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New Methods and Reagents in Organic Synthesis. 14.[1] A Simple Efficient Preparation of Methyl Esters with Trimethylsilyldiazomethane ($TMSCHN_2$) and Its Application to Gas Chromatographic Analysis of Fatty Acids

Trimethylsilyldiazomethane ($TMSCHN_2$), known as a stable and safe substitute for highly toxic and explosive diazomethane in the Arndt–Eistert synthesis and homologation of carbonyl compounds, has smoothly reacted with various carboxylic acids in methanolic benzene solution to give the corresponding methyl esters in excellent yields.

Keywords——trimethylsilyldiazomethane; carboxylic acid; methyl ester; esterification; fatty acid; gas chromatographic analysis

Preparation of methyl esters of carboxylic acids, especially for analytical purposes, has been generally carried out[2] by the reaction of carboxylic acids with (1) methanol in the presence of acidic catalysts such as sulfuric acid, hydrogen chloride, boron trifluoride *etc.* which are mostly corrosive, (2) diazomethane which presents an explosive hazard in addition to its high toxicity, or (3) dimethylformamide dimethylacetal or trimethylphenylammonium hydroxide which requires heating for esterification.

We have reported recently that trimethylsilyldiazomethane ($TMSCHN_2$),[3] which is a stable and safe substitute for hazardous diazomethane, can be efficiently used for the Arndt–Eistert synthesis[4] and homologation of ketones[5] and aldehydes.[6] We now wish to report that the reaction of $TMSCHN_2$ with carboxylic acids in the presence of methanol quickly gives methyl esters in excellent yields at room temperature and the method can be efficiently applied to analytical works such as determination of carboxylic acids by gas chromatography:

$$RCO_2H \xrightarrow[CH_3OH \text{ at room temp.}]{(CH_3)_3SiCHN_2} RCO_2CH_3$$

Table I. Esterification of Carboxylic Acids with $TMSCHN_2$ and Methanol

Run	RCO_2H	Isolated yield of RCO_2CH_3, %
1	2-Nitrobenzoic acid	95
2	Piperonylic acid	Quant.
3	Salicylic acid	Quant.
4	Mesitoic acid	97
5	Picolinic acid	82.5
6	Thiophene-2-carboxylic acid	Quant.
7	Cyclohexanecarboxylic acid	Quant.
8	Palmitic acid	Quant.
9	Oleic acid	Quant.
10	α-Ketoglutaric acid	85
11	Cholic acid	Quant.
12	L-Phenylalanine[a]	42[b]

a) Reaction time was 4 hr.
b) As the hydrochloride. The starting material was recovered in 22% yield.

A general experimental procedure for the new preparation of methyl esters is as follows: To a stirred carboxylic acid (1 mM) in methanol (2 ml)–benzene (7 ml) was added $TMSCHN_2$[7] (1.3 mM) in benzene (1 ml) at room temperature. The mixture was stirred for 30 min at room temperature and concentrated to give the corresponding methyl esters.

The esterification proceeds instantaneously and quantitatively when an excess of $TMSCHN_2$ is used, and the reaction can be easily monitored by the disappearance of the yellow color of $TMSCHN_2$. The utility of the procedure is well demonstrated in Table I. Various carboxylic acids including aromatic, heteroaromatic, alicyclic, and aliphatic ones smoothly undergo the esterification. Salicylic acid and α-ketoglutaric acid easily give the corresponding methyl esters without any change of their phenolic and keto functions, respectively. The basic nitrogen of picolinic acid as well as the double bond of oleic acid is completely intact to $TMSCHN_2$. Cholic acid also undergoes the esterification with the retention of its hydroxylic function. L-Phenylalanine affords the corresponding methyl esters accompanied with some of the starting amino acid.

Although 2-nitrobenzoic acid reacted with $TMSCHN_2$ in 20% methanolic benzene to give methyl 2-nitrobenzoate in 95% yield, replacement of methanol with ethanol resulted in a little decrease of the yield (87%) of the methyl ester and a small amount (5%) of the trimethylsilylmethyl ester[8] was obtained. The latter ester was the main product (86%) when 20% *tert*-butyl alcohol–benzene was used as a reaction solvent. Furthermore, $TMSCHN_2$ does not give any diazomethane when it is treated with 20% ethanolic benzene. These results deny the participation of diazomethane which might be formed from $TMSCHN_2$ by the action of methanol[6] and suggest the reaction mechanisms[8] as follows:

$$RCO_2H \xrightarrow{(CH_3)_3SiCHN_2} RCO_2^-\overset{+}{N}_2CH_2Si(CH_3)_3 \xrightarrow[-(CH_3)_3SiOR']{R'OH}$$

$$RCO_2^-\overset{+}{N}_2CH_2 \xrightarrow{-N_2} RCO_2CH_3$$

The method is applicable to gas chromatographic analysis of fatty acids.[9] Thus, a mixture of six fatty acids (lauric, myristic, palmitic, stearic, arachidic, and behenic acids) is quickly and quantitatively esterified with $TMSCHN_2$ in 20% methanolic benzene at room temperature. The esterification mixture, after adjustment to the desired concentration or solvent, is ready for gas chromatography. Its standard calibration curves relating peak area ratios (each fatty acid/palmitic acid as internal standard) to mole concentrations hold

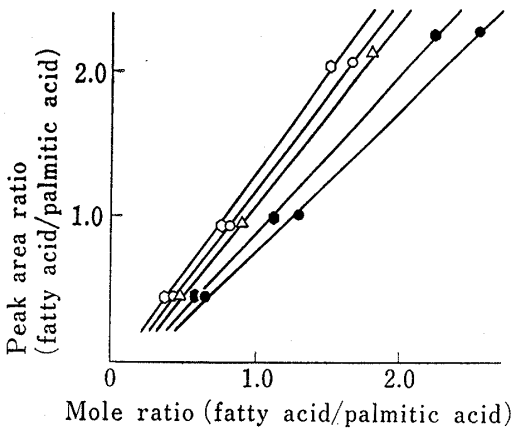

Fig. 1. Standard Calibration Curves for Determination of Fatty Acids

(●): lauric acid, (■): myristic acid,
(△): stearic acid, (○): arachidic acid,
(◯): behenic acid.

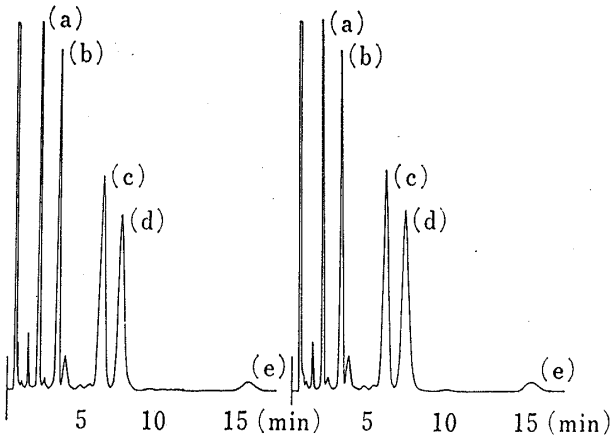

Fig. 2. Gas Chromatograms of Fatty Acid Methyl Esters obtained by the $TMSCHN_2$ (left) and Diazomethane(right) Methods

(15% EGSS-X, lm×5 mmϕ glass column temp., 200°; N_2; FID; chart speed, 10 mm/min).
(a): myristic acid, (b): palmitic acid, (c): oleic acid,
(d): linoleic acid, (e):arachidonic acid.

the linear relationship in a wide range (Fig. 1). Furthermore, the gas chromatogram of methyl esters prepared from a mixture of five fatty acids (myristic, palmitic, oleic, linoleic, and arachidonic acids) by the action of $TMSCHN_2$ and methanol is completely identical with that of methyl esters prepared by the action of diazomethane (Fig. 2). These facts prove an identical potency of $TMSCHN_2$ with diazomethane in the gas chromatographic analysis of fatty acids and promise a non-hazardous routine procedure for the preparation of methyl esters of fatty acids in small quantities.

Esterification of carboxylic acids with $TMSCHN_2$–methanol is conveniently and safely carried out with superior efficiency. Since the reaction quickly proceeds under very mild conditions (room temperature and essentially neutral medium), the method should have a broad applicability not only to preparative but also for analytical works.

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7) $TMSCHN_2$ was prepared by the Seyferth's method,[3] which we slightly modified. The actual reagent used has been a mixture of $TMSCHN_2$ and hexamethyldisiloxane (7: 3), the latter of which has no influence on the reaction.
8) Seyferth and co-workers[3] have reported that the reaction of acetic acid with $TMSCHN_2$ furnished a mixture of methyl acetate, trimethylsilyl acetate, and trimethylsilylmethyl acetate. They have discussed the reaction mechanism, too.
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Quantitative Analysis of Clebopride and Its Metabolites in Rat Blood by Acid Decomposition

6-Chloro-*m*-anisidine was obtained as one of the products by the acidic decomposition of elebopride and its main metabolites (total CP). This degraded product after derivatized with heptafluorobutyric anhydride was measured by use of mass fragmentography. A good calibration curve was obtained in the range 2—80 ng/ml of clebopride in rat blood. The concentration of total CP in rat blood after intravenous (0.1 and 0.5 mg/kg) and oral (0.5 mg/kg) administration was determined by this method.

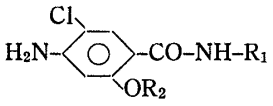
Keywords——clebopride; mass fragmentography; GC/MS; acid decomposition; benzamide drug; pharmacokinetics

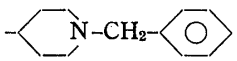
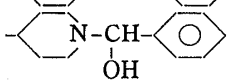
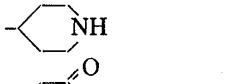
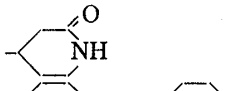
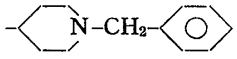
Clebopride (CP; N-(1′-benzyl-4′-piperidyl)-2-methoxy-4-amino-5-chlorobenzamide) is a new benzamide drug with potent antidopaminergic activity.[1] Therapeutic dose of CP is low (0.5 mg/kg). This drug is extensively metabolized as shown in Table I.[2]

Segura *et al.* recently reported an analytical procedure for CP in biological fluids[3]; however this procedure meet only to need for determination of the plasma level following doses more than 10 mg/kg in the experimental animals.

The present report describes mass fragmentographic determination of the unchanged CP and its main metabolites (total CP) in whole blood. This method is based on the fact that not only the unchanged CP but also its main metabolites having 6-chloro-*m*-anisidine (6-CA) ring have to be decomposed with hydrochloric acid to form 6-CA as one of the degraded

Table I. Structures of Clebopride, Its Main Metabolites and Internal Standard



	R_1	R_2
Clebopride		CH_3
Metabolite 1		CH_3
Metabolite 2		CH_3
Metabolite 3		CH_3
Internal standard		CH_2CH_3