Antimycotic Activity of Some Fatty Acid Derivatives

A. F. NOVAK and MARY J. FISHER, Louisiana State University, Baton Rouge, Louisiana, and
SARA P. FORE and H. P. DUPUY, Southern Regional Research Laboratory,[1] New Orleans, Louisiana

Abstract

Petroselinic and ricinoleic acids and a number of their derivatives, together with stearic acid and two oleic acid derivatives which were included for the purpose of comparison, have been screened for antimycotic activity against 22 pathogenic yeasts and molds. A number of the compounds exhibited a wide spectrum of activity, testing either fair or good against all or most of the yeasts and molds, and almost all were fairly active against at least one of the organisms.

Introduction

A LARGE NUMBER of fatty acids and fatty acid derivatives have been shown to exhibit varying degrees of antimicrobial behavior (1–3,8–9,11–13,16, 18,21–26). In a recent screening of a number of fatty acids and some of their derivatives for antimicrobial activity against a group of 12 organisms, which included bacteria, yeasts and molds, ricinoleic acid and several of its derivatives, and petroselinic acid were found to exhibit a considerable degree of activity against one or more of the organisms (18).

In view of the broad antimicrobial spectrum of some of these fatty acid derivatives, this exploratory research project was extended to include additional fatty acid derivatives as well as 22 other species of pathogenic yeasts and molds. The primary objective of such antimicrobial screening tests was to reveal possible pharmaceutical or industrial potentiality of some of these fatty acid derivatives.

In addition, experiments now in progress indicate that some of the compounds are also effective against a number of gram-positive and gram-negative bacteria.

Experimental

Fatty Acid Derivatives. With the exception of those described below, the fatty acid derivatives were prepared for other purposes and have been reported elsewhere (4–7,10,14,19) or were commerical products. 4-(6-Hydroxycaproyl)morpholine (b. 149–151C/2 μ) was prepared from caprolactone and morpholine by the procedure of Dupuy et al. (4). After recrystallization from ethyl acetate at −43C it had a nitrogen content of 6.88% (theory, 6.96%). 4-Petroselinoylmorpholine (nitrogen content, 3.94%; theory, 3.98%), morpholides of essential-oil-free parsley seed fatty acids (nitrogen content, 3.43), and 4-[6(7)-hydroxystearoyl]morpholine (nitrogen content, 3.75%; theory, 3.79%) were prepared from morpholine and the appropriate fatty acids essentially by the amination procedure described by Magne, et al. (15). The former two were purified by chromatography on an activated alumina column employing hexane and a 1:1 hexane-ethanol mixture as solvents, and the latter by washing with dilute alkali followed by crystallization from petroleum ether. A more detailed description of these preparations will be published separately (17).

Test Procedure. The agar plates used for all tests were prepared from Difco Bacto Mycological Agar at pH 7.0. The molds were either streaked onto, or their aqueous suspensions poured onto the hardened agar plates. The filter paper disc method was used for evaluating the liquid samples. Standard-sized paper assay discs saturated with the liquid samples were placed on the surface of the inoculated plates. The solids were introduced directly onto the surface of inoculated plates and tested in the pure form. The area of solid material introduced was comparable to the standard-sized paper assay discs. A min of three experiments employing duplicate plates was used for measuring the antimycotic activity of each compound. Each test plate was incubated at the optimum growing temperature for the organism with which it had been inoculated. The plates were checked after three, five and seven days of incubation, and readings made when max growth had been attained. These show in Table I.

Results and Discussion

In regard to the data in Table I, it should be pointed out that the test which has been applied is for screening purposes only. No attempt has been made to make the test quantitative, and many of the factors which may affect the performance of the compounds under conditions of actual use have not been evaluated. In examining this data is should also be borne in mind that compounds rated ○○ (organism failed to grow on saturated disc or solid) are not necessarily inferior to those rated + (the zone of inhibition was less than one-half cm) or ++ (the zone of inhibition was greater than one-half cm) as the failure to inhibit the growth of an organism beyond the point of actual application to the plate may result from inability to diffuse through the culture medium rather than from low antimycotic activity.

As can be seen from inspection of the data in Table I, a number of the compounds screened in this study show promise as agents against pathogenic yeasts and molds. Of these compounds, 10 showed activity against all 22 molds, and two of these, ethyl 6-hydroxycaproate and 4-ricinoleoylmorpholine, exhibited either fair or good activity against all organisms and hence were, on the whole, comparable to the controls, undecenoic and sorbic acids. With the exception of stearic acid, which was included for comparative purposes, and 6(7)-hydroxystearic acid, all of the compounds screened were fairly active against at least one of the molds, and 10 of them rated either fair or good against 15 or more of the organisms. It is also of interest to note that two of the compounds, ricinoleic and petroselinic acids, which showed varying degrees of activity against all of the other organisms, actually accelerated the growth of *Candida albicans* and that with the exception of one of the standards, sorbic acid, only the lactam of 6-aminocaproic acid was rated good against this mold.

It is impossible to draw any definite conclusions as to the effect of specific functional groups since only a limited number of compounds were screened and there are different factors involved in these screening tests, such as pH changes, solubility, absorption, metabolic degradation, and various combinations of these variables. For example, the esterification of 6-hydroxycaproic acid with ethyl alcohol, the amida-

[1] A laboratory of the So. Utiliz. Res. and Dev. Div., ARS, USDA.

tion with morpholine, or lactonization did not appear to affect the broad antimicrobial spectrum. On the other hand, amidation of 6(7)-hydroxystearic acid, 12-hydroxystearic acid, and ricinoleic acid with morpholine increased antimicrobial activity against some microorganisms, whereas amidation of petroselinic acid with morpholine and the esterification of petroselinic acid and ricinoleic acid with methyl alcohol decreased antimicrobial activity against some microorganisms. An interesting observation, however, is that both petroselinic acid and ricinoleic acid appear to enhance the growth of *Candida albicans*, whereas the morpholides of petroselinic acid and ricinoleic acid inhibit its growth.

In view of the conflicting results as well as the broad antimycotic spectrum of some of these com-

TABLE I

Antimycotic Activity of Some Fatty Acid Derivatives

Compound	Formula[c]	Antimycotic 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
6-Hydroxycaproic acid	$HOCH_2(CH_2)_4COOH$	+	++	+	oo	++	+	++	+	+	++	oo	+	++	+	++	oo	++	+	+	++	++	+
4-(6-Hydroxycaproyl)morpholine	$HOCH_2(CH_2)_4COR$	o	o	oo	+	++	oo	++	+	++	+	+	+	++	+	++	+	++	+	+	+	++	+
Lactone of 6-Hydroxycaproic acid	$\underbrace{OCH_2(CH_2)_4C}O$	oo	++	oo	oo	++	+	++	+	+	++	+	+	++	+	++	+	++	+	+	++	+	+
Ethyl 6-hydroxycaproate	$HOCH_2(CH_2)_4COOCH_2CH_3$	+	++	++	++	++	++	++	++	+	+	++	+	+	+	++	++	++	+	+	+	++	+
Sodium 6-hydroxycaproate	$HOCH_2(CH_2)_4COONa$	+	++	+	+	++	+	oo	+	+	oo	oo	+	+	+	-	oo	oo	+	+	+	+	oo
6-Aminocaproic acid	$NH_2CH_2(CH_2)_4COOH$	-	-	oo	o	oo	o	-	o	o	oo	o	o	oo	oo	o	o	++	oo	o	-	oo	+
Lactam of 6-aminocaproic acid	$\underbrace{NHCH_2(CH_2)_4C}O$	++	oo	oo	+	++	o	+	oo	oo	+	oo	+	oo	o	oo	oo	oo	oo	oo	o	oo	+
Stearic acid	$CH_3(CH_2)_{16}COOH$	-	-	-	oo	oo	-	o	o	-	oo	oo	oo	oo	-	-	-	-	-	o	oo	o	o
6(7)-Hydroxystearic acid	$R'CH(OH)CH(CH_2)_4COOH$	o	o	oo	o	oo	o	oo	oo	o	oo	-	o	oo	-	-	o	oo	o	o	o	-	-
4-(6(7)-Hydroxystearoyl)morpholine	$R'CH(OH)CH(CH_2)_4COR$	o	o	+	oo	+	+	o	+	+	oo	+	+	+	++	++	+	++	+	oo	+	+	+
6,7-Epoxystearic acid	$R'\underbrace{CH-CH}O(CH_2)_4COOH$	-	-	o	-	oo	oo	oo	-	-	oo	oo	-	-	oo	+	oo	o	oo	o	o	+	o
12-Hydroxystearic acid	$R''CH(OH)(CH_2)_{10}COOH$	-	oo	oo	o	oo	oo	-	oo	o	oo	oo	o	oo	o	+	oo	oo	oo	oo	-	-	o
4-(12-Hydroxystearoyl)morpholine	$R''CH(OH)(CH_2)_{10}COR$	-	+	+	+	+	+	+	+	oo	++	+	oo	++	+	-	oo	oo	+	+	++	-	oo
Methyl 12-hydroxystearate	$R''CH(OH)(CH_2)_{10}COOCH_3$	-	oo	-	-	oo	o	o	-	-	oo	o	oo	oo	-	-	-	oo	-	-	oo	-	-
Ricinoleic acid	$R''CH(OH)CH_2CH{=}CH(CH_2)_7COOH$	gf	+	+	+	+	+	o	+	+	++	+	+	++	+	+	+	++	+	+	+	++	+
4-Ricinoleoylmorpholine	$R''CH(OH)CH_2CH{=}CH(CH_2)_7COR$	+	++	++	++	++	+	+	++	++	++	+	++	++	+	+	++	++	++	+	++	++	+
Methyl ricinoleate	$R''CH(OH)CH_2CH{=}CH(CH_2)_7COOCH_3$	o	-	o	+	oo	oo	oo	-	oo	-	oo	o	o	oo	+	+	-	oo	o	-	oo	o
4-(12-Propionoxyoleoyl)morpholine	$R''CH(OOCCH_2CH_3)CH_2CH{=}CH(CH_2)_7COR$	+	oo	oo	oo	++	+	oo	oo	oo	oo	oo	+	oo	oo	+	oo	oo	+	oo	oo	++	o
Petroselinic acid	$R'CH{=}CH(CH_2)_4COOH$	gf	++	+	oo	+	+	+	+	oo	++	oo	+	++	+	+	oo	oo	+	oo	++	+	+
4-Petroselinoylmorpholine	$R'CH{=}CH(CH_2)_4COR$	oo	o	oo	+	oo	o	oo	o	o	+	oo	o	oo	oo	oo	oo	oo	+	++	+	oo	oo
Methyl petroselinate	$R'CH{=}CH(CH_2)_4COOCH_3$	-	oo	-	+	+	oo	+	o	o	+	oo	+	+	+	oo	oo	o	+	++	+	-	o
Petroselinylamine hydrochloride	$R'CH{=}CH(CH_2)_5NH_2{\cdot}HCl$	+	+	+	+	++	+	oo	+	+	+	+	+	+	+	++	+	+	oo	oo	++	+	+
Oleylamine hydrochloride	$CH_3(CH_2)_7CH{=}CH(CH_2)_8NH_2{\cdot}HCl$	+	oo	oo	+	++	++	++	++	+	+	oo	+	+	oo	++	+	oo	+	o	+	+	oo
N-Acetylpetroselinylamine	$R'CH{=}CH(CH_2)_5NHCOCH_3$	oo	oo	oo	oo	+	+	oo	+	oo	+	-	o	oo	o	oo	oo	+	+	o	o	oo	o
N-Acetyloleylamine	$CH_3(CH_2)_7CH{=}CH(CH_2)_8NHCOCH_3$	-	oo	o	+	+	oo	++	o	+	oo	o	+	oo	o	++	+	o	oo	+	-	++	o
Parsley seed acids		o	o	o	o	o	o	oo	-	-	-	-	o	o	-	o	o	-	+	-	+	-	-
Morpholides of parsley seed acids		oo	-	+	+	+	oo	oo	+	oo	+	+	oo	oo	+	+	+	++	oo	+	++	+	++
10-Undecenoic acid	$CH{=}CH(CH_2)_8COOH$	+	++	+	+	++	+	++	+	+	++	+	+	++	+	++	+	++	+	+	+	++	+
Sorbic acid	$CH_3CH{=}CH{-}CH{=}CHCOOH$	++	++	++	++	++	+	++	++	++	++	++	++	++	+	++	++	++	++	++	++	++	++
Morpholine	$HN\underbrace{CH_2CH_2}_{CH_2CH_2}O$	+	++	+	+	++	+	++	++	+	++	++	+	++	++	++	++	++	++	+	++	++	++

a ++ = the zone of inhibition was at least one-half centimeter
+ = the zone of inhibition was less than one-half centimeter
oo = organism failed to grow on saturated disc or solid
o = slight growth of organism on saturated disc or solid
- = no inhibition detectable
gf = promoted growth of organism

b
A = Candida albicans
B = Candida wernecki
C = Epidermophyton floccosum
D = Keratinomyces ajelloi
E = Microsporum canis
F = Microsporum cookii
G = Microsporum gypseum
H = Microsporum nanum
I = Trichophyton concentricum
J = Trichophyton epilans
K = Trichophyton equinum
L = Trichophyton ferrugineum
M = Trichophyton gallinae
N = Trichophyton megnini
O = Trichophyton mentagrophytes var. interdigitales
P = Trichophyton mentagrophytes var. granulare
Q = Trichophyton rubrum
R = Trichophyton Sabouraudi
S = Trichophyton schoenleini
T = Trichophyton sulfurium
U = Trichophyton tonsurans
V = Trichophyton violaceum

c $R = N\underbrace{CH_2CH_2}_{CH_2CH_2}O$, $R' = CH_3(CH_2)_{10}$, $R'' = CH_3(CH_2)_5$

pounds, a more thorough investigation is warranted. It would be of interest to assay the antimicrobial activity of these compounds quantitatively, and also assay the antimicrobial activity of them in commercial products, such as medicinal products, paint films, plastics, and other polymeric materials. The medicinal applications of some of these compounds might prove to be very important. Some of these compounds could serve as plasticizers as well as antimicrobial agents.

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. and Med. *13*, 571–578 (1941).
4. Dupuy, H. P., R. T. O'Connor and L. A. Goldblatt, JAOCS *39*, 411–414 (1962).
5. Fore, S. P., and W. G. Bickford, J. Org. Chem. *26*, 2104–2105 (1961).
6. Fore, S. P., R. L. Holmes and W. G. Bickford, JAOCS *37*, 490–491 (1960).
7. Fore, S. P., T. L. Ward and F. G. Dollear, *Ibid. 40*, 30–33 (1963).
8. 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).
9. Hoffman, C., T. R. Schweitzer and G. Dalby, Food Res. *4*, 539–545 (1939).
10. Holmes, R. L., J. P. Moreau and R. T. O'Connor, JAOCS *39*, 411–414 (1962).
11. Keeney, E. L., L. Ajéllo and E. Lankford, Bull. Johns Hopkins Hosp. *75*, 377–392 (1944).
12. Kiesel, A., Ann. Inst. Pasteur *27*, 391–420 (1913).
13. Kodicek, E., Proc. Intern. Conf., Biochem. Problems Lipids *2*, 401–406 (1955, published 1956).
14. McCutchon, M. A., R. T. O'Connor, E. F. Dupré, L. A. Goldblatt and W. G. Bickford, JAOCS *36*, 115–118 (1959).
15. Magne, F. C., R. R. Mod and E. L. Skau, Ind. Eng. Chem. *50*, 617–618 (1958).
16. Melnick, D., F. H. Luckmann and C. M. Gooding, Food Res. *19*, 44–58 (1954).
17. Mod, R. R., E. L. Skau, S. P. Fore, F. C. Magne, A. F. Novak, H. P. Dupuy, J. R. Orgeto, M. J. Fisher. (Patent Applied For).
18. Novak, A. F., G. C. Clark and H. P. Dupuy, JAOCS *38*, 321–324 (1961).
19. Placek, L. L., and F. G. Dollear, JAOCS *39*, 347–350 (1962).
20. Nathanson, R. B., J. Invest. Dermatol. *35*, 261–263 (1960).
21. Repetto, O. M., Anales asoc. quim. arg. *47*, 326–333 (1959).
22. Sokoloff, B., M. Toyomizu, W. Trauner and G. Renninger, JAOCS *36*, 234–237 (1959).
23. Spoehr, H. A., J. H. C. Smith, H. H. Strain, H. W. Milner and G. J. Hardin, Carnegie Inst. Wash. Pub. No. 586, 67 pp. (1949).
24. Tetsumoto, S., J. Arg. Chem. (Japan) *9*, 388–397 (1933). Bull. Agr. Chem. Soc. (Japan) *9*, 8–19 (1933).
25. Tetsumoto, S., J. Agr. Chem. Soc. (Japan) *9*, 563–567 (1933). Bull. Agr. Chem. Soc. (Japan) *9*, 82–86 (1933).
26. Tetsumoto, S., J. Agr. Chem. Soc. (Japan) *9*, 761–767 (1933).
27. Wyss, O. B., J. Ludwig and R. R. Joiner, Arc. Biochem. *7*, 415–425 (1945).

[Received December 19, 1963—Accepted February 3, 1964]

Studies of the Oxygen Bomb Method for Determining Shortening Stabilities

J. E. BENNETT, Dole Corporation, San Jose, Calif., and M. J. BYER, The Pillsbury Company, Springfield, Ill.

Abstract

The Oxygen Bomb Method as evolved by the studies presented in this paper gives production and quality control laboratories a procedure by which to measure a fifty-hour shortening in one hour and forty-five minutes. The OBM is twice as precise as in the Active Oxygen Method as shown by the comparison of their two sigma error.

Introduction

THE SEARCH for a faster and more accurate test is a never-ending quest. The measurement of the potential stability of edible shortenings is certainly one of the oil industry's slowest and one whose validity is most questioned. This paper describes a faster and more precise procedure by which to measure stability.

A 50-hr shortening by the 97.8C official AOCS Active Oxygen Method (AOM) (1) may be analyzed in 20 hr by the 110C AOM.

Work has been reported using an Oxygen Bomb Method (OBM) (2–6) to reduce the testing time. One of these reports decreased the analysis time for a 50-hr shortening to two hr and 45 min (4).

This paper describes the work accomplished with an interesting apparatus modification which eliminates the limitations of the oil bath, and cuts the analysis time of a 50-hr shortening to one hr and 45 min. These statistical studies show experimental error effects of temp, pressure, day-to-day variation and sample wt. One study examines the correlation between the OBM and the AOM procedures and compares the standard deviation of both methods.

Dispersants have been used to decrease the testing time and improve the OBM end point determination (3,5,6). For the sake of simplicity we did not use a dispersant. End point determinations were not a problem with our test parameters. Two reports were published (5,6) after the analytical work for this paper had been completed. Pohle et al. (6) used a catalyst to decrease testing time.

Experimental Methods

Apparatus: An 8 in. x 6 in. x 24 in. aluminum block was drilled to accommodate two machined ASTM gasoline gum stability bombs, two thermometers and a thermistor. Two 250-w and one 500-w General Electric strip heaters 24 in. long were screwed into machined grooves on the sides of the alumimum block and connected to a Sargent Model S Thermonitor. The temp of the block and bombs are maintained at 135 ± 0.1C by means of the temp controller with its thermistor being installed into a drilled hole. Three inches of 85% magnesia insulation on the top, sides and bottom of the block aid in maintaining temp. The bombs are fitted with air tire stems to allow a regular tire chuck to be used to fill the bombs with oxygen. The bombs are connected to a Bristol recorder by brass connecting tubes. A rupture disc assembly prevents an undue pressure from harming the recorder.

Procedure. The following procedure is one which evolved from the studies included in this paper.

1) A 10-g sample of shortening is weighed into a glass bomb liner, covered with a glass lid and placed into a heated bomb.

2) The bomb is closed and purged ten times to create a relatively pure oxygen atmosphere. A final pressure of 110 psi oxygen is introduced into the bomb.

3) The filled bomb is placed into the 135 ± 0.1C aluminum block and the recorder started.

The stability time is arbitrarily that time that begins at the insertion of the bomb into the block until