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33. **Kyozo Hayashi** : Studies on the Synthesis of Amino Acids by the Schmidt Reaction. VI.[*1] Synthesis of Methionine Sulfoximine Analogs.

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Dogs fed with wheat flour which was treated with nitrogen trichloride often suffer a fit like epilepsy and die. Bentley, *et al.*1) isolated a crystalline substance from gluten treated with nitrogen trichloride and discovered that the toxic substance was a chemical compound having a characteristic structure in which the NH group and O atom were bonded to the S atom. There have been until now only a few reports on syntheses of analogs in this group. Whitehead, *et al.*2) prepared DL-methionine sulfoximine by reaction between DL-methionine and sodium azide in 27% yield.

The author succeeded in the synthesis of several analogs of methionine sulfoximine by the reaction between (2-alkylthioethyl)malonic acid and hydrazoic acid according to the reaction sequence shown in Chart 1 and obtained some new informations about the reaction pathway.

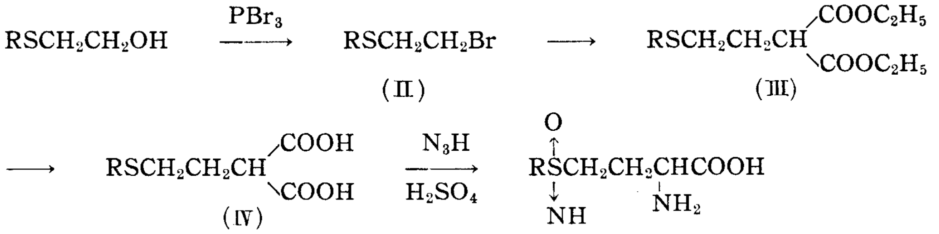

Chart 1.

In this first series, thiol derivatives were condensed with ethylene chlorohydrin according to the method of Steinkopf, *et al.*,3) the 2-alkylthioethanol (I) produced was brominated with phosphorus tribromide, and the bromide (II) thus obtained was condensed with diethyl malonate in the usual way, giving (2-alkylthioethyl)malonate (III), which was hydrolyzed with potassium hydroxide to give (2-alkylthioethyl)malonic acid (IV) as the starting material. The reaction of (IV) with hydrazoic acid was carried out in the same manner as reported previously4), and methionine sulfoximine analogs were obtained in a pure state.

Above experiments showed that the reaction of 1 mole of the starting material (IV) with more than 3 moles of hydrazoic acid gave pure sulfoximine of amino acid, but in the reaction with 1 mole each of (IV) and hydrazoic acid, a mixture of amino acids was

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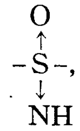
[*1] Part V : This Bulletin, **7**, 912(1959).

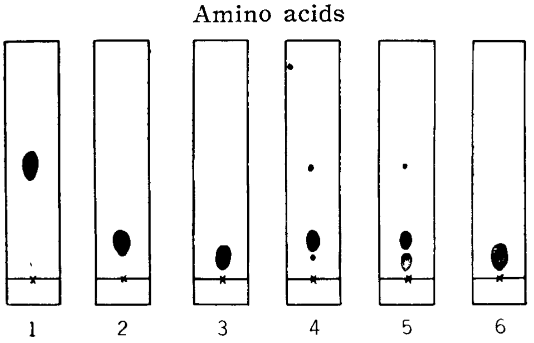
[*2] 1 Yoshida-Konoe-cho, Sakyo-ku, Kyoto (林 恭三).

1) H.R. Bentley, E.E. McDermott, T. Moran, J.K. Whitehead, J. Pace : Proc. Roy. Soc., B. **137**, 402(1950); H.R. Bentley, E.E. McDermott, J.K. Whitehead : *Ibid.*, B. **138**, 265(1951).
2) J.K. Whitehead, H.R. Bentley : J. Chem. Soc., **1952**, 1572.
3) W. Steinkopf, J. Herold, J. Stohr : Ber., **53**, 1009(1921).
4) This Bulletin, **7**, 187(1959).

obtained. This fact means that (IV) has three possible sites of reaction with hydrazoic acid, such as transformation of S atom to sulfoximine,  $\underset{\underset{\displaystyle NH}{\downarrow}}{\overset{\overset{\displaystyle O}{\uparrow}}{-S-}}$, together with conversion of COOH group to NH_2 group.

Therefore, experiments were carried out to ascertain the intermediate reaction products and the mechanism of hydrazoic acid attack on the three reaction points of (IV) to give pure sulfoximine of amino acid. (IV) was reacted with hydrazoic acid in various quantities and the resulting amino acids and acid substances were examined by paper chromatography.

In this experiment, (2-methylthioethyl)malonic acid was used as the starting material and 0.01 mole of (2-methylthioethyl)malonic acid was reacted with 0.011 mole of hydrazoic acid in benzene in the presence of a catalyst (conc. sulfuric acid). The acid layer was diluted with 2~3 volumes of water, adjusted to pH 2~3 with hot saturated aqueous solution of barium hydroxide, the precipitated barium sulfate was filtered off, and the filtrate was passed through a column of Amberlite IR-120. The adsorbed amino acid was eluted with 0.2~0.5*N* ammonia and the eluate was concentrated *in vacuo*. The crystals obtained showed three spots (Rf 0.12, 0.17, 0.46) on the paper chromatogram as shown in Fig. 1 (solvent system used, $BuOH:AcOH:H_2O=4:1:1$). The spot with Rf 0.17, which was the largest of three spots agreed in Rf value with pure DL-methionine sulfoxide. The faint spot at Rf 0.11 agreed with the Rf value of pure DL-methionine sulfoximine, produced by the reaction of 1 mole of (2-methylthioethyl)malonic acid with more than 3 moles of hydrazoic acid. The faint spot at Rf 0.46 was identified as that of DL-methionine by comparison with the Rf values. Thus, the above product was presumed to contain mainly DL-methionine sulfoxide and a little of DL-methionine sulfoximine and DL-methionine.

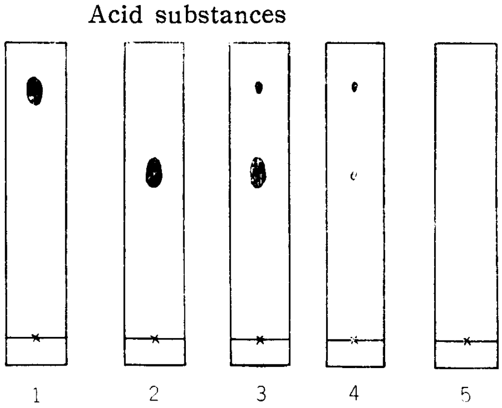


Solvent system : $BuOH:AcOH:H_2O=4:1:1$
Reagent : Ninhydrin
1) DL-Methionine
2) DL-Methionine sulfoxide
3) DL-Methionine sulfoximine
4) 0.01 mole (2-methylthioethyl)malonic acid × 0.011 mole N_3H
5) 0.01 mole (2-methylthioethyl)malonic acid × 0.022 mole N_3H
6) 0.01 mole (2-methylthioethyl)malonic acid × 0.035 mole N_3H

Fig. 1. Paper Chromatogram of the Product from the Reaction between Hydrazoic Acid and (2-Methylthioethyl)malonic Acid

The acid substances were then examined. The effluent from the column of Amberlite IR-120 in the above experiment was extracted with ether and ethyl acetate, the solvent was removed, and the residue was examined by paper chromatography. The paper chromatogram so obtained is shown in Fig. 2. The spot at Rf 0.57 was confirmed as that of (2-methylsulfinylethyl)malonic acid which was obtained in a pure state by the oxidation of (IV) with hydrogen peroxide in acetone containing hydrochloric acid. The other faint spot which was observed at Rf 0.84 agreed with the Rf value of (2-methylthioethyl)malonic acid. From the above results, it is considered that the main product may be (2-methylsulfinylethyl)malonic acid.

Next, 0.01 mole of (2-methylthioethyl)malonic acid was reacted with 0.022 mole of hydrazoic acid and treated in the same manner as above. The paper chromatogram is shown in Fig. 1. The amino acids produced mainly consisted of DL-methionine sulfoxide and



Solvent system : BuOH:AcOH:H_2O=4:1:1
Reagent : Bromothymol Blue
1) Starting material
2) (2-Methylsulfonylmethyl)malonic acid
3) 0.01 mole (2-methylthioethyl)malonic acid × 0.011 mole N_3H
4) 0.01 mole (2-methylthioethyl)malonic acid × 0.022 mole N_3H
5) 0.01 mole (2-methylthioethyl)malonic acid × 0.035 mole N_3H

Fig. 2. Paper Chromatogram of the Product from the Reaction between Hydrazoic Acid and (2-Methylthioethyl)malonic Acid

DL-methionine sulfoximine in about equal quantities plus a trace of DL-methionine. As for acid substances, spots of (2-methylsulfinylethyl)malonic acid and the starting material were faintly observed.

When 0.01 mole of (2-methylthioethyl)malonic acid was reacted with 0.035 mole of hydrazoic acid, only one spot was observed upon chromatographic separation of the reaction mixture. It was identified as DL-methionine sulfoximine according to the method described above. As for acid substances, (2-methylthioethyl)malonic acid and (2-methylsulfinyl)malonic acid were not apparent on the paper chromatogram, and thus it may be concluded that the disappearance of unreacted acid substances is due to the conversion of (2-methylthioethyl)malonic acid into other unknown substance during the reaction.

The product obtained from the reaction between 0.011 mole and 0.022 mole of hydrazoic acid and 0.01 mole of (2-methylthioethyl)malonic acid was treated with an excess of hydrazoic acid and DL-methionine sulfoximine was obtained in a pure state. Further, (2-methylsulfinylethyl)malonic acid, which was obtained by the oxidation of (2-methylthioethyl)malonic acid with hydrogen peroxide, was reacted with the excess of hydrazoic acid and gave DL-methionine sulfoximine.

From the above experiments, it is considered that the reaction proceeds as shown in Chart 2. (2-Methylthioethyl)malonic acid forms a sulfimino-type compound, such as $\underset{\mathrm{NH}}{\underset{\downarrow}{-\mathrm{S}-}}$, but as it was extremely unstable, it was hydrolyzed immediately to sulfoxide. If

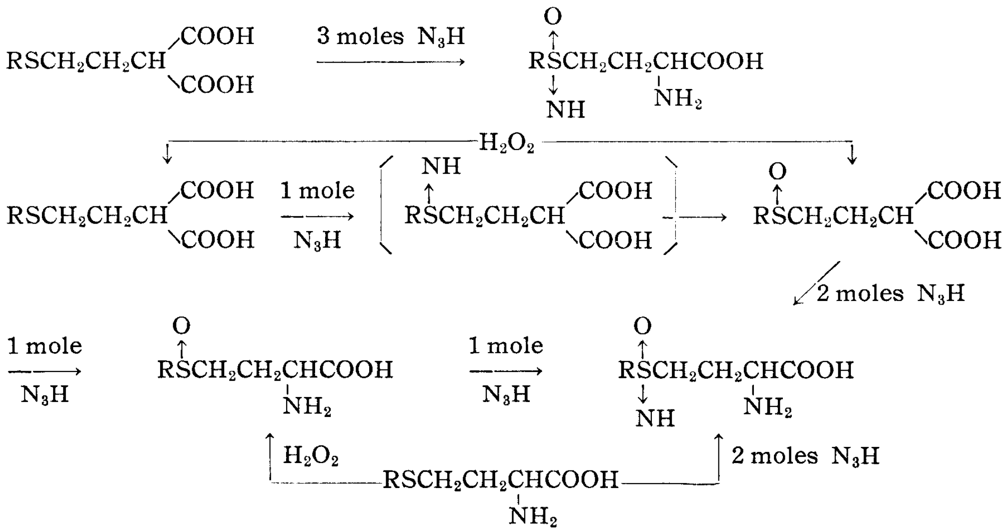


Chart 2. Reaction Mechanism of Formation of Methionine Sulfoximine Analogs

this consideration is correct, ammonia must be formed in its hydrolysis and it was identified as ammonium chloride from the reaction mixture. This sulfinyl compound further reacts with 1 mole of hydrazoic acid to give DL-methionine sulfoxide, which reacts with 1 mole of hydrazoic acid to give DL-methionine sulfoximine.

Further several homologs of methionine sulfoxide and methionine sulfone were prepared from ethionine,[5] propionine,[5,6] butionine,[5] and isoamionine.[5]

Experimental

2-Propylthioethyl Bromide—13 g. of PBr_3 was added slowly to 6.5 g. of 2-propylthioethanol under cooling. After dropwise addition, the mixture was heated on a water bath for 30 min. to complete the reaction and then poured into ice water. The lower oily layer was separated, washed with water, and dried over Na_2SO_4. After the solvent was removed, the residue was distilled *in vacuo*. b.p$_{20}$ 95°. Yield, 9.1 g. *Anal.* Calcd. for $C_5H_{11}BrS$: C, 32.80; H, 6.06. Found : C, 32.11; H, 5.88. All other bromides were prepared analogously.

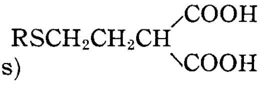
Diethyl (2-Alkylthioethyl)malonate—Diethyl (2-ethylthioethyl)malonate : To a solution of 2.3 g. of metallic Na dissolved in 35 cc. of dehyd. EtOH and 16 g. of diethyl malonate, 18 g. of 2-ethylthioethyl bromide was added. The mixture was refluxed in a water bath until the reaction mixture became neutral and the excess of EtOH was removed. The NaBr crystals formed were filtered off and the residue was fractionally distilled. The yield and b.p. are shown in Table I. All other malonates were prepared analogously.

Table I. Diethyl (2-Alkylthioethyl)malonates (all are colorless oil)

R	b.p. (°C/mm.Hg)	Yield (%)	Analysis (%) Calcd. C	Calcd. H	Found C	Found H
C_2H_5	165~166/23	53	53,21	8.06	53.52	8.24
C_3H_7	170/28	64	54.95	8.45	55.08	8.62
C_4H_9	185/20	42	56.50	8.76	56.65	8.84

(2-Alkylthioethyl)malonic Acid—(2-Ethylthioethyl)malonic Acid : It was obtained in the same manner from 8.5 g. of diethyl (2-ethylthioethyl)malonate, 0.7 g. of KOH, 7.0 cc. of H_2O, and 8 cc. of EtOH. Yield and m.p. are shown in Table II. All other malonic acids were prepared analogously.

Table II. (2-Alkylthioethyl)malonic Acid



$RSCH_2CH_2CH(COOH)_2$

(All are white granular crystals)

R	m.p. (°C) (dccomp.)	Yield (%)	Analysis (%) Calcd. C	Calcd. H	Found C	Found H
C_2H_5	78~79	95	43.75	6.29	44.01	6.24
C_3H_7	72	88	46.60	6.84	45.51	6.68
C_4H_9	67~68	82	49.08	7.32	48.75	7.34

DL-Methionine Sulfoximine—A mixture of 1.8 g. of (2-methylthioethyl)malonic acid, 8 cc. of conc. H_2SO_4, and 10 cc. of $CHCl_3$ was heated on a water bath at 45~50° and 15 cc. of 10.0% hydrazoic acid-benzene solution was added slowly under vigorous stirring. The stirring was continued for further 3 hr. Succeeding procedure was the same as in the previous report. White needles (from hydr. EtOH). The yield and m.p. are shown in Table III.

DL-Ethionine Sulfoximine—A mixture of 2.9 g. of (2-ethylthioethyl)malonic acid, 15 cc. of conc. H_2SO_4, and 40 cc. of $CHCl_3$ was heated on a water bath at 45~50° and 11 cc. of 19.3% hydrazoic acid-benzene solution was added slowly under vigorous stirring. After the addition, stirring was continued for further 3 hr. Succeeding procedure was the same as in the previous report. White amorphous powder (from hydr. EtOH). The yield and m.p. are shown in Table III.

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5) M. D. Armstrong, J. D. Lewis : J. Org. Chem., **16**, 749(1951).

6) C. M. Stevens, C. A. Johnson, R. Watanabe : J. Biol. Chem., **212**, 49(1955).

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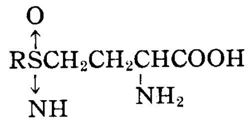
Table III. Methionine Sulfoximine Analogs



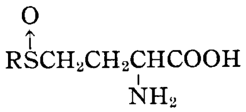
$R\overset{\overset{O}{\uparrow}}{\underset{\underset{NH}{\downarrow}}{S}}CH_2CH_2\underset{NH_2}{CH}COOH$

R	m.p. (°C) (decomp.)	Yield (%)	Analysis (%) Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
CH_3	212	25	33.33	6.71		33.11	6.97	
C_2H_5	197	29	35.40	7.38	13.79	35.30	7.65	13.54
C_3H_7	210~211	25	40.38	7.75	13.46	40.26	7.96	13.32
C_4H_9	214	30	43.23	8.16	12.60	43.39	8.39	12.81

DL-Ethionine Sulfoxide—A mixture of 0.5 g. of DL-ethionine, 0.38 cc. of conc. HCl, 0.45 cc. of 30% H_2O_2, and 2.6 cc. of H_2O was heated at 60° on a water bath for 10 min. The reaction mixture was separated by treatment with ion exchange resin as previously reported. Yield, 0.53 g. m.p.s are shown in Table IV.

DL-Propionine Sulfoxide—It was not obtained by the same manner as DL-ethionine sulfoxide. To a solution of 0.1 g. of DL-propionine, 1.0 cc. of conc. HCl, and 5 cc. of H_2O, 0.5 cc. of 30% H_2O_2 was slowly added under cooling in an ice bath and the mixture was left to stand for 3 hr. The reaction mixture was diluted with 50 cc. of water and treated as above. Recrystallized from hydr. EtOH. Yield and m.p. are shown in Table IV. All other sulfoxides were prepared analogously.

Table IV. Methionine Sulfoxide Analog



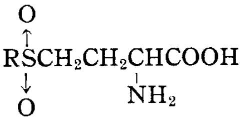
$R\overset{\overset{O}{\uparrow}}{S}CH_2CH_2\underset{NH_2}{CH}COOH$

R	m.p. (°C) (decomp.)	Yield (%)	Appearance	Analysis (%) Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
C_2H_5	227	96	White amorphous powders	40.22	7.31	7.82	40.13	7.22	7.58
C_3H_7	237	70	〃	43.51	7.83	7.25	43.64	7.62	7.06
C_4H_9	235	64	White plates	46.37	8.27	6.76	46.04	8.26	6.72
iso-C_5H_{11}	228~230	80	〃	48.85	8.66	6.51	48.96	8.81	6.33

DL-Ethionine Sulfone—A mixture of 0.5 g. of DL-ethionine, 0.38 cc. of conc. HCl, 1.3 cc. of 30% H_2O_2, and 2.6 cc. of water was heated on a water bath at 90~100° for 2 hr. After the reaction was over, the reaction mixture was treated with ion exchange resin as above. Yield, 0.38 g., white needles (from hydr. EtOH), m.p. 243°.

DL-Propionine Sulfone—Obtained in the same manner as for DL-ethionine sulfoxide from 0.1 g. of DL-propionine, 0.8 cc. of conc. HCl, 0.09 cc. of 30% H_2O_2, and 1.5 cc. of water. Recrystallized from hydr. EtOH. Yield and m.p. are shown in Table V. All other sulfones were prepared analogously.

Table V. Methionine Sulfone Analogs
(All are white plate crystals)



$R\overset{\overset{O}{\uparrow}}{\underset{\underset{O}{\downarrow}}{S}}CH_2CH_2\underset{NH_2}{CH}COOH$

R	m.p. (°C) (decomp.)	Yield (%)	Analysis (%) Calcd. C	Calcd. H	Calcd. N	Found C	Found H	Found N
C_2H_5	243	63	36.92	6.71	7.18	37.16	6.94	7.27
C_3H_7	247	58	40.19	7.23	6.70	40.36	7.02	6.79
C_4H_9	256	73	43.04	7.68	6.28	42.96	7.79	6.42
iso-C_5H_{11}	258	78	45.56	8.07	5.91	45.66	7.87	6.11

(2-Methylsulfonylethyl)malonic Acid and (2-Methylsulfinylethyl)malonic Acid–To 1.5 g. of (2-methylthioethyl)malonic acid dissolved in 15 cc. of acetone, 0.5 cc. of 30% H_2O_2 was added slowly at −18° under chilling. The reaction mixture was left to stand at this temperature for 2 hr. and then overnight in a refrigerator. After the reaction mixture was concentrated *in vacuo*, the residue was extracted with Et_2O and dried over Na_2SO_4. Ether was removed and the residue was recrystallized

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from Et_2O-petr. ether. (2-Methylsulfinylethyl)malonic acid was obtained as white granules, m.p. $147\sim148^\circ$. Yield, 0.3 g. *Anal.* Calcd. for $C_6H_{10}O_5S$: C, 37.12; H, 5.19. Found : C, 37.26; H, 5.32.

The residue from above Et_2O extraction was lyophilized, dissolved in acetone, and treated with activated charcoal. Acetone was removed *in vacuo*, the residue was left to stand in a desiccator for a long time, and crystals that precipitated were recrystallized from acetone-Et_2O, m.p. $114\sim115^\circ$ (decomp.). Yield, 0.8 g. *Anal.* Calcd. for $C_6H_{10}O_6S$: C, 34.29; H, 4.80. Found : C, 34.63; H, 4.91.

Formation of DL-Methionine Sulfoximine from (2-Methylsulfinylethyl)malonic Acid—A mixture of 0.3 g. of (2-methylsulfinylethyl)malonic acid, 2.0 cc. of conc. H_2SO_4, and 5 cc. of benzene was warmed on a water bath at $45\sim50^\circ$ and 1.5 cc. of 9% hyrazoic acid-benzene solution was added slowly under vigorous stirring. Succeeding procedure was the same as for DL-methionine sulfoximine. The crystals so obtained were recrystallized from hydr. EtOH. White amorphous powder, m.p. 212° (decomp.). Yield, 0.1 g. *Anal.* Calcd. for $C_5H_{12}O_3N_2S$: C, 33.33; H, 6.71; N, 15.55. Found : C, 33.51; H, 6.91; N, 15.27.

Formation of DL-Methionine Sulfone from (2-Methylsulfonylethyl)malonic Acid—A mixture of 0.1 g. of (2-methylsulfonylethyl)malonic acid, 1.0 cc. of conc. H_2SO_4, and 3.0 cc. of benzene was heated on a water bath at $40\sim50^\circ$ and 0.5 cc. of 9% hydrazoic acid-benzene solution was added slowly under vigorous stirring. Succeeding treatment with ion exchange resin was the same as for DL-methionine sulfoximine. The crystals obtained were recrystallized from hydr. EtOH to white granules, m.p. $254\sim255^\circ$. Yield, 0.03 g. *Anal.* Calcd. for $C_5H_{11}O_4NS$: C, 33.15; H, 6.12; N, 7.73. Found : C, 33.40; H, 6.38; N, 7.61.

Reaction between (2-Methylthioethyl)malonic Acid and Hydrazoic Acid—Separation and Identification of Amino Acids : The reaction was carried out in the same manner as DL-methionine sulfoximine from 0.89 g. (0.01 mole) of (2-methylthioethyl)malonic acid, 3 cc. of conc. H_2SO_4, and 3.4 cc. of 6.5% hydrazoic acid-chloroform solution. The produced amino acid showed three spots on the paper chromatogram, as shown in Fig. 2. Yield, 0.05 g. These spots were identified respectively as those of DL-methionine (Rf 0.46), DL-methionine sulfoxide (Rf 0.17), and DL-methionine sulfoximine (Rf 0.12) compared with spots of the authentic sample on the paper chromatogram (solvent system BuOH: AcOH:H_2O=4:1:1).

Separation and Identification of Acid Substances : The reaction mixture from above experiment was extracted with Et_2O and AcOEt. The extract was dried over Na_2SO_4 and the solvent was removed. Paper chromatography was carried out on the residue (0.41 g.) and the chromatogram is shown in Fig. 2.

Reaction between (2-Methylthioethyl)malonic Acid and Hydrazoic Acid—Amino Acids: The reaction was carried out in the same manner as described above with 0.01 mole of malonic acid and 0.022 or 0.035 mole of hydrazoic acid. Yields were 0.25 g. and 0.28 g., respectively. Paper chromatogram of these amino acids are shown in Fig. 1.

Acid Substances : Acid substances were separated in the same manner and paper chromatogram so obtained is shown in Fig. 3. Yields were 0.06 g. and zero, respectively.

Identification of Ammonia formed in the Reaction between (2-Methylthioethyl)malonic Acid and Hydrazoic Acid—The reaction was carried out in the same manner as for DL-methionine sulfoximine from (2-methylthioethyl)malonic acid (1.8 g.), conc. H_2SO_4 (5 cc.), benzene (10 cc.), and 7.5% hydrazoic acid-benzene solution (6.5 cc.). The reaction mixture was basified with excess of NaOH, distilled immediately, the distillate was collected in dil. HCl, and concentrated *in vacuo*. EtOH was added to the residue giving white crystals, which were purified by sublimation. *Anal.* Calcd. for NH_4Cl : N, 26.19. Found : N, 25.91.

The author expresses his deep gratitude to Prof. T. Suzuki and Prof. T. Uno for their continued guidance and encouragement during the course of this work, and to the members of the microanalytical center of this Institute for microanalyses.

Summary

Toxic substance, which is contained in wheat flour treated with nitrogen trichloride and causing dogs to suffer a fit like epilepsy, was separated by Bently, *et al.* Although it had been confirmed that this substance is a specific compound having O atom and NH group attached to S atom, its analogs have not yet been synthesized. Therefore, several of its analogs were prepared by the reaction of (2-alkylthioethyl)malonic acid with hydrazoic acid.

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