Antimicrobial Activity and Physical Characteristics of Some N,N-Disubstituted Decanamides

ARTHUR F. NOVAK and JAMES M. SOLAR, Louisiana State University, Baton Rouge, Louisiana 70803, and ROBERT R. MOD, FRANK C. MAGNE, and EVALD L. SKAU, Southern Regional Research Laboratory,[1] New Orleans, Louisiana 70119

Abstract

A number of N,N-disubstituted decanamides have been prepared, characterized and screened for their antimicrobial activity against a number of pathogenic organisms including bacteria both gram positive and negative, yeasts and molds. Several of these compounds exhibited a broad spectrum and high level of activity against all or most of the test organisms. The antimicrobial activity of these compounds has been shown to correlate with their dispersibility and surface tension in an aqueous systems.

Introduction

It is well known that many fatty acids and quaternary nitrogen compounds possess antimicrobial activity (1–17). Recently Novak et al., in a cooperative investigation (18,19), have shown that the morpholino derivative of certain fatty acids also exhibit a broad spectrum of antimicrobial activity against such varied organisms as bacteria, yeasts and molds. In view of these results, the screening program on antimicrobial activity was expanded to encompass a greater variety of N-substituted fatty acid amides. This paper reports on the antimicrobial activity of one such fatty acid amide group, the N-substituted and N,N-disubstituted decanamides.

Experimental Procedures

The decanoic acid used in these preparations was a purified acid obtained from a methyl ester which had been fractionally distilled in a 36 in. Heligrid-packed Podbielniak fractionating column. The decanoyl chloride was a distilled product prepared by reacting this acid with thionyl chloride. The amines used were commercial or research grade products available from research and industrial supply houses.

The substituted amides, with the exception of the two lauramide derivatives, were prepared by one of two methods; either by the interaction at reflux of a mixture of decanoic acid, 1.5 equivalents of amine and sufficient benzene to permit removal of the water of reaction by azeotropic distillation (20), or by the interaction of decanoyl chloride and an equivalent amount of amine in the presence of a slight excess of pyridine. The latter method was used in all preparations involving a volatile amine. The two substituted lauramides were prepared by a procedure involving ester aminolysis (21). Any residual acidity in the amide products was removed by percolation of a hexane solution of the respective amides through an activated alumina column. The acid-free amide was recovered from the percolate.

Densities were determined pycnometrically in a thermostated bath at 30 ± 0.1 C. The refractive indices were determined at 30 C with a precision Bausch and Lomb refractometer using the D sodium line. The melting points were determined on a Fisher-Johns apparatus. Surface tensions were determined by the ring method using a du Nouy tensiometer.

The micro-organisms used were obtained from stock cultures. Difco Bacto Dehydrated Nutrient Agar at pH 6.8, Difco Bacto Dehydrated Yeast Morphological Agar at pH 4.5, and Difco Dehydrated Mycological Agar at pH 7.0 were used to test the inhibition of the bacteria, yeast and mold cultures, respectively. Seeded agar plates were used to measure the antimicrobial activity against bacteria and yeasts. Suspensions of the test organisms were prepared by transferring a loop of spores in the case of spore formers and vegetative cells in the case of nonspore formers into sterile saline. Hardened agar plates were inoculated by placing three drops of the suspension

[1] So. Utiliz. Res. Dev. Div., ARS, USDA.

TABLE I

Elemental Analyses and Properties of Some N,N-Disubstituted Decanamides

	Surface tension[a]	Density 30 C	N^{30}_{L}	mp C[b]	% C		% H		% N	
					Exp.	Theory	Exp.	Theory	Exp.	Theory
N-Butyldecanamide				40.5–41.0	74.21	73.94	12.83	12.55	6.08	6.16
N-Isoamyldecanamide		0.8588	1.4496		74.31	74.56	12.78	12.94	5.39	5.80
N-Cyclohexyldecanamide				77.0–77.5	76.07	75.82	12.27	12.33	5.39	5.53
N,N-Bis(2-ethoxyethyl)decanamide		0.9093	1.4489		67.97	68.51	11.66	11.83	4.42	4.44
N,N-Bis(2-decanoyloxyethyl)decanamide		0.9315	1.4591		71.97	71.84	11.41	11.55	2.62	2.47
N,N-Bis(2-hydroxyethyl)lauramide	24.6	0.9790	1.4708		63.99	66.79	10.81	11.58	4.87	4.87
N,N-Bis(2-decanoyloxyethyl)lauramide		0.9342	1.4582	31.2–32.2	71.89	72.49	11.49	11.68	2.41	2.34
N-Decanoylmorpholine	29.6	0.9608	1.4694		68.45	69.60	11.11	11.27	5.56	5.40
N-Decanoyl-2,6-dimethylmorpholine	35.0	0.9331	1.4638		70.82	71.26	11.48	11.60	5.04	5.21
N-Decanoylpiperidine	37.1	0.9101	1.4688		74.47	75.18	11.85	12.21	5.63	5.85
N-Decanoyl-2-methylpiperidine		0.9085	1.4687		75.16	75.78	12.20	12.31	5.45	5.52
N-Decanoyl-3-methylpiperidine	40.4	0.9009	1.4664		75.01	75.78	11.93	12.31	5.39	5.52
N-Decanoyl-4-methylpiperidine		0.9003	1.4666		75.35	75.78	12.20	12.31	5.42	5.52
N-Decanoyl-4-ethylpiperidine	41.8	0.9013	1.4681		75.69	76.29	12.08	12.44	5.10	5.24
N-Decanoyl-2,6-dimethylpiperidine		0.9079	1.4694		75.95	76.29	12.38	12.44	5.14	5.24
N-Decanoyl-2-methyl-5-ethylpiperidine		0.8989	1.4673		76.49	76.81	12.59	12.55	4.98	4.98
N-Decanoyl-4-nonylpiperidine		0.8836	1.4693		78.69	78.78	12.90	12.96	3.76	3.83
N-Decanoyl-2-(5-nonyl)piperidine		0.8906	1.4697		78.59	78.78	12.70	12.96	3.76	3.83
N-Decanoyl-2-benzylpiperidine		0.9628	1.5072		80.14	80.19	10.71	10.72	4.15	4.26
N-Decanoyl-4-benzylpiperidine	47.7	0.9622	1.5094		80.37	80.19	10.78	10.72	4.20	4.26
N-Decanoylhexamethylenimine		0.9169	1.4726		75.63	75.78	12.43	12.33	5.31	5.53
N-Decanoyl-3-azabicyclo[3.2.2]nonane		0.9551	1.4886		72.83	77.33	10.95	11.81	4.89	5.02
N,N-Di-decanoylpiperazine	43.4			59–60	73.15	73.09	11.68	11.75	7.07	7.09
N-Decanoyl-N'-methylpiperazine	27.4	0.9308	1.4737		69.19	66.04	11.39	11.07	10.83	11.01
N-Decanoyl-1,2,3,4-tetrahydroquinoline		0.9805	1.5212		79.45	79.32	10.17	10.17	4.79	4.87

[a] Dynes/sq. cm at 25.5 C for a 0.1% aqueous dispersion of the compound.
[b] Uncorrected.

TABLE II

Antimicrobial Activity of Some N,N-Disubstituted Decanamides

Sample No.	Compound	Antimicrobial activity[a] Microorganisms[b]																								
		A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U	V	W	X	Y	Z
1	N-Butyldecanamide		+	−	×	+	×	++	××	+	++	+	××	××	+	+	××	××	××	××	+	×	+	××	+		
2	N-Isoamyldecanamide		××	−	×	+	××	++	++	++	++	+	+	××	+	+	+	++	++		++	+	××	++	+		
3	N-Cyclohexyldecanamide			−	×	××	××	××	××	−	××	××	++	××	×	+	×	−	−	×	−	×	×	×	−		
4	N,N-Bis(2-ethoxyethyl)decanamide			−						++									++			+	−	++	++	×	×
5	N,N-Bis(2-decanoyloxyethyl)decanamide		−	×	×	×	+	××	×	×	×	×	××	××	×	××	−	−	×	−	×	××	×	×	××	×		
6	N,N-Bis(2-hydroxyethyl)lauramide	+	++	++	++	+	++	++	++	++	+	++	++	++	++	++	++	++	+	++	+	+	++	++	++		
7	N,N-Bis(2-decanoyloxyethyl)lauramide	××	−	××	×	××	××	××	××	×	+	×	××	××	××	×	×	−	−	××	××	×	−	××	××		
8	N-Decanoylmorpholine	+	+	++	++	+	+	++	+	++	+	+	++	+	+	++	+	++	+	++	++	+	+	++	+		
9	N-Decanoyl-2,6-dimethylmorpholine	××	++	+	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++	++		
10	N-Decanoylpiperidine		×	×	++	+	++	++	++	++	++	+	+	××	+	+	+	++	+	++	+	+	+	++	+		
11	N-Decanoyl-2-methylpiperidine			−						+										+		+	+	++	+	+	−
12	N-Decanoyl-3-methylpiperidine				+			+		+	+									++							
13	N-Decanoyl-4-methylpiperidine				+			+		+	××									++							
14	N-Decanoyl-4-ethylpiperidine				××			+		++	××									+							
15	N-Decanoyl-2,6-dimethylpiperidine				×			×		××	+									××							
16	N-Decanoyl-2-methyl-5-ethylpiperidine				×			×		××	+									×							
17	N-Decanoyl-4-nonylpiperidine				×			−		−	×									−							
18	N-Decanoyl-2(5-nonyl)piperidine				−			×		−	−									−							
19	N-Decanoyl-2-benzylpiperidine			−						−										−		−	−	−	−	−	−
20	N-Decanoyl-4-benzylpiperidine			−						−										−		−	−	−	−	−	−
21	N-Decanoylhexamethylenimine	+	++	××	+	+	++	++	++	+	++	++	+	++	++	++	+	++	++	++	++	++	++	++	+		
22	N-Decanoyl-3-azabicyclo[3.2.2]nonane				××			++		××	++									++							
23	N,N-Didecanoylpiperazine	×	×	×	−	×	×	−	−	−	−	−	×	−	×	−	−	−	×	−	−	×	−	×	×		
24	N-Decanoyl-N′-methylpiperazine	+	+	+	++	+	++	++	++	++	+	++	++	++	+	++	++	++	+	++	++	++	+	++	++		
25	N-Decanoyl-1,2,3,4-tetrahydroquinoline	×	×	××	−	×	××	+	+	+	××	+	××	+	××	+	+	+	+	××	+	×	××	××	××		
	Decanoic acid	+	+	++	++	++	+	++	+	+	+	+	++	+	+	++	+	++	+	++	++	+	++	++	++		
	Sorbic acid	++	++	++	++	++	++	++	+	++	++	++	++	++	++	++	+	++	+	++	++	++	++	++	++		
	Morpholine			+	++	+	+	++	+	++	++	+	++	++	+	++	++	++	++	++	++	+	++	++	++		
	Piperidine			++	++	++	+	++	++	+	++	+	+	++	+	++	+	++	++	+	++	+	++	++	++		
	Piperazine hexahydrate			−	++	+	+	++	+	+	××	××	+	+	××	+	+	+	++	+	+	××	+	++	××		

[a] ++ = The zone of inhibition was at least 0.5 cm beyond disc or cylinder area at 120 hrs.
+ = The zone of inhibition was less than 0.5 cm beyond disc or cylinder area at 120 hrs.
×× = Organism failed to grow on the disc or cylinder area at 120 hrs.
× = Slight growth on the saturated disc or cylinder area at 120 hrs.
− = No inhibition detectable.

[b] A = *Escherichia coli*
B = *Micrococcus pyogenes*
C = *Candida albicans*
D = *Candida wernecki*
E = *Epidermophyton floccosum*
F = *Keratinomyces ajelloi*
G = *Microsporum canis*
H = *Microsporum cookii*
I = *Microsporum gypseum*
J = *Microsporum hanum*
K = *Trichophyton concentricum*
L = *Trichophyton epilans*
M = *Trichophyton equinum*
N = *Trichophyton ferrugineum*
O = *Trichophyton gallinae*
P = *Trichophyton megnini*
Q = *Trichophyton mentagrophytes* var. *interdigitales*
R = *Trichophyton mentagrophytes* var. *granulare*
S = *Trichophyton rubrum*
T = *Trichophyton sabouraudi*
U = *Trichophyton schoenleini*
V = *Trichophyton sulfurium*
W = *Trichophyton tonsurans*
X = *Trichophyton violaceum*
Y = *Cladosporium wernecki*
Z = *Aspergillus flavus*

onto the agar. The micro-organisms were spread over the surface of the plates with sterile glass rods. These plates were employed in the activity estimation against microbial growth. Filter paper discs 6.5 mm in diameter, made from Whatman Number 1 filter paper were used to evaluate the liquid compounds and stainless steel cylinders 5 mm I.D. were used in the case of the solid compounds, Samples 1, 3, 7 and 23 of Table II. The paper discs wetted until they were completely saturated with the test compound or stainless steel cylinders containing the test solid compound were placed on the surface of the agar plates inoculated with the test organisms. To eliminate any errors which could result from an insufficient number of tests, a minimum of three experiments, at different times, employing duplicate plates were made for each compound under test. All plates were incubated at the optimum growing temperature for each organism and readings were taken after 24, 48, 72 and 120 hr periods. The zones of inhibition were compared to those for the controls.

Results and Discussion

The densities, refractive indices and elemental analyses of the various decanoyl derivatives are reported in Table I as are the melting point ranges of the solid derivatives and the surface tension at 25.5 C of 0.1% aqueous dispersions of some selected derivatives.

The antimicrobial activity of these decanoyl derivatives against several species of bacteria, yeasts and molds, under optimum growth conditions of the respective organisms for 120 hr, is reported in Table II. Six of the derivatives, Samples 6, 8, 9, 10, 21 and 24, exhibit a broad spectrum of activity with a sustained high degree of inhibition, giving ++ and + ratings, against all but a few of the test organisms.

The N-decanoyl-2- and -3-methyl piperidines, Samples 11 and 12, although tested on fewer organisms do exhibit an activity pattern which suggests that they may also be broad spectrum antimicrobials. The other decanamides show either a lower degree of activity, a narrower spectrum, or no activity at all. However, even these less active compounds cannot be completely discounted as antimicrobial agents for they could manifest, under less favorable growing conditions for the organisms, a higher level of activity. It is also possible that they could be active against other micro-organisms than those used in the tests.

Whatever structure-activity correlation exists in these derivatives appears to be overshadowed or masked by the effect of dispersibility of the compound in an aqueous medium or of the surfactant characteristics as reflected by the surface tension of an aqueous disperson. That these are real factors becomes apparent when one compares the following pairs of closely related compounds: (a) N,N′-didecanoylpiperazine with N-decanoyl-N′-methylpiperazine, Samples 23 and 24, and (b) N,N-bis(2-decanoyloxyethyl)decanamide with N,N-bis(2-hydroxyethyl) lauramide, Samples 5 and 6. In both pairs, the first named compounds, which are at best poorly dispersible, have little or no antimicrobial activity whereas the last mentioned compounds in both pairs, which are quite dispersible, have a very high and broad activity spectrum. These deductions are substantiated by the surface tensions reported in Table I obtained on 0.1% aqueous dispersions of the compounds in question. The most surface active compounds, those giving the lowest surface tensions, are the most active antimicrobially.

The somewhat better performance of the N-decanoyl morpholine and N-decanoyl-2,6-dimethylmorpholine, Samples 8 and 9, over that of the N-decanoylpiperidine, Sample 10, must also be due, at least in part, to the more hydrophilic and better surfactant character of the morpholides. This effect is also readily demonstrated in the piperidine series where both moieties are individually quite active antimycotics. Here we observe that as we increase the hydrophobicity and decrease the surfactant characteristics, by increasing the number and complexity of the alkyl substituents on the piperidine ring, we decrease the antimycotic activity of the decanoyl derivative.

The broad antimicrobial spectra of several of the N-substituted decanomides suggest potential utility in biostatic products. In view of the established compatibility of these derivatives with polyvinyl chloride (22,23) these decanoyl derivatives may have utility as biostatic additives for plastic compositions.

REFERENCES

1. Bell, T. A., J. L. Etchells and A. F. Borg, J. Bacteriol. *77*, 573–580 (1959).
2. Blum, M. S., A. F. Novak and S. Taber, III, Science *130*, 452–453 (1959).
3. Cowles, P. B., Yale J. Biol. Med. *13*, 571–578 (1941).
4. Garmaise, D. L., R. W. Kay, G. Y. Paris, R. J. Rana, R. Gaudry, H. A. Baker and F. F. McKay, J. Org. Chem. *26*, 857–860 (1961).
5. Hoffman, C., T. R. Schweitzer and G. Dalby, Food Res. *4*, 539–545 (1939).
6. Keeney, E. L., L. Ajello and E. Lankford, Bull. Johns Hopkins Hosp. *75*, 377–392 (1944).
7. Kiesel, A., Ann. Inst. Pasteur *27*, 391–420 (1913).
8. Kodicek, E., Proc. Intern. Conf., Biochem. Probl. Lipids *2*, 401–406 (1955, published 1956).
9. Melnick, D., F. H. Luckmann and C. M. Gooding, Food Res. *19*, 44–58 (1954).
10. Mod, R. R., E. L. Skau, S. P. Fore, F. C. Magne, A. F. Novak, H. P. Dupuy, J. R. Ortego and M. J. Fisher. U.S. Patent 3,285,812.
11. Repetto, O. M., Anales Assoc. Quim. Arg. *47*, 326–333 (1959).
12. Sokoloff, B., M. Toyomizu, W. Trauner and G. Renninger, JAOCS *36*, 234–237 (1959).
13. Spoehr, H. A., J. H. C. Smith, H. H. Strain, H. W. Milner and G. J. Hardin, Carnegie Inst. Wash. Pub. No. 586, 67 p. (1949).
14. Tetsumoto, S., J. Agr. Chem. (Japan) *9*, 388–397 (1933); Bull. Agr. Chem. Soc. (Japan) *9*, 8–19 (1933).
15. Tetsumoto, S., J. Agr. Chem. Soc. (Japan) *9*, 563–567 (1933); Bull. Agr. Chem. Soc. (Japan) *9*, 82–86 (1933).
16. Tetsumoto, S., J. Agr. Chem. Soc. (Japan) *9*, 761–767 (1933).
17. Hueck, H. J., Dorothea M. Adema and J. R. Siegman, Appl. Microbiol. *14*, 308–319 (1966).
18. Novak, A. F., Gladys C. Clark and H. P. Dupuy, JAOCS *38*, 321–324 (1961).
19. Novak, A. F., Mary J. Fisher, Sara P. Fore and H. P. Dupuy, Ibid. *41*, 503–505 (1964).
20. Magne, F. C., R. R. Mod and E. L. Skau, Ibid. *38*, 291–293 (1961).
21. Magne, F. C., R. R. Mod and E. L. Skau, Ibid. *40*, 541–545 (1963).
22. Skau, E. L., R. R. Mod and F. C. Magne, U.S. Patents 3,219,612 and 3,309,332.
23. Mod, R. R., F. C. Magne and E. L. Skau, U.S. Patent 3,309,333.

[Received August 13, 1968]