Electronic Supporting Information

for

Enzymatic cyclizations of squalene analogs with *threo*- and *erythro*-diols at the 6, 7- or 10, 11-positions by recombinant squalene cyclase. Trapping of carbocation intermediates and mechanistic insights into the product and substrate specificities.

Takamasa Abe and Tsutomu Hoshino*

Department of Applied Biological Chemistry, Faculty of Agriculture, and Graduate School of Science and Technology, Niigata University, Ikarashi, Niigata 950-2181, Japan. Fax: +81-25-262-6854; E-mail: hoshitsu@agr.niigata-u.ac.jp

1. NMR data of substrate analogs 15-18.

Threo-**16** and **18**; δ_H ($CDCl_3$, 400 MHz): 1.11 (3H, s, Me), 1.33-1.61 (4H, m, 2 CH_2) 1.60 (9H, s, 3 Me), 1.62 (6H, s, 2 Me), 1.68 (6H, s, 2 Me), 1.95-2.12 (15H, m, 7 CH_2 and 1/2 CH_2), 2.22 (1H, m, 1/2 CH_2), 3.41 (1H, d, *J* 10.4), 5.00-5.15 (4H, m), 5.22 (1H, very broad, triplet like); δ_C ($CDCl_3$, 100 MHz): 15.96 (2C, q), 16.02 (q), 20.95 (q), 17.65 (2C, q), 20.95 (q), 21.98 (t), 25.68 (2C, q), 26.60 (t), 26.72 (t), 28.16 (t), 28.24 (t), 29.50 (t), 36.83 (t), 38.73 (t), 39.70 (2C, t), 74.84 (s), 76.82 (d), 124.1 (d), 124.2 (d), 124.3 (2C, d), 125.1 (d), 131.2 (s), 131.9 (s), 134.9 (s), 135.3 (s). *Threo*-**15** and **17**; δ_H ($CDCl_3$, 400 MHz): 1.11 (3H, s, Me), 1.30-1.56, (4H, m, 2 CH_2), 1.60 (9H, S, 3 Me), 1.62 (3H, s, Me), 1.64 (3H, s), 1.68 (6H, s, 2 Me), 1.93-2.14 (15H, m, 7 CH_2 and 1/2 CH_2), 2.22 (1H, m, 1/2 CH_2), 3.44 (1H, bd, *J* 10.4), 5.05-5.19 (5H, m); δ_C ($CDCl_3$, 100 MHz): 15.99 (2C, q), 16.07 (q), 17.68 (2C, q), 20.89 (q), 21.90 (t), 25.06 (t), 25.69 (2C, q), 26.60 (2C, t), 25.73 (t), 31.41 (t), 38.73 (t), 39.68 (t), 39.70 (t), 39.74 (t), 74.98(s), 76.75 (d), 123.8 (d), 124.1 (d), 124.2 (2C, d), 124.3 (d), 131.3 (s), 131.5 (s), 135.1 (s), 135.7 (s), 136.2 (s).

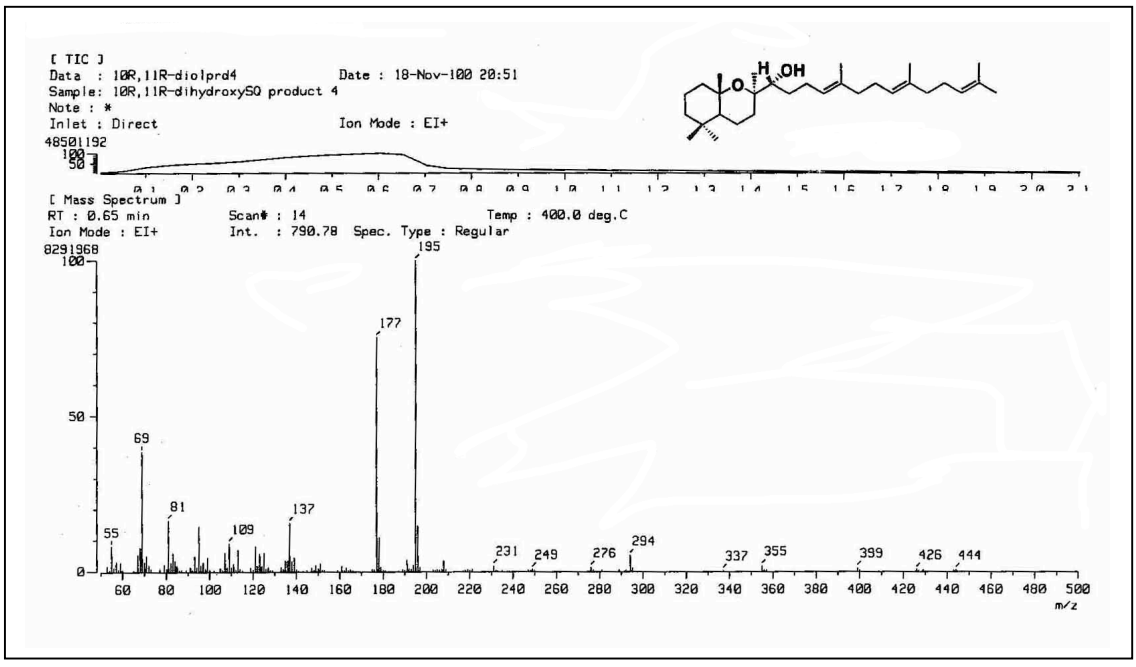
2. NMR data for squalene diols of the racemic mixture of (6*R*, 7*S*)-21 and (6*S*, 7*R*)-22 and that of (10*R*, 11*S*)-19 and (10*S*, 11*R*)-20

(6*R*, 7*S*)-21 and (6*S*, 7*R*)-22: δ_H (C_6D_6, 600 MHz): 1.17 (3H,s), 1.46 (1H, m, 1/2 CH_2), 1.57 (1H, m, 1/2CH_2), 1.69 (3H, s), 1.70 (1H, m, 1/2CH_2), 1.71 (3H, s), 1.73 (9H, s, 3Me), 1.79 (3H, s), 1.80 (3H, s), 1.81 (1H, m, 1/2CH_2), 2.17-2.34 (14H, m, 7 CH_2), 2.38 (1H, m, 1/2 CH_2), 2.43 (1H, m, 1/2 CH_2), 3.41 (bd, *J* 10.3), 53.5 (2H, m), 5.41 (2H, m), 5.47 (1H, broad, triplet like); δ_C (C_6D_6, 150 MHz): 16.11 (q), 16.17 (q), 17.67 (q), 17.72 (q), 22.54 (t), 23.47 (q), 25.82 (2C, q), 27.09 (t), 27.23 (t), 28.69 (t), 28.73 (t), 29.87 (t), 36.57 (t), 37.38 (t), 40.20 (2C, t), 74.35 (s), 78.39 (d), 124.7 (d), 124.8 (d), 124.9 (d), 125.2 (d), 125.6 (d), 131.1 (s), 131.2 (s), 135.0 (s), 135.3 (s), 135.5 (s).

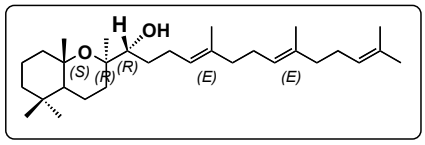
(10*R*, 11*S*)-19 and (10*S*, 11*R*)-20:δ_H (C_6D_6, 400 MHz): 1.15 (3H, s), 1.40-1.66 (3H, m, CH_2 and 1/2 CH_2), 1.69 (6H, s, 2Me), 1.73 (3H, s), 1.75 (3H, s), 1.77 (3H, s), 1.81 (6H, s, 2Me), 1.84 (1H, m, 1/2 CH_2), 2.15-2.35 (14H, m, 7 CH_2), 2.37 (1H, m), 2.44 (1H, m), 3.41 (bd, *J* 10.0), 5.38 (5H, m); δ_C ($CDCl_3$, 100 MHz): 16.05 (q), 16.11 (q), 16.12 (q), 17.73 (2C, q), 22.43 (t), 23.51 (q), 25.64 (t), 25.85 (2C, q), 27.05 (t), 27.12 (t), 27.20 (t), 31.70 (t), 36.39 (t), 40.20 (3C, t), 74.32 (s), 78.30 (d), 124.7 (d), 124.8 (2C, d), 124.90 (d), 125.4 (d), 131.14 (s), 131.3 (s), 135.1 (2C, s), 135.7 (s).

3. EIMS spectra and NMR analyses of all the enzymic products 23-36.

Product 23

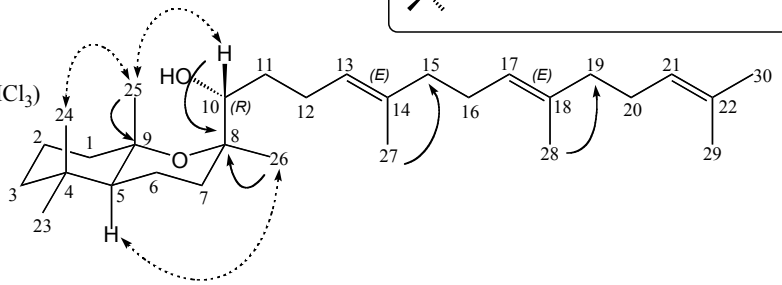


Product **23** (oil) from (10*R*,11*R*)-10,11-DihydroxySQ



$[\alpha]_D^{25} = +26.1 (c\ 0.067, CHCl_3)$

HRFABMS(glycerol)
M+H, Found: 445.4122
Calcd: 445.4046

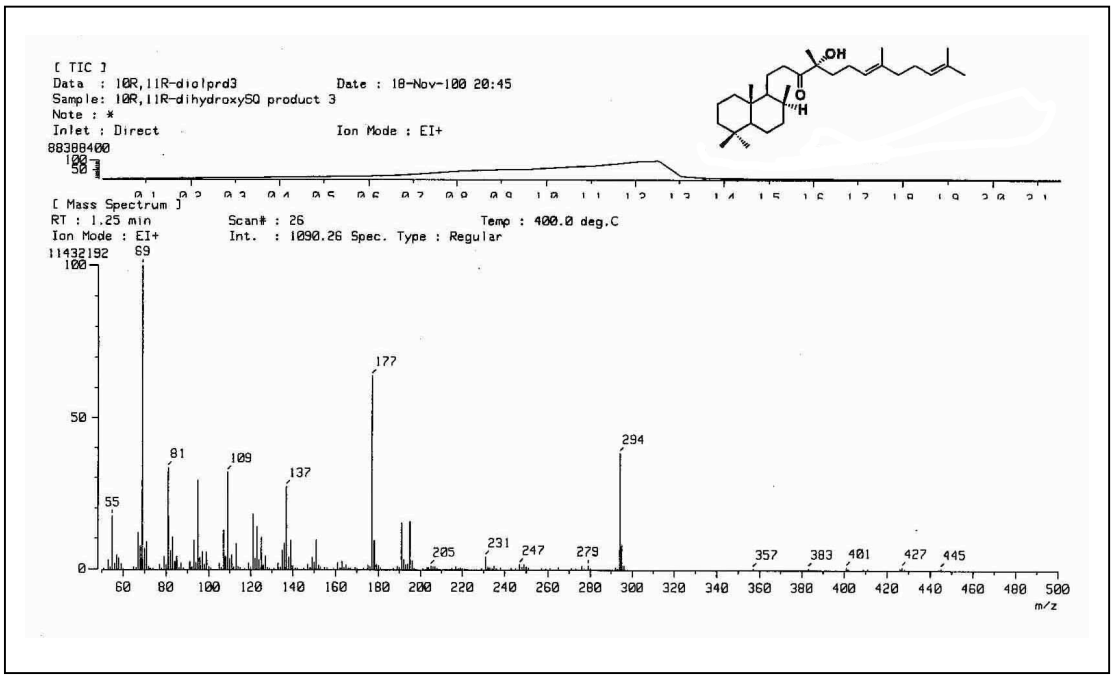


Major HMBC
Major NOE

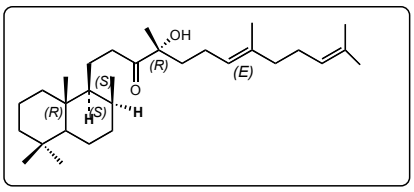
NMR data in C_6D_6, solvent peak : ^1H; 7.28 ppm , ^{13}C; 128.0 ppm

NO.	^1H	^{13}C	NO.	^1H	^{13}C	NO.	^1H	^{13}C	NO.	^1H	^{13}C
1	1.48(m) ; 1.75(m)	**42.54**	9	—	**75.39**	17	5.44 (t, 6.4Hz)	**124.88**	25	1.324 (3H,s)	**23.57**
2	1.34 (m); 1.48(m)	**20.34**	10	3.81 (d, 10.0Hz)	**75.68**	18	—	**134.95**	26	1.286 (3H, s)	**24.29**
3	1.21(dd, 12.8Hz, 4.8Hz) ; 1.34 (m)	**41.72**	11	1.50(m) ; 1.67(m)	**32.52**	19	2.21 (2H,t, 7.2))	**40.33****	27	1.843 (3H, s)	**16.19**
4	—	**33.54**	12	2.51(m) ; 2.72(m)	**26.08**	20	2.27 (2H,t, 7.2))	**27.30***	28	1.743 (3H,s)	**16.11**
5	1.43(1H, m)	**51.57**	13	5.51 (t, 7.0Hz)	**125.18**	21	5.37 (t, 6.4Hz)	**124.93**	29	1.691 (3H, s)	**17.72**
6	1.39 (m); 1.50(m)	**16.07**	14	—	**135.58**	22	—	**131.1**	30	1.807 (3H,s)	**25.84**
7	1.49 (m); 1.89(m)	**31.87**	15	2.22 (2H, t, 7.2)	**40.21****	23	0.903 (3H, s)	**32.14**			
8	—	**76.03**	16	2.33 (2H, t, 7.2)	**27.23***	24	0.775 (3H, s)	**20.68**			

Product 24

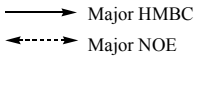


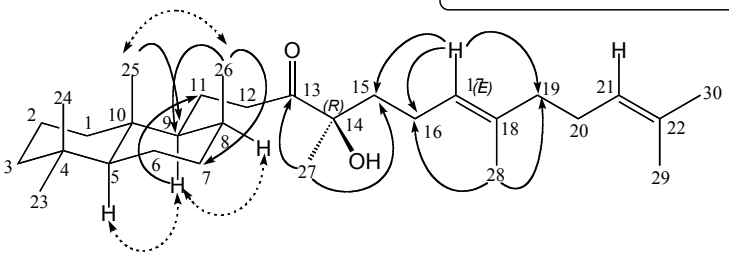
Product **24** (oil) from (10*R*,11*R*)-10,11-DihydroxySQ



$[\alpha]_D^{25} = +8.18$
(c = 0.275, $CHCl_3$)

HRFABMS (glycerol)
M+H, Found: 445.4127
Calcd: 445.4046





NMR data in C_6D_6, solvent peak : ^1H; 7.28 ppm , ^{13}C; 128.0 ppm

NO.	^1H	^{13}C	NO.	^1H	^{13}C	NO.	^1H	^{13}C	NO.	^1H	^{13}C
1	0.93(m); 1.80(m)	**39.72**	9	1.12 (m)	**53.03**	17	5.31(t, 6.8Hz)	**124.07**	25	0.926 (3H, s)	**16.45**
2	1.41(m); 1.55(m)	**18.76**	10	—	**38.75**	18	—	**135.90**	26	0.975 (d,7.2Hz)	**15.44**
3	1.25 (ddd, 12.8, 12.8, 3.6); 1.47(m)	**42.34**	11	1.65(m); 2.03(m)	**20.52**	19	2.18 (2H, t, 6.8Hz)	**40.04**	27	1.320 (3H, s)	**25.75**
4		**33.45**	12	2.30 (m); 2.43(m)	**34.45**	20	2.29 (2H,t, 6.8Hz)	**27.08**	28	1.701 (3H, s)	**16.04**
5	0.905(m)	**56.87**	13	—	**214.25**	21	5.34(t, 6.8Hz)	**124.78**	29	1.685 (3H, s)	**17.71**
6	1.47(m); 1.64(m)	**17.76**	14	—	**78.61**	22	—	**131.25**	30	1.803 (3H, s)	**25.82**
7	1.64(m); 1.75(m)	**34.99**	15	1.86(m); 2.26(m)	**40.16**	23	1.01 (3H, s)	**33.70**			
8	—	**29.82**	16	2.07(m) ; 2.39(m)	**22.70**	24	0.95 (3H, s)	**21.79**			

Product 25