Short Communication

Antiviral and Antimicrobial Activity of 2,4-Diacylphloroglucinols, 2-Acylcyclohexane-1,3-diones and 2-Carboxamidocyclohexane-1,3-diones¹⁾

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Previously, we have reported the isolation and structure elucidation of antimicrobial compounds, chinesin I and II from flowers of *Hypericum chinense* L.²⁾ The structures of chinesins attracted our attention as their partial structure is similar to 1,3-dioxo-2-carboxamide structure of tetracycline.³⁾ Several antimicrobial acylphloroglucinols have been isolated from higher plants, e.g., aspidin,⁴⁾ uliginosin A,⁵⁾ humulon⁶⁾ and lupulon.⁷⁾ The antimicrobial activities of caespitin and its analogues have been reported.⁸⁾ Sessiliflorene, and sessiliflorol A and B are acylphloroglucinols isolated from *Melicope sessiliflora* with inhibitory activity against herpes simplex virus.⁹⁾ Acylphloroglucinols are also known as a plant growth inhibitor.¹⁰⁾ These interesting facts promoted our research about the structure-activity relationship of 2,4-diacylphloroglucinols, 2-acylcyclohexane-1,3-diones and 2-carboxamidocyclohexane-1,3-dione. We report here syntheses of diacylphloroglucinols, 2-acylcyclohexane-1,3-diones and 2-carboxamidocyclohexane-1,3-dione, and their antiviral and antimicrobial activities.

2-Acetylcyclohexane-1,3-dione **4** was synthesized by a Fries rearrangement of 3-acetoxy-2-cyclohexen-1-one, which was prepared from cyclohexane-1,3-dione **2** and acetic anhydride

in pyridine-ethylene chloride (40.2%). The methyl, dimethyl and phenyl analogues (**7**, **10** and **13**) of **4** were synthesized from corresponding cyclohexane-1,3-diones (**6**, **9** and **12**), respectively (26.0–53.4%). 5-Methylcyclohexane-1,3-dione **6** was obtained by condensation of methyl crotonate and ethyl acetoacetate catalyzed with sodium ethoxide in ethanol (53.4%). 5-Phenylcyclohexane-1,3-dione **12** was prepared by a similar condensation from diethyl malonate and 4-phenyl-3-buten-2-one (97.1%).

The 2-carboxamidocyclohexane-1,3-diones (**5**, **8**, **11** and **14**) were synthesized from the corresponding cyclohexane-1,3-diones (**2**, **6**, **9** and **12**) with sodium cyanate in *N,N*-dimethylformamide-water at 100°C (15.5–23.4%).

2,4-Diacylphloroglucinols (**17**, **18**, **19**, **20**, **21** and **22**) were synthesized by a Friedel-Crafts acylation of phloroglucinol with carboxylic acid in boron trifluoride etherate (52.7–78.9%).

Antimicrobial activity was tested by the paper-disk method¹¹⁾ against *Bacillus subtilis* (IFO 3734) and *Escherichia coli* (IFO 3301). The antimicrobial activities were determined by the following formula (Table I):

$$\text{Activity} = (Y - 0.6) / (X - 0.6) \times 100$$

where *X* (mm) is the diameter of the inhibition zone by tetracycline and *Y* (mm) is the diameter of the inhibition zone by the sample. The diameter of the paper disk is 0.6 mm.

All the synthesized compounds showed antimicrobial activity against *B. subtilis*, but weak activity or none against *E. coli*. The activity of the dicarbonyl compounds (**2**, **6**, **9** and **12**) was weaker than corresponding 2-acetylcyclohexane-1,3-diones (**4**, **7**, **10** and **13**). The 2-carboxamidocyclohexane-1,3-diones (**5**, **8**, **11** and **14**) showed stronger activity than the corresponding 2-acetylcyclohexane-1,3-diones (**4**, **7**, **10** and **13**). Clearly, the 2,4-diacylphloroglucinols (**17**, **18**, **19**, **20**, **21** and **22**) were more active than the 2-acetylcyclohexane-1,3-diones and the 2-carboxamidocyclohexane-1,3-diones. The antimicrobial activity of chinesins and 2,4-diacylphloroglucinols is comparable to that of tetracycline. These

Table I. ANTIMICROBIAL ACTIVITY OF CHINESIN ANALOGUES

Compound				Activity		
				<i>B. subtilis</i>	<i>E. coli</i>
1			100 µg/disk 41.5	100 µg/disk 53.7
2	R ₁ =H	R ₂ =H	R ₃ =H	3.5	ND
	3	R ₁ =H	R ₂ =H	R ₃ =CH ₃	69.5	30.2
	4	R ₁ =H	R ₂ =H	R ₃ =COCH ₃	28.2	10.3
	5	R ₁ =H	R ₂ =H	R ₃ =CONH ₂	59.9	ND
	6	R ₁ =CH ₃	R ₂ =H	R ₃ =H	19.5	ND
	7	R ₁ =CH ₃	R ₂ =H	R ₃ =COCH ₃	26.9	ND
	8	R ₁ =CH ₃	R ₂ =H	R ₃ =CONH ₂	71.7	20.4
	9	R ₁ =CH ₃	R ₂ =CH ₃	R ₃ =H	29.9	3.1
	10	R ₁ =CH ₃	R ₂ =CH ₃	R ₃ =COCH ₃	32.4	ND
	11	R ₁ =CH ₃	R ₂ =CH ₃	R ₃ =CONH ₂	53.3	ND
	12	R ₁ =C ₆ H ₅	R ₂ =H	R ₃ =H	32.6	ND
	13	R ₁ =C ₆ H ₅	R ₂ =H	R ₃ =COCH ₃	50.5	12.2
	14	R ₁ =C ₆ H ₅	R ₂ =H	R ₃ =CONH ₂	60.4	ND
	15	R ₁ =R ₂ =H		10 µg/disk ND	100 µg/disk 20.6
16		R ₁ =COC ₆ H ₅	R ₂ =H		28.4	50.3
17		R ₁ =COC ₆ H ₅	R ₂ =COCH ₃		74.8	18.1
18		R ₁ =R ₂ =COCH ₃			91.1	29.2
19		R ₁ =R ₂ =COCH ₂ CH ₃			91.3	27.3
20		R ₁ =R ₂ =COCH(CH ₃) ₂			94.8	51.4
21		R ₁ =R ₂ =COCH ₂ CH ₂ CH ₃			63.9	ND
22		R ₁ =R ₂ =COCH ₂ CH(CH ₃) ₂			74.1	ND
					10 µg/disk	100 µg/disk
Tetracycline				100.0	100.0	
Chinesins				81.8	ND	

results show that dicarbonyl groups on cyclohexane are a necessary structural unit for this activity. An increase in the number of carbonyl groups or enols in a molecule raises the activity. The number of carbons and branching on the acyl groups also influence the activity. As 2,4-diisobutyrylphloroglucinol **20** showed the strongest microbial activity by the primary test, the MIC was measured against various microorganisms by comparing the activity with cefazolin (Table II). Compound **22** was also effective against *Candida albicans* NIH3147.

The antiviral activity of dione **12**, 2-carboxamidocyclohexane-1,3-diones (**5** and **11**) and 2,4-diacylphloroglucinols (**18**, **19** and **20**) was tested at the highest non-toxic dose of the compounds by the inhibition of cytopathic

Table II. MIC OF **20** AGAINST VARIOUS MICROORGANISMS

Microorganism	MIC (µg/ml)	
	20	Cefazolin
<i>Staphylococcus aureus</i>	0.78	0.39
<i>S. aureus</i> ATCC 10537	3.13	0.1
<i>S. epidermidis</i>	> 100	1.56
<i>Micrococcus luteus</i>	6.25	1.56
<i>Bacillus subtilis</i>	3.13	< 0.1
<i>Candida albicans</i> NIH3147	50	> 100
<i>Mycobacterium smegmatis</i> 607	> 100	

effect (c.p.e.) in the development of vesicular stomatitis virus (VSV), herpes simplex virus type I (HSV-I) and polio virus type I (LSc, 2ab: Polio-I) in HEL, RL-33 and FL cells, respectively. Furthermore, VSV yield in HEL

Table III. EFFECT OF 2,4-DIACYLPHLOROGLUCINOLS ON THE REPLICATION OF VESICULAR STOMATITIS VIRUS IN HEL CELL CULTURES^a

Compound	($\mu\text{g/ml}$)	Virus yield Log_{10} p.f.u. (ml)	Cytotoxicity (24 hr)
18	10.0	4.6	(—)
19	5.0	4.4	(—)
20	3.0	4.6	(—)
VSV-control		9.6	

^a HEL cells were seeded in 2-oz prescription bottles and infected at an input m.o.i of 0.01 p.f.u./cell. After adsorption for 1 hr at 37°C, the infected cell monolayers were washed 3 times with PBS, before 5 ml of a maintenance medium containing various concentrations of each compound was added to the bottles, the infected cultures then being incubated at 37°C for 24 hr after infection.

cells was measured in the presence of compounds **18**, **19** and **20**. The infectivity titration of VSV was done by the plaque method in RL-33 cells. No activity was observed for dione **12** and 2-carboxamidocyclohexane-1,3-diones **5** and **11** whereas strong activity was observed for 2,4-diacylphloroglucinols **18**, **19** and **20**, chinesin I and chinesin II against VSV and HSV-I. These suggest that the compounds with an acylphloroglucinol structure should be generally active against VSV and HSV-I. 2,4-Diacylphloroglucinols and chinesins showed no activity against polio-I. It is interesting that these acylphloroglucinols are effective against both the RNA virus with envelope (VSV) and DNA virus with envelope (HSV-I), and ineffective against the RNA virus without envelope (polio-I). It is well

known that tannic acid inactivates such enveloped viruses as HSV, VSV and vaccinia virus. However, chinesins and these acylphloroglucinols did not show any virus inactivating activity. An further investigation of the antiviral activity is in progress.

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