Production of an artifact during methanolysis of lipids by boron trifluoride–methanol

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SUMMARY The production of an artifact by the methanolysis of total lipids of *Ascaridia galli* with boron trifluoride-methanol was dependent on the reaction time and the age of the reagent used. It was shown that this artifact was produced from oleic acid. Methanolysis of total lipids with sodium methoxide was complete in 10 min, and no artifact was produced.

SUPPLEMENTARY KEY WORDS *Ascaridia galli* · sodium methoxide · oleic acid · neutral lipids · phospholipids

LOUGH (1) observed the production of methoxy-substituted fatty acids from unsaturated fatty acids methylated with boron trifluoride-methanol reagent obtained commercially in England. Furthermore, he stated that all methoxy-substituted fatty acids have a carbon number of 21.5. Three different samples of the reagent gave different yields of this artifact from oleic acid (30, 56, and 68%). Morrison and Smith (2) attributed the presence of this artifact to the high concentration of boron trifluoride (50% w/v). They found that 14% (w/v) boron trifluoride–methanol caused no greater loss of unsaturated esters than other reagents which were studied; they suggested that boron trifluoride at a concentration of 14% (w/v) was a satisfactory and generally applicable methanolysis reagent. Contrary to Morrison and Smith (2), in this laboratory the use of 14% (w/v) boron trifluoride–methanol has produced results which support the findings of Lough (1).

A study of the neutral lipids and phospholipids of *Ascaridia galli* revealed an unknown peak on gas–liquid chromatograms of the fatty acid methyl esters. This unknown fraction comprised 25–30% of the total fatty acid methyl esters, and it had a retention time of 2.52 relative to 18:0 and a carbon number of 21.4 on an EGSS-X column (Applied Science Laboratories, Inc., State College, Pa.) (tritium or hydrogen flame detector; cell bath, 225°C, column 186°C, flash heater 204°C). Hydrogenation of the methyl esters (3) showed that this unknown compound was saturated. Methanolysis of the lipid sample had been done according to the method of Morrison and Smith (2) with boron trifluoride–methanol (14% w/v) obtained commercially (Applied Science Laboratories, Inc.). An identical lipid sample was treated with sodium methoxide (4), and chromatograms of the fatty acid methyl esters from both methods of methanolysis were compared. Methanolysis with sodium methoxide yielded fatty acid methyl esters which had approximately the same composition as methyl esters produced by treatment of the lipid with boron trifluoride–methanol except for a marked increase in oleic acid and the complete disappearance of the unknown peak.

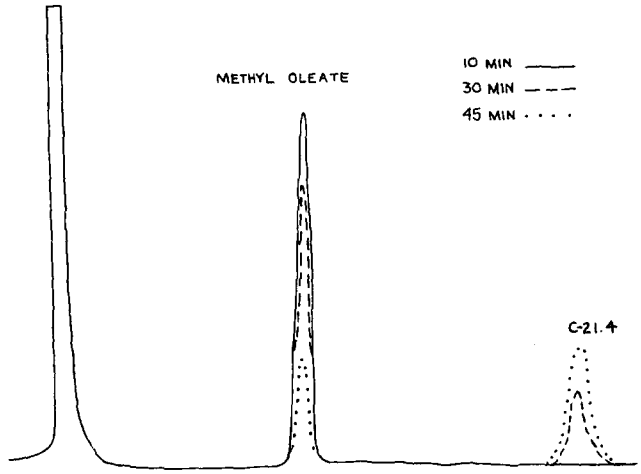

FIG. 1. Superimposed gas–liquid chromatograms of methyl oleate and an unknown peak after esterification of pure oleic acid with boron trifluoride–methanol for 10, 30, and 45 min.

Complete methanolysis of phospolipids with boron trifluoride–methanol according to the procedure of Morrison and Smith (2) requires reaction times up to 90 min. Methanolysis with sodium methoxide, on the other hand, is complete for phosphatides, esters, free fatty acids, etc. in 10 min as seen by thin-layer chromatography (5). To test whether an artifact is produced by boron trifluoride–methanol, chromatographically pure standard oleic acid and triolein (The Hormel Institute, Austin, Minn.) were treated with boron trifluoride–methanol for different lengths of times (2, 10, 30, and 45 min) or with sodium methoxide. Esterification of oleic acid with boron trifluoride–methanol resulted in a gradual increase of the unknown peak and a concomitant decrease of methyl oleate (Fig. 1); only the methyl oleate peak appeared when sodium methoxide was used. Methanolysis of triolein with boron trifluoride–methanol also resulted in two peaks, and it was shown by gas–liquid chromatography that 45 min reaction time produced the greatest reduction of methyl oleate and the greatest amount of artifact from this triglyceride. From these results, it was reasonable to assume that the boron trifluoride reagent altered oleic acid in such a manner as to produce a saturated lipid artifact, and that the amount of artifact produced was a function of the methylation time.

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Since boron trifluoride–methanol reagent has a stated shelf life of 4 months, it was possible that the reagent which had been used was too old (no expiration date appeared on the vials). Therefore, identical lipid samples of *Ascaridia galli* were treated with two different "fresh" lots of boron trifluoride–methanol, one obtained from the distributor (Lawshe Instrument Co., Inc., Bethesda, Md.) and one obtained directly from the manufacturer (Applied Science Laboratories, Inc.). Comparison of the gas–liquid chromatograms of the methyl esters showed that the artifact was present in esters produced by treatment of the reagent received from the distributor, although in greatly reduced quantities. None was apparent in the stock reagent received from the manufacturer. It is, therefore, apparent that the artifact also is a function of the lot and(or) age of the reagent.

Our conclusion is that methanolysis with boron trifluoride–methanol may not always be reliable for analysis of total fatty acids and may alter the fatty acid pattern by as much as a 30% reduction of methyl oleate with an increased production of the artifact.

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References

1. Lough, A. K. 1964. *Biochem. J.* **90:** 4c.
2. Morrison, W. R., and L. M. Smith. 1964. *J. Lipid Res.* **5:** 600.
3. Farquhar, J. W., W. Insull, Jr., P. Rosen, W. Stoffel, and E. H. Ahrens, Jr. 1959. *Nutr. Abstr. Rev.* **17** (Suppl.): No. 8, Pt. II.
4. Marinetti, G. V. 1962. *Biochemistry.* **1:** 350.
5. Dunlap, B. E. 1968. M.S. Thesis. University of Maryland. College Park, Maryland.