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Chemical denitration of nitroglycerin, and conversion of 1,2-dinitroglycerin to 1,3-dinitroglycerin

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Summary

Nitroglycerin (GTN) and two of its partially denitrated analogues [1,2-dinitroglycerin (1,2-GDN) and 1,3-dinitroglycerin (1,3-GDN)] decomposed by denitration with formation of nitrate ions (NO_3^-), but not nitrite (NO_2^-) ions. 1,2-GDN was converted to 1,3-GDN before denitration. The reactions occurred with first-order kinetics, and the rate constants of decomposition were higher with increases in pH and temperature. Arrhenius plots were used in the calculation of thermodynamic constants for the reactions. Activation energies for decomposition of the 3 compounds were not significantly different. The results were contrasted with those from studies of the metabolism of the same compounds, which are converted enzymatically to nitrite ions, the nitrite ions then undergoing oxidation to nitrate ions.

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Introduction

Although nitroglycerin (GTN) has been in use for over 100 years, fundamental questions remain concerning its mode of action (Parratt, 1979). One group of such questions concerns the metabolites of GTN, which is metabolized by denitration to a group of partially nitrated glycerols, and to nitrite (NO_2^-) and nitrate (NO_3^-) ions. The role of the metabolic denitration reaction in the pharmacology of GTN, and whether the metabolites are found in plasma and tissues are unknown, although it is known that the lower glyceryl nitrates possess activity less than that of GTN (Bogaert et al., 1968).

Studies of chemical decomposition of drugs can be useful models for drug metabolism studies. As long ago as 1885, Hay studied the reaction of GTN and KOH, showing formation of potassium nitrate, potassium nitrite, potassium formate, potassium acetate, hydrogen and water. Hay's proposed reaction sequence precluded direct formation of nitrite from nitroglycerin. He hypothesized that stoichiometric formation of nitrate occurred, and that the hydrogen formed reduced the nitrate to nitrite. He used a high temperature (100°C) and a long time (30 min) to be sure that all of the GTN was decomposed. In contrast, Bell (1963) heated a mixture of nitroglycerin and potassium hydroxide in a boiling water bath for 10 min and concluded that only one-third of the nitrate formed was readily converted to nitrite. Another third was reduced more slowly or less completely. There appear to have been no studies of decomposition in relatively mild conditions at neutral or alkaline pH.

Decomposition in acid solutions has been studied. Crew and DiCarlo (1968) showed that 28% denitration occurred in solutions of nitroglycerin heated in 4 N HCl for 6 h. Farmer (1920) also conducted nitroglycerin hydrolysis studies in acid, showing formation of glycerol, and oxalic and formic acids. More recently, Wu et al. (1981a and b) have shown rapid denitration of nitroglycerin in 4 N hydrochloric acid.

Certain aspects of the chemistry involved in the metabolism of GTN and two of its model metabolites in alkaline solutions at relatively low temperatures are presented as a prelude to biological studies with GTN. Specific liquid chromatographic assays were used to determine the influence of temperature and pH on the denitration of GTN, 1,2-dinitroglycerin (1,2-GDN), and 1,3-dinitroglycerin (1,3-GDN). A hitherto unknown reaction, rearrangement of 1,2-GDN to 1,3-GDN, was discovered.

Materials and Methods

Materials

GTN was obtained as a 10% adsorbate on lactose (as a gift from ICI Americas, Wilmington, DE, U.S.A.). Stock solutions of GTN were prepared at a concentration of 1 mg/ml of adsorbate (0.1 mg/ml GTN) in distilled water. Dilutions were made in ethanol for standardization and in water for decomposition experiments. Potassium nitrate, sodium nitrite and other reagents were analytical grade (Fisher Scientific).

Synthesis of partially nitrated glycerols

1,2-GDN and 1,3-GDN were synthesized by refluxing 2,3-dibromo-1-propanol and 1,3-dibromo-1-propanol, respectively, with silver nitrate, and GMN (glycerol mononitrate) was synthesized by partial nitration of glycerol (Dunstan et al., 1965). The reaction products were extracted from the reaction mixtures into ether. The ether extracts were dried over anhydrous magnesium sulphate and ether was removed under reduced pressure. The residues were redissolved in ethanol and the products were purified by thin layer chromatography.

Thin layer chromatography (TLC)

Glass-backed silica gel TLC plates with fluorescent indicator incorporated (20 × 20 cm; 0.25 mm thick; Kodak) were used. Two drops of the ethanol extracts, prepared as described in the previous section, were placed at the two ends of the baseline and in each case a band of the extract was spotted inside the middle 17 cm of the base line. The elution system was either ethyl acetate–*n*-heptane (9 : 1 v/v) (Hodgson and Lee, 1975) for the identification and separation of GMN, or benzene–ethyl acetate (4 : 1 v/v) (Needleman and Krantz, 1965) for the identification and separation of 1,2-GDN and 1,3-GDN. After development, the middle part of the plate was masked with a card and the two sides sprayed with 1% ethanolic diphenylamine (Needleman and Krantz, 1965). The plate was also examined by exposure to ultraviolet light. The zones containing the required products were scraped from the plates and the compounds eluted with ethanol.

Standardization of nitric acid ester solutions

GTN stock solutions, and ethanol extracts of thin-layer plates were standardized for their nitric acid ester content using the colorimetric method of the British Pharmacopoeia, in which NO_3^- ions are removed from the compound to be assayed by means of a hydrolysis reaction and used to nitrate phenol–disulphonic acid. Standardization is by comparison with potassium nitrate. We used this technique without modification except for minor changes in volumes of the samples. Additionally, the calculations allowed for the fact that the different esters liberated different quantities of nitrate ions (Anon., 1973).

Decomposition of the nitric acid esters

GTN, 1,2-GDN and 1,3-GDN were incubated individually, at various temperatures, using a Dubnoff shaker, in aqueous solutions of various pH values. All incubations, except those in which temperature variations were deliberately studied, were at 25°C. The solutions were prepared from 0.1 N HCl (pH 1.0), 0.1 M phosphate buffer (pH 7.4), 0.001 N NaOH (pH 11.0) and 0.1 N NaOH (pH 13.0). In all cases the starting concentration of the nitric acid ester was 10 μg/ml. Once the required reaction time had elapsed, the contents of the tubes were adjusted to pH 7.0 by the addition of pre-determined volumes of 0.1 N NaOH or 0.1 N HCl, and the diluted solutions were assayed for GTN, its partially denitrated analogues, and NO_2^-, and NO_3^- by high-pressure liquid chromatography. The ionic strength of all solutions in the temperature-dependent studies was the same. This was not so in the pH-dependent studies.

High-pressure liquid chromatography (HPLC)

The system consisted of a Waters M-6000A pump, a Waters U6K injection port, a Jasco Uvidec-100-III variable wavelength UV detector, and a Varian Multivolt Model 9176 recorder. Two columns were used for organic nitrite determination. The first was a 20 cm Spherisorb ODS 10 column (LDC) with a mobile phase of 40% methanol–60% water at a flow rate of 1 ml/min. The second was a 25 cm Ultrasil NH_2 10 column (Altex) with a mobile phase of iso-octane (80%)–methylene chloride (13%)–methanol (7%) at 2 ml/min. The pressure at the column head was typically 500–600 psig. The volume of the test solution injected was 20 μl. The temperature was ambient (25°C). The detection wavelength was 210 nm and the absorbance units value for full-scale deflection of the 1 mV recorder was 0.5. The peak heights for the various compounds were measured and compared with responses generated from standard solutions of known concentration in the same type of medium. The calibration curves were linear from 2–10 μg/ml of each compound ($r = 0.99$, 0.99, and 0.96 for GTN, 1,2-GDN, 1,3-GDN, respectively). The lower limit of detection was 10 ng for GTN and 40 ng for 1,2-GDN and 1,3-GDN.

Inorganic nitrite and nitrate were separated on a μBondapak C-18 column. The mobile phase was a mixture of equal quantities of 0.1 M disodium hydrogen phosphate and 0.1 N HCl at pH 2.0, flowing at 2 ml/min. Detection was by UV absorption at 210 nm. Calibration was linear between the lower limit of detection (6.3 ng/ml for potassium nitrate; 25 ng/ml for sodium nitrite) and the upper limits of analytical need (4 mg/ml for potassium nitrate; 20 mg/ml for sodium nitrite).

Results

Fig. 1 shows an HPLC separation of GTN, 1,2-GDN, 1,3-GDN and mixed GMN isomers. IS-DN (ISDN) is isosorbide dinitrate (obtained in the form of a 25% lactose adsorbate as a gift from ICI Americas) used as a marker. This compound was standardized as described earlier for the GTN sample. It gave responses linearly related to concentration, with a lower limit of detection of 100 ng. Fig. 2 shows the HPLC separation of NO_3^- and NO_2^-.

We found initially that GTN decomposed very slowly at pH 7.4. Decomposition occurred slightly faster in an acidic environment (0.1 N HCl), and rapidly at pH values of 11 and 13 (Fig. 3). Similar observations were made with 1,2-GDN and 1,3-GDN. Concentrations of all 3 compounds declined exponentially at pH 11 (Fig. 4). The products of the decomposition of GTN were primarily 1,2-GDN, 1,3-GDN and inorganic nitrate. Small amounts of GMN were detected. These products accounted for all of the decomposition of GTN under the conditions studied. For example, in one experiment at pH 13, 10 min of reaction led to 34.6% decomposition of GTN, and 6.6%, 25.2% and 30.3% formation of 1,2-GDN, 1,3-GDN and inorganic nitrate, respectively. A small amount of GMN was detected qualitatively. In particular, no inorganic nitrite was detected.

1,2-GDN was converted primarily to 1,3-GDN, a hitherto unreported reaction. The chromatographic evidence for this conversion, using the Spherisorb ODS 5

Fig. 1.

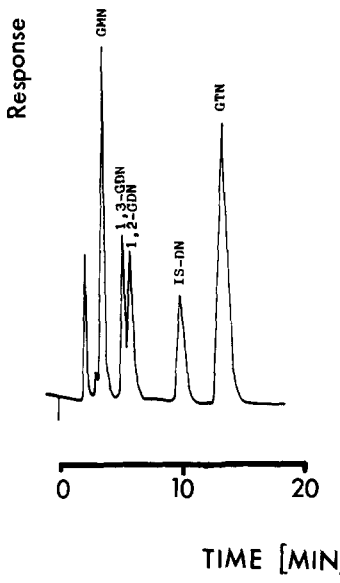

Fig. 2.

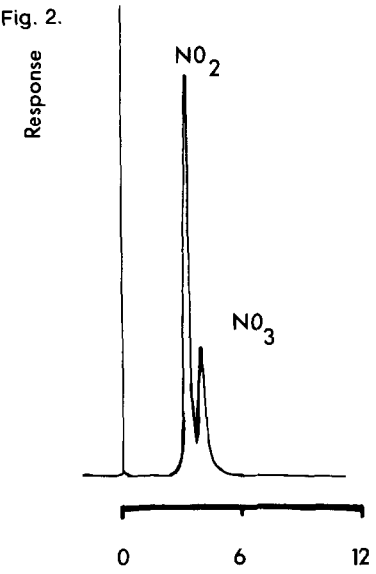

Fig. 1. Liquid chromatogram of a mixture of GTN,IS-DN, 1,2-GDN, 1,3-GDN and GMN at concentrations of 280, 100, 80, 80, and 80 μg/ml, respectively.

Fig. 2. Liquid chromatogram of a mixture of sodium nitrite and potassium nitrate, both at 0.1 mg/ml. The x-axis is marked in retention volume units.

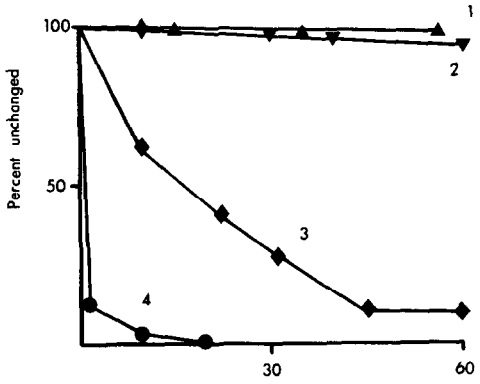

Fig. 3. Disappearance of GTN as a function of time in solutions at pH values of 7.4 (1), 1.0 (2), 11 (3) and 13 (4). Each point is a single determination, and each line is a set of determinations in a single tube. The reaction temperature was 25°C. The graph shows percent of original concentration remaining unchanged at each time point.

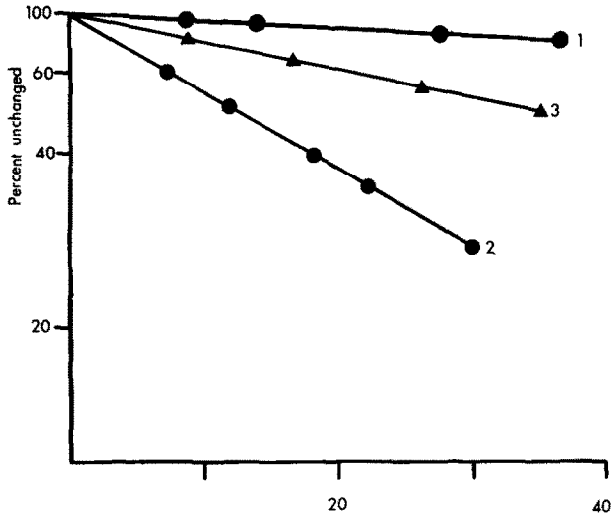

Fig. 4. Exponential disappearance of GTN (1), 1,2-GDN (2) and 1,3-GDN (3) incubated in solutions at pH 11. Each point is the mean of 3–7 determinations, with S.E.M. values less than 5% of the means. The lines were fitted by a linear regression programme. The reaction temperature was 25°C. The graph shows percent of original concentration remaining unchanged at each time point.

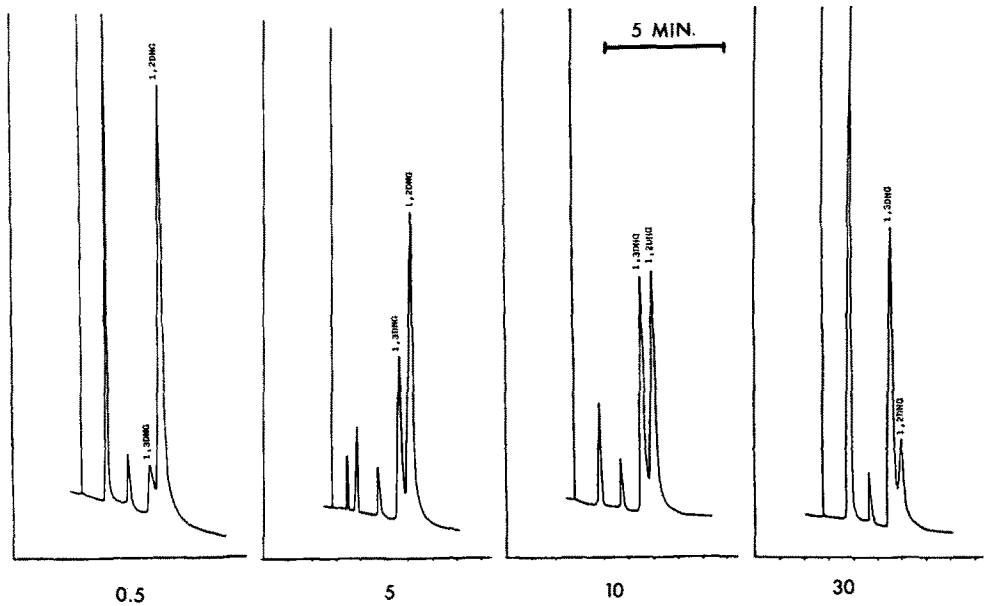

Fig. 5. Chromatographic evidence for conversion of 1,2-GDN (1,2-DNG) to 1,3-GDN (1,3-DNG). The 4 traces show the 1,2-GDN signal decreasing and the 1,3-GDN increasing from 30 s to 30 min after preparation of the solution.

TABLE 1

SLOPE VALUES FOR ARRHENIUS PLOTS, AND CALCULATED VALUES FOR ENERGIES OF ACTIVATION FOR DECOMPOSITION OF GTN, 1,3-GDN AND 1,2-GDN AT pH 10

Compound	Slope ($\times 10^{-3}$)	Energy of activation (J/mol)
GTN	-10.54 ± 0.799	87.7×10^3
1,2-GDN	-11.09 ± 1.300	91.2×10^3
1,3-GDN	-8.69 ± 1.280	72.3 ± 10^3

The slops are given $\pm$S.E. of their estimates. There were no significant differences between the slopes.

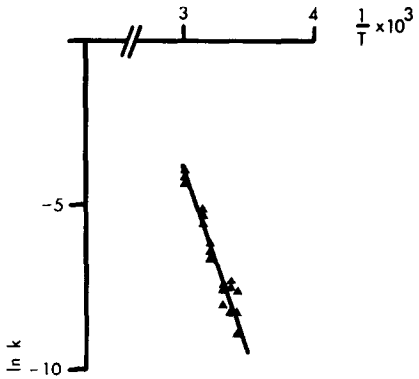

Fig. 6. Arrhenius plot for the decomposition of GTN. The slope was -10.54×10^3, giving an activation energy of 87.7×10^3 J/mol. The correlation coefficient was 0.968.

column, is shown in Fig. 5. Essentially similar data were obtained using the Ultrasil-NH_2 column.

The reactions observed occurred more rapidly at elevated temperatures. The rate constants (k) for disappearance of GTN, 1,2-GDN and 1,3-GDN were measured from exponential decay plots for each compound at 18, 25, 30, 37, 45 and 55°C at pH 11 using a linear regression program and a desk top calculator. Arrhenius plots of ln k against 1/T, where T is absolute temperature, were plotted. The values for the activation energy were calculated from the slopes (Table 1). The Arrhenius plot for GTN decomposition is shown in Fig. 6.

Discussion

The reference materials used included pure GTN/lactose adsorbate, confirmed by standardization to be of USP specification, and standardized reference materials synthesized locally. The GMN sample was a mixture of the two possible isomers. Since this sample was only used to demonstrate specificity in our HPLC systems and formation of GMN from GDN, separation of the two isomers was not conducted.

The organic nitrate HPLC systems were specific for the compounds under investigation. They were essentially the systems of Olsen and Scroggins (1983) and Yu and Goff (1983). Both were extensively validated as stability indicating by their originators. The HPLC separation of NO_3^- and NO_2^- was satisfactory. There was no interference of one ion with the other, and the peaks were symmetrical and sharp. There was no interference of the inorganic ions or the organic nitrates in their respective assays.

The results reported here with GTN contrast with those of Hay (1885) and Bell (1963) in that no nitrite formation was detected. In fact, the probable mechanism for formation of nitrate from nitric acid esters of glycerol in alkaline solutions precludes the formation of nitrite as an intermediate. The work of both Hay and Bell was conducted under conditions which would have allowed reduction of the nitrate formed from the esters to nitrite. Our conditions were closer to the relatively mild conditions prevailing physiologically. The importance of this is that formation of nitrate alone in this work contrasts with metabolism of nitric acid esters in vitro and in vivo, which involves first liberation of nitrite from the esters and then subsequent oxidation of nitrite to nitrate. In fact, our results are a poor model for biochemical denitration for this and other reasons. It should be noted that we detected little decomposition of any of the nitric acid esters at physiological pH. Furthermore, both metabolic denitration and metabolic oxidation of NO_2^- are facilitated by reduced glutathione and occur to some extent when GTN and glutathione, and NO_2^- and glutathione are mixed together in the absence of enzymes (Aburawi and Curry, 1984). The mechanism for denitration of GTN in biological media involves the reduction of the organic nitrate to an unstable organic nitrite, which then hydrolyzes to inorganic nitrite and GDN. Alkali facilitated denitration of GTN with direct formation of NO_3^- thus involves mechanisms totally different from those involved in biochemical changes.

The appearance of 1,2-GDN was transient. 1,2-GDN was rapidly transformed to 1,3-GDN. This transformation may be interpreted as an intramolecular rearrangement with the formation of a transition state. The mechanism is unknown, but it may be analogous to a known rearrangement of glycerol-1- and -2-phosphates (Chargaff, 1942). Glycerol monophosphate in solution is an equilibrium mixture of glycerol-1-monophosphate (87%) and glycerol-2-phosphate (13%). In this case, the intermediate is a cyclic glycerol-1,2-monophosphate. By analogy, in the case of 1,2-GDN, the hydroxyl group of the alkali catalyzing the reaction would attract the proton of the hydroxyl group attached to C3. As a result, the negatively charged oxygen of this group would be attracted to the nitrogen atom of the C2 nitrate group. This has a positive charge induced by the coordinate covalent bond between its nitrogen and one of its oxygen atoms. An unstable cyclic, 1,2-nitro compound would be formed. This would spontaneously convert to 1,3-GDN, possibly with partial back conversion to 1,2-GDN. This potential mechanism is presented as a hypothesis on which to base further work. In particular, no reason can be given at this stage as to why preferential conversion of the intermediate to 1,3-GDN would occur.

The activation energy of a reaction is proportional to the strength of the bond

that suffers breakage (Hirschfelder, 1941). Table 1 shows small differences in the activation energy values for decomposition of the compounds studied. However, linear regression analysis was applied to the graphs of 1/T vs ln k to calculate E_a values. In place of statistically dubious attempts to test for significant differences among the derived values of E_a, the differences between the calculated activation energies were tested for significance by comparing the slopes for each compound with those for the others using *t*-tests. The results of the statistical analysis showed that there were no significant differences between slopes and therefore between the activation energies of the 3 compounds (Table 1). This leads to the conclusion that there is no difference in the thermodynamics of breakage of the bonding of the primary or the secondary nitro-groups.

Acknowledgements

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