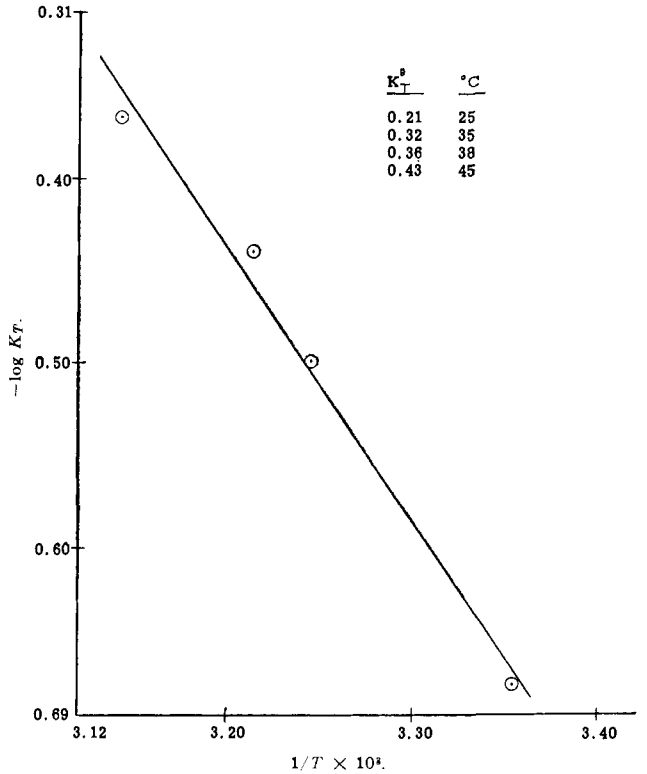

Fig. 2. Effect of temperature on the equilibrium constant (K_T) and the plot of log K_T *vs.* the reciprocal of the absolute temperature.

Additional information about I, together with the results of work on related compounds, will be published at a later date.

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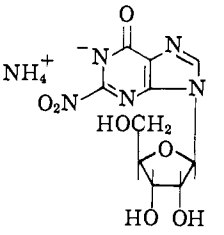
RECEIVED APRIL 13, 1964

Isolation of a 2-Nitropurine from the Reaction of Guanosine with Nitrous Acid

Sir:

It has been long recognized that nitrous acid reacts with those naturally occurring pyrimidines and purines that contain a primary amino group.[1] A great deal of interest has been focused on this reaction recently, with the discovery that nitrous acid, when applied to nucleic acids, is a potent mutagen.[2] Schuster, *et al.*,[3] have stated that the changes produced by nitrous acid within a molecule of ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) were the same as those resulting from the action of nitrous acid upon the simple pyrimidine and purine bases or nucleosides: the amino groups of the adenine, cytosine, and guanine moieties were replaced by carbonyl groups. It was proposed that the first two changes led to mutations and that the third was lethal. More recently, however, various observations have been made which were not encompassed by this scheme. Several workers have noted a cross-linking effect upon the double helix of DNA produced by nitrous acid.[4] The production of deletion mutations by this reagent has also been observed.[5] A synthetic polynucleotide containing uracil and guanine was found to suffer a pronounced loss of activity upon treatment with nitrous acid.[6] In a later communication, Schuster and Wilhelm[7] noted that all of the guanine of tobacco mosaic virus RNA was not being converted to xanthine, but that some was being consumed in an unknown reaction.

In order to obtain some knowledge as to the nature of these side reactions, we reinvestigated the reaction of nitrous acid with the ribonucleosides adenosine, cytosine, and guanosine. No product, other than the expected one, was observed with the first two nucleosides. The deamination of guanosine, however, in acetate buffer, led to the formation of a yellow side product in yields up to 5%. The highest yield was formed when a large excess of nitrite ion was used and the temperature was lowered to 0°. On the basis of the properties reported below, this product has been assigned the structure I, 2-nitroinosine (isolated as its



I

ammonium salt). I could be separated from xanthosine and any unreacted guanosine by paper chromatography (isobutyric acid–ammonia–water, 66:5:29) or, on a larger scale, by anion exchange chromatography on Amberlite CG 400 resin (acetate form). Because of its acidity (see below), 2-nitroinosine adhered firmly to the resin and was eluted with 0.1 *N* HCl and 1 *N* NaCl. It was recovered from that solution by adsorption onto charcoal and elution with ethanol–ammonia–water solution. Evaporation of the eluate gave I as a pale yellow powder. It recrystallized poorly, but an analytical sample could be prepared by repeated washing with cold 95% ethanol. *Anal.* Calcd. for $C_{10}H_{14}N_6O_7$: C, 36.37; H, 4.27; N, 25.45; O, 33.91. Found: C, 36.29; H, 4.40; N (by difference), 25.04; O, 34.27. I was titrated as an ammonium salt. *Anal.* Calcd. equiv. wt.: 330. Found: 320. I did not melt but decomposed when heated above 180°. It was destroyed by heating for 1 hr. in aqueous solution, pH 1.8, at 90°; ultraviolet spectrum: $\lambda_{max}^{pH_1}$ 222 (ϵ 12,000) and 335 mμ (4200); $\lambda_{max}^{pH_7}$ 233 (ϵ 14,400) and 343 mμ (3800). The infrared spectrum of I (KBr) showed a strong band at 6.20 μ (inosine, in alkaline solution, absorbs at 6.27 μ[8]) and bands at 6.40 and 7.38 μ which may be ascribed to the nitro function. Only one dissociation of 2-nitroinosine between pH 1 and 13 was detected by ultraviolet spectrophotometry. This dissociation was found to correspond to a pK_a of 3.3

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(2) A. Gierer and K. W. Mundry, *Nature*, **182**, 1457 (1958).

(3) (a) H. Schuster and G. Schramm, *Z. Naturforsch.*, **13b**, 697 (1958); H. Schuster, *ibid.*, **15b**, 298 (1960); W. Vielmutter and H. Schuster, *ibid.*, **15b**, 304 (1960).

(4) (a) E. P. Geiduschek, *Proc. Natl. Acad. Sci. U. S.*, **47**, 950 (1961); (b) E. E. Horn and R. M. Herriot, *ibid.*, **48**, 1409 (1962).

(5) I. Tessman, *J. Mol. Biol.*, **5**, 442 (1962).

(6) C. Basilio, A. J. Wahba, P. Lengyel, J. F. Speyer, and S. Ochoa, *Proc. Natl. Acad. Sci. U. S.*, **48**, 613 (1962).

(7) H. Schuster and R. C. Wilhelm, *Biochim. Biophys. Acta*, **68**, 554 (1963).

(8) H. T. Miles, *ibid.*, **30**, 324 (1958).

Paper electrophoresis studies showed that 2-nitroinosine exists as an anion at pH values above 4. It migrated at the same rate as xanthosine in glycine buffer at pH 9. Its mobility was enhanced to the same extent as xanthosine by the use of borate buffer at that pH. An aqueous solution of I was decolorized instantly by the addition of sodium hydrosulfite. Two products were produced. One was identified as guanosine, as it coincided with guanosine with respect to the following properties: λ_{max} 250:260 and 280:260 ratio at pH 1, 7, and 11 in the ultraviolet[9]; chromatographic mobility on paper in isobutyric acid–ammonia–water, 66:5:29 (R_f 0.50), and in water (R_f 0.59); electrophoretic mobility in borate buffer, pH 9.1. The other substance (R_f in water 0.88) has an ultraviolet spectrum similar to guanosine but has not yet been identified.

2-Nitroinosine is apparently the first published example of a pyrimidine or purine with a nitro group substituted in an electron-deficient position (2, 4, or 6).[10] It was presumably formed by nucleophilic displacement of a diazonium group by nitrite ion. Several 2-halopyrimidines and purines have been prepared by analogous processes.[11] The acidic dissociation of 2-nitroinosine is an unusually strong one for a heterocyclic ring proton. It is 3×10^5 times as strong as inosine, from which it is formally derived by replacement of a hydrogen by a nitro group. For purposes of comparison it may be noted that the introduction of a nitro group into the 5-position of uracil increases the acidity of that compound by 10^4.

It seems likely that the replacement of the guanine amino group by a nitro group occurs to some extent when nucleic acids are treated with nitrous acid. It is understandable that this product was not observed by Schuster, *et al.*,[3,7] as it would have been destroyed during the acidic hydrolysis used in their procedures. I is not formed in sufficient amounts, however, to account for all of the loss of guanine observed by Schuster and Wilhelm,[7] nor does the structure of I afford an explanation for the observed cross-linking of DNA. It may be that the cross-linking is due to a subsequent reaction of I. An alternative explanation would be that, within the double helix of DNA, nucleophilic displacement of the diazonium ion occurs, but that this is done by a group which is held by hydrogen bonding in the vicinity of the amino group of guanine. An obvious candidate for this role would be the 2-carbonyl group of cytosine, whose ability to participate in intramolecular nucleophilic displacements has been well documented.[12]

Acknowledgment.—This work was supported by a grant (GM 11437-01) from the National Institutes of Health and by an institutional grant to New York University from the American Chemical Society.

(9) E. A. Johnson in "The Nucleic Acids," E. Chargaff and J. N. Davidson, Ed., Vol. 1, Academic Press, New York, N. Y., 1955, p. 508.

(10) Several 8-nitropurines have been prepared: J. W. Jones and R. K. Robins, *J. Am. Chem. Soc.*, **82**, 3773 (1960).

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(12) V. M. Clark, A. R. Todd, and J. Zussman, *J. Chem. Soc.*, 2952 (1951).

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RECEIVED APRIL 29, 1964

Preparation of Optically Active Polyhedral Borane Derivatives

Sir:

$B_{10}H_{10}^{2-}$ has been shown to undergo a wide variety of substitution reactions,[1-5] some of which have led to apically substituted products[1,3] and others of which have led to equatorially substituted products.[4,5] Those reactions which appear to be electrophilic substitutions on $B_{10}H_{10}^{2-}$ are of special interest because the prediction has been made,[6] based on LCAO–MO calculations, that electrophilic substitution reactions on $B_{10}H_{10}^{2-}$ will occur preferentially at an apical position.

We report herein the synthesis, by means of electrophilic substitution reactions, of an equatorially substituted derivative of $B_{10}H_{10}^{2-}$, 2,7(8)-$(CH_3)_3NB_{10}H_8$-CO,[7] and its resolution. This carbonyl derivative was prepared by reaction of oxalyl chloride with 2-$B_{10}H_9N(CH_3)_3^-$ [4] in acetonitrile at room temperature. The product was obtained as a 1:2 mixture of 2,4- and 2,7(8)-$(CH_3)_3NB_{10}H_8CO$(I) in up to 87% yield. *Anal.* Calcd. for $B_{10}H_{17}C_4NO$: B, 53.3; H, 8.45; C, 23.6; N, 6.90; mol. wt., 203. Found: B, 53.4; H, 8.44; C, 23.9; N, 6.91; mol. wt., 201. The carbonyl group of I is chemically similar to the carbonyl groups of 1,10-$B_{10}H_8(CO)_2$.[3] Reaction of the carbonyl derivative, I, with hydroxylamine-O-sulfonic acid[3] followed by methylation[4] (eq. 1) gave $B_{10}H_8[N(CH_3)_3]_2$ (II)

$$(CH_3)_3NB_{10}H_8CO \xrightarrow[2,\ (CH_3)_2SO_4\text{–NaOH}]{1,\ NH_2OSO_3H} B_{10}H_8[N(CH_3)_3]_2 \quad (1)$$

as a mixture of 2,4- and 2,7(8)- isomers as shown by X-ray comparison with authentic 2,4-[4] and 2,7(8)-[8,9] isomers of II.

Treatment of aqueous I with brucine hydrochloride gave brucine-H^+ $(CH_3)_3NB_{10}H_8COOH^-$, from which the highly soluble 2,4- isomer was removed by warm ethanol. Fractional crystallization of the remaining mixture of diastereomeric salts from ethanol–acetonitrile followed by reconversion to I gave (+)2,7-(or 2,8-) $(CH_3)_3NB_{10}H_8CO$,[10] with $[\alpha]^{23}D$ +22° (*c* 1.8, acetone), m.p. 207–208.5°, derived from the less soluble brucine salt, and the (−)enantiomer with $[\alpha]^{23}D$ −14° (*c* 2.42, acetone) derived from the more soluble brucine salt. No observable loss of optical activity occurred on heating (+)-I to 200° for 5 min. Higher

(1) W. H. Knoth, H. C. Miller, D. C. England, G. W. Parshall, E. L. Muetterties, and J. C. Sauer, *J. Am. Chem. Soc.*, **84**, 1056 (1962).

(2) W. H. Knoth, H. C. Miller, J. C. Sauer, J. H. Balthis, Y. T. Chia, and E. L. Muetterties, *Inorg. Chem.*, **3**, 159 (1964).

(3) W. H. Knoth, J. C. Sauer, H. C. Miller, and E. L. Muetterties, *J. Am. Chem. Soc.*, **86**, 115 (1964).

(4) W. R. Hertler and M. S. Raasch, *ibid.*, in press.

(5) W. H. Knoth, J. C. Sauer, D. C. England, W. R. Hertler and E. L. Muetterties, *ibid.*, in press.

(6) R. Hoffman and W. N. Lipscomb, *J. Chem. Phys.*, **37**, 520 (1962).

(7) The numbering system for $B_{10}H_{10}^{2-}$ which is used here is the proposed revision of the ACS Nomenclature Committee, 1963, which is described by R. Adams, *Inorg. Chem.*, **2**, 1087 (1963), see Fig. 1.

(8) W. R. Hertler, *Inorg. Chem.*, in press. 2,7(8)-$B_{10}H_8[N(CH_3)_3]_2$ was prepared by the sodium–alcohol reduction of 2,7(8)-$B_{10}H_8[N(CH_3)_2CH_2Cl]_2$ which was obtained from the reaction of $B_{10}H_{10}^{2-}$ with $ClCH{=}N(CH_3)_2^+Cl^-$. The configurational assignment is based on B^{11} n.m.r. data which, incidentally, do not rule out 2,6(9)- geometry.

(9) The stereochemical assignment for I is based on formation of 2,4-II and 2,7(8)-II and requires the assumption that no stereochemical change results from the reaction of eq. 1. There is ample support for such an assumption in the several reactions of 1,10-$B_{10}H_8(CO)_2$ described in ref. 3.

(10) This appears to be the first preparation of a *stable* optically active boron compound in which the asymmetry (albeit molecular asymmetry) resides on boron. See, however, J. Böeseken and J. Meulenhoff, *Proc. Acad. Sci. Amsterdam*, **27**, 174 (1924).