portion of antiserum it is the antibody molecules with the greatest attraction for the antigen which form the precipitate with it. The argument given above leads to the expectation that the optical isomer effect should be larger in this case than when a large amount of precipitating antigen is used. This expectation is confirmed by the experimental results.

Moreover, in the region of the equivalence zone, with the precipitate containing poor antibody as well as good antibody, the part of the precipitate which is dissolved first on addition of hapten is that containing the poor antibody (with small combining power for the haptenic group of the immunizing antigen and precipitating antigen); the good antibody tends to remain in the undissolved precipitate. Accordingly, by the argument given above, the difference in effect of *d* and *l* haptens would be small at low hapten concentrations and larger at high hapten concentrations, at which a considerable fraction of the precipitate is dissolved. This effect, shown clearly by the middle pair of curves in Fig. 1, leads to a difference in apparent heterogeneity of the antiserum with respect to the isomers.

The small difference shown by the isomeric haptens in the region of slight antigen excess may be the result of the action of the excess of antigen in favoring the formation of soluble complexes involving good antibody molecules.

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## Summary

An antiserum has been prepared by injecting rabbits with an azoprotein made by coupling sheep serum with diazotized *p*-aminosuccinanilic acid, the molecules of which have a plane of symmetry. It has been found that the *d* and *l* isomers of N-(α-methylbenzyl)-succinamic acid differ in their power to inhibit the precipitation of this antiserum by an azoprotein made by coupling ovalbumin with diazotized *p*-aminosuccinanilic acid, the *l* isomer having the greater inhibiting power. This behavior is presumably an effect of the presence of optically active amino acid residues in the antibody molecules.

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# NOTES

## A New Synthesis of 2-Diethylaminoethyl *p*-Aminothiolbenzoate

BY NOEL F. ALBERTSON AND R. O. CLINTON

2-Diethylaminoethyl *p*-aminothiolbenzoate (*Thiocaine*) was first prepared by Hansen and Fosdick,[1] and was shown to have anesthetic activity. Later work[2] indicated this activity to be from four to six times that of procaine hydrochloride, while the toxicity was not proportionately increased. The compound in the form of its hydrochloride was, however, relatively unstable in solution.[3] These facts suggested that a further investigation of its salts was desirable.

The simplest synthesis of this substance would involve reaction between 2-diethylaminoethanethiol and *p*-nitrobenzoyl chloride, followed by reduction of the thiol ester. An attempt to use this method was made by Lischer and Jordan,[4] but they were unable to prepare the thiol. A subsequent failure to prepare it also has been reported.[5] Both of these attempts were based upon the reaction of sodium hydrosulfide with 2-bromotriethylamine hydrobromide. In the present work we have been able to prepare the desired compound in about 40% yield by the reaction of alcoholic sodium hydrosulfide with 2-chlorotriethylamine. A more satisfactory procedure, however, involves the reaction of 2-chlorotriethylamine or 2-chlorotriethylamine hydrochloride with thiourea, followed by alkaline hydrolysis of the isothiouronium salt. The amine is less satisfactory than the hydrochloride, since the tertiary amino group is sufficiently basic to produce partial decomposition of the isothiouronium chloride with resultant loss of product.

Hansen and Fosdick[1] have reported a melting point of 52.0–52.5° for their base. In the present work material of this melting point was obtained in one experiment; however, all other preparations gave a base with melting point 75.0–75.5°. Seeding a melt of the low melting form with the high melting form produced a conversion to the latter. It is therefore evident that the compound exists in dimorphic forms.

A number of new salts of 2-diethylaminoethyl *p*-aminothiolbenzoate were prepared. Of these

(1) Hansen and Fosdick, THIS JOURNAL, **55**, 2872 (1933); U. S. Patent 2,090,756.

(2) Fosdick and Hansen, *J. Pharmacol.*, **60**, 323 (1934); Nolle, *Farm. i. Farmakol.* (U. S. S. R.), **1937**, No. 2, 1; (*Chem. Abstr.*, **34**, 3820 (1940)).

(3) Private communication from Dr. H. L. Hansen.

(4) Lischer and Jordan, THIS JOURNAL, **59**, 1623 (1937).

(5) Cook and Kreke, *ibid.*, **61**, 2971 (1939).

salts, the citrate and the phosphate appear to be the most stable.

### Experimental[6]

**2-Diethylaminoethylisothiouronium Chloride Hydrochloride.**—To a refluxing solution of 156.5 g. of thiourea in 500 ml. of alcohol a solution of 352.2 g. of 2-chlorotriethylamine hydrochloride (m. p. 205–209°) in 1000 ml. of alcohol was added in a thin stream during one-half hour. The resulting clear solution was refluxed for six hours, cooled, and diluted with a mixture of two liters of ethyl acetate and 500 ml. of ligroin. The white precipitate was filtered and air-dried, yielding 444 g. of product. An additional 38 g. was recovered by working up the mother liquor. One crystallization from absolute alcohol–ether gave pure material, m. p. 194–195°, with but little loss; total yield, 94.5%.

*Anal.*[7] Calcd. for $C_7H_{19}N_3Cl_2S$: N, 16.93. Found: N, 17.08.

**2-Diethylaminoethanethiol.**—To a suspension of 248 g. of 2-diethylaminoethylisothiouronium chloride hydrochloride in 400 ml. of water was added a warm solution of 81.2 g. of 98.5% sodium hydroxide in 300 ml. of water. There was an immediate separation of a pink upper oily layer. The mixture was saturated with salt and extracted with three 100-ml. portions of ether. After thorough drying of the combined extracts over anhydrous sodium sulfate, the ether was distilled in a current of nitrogen through an efficient fractionating column. (The compound readily co-distills with ether.) Distillation of the residual oil *in vacuo* gave 103 g. (77.5%) of colorless product; mobile liquid with a nauseating odor, b. p. 65–66° at 23 mm. The pure compound boiled at 74.0° at 32.0 mm.,[8] $n^{20}D$ 1.4670.

*Anal.* Calcd. for $C_6H_{15}NS$: C, 54.08; H, 11.35. Found: C, 54.40; H, 11.46.

The **hydrochloride** formed long slender white needles (from absolute alcohol–ligroin), m. p. 172–173°.[8]

*Anal.* Calcd. for $C_6H_{15}NS{\cdot}HCl$: N, 8.25. Found: N, 8.73.

2-Diethylaminoethanethiol was also prepared from 2-chlorotriethylamine and thiourea by the above method using a one-half hour reflux period. The intermediate **2-diethylaminoethylisothiouronium chloride** was obtained in 85% yield: rosets of long slender white needles, m. p. 192–194° (dec.).

*Anal.* Calcd. for $C_7H_{18}N_3ClS$: N, 19.84. Found: N, 19.72.

Hydrolysis by the above method gave a 76.3% yield of the thiol.

The addition of 2-chlorotriethylamine to an excess of alcoholic sodium hydrosulfide solution under reflux in an atmosphere of hydrogen sulfide gave a 40% yield of the thiol. A large proportion of the 2-chlorotriethylamine was converted to the piperazinium salt.[9] The thiol was always accompanied by a higher boiling fraction, which could be made the major product (up to 80% yield) by using an excess of 2-chlorotriethylamine and conducting the reaction below 25°. This material proved to be **2-diethylaminoethylsulfide**, a colorless mobile liquid, boiling at 64.0° at 0.4 mm., $n^{20}D$ 1.4740. It rapidly absorbed carbon dioxide from the air and became semi-solid.

*Anal.* Calcd. for $C_{12}H_{28}N_2S$: C, 62.01; H, 12.14. Found: C, 62.15; H, 12.37.

The **dihydrochloride** melted at 252–254° (dec.), (lit.[5] m. p. 245.5–247.5°).

*Anal.* Calcd. for $C_{12}H_{28}N_2S{\cdot}2HCl$: C, 47.20; H, 9.90; N, 9.18. Found: C, 46.90; H, 9.66; N, 9.00.

(6) All melting points are uncorrected.

(7) We are indebted to the Misses Patricia Curran and Alice Rainey for the microanalyses.

(8) U. S. Patent 2,342,142 reports b. p. 160–170° for the thiol and m. p. 205° (dec.) for the thiol hydrochloride.

(9) Gough and King, *J. Chem. Soc.*, 2437 (1928); Eisleb, *Ber.*, **74**, 1433 (1941).

**2-Diethylaminoethyl *p*-Nitrothiolbenzoate Hydrochloride.**—2-Diethylaminoethanethiol was treated with *p*-nitrobenzoyl chloride in cold dry benzene; yield 87.8%, m. p. 163–165°. A small portion crystallized from absolute alcohol–petroleum ether formed small prisms with a light yellow color in bulk, m. p. 166–168°.

*Anal.* Calcd. for $C_{13}H_{19}O_3N_2ClS$: C, 48.97; H, 6.01; N, 8.79. Found: C, 49.03; H, 6.03; N, 8.72.

**2-Diethylaminoethyl *p*-Aminothiolbenzoate (Thiocaine).**—2-Diethylaminoethyl *p*-nitrothiolbenzoate hydrochloride was reduced with iron powder essentially by the method of Hansen and Fosdick.[1] However, no additional hydrogen chloride over that present in the salt was used; yield 55.6%, m. p. 74–75°. Recrystallization from benzene–petroleum ether gave long slender white needles, m. p. 74.0–75.5°

*Anal.* Calcd. for $C_{13}H_{20}ON_2S$: C, 61.87; H, 7.99; N, 11.10. Found: C, 61.64; H, 7.90; N, 11.26.

The free base is stable if protected from air and moisture; in the presence of these latter it slowly turns yellow over a period of a few weeks.

**2-Diethylaminoethyl *p*-Aminothiolbenzoate Salts.**—The salts listed below, with the exception of the dihydrochloride, were prepared by admixture of warm solutions of equimolecular amounts of the components in absolute alcohol. The dihydrochloride was prepared by the addition of an excess of anhydrous hydrogen chloride in absolute alcohol to an absolute alcohol solution of the base. The salts were crystallized from absolute alcohol with Nuchar treatment; one crystallization sufficed to render them pure. The yields were nearly quantitative except in the case of the tartrate.

**Dihydrochloride.**—Light yellow hygroscopic needles, m. p. 173–175°. A 0.5% aqueous solution had a *p*H of 2.15 at 25°.[10]

*Anal.* Calcd. for $C_{13}H_{20}ON_2S{\cdot}2HCl$: N, 8.61. Found: N, 8.78.

**Phosphate.**—White needles or blunt prisms, m. p. 170.0–170.5°. A 0.5% aqueous solution had a *p*H of 4.85 at 25°.

*Anal.* Calcd. for $C_{13}H_{20}ON_2S{\cdot}H_3PO_4$: N, 8.00. Found: N, 7.93.

**Citrate.**—Rosets of small white prisms, m. p. 165–166° (dec.). A 0.3% aqueous solution had a *p*H of 3.90 at 25°.

*Anal.* Calcd. for $C_{13}H_{20}ON_2S{\cdot}C_6H_8O_7$: N, 6.30. Found: N, 6.31.

**Tartrate.**—Rosets of large white needles, m. p. 79–80°. A 0.5% aqueous solution had a *p*H of 3.68 at 25°.

*Anal.* Calcd. for $C_{13}H_{20}ON_2S{\cdot}C_4H_6O_6$: N, 6.96. Found: N, 6.96.

(10) We are indebted to Mr. George Bronell for these determinations.

RESEARCH LABORATORIES
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## The Purification of Phenothiazine

BY BRUCE E. BAKER AND LEO BRICKMAN

The discovery that phenothiazine is an excellent anthelmintic in sheep,[1] cattle[2] and poultry[3] as well as an effective insecticide[4] has renewed interest in this compound and its derivatives. The commercial material as usually obtained is a dark green powder melting 170–175°. The value set

(1) Swales and Collier, *Can. J. Research*, **18D**, 279 (1940).

(2) Knipling, *J. Econ. Entomol.*, **31**, 315 (1938).

(3) Roberts, *Austral. Vet. J.*, **16**, 172 (1940).

(4) Campbell, Sullivan, Smith and Haller, *J. Econ. Entomol.*, **27**, 1176 (1934).