CHAPTER IV

RESULTS AND DISCUSSION

1. Isolation of strains

Twenty two soil samples were collected from Prajuabkirikhan, Chumporn, Chonburi, Ratchaburi and Chantaburi provinces. From these soils, bacteria in the group of actinomycetes was selected by using PCA and SCA medium. A total of 33 isolates of *Micromonospora* were isolated and collected on YMA for stock culture in cold room at 4°C. Sources of soil sample and isolate number are shown in Table 5.

2. Primary screening of isolates for antibiotic production

Antibacterial activity of all isolates were shown in Table 6. All strains exhibited activity against Gram positive bacteria *S. aureus* ATCC 25923 and *B. subtilis* ATCC 6633 but little or no activity towards the Gram negative bacterium *E. coli* ATCC 25922. We have selected 3 strains of *Micromonospora* spp.JSM5-1, JSM1-3 and A-25 for further study because they exhibited a broad spectrum of activity with high potency of inhibition.

3. Identification of strain

3.1 Morphological and cultural characteristics

The morphological and cultural characteristics of *Micromonospora* strains isolated in this study on YMA are shown in Table 7. Examination of all strains by light microscopy after growth on YMA revealed the presence of single spores on short sporophore of the vegetative mycelia, and mycelial pigments on YMA were initially orange and then changed from orange to dark brown or black during the incubation term.

The cultural characteristics of *Micromonospora* spp. JSM1-3, JSM5-1 and A-25 on various media are shown in Table 8. All strains grew better on Oatmeal and Yeast extract-malt extract agar (YMA) than Tyrosine and Nutrient agar medium. In particular for Oatmeal agar, vegetative mycelia raised from the agar surface. None of them grew on Glycerol-asparagine agar.

Scanning electron micrograph indicated that spores of these *Micromonospora* spp. are borne singly at the ends of the sporophores branching from vegetative hypha. Spores were 0.8-1.2 μm in diameter and spherical shape. A scanning electron micrograph of spores of *Micromonospora* strain JSM5-1 from 10 days culture on YMA is shown in Figures 1a and 1b.

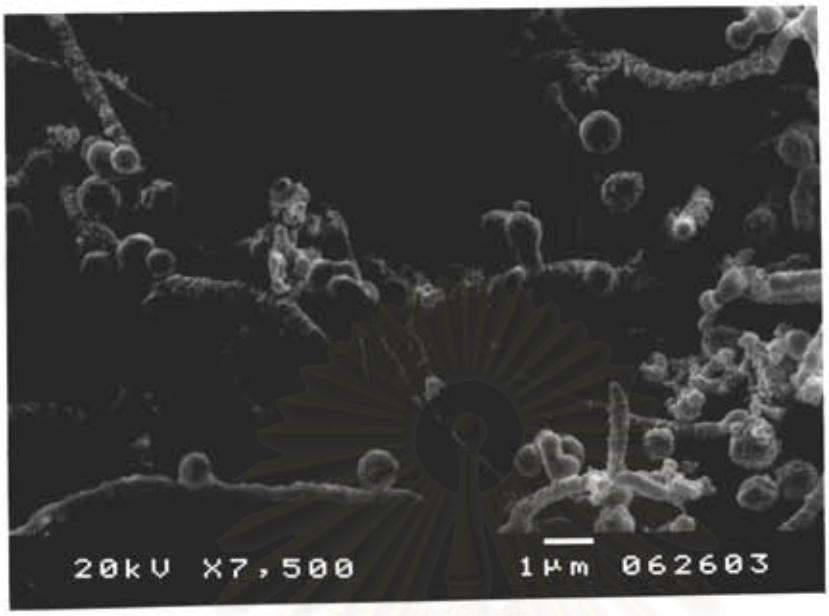
The colonial appearance on YMA of *Micromonospora* spp. JSM1-3, JSM5-1 and A-25 compared with *Micromonospora chalcea* KA-579 are shown in Figure 2.

The general growth habitat, production of single spore on short sporophore, morphology and structure of spore, placed these isolates in the genus *Micromonospora*

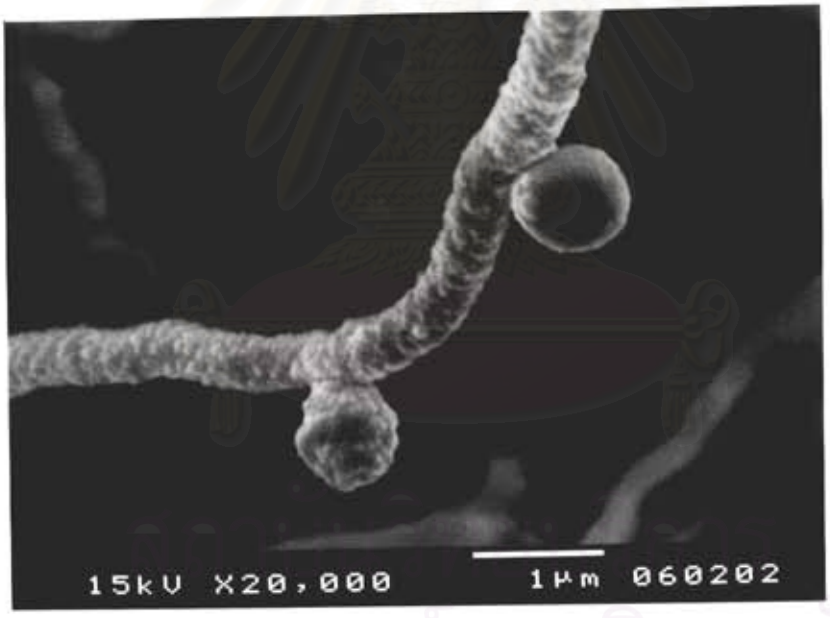
3.2 Biochemical and Physiological characteristics

Physiological characteristics and utilization of carbon sources of all strains are given in Table 9 and 10, respectively.

Physiological characteristics of strains JSM 5-1, JSM 1-3, and A-25 are summarized in Table 11. Reduction of nitrate, hydrolysis of starch, production of H_2S, production of melanin pigment and peptonization of milk are negative. Liquefaction of gelatin and coagulation of milk are positive. Sodium chloride tolerance of strain JSM5-1 is 4 %.



a



b

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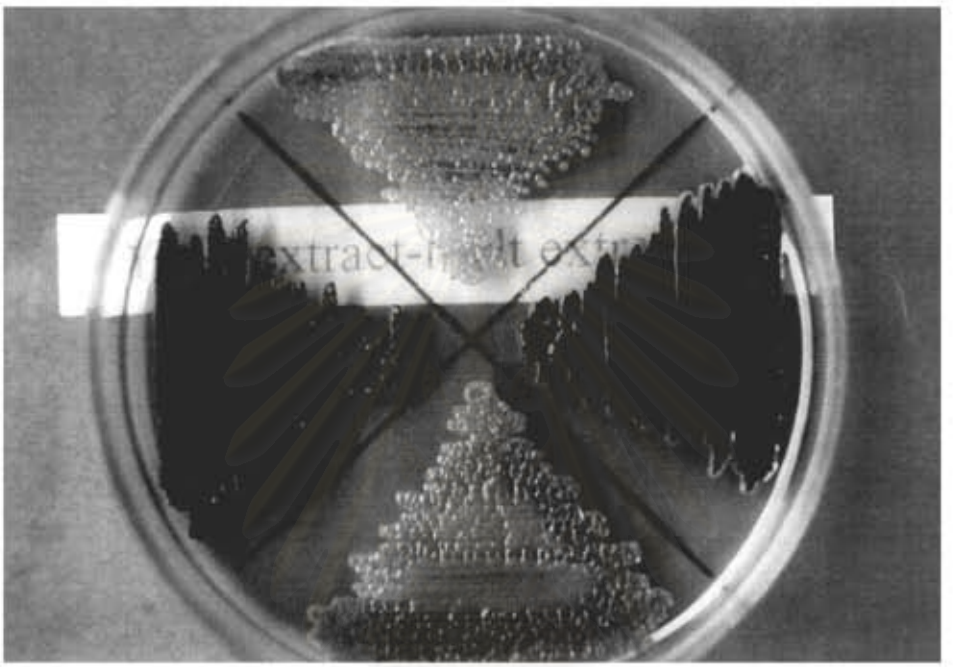
Figure 1 Scanning electron micrograph of spore-bearing substrate mycelium of *Micromonospora* sp. JSM5-1 on YMA for 10 days, (a, x7,500 b, x20,000)

(2)

(1)



(3)

(1)

Figure 2 The colonial appearance of the selected strains JSM5-1 (1), JSM1-3 (2), A-25 (3) and *Micromonospora chalcea* KA 579 (4)

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4. Antibiotic production in liquid culture

4.1 Antibiotic production of 3 strains in various media

The strains JSM5-1, JSM1-3 and A-25 were fermented in seven kinds of production medium. Antibacterial activity of 3 strains on various media are shown Table 12.

4.2 Effect of pH and temperature on antibiotic production

Effects of pH and temperature on antibacterial activity of strain JSM5-1 are shown in Tables 14 and 15, respectively. Strain JSM5-1 produced the highest activity of antibiotic against test organisms at day 5. pH 7 gave the widest inhibition zone. The optimum temperature for antibiotic production was 28 °C (Table 14).

5. Antibacterial activity of extracts and structure determination of compound JM-1

Antibacterial activities of the extracts from 3 strains were shown in Table 15. The ethyl acetate extract of strain JSM5-1 exhibited activity against all tested organisms (Table 16). The butanol and methanol extract exhibited little or no activity. The purified compound (JM-1) show antibacterial activity against *Staphylococcus aureus* MRSA I (inhibition zone, 9.0 mm), *Bacillus subtilis* ATCC 6633 (inhibition zone, 9.5 mm), *Salmonella typhi* ATCC 14028, *Pseudomonas aeruginosa* ATCC 27853.

Table 5 Sources of soil samples and isolates

Samples no.	Sources	Isolates
1. marine soil	Koa Pee-Pee, Krabi	MA-1
2. marine soil	Koa Pee-Pee, Krabi	MA-2
3. marine soil	Hua-Hin, Prachuap Khiri Khun	MB2-1, MB2-2, MB2-3
4. marine soil	Koa Seechung, Chonburi	029-2P
5. marine soil	Hua-Hin, Prachuap Khiri Khun	JSM1-1, JSM1-2, JSM1-3
6. marine soil	Chumporn	JSM2-1
7. marine soil	Chumporn	JSM3-1
8. marine soil	Chumporn	JSM5-1
9. marine soil	Koa Seechung, Chonburi	026-1P
10. marine soil	Koa-Seechung, Chonburi	028-2P
11. marine soil	Hua-Hin, Prajuabkirikhun	P1-1
12. soil	Tepsathit, Chaiyaphum	MC1-1
13. soil	Tepsathit, Chaiyaphum	MC2-2, MC2-3
14. soil	Tepsathit, Chaiyaphum	MC5-1
15. soil	Tepsathit, Chaiyaphum	MC7-1,MC7-2, MC7-3
16. soil	Tepsathit, Chaiyaphum	MC8-2, MC8-3
17. soil	Tepsathit, Chaiyaphum	MC10-1
18. soil	Tepsathit, Chaiyaphum	MC12-2
19. soil	Khao Kanjun, Ratchaburi	R1-1, R1-2, R1-3
20. soil	Na-yai-arm, Chantaburi	CB2-2
21. soil	Na-yai-arm, Chantaburi	CB5-1, CB5-2
22. soil	Chaiyapum	A-25

Table 6 Antibacterial activities of *Micromonospora* isolates

Isolates	Inhibition zone (mm)		
	S. aureus ATCC 25923	*B. subtilis* ATCC 6633	*E. coli* ATCC 25922
MA-1	++	+++	++
MA-2	+++	+++	++
MB2-1	+	+++	++
MB2-2	++	++	++
MB2-3	++	+++	+
MC1-1	++	+++	++
MC2-2	++	++	++
MC2-3	++	++	++
MC5-1	+	+++	+
MC7-1	++	++	++
MC7-2	+++	+++	+
MC7-3	-	++	+
MC8-2	++	-	+
MC8-3	+++	-	+
MC10-1	+++	+++	++
MC12-2	+++	++	+
029-2P	++	++	++
A-25	+++	+++	++
JSM1-1	++	+	+

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Table 6 (continued)

Isolates no.	Inhibition zone (mm)		
	S. aureus ATCC 25923	*B. subtilis* ATCC 6633	*E. coli* ATCC 25922
JSM1-2	++	-	+
JSM1-3	+++	+++	++
JSM2-1	++	-	+
JSM3-1	+++	-	++
JSM5-1	+++	+++	++
R1-1	++	+++	-
R1-2	+	+-	+
R1-3	+	-	+
CB2-2	+	+-	+
CB5-1	++	+++	+
CB5-2	+	-	+
026-1P	++	-	++
028-2P	++	+	++
P1-1	++	+-	++

-, no activity; +-, little activity; +, 0-10.5 mm; ++, 10.-16.5 mm; +++, 16.6 mm

Table 7 Morphological and cultural characteristics of *Micromonospora* species on YMA

Isolates no.	Spore size (μm)	Spore color	Colony color	
			upper colony	reverse colony
MA-1	0.8-1.5	orange-black	orange-black	orange-black
MA-2	0.8-1.2	orange	orange	orange
MB2-1	0.8-1.0	green-gray	green-gray	green-gray
MB2-2	0.6-1.0	black-gray	green-black	green-black
MB2-3	0.8-1.0	dark brown	orange-brown	orange-brown
MC1-1	0.6-0.8	dark brown	dark brown	dark brown
MC2-2	0.8-1.0	dark brown	orange-black	orange-black
MC2-3	0.8-1.0	dark brown	dark brown	dark brown
MC5-1	0.8-1.0	dark brown	dark brown	dark brown
MC7-1	0.8-1.0	green-gray	black	black
MC7-2	0.8-1.0	yellow-orange	yellow-orange	yellow-orange
MC7-3	0.8-1.0	green-gray	green-gray	green-green
MC8-2	0.8-1.2	green-gray	dark brown	dark brown
MC8-3	0.8-1.2	yellow-orange	orange-brown	orange-brown
MC10-1	0.8-1.2	green-gray	orange brown	orange brown
MC12-2	0.8-1.0	green-black	black	black
029-2P	0.8-1.0	green-gray	green-black	green-black
A-25	0.8-1.2	dark brown	black	black
JSM1-1	0.6-1.0	black	brown	brown

Table 7 (continued)

Isolates	Spore size (μm)	Spore color	Colony color	
			upper colony	reverse colony
JSM1-2	0.8-1.5	green-black	dark brown	dark brown
JSM1-3	0.8-1.0	dark brown	dark brown	dark brown
JSM2-1	0.8-1.0	yellow-orange	yellow-orange	yellow-orange
JSM3-1	0.8-1.2	dark brown	brown	brown
JSM5-1	0.8-1.2	dark brown	dark brown	dark brown
R1-1	0.8-1.2	black-orange	orange	orange
R1-2	0.8-1.0	green-gray	green-gray	green-gray
R1-3	0.8-1.0	green-gray	green-gray	green-gray
CB2-2	0.8-1.2	green-black	green-black	green-black
CB5-1	0.8-1.2	dark brown	green-black	green-black
CB5-2	0.8-1.2	orange brown	orange-brown	orange-brown
026-1P	0.8-1.2	dark brown	brown	brown
028-2P	0.8-1.2	brown	brown	brown
P1-1	0.8-1.0	dark brown	orange-brown	orange-brown

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Table 8 Cultural characteristics of *Micromonospora* strains on different media for 10 days

Isolates no.	Medium	Growth	Colony color	
			upper colony	lower colony
A-25	YM	++	green-black	green-black
	Tyrosine	+	orange-brown	orange-brown
	Oatmeal	+++	orange-brown	orange-brown
	Nutrient	+	green-black	green-black
	GA	-	-	-
JSM1-3	YM	+	pale-brown	pale-orange
	Tyrosine	+-	pale-brown	pale-orange
	Oatmeal	+++	orange	pale-orange
	Nutrient	+	orange-brown	pale-orange
	GA	-	-	-
JSM5-1	YM	++	dark-brown	brown
	Tyrosine	+-	-	-
	Oatmeal	+++	orange-brown	pale-orange
	Nutrient	+	orange-brown	brown
	GA	-	-	-
M. chalcea KA-579	YM	++	orange	orange
	Tyrosine	+	orange	orange
	Oatmeal	+++	orange	orange
	Nutrient	+	orange	orange
	GA	-	-	-

-, no growth; +, poor; ++, moderate; +++, good

Table 9 Utilization of various carbon sources by *Micromonospora* isolates

Isolates	None	Glu	Rib	Rha	Raff	Mel	Man	Gly
MA-1	-	+	-	-	-	-	-	-
MA-2	-	+	-	-	-	-	-	-
MB2-1	-	+	-	+	+	+	-	-
MB2-2	-	+	-	-	-	-	-	-
MB2-3	-	+	+	+	+	+	+	-
MC1-1	-	+	-	-	-	-	-	-
MC2-2	-	+	-	-	-	-	-	-
MC2-3	-	+	-	-	-	+	+	-
MC5-1	-	+	-	-	+	-	+	-
MC7-1	-	+	-	-	-	-	-	-
MC7-2	-	+	-	-	-	-	-	-
MC7-3	-	+	-	-	-	-	-	-
MC8-2	-	+	-	-	+	+	-	-
MC8-3	-	+	-	-	-	-	-	-
MC10-1	-	+	-	-	-	-	-	-
MC12-2	-	+	-	-	-	-	-	-
029-2P	-	+	-	+	+	+	+	+

Glu, Glucose; Rib; Ribose; Rha, Rhanose; Raff, Raffinose

Mel; Melibiose, Man, Mannitol; Gly, Glycerol

* -, not utilized, +; utilized

Table 9 (continued)

Isolates	None	Glu	Rib	Rha	Raff	Mel	Man	Gly
JSM1-1	-	+	-	-	-	-	-	-
JSM1-2	-	+	-	+	+	+	+	+
JSM1-3	-	+	-	-	-	-	-	-
JSM2-1	-	+	-	-	-	-	+	-
JSM3-1	-	+	-	+	+	+	-	-
JSM5-1	-	+	-	-	-	-	-	-
R1-1	-	+	-	-	-	-	-	-
R1-2	-	+	-	-	-	-	-	-
R1-3	-	+	-	-	-	-	-	-
CB2-2	-	+	-	-	+	+	+	-
CB5-1	-	+	-	-	-	-	-	-
CB5-2	-	+	-	-	-	-	-	-
026-1P	-	+	-	-	-	-	-	-
028-2P	-	+	-	+	+	+	-	+
P1-1	-	+	-	-	+	-	-	-

Glu, Glucose; Rib, Ribose; Rha, Rhanose; Raff, Raffinose

Mel, Melibiose; Man, Mannitol; Gly, Glycerol

* -, not utilized; +, utilized

+, utilized

Table 10 Biochemical and Physiological characteristics of *Micromonospora* isolates

Isolates	reduce NO_3	Starch	H_2S	Melanin	Gelatin	Czapex	% NaCl
MC7-3	+	+	-	+	+	+	4
R1-2	+	+	-	+	+	+	4
R1-3	+	+	-	+	+	+	3
A-25	+	+	-	+/-	+	+	4
MB2-1	+	+	-	+/-	+	+	5
JSM1-2	+	+	-	+/-	+	+	6
028-2P	+	+	-	+/-	+	-	6
MB2-3	+	+	-	-	+	+	6
029-2P	+	+	-	-	+	+	6
MC12-2	+	+	-	-	+	+	2
CB5-1	+	+	-	-	+	+	4
MC7-1	+	w	-	-	+	+	4
MC5-1	+	w	-	-	+	+	4
CB2-2	+	w	-	-	+	+	4
MC2-2	+	w	-	-	+	+	3
026-1P	+	+	-	-	+	-	5
JSM1-1	+	w	-	-	-	-	4

Table 10 (continued)

Isolates	reduce NO_3	Starch	H_2S	Melanin	Gelatin	Czapex	% NaCl
MB2-2	-	+	-	+	+	+	4
MC7-2	-	w	-	+	+	+	4
MC10-1	-	+	-	+	+	+	3
MA-2	-	+	-	-	+	+	3
MC1-1	-	+	-	-	+	+	3
MC8-2	-	+	-	-	+	+	4
JSM3-1	-	+	-	-	+	+	4
MA-1	-	+	-	-	+	+	5
P1-1	-	w	-	-	+	+	5
MC2-3	-	w	-	-	+	+	4
JSM2-1	-	w	-	-	+	+	4
MC8-3	-	+	-	-	+	-	2
CB5-2	-	+	-	-	-	-	3
R1-1	-	-	-	-	+	+	4
JSM1-3	-	-	-	-	+	-	4
JSM5-1	-	-	-	-	+	-	4

+, positive

-, negative

w, weakly

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Table 11 Biochemical characteristics of *Micromonospora* sp. JSM5-1, JSM1-3, and A-25

Reactions	Response		
	JSM5-1	JSM1-3	A-25
Growth on Czapex's agar	-	-	+
milk coagulation	+	-	-
milk peptonization	-	+	+
Carbon utilization :			
D-glucose	+	+	+
ribose	-	-	-
Rhamnose	-	-	-
Raffinose	-	-	+
melibiose	-	-	-
Manitol	-	-	+
Glycerol	-	-	-
Xylose	+	-	+
Galactose	+	+	-
Arabinose	+	+	+
Trehalose	+	+	+
Cellobiose	-	+	+
Fructose	+	-	+

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Table 12 Antibacterial activity of the selected isolates in different media

media	Inhibition zone (mm)								
	S. aureus ATCC 25923			*B. subtilis* ATCC 6633			*E.coli* ATCC 25922		
	JSM5-1	A-25	JSM1-3	JSM5-1	A-25	JSM1-3	JSM5-1	A-25	JSM1-3
GP	17.2	33.1	28.0	18.7	31.7	30.1	-	-	-
GM	-	-	-	-	-	-	-	-	-
GN	-	29.3	27.8	-	29.2	28.0	-	17.4	-
GS	32.75	-	+-	+-	-	+-	-	23.8	20.0
SY	-	25.3	25.9	-	25.2	26.1	-	-	+-
SS	16.5	27.0	+-	+-	25.5	+-	-	-	16.0
DS	-	21.0	-	-	19.6	-	-	-	-

+-, weakly

GP, Glycerol peptone medium

GM, Glucose molasses medium

GN, Glucose NaCl medium

GS, Glucose soybean medium

SY, Sucrose yeast extract medium

SS, Sucrose soybean medium

DS, Dextrin soybean medium

Table 13 Effect of pHs on antibacterial activity of *Micromonospora* sp. JSM 5-1

initial pH	final pH		Inhibition zone (mm)	
	5 days	7 days	5 days	7 days
6.25	6.60	6.55	27.2	27.15
6.40	6.82	6.53	29.0	26.3
7.01	6.86	6.70	32.75	30.1
7.51	7.44	6.79	29.8	27.9
8.08	7.67	7.94	27.3	27.85

Table 14 Effect of temperatures on antibacterial activity of *Micromonospora* sp. JSM5-1

Temperature(°C)	Inhibition zone(mm)	
	S. aureus ATCC 25923	*B.subtilis* ATCC 6633
28°C	28.1	+-
30°C	15.6	+-
33°C	+-	+-

+-, weakly

Table 15 Antibacterial activity of extracts from *Micromonospora* sp. JSM 5-1, JSM1-3 and A-25

Isolates	Extracts	Inhibition zone (μm)		
		S.aureus ATCC 25923	*B. subtilis* ATCC 6633	*E. coli* ATCC 25922
A-25	Ethyl acetate	9.5	9.2	8.0
	Butanol	-	9.0	-
	Methanol	-	-	-
JSM1-3	Ethyl acetate	9.0	9.2	+-
	Butanol	+-	-	-
	Methanol	-	-	-
JSM5-1	Ethyl acetate	10.9	15.3	10.5
	Butanol	9.9	13.0	+-
	Methanol	-	+-	+-

+-, weakly

Table 16 Antibacterial activity of ethyl acetate extract from *Micromonospora* spp.

Test organisms	Inhibition zone (mm)					
	5 mg/disc			10 mg/disc		
	JSM5-1	A-25	JSM1-3	JSM5-1	A-25	JSM1-3
Staphylococcus aureus ATCC 25923	11.3	11.2	+-	12.4	13.3	8.6
S. aureus MRSA I	14.5	12.7	+-	19.7	15.1	9.1
S. aureus MRSA II	13.5	14.3	7.2	14.9	17.9	9.9
S. epidermedis	14.3	14.5	11.8	16.9	20.1	15.5
Bacillus subtilis ATCC 6633	16.9	11.2	+-	18.1	10.5	8.8
Enterococcus feacalis ATCC 25912	11.8	-	+-	17.3	11.1	10.7
Escherichia coli ATCC 25922	14.6	8.6	-	15.9	11.3	7.8
Pseudomonas aeruginosa ATCC 27853	10.2	+-	+-	14.2	7.8	7.3
Samonella typhi ATCC 14028	10.1	+-	+-	13.0	8.6	8.2
Alcaligenes sp.	11.9	15.6	+-	15.1	17.8	11.0

+-, weakly

A number of actinomycetes bacteria were isolated from the soil of marine and terrestrial sources collected from different provinces in Thailand. In the primary screening, a total of 33 strains exhibited antibacterial activities against the gram-positive bacteria, *S. aureus* ATCC 25923, *B. subtilis* ATCC 6633, and the gram-negative bacterium, *E. coli* ATCC 25922. The isolated strains produced dark hue with yellow, orange, green, brown, and black colonies, single spore on short sporophores of the vegetative mycelia. They showed different responses to carbon sources, reduction of nitrate, hydrolysis of starch and gelatin, hydrogen sulfide, growth on Czapex medium, and NaCl tolerance. The colonial appearance of these strains showed the moist colony the same as *Micromonospora chalcea* KA 579. They were all identified as *Micromonospora* species. (Holt, 1989). These strains are widely distributed in marine and terrestrial soils. (Kutzner, 1981).

The selected strains, JSM 5-1, JSM 1-3, and A-25 were fermented in different media, GP, GM, GN, GS, SY, SS, and DS. The results of fermentation in GS medium of JSM 5-1 showed high antibacterial activity against *S. aureus* ATCC 25923. Different pHs and temperatures had effects on growth and antibacterial activity of the tested strains. Strains JSM 5-1 showed high antibacterial activity when cultivated at pH 7.0 and 28°C.(Ishigami et al., 1994 ; Konishi et al., 1991 ; Wu et al., 1987).

The ethyl acetate extract of strain JSM 5-1 exhibited antibacterial activity against the strain of *Staphylococcus aureus, S. epidermidis, B. subtilis, Enterococcus faecalis, E. coli, Pseudomonas aeruginosa, Salmonella typhi,* and *Alcaligenes* sp.

The fermentation broth of *Micromonospora* sp. JSM 5-1 were centrifuged to give filtrate and mycelium. The filtrate was partitioned with ethyl acetate and butanol, respectively. The ethyl acetate extract was then separated by repetitive chromatography to afford one compound (JM-1).

The structure of JM-1 compound was determined through interpretation of their MS and NMR data, and subsequencely confirmed by comparison of these values with those reported in the literature.

Structure determination of compound JM-1

The electron impact mass spectrum of compound JM-1 (Figure 3) exhibited a molecular ion at m/z 270, consistent with a molecular formula of $C_{15}H_{10}O_5$.

Compound JM-1 could be assigned as the known compound genistein by analysis of its ^1H and ^{13}C NMR spectral properties. Its ^1H and ^{13}C NMR data (DMSO-d_6) have been earlier reported (Harborne, 1982). In this investigation, the NMR studies were done in DMSO-d_6.

From the ^1H NMR spectrum (Figure 4) compound JM-1 contained seven aromatic methine protons at δ 6.21(1H,d, J=1.3), 6.39 (1H,d, J=1.3), 6.81 (2H, d, J=8.5), 7.37 (2H, d, J=8.5), 8.32 (1H, s), and one H-bonded hydroxyl group at δ 12.95 (1H, s). The most downfield signal at δ 12.95 (1H, s) was assigned to 5-OH group because of its intramolecular hydrogen bonding to the C-4 carbonyl oxygen.

The ^{13}C NMR spectrum (Figure 5) showed fifteen carbons. These spectral data suggested the presence of one carbonyl group, seven methine carbons and seven quaternary carbons. The most downfield carbon signals was assigned to the carbonyl group.

Table 17 The ^{1}H NMR assignments of compound JM-1 (in DMSO-d_6) and genistein* (in DMSO-d_6)

Position	δ_C (ppm) (multiplicity, J in Hz)	
	Compound JM-1	*genistein
C-6	6.21 (d,J = 1.3)	6.2
C-8	6.39 (d,J = 1.3)	6.4
C-3' and C-5'	6.81(d,J = 8.5)	6.8
C-2' and C-6'	7.37(d,J =8.5)	7.45
C-2	8.32	8.3
C-5	12.95	12.96

* From Ganguly, 1970

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Table 18 The ^{13}C NMR assignments of compound JM-1 (in DMSO-d_6) and genistein* (in DMSO-d_6)

Carbon	δ_C (ppm)	
	Compound JM-1	*genistein
2	154.36	153.6
3	122.70	122.4
4	180.62	180.2
5	162.41	162.1
6	99.40	98.6
7	164.79	164.3
8	94.09	93.7
9	157.81	157.5
10	104.85	104.6
1'	121.64	121.4
2', 6'	130.56	130.0
3', 5'	115.46	115.2
4'	158.02	157.6

* From Harborne and mabry, 1982.

Comparison of its ^1H and ^{13}C NMR spectra with reported data (Harborne and marby , 1982 and Ganguly, 1970) indicated that compound JM-1 was genistein.

Genistein has been reported from *Micromonospora halophytica* (Ganguly,1970). It has been found in culture filtrates of *Aspergilli* and *Streptomycetes griseus* (Goodfellow *et al.*, 1988 ; Kenichi *et al.*, 1981.). In *Streptomyces griseus*, it was shown that genistein came from the medium containing soybean (Kenichi *et al.*, 1981). *Micromonospora* sp. exhibited activity against bacteria. Genistein demonstrated a side range of activity such as antifungal, antibacterial, mosquitocidal, menatocidal activity, and inhibition of topoisomerase (Chang *et al.*, 1995.).

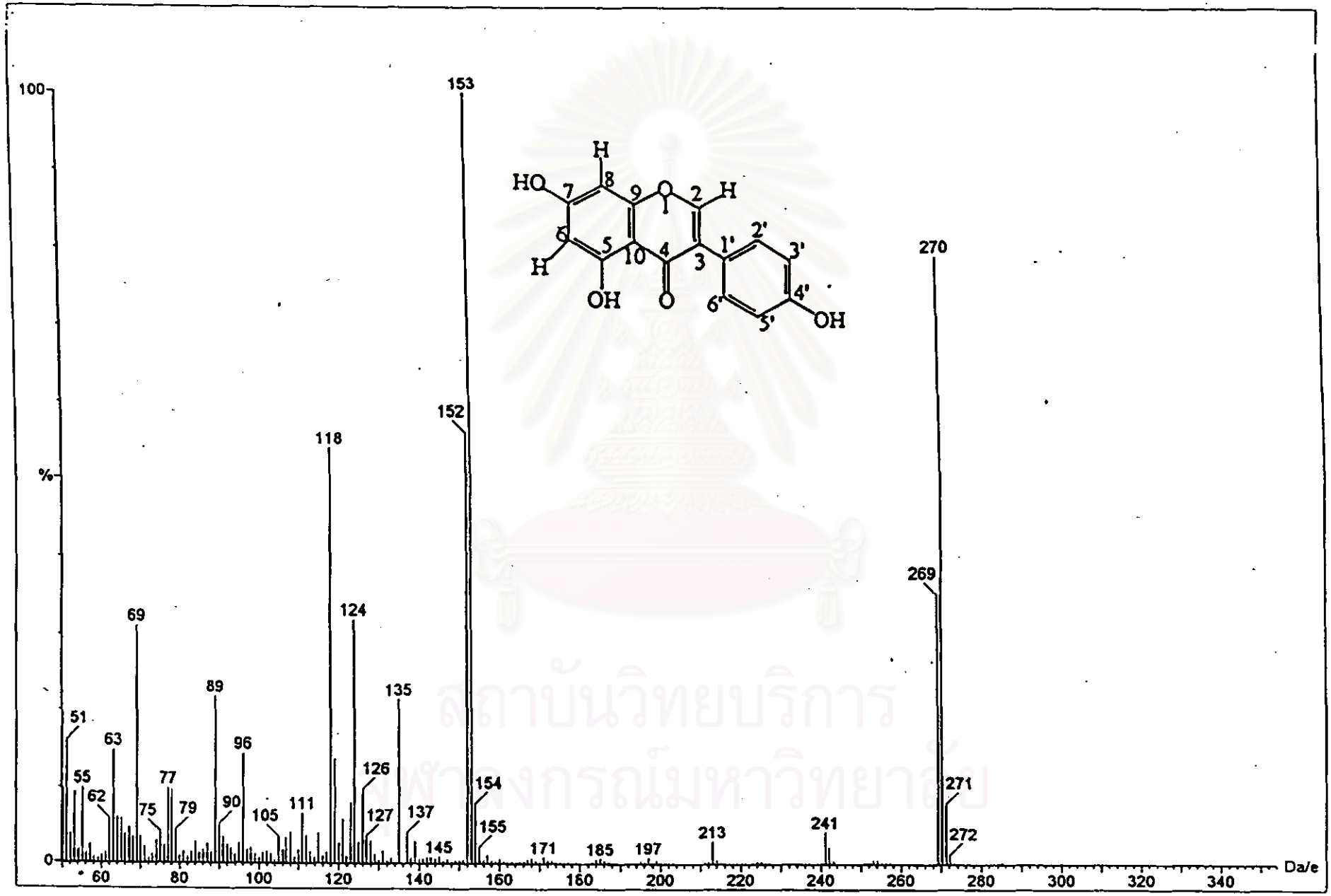

Figure 3. EI mass spectrum of compound JM-1

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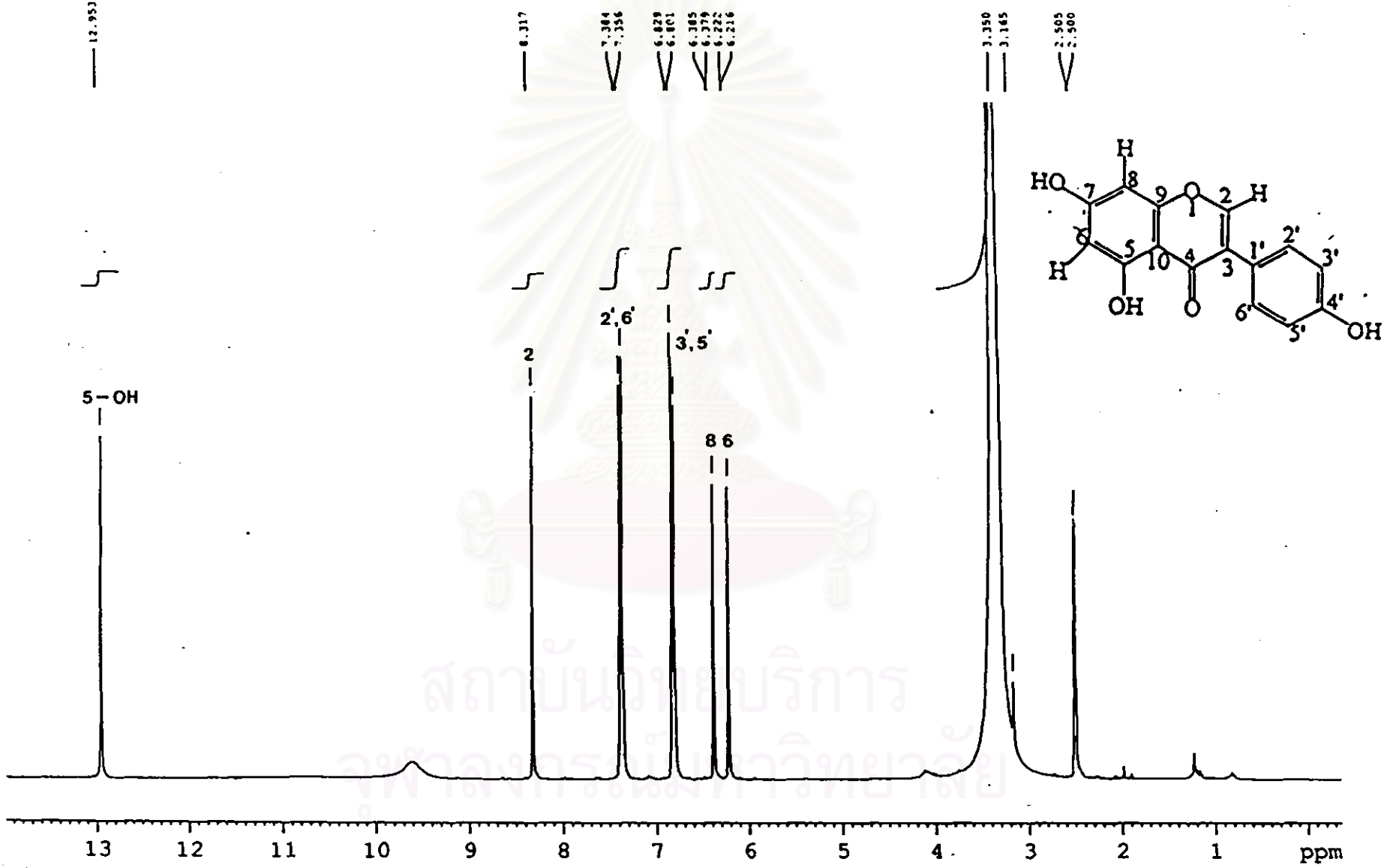

Figure 4 ^{1}H NMR spectrum (300 Mhz) of compound JM-1(in DMSO-d_6)

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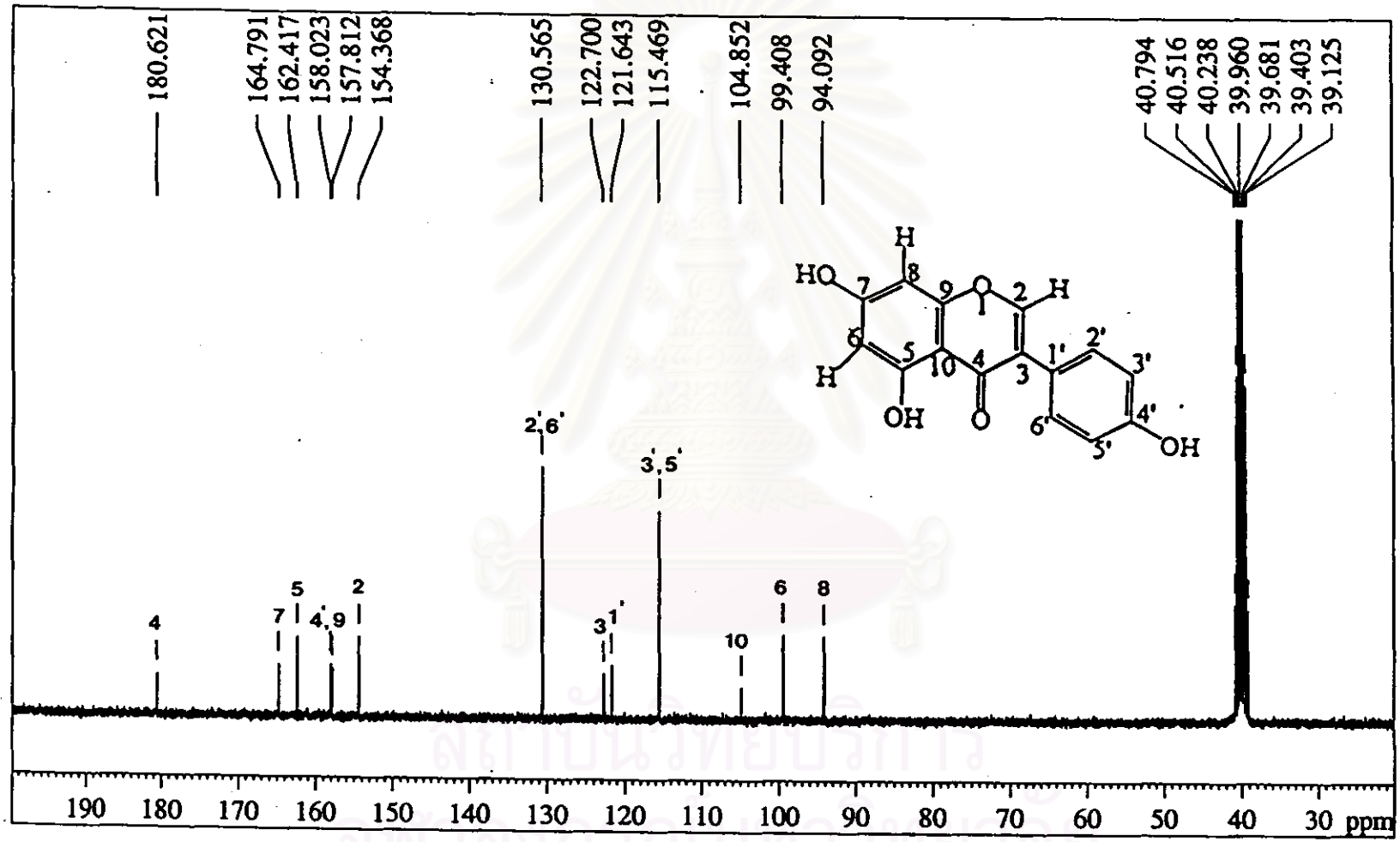

Figure 5 ^{1}C NMR spectrum (300 Mhz) of compound JM-1(in DMSO-d_6)

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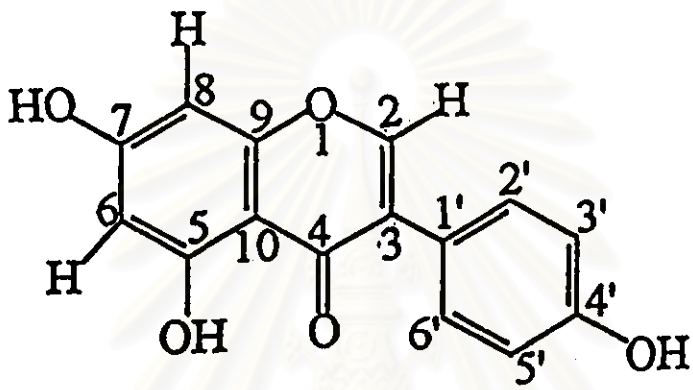

Figure 6 Structure of compound JM-1

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