Supplementary Material (ESI) for Organic and Biomolecular Chemistry

Electronic Supplementary Information (ESI)

Production of epoxydammaranes by the enzymatic reactions of (3*R*)- and (3*S*)-2,3-squalene diols and 2,3:22,23-dioxidosqualenes with recombinant squalene cyclase. Mechanistic insight into the polycyclization reactions and evidence for the intermediacy of the 17-*epi*-dammarenyl cation

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1. Analytical methods

NMR spectra were recorded in C_6D_6 on a Bruker DMX 600 or DPX 400 spectrometer, the chemical shifts being relative to the solvent peak δ_H 7.280 and δ_C128.0 ppm as the internal reference for ^{1}H- and ^{13}C NMR spectra, respectively. NMR data of some compounds are measured in acetone-d_6 or in $CDCl_3$. The chemical shifts were given relative to the solvent peaks: δ_H 2.040 and δ_C 29.8 ppm for acetone d_6. In the case of the $CDCl_3$ solutions, the chemical shifts given to TMS peak (0.00 ppm). The coupling constants *J* are given in Hz. GC analyses were done on a Shimadzu GC-8A chromatograph equipped with a flame ionization detector (DB-1 capillary column (0.53 mm × 30 m). GC-MS spectra were on a JEOL SX 100 spectrometer under electronic impact at 70 eV with a DB-1 capillary column (0.32 mm x 30 m). Isolation of some products was performed by a Hitachi LC system equipped with L-7100 pump and L-7405 UV detector, the product elution being monitored at 210 nm. HR-EIMS was performed by direct inlet system. Specific rotation values were measured at 25°C with a Horiba SEPA-300 polarimeter.

2. Incubation conditions, GC and HPLC analyses

Standard culture of *E. coli* and incubation conditions were performed according to our published protocols. [1] The cell-free extract was prepared as follows. One liter culture of *E. coli* encoding the native SHC was harvested by centrifugation and to the collected pellets was added 50 cm^3 of citrate buffer solution (pH 6.0, 50 mM), and then subjected to ultrasonication to disrupt the cells. The supernatant was used for the incubations after removing the cell debris by centrifugation. One cm^3 of the supernatant contains *ca.* 200 μg of the pure SHC. One mg of the substrate analogs and 20 mg of Triton X-100 were emulsified with 1.0 ml of distilled water and 3.0 ml of Na-citrate buffer solution (pH 6.0, 0.5 M). To the solution was added 2.0 ml of the cell-free extract and then incubated at 60°C for 12 h. To terminate the enzyme reaction, 6 ml of 15%KOH/MeOH was added and heated at 80°C for 30 min. The lipophilic enzymic products and the substrate analogs, which remained unreacted, were extracted four times with hexane (5 ml) from the incubation mixtures, and the quantities of the products and the starting materials were estimated by GC analyses with a DB-1 capillary column (30 m in length, J & W Scientific, USA). GC conditions: injection temp. 280°C, column temp., 280°C and carrier pressure, 1.0 Kg/cm^2. In the case of diepoxides **20**-**22**, the product distribution was estimated after acetylation with Ac_2O/Py at room temperature. The GC condition was the same as that of

the diol. HPLC conditions: a Mightysil Si 60 (5 μm) column and detection at 210 nm.

3. Syntheses of (3*R*)- 18and (3*S*)-2,3-oxidosqualene 19

These compounds were synthesized the according published methods.[2] Treatment of the chiral ligand of $(DHQ)_2PHAL$ with **1** gave **14**, **16** and **18**, while that of $(DHQD)_2PHAL$ afforded **15**, **17** and **19**. Separation of **18** and **19** from other diols was easily done by a SiO_2 column chromatography eluting with hexane: EtOAc (100: 15), because the R_f value of **18** or **19** was relatively lower than those of **14**-**17**. ^{1}H NMR (C_6D_6, 400 MHz); δ_H 5.41 (m, 5H), 3.33 (bd, *J* 10.4, 1H), 2.43 (m, 1H), 2.3-2.1 (m, 17H), 1.98 (bs, 1H, O*H*), 1.81 (s, 3H), 1.74(s, 3H), 1.73 (s, 6H), 1.71 (s, 3H), 1.69 (s, 3H), 1.62-1.45 (m, 2H), 1.152 (s, 3H), 1.145 (s, 3H). ^{13}C NMR (C_6D_6, 100.6 MHz); δ_H 135.25 (s), 135.19 (s), 135.06 (s), 134.96 (s), 131.09 (s), 125.09 (d), 124.97 (d), 124.93 (d), 124.82 (d), 124.79 (d), 78.26 (d), 72.53 (s), 40.20 (2C, t), 40.16 (t), 37.22 (t), 30.12 (t), 28.73 (2C, t), 27.21 (t), 27.09 (t), 27.00 (t), 26.38 (q), 25.84 (q), 23.55 (q), 17.72 (q), 16.16 (q), 16.10 (2C, q), 16.03 (q). The NMR data of **18** was superimposable on that of **19**, because of enantiomeric relationship between them. EIMS of **18** and **19** (%); *m/z* 69 (46), 81 (100), 95 (43), 109 (37), 135 (37), 153 (42), 411 (3); 426 (4), 444 (M^+, 2). The specific rotations, $[\alpha]_D^{25}$, **18** and **19** were +10.9° (*c* 2.1, $CHCl_3$, lit.[3, 4] +10.7 or + 11.4) and -10.7 (*c* 2.2, $CHCl_3$ lit.[3, 4] -10.7 or -11.8), respectively, indicating that the enantiomeric excess of **18** and **19** was close to 100%.

4. Preparation of 2,3:22,23-dioxidosqualene 20-22

The three-necked flask containing **1** (1.0 g) dissolved in 50 cm^3 of THF was cooled at 0°C, and water was added under N_2 atmosphere until the solution became slightly cloudy. To the flask was added NBS (*N*-Bromosuccinimide, 1 g) in a portion and stirred for 3 h. The reaction mixture was poured into ice water. The product and unreacted **1** were extracted with hexane (50 ml x 3) and then dried over anhydrous Na_2SO_4. The desired dibromohydrin of **1** was purified with SiO_2 column chromatography eluting with hexane:EtOAc (100:2~100:10), affording 270 mg (18% yield). The stirred solution of K_2CO_3 (204 mg) in MeOH (30 cm^3) was added in a portion to the dibromohydrin for 2 h, and then poured into ice water. The desired oxidosqualene was extracted with hexane and subjected to a SiO_2 column chromatography with hexane:EtOAc (100:1~100:3) to give diastereomers **20**-**22** in a yield of 180 mg (90 %). ^{1}H NMR (C_6D_6, 600 MHz); δ_H 5.39 (br s, 2H), 5.34 (br t, *J* 6.6, 2H), 2.77(t, *J* 6.1, 2H), 2.30-2.15(m, 8H), 1.75-1.6 (m, 2H), 1.71 (s, 6H), 1.65 (s, 6H), 1.26 (s, 6H), 1.22 (s, 6H). ^{13}C NMR (C_6D_6, 150.9 MHz); δ_H 135.0 (s,

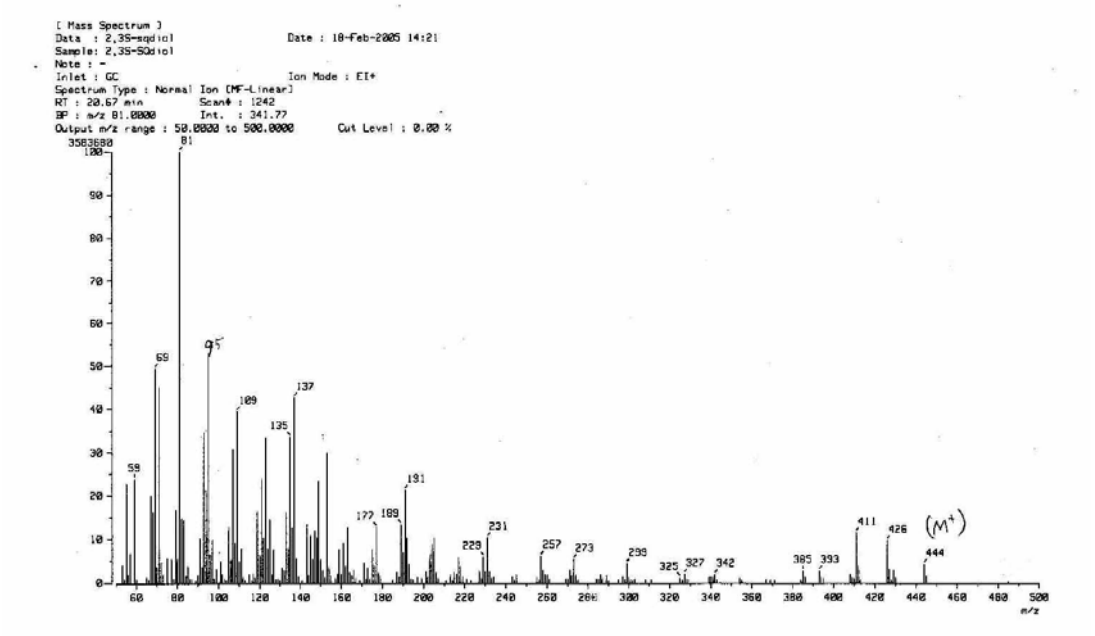
2C), 134.3(s, 2C), 125.1 (d, 2C), 124.9 (d, 2C), 63.45 (d, 2c), 57.29 (s, 2C), 40.11 (t, 2C), 36.8 (t, 2C), 28.71 (t, 2C), 28.00 (t, 2C), 27.04 (t, 2C), 24.98 (q, 2C), 18.90 (q, 2C), 16.15 (q, 2C), 16.06 (q, 2C). EIMS (%): *m/z* 69 (45), 81 (95), 93 (92), 107 (68), 135 (100), 203 (32), 406 (15), 424 (20), 442 (M^+, 2).

5. Purification methods of the enzymic products 31-42

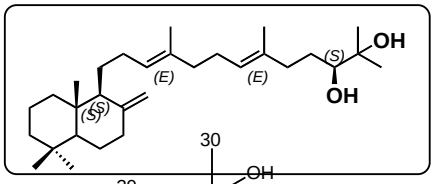
The reaction mixture of **18**, obtained by the large scale incubation, was subjected to a SiO_2 column chromatography eluting with hexane: EtOAc (100:3~100:20) to afford three fractions with low, medium and high polarity, the R_f values on SiO_2 TLC being 0.65, 0.42 and 0.18 [hexane: EtOAc (100:20)], respectively. To obtain pure **33**, the low polar fraction containing **33** was subjected to HPLC (hexane: 2-PrOH=100:0.15). A repetitive SiO_2 column chromatography (hexane: EtOAc=100:1) of the medium polar fraction afforded **34** in a pure state. Separation of high polar products **31** and **32** was done by 5% $AgNO_3$-impregnated SiO_2 column chromatography (hexane: EtOAc= 100:10~100:15), **31** being followed by **32**, then each fraction was subjected to normal phase HPLC with *n*-hexane:2-PrOH (100:0.8) gave pure **31** and **32**. Isolation of products **35**-**38** from diol **19** was carried out in a similar way as that of the products from **18**. The reaction mixture of **19** was divided into two fractions (low and high polar products) by a SiO_2 column chromatography [hexane: EtOAc (100:3~100:20)]. **37** and **38** included in the low polar fraction were separated by the repetitive SiO_2 column chromatography [hexane: EtOAc=100:2], the R_f values of **37** and **38** being 0.70 and 0.58 on a SiO_2 TLC, respectively. Separation of **35** and **36** included in a high polar fraction was achieved by 5% $AgNO_3$-SiO_2 column chromatography in a similar way as that of **31** and **32**. Products **39**-**42** from diastereomeric mixture **20**-**22** were isolated as follows. After removing an excess of Triton X-100 by passing a short SiO_2 column, the residues were chromatographed over SiO_2 eluting with hexane-EtOAc (100:2). The purified fraction was nearly homogeneous on SiO_2 TLC. However, normal phase HPLC analysis of the acetylated products [hexane: 2-PrOH (100:0.4)], prepared with Ac_2O/Py, revealed that four products **39**-**42** were mixed. Each of the products was isolated by HPLC as shown in Fig. 4B.

6. Spectroscopic data of all the isolated products.

Product 31



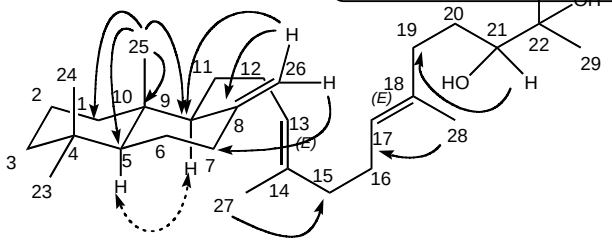
Product **31** (oil) from diol **18**



$[\alpha]_D^{25}$=+79.6 (EtOH)
c=0.40HREIMS: *m/z* (M^+), calcd. for $C_{30}H_{52}O_2$, 444.3967; found, 444.3972.

HMBC

NOE



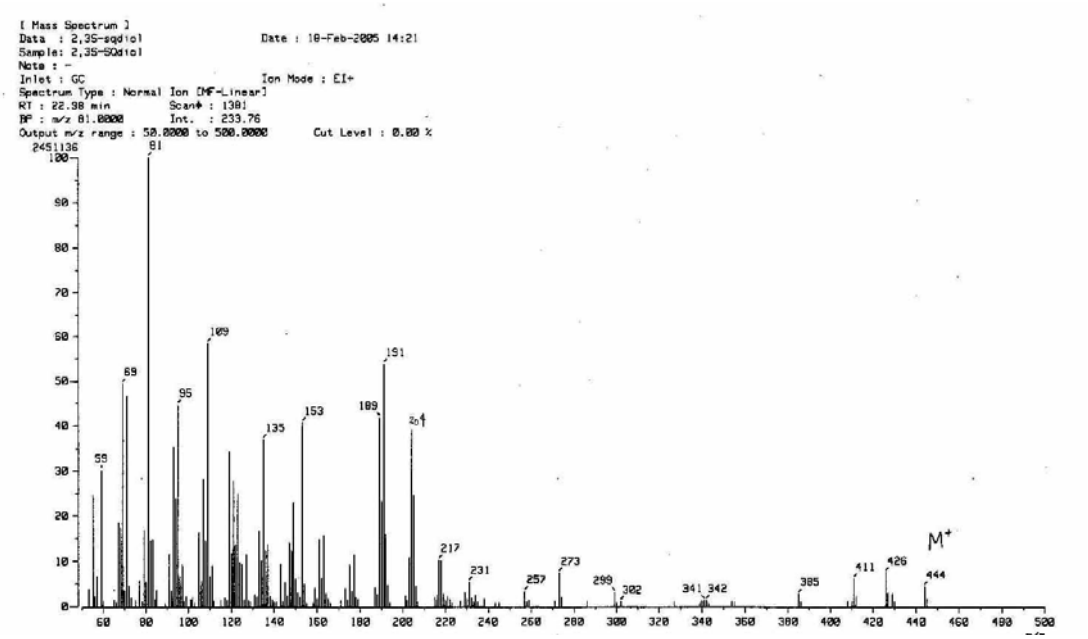
600 MHz

NMR data, δppm, in C_6D_6 relative to C_6D_6 : ^{1}H ; 7.28ppm, ^{13}C; 128.0ppm

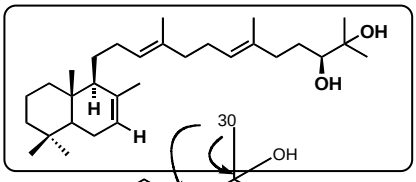
NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C
1	1.08 (m); 1.83(m)	**39.31**	9	1.78 (m)	**56.52**	17	5.46 (br s)	**125.13**	25	0.868 (3H, s)	**14.75**
2	1.55 (m); 1.66(m)	**19.76**	10	—	**39.84**	18	—	**135.29**	26	4.84 (br s); 5.10(br s)	**106.66**
3	1.27 (m); 1.49(m)	**42.43**	11	1.62(m) ; 1.69(m)	**24.24**	19	2.21(m) ; 2.44(m)	**37.24**	27	1.770 (3H, s)	**16.16**
4	—	**33.66**	12	2.15(m) ; 2.45(m)	**27.43**	20	1.50(m) ; 1.61(m)	**30.13**	28	1.725 (3H, s)	**16.06**
5	1.14(m)	**55.65**	13	5.46 (br s)	**125.80**	21	3.35 (dd, 10.4, 1.8Hz)	**78.28**	29	1.148 (3H, s)	**26.40**
6	1.41(m) ; 1.75(m)	**24.76**	14	—	**134.95**	22	—	**72.56**	30	1.154 (3H, s)	**23.58**
7	2.11(m); 2.52(m)	**38.73**	15	2.26 (2H, t, 7.8Hz)	**40.21**	23	0.965 (3H,s)	**33.72**			
8	—	**148.87**	16	2.34 (2H, dt, 7.6, 7.6Hz)	**27.13**	24	0.921 (3H, s)	**21.89**			

The assignments of H-29, H-30, C-29 and C-30 are exchangeable.

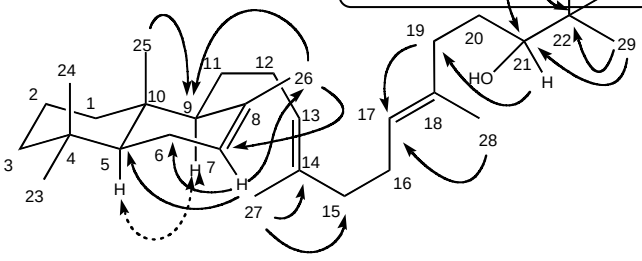
Product 32



Product 32 (oil) from diol 18



$[\alpha]_D^{25}$=-33.3 (EtOH), *c*=0.41
HRMS M$^+$, calcd. for $C_{30}H_{52}O_2$, 444.3967; found,444.3966.



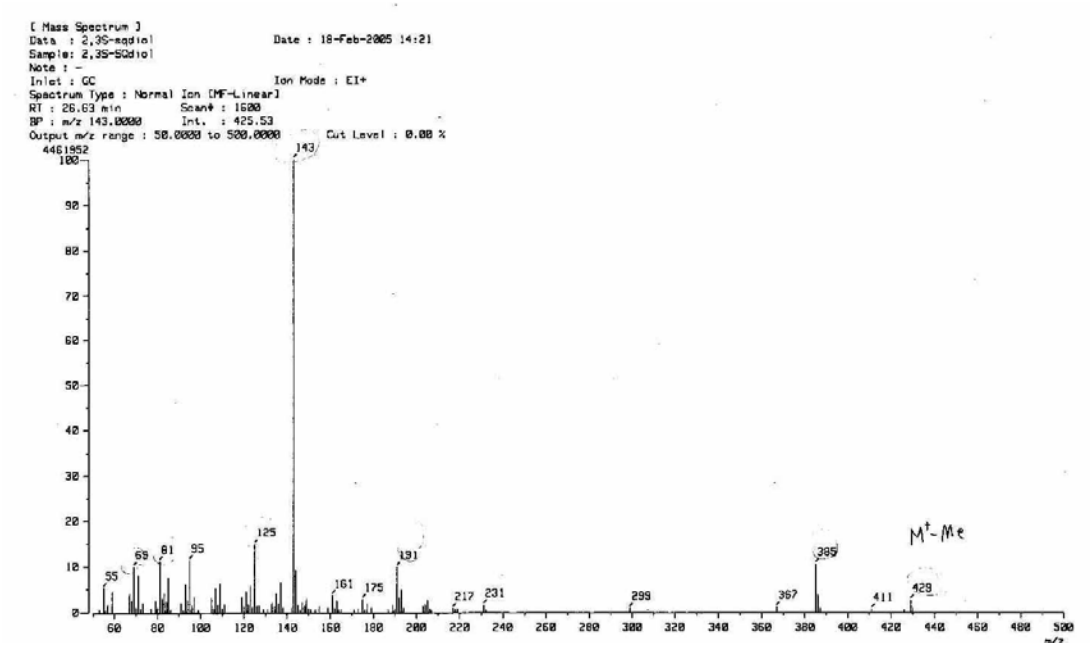
600 MHz

NMR data, δppm in C_6D_6 relative to C_6D_6: ^{1}H ; 7.28ppm , ^{13}C ; 128.0ppm

NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C
1	1.08(m) ; 1.98(m)	**39.49**	9	1.79(m)	**54.57**	17	5.46 (bt, 6.8Hz)	**125.07**	25	0.955 (3H, s)	**13.77**
2	1.55(m) ; 1.65(m)	**19.21**	10	—	**37.02**	18	—	**135.34** *a*	26	1.916 (3H, s)	**22.46**
3	1.27(ddd, 14,13.9, 3.9Hz) ; 1.51(m)	**42.64**	11	1.44(m) ; 1.65(m)	**27.74**	19	2.21(m) ; 2.45(m)	**37.23**	27	1.773 (3H, s)	**16.24**
4	—	**33.08**	12	2.21(m) ; 2.42(m)	**30.76**	20	1.50(m) ; 1.61(m)	**30.14**	28	1.731 (3H, s)	**16.06**
5	1.32(dd, 11.9, 4.9Hz)	**50.37**	13	5.45 (bt, 6.8Hz)	**125.52**	21	3.34 (bd, 9.5Hz)	**78.28**	29	1.145 (3H, s) *b*	**26.39** *c*
6	1.99(m) ; 2.06(m)	**24.20**	14	—	**134.89**	22	—	**72.54**	30	1.150 (3H, s) *b*	**23.58** *c*
7	5.58(bs)	**122.54**	15	2.25 (2H, t, 7.4 Hz)	**40.17**	23	0.980 (3H, s)	**33.35**			
8	—	**135.39** *a*	16	2.34 (2H,m)	**27.09**	24	1.000 (3H, s)	**22.03**			

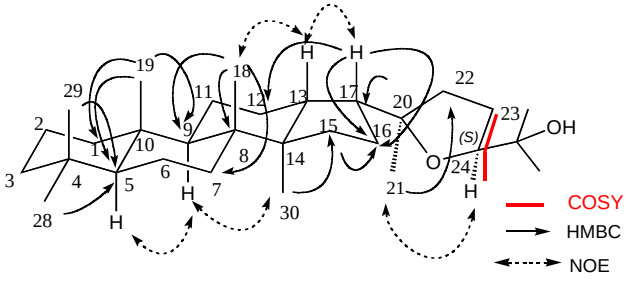
The signals of a~c are exchangeable in respect to the same latters.

Product 33



*NMR data of **33** in C_6D_6.*

Product 33 from 2,3S-diol 18 in C_6D_6



$[\alpha]_D^{25}$=-72.5 (EtOH)
c=0.090

HREIMS: *m/z* (M^+-Me), calcd. for $C_{29}H_{49}O_2$, 429.3733; found, 429.3730.

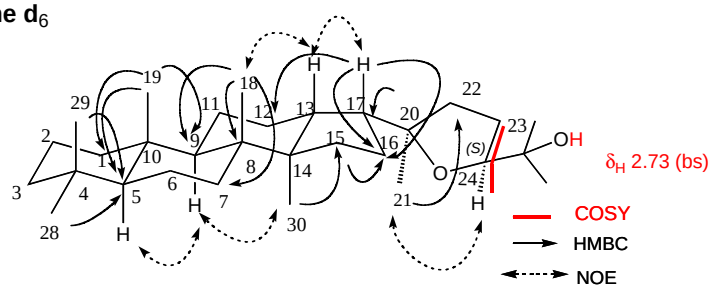
600 MHz NMR data in C_6D_6 (the solvent peak; δ_H 7.28, δ_C128.0 ppm)

NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C
1	0.87 (m); 1.75(m)	40.81	9	1.47 (m)	51.15	17	2.16(m)	48.66	25	—	71.28
2	1.38(m); 1.50(m) c	19.12 b	10	—	37.69	18	1.15 (3H,s)	16.13	26	1.276(3H,s) d	25.29 a
3	1.30 (m); 1.52(m)	43.81	11	1.20(m); 1.66(m)	22.65	19	1.01 (3H,s)	16.47	27	1.448 (3H,s) d	27.78 a
4	—	33.54	12	1.549(m);1.93(m)	26.21	20	—	85.31	28	1.039 (3H, s)	33.63
5	0.919 (bd, 12.5Hz)	57.29 b	13	2.07 (m)	43.81	21	1.273 (3H,s)	25.23 a	29	0.998 (3H,s)	21.76
6	1.50 (m); 1.66(m) c	19.08 b	14	—	49.43	22	1.48 (m); 1.74 (m)	38.22	30	1.151(3H,s)	17.20
7	1.38 (m); 1.68 (m)	35.63	15	1.22(m); 1.58 (m)	32.72	23	1.74(m); 1.91(m)	25.79			
8	—	41.17	16	1.61(m); 1.82(m)	27.08	24	3.74(t, 7.3, 7.3)	83.98			

The signals of a~d are exchangeable in respect to the same letters.

*NMR data of **33** in Acetone d_6*

Product 33 from 2,3*S*-diol 18
in acetone d_6



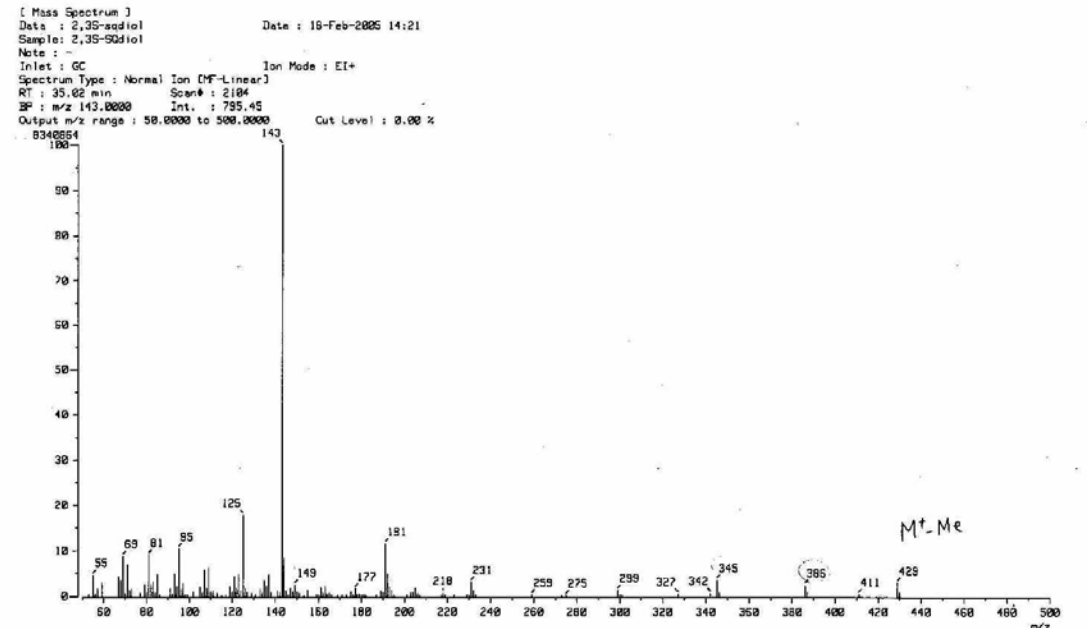
600 MHz

NMR data in C_6D_6 (the solvent peak; δ_H 2.04, δ_C 29.80 ppm)

NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C
1	0.87(m); 1.66(m)	**41.32**	**9**	1.43(dd,12.8, 3.0)	**51.70**	**17**	2.25 (m)	**49.20**	**25**	—	**71.62**
2	1.36(m); 1.50(m) [a]	**19.44** [b]	**10**	—	**38.17**	**18**	0.967(3H,s)	**16.29**	**26**	1.098(3H,s) [c]	**26.66** [d]
3	1.16(m);1.35(m)	**42.87**	**11**	1.20(m);1.58(m)	**23.09**	**19**	0.859(3H, s)	**16.62**	**27**	1.128(3H,s) [c]	**26.33** [d]
4	—	**33.94**	**12**	1.45(m);1.90(m)	**26.66**	**20**	—	**85.75**	**28**	0.848(3H, s)	**33.75**
5	0.82(m)	**57.75**	**13**	2.10(m)	**44.36**	**21**	1.183(3H,s)	**25.21**	**29**	0.815(3H,s)	**21.83**
6	1.42(m);1.63(m) [a]	**19.34** [b]	**14**	—	**49.95**	**22**	1.56(m); 1.72(m)	**38.45**	**30**	1.023(3H,s)	**17.42**
7	1.26(m);1.59(m)	**36.04**	**15**	1.06(m); 1.45(m)	**33.13**	**23**	1.82(m);1.90(m)	**26.20**	OH	2.73 (brs)	
8	—	**41.63**	**16**	1.62(m);1.72)m)	**27.28**	**24**	3.715(t, 7.3,7.3)	**84.60**			

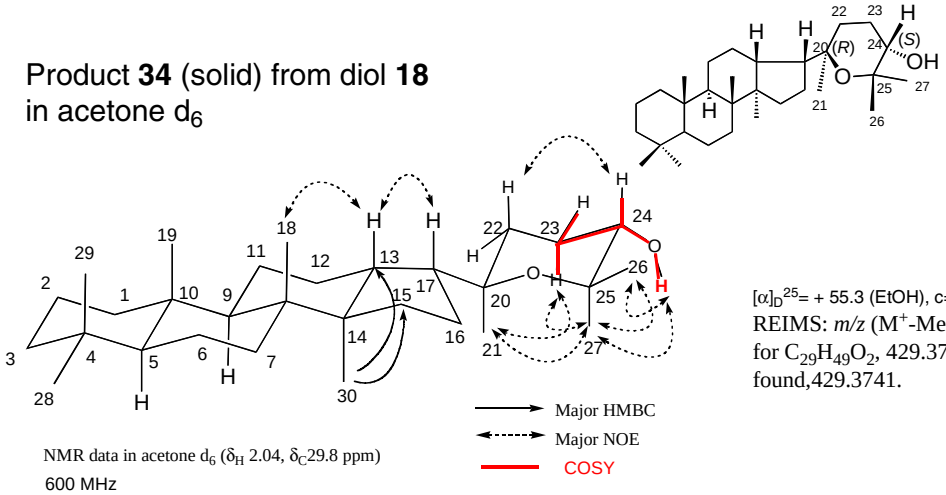
The signasl of a~d are exchangeable in respect to the same letters.

Product 34



*NMR data of **34** in acetone d_6*

Product **34** (solid) from diol **18** in acetone d_6



$[\alpha]_D^{25}$= + 55.3 (EtOH), c=0.09H.
REIMS: *m/z* (M^+-Me), calcd. for $C_{29}H_{49}O_2$, 429.3733; found,429.3741.

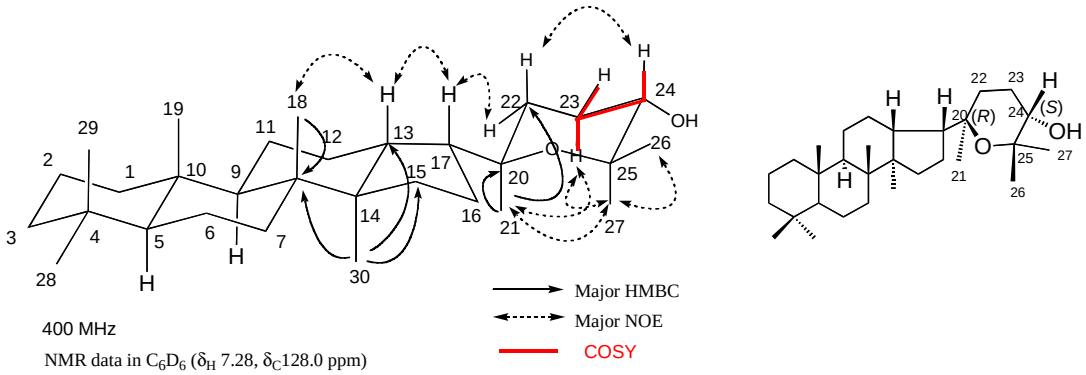
NMR data in acetone d_6 (δ_H 2.04, δ_C29.8 ppm)
600 MHz

NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C
1	0.87(m) ; 1.64(m)	**41.33**	9	1.44(m)	**51.62**	17	1.93 (ddd, 9.3, 8.5,7.8)	**51.41**	25	—	**75.83**
2	1.41(m) ; 1.62(m)	**19.21**[a]	10	—	**38.14**	18	0.946(3H, s)	**16.28**	26	1.151 (3H, s)	**22.49**
3	1.15(m); 1.35(m)	**42.89**	11	1.17(m) ; 1.56(m)	**23.22**	19	0.853(3H, s)	**16.61**	27	1.179 (3H,s)	**30.49**
4	—	**33.93**	12	1.56(m) ; 2.02(m)	**26.95**	20	—	**75.83**	28	0.845 (3H,s)	**33.75**
5	0.82(m)	**57.80** [a]	13	2.04(m)	**44.83**	21	1.284 (3H,s)	**27.53**	29	0.813(3H,s)	**21.83**
6	1.39(m) ; 1.51(m)	**19.12**	14	—	**49.74**	22	1.68(m,ax); 1.32(m, eq)	**35.05**	30	1.034(3H, s)	**17.29**
7	1.23(m) ; 1.59(m)	**36.07**	15	1.03(m); 1.38(m)	**33.11**	23	1.60(m, ax) ; 1.78(m,eq)	**25.89**	OH	3.78 (very broad;d, 4.4Hz)	
8	—	**41.61**	16	1.40(m);1.59(m)	**27.71**	24	3.28(ddd,11.5, 4.4, 4.4Hz)	**75.36**			

a: Assignments of C2 and C6 are exchangeable.

*NMR data of **34** in C_6D_6.*

Product **34** (solid) from diol **18** in C_6D_6

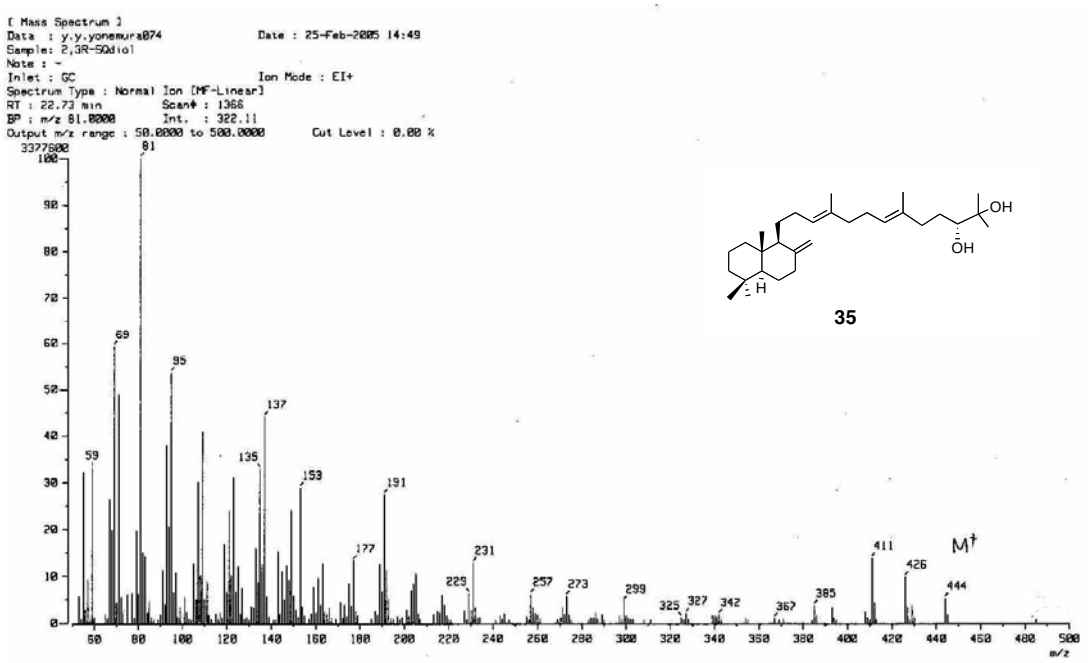


400 MHz
NMR data in C_6D_6 (δ_H 7.28, δ_C128.0 ppm)

NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C
1	0.94(m) ; 1.78(m)	**40.89**	9	1.62(m)	**51.17**	17	1.91 (m)	**51.86**	25	—	**75.07**
2	1.50(m) ; 1.75(m)	**19.21**[b]	10	—	**37.74**	18	1.123(3H, s)	**16.19**	26	1.32 (3H, s)	**22.02**
3	1.28(m); 1.52(m)	**42.54**	11	1.35(m) ; 1.74(m)	**22.88**	19	1.03 (3H,s)	**16.49**	27	1.419(3H,s)	**30.11**
4	—	**33.56**	12	1.77(m) ; 2.18(m)	**26.58**	20	—	**75.32**	28	1.03 (3H,s)	**33.65**
5	0.973(dd, 12.0, 2.4)	**57.42**	13	2.14(m)	**44.33**	21	1.336 (3H,s)	**27.25**	29	1.004(3H,s)	**21.78**
6	1.55(m) ; 1.78(m)	**19.12**[b]	14	—	**49.38**	22	1.22(m); 1.68(m)	**34.63**	30	1.29(3H, s)	**17.14**
7	1.44(m) ; 1.78(m)	**35.78**	15	1.30(m); 1.58(m)	**32.84**	23	1.46(m, ax) ; 1.68(m,eq)	**25.57**			
8	—	**41.23**	16	1.66(m);1.74(m)	**27.49**	24	3.22(dd,11.5, 4.4Hz)	**75.32**			

Assignments of carbon and proton signals at C2 and C6 are exchangeable.

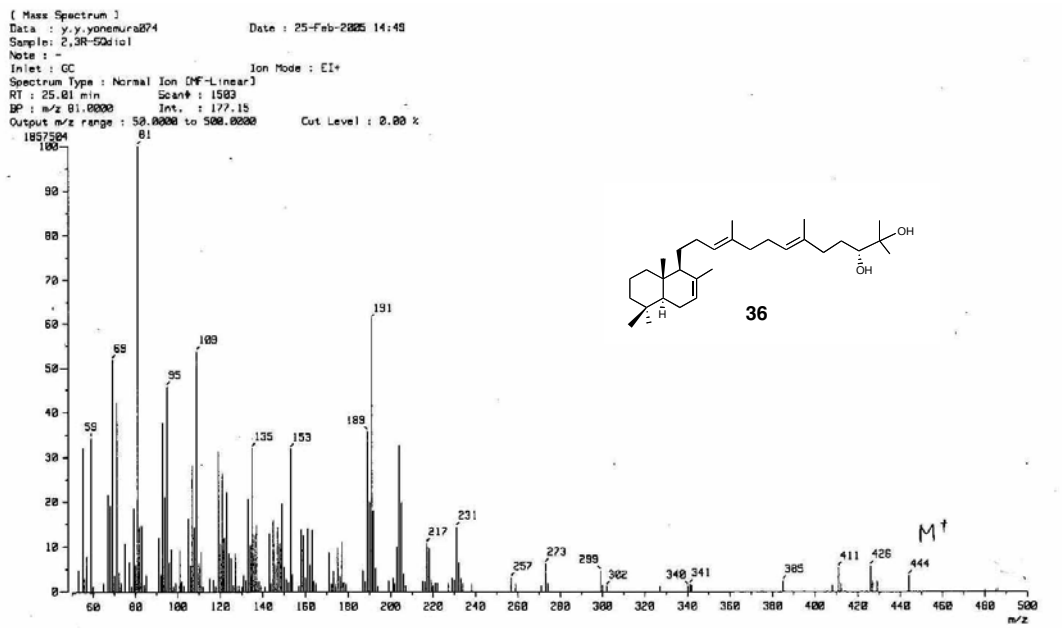
Product 35



Product **35** from diol **19**

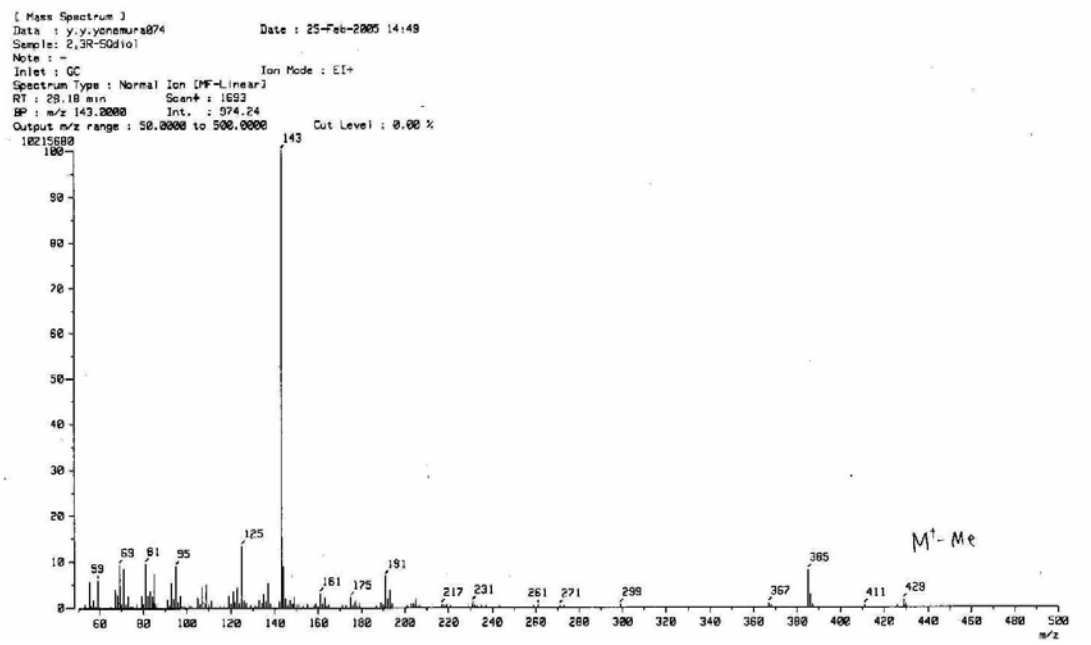
The NMR signals in C_6D_6 were identical to those of product **31**. $[\alpha]_D^{25}$=+22.8 (EtOH), c=0.28. HREIMS: *m/z* (M^+), calcd. for $C_{30}H_{52}O_2$, 444.3967; found, 444.3961.

Product 36



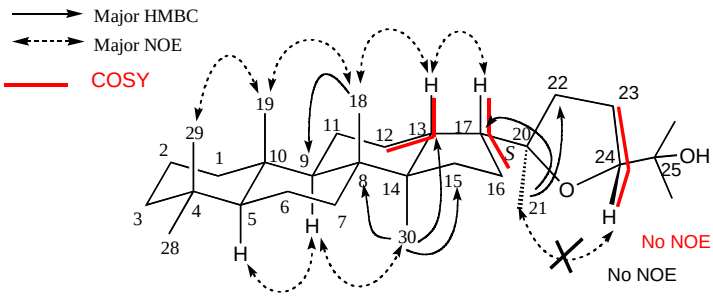
Product **36** from diol **19.** The NMR signals were the same as those of product **32**. $[\alpha]_D^{25}$=+4.08 (EtOH), c=0.29. HREIMS: *m/z* (M^+), calcd. for $C_{30}H_{52}O_2$, 444.3967; found, 444.3979.

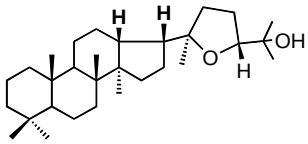
Product 37



NMR data of Product **37** in C_6D_6.

Product 37 (oil) from 19 in C_6D_6





$[\alpha]_D^{25}$= -22.8 (EtOH), c=0.38. HREIMS: *m/z* (M^+-Me), calcd. for $C_{29}H_{49}O_2$, 429.3733; found, 429.3717.

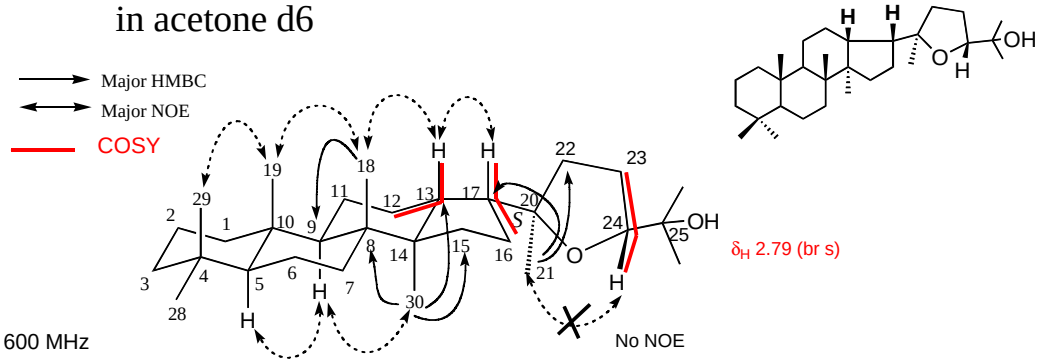
NMR data in C_6D_6 (δ_H 7.28, δ_C128 ppm)

NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C
1	0.96(m) ; 1.77 (m)	**40.89**	9	1.57(m)	**51.15**	17	2.17(m)	**48.73**	25	—	**69.83**
2	1.52(m); 1.72(m) [a]	**19.16** [b]	10	—	**37.74**	18	1.110(3H,s)	**16.15**	26	1.231 (3H,s) [c]	**24.64** [d]
3	1.31(m); 1.54 m)	**42.48**	11	1.26(m); 1.70(m)	**22.76**	19	1.027(3H, s)	**16.49**	27	1.405(3H,s) [c]	**28.36** [d]
4	—	**33.55**	12	1.56(m);1.96 (m)	**25.80**	20	—	**85.51**	28	1.048(3H,s)	**33.68**
5	0.96(brd, 12.4 Hz)	**57.36**	13	2.10 (ddd, 11.5,10.8,3.2Hz)	**44.03**	21	1.231 (3H,s)	**28.65**	29	1.008 (3H, s)	**21.78**
6	1.52(m); 1.63(m) [a]	**19.10** [b]	14	—	**49.36**	22	1.48 (m); 1.85(m)	**38.28**	30	1.196 (3H, s)	**16.65**
7	1.43(m); 1.78(m)	**35.73**	15	1.26(m);1.57(m)	**32.76**	23	1.56(m);1.96 (m)	**26.48**			
8	—	**41.20**	16	1.61 (m);1.82(m)	**27.99**	24	3.67 (dd, 10.5,5.0Hz)	**87.59**			

The signals of a~d are exchangeable in espect to the same letters.

*NMR data of Product **37** in Acetone d_6*

Product 37 (oil) from 19
in acetone d6

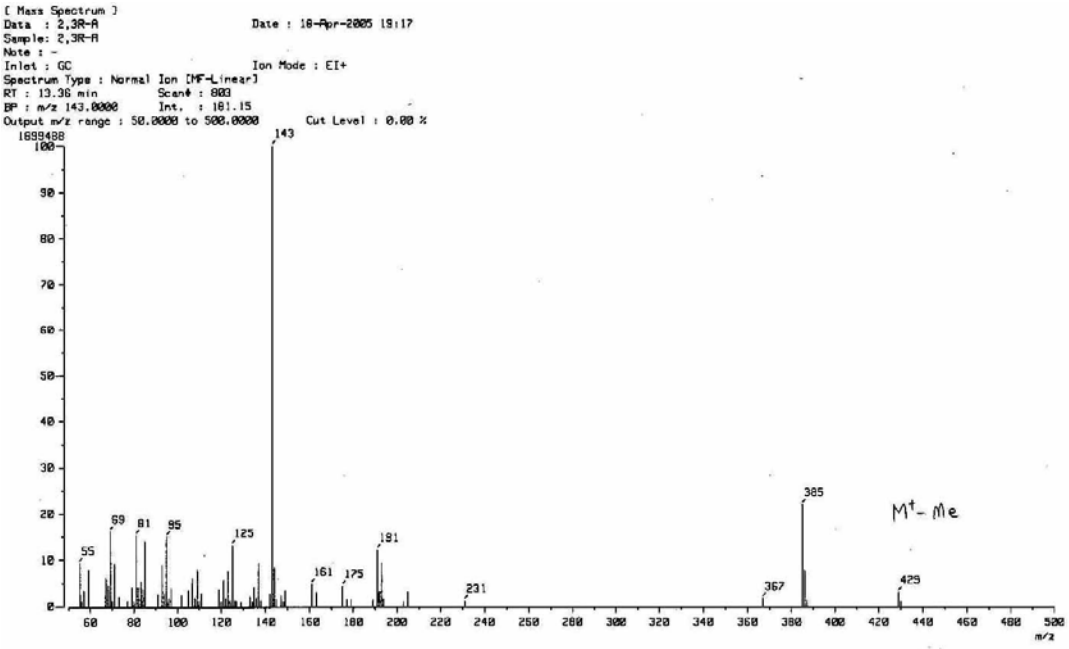


NMR data in acetone d_6 (δ_H 2.04, δ_C29.8 ppm)

NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C
1	0.88(m) ; 1.66 (brd, 13.0 Hz)	**41.30**	9	1.44 (m)	**51.64**	17	2.25 (m)	**49.30**	25	—	**70.44**
2	1.38(m); 1.62(m) [a]	**19.43** [b]	10	—	**38.13**	18	0.970(3H,s)	**16.30**	26	1.055 (3H,s) [c]	**25.84** [d]
3	1.15(m); 1.36 (brd, 12.8 Hz)	**42.83**	11	1.20(m); 1.58(m)	**23.17**	19	0.861 (3H,s)	**16.38**	27	1.159(3H,s) [c]	**27.28** [d]
4	—	**33.91**	12	1.52(m);1.93 (m)	**26.78**	20	—	**86.01**	28	0.851(3H,s)	**33.75**
5	0.82(m)	**57.75**	13	2.10 (ddd, 11,10,3.2Hz)	**44.53**	21	1.188(3H, s)	**28.87**	29	0.818(3H,s)	**21.85**
6	1.41(m); 1.54(m) [a]	**19.33** [b]	14	—	**49.80**	22	1.58 (m); 1.91(m)	**38.65**	30	1.024 (3H, s)	**16.92**
7	1.26(m); 1.61(m)	**36.04**	15	1.05(m); 1.44(m)	**33.08**	23	1.75 (m); 1.84(m)	**26.37**	OH	2.79 (br s)	
8	—	**41.62**	16	1.54 (m);1.77(m)	**28.25**	24	3.71 (dd, 10.5,5.2Hz)	**88.16**			

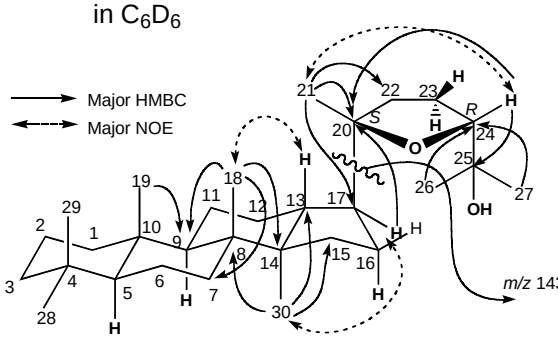
The signals of a~d are echangeable in respect to the same letters.

Product 38



*NMR data of **38** in C_6D_6.*

Product 38 (solid) from 19
in C_6D_6



$[\alpha]_D^{25}$=+35.9 (EtOH)
c=0.04, cf.
lit.$[\alpha]_D$=+19.3 (EtOH)
c=0.04
HREIMS: *m/z* (M^+-Me), calcd. for $C_{29}H_{49}O_2$, 429.3733; found, 429.3717.

^{1}H- and ^{13}C-Chemical Shift (δ) in $CDCl_3$

	our experiment	reference value
H-18	0.954	0.953
H-19	0.835	0.834
H-21	1.128	1.128
H-26	1.118	1.118
H-27	1.208	1.209
H-28	0.846	0.846
H-29	0.802	0.801
H-30	0.881	0.881
C-17	**49.59**	**49.57**
C-20	**86.49**	**86.47**
C-21	**23.49**	**23.50**
C-24	**83.33**	**83.32**
C-25	**71.46**	**71.44**
C-26	**27.49**	**27.49**
C-27	**24.26**	**24.27**

Reference;Yamashita *et al*, *Phytochemistry*, **49**, 2461-2466 (1998)

600 MHz

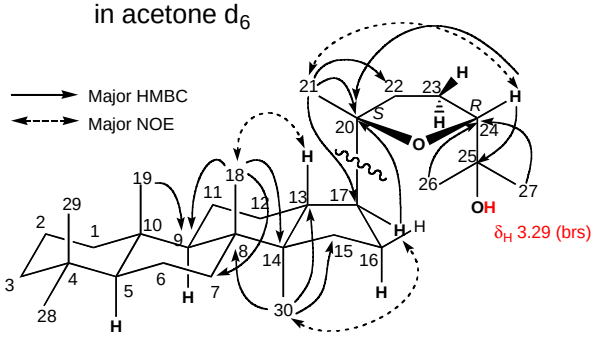
NMR data, δppm in C_6D_6 relative to C_6D_6 : ^{1}H ; 7.28ppm , ^{13}C ; 128.0ppm

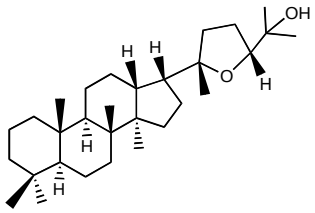
NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C
1	0.873(m); 1.70(m)	**40.85** *a*	**9**	1.48 (m)	**51.30**	**17**	1.94(m)	**50.04**	**25**	—	**71.08**
2	1.52(m);1.73(m)	**19.05** *a*	**10**	—	**37.70**	**18**	1.111 (3H, s)	**15.69**	**26**	1.263 (3H, s) *c*	**25.04** *d*
3	1.28(m) ; 1.50(m)	**42.43**	**11**	1.30(m) ; 1.64(m)	**21.76**	**19**	0.972 (3H, s)	**16.45**	**27**	1.434 (3H, s) *c*	**27.77** *d*
4	—	**33.52**	**12**	1.33(m) ; 1.98(m)	**27.83**	**20**	—	**86.27**	**28**	1.029 (3H, s)	**33.63**
5	0.909 (dd, 13.4, 2.6Hz)	**57.31** *a*	**13**	1.77(m)	**43.25**	**21**	1.222 (3H, s)	**23.66**	**29**	0.982 (3H, s) *e*	**21.76** *f*
6	1.48(m);1.64(m)	**18.97** *a*	**14**	—	**50.37**	**22**	1.55(m) ; 1.79(m)	**36.19** *b*	**30**	0.987 (3H, s) *e*	**16.70** *f*
7	1.38(m) ;1.68(m)	**35.64**	**15**	1.18(m) ;1.60(m)	**31.80** *b*	**23**	1.72(m) ;1.92(m)	**26.02**			
8	—	**40.85**	**16**	1.66(m) ; 1.91(m)	**26.26** *b*	**24**	3.75 (t, 7.3Hz)	**83.69**			

The signals of *a*~*f* are exchangeable in respect to the same letters.

*NMR data of **38** in Acetone d_6*

Product 38 (solid) from 19
in acetone d_6





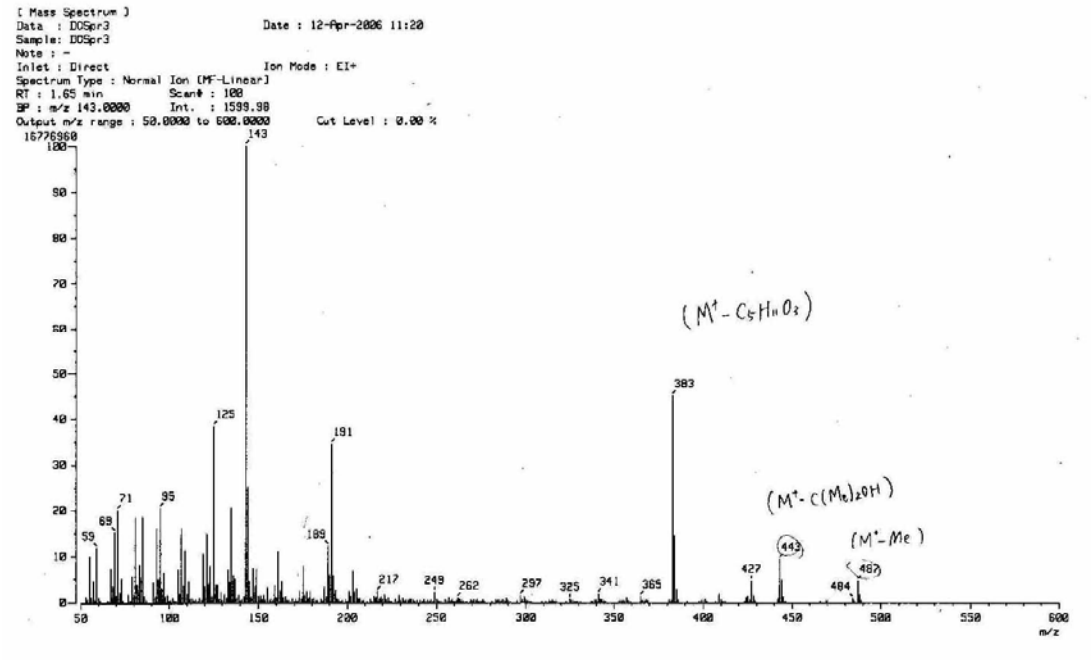
600 MHz

NMR data, δppm in acetone-$_6$ relative to acetone d6 : ^{1}H ; 2.04 , ^{13}C ; 29.8 ppm

NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C
1	0.84(m); 1.66(m)	**41.33**	**9**	1.41(m)	**51.75**	**17**	1.78(m)	**50.56**	**25**	—	**71.49**
2	1.38(m);1.62(m) *a*	**19.31** *b*	**10**	—	**38.17**	**18**	0.985(3H,s)	**15.89**	**26**	1.082(3H,s)	**26.73**
3	1.13(m);1.32(m)	**42.83**	**11**	1.20(m);1.52(m)	**22.09**	**19**	0.865(3H,s)	**16.61**	**27**	1.086(3H,s)	**26.29**
4	—	**33.73**	**12**	1.20(m);1.85(m)	**28.00**	**20**	—	**86.68**	**28**	0.847(m)	**33.91**
5	0.82 (m)	**57.72**	**13**	1.63(m)	**43.56**	**21**	1.096(3H,s)	**23.45**	**29**	0.815(3H,s)	**21.82**
6	1..40(m);1.52(m) *a*	**19.28** *b*	**14**	—	**50.78**	**22**	1.61(m);1.68(m)	**36.36**	**30**	0.896(3H,s)	**16.81**
7	1.26(m); 1.57(m)	**36.01**	**15**	1.06(m);1.51(m)	**32.09**	**23**	1.80(m);1.88(m)	**26.33**	OH	3.29 (br s)	
8	—	**41.33**	**16**	1.50(m);1.66(m)	**26.33**	**24**	3.71 (t, 7.3Hz)	**84.21**			

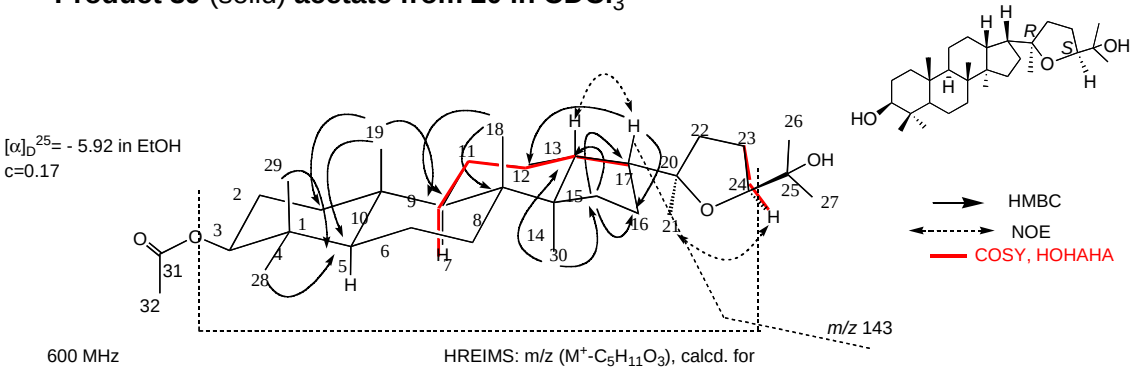
The proton and carbon signals of *a* and *b* are exchangeable.

Product 39



*NMR data of **39** acetate in $CDCl_3$.*

Product 39 (solid) **acetate from 20 in $CDCl_3$**



$[\alpha]_D^{25}$= - 5.92 in EtOH
c=0.17

600 MHz

HREIMS: m/z (M^+-$C_5H_{11}O_3$), calcd. for $C_{27}H_{43}O$, 383.3314; found, 383.3304.

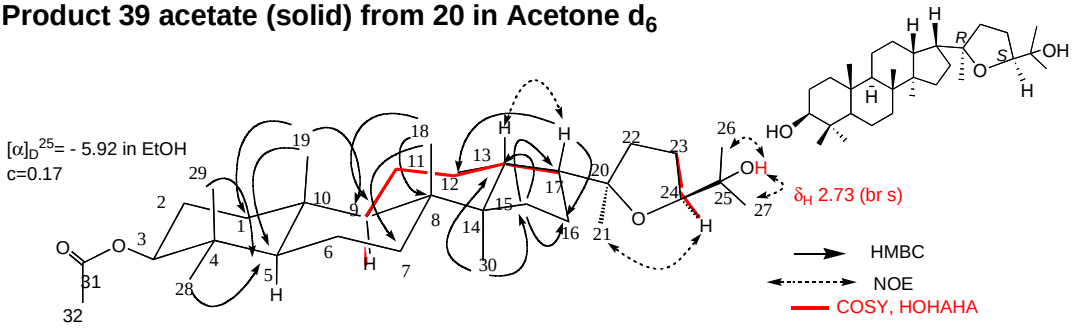
NMR data in $CDCl_3$ (TMS, 0.0 ppm)

NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C
1	1.04 (dd, 12.5,12.5,4.5Hz);1.68(m)	38.68	9	1.34(dd, 12.7, 2.6Hz)	50.65	17	2.23(ddd,8.9, 9.0,11.0Hz)	48.59	25	——	71.55
2	1.61(2H,m)	23.71	10	——	37.90	18	0.935(3H,s)	15.82 a	26	1.119(3H,s) d	24.46 c
3	4.48(dd,11.1, 5.1Hz)	80.94	11	1.17(m);1.53(m)	22.51 b	19	0.846(3H,s)	16.46	27	1.217(3H,s) d	27.43 c
4	——	37.06	12	1.43(m);1.86(m)	25.76	20	——	85.45	28	0.854(3H,s)	27.94 a
5	0.84(m)	55.96	13	2.07(m)	43.49	21	1.189(3H,s)	24.88	29	0.846(3H,s)	16.27
6	1.43(m);1.53(m)	18.23	14	——	49.16	22	1.6(m); 1.73(m)	37.93	30	0.963(3H,s)	16.90
7	1.26(bd,12.7Hz)	35.21	15	1.10(m);1.43(m)	32.44	23	1.77(m);1.84(m)	25.63 b	31	——	170.9
8	——	40.75	16	1.75(m); 1.58(m)	26.85	24	3.714(t, 6.8 Hz)	83.43	32	2.044(3H,s)	21.30

The carbon signals of a~c are exchangeable each other in respect to the same letters.
The protons desinated as *d* is interchangeable.

*NMR data of **39** acetate in Acetone d_6.*

Product 39 acetate (solid) from 20 in Acetone d_6



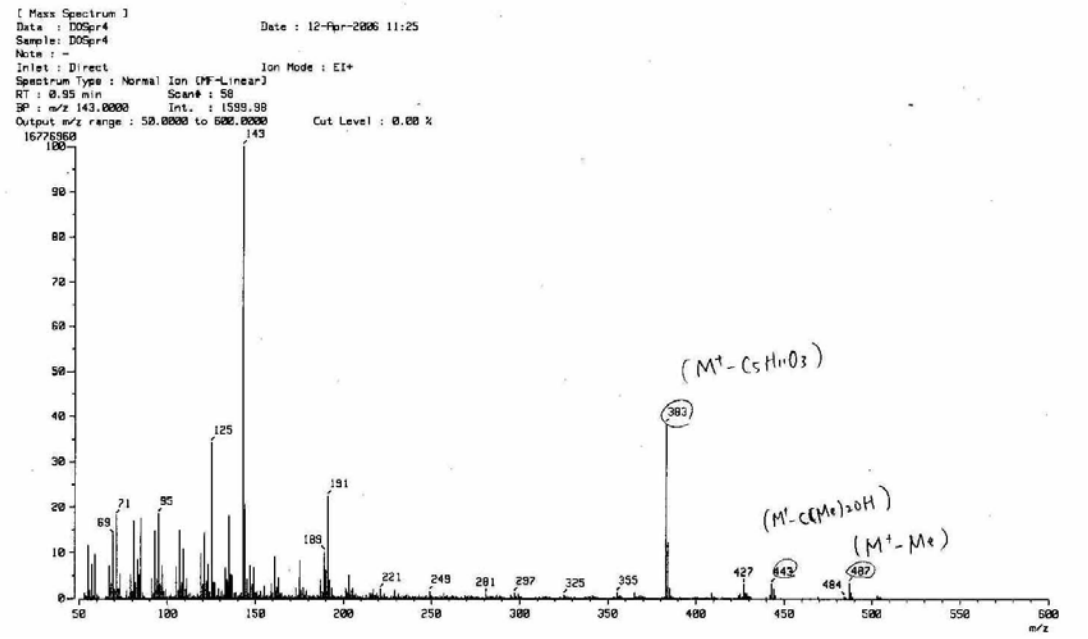
600 MHz

NMR data in acetone d6 relative to the solvent peak (δ_H, 2.04, δ_C 29.8 ppm)

NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C
1	1.06(m);1.68(m)	**39.33**	**9**	1.46(m)	**51.39**	**17**	2.25(m)	**49.19**	**25**	——	**71.60**
2	1.62(2H, m)	**24.42**	**10**	——	**38.53**	**18**	0.971(3H,s)	**16.19**^{a}	**26**	1.09 (3H, s)	**26.71**^{b}
3	4.43(dd,11.3, 5.0Hz)	**80.99**	**11**	1.21(m);1.57(m)	**23.22**	**19**	0.887(3H,s)	16.83	**27**	1.13(3H,s)	**26.32**^{b}
4	——	**37.79**	**12**	1.46(m); 1.90(m)	**26.58**	**20**	——	**85.75**	**28**	0.841(3H,s)	**28.24**^{a}
5	0.91(dd, 11.7, 2.5Hz)	**55.63**	**13**	2.11(m)	**44.41**	**21**	1.183 (3H,s)	**25.19**	**29**	0.852 (3H,s)	**16.65**
6	1.47(m); 1.52(m)	**18.98**	**14**	——	**49.89**	**22**	1.55(m); 1.71(m)	**38.43**	**30**	1.030(3H,s)	**17.37**
7	1.26(m);1.61(m)	**35.96**	**15**	1.08(m);1.45(m)	**33.17**	**23**	1.81(m);1.86(m)	**26.18**	**31**	——	170.9
8	——	**41.43**	**16**	1.63(m);1.73(m)	**27.27**	**24**	3.72 (t, 7.3 Hz)	**84.59**	**32**	1.979(3H,s)	**21.04**

The carbon signals of a and b are exchangeable each other.

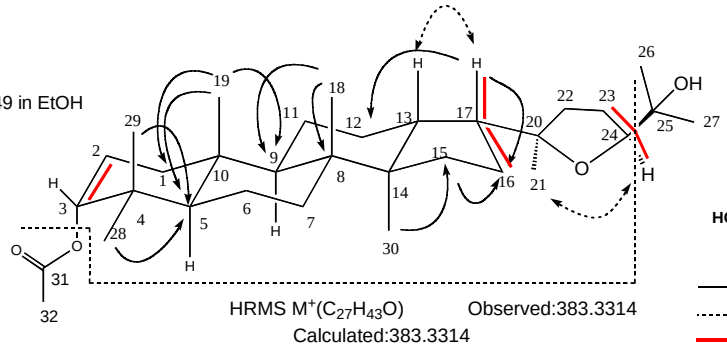
Product 40

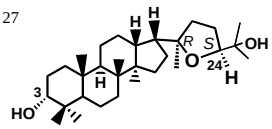


*NMR data of **40** acetate in $CDCl_3$.*

Product 40 acetate (oil) from 21 in $CDCl_3$

$[\alpha]_D^{25}$= - 13.2749 in EtOH
c=0.11





HMBC
NOE
COSY

600 MHz

NMR data in $CDCl_3$ (TMS, 0.0 ppm)

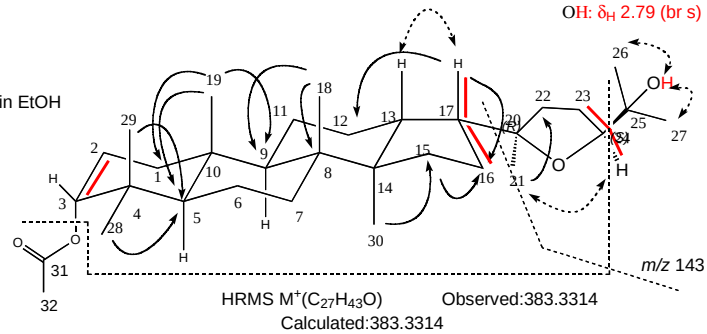
NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C
1	1.15(m);1.42(m)	34.25	9	1.44(m)	50.52	17	2.25(m)	48.52	25	——	71.59
2	1.57(m);1.87(m)	22.91	10	——	37.17	18	0.946(3H,s)	16.02 b	26	1.223(3H,s) d	27.45 c
3	4.62(bs)	78.42	11	1.16(m); 1.58(m)	22.35 a	19	0.852(3H,s)	15.87 b	27	1.104 (3H,s) d	24.43 c
4	——	36.77	12	1.57(m);1.85(m)	25.77 a	20	——	85.45	28	0.835(3H,s)	27.86
5	1.23(m)	50.77	13	2.06(m)	43.51	21	1.989(3H,s)	25.01	29	0.881(3H,s)	21.73
6	1.42(2H, m)	18.22	14	——	49.28	22	1.60(m); 1.76(m)	37.94	30	1.024(3H,s)	17.13
7	1.26(m);1.59(m)	35.12	15	1.10(m);1.45(m)	32.46	23	1.76(m);1.87(m)	25.70 a	31	——	170.8
8	——	40.94	16	1.58(m);1.75(m)	26.83	24	3.72 (t, 7.2Hz)	83.49	32	2.09(3H,s)	21.40

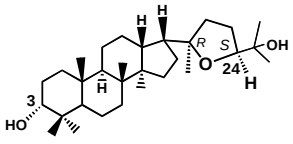
The signals of a~d are exchangeable each other in respect to the same letters.

*NMR data of **40** acetate in Acetone d_6.*

Product 40 acetate (oil) from 21 in acetone d_6

$[\alpha]_D^{25}$= - 13.27 in EtOH
c=0.11





HMBC
NOE
COSY

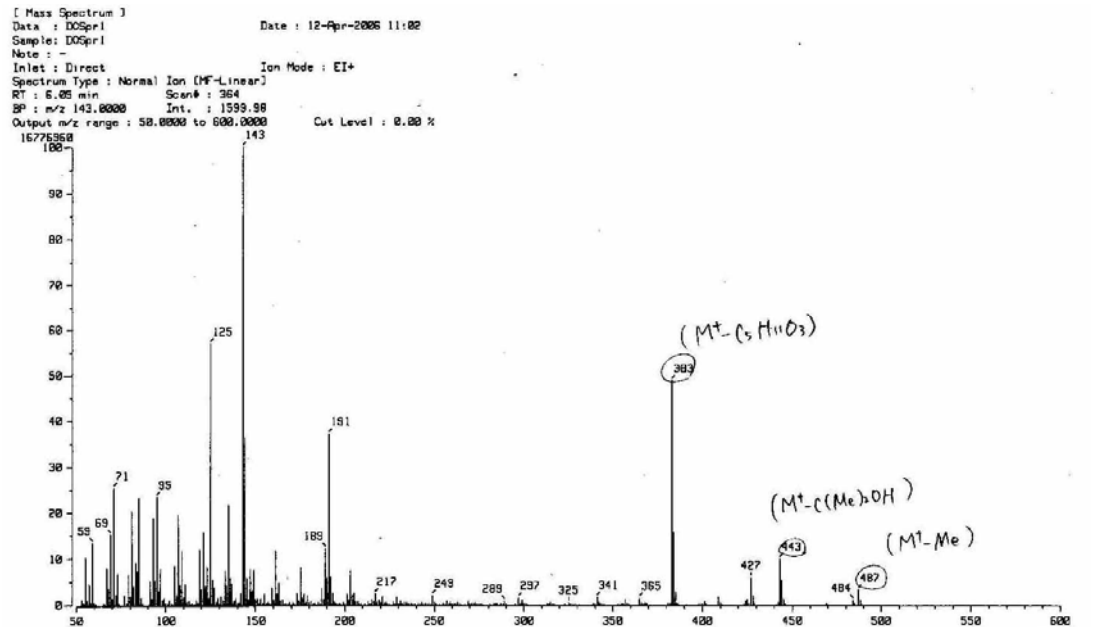
600 MHz

NMR data in acetone d6 relative to the solvent peak (δ_H, 2.04, δ_C 29.8 ppm)

NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C
1	1.23(m);1.43(m)	34.99	9	1.50 (m)	51.41	17	2.25(m)	49.16	25	——	71.61
2	1.50(m);1.90(m)	23.53	10	——	37.89	18	0.980(3H,s)	16.23	26	1.054(3H,s) b	26.71 c
3	4.56 (br s)	78.42	11	1.21(m); 1.58(m)	23.04	19	0.893 (3H,s) a	19.40	27	1.133(3H,s) b	26.34 c
4	——	37.41	12	1.46(m);1.90(m)	26.58	20	——	85.75	28	0.832(3H,s)	28.27
5	1.31(m)	51.63	13	2.11 (m)	44.36	21	1.191(3H,s)	25.23	29	0.898(3H,s) a	22.05
6	1.45(m)	18.89	14	——	49.94	22	1.58(m);1.74(m)	38.44	30	1.044(3H,s)	17.50
7	1.27(m);1.62(m)	35.92	15	1.08(m);1.45(m)	33.13	23	1.82(m);1.90(m)	26.19	31	——	170.5
8	——	41.59	16	1.63(m);1.74(m)	27.25	24	3.72 (t, 7.2 Hz)	84.63	32	2.00 (3H,s)	21.10

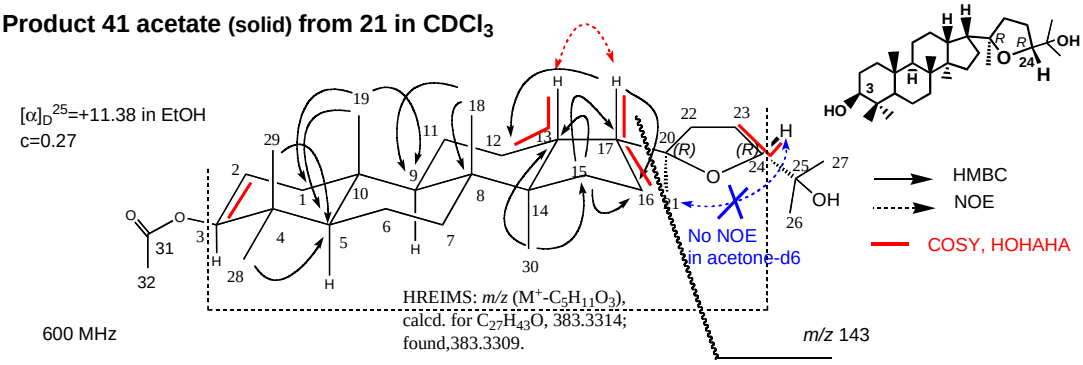
The signals of a~c are exchangeable in respect to the same letters.

Product 41



*NMR data of **41** acetate in $CDCl_3$.*

Product 41 acetate (solid) from 21 in $CDCl_3$

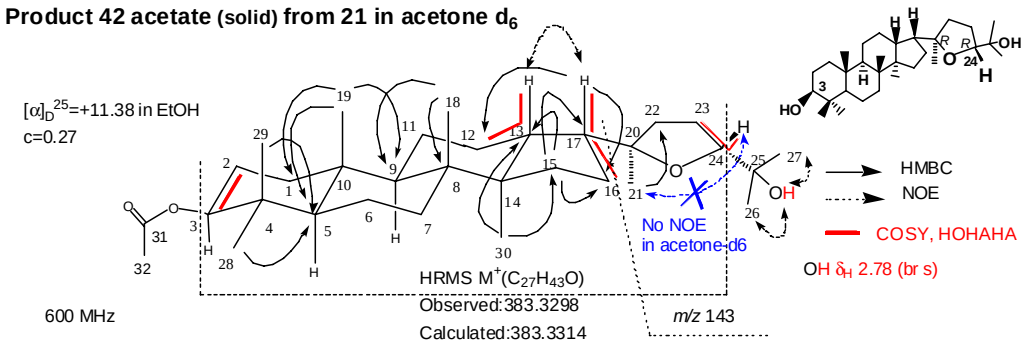


NMR data in $CDCl_3$ (TMS, 0.00 ppm)

NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C
1	1.048(m); 1.69(m)	38.70	9	1.37(dd, 12.7, 2.7Hz)	50.63	17	2.24(m)	48.56	25	—	70.12
2	1.64(2H,m)	23.73	10	—	37.08	18	0.936(3H,s)	15.83 c	26	1.099(3H,s) b	24.02 b
3	4.48(dd, 11.1, 5.2Hz)	80.95	11	1.17(m); 1.55(m)	22.62 d	19	0.857(3H,s)	16.30	27	1.200(3H.s) b	28.48 b
4	—	37.90	12	1.47(m);1.93(m)	25.86	20	—	85.63	28	0.847(3H,s)	28.04 a
5	0.84(m)	55.98	13	2.07(m)	43.73	21	1.200(3H.s)	27.95 a	29	0.847(3H,s)	16.36 c
6	1.44(m); 1.52(m)	18.32	14	—	49.05	22	1.63(m);1.90(m)	37.90	30	0.965(3H,s)	16.47 c
7	1.26(bd,12.4);1.57(m)	35.25	15	1.08(m);1.42(m)	32.41	23	1.72(m); 1.85(m)	25.92 d	31	—	170.9
8	—	40.76	16	1.49(m); 1.76(m)	27.73	24	3.71(dd, 10.7, 5.2Hz)	86.95	32	2.04(3H,s)	21.31

The signals of a~d are exhangeable.

*NMR data of **41** acetate in Acetone d_6.*

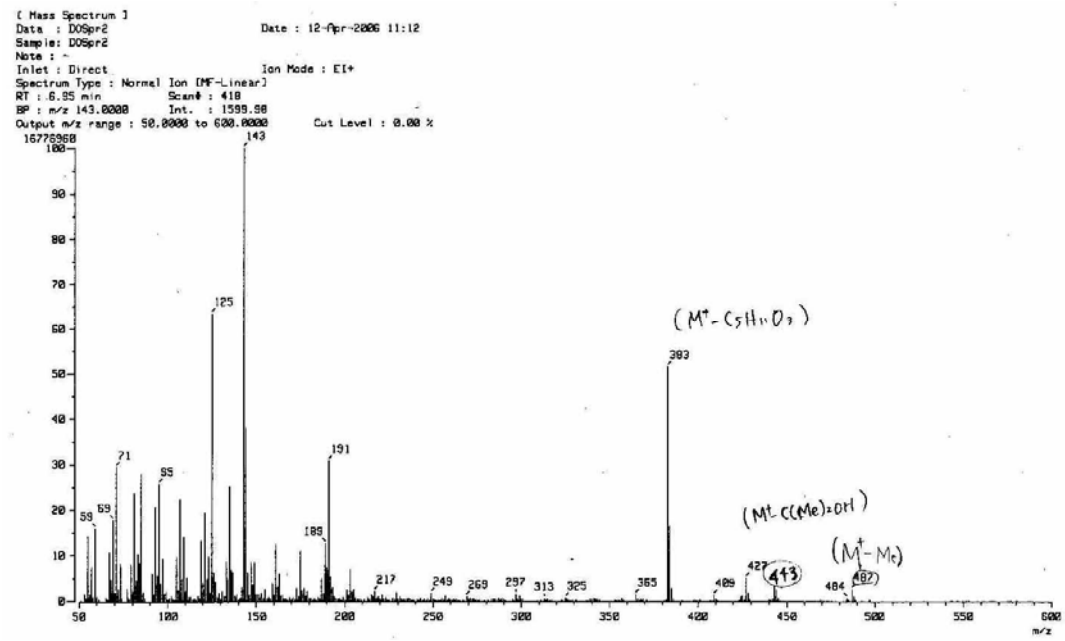


NMR data in acetone d6 relative to the solvent peak (δ_H, 2.04, δ_C 29.8 ppm)

NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C
1	1.08(m);1.70(m)	39.35	9	1.47(m)	51.39	17	2.25(m)	49.33	25	—	70.39
2	1.61(2H, m)	24.44	10	—	37.79	18	0.974(3H,s)	16.21	26	1.049(3H,s) [b]	25.82[c]
3	4.43(dd, 11.4, 5.0Hz)	80.99	11	1.21(m);1.56(m)	23.33	19	0.889(3H,s)	16.66	27	1.134(3H,s) [b]	27.36[c]
4	—	38.53	12	1.53(m);1.94(m)	26.75	20	—	86.01	28	0.843(3H,s)	28.25
5	0.915(dd, 11.6, 2.3 Hz)	55.65	13	2.08(m)	44.63	21	1.189(3H,s)	28.89	29	0.855(3H,s)	16.88[a]
6	1.48(m);1.55(m)	19.00	14	—	49.77	22	1.57(m);1.92(m)	38.64	30	1.031(3H,s)	16.84[a]
7	1.28(m);1.64(m)	35.99	15	1.07(m);1.43(m)	33.14	23	1.74(m);1.83(m)	26.36	31	—	170.67
8	—	41.46	16	1.53(m);1.77(m)	28.27	24	3.71(dd, 10.6, 5.2)	88.24	32	1.980(3H,s)	21.05

The signals of a~c exchangeable in respect to the same letters.

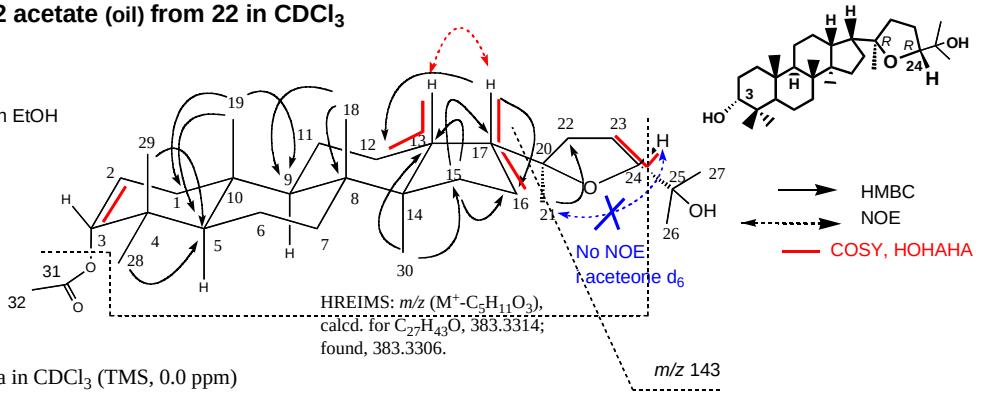
Product 42



*NMR data of **42** acetate in $CDCl_3$*

Product 42 acetate (oil) from 22 in $CDCl_3$

$[\alpha]_D^{25}$= - 17.3 in EtOH
c=0.36



600 MHz

NMR data in $CDCl_3$ (TMS, 0.0 ppm)

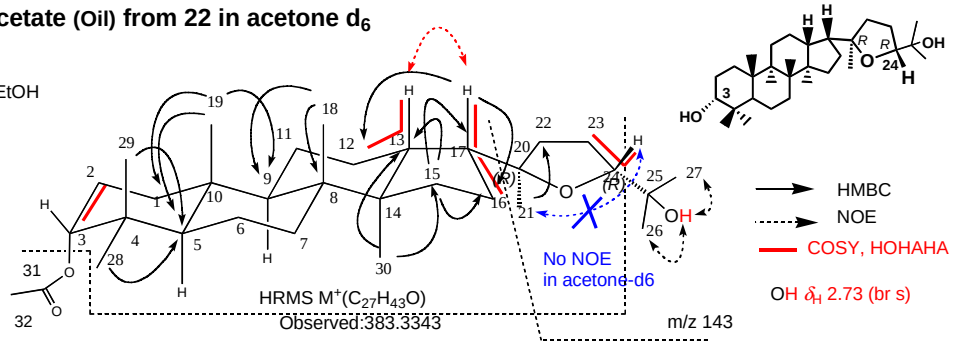
NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C
1	1.17(m); 1.42(m)	34.25	9	1.46(m)	50.49	17	2.24(m)	48.55	25	—	70.15
2	1.58(m);1.78(m)	22.92	10	—	37.18	18	0.947(3H,s)	15.87	26	1.103(3H,s) [a]	23.98[b]
3	4.62(bs)	78.42	11	1.17(m);1.58(m)	22.44	19	0.855(3H,s)	16.04	27	1.208(3H,s) [a]	28.88[b]
4	—	36.77	12	1.46(m);1.95(m)	25.86 [c]	20	—	85.64	28	0.834(3H,s)	28.00
5	1.23(m)	50.78	13	2.07(m)	43.75	21	1.208(3H,s)	28.00	29	0.882(3H,s)	21.73
6	1.42(2H,m)	18.25	14	—	49.16	22	1.62(m);1.92(m)	38.01 [c]	30	1.023(3H,s)	16.53
7	1.26(m);1.61(m)	35.15	15	1.08(m);1.42(m)	32.42	23	1.73(m);1.83(m)	25.98	31	—	170.8
8	—	40.94	16	1.50(m);1.76(m)	27.65	24	3.72(dd, 10.8,5.1Hz)	87.05	32	2.089(3H,s)	21.40

The signals of a~c are exchangeable in respect to the same letters.

*NMR data of **42** acetate in Acetone d_6.*

Product 42 acetate (Oil) from 22 in acetone d_6

$[\alpha]_D^{25}$= - 17.3 in EtOH
c=0.36



600 MHz

Calculated:383.3314

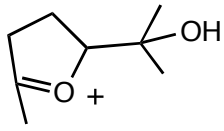
NMR data in acetone d6 relative to the solvent peak (δ_H, 2.04, δ_C 29.8 ppm)

NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C	NO.	^{1}H	^{13}C
1	1.22(m);1.43(m)	34.99	9	1.51(m)	51.40	17	2.25(m)	49.31	25	—	70.43
2	1.51(m); 1.90(m)	23.54	10	—	37.89	18	0.981(3H,s)	16.25	26	1.056(3H,s)[a]	25.79[b]
3	4.56 (br s)	78.43	11	1.22(m); 1.58(m)	23.14	19	0.895(3H,s)	16.41	27	1.139(3H,s)[a]	27.34[b]
4	—	37.41	12	1.53(m);1.94(m)	26.76	20	—	86.03	28	0.832(3H,s)	28.27
5	1.31(2H, m)	51.64	13	2.11(m)	44.59	21	1.193(3H,s)	28.90	29	0.898(3H,s)	22.06
6	1.45(m)	18.91	14	—	49.83	22	1.57(m); 1.92(m)	38.68	30	1.046(3H,s)	17.00
7	1.27(m);1.63(m)	35.95	15	1.07(m);1.44(m)	33.11	23	1.76(m);1.85(m)	26.38	31	—	170.5
8	—	41.63	16	1.54(m);1.76(m)	28.23	24	3.72 (dd, 10.6, 5.3Hz)	88.28	32	2.00(3H,s)	21.10

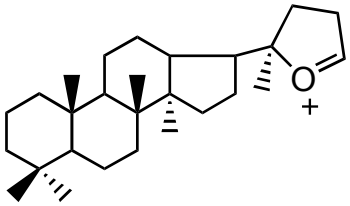
The signals of a~b are exchangeable in respect the same letters.

7. Major Fragment ions of Enzymatic Products in EI-MS of Products 33,34 and 37-42

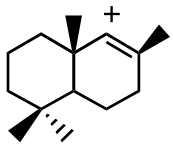
Products 33, 37, 40



m/z 143

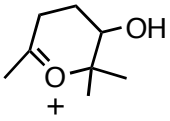


m/z 385

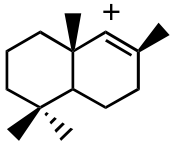


m/z 191

Product 34

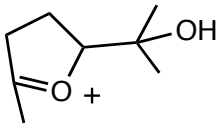


m/z 143

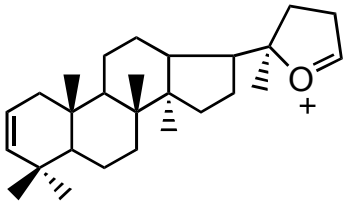


m/z 191

Acetates of Products 39-42

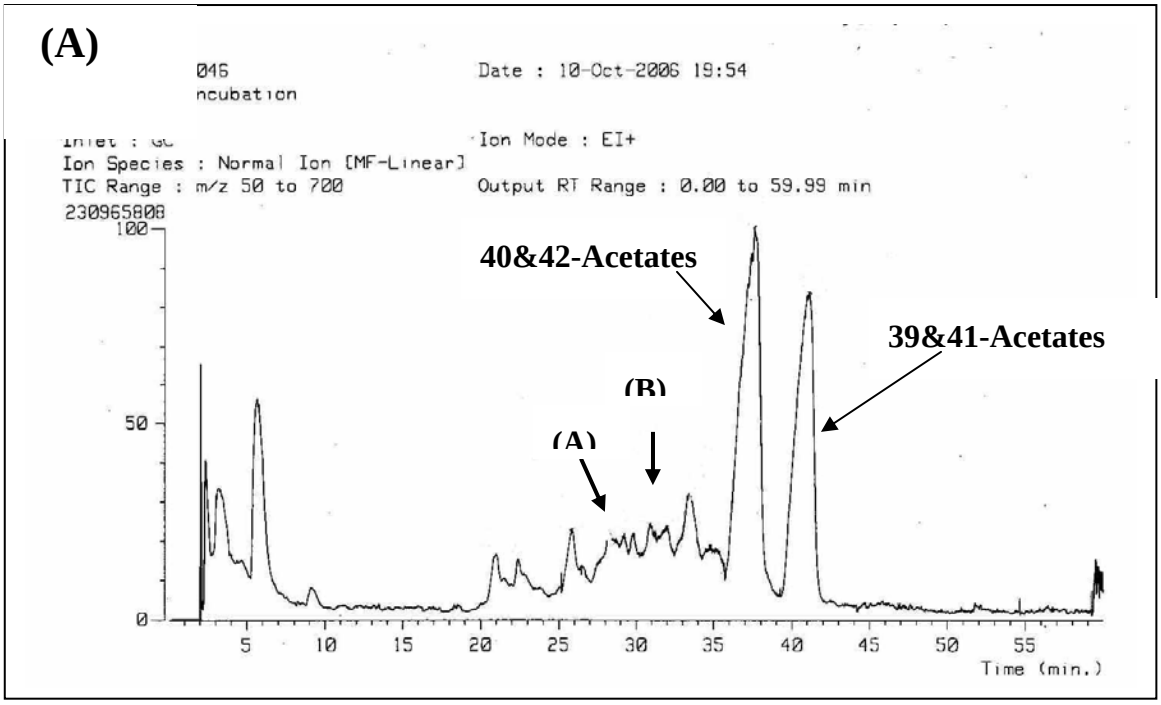


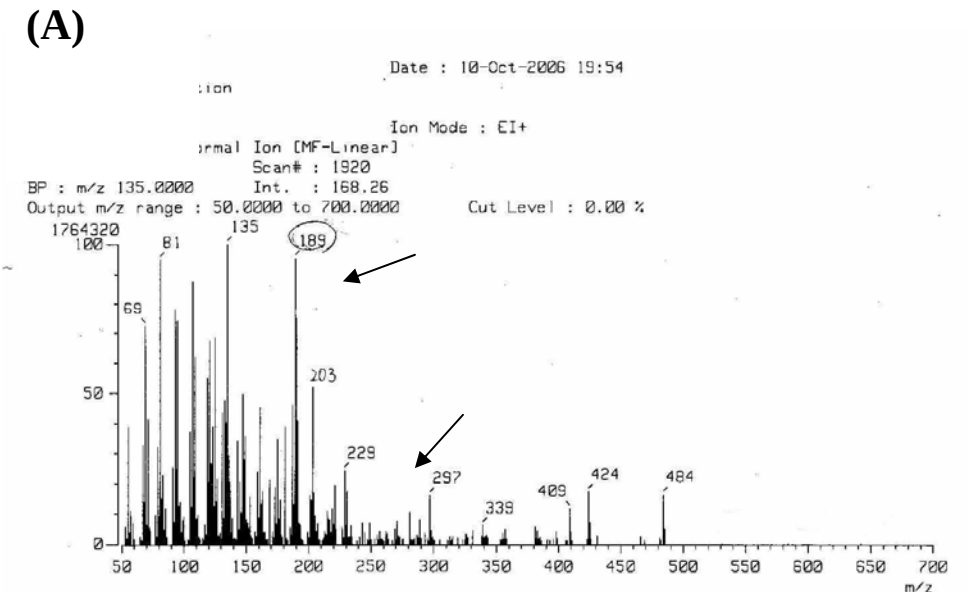
m/z 143

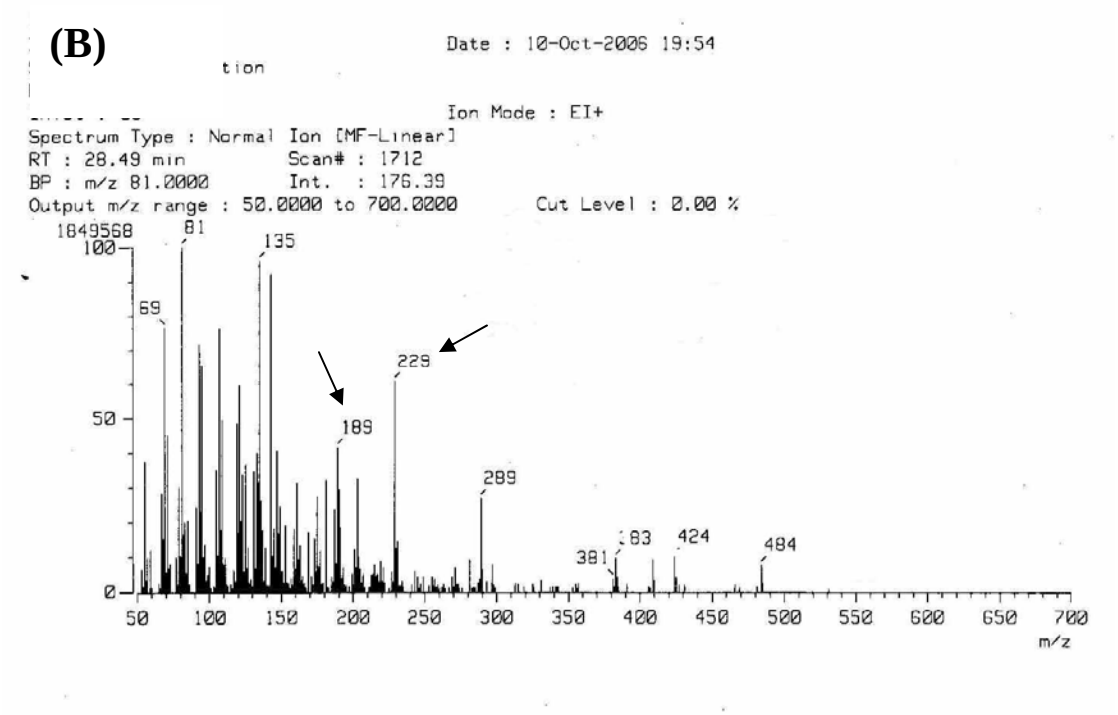


m/z 383

8. GCMS of acetate mixtures obtained by incubating diastereomeric mixture of dioxidosqualene 20-22 (Minor Peaks)

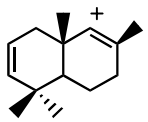




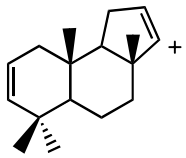


The ions of *m/z* 189 and *m/z* 229 suggest the following fragments. *m/z* 229 is characteristic of a 6/6/5-fused tricyclic skeleton. Probably, these minor product peaks would contain bi- and tricycic compounds. It should be noted that (B) and (C) do not show the EIMS spectra of pure products, because many peaks are overlapped in the GCMS trace (A).

Fragment ions of Minor GCMS Peaks from dioxidosqualene **20-22**



m/z 189



m/z 229

7. References

1) a) Sato, T. and T. Hoshino, *Biosci. Biotechnol. Biochem*., 1999, **63**, 1171. b) T. Sato, M. Kouda and T. Hoshino, *Biosci. Biotechnol. Biochem*., 2004, **68**, 728-738.

2) a) J-L. Abad, J. Casa, F. Sanchez-Baeza and A. Messeguer, *J. Org. Chem.*, 1995, **60**, 3648-3656. b) G.A. Crispino and K. B. Sharpless, *Tetrahedron Lett*., 1992, **33**, 4273-4274.

3) R.B. Boar and K. Damps, *J. Chem. Soc., Perkin Trans. 1*, **1997**, 709-712.

4) J-Luis Abad and F. Camps, *Tetrahedron*, 2004, **60**, 11519–11525.