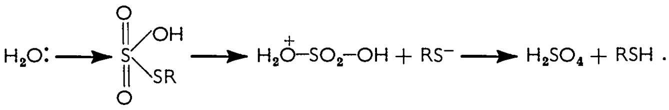
416. *New Syntheses of Disulphides from Bunte Salts.*

By Brian Milligan and J. M. Swan.

Disulphides are obtained in high yield by heating salts of *S*-alkyl(or aryl) hydrogen thiosulphates (Bunte salts) in acid solutions containing thiourea, substituted thioureas, iodide ions, or thiocyanate ions. A general mechanism for this new class of reaction is proposed. By an extension of the reaction, symmetrical and unsymmetrical disulphides are obtained by the reaction of Bunte salts with sulphenyl chlorides or sulphenyl thiocyanates.

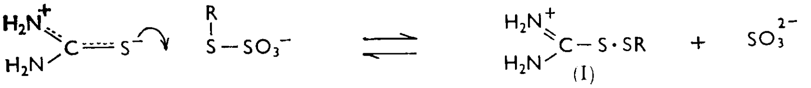
The acid hydrolysis of a Bunte salt (the salt of an *S*-alkyl or *S*-aryl hydrogen thiosulphate) generally yields the corresponding thiol and sulphuric acid.[1] No mechanistic study of this reaction has been reported, but it seems likely that the " outer " sulphur atom of the undissociated acid undergoes nucleophilic attack by water:

$$RS{\cdot}SO_3^- + H^+ \rightleftharpoons RS{\cdot}SO_3H \qquad \text{(1a)}$$



(1b)

In most reactions of Bunte salts, however, the primary step is attack by a nucleophile at the " inner " sulphur atom, with ejection of sulphite. Thus we expected that in neutral or acid solution a Bunte salt might react with thiourea, according to equation (2), to give the mixed disulphide cation (I). This reaction might well be promoted in acid solution by conversion of sulphite into sulphur dioxide. Surprisingly, sodium *S*-benzyl thiosulphate and thiourea in hot acid solution gave dibenzyl disulphide, sulphur dioxide, and sulphuric acid. High yields were obtained even when relatively small amounts of thiourea were used (0·05—0·1 mole per mole of Bunte salt). However, very small amounts of thiourea (0·005 mole per mole of Bunte salt) were not effective. Optimum yields (58—100% of disulphide in 5 examples) were obtained with equimolecular amounts of Bunte salt and thiourea in N-hydrochloric acid at 100° (1 hour). Acetic acid could also be used as solvent, but yields of disulphide were then lower.



(I)

(2)

When sodium *S*-benzyl thiosulphate was heated with an equimolar amount of thiourea in N-hydrochloric acid, the yields of dibenzyl disulphide, sulphur dioxide, and sulphate were 96%, 94%, and 94% respectively, calculated on the basis of equation (3).

$$2RS{\cdot}SO_3^- \longrightarrow RS{\cdot}SR + SO_2 + SO_4^{2-} \qquad \text{(3)}$$

In the absence of thiourea, in either N-hydrochloric acid or acetic acid, toluene-ω-thiol was obtained in 47—66% yield; no disulphide was formed. Further, this thiol and the Bunte salt together in hot acetic acid failed to yield any disulphide, although the reaction proceeds in the pH range 5—9. However, reaction of equimolar amounts of toluene-ω-thiol, sodium *S*-benzyl thiosulphate, and thiourea in acetic acid gave dibenzyl disulphide in 91% yield, based on both thiol and Bunte salt.* No disulphide was formed if the Bunte salt was omitted. Similar results were obtained in experiments with potassium *S*-phenyl

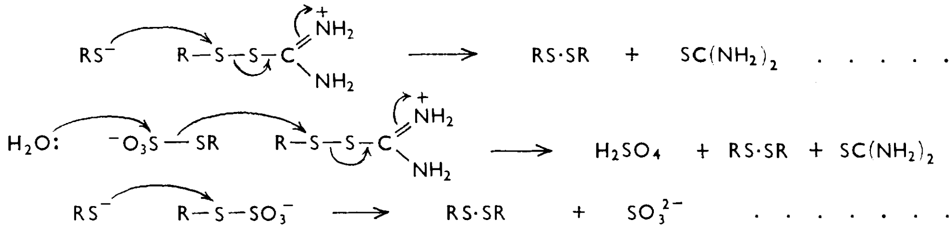
---

* This reaction was suggested by a Referee.

[1] See, *e.g.*, Bunte, *Ber.*, 1874, **7**, 646; Price and Twiss, *J.*, 1909, **95**, 1725; Weiss and Sokol, *J. Amer. Chem. Soc.*, 1950, **72**, 1687; Schöberl and Kawohl, *Monatsh.*, 1957, **88**, 478; El-Heweki and Taeger, *J. prakt. Chem.*, 1958, **7**, 191.

thiosulphate and benzenethiol. However, in the reaction of cysteine and *S*-sulphocysteine a similar yield of disulphide (65—71%) was obtained both in the presence and the absence of thiourea.

These results suggest that reactions (4—6) may be involved in the formation of disulphides from Bunte salts in the presence of thiourea:



$$RS^- + R{-}S{-}S{-}\overset{+}{C}(NH_2)_2 \longrightarrow RS{\cdot}SR + SC(NH_2)_2 \quad (4)$$

$$H_2O: \; {}^-O_3S{-}SR \; R{-}S{-}S{-}\overset{+}{C}(NH_2)_2 \longrightarrow H_2SO_4 + RS{\cdot}SR + SC(NH_2)_2 \quad (5)$$

$$RS^- \; R{-}S{-}SO_3^- \longrightarrow RS{\cdot}SR + SO_3^{2-} \quad (6)$$

Equation (6) seems to be important only in the reaction of *S*-sulphocysteine. Equation (5) is shown as a concerted process involving the solvent, the intermediate (I), and a second mole of Bunte salt, and is essentially equivalent to equation (4) where the thiol is produced by acid hydrolysis according to equation (1).*

Evidence to support these formulations was obtained when the reaction of sodium *S*-benzyl thiosulphate and an equimolar amount of thiourea in N-hydrochloric acid was effected with passage of a rapid stream of nitrogen through the solution; the yield of disulphide fell to 39%, the yields of sulphur dioxide and sulphate, again calculated on the basis of equation (3), being 140% and 34%. Rapid removal of sulphur dioxide would be expected to facilitate reaction (2), so that a much smaller amount of unchanged Bunte salt would be available for the secondary reactions. On the other hand, when only 0·05 mole of thiourea per mole of Bunte salt was used, a nitrogen stream had little effect. This is in accordance with expectation, since with relatively small amounts of thiourea the reaction can proceed to completion only if the thiourea used in step (2) is regenerated, as in steps (4) or (5). Failure of the reaction when very small amounts of thiourea were used can be attributed to an increased importance of reaction (1) compared with reaction (2)

It was also found that iodide or thiocyanate ions could replace thiourea in the conversion of Bunte salts into disulphides, the overall reaction again being given by equation (3). With equimolar amounts of sodium *S*-benzyl thiosulphate and potassium iodide in acetic acid, the yields of disulphide, sulphur dioxide, and sulphate were 90%, 84%, and 82% respectively; lower yields were obtained in N-hydrochloric acid. When equimolar amounts of thiol, Bunte salt and potassium iodide were heated in acetic acid, high yields of disulphides were obtained. Hence the following can be suggested for the reaction of Bunte salts with potassium iodide, analogous to the reaction with thiourea:

$$RS{\cdot}SO_3^- + I^- \longrightarrow RSI + SO_3^{2-} \quad . \; . \; . \; . \; . \; . \; . \; . \; . \; . \quad (7)$$

$$H_2O + RS{\cdot}SO_3^- + RSI \longrightarrow RS{\cdot}SR + I^- + H_2SO_4 \quad . \; . \; . \; . \; . \; . \; . \quad (8)$$

and/or

$$RS^- + RSI \longrightarrow RS{\cdot}SR + I^- \quad . \; . \; . \; . \; . \; . \; . \; . \; . \; . \quad (9)$$

Similar mechanisms involving sulphenyl thiocyanates probably apply to the reaction of Bunte salts with thiocyanate, which in general gave less satisfactory yields of disulphides. In the reaction of Bunte salts with iodide it can also be suggested that the intermediate sulphenyl iodide [reaction (7)] may decompose to disulphide and iodine,[3] the latter then being reduced by sulphite to give iodide and sulphate. In this case the overall reaction would still be that shown in expression (3), the iodide having a catalytic function only.

---

* In reactions (4) and (5) thiourea is eliminated by fission of an S–S bond. Saville [2] has shown recently that thiourea can be eliminated from thiouronium salts by fission of the C–S bond.

[2] Saville, *Proc. Chem. Soc.*, 1961, 214.

[3] Fuson, Price, Bauman, Bullitt, Hatchard, and Maynert, *J. Org. Chem.*, 1946, **11**, 469.

Consideration of reaction (8) suggested that disulphides might also be obtained on reaction of sulphenyl halides (or sulphenyl thiocyanates) with Bunte salts in acid solution. In fact, *o*-nitrobenzenesulphenyl chloride and thiocyanate both react readily with Bunte salts in N-hydrochloric or acetic acid, giving unsymmetrical disulphides, although better yields were obtained in *NN*-dimethylformamide. In the last solvent the concerted process shown in expression (8) is probably more likely than hydrolysis followed by reaction as in (9).

A recent method for preparing water-soluble derivatives from insoluble proteins such as keratin involves reaction of the disulphide groups with ammoniacal cupric sulphite, which converts each cystyl residue into two *S*-sulphocysteinyl residues.[4] Disulphides can be formed from such Bunte salts by a variety of oxidative procedures, and also by reaction with thiols, giving mixed disulphides which may then undergo further interchange reactions.[4,5] We expected that reaction of *S*-sulphopeptides or *S*-sulphoproteins with thiourea or iodide in acid solution might convert them into their original, or new, disulphide forms, provided these reactions could be modified so as to avoid hydrolysis of peptide bonds. However, there was virtually no reaction of *S*-sulphocysteine with thiourea or potassium iodide in N-hydrochloric or acetic acid at room temperature, but with thiourea in formic acid, after 2—3 days, most of the Bunte salt was converted into the mixed disulphide, *S*-amidinothiocysteine [I; $R = CH_2{\cdot}CH(NH_2){\cdot}CO_2H$], only a small amount of cystine being formed. With potassium iodide in formic acid, *S*-sulphocysteine was largely converted into cystine after two days, but free iodine was also formed. Iodine and *S*-sulphocysteine in formic acid gave cystine slowly, but we believe that the main reaction involves participation by iodide ions. Ethylenethiourea (2-thioimidazolidine) and *NNN'N'*-tetramethylthiourea also reacted with *S*-sulphocysteine in formic acid and the main products again appeared, from their electrophoretic behaviour, to be the corresponding mixed disulphides.

The peptides, *S*-sulphocysteinylglycine, glycyl-*S*-sulphocysteine, glycyl-*S*-sulphocysteinylglycine, and *S*-sulphoglutathione reacted with thiourea, ethylenethiourea, and *NNN'N'*-tetramethylthiourea more rapidly than did *S*-sulphocysteine, the main products again being mixed disulphides. With potassium iodide, the expected disulphides were formed in about 50% yield in 20 hr.; using a large excess of iodide gave good yields of disulphide in 2—3 days.

EXPERIMENTAL

Reaction of Thiosulphates with Thiourea.—(i) The thiosulphate (10 mmoles) and thiourea (10 mmoles) were heated in N-hydrochloric acid (50 ml.) at 100° for 1 hr., and the resulting disulphide was then filtered off from the cooled mixture. (ii) The reaction was carried out in the same way with acetic acid (25 ml.) as solvent. The mixture was diluted with water (75 ml.), and the product filtered off. Yields and m. p.s of crude products are shown in the annexed Table.

		Method (i)		Method (ii)		Recorded
Thiosulphate	Disulphide	Yield	M. p.	Yield	M. p.	m. p.
Na *S*-benzyl	Dibenzyl	97	70°	91	69°	71—72° [6]
		90 *	70	32 *	70	
		10 †	66—67			
		59 ‡	70			
		65 §	70	49 ¶	70	
Na *S*-phenyl	Diphenyl	58	60			61 [7]
Na *S*-cyclohexylcarbamoylmethyl	Di(cyclohexylcarbamoylmethyl)	88	163—164			165 [8]
Sodium *S*-*p*-nitrobenzyl	Di-(*p*-nitrobenzyl)	100	125	38	126	126·5 [9]
S-Sulphocysteine	Cystine	77				

* 0·5 mmole thiourea. † 0·05 mmole thiourea. ‡ Reaction time, 10 min. § In 0·1N-HCl. ¶ In 98—100% formic acid.

---

[4] Swan, *Nature*, 1957, **180**, 643; *Austral. J. Chem.*, 1961, **14**, 69.
[5] Dixon and Wardlaw, *Nature*, 1960, **188**, 721.

Reaction of Sodium S-*Benzyl Thiosulphate with Thiourea*.—A solution of sodium *S*-benzyl thiosulphate (10 mmoles) and thiourea (10 mmoles) in N-hydrochloric acid (50 ml.) was heated on a steam-bath, 0·25N-sodium hydroxide being used to trap any sulphur dioxide expelled. After 1 hr. the mixture was cooled, and the sulphur dioxide still present was displaced into the trap in a stream of nitrogen. The disulphide was extracted with ethyl acetate (2 × 25 ml.), and sulphate in the aqueous phase was estimated gravimetrically as barium sulphate. The contents of the trap were analysed iodometrically for sulphite. Yields of dibenzyl disulphide, sulphate, and sulphur dioxide were 4·8, 4·7, and 4·7 mmoles, respectively. If nitrogen was bubbled continuously through the system dibenzyl disulphide (1·9 mmoles), sulphate (1·7 mmoles), and sulphur dioxide (7·0 mmoles) were formed.

When the reaction was carried out without passage of nitrogen, with only 0·5 mmole of thiourea, yields of disulphide, sulphate, and sulphur dioxide were 4·2, 4·6, and 4·0 mmoles, respectively. When nitrogen was passed continuously the yields were 3·6, 4·1, and 4·4 mmoles.

Reaction of Thiosulphates with Thiols and Thiourea.—(i) Sodium *S*-benzyl thiosulphate (2·26 g.), toluene-ω-thiol (1·24 g.), and thiourea (0·76 g.) were heated in acetic acid (25 ml.) at 100° for 1 hr., giving dibenzyl disulphide (2·24 g., 91% based on both Bunte salt and thiol), m. p. 67—68°. When thiourea was omitted from the mixture, no disulphide was obtained.

(ii) Reaction of equimolar amounts of potassium *S*-phenyl thiosulphate, benzenethiol, and thiourea in the same way, followed by crystallization of the crude product from methanol, gave diphenyl disulphide (84%), m. p. 60°. No disulphide was obtained in the absence of thiourea.

(iii) Similar reaction of sodium *S*-*o*-nitrophenyl thiosulphate (1·28 g.), toluene-ω-thiol (0·625 g.), and thiourea (0·38 g.) gave a crude product from which di-*o*-nitrophenyl disulphide (0·26 g.) and benzyl *o*-nitrophenyl disulphide (0·33 g.) were obtained by fractional crystallization from methanol.

(iv) Equimolar amounts of the monosodium salt of *S*-sulphocysteine, cysteine, and thiourea in acetic acid at 100° for 1 hr. gave cystine (71%). In the absence of thiourea the yield was 65%.

Reaction of Thiosulphates with Ammonium Thiocyanate.—(i) Sodium *S*-benzyl thiosulphate (2·26 g.) and ammonium thiocyanate (0·76 g.) were heated in acetic acid (25 ml.) at 100° for 1 hr. Dibenzyl disulphide (0·39 g., 32%), m. p. 71°, separated after addition of water.

(ii) When N-hydrochloric acid was used instead of acetic acid, as in (i), the crude product contained some thiol. However, after one crystallization from ethanol, dibenzyl disulphide (50%), m. p. 68—69°, was obtained.

(iii) Reaction of sodium *S*-*o*-nitrophenyl thiosulphate and ammonium thiocyanate in N-hydrochloric acid gave di-*o*-nitrophenyl disulphide (71%), m. p. 191° (from benzene).

Reaction of Thiosulphates with Potassium Iodide.—(i) The thiosulphate (10 mmoles) and potassium iodide (10 mmoles) were heated at 100° for 1 hr. in acetic acid (25 ml.). The product was filtered off after dilution with water. Yields and m. p.s of crude products are shown in the annexed Table.

Thiosulphate	Disulphide	Yield	M. p.	M. p. (lit.)
Na *S*-benzyl	Dibenzyl	98	71°	71—72° [6]
		78 *	68	
K *S*-phenyl	Diphenyl	89	60—61	61 [7]
Na *S*-*p*-nitrobenzyl	Di-*p*-nitrobenzyl	90	126	126·5 [9]
Na *S*-cyclohexylcarbamoylmethyl	Di(cyclohexylcarbamoylmethyl)	87	164	165 [8]

* KI 0·5 mmole.

(ii) Sodium *S*-benzyl thiosulphate (2·26 g.) and potassium iodide (1·66 g.), heated at 100° for 1 hr. in N-hydrochloric acid (50 ml.), gave a crude product containing thiol. Crystallization from ethanol gave dibenzyl disulphide (0·78 g., 56%), m. p. 69—70°. When 0·5 mmole of potassium iodide was used the yield of dibenzyl disulphide was only 15%.

Reaction of Sodium S-*Benzyl Thiosulphate with Potassium Iodide*.—Sodium *S*-benzyl thiosulphate (10 mmoles) and potassium iodide (10 mmoles) in acetic acid (25 ml.) were heated at 100° for 1 hr., any sulphur dioxide expelled being trapped in 1% sodium hydroxide solution. After flushing with nitrogen for 30 min., the contents of the trap were analysed iodometrically

---

[6] Hofmann, *Ber.*, 1887, **20**, 4.

[7] Stenhouse, *Annalen*, 1869, **149**, 247.

[8] Milligan and Swan, *J.*, 1959, 2969.

[9] Price and Twiss, *J.*, 1908, **93**, 1401.

for sulphite. Water (50 ml.) was added to the reaction mixture, dibenzyl disulphide was filtered off, and the filtrate then analysed gravimetrically for sulphate. The yields of dibenzyl disulphide, sulphate, and sulphur dioxide were 4·5, 4·1, and 4·2 mmoles, respectively.

Reaction of Thiosulphates with Thiols and Potassium Iodide.—A mixture of sodium *S*-benzyl thiosulphate (2·26 g.), toluene-ω-thiol (1·24 g.), and potassium iodide (1·66 g.) was heated at 100° for 1 hr. in acetic acid (25 ml.), giving dibenzyl disulphide (2·41 g., 98% based on both Bunte salt and thiol), m. p. 65—68°. Similar treatment of equimolar amounts of potassium *S*-phenyl thiosulphate, benzenethiol, and potassium iodide gave diphenyl disulphide (92%), m. p. 59°.

Reaction of Thiosulphates with o-*Nitrobenzenesulphenyl Chloride.*—(i) *o*-Nitrobenzenesulphenyl chloride (0·95 g.) was dissolved in a solution of sodium *S*-benzyl thiosulphate (1·13 g.) in *NN*-dimethylformamide (15 ml.) at room temperature. After 15 min. addition of water gave benzyl *o*-nitrophenyl disulphide (1·34 g., 96%), m. p. 54° (from methanol) (lit.,[10] 54°).

(ii) *o*-Nitrobenzenesulphenyl chloride (1·9 g.) and sodium benzyl thiosulphate (2·26 g.) were heated in acetic acid (30 ml.) at 100° for 1 hr. The resulting oil gave benzyl *o*-nitrophenyl disulphide (1·01 g., 36%), m. p. 52—53° (from methanol). Using N-hydrochloric instead of acetic acid gave benzyl *o*-nitrophenyl disulphide (1·39 g.) and di-(*o*-nitrophenyl) disulphide (0·4 g.).

(iii) Reaction of *o*-nitrobenzenesulphenyl chloride and potassium *S*-phenyl thiosulphate as in (i) gave *o*-nitrodiphenyl disulphide (61%), m. p. 54—55° after two crystallizations from light petroleum (lit.,[11] 55°).

Reaction of Thiosulphates with o-*Nitrobenzenesulphenyl Thiocyanate.*—*o*-Nitrobenzenesulphenyl thiocyanate was prepared by shaking the corresponding chloride with potassium thiocyanate in benzene.[12]

(i) Benzyl *o*-nitrophenyl disulphide (0·68 g., 98%), m. p. 54°, was obtained by reaction of sodium *S*-benzyl thiosulphate (0·57 g.) and *o*-nitrobenzenesulphenyl thiocyanate (0·53 g.) in *NN*-dimethylformamide (10 ml.) at room temperature for 15 min. Reaction in acetic acid at 100° for 1 hr. gave a 37% yield. Similar reaction in N-hydrochloric acid gave the mixed disulphide in 45% yield.

(ii) Reaction of potassium *S*-phenyl thiosulphate and *o*-nitrobenzene sulphenyl thiocyanate

Bunte salt	Other reactant *	Reaction time (hr.)	Relative amounts † of		
			Bunte salt	Mixed disulphide	Symmetrical disulphide
S-Sulphocysteine	A	60	+	+++	Trace
	B	60	++	—	++
	C	60	++	+	+
	D	60	+	—	+++
Glycyl-*S*-sulphocysteine	A	20	Trace	++++	—
	B	20	+	++	+
	C	20	+	+++	Trace
	D	20	++	—	++
S-Sulphocysteinylglycine	A	20	+	+++	—
	B	20	++	++	Trace
	C	20	+++	+	Trace
	D	20	+++	—	+
	D	70	++	—	++
	D ‡	50	Trace	—	++++
Glycyl-*S*-sulphocysteinylglycine	A	20	Trace	++++	Trace
	B	20	+	+++	Trace
	C	20	+	+++	Trace
	D	20	+	—	+++
S-Sulphoglutathione	A	20	+	+++	Trace
	B	20	+	+++	Trace
	C	20	+	+++	Trace
	D	20	++	—	++

* The reactants were thiourea (A), ethylenethiourea (B), *NNN′N′*-tetramethylthiourea (C), and potassium iodide (D).

† Judged by the size and density of spots on electrophoretograms. ‡ KI 2 mmoles.

---

[10] Footner and Smiles, *J.*, 1925, **127**, 2887.

[11] Lecher, *Ber.*, 1920, **53**, 577.

[12] Lecher and Simon, *Ber.*, 1921, **54**, 532.

in *NN*-dimethylformamide gave *o*-nitrodiphenyl disulphide (36%), m. p. 54° (from light petroleum) (lit.,[11] 55°).

Reaction of S-*Sulpho*-L-*cysteine and Related Peptides*.—Glycyl-*S*-sulpho-L-cysteine, *S*-sulpho-L-cysteinylglycine, glycyl-*S*-sulpho-L-cysteinylglycine, and *S*-sulphoglutathione were prepared from bisglycyl-L-cystine, L-cystylbisglycine, bisglycyl-L-cystylbisglycine, and glutathione, respectively, by passing a slow current of air through solutions of the peptides (0·5 mmole) in aqueous 0·1N-ammonia (20 ml.) containing sodium sulphite (5 mmoles) and a trace of copper chloride. After 3 hr. the solutions were evaporated *in vacuo*, and the residues were dissolved in water and passed down a Dowex-50 ion-exchange column (H^+ form). The eluates were evaporated to dryness and the residues dissolved in 0·1N-ammonia. These solutions were evaporated again, to give the ammonium salts which were used without further purification.

S-Sulpho-L-cysteine and the above *S*-sulphopeptides (0·05 mmole) were allowed to react with thiourea, ethylenethiourea, *NNN'N'*-tetramethylthiourea, or potassium iodide (0·2 mmole) in "AnalaR" 98—100% formic acid (0·5 ml.) at room temperature. Aliquot parts were withdrawn at intervals and spotted on paper electrophoretograms (Whatman 3MM filter paper) which were run at 300 v for 3—4 hr. with a pyridine–acetic acid buffer (pH 3·7). Products were located by the ninhydrin reaction. The starting materials migrated towards the anode; symmetrical disulphides moved slowly, and intermediate products (mixed disulphides) rapidly, towards the cathode. Results are tabulated.

A sample of *S*-amidino-L-cysteine hydrochloride was prepared by Toennies's method.[13] Its electrophoretic behaviour and brown colour with ninhydrin were identical with those of the major product of the reaction of *S*-sulphocysteine and thiourea in formic acid.

DIVISION OF PROTEIN CHEMISTRY (B. M.) AND DIVISION OF ORGANIC CHEMISTRY (J. M. S.),
COMMONWEALTH SCIENTIFIC AND INDUSTRIAL RESEARCH ORGANIZATION,
MELBOURNE, AUSTRALIA.

[*Received, October* 25*th*, 1961.]

---

[13] Toennies, *J. Biol. Chem.*, 1937, **120**, 297.