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# Methionine Studies. VII. Sulfonium Derivatives[1]

BY GERRIT TOENNIES AND JOSEPH J. KOLB

The amino acid methionine and its derivatives can react as sulfides with organic halides to form sulfonium salts.[2] In the present paper compounds of this type and some of the factors governing their formation are described.

The reactions involved are

$$(1)\quad HOOCCH(NH_2)CH_2CH_2SCH_3 + RX \longrightarrow HOOCCH(NH_2)CH_2CH_2S(CH_3)(R)^+X^-$$

$$(2)\quad HOOCCH(NHAc)CH_2CH_2SCH_3 + RX \longrightarrow HOOCCH(NHAc)CH_2CH_2S(CH_3)(R)^+X^-$$

$$(3)\quad HOOCCH(NH_2)CH_2CH_2S(CH_3)(R)^+X^- + (CH_3CO)_2O + HCOOH \longrightarrow HOOCCH(NHCHO)\text{-}CH_2CH_2S(CH_3)(R)^+X^- + 2CH_3COOH$$

R having been represented by $CH_3$—, $CH_2$=$CHCH_2$—, $C_6H_5CH$=$CHCH_2$— and $HOOCCH_2$—, X by Cl, Br and I, and Ac by $CH_3CO$— and HCO—.

## Experimental[3]

1. **Alkyl Halide Derivatives of Methionine.** (a) **Methioninemethylsulfonium Iodide.**—A mixture of 40 mM. of methionine, 65 cc. of 89% formic acid, 20 cc. of acetic acid and 10 cc. (160 mM.) of methyl iodide is kept in a dark place at about 25° for three days. The progress of the reaction may be followed by titration of the iodide with 0.05 *N* silver nitrate using erythrosin as an adsorption indicator (2 drops of an 0.5% alcoholic solution added to 1 cc. of the reaction mixture[4] and 1 cc. of acetic acid). The solution is distilled *in vacuo* to a sirup weighing 30 to 40% more than the theoretical amount of the sulfonium iodide. Digestion with 40 cc. of methanol produces a granular precipitate. After filtering and washing with methanol and acetone it is dissolved in 30 cc. of warm 50% ethanol and recrystallized by the addition of 100 cc. of ethanol; yield 75%; dec. pt. about 150°.

*Anal.* Calcd. for $C_6H_{14}O_2NSI$: C, 24.7; H, 4.8; N, 4.8; S, 11.0; eq. wt., 291.2. Found: C, 25.0; H, 4.7; N, 4.6; S, 10.8; eq. wt., 290.3, 292.0 ($I^-$, erythrosin).

(b) **Methioninemethylsulfonium bromide** is similarly obtained from 40 mM. of methionine, 50 cc. of formic acid, 50 cc. of acetic acid and 160 mM. of methyl bromide. One cc. of the reaction mixture, 10 cc. of water, and 3 drops of an 0.5% aqueous eosin yellow solution are used for titration of bromide; yield 72%; dec. pt. about 140°.

*Anal.* Calcd. for $C_6H_{14}O_2NSBr$: C, 29.5; H, 5.8; N, 5.7; S, 13.1; eq. wt., 244.2. Found: C, 28.8; H, 5.7; N, 5.6; S, 12.9; eq. wt., 244.3 ($Br^-$, eosin).

The preceding two salts are soluble in water and formic acid, insoluble in other organic media.

(c) **Methioninemethylsulfonium chloride** could not be obtained crystalline. Preparation of an aqueous solution, from the iodide, has been described elsewhere.[5]

(d) **Methioninemethylsulfonium acetate** may be obtained from an aqueous solution of the iodide by reaction with mercuric acetate. The resulting sirupy evaporation residue corresponds in yield and equivalent weight (acidity, by methylate titration[6]) to a monohydrate. On storage *in vacuo* it undergoes slow decomposition.

2. **Alkyl Halide Derivatives of N-Acylmethionine.** (a) **N-Formylmethioninemethylsulfonium Iodide.**—To 50 mM. of methioninemethylsulfonium iodide in 40 cc. of 89% formic acid, 35 cc. of 10.1 *M* acetic anhydride is added.[7] After about five minutes a vigorous reaction occurs which must be controlled by cooling. Vacuum distillation, after several hours of standing, leaves a sirupy residue. Its solution in 20 cc. of warm methanol deposits a granular precipitate on gradual addition of 300 cc. of acetone; yield 60–70%.

The same compound is obtained, in 60% yield, from methionine (20 mM. in 16 cc. of formic acid) by acetylation[7] (14 cc. of acetic anhydride), followed after half an hour by alkylation (40 mM. of methyl iodide), and after one day by vacuum distillation, digestion with 5 cc. of ethanol, and precipitation with acetone.

*Anal.* Calcd. for $C_7H_{14}O_3NSI$: eq. wt., 319. Found: eq. wt., 321, 326, 326 (acidity, phenolphth.), 322, 322, 324 ($I^-$, erythrosin).

(b) **N-Formylmethioninemethylsulfonium bromide** is analogously prepared from the methylsulfonium bromide (20 mM., 15 cc. formic acid, 13.5 cc. acetic anhydride). The evaporation sirup is dissolved in 15 cc. of hot ethanol. Crystallization is best induced by inoculation with a separately prepared test-tube sample, and brought to completion by careful addition of acetone (180 cc.); yield 80%.

*Anal.* Calcd. for $C_7H_{14}O_3NSBr$: eq. wt., 272. Found: eq. wt., 282 (acidity), 273 (bromide).

(c) **N-Formylmethionineallylsulfonium Bromide.**—A solution of 25 mM. of N-formylmethionine,[7] 34 cc. of 89% formic acid, 8.5 cc. of acetic acid and 50 mM. of allyl bromide gave on vacuum distillation after one day, and drying *in vacuo* over sodium hydroxide, an amber-colored sirup, the weight of which was 111% of the theoretical. A series of digestions with ether, continued until methylate titration[6] of the washings showed absence of acid, reduced the weight to 97% of the theoretical, and the total acidity of the washings, calculated as formic acid, accounted substantially for the loss. The compound could not be crystallized.

*Anal.* Calcd. for $C_9H_{16}O_3NSBr$: neut. eq., 298. Found: neut. eq., 299.

(d) **N-Acetylmethionineallylsulfonium Bromide.**—The crystalline material obtained from 125 mM. of acetylmethionine,[7] 200 cc. of acetic acid and 250 mM. of allyl bromide by distillation after two days standing and ethyl acetate digestion of the residue is impure. The pure compound was obtained by the following device: 25 g. of the crude product suspended in 71 cc. of propionic acid was brought into solution by the addition of 531 mM. of water. After filtration, and washing with 4 cc. of propionic acid, a total of 72 cc. of propionic anhydride[8] (110% of the amount equivalent to the water) was added in portions daily during nine days. Slow separation of the sulfonium salt is caused by the disappearance of water resulting from the acid-catalyzed[8] hydration of the anhydride. Oily separations become readily crystalline on seeding (the same process on a centigram scale yields crystals on standing when the corresponding amount of the anhydride is added

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[1] Aided by a grant in memory of Emma M. S. Althouse. For the preceding paper of this series *cf.* Toennies and Kolb, *J. Biol. Chem.*, **140**, 131 (1941). Original manuscript received June 14, 1944.

[2] Toennies, *J. Biol. Chem.*, **132**, 455 (1940); **133**, CII (1940).

[3] Only the *dl*-form of methionine has been used in this work. C, H, N and most of the S determinations have been made by Mr. William Saschek, College of Physicians and Surgeons, Columbia University, New York, N. Y., whose aid is gratefully acknowledged.

[4] The sulfonium, rather than ammonium, nature of the reaction product is indicated by absence of an analogous reaction between methyl iodide and alanine; and also by the failure of the reaction product to react as a sulfide with hydrogen peroxide (Kolb and Toennies, *Ind. Eng. Chem.*, *Anal. Ed.*, **12**, 723 (1940)).

[5] Bennett, *J. Biol. Chem.*, **141**, 573 (1941); Handler and Bernheim, *ibid.*, **150**, 335 (1943).

[6] Lavine and Toennies, *ibid.*, **101**, 727 (1933).

[7] Kolb and Toennies, *ibid.*, **144**, 193 (1942).

[8] For analysis of anhydrides by methylate titration, *cf.* Toennies and Elliott, THIS JOURNAL, **59**, 902 (1937).

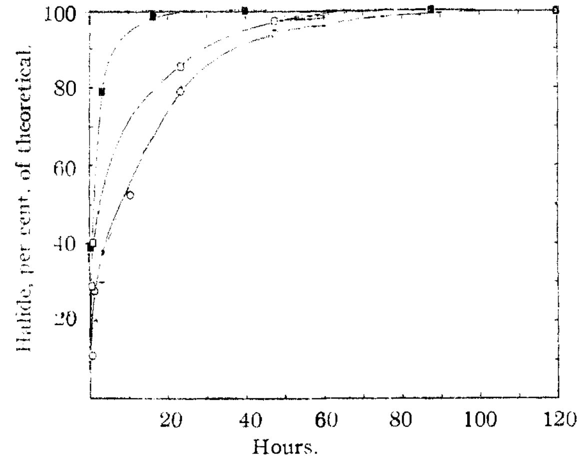

Fig. 1.—Reaction of haloacetic acids with methionine in 89% formic acid, at 26–28°. In the iodoacetic acid reaction, 1-cc. samples were titrated directly with 0.05 *N* silver nitrate in the presence of erythrosin (*cf.* section 1a). In the bromoacetic acid reactions 1-cc. samples were added to 50 cc. of water and 2 drops of eosin solution (*cf.* section 1b) and titrated. Methionine, 0.04 *M*; ○, $ICH_2COOH$, 0.82 *M*; □, $BrCH_2COOH$, 0.86 *M*; ■, $BrCH_2COOH$, 4.01 *M*.

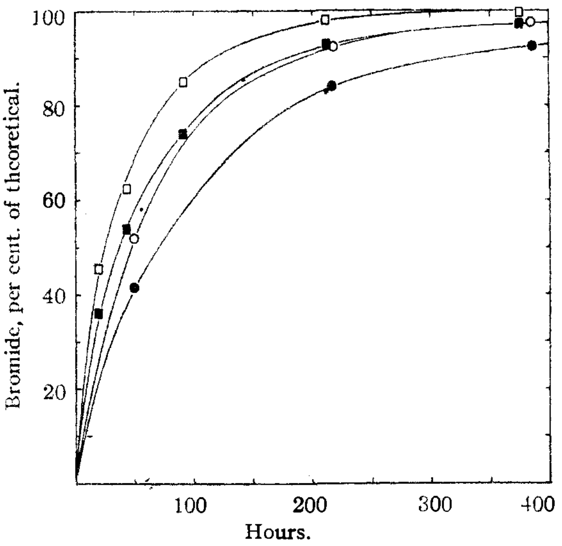

Fig. 2.—The effect of water and acetic acid on the reaction of methionine and bromoacetic acid in formic acid at room temperature (25–28°). For analytical technique see note under Table I.

| Symbol → | □ | ■ | ○ | ● |
|---|---|---|---|---|
| Medium | HCOOH | HCOOH + $CH_3COOH$ | HCOOH | HCOOH + $CH_3COOH$ |
| $CH_3COOH$, *M* | — | 6.0 | — | 6.6 |
| $H_2O$, *M* | 7 | 7 | 2 | 2 |
| Methionine, *M* | 0.18 | 0.18 | 0.20 | 0.20 |
| $BrCH_2COOH$, *M* | 0.36 | 0.36 | 0.40 | 0:40 |

in one portion) and rubbing. After a total of twelve days the crystals were filtered and washed four times with 4 cc. of acetic acid and ten times with 50 cc. of ether; the overall yield was 60%; dec. pt. 118–122°.

*Anal.* Calcd. for $C_{10}H_{18}O_3NSBr$: C, 38.4; H, 5.8; N, 4.5; S, 10.3; allyl, 13.2; eq. wt., 312.2. Found: C, 38.3; H, 5.7; N, 4.8; S, 10.3; allyl, 12.8 (bromination); eq. wt. (acidity), 312.2 ± 1.4, ($Br^-$) 312.4 ± 1.5 (4 determinations each).

(e) **N-Acetylmethioninecinnamylsulfonium Chloride.**—An acetic acid solution 0.50 *M* in N-acetylmethionine and 0.88 *M* in cinnamyl chloride showed after five days the expected chloride value. Acetic acid was removed by vacuum distillation, and cinnamyl chloride by ether extraction. The sulfonium chloride could only be obtained in the form of a glassy residue; nor could the bromide or iodide be crystallized. However, an aqueous solution 0.045 *M* in the sulfonium bromide and 0.07 *M* in ammonium reineckate yielded (50%) crystalline N-acetylmethioninecinnamylsulfonium reineckate.

*Anal.* Calcd. for $C_{16}H_{22}O_3NS^+Cr(SCN)_4(NH_3)_2^- + H_2O$: SCN, 36.0; $H_2O$, 2.8. Found: SCN,[9] 36.0, 36.0, 36.1; $H_2O$ (78°, *in vacuo*) 2.5.

In contrast to the unacylated sulfonium derivatives the N-acylated derivatives are soluble in the lower alcohols.

**3. Reaction Rates.**—While the greater similarity between the solubilities of haloacetic acids and their reaction products with methionine or acylmethionines makes the isolation of the latter more difficult than that of the alkyl sulfonium derivatives, it makes them more suitable

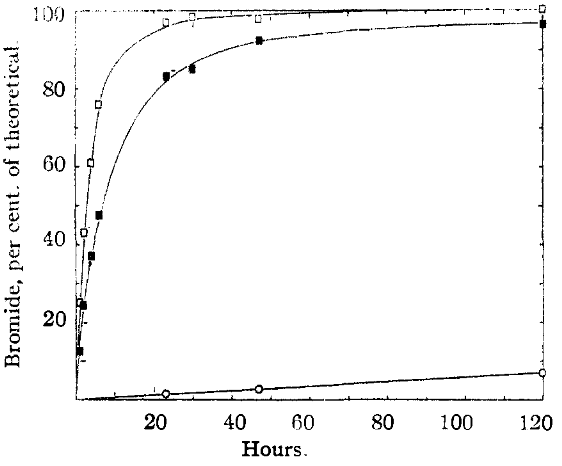

Fig. 3.—The reactions of methionine and N-formylmethionine with allyl bromide at room temperature (21–26°). To 3-cc. samples, 10 cc. of ether was added and 25 cc. of a cold solution 0.02 *M* in $FeNH_4(SO_4)_2$ and 1 *M* in $HNO_3$, and titration was carried out as noted under Table I. Removal of allyl bromide from the aqueous phase by ether eliminates its hydrolysis as a possible source of titration error. The values for the upper two curves were obtained by deducting from the net values those of the allyl bromide control.

| Symbol → | ○ | ■ | □ |
|---|---|---|---|
| HCOOH, *M* | 13 | 13 | 13 |
| $CH_3COOH$, *M* | 6.5 | 6.5 | 6.5 |
| $H_2O$, *M* | 7 | 7 | 7 |
| $CH_3COONa$, *M* | 0.20 | 0.20 | 0.20 |
| Allyl bromide, *M* | 0.20 | 0.20 | 0.20 |
| Methionine, *M* | — | 0.10 | — |
| Formylmethionine, *M* | — | — | 0.10 |

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(9) Carlsohn and Neumann, *J. prakt. Chem.*, **147**, 38 (1936).

TABLE I

EFFECT OF ACIDITY LEVEL AND MEDIUM ON THE REACTION OF METHIONINE AND BROMOACETIC ACID

These reactions were run simultaneously. The temperature was 25–28°. Bromide ion was determined as follows: to 1 cc. of the reaction mixture add 25 cc. of $H_2O$, 3 cc. of 7.5 *M* $HNO_3$ (which has been boiled to remove free nitrogen oxides) and 0.5 cc. of a 1 *M* solution of $FeNH_4(SO_4)_2$ in 0.1 *N* $HNO_3$. Cool and add 0.1 or 0.2 cc. of 0.05 *N* KSCN, titrate to colorless with 0.05 *N* $AgNO_3$ and complete titration with 0.05 *N* KSCN. The behavior of analogous reaction mixtures in which alanine was substituted for methionine showed that there are no significant blank values under these conditions.

| | | $HClO_4 \longrightarrow M$ | 0.44 | 0.33 | 0.22 | .... | .... | .... |
|---|---|---|---|---|---|---|---|---|
| | | $CH_3COONa \longrightarrow M$ | .... | .... | .... | .... | 0.10 | 0.20 |
| Medium: HCOOH | | | | | | | | |
| + $CH_3COOH$ | 6.6 *M* | 49 hr., % | 48 | 45 | 47 | 41 | 42 | 44 |
| + $H_2O$ | 2 *M* | 218 hr., % | 87 | 86 | 84 | 84 | 86 | 88 |
| Methionine | 0.20 *M* | 385 hr., % | 92 | 92 | 91 | 93 | 95 | 96 |
| $BrCH_2COOH$ | 0.40 *M* | | | | | | | |
| Medium: HCOOH | | | | | | | | |
| + $CH_3COOH$ | 6.0 *M* | 44 hr., % | 59 | 57 | 57 | 54 | 57 | 58 |
| + $H_2O$ | 7 *M* | 93 hr., % | 78 | 75 | 75 | 74 | 76 | 78 |
| Methionine | 0.18 *M* | 213 hr., % | 95 | 96 | 96 | 93 | 94 | 96 |
| $BrCH_2COOH$ | 0.36 *M* | 376 hr., % | 97 | 100 | | 98 | 99 | 102 |

for the study of kinetic factors, because no problems of vapor pressure are encountered over a wide range of solvent composition and temperature. Figure 1 shows examples of reactions between methionine and monoiodo- and monobromoacetic acid. The latter seems to be somewhat more reactive than the former. Monochloroacetic acid, on the other hand, is of extremely low reactivity. A solution of 0.08 *M* methionine and 0.16 *M* monochloroacetic acid in water (which favors sulfonium reactions, see below) containing 0.05 *M* perchloric acid showed a half-time of reaction of approximately fifteen hours at 74°. The results shown in Fig. 2 indicate that among different media the sulfonium reaction is increasingly favored in the following order: acetic acid, formic acid, water, *i. e.*, in the order of increasing dielectric constant.[10] The accelerating effect of water is further borne out by the data of Table I. These data also indicate that in the formeous-aceteous media both strong acids and strong bases (sodium acetate) slightly but definitely catalyze sulfonium formation.

A comparison of the reactivities, with allyl bromide, of methionine and N-formylmethionine, is shown in Fig. 3. Interference of the positive nitrogen charge ($HOOC—CH(NH_3^+)—CH_2—CH_2—S—CH_3$) with the establishment of the positive sulfur charge could explain the slower reaction of the free amino acid.

## Summary

A number of alkyl sulfonium salts derived from methionine and N-acyl-substituted methionine have been isolated and certain factors governing their formation have been studied.

PHILADELPHIA 30, PA. RECEIVED FEBRUARY 23, 1945

(10) *Cf.* Bost and Everett, THIS JOURNAL, **62**, 1752 (1940).

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[CONTRIBUTION FROM THE WESTERN REGION, BUREAU OF MINES, U. S. DEPARTMENT OF THE INTERIOR, BERKELEY, CALIFORNIA]

# Heats of Formation of $NH_4Al(SO_4)_2·12H_2O$ and $NH_4Al(SO_4)_2$[1]

BY FRANK E. YOUNG[2]

Several recent thermochemical investigations at the Pacific Experiment Station of the Bureau of Mines have dealt with aluminum compounds of current metallurgical interest. Previous papers by the author described new determinations of the heats of formation of two hydrates of aluminum nitrate,[3] anhydrous and hexahydrated aluminum sulfate, and anhydrous and dodecahydrated potassium aluminum sulfate.[4] Other papers reported low-temperature specific heats, entropies, and high-temperature heat contents of some of these and related compounds.[5] The present paper, a continuation of this work, presents new determinations of the heats of formation of nearly anhydrous (1.80% water) and dodecahydrated ammonium aluminum sulfate and gives an estimate for the completely dehydrated compound.

### Materials and Method

J. T. Baker analyzed ammonium sulfate containing 0.02% impurities was dried at 62° and stored over Dehydrite until used. The average of four sulfate analyses[6] indicated 99.96% purity.

Mallinckrodt "Analytical Reagent" ammonium aluminum sulfate dodecahydrate was used without drying or other treatment. Analyses for alumina showed 99.78% purity. Correction was made in the thermal results for the 0.20% alkali sulfate found to be present.

"Anhydrous" ammonium aluminum sulfate[7] was pre-

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(2) Chemist, Pacific Experiment Station, Bureau of Mines.

(3) Young, THIS JOURNAL, **66**, 777 (1944).

(4) Young, *ibid.*, **67**, 257 (1945).

(5) (a) Moore and Kelley, *ibid.*, **64**, 2949 (1942); (b) Shomate and Kelley, *ibid.*, **66**, 1490 (1944); (c) Shomate and Naylor, *ibid.*, **67**, 72 (1945); (d) Shomate, *ibid.*, **67**, 765 (1945).

(6) These analyses were made by E. H. Huffman, formerly metallurgist, Pacific Experiment Station, Bureau of Mines.

(7) This material was prepared and analyzed by A. E. Salo, metallurgist, Pacific Experiment Station, Bureau of Mines.