On methylation of unsaturated acids using boron trihalide–methanol reagents

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SUMMARY Methanolysis of unsaturated fatty acids and triglycerides was carried out with boron trihalide–methanol reagents of various ages. Boron trichloride–methanol produced esters apparently free from contaminants; boron trifluoride caused some loss of highly unsaturated esters after 90 min at 100 or 120°C in Teflon-lined screw-cap vials and at 120°C in ampoules. The losses were more marked when the reactions were carried out in vinyl-lined screw-cap vials. Noticeable losses did not occur with any of the vessel types when BCl_3–CH_3OH was the methylating agent. Long-term studies at 80°C further demonstrated that BCl_3–CH_3OH caused less loss of unsaturated acid than did BF_3–CH_3OH.

SUPPLEMENTARY KEY WORDS methyl esters · boron trifluoride–methanol · boron trichloride–methanol · unsaturation · fatty acids · triglycerides · methoxy esters

Boron trihalides (especially boron trifluoride) in methanol are widely used reagents for preparation of methyl esters of carboxylic acids. Morrison and Smith (1) carried out an extensive study to determine the conditions necessary for conversion of various lipids to methyl esters with the BF_3 reagent. Other investigators have used differing esterification conditions, such as longer reaction times (2–4), reaction temperatures up to 160°C (3), and BF_3 concentrations up to 50% (5).

Reports of formation of artifacts from unsaturated acids by BF_3–CH_3OH have appeared. Lough (5) identified the artifact produced by 50% BF_3 as a methoxy ester. This identification has been supported by Coppock, Daniels, and Eggitt (6). Recently Fulk and Shorb (7) reported that an artifact is formed when oleic acid-containing preparations are treated with BF_3–CH_3OH. This artifact had retention characteristics similar to the methoxy esters. The authors suggested that artifact formation was a function of the reaction time at 100°C and the lot or age of the reagent, or both.

Artifacts resembling fatty acid esters have reportedly been formed from HCl in dry CH_3OH (8). Stein, Slawson, and Mead (9) have briefly reviewed the formation of artifacts during other methylation procedures.

Screw-cap vials have been widely used as methylation reaction vessels. The caps used are generally lined with Teflon (5), but sometimes the nature of the liner is not stated (3). Kishimoto and Radin (10) found that, for methylation with HCl–CH_3OH, Teflon-lined caps were not uniformly leakproof.

Since in our laboratory we use boron trihalide–methanol reagents for methylation of a variety of lipids and are concerned about the possible loss of unsaturated acids, with or without the formation of artifacts, we investigated the effects of the age of the methylation reagents, reaction temperature and time, and type of reaction vessel on the recovery of unsaturated fatty acids and artifact formation.

Materials. Trilinolenin and oleic, linoleic, and linolenic acids were obtained from Sigma Chemical Co., St. Louis, Mo. Palmitoleic acid and methyl elaidate were from Applied Science Laboratories Inc., State College, Pa. Ethyl heneicosanoate was synthesized by the author. Various lots of BF_3–CH_3OH and BCl_3–CH_3OH were used and will be described where used. The Teflon-lined screw-cap vials were Kimble no. 45066-A; the vinyl-lined screw-cap vials were Kimble no. 60910.

Results. The formation of artifact with various methylation reagents was studied as follows. Approximately 4 mg of palmitoleic, oleic, linoleic, and linolenic acids and methyl elaidate were added individually to glass ampoules, and approximately 1 ml of one of the methylating reagents was added. Boron trifluoride–methanol reagent was obtained from Supelco, Inc., Bellefonte, Pa. One lot of this reagent was used 5.5 months after purchase. Another lot from the same supplier was on hand about a year. A dated 3.5-year-old bottle of boron trichloride–methanol reagent from Applied Science Laboratories was also used. The ampoules were flushed with nitrogen and sealed. The esterification was carried out at 120°C for 90 min. Esters were extracted with hexane, washed with H_2O, and dried over anhydrous Na_2SO_4. They were analyzed on a Barber-Colman 5000 equipped with a 7.5% DEGS column 8 ft in length. Column temperature was 190°C, and nitrogen carrier gas flow rate was 70 ml/min. Hydrogen flame detectors were used.

The 3.5-year-old BCl_3–CH_3OH solution was used to esterify palmitoleic, oleic, and linolenic acids. It produced ester preparations in which full-scale peaks of the major component were accompanied by only very small additional peaks (Fig. 1*A*). These peaks were usually 1% of chart width or less and corresponded to the impurities expected in the acid. BF_3–CH_3OH reagent was used for methanolysis of all of the free acids and methyl elaidate. Both the 5.5-month- and 1-yr-old BF_3–CH_3OH solutions produced larger quantities and larger numbers of additional peaks than the BCl_3–CH_3OH reagent (Fig. 1*B*). Some of these peaks correspond to impurities which would be expected to occur with the acids, but each also had material which eluted

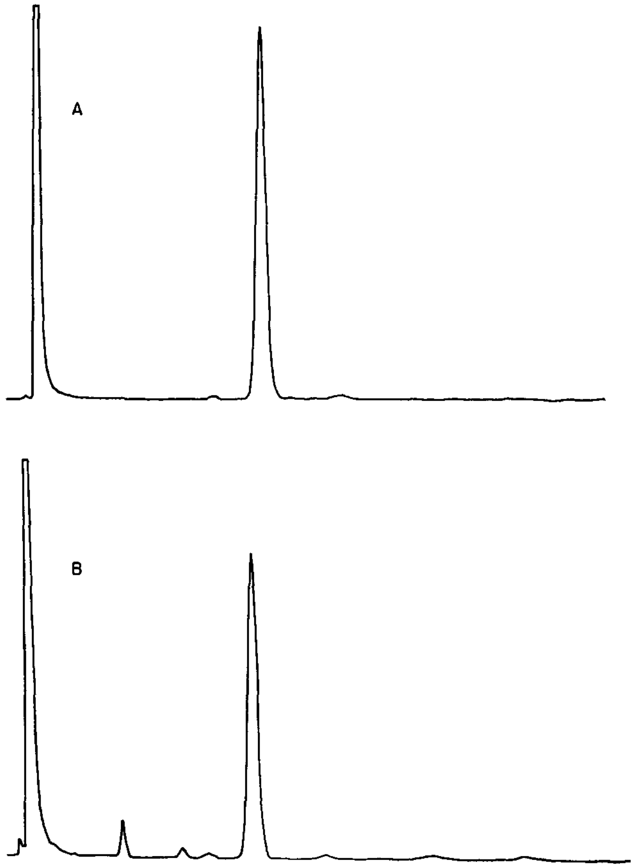

FIG. 1. Linoleic acid was methylated with boron trichloride (3.5 yr old) or boron trifluoride (5.5 months or 1 yr old) at 120°C for 90 min in glass ampoules under a nitrogen atmosphere. Part *A* shows the ester produced with the BCl_3–CH_3OH. A chromatogram typical of those from BF_3–CH_3OH, regardless of the age of the reagent, is shown in part *B*.

later than the major component. These later-eluting compounds could correspond to the methoxy esters of Lough (5). However, it must be emphasized that even under these strenuous reaction conditions of 120°C for 90 min the quantities of these materials do not approach the artifact reported by Fulk and Shorb (7).

To determine the effect of the reaction vessel on the production of artifacts, trilinolenin was methylated with 5.5-month-old BF_3–CH_3OH for 90 min at 120°C under nitrogen in glass ampoules, Teflon-lined screw-cap vials, and vinyl-lined screw-cap vials. The esters were recovered and chromatographed as before. As can be seen in Fig. 2*A*, esters from the ampoules produced only a slight base line rise with a retention time considerably longer than the linolenate. The methyl linolenate peak had a leading shoulder which was present on all chromatograms of this ester. Since the supplier did not state the content of positional isomers, the shoulder was assumed to arise from partial resolution of positional isomers of methyl octadecatrienoates.

FIG. 2. Trilinolenin was methylated with 5.5-month-old boron trifluoride–methanol for 90 min at 120°C in a glass ampoule (*A*), a Teflon-lined screw-cap vial (*B*), or a vinyl-lined screw-cap vial (*C*). The destruction of the linolenic acid increased with decreasing inertness and tightness of the reaction vessel.

Hofstetter, Sen, and Holman (11) have determined that methyl γ-linolenate has an equivalent chain length 0.4 less than that of methyl α-linolenate on DEGS.

Fig. 2*B* shows the esters from the Teflon-lined screw-cap vials to contain a larger number of extraneous products, including a major component at carbon number 21.9.

As can be seen in Fig. 2*C*, there was almost complete destruction of the linolenic acid when vials with caps lined with vinyl were used; however, no artifact with carbon number 21.9 which might correspond to the methoxy ester was found. The less satisfactory performance of the screw-cap vials may be due to the inability to prevent loss of CH_3OH, so that with time the concentration of BF_3 increases.

The amount of artifact shown in Fig. 2*B* was not routinely found, and seemed to depend on the lot of

BF_3–CH_3OH used. The experiment was repeated using reagents which were 2 and 5 months old (both from Supelco). The 5-month reagent produced a series of small peaks preceding the methyl linolenate, but only a broad, diffuse later peak. A blank reaction mixture (containing no lipid) had these same peaks, except for the broad later peak. The 2-month-old reagent did not produce noticeable extraneous peaks. These reagents were used in a repeat of this experiment 4 months later with similar results. No large new peaks appeared, and the sizes of the small peaks were not substantially changed. Thus, age of reagent alone does not account for the formation of artifact.

In view of the conversion of linolenic acid to other products, it was desirable to study further the losses under various esterification conditions. A solution containing 5.2 mg of linolenic acid and 4.9 mg of stearic acid per ml of benzene was prepared. Reaction mixtures were prepared by adding 0.2 ml of this mixture to an ampoule, a screw-cap vial with Teflon cap liner, or a screw-cap vial with vinyl cap liner. Methylation was carried out with BCl_3–CH_3OH solutions 2 months and 4 yr old and with BF_3–CH_3OH solutions 2 months, 5 months, and approximately 2 yr old. Reactions were carried out in the three types of vessels described previously at 100°C and 120°C for 15, 30, and 90 min. In another study these fatty acids were esterified at 80°C for up to 10 days using 2-month BCl_3–CH_3OH and BF_3–CH_3OH solutions in ampoules and Teflon-lined screw-cap vials.

The ratio of the area of the linolenate peak to that of the stearate peak was determined for each reaction. Since the reaction mixture contained linolenate and stearate in amounts of 5.2 and 4.9 mg, respectively, this ratio should be 1.06. The experimental ratios (averages of at least duplicate determinations) are shown in Table 1. That none of the ratios is equal to the theoretical could be partially explained by the fact that the acids used were not stated to be anhydrous.

A lower ratio was consistently found for the 2-yr BF_3–CH_3OH. A number of extraneous peaks, with retention times up to 9.2 times longer than that of methyl stearate, were consistently found with this reagent. One of these extraneous peaks had a retention identical to stearic acid, thus increasing the size of the stearic acid peak and lowering the ratio. These peaks were present in blanks as well as in the unheated reagent. This reagent was the only one used that was packaged in tear-away sealed bottles which incorporate rubber plugs. Thus, the possibility exists that these peaks resulted from the long-term exposure of the reagent to the rubber plug.

No loss of linolenate was apparent at 15 or 30 min regardless of reagent, temperature, or reaction vessel. At 90 min, however, the BF_3–CH_3OH reagents caused some loss of linolenate at both temperatures in the Teflon-lined screw-cap vials and at 100°C in the vinyl-lined screw-cap vials, with essentially complete loss at 120°C in the latter type of vessel.

Noticeable losses with the BCl_3–CH_3OH generally did not occur. The results of the long-term study are shown in Fig. 3. Again, it is apparent that BF_3–CH_3OH caused greater loss of linolenate than did the BCl_3–CH_3OH. With the BF_3–CH_3OH, the type of reaction vessel did not have much effect, while a greater effect is seen in the case of the BCl_3–CH_3OH. The BCl_3–

TABLE 1 Relative Recovery of Methyl Linolenate and Methyl Stearate

		Reaction Time					
Vessel	Reagent and Age	15 min		30 min		90 min	
Temperature, °C		100	120	100	120	100	120
Glass ampoule	BCl_3, 4 yr	0.93	0.91	0.95	0.95	0.94	0.96
	BCl_3, 2 mo	0.94	0.93	0.99	0.92	1.01	0.94
	BF_3, 2 yr	0.86	0.84	0.92	0.82	0.89	
	BF_3, 5 mo	0.94	0.87	0.89	1.01	0.94	0.94
	BF_3, 2 mo	0.99	0.89	0.98	0.92	0.92	0.89
Teflon screw cap	BCl_3, 4 yr	0.92	1.02	1.00	0.93	1.00	0.88
	BCl_3, 2 mo	0.94	1.00	1.02	0.90	0.90	0.86
	BF_3, 2 yr	0.82	0.95	0.88	0.85	0.74	0.85
	BF_3, 5 mo	0.93	0.90	0.99	0.94		0.68
	BF_3, 2 mo	0.94	0.90	0.95	0.93	0.78	0.64
Vinyl screw cap	BCl_3, 4 yr	0.93	0.91	0.87	0.96	0.95	0.92
	BCl_3, 2 mo	0.93	0.96	0.99	0.91	0.96	0.90
	BF_3, 2 yr	0.84	0.86	0.82	0.90	0.82	0.16
	BF_3, 5 mo	0.91	0.98	1.00	0.97	0.34	0.06
	BF_3, 2 mo	0.98	0.97	0.94	0.94	0.50	0.00

A solution containing 1.04 mg of linolenic and 0.98 mg of stearic acid in 0.2 ml of benzene was methylated with 1 ml of the reagents above under the conditions indicated. The ratios are of the area of the methyl linolenate peak to the area of the methyl stearate peak. Each value represents at least two determinations.

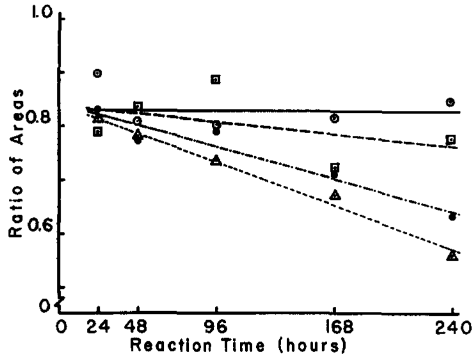

Fig. 3. The solution of linolenic acid and stearic acid described in Table 1 was esterified with BCl_3–CH_3OH and BF_3–CH_3OH (both 2 months old) in ampoules and Teflon-lined screw-cap vials for up to 10 days at 80°C. The points shown represent the averages of the ratios of area of methyl linolenate peak to area of methyl stearate peak from duplicate experiments. The line —⊙— represents BCl_3–CH_3OH in ampoules, —⊡— represents BCl_3–CH_3OH in screw-cap vials, – –△– – represents BF_3–CH_3OH in ampoules, and —·●—· represents BF_3–CH_3OH in screw-cap vials. The slopes of the lines were determined by the method of least squares.

CH_3OH reaction mixtures in the Teflon-lined screw-cap vials went dry much more frequently than the BF_3–CH_3OH mixtures in similar vessels. This probably accounts for much of the scatter observed for that reagent in those vessels.

This long-term study is of interest in view of the reports of Hyun, Vahouny, and Treadwell (3) and of Marshall, Erickson, and Folsom (4) that long periods are required for production of methyl esters from sterically hindered acids. It is necessary to know what would become of any highly unsaturated acids which might be present if these procedures are to be applied to biological materials. It appears that BCl_3–CH_3OH would be the reagent of choice for such procedures and that ampoules would be the reaction vessel of choice.

During the late stages of this investigation, a limited quantity of 3.5-yr-old BF_3–CH_3OH, packaged in ampoules (Applied Science), became available. This material was used for methylations in ampoules at 80°C for 24 hr and in Teflon-lined screw-cap vials at 120°C for 90 min and at 80°C for 24 hr. At 80°C for 24 hr, the ratio of linolenate to stearate was approximately 10% lower from the ampoules and 20% lower from the vials than when the 2-month-old reagent was used. At 120°C for 90 min, the linolenate was always quantitatively destroyed. In no instance, however, were measurable quantities of methoxy ester produced, as found by Lough (5) or Fulk and Shorb (7).

Conclusions. These results indicate that although BF_3–CH_3OH is capable of producing products other than the desired methyl esters from fatty acids under very harsh conditions, highly unsaturated fatty acids are not destroyed under the more conventional methylation conditions if the reaction is carried out under inert conditions. Aging of the BF_3–CH_3OH solution did not seem to lead to the production of artifacts, in contrast to the suggestion of Fulk and Shorb (7). Under no conditions were artifacts formed in the quantities observed by these authors. However, various reagent lots did contain extraneous material, and these results cannot rule out the possibility of lot variations which might cause artifact formation. BCl_3–CH_3OH and ampoules appear to be the reagent and vessel of choice to avoid destruction of highly unsaturated fatty acids under prolonged or harsh reaction conditions.

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