9. Futterman, S., and J. S. Andrews, J. Biol. Chem. *239*, 81–84 (1964).
10. Huang, H. S., and D. S. Goodman, Fed. Proc. *23*, 270 (1964).
11. Booth, V. H., Phytochem. *3*, 229–234 (1964).
12. Kinumaki, T., H. Taguchi and K. Iwasaki, J. Vitaminol. (Kyoto) *9*, 160–167 (1963).
13. Wolfe, D. A., P. Venkato Rao and D. G. Cornwell, Fed. Proc. *23*, 376 (1964).
14. Lowery, O. H., H. R. Roberts, K. Y. Leiner, M. L. Wu and L. A. Farr, J. Biol. Chem. *207*, 1–17 (1954).
15. Abell, L. A., R. B. Levy, B. B. Brodie and F. E. Kendall, J. Biol. Chem. *195*, 357–366 (1952).
16. A.O.C.S. Official Method Ca 5–40.
17. McCarthy, R. D., and A. H. Duthrie, J. Lipid Res. *3*, 117–119 (1962).
18. Holla, K. S., L. A. Horrocks and D. G. Cornwell, J. Lipid Res. *5*, 263–265 (1964).
19. Luddy, F. E., R. A. Barford and R. W. Riemenschneider, JAOCS *37*, 447–451 (1960).
20. Jantzen, E., and H. Andreas, Chem. Ber. *92*, 1427–1437 (1959).
21. Mangold, H. K., and R. Kammereck, Chem. Ind. (London), 1032–1034 (1961).
22. Horrocks, L. A., D. G. Cornwell and J. B. Brown, J. Lipid Res. *2*, 92–94 (1961).
23. Gruger, E. H., Jr., P. W. Nelson and M. E. Stansby, JAOCS *41*, 662–667 (1964).
24. Ackman, R. G., JAOCS *40*, 558–564 (1963).
25. *Ibid*, JAOCS *40*, 564–567 (1963).
26. Fisher, L. R., Rappt. Proces-Verbaux Reunions, Conseil Perm. Intern. Exploration Mer. *153*, 129–136 (1961).
27. Kodama, A. M., and N. Pace, J. Appl. Physiol. *19*, 863–867 (1964).
28. Reiser, R., B. Stevenson, M. Kayama, R. B. R. Choudhury and D. W. Hood, JAOCS *40*, 507–513 (1963).
29. Kayama, M., and Y. Tsuchiya, Tohoku J. Agr. Res. *13*, 229–235 (1962).
30. Ackman, R. G., J. Fisheries Res. Board Can. *21*, 247–254 (1964).
31. Shuster, C. Y., J. R. Froines and H. S. Olcott, JAOCS *41*, 36–41 (1964).

[Received December 28, 1964—Accepted February 19, 1965]

Methods for the Determination of Cyclopropenoid Fatty Acids. VI. A Direct Infrared Absorption Method

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Abstract

A simple rapid method for the estimation of the cyclopropenoid content of glycerides and methyl esters is described based upon the measurement of the characteristic infrared absorptivity of cyclopropenoids at 9.9 μ. Autoxidation products do not interfere, and the sample can be recovered. Equations are given for the calculation of the cyclopropenoid content of both glycerides and methyl esters.

Introduction

WHILE INVESTIGATING METHODS of isolation of cyclopropenoid methyl esters a need arose for a simple rapid method for estimating the cyclopropenoid content of concs. In general, available methods are either unsuitable or require some form of pretreatment of the sample. An infrared (IR) method based upon the characteristic absorptivity of cyclopropenoids at 9.9 μ was used by Varma et al. (1) in an attempt to measure the sterculic acid content of *Sterculia foetida* oil. However, the method involved converting the oil to the free acids and comparing their absorptivity in CS_2 solution with that of a standard. Their standard was a supposedly pure sample of sterculic acid. This procedure cannot be expected to give reliable results since pure sterculic acid is extremely unstable and polymerizes rapidly at room temp (2). Moreover, cyclopropenoid fatty acids are reported to react with carbon disulfide even at room temp (3). The present report deals with a simple rapid method of analysis for glycerides and methyl esters based upon the direct measurement of the 9.9 μ absorptivity and calibrated against a series of standards of accurately known cyclopropenoid concn.

Materials

Sterculia foetida oil, the fatty acids of which contain about 50% of sterculic acid and a small amt of malvalic acid, was used as a source of cyclopropenoid fatty acids for testing the applicability of the method. *Sterculia foetida* seed meats were extracted with several portions of petroleum ether (bp 30–60C) in an explosion-proof Waring Blendor at room temp. The extracts were combined, filtered, and freed from solvent on a rotary evaporator with a nitrogen leak at reduced pressure. Methyl esters were prepared from the oil by methanolysis catalyzed by sodium methoxide (5). The methyl sterculate concn of a portion of the *Sterculia foetida* methyl esters was increased by removal of the saturated esters by urea clathration. The oil, the methyl esters, and the methyl ester conc were passed in petroleum ether solution through an activated alumina column to remove interfering substances, dried over anhydrous sodium sulfate, stripped of solvent on a rotary evaporator at reduced pressure, and their cyclopropenoid acid content determined by HBr titration (4,5) just prior to their use in connection with the calibration measurements. The cyclopropenoid contents of the oil, the methyl esters, and the methyl ester conc so obtained were 52.05, 48.55, and 72.24%, respectively, calculated as sterculic acid.

Experimental and Results

Two series of methyl ester mixtures of accurately known cyclopropenoid content were prepared and used to establish a calibration curve. The first was prepared from the *Sterculia foetida* methyl esters, and the second from the methyl sterculate conc, by mixing with methyl oleate. The IR absorption curves of these mixtures from 9.0 to 10.7 μ were obtained on a Perkin-Elmer Model 21 IR spectrophotometer. The slits were fixed at 151 μ, and the instrument adjusted to have a slight drift upward. The scan was made at a speed of 1 μ per 3 min with a gain of 6, a response of 1, and a suppression of 0. The samples were analyzed in carbon tetrachloride using matched KBr cells of 0.5 mm length at a concn suf-

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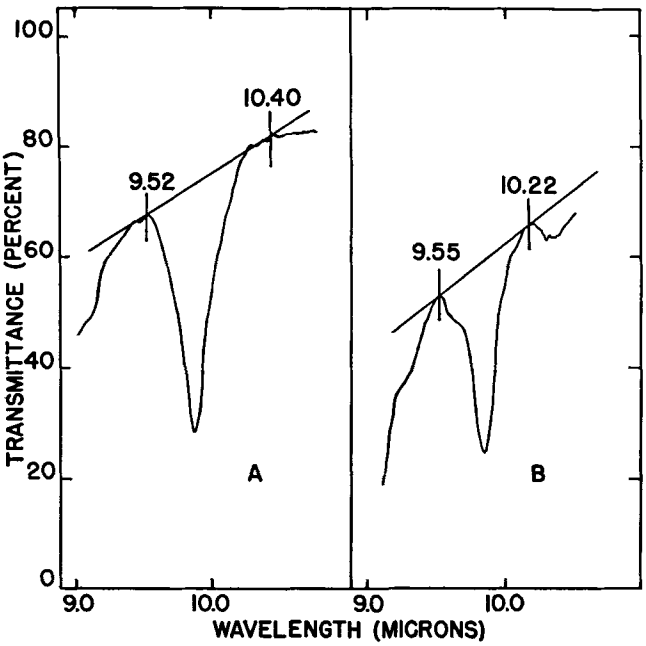

FIG. 1. Typical IR spectra (in CCl_4) illustrating background correction for (A) methyl esters and (B) glycerides.

ficient to give between 20 and 70% transmission at the max, at about 9.9 μ. Pure solvent was used in the reference beam. To correct for background absorption, a baseline was drawn intersecting the curve at 9.52 and 10.40 μ (Fig. 1). The difference between the measured absorbance at the wavelength of max absorption and the baseline at the same wavelength was used to calculate the corrected absorptivity, a; that is, the corrected absorbance divided by the concn in grams per liter and the cell length in centimeters. A similar calibration curve was constructed for the glycerides using a series of *Sterculia foetida*-corn oil mixtures. The IR absorptivities were obtained by the above procedure except that the baseline was drawn through the curve at 9.55 and 10.22 μ. The absorptivities (Table I) plotted against the sterculic acid contents of the samples, fell on straight lines represented by equations (1) and (2), obtained by the method of least squares, for the methyl esters and the glycerides, respectively

$$x = \frac{a - 0.049}{0.00290} \qquad [1]$$

$$x = \frac{a + 0.012}{0.00340} \qquad [2]$$

where x is the percentage of cyclopropenoid moiety as sterculic acid and a is the corrected absorptivity at the 9.9 μ absorption max. This absorptivity may of course vary slightly with the particular spectrophotometer used. It was noted that the proximity of the 9.8 μ methyl ester band resulted in a shift of the max absorption wavelength from 9.90 μ at 72.24% sterculic acid to 9.86 μ at 5.97%. The average deviation for the glycerides, $\pm$0.29, was considerably less than that of the methyl esters, $\pm$0.62.

This method is applicable in the presence of autoxidation products and other substances which interfere with the stepwise HBr titration procedure (5). Absorptivity measurements were made on a series of highly autoxidized samples without prior alumina-column treatment (Table I). The methyl sterculate conc (Sample 1), and two mixtures of

TABLE I

Analyses of Mixtures of *Sterculia foetida* Methyl Esters with Methyl Oleate and *Sterculia foetida* Oil with Corn Oil

Sample No.	% Sterculic acid theory[a]	Corrected absorptivity	% Sterculic acid calculated	Deviation
Calibration series (esters)[b]				
1	72.24	0.257	71.72	−0.52
2	72.24	0.258	72.07	−0.17
3	55.88	0.214	56.90	+1.02
4	48.55	0.188	47.93	−0.62
5	48.55	0.185	46.90	−1.35
6	35.10	0.154	36.20	+1.10
7	34.35	0.150	34.83	+0.48
8	29.73	0.134	29.31	−0.42
9	24.14	0.119	24.14	0.00
10	22.82	0.114	22.41	−0.41
11	14.58	0.090	14.14	−0.44
12	13.31	0.085	12.41	−0.90
Test series (esters)				
13	58.61	0.215	57.24	−1.37
14	37.86	0.163	39.31	+1.45
15	19.11	0.106	19.65	+0.54
16[c]	6.67	0.066	5.86	−0.81
Calibration series (Glycerides)[d]				
17	52.05	0.165	52.06	+0.01
18	52.05	0.166	52.35	+0.30
19	36.49	0.113	36.76	+0.27
20	36.44	0.110	35.88	−0.56
21	27.32	0.082	27.65	+0.33
22	26.70	0.078	26.47	−0.23
23	15.31	0.041	15.59	+0.27
24	14.97	0.038	14.71	−0.26

[a] Determined by HBr titration.
[b] Samples 1, 2, 3, and 8 were prepared using methyl sterculate conc (72.24% sterculic acid). The remaining samples in this series were prepared using *Sterculia foetida* composite methyl esters (48.55% sterculic acid).
[c] *Lavatera trimestris* methyl esters. Cyclopropenoid is methyl malvalate, calculated as sterculic acid.
[d] Samples were prepared from *Sterculia foetida* oil (52.05% sterculic acid).

the conc with methyl oleate were allowed to undergo autoxidation at room temp for 10 days, during which time the sterculic acid content of the conc decreased; for example, from 72.24% to 58.61%. The cyclopropenoid content of these oxidized samples (Samples 13–15) and of a sample of the methyl esters of *Lavatera trimestris* seed oil (Sample 16), which contains both cyclopropenoid and epoxy fatty acids, were accurately determined by a new modification of the stepwise HBr titration procedure (6). It is particularly applicable at high cyclopropenoid concns and in the presence of large amts of interfering substances. The "sterculic acid" contents calculated from equation [1] are in good agreement with the true values obtained by the HBr titration procedure, the average deviation being $\pm$ 1.04%.

The IR method has a number of advantages over other available methods of analysis of glycerides and methyl esters. It is rapid and requires but 30 to 300 mg of sample material depending upon the cyclopropenoid content. The sample requires no pretreatment and can be recovered. The method will be particularly useful at high cyclopropenoid concns, and when the higher accuracy of the stepwise HBr titration procedure is not necessary; e.g., as a control in the preparation of cyclopropenoid concs.

ACKNOWLEDGMENT

J. A. Harris performed the HBr titration analyses on the autoxidized samples.

REFERENCES

1. Varma, J. P., Sharda Dasgupta, Bhola Nath and J. S. Aggarwal, JAOCS *34*, 452 (1956).
2. Nunn, R. J., Chem. Soc. *1952*, 313.
3. Faure, P. K., Nature *178*, 372 (1956).
4. Harris, J. A., F. C. Magne and E. L. Skau, JAOCS *40*, 718 (1963).
5. Harris, J. A., F. C. Magne and E. L. Skau, *Ibid. 41*, 309 (1964).
6. Magne, F. C., J. A. Harris and E. L. Skau, manuscript in preparation.

[Received November 23, 1964—Accepted February 26, 1965]