Preparation of the Copper Salt

A stirred mixture of the acid (0.01 mole), sodium carbonate (0.005 mole), and water (100 ml) was heated until the acid dissolved. Upon the addition of a solution of cupric sulphate pentahydrate (0.005 mole) in 50 ml of water, the blue cupric salt of the indole-2-carboxylic acid precipitated. The solid was washed thoroughly with water, air-dried, and then given a final drying in a vacuum desiccator over calcium chloride.

The Procedure for Decarboxylation

A mixture of the indole-2-carboxylic acid (0.01 mole) and its copper salt (0.0004 mole) in 10 ml of synthetic quinoline was heated until carbon dioxide began to evolve. The mixture was kept at this temperature until gas evolution ceased (1.5–5 hours). The cooled solution was taken up in ether and the ether solution was washed several times with 1 *N* hydrochloric acid, once with water, twice with sodium carbonate solution, and finally with water. When the dried (Na_2SO_4) ether solution was freed from solvent a solid was obtained. This was further purified, if necessary, by passage through a short column of neutral alumina, using methylene dichloride as eluant.

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Department of Chemistry,
University of Alberta,
Edmonton, Alberta.

N,N-BRIDGED DERIVATIVES OF ADENINE[1]

Sumner H. Burstein and H. J. Ringold

The reaction of 6-bis(2-hydroxyethyl)aminopurine (I*a*) with thionyl chloride was reported in 1957 by DiPaco and Tauro (1) to yield the adenine mustard derivative (II). Huber (2) in an earlier publication reported that the identical reaction gave an ionic halogen-containing product to which he assigned the dimeric piperazinium chloride structure (III). A 1960 United States patent of Lyttle and Petering (3) stated that the product derived from I*a* and thionyl chloride contained 1 equivalent of ionic halogen and

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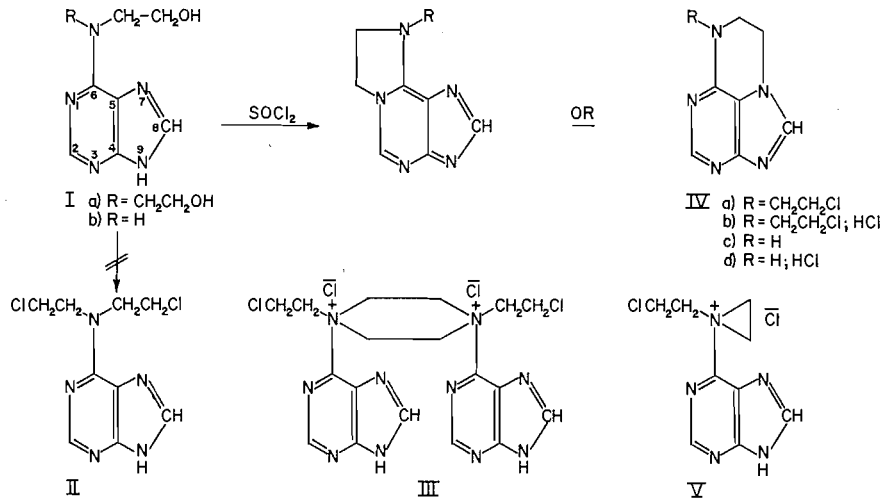
had probably undergone internal cyclization to the 1- or to the 7-nitrogen position. We have had occasion to prepare what appears to be the same product obtained by the three groups enumerated above and wish to record our observations and conclusions regarding its structure.



The reaction of 6-bis(2-hydroxyethyl)aminopurine (I*a*) with thionyl chloride, either at room temperature or under reflux, gave a product (IV*b*) of melting point 254–256° (decomp.) analyzing for $C_9H_{11}Cl_2N_5$. The compound, which was readily soluble in water, reacted instantaneously with excess silver nitrate, forming 1 equivalent of silver chloride. Titration with 0.1 *N* aqueous sodium hydroxide demonstrated a pK_a of 6.8 and when alkali was added to a pH of 12, followed by retitration with 0.1 *N* HCl, the titration curve did not show any major deviation. This behavior appeared to be indicative of a hydrochloride. When IV*b* was treated at room temperature with sodium acetate a product (IV*a*), which had lost a mole of hydrogen chloride, was isolated. The latter was readily reconverted to IV*b* by treatment with anhydrous hydrogen chloride in ethanol. This conclusively establishes that IV*b* is a hydrochloride and therefore eliminates the piperazinium structure (III), the conventional nitrogen mustard structure (II), as well as the quaternary aziridine structure (V). The latter could have been considered a structural possibility on the basis of ionic halogen content.

Thus it is evident that IV*b* is, in fact, a product that has undergone self-alkylation[2] on either the 1- or 7-nitrogen function and that IV*a* is simply the cyclized hydrogen-chloride-free product.

Before discussing the attempt to resolve the question of 1- or 7-cyclization, the behavior of the "one-armed" compound (I*b*) is pertinent at this point. When 6-(2-hydroxyethyl)-aminopurine (I*b*) (2, 4) (prepared by the reaction of 6-chloropurine with ethanolamine) was reacted with thionyl chloride a product, $C_7H_8ClN_5$ (IV*d*), was obtained. This compound exhibited an acidity parallel to IV*b*, the pK_a being 7.0. Further, the titration curve of IV*d* was very similar to that of IV*b* and neutralization with sodium acetate or alkali resulted in the loss of hydrogen chloride and formation of a halogen-free product (IV*c*). The latter was readily reconverted to the original hydrochloride. From these character-

[2]*W. T. Caldwell and S. Toukan, Temple University, have reached similar conclusions in an independent study (private communication from Professor Caldwell).*

istics as well as from the similar ultraviolet spectral curves of the two series of compounds, it may be concluded that the same type of cyclization has occurred with both the "one-armed" and "two-armed" substances. Further, the cyclization of I*b* establishes that a quaternary aziridine, V, is not an intermediate in the formation of IV*b*.

The n.m.r. of IV*a* in deuterochloroform solution showed adenine ring hydrogen peaks at $\tau = 1.79$ and 2.21, while N-benzyltriacanthine (5), a γ,γ-dimethylallyl 7-substituted adenine derivative, showed ring hydrogens at $\tau = 2.05$ and 2.23 (5). Assignment of the 2.21–2.23 position to the unperturbed 2-proton would be consistent with cyclization to the 7-position, the 8-proton being shifted various degrees by the different substituents attached to the 7-nitrogen.

The ultraviolet spectra of our products, however, are not in accord with that expected for a 7-substituted adenine. Triacanthine and 7-D-ribofuranosidoadenine (6) are reported to exhibit $\lambda_{max}^{neutral}$ 273 mμ, ϵ 13,000 and a $\lambda_{max}^{pH\ 1}$ for triacanthine of 277 mμ, ϵ 18,000. Compounds IV*a* and IV*c* respectively exhibit $\lambda_{max}^{H_2O}$ 264 mμ and 270 mμ and undergo a hypsochromic displacement of 2 mμ rather than a bathochromic shift in going from water to 0.1 *N* hydrochloric acid. It is possible that the presence of the third ring in IV is modifying the spectrum but at the present time neither the 1- nor 7-cyclized structure can be discarded. In this connection it should be noted that Ramage and Trappe (7) reported the cyclization of 2-chloro-4-methyl-5-amino-6(2-chloroethyl)aminopyrimidine to a five- rather than to a six-membered ring, while Chu, Harris, and Mautner (8) prepared 8-bis-(β-chloroethyl)-aminoadenine hydrochloride without internal cyclization having occurred, although self-alkylation to either the 7- or 9-position would have led to a new five-membered ring.

Compounds IV*a* and IV*c* were found to be non-toxic in the mouse at a daily dose of 500 mg/kg for a period of 1 week[3] and have not demonstrated any significant antitumor activity.[4]

EXPERIMENTAL

Melting points are uncorrected and ultraviolet spectral determinations were made with a Carey model 14 recording spectrophotometer. We are grateful to Mr. T. A. Wittstruck for the n.m.r. determination. Elementary analyses were performed by Midwest Microlab, Inc., 7838 Forest Lane, Indianapolis 20, Indiana.

6-Bis(2-hydroxyethyl)aminopurine (Ia)

A mixture of 6-chloropurine[5] (5.0 g), diethanolamine (10 ml), and absolute ethanol (50 ml) was heated for 8 hours under reflux and then cooled overnight at 0°, yielding 6.5 g of I*a*, m.p. 219–221° (reported 205° (1), 216–218° (2), 228–231° (3)); $\lambda_{max}^{H_2O}$ 213 and 276 mμ, ϵ 16,500 and 18,300; $\lambda_{max}^{0.1\ N\ HCl}$ 284 mμ, ϵ 16,400. The constants were unchanged after further crystallization from 95% ethanol. Anal. Calc. for $C_9H_{13}N_5O_2$: C, 48.42; H, 5.87; N, 31.37. Found: C, 48.28; H, 5.99; N, 31.23.

Reaction of Ia with Thionyl Chloride – Preparation of IVb

(a) With Heating

The bis-hydroxyethyl derivative (I*a*) (5.0 g) was heated for 16 hours, with stirring, in boiling, freshly distilled thionyl chloride (100 ml). The solvent was removed *in vacuo* and the residue stirred with absolute ethanol (50 ml). The product, 5.0 g, exhibited m.p. 250° (decomp.) while the analytical specimen from absolute ethanol melted at 254–256° (decomp.); $\lambda_{max}^{H_2O}$ 215 and 268 mμ, ϵ 18,500 and 13,100; $\lambda_{max}^{0.1N\ HCl}$ 215 and 268 mμ, ϵ 18,500 and 13,400. (Reported m.p. 245° (1), 243–247° (2), 253–255° (3).) Titration with 0.1 *N* sodium hydroxide gave pK_a 6.8. Anal. Calc. for $C_9H_{11}Cl_2N_5$: C, 41.56; H, 4.26; N, 26.93; Cl, 27.26. Found: C, 41.53; H, 4.34; N, 27.04; Cl, 27.29.

(b) Without Heating

A suspension of I*a* (480 mg) in thionyl chloride (15 ml) was stirred for 14 hours at room temperature. The precipitate was filtered through a sintered-glass funnel and washed with benzene and then absolute

[3]*Toxicity studies by Dr. R. I. Dorfman of this foundation.*
[4]*Antitumor assays by the Cancer Chemotherapy National Service Center.*
[5]*We wish to thank Burroughs Wellcome and Co. for a generous gift of material.*

ethanol, yielding 600 mg of IV*b*, m.p. 246–248°, whose ultraviolet and infrared spectra were identical with the product obtained in (*a*). Recrystallization from absolute ethanol gave 453 mg of product, m.p. 254° (decomp.), whose infrared spectrum was unchanged.

Reaction of IVb with Silver Nitrate

A solution of IV*b* (65 mg) in water (10 ml) was reacted with silver nitrate solution (2.0 equiv. in 5 ml of water) containing a drop of nitric acid, causing instantaneous formation of a precipitate. The silver chloride was rapidly filtered and then washed with ethanol–acetone and dried. Weight 32 mg (0.89 equiv.).

Neutralization of IVb – Preparation of IVa

Sodium acetate trihydrate (500 mg) was added to a solution of hydrochoride IV*b* (500 mg) in water (5 ml). The solvent was removed at room temperature *in vacuo* and the residue extracted with chloroform and crystallized several times from chloroform–hexane to yield an analytical specimen of IV*a*, m.p. 310–315°; $\lambda_{max}^{H_2O}$ 214 and 270 mμ, ϵ 18,300 and 12,700; $\lambda_{max}^{0.1N\,HCl}$ 215 and 268 mμ, ϵ 19,700 and 14,200; n.m.r. ($CDCl_3$) 1.79, 2.21, 5.50, 5.61, 5.71, 5.78, 5.97, and 6.06 τ. Anal. Calc. for $C_9H_{10}ClN_5$: C, 47.79; H, 4.69. Found: C, 47.92; H, 4.81.

Reconversion of IVa to IVb

Hydrogen chloride was bubbled for 30 minutes through a stirred suspension of IV*a* (50 mg) in absolute ethanol (5 ml). Ether was added and the precipitate was filtered, washed with ether, and recrystallized from methanol, yielding the hydrochloride IV*c*, m.p. 254° (decomp.), whose infrared spectrum was identical with the product described above.

6-(2-Hydroxyelhyl)aminopurine (Ib)

A mixture of 6-chloropurine (5.0 g) and 2-aminoethanol (10 ml) in absolute ethanol (50 ml) was heated for 3 hours under reflux and then cooled, yielding 5.14 g of I*b*, m.p. 250–252°. An analytical specimen obtained from ethanol exhibited m.p. 255–257° (reported 247–249° (2)); $\lambda_{max}^{H_2O}$ 267 mμ, ϵ 15,000, $\lambda_{max}^{0.1N\,HCl}$ 273 mμ, ϵ 14,500. Anal. Calc. for $C_7H_9N_5O$: C, 46.91; H, 5.06. Found: C, 46.73; H, 5.20.

Reaction of Ib with Thionyl Chloride – Preparation of IVd

A stirred suspension of I*b* (2.0 g) in freshly distilled thionyl chloride (50 ml) was boiled for 16 hours under anhydrous conditions. The thick paste was cooled, filtered, and the precipitate washed with benzene and then suspended in absolute ethanol and stirred for a few minutes. The collected precipitate of IV*d* weighed 1.7 g and exhibited m.p. 305–309° (decomp.). Crystallization from methanol gave an analytical sample, m.p. 306–310°, whose pK_a (titration with 0.1 *N* aqueous sodium hydroxide) was 7.0. Anal. Calc. for $C_7H_8ClN_5$: C, 42.54; H, 4.08; N, 35.45; Cl, 17.95. Found: C, 42.87; H, 4.08; N, 35.70; Cl, 17.93.

Reaction of IVd with Silver Nitrate

A solution of IV*d* (200 mg) in water (20 ml) was treated with silver nitrate (180 mg, 1.05 equiv.) in 5 ml of water. A few drops of dilute nitric acid were added and the precipitate, which had formed immediately after the silver nitrate addition, was removed by centrifugation, washed, and dried, yielding 137 mg (0.95 equiv.) of silver chloride.

Neutralization of IVd – Preparation of IVc

Solid sodium bicarbonate (200 mg) was added to a clear ice-cold solution of 200 mg of IV*d* in water (2 ml). Carbon dioxide was liberated and a crystalline product deposited. Collection of V*a* and recrystallization from methanol yielded 102 mg of pure product, m.p. 295–296°. When sodium acetate (400 mg) was substituted for bicarbonate the identical product was obtained. $\lambda_{max}^{H_2O}$ 212 and 264 mμ, ϵ 17,500 and 10,400. $\lambda_{max}^{0.1N\,HCl}$ 211 and 262 mμ, ϵ 18,900 and 11,600. Anal. Calc. for $C_7H_7N_5$: C, 52.16; H, 4.38; N, 43.46. Found: C, 51.94; H, 4.43; N, 43.27.

Reconversion of IVc to IVd

Treatment of IV*c* with hydrogen chloride exactly as described above for IV*a* to IV*b*, gave the hydrochloride, m.p. 305–309° (decomp.), identical with the original sample.

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