

Synthesis of a New Phosphorylating Agent, 2-Methylthio-4H-1,3,2-benzodioxaphosphorin 2-Oxide (MTBO) from its Thiono Isomer'

Eto, Masayoshi
九州大学農学部食糧化学工学科食糧化学講座

Iio, Masayoshi
Food Chemistry Institute, Faculty of Agriculture, Kyushu University

Omura, Hirohisa
Food Chemistry Institute, Faculty of Agriculture, Kyushu University

Eto, Morifusa
Laboratory of Pesticide Chemistry, Faculty of Agriculture, Kyushu University

<https://doi.org/10.5109/23648>

出版情報：九州大学大学院農学研究院紀要. 22 (1/2), pp.25-39, 1977-10. Kyushu University
バージョン：
権利関係：

Synthesis of a New Phosphorylating Agent, 2-Methylthio-4*H*-1,3,2-benzodioxaphosphorin 2-Oxide (MTBO) from its Thiono Isomer'

Masayoshi **Eto**,² Masayoshi **Iio**,³ Hirohisa **Omura**⁴
and Morifusa **Eto***

Food Chemistry Institute and *Laboratory of Pesticide Chemistry,
Faculty of Agriculture, Kyushu University 46-09
and *46-02, Fukuoka 812

(Received July 19, 1977)

Optimum condition in the synthesis of MTBO was found by the examination and improvement of thiono-thiol isomerization of salithion. For the isomerization of salithion were used acetone as the solvent, sodium iodide as the catalyst, potassium carbonate as the acceptor of iodide ion, and methyl iodide as the methylating agent. MTBO was crystallized from ether to give pure crystals which are stable on storage for a long time. Salithion was readily demethylated by potassium dimethyldithiocarbamate to give potassium saligenin cyclic phosphorothioate (KSCP). KSCP was S-methylated by methyl iodide to give MTBO in high yield.

INTRODUCTION

Discovery of saligenin cyclic phosphate as a biologically active metabolite of tri-*o*-tolyl phosphate (Casida *et al.*, 1961; Eto *et al.*, 1962) has led to the extensive studies on synthesis and chemical, biochemical, and biological properties of many related compounds (Eto, 1969). One of them, 2-methoxy-4*H*-1,3,2-benzodioxaphosphorin 2-sulfide (salithion) (4) has been produced in a large quantity and practically used as an insecticide (Eto *et al.*, 1963).

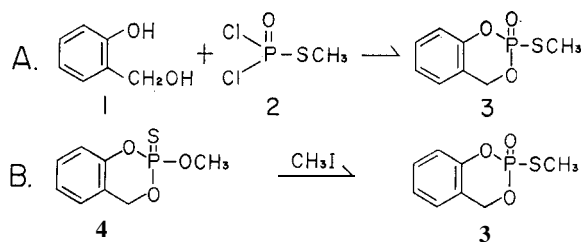
Its thiol isomer, 2-methylthio-4*H*-1,3,2-benzodioxaphosphorin 2-oxide (MTBO) (3) has been recently developed as a phosphorylating agent (Eto *et al.*, 1971; Sasaki *et al.*, 1973; Iio *et al.*, 1973) for the synthesis of various phosphate esters of biological interest, such as 3':5'-cyclic nucleotides (Eto *et al.*, 1974), 2':3'-cyclic nucleotides (Eto *et al.*, 1971; Iio *et al.*, 1975), ribonucleoside 5'-S-methyl phosphorothiolates (Eto *et al.*, 1974), triose reductone 2-phosphate (Shinohara *et al.*, 1975), ribonucleoside 5'-phosphates (Eto, 1975), ribonucleoside 5'-pyrophosphates (Iio *et al.*, 1977), and cytokinin-like nucleotides (Eto *et al.*, 1976).

¹ A new phosphorylating agent, 2-methylthio-4 *H*-1, 3,2-benzodioxaphosphorin 2-oxide. Part VI. For Part V see reference (Iio *et al.*, 1977).

² Present address: Pesticide Chemistry and Toxicology Laboratory, College of Natural Resources, University of California, Berkeley, California, USA.

³ Present address: Kumamoto Women's University, Ooe, Kumamoto.

⁴ Reprint requests should be addressed to this author.



Scheme 1. Synthesis of MTBO.

MTBO was initially synthesized by condensation of saligenin (1) and *S*-methyl phosphorodichloridothiolate (2) whose synthesis is relatively difficult (Scheme 1-A) (Kobayashi *et al.*, 1969). Synthesis of MTBO was attempted by isomerization of salithion applying Pistschimuka reaction (Scheme 1-B) (Eto *et al.*, 1971; Sasaki *et al.*, 1973; Iio *et al.*, 1973) and by demethylation of salithion with potassium dimethyldithiocarbamate followed by *S*-methylation of demethylated salithion with methyl iodide (Scheme 3) (Sasaki *et al.*, 1974; Eto, 1975).

The present paper describes the results of examination of optimum conditions for these methods for the synthesis of MTBO.

RESULTS AND DISCUSSION

I) Isomerization of salithion into MTBO by Pistschimuka reaction

Effect of dimethylformamide (DMF) or dimethylacetamide (DMA) on the isomerization of salithion

We first reexamined the isomerization of salithion to MTBO applying the Pistschimuka reaction which had been attempted by Sasaki *et al.* in polar solvents such as DMF or DMA (Eto *et al.*, 1971; Sasaki *et al.*, 1973). Table 1 shows the effect of DMF or DMA on the isomerization of salithion.

In the presence of potassium carbonate, the use of acetone as the solvent gave MTBO in about 50 % yield and a by-product II (*R_f*=0.73), but unchanged salithion remained (28 %) (A). Addition of DMF made unchanged salithion disappear (B). The filtrate of the reaction mixture B was concentrated and checked by TLC, which showed that the yield of decomposition product increased because of degradation of MTBO during concentration procedure. In the absence of potassium carbonate, the isomerization hardly proceeded (C). Decreasing the amount of acetone increased the decomposition product (D). However, the use of larger amount of acetone than in B had no significant effect (E). Benzene was used instead of acetone, because benzene well dissolves salithion and is inert in decomposition reaction of MTBO, but it suppressed the isomerization of salithion as well as decomposition of the product (F). Unchanged salithion remained by the use of DMF as the sole solvent on standing at 37°C for 12 hr (G). Since potassium carbonate not only accelerates the isomerization of salithion but also may decompose MTBO on account of its basicity, neutral salts such as potassium bromide and chloride were used. By the use of potassium bromide and DMA (I) or potassium chloride and DMF (J),

Table 1. Effect of DMF or DMA on the isomerization of salithion.

| | Solvent (ml) | Salt | Reaction temperature (°C) | Re-action time (hr) | Yield(%) ^{a)} | | |
|---|------------------------|--------------------------------|---------------------------|---------------------|------------------------|--------------------|---|
| | | | | | MTBO | Salithion | Decomposition product I (R _f =0) II (R _f =0.73) ^{b)} |
| A | Acetone (5) | K ₂ CO ₃ | 56 | 3 | 53 | 28 | 6 |
| B | Acetone (5) DMF (0.3) | K ₂ CO ₃ | 56 | 5 | 90 | 0 | 10 |
| | | | | | 59 ^{c)} | 0 | 41 |
| C | Acetone (5) DMF (0.3) | | 56 | 3 | 15 | 75 | 10 |
| D | Acetone (1) DMF (0.3) | K ₂ CO ₃ | 56 | 2.5 | 14 | 0 | 86 |
| E | Acetone (15) DMF (0.3) | K ₂ CO ₃ | 50 | 7 | 86 | 4 | 10 |
| F | Benzene (5) DMF (1.2) | K ₂ CO ₃ | 56 | 4 | 54 | 44 | 2 |
| G | DMF (3) | K ₂ CO ₃ | 37 | 12 | 72 | 14 | 14 |
| H | Acetone (5) DMA (0.4) | KBr | 37 | 4 | 53 | 38 | 9 |
| I | DMA (5) | KBr | 56 | 5 | 41 | 23 ^{d)} 0 | 59 |
| J | I DMF (10) | KCl | 48 | 12 | 60 | 52 ^{d)} 0 | 40 |
| K | - | KBr | 37 | 12 | 7 | 91 | 2 |
| L | - | KCl | 37 | 12 | 10 | 89 | 1 |

The composition of reaction mixture: salithion (4.67 mmoles), methyl iodide (5.64 mmoles), salt (4.67 mmoles), solvent (ml).

^{a)} Estimated from TLC. ^{b)} This compound may be o-(a-methylthio)cresol.

^{c)} After filtration and concentration. ^{d)} Isolated yield.

salithion disappeared. DMF and DMA evidently catalyze the isomerization (Scheme 2-A), because the reaction hardly proceeds in the absence of the solvents (K., L).

MTBO was isolated in 35.4 % yield after the isomerization of salithion with DMF and potassium chloride, followed by water wash.

The use of potassium iodide as the salt more rapidly promoted the isomerization: MTBO was produced even by standing at room temperature for 24 hr and isolated in 45% yield after water wash.

Effect of various salts on the isomerization of salithion

DMF is effective as a catalyst for the isomerization of salithion, but may promote the decomposition of MTBO. DMF is difficult to remove because of its high boiling point. In the isolation procedure, evaporating the reaction mixture to complete dryness changes MTBO and the decomposition product I (R_f=0) into a solid insoluble in chloroform. In order to remove residual DMF and the decomposition product, concentration was stopped just before the reaction mixture dried up and the residue was dissolved in chloroform, and washed with water. MTBO contained a trace of DMF even after the water wash. Therefore, solvents with lower boiling point were desired to avoid this difficulty. Acetone satisfies this requirement, but gives MTBO in low yield (Table 1-A). The solvents and the salts listed in Table 2 were then examined.

By the use of 3-pentanone and lithium iodide, the yield of MTBO was 94 % by estimation from the relative area ratio of TLC spots but the reaction mixture gave two dark brown layers, suggesting that the actual yield of MTBO was lower than the estimation. The use of lithium iodide or barium iodide in acetone gave only the decomposition product.

Effect of 1,3-dimethylthiourea (DMTU) and 1,3-dimethylurea (DMU) on the isomerization of salithion

Table 2. Effect of various salts on the isomerization of salithion.

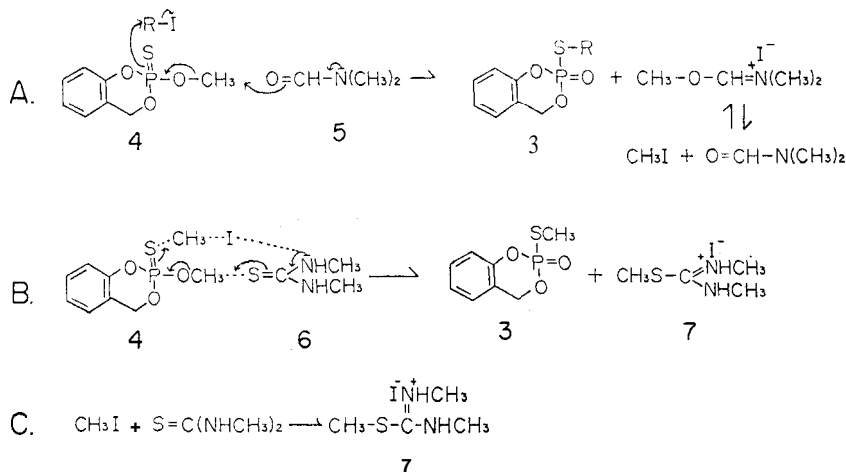
| Solvent | Salt | MTBO | Salithion | Yield (%) ^{a)} | |
|---------------|--------------------------------|------|-----------|---|----------------------------------|
| | | | | Decomposition product I (<i>R_f</i> =0) | II (<i>R_f</i> =0.73) |
| A 3-Pentanone | K ₂ CO ₃ | 49 | 30 | 2 | 18 |
| B 3-Pentanone | KI | 51 | 44 | 5 | |
| C 3-Pentanone | LiI | 94 | 0 | 6 | |
| D 3-Pentanone | LiCl | 37 | 22 | 5 | |
| E Acetone | LiI | 0 | 0 | 100 | |
| F Acetone | LiCl | 60 | 30 | 10 | |
| G Acetone | KHCO ₃ | 61 | 35 | 3 | |
| H Acetone | BaI ₂ | 0 | 0 | 100 | |

The composition of reaction mixture : salithion (4.67 mmoles), methyl iodide (5.64 mmoles), salt (4.67 mmoles), and solvent (3 ml).

Reaction temperature, 37°C; Reaction time, 12 hr.

^{a)} Estimated from TLC.

DMF (5) promotes the isomerization reaction by demethylation of salithion (Scheme Z-A) (Sasaki et al., 1973). We searched for compounds which have the similar structure as DMF and are soluble in acetone and water, but insoluble in ether to be removable by water wash. Since 1,3-dimethylthiourea (DMTU) (6) and 1,3-dimethylurea (DMU) satisfy the above requirements, they were examined for the effect on the isomerization, as shown in Table 3.

**Scheme 2.** Proposed mechanism of the isomerization of salithion by (A) DMF or (B) DMTU, and (C) reaction of DMTU with methyl iodide.

Addition of methyl iodide to the mixture containing DMTU gave white crystals (7) immediately. Standing the mixture at the room temperature for 0.5 hr produced MTBO in 63% yield (A). By heating it at 56°C for 3.5 hr, salithion disappeared and MTBO was formed almost quantitatively (A). The reaction mixture containing DMU needs heating for the isomerization (B). The use of potassium carbonate with DMTU or DMU accelerated the decom-

Table 3. Effect of DMTLJ or DMU on the isomerization of salithion.

| Additive | Reaction temperature (°C) | Reaction time (hr) | Yield (%) ^{a)} | | |
|---------------------------------------|---------------------------|--------------------|-------------------------|-------------------|---|
| | | | MTBO | Salithion | Decomposition product I (Rf=0) II (Rf=0.73) |
| A DMTU | 25 | 0.5 | 63 | 37 | |
| | 56 | 3.5 | 99 | 0 | 1 |
| B DMIJ | 25 | 0.5 | 0 | 100 | 0 |
| | 56 | 7.0 | 77 | 32 | |
| C DMTU K ₂ CO ₃ | 25 | 0.5 | 56 | 14 | 0 |
| | 56 | 3.0 | 0 | 0 | 25 |
| D DMU K ₂ CO ₃ | 25 | 0.5 | 63 | 100 | 0 |
| | 56 | 3.5 | 0 | 22 | 15 |
| | 56 | 7.0 | 0 | 0 | 100 |
| E DMTU -CH ₃ I | 25 | 0.5 | 0 | 100 | 0 |
| | 25 | 0.5 | 13 | 73 | 13 |
| | 56 | 1.0 | 65 | 26 | 9 |
| F DMTU NaI-CH ₃ I | | 0.33 | 65 | 32 | 3 |
| G DMTU NaI-CH ₃ I | 56 | 0:08 | | | |
| | 56 | 0.66 | 80 | 15 ^{'''} | 0 |
| | | | | | 20 |

The composition of reaction mixture: A, B, C, D, and E, salithion (4.67 mmoles), methyl iodide (5.64 mmoles), DMTLJ or DMU (9.34 mmoles), salt (4.67 mmoles), and acetone (5 ml); F and G, DMTU (4.67 mmoles), other reagents are the same as the above.

^{a)} Estimated from TLC. ^{b)} Isolated yield.

position to make the yield of MTBO lower (C, D). The addition of methyl iodide to the mixture after treatment of salithion with DMTU (E) showed no effect on the isomerization, indicating that DMTU has no ability to demethylate salithion by itself. Thus, we propose a concerted reaction shown in Scheme Z-B. Sodium iodide was used with DMTU, inasmuch as iodide ion is effective to the demethylation of salithion. White crystals began to appear on reflux for 20 min without adding methyl iodide (F). These crystals may be the compound 7. MTBO was produced in 65 % yield without addition of methyl iodide (F). The methyl group removed by iodide ion was supposed to be used for the isomerization as methyl iodide. After salithion was refluxed together with DMTU and sodium iodide for 5 min, methyl iodide was added to give MTBO in 80 % yield. After extraction with chloroform followed by water wash, MTBO was isolated in only 13 % yield.

Addition of methyl iodide to the mixture of salithion, DMTU, and acetone produces white crystals, which are of *N,N',S*-trimethylthioformamidium iodide (7) made from DMTU and methyl iodide (Scheme 2-C).

Effect of sodium iodide and potassium carbonate on the isomerization of salithion

The use of the compounds requiring water wash for the removal has disadvantage that the procedure may decompose MTBO. Acetone is appropriate as the solvent, sodium iodide as the catalyst which is soluble in acetone and insoluble in chloroform, and potassium carbonate as the reagent which prevents the reaction mixture from coloring by release of iodine molecule which oxidizes MTBO.

Table 4 shows that sodium iodide promotes not only the isomerization but also the decomposition (A). Decreasing the amount of sodium iodide dimin-

Table 4. Effect of sodium iodide and potassium carbonate on the isomerization of salithion.

| Additive | Reaction time (hr) | Yield (%) ^{a)} | | |
|--|--------------------|-------------------------|-------------------|-----------------------|
| | | MTBO | Salithion | Decomposition product |
| A NaI (5) | 0.5 | 70 | 17 | 13 |
| | 1.0 | 57 | 4 | 40 |
| | 1.5 | 36 | 0 | 64 |
| B NaI (0.5) | 0.5 | 42 | 52 | 6 |
| | 5:0 | 56 | 36 | 9 |
| | 1.0 | 64 | 29 | 7 |
| C NaI (1) K ₂ CO ₃ (10) | 1.5 | 72 | 13 | 9 |
| | 4.0 | 90 | 1 | 9 |
| | 4.0 | 86 | 6 | 9 |
| D NaI (30) K ₂ CO ₃ Acetone (450 ml) | 5:0 | 85 | 40 ^{b)} | 14 |
| E NaI (10) K ₂ CO ₃ Acetone (150 ml) | 3.5 | 86 | 12 | 2 |
| | 4.5 | 86 | 11 | 3 |
| F NaI (10) K ₂ CO ₃ Acetone (80 ml) | 3.5 | 73 | 21 | 6 |
| | 5.0 | 85 | 37 ^{'''} | 4 |
| G NaI (15) K ₂ CO ₃ CH ₃ I (225) Acetone (150 ml) | 3.0 | 66 | 27 | 7 |
| | 4.5 | 81 | 14 | 5 |
| | 6.0 | 93 | 32 ^{b)} | 6 |

The composition of reaction mixture: A, B, and C, salithion (5 mmoles), methyl iodide (6 mmoles), acetone (15 ml), and salt (mmoles); D, E, F, and G, salithion (150 mmoles), K₂CO₃ (300 mmoles), methyl iodide (180 mmoles), NaI (mmoles), and acetone (ml). Reaction temperature, 56°C.

^{a)} Estimated from TLC. ^{b)} Isolated yield.

ishes the rate of the isomerization (B). The use of potassium carbonate in addition to sodium iodide gave a precipitate after 1 hr. MTBO was produced in good yield after 4 hr (C). Preparation of MTBO on the scale of 30 times as large as C gave the similar result. After cooling and evaporating the mixture to dryness, the residue was extracted with chloroform. Chloroform-soluble fraction was evaporated to dryness to isolate MTBO in 40 % yield. Examination of different amounts of compositions of the reaction mixture gave the result in Table 4 (E, F, and G). The conditions where the isomerization completely proceeds were found.

The use of sodium iodide and potassium carbonate has a great advantage that does not need a washing with water in order to isolate MTBO.

The purification of MTBO

MTBO is highly reactive and is subject to hydrolysis. The decomposition products of MTBO are acidic enough to catalyze the decomposition. Therefore, it is necessary to purify MTBO in order to store it stably. One of the purification methods is distillation (Kobayashi *et al.*, 1969) which requires high temperature (144–5°C at 0.1 mmHg), often resulting in the decomposition particularly in the case of contamination by DMF. The purification of MTBO was attempted by two methods, column chromatography and crystallization.

Silica gel column chromatography was carried out with chloroform as the solvent. However, it was unsuitable for the purification of MTBO, inasmuch as it decomposed MTBO to give a new decomposition product (*R_f*=0.73) instead of ef-

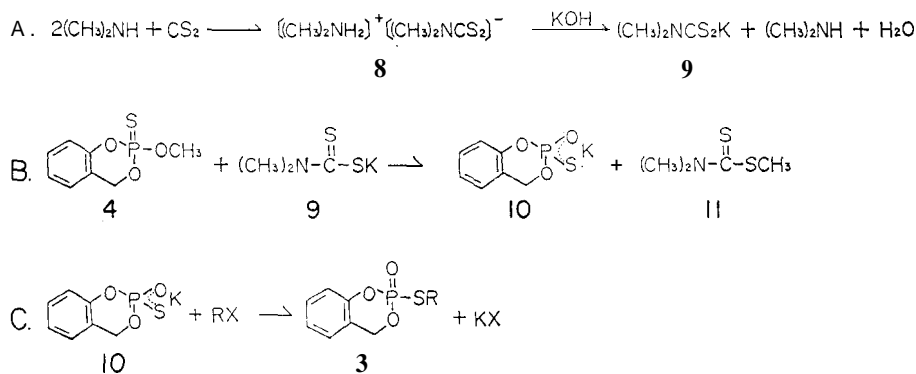
fectiveness in the separation of MTBO from salithion or the decomposition product I ($R_f=0$). It was suggested that the decomposition product of $R_f=0.73$ is o-(a-methylthio)cresol which was found as one of the reaction products of MTBO with ethanol (Sasaki *et al.*, 1973) because of its R_f value and its positive tests indicating existence of phenolic OH and methylthio groups. The decomposition of MTBO may be caused by the catalytic action of silica gel as an acid.

MTBO was able to be crystallized from ethanol. Crystallization of MTBO was attempted in the following solvents: acetone, benzene, chloroform, ethyl acetate, ether, and hexane. Ether was the most appropriate solvent of them for the crystallization. MTBO was crystallized from ether as white scaly crystals. Elemental analysis and NMR spectrum indicated MTBO remained intact after the recrystallization procedure. The existence of a group of S-methyl phosphorothiolate, phenyl protons, and methylene protons whose spin couples with that of phosphorus atom was confirmed by NMR spectroscopy (Fig. 1-C).

Since the decomposition product I ($R_f=0$) is insoluble in ether, it is readily removed by the procedure of the crystallization. MTBO purified by this method is considerably stable: on storage for 2 months at room temperature (below 20°C) in a desiccator, MTBO gave no decomposition product insoluble in ether and was pure on the basis of TLC, and its crystalline shape did not change. Therefore, colder and dry storage makes MTBO be kept intact for a longer time.

II) Synthesis of MTBO from demethylated salithion

It is suggested that the separation of the step of the demethylation of salithion from that of the synthesis of MTBO by S-methylation of demethylated salithion makes the yield of MTBO higher. Demethylation of salithion was attempted by the use of dimethylthiourea and sodium iodide, but demethylation product was not isolated. Demethylation of salithion with potassium dimethyldithiocarbamate (Scheme 3-B) and S-alkylation of demethyl salithion (Scheme 3-C) was performed by Sasaki *et al.* (1974). This procedure was examined.



Scheme 3. (A) Synthesis of potassium dimethyldithiocarbamate, (B) demethylation of salithion by potassium dimethyldithiocarbamate, and (C) S-alkylation of demethylated salithion.

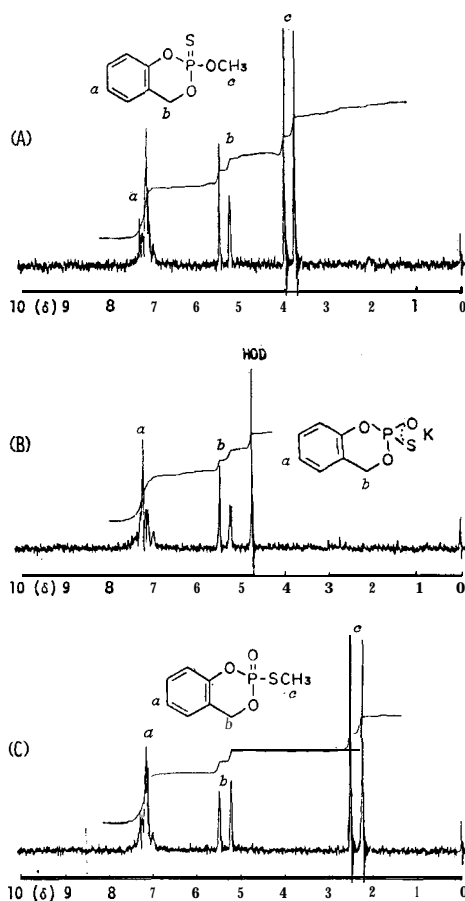


Fig. 1. NMR spectra of (A) salithion, (B) KSCP, and (C) MTBO.

Demethylation of salithion with dimethyldithiocarbamate

Salithion reacted with potassium dimethyldithiocarbamate (9) in ethanol to give crystals of potassium saligenin cyclic phosphorothioate (KSCP) (10) as shown in Scheme 3. The structure of KSCP was characterized by elemental analysis and by NMR spectra. The NMR spectrum of KSCP (Fig. 1-B) is almost the same as that of salithion (Fig. 1-A) except the absence of the signals of the protons of P-O-CH₃, (δ 3.89, doublet, $J=14$ Hz). The intact retention of the benzodioxaphosphorin ring structure was confirmed by the spin-spin coupling between the methylene protons of the benzyl group and phosphorus atom ($J=15$ Hz).

S-Methylation of demethylated salithion with methyl iodide

Potassium saligenin cyclic phosphorothioate (KSCP) (10) reacted with methyl iodide in acetone to form MTBO, being accompanied by precipitation of potassium iodide as shown in Scheme 3-C.

When acetone was used as the solvent, MTBO was isolated in good yield (87.5%) after extraction with ether. It was confirmed by TLC that the product was pure MTBO. The use of ether instead of acetone as the solvent in the same reaction gave MTBO in only 6% yield. Acetone was used in the following experiments, though acetone dissolves a small amount of potassium iodide which promotes decomposition of MTBO.

Although anhydrous potassium carbonate which removes water in the solvent and plays as an acceptor of iodide ion was added to the same reaction system, no good effect on S-methylation of KSCP was observed: the yield of MTBO was 54%.

The relationship between reaction time and the yield of MTBO at room temperature (27.5°C) was examined by weighing the yield of MTBO at different reaction times.

Figure 2 shows that the yield of MTBO attains to the maximum 89 % at about 30 min. The amount of unchanged material was considerably great within 20min. The yield of MTBO lowered on account of the decomposition after 40 min.

The preparative scale of the reaction system also gave MTBO in 83.3 % yield.

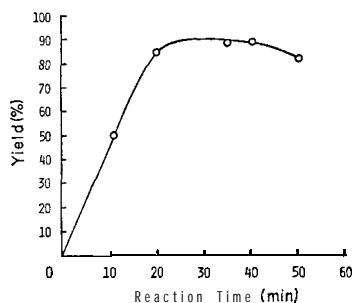


Fig. 2. Relationship between yield of MTBO and time of reaction of KSCP with methyl iodide.

CONCLUSION

In the thiono-thiol isomerization of salithion to MTBO applying Pistschi-muka reaction, the use of acetone as the solvent gave MTBO in low yield, but the use of DMF incereased the yield. However, the use of DMF has a disadvantage that DMF is difficult to remove from the reaction mixture: the evaporation of the reaction mixture containing DMF to dryness changes MTBO into a material insoluble in the solvent such as chloroform. This fact suggests that MTBO and the decomposition product of MTBO polymerize by catalytic action of DMF under concentrated and heated conditions. Therefore, in order to remove DMF without decomposition of MTBO, the reaction mixture needs to be carefully concentrated to avoid complete dryness, dissolved in chloroform, and washed with water. The water wash promotes the hydrolysis of MTBO.

Since MTBO obtained by the above procedure is contaminated by a small amount of its acidic decomposition products which promote the breakdown of MTBO, it is not sufficiently stable on storage for a long time.

In order to store MTBO stably it is desired to obtain MTBO in dry and pure state. The use of the combination of various catalysts and acetone as a readily removable solvent was examined in order to proceed the isomerization smoothly. 1,3-Dimethylthiourea is effective as the catalyst but needs the water wash to remove it. Sodium iodide accelerates the isomerization. Potassium carbonate is efficient as an acceptor of iodide ion, preventing the coloring of the reaction mixture by liberation of iodine molecule. The use of combination of sodium iodide and potassium carbonate stimulates the isomerization in acetone. MTBO was extracted with chloroform from the evaporated residue of the reaction mixture. The product is sufficiently usable as a phosphorylating agent, but unsuitable for the storage for a long time. Recrystallization of MTBO from ether was found to be the most appropriate for purification: the decomposition product of MTBO is hardly soluble in ether, and MTBO is obtained as white crystals from ether.

An alternative method for the synthesis of MTBO is more effective: salithion is demethylated with potassium dimethyldithiocarbamate to give potassium saligenin cyclic phosphorothioate (KSCP) followed by S-methylation of KSCP to form MTBO in good yield. Recrystallization of MTBO from ether is efficient also in this case. This stepwise method gives a few advantages as follows: 1) MTBO is readily prepared in high yield because each step of reactions proceeds smoothly under mild conditions; 2) pure MTBO preparation is easily obtained without contamination of salithion; and 3) the related compounds of MTBO can be easily prepared by the reaction of KSCP with other alkyl halides as alkylating agents.

Thus, MTBO was readily prepared in pure form from the thiono-isomer, salithion, by thiono-thiol isomerization.

EXPERIMENTAL

General method

i) Materials

2-Methoxy-4 *H*-1,3,2-benzodioxaphosphorin 2-sulfide, salithion (purity 94%), supplied by Sumitomo Chemical Co., was recrystallized from methanol and from ether. Dimethylformamide (DMF) was dried by co-distillation with benzene followed by distillation. Acetone was dried over anhydrous calcium sulfate and distilled. Methyl iodide was purified by distillation. Other reagents were of reagent grade.

ii) Detection of the compounds

MTBO, salithion, and their decomposition products were detected on thin-layer chromatogram (TLC). Thin-layer chromatography was performed with microchromatoplate (2.5 × 7.5 cm) of silica gel G (Merck) by the use of the solvent system, mainly a mixture of chloroform and ether (9 : 1 v/v). The *R_f* values of MTBO and salithion in the solvent system are 0.95 and 0.50, respec-

tively. Their *R_f* values in other solvent systems are listed in Table 5. MTBO and related compounds were detected by palladium chloride reagent (Niessen *et al.*, 1962) (compounds having partial structure of P-SCH₃ or P=S are visualized as yellow or dark brown spots, respectively) and by heating at 100°C for 5 min after spraying alcoholic potassium hydroxide followed by diazotized sulfanilic acid (compounds having phenolic OH group produce yellow spots). Amines were detected as blue spots by cobalt-thiocyanate reagent (Lane, 1965).

iii) Spectroscopic measurements

NMR spectra were measured with a Hitachi Parkin Elmer R-20 High Resolution NMR Spectrometer (60 MHz). UV spectra were obtained with a Shimadzu spectrometer MPS-50.

iv) Determination of the yields of MTBO

The yields of MTBO were determined by weighing after isolation. The state of the progress of the reaction was observed by the yields estimated from relative ratio of areas of spots of the products on TLC of the reaction mixture after visualizing by diazotized sulfanilic acid. This sufficiently satisfied the aim of examination of optimum conditions, though actual yields were lower than the estimated yields in such a case as precipitation occurred in the reaction mixture.

Effect of dimethylformamide (DMF) or dimethylacetamide (DMA) on the isomerization of salithion

A mixture of salithion (1.0 g, 4.67 mmoles), methyl iodide (0.35 ml, 5.64 mmoles), the salt (4.67 mmoles) listed in Table 1, and the solvent was allowed to stand at designated temperature. After a definite time, the reaction mixture was subjected to TLC. The yields estimated and isolated are listed in Table 1.

Synthesis of MTBO with potassium chloride and DMF

A mixture of salithion (30 g, 0.139 mole), potassium chloride (10.3 g, 0.138 mole), methyl iodide (12.9 ml, 0.208 mole), and DMF (300 ml) was heated at 55°C for 8 hr. After observed formation of MTBO and disappearance of salithion by TLC, the reaction mixture was filtered and the filtrate was concentrated *in vacuo*. The residue was dissolved in chloroform again. The chloroform solution was washed successively with ice water, 0.1% NaOH solution, and then ice water. Resulting emulsion was centrifuged. The chloroform layer was separated, dried over anhydrous sodium sulfate at 5°C overnight, and filtered. The filtrate was evaporated to dryness *in vacuo* to give 10.6 g of MTBO (in 35.4 % yield). It was observed that the MTBO preparation contained neither decomposition product I (*R_f*=0) nor salithion but a trace of DMF.

Synthesis of MTBO with potassium iodide and DMF

A mixture of salithion (20 g, 0.0925 mole), potassium iodide (3 g, 0.018 mole), methyl iodide (9 ml, 0.145 mole), and DMF (20 ml) was kept at room temperature (25°C) for 24 hr. TLC showed the formation of MTBO (70 %), and existence of unchanged salithion (10 %) and decomposition product (20 %). The mixture was carefully concentrated to avoid complete dryness *in vacuo* and dissolved in chloroform (50 ml). Chloroform solution was washed with water 4 times and dried over anhydrous sodium sulfate. After filtration, the filtrate

was evaporated at reduced pressure to give MTBO (8.95 g) in 45 % yield. The residue was solidified on cooling. MTBO was recrystallized from ethanol at -15°C to give MTBO (4.33 g, 21.7%) pure on the basis of TLC.

Effect of various salts on the isomerization of salithion

A mixture of salithion (1 g, 4.6 mmoles), methyl iodide (0.35 ml, 5.6 mmoles), the salt (4.6 mmoles) listed in Table 2, and the solvent (3ml) was allowed to stand at 37°C for 12 hr. The mixture was checked by TLC.

Effect of 1,3-dimethylthiourea (DMTU), and 1,3-dimethylurea (DMU) on the isomerization of salithion

A mixture of salithion (4.67 mmoles), methyl iodide (5.64 mmoles), DMTU or DMU (9.34 mmoles), potassium carbonate or sodium iodide (4.67 mmoles), and acetone (5ml) was kept at the temperature listed in Table 3. The yield was estimated from TLC.

N,N',S-Trimethylthioformamidium iodide (9)

DMTU (8) (1.042 g, 10 mmoles) was dissolved in acetone (5 ml). To the solution was added methyl iodide to form white crystals (9) in 2 min. White needle-like crystals (2.31 g, 94.3%) were obtained after filtrated, washed with acetone and dried in *vacuo*.

Anal. Found : C, 19.70 ; H, 4.42 ; N, 11.32. Calcd. for $C_4H_{11}N_2SI$: C, 19.50 ; H, 4.51; N, 11.40 %.

Purification of MTBO by silica gel chromatography

i) Search for the solvent

The solvents were sought from the *R_f* values of MTBO, *o*-(α -methylthio)-cresol, and salithion in TLC with silica gel G microchromatoplate (Table 5).

Table 5. *R_f* values of MTBO and related compounds in TLC.

| Solvent | MTBO | <i>o</i> -(α -Methylthio)- cresol | Salithion |
|----------------------------|------|--|-----------|
| Chloroform | 0.18 | | 0.84 |
| Benzene | 0 | 0.32 | 0.70 |
| Hexane | 0 | 0 | 0 |
| Ether | 0.61 | | 0.93 |
| Acetone | 0.93 | 0.93 | 0.93 |
| Ethyl acetate | 0.44 | | 0.91 |
| Chloroform-benzene (9 : 1) | 0.14 | 0.45 | 0.83 |
| Chloroform-Ether (9 : 1) | 0.50 | 0.73 | 0.95 |
| Chloroform-Hexane (9 : 1) | 0 | 0.33 | 0.74 |
| Chloroform-Benzene (1 : 1) | 0.09 | 0.38 | 0.74 |
| Chloroform-Acetone (9 : 1) | 0.63 | 0.73 | 0.89 |
| Benzene-Acetone (9 : 1) | 0.36 | 0.59 | 0.75 |

ii) Synthesis of MTBO and silica gel chromatography

A mixture of salithion (1 g), methyl iodide (0.8g), potassium carbonate (0.75g), DMF (1.28ml), and acetone (10ml) was refluxed for 4 hr. After check of formation of MTBO by TLC, the mixture was filtered and filtrate was evaporated to dryness. The residue was dissolved in chloroform and applied to a column (1.1 x 12.5 cm) of silica gel (0.05-0.2 mm, 5 g) packed by dry method.

Chloroform was made to flow through the column. The 4 ml-fractions were collected. All the fractions were checked by TLC.

Crystallization of MTBO

i) Search for the solvents for crystallization

MTBO was dissolved in solvents to examine the solubility and stability of MTBO for the solvents. The stability was checked by TLC after standing the solution at room temperature for 1 day.

ii) Crystallization of MTBO from ether

Crude MTBO (12.96 g) prepared by the method in Table 4-D was dissolved in ether (75ml) by heating under reflux. Insoluble material was filtered off. The filtrate was chilled at -15°C to give white scaly crystals. The crystals were collected by filtration and dried over phosphorus pentoxide *in vacuo* to give 5.2 g of MTBO. MTBO was readily crystallized from ether by adding small amount of this crystals as seed. It has a melting point of 44°C , and is pure on the basis of TLC.

Anal. Found: C, 44.70; H, 4.47. Calcd. for $\text{C}_8\text{H}_9\text{PO}_3\text{S}$: C, 44.50; H, 4.20 %

NMR $\delta_{\text{TMS}}^{\text{CDCl}_3}$: 2.40 (3H, doublet, $J=16.5\text{ Hz}$, P-S-CH_3), 5.37 (2H, doublet, $J=16.5\text{ Hz}$, $J'=2\text{ Hz}$, $\text{Ph-CH}_2\text{-O-P}$), 6.9-7.5 (4H, Ph-H).

Establishment of the method for preparation of MTBO by Pistschimuka reaction

A mixture of salithion (64.8 g, 300 mmoles), potassium carbonate (82.6 g, 600 mmoles), sodium iodide (3 g, 20 mmoles), methyl iodide (22.5 ml, 360 mmoles), and acetone (300 ml) is refluxed for 4 hr. The reaction mixture is cooled and evaporated to dryness. To the residue is added ether (100 ml) and refluxed. The adhesive precipitate is filtered off. The filtrate is chilled at -15°C and is allowed to stand after seeding of MTBO to form white crystals. The crystals are collected by filtration and dried *in vacuo* to give 15.4 g (23.8 %) of MTBO.

Preparation of potassium dimethyldithiocarbamate

Dimethylammonium dimethyldithiocarbamate (8) was readily prepared only by mixing dimethylamine with carbon disulfide (2 : 1, moles/mole) (Brown and Harris, 1949).

Anal. Found: C, 35.91; H, 8.43; N, 16.86. Calcd. for $\text{C}_5\text{H}_{14}\text{N}_2\text{S}_2$: C, 36.11; H, 8.49; N, 16.84%.

The potassium salt (9) was obtained by adding an equivalent aqueous potassium hydroxide solution to the aqueous solution of 8 followed by evaporation to dryness. It was observed that co-evaporation of the residue with isopropanol gives hygroscopic crystals of 9 but no crystals with ethanol.

Anal. Found : C, 22.67 ; H, 4.64 ; N, 8.85. Calcd. for $\text{C}_3\text{H}_6\text{NS}_2\text{K}$: C, 22.62 ; H, 3.77; N, 8.79%.

Potassium saligenin cyclic phosphorothioate (KSCP) (10)

Salithion (118.89 g, 0.55 mole) was dissolved in ethanol (250ml) on gentle heating and cooled to room temperature. Potassium dimethyldithiocarbamate (9) (79.7 g, 0.5 mole) was also dissolved in ethanol (150 ml) in the same man-

ner. The solution of salithion was added dropwise over a period of 40min to the solution of 9 with stirring at 40°C. White crystals were produced in 20 min from start of the addition. The reaction mixture was further stirred at 40°C for an additional 1 hr. The precipitate was collected by filtration. The cake was washed twice with chloroform to remove unchanged salithion. Drying the cake *in vacuo* gave white powdery crystals of KSCP (99.2 g, 82.7 %). mp. 204-209°C

Anal. Found: C, 35.14; H, 2.90; P, 13.20. Calcd. for $C_7H_6O_3PSK$: C, 34.99; H, 2.52; P, 12.89%.

UV $\lambda_{max}^{H_2O}$ nm (E): 268 (1150) and 275 (1040) at pH 2, 7 and 12.

NMR $\delta_{DSS}^{D_2O}$: 5.33 (2 H, doublet, $J=15$ Hz, $J'=2$ Hz, Ph-CH₂-O-P), 6.9-7.4 (4 H, Ph-H)

NMR spectrum of salithion

$\delta_{TMS}^{CDCl_3}$: 3.89 (3 H, doublet, $J=14$ Hz, P-O-CH₃), 5.35 (2 H, doublet, $J=14$ Hz, $J'=1$ Hz, Ph-CH₂-O-P), 6.9-7.4 (4 H, Ph-H).

S-Methylation of demethylated salithion with methyl iodide

i) Examination of the reaction solvent

A mixture of KSCP (2.4 g), methyl iodide (0.68 ml), and acetone (5 ml) was stirred for 35min at room temperature (27.5°C) to give a white precipitate of potassium iodide (1.28 g). After the precipitate was filtered off, the filtrate was evaporated to dryness at reduced pressure. The residue was refluxed with ether. The ether-soluble portion of the solution was evaporated to dryness to give 1.89 g of the product. It was confirmed by TLC that the product was pure MTBO.

ii) Effect of potassium carbonate

Anhydrous potassium carbonate (1.38 g) was added to the same reaction system described in i) in order to examine the effect of potassium carbonate.

iii) Determination of reaction time

The relationship between reaction time and the yield of MTBO at room temperature (27.5%) was examined by weighing the yield of MTBO at different reaction times by the use of the same reaction composition described in i).

iv) The reaction system on preparative scale

A mixture of KSCP (24 g), methyl iodide (8.40 ml), and acetone (50 ml) was stirred for 30min at room temperature, followed by the same procedure to give MTBO (18 g) in 83.3 % yield.

REFERENCES

- Brown, H. C. and R. H. Harris 1949 Action of carbon disulfide on trimethylamine and trimethylphosphine. *J. Amer. Chem. Soc.*, 71: 2751-2753
- Casida, J. E., M. Eto and R. L. Baron 1961 Biological activity of a tri-o-cresyl phosphate metabolite. *Nature* 191: 1396-1397
- Eto, M. 1969 Specificity and mechanism in the action of saligenin cyclic phosphorus esters. *Residue Reviews*, 25: 187-200
- Eto, M., J. E. Casida and T. Eto 1962 Hydroxylation and cyclization reactions involved

- in the metabolism of tri-o-cresyl phosphate. *Biochem. Pharmacol.*, 11: 337-352
- Eto, M., Y. Kinoshita, T. Kato and Y. Oshima 1963 Saligenin cyclic methyl phosphate and its thiono analogue : New insecticides related to the active metabolite. *Nature*, 200: 171-172
- Eto, M., M. Sasaki, M. Iio, M.-Y. Eto and H. Ohkawa 1971 Synthesis of 2-methylthio-4H-1,3,2-benzodioxaphosphorin-2-oxide by thiono-thiol conversion and its use as phosphorylating agent. *Tetrahedron Lett.*, 1971: 4263
- Eto, M.-Y. 1975 Studies on the synthesis of biochemically important phosphate esters- Development of a new phosphorylating agent, 2-methylthio-4H-1,3,2-benzodioxaphosphorin-a-oxide (MTBO). Doctoral Dissertation, Kyushu University.
- Eto, M.-Y., M. Iio, Y. Kobayashi, H. Omura and M. Eto 1974 Synthesis of ribonucleoside 5'-S-methyl phosphorothiolates and ribonucleoside 3': 5'-cyclic phosphate from nucleosides applying a new phosphorylating agent, MTBO. *Agric. Biol. Chem.*, 38: 2081-2092
- Eto, M.-Y, M. Iio, H. Omura and M. Eto 1976 Synthesis of nucleotides with a new phosphorylating agent, 2-methylthio-4H-1,3,2-benzodioxaphosphorin-2-oxide (MTBO) (II). Synthesis of cytokinin-like compounds. *Nucleic Acids Research* 1976 Special Publication No. 2, 565-568
- Iio, M., M.-Y. Eto, M. Eto and H. Omura 1973 Reaction of MTBO with alcohols. *Agric. Biol. Chem.*, 37: 115-121
- Iio, M., Y. Kobayashi, M.-Y. Eto, K. Yano, H. Omura and M. Eto 1975 Synthesis of 2',3'-cyclic ribonucleotide derivative from ribonucleoside with phosphorylating agent, MTBO, and its application to synthesis of oligonucleotide. *J. Fuc. Agr., Kyushu Univ.*, 19: 73-81
- Iio, M., K. Yano, M.-Y. Eto, H. Omura and M. Eto 1977 Chemical conversion of ribonucleoside 5'-S-methyl phosphorothiolates into ribonucleotide anhydrides including adenosine 5'-triphosphate and uridine diphosphate glucose. *Agric. Biol. Chem.*, 41: 155-160
- Kobayashi, K., M. Eto, Y. Oshima, T. Hirano, T. Hosoi and S. Wakamori 1969 Synthesis and biological activities as insecticides and fungicides of saligenin cyclic phosphorothiolates. *Botyu-Kagaku*, 34: 165-170
- Lane, E. S. 1965 Thin-layer chromatography of long-chain tertiary amines and related compounds. *J. Chromatog.*, 18: 426-430
- Niessen, H., H. Tietz und H. Frehse 1962 Papierchromatographische Trennung aromatischer Phosphorsäureester-Insectizide. *J. Chromatog.*, 9: 111-113
- Sasaki, M., H. Ohkawa and M. Eto 1973 The conversion of saligenin cyclic methyl phosphorothionate into the thiolate analogs and a new rearrangement reaction. *J. Fuc. Agr., Kyushu Univ.*, 17: 173-180
- Sasaki, M. and D. Mukai 1974 Saligenin cyclic thiophosphate alkali metal salts. *Chem. Abst.*, 81: 135702 y