contained 11.9 μmoles linoleic acid and 2.8 ml 0.1% Tween 20 in 0.1 M borate buffer, pH 9.0. The reaction was started by the addition of 0.2 ml commercial (Nutritional Biochemicals, Cleveland, Ohio) soybean lipoxygenase (0.25 mg/ml in 0.01 M phosphate buffer pH 7.0) and a serum stopper pierced by a 16 gauge needle was fitted quickly on the reaction vessel. This ensured that no pressure changes occurred in the vessel, and the needle was removed immediately after the serum stopper was secured. Under these conditions it required 1.5-2 min for the available oxygen in the reaction medium to be depleted by the lipoxygenase, and oxygen consumption was linear with time and enzyme concentration. At a predetermined time after achieving anaerobic conditions, a 5 ml gas volume was withdrawn with an air-tight syringe and injected into a Varian Aerograph Gas Chromatograph model 1840. A Chromosorb 102 column (6 ft x 1/8 in. stainless steel), operated isothermally at 140 C, was used to separate the volatile compounds. Under the above assay conditions the major peak previously had been identified as pentane by combined gas chromatographic mass spectral analysis (1,3). Peak areas were integrated using an Infotronics CRS-100 digital readout system, and pentane data are presented as integrator area units. Chromatograms of typical and control (heat inactivated enzyme) reactions show that pentane was not produced without native enzyme (Fig. 2). Pentane production using this apparatus was linear 5-20 min (Fig. 3), the delay in linear production being the time necessary for depletion of oxygen from the system. These results confirm the observation of Garssen, et al. (5) in regard to the necessity of anaerobic conditions for the production of pentane at pH 9. Previously, we reported (4) that pentane production is linear with enzyme concentration and has an optimum substrate concentration of 2.1 mM and an optimum pH of 9.

Garssen, et al., (5) and Johns, et al., (4) have found pentane, and St. Angelo, et al., (6) has found hexanal to be the principal secondary reaction products formed by the enzymatic oxidation of linoleic acid by soybean and peanut lipoxygenase, respectively. Peanut (6) and soybean (7) lipoxygenases have been reported to be specific for the C-13 position of linoleic acid, which indicates that the specificity of the secondary reaction is different for the two enzymes. The technique described in this paper could, thus, prove useful in determining whether only hexanal is produced by the peanut enzyme.

This method is rapid, requiring only 20-25 min for each lipoxygenase and pentane assay, and can be easily used or modified for use in measuring any reaction or sequence of reactions where oxygen is consumed or liberated and a volatile product is formed.

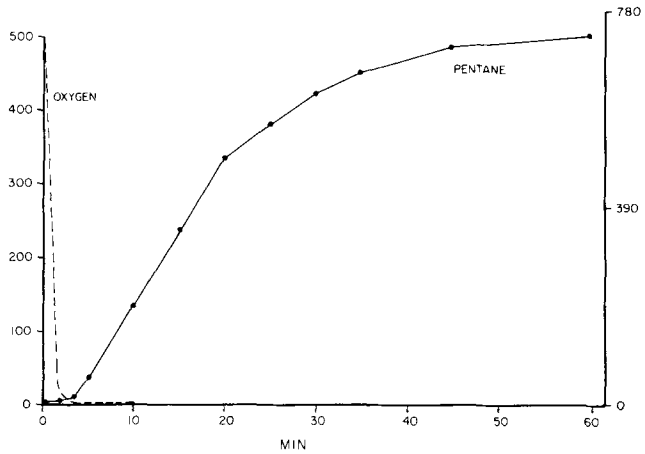

FIG. 3. Pentane production and oxygen consumption as a function of time. (————) pentane: area units x 10^{-3}, (-----) nmoles oxygen.

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REFERENCES

1. Pattee, H.E., J.A. Singleton, E.B. Johns and B.C. Mullin. J. Ag. Food Chem. 18:353-356 (1970).
2. Mitsuda, H., K. Yasumoto, A. Yamamoto and T. Kusano. Agr. Biol. Chem. 31:115-118 (1967).
3. Pattee, H.E., J.A. Singleton and W.Y. Cobb. J. Food Sci. 34:625-627 (1969).
4. Johns, E.B., H.E. Pattee and J.A. Singleton. J. Ag. Food Chem. 21:570-573 (1973).
5. Garssen, G.J., J.F.G. Vliegenthart and J. Boldingh. Biochem J. 122:327-332 (1971).
6. St. Angelo, A.J., H.P. Dupuy and R.L. Ory. Lipids 7:793-795 (1972).
7. Dolev, A., W.K. Rohwedder and H.J. Dutton. Lipids 2:28-32 (1967).

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Infrared Absorption of Methyl *cis*-9,*trans*-11-, and *trans*-10,*cis*-12-Octadecadienoates

ABSTRACT

The ratio of absorptivity at 10.2 μm and 10.6 μm differs between methyl *cis*-9,*trans*-11-, and *trans*-10,*cis*-12-octadecadienoates. For the *cis*-9,*trans*-11-ester, $a_{10.2\ \mu m}/a_{10.6\ \mu m}$ is in the range of 1.1-1.2; for the *trans*-10,*cis*-12-ester, it is 1.3-1.4. These differences in absorptivities are great enough to affect significantly compositions calculated from IR absorption.

INTRODUCTION

The two bands at 10.2 μm and 10.6 μm in the IR absorption spectrum of conjugated *cis,trans*-linoleate isomers have been well established since they were reported by Jackson, et al., (1) in 1952 and have been used for quantitative determination of conjugated *cis,trans*-and *trans,trans*-isomers (2). This quantitative procedure, which has been described in a number of reviews (3-7), assumes that the absorptivities at the maximum of the two bands are the same for all the *cis,trans*-isomers in the sample. Qualitatively the ratio of absorptivities of the two bands $a_{10.2\ \mu m}/a_{10.6\ \mu m}$ also has been used as an indication of purity. Chipault and Hawkins (2), finding a higher ratio for the *trans*-10,*cis*-12 isomer than the *cis*-9,*trans*-11, stated that, although the two compounds may have different

TABLE I

Properties of Methyl *trans*-10,*cis*-12- and *cis*-9,*trans*11-Octadecadienoates

Isomer	$a_{10.2\ \mu m}$	$a_{10.6\ \mu m}$	$\frac{a_{10.2\ \mu m}}{a_{10.6\ \mu m}}$	$a_{233\ nm}$	mp	
trans-10,*cis*-12	0.390	0.284	1.37	88.2	-9.4,	-10.5
cis-9,*trans*-11	0.424	0.382	1.11	91.9	-25,	-26

absorptivities, the more likely possibility was that the *trans*-10,*cis*-12 compound contained small amounts of the *trans,trans*-isomer. Although one would expect no two compounds to have exactly the same absorptivities, little other information is available even for these two isomers derived from the common *cis*-9,*cis*-12 linoleic acid (8).

EXPERIMENTAL PROCEDURES

Since gas chromatography separates conjugated *trans, trans*-esters from the corresponding *cis,trans*-and *cis,cis*-stereoisomers (9,10), the presence of a *trans,trans*-impurity now can be determined. We at the Northern Laboratory reported a value for $a_{10.2\ \mu m}/a_{10.6\ \mu m}$ of 1.30 (11) for methyl *trans*-10,*cis*-12-octadecadienoate, free of *trans, trans*-impurity. We not only confirmed this high value on other samples, but also have found a lower value for the *cis*-9,*trans*-11-isomer. IR absorptivities and other data for samples of the two isomers are given in Table I. IR absorptivities in the table were run in a carbon disulfide solution; absorbances were measured from a base-line tangent to the curve at ca. 9.5 μm and 10.9 μm. UV absorption was measured in isooctane solution. Melting points were run in capillary tubes in a dry ice-cooled bath. Differences in the absorptivity of the 10.6 μm band are such that an absorptivity calculated as 50% *cis*-9,*trans*-11 actually might be 67% *trans*-10,*cis*-12.

Values for the ratio $a_{10.2\ \mu m}/a_{.0.6\mu m}$ were much more reproducible than the individual absorptivities. Six different samples of methyl *trans*-10,*cis*-12-octadecadienoates, including the sample in Table I, gave a range for $a_{10.2\ \mu m}/a_{10.6\ \mu m}$ from 1.30-1.39 with an average of 1.363. Six samples of methyl *cis*-9,*trans*-11-octadecadienoates, including the sample in Table I and some less pure samples from low-temperature crystallization and from dehydrated ricinoleic acid, gave a range for $a_{10.2\ \mu m}/a_{10.6\ \mu m}$ from 1.11-1.18 with an average of 1.147. Statistical analysis of all our data gave a standard deviation of 0.027 for $a_{10.2\ \mu m}/a_{10.6\ \mu m}$ and 95% confidence limits of ±0.058.

The *trans*-10,*cis*-12-ester was isolated by low-temperature crystallization from alkali-isomerized methyl linoleate (11). The *cis*-9,*trans*-11-ester was prepared by partial reduction of eleostearic acid with hydrazine (12); the diene fraction as methyl esters was separated by countercurrent distribution (13); and *cis*-9,*trans*-11-octadecadienoate from the α-eleostearic acid was separated from *trans*-9-*trans*-11-octadecadienoate from the β-eleostearic acid and from other diene isomers by argentation countercurrent distribution (14). Gas chromatography showed the absence of *trans,trans*-conjugated esters. Other samples of *cis*-9,*trans*-11-isomer from the filtrate of the *trans*-10,*cis*-12 preparation contained impurities, and samples from dehydration of ricinoleic acid showed some double bond migration.

Double bond positions were determined by partial reduction with diimide generated from potassium azodicarboxylate (15). This procedure is convenient for reducing small samples intended for chromatographic separation of methyl esters. When methyl esters are reduced with hydrazine, some hydrazide forms; and, when acids are reduced with hydrazine, free acids must first be prepared and reesterified. The monoene fraction was separated on a rubber column (16); the *cis*-and *trans*-monoenes were separated on a silver resin colum (17). Double bond positions of the *cis*-and *trans*-monoenes were determined by ozonization (18). The *trans*-10,*cis*-12 sample was estimated to be greater than 95% pure with *cis*-9,*trans*-11 as the principal inpurity. The *cis*-9,*trans*-11 sample yielded only *cis*-9 and *trans*-11 monoenes.

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REFERENCES

1. Jackson, J.E., R.F. Paschke, W. Tolberg, H.M. Boyd, and D.H. Wheeler, JAOCS 29:229 (1952).
2. Chipault, J.R., and J.M. Hawkins, Ibid. 36:535 (1959).
3. O'Connor, R.T., in "Encyclopedia of Spectroscopy," Edited by G.L. Clark, Reinhold Publishing, New York, N.Y., 1960, p. 420.
4. Rao, C.N.R., "Chemical Applications of Infrared Spectroscopy," Academic Press, New York, N.Y., 1963, p. 448.
5. Chapman, D., "The Structure of Lipids by Spectroscopic and X-Ray Techniques," John Wiley and Sons, New York, N.Y., 1965, p. 83.
6. Chapman, D., JAOCS 42:353 (1965).
7. O'Connor, R.T., in "Fatty Acids," Second Edition, Part 5, Edited by K.S. Markley, Interscience Publishers, New York, N.Y., 1968, p. 3355.
8. Nichols, P.L., Jr., S.F. Herb, and R.W. Riemenschneider, J. Amer. Chem. Soc. 73:247 (1951).
9. Keppler, J.G., in "The Chemistry and Technology of Edible Oils and Fats," Edited by J. Devine and P.N. Williams, Pergamon Press, New York, N.Y., 1961, p. 91-92.
10. Body, D.R., and F.B. Shorland, JAOCS 42:5 (1965).
11. Scholfield, C.R., and S. Koritala, Ibid. 47:303 (1970).
12. Mikolajczak, K.L., and M.O. Bagby, Ibid. 42:43 (1965).
13. Scholfield, C.R., J. Nowakowska, and H.J. Dutton, Ibid. 37:27 (1960).
14. Scholfield, C.R., E.P. Jones, R.O. Butterfield, and H.J. Dutton, Anal. Chem. 35:1588 (1963).
15. Hamersma, J.W., and E.I. Snyder, J. Org. Chem. 30:3985 (1965).
16. Hirsch, J., Colloq. Int. Centre Nat. Rech. Sci. (Paris) 99:11 (1961).
17. Emken, E.A., C.R. Scholfield, and H.J. Dutton, JAOCS 41:388 (1964).
18. Johnston, A.E., and H.J. Dutton, Ibid. 49:98 (1972).

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