bacco seed oils partially gelled during the process. The films of all the treated oils dried in wrinkled patterns.

REFERENCES

1. Gardner, H. A., U. S. Patent 1,484,014 (Feb. 19, 1924); British Patent 316,732 (1928).
2. Münzel, F., Swiss Patent 193,931 (April 1, 1938).
3. Colbeth, I. M., U. S. Patent 2,388,122 (Oct. 30, 1945).
4. Farmer, E. H., Koch, H. P., and Sutton, D. A., J. Chem. Soc., 541–547 (1943).
5. Moore, T., Biochem. J., *31*, 141–148 (1937).
6. Kass, J. P., and Burr, G. O., J. Am. Chem. Soc., *61*, 3292–3294 (1939).
7. Brode, W. R., Patterson, J. W., Brown, J. B., and Frankel, J., Ind. Eng. Chem., Anal. Ed., *16*, 77–80 (1944).
8. Teeter, H. M., Bechmann, R. C., Bell, E. W., and Cowan, J. C., Ind. Eng. Chem., *41*, 849–852 (1949).
   Teeter, H. M. Gast, I. F., Raleigh, D., and Woods, L. C., J. Am. Chem., Soc., *73*, 2302–2303 (1951).
   Teeter, H. M. and Jackson, J. E.. J. Am. Oil Chemists' Soc., *26*, 535–540 (1949).
   Teeter, H. M., and Bell, E. W., *ibid.*, *27*, 102–105 (1950).
   Teeter, H. M., and Myren, R. A., *ibid.*, *29*, 367–70 (1952).
9. Arnold, R. T., U. S. Patent, 2,698,857.
10. Ziegler, K., Spaeth, A., Schaef, E., Schumann, W., and Winkelmann, E., Ann., *551*, 80–88 (1942).
11. Teeter, H. M., J. Am. Oil Chemists' Soc., *25*, 243–248 (1948).
12. Schmid, H., and Lehmann, A., Helv. Chem. Acta, *33*, 1494–1498 (1950).
13. Gunstone, F. D., Quarterly Reviews, *7*, 175–197 (1953).
14. Sutton, D. A., and Dutta, J., J. Chem. Soc., 939–944 (1949).
15. Clemo, G. R., and Stevens, R., *ibid.*, 4684–4685 (1952).
16. Bergstrom, S., and Hansson, G., Acta Chim. Scand., *4*, 435–443 (1950).
17. Nanavati, D. D., Nath, B., and Aggarwal, J. S., Chem. and Ind., 82–3 (1956).
18. Waters, W. A., J. Chem. Soc., 2007–2016 (1937).
19. Bloomfield, G. F., *ibid.*, 114–120 (1944).
20. Grigor, J., MacInnes, P. M., McLean, J., and Hogg, J. P., *ibid.*, 1069–1071 (1955).
21. Hatt, H. H., and Szumer, A. Z., Chem. and Ind., 962–963 (1954).
22. Plank, R. W., Pack, F. C., and Goldblatt, L. A., J. Am. Oil Chemists' Soc., *30*, 417–419 (1953).
23. A.O.C.S. Tentative Method, Ka 12-55.

[Received January 3, 1957]

Methyl Esters Directly from Acidulated Soapstock

PAUL H. EAVES, J. J. SPADARO, and E. A. GASTROCK,
Southern Regional Research Laboratory,[1] New Orleans, Louisiana

THE ESTERS of fatty acids are usually prepared by alcoholysis of triglycerides or by esterification of fatty acids. As both of these commodities are in the 13¢ to 15¢-per-pound price range, esters prepared from them would necessarily be high in price. The use of acidulated cottonseed oil refinery soapstock (or foots) "as is" as a primary source of fatty acids for the preparation of fatty acid esters was suggested by the low cost of foots fatty acids (about 5¼¢ per pound) as compared to the distilled acids, by the fact that foots are normally in surplus and difficult to dispose of, and by the opportunity of making the fatty acid esters available at low cost for commercial uses.

Pack and Goldblatt (3) have reported a method of preparing benzyl esters by reacting the respective chlorides with dehydrated raw soapstock in an inert solvent media. However no work is known in which acidulated soapstock has been employed directly "as is" as the primary source of fatty acids for the preparation of the fatty acid esters. This paper is a report of the results of an investigation conducted to determine the conditions required to esterify *in situ* the fatty acids of acidulated foots and to develop a practical process for producing fatty acid methyl esters directly from acidulated cottonseed foots.

Experimental

Materials. Four lots of commercial grade acidulated soapstock (or foots) were used in this investigation. One lot had been hydrolyzed by the Twitchell process. All of the acidulated foots were from raw soapstocks produced by the continuous alkali-refining method. The analyses of the foots, given in Table I, show that their total fatty acids (T.F.A.) content varied only from about 91% to 94% but that their free fatty acids content ranged from 70% to 94%. This variation in free fatty acids was caused by the variation in neutral oil content, ranging from the unusually low value of 0.2% in the hydrolyzed foots to the rather high 23.2% in Lot A.

TABLE I
Analyses of Soapstocks

Lot	Description	Total fatty acids	Oxidized fatty acids	Free fatty acids	Neutral oil	Gossypol
		%	%	%	%	%
A	Acidulated	92.6	4.6	70.4	23.2	......
B	Acidulated	91.3	5.4	79.1	12.4	0.12
C	Hydrolyzed[a]	94.3	5.0	94.1	0.2	0.02
D	Acidulated	91.3	6.0	76.0	16.1	......

[a] Hydrolyzed by the Twitchell Process.

The methanol used was reagent grade absolute and was of synthetic origin.

Acidic catalysts were used because of their tolerance for small amounts of water, such as are found in most acidulated soapstocks. Hydrogen chloride was used as the catalyst in the first series of tests. The catalyst for all of the remaining tests was a crude form of Twitchell reagent in an excess of sulfuric acid. This catalyst was prepared by reacting together one mole of naphthalene, one mole of distilled cottonseed fatty acids (mixed), and 6 moles of 100% sulfuric. The resulting mixture of naphthalenestearosulfonic and sulfuric acid was used "as is." The sulfuric acid was probably the major catalytic agent with its activity enhanced by the emulsifying action of the sulfonic acid.

Methods of Analysis and Evaluation. The applicable methods of the American Oil Chemists' Society (1) were employed in determining total and oxidized fatty acids and unsaponifiable matter. Gossypol was determined by the method of Pons, Hoffpauir, and O'Connor (4) and neutral oil by the method of Linteris and Handschumaker (2) as modified by the American Oil Chemists' Society.

Free fatty acids in the distilled esters were determined by titration with 0.1 N alkali. The free fatty acids of the soapstocks were calculated on the basis of their content of total fatty acids and neutral oil.

---

[1] One of the laboratories of the Southern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

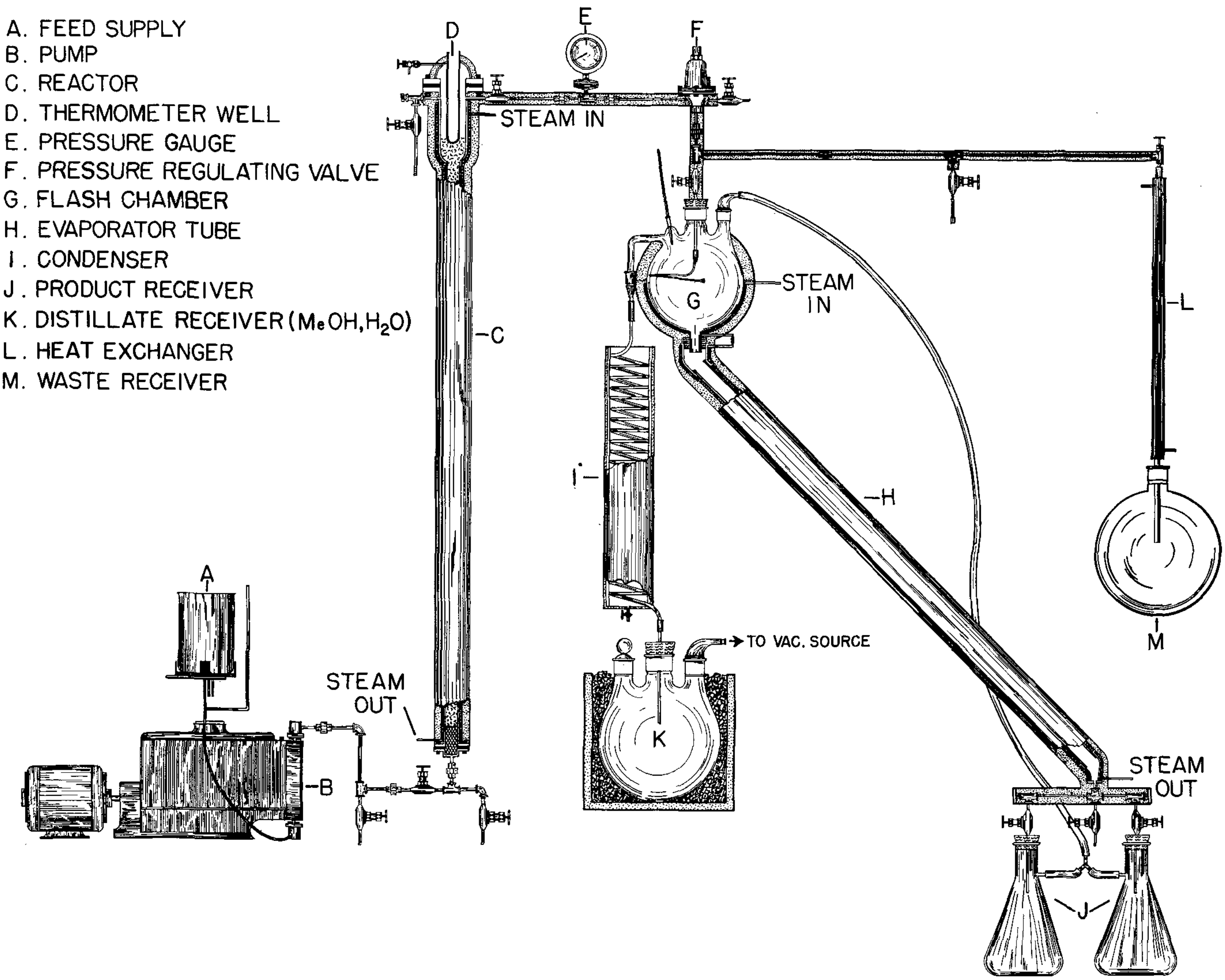

Fig. 1. Pilot scale continuous reactor.

The yields of methyl esters were found by distilling weighed quantities of the reactor output products and correcting the weights of the distillates for free fatty acids. The percentage of the total fatty acids of the soapstock converted to methyl esters was calculated from the weight of fatty acids in a given quantity of input soapstock and the weight of fatty acids found combined as methyl esters in the equivalent quantity of processed material. The percentages of conversion of the total fatty acids determined by the foregoing methods are considered by the authors to be correct to within about 1% of the values reported.

Equipment and Procedures. An all-stainless steel packed column type of continuous reactor which had been designed and fabricated for the purpose was used in these tests. The reactor and auxiliary equipment are shown by Figure 1. The reactor vessel proper consisted of a 1-in. pipe 4 ft. in length provided with a steam jacket and packed with ½ in. Berl saddles. The net volumetric capacity of the packed column was 720 cc.

The auxiliary equipment included a feed supply tank, a small-capacity, high-pressure (450 p.s.i.g.), variable-rate, positive-displacement pump, a pressure gauge, a variable-pressure discharge valve, an evaporator and condenser, and suitable receiving vessels.

In operation the temperature was controlled by regulating the steam pressure to the reactor jacket, the pressure in the reactor by means of the discharge pressure-control valve, and the time of retention of the material in the reactor by the rate of feed input.

The evaporator consisted of a flash chamber supplemented by a falling film tube. Both were heated by external steam coils and were operated at an absolute pressure of about 110 mm. of mercury. The hot material discharged from the reactor *via* the discharge-pressure regulating valve directly into the evacuated flash chamber where the major portion of the excess methanol and the water formed by the reaction was removed by flash evaporation. The remainder, if any, was evaporated from the material as it trickled down the wall of the tube.

Flash evaporation served two important purposes. The first of these was to terminate the reaction by almost instantaneous removal of methanol and water. The second was that the output product, being free of methanol and water, could be either distilled without further treatment or fresh methanol could be added back and the material re-passed through the reactor to increase the yield of esters.

In conducting the tests, the proportioned mixtures of foots, methanol, and catalyst were prepared in 1,000-g. batches as required for use. The reactor was then filled and brought into initial operating equilibrium with respect to retention time, temperature, and pressure; the output product was collected in open vessels and discarded. The output product stream was then diverted to the evaporator, and full equilibrium, including hold-up in the evaporator, was established. After establishment of full-equilibrium conditions the contents of the reactor were displaced a minimum of five times; this output material also was discarded. The receiving vessel was then changed, and a weighed quantity of mixture was passed through the reactor. The output product collected while passing the weighed quantity of mixture through the reactor was taken to be equivalent to the quantity of input feed mixture. All yield data were based on the foregoing input-output equivalence.

For the tests in which the same material was passed through the reactor twice the initial mole ratio of methanol to total fatty acids, 5 to 1, was restored by adding the required quantity of fresh methanol back to the methanol and water-free product from the first pass. No additional catalyst was used for the second pass. The same procedures were followed in establishing operating equilibrium for the second as for the first pass.

The 5 to 1 mole ratio of methanol to total fatty acids, equivalent to about 35% methanol and 65% foots by weight, provided an adequate excess of methanol and was the lowest which would give complete solution of the foots. With the exception of the tests reported in Tables II and III the same conditions of temperature and pressure, 120°C. and 150 p.s.i.g., respectively, were used throughout. The catalyst was proportioned as a percentage of the total fatty acids and for all of the tests it was dissolved in methanol before mixing with the foots.

Results and Discussion

The results of the first series of tests, which were conducted for the purpose of comparing qualitatively the effects of pressure, retention time, and temperature on the rate and degree of conversion of the fatty acids in the foots to methyl esters, are given in Tables II and III. The foots used in these tests was from Lot A with hydrogen chloride as the catalyst.

The data in Table II show that at comparable temperatures and residence times there was no significant difference in the degree of conversion of the total fatty acids of the foots to methyl esters which could be attributed to the effect of differences in pressure. At 0, 100, and 300 p.s.i.g., with residence times of 60, 55, and 47 minutes, respectively, the percentage of fatty acids converted to esters was essentially the same. The same was true for the two tests in which short residence times, 10 min., with high and low pressures of 100 and 300 p.s.i.g., were employed. Prolonging the reaction time increased the percentage conversion of the fatty acids although the order of increase was small, being only 5% to 6% for a 5- to 6 fold lengthening of retention time.

TABLE II

Effect of Varying Pressure and Retention Time, with Other Conditions Constant,[a] on the Percentage of the Total Fatty Acids Esterified

Test	Conditions			Total fatty acids esterified
	Temperature	Retention time	Pressure	
No.	*°C.*	*min.*	*p.s.i.g.*	*%*
1	70	60	0	78.6
2	70	10	100	73.4
3	69	10	300	74.9
4	71	55	100	80.1
5	72	47	300	78.7

[a] Material, foots Lot A. Catalyst; hydrogen chloride, 3% of total fatty acids. Mole ratio of methanol to total fatty acids, 5 to 1.

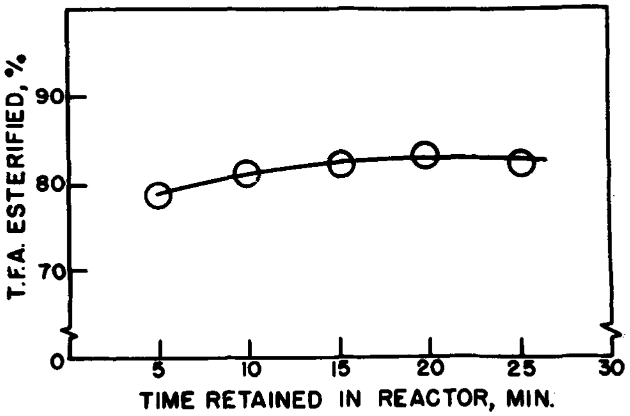

FIG. 2. Effect of reaction time on the percentage of total fatty acids esterified.

The effects of high and low temperatures at comparable retention times and pressures are given in Table III. At 100 p.s.i.g., with a residence time of 10 min., conducting the reaction at 110°C. as compared to 70° resulted in increasing the percentage of total fatty acids converted to esters from 73.4% to 81.7%. At similar conditions, but at 300 p.s.i.g., the increase was from 74.9% to 79.4%. In view of the short retention time of 10 min. these results are significant. The fact that the percentage of conversion of total fatty acids to esters was not increased by the use of the higher pressure in conjunction with the higher temperature, as compared with the use of the lower pressure, indicates that only enough pressure to maintain the methanol liquid at the temperature employed is needed.

The results of a series of tests which were made to establish the effects of varying retention time, with temperature and pressure constant at 120°C. and 150 p.s.i.g., respectively, are shown by Figure 2. The foots used in this series was Lot D (91.3 TFA, 16.1 neutral oil) with 3% of crude Twitchells reagent as the catalyst.

The results indicated that extending the reaction time beyond 10 to 13 min. did not appreciably increase the percentage of the total fatty acids converted to esters. At 5 min. conversion was 79% and at 10 min. had increased to only 81%. After about 13 min. the curve tended to level off at about 82–83% and thereafter there was no significant increase.

TABLE III

Effect of Varying Temperature and Pressure, with Other Conditions Constant,[a] on the Percentage of Total Fatty Acids Esterified

Test	Conditions			Total fatty acids esterified
	Temperature	Retention time	Pressure	
No.	*°C.*	*min.*	*p.s.i.g.*	*%*
2	70	10	100	73.4
6	110	10	100	81.7
3	69	10	300	74.9
7	110	10	300	79.4

[a] Material, foots Lot A. Catalyst; hydrogen chloride, 3% of total fatty acids. Mole ratio of methanol to total fatty acids, 5 to 1.

The relationship between the percentage of the total fatty acids of the foots which were present in the form of neutral oil and the percentage of the total fatty acids which were converted to methyl esters is shown by Figure 3. The data show that, when essentially all of the total fatty acids were in the free form, about 90% were converted to esters. As the percentage of the total fatty acids present in the form of neutral oil increased and approached 15%, the percentage of total fatty acids converted to esters decreased from 90% to about 81–82%. At about this point, with approximately 15% or more of the total fatty acids in the form of neutral oil, further increases in the proportion of the total fatty acids present as neutral oil did not appear to have any adverse effect on the percentage of the total fatty acids converted to esters.

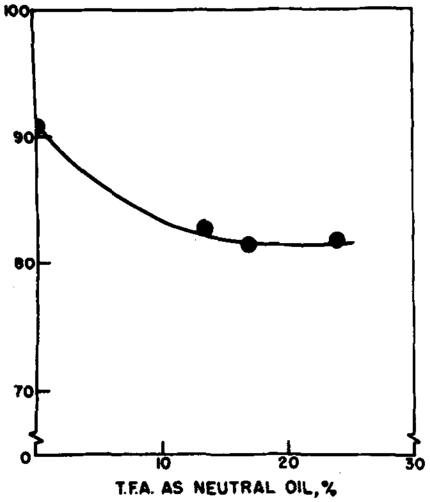

FIG. 3. Effect of the neutral oil content of the foots on the percentage of their total fatty acids esterified.

The results of a series of tests which were conducted to determine the effect of varying percentages of catalyst and of reacting the same material a second time are shown by Figure 4. For this series of tests acidulated foots from Lot B, containing 12.4% of neutral oil, was used. The reaction conditions employed were temperature, 120°C.; retention time, 10 min.; and pressure, 150 p.s.i.g.

The data show that, by increasing the percentage of catalyst used, the reaction is carried more nearly to completion, *i.e.*, with 1% catalyst, about 77% of the total fatty acids were converted to esters; with 3%, about 83%; and with 5%, about 86%. When the methanol to total fatty acids ratio of the above methanol- and water-free products was restored (no additional catalyst used) and the reconstituted mixtures were passed through the reactor a second time, the percentage of total fatty acids converted to esters was increased by from 5% to 13%. The greater increase was with the lower percentages of catalyst.

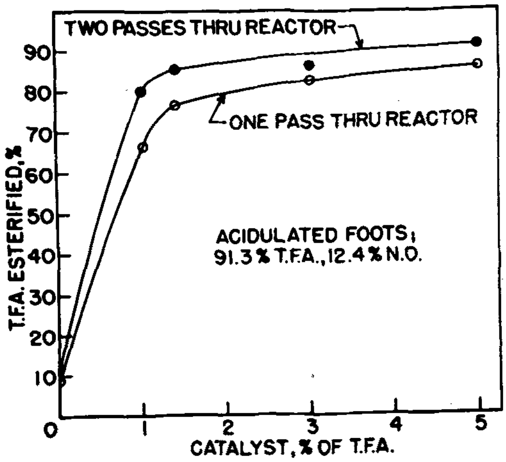

FIG. 4. Effect of catalyst concentration and of two reaction stages on the esterification of the total fatty acids of acidulated foots.

Figure 5 shows the results obtained when the foregoing tests were replicated by using foots from Lot C, which had been hydrolyzed by Twitchellizing to reduce its content of neutral oil to about 0.2%. With two exceptions the results of those tests followed the same general pattern as those for the similar tests that used acidulated foots. The exceptions were a) that the percentages of the total fatty acids converted to esters were appreciably higher and b) that no significant increase in total fatty acids conversion resulted from increasing the percentage of catalyst from 3% to 5%. With 1% catalyst the twice-processed output product gave 91% conversion of total fatty acids to esters as compared to 88% for the material processed once. With 3% catalyst the single-pass reaction gave about 91% conversion of the total fatty acids to esters while the percentage conversion was about 96% for the two-pass reaction.

Summary

A method has been developed for utilizing low-cost acidulated refinery soapstock directly as raw material for producing the mixed methyl esters of cottonseed oil fatty acids. The conditions determined as optimum for producing the esters were as follows: temperature, 110°C. to 120°C.; pressure, 150 p.s.i.g.; reaction time, 10 to 15 min.; catalyst, acidic, proportioned as 3% to 5% of the fatty acids of the foots; and ratio of reactants, 5 moles of methanol to 1 of fatty acids.

Processing acidulated foots containing 12.4% of neutral oil at the foregoing conditions resulted in esterifying 83% to 86% of the fatty acids. Reprocessing the same material after dehydrating it and restoring the initial methanol to fatty acids ratio increased the percentage of fatty acids esterified by about 5%.

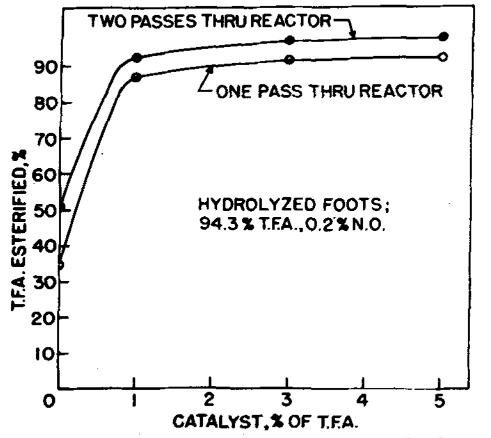

FIG. 5. Effect of catalyst concentration and of two reaction stages on the esterification of the total fatty acids of hydrolyzed acidulated foots.

Following the same procedure but using foots which had been hydrolyzed to reduce their neutral oil content to less than 1%, one-pass processing esterified 91% to 92% of the fatty acids, with a second pass increasing the percentage of fatty acids esterified to 96% or 97%.

Acknowledgment

The authors wish to express their appreciation to J. M. Funderburke for preparing the drawing of the reactor. They also thank S. M. Stark Jr. and J. A. Harris for conducting the analyses.

REFERENCES

1. American Oil Chemists' Society, "Official and Tentative Methods," edited by V. C. Mehlenbacher and T. H. Hopper, 2nd. edition, rev. to 1957, Chicago, 1946–57.
2. Linteris, L., and Handschumaker, E., J. Am. Oil Chemists' Soc., *27*, 260–263 (1950).
3. Pack, F. C., and Goldblatt, L. A., J. Am. Oil Chemists' Soc., *32*, 551–553 (1955).
4. Pons, W. A. Jr., Hoffpauir, C. L., and O'Connor, R. T., J. Am. Oil Chemists' Soc., *28*, 8–12 (1951).

[Received August 7, 1958]

The Fungicidal Activity of the Unsaturated Fatty Acids and Quaternary Salts Prepared from Fish Oils[1]

BORIS SOKOLOFF, MASAMICHI TOYOMIZU, WALTER TRAUNER, and GEORGE RENNINGER **Southern Bio-Research Institute, Florida Southern College, Lakeland, Florida**

POLLOCK (1) and Axelrod *et al.* (2) have reported that fatty acids with three double bonds in a molecule exert an inhibitory influence upon some micro-organisms. This observation was confirmed by Laser (3) and Wyss (4). As a rule, only gram-positive organisms are inhibited by the action of unsaturated fatty acids, according to Kodicek (5) and Dubos (6). These investigators have reported that oleic, linoleic, and linolenic acids inhibited *Lactobacillus helveticus* and other gram-positive bacteria. No such effect was evidenced in regard to *E. coli* and *Proteus vulgaris.* It appears that oxidation of unsaturated fatty acids increases their antibiotic activity. Tomiyasu *et al.* (7) found that unsaturated fatty acids or highly unsaturated fatty acid fractions from fish oils did not inhibit the growth of *Debaryomyces membranefaciens,* but by oxidation they manifested an activity against this micro-organism. Ferlin *et al.* (8) and Karbinos *et al.* (9) have suggested that this phenomenon depends on the formation of pelargonic acid, a by-product of the oxidation of fatty acids.

Apparently nonionic surface-active compounds have little, if any, effect on microbial metabolism and do not show any antibiotic activity. Baker *et al.* (10) and Hotchkiss (11) were unable to disclose any inhibitory effect of such compounds in spite of the fact that they were effective as depressants of surface tension. According to Baker *et al.* (12), the cationic surface-active compounds at a physiologic pH are more biologically active than the anionic compounds. Furthermore Baker *et al.* (13), Birkeland *et al.* (14), Ordal and Borg (15), and others have reported that cationic surface-active compounds exerted the same activity against both gram-positive and gram-negative organisms while the anionic surface-active compounds showed a rather selective activity.

Among the cationic surface-active compounds, quaternary ammonium compounds were found to be biologically effective, even in high dilutions. Numerous investigations have been conducted on the antibacterial and antifungal activities of these compounds. Valko and DuBois (16) found that some quaternary ammonium compounds were effective against *Staphylococcus aureus* and *E. typhosa* in concentrations from 1:10,000 down to 1:19,000. Heineman (17), Dunn (18), Howard and Keil (19), Lawrence (20) and others have reported that some of the quaternary compounds possess fungicidal and fungistatic action toward some of the pathogenic fungi in highly diluted concentrations.

In the present study the fungicidal activities of the unsaturated fatty acids prepared from fish oils and their quaternary salts were investigated.

Experimental

Method of Testing Antifungal Activity of Fatty Acids. Several methods of testing fatty acids for their antifungal activity were investigated in the present study. Both the partition method on agar-agar plates and the paper-disc method were used in some tests. However it was found that the method of two-fold dilution, with Sabouraud's peptone-dextrose medium at the pH 6.1, was the most satisfactory and reliable. The fatty acids and fatty acid fractions were emulsified according to the following formula: 0.6 ml. Tween 20; 0.3 ml. propylene glycol; and 9.1 ml. medium. Their activity was tested on three fungi: *Candida albicans, Aspergillus niger,* and *Rhyzopus nigricans.* One drop of the suspension of fungi culture was inoculated into 5 ml. of the mixture, containing various degrees of dilution of the fatty acids. After 48 hrs. the growth of the organism was determined by turbidity of the medium and microscopic examination. The antifungal activity of the material was expressed by the minimum amount of sample for a complete inhibition of growth of the organism.

Material

The following fatty acids and unsaturated fatty acid fractions from fish oil were tested on *Candida albicans:*

a) four saturated fatty acids: caproic, undecanoic, myristic, and palmitic;
b) four unsaturated fatty acids:[2]

---

[1] Aided by a grant from the Collett-Week Company, Ossining, N. Y.