was used to calculate the extent of isotopic label incorporated (column 4, Table I). It is seen from the result of experiments 1–4 that catalytic isotopic exchange at 100° takes place in acidic medium or in an aqueous solution of the purine base. The recognized lability[3] of H in $-NH_2$ and $=NH$ leaves only the 8-position in guanine as the site of the isotopic label. The result in column 4 (expt. 5) indicates that the isotopic exchange did not go to completion under the experimental conditions. In adenine, the 2 and 8 positions may be sites for non-labile hydrogen. The isotopic content of the product is equivalent to slightly less than one equivalent atom position (method of calculation described below). The results (0.8–0.9) can signify either (a) substantially complete exchange at either the 2- or 8-positions or (b) partial exchange at both the 2- or 8-positions. Either possibility does not decrease the usefulness of the product for analyses using isotopic dilution techniques and for studies in which the adenine is not transformed into other compounds. Its usefulness in tracer metabolism studies depends on the location of the hydrogen isotope label. This method has been utilized to prepare adenine with tritium activity equal to 0.5 mc. per millimole using the conditions stated in experiment 3. The catalytic procedure described above will probably be useful for introducing isotopic hydrogen into other compounds having purine ring structures.

TABLE I

EXTENT OF ISOTOPIC EXCHANGE IN PREPARATION OF LABELLED ADENINE AND GUANINE[a]

Expt.	Compound	Medium	Equivalent no. of labeled atom positions
1	Adenine	70% AcOH + AcOT (no catalyst)	0
2	Adenine	70% AcOH + AcOT	0.8 ± 0.1
3	Adenine	H_2O + HTO	.85 ± .02
4	Adenine	D_2O	.91 ± .02
5	Guanine	H_2O + HTO 0.1 *N* HCl	.6 ± .1

[a] 18 hours at 100° for each run. Pt catalyst in all media except expt. no. 1.

Experimental

The platinum catalyst (250 mg.) was reduced in the reaction medium (Table I) with ordinary hydrogen gas at room temperature. The reaction mixture contained the reduced catalyst and 500 mg. of adenine or guanine in a total volume of 25 ml. After the contents had been frozen, the reaction tube was sealed off under vacuum. The tube was agitated at 100° for 18 hours. In experiment 4, the catalyst was reduced with 99.7% D_2 in 99.8% D_2O. The water used to prepare the media in experiments 1, 2, 3 and 5 had a tritium atom fraction of approximately 10^{-9}. The tritium was obtained from the Isotopes Division, U. S. Atomic Energy Commission.

The product in experiment 2, after heating with *N* sodium hydroxide for several minutes, was precipitated from hot solution as the hydrochloride. The products in experiments 3 and 4 were purified by recrystallization of the free base from ordinary water. The tritium content of the adenine was unchanged after standing in 0.01 *N* HCl solution for two days at room temperature and after boiling in *N* NaOH for five minutes. The product in experiment 5 was dissolved in hot *N* sodium hydroxide and precipitated as the sulfate. Deuterium or tritium bonded to nitrogen and oxygen would be replaced by ordinary hydrogen during the above purification steps. The purity of the products was checked by ultraviolet spectrophotometry (Beckman model DU instrument) using transmittance ratios at 250, 280 and 290 relative to 260 mμ. The infrared spectrum of the adenine prepared in experiment 1 was compared with that of a purified stock sample using a Model C Perkin–Elmer infrared spectrometer and rock-salt prism. The samples were prepared as a Nujol mull on a rock-salt window. The spectra were identical.

Isotopic Analyses.—The dried adenine and guanine samples were burned in a combustion train in which nitrogen oxides were reduced to nitrogen gas over copper at approximately 500°. The water was converted to hydrogen gas over zinc at 420°. Deuterium measurements were made using a dual collector Nier-type hydrogen mass spectrometer. Tritium measurements were made using a hydrogen–methane gas mixture in the proportional region as previously described by the authors.[4] The numerical values in column 4, Table I are equal to nf where n is the number of hydrogen atom positions undergoing exchange and f is the ratio of the D or T isotopic abundance in the n-positions to that in the aqueous exchange medium. For example, in experiment number 3, the tritium abundance in the medium was expressed as 9.6×10^5 counts per minute per standard volume of hydrogen gas, while the tritium abundance in the adenine prepared was 1.64×10^5 counts per minute per standard volume of hydrogen gas obtained from the combustion of the product. Taking the 5 hydrogen atoms into account $(9.6 \times 10^5)\ nf/5 = 1.64 \times 10^5$ and $nf = 0.85$. The result may be interpreted as showing the presence of one atom position undergoing catalytic exchange with $f = 0.85$ or possibly two exchangeable atom positions with average $f = 0.42$. Degradation studies would be required to resolve this question.

(3) M. Kamen, "Radioactive Tracers in Biology," 2nd ed., Academic Press, Inc., New York, N. Y., Chap. VII, 1951.

(4) M. L. Eidinoff, J. E. Knoll, D. K. Fukushima and T. F. Gallagher, THIS JOURNAL, **74**, 5280 (1952).

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Low Temperature Pyrolysis of Boron Trifluoride–Mannich Base Complexes. 2-Nitro-1-alkenes[1]

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Recently Blomquist and Shelley[2] have demonstrated that Mannich base hydrochlorides derived from nitroalkanes undergo thermal decomposition[3] to yield the corresponding 2-nitro-1-alkenes. These investigators carried out the pyrolysis reaction at relatively high temperatures and this is certainly disadvantageous when heat-labile intermediates or

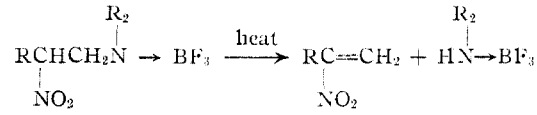
$$RCHCH_2NR'_2\cdot HCl \xrightarrow{\text{heat}} RC{=}CH_2 + R'_2NH\cdot HCl$$
(NO_2 on the left reactant and on the right product)

unstable products are present in the reaction mixture. We have established that the use of boron trifluoride complexes of these Mannich bases effectively reduces the temperature necessary for carrying out the reaction by as much as 100°. This is in agreement with electronic theory which predicts that the carbon–nitrogen bond in this system will be weaker than in the corresponding hydrochloride. Furthermore the yields of nitroölefins obtained (80–90%) were as good as and in most

(1) This work was carried out under Army Ordnance Contract W-01-021-ORD-334.

(2) A. T. Blomquist and T. H. Shelley, Jr., THIS JOURNAL, **70**, 147 (1948).

(3) H. R. Snyder and W. E. Hamlin (*ibid.*, **72**, 5072 (1950)) utilized similar Mannich bases as nitroalkene precursors for alkylating nitroparaffins.



cases better than those reported with the Mannich base hydrochlorides.

In the initial experiments the boron trifluoride complexes were prepared from gaseous boron trifluoride and isolated prior to their pyrolysis (Experimental procedure B). It was found, however, that these complexes were not very stable and frequently decomposed on standing. Accordingly the boron fluoride adducts were prepared *in situ* by addition of an equivalent amount of boron trifluoride etherate to a solution of the Mannich base in an inert solvent. The resulting mixture was then heated under reduced pressure until pyrolysis was complete and the nitroölefin was removed by distillation as it was formed. The yields obtained with this procedure (procedure A) were much better and more reproducible than those obtained using procedure B. The nitroalkenes were identified by determination of physical constants, examination of infrared spectra, and conversion to the *p*-toluidine derivatives.

Experimental[4,5]

Mannich Bases of Nitroalkanes.—The Mannich bases were all prepared according to the directions of Blomquist and Shelley.[2] The physical constants of N-(2-nitropropyl)-piperidine (b.p. 87° (1 mm.), $n^{20}D$ 1.4469) which were determined and reported by these investigators were not confirmed in this Laboratory. Our values for this compound are: b.p. 67–68° (1–1.5 mm.), $n^{20}D$ 1.4650. This nitroamine was also converted to the picrate, m.p. 127–127.5° (recryst. from ethanol).

Anal. Calcd. for $C_{14}H_{19}N_5O_9$: C, 41.89; H, 4.74; N, 17.45. Found: C, 41.68; H, 4.99; N, 17.47.

2-Nitro-1-alkenes.—A summary of the pyrolysis experiments performed by both procedure A (use of inert diluent without isolation of the boron trifluoride complex) and procedure B (pyrolysis of the isolated boron trifluoride complex) is presented in Table I. The preparations of 2-nitropropene by procedure A and 2-nitro-1-butene by procedure B are described in detail. The nitroölefins obtained directly from the pyrolysis reactions were sufficiently pure for most purposes.

2-Nitropropene (Procedure A).—To a stirred solution of 17.2 g. (0.1 mole) of freshly distilled N-(2-nitropropyl)-piperidine in 150 ml. of di-2-ethylhexyl phthalate was added 15.6 g. (0.105 mole) of boron trifluoride etherate. A viscous white liquid separated out on the walls of the flask immediately. The ethyl ether formed in this reaction was removed under reduced pressure at room temperature. The mixture was then heated to 85° at a pressure of 1 mm. with continuous stirring. At this temperature the solution darkened and the 2-nitropropene began to form. The nitroolefin was distilled into a Dry Ice–acetone-cooled receiver as fast as it was formed. The solution was heated to 105° and was maintained at this temperature for one hour. There was obtained in the distillate receiver 6.7 g. (77%) of light green solid which melted on warming to room temperature, $n^{20}D$ 1.4296 (lit.[6] $n^{23}D$ 1.4292). The *p*-toluidine derivative of this nitroölefin was prepared and melted at 81–82° (lit.[2] 81.5–82.5°). The infrared spectrum of 2-nitropropene showed a conjugated nitro band at 1525 cm.$^{-1}$.

2-Nitro-1-butene (Procedure B).—A solution of 34.8 g. (0.2 mole) of freshly distilled N-(2-nitrobutyl)-diethylamine in 150 ml. of dry petroleum ether was saturated with gaseous boron trifluoride at 0–5°. The solid which separated was collected on a filter and washed with petroleum ether. It was then dried in a vacuum desiccator; yield 46.5 g. (96%) of slightly yellow solid, m.p. 50–53°. The boron trifluoride adduct (24.2 g., 0.1 mole) was then heated under a pressure of 1 mm. in a distillation apparatus with a distillate receiver packed in Dry Ice–acetone. Decomposition was observed at 35°. The distillation pot was slowly heated to 60° and was maintained at this temperature for one hour. There was obtained in the receiver 6.3 g. (63%) of 2-nitro-1-butene, $n^{20}D$ 1.4258 (lit.[2] $n^{20}D$ 1.4256). The *p*-toluidine adduct of this nitroölefin was prepared and melted at 68.5–69.5° (lit.[2] m.p. 67.5–68.5°). The infrared spectrum of 2-nitro-1-butene showed a conjugated nitro band at 1522 cm.$^{-1}$.

TABLE I

PYROLYSIS OF MANNICH BASE–BORON TRIFLUORIDE COMPLEXES

Boron trifluoride complex	Nitroolefin, 2-nitro-	Procedure A Yield, %	Procedure A Pyrolysis temp., °C.	Procedure B Yield, %	Procedure B Pyrolysis temp., °C.
N-(2-Nitropropyl)-piperidine	Propene	77	105	48	70
N-(2-Nitrobutyl)-diethylamine	1-Butene	78	100	62	60
N-(2-Nitrobutyl)-dimethylamine	1-Butene	86	100	72	90
N-(2-Nitropentyl)-diethylamine	1-Pentene	90	110	40	80

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(4) All melting and boiling points are uncorrected.

(5) We are indebted to Dr. Keith S. McCallum and Mr. Al Kennedy for infrared interpretations and microcombustion data. The Commercial Solvents Corporation generously supplied us with a sample of 1-nitrobutane.

(6) A. T. Blomquist, W. J. Tapp and J. R. Johnson, THIS JOURNAL, 67, 1519 (1945).

Optical Enantiomorphs of α-Aminoadipic Acid

BY JESSE P. GREENSTEIN, SANFORD M. BIRNBAUM AND M. CLYDE OTEY

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A resolution of racemic α-aminoadipic acid into its optical enantiomorphs has not been recorded. Borsook, *et al.*, reported a preparation of L-α-aminoadipic acid with $[\alpha]^{25}D$ +33.9° for a 5.49% solution in 6 *N* HCl by treating carbobenzoxy-DL-α-aminoadipic acid with aniline and papain, followed by hydrolysis of the anilide and removal of the carbobenzoxy group from the separated L-enantiomorph.[1] No mention was made of the D-enantiomorph.

We have resolved the racemic aminodicarboxylic acid into its optical enantiomorphs by the acylase procedure developed in this Laboratory.[2,3] Chloroacetyl-DL-α-aminoadipic acid was prepared and subjected at *p*H 7.0 to the asymmetric action of hog kidney acylase I to yield L-α-aminoadipic acid and chloroacetyl-D-α-aminoadipic acid. The latter compound yielded D-α-aminoadipic acid after HCl hydrolysis followed by adjustment to *p*H 3.2. For 2% solutions in 5 *N* HCl the $[\alpha]^{25}D$ for the L-isomer was +25.0°, and for the D-isomer −25.0°. The $[\alpha]^{25}D$ for a 6% solution of the D-isomer in 6 *N* HCl was −24.9°. L-α-Aminoadipic acid was

(1) H. Borsook, C. L. Deasy, A. J. Haagen-Smit, G. Keighley and P. H. Lowy, *J. Biol. Chem.*, **176**, 1383 (1948).

(2) S. M. Birnbaum, L. Levintow, R. B. Kingsley and J. P. Greenstein, *ibid.*, **194**, 455 (1952).

(3) J. P. Greenstein, S. M. Birnbaum and L. Levintow, in "Biochemical Preparations," Vol. III, in press.