crystallized from hot water; colorless plates, m. p. 150° Samples of 5-bromodioxindole prepared by procedures A, B and C yielded identical samples of the acetyl derivative (m. p. and mixed m. p. 150°).

Anal. Calcd. for $C_{10}H_8O_3NBr$: N, 5.18. Found: N, 5.12, 5.14.

5-Bromoisatin-β-phenylhydrazone.—A mixture of 2.28 g. of 5-bromodioxindole, 25 cc. of ethyl alcohol and 5 g. of phenylhydrazine was heated at reflux temperature for thirty minutes. The product which separated on cooling was crystallized from ethyl alcohol; orange-yellow needles, m. p. 271–272°. A mixture with an authentic sample of 5-bromoisatin-β-phenylhydrazone exhibited the same m. p.

5,7-Dibromodioxindole (II) A.—By the procedure of Baeyer and Knop.[1] Nearly colorless crystals possessing a slight red tinge, m. p. about 260° (depends on rate of heating).[4]

B.—A solution of 16 g. of bromine (0.1 mole) in 50 cc. of water containing 24 g. of potassium bromide was added slowly to a boiling solution of 7.45 g. (0.05 mole) of dioxindole in 250 cc. of water. The nearly colorless product was crystallized from hot ethyl alcohol from which it separated as colorless microscopic needles which rapidly acquired a reddish tinge on exposure to air. The substance melts at about 260°, the melting point depending on the rate of heating since considerable decomposition takes place below the melting point. The substance is rapidly oxidized to 5,7-dibromoisatin by boiling its solution in glacial acetic acid in contact with air.

C. From 5,7-Dibromoisatin.—5,7-Dibromoisatin (0.02 mole) was suspended in 200 cc. of water and the mixture heated to boiling and 3 g. of sodium hydrosulfite added. The reaction mixture was cooled and the product collected. Nearly colorless microscopic needles were obtained identical with samples prepared by procedures A and B.

Anal. Calcd. for $C_8H_5O_2NBr_2$: N, 4.56. Found: N, 4.60, 4.76.

3-Acetyl-5,7-dibromodioxindole.—A mixture of 2 g. of 5,7-dibromodioxindole with 10 cc. of acetic anhydride was heated at reflux temperature for thirty minutes. The product which separated on cooling was crystallized from glacial acetic acid; colorless microscopic needles, m. p. 226–227°. Mixtures of samples prepared from samples of dibromodioxindole prepared by procedures A, B and C exhibited the same m. p.

Anal. Calcd. for $C_{10}H_7O_3NBr_2$: N, 4.00. Found: N, 4.05, 4.23.

5,7-Dibromoisatin-β-phenylhydrazone.—A solution containing 1.5 g. (0.005 mole) of 5,7-dibromodioxindole and 5 g. of phenylhydrazine in 25 cc. of alcohol was heated at reflux temperature for thirty minutes. The product which separated on cooling was crystallized from glacial acetic acid; orange-yellow needles, m. p. 301–302°. A mixture with an authentic sample of 5,7-dibromoisatin-β-phenylhydrazone exhibited the same m. p.

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Summary

The monobromo and dibromodioxindoles of Baeyer and Knop have been shown to be 5-bromodioxindole and 5,7-dibromodioxindole, respectively.

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(4) Baeyer and Knop, ref. 1, reported 170°.

[CONTRIBUTION FROM THE LANKENAU HOSPITAL RESEARCH INSTITUTE]

Methionine Studies. VIII. Regeneration of Sulfides from Sulfonium Derivatives[1]

BY GERRIT TOENNIES AND JOSEPH J. KOLB

Derivatives of methionine containing a sulfonium function have been described.[2] Methionine is distinguished among the known natural amino acids by the capacity to form sulfonium salts, and the possibility of utilizing this distinction for the isolation of methionine from protein hydrolysates seemed worth considering as existing methods are far from satisfactory.[3,4] If in a protein hydrolysate all amino groups were acylated[5] and the resulting mixtures were subjected to the action of a sulfonium-forming reagent, the only cation among the amino acid derivatives should be that of the sulfonium salt formed from the methionine derivative.[6] Isolation of this singular ion as a suitable salt could then serve as a basis for the isolation of methionine if it were possible to regenerate the amino acid from the sulfonium combination. The present paper deals with studies bearing on the question of regeneration.

The potential reversibility of the sulfonium ion forming reaction[7] $R_2S + RX \rightleftarrows R_3S^+ + X^-$ theoretically permits the derivation of three different sulfides from a methionine sulfonium ion (or of two if a methyl group is the addend). Regeneration of the methionine skeleton would require preferential removal of the added radical (or of a methyl group). Actually, in the case of N-formylmethioninemethylsulfonium acetate, spontaneous removal of the 4-carbon chain, *i. e.*, loss of the methionine skeleton, was observed

$$HOOCCH(NHCHO)CH_2CH_2S(CH_3)_2^+ + CH_3COO^- \longrightarrow CH_3COOH + (CH_3)_2S + \underbrace{COCH(NHCHO)CH_2CH_2O} \quad (1)$$

However, the analogous reaction was not encountered with the corresponding halide salts, nor with acetate or halides of the sulfonium ion with an unsubstituted amino group, although the volatile dimethyl sulfide is a potential product in all these

(1) Aided by a grant in memory of Emma M. S. Althouse. Original manuscript received June 14, 1944.

(2) Toennies and Kolb, THIS JOURNAL, **67**, 849 (1945).

(3) Toennies and Kolb, *J. Biol. Chem.*, **126**, 367 (1938).

(4) Simmonds, Cohn, Chandler and du Vigneaud, *ibid.*, **149**, 519 (1943).

(5) The amino groups of the α-amino acids, with the exception of some groups of the diamino acids, are readily formylated by formic acid–acetic anhydride mixtures: Kolb and Toennies, *ibid.*, **144**, 193 (1942).

(6) Toennies, *J. Biol. Chem.*, **132**, 455 (1940); **133**, CII (1940).

(7) *Cf.* Ray and Levine, *J. Org. Chem.*, **2**, 267 (1937).

cases. This differential behavior of the formylated sulfonium acetate seems explainable in terms of relative proton affinities (acid dissociation constants).

The following tabulation shows the approximate pK values of the acid groups involved, and the predominating distribution of charges, for four different types of compounds.

I	S^+ NH_3^+ (9) COO^-(2)	X^-(< 0)	II	S^+ NH_3^+ (9) COO^-(2)	CH_3COO^-(5)
III	S^+ NHAcyl COOH(2)	X^-(< 0)	IV	S^+ NHAcyl COO^-(2)	CH_3COOH(5)

Evidently only types III and IV can be titrated as acids.[2] The juxtaposition of the opposite charges in type IV would account for the ring-closing tendency, consummated, with formation of a 5-ring, through acquisition of the C-S electron pair by the sulfur, *i. e.*, formation of dimethyl sulfide, according to equation 1. In types I and II this tendency would be offset by the presence of the N-cation. Under alkaline conditions compounds of types I, II and III may be expected to behave similarly to type IV, while on the other hand an acid reaction should suppress the tendency of type IV to decompose into lactone and volatile sulfide. Observations indicated that relatively high stability or spontaneous formation of the volatile sulfide, depending on acidity factors, are the actual alternatives.

Other approaches to the regeneration problem were guided by gleanings from the more fully investigated chemistry of quaternary ammonium compounds. Evidence of the labilizing effect of a 2,3 double bond upon the C–N linkages[8,9] led to the consideration of allyl derivatives of N-acylated methionine, which were found readily accessible.[2] Removal of the allyl group by catalytic hydrogenation[9] was not possible, the experiments providing clear-cut evidence of catalyst-poisoning by sulfonium ion. However, reductive cleavage could be accomplished in the case of N-acetylallylsulfonium ion by means of nascent hydrogen, according to the equation

$$R{-}S(CH_3)(CH_2CH{=}CH_2)^+ + H_2 \longrightarrow R{-}SCH_3 + CH_3CH{=}CH_2 + H^+ \quad (2)$$

while the best results (80 to 90% yield) were obtained by reaction of allyl derivatives with thiocyanate ion

$$HOOCCH(NHAc)CH_2CH_2S(CH_3)(CH_2CH{=}CH_2)^+ + SCN^- \longrightarrow HOOCCH(NHAc)CH_2CH_2SCH_3 + CH_2{=}CHCH_2SCN \quad (3)$$

a reaction modeled after the analogous cleavage of the quaternary ammonium ion of thiamin.[10] The alternate equations

$$HOOCCH(NHAc)CH_2CH_2S(CH_3)(CH_2CH{=}CH_2)^+ + SCN^- \longrightarrow HOOCCH(NHAc)CH_2CH_2SCH_2CH{=}CH_2 + CH_3SCN \quad (4)$$

$$HOOCCH(NHAc)CH_2CH_2S(CH_3)(CH_2CH{=}CH_2)^+ + SCN^- \longrightarrow CH_3SCH_2CH{=}CH_2 + HOOCCH(NHAc)CH_2CH_2SSCN \quad (5)$$

apparently represent secondary reaction paths. However, in the analogous reaction with hydrogen sulfide[10,11] the path corresponding to equation 5, involving formation of the volatile methylallyl sulfide, seems to predominate.

Exploratory work on the application of these findings showed that N-formylmethionineallylsulfonium ion can be obtained from crude protein hydrolysates as the reineckate and that regeneration of optically active methionine from the latter is possible by means of the reaction with thiocyanate, although for the present the over-all yields are low.

Experimental

1. **Analytical.**—In order to study the formation of sulfides from sulfonium ions containing allyl groups analytical differentiation of sulfonium, sulfide and allyl groups is necessary. The reactions with hydrogen peroxide, iodate and bromine, as well as the iodometric methionine method of Lavine,[12] have provided the needed tools. Hydrogen peroxide in 2 M perchloric acid oxidizes the sulfide group to sulfoxide in N-acylated methionine, as it does in methionine,[13] while neither allyl double bond nor sulfonium configuration are affected unless bromide ion is also present. To remove the latter it is sufficient to add an excess of silver acetate in perchloric acid since silver bromide or silver ion is without effect. The allyl group is determined by bromination. For its differentiation from sulfide two possibilities have been used. Either (procedure A) the sulfide is converted to sulfone, prior to the allyl determination, by hydrogen peroxide and molybdate in acid solution,[14] and the excess peroxide is decomposed by silver oxide at alkaline reaction, or (procedure B) the allyl bromination is carried out with simultaneous oxidation of sulfide to sulfone, and the allyl value is obtained by subtraction based on the separately determined sulfide value.

Procedure A.—To an aliquot of the unknown in a 25-cc. volumetric flask add silver acetate and perchloric acid in amounts sufficient to make the solution about 0.1 N acid and to leave a silver ion concentration of approximately 0.01 N after precipitation of halide. Filter after ten minutes through a dry filter. In another 25-cc. flask make 20 cc. of the filtrate to volume after adding 0.5 mM of molybdate, and hydrogen peroxide in about three times the molar amount of the maximum anticipated sulfide content. After allowing one night for complete oxidation to the sulfone, remove two 10-cc. aliquots. To one of them add just enough 2 N sodium hydroxide to cause separation of silver oxide and to the other add approximately twice as much. After allowing one hour with occasional shaking for the decomposition of unused hydrogen peroxide (catalyzed by silver oxide) acidify with perchloric acid and determine allyl with bromide-bromate.[15] Incomplete decomposition of hydrogen peroxide would be revealed by disagreement between the two determinations.

(8) v. Braun, Kühn and Goll, *Ber.*, **59**, 2330 (1926); Stevens, Creighton, Gordon and MacNicol, *J. Chem. Soc.*, 3193 (1928); Emde, *Helv. Chim. Acta*, **15**, 1330 (1932); Davies and Cox, *J. Chem. Soc.*, 614 (1937).

(9) Emde and Kull, *Arch. Pharm.*, **274**, 173 (1936); Achmatowicz and Lindenfeld, *Rocznìki Chem.*, **18**, 75 (1938).

(10) Snyder and Speck, THIS JOURNAL, **61**, 668 (1939).

(11) Bost and Schultze, *ibid.*, **64**, 1165 (1942).

(12) Lavine, *J. Biol. Chem.*, **151**, 281 (1943).

(13) Kolb and Toennies, *Ind. Eng. Chem., Anal. Ed.*, **12**, 723 (1940).

(14) Toennies and Kolb, *J. Biol. Chem.*, **140**, 131 (1941).

(15) A solution 0.01 M in the allylsulfonium salt, 0.07 M in potassium bromide, 0.2 M in perchloric acid and 0.03 N in sodium bromate showed iodometrically consumption of 97.4, 96.8 and 96.7% of one Br_2 per molecule, after one-half, one and two hours, respectively.

Procedure B ("allyl-plus sulfide").—The reaction mixtures consist of 5 cc. of the solution to be determined (or a blank), 1 cc. of concentrated hydrochloric acid, 5 cc. of 0.1 *N* potassium iodate and 0.25 cc. of 1 *M* potassium bromide. Bromination and oxidation are both complete after one hour as long as iodate is used in at least 100% excess. However, the results are precise only if the methionine amino group is acylated. With pure methionine the results were 102.8, 105.6 and 106.9% of the theoretical after one-half, one and two hours, respectively. The excess oxidation could be prevented by acetylation[16]: 0.617 mM of methionine went into solution when left overnight with 6.25 cc. of an acetic acid solution 0.1 *M* in sodium acetate and 0.3 *M* in acetic anhydride. After dilution with water to 50 cc., aliquots were used for determination as above, and values of 100.5, 100.3 and 101.2% were obtained. A known mixture of methionine acetylated in solution and acetylmethionineallylsulfonium bromide gave 99.1, 99.5 and 99.7% of the expected (two O atoms per sulfide, one O atom per allyl) value. Elimination of the basicity of the amino group by acetylation apparently prevents an irreversible intrusion of bromine cation into the reversible amine–proton equilibrium ($RNH_3^+ + Br_2 \rightarrow RNH_2Br^+ + HBr$).

2. **Dissociation.**—(a) When the filtrate resulting from treating a solution of 10 mM of formylmethioninemethylsulfonium iodide with an equivalent amount of mercuric acetate was evaporated and dried *in vacuo*, a sirupy residue of 1.12 g. remained. While its direct titration gave no permanent end-point, addition of excess alkali and back titration with acid after one-half to one and one-half hours gave equivalent weights of 148 (methyl red) and 142 (phenolphthalein). The sulfur content was 0.61%. These data are indicative of an impure *α-formamido-γ-butyrolactone* (theoretical yield 1.29 g., equivalent weight 129, sulfur none), formed presumably according to equation 1. Five years later the sirup was found to have crystallized; apparently due to hydrolytic processes, since digestion with methanol left, in 50% yield, α-amino-γ-hydroxybutyric acid; equivalent weight, basic[17] 118, acid[18] 119, calcd. 119, m. p. 178–179° (lit.[19] 176–177°). The hydroxyamino acid was further identified by conversion (evaporation of the hydrochloric acid solution) to α-amino-γ-hydroxybutyrolactone hydrochloride; equivalent weight, methylate titration[18] 136, chloride 141, calcd. 137.5, m. p. 196–198° (lit.[19] 198–200°).

(b) An 0.1 *M* acetylmethionineallylsulfonium bromide (type III, above) solution in 0.2 *M* sodium hydroxide contained, after one and one-half and twenty hours of standing, 1 and 8%, respectively, of the maximum potential sulfide value while the allyl content was 72 and 23%, respectively. The volatile methylallyl sulfide (equation 5) seems to be the principal sulfur-carrying reaction product.

(c) Two hours of boiling in 0.5 *M* sulfuric acid of 0.2 *M* solutions of either methionineallylsulfonium bromide or its N-formyl derivative produced only 26% of the theoretical methionine[12] value.

3. **Catalytic Hydrogenation.**—The hydrogenations were carried out at 25–30°, with rapid shaking and at atmospheric pressure, in a hydrogenation flask with side vessel.[20] Under these conditions 4 mM of maleic acid in 10 cc. of acetic acid, and in the presence of either 25 mg. of platinum catalyst[21] or 150 mg. of palladinized barium sulfate,[22] consumed hydrogen at rates of 6 to 9 cc. per minute. However, hydrogenation of 0.5 mM of N-acetylmethionineallylsulfonium bromide in 5 cc. of acetic acid in the presence of platinum ceased after 4 cc. of hydrogen had been taken up in six minutes, and was not resumed when maleic acid was added from the side-vessel; also hydrogenation of maleic acid was halted by the addition of either methioninemethylsulfonium bromide or N-formylmethioninemethylsulfonium bromide. The catalysts could not be reactivated by either air[23] or ferrous chloride,[24] and since the inhibitory properties remained unchanged by double recrystallizations, with large losses, of N-formylmethioninemethylsulfonium bromide and N-acetylmethionineallylsulfonium bromide, the conclusion that the sulfonium ion *per se* is a strong poison for noble metal catalysts[25] seems justified.

4. **Reduction with Nascent Hydrogen.**—Four-tenths mM of acetylmethionineallylsulfonium ion and 0.001 mE Ag^+ (excess from removal of bromide) in 14 cc. of 1.5 *M* perchloric acid were left overnight with 300 mg. of zinc. Peroxidimetric sulfide determinations and allyl-plus-sulfide determinations on the filtrate (made to 25 cc.) showed oxygen consumptions of 0.0125 and 0.0279 milliatoms per cc., respectively, corresponding to a 78% conversion of the sulfonium group (initial value 0.0160 *M*) to the methionine sulfide group. The allyl-plus-sulfide value indicates that 18% of the initial allyl is present, so that 96% of the starting material can be accounted for in terms of equation 2.[26] Repetition of this experiment yielded values of 74 and 24%, respectively, for sulfide and allyl. When sulfuric instead of perchloric acid was used the values were 66 and 31%.

5. **Reaction with Hydrogen Sulfide.**—Treatment of 0.05 *M* solutions of acetylmethionineallylsulfonium bromide and acetate (obtained by silver acetate from the bromide solution) with a stream of hydrogen sulfide at 100° for two hours gave sulfide and net allyl values of 3 and 22, and 22 and 13%, respectively, indicating a more rapid reaction in the less acid solution and the probability of a volatile allyl sulfide, rather than the desired acetylmethionine, being formed in the main reaction path.

6. **Reaction with Thiocyanate.**—Both the N-formyl and N-acetyl derivatives of the allylsulfonium bromide were used and the progress of the reaction (equations 3, 4, 5) was followed by argentometric determination of thiocyanate ion remaining (by measuring silver nitrate consumed and correcting for bromide present), peroxidimetric determination of sulfide formed (after precipitation of thiocyanate by silver ion and filtration), and determination of allyl double bonds by one of the two procedures detailed (section 1). In exploratory experiments, series of 2-cc. samples of potassium thiocyanate (approximately 0.2 *M*), in the presence and absence of an equimolar amount of acetylmethionineallylsulfonium bromide, were heated in long tubes in a boiling water-bath, under different conditions of acidity. The results (Table I) show that acidity favors the reaction between sulfonium ion and thiocyanate and that hydrolysis of the latter becomes significant only below *p*H 1.

For further investigation reaction mixtures were heated at 100° under a reflux condenser, and with air (purified by passing it through 5% silver nitrate solution and glass wool) being bubbled through the solution in order to expel the allyl thiocyanate formed. These experiments are summarized in Table II. The general similarity in the figures for "sulfide formed" and "thiocyanate disappeared" (if allowance is made for the sources of error that tend to make the latter value too high, *viz.*, hydrolysis of thiocyanate and correction for bromide) and the low allyl values accord with the main reaction path represented by

(16) Kolb and Toennies, *J. Biol. Chem.*, **144**, 193 (1942).

(17) Toennies and Callan, *ibid.*, **125**, 259 (1938).

(18) Lavine and Toennies, *ibid.*, **101**, 727 (1933).

(19) Feofilaktov and Onishchenko, *J. Gen. Chem.* (U. S. S. R.), **9**, 304, 314 (1939); *C. A.*, **34**, 378 (1940).

(20) Fischer and Baer, *Ber.*, **65**, 337 (1932).

(21) Adams and Shriner, THIS JOURNAL, **45**, 2171 (1923).

(22) Gattermann and Wieland, "Laboratory Methods of Organic Chemistry," transl. from 22nd German ed., McCartney, New York, N. Y., 1934, p. 369.

(23) Willstätter, *Ber.*, **54**, 113 (1921).

(24) Carothers and Adams, THIS JOURNAL, **45**, 1071 (1923).

(25) These experiments were done in 1940. We have not investigated the recently discovered merits of polyvinyl alcohol (Kavanagh, *ibid.*, **64**, 2721 (1942)) as a carrier for palladium in the hydrogenation of sulfur compounds.

(26) In the alternative involving formation of the allyl analog of N-acetylmethionine (analogous to equation 4) sulfide would increase and allyl remain stationary, while in the third alternative, formation of the volatile methylallyl sulfide (analogous to equation 5), allyl would decrease and no sulfide would accumulate in solution.

TABLE I

DISAPPEARANCE OF THIOCYANATE IN THE PRESENCE AND ABSENCE OF SULFONIUM ION

	Thiocyanate ion disappeared, % of initial amount								
	In the presence of sulfonium ion			In the absence of sulfonium ion					
				Concn. of sulfuric acid, moles per liter					
Hours at 100°	In 0.05 *M* H_2SO_4	In H_2O	At *p*H 5.2 (acetate buffer)	0.00	0.01	0.05	0.10	0.50	1.0	2.0
1/2	25	24	7							
1	50	40								
2	81	66	10							
5	93	77			0.9	1.7	2.8	40		
18		93		0.1				96	97	100

TABLE II

THE REACTION OF N-ACYLMETHIONINEALLYLSULFONIUM ION WITH THIOCYANATE

Experiment no.	1	2	3	4	5	6	7	8	9
N-Acylmethionineallylsulfonium[a] bromide, *M*	0.1	0.5	1.0	0.1	1.0	1.0	0.1	0.1	0.1
KSCN, *M*	0.2	1.0	1.9	0.2	2.0	8.0	0.2	0.2	0.2
H_2SO_4, *M*	0	0	0	0	0	0	0.2	0.2	0.1
Reaction time at 100°, hr.	24	20	24	105	47	8	8	24	8
Amount of initial bromide[b] remaining, %	97						87	97	
Amount of initial allyl remaining, %				8	10	6	9	9	
Extent to reaction 3 according to:									
thiocyanate disappeared, %	72	86	97	73	95	129	105	114	91
sulfide formed, %	68	88	90	64	83	49	82	92	91

[a] In experiments no. 1–8 the acetyl compound, and in no. 9 the formyl compound was used. [b] Bromide was determined by Volhard titration after selective oxidation of thiocyanate according to Rosanoff and Hill, THIS JOURNAL, 29, 1467 (1907).

equation 3. The device of increasing the bimolecular completion rate of this reaction by increasing concentrations appears to be of limited value, while a moderate acidification with sulfuric acid seems useful. The relative uniformity of the final allyl values suggests that at least in part the reaction goes in the direction of the alternative pair of products, allyl-S-acylamino acid and methyl thiocyanate (equation 4), while cases where "sulfide formed" is markedly lower than "thiocyanate disappeared" may indicate intrusion of the third alternative (equation 5).

7. Isolation of Methionine from Hydrolysates.—Extensive exploratory work has disclosed a number of difficulties arising in the practical application of the steps outlined, and possible means for meeting these difficulties that have suggested themselves remain to be developed. The pertinent results, demonstrating that precipitation of a methionine sulfonium derivative from a protein hydrolysate and its reconversion into optically active methionine is possible, may be summarized as follows.

A crude sodium chloride–amino acid fraction[27] isolated from an egg albumin hydrolysate contained 58% of the available methionine. Formylation[5] of this mixture, removal of excess acetic anhydride by water and perchloric acid,[28] reaction with allyl bromide in the acetic–formic acid medium,[2] precipitation with ammonium reineckate in an aqueous (0.1 *M* H_2SO_4) medium, and removal of reineckate from the resulting precipitate by ethyl acetate extraction, resulted in an aqueous solution containing allyl sulfonium ion corresponding to 70% of the methionine present in the salt fraction. By reaction with thiocyanate, followed by acid hydrolysis (to remove the formyl group), methionine[12] was formed in 65% yield. Because of the presence, up to this point, of large amounts of foreign matter, its isolation was difficult. The best results were obtained by removal of basic amino acids by resin adsorption,[29] followed by precipitation with mercuric chloride.[3] From the mercuric chloride precipitate 66% of the expected methionine could be separated, in 70 to 80% purity. Recrystallizations yielded combined fractions, in 84% yield, of the following characteristics: methionine content peroxidimetrically[13] 93%, iodometrically[12] 91%; $[\alpha]^{25}D$ −21.1°, −21.0° (1.3% in 0.2 *M* HCl).

The optical rotations found are substantially those reported for *l*-methionine,[30] but the fact that the purity, calculated as methionine, remained short of 100%, could be an indication of the presence of the allyl analog of methionine (*cf.* equation 4). For methionine the oxygen consumed in an allyl-plus-sulfide determination with preceding acetylation (*cf.* section 1) is exactly twice that used in a peroxidimetric sulfide determination, while in the allyl analog this ratio would be 3. Application of this criterion to several fractions yielded values of 2.1 ± 0.1 and 2.06 ± 0.02, indicating that the allyl analog, if present, comprises less than 10% of the total sulfide.

The exploratory findings suggest that substantially better results may be expected from applying the reactions outlined to the neutral amino acids remaining after removal of the basic and acid groups by acid and basic adsorbents.[31]

Summary

Analytical procedures suitable for the differentiation of sulfonium, sulfide and allyl groupings, in methionine sulfonium derivatives have been developed, and the generation of sulfides from such derivatives has been studied. It has been shown that the methionine type of sulfide is preferentially regenerated from allylsulfonium derivatives of N-acylated methionine by reaction with nascent hydrogen or thiocyanate ion. The applicability of these reactions to the isolation of methionine from protein hydrolysates has been established in principle.

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(27) Hill and Robson, *Biochem. J.*, **28**, 1008 (1934).
(28) Sakami and Toennies, *J. Biol. Chem.*, **144**, 203 (1944).
(29) Block, *Proc. Soc. Expll. Biol. Med.*, **51**, 252 (1942).
(30) Toennies, *Growth*, **1**, 337 (1937).
(31) Schramm and Primosigh, *Ber.*, **76**, 373 (1943).