

THE PYRIDINE DENITRATION OF MANNITOL HEXANITRATE

by

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A Thesis submitted in Partial Fulfilment of
the Requirements for the Degree of
Master of Science
in the
Department of Chemistry

We accept this thesis as conforming to the
standard required from candidates for the
degree of Master of Science.

Members of the Department of Chemistry.

The University of British Columbia

April, 1953.

ABSTRACT

The products of the reaction of excess anhydrous pyridine with D-mannitol hexanitrate at 30°C. have been analysed and five of the major components identified. A gas consisting of nitric oxide, nitrous oxide and nitrogen was evolved and D-mannitol-1,2,4,5,6-pentanitrate and pyridinium nitrate were recovered from the reaction mixture. Twelve other non-nitrogenous unidentified components were detected in the reaction mixture by paper-partition chromatography. Establishment of a nitrogen balance for the reaction indicated that complete removal of nitrogen from about 2 moles of pyridine and from about 0.25 moles of the hexanitrate occurred during the formation of 0.75 moles of mannitol pentanitrate.

ACKNOWLEDGEMENTS

The writer wishes to express his sincere thanks to

Dr. L. D. Hayward

for his encouragement and willing assistance in the direction of this investigation.

Grateful acknowledgement is made also to the National Research Council of Canada for a summer grant.

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INTRODUCTION

Since the first discovery of the pyridine denitration of mannitol and dulcitol hexanitrate to the corresponding pentanitrate no attempts have been made to determine the mechanism of the reaction. However, since the reaction has recently been shown to result in specific denitration of both mannitol- and dulcitol-hexanitrate in the three (or chemically equivalent 4-) position, it has become important as a possible method of making available/^aspecific secondary hydroxyl group in polyhydric alcohols and possibly also in sugar molecules. This specificity coupled with the desirable characteristics of nitrate groups as carbohydrate "blocking groups" suggests the use of the carbohydrate nitrates as intermediates in sugar syntheses. Further development toward this goal required the investigation of the mechanism of the pyridine-carbohydrate nitrate reaction.

The present work describes the isolation and identification of the major by-products of the reaction of pyridine with mannitol hexanitrate, together with preliminary studies on the rate of the reaction.

HISTORICAL INTRODUCTION

The pyridine denitration of mannitol hexanitrate was first discovered by Wigner in 1903 (27) when he found that pyridine was a more selective denitrating agent than Tichanowitsch's ammoniacal ether (21). The latter reagent was stated by Wigner to cause some deep-seated decomposition as a result of its action as a caustic alkali. Pyridine, however, did not show this undesirable secondary effect. The method described was

to dissolve the mannitol hexanitrate in six times its weight of pyridine with cooling, when necessary, to prevent the reaction from becoming too vigorous. The reaction was accompanied by the evolution of large amounts of "nitrous vapor" and by a gradual change in the solution from colorless to bright yellow. The crystalline pentanitrate was isolated by precipitating with water and was purified by crystallization from aqueous ethanol.

No further investigation of the reaction was attempted until Hayward, in 1951, showed by methylation, denitration and periodate oxidation that the partial denitrating action of the pyridine is specific to the 3- (or equivalent 4-) position of the mannitol hexanitrate molecule (8).

McKeown, in 1952, showed that the 3- (or 4-) position is also the point of attack of pyridine on dulcitol hexanitrate (10).

The action of pyridine and other weak bases on nitrated carbohydrate derivatives has been investigated by several workers. Walter (25) in 1911, noted that guncotton, soaked with one of dimethylaniline, phenylhydrazine, o- or p-toluidine or naphthylamine, and left in the dark, suffered a gradual change in color indicating decomposition.

Angeli (1) found that pyridine-moistened nitrocellulose gives an 80% yield of the original weight of nitrocellulose with a nitrogen content reduced from an original 12% to 9-10% indicating decided decomposition.

Giannini (5) extended this work in 1924. He found that a gas containing carbondioxide, nitric oxide, nitrous oxide and nitrogen, was given off and that the carbohydrate product, after 45 days in contact with

pyridine, had approximately the molecular weight of a biose unit. In 1944 Gladding and Purves (6) found that pure, dry pyridine caused a vigorous decomposition of dissolved, stabilized guncotton at 100°C. Nitrogen dioxide was evolved as a volatile pyridine complex which readily crystallized above the solution on cooling.

Ryan and Casey (15) studied the effect of primary, secondary and tertiary amines on various carbohydrate nitrate esters. Dimethyl aniline reacted with mannitol hexanitrate at an elevated temperature to evolve a gas consisting of 70% Nitrous oxide and 30% nitrogen.

Much work has recently been done on the action of hydroxylamine, methoxyamine and their corresponding hydrochlorides on pyridine solutions of carbohydrate nitrates.

The reactions observed were assumed to be caused by the ammonia derivative with the pyridine acting merely as solvent.

In 1946 Segall (18) found that excess hydroxylamine in pyridine at room temperature, acted on cellulose trinitrate to give a cellulose dinitrate with the evolution of one mole of nitrogen per mole of anhydroglucose. The nitrate groups attacked proved to be secondary in nature. Unlike other cellulose nitrates, the product was stable to pyridine. Methoxyamine acted similarly except that no nitrogen was evolved. With excess hydroxylamine hydrochloride the product appeared to be a cellulose ketoxime dinitrate and the gas evolved consisted of 85% nitrous oxide and 15% nitrogen.

Hayward (7) followed up this work by investigating the action of hydroxylamine in pyridine on methyl- α - and β -D-glucopyranoside tetranitrates. In preliminary experiments he found that an alcoholic solution

of hydroxylamine had little or no effect on methyl- α - or β -D-glucoside tetranitrate. When the compound was dissolved in anhydrous pyridine, highly colored products were rapidly formed but no gas was evolved. The α -isomer was recovered in 76% yield after four and one half hours, and in 60% yield after sixteen hours. A vigorous exothermic reaction ensued on addition of hydroxylamine in anhydrous pyridine to methyl- β -D-glucoside tetranitrate. Nitrogen gas was evolved in the ratio of 1.3 moles per mole of tetranitrate, and the product contained methyl- β -D-glucoside-2,3,6-trinitrate (53%), methyl- β -D-glucoside-3,6-dinitrate (33%) and unidentified methyl- β -D-glucoside trinitrate.

The action of hydroxylamine hydrochloride in pyridine on methyl- β -D-glucopyranoside tetranitrate, investigated by Rooney (15), was slower and more complex. The gas evolved was 87% nitrous oxide and 30% nitrogen, and the carbohydrate products appeared to be a mixture of partially denitrated methyl glucoside and completely denitrated polyoxime products. Methyl- β -D-glucopyranoside-2,3,6-trinitrate and a substance believed to be methyl- β -D-glucopyranoside-2,6-dinitrate were isolated.

An investigation of the action of quinoline on methyl- β -D-glucoside tetranitrate by Swan (20) showed that a partial denitration occurs with the evolution of a gas.

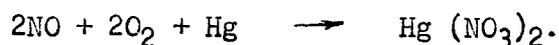
DISCUSSION OF RESULTS

The reaction of mannitol hexanitrate with pyridine (denitration reaction), carried out in air, led to the evolution of a brown gas and the formation of a thin film of nearly colourless crystals on the walls of the glass vessel above the solution. This crystalline material (m.p. 116 - 117°C. after crystallization from methanol) was tentatively identified as

pyridinium nitrate by means of a mixed melting point with an authentic sample of pyridinium nitrate. When, however, the reaction was carried out in the absence of air in the Toricellian vacuum of a Lunge Nitrometer, the gas evolved was colourless and no crystalline material was deposited. The gas turned red-brown on admission of commercial oxygen, but did not deposit any solid material. Consideration of the reaction indicated that the following components could be anticipated in the gaseous product: nitric oxide, nitrous oxide, carbon dioxide, carbon monoxide, nitrogen and pyridine vapour. Since the gas was colourless but reacted with oxygen to give a red-brown gas, nitric oxide must have been present and nitrogen dioxide and oxygen absent. The alkaline, oxidizing conditions prevailing in the reaction mixture made the formation of gaseous ammonia, hydrogen cyanide, formic acid, formaldehyde and hydrocarbons unlikely. Water-vapour was shown to be absent when no solid was deposited on admission of oxygen to the gas.

A method for the analysis of such a mixture was not available in the literature. However, several reagents, described as absorbents for nitric oxide were checked, in the Orsat apparatus, for absorption of commercial nitrous oxide. These were 15% aqueous sodium sulphite, 1% in potassium hydroxide (4), 20% potassium hydroxide (11), 0.1 normal sulphuric acid 1% in potassium permanganate (23) and 0.1 normal potassium hydroxide 1% in potassium permanganate (9). In each case the nitrous oxide was slowly absorbed. Aqueous ferrous sulphate, recommended by Scott (17) was not tested because the unstable complex formed with nitric oxide is reported to have an appreciable vapour pressure of nitric oxide in equilibrium with it (26).

The nitrogen dioxide formed by reaction of oxygen with the gas was found to react fairly readily with mercury if sufficient surface was available for reaction. The disappearance of the characteristic colour of the gas was taken as evidence for the completion of the reaction:



Carbon monoxide, if present, would, of course, be oxidized to carbon dioxide during this reaction. The possible components then remaining would be nitrous oxide, carbon dioxide, nitrogen, pyridine vapour and the excess oxygen. The nitrous oxide was absorbed in 95% ethanol (18) which would also absorb the pyridine vapour. The carbon dioxide was absorbed in 40% potassium hydroxide and the excess oxygen in alkaline pyrogallol (24). The percentage of pyridine in the gas could be calculated (from the ratio of its vapor pressure at the temperature of the reaction to the atmospheric pressure)^{and} subtracted from the percentage of the gas absorbed in ethanol to give the true percentage nitrous oxide. However, since its concentration was small and fairly constant (2.5 - 2.8%), the correction was not considered necessary and the total gas absorbed in ethanol was calculated as nitrous oxide. The absorption of oxygen by ethanol was found to be nil within the experimental error. (The volume of the gas increased due to the pressure of ethanol vapour but returned to the original when the latter was absorbed in 40% potassium hydroxide).

In a test with commercial nitrous oxide ethanol was found to absorb 98.5% and 98.5%, the absorption taking about 1½ hours. In the analysis of the oxygen-treated gas, a repass through the ethanol pipette was required after absorption in potassium hydroxide to establish how much of the gas absorbed was ethanol. Within the limits of the experimental

error (about 1.5%) the loss in volume of the gas on passage through potassium hydroxide was due to absorption of ethanol vapour.

The molecular weight of the gas unabsorbed during the analysis, determined by the vapour density method (3), was found to vary between 28.5 and 29.5. The variable discrepancy between these values and the theoretical value for nitrogen was attributed to contamination of the unabsorbed gas with pyridine. The composition of the gas was found to vary considerably from run to run in spite of extreme care in attempting to control the reaction conditions. The rate of evolution of the gas during each reaction was also found to vary. It appeared that the observed variations might be the result of a varying water content of the hygroscopic pyridine. However, use of pyridine dried over barium oxide and freshly fractionally distilled through a 20 inch Widmer column (boiling range 115.0 - 115.5°C corr.) did not lead to reproducible gas compositions or rate curves. In Fig. 1. are plotted moles of gas per mole of mannitol hexanitrate for several runs using anhydrous pyridine.

To check the possibility that the absolute number of moles of nitrogen evolved in the various forms was a constant quantity, the rate and analytical data were calculated to gram atoms of nitrogen per mole of mannitol hexanitrate. The resulting plots, while not reproducible (as is shown in Fig. 2) were more closely grouped than the plots of moles of total gas evolved (Fig. 1).

The effect of water on the reaction was further tested. Denitrations were carried out with the addition of 0.0014 mole of water in one case and 0.0028 mole in another. A marked decrease in the total amount of nitrogen evolved resulted. In the first case the yield of gas

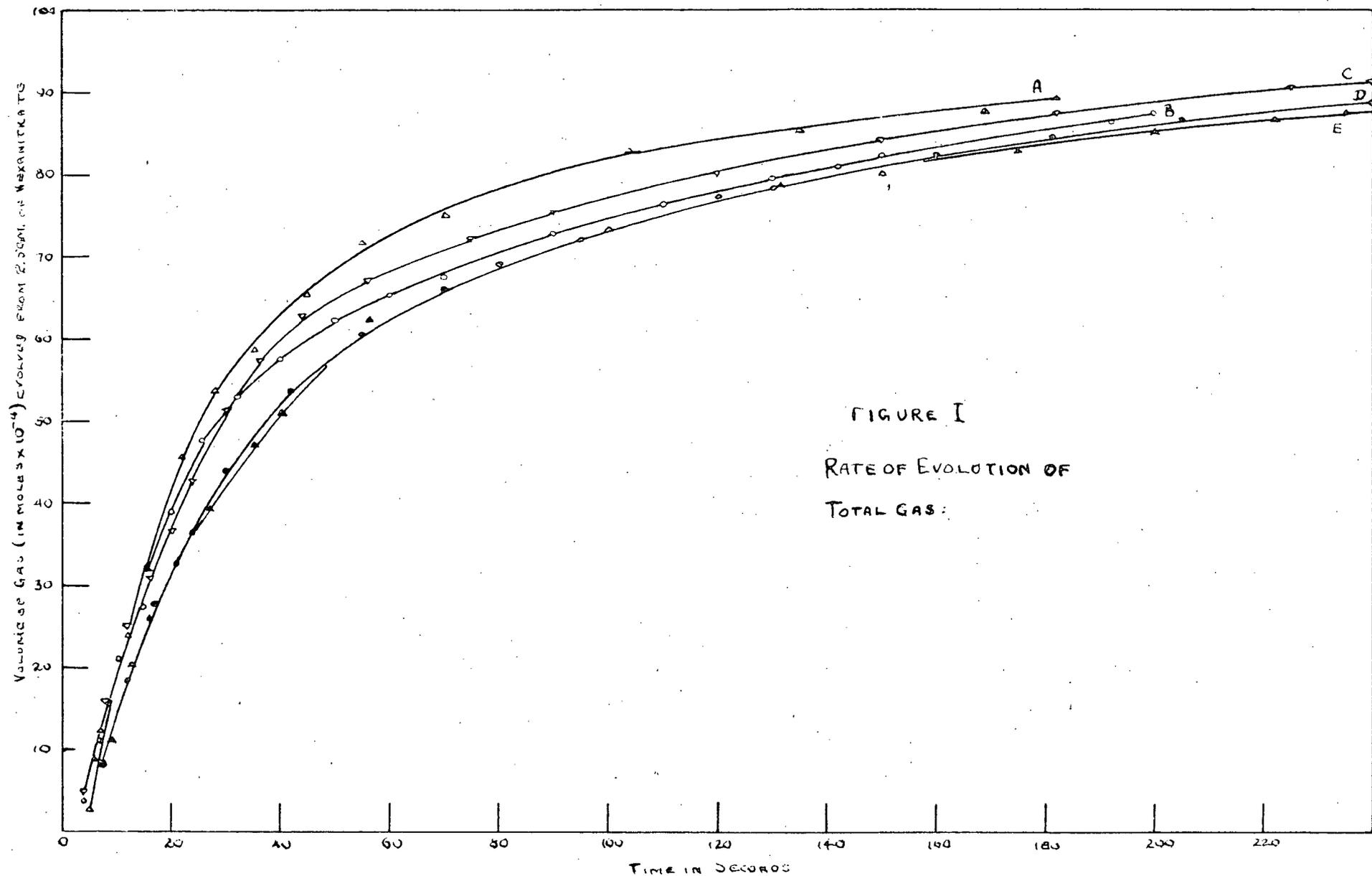


FIGURE I
RATE OF EVOLUTION OF
TOTAL GAS:

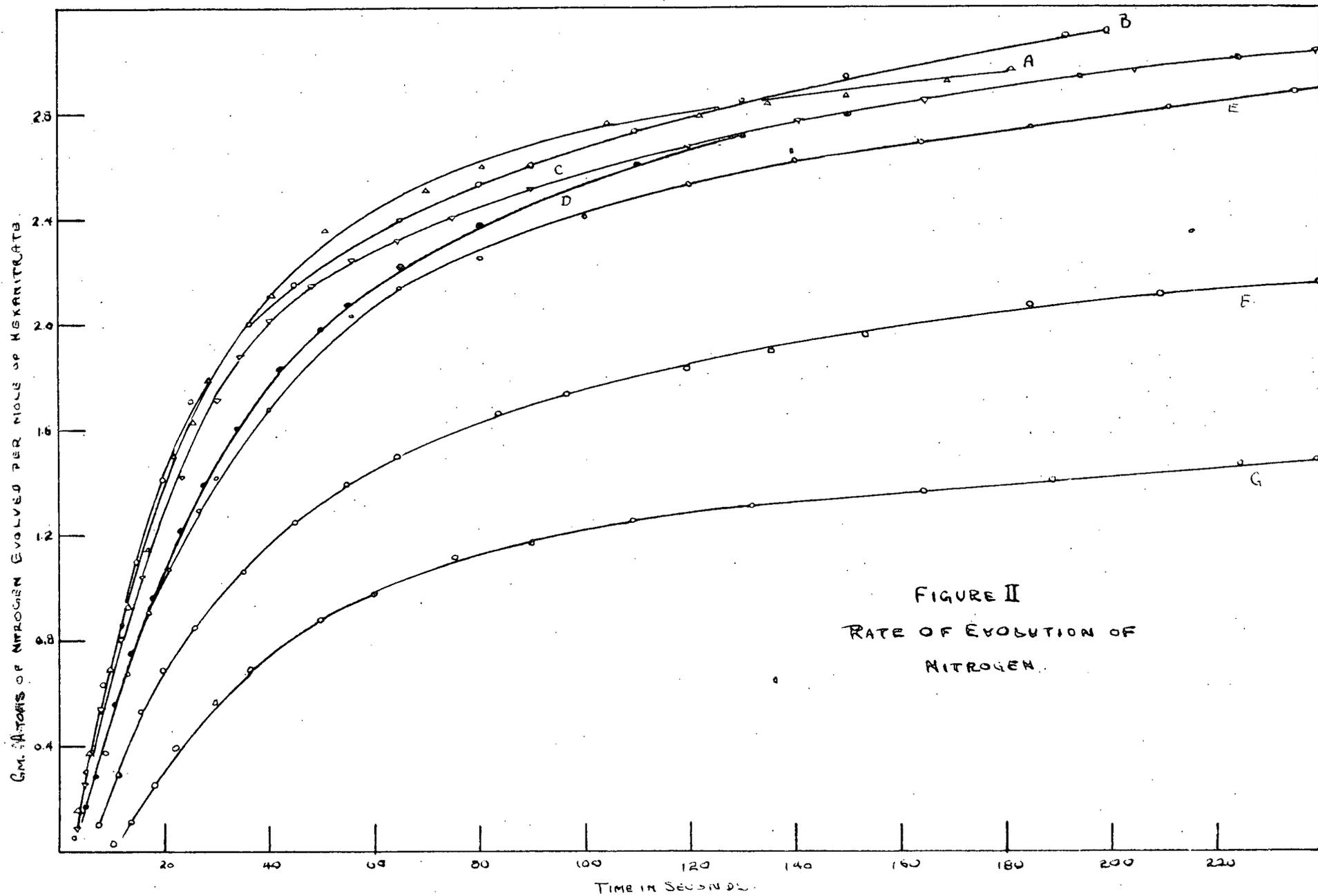


FIGURE II
RATE OF EVOLUTION OF
NITROGEN.

calculated as nitrogen was reduced by about one quarter and in the second by about one half of the average yield from an anhydrous reaction.

The results are plotted in Fig. 2, ^{plots F and G}. The composition of the gas was not significantly altered, as can be seen by reference to Table I, but the yield of crude mannitol pentranitrate was apparently increased. However, since the nitrogen evolved during these reactions was significantly less than that evolved during an "anhydrous" reaction and the colour of the reaction mixture diluted to 250 ml. was much lighter, it is indicated that the reaction did not proceed as far in these cases as it did under anhydrous conditions. Therefore, it is likely that the isolated products contained unreacted mannitol hexanitrate.

The water soluble reaction products, (residue) after reduction to dryness, were extracted with acetone (about 0.5% by weight did not dissolve) and subjected to fractional crystallization from that solvent. While a small sample of pyridinium nitrate was isolated by this method, no further separation was possible. The pyridinium nitrate was eliminated from the residue by neutralization with potassium hydroxide but it was found that the coloured components in the resulting residue were no more easily separable from potassium nitrate by fractional crystallization than they had been from pyridinium nitrate. A change in the colour of the aqueous solution of the residue from yellow-orange to brick-red during the neutralization, indicated that a reaction in addition to neutralization had taken place.

Pure pyridinium nitrate was isolable from the residue by removal of the coloured components with activated charcoal.

Paper-partition chromatography of the residue separated it into

TABLE I
RESULTS OF THE ANALYSES OF THE GASEOUS PRODUCT

Run	Time of Reaction hrs. min.	Vol. of water added	Composition of Evolved Gas			Gm. Atoms N ₂ evolved in 4 hrs.	weight M.P.N.*
			% N ₂	% NO	% N ₂		
A	3	nil	67.5	14.8	17.9	2.98 (3 hr)	1.398 g.
			67.3	14.7	18.2		
B	3 20	nil	66.3	15.2	18.5	3.12 (3 hr. 20 min.)	1.61 g.
C	4 30	nil	65.7	15.3	20.0	3.04	1.48 g.
D	5	nil	68.7	9.8	21.5	3.04	1.61 g.
E	4	nil	63.8	18.2	18.1	2.92	1.64 g.
F	4	0.025 ml.	63.5	16.4	20.3	2.16	1.75 g.
G	4	0.05 ml.	60.8	16.5	22.8	1.49	1.72

* Mannitol Pentanitrate.

fourteen components with Rf values ranging from 0.0 to 0.9. The developing solvent had the composition butanol 40%, water 49%, ethanol 10% and ammonia 1% by volume. The spots were detected by their ultraviolet fluorescence and by development with a 1% solution of aniline trichloroacetate in glacial acetic acid. Interpretation of the spots was made difficult by their number, by considerable trailing of some of the components and by the relatively high proportion of pyridinium nitrate in the residue.

A very large spot with an Rf value of 0.35 was identified, by comparison with a standard, as pyridinium nitrate. Mannitol pentanitrate gave a spot with Rf value 0.9 as identified by comparison with a standard. This spot did not fluoresce appreciably but was detected as a salmon pink spot on treatment with aniline trichloroacetate. The Rf. values of the spots and their color are given in Table II.

The components of the neutralized residue did not separate very well on partition chromatographing, but seven spots appeared under ultraviolet irradiation and one on developing with aniline trichloroacetate. One of these spots was probably produced by potassium nitrate and another by mannitol pentanitrate. Three of the spots appear to be due to compounds also present in the untreated residue and the rest to compounds produced by the action of the potassium hydroxide. Adsorption chromatography of the acetone soluble portion of the residue on silica gel using acetone as the eluting solvent, led to six fractions which were detected by their reaction or non-reaction with diphenylamine reagent. Most of the residue was collected in the first and fourth fractions, the first containing a small quantity of brown gum and the fourth, heavy yellow needles contaminated with an orange syrup. However, it appeared that the method would be useful for

TABLE II
PAPER-PARTITION CHROMATOGRAPHIC ANALYSIS
OF RESIDUE FRACTIONS

Rf Value	Color	Total Residue	Total Neutralized Residue	ClO_4^- precipitated fraction	ClO_4^- soluble fraction	Dialyzeable fraction acetone sol.	Dialyzeable fraction acetone in sol.	Fraction retained by I-R-45	Fraction unaffected by I-R-45
0.	yellow-orange	x	x	x			x		x
.016	yellow	x	x		x		x	xTr	x
.07	orange		x	x			x	xTr	x
.09	orange	x							
.10	sky blue	x							
.15	yellow (ATCA)		x	x					
.18	orange		x			xTr	x		x
.19	blue	x							
.19	orange							x	
.20	orange	x							
.22	orange	x							
.24	sky blue	x					x		
.25	light orange		x	x					x
.26	light orange						x		
.28	yellow	x				x			
.29	blue						x		
.32	yellow						x		
.35	quench	x				x	xTr		
.35	blue								x
.40	blue	x				x			x
.44	yellow	x				x	xTr		
.50	light orange		x						x
.63	sky blue	x							
.9	salmon pink	x	x		x	x			x

Tr = trace.

The colors appeared under ultra-violet irradiation except where aniline trichloroacetate (ATCA) is specified, in which case development of the chromatogram with that reagent was required.

resolution of the residue only after the pyridinium nitrate had first been removed.

A quantitative method for determination of pyridine was sought in order that both the amount of pyridine used in the reaction and the amount in the form of pyridinium nitrate could be determined.

The method of Spacu (19), designed for the estimation of copper, was found to give low yields (96%) of the green pyridine complex with copper thiocyanate ($\text{Cu}(\text{C}_5\text{H}_5\text{N})_2(\text{CNS})_2$) when 1 mole potassium thiocyanate and 0.5 molar cupric sulphate were added in slight excess to a standard 0.1 molar pyridine solution. Addition of further precipitating reagents to the solution lead to precipitation of white potassium thiocyanate. The method was therefore considered unsatisfactory.

Precipitation of the pyridine from a glacial acetic acid solution of the residue as pyridinium perchlorate would have the advantage that an excess of precipitant could be removed from the filtrate by precipitation with potassium acetate. Thus the remaining residue would be relatively free from salts. The method was therefore applied to the residue in spite of the fact that it gave only 98% of the theoretical yield with a standard pyridinium nitrate solution. The resulting precipitate was a bright yellow instead of white as expected. The indication was that coloured cations were present in the solution of the residue that precipitated with perchlorate anions. On solution of the precipitate in acetic acid, removal of perchlorate with potassium acetate and cautious reduction to dryness, a part white, part brown powder was obtained (weight about 0.3 gm.). The white material was assumed to be potassium acetate.

The perchlorate ions were similarly removed from the yellow

filtrate and the solution evaporated. Acetone extraction removed the coloured components from the resulting light brown solid (assumed to be mostly potassium acetate) and evaporation of the remaining solution left 0.1 gm. of dark orange-brown, amorphous, hygroscopic material.

The spots produced by paper-partition chromatography of the perchlorate-precipitated fraction and the soluble fraction, corresponded to those produced by chromatography of the potassium hydroxide neutralized residue. The fraction precipitated with perchlorate ions gave five spots, one of which appeared to be potassium acetate.

The unprecipitated fraction of the residue showed two spots, one being mannitol pentanitrate, and a faint spot for potassium acetate.

Since the denitration reaction could involve polymerization of the pyridine, it was thought that dialysis of the residue might lead to some separation. Preliminary tests showed that pyridinium nitrate easily passed through cellophane dialysis tubing.

Almost the entire residue passed through the pores of the membrane during dialysis, leaving only 0.02 gm. of solid material from an original 0.8 gm. of residue. The solid material in the dialysing solution could be extracted with acetone which dissolved out the pyridinium nitrate, mannitol pentanitrate and three other components as shown by papergrams of the two resulting fractions.

The Rf values of some of the spots (10 appeared under ultraviolet) produced by the acetone insoluble fraction did not correspond to those of the residue, but it appeared that these values were dependent on the amount of pyridinium nitrate present. The method of dialysing might, however, have produced some changes in the residue.

The fact that acetone dissolved only part of the dialysed material but practically all of the original residue supported the latter view.

The solid remaining in the dialysis bag remained at the point of application when subjected to paper-partition chromatography.

Pyridinium nitrate was found to be nearly completely removed from a standard aqueous solution by the weakly basic anion exchange resin Amberlite I-R-45. On application of the method to the residue, however, it was found that the resin retained a part of the residue which could be recovered from the regenerating bicarbonate solution by reduction to dryness and extraction with acetone. The material recovered in this way (0.32 gm.) appeared to be mostly colorless crystals of sodium bicarbonate on which was deposited a small quantity of very dark brown material. The treated residue solution yielded 0.11 gm. of hard, amorphous, fairly dark orange-brown material which gave nine spots on a paper-partition chromatogram, one of which corresponded to mannitol pentanitrate. No spot appeared for pyridinium nitrate. The material retained by the resin gave one spot corresponding to one of those from the neutralized residue and two indistinct spots for components readily detectable in the fraction unaffected by I-R-45. The results of the chromatography experiments are summarized in Table II.

An estimate of the amounts of pyridine remaining after reaction and pyridinium nitrate produced was obtained by titration of the reaction mixture after removal of the mannitol pentanitrate and dilution with water to 250 ml. End points were detected with a calibrated Beckman model "G" pH meter equipped with standard glass and saturated calomel electrodes. A 25 ml. aliquot of the solution was first titrated with 0.2 - 0.3 N.

potassium hydroxide for nitrate ion and then with 0.6 normal sulphuric acid for pyridine. The pyridine titrated thus included that originally bound as pyridinium nitrate. The accuracy, as determined by analysis of standard samples, was $\pm 0.3\%$ for both titrations. The loss of the volatile pyridine involved in the manipulation of the reaction mixture was found to be $1.1\% \pm 0.2\%$ by subjecting a pyridine sample to the transferring and filtering required of the reaction mixture. After determination of the volume of potassium hydroxide solution required, another 25 ml. aliquot of the solution was treated with a known excess of potassium hydroxide and reduced to dryness for determination of the weight of pyridinium nitrate-free residue.

With these data it was possible to make a rough calculation of the composition and weight of the reaction products to be expected in the residue and to compare the calculated weight of residue to that found. The calculations showed that there was essentially no nitrogen to be expected in the residue other than that determined as nitrate ion, that carbon and hydrogen were present in the approximate ratio of 1:1 and that oxygen was present in very small quantity. This composition is inconsistent with the character of the residue since it indicates the presence of only hydrocarbon material. However, in calculating the number of moles of oxygen in the residue, it was assumed that all of the oxygen as nitrate ion came from the reactants, while the probability is that it came in large part, from the water into which the reaction products were poured. When it was assumed that the acidic material titrated was produced from nitric oxide after the reaction, the ratio of carbon to oxygen became approximately 3:1 and the water solubility of the residue was then consistent with the

calculations. Approximately two moles of pyridine were used in the reaction for each mole of mannitol hexanitrate added. The discrepancy between the calculated and experimental values for the weight of residue must be attributed to the presence in the reaction mixture of a volatile, pyridine and water soluble fraction which would be lost in the evaporation of the neutralized residue to dryness. The results of the volumetric analyses for three denitration runs are given in Table III. Table IV. gives the composition of the residues calculated from the analytical data. The value for pyridine added was corrected for losses in manipulation. The experimentally determined weight of residue given in Table IV. was that obtained from the weight of the neutralized water soluble reaction products by subtracting the known weights of potassium nitrate and potassium hydroxide. The calculated weight of residue is the weight of pyridinium nitrate-free residue.

CONCLUSIONS

The principal reaction which occurred on solution of mannitol hexanitrate in pyridine resulted in the replacement of the number three nitrate ester group of the hexanitrate by hydroxyl. Since no inversion or racemization occurred, the replacement probably involved substitution of a proton for a nitronium ion. The source of this proton remains unknown since the results of the present investigation indicated profound decomposition of both the pyridine used in the reaction and the mannitol hexanitrate not recovered as the pentanitrate. Paper-partition chromatography indicated that no less than fourteen solid products were formed during the reaction and analysis of the gaseous product showed that it contained three components. The substitution of the nitronium

TABLE IV

MATERIAL BALANCE BASED ON THE ANALYSIS OF THE
AQUEOUS AND GASEOUS REACTION PRODUCTS

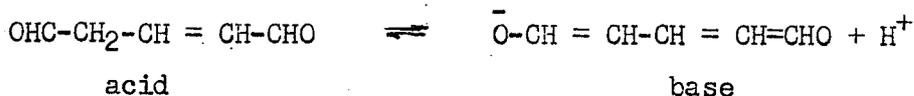
Run	E	F	G
	moles	môles	moles
Nitrogen in the residue	-0.001	0.002	0.004
Carbon in the residue	0.064	0.065	0.055
Hydrogen in the residue	0.063	0.063	0.053
Oxygen in the residue	0.019	0.016	0.019
Calculated weight of residue	1.13 gm.	1.13 gm.	1.07 gm.
Determined weight of residue	0.097 gm.	0.046 gm.	0.102 gm.

ion into the pyridine ring with ejection of a proton appeared unlikely in view of the extreme conditions required for the nitration of pyridine (12).

Nitrogen was recovered in simple molecules far in excess of one gram atom per mole of mannitol pentanitrate produced (six gram atoms of nitrogen per mole of mannitol pentanitrate were produced in the case of the anhydrous reaction). In fact, the calculations based on the analyses of the reaction products from the anhydrous denitration account for all the nitrogen content of the reactants in mannitol pentanitrate unreacted pyridine, nitric oxide, nitrous oxide, nitrogen and nitrate ion.

In the two denitrations done with the addition of water to the reactants, the calculations showed that a small amount of nitrogen was present in the unidentified products. However, an increased yield of water-insoluble product was obtained from these two reactions (Table III) and this increase could be accounted for by assuming the presence of unreacted hexanitrate. Thus a nitrogen balance was achieved in the three cases which indicated the unidentified products to be nitrogen-free. The hexanitrate not recovered as pentanitrate must then have been completely denitrated, probably with further oxidative degradation. The pyridine used in the reaction must also have been degraded at least to the extent of ring opening to eliminate the nitrogen.

It may be conjectured that these products were low molecular weight unsaturated alcohols, aldehydes and acids. One possible component is glutaconaldehyde (27). It has an empirical formula ($C_5H_6O_2$) consistent with the calculated composition of the residue (C_3H_3O) and has two forms depending on the pH: in acid solution it has the yellow-brown dialdehyde structure; in basic media it is in the form of the dark red enolate ion.



The presence of this compound or similar ones in the reaction product would account for the observed colour change on neutralization of the residue and for the extraction of a portion of the residue by an anion exchange resin.

The major problem in the resolution of the solid residue was the removal of the very large quantity of pyridinium nitrate. Very mild conditions were required because the expected components would be subject to ready polymerization and decomposition. Alkaline solutions had to be avoided because of the possibility of aldol type condensations and Cannizano disproportionations. Of the methods utilized in this work, treatment of the residue in aqueous solution with the weakly basic anion exchange resin amberlite I-R-45 appeared to be the one most suited for the removal of the pyridinium nitrate. In addition, it separated at least one coloured anionic component from the rest of the residue. The treated residue could then be subjected to adsorption chromatography on unactivated silica gel using acetone followed by methanol as the eluting solvent. The fluorescence of the fractions under ultraviolet irradiation should serve to differentiate them. The number of components in each fraction could be determined by application of paper-partition chromatography. Further work should be done on the total analyses of the reaction products. This should include analysis of the crude mannitol pentanitrate and of the neutralized residue for nitrogen. An investigation to ascertain the identity of the components evaporated during the reduction of the neutralized residue to dryness should also be undertaken.

EXPERIMENTAL

Special Precautions

The explosive character of mannitol hexa- and pentanitratates made it essential that special precautions be taken in handling these compounds and the solid byproducts of the denitration reaction. The mannitol was nitrated in 5 gram lots and no more than the product of one nitration was stored in the dry state at one time. Distillations were carried out on a steam bath at reduced pressure to a minimum volume of residue of approximately 25 ml. Further drying was accomplished at room temperature in a vacuum desiccator.

Materials and Methods

Nitric Acid. Red fuming nitric acid supplied by Baker and Adamson was used for all nitrations.

Pyridine. A.R. pyridine supplied by The British Drug Houses was used for the denitrations.

Mannitol. D-Mannitol supplied by the Schering-Kahlbaum Company, Berlin, Germany, melting point 166-167°C., $[\alpha]_D^{20} = 0.53^\circ$ (C, 7.00) H₂O was used in the preparation of mannitol hexanitrate.

D-Mannitol-1,2,3,4,5,6-hexanitrate was prepared in five gram lots as described by Patterson and Todd (14).

Diphenylamine Reagent used in testing for nitrate was prepared by the method of Mulliken (13).

Ion Exchange Resin. Amberlite I-R-45 anion exchange resin supplied by Rohm and Haas Company, The Resinous Products Division, was used for the removal of anions.

Dialysis Tubing. Cellophane $1\frac{1}{8}$ inch Osmosis Membrane supplied by Central Scientific Company, was used in six inch lengths, tied off at the bottom with a rubber band, for all dialysis experiments.

Paper-Partition Chromatographic Paper. Watman No.1. Paper, supplied by W. and R. Baker, Ltd. was used for the paper-grams.

Silica Gel. Technical Dessigel was ground in a corn mill and sieved. The 80-115 mesh portion was used.

Paper-Partition Chromatography. Spots of the material to be analyzed were applied to the 50 cm. paper strip 8 cm. from the top and at intervals of 3 cm. The concentration of the material on each spot was built up by repeated application of the solution of the material from a capillary-tipped pipette, the spots allowed to dry between each application. The chromatogram was developed by suspending it from a trough of the developing solvent (40% butanol, 49% water, 10% ethanol and 1% ammonia by volume) in an insulated chromatography tank with a light-fitting cover. After allowing the developing process to proceed for twenty-four hours, the chromatogram was removed from the tank and allowed to dry. It was then investigated in a photographic dark-room under ultra-violet irradiation. Spraying of the chromatogram with 1% aniline trichloroacetate in glacial acetic acid by means of an atomizer then followed. The chromatogram was allowed to dry and then heated in an oven for three minutes. Spots produced by the aniline trichloroacetate appeared the following day.

Preliminary Experiments

Mannitol hexanitrate (5 grams) was dissolved in 30 ml. of pyridine in a 250 ml. erlenmayer flask (7). The solution changed from

colourless to yellow-orange in twenty minutes. A frosting of crystals on the walls of the flask above the solution had also appeared by this time. In one half hour the container had filled with a red brown gas. After this time external cooling of the mixture, to keep the temperature below 35°C., was no longer necessary. After 24 hours the solution (now red-brown) was poured into 250 ml. of distilled water. A white, curdy precipitate of mannitol-1,2,4,5,6-pentanitrate immediately deposited and was filtered off after two hours, leaving a yellow-orange solution. Wash-water (200 ml.) added to the main filtrate caused further precipitation but another 100 ml. used for further washing after refiltering the mother-liquor induced no precipitation.

The pentanitrate product, after two crystallizations from ethanol-water, melted correctly at 81-82°C. (27). The yield of crude product was 3.1 gm. (69% of theory).

Determination of the Rate of Gas Evolution

The reaction was carried out in a Lunge Nitrometer equipped with a burette calibrated to read in moles $\times 10^{-4}$ at 20°C. The weight of mannitol hexanitrate used in each case was 2.500 ± 0.001 gm. The temperature of the reaction mixture was controlled to 30 ± 2 °C. by means of a jacket surrounding the reaction chamber. The mannitol hexanitrate was placed in the cup and washed into the reaction chamber with pyridine. Evolution of the colorless gas started in three to six minutes. The rate of gas evolution is plotted in Fig. 1.

Analysis of the Evolved Gas

All estimations of gas composition were done by the volumetric method using a Fischer Technical model Orsat apparatus. The retaining

liquid was water saturated with the gas to be analysed. Results are tabulated in Table I.

After removal of the reaction mixture from the Lunge Nitrometer, the reaction chamber was rinsed and dried with acetone.

A volume of commercial oxygen (approximately one half the volume of the evolved gas) was then admitted to the reaction chamber and transferred to the burette. The gas became deep brown in contact with the oxygen. The mercury levelling bulbs were so adjusted that mercury passed from the reaction chamber through the gas. In about two hours the gas was colorless, indicating complete reaction of the nitric oxide with oxygen and mercury.

After noting the residual volume of the gas, it was transferred to the Orsat apparatus equipped with pipettes containing 95% ethanol, 40% potassium hydroxide and alkaline pyrogallol solutions, for absorption of nitrous oxide, ethanol and oxygen respectively.

The percentage of nitrous oxide was given by

$$a \times \frac{V_2}{V_1}$$

where a = percent of residual gas absorbed by ethanol
and potassium hydroxide

V_1 = volume of gas before admission of oxygen

V_2 = volume of residual gas.

The percentage of nitrogen in the gas was given by

$$b \times \frac{V_2}{V_1}$$

where b = percentage of residual gas unabsorbed.

The percentage of nitric oxide was given by

$$\frac{(V_1 - V_2 + \frac{C}{100} V_2)}{V_1} \times 100$$

where c = percentage volume of gas absorbed in alkaline pyrogallol.

The original gas volume was corrected for pyridine vapor in accordance with Van der Meulen and Mann (22).

The molecular weight of the gas remaining unabsorbed after being passed through the various solutions was determined as described by Daniels, Mathews and Williams (3). The unabsorbed gas was transferred from the Orsat apparatus into a previously evacuated and weighed glass bulb of known volume (11.00 ml.). The pressure and temperature of the gas in the bulb were recorded and the bulb then closed by means of a stopcock, wiped with a moist then a dry chamois cloth, allowed to stand in the balance case for ten minutes, then weighed. The weight and pressure of the gas were corrected for the weight and pressure of water present, assuming saturation, and the molecular weight calculated from the equation

$$M = g \frac{TR}{pV}$$

where M = the molecular weight.

g = the corrected weight of gas

T = temperature in degrees Kelvin

R = the universal gas constant

p = the corrected pressure of the gas

V = the volume.

Results from several different runs were:

1. 29.3, 29.2
2. 28.7
3. 28.6
4. 29.1
5. 29.6

Fractionation of the Water Soluble Reaction Products

The filtrate was concentrated under reduced pressure on the steam bath to approximately 25 ml. of clear orange liquid, transferred to a small beaker and dried in vacuo over phosphorous pentoxide and potassium hydroxide pellets. The residue so obtained was dark orange-brown in color and semi-crystalline, readily dissolved in cold water, dissolved almost completely in methanol, ethanol and acetic acid, and to the extent of about three quarters in hot acetone. It was partly soluble in chloroform and slightly soluble in ether, benzene and petroleum ether.

The residue (2.1g) was extracted in a Wazitski extractor with acetone until fresh acetone did not become colored. The undissolved material was almost black and weighed 0.12g. Fractional crystallization of the extract led to the isolation of 0.02 g. of near white needles that melted at 115.5-117°C unchanged in admixture with pure pyridinium nitrate prepared by the action of concentrated nitric acid on pyridine and purified to constant melting point (116-117.5°C.) by recrystallization from methanol.

The colored components could not be separated from the colorless crystals which appeared to make up the bulk of the residue.

A portion of the residue (0.86 g.) was dissolved in 60 ml. of water and neutralized to pH 9 (pH paper) with 7.5 ml. of 10% potassium

hydroxide (original pH = 4). The color of the solution changed during the addition from orange-brown to brick-red. A distinct odor of pyridine was noted.

The solution was evaporated on the steam bath under reduced pressure to 25 ml. and further evaporated to dryness in a vacuum desiccator, leaving heavy red-black needles. This residue was insoluble in petroleum ether, benzene and ether, slightly soluble in chloroform, more soluble in acetone, ethanol and methanol, and completely soluble in water. On successive extraction in a Wazitskic extractor with acetone (yellow solution), methanol (dark red solution) and ethanol (almost colorless solution), three fractions were obtained, the major one being the acetone soluble fraction. Fractional crystallization appeared, in separating the components of the acetone soluble fraction, to be no more efficient than it had been in the case of the untreated residue and the method was abandoned.

Isolation of Pyridinium Nitrate

A portion of residue weighing 0.78 g. was dissolved in 50 ml. of water, heated to boiling with 1 gm. of activated charcoal, and filtered through Kieslguhr. The yield of almost colorless needles obtained from the solution after drying and recrystallizing from acetone was 0.24 g. (31% of the weight of the residue), m.p. 115-116.2°; m.p. in admixture with pure pyridinium nitrate 115.5 to 117.2°.

Adsorption Chromatography of the Acetone-Soluble Residue

Unactivated 80-115 mesh silicagel (50 gm.) assaislurrycin acetone was introduced into a column 1.3 cm. in diameter and plugged at the constricted end with glass wool. The height of the resulting column of

silicagel was 52 cm. The column was flushed with acetone until the effluent was clear. The acetone extract of 0.8 gm. of residue (volume 22 ml.) was added to the column and collection of 8 ml. fractions commenced. The flow-rate was 8 ml. in two and a half minutes. The separation of the fractions was detected by testing the 8 ml. portions of effluent with diphenylamine reagent (13) on a spot-plate. When, after collection of 145 - eight ml. portions, the portions showed only a faint, gradually decreasing test to diphenylamine reagent, methanol was added to the eluting solvent in gradually increasing proportions until acetone was eliminated completely. In this manner two further fractions were obtained. A total of 180 - eight ml. portions of effluent was collected, which yielded six fractions. The first was an orange-brown gummy material, the second a very small quantity of yellow oil, the third a small quantity of orange-brown semicrystalline material, the fourth contained heavy light yellow needles in admixture with an orange gum, the fifth a light brown semi-crystalline material and the sixth an orange oil.

Determination of Pyridine and Pyridinium Nitrate in the Residue

(a) Precipitation as the Copper Thiocyanate Complex (19)

- (i) Estimation of Pyridine. A 50 ml. aliquot of 0.101 molar aqueous pyridine was treated with 7 ml. each of one molar aqueous potassium thiocyanate and 0.5 molar aqueous cupric sulphate. A green flocculent precipitate appeared which was filtered after one hour on a fine sintered glass funnel. The precipitate was dried in vacuo. Yield 0.82 gm. (96%). A second run gave 0.81 g. of product (95%). Addition of further

precipitating reagents to the filtrate gave a white precipitate presumably of copper thiocyanate.

(ii) Estimation of Pyridine in Pyridinium Nitrate. A solution of 0.58 gm. of pyridinium nitrate dissolved in 100 ml. of water was neutralized to pH 10 (alkacid paper). The pyridine-water azeotrope was distilled off under reduced pressure in an all glass apparatus into a 100 ml. receiver cooled in a brine bath at -10°C . The receiver was backed up by a dry ice trap. The contents of the receiver and trap were washed into a beaker and the pyridine estimated by precipitation as the copper thiocyanate complex. The yield was 80% of theory. Two repetitions of this procedure gave yields of 91.3% and 91.3% of theory.

(b) Precipitation as Pyridinium Perchlorate

To 0.62 gm. of pyridinium nitrate dissolved in 10 ml. of glacial acetic acid was added a 1 N. solution of perchloric acid in acetic acid until precipitation of the white pyridinium perchlorate was complete.

The precipitate was filtered and dried in vacuo. Yield 0.75 gm. (98%). About 0.7 gm. of residue was dissolved in 10 ml. of glacial acetic acid, filtered from a very small quantity of medium-brown insoluble material and treated with 1 N. perchloric acid in glacial acetic acid. The solution was made up to 125 ml. with ether to induce further precipitation. The precipitate was filtered and dried in vacuo. Yield 0.73 g. of heavy yellow needles. The mother liquor was yellow in color.

The precipitate was dissolved in 100 ml. of glacial acetic acid and treated with 7 ml. of 10% potassium acetate in glacial acetic acid. The fine white precipitate of potassium perchlorate was filtered off and the solution evaporated under reduced pressure. The solid material remaining was part white, part brown powder. The white material probably was potassium acetate.

The filtrate from the perchlorate precipitation, after precipitation of excess perchlorate with potassium acetate in acetic acid, was concentrated under reduced pressure and dried in vacuo. The solid, light brown residue was extracted with acetone until the remaining solid was almost colorless. The acetone solution was evaporated to dryness (the last stage in vacuo) leaving 0.1 gm. of dark orange-brown amorphous hygroscopic material which was then chromatographed on paper (Table II).

(c) Separation by Dialysis

(i) Dialysis of Pyridinium Nitrate. A dialysis bag was charged with 1 gm. of pyridinium nitrate in 100 ml. of water and the whole suspended in a beaker of water. Water was constantly admitted to the beaker from the bottom. In a twenty-four hour period the solution in the dialysis bag gave a negative test for nitrate to diphenylamine reagent and had no pyridine odor.

(ii) Dialysis of the Residue. A solution of 0.8 gm. of residue in 100 ml. of water was dialysed in a liquid-liquid extractor. The color of the solution in the bag did not appear to diminish but the extracting liquid became yellow-orange. After twenty-four hours no more color was extracted from the bag, the contents

of which gave a negative test to diphenylamine reagent. Both bag contents and dialysing solution were reduced to solid residues by the usual procedure. The bag residue weighed 0.01 gm., was dark brown in color and difficultly soluble in water. The solid from the dialysing solution was light orange-brown in color and had more crystalline character than the original residue. It could be separated, by extraction with acetone, into a partly crystalline yellow-orange acetone soluble fraction and an orange-brown amorphous material.

(d) Separation by Ion Exchange

- (i) Preliminary Tests. In preliminary tests, Amberlite I-R-4B, anion exchange resin was found to "throw" its color badly and so was substituted with Amberlite I-R-45.

Approximately 20 gm. (damp) of Amberlite I-R-45 anion exchange resin was stirred for twenty minutes with a solution of one gram of pyridinium nitrate dissolved in 100 ml. of water. The mixture was then filtered on a coarse sintered glass funnel and the resin washed with water. The resin was then regenerated by washing with 5% NaHCO_3 (200 ml.) and then water until the washings were neutral to pH-paper. Filtrate and resin were then recombined and the process repeated twice. After two treatments the solution gave only a faint test with diphenylamine reagent and the third treatment had no noticeable effect.

- (ii) Treatment of the Residue with Amberlite I-R-45 Resin. A portion of the resin (0.68 gm.) was treated in the preceding

manner except that the sodium bicarbonate regenerating solution was saved since it was yellow in color. On the third regeneration the regenerating solution was not colored.

The treated solution was evaporated to dryness, leaving a hard, amorphous, fairly dark orange-brown residue weighing 0.11 gm. The combined regenerating solutions were reduced to dryness. The solid residue (mostly sodium bicarbonate) was then extracted with acetone until it was colorless. On evaporation of the acetone, 0.32 gm. of very dark brown material was obtained. The crystalline character appeared to be attributable to colorless crystals of sodium bicarbonate. In any case, the dark material was only a very small part of the total.

(e) Titrimetric Determination

(i) Preliminary Tests. A 250 ml. aqueous solution was prepared, containing 1.340 gm. of pyridinium nitrate and 17.55 gm. of pyridine. Aliquots of 25 ml. of this solution were titrated first with standard 0.2 N. potassium hydroxide from a 5 ml. burette for estimation of nitrate, and then with standard 0.6 N Sulfuric acid for estimation of total pyridine (free pyridine added plus pyridine freed from pyridinium nitrate). The end points were detected with the aid of a Beckman model "G" pH meter equipped with a standard glass electrode and a fibre-tipped calomel electrode containing saturated potassium chloride solution. The end point in the potassium hydroxide titration occurred at a pH of about 9.2 with a maximum change

in pH per drop of potassium hydroxide solution (0.033 ml.) of about 0.35 pH units. The end point in the titration with sulphuric acid occurred at a pH of about 3.05 with a maximum change in pH per four drops of sulphuric acid solution (0.20 ml.) of about 0.16 pH units. The determined weight of pyridinium nitrate was 0.1343 (100.2%) and 0.1347 gm. (100.4%).

The determined weight of pyridine (actual weight was 18.2 gm.) was 18.1 gm. and 18.1 gm. (99.5%).

The pyridine lost in manipulation of the reaction mixture was determined as follows:

Pyridine (15 ml.) was subjected to the entire procedure of the denitration and preparation of the aqueous reaction products for titration. The theoretical weight of pyridine titratable then should be 17.7 gm. The results were as follows:

Run 1.	17.52	Run 2.	17.52
	17.49		17.52
	17.48		17.59
	<u>17.48</u>		<u>17.59</u>
average =	17.49	average =	17.54
	= 17.5		= 17.5

pyridine lost = 0.2 gm. (1.1%) 0.2 gm. (1.1%).

deviation between the results of the two runs is 0.05 ml. (0.28%).

(ii) Volumetric Analysis of the Water Soluble Reaction Products.

The denitration reaction was carried out as usual in

the Lunge Nitrometer. Special care was taken in handling the pyridine and the reaction mixture to prevent loss in manipulation. The reaction was allowed to proceed for four hours and the rate of evolution of the gas was recorded. After transferring the reaction mixture to a 250 ml. groundglass stoppered erlenmeyer flask containing 200 ml. of water, the reaction chamber was rinsed with three one-ml. portions of pyridine delivered from a one-ml. pipette and then with 10 ml. of water in three portions. The mixture was then allowed to stand for at least two hours, the precipitate of pentanitrate was filtered and washed on a previously weighed coarse sintered glass funnel and the filtrate transferred to a 250 ml. volumetric flask and diluted to the mark. Twentyfive ml. aliquots of this solution were then titrated as described in the preceding section. One 25 ml. aliquot was treated with a measured excess of potassium hydroxide and evaporated to dryness in a desiccator for determination of the weight of residue. The results are tabulated in Table III.

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