Antimicrobial Properties of Some Erucic Acid-Glycolic Acid Derivatives

A.V. Bailey, A.J. De Lucca II and J.P. Moreau*
Southern Regional Research Center, ARS/USDA, 1100 Robert E. Lee Boulevard, P.O. Box 19687, New Orleans, Louisiana 70179-0687

Twelve fatty acid derivatives of glycolic acid containing erucic acid, or other selected vegetable oil fatty acids, were synthesized. These were screened for antimicrobial activity against a gram-positive bacterium, *Staphylococcus aureus*; a gram-negative bacterium, *Escherichia coli*; a mold, *Penicillium notatum*, and a yeast, *Candida utilis*. All of the compounds inhibited at least one of these organisms. These compounds were derivatives of glycolic acid prepared by esterification of the carboxyl function of glycolic acid with a long-chain fatty alcohol and reaction of the hydroxyl function of glycolic acid with a long-chain fatty acyl group.

Long-chain fatty acids, fatty acid esters and many other fatty acid-derived compounds exhibit some degree of antimicrobial activity (1-8). In the course of our research on industrial uses of fats and oils, a number of long-chain fatty acid derivatives of glycolic acid were prepared. The compounds were synthesized by esterification of the carboxyl function of the glycolic acid with a long-chain fatty alcohol followed by reaction of the hydroxyl function of the glycolic acid with a long-chain fatty acyl group as shown by reactions (1) and (2).

$$HO\text{-}CH_2\text{-}C(O)\text{-}OH + R\text{-}OH \rightarrow \underset{I}{HO\text{-}CH_2\text{-}C(O)\text{-}OR} + H_2O \quad [1]$$

$$I + R'\text{-}C(O)\text{-}Cl \rightarrow R'\text{-}C(O)\text{-}OCH_2\text{-}C(O)\text{-}OR + HCl \quad [2]$$

Some of the resulting long-chain fatty glycolate esters were tested as lubricants for knitting yarns with good success (9). This paper is a report of the preparation of these compounds and the results from screening these compounds for antimicrobial activity.

MATERIALS AND METHODS

Glycolic acid was a technical grade Eastman product. Butyl, octyl, 2-ethylhexyl, decyl and hexadecyl alcohols were commercial products. 13-Docosenoic (erucic) acid and 13-docosenol were obtained from the Henkel Corp. 11-Eicosenol was prepared in our lab by the Bouveault-Blanc (10,11) reduction of jojoba oil. 9-Hexadecenoic (palmitoleic) acid, 6-octadecenoic (petroselinic) acid, 9-octadecenoic (oleic) acid, and the whole soybean acids were prepared in our lab from the fractionally distilled methyl esters. Acid chlorides were also prepared, as reported by Bauer (12).

Preparation of carbobutoxymethyl erucate. To 300 g (4.0 mol) of butyl alcohol containing 5 g of p-toluenesulfonic acid in 300 ml of benzene at reflux was added 400 g (4.0 mol) of glycolic acid (70% in water), drop by drop. The water of reaction and water from the glycolic acid solution were azeotropically removed and collected in a Dean-Stark trap. The reflux was continued until all of the water had vbeen removed. The reaction solution was allowed to cool, washed with water, dried over anhydrous sodium sulfate and the benzene removed at reduced pressure using a rotary evaporator. Butyl glycolate was obtained by fractional distillation. Erucoyl chloride was added drop by drop to an equivalent weight of butyl glycolate in five volumes of benzene containing an equivalent weight of pyridine. After the heat of reaction had subsided, the pyridine hydrochloride was filtered off and the solution successively washed with two portions of aqueous hydrochloric acid and then with water until free of mineral acid. The benzene solution was dried over anhydrous sodium sulfate, filtered and percolated through a column of activated alumina to remove residual acidity and also to lower the color level.

The other glycolic acid derivatives were prepared by the same procedure with substitution of the appropriate alcohol and the appropriate acid chloride. The structure of the compounds was confirmed by nuclear magnetic resonance (NMR) and infrared spectroscopy (IR).

IR spectra were obtained with a conventional spectrophotometer (Perkin Elmer Model 337). NMR measurements were made on a Varian Associates Model operated at 60 MHz at normal probe temperature. Densities of the liquids were determined pycnometrically in a bath thermostatically controlled to $\pm$ 0.1°C. Refractive indices were determined at 30°C with a precision Bausch and Lomb refractometer with the sodium D line. Kinematic viscosities were determined in thermostated baths using calibrated Oswald viscometers as modified by Zeitfuchs (13,14). Viscosity index values were obtained from the American Society for Testing and Materials Viscosity Index Tables calculated from kinematic viscosity, ASTM Data Series, DS 39a (15).

Four organisms were used to study the ability of the test compounds to inhibit microbial growth: *Escherichia coli* (ATCC 8739), *Candida utilis* (ATCC 22023), *Staphylococcus aureus* (ATCC 6538), and *Penicillium notatum* (ATCC 11625). Both the *Staph. aureus* and *E. coli* were grown on nutrient agar (Difco, Detroit, MI), while *C. utilis* was cultivated on YM medium (Difco) and *P. notatum* was grown on potato dextrose agar (Difco). The cultures were incubated at 30°C.

Except for the Pencillium culture, 24-hr-old slant cultures of the microorganisms were used to prepare suspensions for plate inoculations. The Penicillium culture was used after spores developed in approximately 7 days. One loopful (3.2 mm loop) of spores, or of vegetative cells of the nonsporeformers, was removed from the cultures and placed in 5 ml of sterile phosphate buffer with 0.1% peptone solution. The suspensions served as the inocula for the determination of antimicrobial activity.

Agar plates were inoculated with the appropriate inoculum by placing 3 drops on the agar surface and spreading them uniformly with a sterile, bent glass rod. Sterile blank discs (Difco, 6.5 mm) were saturated with the test compound and single discs were aseptically placed in the center of each test plate. The solid test sample (Table 1,

*To whom correspondence should be addressed.

TABLE 1

Properties of Fatty Glycolic Acid Derivatives

No.	Compound	Density[a]	Ref. Index[b]	Viscosity[c]
1.	Carbobutoxymethyl erucate	0.9100	1.4527	176.1
2.	Carbooctyloxymethyl erucate	0.8786	1.4517	158.7
3.	Carbo-2-ethylhexyloxymethyl erucate	0.8956	1.4638	160.2
4.	Carbodecyloxymethyl erucate	0.8982	1.4532	165.2
5.	Carbohexadecyloxymethyl erucate	(solid: m.p. 68°C)		
6.	Carbo-11-eicosenyloxymethyl erucate	0.8452	1.4631	159.7
7.	Carboerucyloxymethyl erucate	0.8808	1.4616	155.7
8.	Carboerucyloxymethyl palmitoleate	(solid: m.p. 30°C)		
9.	Carboerucyloxymethyl oleate	0.8763	1.4595	167.8
10.	Carboerucyloxymethyl petroselinate	0.8822	1.4595	154.1
11.	Carbodecyloxymethyl (whole soybean acids)-ate	[d]	[d]	[d]
12.	Carboerucyloxymethyl (whole soybean acids)-ate	[d]	[d]	[d]

[a] d_4^{30}

[b] n_D^{30}

[c]Kinematic index values.

[d]No data.

TABLE 2

Antimicrobial Activity of Glycolic Acid Derivatives

		Antimicrobial activity[a] Microorganism[b]			
No.	Compound	A	B	C	D
1.	Carbobutoxymethyl erucate	00	0	0	00
2.	Carbooctyloxymethyl erucate	00	+	0	+
3.	Carbo-2-ethylhexyloxymethyl erucate	00	+	00	+
4.	Carbodecyloxymethyl erucate	00	0	0	00
5.	Carbohexadecyloxymethyl erucate	00	00	00	00
6.	Carbo-11-eicosenyloxymethyl erucate	00	0	0	00
7.	Carboerucyloxymethyl erucate	00	0	0	00
8.	Carboerucyloxymethyl palmitoleate	00	0	0	00
9.	Carboerucyloxymethyl oleate	00	00	+	00
10.	Carboerucyloxymethyl petroselinate	00	0	00	00
11.	Carbodecyloxymethyl (whole soybean acids)-ate	+	+	+	+
12.	Carboerucyloxymethyl (whole soybean acids)-ate	00	00	00	00
	Control[c]	00	0	0	0

[a]+, Zone of inhibition less than 5 mm beyond disc area at 120 hr; 00, organism failed to grow on disc area at 120 hr; 0, slight growth on the disc area at 120 hr.

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

[c]Phosphate buffer with 0.2% peptone, pH 7.0.

#5) was cut from a film with a sterile cork borer (6.5 mm). The sample disc was then aseptically placed on the agar surface. No carrier solvent was used. Three experiments were made at separate times with duplicate plates for each compound tested. Fresh inocula were prepared prior to each experimental run. All plates were incubated at 30°C, and the size of any inhibitory zones was recorded at 24, 48, 72, 96 and 120 hr after inoculation. The results of 120 hr were averaged for the respective samples.

RESULTS AND DISCUSSION

Density, refractive index and viscosity of the compounds used in this evaluation are shown in Table 1. Three groups of glycolic acid derivatives are represented:

- Compounds in which the carboxyl function was esterified with various alcohols, (butyl, octyl, ethylhexyl, decyl, hexadecyl, 11-eicosenyl, erucyl), and erucic acid reacted with the hydroxyl moiety. These are compounds #1-7, respectively.

- Compounds in which the carboxyl function was esterified with erucyl alcohol and different mono-unsaturated fatty acids—palmitoleic, oleic and petroselinic were attached to the hydroxyl moiety. These are compounds #8-10, respectively.
- Two compounds in which the carboxyl function was esterified with decyl and erucyl alcohols and whole soybean fatty acids were attached to the hydroxy moiety. These are compounds #11 and 12, respectively.

All compounds but #11 contained the long chain mono-unsaturated C_{22} fatty group, either on the acid side or alcohol side of glycolic acid.

The 12 compounds and control listed in Table 2 were screened for antimicrobial activity against a gram-positive bacterium, *Staphlococcus aureus*; a gram-negative bacterium, *Escherichia coli*; a yeast, *Candida utilis*, and a mold, *Penicillium notatum*. Only one compound (#11) displayed inhibitory activity against all four organisms. Two compounds (#2 and #3) showed inhibition against two microorganisms each, while only one compound (#9) displayed inhibition against a single organism. However, all of the compounds indicated some inhibitory effect against at least one of the four organisms used in the test. Compounds marked 00 in Table 2 are not necessarily inferior in inhibitory activity to those rated + because the zones of inhibition studied may be a function of the ability of the compounds to diffuse through the medium beyond the point of contact with the agar surface rather than from an intrinsic inability to retard microbial growth.

There did not seem to be much difference in the inhibitory effects of the compounds that could relate to the mode of attachment of the acyl moiety. The decyl alcohol with the whole soybean fatty acids was strongly inhibitory to all four of the organisms. Both the straight and branched chain eight carbon-atom compounds with erucic acid were effective against two each of the organisms. A simple screening technique was used for this investigation to obtain general information on whether the compounds tested had antimicrobial properties that might be useful in commercial products. The longer chain compounds might be more effective in nonaqueous media than under the aqueous conditions of the screening test. The test results reported in this paper did show, however, that these types of compounds can have inhibitory effects against the four organisms tested. Further testing of these compounds as biostatic agents should be considered.

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