

Supporting information I

The spectral data (NMR, MS) showing the purity of sitosterol oxidation product standards

The purity of sitosterol oxidation product standards was tested by NMR and GC-MS with reference to the previous reports (McCarthy, F. O., *et al.* Org Biomol Chem. 2005, 3(16):3059-3065, Zhang. X., *et al.* Steroids. 2005, 70(13):886-895, Kimura, *et al.* Chem Pharm Bull. 1995, 43(10):1813-1817).

Final products were TMS derived in 100 μ L MSHFBA with 5% 1-MIM, and incubated at 70°C for 20 min before tested by GC-MS.

The GC-MS system was a 7890A-5973N GC-MS system with quadrupole technology supplied with split/splitless injection and 7683B sample injector (Agilent Technologies, US). GC-MS conditions were listed as follows: AB-5MS (30m $\times$ 0.25mm $\times$ 0.25 μ m, Abel Industries, USA) supplied with a 2 m guard column. Carrier gas: Helium, 1.2 mL/min. The oven temperature was initially set at 100°C for 1 min, then raised to 200 °C at 50 °C/min rate, to 250 °C at 20 °C/min rate, to 300 °C at 1.5 °C/min rate gradually, and held for 15 min. Injection was hot splitless at 300 °C, the ion source temperature was set at 250°C, and the transfer line was at 300°C. MS: Scan (m/z 50-600).

NMR spectra of the final products were recorded on a Bruker DMX-500 spectrometer (¹H NMR at 500MHz) with CDCl₃ or pyridine-d₅ as the solvent at ambient temperature unless otherwise stated.

List of spectral data:

1. 6 β -hydroxysitosterol

Fig. 1-1 Total ion chromatogram of 6 β -hydroxysitosterol;

Fig. 1-2 Fragmentation pattern of 6 β -hydroxysitosterol;

Fig. 1-3 ¹H-NMR data of 6 β -hydroxysitosterol;

Table 1 Characterization data summary of 6 β -hydroxysitosterol.

2. 7-ketositosterol

Fig. 2-1 Total ion chromatogram of 7-ketositosterol;

Fig. 2-2 Fragmentation pattern of 7-ketositosterol;

Fig. 2-3 ^1H -NMR data of 7-ketositosterol;

Table 2 Characterization data summary of 7-ketositosterol.

3. Stigmastane-3 β ,5 α ,6 β -triol

Fig. 3-1 Total ion chromatogram of stigmastane-3 β ,5 α ,6 β -triol;

Fig. 3-2 Fragmentation pattern of stigmastane-3 β ,5 α ,6 β -triol;

Fig. 3-3 ^1H -NMR data of stigmastane-3 β ,5 α ,6 β -triol;

Table 3 Characterization data summary of stigmastane-3 β ,5 α ,6 β -triol.

4. 7 α / β -hydroxysitosterol

Fig. 4-1 Total ion chromatogram of 7 α / β -hydroxysitosterol;

Fig. 4-2 Fragmentation pattern of 7 α -hydroxysitosterol;

Fig. 4-3 Fragmentation pattern of 7 β -hydroxysitosterol;

Fig. 4-4 ^1H -NMR data of 7 α / β -hydroxysitosterol;

Table 4 Characterization data summary of 7 α / β -hydroxysitosterol.

5. 5 α ,6 α /5 β ,6 β -epoxysitosterol

Fig. 5-1 Total ion chromatogram of 5 α ,6 α /5 β ,6 β -epoxysitosterol;

Fig. 5-2 Fragmentation pattern of 5 α ,6 α -epoxysitosterol;

Fig. 5-3 Fragmentation pattern of 5 β ,6 β -epoxysitosterol;

Fig. 5-4 ^1H -NMR data of 5 α ,6 α /5 β ,6 β -epoxysitosterol;

Table 5 Characterization data summary of 5 α ,6 α /5 β ,6 β -epoxysitosterol.

1. 6 β -hydroxysitosterol (6 β -HS),

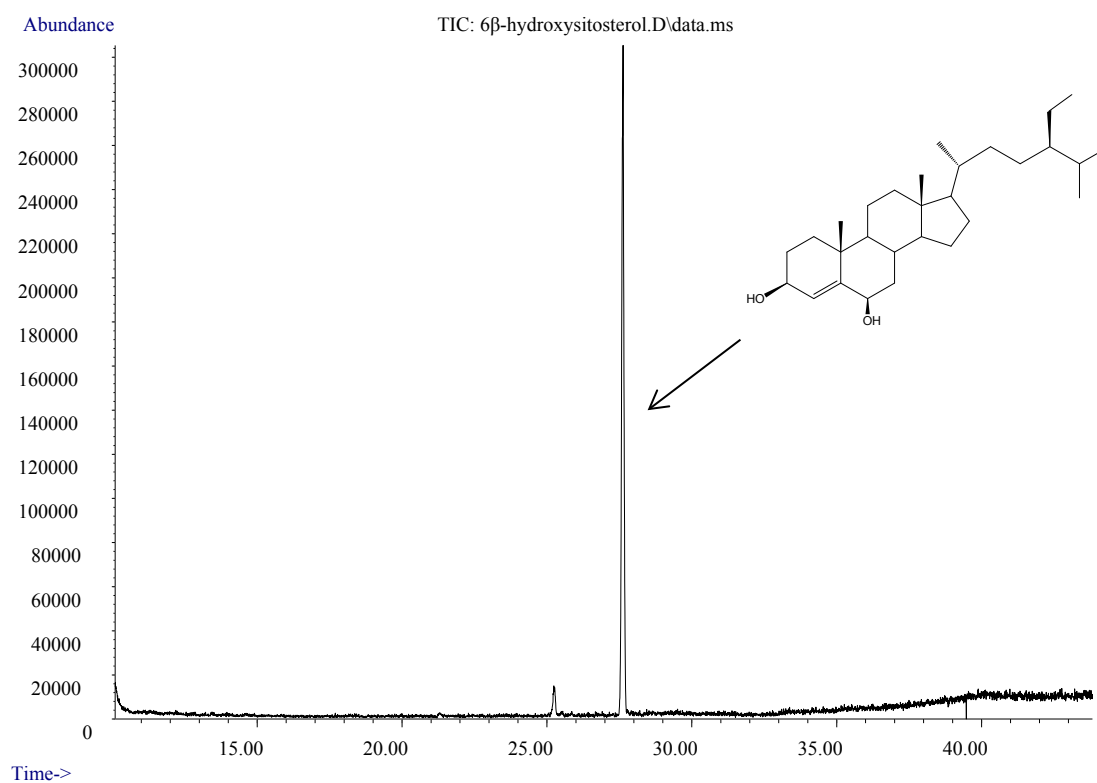


Fig.1-1 Total ion chromatogram of 6 β -hydroxysitosterol

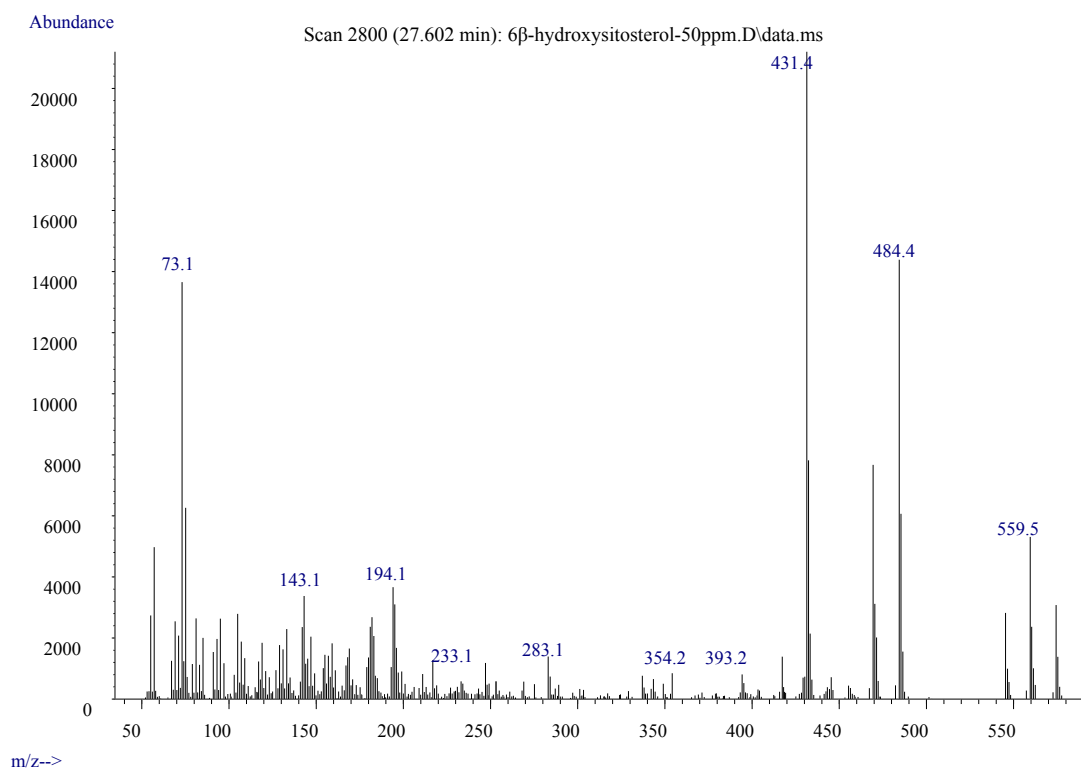


Fig. 1-2 Fragmentation pattern of 6 β -hydroxysitosterol

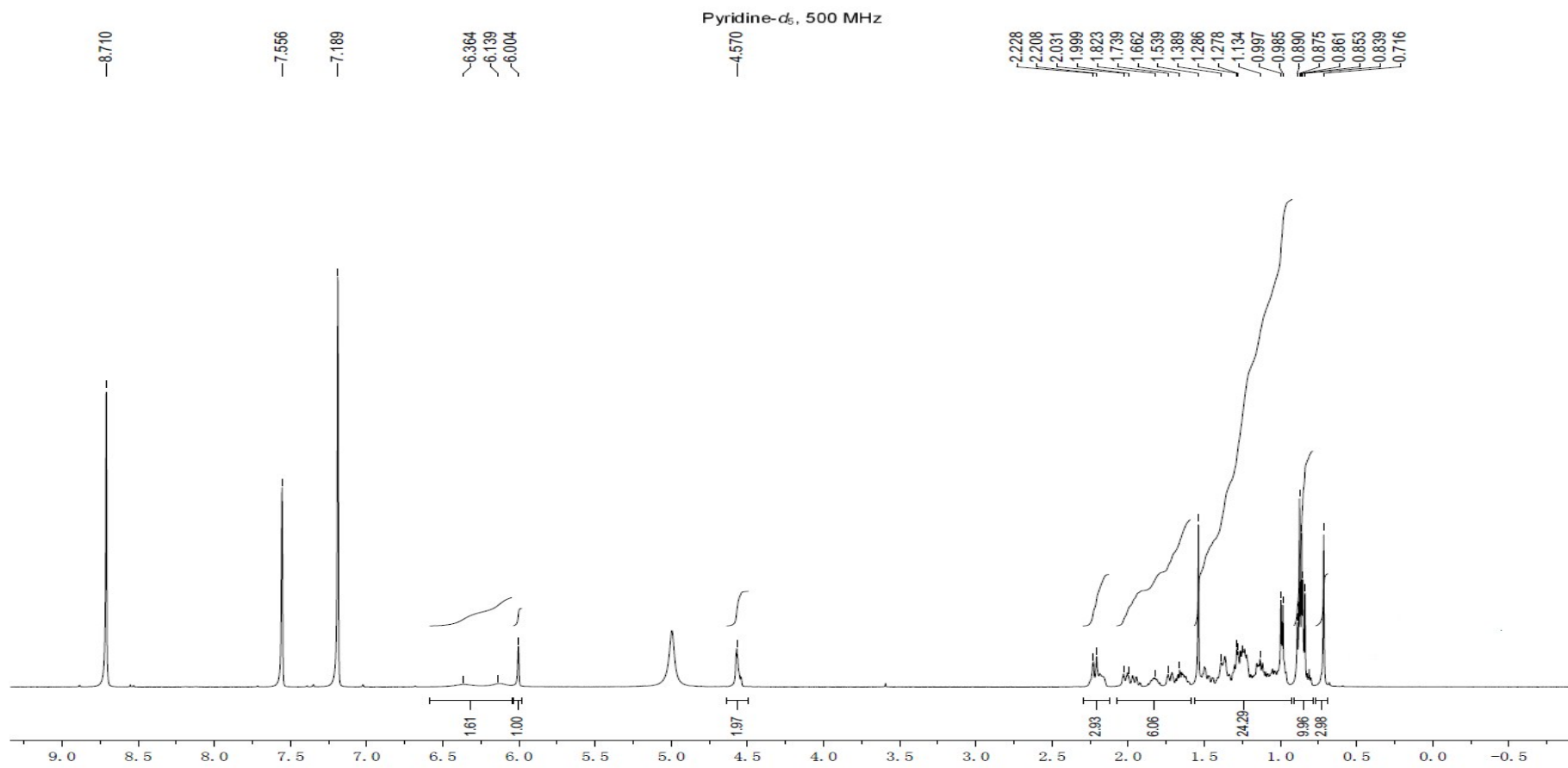


Fig. 1-3 ^1H -NMR data of 6 β -hydroxysitosterol

Table 1 Characterization data summary of 6β-hydroxysitosterol

Analytical Test	Results
Identification by ¹ H-NMR, GC-MS	Consistent with the above structure
Purity tested by GC-MS	97.0%

2. 7-ketositosterol (7-KS),

Abundance

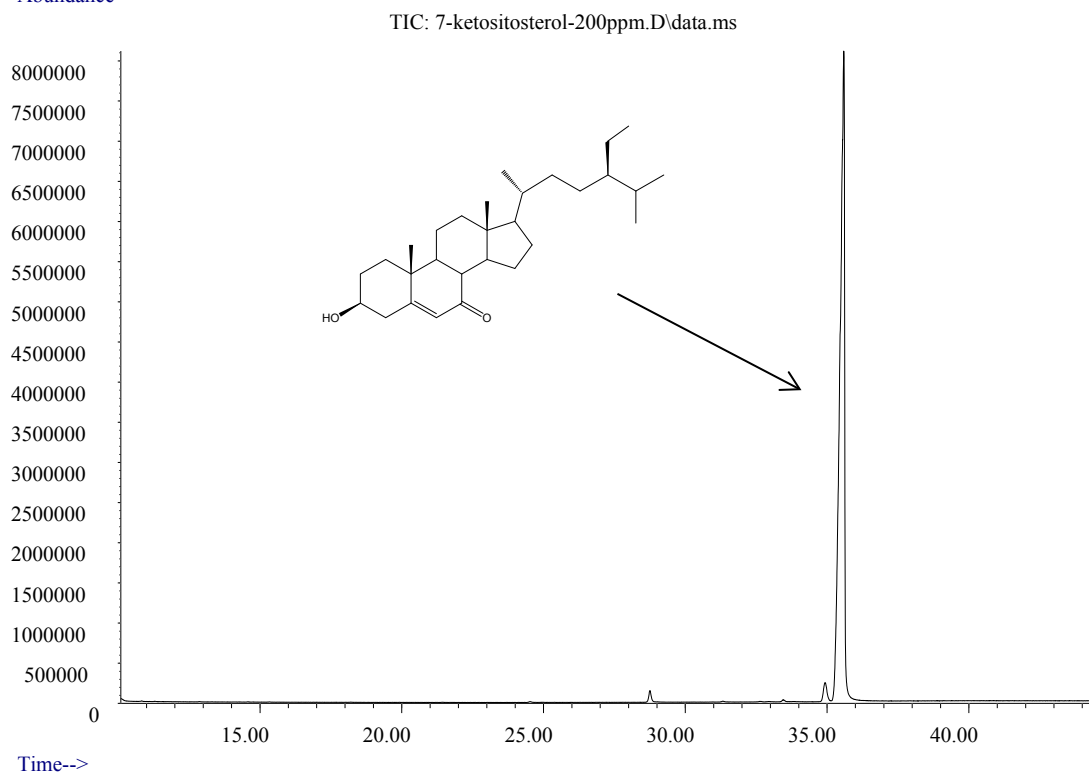


Fig. 2-1 Total ion chromatogram of 7-ketositosterol

Abundance

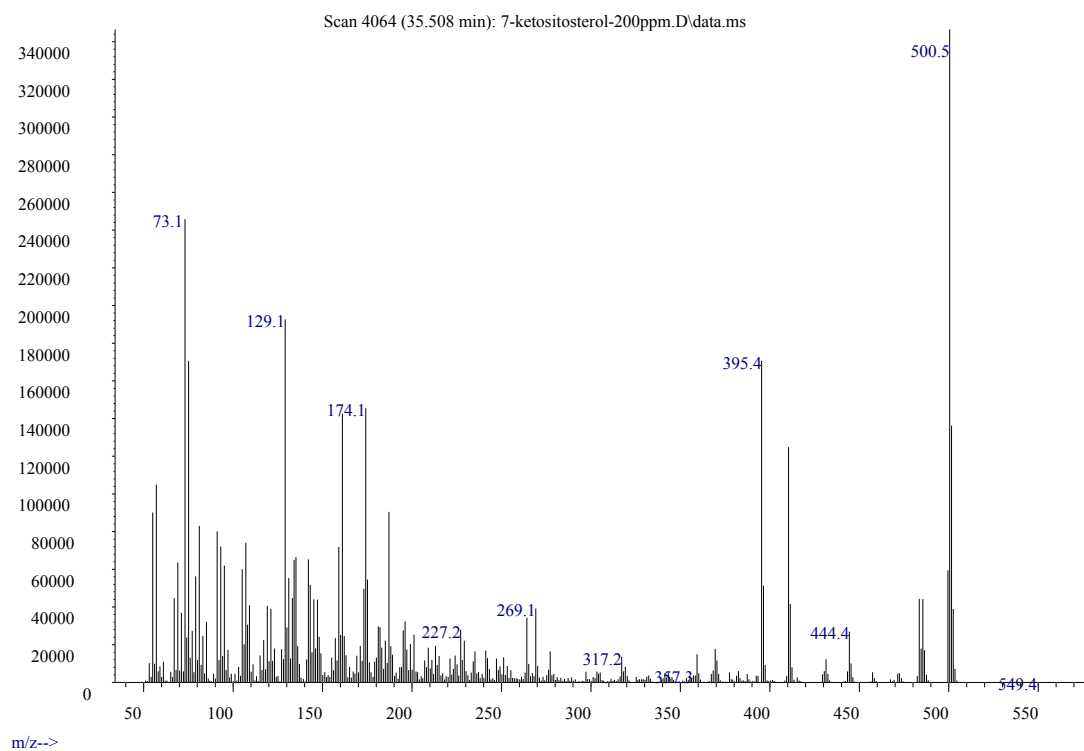


Fig. 2-2 Fragmentation pattern of 7-ketositosterol

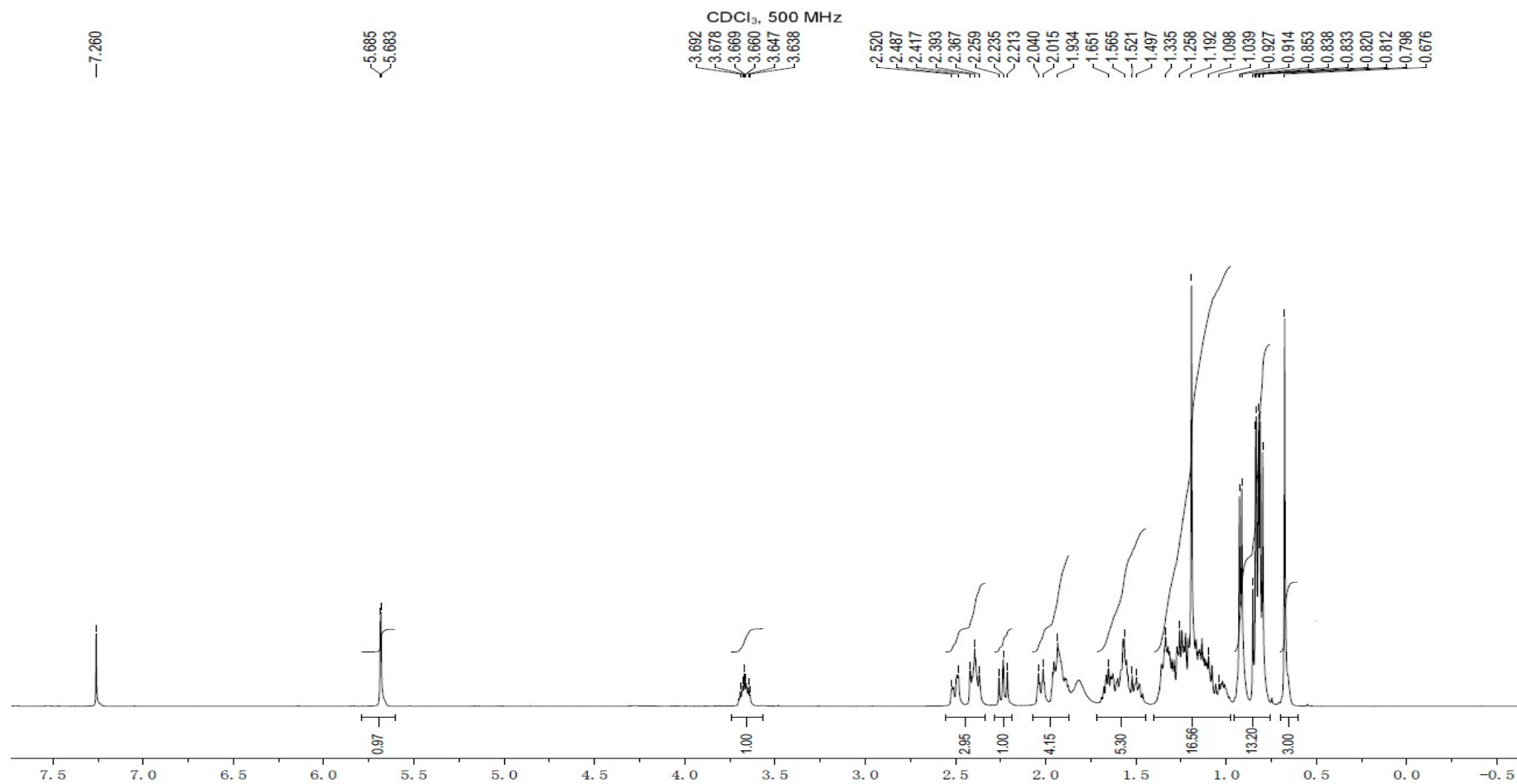


Fig. 2-3 ¹H-NMR data of 7-ketositolsterol

Table 2 Characterization data summary of 7-ketositosterol

Analytical Test	Results
Identification by ¹ H-NMR, GC-MS	Consistent with the above structure
Purity tested by GC-MS	98.0%

3. stigmastane-3 β ,5 α ,6 β -triol (TS),

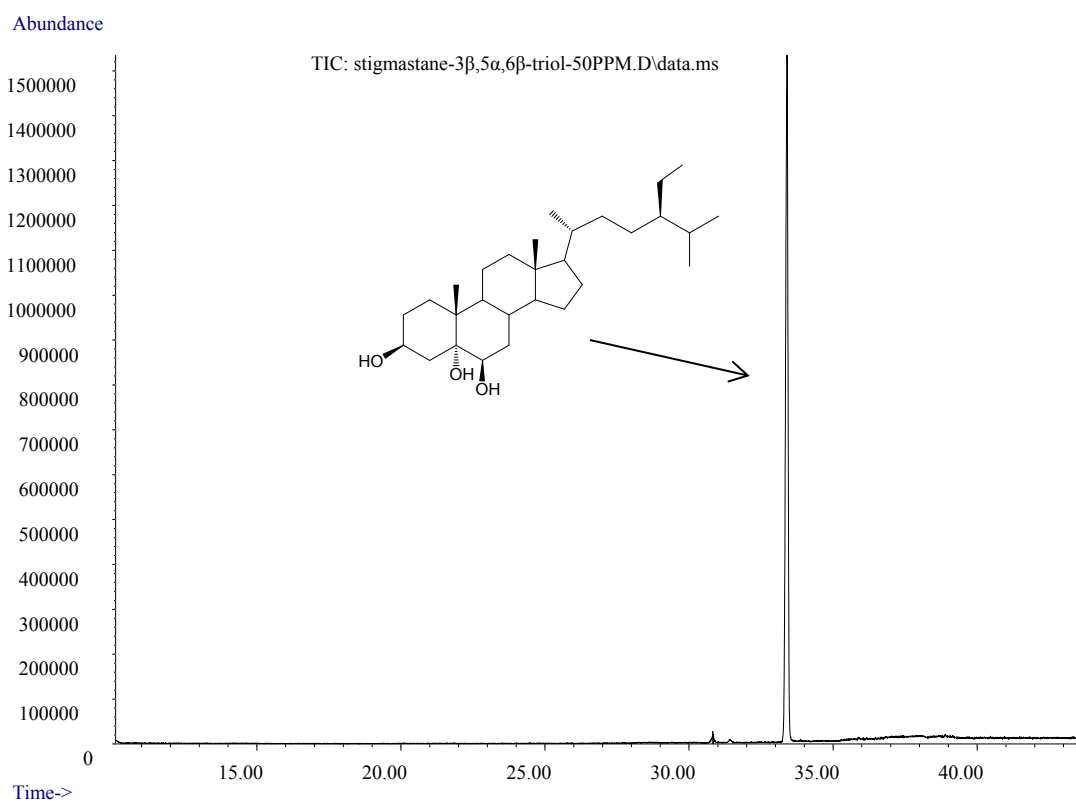


Fig. 3-1 Total ion chromatogram of stigmastane-3 β ,5 α ,6 β -triol

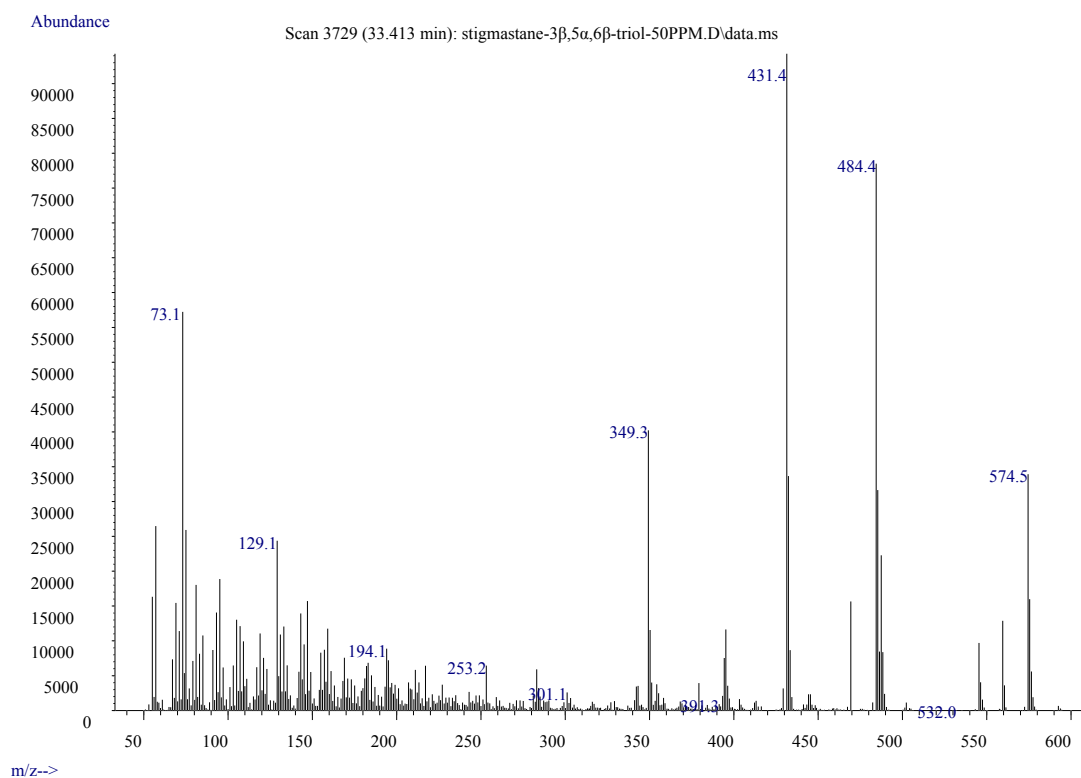


Fig. 3-2 Fragmentation pattern of stigmastane-3 β ,5 α ,6 β -triol

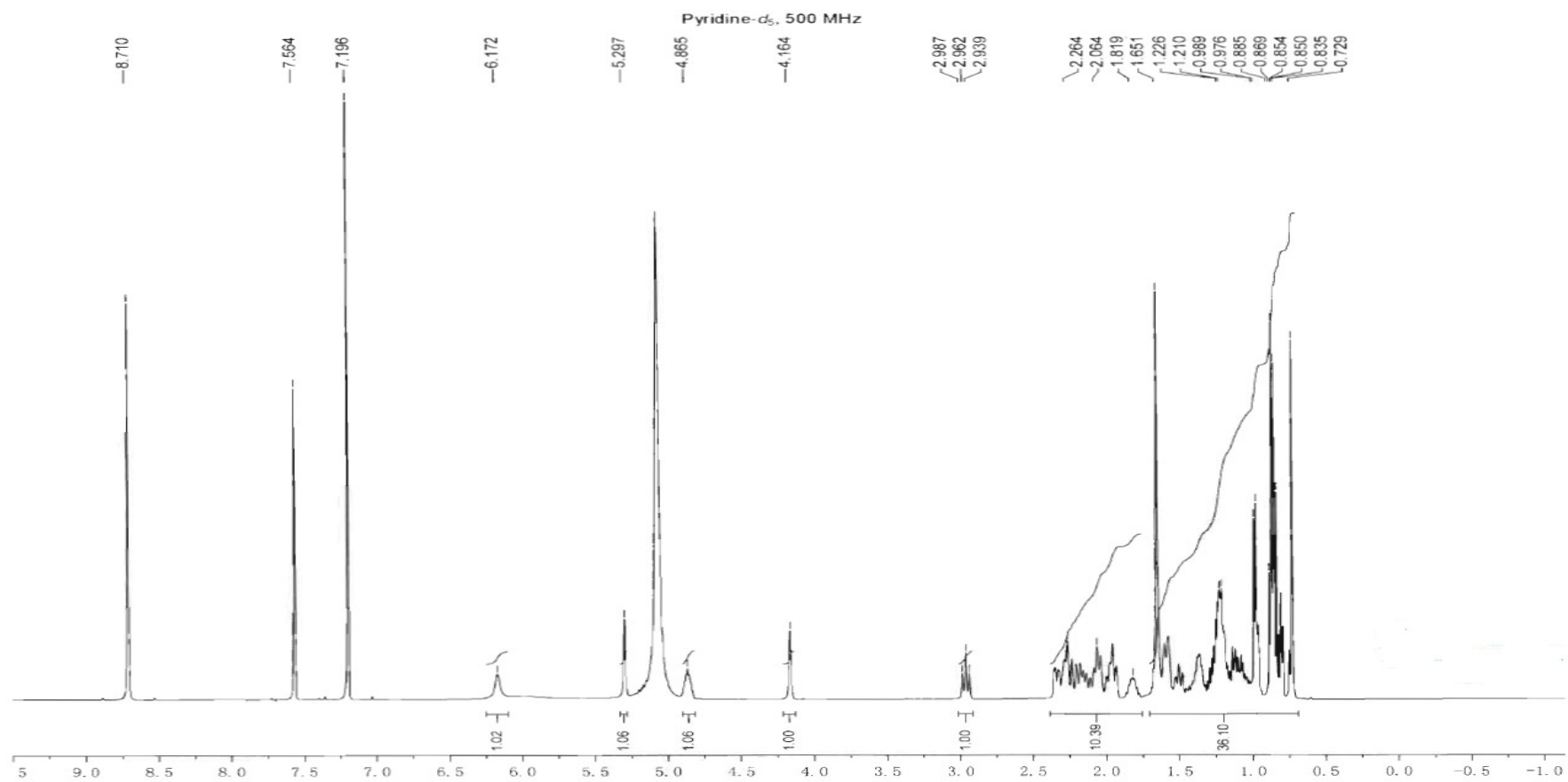
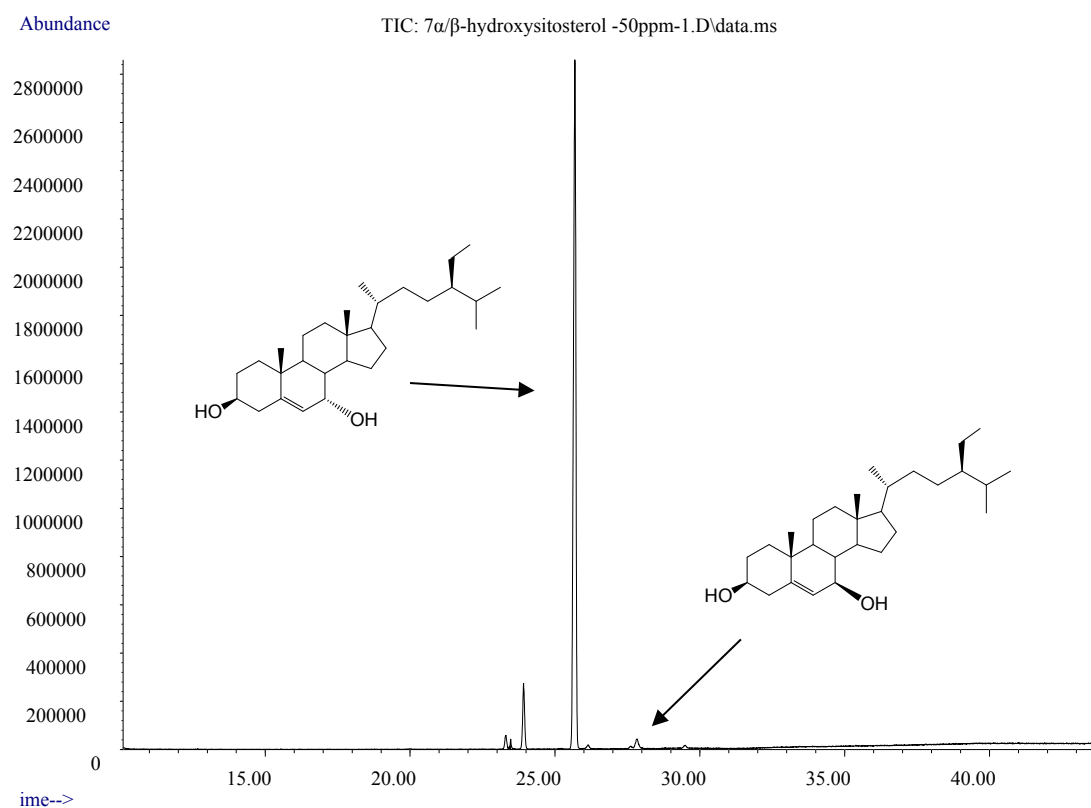


Fig. 3-3 ^1H -NMR data of stigmastane- $3\beta,5\alpha,6\beta$ -triol

Table 3 Characterization data summary of stigmastane-3 β ,5 α ,6 β -triol

Analytical Test	Results
Identification by ¹ H-NMR, GC-MS	Consistent with the above structure
Purity tested by GC-MS	98.0%

4. 7 α / β -hydroxysitosterol (7 α / β -HS),



Abundance

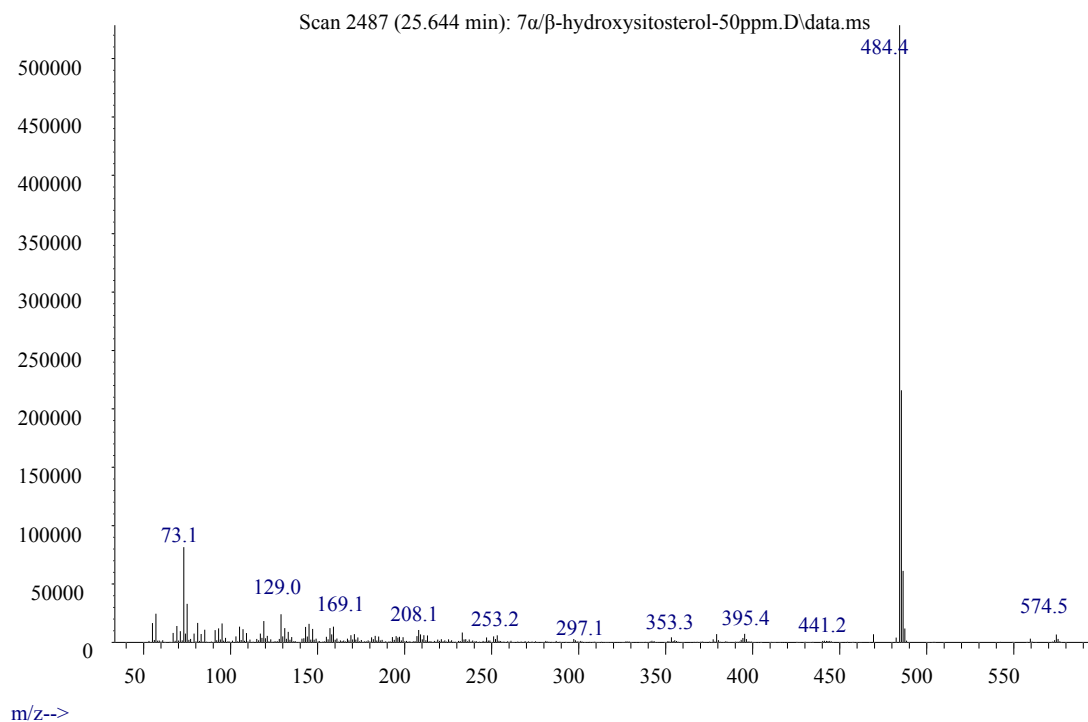


Fig. 4-2 Fragmentation pattern of 7 α -hydroxysitosterol

Abundance

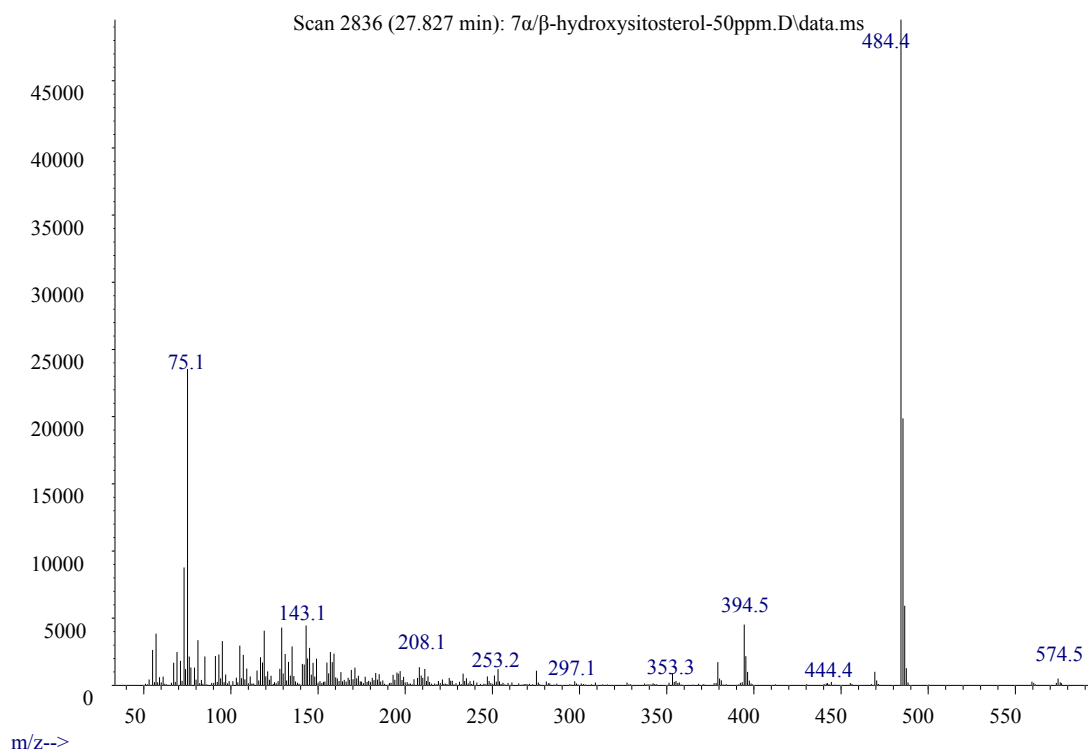


Fig. 4-3 Fragmentation pattern of 7 β -hydroxysitosterol

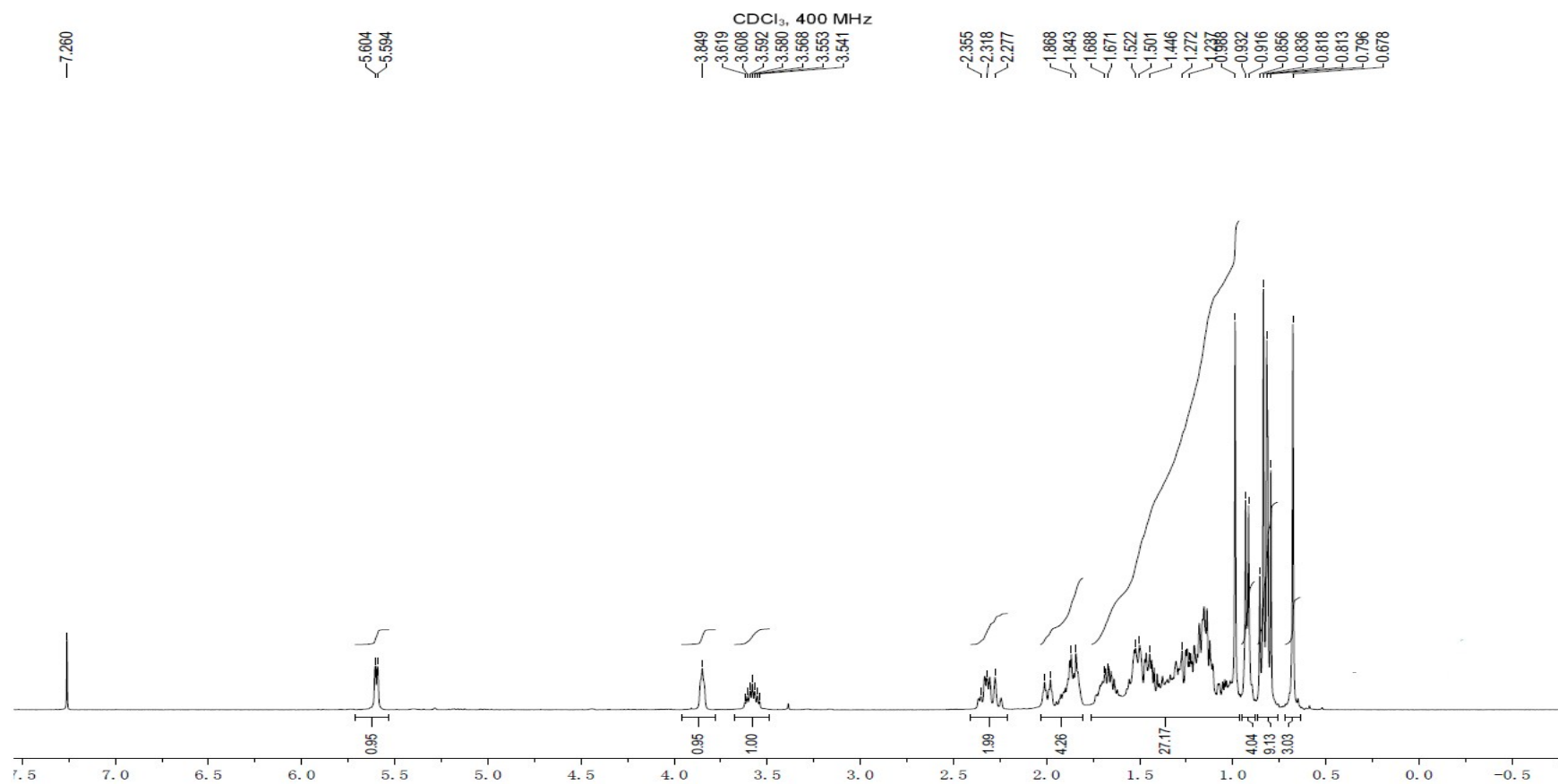


Fig. 4-4 ^1H -NMR data of $7\alpha/\beta$ -hydroxysitosterol

NMR spectra were recorded on a Bruker AVANCE III 400 spectrometer (^1H NMR at 400MHz) with CDCl_3 as the solvent at ambient temperature

Table 4 Characterization data summary of 7 α /7 β -hydroxysitosterol

Analytical Test	Results
Identification by ¹ H-NMR,GC-MS	Consistent with the above structure
Purity tested by GC-MS	95.0% (94% 7 α -HS, 1% 7 β -HS)

5. 5 α ,6 α /5 β ,6 β -epoxysitosterol (α/β -ES)

Abundance

TIC: 5 α ,6 α /5 β ,6 β -epoxysitosterol-50ppm.D\data.ms

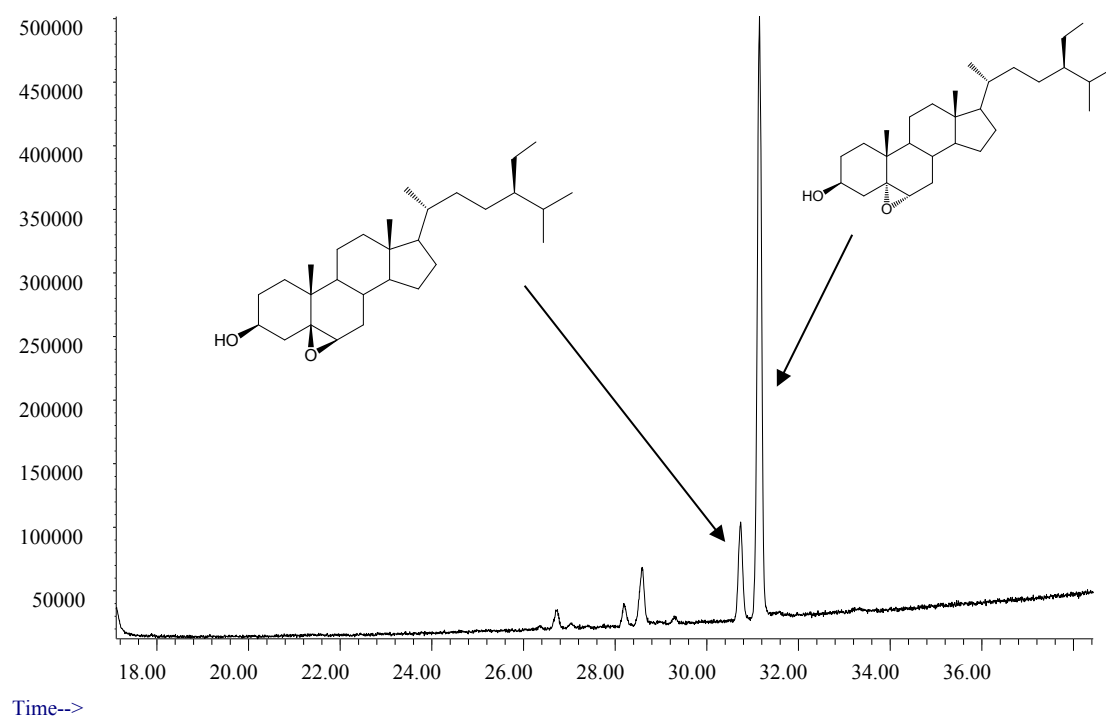


Fig. 5-1 Total ion chromatogram of 5 α ,6 α /5 β ,6 β -epoxysitosterol

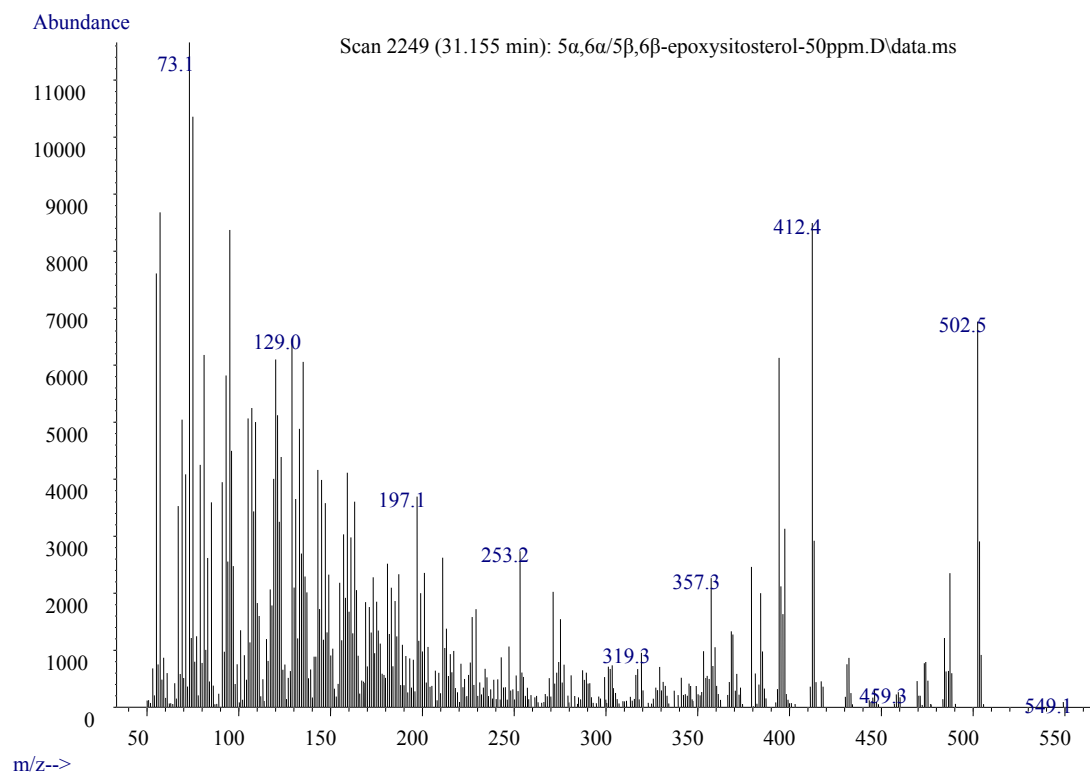


Fig. 5-2 Fragmentation pattern of 5 α ,6 α -epoxysitosterol

Abundance

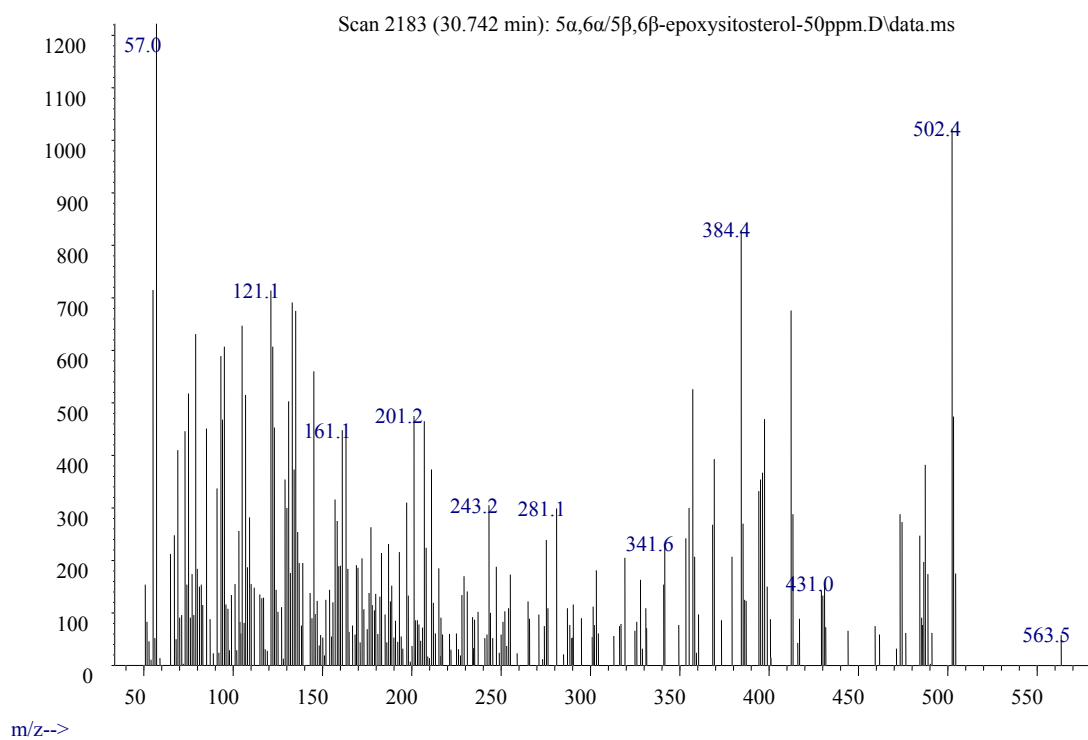


Fig. 5-3 Fragmentation pattern of 5 β ,6 β -epoxysitosterol

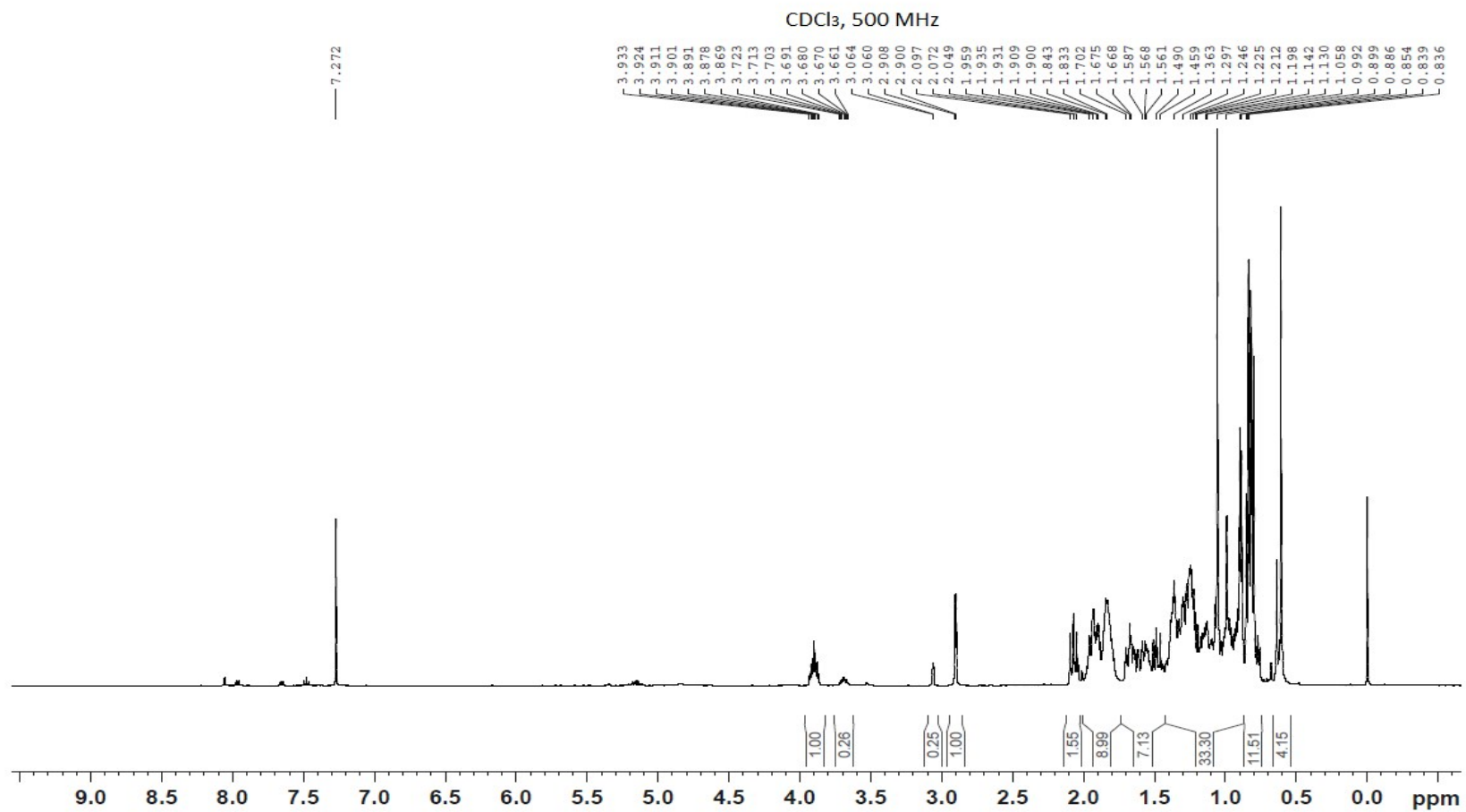


Fig. 5-4 ¹H-NMR data of of 5 α ,6 α /5 β ,6 β -epoxysitosterol

Table 5 Characterization data summary of 5 α ,6 α /5 β ,6 β -epoxysitosterol

Analytical Test	Results
Identification by ¹ H-NMR and GC-MS	Consistent with the above structure
Purity tested by GC-MS	90.0%(75% α -ES, 15% β -ES)

Supporting information II

SIM parameters (29.70 min-32.8 min)

Group 20

Group ID : 7 β -HS and 7-KC
Resolution : low
Group start time : 29.70
Draw 1 ion : 484.40
Draw 2 ion : 472.40
Ion/dwell time in Group (m/z, dwell time) (m/z, dwell time) (m/z, dwell time)
(367.30, 38) (382.40, 38) (457.40, 38)
(469.40, 38) (472.40, 38) (484.40, 38)
(559.50, 38) (574.50, 38)

Group 21

Group ID : ES, 7-KB and TCam
Resolution : low
Group start time : 30.30
Draw 1 ion : 502.50
Draw 2 ion : 484.40
Ion/dwell time in Group (m/z, dwell time) (m/z, dwell time) (m/z, dwell time)
(379.40, 25) (394.40, 25) (412.40, 25)
(417.40, 25) (455.40, 25) (469.40, 25)
(470.40, 25) (473.40, 25) (484.40, 25)
(487.40, 25) (502.40, 25) (560.50, 25)

Group 22

Group ID : TSt
Resolution : low
Group start time : 31.65
Draw 1 ion : 429.40
Draw 2 ion : 482.40
ion/dwell time in Group (m/z, dwell time) (m/z, dwell time) (m/z, dwell time)
(429.40, 75) (467.40, 75) (482.40, 75)
(572.40, 75)

Group 23

Group ID : 7-KCam
Resolution : low
Group start time : 32.80
Draw 1 ion : 486.40
Draw 2 ion : 381.40
Ion/dwell time in Group (m/z, dwell time) (m/z, dwell time) (m/z, dwell time)

(381.30, 75) (396.30, 75) (471.40, 75)
(486.40, 75)