sition. As noted before (14) epoxy esters give a lower response than nonoxygenated esters when a $\beta$-ionization detector is used. In addition, any dihydroxy esters, such as occur in the esters from *Vernonia* oil, will not be measured. The effect of both these factors is to give high values for the nonoxygenated esters (cf. Table I).

Most of the methods described for the isolation of epoxy acids or esters are equally, or more suitable for the isolation of hydroxy or other oxygenated derivatives. Thus a wide range of methods is available for the isolation of epoxy and other oxygenated acids and esters, and some of the methods, in conjunction with gas-liquid chromatography, may be used to give more accurate analyses of the mixed esters of oils containing these derivatives than are possible with older analytical methods.

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# Antimicrobial Activity of Some Ricinoleic and Oleic Acid Derivatives

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Ricinoleic and oleic acid derivatives were screened for their antimicrobial activity, under optimum growing-conditions, against several species of bacteria, yeasts, and molds. Several ricinoleic acid derivatives and petroselinic (iso-oleic) acid exhibited considerable activity; in fact, their activity against some micro-organisms was comparable to sorbic and 10-undecenoic acid, known antimicrobial agents, as indicated by this test.

THE ANTIMICROBIAL ACTIVITY of various types of fatty acids or fatty acid derivatives has been studied by a number of investigators, using different techniques and organisms. According to Kiesel (10), the antimycotic action of saturated fatty acids increases as the number of carbon atoms in the chain increases up to eleven, and the branched-chain fatty acids are less active than straight-chain fatty acids of equal molecular weights. Tetsumoto (20,21,22) observed that the unsaturated fatty acids are more antimycotic than the corresponding saturated fatty acids, that the normal fatty acids are more active than the isomeric acids, and that the activity is due to the undissociated molecule, not the anion. The fungistatic activity of an organic acid varies with the pH of the test medium; the activity is roughly proportional to the concentration of the nonionized molecules (8). Cowles (4) found the bactericidal action of fatty acids to be greater at low pH values and the long-chain fatty acids to be more active than the short-chain fatty acids. Keeney *et al.* (9) stated that the long-chain fatty acids are more fungicidal than the short-chain fatty acids. Wyss *et al.* (24) found that the antimycotic action of fatty acids increases with chain length up to 12 carbon atoms, that the unsaturated fatty acids are slightly more active than the corresponding saturated fatty acids, and that fatty acids possessing an odd number of carbon atoms are no more active than the even-numbered homologs. The results obtained by Spoehr *et al.* (18) indicated that fatty acids with 16 or fewer carbon atoms exhibit antibacterial activity; that oleic, elaidic, linoleic, $\beta$-eleostearic, and $\beta$-licanic acids lacked antibacterial power but acquired it on photo-oxidation; that stearic acid lacked antibacterial power even after photo-oxidation; and that glycerides lacked antibacterial power even though the respective fatty acids were antibacterial. McGowan *et al.* (15) suggested that the fungistatic activity of substituted, unsaturated fatty acids is associated with the tendency of the substituents to withdraw electrons from the ethylenic bond. Stedman (19) found that propionic, undecylenic, and caprylic acids exhibit superior antimicrobial action at acidic pH's. Melnick *et al.* (16). reported that $\alpha,\beta$-unsaturated fatty acids, such as sorbic acid, are normal transitory metabolites in the oxidation of saturated fatty acids by molds; however high initial concentrations can inhibit the dehydrogenase enzyme system in molds. Inhibition of this important enzyme system is held responsible for the fungistatic or fungicidal activity of sorbic acid. The bacteriostatic action of unsaturated fatty acids was observed by Kodicek (11) to increase as the number of *cis* ethylenic bonds increases. It was noted also

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TABLE I

The Antimicrobial Activity of Some Ricinoleic and Oleic Acid Derivatives

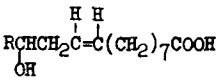
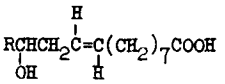
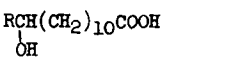
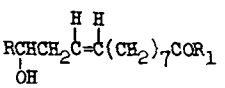
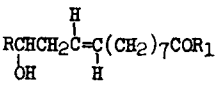
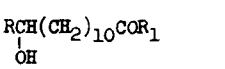
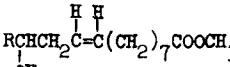
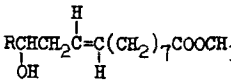
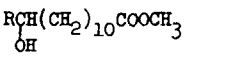
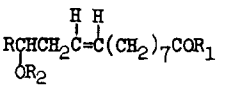
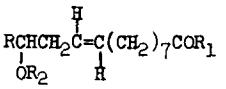
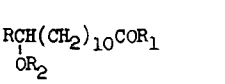
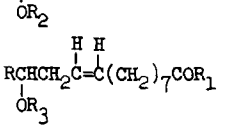
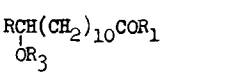
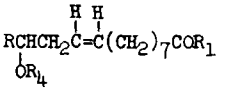
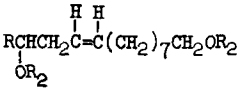
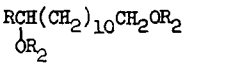
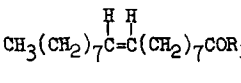
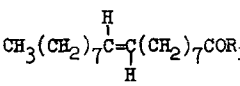
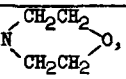
| Compound | Structure [a] | Antimicrobial activity [b] micro-organisms [c] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | F | G | H | I | J | K | L |
| Ricinoleic acid |  | ++ | - | - | - | - | + | ++ | o | + | + | ++ | ++ |
| Ricinelaidic acid |  | + | - | - | - | - | + | - | - | o | - | o | - |
| 12-Hydroxystearic acid |  | - | - | - | - | - | - | - | - | - | - | o | - |
| 4-Ricinoleoylmorpholine |  | ++ | + | - | - | o | ++ | - | ++ | + | + | oo | ++ |
| 4-Ricinelaidoylmorpholine |  | + | + | - | - | - | ++ | ++ | - | o | + | o | o |
| 4-(12-Hydroxystearoyl)morpholine |  | + | + | + | - | - | + | - | - | - | - | - | - |
| Methyl ricinoleate |  | - | - | - | - | - | - | - | o | - | - | - | ++ |
| Methyl ricinelaidate |  | + | + | + | - | o | + | - | - | - | - | - | o |
| Methyl 12-Hydroxystearate |  | ++ | - | ++ | - | + | + | o | - | o | + | o | - |
| 4-(12-β-Cyanoethoxyoleoyl)morpholine |  | + | + | - | - | o | + | - | ++ | o | + | + | o |
| 4-(12-β-Cyanoethoxyelaidoyl)morpholine |  | + | - | - | - | - | - | - | + | - | - | - | + |
| 4-(12-β-Cyanoethoxystearoyl)morpholine |  | - | - | - | - | - | + | - | o | - | - | - | o |
| 4-(12-Acetoxyoleoyl)morpholine |  | - | - | - | - | - | - | - | - | - | - | - | o |
| 4-(12-Acetoxystearoyl)morpholine |  | + | - | - | - | - | + | - | - | - | - | - | oo |
| 4-(12-Propionoxyoleoyl)morpholine |  | + | + | - | - | - | ++ | - | - | - | - | - | oo |
| 1,12-Bis(β-cyanoethoxy)-cis-9-octadecene |  | + | + | + | - | - | - | - | - | - | - | - | o |
| 1,12-Bis(β-cyanoethoxy)octadecane |  | - | - | - | - | - | - | - | - | - | - | - | - |
| 4-Oleoylmorpholine |  | + | - | - | - | o | + | - | - | - | - | ++ | - |
| 4-Elaidoylmorpholine |  | + | - | - | - | - | - | - | o | - | - | - | - |
| 4-Stearoylmorpholine | $CH_3(CH_2)_{16}COR_1$ | - | + | - | - | - | + | - | o | o | + | o | o |

TABLE I (Concluded)

| Compound | Structure [a] | Antimicrobial activity [b] micro-organisms [c] | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A | B | C | D | E | F | G | H | I | J | K | L |
| Oleic acid | $CH_3(CH_2)_7\overset{H}{\underset{}{C}}=\overset{H}{\underset{}{C}}(CH_2)_7COOH$ | + | - | - | - | - | - | - | - | - | - | - | - |
| Elaidic acid | $CH_3(CH_2)_7\overset{H}{\underset{}{C}}=\underset{H}{\overset{}{C}}(CH_2)_7COOH$ | + | - | - | - | - | - | - | o | - | - | - | oo |
| Stearic acid | $CH_3(CH_2)_{16}COOH$ | + | - | - | - | - | - | - | - | - | - | o | o |
| Petroselinic acid | $CH_3(CH_2)_{10}\overset{H}{\underset{}{C}}=\overset{H}{\underset{}{C}}(CH_2)_4COOH$ | ++ | + | - | - | - | + | + | o | o | + | ++ | ++ |
| Petroselaidic acid | $CH_3(CH_2)_{10}\overset{H}{\underset{}{C}}=\underset{H}{\overset{}{C}}(CH_2)_4COOH$ | - | - | - | - | - | - | - | - | - | - | - | - |
| Sorbic acid | $CH_3\overset{H}{\underset{}{C}}=\overset{H}{\underset{}{C}}\overset{}{\underset{H}{C}}=\overset{}{C}COOH$ | ++ | ++ | ++ | + | ++ | ++ | + | ++ | + | + | ++ | ++ |
| 10-Undecenoic acid | $CH_2=CH(CH_2)_8COOH$ | ++ | ++ | ++ | + | ++ | ++ | + | ++ | + | + | - | ++ |
| Vinyl 10-undecenoate | $CH_2=CH(CH_2)_8COOCH=CH_2$ | - | - | - | - | - | - | - | o | - | - | - | - |

a/ R = $CH_3(CH_2)_5$, $R_1$ =  , $R_2$ = $CH_2CH_2CN$, $R_3$ = $CH_3CO$, $R_4$ = $CH_3CH_2CO$.

b/ ++ = good, + = fair, oo = organism failed to grow over compound, o = very slight growth over the compound, and - = none.

c/ A = Micrococcus pyogenes, B = Escherichia coli, C = Saccharomyces cerevisiae, D = Candida stellatoidea, E = Torulopsis sp., F = Neurospora sp., G = Alternaria sp., H = Mucor sp., I = Hormodendrum sp., J = Geotrichum sp., K = Penicillium sp., L = Aspergillus sp.

that branched or straight-chain, saturated fatty acids of 12 to 14 carbon atoms are bacteriostatic.

The antimicrobial action of sorbic acid is related to the concentration of the undissociated acid (1). There are indications that sorbic acid is an inhibitor of sulfhydryl enzymes (23). According to Sokoloff *et al.* (17), the oxidation of highly-unsaturated, long-chain fatty acids increases their antifungal activity. The quaternary ammonium derivatives of long-chain fatty acids are very active against fungi; the unsaturated fatty acid derivatives are the most active.

Since it has recently been shown that 10-hydroxy-*trans*-2-decenoic acid (3) possesses antimicrobial activity (2), ricinoleic (12-hydroxy-*cis*-9-octadecenoic) acid derivatives were screened to determine their potentiality as antimicrobial agents. Oleic (*cis*-9-octadecenoic) acid derivatives were screened for comparative purposes.

## Experimental

*Materials.* Ricinoleic and oleic acid derivatives tested for their antimicrobial activity were prepared in the course of other investigations (5,6,7,13,14). The micro-organisms used were obtained from stock cultures. Difco Bacto Dehydrated Stock Culture Agar at pH 7.0, Difco Bacto Dehydrated Yeast Morphology Agar at pH 4.5, and Difco Dehydrated Mycological Agar at pH 7.0 were selected to test the inhibition of the bacteria, yeast, and mold cultures, respectively.

*Procedure.* Ricinoleic and oleic acid derivatives were screened for their antimicrobial activity against two bacteria—a gram positive, *Micrococcus pyogenes,* and a gram negative, *Escherichia coli;* several yeasts —*Saccharomyces cerevisiae, Candida stellatoidea,* and *Torulopsis* sp.; and several molds—*Neurospora* sp., *Alternaria* sp., *Mucor* sp., *Hormodendrum* sp., *Geotrichum* sp., *Penicillum* sp., and *Aspergillus* sp.

Seeded agar plates were used to measure the antimicrobial activity against bacteria and yeasts. The filter paper disc method was used to evaluate the liquid compounds, and the cylinder plate method was used to evaluate the solid compounds. Standard-size paper discs or uniform-size stainless steel cylinders were placed on the surface of the previously inoculated plates. The liquid compounds were pipetted onto the discs, and the solid compounds were introduced into the stainless steel cylinders. Streaked plates were used to measure the activity against molds. Hardened agar plates were streaked with the test mold before the compounds were added onto specified areas of the plates. To eliminate any errors which could result from an insufficient number of tests, a minimum of three experiments employing duplicate plates was used for measuring the antimicrobial activity of each compound.

All plates were incubated at the optimum growing-temperature for each organism. Readings were made after 24, 48, 72, and 120 hrs., respectively. Zones of inhibition were compared to those of the controls.

### Results and Discussion

Of the ricinoleic and oleic acid derivatives screened for their antimicrobial activity against several species of bacteria, yeasts, and molds under optimum growing-conditions, such as pH, nutrition, incubation temperature, and moisture, several exhibited considerable activity (Table I). In general, these compounds did not exhibit as broad a spectrum as sorbic and 10-undecenoic acids, common antimicrobial agents. Ricinoleic acid, 4-ricinoleoylmorpholine, 4-ricinelaidoylmorpholine, methyl 12-hydroxystearate, 4-(12-propionoxyoleoyl)morpholine, and petroselinic (iso-oleic) acid in several cases however were comparable to these positive controls as indicated by this test. In many cases, the various compounds exhibited only a slight degree of inhibition, that is, the micro-organisms merely failed to grow over the compound. Even though some of the compounds exhibited only slight activity, they should not be ruled out as antimicrobial agents. Under less favorable growing-conditions, such as that found in paint films, polymers, and copolymers, the activity might be increased considerably. They may be effective also against other types of micro-organisms.

Since there are different factors involved, such as solubility, absorption, metabolic degradation, etc., molecular structure of the compounds studied cannot be associated with antimicrobial activity. Even with this limited number of compounds and micro-organisms, observations indicate that it is impossible to make any generalization concerning the antimicrobial activity imparted by various functional groups in the molecule. The β-hydroxy-*cis*-ene system in ricinoleic acid and 4-ricinoleoylmorpholine appears to impart activity to the molecule whereas it appears to decrease the activity in methyl ricinoleate. The β-hydroxy-*trans*-ene system appears to impart activity to 4-ricinelaidoylmorpholine but very little to ricinelaidic acid or methyl ricinelaidate. On the other hand, methyl 12-hydroxystearate and petroselinic (iso-oleic) acid exhibited considerable activity even though neither one of these compounds possesses a β-hydroxy-ene system.

Cyanoethylation or esterification of the hydroxyl group in 4-ricinoleoylmorpholine, 4-ricinelaidoylmorpholine, and 4-(12-hydroxystearoyl)morpholine appears to alter the activity of the molecule, reducing it in most cases, but no generalization can be made.

Similarly, converting the carboxyl of ricinoleic, ricinelaidic, and 12-hydroxystearic acids to morpholino or methyl ester groups appears to alter the antimicrobial activity of the molecule but not systematically.

Some of these compounds might be of commercial interest since they possess such broad antimicrobial spectra. For example, 4-ricinoleoylmorpholine should probably be of interest in paint films, plastics, and other polymeric materials for its antimicrobial as well as its plasticizer properties (12). In addition, some of the compounds tested may have medicinal applications.

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# ABSTRACTS . . . .

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## • Fats and Oils

THE ISOLATION OF CIS-$\Delta^9$-HEPTADECENOIC ACID FROM BUTTERFAT. R.P. Hansen, F.B. Shorland, and N. June Cooke (Fats Res. Lab., Dept. of Sci. and Ind. Res., Wellington, New Zealand). *Biochem. J.* **77**, 64–6 (1960). *cis*-$\Delta^9$-Heptadecenoic acid was isolated from butterfat in a concentration of approximately 0.06% of the total weight of fatty acids. According to the authors, odd-numbered unsaturated fatty acids have not previously been found in butterfat.

THE CHEMICAL NATURE OF THE TOXIC COMPOUNDS CONTAINING FLUORINE IN THE SEEDS OF DICHAPETALUM TOXICARIUM. R.A. Peters, R.J. Hall, and P.F.V. Ward (A.R.C. Inst. of Animal Physiol., Babraham, Cambridge) and N. Sheppard. *Biochem. J.* **77**, 17–23 (1960). Fluoro-oleic acid from the seeds of *D. toxicarium* (Sierra Leone) has been isolated in pure form and its structure proved to be ω-fluoro-*cis*-$\Delta^9$-octadecenoic acid. During the final stages of purification, another fluoro-fatty acid of much higher melting point was isolated in small amounts. It contained no double bond and possibly 17 carbon atoms, but behaved biochemically as a long-chain fatty acid with an even number of carbons. Both acids were found to significantly inhibit citrate metabolism in guinea pig kidney particles.

THE FATTY ACID COMPOSITION OF HUMAN DEPOT FAT. K.J. Kingsbury, S. Paul, A. Crossley, and D.M. Morgan (St. Mary's Hospital, London, W. 2). *Biochem. J.* **78**, 541–50 (1961). The fatty