by heating dimethylene-blue chromate in dilute acid without access of air. The dye is extremely soluble in the form of the chloride but may be salted out readily as the bromide or iodide or in the form of a zinc chloride double salt. It is unstable and tends to decompose when heated.

3. Asymmetric dimethylthionine or methylene azure A of Kehrmann is obtained by heating methylene blue and potassium dichromate in dil. hydrochloric acid or, in a purer form, by heating the chromate of methylene azure B with dilute acid. It is less soluble than the trimethyl derivative and may be salted out readily in the form of the chloride.

4. Dimethylthionoline, or methylene violet of Bernthsen, may be prepared from methylene blue, from methylene azure B or A by heating in dilute alkaline solution in the presence of a complex soluble ammoniacal salt of silver or copper or in the presence of alkaline zincate or chromate. The metal evidently enters into the formation of a very soluble dye ion and tends to protect the respective free base from precipitation until hydrolysis has separated the metal amine group from the dimethylthionoline residue with precipitation of the latter. Better yields are obtained from methylene blue than from the azures.

5. These reactions lend support to the *para*-quinoid conception of Bernthsen and of Hantzsch in regard to the structural formula of the methylene blue dyes.

NEW YORK, N. Y.

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF MISSOURI]

ALPHA-NAPHTHYLISOCYANATE AS A REAGENT FOR ALCOHOLS

BY V. T. BICKEL[1] WITH H. E. FRENCH

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In general the use of reagents for the determination of alcohols is limited to the primary and secondary alcohols. Tertiary alcohols either yield unusual substances or do not react. The literature, however, mentions the formation of urethans from α-naphthylisocyanate and two tertiary alcohols.[2] It seemed probable, therefore, that this reagent might be more general in its application. Because of its high molecular weight the naphthylisocyanate was found to be relatively stable in water, reacting very slowly at room temperature. Its high boiling point also seemed a desirable feature, in that it permitted the use of higher temperatures with the less reactive alcohols. For these reasons α-naphthylisocyanate was chosen for this investigation.

[1] This communication is an abstract of a thesis submitted by V. T. Bickel in partial fulfilment of the requirements for the degree of Master of Arts at the University of Missouri.

[2] Neuberg and Kansky, *Biochem. Zeit.*, **20**, 446 (1909).

In the reactions carried out, the only by-product obtained was di-α-naphthylurea. This was found to be exceedingly insoluble in boiling ligroin (b. p., 100–120°). The urethans, on the other hand, were readily soluble in the hot ligroin, and on cooling the solutions, they crystallized well, and had sharp melting points. Usually two crystallizations were sufficient to prepare the substances for analysis.

In general, the reactions with primary alcohols went very well, often without the application of heat and giving very good yields of the urethans. We were unable to get citronellol to react. Ethylene and trimethylene glycols gave fair yields of the corresponding urethans, both hydroxyl groups reacting in each case. Glycerol readily gave a good yield of the urethan, all three of the hydroxyl groups reacting. The presence of considerable water did not interfere with the formation of the desired product. Ethylene and trimethylene-chlorohydrins also gave good yields of the urethans, the latter reacting in the cold. The corresponding bromohydrins produced slightly smaller yields of the urethans.

Secondary alcohols also reacted well with the reagent but it was always necessary to heat the reaction mixtures and the yields were somewhat smaller than those from the primary alcohols. Results from the tertiary alcohols were not satisfactory. Neither triphenyl carbinol nor diethylmethyl carbinol formed a urethan. In both cases dinaphthylurea was obtained but, since the amounts were small, it is unlikely that the reagent was destroyed by moisture. Urethans from *tert.*-butyl and *tert.*-amyl alcohols with α-naphthylisocyanate are reported in the literature in yields of 34 and 3.5%, respectively.[2]

The presence of moisture had a varying effect on the formation of the urethans. In the cases of those alcohols that reacted without the application of heat, moisture did not seriously interfere with the reaction. Both ethyl alcohol and *iso*-amyl alcohol were salted out of water solution with anhydrous potassium carbonate, and without further drying they gave good yields of the urethans. Under the same conditions phenylisocyanate failed to form urethans, diphenylurea only being obtained. With alcohols requiring heat to cause reaction, the presence of moisture is decidedly harmful. Diethyl carbinol and methylhexyl carbinol did not yield urethans until they had been dried over anhydrous copper sulfate and the reaction flasks had been dried by heat. From the standpoint of laboratory manipulation in organic qualitative analysis this is a disadvantage. It is not so serious, however, since those alcohols of lower molecular weight and, therefore, greater solubility in water, react well even in the presence of moisture.

For the identification of the tertiary alcohols it seemed possible that the *sym.*-tribromophenol ethers might serve. The procedure used consisted in converting the alcohol into the chloride and condensing with the sodium

salt of *sym.*-tribromophenol. Triphenyl carbinol gave a very good yield of the ether, but trimethyl carbinol halide failed to react. As a general reagent for tertiary alcohols, this substance is not satisfactory. Apparently the presence of so-called negative groups on the carbon holding the halogen is necessary for this reaction to take place, at least in the cases of the tertiary alcohols.

Experimental Part[3]

In the preparation of the urethans, from 2 to 3 g. of the alcohol was used with a slight excess of the α-naphthylisocyanate. The reactions were

TABLE I

PREPARATION OF URETHANS

Alcohol	Urethan M. p., °C.	Urethan Formula	Anal., N, % Calcd.	Anal., N, % Found
Methyl	124	$C_{12}H_{11}O_2N$	6.96	7.01
Benzyl	134.5	$C_{18}H_{15}O_2N$	5.05	5.05
Cinnamyl	114	$C_{20}H_{17}O_2N$	4.62	4.56
Phenylethyl	119	$C_{19}H_{17}O_2N$	4.81	4.74
Lauryl	80	$C_{23}H_{33}O_2N$	3.94	4.01
n-Amyl	68	$C_{16}H_{19}O_2N$	5.44	5.62
Furfuryl	129–130	$C_{16}H_{13}O_3N$	5.24	5.29
m-Xylyl	116	$C_{19}H_{17}O_2N$	4.81	4.83
o-Methoxybenzyl	135–136	$C_{19}H_{17}O_3N$	4.56	4.45
Ethylene glycol	176	$C_{24}H_{20}O_4N_2$	7.00	7.00
Trimethylene glycol	164	$C_{25}H_{22}O_4N_2$	6.76	6.68
Glycerol	191–192	$C_{36}H_{29}O_6N_3$	7.01	7.06
Ethylene-bromohydrin	86– 87	$C_{13}H_{12}O_2NBr$	4.76	4.85
Trimethylene-bromohydrin	73– 74	$C_{14}H_{14}O_2NBr$	4.54	4.87
Ethylene-chlorohydrin	101	$C_{13}H_{12}O_2NCl$	5.61	5.63
Trimethylene-chlorohydrin	76	$C_{14}H_{14}O_2NCl$	5.31	5.38
Phenylmethyl carbinol	106	$C_{19}H_{17}O_2N$	4.81	4.80
Phenylethyl carbinol	102	$C_{20}H_{19}O_2N$	4.59	4.50
Menthol	119	$C_{21}H_{27}O_2N$	4.30	4.13
Borneol	127	$C_{21}H_{25}O_2N$	4.33	4.33
Isoborneol	130	$C_{21}H_{25}O_2N$	4.33	4.25
Cholesterol	160	$C_{35}H_{53}O_2N$	2.52	2.58
Benzoin	140	$C_{25}H_{19}O_3N$	3.67	3.64
Diphenyl carbinol	135–136	$C_{24}H_{19}O_2N$	3.96	3.81
Cyclohexanol	128–129	$C_{17}H_{19}O_2N$	5.20	5.17
2-Methylcyclohexanol	154–155	$C_{18}H_{21}O_2N$	4.94	4.84
3-Methylcyclohexanol	122	$C_{18}H_{21}O_2N$	4.94	4.86
4-Methylcyclohexanol	159–160	$C_{18}H_{21}O_2N$	4.94	4.92
Methylhexyl carbinol	63– 64	$C_{19}H_{25}O_2N$	4.68	4.78
Diethyl carbinol	71– 72	$C_{16}H_{19}O_2N$	5.44	5.20
Triphenyl carbinol	No reaction			
Diethylmethyl carbinol	No reaction			
Citronellol	No reaction			

[3] The urethans of several of the alcohols of low molecular weight are described in the literature. See Ref. 2.

carried out by simply mixing the alcohol and the reagent. When no solid appeared in the cold, heat was applied for a few minutes. When, on cooling, no solid separated, or if the reaction mixture were a semi-liquid, the heating was repeated. In a few cases ligroin was used as a solvent for the reaction, but in general no solvent was used. When the reaction was complete, the contents of the flask were extracted with boiling ligroin (b. p., 100–120°), which readily dissolved the urethans, leaving the dinaphthylurea undissolved. Nitrogen was determined by the Kjeldahl method. Table I summarizes the experimental work on the urethans.

In the preparation of urethans from cinnamyl alcohol, menthol, borneol, isoborneol, cholesterol and benzoin, the alcohol was first dissolved in 10 cc. of ligroin, to which solution the reagent was added. In all other cases no solvent was used.

Urethans from ethylene glycol and glycerol were crystallized from alcohol, the rest from ligroin (b. p., 100–120°).

The reactions with ethylene- and trimethylene-bromohydrins yielded sticky, jelly-like masses which, however, crystallized well from ligroin. The urethans from methylhexyl carbinol and diethyl carbinol were so soluble in ligroin that purification for analysis was difficult.

Triphenyl Carbinol and Sodium *sym.*-Tribromophenolate.—Ten g. of triphenyl carbinol was converted to the chloride by means of a slight excess of phosphorus pentachloride. The chloride was then dissolved in benzene and treated with half a molecular quantity excess of the sodium salt of *sym.*-tribromophenol. After being refluxed for an hour the reaction mixture was washed with dil. aqueous sodium hydroxide and recrystallized from acetic acid and then from alcohol; m. p., 74–75°.

Anal. Calcd. for $C_{25}H_{17}OBr_3$: Br, 41.85. Found: 41.80.

***tert.*-Butyl Bromide and Sodium *sym.*-Tribromophenolate.**—The two substances were refluxed as described in the preceding experiment, but no ether was produced. A second trial gave similar results.

Summary

1. The use of α-naphthylisocyanate as a reagent for alcohols was investigated.

2. The reagent was found to react well with primary and secondary alcohols, forming urethans in good yields. These were found to crystallize well and melt sharply. Tertiary alcohols do not react well.

3. The presence of moisture is not detrimental to the reaction with alcohols of low molecular weight, or with those which do not require heat for the formation of the urethan. In cases where the reaction mixtures must be heated, moisture seriously interferes with the reaction.

4. The urethans are very easily separated from dinaphthylurea, the only by-product of the reaction, because of the great insolubility of the latter and the very considerable solubility of the former in hot ligroin.

5. The formation of ethers from sodium *sym.*-tribromophenolate and the

chlorides of the tertiary alcohols is not a satisfactory reaction, since the purely aliphatic alcohols do not react.

COLUMBIA, MISSOURI

[CONTRIBUTION FROM THE OFFICE OF PLANT PHYSIOLOGICAL INVESTIGATIONS, BUREAU OF PLANT INDUSTRY, UNITED STATES DEPARTMENT OF AGRICULTURE]

THE FORMOL TITRATION OF CERTAIN AMINO ACIDS

BY S. L. JODIDI

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The formol titration method of Sörensen[1] has been found to be of great usefulness. Therefore, it seemed desirable, if not imperative, to learn as much as possible about its accuracy or inaccuracy.

The primary cleavage products which proteins yield under the influence of acids, alkalies, enzymes or micro-organisms, are ordinarily referred to as amino acids, for the sake of convenience. Actually, however, they represent a variety of compounds, namely *amino acids* proper, that is, compounds which contain in their molecule one amino and one carboxyl group, such as leucine and alanine, *diamino acids* which contain two amino and one carboxyl groups, such as lysine and ornithine, *hydroxy-amino acids*, such as serine, *imino acids* which contain an imino group instead of the amino group, as is the case with proline and hydroxyproline, *amino-dicarboxylic acids* which contain one amino and two carboxyl groups, such as aspartic and glutaminic acids, *cyclic* amino acids such as tryptophan, and amino acids which contain groups other than those enumerated above, like arginine (guanidine group). Of these compounds, as was pointed out in a previous paper,[2] only the genuine amino acids, containing one amino and one carboxyl group, can accurately be estimated by the formol titration method. It may be added here that also the formol titration of hydroxy-amino acids as well as of amino-dicarboxylic acids yields accurate results, while the other "amino" acids show a greater or less deviation from calculated results. In this paper the titration of several amino acids according to Sörensen's method is given, the formol titration of which has as yet not been reported in the literature as far as the writer is aware. For details of the operations incidental to the formol titrations made, which cannot be given here, the reader is referred to the papers of Sörensen[1] and his associates.

Experimental Part

Cystine is the disulfide of cysteine which is analogous to serine, the latter having the hydroxyl group instead of the sulfhydryl (SH) group.

[1] Sörensen, *Biochem. Z.*, **7**, 45, 407 (1907); *Z. physiol. chem.*, **63**, 27 (1909); **64**, 120 (1910).

[2] Jodidi, THIS JOURNAL, **40**, 1031 (1918).