NOTE

トリメチルアミンオキシドの迅速比色定量法

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RAPID SPECTROPHOTOMETRIC METHOD FOR TRIMETHYLAMINE OXIDE DETERMINATION

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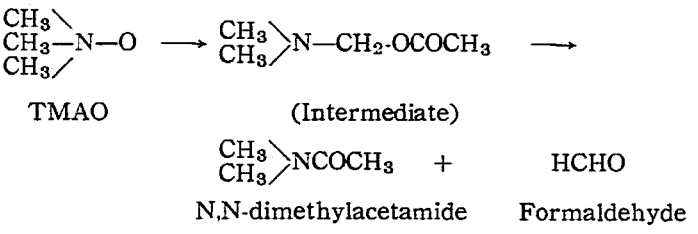
During the course of studies on the fate of trimethylamine oxide (TMAO) in marine products, a method for its determination was necessary which is rapid and simple without sacrificing accuracy. It was found in our laboratory that TMAO also suffers a Polovinski reaction(1), a specific intramolecular rearrangement of methyl-N-oxides in hot acetic anhydride, to yield formaldehyde and N,N-dimethylacetamide stoichiometrically. When the reaction mixture is subjected to MacFadyen's chromotropic acid method for formaldehyde determination(2) after the hydrolysis of acetic anhydride by adding water, the reaction can be applied to the microdetermination of TMAO.



TMAO　　(Intermediate)

N,N-dimethylacetamide　　Formaldehyde

The recommended procedure is as follows:

1. Add 5 ml. acetic anhydride to 0.5 ml. of the practically neutral aqueous extract contained in a glass reaction tube, mix, and heat in a boiling water-bath for 1 hour under an air condenser (a 0.5×40 cm glass tubing is sufficient).
2. After cooloing, add 5 ml. of water in the tube and mix vigorously.
3. Take 1 ml. of the reaction mixture and add 3 ml. of the chromotropic acid reagent (see "Experimental") and mix. In heating in a boiling water bath for 1 hour, purple color developes.
4. After cooling the mixture, read the density of color at 580mμ.
5. The blank experiment is required in which TMAO is replaced with water.

Standard curve: The color developed adheres the Beer's law in the range of 0.25 to 1.5 μmole/ml. TMAO. (Fig. 1).

The quantitative formation of formaldehyde is shown in Table I, in which are listed absorbances at 580mμ obtained when the present procedure was adopted to both the TMAO and formaldeldehyde-sodium bisulfite solutions.

Stability of color and its identity: The color developed was found to be stable at least for 1 day. The analysis of the absorption curve demonstrated that no interfering

substances with the color developement and its stability are formed throughout the procedure.

Comparison with Dyer method(3-5), which is known to be rather time-consuming and requires technical skill, was made by pursuing the variation of TMAO content during chill storage of shrimps with both procedures (Table Ⅱ). The present procedure was found to have advantage in time and in labor.

Interferences: Equimolar biogenic amines tested were found not to interfere with the colorimetry of TMAO (Table Ⅲ).

Determination of TMAO in the presence of excess formaldehyde: TMAO content is successfully recovered from formaldehyde solutions by taking the difference of formaldehyde contents in the acetic anhydride-treated and untreated reaction mixtures (Table Ⅳ).

Experimental

Acetic anhydride is required to be free from most of its decomposition products which may converted to formaldehyde during the acid treatment with chromotropic acid and give a high blank value(6). Commercial acetic anhydride was boiled under reflux with a few drops of sulfuric acid, and distilled repeatedly. A fraction distilled at 137°–139° is collected.

TMAO, trimethylamine hydrochloride, dimethylamine hydrochloride, and chromotropic acid were recrystallized from alcohol and water.

"*Chromotropic acid reagent*" was prepared according to MacFadyen(2). To 100 ml. of the 1% aqueous solution of chromotropic acid, gradually added 24N sulfuric acid.

Formaldehyde: Commercial "formalin" was used.

"*Formaldehyae-sodium bisulfite*" was synthesized from formalin and sodium bisulfite.

要　　旨

ポロビンスキー反応（メチル-N-オキシドの分子内転位反応）を用いて，TMAO から定量的に生成するホルムアルデヒドを比色定量することによって，TMAO を直接定量することができる．次のような操作が推奨される．

1．0.5 ml のほぼ中性の TMAO 溶液（抽出液など）をガラス製の反応管に入れ，これに 5 ml. の無水酢酸を添加して，空冷管（0.5×40cm のガラス管）を付して 1 時間沸とう水中に保つ．

2．冷却後 5 ml の水を添加してはげしく混和する．

3．1 ml をとって 3 ml のクロモトロープ酸試薬（1%クロモトロープ酸の約 20N 硫酸溶液）を加え，混和して，沸とう水浴中に 1 時間保つ．

4．生ずる紫色を 580mμ で比色する．

5．同時に盲験（無水酢酸の代りに水を用いる）を行う．

発色値は TMAO 濃度に比例して Beer の法則を満足する（Fig. 1）．

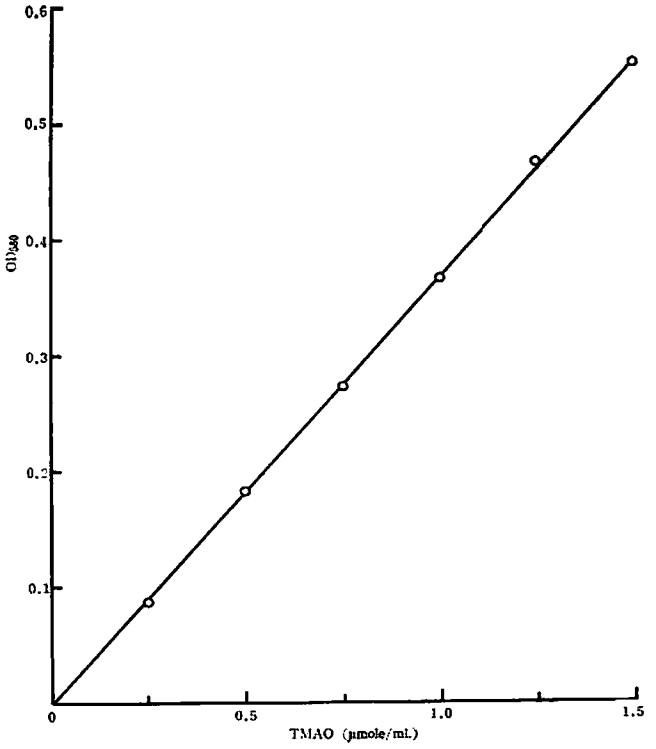


Fig. 1 Standard curve for TMAO determination.

TMAO およびホルムアルデヒドの等モル溶液について本法を実施するとおのおのほぼ同値となり，TMAO からのホルムアルデヒドの定量的生成が認められる (Table Ⅰ).

発色は少くとも一昼夜安定である．シュリンプの貯蔵中の変化を Dyer の方法と本法とで追跡するとほぼ一致を示す．(Table Ⅱ)

トリプタミンを除き，他の生体アミン類の存在は本法を妨害しない (Table Ⅲ).

無酢処理を実施したものと実施しないものについてクロモトロープ酸処理を施すことによって，前者から TMAO，FA 量

Table Ⅰ Comparison of Color Developement from TMAO and Formaldehyde Solutions.

Concentration	TMAO (a)	HCHO* (b)	a/b
μmole/ml.	OD_{580}	OD_{580}	
0.5	0.183	0.180	1.09
1.0	0.365	0.370	0.98
1.5	0.550	0.552	1.00

* Formaldehyde-sodium bisulfite.

"Recommended procedure" for TMAO determination was adopted to TMAO and formaldehyde-sodium bisulfite solutions.

Table Ⅱ Comparison with Dyer's Method: Variation in TMAO Content during Chill Storage of Shrimp.

Storage for	Present method	Dyer's method
Day	mg/g*	mg/g*
0	7.65	6.59
1	6.87	6.41
2	3.06	2.97
3	2.94	2.89
4	2.82	2.77
8	2.46	2.22

* Milligrams per g tissue homogenate.

Table Ⅲ Interferences.

Amines added**	Absorbance OD_{580}	%
None*	0.375	100
TMA	0.377	100.5
DMA	0.366	97.6
Methylamine	0.403	107.4
Ammonia	0.359	95.8
Ethylamine	0.375	100.0
Cadaverine	0.359	95.7
Putrescine	0.373	99.5
Histamine	0.387	103.2
Tyramine	0.331	88.3
Tryptamine	0.081	21.6

* 1 *μmole*/ml TMAO
**1 *μmole*/ml.

の和，後者からは FA 量のみが知られるので，その差から，FA 混在試料の TMAO 量が算出できる (Table Ⅳ).

Table Ⅳ Simultaneous Determination of TMAO and Formaldehde.

Solution	Color developed with heat treatment with		(a-b)
	Acetic anhydride (a)	Water (b)	
	OD_{580}	OD_{580}	OD_{580}
TMAO	0.440	0.002	0.438
HCHO	0.625	0.645	0.020
TMAO+HCHO	1.100	0.650*	0.450**

* Color due to formaldehyde.

**Color due to TMAO.

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