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Antimicrobial Compounds, Chinesin I and II from Flowers of Hypericum chinense L.

Masashi NAGAI and Masahiro TADA*
Tokyo University of Agriculture and Technology,
Laboratory of Bio-organic Chemistry, Fuchu, Tokyo 183

Two new antimicrobial compounds against Gram-positive bacteria, chinesin I and II, were isolated from the flowers of Hypericum chinense L. The structures of chinesin I and II were elucidated on the basis of spectral and chemical evidences. Chinesin I is relatively active in the cytotoxicity test with Hela.

There are many reports on the antimicrobial constituents of higher plants. Schönbeck investigated the antibiotic properties of individual part of a plant body separately and found that the flowers of many plants showed especially high antibiotic activities, followed by leaves, roots and lastly branches.[1] So far as we know, there are few reports on biological active compounds from flowers.[2] In the course of our chemical investigation on the self-defense mechanism of plants, we examined antibacterial activities of methanol extracts from various plants by the paper-disk method against Escherichia coli and Bacillus subtilis, and found that almost all plants showed anti-Bacillus activities and especially the flowers of Hypericum chinense L. (Guttiferae) (Japanese name: byouyanagi) exhibited strong activity. The plants belonging to Guttiferae family are well known folk medicines for the external wound in Japan.[3] We report here the isolation and the structure elucidation of the antibacterial compounds from the flowers of Hypericum chinense L (Guttiferae).

The methanol extract from the flowers of H. chinense (2.9 kg, fresh weight) collected in the campus of Tokyo University of Agriculture and Technology, in June was concentrated in vacuo and the residue was partitioned with ethyl acetate and water. The antimicrobial activity was tested by paper-disk method against B. subtilis (IFO 3734) for each fraction. The activity was found in the ethyl acetate phase. The ethyl acetate phase was chromatographed on silica gel with hexane-ethyl acetate-methanol. The active fractions were purified by HPLC on silica gel with hexane-ethyl acetate and on ODS with methanol-water to afford two antimicrobial compounds which were named as chinesin I (1) and chinesin II (2), both as labile colorless oils.

Chinesin I (1) (4 g: calcd yield) liquid, $C_{27}H_{40}O_5$ (MS: M^+ m/z found 444.2847, calcd 444.2820), $[\alpha]_D$ 69°(c 0.12, MeOH), UV λ_{max} (EtOH) 224 nm (ε $1.5*10^4$), 354 nm (ε $1.2*10^4$),IR (NaCl) 3362(br.), 3070, 2964, 2930, 2874, 1638, 1570, 1506, 1456, 1373, 1198, 889 cm^{-1}. The ^{13}C-NMR spectrum (Table 1) of chinesin I showed the existence of four carbonyl groups (or enols) (δ 207.6,

197.1, 189.9, 174.9) and a tertiary alcohol (δ 81.1). The IR spectrum of chinesin I suggested that all the carbonyl groups were conjugated. In the ^{1}H-NMR spectrum (Table 1) of chinesin I, three OH protons were observed at δ 3.51 (br.s, 1H), 10.52 (br. s, 1H) and 19.23 (s, 1H), which lead to the assumption that two of the four carbonyl groups should exist predominantly in the enol forms.[5)]

Table 1. NMR data[4)] of chinesin I and chinesin II

	Chinesin I				Chinesin II	
C	^{13}C(INEPT)	^{1}H(J ,Hz)	COSY	^{13}C-^{1}H COSY (long range)	^{13}C(INEPT)	^{1}H(J ,Hz)
1	189.9			1-OH,7H(a)	189.9	
2	106.4			1-OH	105.7	
3	197.1			17H(a),22H	197.0	
4	53.2			17H(a),22H	53.1	
5	174.9			7H(b),22H	175.0	
6	108.4			1-OH,7H(b)	108.2	
7	21.2(CH_2)	a)2.16dd(15,3)	8H		21.1(CH_2)	a)2.13dd(15,12)
		b)2.70dd(15,11)	8H			b)2.67dd(15,3)
8	50.2(CH)	1.80ddd(11,11,3)	7H,12H	16H	50.1(CH)	1.76ddd(12,12,3)
9	81.1			7H(b),16H	81.0	
10	43.3(CH_2)	a)1.87m	11H	16H	43.2(CH_2)	a)1.85m
		b)1.98m	11H			b)1.95m
11	28.6(CH_2)	a)1.54m	10H,12H		29.4(CH_2)	a)1.50m
		b)1.86m	10H,12H			b)1.81m
12	54.1(CH)	2.49ddd(11,10,7)	8H,11H	14H,15H	54.1(CH)	2.46ddd(12,11,7)
13	146.2			15H	146.2	
14	111.6(CH_2)	a)4.83qd(2,1)	15H	15H	111.6(CH_2)	a)4.80qd(2,1)
		b)4.88qd(1,1)	15H			b)4.83qd(1,1)
15	18.5(CH_3)	1.78dd(2,1)	14H	14H	18.4(CH_3)	1.74dd(2,1)
16	29.2(CH_3)	1.27s			29.1(CH_3)	1.23s
17	37.5(CH_2)	a)2.54dd(15,7)	18H	22H	37.7(CH_2)	a)2.50dd(15,8)
		b)2.67dd(15,7)	18H			b)2.64dd(15,8)
18	118.8(CH)	4.77t(7)	17H	20H,21H	118.7(CH)	4.74t(8)
19	134.5			20H,21H	134.6	
20	17.9(CH_3)	1.56s			17.9(CH_3)	1.53s
21	25.8(CH_3)	1.54s			25.8(CH_3)	1.53s
22	24.6(CH_3)	1.37S			24.6(CH_3)	1.33s
23	207.6			1-OH,24H, 25H,26H(a)	208.0	
24	42.1(CH)	3.89qt(7,7)	25H,26H	25H,27H	35.7(CH)	4.00qq(7,7)
25	16.9(CH_3)	1.11d(7)	24H		18.7(CH_3)	1.11d(7)
26	26.3(CH_2)	a)1.40qdd(7,14,7)	24H,27H	25H,27H	19.1(CH_3)	1.10d(7)
		b)1.75qdd(7,14,7)	24H,27H			
27	11.9(CH_3)	0.91t(7)	26H			
1-OH		19.23s				19.14s
5-OH		10.52br				10.50br.s
9-OH		3.51br				3.50br.s

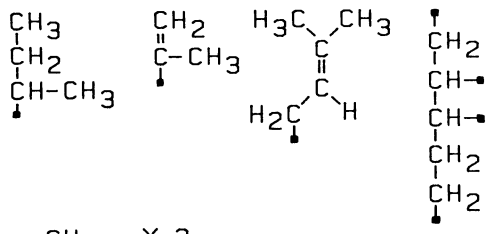


Fig.1.

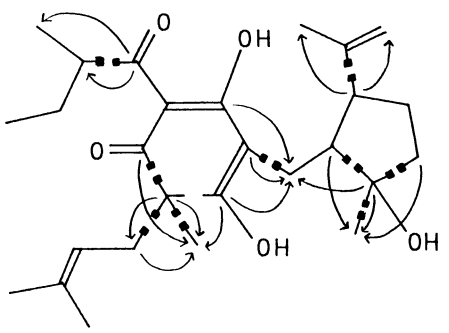


Fig.2.

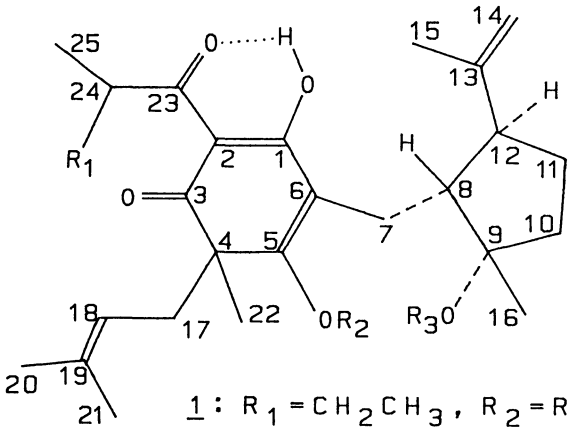
In fact the UV absorption (354 nm) of chinesin I is quite similar to the absorption of 1,3,3',5-tetraketone conjugated system of humulone[5] (355 nm) which was isolated from hop (Humulus lupulus).

Treatment of chinesin I with etherial diazomethane yielded mono-methylated compound (3) and di-methylated compound (4). The ^{1}H-NMR spectrum of compound 3 showed the signals of methoxy protons at δ 3.32 (s,3H) and two acidic protons at δ 10.15 (s, 1H) and 19.23 (s, 1H). In the ^{13}C-NMR spectrum, the signals due to the carbons C-8, C-9, C-10, and C-16 were shifted larger than the other carbons by the methylation of chinesin I.

The ^{1}H-^{1}H and ^{1}H-^{13}C shift correlated 2D NMR[6,7] and INEPT[8] experiments of chinesin I (1) and the methyl ether (3) (Tables 1 and 2) clearly showed the presence of several partial structures which were separated by quaternary carbons or di-substituted sp^{2} carbons (drawn in the Fig. 1).

Table 2. NMR data[4] of the methyl ether 3

C	^{13}C	^{1}H(J ,Hz)	NOESY	C	^{13}C	^{1}H(J ,Hz)	NOESY
1	189.8			15	18.4	1.76dd(2,1)	8H
2	106.3			16	23.4	1.17	5-OH,8H,9-OCH$_3$
3	196.5			17	37.4	a)2.50dd(14,7)	20H,22H
4	53.0					b)2.69dd(14,7)	20H,22H
5	174.7			18	118.9	4.76t(7)	
6	108.3			19	134.4		
7	20.5	a)2.13dd(15,12)	5-OH	20	17.9	1.57s	17H,22H
		b)2.65dd(15,3)	12H	21	25.8	1.55s	
8	51.6	1.85ddd(12,11,3)	15H,16H	22	24.6	1.35s	17H,20H,5-OH
9	85.7			23	207.3		
10	36.0	a)1.60ddd(13,8,4)	9-OCH$_3$	24	41.9	3.88qt(7,7)	27H
		b)2.05td(6,13)	9-OCH$_3$	25	16.8	1.10d(7)	27H
11	28.5	a)1.50m		26	26.4	a)1.38qdd(7,12,7)	
		b)1.77m				b)1.73qdd(7,12,7)	
12	53.7	2.43td(8,11)	7H(b)	27	11.9	0.91t(7)	24H,25H
13	146.3			1-OH		19.23s	
14	111.6	a)4.81qd(1,1)		5-OH		10.15s	7H(a),9-OCH$_3$,
		b)4.85qd(2,1)					16H,22H
				9-OCH$_3$	49.3	3.32s	5-OH,10H,16H



1: R_1=CH_2CH_3, R_2=R_3=H
2: R_1=CH_3, R_2=R_3=H
3: R_1=CH_2CH_3, R_2=H, R_3=CH_3
4: R_1=CH_2CH_3, R_2=R_3=CH_3

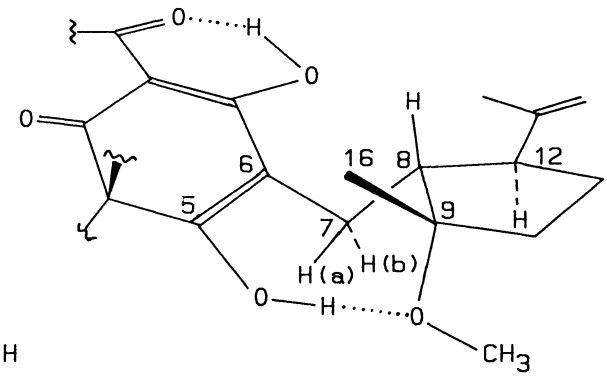


Fig.3

The connection of the partial structures with the quaternary carbons and di-substituted sp^2 carbons was clarified by ^1H-^{13}C shift correlated 2D NMR experiment for long range coupling (J_{C-H}=10 Hz and 5 Hz) of chinesin I (Table 1 and Fig. 2). These observations led to the structure (1) for chinesin I. The stereochemistry of chinesin I was inferred from the result of NOESY[9] experiment of chinesin I methyl ether (3)(Table 2). The NOE between 5-OH and 16-H, between 5-OH and 7-H(a) and between 5-OH and 9-OCH_3 of the compound 3 suggested the existence of the intramolecular hydrogen bonding between 5-OH and 9-OCH_3. The conformation of the cyclopentane ring of the compound 3 thus should be rigid (Fig. 3). The coupling constant between 8-H and 12-H (11 Hz) gave us the conjecture that the dihedral angle between 8-H and 12-H should be about 180°. The NOE between 8-H and 15-H, between 8-H and 16-H and between 7-H(b) and 12-H of the compound 3 showed that 8-H, 16-C and 13-C should be on the same side of the cyclopentane ring. The stereochemistry at C-4 and C-24 of chinesin I was not determined.

Another antimicrobial compound, chinesin II, was obtained from the same extract. The spectral data of chinesin II showed that chinesin II has a methyl group at C-24 instead of the ethyl group of chinesin I (Table 1).

Chinesin I and II have moderate activities against Gram-positive bacteria (Table 4) and no activity against Gram-negative bacteria. The cytotoxic activity of chinesin I to Hela was relatively strong [ID_{50} (μg/ml) 0.52]. Further studies on the activity of chinesin I against plant pathogens are in progress.

Table 3. Antibacterial activities of chinesin I

Gram-positive bacteria	MIC (μg/ml)
Staphylococcus aureus	3.13
Staphylococcus epidermidis	>100
Micrococcus luteus	6.25
Batillus subtilis	3.13

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References

1) F. Schönbeck, Phytopathol. Z., 59, 205 (1967).
2) J. W. Mitchell, N. Mandava, J. F. Worley, J. R. Plimmer,and M. V. Smith, Nature, 225, 1065 (1970).
3) W. L. Parker and F. Johnson, J. Am. Chem. Soc., 90, 4716, 4724 (1968).
4) These data (Tables 1 and 2) were taken on JEOL JMN-GX270 and GX400 FT-NMR spectrometers in $CDCl_3$ with TMS as an internal standard.
5) D. De Keukeleire and M. Verzele, Tetrahedron 26, 385 (1970).
6) W. P. Aue, E. Bartholdi, and R. R. Ernst, J. Chem. Phys., 64, 2229 (1976).
7) T. Kikuchi, S. Kadota, S. Matsuda, and H. Suehara, Tetrahedron Lett., 25, 2526 (1984).
8) D. M. Doddrell and D. T. Pegg, J. Am. Chem. Soc., 102, 6388 (1980); R. Freeman and G. A. Morris, J. Chem. Soc., Chem. Commun., 1978, 64.
9) B. H. Meier and R. R. Ernst, J. Am. Chem. Soc., 101, 6441 (1979).

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