[CONTRIBUTION FROM THE SCHOOL OF PHARMACY AND THE MORLEY CHEMICAL LABORATORY OF WESTERN RESERVE UNIVERSITY]

THE CONDENSATION OF ALDEHYDES WITH ORTHO-AMINOTHIOPHENOLS, BENZOTHIAZOLINES AND BENZOTHIAZOLES

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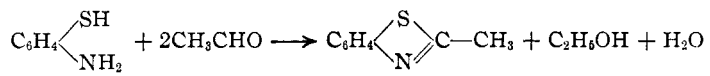
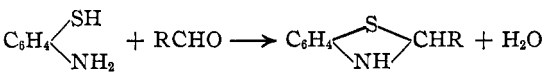
Hofmann[1] obtained benzothiazoles by the action of acids, acid chlorides and acid anhydrides upon *o*-aminothiophenol. Upon condensing certain aldehydes with *o*-aminothiophenol, he also obtained benzothiazoles.[2] He formulated the change as involving two molecules of aldehyde, one of which was reduced to the alcohol in the process.



In no case however was he able to show that an alcohol was actually formed.

Green and Perkin[3] also obtained a benzobisthiazole by condensing benzaldehyde with *p*-phenylenediamine-2,5-di-(thiosulfonic acid).

Claasz[4] condensed a number of aldehydes with *o*-aminothiophenol hydrochloride and obtained products which he described as benzothiazolines.



Since he obtained practically quantitative yields of product upon treating one mole of *o*-aminothiophenol with one mole of aldehyde, he pointed out that Hofmann's formulation of the reaction could not be correct and concluded that his products were benzothiazolines. In the cases where the corresponding benzothiazoles had previously been prepared, the melting points of Claasz' benzothiazolines were close to them and the melting points which he reported were in most cases not sharp. In no case did he establish his products as benzothiazolines by mixed melting points with the corresponding benzothiazoles.

Bogert and Stull[5] repeated a portion of Claasz' work and, upon purifying the condensation products, obtained benzothiazoles. They therefore characterized Claasz' benzothiazolines as impure benzothiazoles. In order to account for the formation of benzothiazoles from *o*-aminothiophenol and aldehydes they proposed two alternative mechanisms.

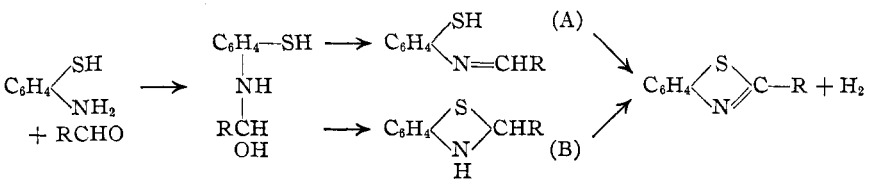
[1] Hofmann, *Ber.*, **12**, 2359 (1879); **13**, 8 (1880).

[2] Hofmann, *ibid.*, **13**, 1236 (1880).

[3] Green and Perkin, *J. Chem. Soc.*, **83**, 1207 (1903).

[4] Claasz, *Ber.*, **45**, 1031 (1912); **49**, 1141 (1916).

[5] Bogert and Stull, THIS JOURNAL, **47**, 3078 (1925).



As evidence in favor of (A) they point out that *o*-aminothiophenol will not condense with ketones or their dichlorides.

In the course of an investigation in this Laboratory dealing with the use of substituted *o*-aminothiophenols as reagents for the identification of aldehydes, we have condensed a number of aldehydes, both aliphatic and aromatic, with 2-amino-4-chlorothiophenol hydrochloride and in every case have obtained a product different from the benzothiazole obtained by the action of acid chlorides or acid anhydrides. These products were obtained as crystalline solids of sharp melting point. In some cases the melting points lay within a few degrees of the corresponding benzothiazole. In each such case a mixed melting point with the benzothiazole showed them to be different.

These substances resemble benzothiazolines as described by Claasz with the exception that they are insoluble in mineral acid. Analysis gave values required by the two possible types (A) and (B). Since they are insoluble in aqueous alkali they cannot be thiophenols as represented by (A). They are easily oxidized to benzothiazoles by warming with ferric chloride in alcohol. These facts show that these products are benzothiazolines (B).

Previous work in this field has been practically confined to the use of aromatic aldehydes. In the present work two benzothiazolines were prepared from aryl aldehydes, and both were found to be easily oxidized by the air to the benzothiazole upon crystallizing them from certain organic solvents such as alcohol or carbon tetrachloride. This would indicate that where benzothiazoles are obtained from the action of an aldehyde upon an *o*-aminothiophenol, a benzothiazoline is first formed and is subsequently oxidized to a benzothiazole, either in the process of preparation or purification. Whether the products which Claasz describes are benzothiazoles or benzothiazolines cannot be stated from the evidence at hand. However, in the light of our results it is quite possible that they are benzothiazolines, which in the process of purification employed by Bogert and Stull, were oxidized to benzothiazoles.

Experimental

Preparation of Benzothiazoles.—The action of an acid chloride or acid anhydride upon an *o*-aminothiophenol has been shown by several investigators to be a general method for the preparation of benzothiazoles.[1,2,6] The method employed in this work

[6] Papers of Bogert, THIS JOURNAL (1924–1927).

had previously been used in this Laboratory for the preparation of benzothiazoles,[7] and gave very satisfactory results. A mixture of one mole of 2-amino-4-chlorothiophenol hydrochloride and one mole of the acid chloride or acid anhydride was dissolved in dimethylaniline and the solution boiled for about thirty minutes. The benzothiazole was precipitated by acidifying the cold solution. Yields from 60 to 90% were obtained, depending upon the acid chloride or acid anhydride employed.

Preparation of Benzothiazolines.—After trying a number of different condensing agents, the following general method was found to give the best results. One mole of

TABLE I

MELTING POINTS OF CERTAIN 2-DERIVATIVES OF 5-CHLOROBENZOTHIAZOLE AND 5-CHLOROBENZOTHIAZOLINE[8]

Name	M. p., °C.	Mixed m. p., °C.	Solvent
5-Chlorobenzothiazole	106		Alcohol
5-Chlorobenzothiazoline	168–169		Chloroform
2-Methyl-5-chlorobenzothiazole[7]	68– 69	40–51	Alcohol
2-Methyl-5-chlorobenzothiazoline	61		Carbon tetrachloride and ligroin
2-Ethyl-5-chlorobenzothiazole	56– 57	30–44	Alcohol
2-Ethyl-5-chlorobenzothiazoline	60		Carbon tetrachloride and ligroin
2-Hexyl-5-chlorobenzothiazoline	51– 52		Alcohol
2-Phenyl-5-chlorobenzothiazole[7]	139	108–115	Alcohol
2-Phenyl-5-chlorobenzothiazoline	127		Chloroform and ligroin
2-(*o*-Chlorophenyl)-5-chlorobenzothiazole	136–137		Alcohol
2-(*o*-Chlorophenyl)-5-chlorobenzothiazoline	81		Alcohol

TABLE II

ANALYTICAL DATA

Name	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Chlorine, % Calcd.	Chlorine, % Found
5-Chlorobenzothiazole	C_7H_4NClS	49.52	49.82	2.37	2.46	20.92	21.08
5-Chlorobenzothiazoline	C_7H_6NClS	48.92	49.33	3.53	3.67	20.68	20.44
2-Methyl-5-chlorobenzothiazoline	C_8H_8NClS	51.70	51.79	4.32	4.44	19.11	18.74
2-Ethyl-5-chlorobenzothiazole	C_9H_8NClS	54.63	54.33	4.08	4.33	17.95	17.98
2-Ethyl-5-chlorobenzothiazoline	$C_9H_{10}NClS$	54.08	54.24	5.05	4.98	17.79	17.83
2-Hexyl-5-chlorobenzothiazoline	$C_{13}H_{18}NClS$	60.99	61.06	7.10	7.12		
2-Phenyl-5-chlorobenzothiazoline	$C_{13}H_{10}NClS$	63.03	63.10	4.04	3.91		
2-(*o*-Chlorophenyl)-5-chlorobenzothiazole	$C_{13}H_7NCl_2S$	55.71	56.12	2.52	2.29	25.32	25.46
2-(*o*-Chlorophenyl)-5-chlorobenzothiazoline	$C_{13}H_9NCl_2S$	55.28	55.14	3.21	3.35	25.14	25.14

[7] Lankelma and Knauf, THIS JOURNAL, **53**, 311 (1931).

[8] The numbering suggested for the benzothiazole nucleus by Bogert and Abrahamson, *ibid.*, **44**, 826 (1922), is used here for both the benzothiazole and the benzothiazoline nuclei.

2-amino-4-chlorothiophenol was dissolved in pyridine and one mole of aldehyde added dropwise to the warm solution. The mixture was finally heated from two to thirty minutes on the water-bath, the aromatic and the higher aliphatic aldehydes requiring the longer heating time.[9] The benzothiazoline was precipitated by acidifying the mixture; yields, 70–90%.

Oxidation of Benzothiazolines to Benzothiazoles.—The alkyl benzothiazolines could be crystallized unchanged from various solvents. 2-Phenyl-5-chlorobenzothiazoline, however, was converted to the benzothiazole upon two or three crystallizations from alcohol. Similarly 2-(*o*-chlorophenyl)-5-chlorobenzothiazoline was converted to the benzothiazole upon crystallization from acetone or carbon tetrachloride. Any of the benzothiazolines are oxidized to the benzothiazole in good yield by warming for a few minutes with a slight excess of ferric chloride in alcohol.

Summary

1. Aldehydes condense with 2-amino-4-chlorothiophenol to give benzothiazolines.

2. Benzothiazolines are readily oxidized to benzothiazoles.

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THE MOLECULAR WEIGHT OF INSULIN

BY BERTIL SJÖGREN AND THE SVEDBERG

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The eminent physiological importance of the pancreas hormone insulin has in recent years, and especially since the isolation of crystalline insulin by Abel,[1] made it the subject of numerous chemical investigations. The experimental material so far collected decidedly indicates that insulin is of protein nature.[2] It gives several of the characteristic protein reactions,[3] its empirical composition resembles that of the proteins,[3] it is an amphoteric electrolyte possessing an isoelectric point at about P_H 5,[3] which is in the same region where the isoelectric points of many proteins are situated and it shows a light absorption in the ultraviolet at exactly the same place as most of the proteins with a maximum at 270 mμ.[4]

At the suggestion of Dr. H. Jensen of The Johns Hopkins University, Baltimore, we have undertaken an ultracentrifugal study of insulin along the same lines as already followed in this Laboratory for the determination

[9] The formaldehyde employed was a 30–35% aqueous solution; the other aldehydes used were dried in the process of purification.

[1] J. J. Abel, *Proc. Nat. Acad. Sci.*, **12**, 132 (1926).

[2] H. Jensen and A. M. De Lawder, *Z. physiol. Chem.*, **190**, 262 (1930).

[3] J. J. Abel, E. M. K. Geiling, C. A. Rouiller, F. K. Bell and O. Wintersteiner, *J. Pharmacol.*, **31**, 65 (1927).

[4] W. Graubner, *Z. Ges. Exp. Medizin*, **63**, 527 (1928).