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APPENDICES

APPENDIX A

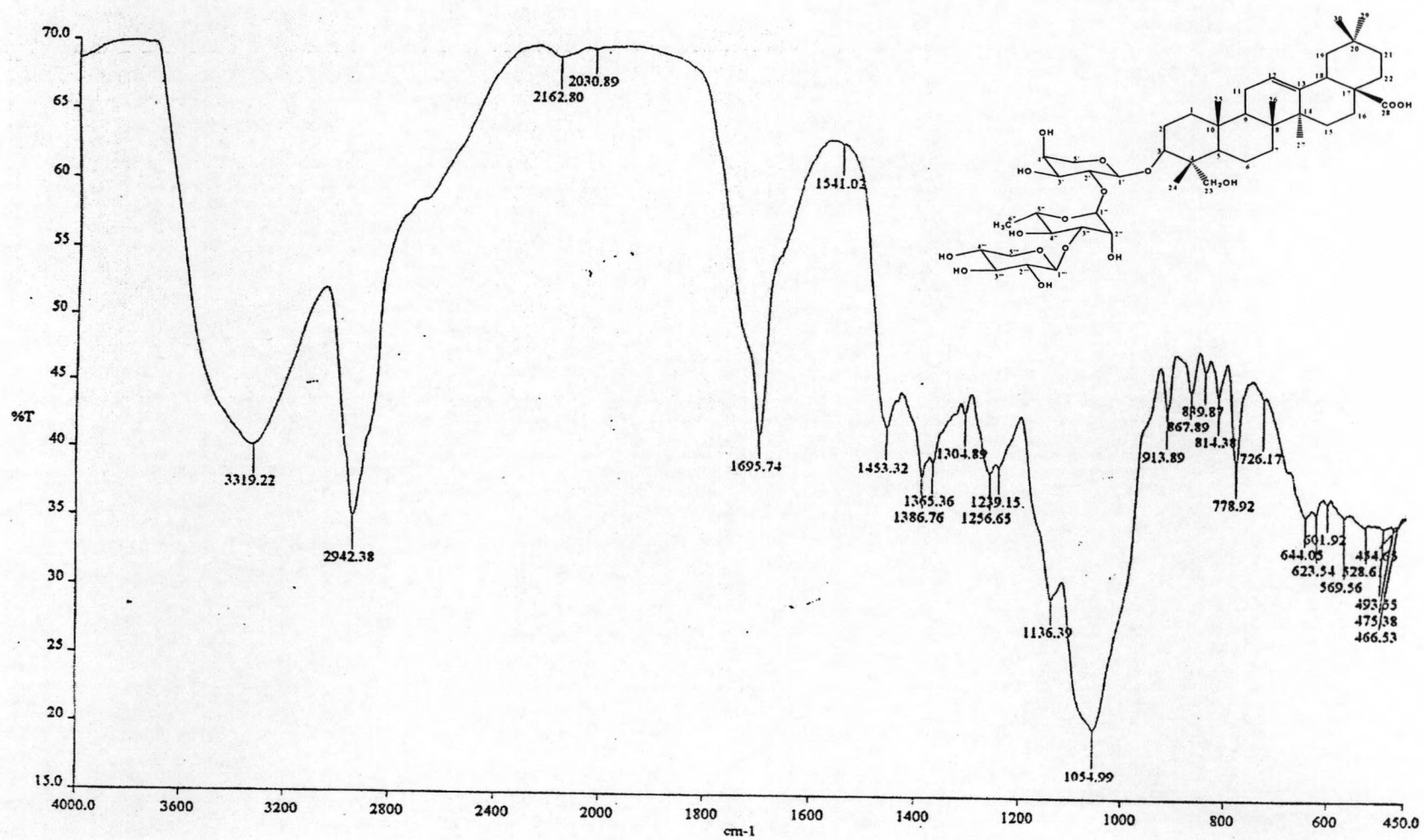


Figure 11 The IR spectrum of Compound Sp1 (KBr disc)

S3ms2 - ESI - dg 881 - scan 600-1000 - EC 35 eV
s3ms2dg881 133 (2.486) Sm (Mn, 4x1.00); Cm (117:181-90:110)

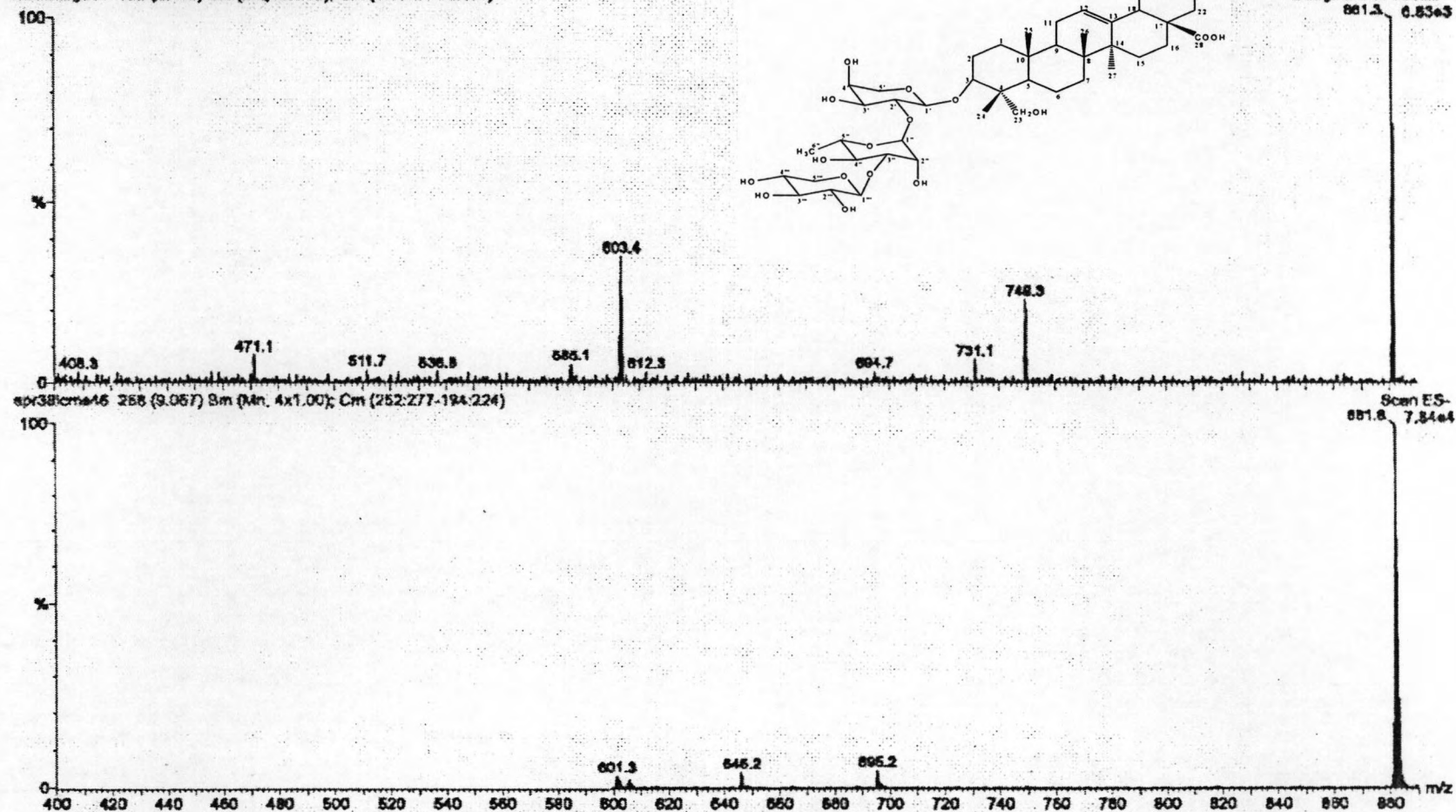


Figure 12 The EI-MS spectrum of Compound Sp1

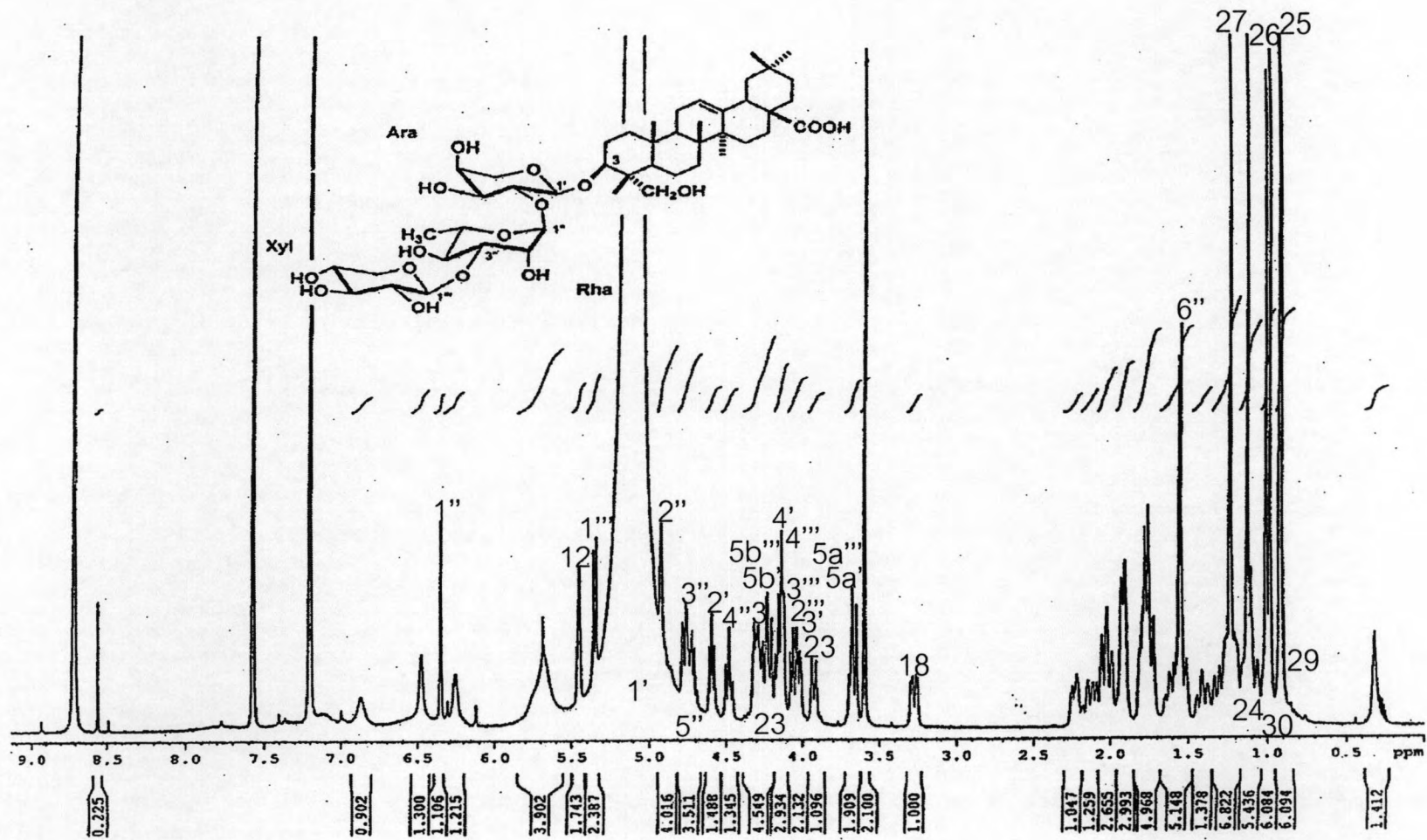


Figure 13 The 400 MHz ¹H-NMR spectrum of Compound Sp1 (in pyridine-d₅)

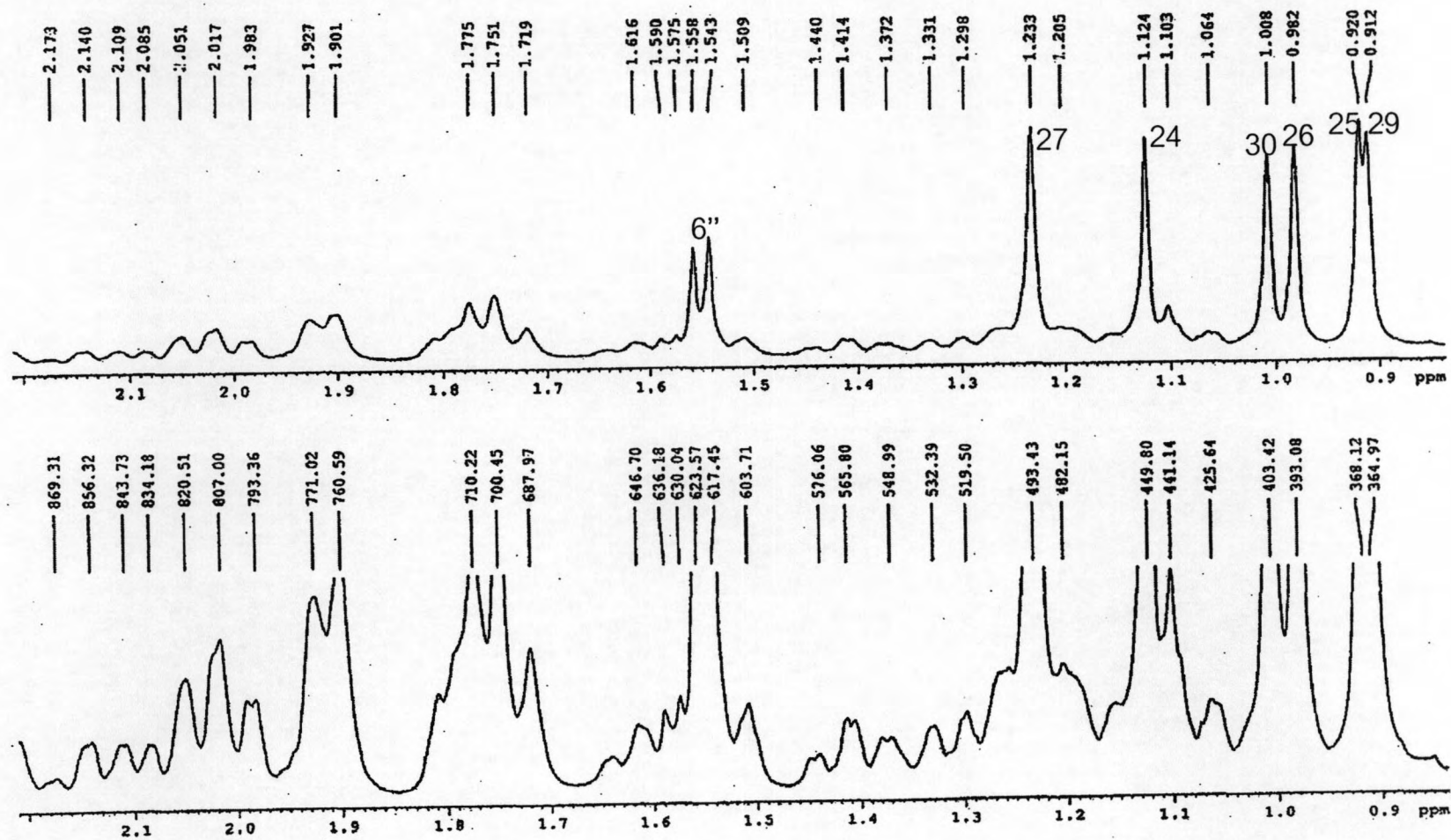


Figure 14 The expansion of $^1\text{H-NMR}$ spectrum of Compound Sp1 (in $\text{pyridine-}d_5$)

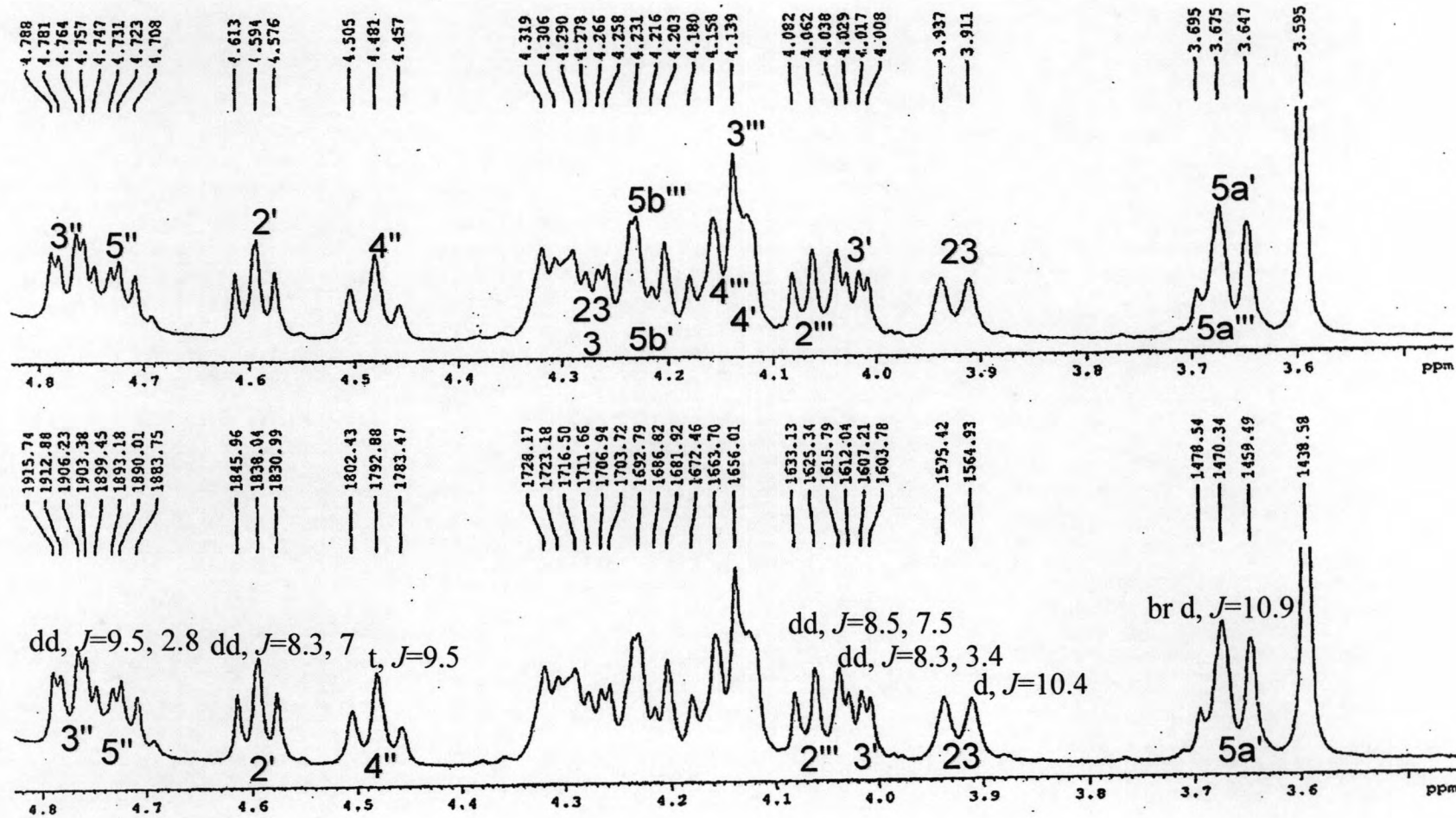


Figure 15 The expansion of ¹H-NMR spectrum of Compound Sp1 (in pyridine-d₅)

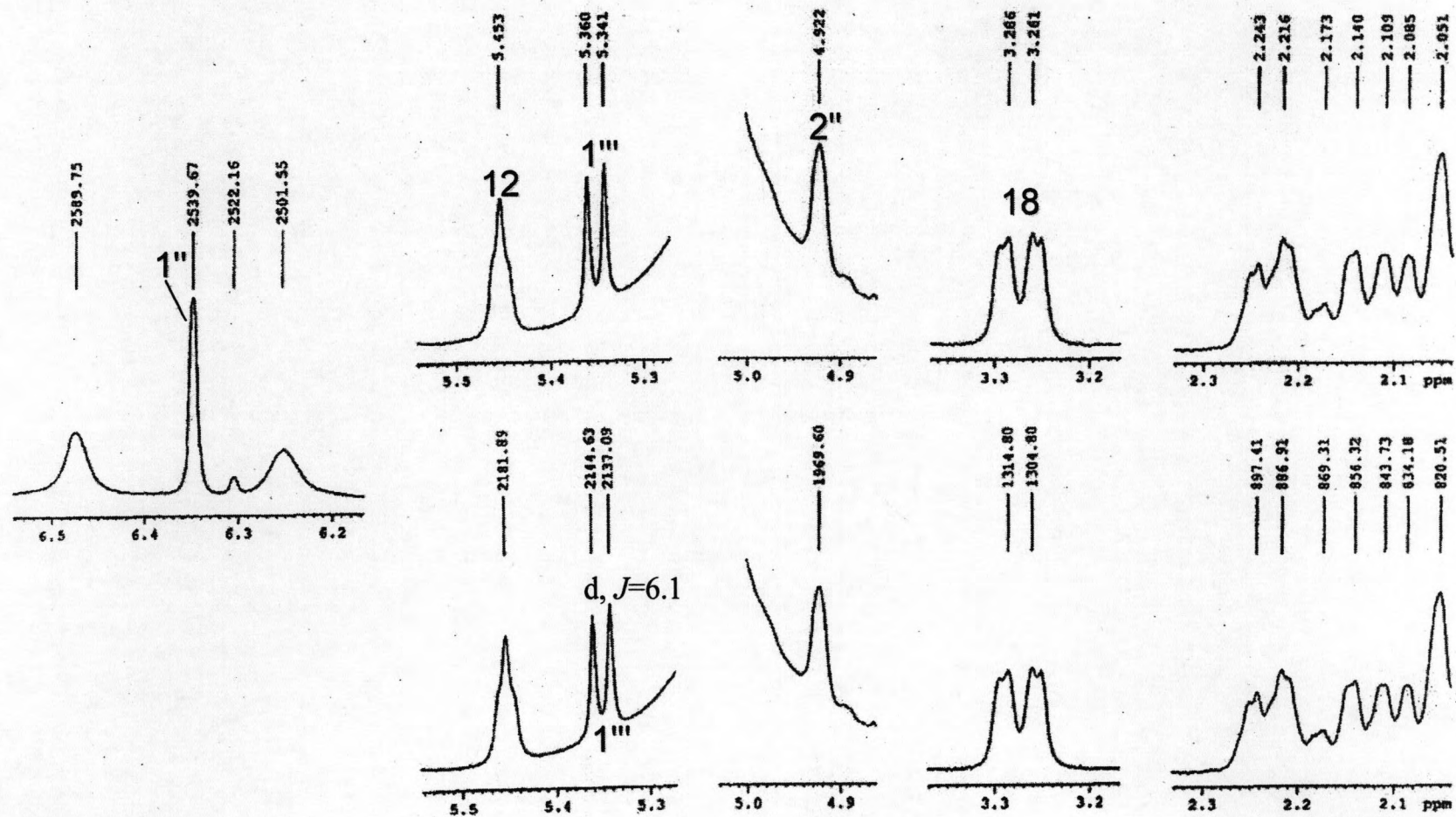


Figure 16 The expansion of $^1\text{H-NMR}$ spectrum of Compound Sp1 (in $\text{pyridine-}d_5$)

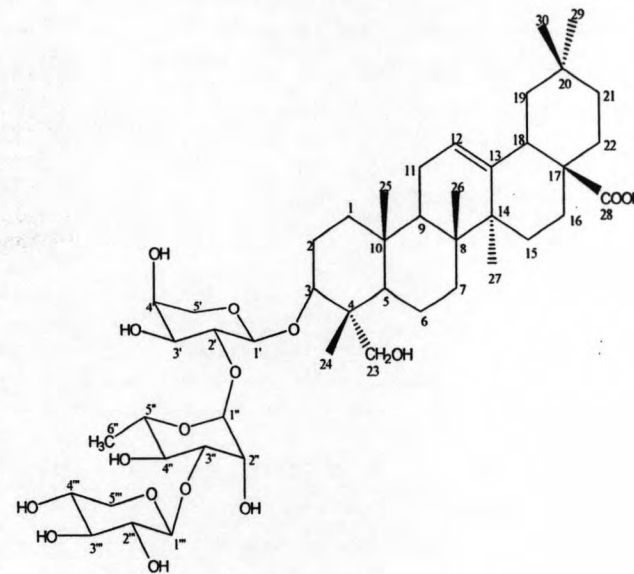
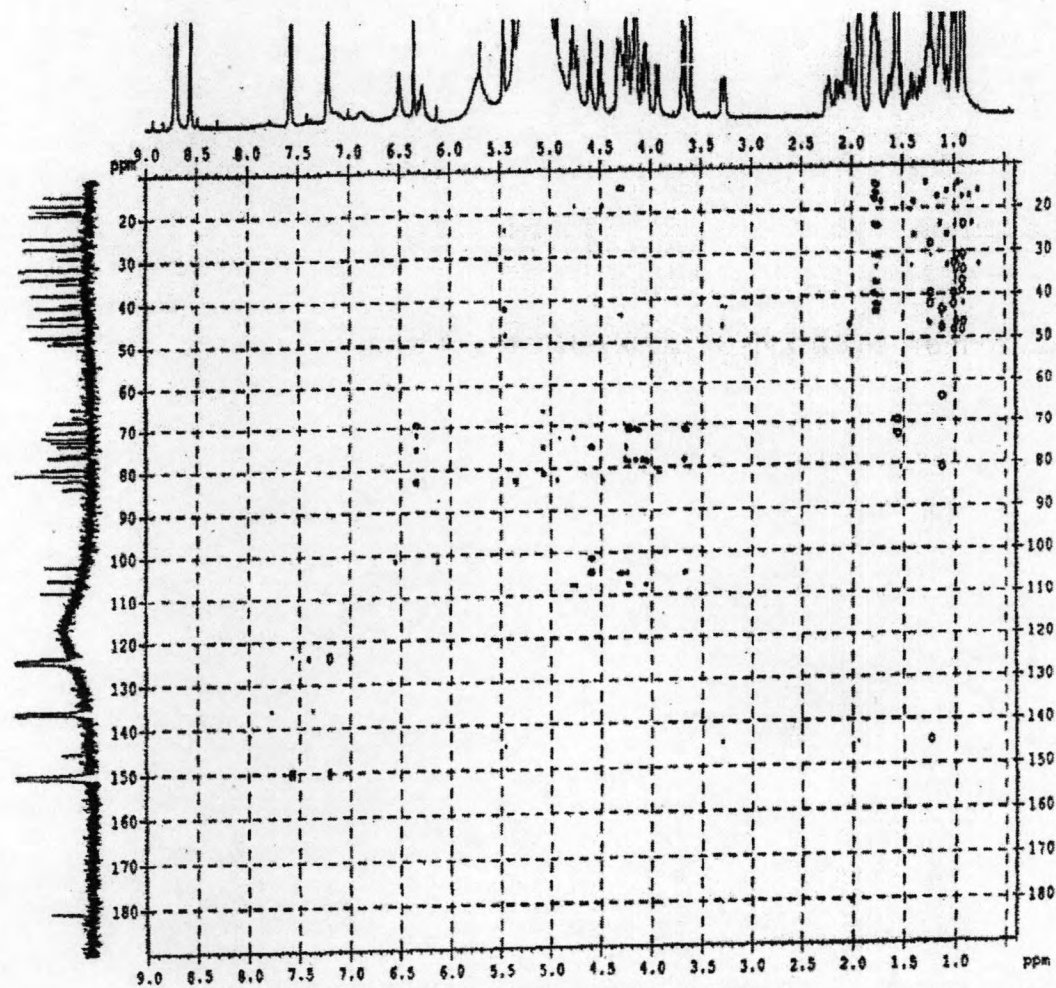


Figure 18 The 400 MHz HMBC spectrum of Compound Sp1 (in pyridine- d_5)

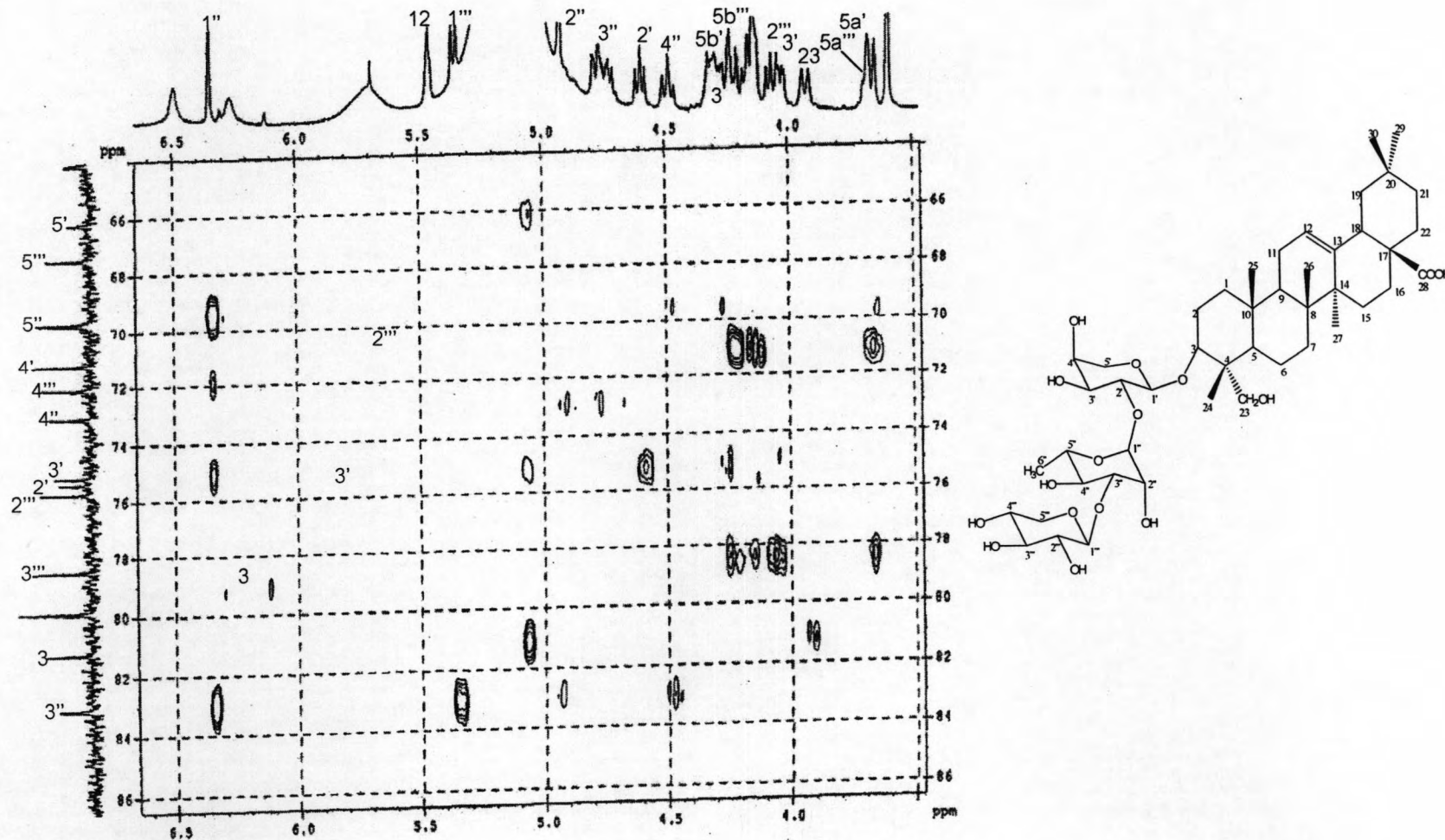


Figure 19 The expansion of HMBC spectrum of Compound Sp1 (in pyridine- d_5)

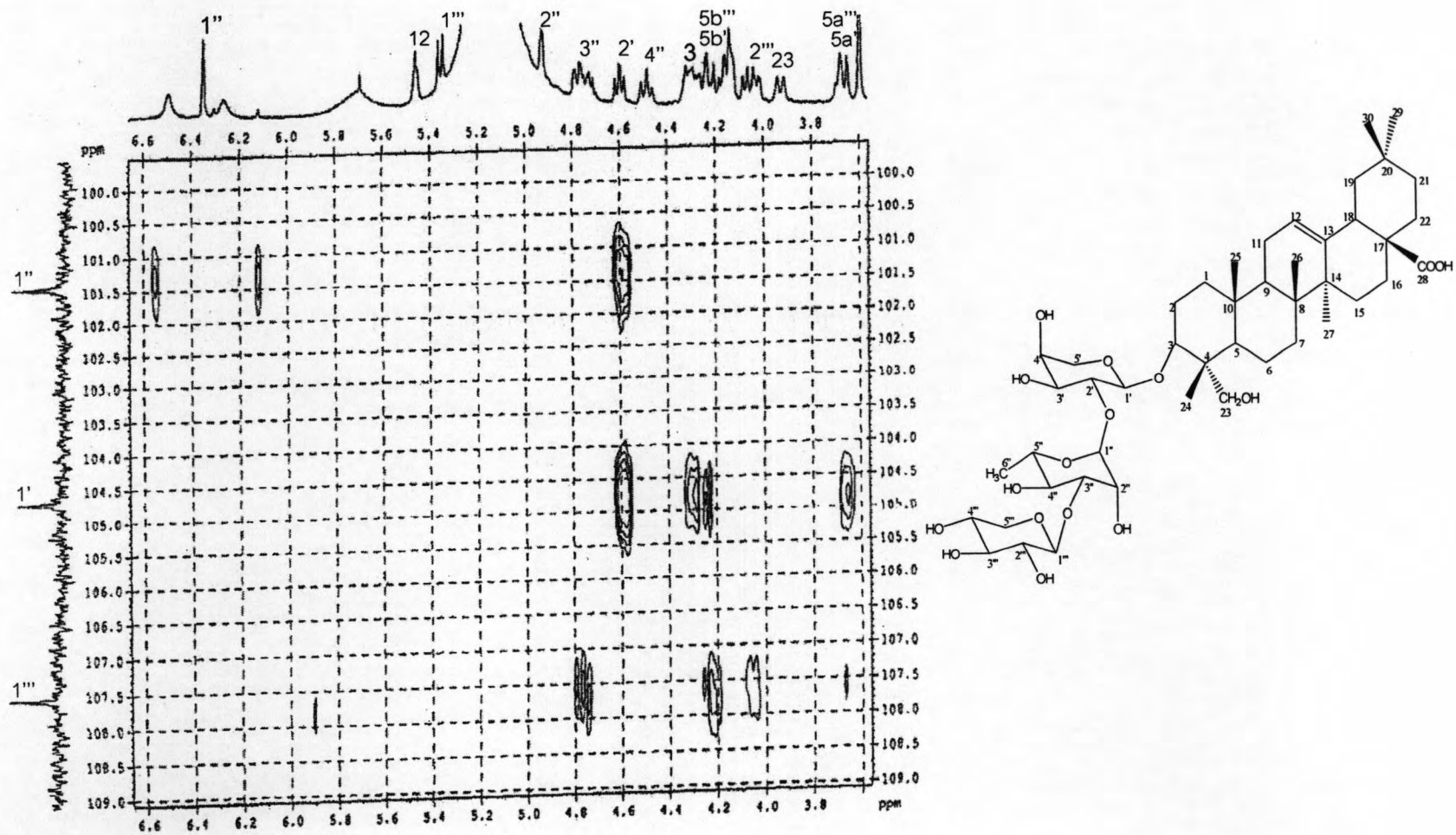


Figure 20 The expansion of HMBC spectrum of Compound Sp1 (in pyridine- d_5)

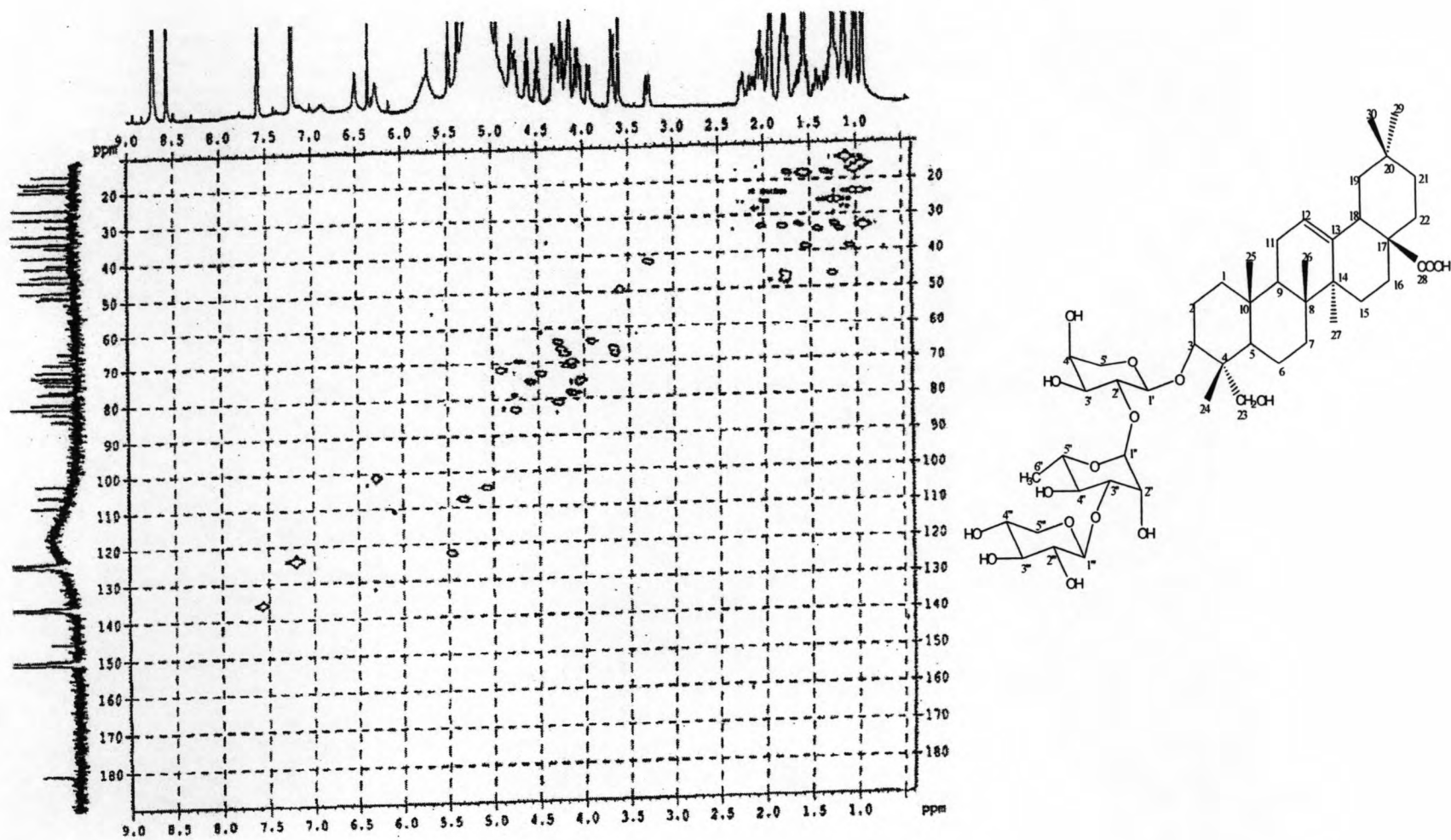


Figure 21 The 400 MHz HMQC spectrum of Compound Sp1 (in pyridine-*d*₅)

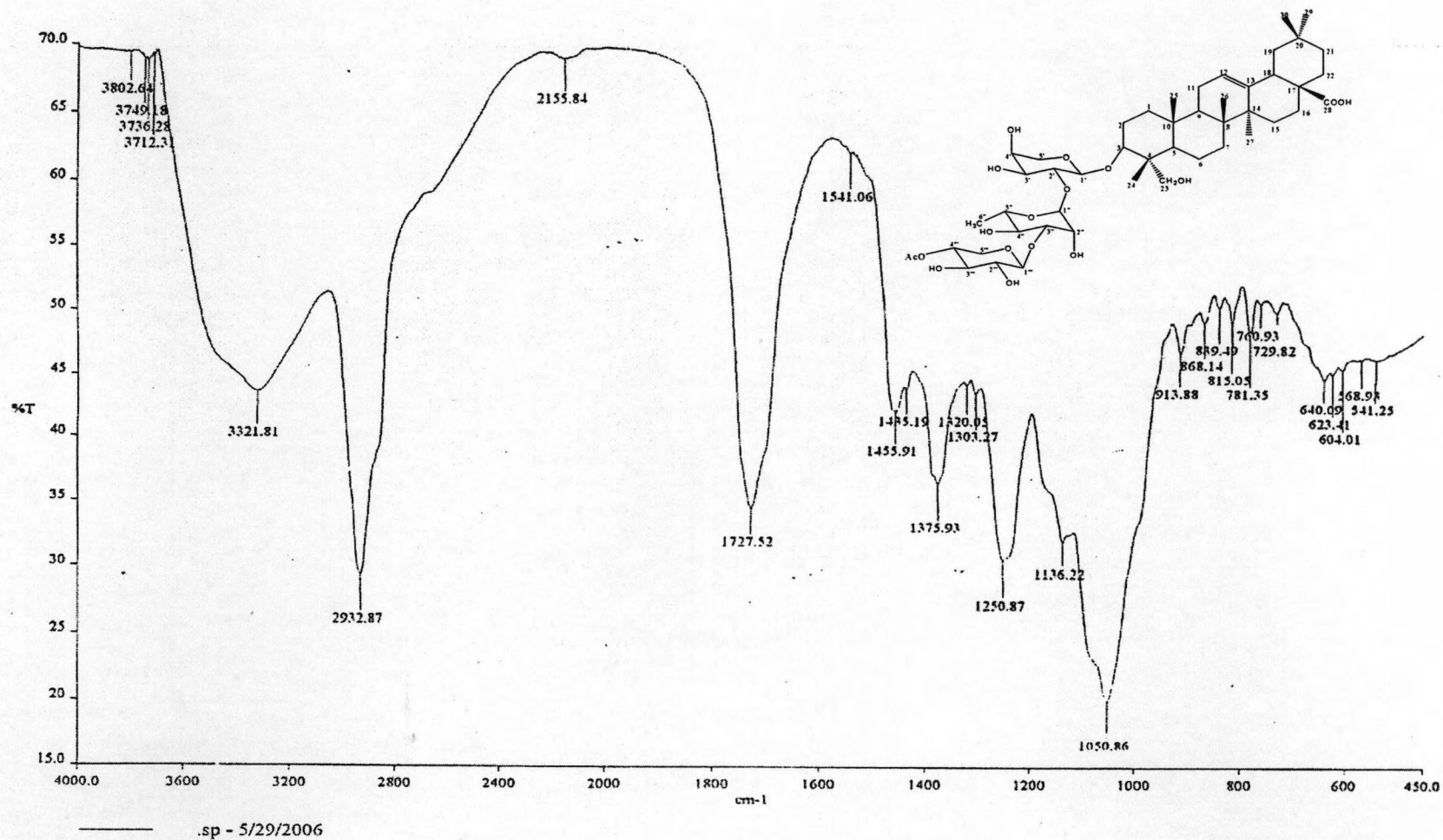
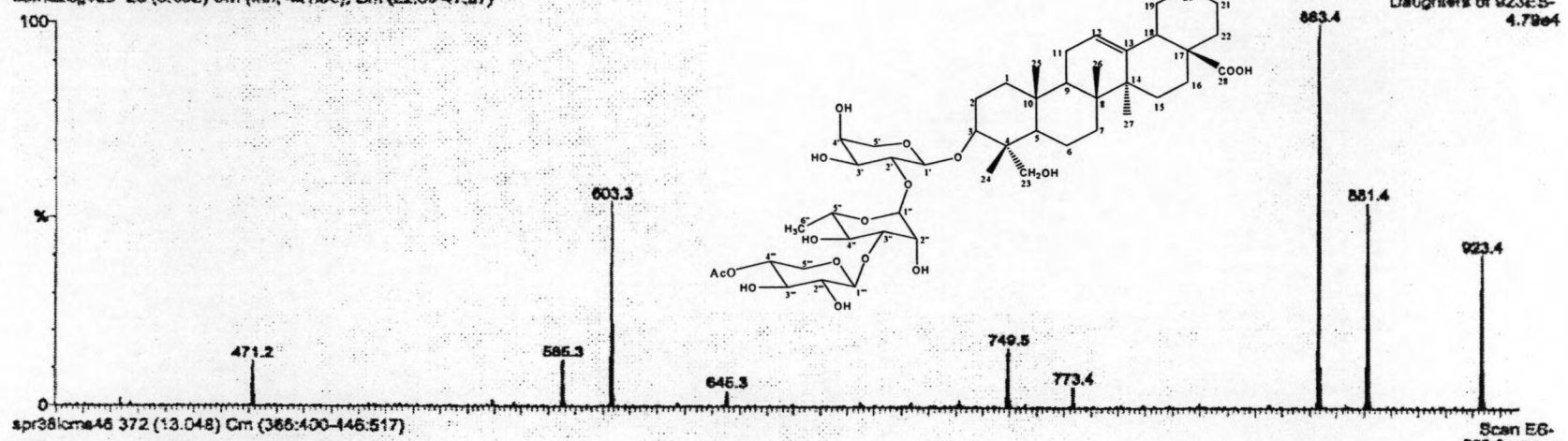


Figure 22 The IR spectrum of Compound Sp2 (KBr disc)

spr 38 - scan 800-1600 - ESI- Icms 48(0)61(28)70(3
 u3ma2dq923 28 (0.632) Sm (Min, 4x1.00); Cm (22:39-47:57)



spr38 Icms 46 372 (13.048) Cm (366:400-446:517)

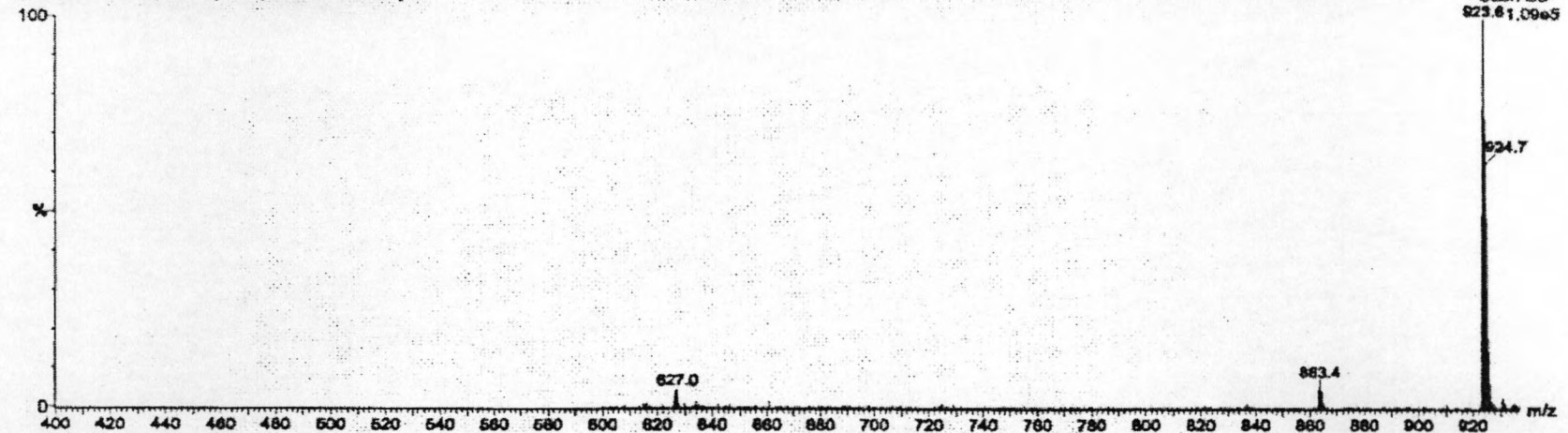


Figure 23 The EI-MS spectrum of Compound Sp2

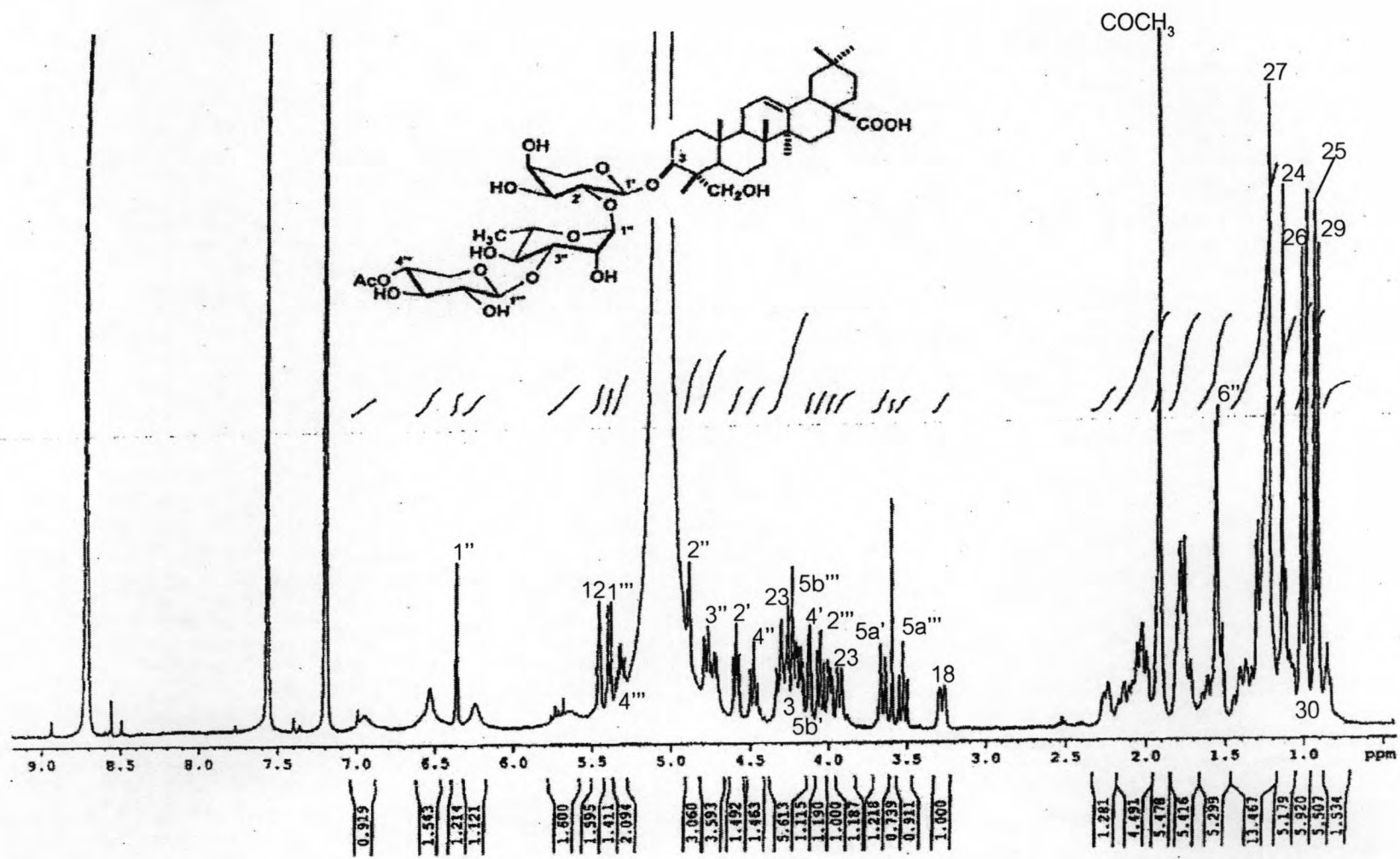


Figure 24 The 400 MHz ¹H-NMR spectrum of Compound Sp2 (in pyridine-*d*₅)

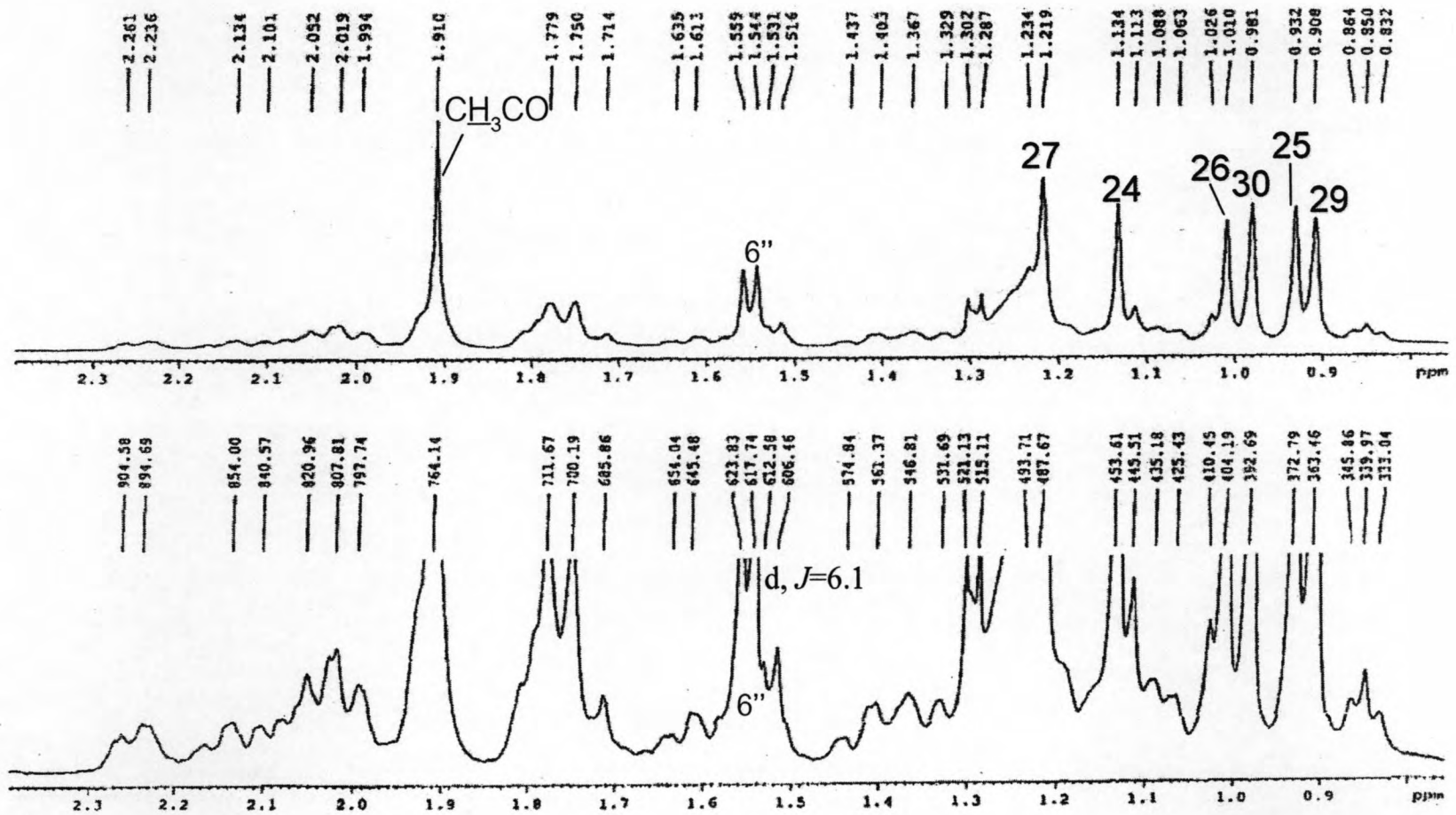


Figure 25 The expansion of ¹H-NMR spectrum of Compound Sp2 (in pyridine-d₅)

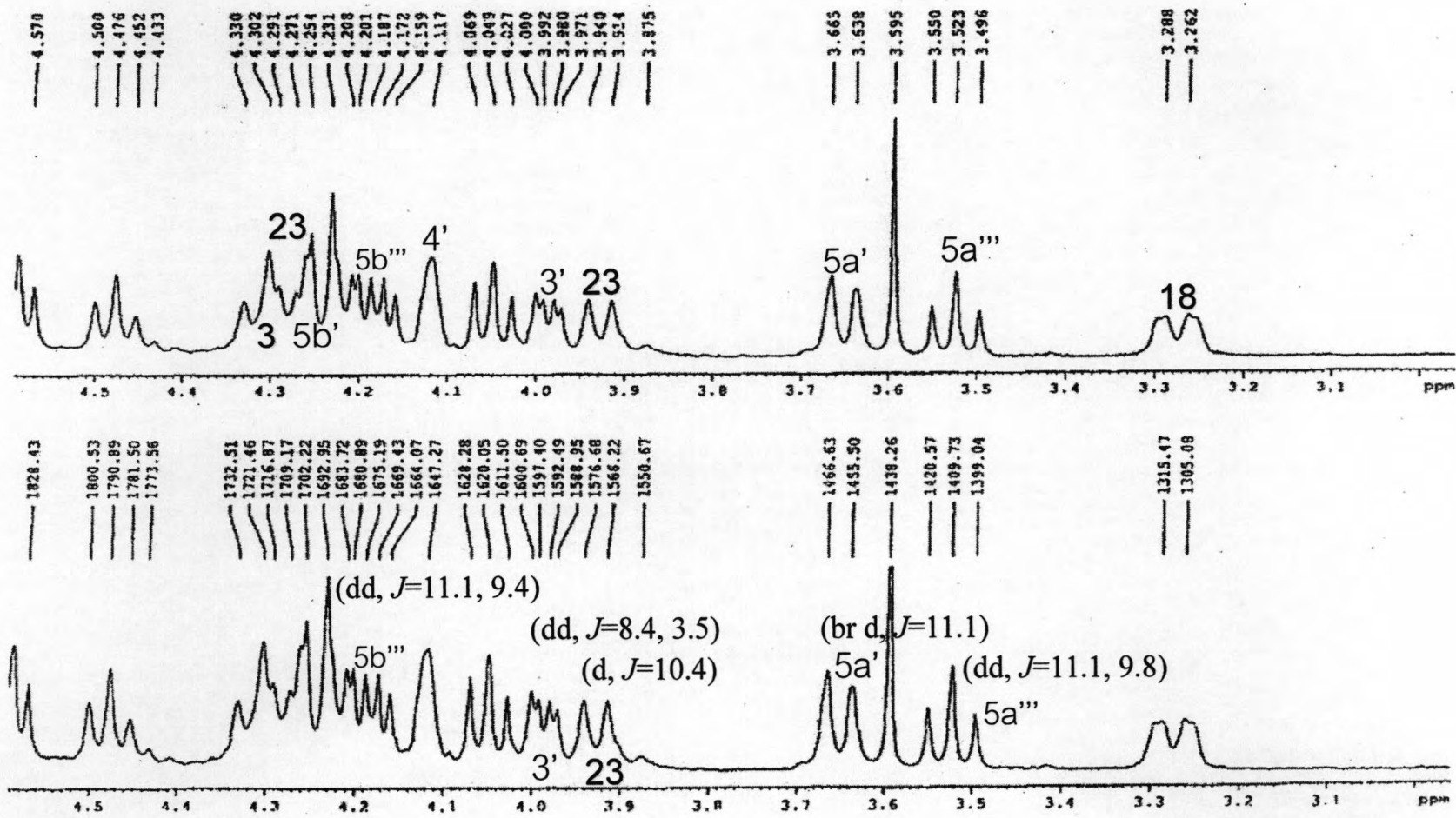


Figure 26 The expansion of $^1\text{H-NMR}$ spectrum of Compound Sp2 (in pyridine- d_5)

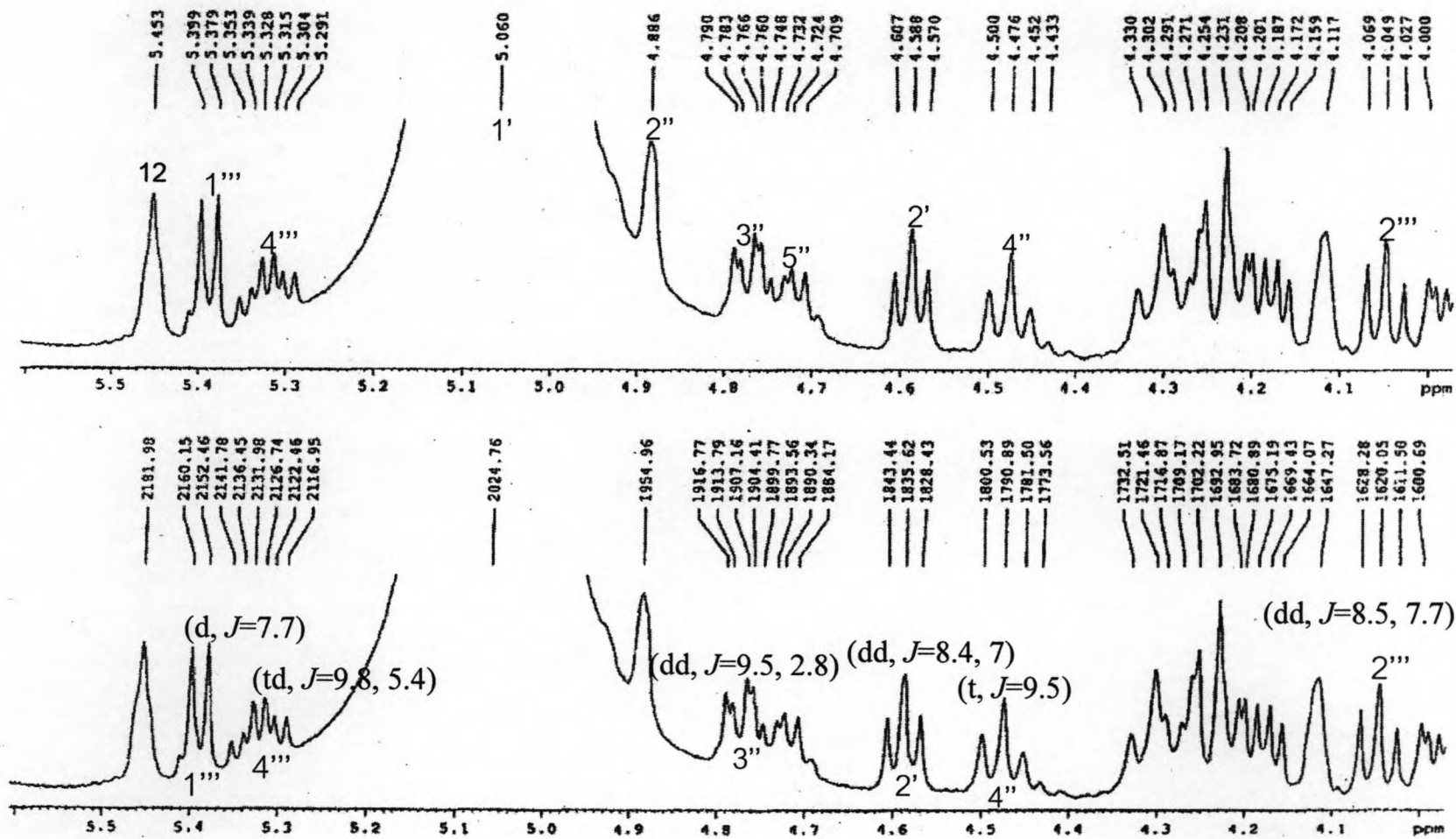


Figure 27 The expansion of ¹H-NMR spectrum of Compound Sp2 (in pyridine-d₅)

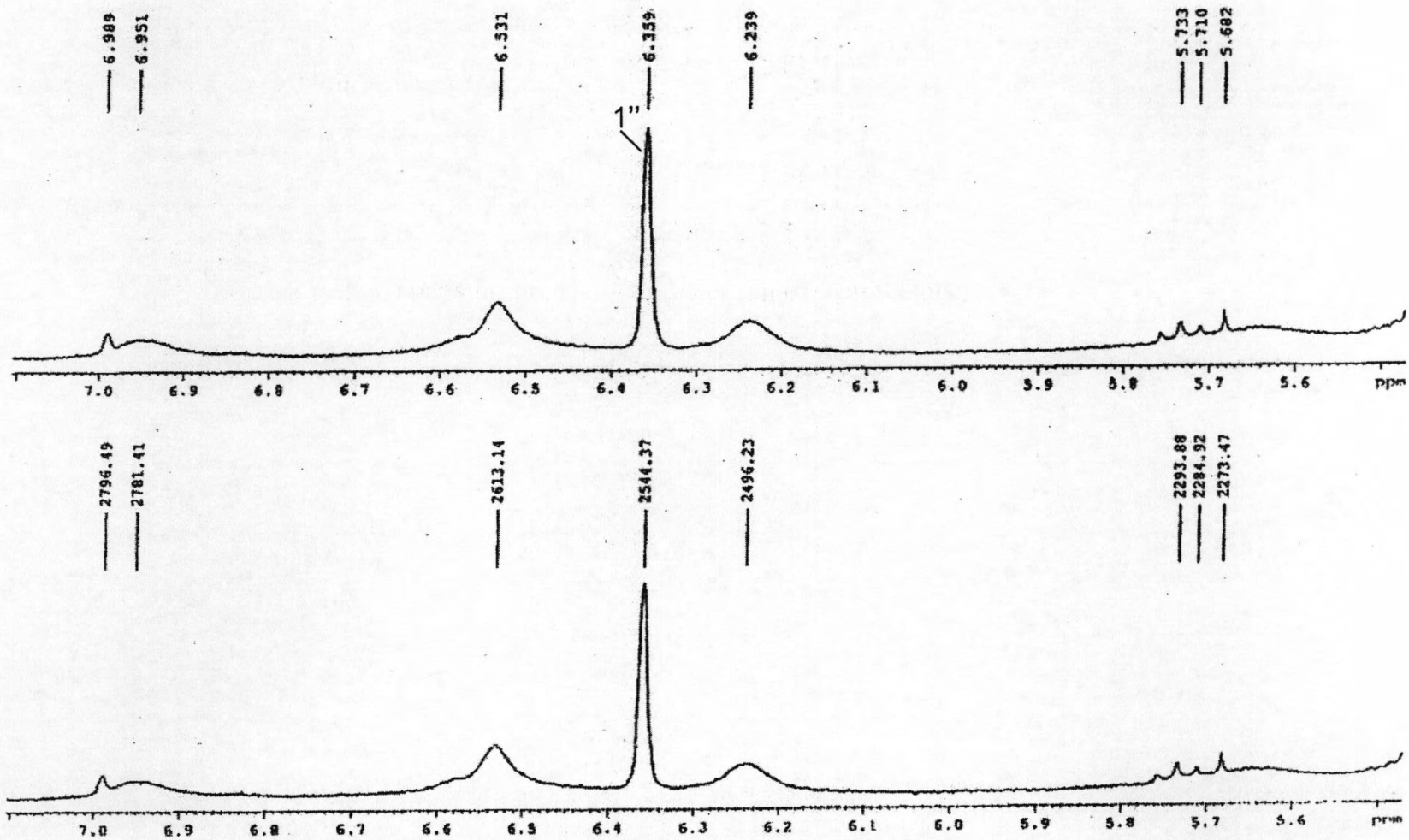


Figure 28 The expansion of $^1\text{H-NMR}$ spectrum of Compound Sp2 (in pyridine- d_5)

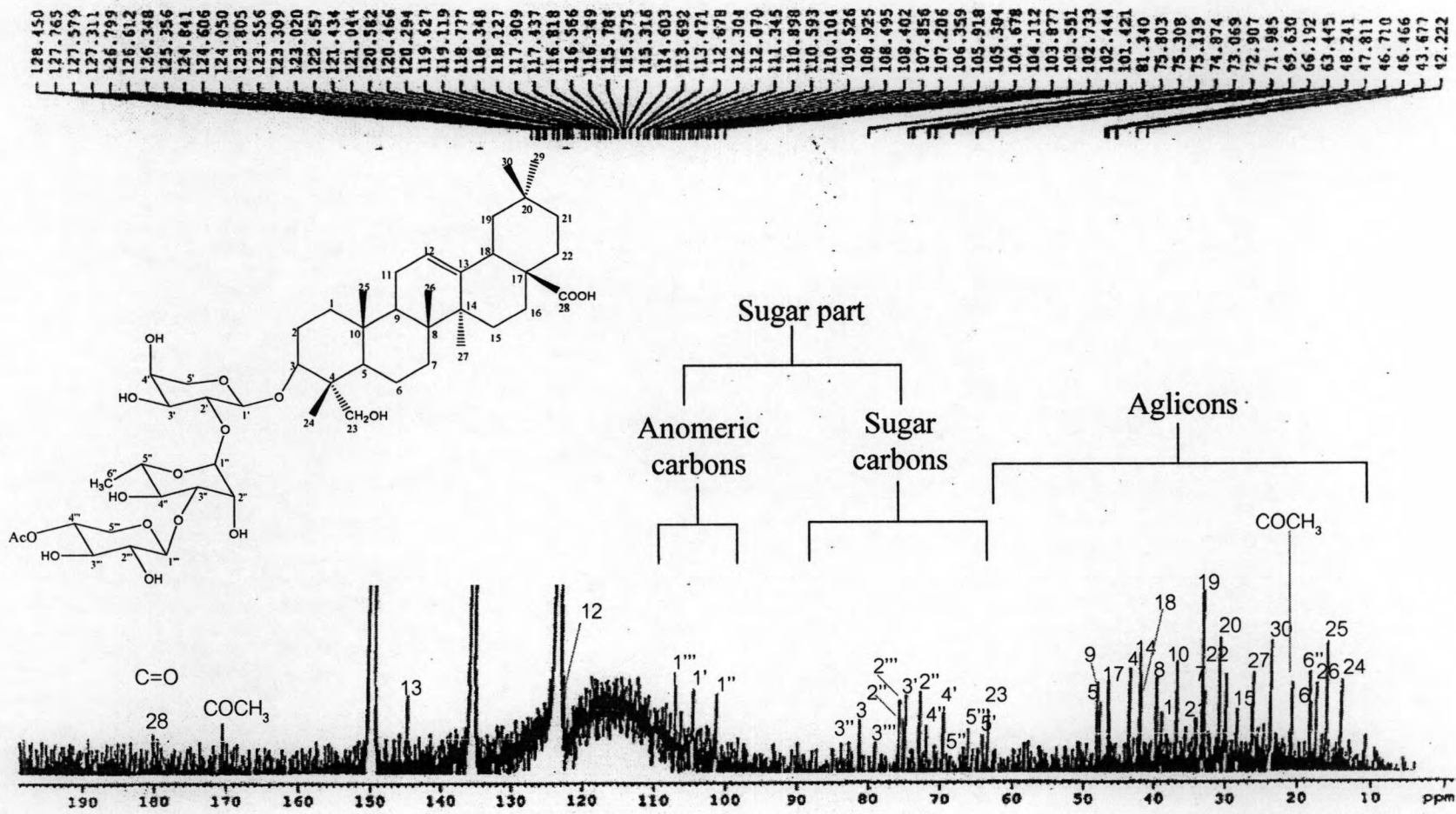


Figure 29 The 100 MHz ¹³C-NMR spectrum of Compound Sp2 (in pyridine-d₅)

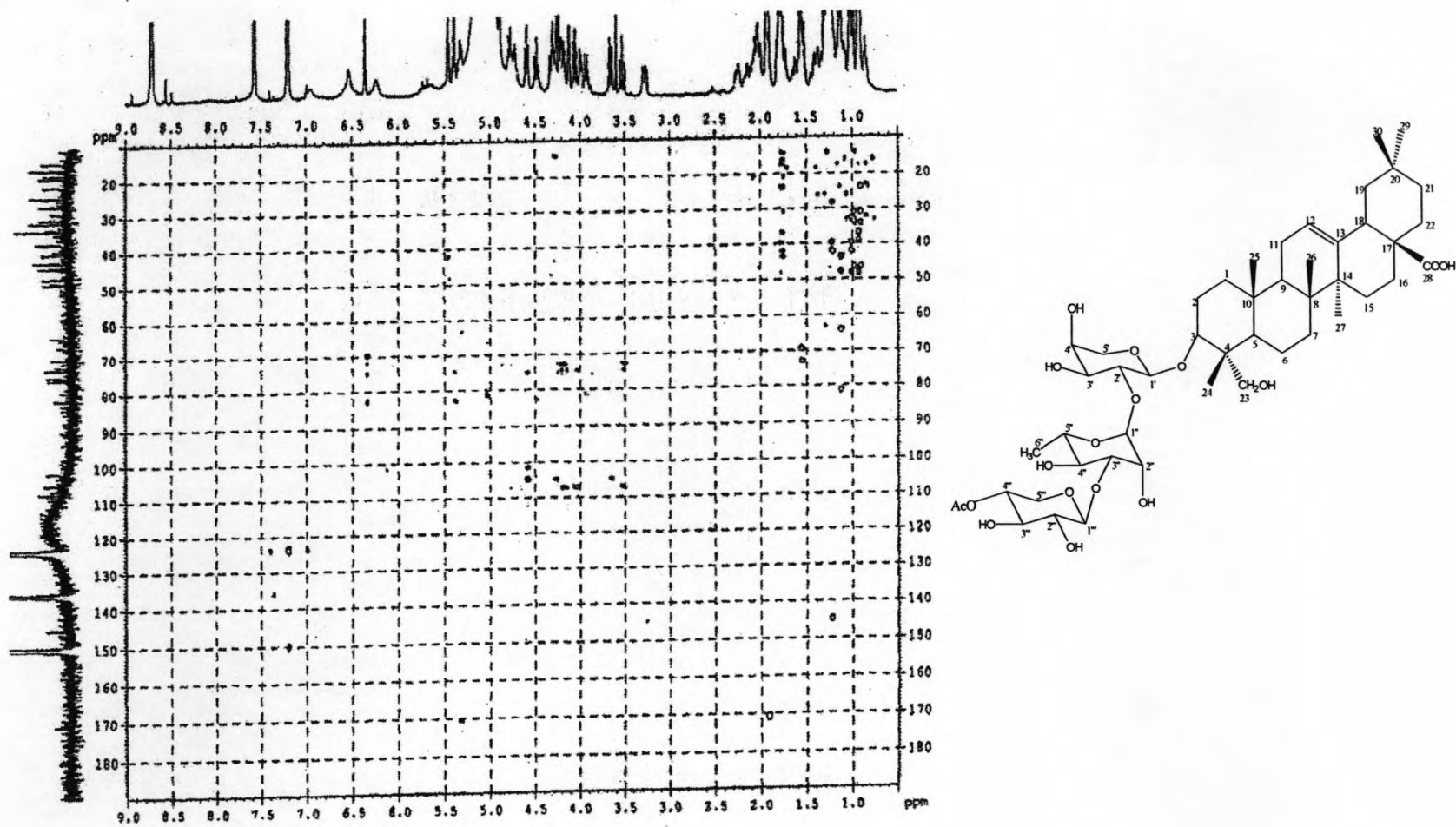


Figure 30 The 400 MHz HMBC spectrum of Compound Sp2 (in pyridine-*d*₅)

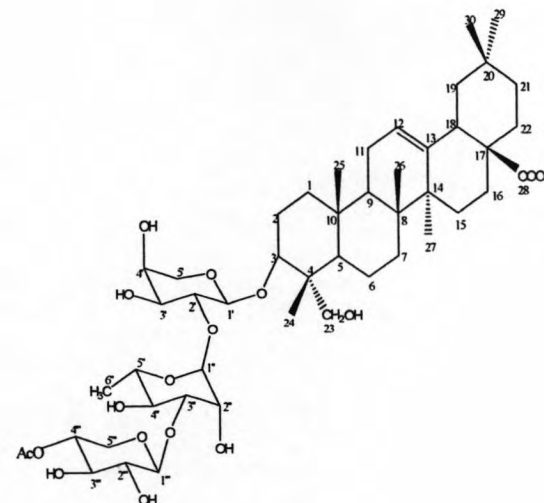
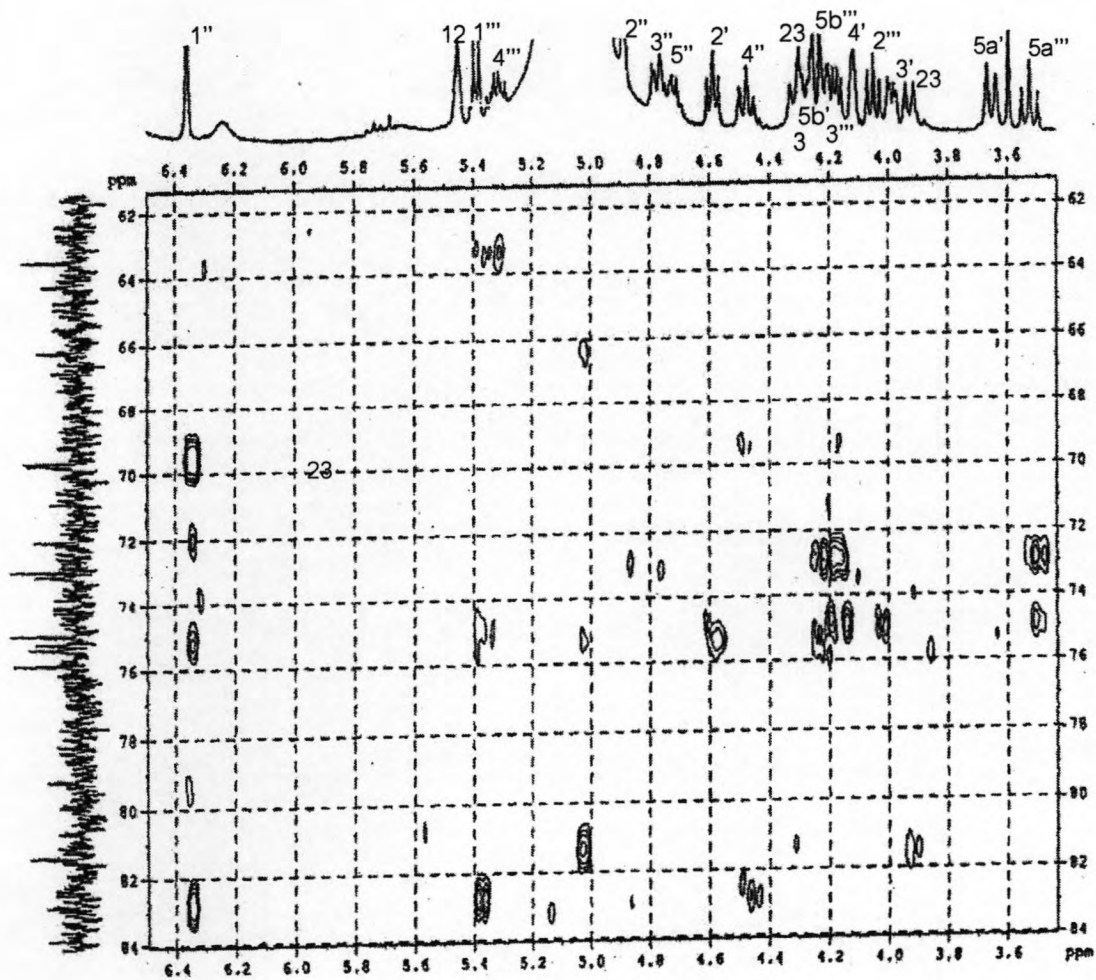


Figure 31 The expansion of HMBC spectrum of Compound Sp2 (in pyridine- d_5)

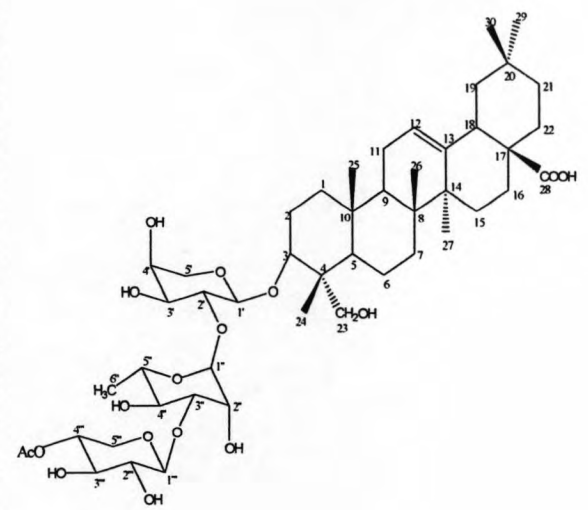
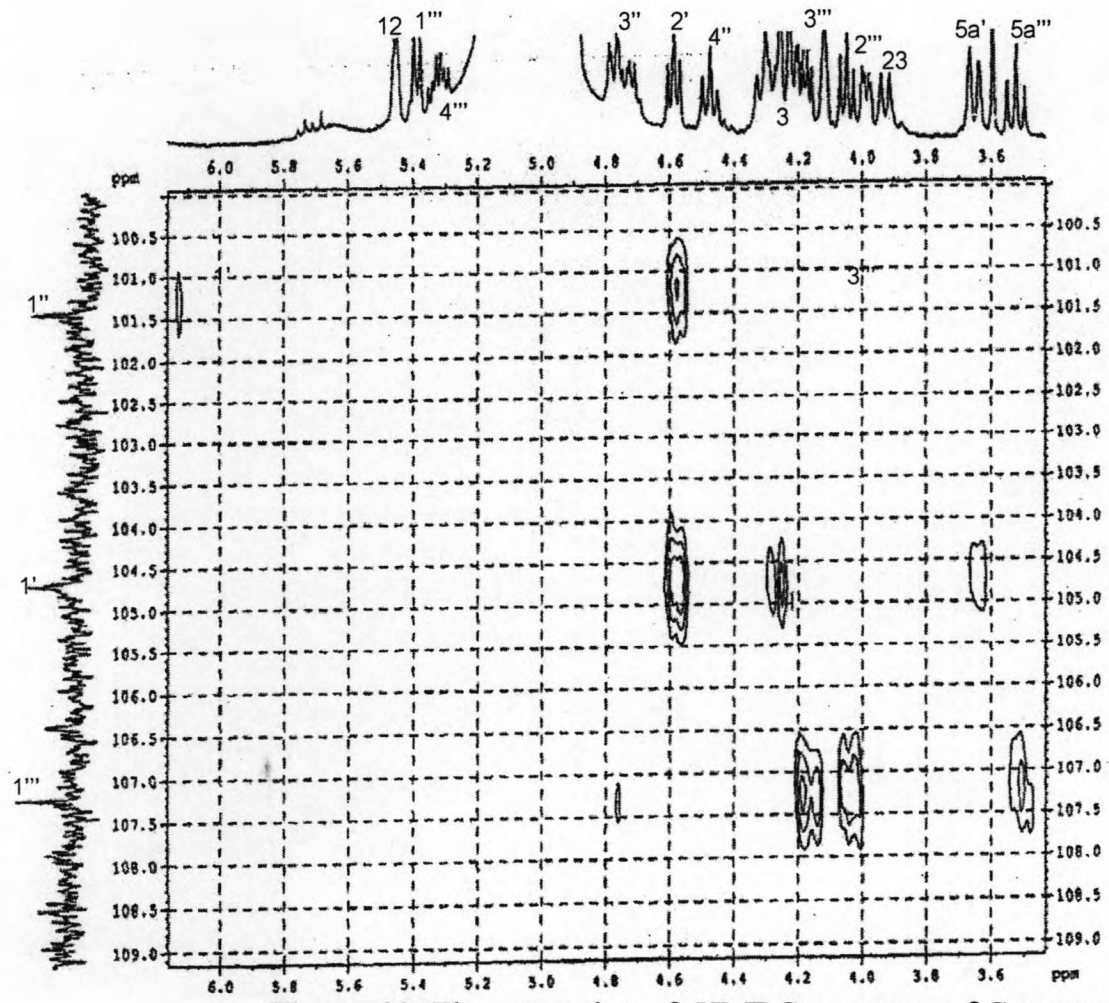


Figure 32 The expansion of HMBC spectrum of Compound Sp2 (in pyridine- d_5)

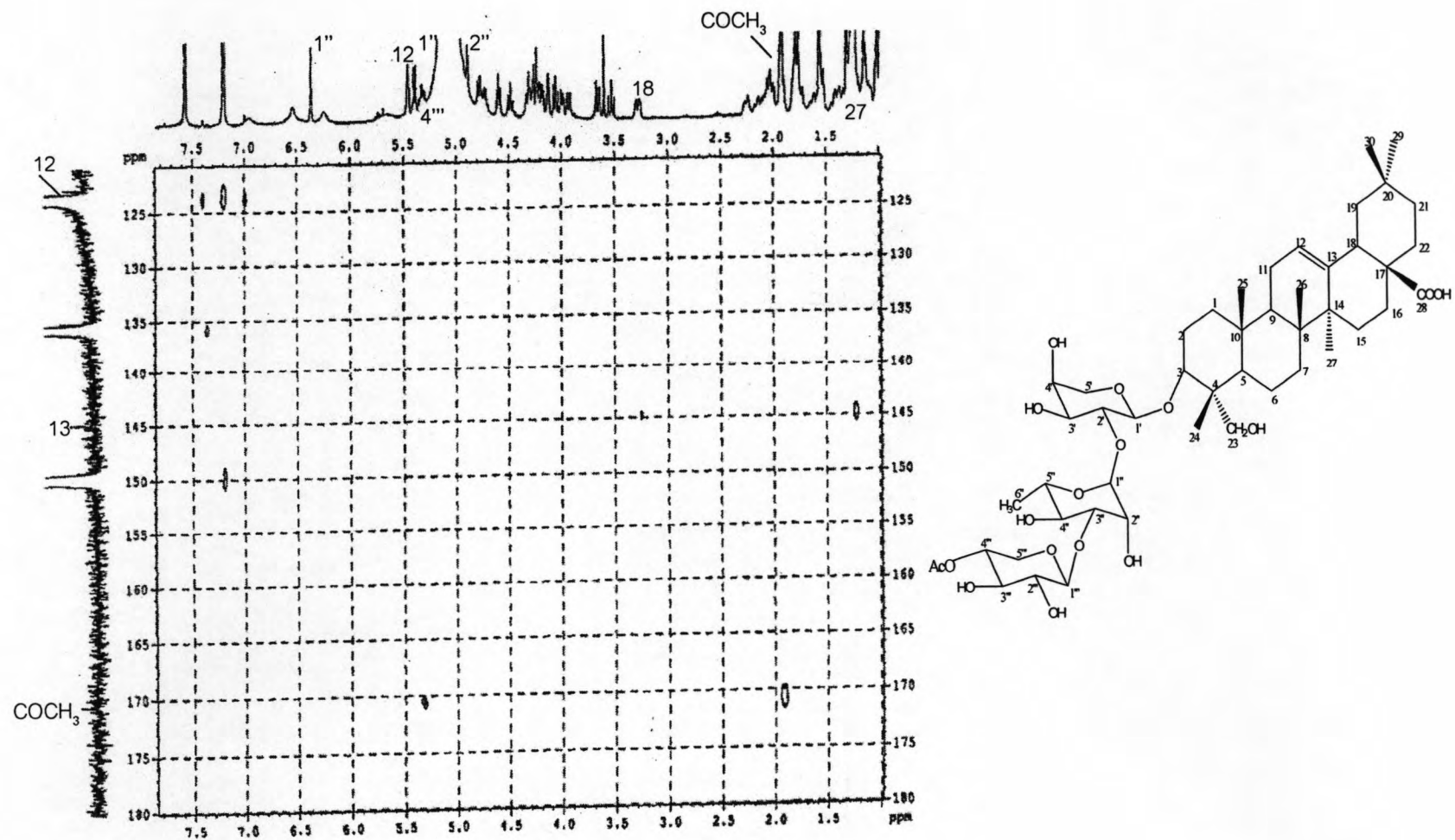


Figure 33 The expansion of HMBC spectrum of Compound Sp2 (in pyridine- d_5)

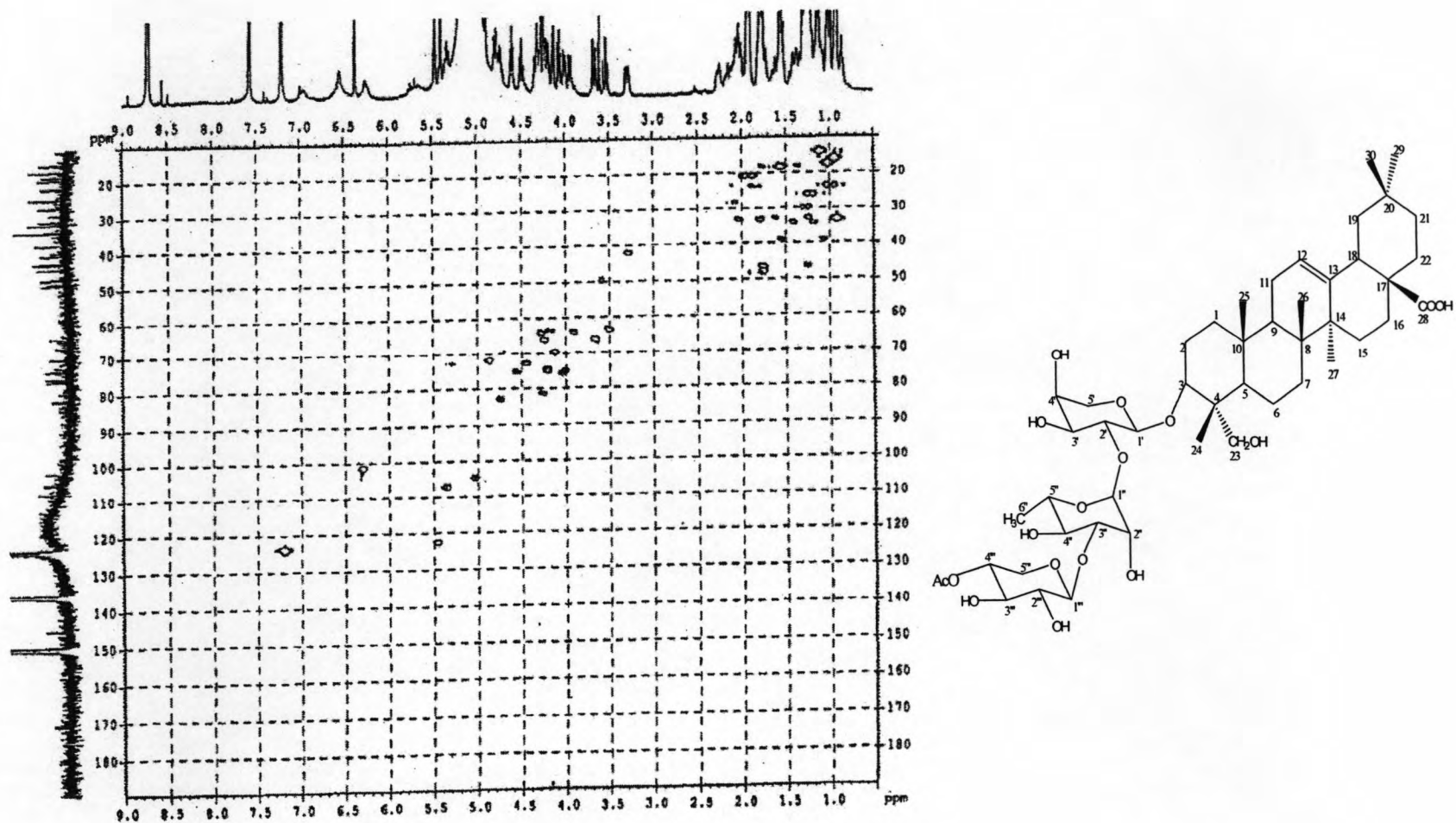


Figure 34 The 400 MHz HMQC spectrum of Compound Sp2 (in pyridine-*d*₅)

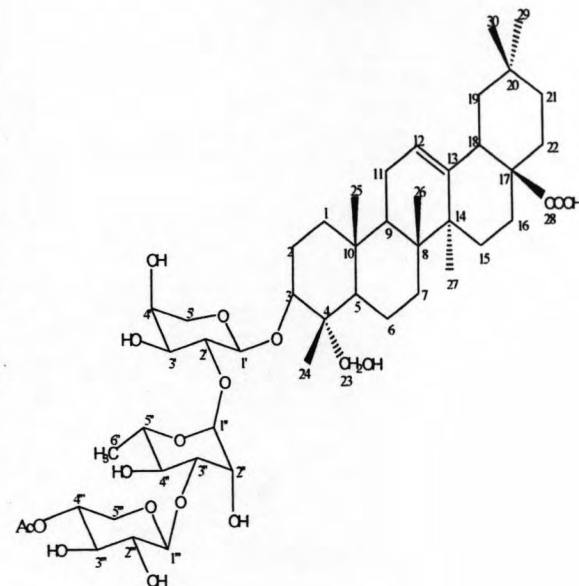
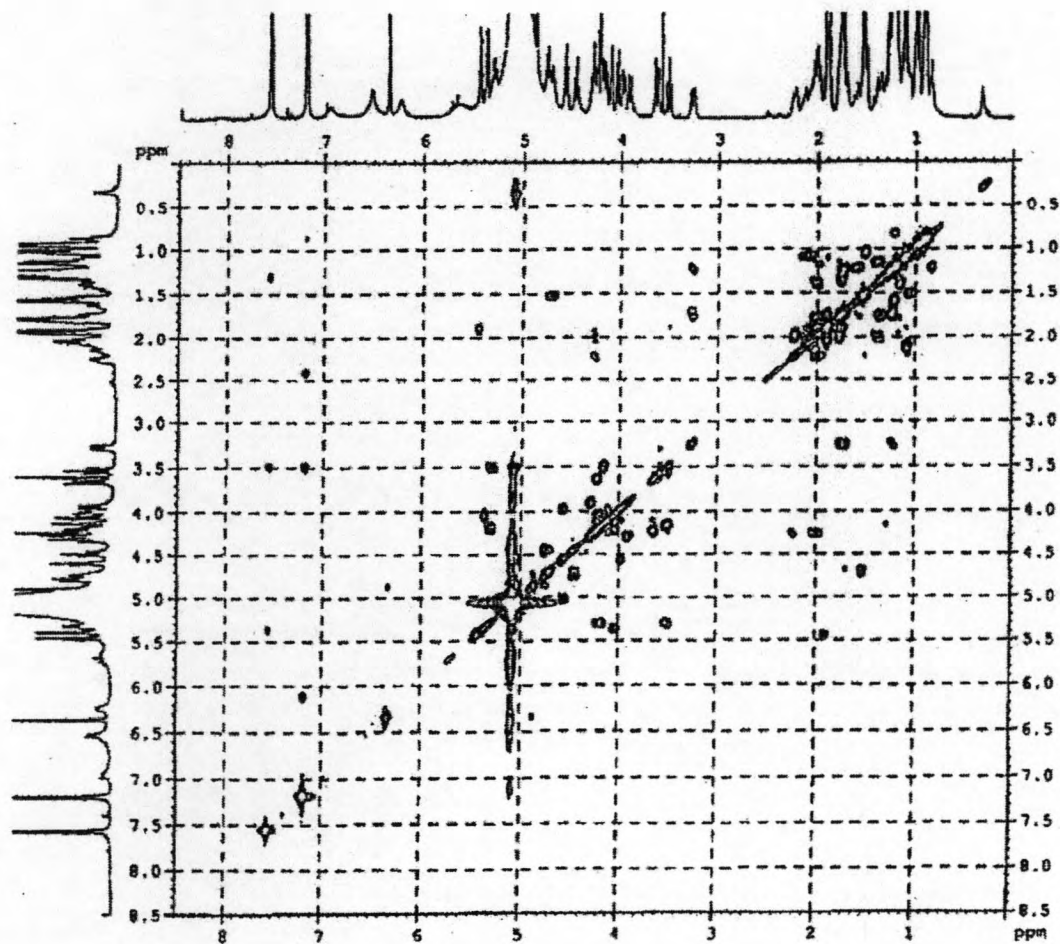


Figure 35 The 400 MHz ^1H - ^1H COSY spectrum of Compound Sp2 (in pyridine- d_5)

APPENDIX B
Molluscicidal activity test

Table 38 The results of molluscicidal activity testing of methanol extract against *P. canaliculata* at 24 hours intervals.

Concentration (ppm)	Total snails	Death snails	Mean of mortality	LC ₅₀ (ppm)	LC ₉₀ (ppm)
1.00	60	0.00	0.00±0.00	11.19 (9.71-12.72)	19.82 (17.72-22.79)
5.00	60	19.00	6.33±1.53		
10.00	60	32.00	10.67±3.51		
15.00	60	40.00	13.33±1.53		
20.00	60	50.00	16.67±1.15		
25.00	60	60.00	20.00±0.00		
0 (control)	60	0.00	0.00		

95 % confidence Limits of LC₅₀ and LC₉₀

Mean±S.E., calculated from Triplicate (each per 20 snails)

Table 39 The results of molluscicidal activity testing of aqueous layer against *P.canaliculata* at 24 hours intervals.

Concentration (ppm)	Total snails	Death snails	Mean of mortality	LC ₅₀ (ppm)	LC ₉₀ (ppm)
1	60	0.00	0.00±0.00	9.62 (7.49-11.76)	18.80 (16.11-23.46)
5	60	32.00	10.67±1.53		
10	60	37.00	12.33±2.08		
15	60	44.00	14.67±1.53		
20	60	54.00	16.67±2.08		
25	60	60.00	20.00±0.00		
0 (control)	60	0.00	0.00		

95 % confidence Limits of LC₅₀ and LC₉₀

Mean±S.E., calculated from Triplicate (each per 20 snails)

Table 40 The results of molluscicidal activity testing of fraction A-1 against *P. canaliculata* at 24 hours intervals.

Concentration (ppm)	Total snails	Death snails	Mean of mortality	LC ₅₀ (ppm)	LC ₉₀ (ppm)
1	60	0.00	0.00±0.00	8.47 (6.24-10.69)	16.86 (14.08-21.61)
5	60	37.00	12.33±1.20		
10	60	40.00	13.33±1.20		
15	60	46.00	15.33±1.20		
20	60	55.00	18.33±1.20		
25	60	60.00	20.00±0.00		
0 (control)	60	0.00	0.00		

95 % confidence Limits of LC₅₀ and LC₉₀

Mean±S.E., calculated from Triplicate (each per 20 snails)

Table 41 The results of molluscicidal activity testing of fraction B-6 against *P.canaliculata* at 24 hours intervals.

Concentration (ppm)	Total snails	Death snails	Mean of mortality	LC ₅₀ (ppm)	LC ₉₀ (ppm)
1	60	0.00	0.00±0.00	6.21 (5.67-6.79)	10.59 (9.73-11.73)
3	60	20.00	6±0.57		
5	60	26.00	9±0.57		
8	60	38.00	12.67±0.33		
10	60	48.00	16.67±0.88		
15	60	60.00	20.00±0.00		
0 (control)	60	0.00	0.00		

95 % confidence Limits of LC₅₀ and LC₉₀

Mean±S.E., calculated from Triplicate (each per 20 snails)

Table 42 The results of molluscicidal activity testing of fraction C-1 against *P.canaliculata* at 24 hours intervals.

Concentration (ppm)	Total snails	Death snails	Mean of mortality	LC ₅₀ (ppm)	LC ₉₀ (ppm)
1	60	0.00	0.00±0.00	5.54 (4.66-6.46)	9.56 (8.35-11.44)
3	60	14.00	4.67±1.20		
5	60	41.00	13.67±1.45		
8	60	46.00	15.33±1.45		
10	60	50.00	16.67±1.45		
15	60	60.00	20.00±0.00		
0 (control)	60	0.00	0.00		

95 % confidence Limits of LC₅₀ and LC₉₀

Mean±S.E., calculated from Triplicate (each per 20 snails)

Table 43 The results of molluscicidal activity testing of fraction D-4 against *P.canaliculata* at 24 hours intervals.

Concentration (ppm)	Total snails	Death snails	Mean of mortality	LC ₅₀ (ppm)	LC ₉₀ (ppm)
1	60	0.00	0.00±0.00	4.44 (3.85-5.00)	7.24 (6.53-8.23)
3	60	19.00	6.33±1.20		
5	60	44.00	14.67±1.86		
7	60	53.00	17.67±0.88		
9	60	55.00	18.33±0.33		
10	60	60.00	20.00±0.00		
0 (control)	60	0.00	0.00		

95 % confidence Limits of LC₅₀ and LC₉₀

Mean±S.E., calculated from Triplicate (each per 20 snails)

Table 44 The results of % mortality of compound Sp1 at 24, 48 and 72 h. against *P. canaliculata*.

Concentration(ppm)	% mortality at 24 h.	% mortality at 48 h.	% mortality at 72 h.
1	0.00	3.33	13.33
3	31.67	53.33	73.33
5	76.67	86.67	91.67
7	83.33	96.67	100
9	98.33	100	-
10	100	-	-
0 (control)	0.00	0.00	0.00

calculated from Triplicate (each per 20 snails)

Table 45 The results of molluscicidal activity testing of compound Sp1 against *P.canaliculata* at 24 hours intervals.

Concentration (ppm)	Total snails	Death snails	Mean of mortality	LC ₅₀ (ppm)	LC ₉₀ (ppm)
1	60	0.00	0.00±0.00	4.34 (3.78-4.88)	7.21 (6.52-8.15)
3	60	23.00	7.67±0.67		
5	60	44.00	14.67±0.88		
7	60	51.00	17.00±0.58		
9	60	56.00	18.67±0.67		
10	60	60.00	20.00±0.00		
0 (control)	60	0.00	0.00		

95 % confidence Limits of LC₅₀ and LC₉₀

Mean±S.E., calculated from Triplicate (each per 20 snails)



Table 46 The results of % mortality of compound Sp2 at 24, 48 and 72 h. against *P. canaliculata*.

Concentration(ppm)	% mortality at 24 h.	% mortality at 48 h.	% mortality at 72 h.
1	0.00	6.67	18.33
3	36.67	56.67	83.33
5	76.67	93.33	96.67
7	86.67	96.67	100
9	93.33	100	-
10	100	-	-
0 (control)	0.00	0.00	0.00

calculated from Triplicate (each per 20 snails)

Table 47 The results of molluscicidal activity testing of compound Sp2 against *P. canaliculata* at 24 hours intervals.

Concentration (ppm)	Total snails	Death snails	Mean of mortality	LC ₅₀ (ppm)	LC ₉₀ (ppm)
1	60	0.00	0.00±0.00	4.28 (3.62-4.92)	7.08 (6.30-8.22)
3	60	22.00	7.33±1.45		
5	60	46.00	15.33±0.88		
7	60	52.00	17.33±0.33		
9	60	56.00	18.67±0.88		
10	60	60.00	20.00±0.00		
0 (control)	60	0.00	0.00		

95 % confidence Limits of LC₅₀ and LC₉₀

Mean±S.E., calculated from Triplicate (each per 20 snails)

PROBIT ANALYSIS

The LC_{50} value was determined by Probit Analysis using SPSS version 15 for window at 95% confidence interval. This is the example of the result from probit analysis for LC_{50} value (24 hours) of saponin crude extract from *Sapindus rarak* fruits in small snails (2.0-2.5 cm.)

***** PROBIT ANALYSIS*****

DATA Information

21 unweighted cases accepted.

0 cases rejected because of missing data.

3 cases are in the control group.

MODEL Information

ONLY Normal Sigmoid is requested.

***** PROBIT ANALYSIS*****

Parameter Estimates (PROBIT model: (Probit(p)) = Intercept +BX):

Regression Coeff	Standard Error	Coeff./S.E.
CONC .153	.012	12.357
Intercept	Standard Error	Intercept/ S.E.
- 1.294	.132	- 9.779

Pearson Goodness- of -Fit Chi Square = 70.297 DF = 19 P = .000

Since Goodness-of-Fit Chi square is significant, a heterogeity

Factor is used in the calculation of confidence limits.

***** PROBIT ANALYSIS*****

Observed and Expected Frequencies

Cell Counts and Residuals

	Number	Concen	Number of Subjects	Observed Responses	Expected Responses	Residual	Probability
PROBIT	1	.000	20	0	1.957	-1.957	.098
	2	.000	20	0	1.957	-1.957	.098
	3	.000	20	0	1.957	-1.957	.098
	4	1.000	20	0	2.538	-2.538	.127
	5	1.000	20	0	2.538	-2.538	.127
	6	1.000	20	0	2.538	-2.538	.127
	7	5.000	20	13	5.961	7.039	.298
	8	5.000	20	14	5.961	8.039	.298
	9	5.000	20	10	5.961	4.039	.298
	10	10.000	20	15	11.849	3.151	.592
	11	10.000	20	14	11.849	2.151	.592
	12	10.000	20	11	11.849	-.849	.592
	13	15.000	20	16	16.816	-.816	.841
	14	15.000	20	17	16.816	.184	.841
	15	15.000	20	13	16.816	-3.816	.841
	16	20.000	20	19	19.219	-.219	.961
	17	20.000	20	20	19.219	.781	.961
	18	20.000	20	16	19.219	-3.219	.961
	19	25.000	20	20	19.884	.116	.994
	20	25.000	20	20	19.884	.116	.994
	21	25.000	20	20	19.884	.116	.994

***** PROBIT ANALYSIS *****

Confidence Limits for Effective Concen

Confidence Limits

	Probability	95% Confidence Limits for Concen		
		Estimate	Lower Bound	Upper Bound
PROBIT ^a	.010	-6.759	-14.576	-2.573
	.020	-4.974	-11.981	-1.175
	.030	-3.842	-10.341	-.282
	.040	-2.991	-9.112	.394
	.050	-2.298	-8.116	.948
	.060	-1.708	-7.270	1.422
	.070	-1.191	-6.531	1.839
	.080	-.728	-5.871	2.215
	.090	-.307	-5.273	2.559
	.100	.080	-4.724	2.877
	.150	1.685	-2.472	4.216
	.200	2.960	-.715	5.312
	.250	4.054	.762	6.283
	.300	5.036	2.057	7.186
	.350	5.947	3.223	8.057
	.400	6.811	4.294	8.919
	.450	7.647	5.292	9.791
	.500	8.469	6.235	10.688
	.550	9.292	7.138	11.626
	.600	10.127	8.016	12.618
	.650	10.991	8.883	13.684
	.700	11.902	9.759	14.845
	.750	12.884	10.667	16.135
	.800	13.978	11.642	17.608
	.850	15.253	12.741	19.363
	.900	16.858	14.083	21.611
	.910	17.245	14.401	22.159
	.920	17.666	14.746	22.757
	.930	18.129	15.122	23.416
	.940	18.646	15.541	24.155
	.950	19.236	16.015	25.000
	.960	19.929	16.569	25.996
	.970	20.780	17.246	27.225
	.980	21.913	18.140	28.864
	.990	23.697	19.538	31.458

a. A heterogeneity factor is used.

VITA

Miss Tatsaneewan Faysoon was born on Tuesday 28th August, 1979, in Burirum, Thailand. She received her Bachelor's degree of Science in Pharmacy in 2003 from the Faculty of Pharmaceutical Sciences, Rungsit University, Thailand.

