FISHERIES AND MARINE SERVICE

Translation Series No. 4238

Some problems of the preparation of fatty acid methyl ester using boron trifluoride-methanol reagent

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Original title: Sanfukkahoso-metanoru shiyaku ni yoru Shibosan no mechiruesuteruka ni okeru mondaiten

From: Yukagaku 24(1): 59-61, 1975

Translated by the Translation Bureau (PS)
Multilingual Services Division
Department of the Secretary of State of Canada

Department of Fisheries and the Environment
Fisheries and Marine Service
Halifax Laboratory
Halifax, N.S.

1978

8 pages typescript

DEPARTMENT OF THE SECRETARY OF STATE
TRANSLATION BUREAU

MULTILINGUAL SERVICES
DIVISION

CANADA

SECRÉTARIAT D'ÉTAT
BUREAU DES TRADUCTIONS

DIVISION DES SERVICES
MULTILINGUES

F 0mA 4234

TRANSLATED FROM – TRADUCTION DE	INTO – EN
Japanese	English

AUTHOR – AUTEUR

Hajime Seino, Naoko Chihara and Shoichiro Watanabe

TITLE IN ENGLISH – TITRE ANGLAIS

Some Problems of the Preparation of Fatty Acid Methyl Ester using Boron Trifluoride-Methanol Reagent

TITLE IN FOREIGN LANGUAGE (TRANSLITERATE FOREIGN CHARACTERS)
TITRE EN LANGUE ÉTRANGÈRE (TRANSCRIRE EN CARACTÈRES ROMAINS)

Sanfukkahoso-metanoru shiyaku ni yoru Shibosan no mechiruesuteruka ni okeru mondaiten

REFERENCE IN FOREIGN LANGUAGE (NAME OF BOOK OR PUBLICATION) IN FULL. TRANSLITERATE FOREIGN CHARACTERS.
RÉFÉRENCE EN LANGUE ÉTRANGÈRE (NOM DU LIVRE OU PUBLICATION), AU COMPLET, TRANSCRIRE EN CARACTÈRES ROMAINS.

Yukagaku

REFERENCE IN ENGLISH – RÉFÉRENCE EN ANGLAIS

Journal of Japan Oil Chemists' Society

PUBLISHER – ÉDITEUR	DATE OF PUBLICATION / DATE DE PUBLICATION			PAGE NUMBERS IN ORIGINAL / NUMÉROS DES PAGES DANS L'ORIGINAL
	YEAR / ANNÉE	VOLUME	ISSUE NO. / NUMÉRO	59–61
PLACE OF PUBLICATION / LIEU DE PUBLICATION				NUMBER OF TYPED PAGES / NOMBRE DE PAGES DACTYLOGRAPHIÉES
	1975	24	1	8

REQUESTING DEPARTMENT / MINISTÈRE-CLIENT: Environment

BRANCH OR DIVISION / DIRECTION OU DIVISION: Fisheries

PERSON REQUESTING / DEMANDÉ PAR: Allan T. Reid

YOUR NUMBER / VOTRE DOSSIER N°:

DATE OF REQUEST / DATE DE LA DEMANDE: 27/4/78

TRANSLATION BUREAU NO. / NOTRE DOSSIER N°: 1486819

TRANSLATOR (INITIALS) / TRADUCTEUR (INITIALES): P.S.

MAY - 5 1978

UNEDITED TRANSLATION
For information only
TRADUCTION NON REVISÉE
Information seulement

SOS-200-10-6 (REV. 2/68)
7530-21-029-5333

Secretary of State / Secrétariat d'État

TRANSLATION BUREAU / BUREAU DES TRADUCTIONS

MULTILINGUAL SERVICES DIVISION / DIVISION DES SERVICES MULTILINGUES

Fd mar 4 2 3 F

CLIENT'S NO. / N° OU CLIENT	DEPARTMENT / MINISTÈRE	DIVISION/BRANCH / DIVISION/DIRECTION	CITY / VILLE
	Environment	Scientific Info. & Publications Fisheries	
BUREAU NO. / N° DU BUREAU	**LANGUAGE / LANGUE**	**TRANSLATOR (INITIALS) / TRADUCTEUR (INITIALES)**	
1486819	Japanese	PS	MAY - 5 1978

Some Problems of the Preparation of Fatty Acid Methyl Ester Using Boron Trifluoride-Methanol Reagent

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The procedure for preparation of fatty acid methyl ester using boron trifluoride-methanol reagent was examined by varying the reaction conditions. Byproducts were observed to occur during the esterification of oleic and linolic acids. The amounts of these byproducts depended on the relative amounts of the unsaturated fatty acids against the amount of boron trifluoride.

One of the byproducts formed from oleic acid was confirmed to be a methoxy derivative of methyl stearate. The another formed from oleic acid was believed as methyl substituted methyl stearate.

1. Foreword

The fatty acid methyl esterification method based on trifluoride-methanol reagent was studied by Mitchell et al[1] and Metcalfe et al[2)3)]. It is presently adopted as official method by the AOCS and widely used as sample preparation method for the analysis of fatty acids by GC. Yet it has been reported that when performing esterification by this method, byproducts induced from the unsaturated acids are sometimes generated depending on the reaction conditions and that sometimes the unsaturated acid methyl decreases[4)5)]. The conditions and the extent of byproduct generation or unsaturated acid loss have not yet been made quite clear. Our main purpose in this report is to show the range utilizable by this method as method to prepare samples for GC. Using trifluoride methanol

reagents of various concentrations we varied the conditions of fatty acid esterification. We will report here the results of our examination regarding the degree of esterification and of byproducts generation.

2. Experiment

2.1 Material

2.1.1 Fatty acids: the samples are lauric acid, palmitic acid, stearic acid, oleic acid and linolic acid. We methyl-esterified these acids with diazomethane and examined the purity by GC. In all cases, it was above 99%.

2.1.2 Trifluoride-methanol reagents: we diluted trifluoride-methanol complex of the Morita Chemical Industries (concentration 68%) with methanol and used it at the respective concentrations of 50%, 25%, 12.5%, 6.25%. It will be abbreviated hereafter at BF_3-methanol.

2.2 Method of the Experiment

For the methyl-esterification, we followed roughly the AOCS method Ce-2-66 and proceeded by changing such things as the BF_3-methanol concentration, the volume of sample gathered, the reaction time. We measured the acid value, the IR and the UV spectra of the products obtained, examined the degree of esterification and of isomerization and examined by GC the degree of the byproducts generated. With regard to the esterification products of oleic acid, we isolated the byproducts generated by apportioning GC and examined their structure by IR, NMR, and mass spectrum. We used the following apparatuses.

GC: Hitachi Gas Chromatograph Type 063

IR: Hitachi Infrared Spectrophotometer Type 215

NMR: Hitachi R-24 type Nuclear Magnetic Resonator

MS: Nippon Denshi JMS-OIS mass analyzer

3. Results of the Experiment

3.1 Amount of free fatty acids and degree of isomerization in the esterification products.

We took 1 g sample of every acid and adding to each 12 ml of BF_3-methanol at various concentrations, carried out the reactions. We measured the acid values of the products obtained and found the contents of free acids. The results showed that regardless of the concentration of BF_3-methanol used, the amount of free fatty acid in the products was extremely small (<0.8%).

We measured the IR spectrum of the esterified oleic acid. The examination of the degree of trans-isomerization showed some trans-isomerization when using 25% BF_3-methanol and an approximate 5% degree of trans-isomerization when using 50% BF_3-methanol. We also measured the UV spectrum of the esterification products of linolic acid and examined the contents of conjugate diene. We saw almost no conjugation.

3.2 Decrease in unsaturated acids and generation of byproducts.

We prepared 1 g samples by mixing roughly equal amounts of each fatty acid. Each sample was esterified with 12 ml of BF_3-methanol at various concentrations. We performed the GC analysisof the products. The results are shown in Table 1.

As evident from the Tables, whatever the concentration of BF_3-methanol utilized, the peak of unsaturated acid is lower than that of the saturated one and we note that the tendency grows sharper as the BF_3 concentration rises. Particularly when using 50% BF_3-methanol, the peaks of oleic and of linolic acids are almost lost. On the other hand an unknown peak is seen at the ECL value 17.2. We further esterified 1 g of oleic acid with 12 ml of 12.5% BF_3-methanol and performed the GC of the products obtained.

We noted that as shown in Fig. 1, there are two peaks (ECL values: 17.2, 21.4) although very small besides the oleic acid methyl peak. These byproducts will be called hereafter X and Y.

3.3 The effect of the amount of sample gathered and of the reaction time upon the generation of byproducts.

In order to examine in further details the byproducts generated by the esterification of unsaturated acids, we used each time 12 ml of 50% BF_3-methanol and esterified separately 500 mg and 200 mg of oleic acid as well as 200 mg of linolic acid. Fig. 2 shows the results of the GC of the products.

X and Y peaks are noted outside oleic acid methyl when using 500 mg of oleic acid. In the case of 200 mg, the oleic acid methyl and the Y peaks are almost entirely lost and X only was seen. As the /61 graph shows, both X and Y seem to be constituted by components of more than two kinds. We were unable to separate them entirely by GC using EGS, DEGS, SE-30 as stationary phases. In the case of linolic acid, a byproduct peak was seen at the ECL value 22.5 with a 500 mg sample. This byproduct will be called hereafter Z. Even when decreasing the sample to 200 mg, no byproduct peak was seen except for some increase in Z.

After this, in order to examine the effect of the reaction time, we took 1 g each of oleic acid and of linolic acid. Using 12 ml of 12.5% BF_3-methanol, we esterified while extending the reaction time to 10 min, 20 min, 30 min and examined the products by GC. The result is that in the case of oleic acid, the byproduct Y increased a little at a time when the reaction time was extended. However we noted almost no increase of X. Almost no generation of byproducts was noted in the case of linolic acid.

3.4 Isolation and analysis of byproducts

We obtained separately the byproducts X,Y by apportioning GC from samples prepared by esterifying 500 mg of oleic acid with 50% BF_3-methanol. The separate obtention conditions were as follows.

Column: steel, ϕ5mmX2m

Filter: X collection 30%-DEGS/Chromosorb W (60-80 mesh)

Y collection 30%-SE-30/Chromosorb W (60-80 mesh)

Column Temperature: X collection 218°C

Y collection 160°C

We measured the IR spectrum of X, Y collected. With Y, the absorption due to the C-H shrinkage vibration of the double bonds around 3,000 cm^{-1} noted in oleic acid methyl, is lost. At 1,270 cm^{-1}, 1.100 cm^{-1} and 1.030 cm^{-1} we noted an absorption not seen in oleic acid methyl. Except for the loss of absorption around 3.000 cm^{-1}, there was with X no large difference as compared to the measurement results of oleic acid methyl.

We compared the NMR spectra of X, Y to that of oleic acid methyl. We noted that the proton signal attached to the double bond carbon around the δ value 5.3 ppm originating with oleic acid methyl common to both X, Y and the proton signal at the α position vs. the double bonds around 2.0 ppm had been lost.

The mass spectrum measurement of X showed a peak considered to be due to molecular ions at m/e 312.

4. Discussion

Even when using oleic acid only as sample and carrying out the reaction according to the AOCS regulation, byproducts are observed although in an extremely small amount. When the BF_3 concentration is high and the amount of oleic acid small vs. the BF_3 volume, the amount of the byproduct

X increases. Particularly when using 12 ml of 50% BF_3-methanol vs. 200 mg of oleic acid, the oleic acid methyl and the byproduct Y are completely lost and X only is observed. When linolic acid is used as sample, only one kind of byproduct was observed within the range of our examination. When the sample was a mixed item with equal volumes of the various fatty acids, a decrease in oleic and in linolic acids was observed even when using 12.5% BF_3-methanol according to the AOCS regulations. In the case of a 50% solution, the unsaturated fatty acids were almost entirely lost. In such case, only one kind of byproduct was observed by GC but it seems also that there is a production of polymerized matters not detectable by GC.

Y is one of the byproducts generated through the esterification of oleic acid by highly concentrated BF_3-methanol. The fact that its ECL value by GC is 21.4 and the fact that no double bond absorption is observed with IR and that there is a C-O shrinkage absorption at 1.100 cm^{-1} agree with the experimental results of Lough[4], Fulk et al[5]. Y is thought to be methoxystearic acid methyl. Shoulders are observed in the peak of Y with GC. It seems that it might be based on isomers due to the position reached by the methoxy- group.

X does not figure as byproduct in the reports of Lough[4], Fulk et al[5]. As can be seen from Fig. 2, it is a mixed matter containing more than 2 kinds of components. As these could not be collected separately, their structure could not be determined. Yet as no double bonds were observed by IR, NMR and as there were no peaks that could be estimated on the basis of molecular ions at m/e 312 as a result of mass spectrum, the estimation is that in the double bonds of oleic acid methyl of the methyl group, they could be a mixture of additional compounds. With respect to the byproduct Z from linolic acid, isolation was difficult and no analysis possible.

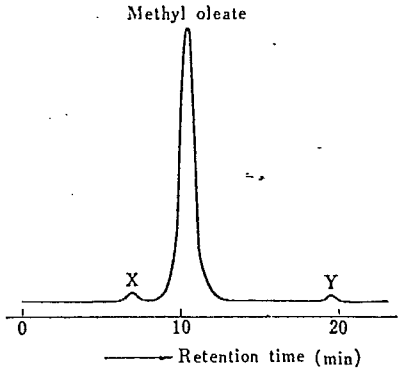
X and Z are presently being examined.

As we have explained above, if the volume of BF_3 vs. the volume of unsaturated acids is large when methyl-esterifying fatty acid mixtures containing unsaturated acids with BF_3-methanol, then a fairly large generation of byproducts is induced from the unsaturated acids and the unsaturated acid methyl decreases. Consequently when analyzing this by GC, the results are inaccurate. This must be kept in mind. Further examination is needed with regard to the quantitative relationship. Study is presently in progress.

(Received June 26, 1974)

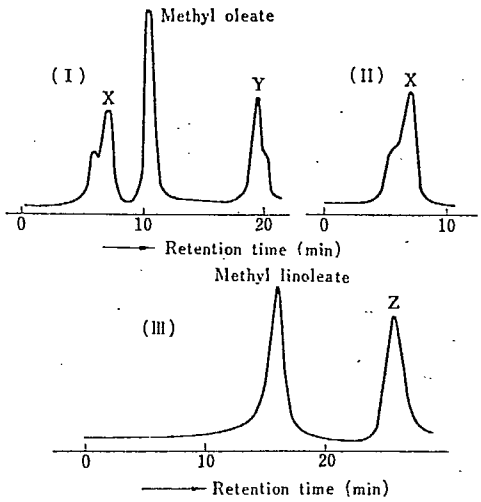
Table-1 Analytical values of esterification products by gas chromatography.

BF_3 concentration (%) \ Fatty acid / Mixed ratio (%)	C_{16} (%)	C_{18} (%)	Artifact (ECL=17.2)	C_{18} (%)	C_{18}' (%)	C_{18}'' (%)
	19.91	19.74	0	19.82	20.41	20.13
6.25	24.0	22.8	nil	22.1	17.8	13.3
12.50	23.2	22.4	nil	21.6	18.1	14.7
25.00	23.3	23.1	nil	22.6	17.0	14.0
50.00	33.7	32.9	2.1%	31.3	nil	nil



Conditions GC
Column : 25%-DEGS/ChromosorbW (60~80 mesh), 3 mm×2 m
Column temp. : 210°C
Carrier gas : He, 40 ml/min.
Detector : TCD
Appratus : Hitachi-063
Esterification
Concentration of BF_3 : 12.5%
Sample size : 1 g

Fig.-1 Gas chromatogram of the esterification product of oleic acid.



Conditions GC
Column : 25%-DEGS/Chromosorb W (60~80 mesh), 3 mm×2 m
Column temp. : 210°C
Carrier gas : He, 40 ml/min.
Detector : TCD
Apparatus : Hitachi-063
Esterification
Concentration of BF_3 : 50%
Sample size : (I) 500 mg of oleic acid (II) 200 mg of oleic acid & (III) 200 mg of linoleic acid.

Fig.-2 Gas chromatograms of the esterification products of unsaturated fatty acids.

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