for the ethyl structural unit, the β-methylene protons of the ring occurred as a distinct unit at 8.3, and the remaining methylene proton absorption occurred in the region 6.8–7.7. The integration supports the assignment of structure based on the presence of 5 mole %, or less, of triethylphosphate.

Anal. Calcd for $C_{10}H_{23}N_2O_3P$: C, 47.99; H, 9.26; N, 11.20; P, 12.38. Found: C, 47.81; H, 9.31; N, 11.32; P, 12.52.

Kinetic Experiments.—Freshly prepared solutions of **1** or **2** and of pyrolidine were pipetted into reaction tubes. These were then placed into a constant temperature bath and periodically withdrawn. The concentration at time t (the first sample was taken as $t = 0$) was determined by transferring the contents of the tube with a little ethanol into a mixture of cracked ice and 1 equiv of $HClO_4$ which neutralized the amines present (pH was characteristically 7–8). Aqueous $Na_2S_2O_3$ (15 ml of 0.5 *M*) was added directly, 13 ml of 1.2 *M* NaOAc, and an amount of $HClO_4$ (0.72 g) which brought the pH of the mixture to 4–5. The concentrations of **1** or **2** and pyrrolidine in the reaction tubes (10-ml aliquots) were *ca.* 0.3 and 1 *M*, respectively. The next day the remaining thiosulfate was titrated with standard iodine to the starch end point. This end point showed a tendency to fade and was yellow-brown rather than deep blue, but it was sufficiently definite that reproducibility was good. Bimolecular rate constants were calculated from the integrated form of the second-order rate equation for each pair of samples. The method of weighted averages based on probable random error for each constant was used to determine the final value of the constant.[7]

Acknowledgment.—The author wishes to express his gratitude to Dr. Alexej B. Bořkovec and Dr. Nobel Wakabayashi of this division for generously reading and commenting upon this manuscript.

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Nitration and Denitration in Hydrogen Fluoride

JOHN LENARD[1]

Department of Biology, University of California at San Diego, La Jolla, California

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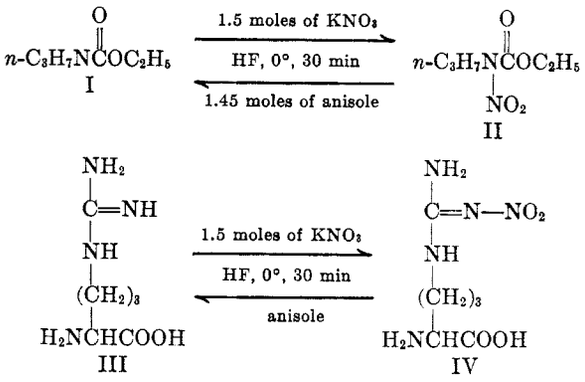
Recently, Sakakibara and Shimonishi[2] have shown that treatment of the synthetic, fully blocked peptide oxytocin with anhydrous hydrogen fluoride and excess anisole under mild conditions effectively removes the N-carbobenzyloxy, S-benzyl, and S-*p*-methoxybenzyl protecting groups from the peptide. Similar conditions have also been successfully applied to a wide variety of other peptide blocking groups.[3]

In the course of applying this procedure to the simultaneous unblocking and removing from the resin of bradykinin synthesized by the Merrifield solid-phase method,[4] it was observed that during the HF reaction the N-nitro group was quantitatively removed from the nitroarginine residues of the peptide.[5]

The transfer of the nitro group in HF from nitroarginine residues to anisole suggested that HF might be a suitable medium not only for denitration of a variety of compounds, but for nitration as well. This possibility was supported by the observations of Del Greco and Gryder,[6] who showed in an infrared and Raman spectral study that solutions of KNO_3 or HNO_3 in anhydrous HF contain appreciable concentrations of the nitrating species, NO_2^+.

This report describes the nitration, in HF under very mild conditions and in high yield, of a carbamate, N-*n*-propyl ethyl carbamate (I), and a guanidine derivative, L-arginine (III). The quantitative denitration of the corresponding N-nitro compounds (II and IV, respectively) by HF and anisole is also described.



Nitration and denitration of both carbamates and guanidines in sulfuric acid has been previously reported.[7] The use of HF for these reactions affords the advantages of very high yields, mild reaction conditions, ease of removal of HF (bp 19.5°), and apparent lack of side reactions.

Experimental Section

Reagents.—HF was obtained from the Matheson Chemical Co. It was purified on a vacuum line (see below) by distillation into a vessel containing cobalt trifluoride as drying agent. It was distilled directly from this vessel into the reaction vessel.

N-*n*-propyl ethyl carbamate (I) and N-*n*-propyl-N-nitro ethyl carbamate (II) were a gift of Mr. Lester A. Dolak, The Johns Hopkins University. The synthesis of these compounds has been reported.[8] L-Arginine (III) was obtained from Calbiochem. L-Nitroarginine (IV) was obtained from Cyclo Chemical Co., and was also prepared as described below. No differences in the denitration of these two samples were observed. Anisole, *o*-nitroanisole, and *p*-nitroanisole were products of Matheson Coleman and Bell.

Apparatus.—Experiments with HF were carried out on a monel vacuum line fitted with Hoke nickel diaphragm valves.[9] The reaction vessels (1.5 × 15 cm) were translucent Fluorothene tubes attached to the line by standard S.A.E. refrigeration flare fittings.

Vapor phase chromatography was carried out on an Aerograph 200 instrument. Chromatography of the amino acids was performed on a Beckman automatic amino acid analyzer, as modified by Dus, *et al.*[10]

Reactions in HF.—HF was introduced into the reaction vessel containing the reagents by freezing the vessel in liquid nitrogen and distilling the desired amount of HF into the vessel under vacuum from an oil pump. After allowing the mixture to stand for 30 min at 0°, the HF was removed by evaporation while stirring with a Teflon-coated magnetic stirring bar. A water aspirator rather than an oil pump was used to remove HF from reactions containing **I** and **II** owing to the volatility of these substances. After evaporation of the HF in this manner for about 30 min, the remaining HF and HNO_3 were removed by storing the vessel overnight in an evacuated desiccator containing NaOH.

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Nitration of I.—To 0.2 ml of I (191 mg, 1.46 mmoles) and 221 mg (2.2 mmoles) of KNO_3 was added 2–3 ml of HF, and the reaction was conducted as described above. The infrared spectrum of the reaction product showed a double carbonyl band, at 5.65 and 5.75 μ, as expected for II.[11] The infrared spectrum of I exhibits a broad carbonyl absorption at 5.8–6.0 μ.

Analysis by vapor phase chromatography was performed on a 3% Carbowax 20 M column, 15 ft × 1/8 in. at 120°. Under these conditions I has a retention time of 1.0 min, while II is retained for 1.8 min. The reaction mixture showed only these two peaks. The ratio of II to I was 93:7.

Denitration of II.—A mixture of 0.2 ml of II (225 mg, 1.27 mmoles) and 0.2 ml of anisole (199 mg, 1.84 mmoles) was allowed to react in 2–3 ml of HF as described above. The reaction mixture exhibited an infrared absorption band at 5.8–6.0 μ, the expected carbonyl absorption band for I. Vapor phase chromatography under the conditions detailed above showed a peak at 0.3 min, attributable to anisole, and a peak at 1.0 min owing to I. No peak was detected at 1.8 min, the position expected for II.

The nitroanisoles are retained on the column under the conditions used to separate I and II. They were separated on a 20% Carbowax 20 M column, 9 ft × 1/8 in. at 200°. Under these conditions *o*-nitroanisole appears at 22 min and *p*-nitroanisole at 25 min. These two compounds were both identified in the reaction mixture by their retention times. The ratio of *o*-nitroanisole to *p*-nitroanisole was 70:30.

Nitration of III.—To 348 mg of III (2 mmoles) and 303 mg (3 mmoles) of KNO_3 was added 7 ml of HF. The reagents were dissolved in the HF by stirring and allowed to react as described above. Amino acid analysis of the reaction mixture showed that quantitative conversion to nitroarginine had occurred. No arginine was detected, nor were any other ninhydrin-positive products except nitroarginine. The product was then recovered from water (adjusted to pH 6 with NH_4OH and acetic acid) in 83% yield, and recrystallized once from hot water: $[\alpha]^{25}D$ +26.2° (*c* 2.9, 1 *N* HCl), λ_{max} 271.6 mμ (ϵ 15,000) in dimethylformamide–0.2 *N* HCl (1:1); lit. $[\alpha]^{25}D$ +24.3° (*c* 4.12, 2 *N* HCl),[12] 271.6 mμ (ϵ 15,595) in dimethylformamide–0.2 *N* HCl (1:1).[13]

Denitration of IV.—A mixture of 58.1 mg of IV (26.5 μmoles) and 0.1 ml of anisole (100 mg, 0.92 mmole) was allowed to react in 2–3 ml of HF as described above. The reaction mixture was taken up in 25 ml of 0.01 *N* HCl, chilled, and filtered to remove traces of insoluble material. Some of this solution (0.5 ml) was subjected to automatic amino acid analysis. Only one peak appeared, in the position normally occupied by III. Calculation of its area showed it to contain 0.51 ± 0.02 μmole (97 ± 4%) of III.

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Synthesis of 9-(2′-Deoxy-β-D-ribopyranosyl)adenine

NORIKO NAGASAWA, IZUMI KUMASHIRO, AND TADAO TAKENISHI

Central Research Laboratories, Ajinomoto Company, Inc., Kawasaki, Japan

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In the previous paper[1] 9-(tetrahydro-2-pyranyl)-adenine was shown to be prepared easily from adenine and 2,3-dihydro-4H-pyran. In the present work, by using 3,4-di-O-acetyl-D-arabinal in place of 2,3-dihydro-4H-pyran, 9-(2′-deoxy-β-D-ribopyranosyl)adenine was obtained.

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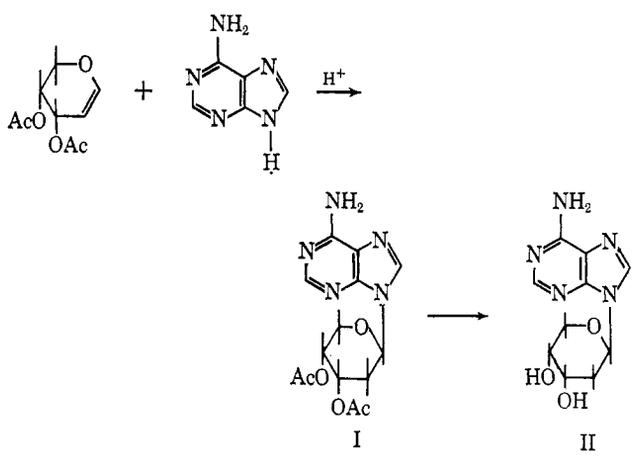
Adenine was treated with 3,4-di-O-acetyl-D-arabinal in dimethyl sulfoxide in the presence of a small excess of hydrogen chloride. The product was separated on an alumina column and 9-(2′-deoxy-3′,4′-di-O-acetyl-D-ribopyranosyl)adenine (I) was obtained. The sugar moiety of I was assigned to position 9 by comparison of its ultraviolet absorption spectra with those of 9-substituted adenines. The ultraviolet absorption spectra of I at pH 1 and 11 show maxima at 257 and 260.5 mμ, respectively, very similar to those of adenosine at 259 (pH 1) and 261 (pH 11) and 9-methyladenine at 260 (pH 1) and 260 (pH 11), in contrast to those of 7-methyladenine at 272 (pH 1) and 270 (pH 11) and 3-methyladenine at 274 (pH 1) and 273 mμ (pH 11).[2] Deacetylation of I gave a compound (II), mp 267–268°, $[\alpha]^{23}D$ −16.5 (*c* 0.53, water). Compound II was shown to be identical with 9-(2′-deoxy-β-D-ribopyranosyl)adenine prepared by Zinner[3] [mp 262–264°, $[\alpha]^{20}D$ −17.8 (*c* 0.58, water)] and by Robins[4] [mp 266–267°, $[\alpha]^{26}D$ −17.0 (*c* 0.6, water)]. It is of interest that the 9-β derivative (I) was the predominant isomer formed from adenine and 3,4-di-O-acetyl-D-arabinal with hydrochloric acid. When 6-chloropurine was treated with glycal in the presence of *p*-toluenesulfonic acid, Robins[5] found that the α anomer, 6-chloro-9-(2′-deoxy-3′,4′-di-O-acetyl-α-D-ribopyranosyl)purine, was the main product.



I II

Experimental Section

9-(2′-Deoxy-3′,4′-di-O-acetyl-β-D-ribopyranosyl)adenine (I).—Four grams of adenine (0.03 mole) was dissolved in 40 ml of dimethyl sulfoxide with 7 ml of a solution of hydrogen chloride in dry dioxane (5 *N*). To the solution 8 g of 3,4-di-O-acetyl-D-arabinal[6] was added at 50° with stirring. The mixture was kept at 50° for 15 hr, made basic with ammonium hydroxide, and concentrated under reduced pressure. The residue was treated with acetone. After the residual adenine (3 g, 0.02 mole) was filtered, the filtrate was applied to an alumina column and eluted with acetone–ethanol (ethanol: 0, 5, 10, 50%). From the fraction of acetone, a yellow oil was obtained. From the fraction of 10% ethanol–acetone, 0.7 g (0.002 mole) of I was obtained. Recrystallization of I from ethanol gave colorless needles, mp 218.5–219°. Ultraviolet absorption at pH 1 showed λ_{max} 257 mμ (ϵ 14,800); at pH 11 it showed λ_{max} 260.5 mμ (ϵ

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