



Supplemental Figure 1. Sterol biosynthesis pathways in Eukarya as adapted from Bouvier F. et al. 2005. *Prog. Lipid Res.* **44**: 357-429 and Desmond E. and S. Gribaldo, 2010. *Genome Biol. Evol.* **1**: 364-381. Note- alternate routes can develop as reported by Fliesler, S. J. and L. Bretillon, *J. Lipid Res.* 2010. **51**: 3399-3413, and Porter, F. D. and G. E. Herman. 2011. *J. Lipid Res.* **52**: 6-34.

Supplemental Table 1. ¹HNMR signals of sterols isolated from *Chlamydomonas reinhardtii*

Sterol	Structure ^a	α_c ^b	H-18	H-19	H-21	H-26	H-27	H-28	H-29	Olefinic Protons
Ergosta-5,7,22E-trienol (C ₂₈)	14	0.90	0.632 (s)	0.948 (s)	1.038 (d)	0.844 (d)	0.821(d)	0.919(d)		H6, 5.573 (dd) H7, 5.380 (m) H22, 5.167 (d) H23, 5.235 (d)
Porifersta-5,7,22E-trienol (C ₂₉)	16	1.05	0.644 (s)	0.957 (s)	1.059 (d)	0.857 (d)	0.846 (d)		0.828 (t)	H6, 5.573 (dd) H7, 5.380 (m) H22, 5.167 (d) H23, 5.235 (d)
Ergosta-5,7-dienol (C ₂₈)	12	1.01	0.616 (s)	0.944 (s)	0.941 (d)	1.636 (s)	0.776 (s)	0.857 (d)	-	H6, 5.573 (dd) H7, 5.380 (m)
Porifersta-5,7-dienol (C ₂₉)	15	1.13	0.618 (s)	0.943 (s)	0.95 (d)	0.812 (d)	0.831 (d)		0.857 (t)	H6, 5.573 (dd) H7, 5.380 (m)
Ergosta-5,7, 25(27)- trienol (C ₂₈)	21	0.76 ^c	0.621 (s)	0.951 (s)	0.945 (d)	1.648 (s)	4.672 (brd)	1.006 (d)		H6, 5.573 (dd) H7, 5.380 (m) H27, 4.672 (brd)
Ergosta-7,25(27)-dienol (C ₂₈)	8	0.85	0.527 (s)	0.793 (s)	0.916 (d)	1.636 (s)	4.672 (brd)	0.993 (d)		H7, 5.155 (dd) H27, 4.672 (brd)
Porifersta-5,7,25(27)- trienol (C ₂₉)	23	0.85	0.612 (s)	0.942 (s)	0.939 (d)	1.567 (s)	4.672 (brs)		0.803 (t)	H6, 5.573 (dd) H7, 5.380 (m) H27, 4.672 (brd)
Porifertsa-5,7,22E,25(27)- tetraenol (C ₂₉)	24	0.76 ^c	0.63 (s)	0.945 (s)	1.038 (s)	1.645 (s)	4.694 (t) 4.709 (t)		0.835 (t)	H6, 5.569 (dd) H7, 5.387 (m) H22, 5.221 (m) H23, 5.221 (m)

^aStructures of sterols are shown in Figure 7. ¹HNMR was performed at 500 MHz with samples referenced to chloroform δ 7.265ppm; s, singlet, d, doublet, brd, broad doublet, m, multiplet. ^bThe α_c values correspond to the chromatographic times of the C24-alkylated sterol relative to cholesterol in HPLC. ^cCompounds first collected on the Luna C₁₈- column then purified using the TSK-Gel C₁₈- column as described in Methods.