 (100), 268 (43), 251 (92), 235 (66), 209 (46)
Ergosta-8,22-dien-3-ol	398 [M] (37), 383 (21), 380 (2) 299 (6), 273 (40), 271 (41), 255 (23), 246 (37), 69 (100)
Ergosta-5,8,14,22-tetraen-3-ol	394 [M] (42), 379 (5), 361 (100), 297 (8), 268 (28)
Cholesta-8,14,22-trien-3-ol	382 [M] (100), 367 (35), 349 (41), 297 (53), 270 (18), 255 (28), 218 (32)
Zymosterol	384 [M] (97), 369 (75), 366 (2), 351 (13), 273 (10), 271 (22), 231 (22), 229 (23), 213 (42), 69 (100)
Ergosterol	396 [M] (96), 381 (4), 378 (5), 363 (100), 337 (41), 271 (17), 253 (43), 211 (36), 157 (37) 143 (52)
Ergosta-7,22-dien-3-ol	398 [M] (37), 383 (13), 300 (16), 271 (100), 255 (32), 213(17)
Ergosta-5,7,22,24 (28)-tetraen-3-ol	394 [M] (49), 376 (7), 361 (78), 271 (18), 269 (29), 253 (46), 251 (58), 237 (41), 211 (45) 159 (43), 157 (57),143 (92) 81 (100)
Ergosta- 8,14,24 (28)-trien-3-ol	396 [M] (61), 381 (47), 378 (9), 363 (33), 353 (19), 311 (29), 297 (48), 270 (28), 269 (28), 255 (38), 55 (100)
Ergosta-8,14-dien-3-ol	398 [M] (100), 383 (49), 380 (2), 365 (13), 299 (2), 271 (15), 238 (11)
Fecosterol	398 [M] (100), 383 (60), 380 (3), 365 (12), 314 (6), 285 (18), 245 (23), 227 (34)
Ergosta-8-en-3-ol	400 [M] (100), 385 (47), 367 (7), 273 (35), 255 (17), 246 (13), 231 (22), 229 (31), 213 (36), 147 (35)
Ergosta-8,14-dien-3-one	396 [M] (100), 381 (34), 269 (56), 255 (37), 159 (32)
Episterol	398 [M] (33), 383 (12), 365 (18), 314 (29), 299 (7), 271 (100)
Lanosterol	426 [M] (58), 411 (87), 393 (47), 259 (21), 109 (61), 69 (100)
4,4 Dimethyl Cholesta-8,14,22-trien-3-ol	410 [M] (58), 395 (33), 377 (16), 325 (43), 313 (21), 298 (22), 283 (29), 246 (32), 69 (100)
4,4-Dimethyl Zymosterol	412 [M] (62), 397 (28), 394 (7), 379 (15), 299 (10), 259 (17), 135 (54), 69 (100)
4,4-Dimethyl Fecosterol	426 [M] (100), 411 (25), 408 (7), 393 (14), 342 (7), 327 (17), 299 (37), 281 (31),

^a Sterols are listed from lowest to highest retention times.