understanding. In short there is no coherent account of how to tell one smell from another. In that sense, the equation (3) could be regarded as a new step to approach the mechanism of classification of odors.

Finally, in view of the fundamental likeness found in the response behavior of single olfactory receptors in insect antennae[2,7], and in the vertebrate olfactory mucosa[3,5], the equation (1) would appear to be the "quality coding equation" basic for the odor quality coding in the invertebrate as well as the vertebrate.

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Method for the Determination of Residues of Meobal® (3, 4-Dimethylphenyl *N*-Methyl Carbamate) in Rice Grains. Seizo Sumida, Masahiro Takaki, and Junshi Miyamoto (Research Department, Pesticides Division, Sumitomo Chemical Co., Ltd., Konohana-ku, Osaka, Japan) Received July 14, 1970, *Botyu-Kagaku* 35, 72, 1970.

9. 玄米中のメオバール® (3, 4-dimethylphenyl *N*-methyl carbamate) の残留分析

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玄米中のメオバールのガスクロマトグラフィーによる残留分析法を報告する．粉砕した玄米をジクロルメタン-アセトン-水の混合液で抽出し，抽出物をカラム及び薄層クロマトグラフィーでクリーンアップする．次に，得られた試料中のメオバールの加水分解及びジニトロフェニル化を同時的に行わせ DNP-メチルアミン (2, 4-dinitrophenyl methylamine) を得る．DNP-メチルアミンをエレクトロンキャプチャーガスクロマトグラフィーにより定量する．回収率は 0.5ppm レベルで 87% であった．本法によれば，0.01ppm 又はそれ以下までのメオバールの残留量を分析できる．

Meobal® (3, 4-dimethylphenyl *N*-methyl carbamate) is an insecticidal compound developed by Sumitomo Chemical Co., Ltd. for pest control mainly of rice plant. From the standpoint of public hygiene, it is quite important to have information on the residues of this compound left in the rice grains. Methods had been reported for the determination of *N*-methyl carbamates in a number of plants,[1,2] but they failed to give consistent results with rice grains, as tested in our laborotory. Under these circumstances, an attempt was made to develop a new method to serve our purposes.

We have reported elsewhere[3] that microquantities of *N*-methyl carbamates can be determined by converting them to 2, 4-dinitrophenyl methylamine (DNP-MA) in a novel fashion, *i.e.*, *N*-methyl carbamate is heated at 100°C and at pH 9 in the presence of 2,4-dinitro-1-fluorobenzene (DNFB). The carbamate breaks down under these

conditions to give methylamine which is trapped as DNP-MA by instantaneous reaction with DNFB. It was found in the present investigation that, based on the reaction conditions mentioned above, Meobal residues in rice grains can be determined by electron capture gas chromatography after an appropriate clean-up procedure.

Methods

Extraction:

Reagents used were all analytical grade throughout the investigation. Unpolished rice grains were mechanically ground to powder (30 mesh all through) with a rice grinder (Kiya Seisakusho, Tokyo). One hundred grams of the rice powder were soaked overnight in the mixture of water (75m*l*) and acetone (100m*l*) at room temperature. Then, it was agitated mechanically for 10 min with a shaker (Model KM, Iwaki K. K., Tokyo). One hundred and fifty m*l* of dichloromethane were added to the mixture, and the mixture shaken for another 10min. The mixture was then filtered through two layers of filter paper (Toyo No. 2, Toyo Roshi, Tokyo) under reduced pressure. The filtrate was transferred to a 500m*l* separatory funnel and the lower phase (organic) was taken. The residue on the filter paper was washed with 50 m*l* of dichloromethane which was then transferred to the separatory funnel to wash the upper phase (aqueous). The separatory funnel was shaken mechanically for 5 min and the lower phase (organic) taken. The combined organic phase was dehydrated by brief treatment with 100g of anhydrous sodium sulphate and then filtered through Toyo No. 2 filter papar. The residue (sodium sulphate) was washed with 15 m*l* of dichloromethane. The combined filtrate was evaporated to dryness at 30°C under reduced pressure, and the residue was dissolved in a minimal amount of *n*-hexane/chloroform (3/2, by vol.) for column chromatography.

Column chromatography:

Equal weights of silicic acid (100 mesh, Mallinckrodt Chemical, St. Louis, U. S. A.) and Hyflo supercel (Johns-Manville, Lompoc, U. S. A.) were thoroughly mixed and activated at 120°C overnight. Twenty grams of the activated material was packed in a column 2.7cm in diameter with 150 m*l* of *n*-hexane/chloroform (3/2, by vol.). Then, the rice extract was carefully applied to the column and the column eluted with 350 m*l* of the same solvent. The first 150 m*l* fraction was discarded and the second fraction (200 m*l*) was collected. The eluate was evaporated to dryness at 30°C in a stream of air, and the residue dissolved in a minimal amount of dichloromethane for thin layer chromatography (TLC).

TLC:

The sample was applied in a band to a TLC plate coated with Kieselguhr HF (Merck, Darmstadt, Germany) which had been pre-activated at 120°C for 2 hours. Standard Meobal was spotted as a reference on both sides of the plate. The plate was developed in a tank lined with filter paper in a system, *n*-hexane/ethyl acetate (3/2, by vol.) The developed plate was placed under a UV lamp to visualize the standard Meobal spots. The area corresponding to the standard spots was marked in a broad band, and scraped from the plate to a small column. The column was eluted with 30ml of methanol/chloroform (1/9, by vol.). The eluate was evaporated to dryness at 30°C in a stream of air.

Dinitrophenylation reaction:

The dried material was dissolved in two portions of 1 m*l* of dioxane, and transferred to a 20 m*l* glass-stoppered test tube. Twenty μl of DNFB (Wako Chemical, Osaka) and 5 m*l* of saturated aqueous sodium borate solution were added to the tube in that order. The reaction mixture was tipped well and heated at 100°C for 30min. Then, 1m*l* of saturated glycine solution (pH adjusted to 9.0 with HCl) was added, mixed well and heating was continued for another 10 min. The mixture was then rapidly cooled with ice, and extracted with one 4 m*l* and two 2 m*l* portions of benzene by vigorous shaking by hand. The combined benzene extract was washed with 8 m*l* of 0.1 N sodium carbonate. In case emulsion was formed, a small amount of anhydrous sodium sulphate was added to break it. The benzene phase was taken and the aqueous phase was back-extracted with 2 ml of benzene. An appropriate amount of ethyl *p*-cyanophenyl phenyl phosphonothioate (Surecide®, Sumitomo Chemical) was added to the combined benzene extract as an

internal standard. The mixture was evaporated to thorough dryness at 30°C in a stream of air. The dried material was dissolved in an appropriate amount of benzene and an aliquat subjected to electron capture gas chromatography (GLC).

GLC:

GLC was performed with a Varian aerograph, model 1800 (Walnut Creek, U.S.A.), with a tritium electron capture detector. The column was 4% XE-60 on 100–120 mesh Chromosorb W, AW/DMCS (Nishio Kogyo, Tokyo) packed in a 1.5m long glass tubing with an internal diameter of 1.7mm. The temperature was 185°C for the column and 210°C for the detector.

The flow rate of nitrogen carrier gas was 167 ml/min. The amount of DNP-MA in a sample was determined by comparing the peak area with that of the internal standard and by reference to a calibration curve prepared by plotting weight ratios against peak area ratios of DNP-MA to the internal standard. The original amount of Meobal was calculated by dividing that of DNP-MA formed by a factor of 1.10, according to the formula described below.

$$\text{Amount of Meobal present} = \text{Amount of DNP-MA formed} \times \frac{\text{mol. wt of Meobal}}{\text{mol. wt of DNP-MA}}$$

Results and Discussion

A typical chromatogram obtained from a sample fortified at the 0.5 ppm level is presented in Fig. 1. Peaks of DNP-MA and Surecide (broken line) are superimposed on the chromatogram of the control sample (solid line). No interfering peak was observed in the rice samples. According to the method, the lowest limit of sensitivity is 0.01 ppm or less.

The overall recovery of this method was 84, 90, 87, and 87% (average, 87%) when 50 μg of Meobal was added to 100g of powdered rice grains (0.5 ppm). Recoveries for each individual steps of the procedure were monitored by using ^{14}C-Meobal labeled at 4-CH_3 of the xylenol moiety. The recoveries after the extraction, column chromatography, and TLC were 100, 95, and 95%, respectively, when the sample was fortified at the 0.5 ppm level. The rice extract obtained after the clean-up steps was used to study the optimal conditions for the dinitrophenylation reaction. Some lipid material in the rice sample might

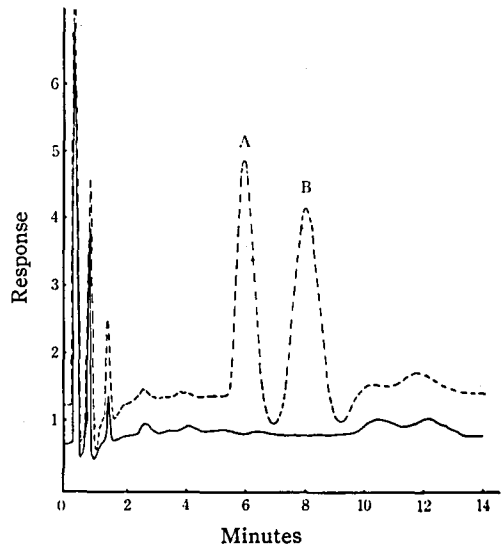

Fig. 1. Gas chromatogram of the rice extract fortified with Meobal at the 0.5ppm level.

The conditions of GLC were the same as described in the text. Solid line indicates the chromatogram of the control sample, and broken line that of the fortified sample. A; DNP-methylamine derived from Meobal and B; ethyl *p*-cyanophenyl phenyl phosphonothioate added as an internal standard.

Table 1. Effect of the amount of added DNFB on the yield of DNP-MA in the presence of the cleaned-up rice extract.

Amount of added DNFB	Yield of DNP-methylamine
0μl	0%
20	97
40	90
70	91
100	80

The rice extract used in this experiment was obtained from the rice grains which were known to contain no Meobal residues (control). The extraction and clean-up steps are described in the text. The conditions for dinitrophenylation reaction were the same as described in the text, except that the amount of added DNFB was varied, and that 50 μg of Meobal was added to the reaction mixture.

react with DNFB. If the consumption of DNFB were made in this way to a significant degree, then the optimal conditions for the reaction would be different from those reported earlier.[3] Actually, however, the reaction yield was the highest (97%) when 20 μl of DNFB was added as shown in Table 1. This shows that the optimal conditions do not change considerably if the rice sample is sufficiently cleaned up.

Since *N*-methyl carbamates are known to be thermolabile, the temperature was kept at 30°C throughout the procedure when the Meobal-containing solutions were evaporated to dryness. Actually, when the evaporation was performed at tempreature above 40°C, the recovery of Meobal was reduced by 20% or more.

This method was developed particularly for the residue analysis of Meobal in rice grains. However, since the dinitrophenylation reaction is applicable to *N*-methyl carbamates in general,[3] this method with some modifications may probably be applied for the analysis of other *N*-methyl carbamates in other crops. Considering the variation in the lipid composition of various plant materials,[4] the clean-up procedure, especially column chromatography, must be suitably modified for successful application.

A limitation of the present method would be that a mixture of *N*-methyl carbamates must be separated prior to the dinitrophenylation reaction in order for the individual carbamates to be analysed. Particularly when its metabolites[5] are to be analysed together with intact Meobal, the prior separation will be essential. This is left as our future project.

Summary

A gas chromatographic method is described for the residue determination of 3,4-dimethylphenyl *N*-methyl carbamate (Meobal®) in rice grains. Powdered rice grains were subjected to extraction with a dichloromethane-acetone-water mixture. The extract was cleaned up by a combination of column and thin layer chromatography. Meobal in the cleaned-up sample was converted to 2,4-dinitrophenyl methylamine (DNP-MA) by simultaneous hydrolysis and dinitrophenylation and the DNP-MA was determined by electron capture gas chromatography. Recovery was 87% when the sample was fortified at the 0.5 ppm level. The lowest limit of sensitivity was 0.01 ppm or less.

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抄　　録

Grass Grub Beetle (コガネムシの一種) の性誘引物質

Sex Attractant of the Grass Grub Beetle.
R. F. Henzell, M. D. Lowe, *Science* 168, 1005 (1970).

コガネムシの一種 grass grub beetle *Costelytra zealandica* (White) の幼虫は，ニュージランドにおける牧草の害虫の一つである．この成虫の雌が性誘引物質を発散することは，すでに知られていた．今回1500匹の処女雌の腹部を洗滌することによりこれを抽出した．これを濃縮後昇華精製し，ついで層薄クロマトグラフィーで Rf 値を比較した結果，フェノールと一致した．さらにペーパークロマトグラフィー，ガスクロマトグラフィーでも誘引物質はフェノールであることが確認された．

室内での生物検定には，パラフィン製の擬似体に一定量のフェノールを塗布して10匹の雌の入った容器に置いて数分後の交尾行動によって判定した．それによると 0.1μg でも 80% の雄が反応することがわかった．さらに野外試験で一夜に 100ppm のフェノール水溶液が71匹の雄を誘引した．しかし，近くにおいた水のみを入れたトラップには一匹もオスは捕われなかった．　　(高橋正三)