TABLE III

Molar Calibration Factors Calculated for Simple Monoacid Triglycerides on a 1.83 m x 2.5 mm I.D. Column Containing 2% OV-17 on Supelcoport 100/120 mesh.

No. of acyl carbon atoms	Molar calibration factor
36	1.00
42	0.88
48	0.85
54	0.93

lower sample weights can be used and the method may lend itself to the analysis of lipids from body fluids.

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Preparation of Some Fatty Glycolic Acid Derivatives and Screening for Antimicrobial Activity

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ABSTRACT

Nine glycolic acid derivatives were prepared and screened as primary plasticizers for polyvinyl chloride (PVC) and for antimicrobial activity. Two types of compounds were represented: (a) those with a methyl or ethyl ester grouping at the carboxyl function of glycolic acid and a fatty acyl group at the hydroxyl function; and (b) those with three acyl groups attached at the hydroxyl functions of the amide, resulting from the reaction of glycolic acid and diethanolamine. None of the compounds was compatible with PVC at the 35% level of incorporation, and the compounds were not further evaluated as plasticizers. They were screened for antimicrobial activity against a gram-positive bacterium, *Staphylococcus aureus;* a gram-negative bacterium, *Escherichia coli;* a yeast, *Candida utilis;* and a mold, *Penicillium* sp. Six of the compounds inhibited two or more of the test organisms suggesting some of these materials merit further testing as biostatic agents.

INTRODUCTION

During an investigation of potentially useful derivatives of vegetable oils, a number of new fatty derivatives of glycolic acid were prepared for evaluation as plasticizers. They failed as primary plasticizers for polyvinyl chloride (PVC) at the usual (35%) level of incorporation. When evaluated according to the procedure described (1), the compounds either did not mill with the PVC or showed exudation within a short time after being incorporated into the polymer. The compounds were not further investigated as plasticizers; however, they were screened for antimicrobial activity. Other research has shown that many fatty compounds have antimicrobial activity (2-9). This paper is a report of the preparation of the compounds and the screening results.

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EXPERIMENTAL PROCEDURES

Glycolic acid, diethanolamine and the acid chlorides used were commercial products. Intermediates and final products were characterized by infrared (IR) and nuclear magnetic resonance (NMR) spectral analyses (10). Densities of the liquids were determined pycnometrically in a bath thermostatically controlled to ± 0.1 C. Melting points of the solids are uncorrected and were determined by immersing the bulb of a thermometer directly in the partially melted material while it was maintained in a water bath at a temperature slightly above the melting point. Refractive indices were determined at 30 C with a precision Bausch and Lomb refractometer, with the D sodium line. Isolated yields were 90% or more of the theoretical.

Preparations

Methyl and ethyl glycolate. Ca. 500 g of glycolic acid (70% in water) was dried by azeotropic distillation of the water from a benzene solution with a Dean-Stark trap. The methyl and ethyl esters were each prepared in situ by refluxing with methyl or ethyl alcohol for 72 hr. The purified esters were obtained by fractional distillation.

Carbomethoxymethyl palmitate. Palmitoyl chloride (55 g, 0.2 mol) was added to a stirred solution of methyl glycolate (18 g, 0.2 mol) in pyridine (20 ml). The precipitated pyridine hydrochloride was filtered and washed with benzene; the resulting benzene solution was water-washed, dried over sodium sulfate and the solvent stripped off with a rotary evaporator, leaving a quantitative yield of product.

Carbomethoxymethyl oleate, carboethoxymethyl oleate, carboethoxymethyl hydrocinnamate, and *bis*(carbomethoxymethyl) adipate were prepared by the same procedure used for the palmitate, but the appropriate acid chloride and glycolate were substituted.

N,N-bis(Hydroxyethyl)hydroxyacetamide. A solution of freshly prepared sodium methoxide in methanol (3.6 g sodium in 50 ml methanol) was added to diethanolamine (105 g, 1 mol) and the methanol was removed by vacuum distillation. Methyl glycolate (90 g, 1 mol) was then added dropwise while the stirred mixture was maintained at 60 C and 60 mm pressure (11). The methanol that formed during the reaction was collected in a dry-ice trap. The reaction product was neutralized with dilute HCl and the water was removed by azeotropic distillation with benzene. The reaction product was stripped at high vacuum.

N,N-bis(Pelargonoyloxyethyl)pelargonoyloxyacetamide. Pelargonoyl chloride (106 g, 0.6 mol) was added dropwise with stirring to a solution of N,N-*bis*(hydroxyethyl)hydroxyacetamide (32.6 g, 0.2 mol) in pyridine (60 ml). The precipitated pyridine hydrochloride was filtered and washed with benzene. The resulting benzene solution was water-washed, dried over sodium sulfate and passed through an activated alumina column to remove any acid. The benzene was stripped off on a rotary evaporator. The yield of product was essentially quantitative.

The analogous lauroyl, oleoyl and trimethylacetyl derivatives were prepared in a similar manner to that described for the pelargonoyl derivative.

Antimicrobial Testing

The purpose of the simple screening technique was to obtain general information on whether the compounds tested have antimicrobial properties that might be useful in commercial products. Results reported in this paper are merely an indication that these compounds possess antimicrobial properties and merit further testing.

Difco Bacto dehydrated nutrient agar at pH 6.8, Difco Bacto dehydrated yeast mycological agar at pH 4.5 and Difco dehydrated mycological agar at pH 7.0 were used to test inhibition of the bacteria, yeast and mold cultures, respectively. The microorganisms were from stock cultures: *Staphylococcus aureus,* ATCC 12692; *Escherichia coli,* ATCC 25922; *Candida utilis,* ATCC 2048; and *Penicillium* sp. The *Penicillium* is a stock culture of the Louisiana State University Food Science Department and was isolated from contaminated food.

After the stock cultures were incubated for 48 hr at room temperature, suspensions of the microorganisms were prepared. One loopful (3.2 mm loop) of spores, or of vegetative cells of nonsporeformers, was removed from the cultures and placed in 5 ml sterile 0.5% saline solution. The suspension served as the inoculum for the determination of antimicrobial activity.

Agar plates were inoculated by placing 3 drops of the suspension on the agar. Microorganisms were spread over the surface of the plates with sterile glass rods. Paper discs (6.5 mm diam) made from Whatman No. 1 filter paper were used in the evaluation of the liquid compounds, and stainless steel cylinders (5 mm id) were used for the solid compounds (samples 1, 7 and 9 of Table I). The paper discs, saturated with the liquid test compound, were placed on the surface of agar plates inoculated with test organisms. Solid compounds were placed in stainless steel cylinders in direct contact with the inoculated plates. No carrier solvent was employed. At least 3 experiments were made at different times, duplicate plates for each compound were tested. All plates were incubated at the optimal temperature for each organism, 37 C for *S. aureus* and *E. coli,* and 30 C for the other organisms. Readings were taken after 24, 28, 72 and 120 hr.

RESULTS AND DISCUSSION

The nine compounds listed in Table I were screened for activity against a gram-positive bacterium, *Staphylococcus aureus;* a gram-negative bacterium, *Escherichia coli;* a yeast, *Candida utilis;* and a mold, *Penicillium* sp. All of the compounds showed some inhibitory effect against the four microorganisms used in the test and six compounds effectively inhibited two or more of the organisms. Compounds rated oo in Table I are not necessarily inferior to those rated + or ++, because failure to inhibit the growth of an organism beyond the point of actual application to the plate may result from the inability of the compound to diffuse through the culture medium, rather than from low

TABLE I

Antimicrobial Activity and Physical Properties of Glycolic Acid Derivatives

No.	Compound	Antimicrobial activity[a] Microorganism[b] A	B	C	D	n_D^{30}	d_4^{30}
1	Carbomethoxymethyl palmitate[c]	o	o	o	o	d	d
2	Carbomethoxymethyl oleate	o	oo	o	o	1.4530	0.9305
3	Carboethoxymethyl oleate	+	+	o	o	1.4504	0.9237
4	Carboethoxymethyl hydrocinnamate	oo	oo	+	oo	1.4900	1.0919
5	*bis*(Carbomethoxymethyl) adipate	+	+	++	++	1.4445	1.1961
6	N,N-*bis*(Pelargonoyloxyethyl)pelargonoyloxyacetamide	oo	oo	+	oo	1.4537	0.9745
7	N,N-*bis*(Lauroyloxyethyl)lauroyloxyacetamide[e]	o	o	oo	oo	1.4597	d
8	N,N-*bis*(Oleoyloxyethyl)oleoyloxyacetamide	o	o	o	o	1.4731	0.9383
9	N,N-*bis*(Trimethylacetyloxyethyl)trimethylacetyloxyacetamide[f]	+	o	++	+	d	d
	Controls: Sorbic acid	++	++	++	++		
	10-Undecenoic acid	++	++	++	--		
	No inhibitor used	--	--	--	--		

[a] ++ = Zone of inhibition at least 5 mm beyond disc or cylinder area at 120 hr. + = Zone of inhibition less than 5 mm beyond disc or cylinder area at 120 hr. oo = Organism failed to grow on disc or cylinder area at 120 hr. o = Slight growth on the disc or cylinder area at 120 hr. -- = No inhibition detected.

[b] A = *Staphylococcus aureus;* B = *Escherichia coli;* C = *Candida utilis;* D = *Penicillium* sp.

[c] mp = 33.8 C.

[d] No data.

[e] mp = 29.6 C.

[f] mp = 51.4 C.

antimicrobial activity. In practice, a compound would only be used in an environment in which it was effective.

Two types of compounds are represented in Table I: (a) diesters in which the ester groupings are in close proximity, being separated by only a methylene group; and (b) triester amides in which the amide moiety and one of the ester groupings are separated by a methylene group, and the other two ester groupings are further removed from the amide moiety. Both types of compounds had examples that inhibited two or more of the microorganisms. The most inhibitory compound under the test conditions was the diester of adipic acid with methyl glycolate (No. 5 in Table I). It was strongly inhibitory against all of the organisms. The long chain compounds might be more effective in nonaqueous media than under the aqueous conditions of the screening test. The test results, however, show that all of the compounds have some inhibitory effect on the four microorganisms used in the test, and six of them inhibited two or more in the area of application of the compound. Thus, some of the compounds merit further testing as biostatic agents.

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Quantitative Analysis of Fatty Acids and Sterols in Malagasy Rice Bran Oils

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ABSTRACT

The fatty acid and sterol compositions of six Malagasy rice bran oils were evaluated. Investigation by gas liquid chromatography (GLC) using Carbowax 20 M revealed 10 fatty acids, mainly palmitic (16-20%) oleic (41-44%) and linolenic (31-37%) acids. An OV 17 column was used to separate eight sterols, mainly β-sitosterol (53-59%), campesterol (16-26%) and stigmasterol (10-13%). No significant variation for the fatty acid and sterol contents was observed among the rice varieties studied.

INTRODUCTION

Rice bran is an interesting raw material that could be used for edible oil production in Madagascar: paddy rice crops yearly exceed 2,000,000 t and the need for a rice bran oil extraction plant is being evaluated. Feasibility studies previously were reported (1-4) and extraction plants already exist in various countries.

Our laboratories are working on the composition and properties of the oil extracted from some local rice varieties. We studied 6 varieties which are widely grown in Madagascar: japonica, vory, lava, alicumbo, angika and makalioka. Preliminary results were obtained for laboratory neutralized and bleached oils.

EXPERIMENTAL PROCEDURES

Fatty acid methyl esters were prepared by saponification of triglycerides and acid catalyzed methylation according to Wolff (5). A Girdel Model 30 gas chromatograph equipped with a flame ionization detector was used for the analyses. The column employed was a 100-m-long, 0.25-mm-i.d. glass capillary column coated with Carbowax 20 M. The oven temp was kept at 200 C.

TABLE I

Moisture, Fat and Unsaponifiable Matter Content in Rice Bran

Rice variety	Moisture content (%)	Fat content (%)	Unsaponifiable matter (%)
Japonica	15.8	13.9	5.2
Vory	13.7	14.6	5.3
Lava	12.3	11.2	5.7
Alicumbo	15.4	14.9	6.0
Angika	16.8	11.9	5.5
Makalioka	10.3	10.0	6.0