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Relationship Between the Structures of Fatty Acid Amide Derivatives and Their Antimicrobial Activities

SATOSHI ŌMURA, MICHIKO KATAGIRI, JUICHI AWAYA, TOKIO FURUKAWA, IWAO UMEZAWA, NOBUHIRO ŌI, MASAKAZU MIZOGUCHI, BUNYA AOKI, AND MINORU SHINDO

Kitasato Institute, Minato-ku, and Chugai Seiyaku, Toshima-ku, Tokyo, Japan

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The structure-activity relationships of derivatives of the antibiotic cerulenin were investigated by chemically modifying dodecanoic acid, its skeletal backbone. The dimethylamide derivatives were active against both gram-positive and -negative bacteria, and fungi. Among the compounds having modified groups at positions C2 and C4, the most active were those with a carbonyl group at C4 and a double bond at C2. The dimethylamide and pyrrolidine amide derivatives of this structure type were the most active. Activity against bacteria and yeast increased with the number of carbon atoms in the skeleton, with the maximum activity being observed at C=12. No significant differences in activity against fungi were observed with change in chain length.

The mechanism of action of the antibiotic, cerulenin, (2S)(3R)2,3-epoxy-4-oxo-7,10-dodecadienoyl amide, has been attributed to the inhibition of cellular lipid biosynthesis (8-10, 13). Being interested in the biological properties of this acid amide derivative, we have further investigated the antibacterial and antifungal properties of a number of acid amide derivatives. The antibacterial and antifungal activities of fatty acids and their derivatives have been reported on (2, 4-7, 11, 12, 14), but no systematic study has been reported on amide derivatives.

The dihydro derivative of cereulenin was inactive. The tetrahydro derivative, however, retained some activity. Dimethylamide and pyrrolidine amide derivatives with a 4-oxo-2-ene moiety proved to be the most active.

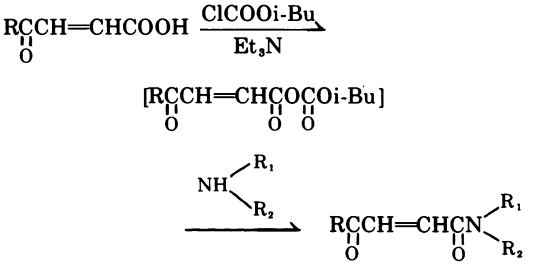
MATERIALS AND METHODS

The antimicrobial activity of the derivatives was determined by an agar dilution method and was expressed as minimal inhibitory concentration. Test organisms were *Bacillus subtilis* PCI 219, *Staphylococcus aureus* FDA 209P, and *Mycobacterium smegmatis* ATCC 607 as gram-positive bacteria, and *Escherichia coli* NIHJ and *Pseudomonas aeruginosa* P-3 as gram-negative bacteria. The cells were cultured in peptone water for 18 to 24 h and then used as inoculum. Nutrient agar was used for plate assays, and determinations were made after incubation at 37 C for 24 h.

Candida albicans was used as an assay organism for yeast activity, and *Piricularia oryzae* and *Tricophyton interdigitale* (or *Tricophyton rubrum*) were used for the detection of antifungal activity. Suspensions in physiological saline were inoculated into potato agar assay plates and incubated for 72 h at 27 C.

The compounds were synthesized by reacting the appropriate fatty acids derivative with an amine. Only *trans* compounds were synthesized in this study. General procedures are given below. Melting points or boiling points of the compounds are given in Table 2 through 6. Those compounds whose melting or boiling points had not been determined were identified by infrared light (Hitachi EPI-G3 infrared spectrophotometer), nuclear magnetic resonance (Hitachi 20A 60-mHz nuclear magnetic resonance spectrometer), or elementary analysis.

The general procedure was as shown:



4-Oxo-2-ene-fatty acid (1) (0.0024 mol) and triethylamine (0.0024 mol) were dissolved in 10 ml of tetrahydrofuran, and isobutyl chloroformate (0.0024 mol) was added dropwise with constant stirring at −5 to −10 C. Stirring was continued at the same temperature for 30 min, and 0.0024 mol of an amine in 10 ml of tetrahydrofuran was added dropwise to the above solution. The solution was stirred at −5 to −10 C for 30 min and then at room temperature for 2 h. Amine was also added in aqueous solution, in which case the addition was faster. The precipitated triethylamine hydrochloride was filtered off and the filtrate was

evaporated in vacuo. The residue was dissolved in ether (100 ml), and the ethereal solution was washed with 5 % sodium carbonate and then with water. The solution was dried over anhydrous sodium sulfate and the solvent was evaporated in vacuo. The residue was subjected to silica gel chromatography using benzene-chloroform-ether (1:1:1) as eluant. The appropriate fraction was evaporated in vacuo, and the product was crystallized from petroleum ether or acetone.

RESULTS

The properties of the compounds obtained by reduction of cerulenin are summarized in Table 1.

The dihydro compound, in which the epoxy portion had been cleaved, showed little antimicrobial activity. The tetrahydro compound, however, in which two double bonds had been reduced but the epoxy group retained, possessed some activity. This suggests that the epoxy group may have some effect on the activity and that the contribution of the double bond may be marginal.

Of those derivatives of dodecanoic acid, the fundamental backbone of cerulenin, that we had occasion to synthesize (Table 2), only the dimethylamide derivative possessed some activity. Its activity was about 1/10 to 1/20 that of cerulenin.

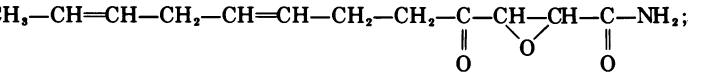
Compounds similar to cerulenin but modified in the C2-C4 positions were also synthesized, and their antimicrobial activity was determined (Table 3). The compounds modified only at C4, i.e., CM-41 and CM-36, showed a complete loss of activity, whereas those functionalized only at C2, i.e., CM-12 and CM-18, had some activity. Compounds functionalized at both C2 and C4, in particular those with the partial structure $-\overset{4}{\underset{\underset{O}{\|}}{C}}\overset{3}{C}H=\overset{2}{C}H-$, displayed good activity. Variation of the amide moiety gave activity only for those compounds possessing the grouping $-\overset{4}{\underset{\underset{O}{\|}}{C}}-\overset{3}{C}H=\overset{2}{C}H-$.

Derivatives of 4-oxo-dodec-2-en-oic acid are listed in Table 4. Insertion of the moiety

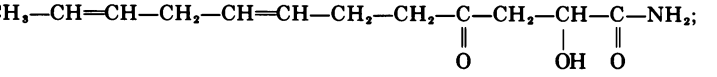
TABLE 1. *Minimal inhibitory concentrations (MIC) of cerulenin derivatives*

No.	Compound[a]	Melting point (C)	MIC (μg/ml)[b]							
			B.s.	S.a.	M.	E.c.	P.a.	C.a.	P.o.	T.i.
1	Cerulenin	93-94	1.56	100	1.56	12.5	>100	1.56	3.12	12.5
2	Dihydro cerulenin	128-130	100	100	>100	>100	>100	>100	>100	>100
3	Tetrahydro cerulenin	85.5-86.5	12.5	100	>100	>100	>100	50	6.25	25
4	Hexahydro cerulenin	135-136	>100	>100	>100	>100	>100	>100	>100	>100

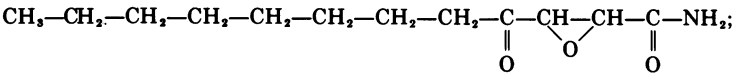
[a] Cerulenin:



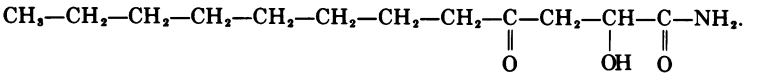
dihydro cerulenin:



tetrahydro cerulenin:



hexahydro cerulenin:



[b] B.s., *Bacillus subtilis* PCI 219; S.a., *Staphylococcus aureus* FDA 209P; M., *Mycobacterium smegmatis* ATCC 607; E.c., *Escherichia coli* NIHJ; P.a., *Pseudomonas aeruginosa* P-3; C.a., *Candida albicans;* P.o., *Piricularia oryzae;* T.i., *Tricophyton interdigitale* (or *T. rubrum*).

TABLE 2. *Minimal inhibitory concentration (MIC) of dodecanoic acid derivatives*

No.	Compound[a]	Melting or boiling point (C)	MIC (μg/ml)[b]							
			B.s.	S.a.	M.	E.c.	P.a.	C.a.	P.o.	T.i.
5 (CM-01K)	$R-CH_2-CH_2-CH_2-COOH$	44	>100	>100	>100	>100	>100	>100	>100	>100
6 (CM-9K)	$R-CH_2-CH_2-CH_2-COOC_2H_5$	$b_{1.5}$ 104-106	>100	>100	>100	>100	>100	>100	>100	
7 (CM-7K)	$R-CH_2-CH_2-CH_2-CONH_2$	102	>100	>100	>100	>100	>100	>100	>100	
8 (CM-5K)	$R-CH_2-CH_2-CH_2-CO-N(CH_3)H$	68-69	>100	>100	>100	>100	>100	>100	>100	
9 (CM-8K)	$R-CH_2-CH_2-CH_2-CO-N(C_6H_5)H$	76-77	>100	>100	>100	>100	>100	>100	>100	
10 (CM-6K)	$R-CH_2-CH_2-CH_2-CO-N(CH_3)CH_3$	$b_{1.5}$ 137-138	15.6	15.6	31.2	31.2	>100	62.5	31.2	

[a] R, $-(CH_2)_7CH_3$.
[b] Abbreviations are as in Table 1.

$$-\overset{4}{\underset{\underset{O}{\|}}{C}}-\overset{3}{CH}=\overset{2}{CH}-$$ into dodecanoic acid enhanced activity. The methylamide (CM-105) and dimethylamide (CM-55) derivatives of this compound, i.e., 4-oxo-dodec-2-en-oic acid, further increased the antimicrobial activity.

The dimethylamides (CM-55 and CM-6K) proved to be the most active compounds in both the dodecanoic acid and the 4-oxo-dodec-2-en-oic acid series (Tables 2 and 4, respectively). Each compound modified at the C2-C4 positions as shown in Table 3 were modified further by amide end group as shown in Table 4. All those amide derivatives prepared by us of C4 functionalized dodecanoic acid were inactive, and only the dimethylamide derivative of C2 functionalized dodecanoic acid possessed some activity.

In conclusion, the compound possessing the partial structure

$$-\underset{\underset{O}{\|}}{C}CH=CH\underset{\underset{O}{\|}}{C}N\begin{matrix}CH_3\\CH_3\end{matrix}$$

was found to be the most active. The activity of *N*-mono-substituted amides of 4-oxo-dodec-2-en-oic acid (Table 5) peaked with the *N*-propyl derivative (CM-114). The *N*-benzyl, *N*-phenyl, and *N*-cyclohexyl derivatives were inactive. The *N*-benzyl, *N*-methyl derivative (CM-100), however, possessed good activity against both bacteria and fungi. When both hydrogen atoms of the amide group (Table 5) were substituted with alkyl groupings, the activity decreased with the number of C atoms. The dibenzylamide (CM-112) was ineffective against bacteria but effective against fungi (*P. oryzae*, *T. interdigitale*).

The activity of the cycloalkyl amides decreased with increasing ring size. The pyrrolidine amide (CM-115) was as effective as the dimethylamide (CM-55); these two compounds had the best antimicrobial spectrum of all the derivatives prepared by us.

Variation in chain length of compounds of the general type

$$CH_3(CH_2)_nCCH=CHCN\begin{matrix}CH_3\\CH_3\end{matrix}$$
$$\qquad\qquad\quad \| \qquad\quad \|$$
$$\qquad\qquad\quad O \qquad\quad O$$

was also undertaken (Table 6). Increased activity against bacteria and yeast was observed with an increase in the number of carbon atoms, the derivatives with $n = 7$ (cerulenin chain length) being the most effective. Activity decreased rapidly at $n = 10$. There was no appreciable effect of alkyl chain length on antifungal activity. The toxicity of the most active derivative, CM-55, against mice was: (i) for intraperitoneal injection, mean lethal dose of 70 mg/kg (211 mg of cerulenin per kg); (ii) for subcutaneous injection, mean lethal dose of 250 mg/kg (245 mg of cerulenin per kg); (iii) for oral administration, mean lethal dose of 660 mg/kg (547 mg of cerulenin per kg).

DISCUSSION

The antimicrobial activity of fatty acids and

TABLE 3. *Minimal inhibitory concentration (MIC) of N,N-dimethyl dodecanamide modified at C2-C4*

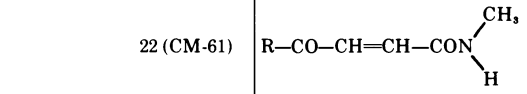
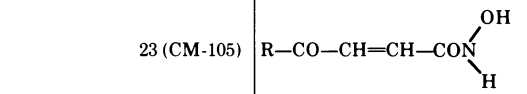
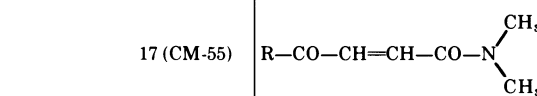
No.	Compound[a]	Melting or boiling point (C)	MIC (μg/ml)[b]							
			B.s.	S.a.	M.	E.c.	P.a.	C.a.	P.o.	T.i.
11 (CM-6K)	R—CH_2—CH_2—CH_2—CO—R′	$b_{1.5}$ 137–138	15.6	15.6	31.2	31.2	>100	62.5	31.2	
12 (CM-12)	R—CH_2—CH=CH—CO—R′	$b_{1.5}$ 125–126	31.2	31.2	7.8	62.5	>100	>100	15.6	
13 (CM-18)	R—CH_2—CH(O)CH—CO—R′		62.5	>100	31.2	>100	>100	>100	>100	
14 (CM-23)	R—CH_2—CH(OH)—CH(OH)—CO—R′		62.5	>100	62.5	>100	>100	>100	>100	
15 (CM-41)	R—CO—CH_2—CH_2—CO—R′	b_2 82–91	>100	>100	>100	>100	>100	>100	>100	>100[c]
16 (CM-36)	R—C(OCH_2—CH_2O)—CH_2—CH_2—CO—R′		>100	>100	>100	>100	>100	>100	100	100[c]
17 (CM-55)	R—CO—CH=CH—CO—R′	41–42	6.25	3.12	6.25	3.12	>100	12.5	6.25	6.25[c]
18 (CM-54)	R—C(OCH_2—CH_2O)—CH=CH—CO—R′		>100	100	50	>100	>100	>100	>100	50

[a] R, $-(CH_2)_7CH_3$; R′, $-N(CH_3)_2$

[b] Abbreviations are as in Table 1.

[c] *Trichophyton rubrum* was used.

TABLE 4. *Minimal inhibitory concentration (MIC) of 4-oxo-dodec-2-en-oic acid derivatives*

No.	Compound[a]	Melting or boiling point (C)	MIC (μg/ml)[b]							
			B.s.	S.a.	M.	E.c.	P.a.	C.a.	P.o.	T.i.[c]
19 (CM-50K)	R—CO—CH=CH—COOH	108-109	25	50	>100	50	>100	100	>100	50
20 (CM-45-3)	R—CO—CH=CH—CN		>100	50	50	25	>100	>100	25	6.25
21 (CM-62)	R—CO—CH=CH—$CONH_2$	138-139	50	50	25	12.5	>100	>100	25	12.5
22 (CM-61)		119-120	50	12.5	12.5	25	>100	>100	>100	<3.12
23 (CM-105)		150-151	>100	>100	>100	>100	>100	>100	100	25
17 (CM-55)		41-42	6.25	3.12	6.25	3.12	>100	12.5	6.25	6.25

[a] R, $-(CH_2)_7CH_3$.
[b] Abbreviation are as in Table 1.
[c] *Trichophyton rubrum* was used.

TABLE 5. *Minimal inhibitory concentration (MIC) of 4-oxo-dodec-2-enamide derivatives*

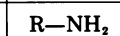
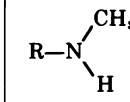
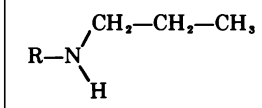
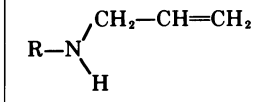
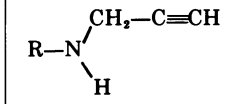
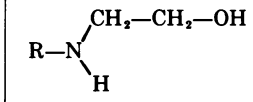
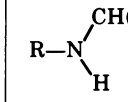
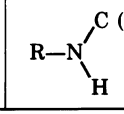
No.	Compound[a]	Melting point (C)	MIC (μg/ml)[b]							
			B.s.	S.a.	M.	E.c.	P.a.	C.a.	P.o.	T.i,
21 (CM-62)		138-139	50	50	25	12.5	>100	>100	25	12.5[c]
22 (CM-61)		119-120	50	12.5	12.5	25	>100	>100	>100	<3.12
24 (CM-114)		116	100	1.56	25	1.56	>100	>100		100[c]
25 (CM-107)		111.5-112	>100	3.12	50	3.12	>100	>100	>100	12.5[c]
26 (CM-119)		134-135	>100	50	>100	25	>100	>100	>100	>100[c]
27 (CM-108)		119-120	12.5	0.8	25	0.8	>100	>100	12.5	12.5[c]
28 (CM-109)		116-117	>100	0.8	6.25	100	>100	100	100	1.56[c]
29 (CM-110)		62	6.25	1.56	25	1.56	>100	>100	3.12	25[c]

TABLE 5—*Continued*

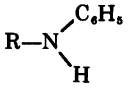
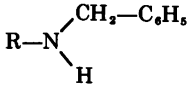
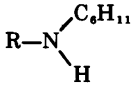
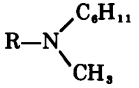
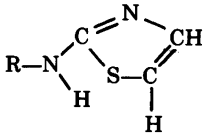
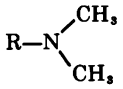
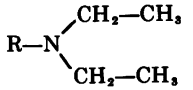
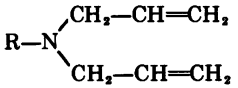
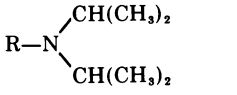
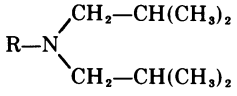
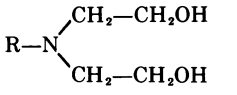
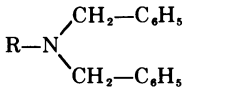
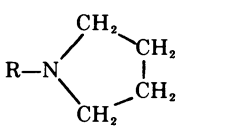
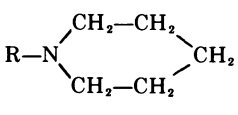
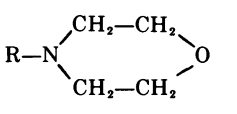
30 (CM-64)		224–225	>100	>100	>100	>100	>100	>100	>100	>100
31 (CM-106)		138–139	>100	>100	>100	>100	>100	>100	>100	>100
32 (CM-103)		130–133	>100	>100	>100	>100	>100	>100	>100	>100
33 (CM-100)			12.5	3.12	12.5	6.25	>100	>100	6.25	12.5[c]
34 (CM-117)		169–170	>100	>100	>100	>100	>100	>100	>100	>100[c]
17 (CM-55)		41–42	6.25	3.12	6.25	3.12	>100	12.5	6.25	6.25[c]
35 (CM-102)			12.5	3.12	12.5	3.12	>100	100	12.5	6.25[c]
36 (CM-101)			12.5	6.25	3.12	6.25	>100	>100	12.5	12.5[c]

TABLE 5—*Continued*

No.	Compound[a]	Melting point (C)	MIC (μg/ml)[b]							
			B.s.	S.a.	M.	E.c.	P.a.	C.a.	P.o.	T.i.
37 (CM-111)			>100	6.25	12.5	12.5	>100	>100	>100	>100[c]
38 (CM-104)			>100	>100	>100	>100	>100	>100	>100	>100[c]
39 (CM-113)			100	50	100	50	>100	>100		50[c]
40 (CM-112)			>100	>100	>100	>100	>100	>100	3.12	6.25[c]
41 (CM-115)			6.25	1.56	12.5	1.56	>100	25		1.56[c]
42 (CM-116)			12.5	3.12	25	1.56	>100	100		6.25[c]
43 (CM-63)		182–183	100	50	25	25	>100	>100	50	50[c]

[a] R, —CO—CH=CH—CO—$(CH_2)_7 \cdot CH_3$.

[b] Abbreviations are as in Table 1.

[c] *Trichophyton rubrum* was used.

TABLE 6. *Minimal inhibitory concentration (MIC) comparison of N,N-dimethyl-4-oxo-2-ene fatty acid amides*

No.	Compound[a]	Melting point (C)	MIC (μg/ml)[b]							
			B.s.	S.a.	M.	E.c.	P.a.	C.a.	P.o.	T.i.
44 (CM-121)	$CH_3(CH_2)_2R'$		>100	100	>100	25	>100	>100	3.12	25[c]
45 (CM-124)	$CH_3(CH_2)_3R'$		100	25	100	6.25	>100	>100	0.8	12.5[c]
46 (CM-122)	$CH_3(CH_2)_4R'$		50	50	100	12.5	>100	50	6.25	6.25[c]
47 (CM-126)	$CH_3(CH_2)_5R'$		50	25	100	12.5	>100	50	3.12	6.25[c]
48 (CM-129)	$CH_3(CH_2)_6R'$	31-32	25	6.25	6.25	6.25	>100	12.5	1.56	12.5
17 (CM-55)	$CH_3(CH_2)_7R'$	41-42	6.25	3.12	6.25	3.12	>100	12.5	6.25	6.25[c]
49 (CM-125)	$CH_3(CH_2)_{10}R'$		25	12.5	25	12.5	>100	25	3.12	6.25[c]

[a] R', $-CO-CH=CH-CO-N(CH_3)(CH_3)$

[b] Abbreviations are as in Table 1.

[c] *Trichophyton rubrum* was used.

their derivatives has long been known (2-7, 14), and their effect has been ascribed to a combination of concentration, pH of the medium, chain length of the fatty acid, and the presence or absence of serum (3). Chain length in particular has a dramatic effect on activity. In general, the activity increases with an increase in chain length, reaching a maximum at C-12 (4). This is in agreement with the results of our studies with the antibiotic cerulenin. Furthermore, the activity of unsaturated fatty acid derivatives is higher than that of the saturated ones, and the *cis* form is more active than the *trans* (4-6). It would be interesting, therefore, to examine the *cis* form of the compounds reported in this study. The effect of introduction of unsaturation between C5 and C11 as in cerulenin would also be interesting.

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