ents, probably silicates. The necessity of devising methods for the purification of the above products was eliminated when it was found that acetyl-DL-tryptophan could be hydrolyzed to DL-tryptophan in practically quantitative yields by heating aqueous solutions of the acetyl compound at 200° for six to seven hours. The following procedure was used for the conversion of III to V. III (1.5 g.) was heated with 20 ml. of water, contained in an unsealed bomb tube, at the refluxing temperature for 30 min., the tube was sealed, the tube and contents heated at 200° for six to seven hours, the hydrolysate decolorized with Norite, evaporated to dryness *in vacuo*, and the residue dried over phosphorus pentoxide. The residue was extracted with 15 ml. of boiling ethanol to give 0.88 g. (84%) of V, dec. p. 262–264°. This product was recrystallized from 30% aqueous ethanol to give V, dec. p. 264–265°.

*Anal.* Calcd. for $C_{11}H_{11}O_2N_2F$ (222.2): C, 59.5; H, 5.0; N, 12.6. Found: C, 59.4; H, 5.0; N, 12.6.

A small amount of IV was recovered from the ethanol extract.

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## Studies on Nitrogen Trichloride Treated Prolamines. IV. Isolation of the Neurotoxic Principle

BY L. REINER, F. MISANI, T. W. FAIR, P. WEISS AND M. G. CORDASCO

In a recent publication Bentley, *et al.*,[1] announced the isolation of the toxic factor from nitrogen trichloride treated zein. We have also succeeded in isolating the toxic principle in a crystalline form and the purpose of this note is to report the procedure used and to describe the progress made in the characterization of this material.

The treatment of zein and the initial steps of purification with cation exchanger (Duolite $C_1$) were those reported previously.[2] For further purification the following procedure was adopted: The material (toxicity 0.5 g./kg.)[3] was hydrolyzed by refluxing with 5 *N* hydrochloric acid for four hours. After removal of the acid it was extracted repeatedly with butanol from an aqueous solution containing 5% trichloroacetic acid. Subsequently, it was precipitated at an acid *p*H value with mercuric acetate, the mercury and hydrogen sulfide removed and the concentrated solution treated with picric acid. In some instances a second mercury precipitation was carried out after removal of the picric acid. This was followed by treatment with Duolite to bring the *p*H to 3.7. The material dried *in vacuo* possessed a toxicity of about 30 mg./kg. The solid was extracted with hot methanol. On concentrating and cooling the methanol solution solids appeared, which after crystallization from aqueous methanol were toxic at 5 mg./kg. Recrystallization from water yielded rod-shaped crystals (see Fig. 1). They produced typical convulsions in rabbits at a dose of 1–1.5 mg./kg. and in mice by intraperitoneal injection at about 40 mg./kg. The material decomposed slowly at 220° and more rapidly at 240° without melting completely up to 350°.

Fig. 1.

The Pauly and biuret tests were negative. The compound contained sulfur but no –SH, –SS– or chlorine. A bluish purple dye was formed with ninhydrin. Paper chromatograms (ascending) revealed a single spot with $R_f$ values of 0.04, butanol–acetic acid; 0.30, lutidine–collidine–ethanol; 0.62, phenol. After refluxing with 2 *N* sodium hydroxide for twenty-four hours the reaction with ninhydrin was negative. Refluxing for twenty-four hours with 5 *N* hydrochloric acid yielded two additional spots and almost complete destruction of the toxicity. There was, however, no increase in the alpha amino nitrogen content after hydrolysis[4] and no liberation of ninhydrin-positive material after hydrolysis of the dinitrophenyl derivative.

Analysis by Dr. Richard Baltzly (Wellcome Research Laboratories) gave C, 33.31; H, 6.57; N, 15.65; S, 16.15.[5] A compound having the formula $C_5H_{12}N_2O_3S$ calculates for C, 33.32; H, 6.71; N, 15.55; S, 17.79; mol. wt., 180.2. Molecular weight determination by freezing point lowering of water gave the value of 187. Electrometric titration revealed only two ionizing groups between the *p*H values of 2.0 and 9.5 having the *pK* values of 2.5 and 8.4, respectively; thus only one of the nitrogens is present as a free amino group. The

(1) Bentley, McDermott, Pace, Whitehead and Moran, *Nature*, **164**, 438 (1949).

(2) Reiner, Weiss, Misani, Cordasco and Fair, *Federation Proc.*, **8**, 241 (1949).

(3) Toxicity values reported in this communication were obtained in rabbits, orally, unless stated otherwise.

(4) Determined by Dr. James B. Allison, Rutgers University.

(5) The low sulfur value may be due to a slight contamination by sulfur-free amino acids. Analysis of a second batch was in agreement with the first, except that sulfur was higher, S, 17.81.

minimum molecular weight calculated from the neutralization equivalent was 175.

The evidence presented suggests that the compound is not a peptide but a derivative of methionine. The chromatograms of acid-hydrolyzed material apparently contradicts this view. We found however that methionine sulfoxide also yields two additional chromatographic spots after refluxing with 5 *N* hydrochloric acid. Only one of these is similar to one of those obtained from the toxic principle. It is also significant that methionine in large doses (reversal ratio about 1:1000) prevented the development of seizures in rabbits.

**Acknowledgment** is due to Dr. H. K. Parker and Mr. K. L. Fortmann for the treatment of the zein.

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## The Reduction of Some 9,10-Dihydroxy-9,10-dihydro Derivatives of Phenanthrene and Anthracene

BY ROBERT W. RIMMER,[1] ROBERT G. CHRISTIANSEN,[2] ROBERT K. BROWN AND REUBEN B. SANDIN

The reduction of triarylcarbinols, by agents such as vanadous and titanous chlorides, to give hexa-arylethanes has been reported by Conant and co-workers.[3] The present report is a study of the action of stannous chloride on certain dihydrodiols of phenanthrene and anthracene. 9,10-Dimethyldihydrophenanthrenediol is reduced to 9,10-dimethylphenanthrene by stannous chloride, while 9,10-dimethyl-3,6-dimethoxyphenanthrene is obtained from the corresponding dihydrodiol. The 9,10-diethyl and 9,10-diphenyldihydrodiol derivatives of phenanthrene, however, undergo pinacol rearrangement, giving 9,9-diethyl and 9,9-diphenyl-10-phenanthrone. Stannous chloride also reduces a number of 9,10-dialkyldihydroanthracenediols and 9,10-dimethyl-9,10-dihydroxy-9,10-dihydro-1,2-benzanthracene to the corresponding hydrocarbons.

The demethylation of 9,10-dimethyl-3,6-dimethoxyphenanthrene gives 9,10-dimethyl-3,6-dihydroxyphenanthrene, a compound that is structurally related to 2,3-bis-(*p*-hydroxyphenyl)-2-butene.[4] The phenanthrene was therefore tested for hormone activity but there was no evidence of estrogenic activity at the high dose of 3740 γ.

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### Experimental[5]

The 9,10-dihydro-9,10-diols, with the exception of compound I, were prepared by published procedures.

**Reduction of Diols.**—The general procedure was as follows: a mixture of 1.0 g. of diol in 25 ml. of glacial acetic acid, 10 g. of stannous chloride and 10 ml. of concentrated hydrochloric acid was refluxed for one hour. The cooled solution was poured into 500 ml. of water and after standing overnight, the hydrocarbon was filtered, washed with water and dried. The picrate was made and crystallized from alcohol. The pure hydrocarbon was regenerated from the picrate by treatment with ammonia.

TABLE I

ACTION OF STANNOUS CHLORIDE ON 9,10-DIHYDROXY-9,10-DIHYDRO DERIVATIVES OF PHENANTHRENE AND ANTHRACENE

| Compound used in reaction<br>Substituted 9,10-dihydrophenanthrene | Product isolated | Yield, % | M. p., °C. |
|---|---|---|---|
| 9,10-Dimethyl-9,10-dihydroxy[a] | 9,10-Dimethylphenanthrene[b] | 60 | 144[c] |
| 9,10-Diethyl-9,10-dihydroxy[a] | 9,9-Diethyl-10-phenanthrone | | 64 |
| 9,10-Diphenyl-9,10-dihydroxy[d] | 9,9-Diphenyl-10-phenanthrone | | 198 |
| 3,6-Dimethoxy-9,10-dimethyl-9,10-dihydroxy | 3,6-Dimethoxy-9,10-dimethylphenanthrene[e] | 50 | 138 |
| Substituted 9,10-dihydroanthracene | | | |
| 9,10-Dimethyl-9,10-dihydroxy[f] | 9,10-Dimethylanthracene | 50 | 178–179 |
| 2,9,10-Trimethyl-9,10-dihydroxy[f] | 2,9,10-Trimethylanthracene | 35 | 96 |
| 9,10-Diethyl-9,10-dihydroxy[f] | 9,10-Diethylanthracene | 25 | 145 |
| 9,10-Dimethyl-9,10-dihydroxy-1,2-benz[g] | 9,10-Dimethyl-1,2-benzanthracene | 25 | 122–123 |

[a] Zincke and Tropp, *Ann.*, **362**, 242 (1908). [b] The picrate melted at 192°. Bradsher and Amore, THIS JOURNAL, **66**, 1280 (1944), report 193–194°. *Anal.* Calcd. for $C_{22}H_{17}O_7N_3$: C, 60.7; H, 3.9. Found: C, 61.2; H, 4.2. [c] Bradsher and Amore, *ibid.*, report 142.5–143°. [d] Werner and Grob, *Ber.*, **37**, 2887 (1904). [e] *Anal.* Calcd. for $C_{18}H_{18}O_2$: C, 81.2; H, 6.8. Found: C, 81.1; H, 6.8. The picrate melted at 186–187°. *Anal.* Calcd. for $C_{24}H_{21}O_9N_3$: C, 58.2; H, 4.3. Found: C, 58.3; H, 4.5. [f] Bachmann and Chemerda, *J. Org. Chem.*, **4**, 583 (1939). [g] Bachmann and Bradbury, *ibid.*, **2**, 175 (1938).

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(3) Conant and Sloan, THIS JOURNAL, **47**, 572 (1925); Conant, Small and Taylor, *ibid.*, **47**, 1959 (1925); Conant and Bigelow, *ibid.*, **53**, 676 (1931).

(4) Dodds, Goldberg, Lawson and Robinson, *Nature*, **141**, 247 (1938); Dodds, Goldberg, Lawson and Robinson, *Proc. Roy. Soc. (London)*, **B127**, 140 (1939); Dodds, Goldberg, Grunfeld, Lawson, Saffer and Robinson, *ibid.*, **B132**, 83 (1944).

(5) All melting points are uncorrected.