REACTIONS OF NITRO SUGARS

II.[1] THE CONVERSION OF METHYL 6-DEOXY-6-NITRO-HEXOPYRANOSIDES INTO DEOXYNITROINOSITOLS BY ALKALINE GLYCOSIDE CLEAVAGE

HANS H. BAER AND WERNER RANK
Department of Chemistry, University of Ottawa, Ottawa, Canada
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ABSTRACT

Methyl 6-deoxy-6-nitro-D-glucopyranoside and methyl 6-deoxy-6-nitro-L-idopyranoside under mild alkaline conditions undergo fission of the pyranoside ring followed by loss of the aglycon and cyclization of the resulting nitro sugars to a mixture of *scyllo* and *myo-1* deoxynitroinositols. A mechanism for the alkaline glycoside cleavage is proposed in which a 5,6-dideoxy-6-nitro-aldos-5-ene is an intermediate. The susceptibility to attack by base of such nitroolefins has been studied using 3-*O*-acetyl-5,6-dideoxy-1,2-*O*-isopropylidene-6-nitro-α-D-*xylo*-hexofuranos-5-ene. It has been shown that this compound, when treated with methanolic sodium hydroxide, is methoxylated in position 5 and that this methoxylation occurs more rapidly than de-*O*-acetylation. The significance of this reaction with regard to alkaline acetal and ketal cleavages in the nitro sugar series is discussed.

INTRODUCTION

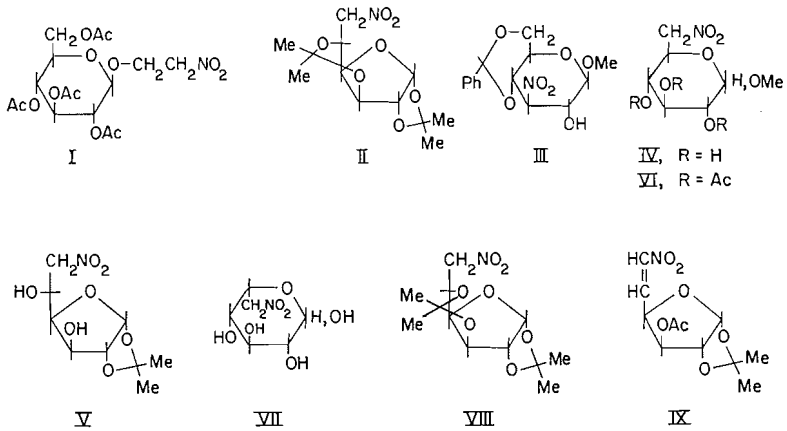
Lability towards alkali of acetals and ketals that bear a primary or secondary nitro group in the β-position has been observed on several occasions. Thus, Helferich and Hase (1) found that the base-catalyzed deacetylation of 2-nitroethyl β-D-glucopyranoside tetraacetate (I) was attended by fission of the glycosidic linkage; Fischer and Baer (2) observed a facile, alkaline removal of the 3,5-*O*-isopropylidene group in 6-deoxy-1,2:3,5-di-*O*-isopropylidene-6-nitro-α-D-glucofuranose (II)[2]; similarly, methyl 4,6-*O*-benzylidene-3-deoxy-3-nitro-β-D-glucopyranoside (III) readily loses benzaldehyde upon treatment with alkali (4); and finally, bis(2-nitrobutoxy)methane is cleaved by aqueous sodium hydroxide at 38° to 2-nitro-1-butanol and formaldehyde (5).

In view of these facts it appeared conceivable that alkyl hexopyranosides bearing a nitro group in the 6-position, e.g. IV, might represent a novel class of alkali-sensitive glycosides (cf. ref. 6), which would differ from Helferich and Hase's labile glycoside in that the activating substituent is located in the sugar moiety rather than the aglycon. If fission occurs it would be expected to take place between the ring oxygen and C-5 rather than between the lactol oxygen and aglycon. Compounds of this type were therefore prepared, and their predicted alkali cleavage was verified.

MATERIALS

Heating of 6-deoxy-1,2-*O*-isopropylidene-6-nitro-α-D-glucofuranose (V) under reflux in methanol containing 1% of hydrogen chloride produced a syrupy α,β mixture of methyl 6-deoxy-6-nitro-D-glucopyranosides (IV). Acetylation of this material produced a crystalline mixture of the 2,3,4-tri-*O*-acetates (VI) from which the pure α-anomer was obtained after lengthy recrystallizations. Since the anomeric configuration was not regarded as being of prime relevance to an initial study of the present problem, the mixtures IV and

[1] *For Part I, see ref. 8.*
[2] *Under the conditions employed (action of dilute methanolic sodium hydroxide for 30 min at room temperature) the 5-epimeric, L-idose analog of II (VIII) appeared more stable. However, recent investigations have shown that in this case complete loss of the 3,5 acetone group occurs within 3 h at 50° (3).*



Formula VII should have OMe instead of OH at position 1.

VI were used in the subsequent work. Similarly, α,β mixtures of methyl 6-deoxy-6-nitro-L-idopyranoside (VII) were prepared. This was done best by methanolysis of crystalline 6-deoxy-1,2:3,5-di-*O*-isopropylidene-6-nitro-β-L-idofuranose (VIII). Glycosidation of free 6-deoxy-6-nitro-L-idose was also possible although some difficulty arose here because of an interesting instability of that sugar, which will be dealt with in an accompanying communication. Finally, it was convenient to subject to methanolysis the readily available, crystalline mixture of V and its 5-epimer, 6-deoxy-1,2-*O*-isopropylidene-6-nitro-β-L-idofuranose. The D-*gluco*-L-*ido* glycoside mixture so obtained (IV + VII) consumed 2 moles of periodate as required for hexopyranosides.

In the course of the studies described below it became desirable to prepare, as a model compound, 3-*O*-acetyl-5,6-dideoxy-1,2-*O*-isopropylidene-6-nitro-α-D-*xylo*-hexofuranos-5-ene (IX). Acetylation of V gave a syrup that was considered to be largely the 3,5-di-*O*-acetate. The infrared spectrum revealed, however, that the acetylation was accompanied to some extent by the generation of a nitroolefin grouping. Since the nitroolefin (IX) rather than a pure diacetate of V was the goal, the crude acetylation product was subjected to a Schmidt–Rutz reaction which produced crystalline IX in 56% yield.[3]

RESULTS AND DISCUSSION

The Alkaline Conversion of the Nitro Glycosides into Nitro Inositols

When the *gluco-ido* glycoside mixture IV + VII was heated for 20 min at 98° in 1.1 equivalents of aqueous $N/100$ sodium hydroxide, the methoxyl content decreased by 90%. Paper chromatography revealed the disappearance of the fast-traveling methyl glycosides and the formation of two slow-moving products. These products also appeared, in the course of several days, when a similar reaction was run at 20°. Treatment of the L-idosides (VII) and of the D-glucoside triacetates (VI) with 1 and 4 equivalents, respectively, of $N/100$ sodium hydroxide for 7 h at 60° resulted in their complete conversion into the same slow-moving products whose chromatographic spot intensities were all approximately equal.

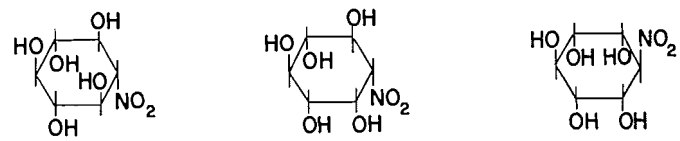
The two reaction products were identified, by co-chromatography with authentic samples, as *scyllo*-deoxynitroinositol (X) (R_{rh} 0.70) and D,L-*myo*-1-deoxynitroinositol (XI) (R_{rh} 0.65). The *scyllo* isomer was isolated in crystalline form.

[3]*The analogous 1,2-O-cyclohexylidene derivative has recently been obtained in amorphous condition (7). Partial olefin formation in the acetylation step is not surprising in view of what has been found in similar systems (cf. ref. 8).*

The conversion of methyl 6-deoxy-6-nitro-α,β-D-glucopyranoside (IV) into deoxynitroinositols at 25° was followed by ultraviolet spectroscopy. This was possible by virtue of a change in absorbance of the nitronate peak that is associated with the reaction. The nitro glycoside (IV), in a buffer solution of pH 10,[4] has an initial ϵ value of 10 000 at λ_{max} 242.5 mμ, whereas nitro inositol (X) shows ϵ 3 000 at λ_{max} 250 mμ under the same conditions and attains an extinction comparable to that of IV only at increased pH (with λ_{max} shifting to 248 mμ) (Fig. 1). This difference evidently reflects a lower acidity of X as compared to IV, which causes a smaller proportion of the absorbing species, the nitronate ion, to exist in equilibrium with undissociated nitro compound at pH 10. In Fig. 2 is depicted the decrease in absorbance that occurred in a 0.004 *M* solution of IV at pH 10 in the course of 11 days, after which time ϵ had reached a value (4 000) close to that of X-nitronate. As with the latter, addition thereafter of sodium hydroxide to pH 12 produced a peak of λ_{max} 247 mμ and ϵ 10 000. The half-life of IV under the conditions employed was about 28 h.

When a similar reaction was carried out at pH 12.6 the picture was similar in the early stages (Fig. 3). Later on a broad, relatively weak absorption developed gradually in the near ultraviolet, which must have arisen from the formation of products other than nitro inositols.[5]

It has been demonstrated by Grosheintz and Fischer (9) that 6-deoxy-6-nitro-D-glucose and -L-idose undergo base-catalyzed cyclizations in which both yield three stereoisomeric deoxynitroinositols later shown (10, 11) to possess *scyllo* (X), D,L-*myo-1* (XI), and *muco-3*



(XII) configurations. Lichtenthaler's (11) detailed studies established that the *muco* isomer arises by kinetic control; when allowed to remain in an alkaline medium it epimerizes to a thermodynamically more stable equilibrium mixture of the *scyllo* and *myo* compounds. It is obvious from our findings, therefore, that the methyl pyranosides of 6-deoxy-6-nitro hexoses readily undergo base-catalyzed deglycosidation to the free sugars, which do not accumulate but cyclize rapidly to inositol derivatives. The process is visualized to proceed via a nitroolefin hemiacetal (XIII) which instantly loses methoxide ion to furnish a nitroolefin aldose (XIV). The latter is attacked by hydroxyl ion, giving hexose nitronate (XV) predisposed to Fischer cyclization.

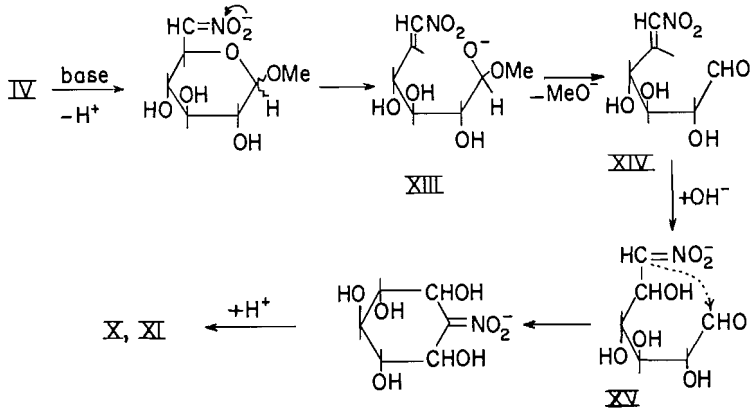
As far as the β-elimination of an acetal oxygen and the formation of a nitroolefin intermediate are concerned, the mechanism suggested here is the same as that proposed (2) in the alkaline removal of the 3,5-*O*-isopropylidene group from 6-deoxy-1,2:3,5-di-*O*-isopropylidene-6-nitro-α-D-glucofuranose (II). In this latter case the nitroolefin presumed to be primarily engendered was the 1,2-acetone derivative of XIV; it evidently added methoxide ion producing two stable, stereoisomeric methyl ethers.[6]

To assess the susceptibility of the hypothetical nitroolefin intermediates to nucleophile

[4]*The actual pH of an unbuffered M/10 sodium IV-nitronate solution was found to be 9.8.*

[5]*The spectral measurements were taken at pH 10 to ensure comparability with the reaction illustrated in Fig. 2. After 11 days no peak in the 247 mμ region was brought forth by measuring at pH 12, but only a slight intensity increase in the long wavelength area.*

[6]*The reaction was carried out in methanol solution (2). Cyclization of the products to inositol derivatives was, of course, prevented by the 1,2-acetone group that is alkali-stable under the conditions employed.*



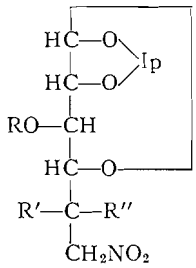
attack, the behavior toward methanolic sodium hydroxide of crystalline 3-*O*-acetyl-5,6-dideoxy-1,2-isopropylidene-6-nitro-α-D-*xylo*-hexofuranos-5-ene (IX) was studied. These experiments, which are described in the next section, clearly proved the extreme ease with which such olefins are liable to add base. As will be seen, the results are in full accord with the mechanism discussed above and earlier (2), and they suggest in addition that in the sequence IV → XIV → XV the ring cleavage is the rate-determining step.

The Action of Base upon Nitroolefin IX

3-*O*-Acetyl-5,6-dideoxy-1,2-*O*-isopropylidene-6-nitro-α-D-*xylo*-hexofuranos-5-ene (IX), owing to its nitroolefin structure, exhibits high-intensity absorption in neutral and acidic solution: λ_{max} 230 mμ (ε 10 500) in cyclohexane; and λ_{max} 234 mμ (ε 11 500) in methanol with and without addition of sulfuric acid. The addition of *excess* sodium hydroxide to a 10^{-4} *M*, methanolic solution caused an instantaneous bathochromic shift to 244–245 mμ (Fig. 4). When the amount of alkali added was limited to two equivalents,[7] however, the 234 mμ peak disappeared in the course of 90 min with a half-life of ca. 12 min (at 26°). Addition of excess hydroxide after 90 min then produced a new peak at 245 mμ (ε 9 500) (Fig. 5). Now the experiment was repeated over an extended period of time with aliquots being withdrawn at intervals. When the reaction was arrested in the aliquots by acidification with acetic acid or by cation exchange the absorption ceased to decrease, thus providing a measure for the amount of remnant nitroolefin. Thin-layer chromatography revealed the nature of the reaction (Fig. 6). Paralleling the decrease in ultraviolet absorbance, the spot of starting material IX (R_f 0.73) disappeared with concomitant appearance of a spot (R_f 0.65) attributable to 3-*O*-acetyl-6-deoxy-1,2-*O*-isopropylidene-5-*O*-methyl-6-nitro-D-glucofuranose (XVI) and -L-idofuranose (XVII), which were not separated. As the reaction continued the new spot gave way to one of R_f 0.53 that could be resolved into two spots by use of a different solvent system. These spots were indistinguishable from those given by 6-deoxy-1,2-*O*-isopropylidene-5-*O*-methyl-6-nitro-D-glucofuranose (XVIII) and -L-idofuranose (XIX) that had been obtained (2) by treatment with methanolic alkali of the diacetone compound II, and the ratio of XVIII and XIX, roughly estimated at 4:1, was found to be the same in both cases. This can be regarded as proof for the nitroolefin structure being an intermediate in the ketal fission and methoxylation of II as earlier proposed.

From an inspection of Figs. 5 and 6 it follows, moreover, that methoxylation of the

[7]*One equivalent to be consumed by saponification of the acetyl group.*



XVI, R = Ac, R′ = H, R″ = OMe
XVII, R = Ac, R′ = OMe, R″ = H
XVIII, R = R′ = H, R″ = OMe
XIX, R = R″ = H, R′ = OMe

olefinic bond in IX occurred at a rate markedly faster than the de-*O*-acetylation which was quite incomplete when, after 1 h, virtually all of IX had reacted. This conclusion was supported by the results of infrared spectroscopy, confirming the ultraviolet and chromatographic data. When the reaction was done at a higher initial concentration of IX (3.65×10^{-2} *M*), the ultraviolet and thin-layer patterns were fully analogous to those in Figs. 5 and 6, although the speed of reaction was considerably greater: after only 20 and 90 s, respectively, the 234 mμ-peak had decreased by 45 and 90%, while on the chromatograms (Fig. 7) a slow spot corresponding to deacetylated material was already visible after 90 s and became the sole spot within 1 h. The infrared spectra also indicate the rapid conversion of the nitroolefin IX into the nitroalkane acetates (XVI + XVII) and the deacetylation of the latter to XVIII and XIX. Figure 7*b* (20 s) shows a reduction by about half, of the (weak) C=C absorption at 1 680 cm^{-1} and, more significantly, the emergence of a nitroalkane peak (1 555 cm^{-1}) in addition to the olefinic nitro peak (1 530 cm^{-1}). Figure 7*c* (90 s) indicates a far-reaching though incomplete replacement of IX by XVI + XVII, since the C=C frequency has now vanished and the originally strong olefinic NO_2 peak is reduced to a shoulder of the newly generated nitroalkane peak. The change in the intensity ratio of the carbonyl and nitro absorptions and the occurrence of a (yet small) hydroxyl band in Fig. 7*c* bears out the beginning of the deacetylation which progresses (Fig. 7*d*,*e*), and after 1 h (Fig. 7*f*) appears to be complete.

EXPERIMENTAL

Paper Chromatography

Paper chromatography was performed using the descending technique on Whatman No. 1 paper with 1-butanol – acetic acid – water (4:1:5 (v/v), upper layer; the lower layer was placed in the bottom of the tank). The spots were made visible by spraying the chromatogram with ammoniacal silver reagent (0.1 *N* $AgNO_3$, 5 *N* NH_3, 2 *N* NaOH, 1:1:2) and heating it over steam. L-Rhamnose served as a reference standard (R_{rh} = 1). Authentic deoxynitroinositols X (R_{rh} 0.70), XI (R_{rh} 0.65), and XII (R_{rh} 1.04) were prepared according to Lichtenthaler (11; cf. also ref. 13).

Thin-Layer Chromatography

Thin-layer chromatography was done on Silica Gel G (E. Merck AG, Darmstadt) activated for 1 h at 110°, with cyclohexane – ether (1:6) (solvent A) and chloroform – acetone (4:1) (solvent B) as irrigating systems. The spots were detected with 1% ceric sulfate in 10% sulfuric acid.

Spectroscopy

Infrared spectra were taken using a Perkin–Elmer Infracord instrument.

Ultraviolet spectra were recorded with a Perkin–Elmer spectrophotometer Model 202.

Methyl 6-Deoxy-6-nitro-α,β-D-glucopyranosides (IV)

A solution of 500 mg of 6-deoxy-1,2-*O*-isopropylidene-6-nitro-α-D-glucofuranose (V) (12) in 50 ml of methanol containing 1% by weight of hydrogen chloride was refluxed for 4 h. Upon cooling of the solution to 25° the hydrogen chloride was removed by anion exchange with Dowex-1-X8 resin (carbonate form) and the filtrate was evaporated *in vacuo*, with 2 portions of methanol being added near the end. There was obtained 425 mg of a colorless syrup (IV) which, on paper chromatography, showed two spots (R_{rh} 1.48 and 1.62) and was free from starting material (R_{rh} 1.88).

Methyl 6-Deoxy-6-nitro-2,3,4-tri-O-acetyl-α,β-D-glucopyranosides (VI)

Glycoside mixture IV (340 mg) and anhydrous sodium acetate (340 mg) in 7 ml of acetic anhydride were heated for 1 h on a steam bath. The mixture was allowed to cool and then stirred into 70 ml of ice water, whereby an ochra-colored, crystalline product separated out. The crude product was exhaustively washed with water and dried; yield 448 mg. After one recrystallization from ethanol the acetate mixture (VI) was obtained as colorless needles melting at 148–151° and giving a correct elemental analysis.

Anal. Calcd. for $C_{13}H_{19}NO_{10}$ (349.3): C, 44.70; H, 5.48; N, 4.01. Found: C, 44.87; H, 5.62; N, 4.20.

A part of the acetate mixture VI (200 mg) was recrystallized eight more times. The melting point was 173–175° ($[\alpha]_D^{23}$ + 103.6° in chloroform) after the fifth, and 179–180° after the eighth and ninth recrystallizations. The final rotation was $[\alpha]_D^{23}$ +145° (*c*, 1 in chloroform). This product (21 mg) was considered to be the pure α-anomer. Its infrared spectrum was in agreement with the assigned structure: no hydroxyl absorption; 1 740 cm^{-1} (acetyl C=O); 1 550 and 1 370 cm^{-1} (asym. and sym. C—NO_2).

Methyl 6-Deoxy-6-nitro-α,β-L-idopyranosides (VII)

A solution of 200 mg of 6-deoxy-1,2:3,5-di-*O*-isopropylidene-6-nitro-β-L-idofuranose (VIII) (12) in 20 ml of methanol containing 1% by weight of hydrogen chloride was heated under reflux for 5 h. The reaction mixture was worked up as described above for IV. A colorless syrup of VII (150 mg; 97%) was obtained which gave two chromatographic spots (R_{rh} 1.50 and 1.65); no starting material and no slow-moving by-products (deoxynitroinositols, cf. ref. 13) were detectable. The product (VII) had a methoxyl content of 13.0% (calcd. 13.9).

Mixture of IV and VII

A crystalline mixture (2 g) of monoacetone compound V and its β-L-*ido* isomer (12) was methanolyzed for 4 h under reflux with 200 ml of 1% methanolic hydrogen chloride. Work-up as described above for IV afforded 1.7 g (95%) of a colorless syrup showing a methoxyl content of 13.15% (calcd. 13.9). Paper chromatography gave the same pattern as the glycosides IV and the glycosides VII.

A sample of the mixture IV + VII (22.3 mg; 0.1 mmole) was oxidized with sodium periodate, and the consumption of oxidant was followed by titration (14). The consumption, in molar equivalents of $NaIO_4$, was 0.93 (5 min) → 1.32 (10 min) → 1.48 (30 min) → 1.75 (70 min) → 2.02 (130 min), and did not increase further.

3-O-Acetyl-5,6-dideoxy-1,2-O-isopropylidene-6-nitro-α-D-xylo-hexofuranos-5-ene (IX)

To a suspension in anhydrous ether (15 ml) of 400 mg of monoacetone compound V (12) was added pyridine (2.4 ml) and acetic anhydride (0.9 ml). The reaction mixture was kept in a refrigerator for 18 h and then extracted three times with 3 ml of water, twice with 2 ml of 10% acetic acid, and twice with 2 ml of water. Finally, the solution was dried over anhydrous sodium sulfate and evaporated with several additions of xylene to give a colorless syrup (390 mg). The infrared spectrum of this syrup exhibited in addition to the expected characteristics of a V-diacetate (acetyl C=O at 1 755 cm^{-1}; C—NO_2 at 1 560 cm^{-1}), bands attributable to the olefin IX (olefinic NO_2 at 1 535 cm^{-1} and C=C at 1 670 cm^{-1}).

The above syrup was dissolved in 6 ml of dry benzene and the solution was heated under reflux for 5 h in the presence of sodium bicarbonate (600 mg). After it had been cooled and filtered the solution was evaporated, leaving a yellow syrup in which according to an infrared spectrum the nitroolefin predominated, but some nitroalkane was still present. The syrup was extracted twice with 10 ml of hot cyclohexane which upon evaporation gave a partly crystalline residue. This residue was taken up in a small amount of ether from which on standing IX crystallized in long prisms, and these were isolated and washed with ice-cold ether. The yield was 179 mg (56%); m.p. 106–107°. The melting point was raised to 109–110° by one recrystallization from ether and then remained constant. $[\alpha]_D^{23}$ +6.5° (*c*, 1 in chloroform). Ultraviolet absorption: λ_{max} 230 mμ (ε 10 500) in cyclohexane; 234 mμ (ε 11 500) in methanol, unchanged on addition of *N*/10 sulfuric acid (1 drop per milliliter). A 60-Mc nuclear magnetic resonance spectrum in deuterochloroform exhibited peaks corresponding to 3 protons each at 8.65 τ and 8.46τ (the two isopropylidene methyls) and at 7.95τ (acetoxy). A signal at 2.79τ that corresponded to two protons was assigned to the olefinic H-5 and H-6. The H-1, H-2, H-3, and H-4 protons gave doublets in the 4–5.3τ region.

Anal. Calcd. for $C_{11}H_{15}NO_7$ (273.3): C, 48.35; H, 5.53; N, 5.13. Found: C, 48.60; H, 5.63; N, 5.15.

Action of Base upon the Nitro Glycosides

An accurately weighed sample (4.70 mg) of the glycoside mixture IV + VII was introduced in the disconnected reaction flask of a micro methoxyl determination apparatus. *N*/100 sodium hydroxide (2.32 ml, 1.1 molar equivalent) was added and the loosely stoppered flask was heated in a steam bath for 20 min. Upon cooling of the solution, carbon dioxide was passed through the inlet tube for 10 min, and the solution was then freeze-dried. Water (1 ml) was added and the freeze-drying was repeated. Methoxyl determination gave 1.34% of OCH_3 corresponding to 9.65% of the theoretical value (and to 10.4% of the value found) for the starting glycoside.

In a similar experiment 12 mg of glycoside was heated for 20 min at 98° in an equivalent amount of *N*/100 sodium hydroxide; the solution was then deionized with Rexyn RG-50 (H^+), concentrated *in vacuo*, and chromatographed. It gave strong spots at R_{rh} 0.66 and R_{rh} 0.71, but no spots in the glycoside area of R_{rh} 1.5–1.65.

A sample of methyl L-idosides VII was dissolved in 1 molar equivalent of $N/100$ sodium hydroxide and heated in a closed vessel for 7 h at 60°. After deionization, chromatography gave spots of approximately equal intensity of X and XI; the glycoside spots had disappeared.

A 10-mg sample of the methyl D-glucoside triacetates VI was treated in the same way but with 4 molar equivalents of base. The material, which was difficultly soluble in the cold, dissolved within a few minutes at 60° as deacetylation proceeded. The results of chromatography were the same as with VII. From a 100-mg sample of VI that was treated analogously a syrup was obtained upon deionization and repeated evaporation with water and then with ethanol. When allowed to stand at 4°, a concentrated solution of the syrup in ethanol deposited crystals of *scyllo* deoxynitroinositol (X). The identity of the product was supported by paper chromatography and infrared spectrum. The melting point was 210–220°; reported: 215° decomp. (9); 224–225° for recrystallized product (11).

Ultraviolet Spectroscopic Observations

Nitroglycoside IV (4.46 mg, 0.02 mmole) was dissolved in 5.0 ml of buffer (pH 10) giving a 4×10^{-3} M

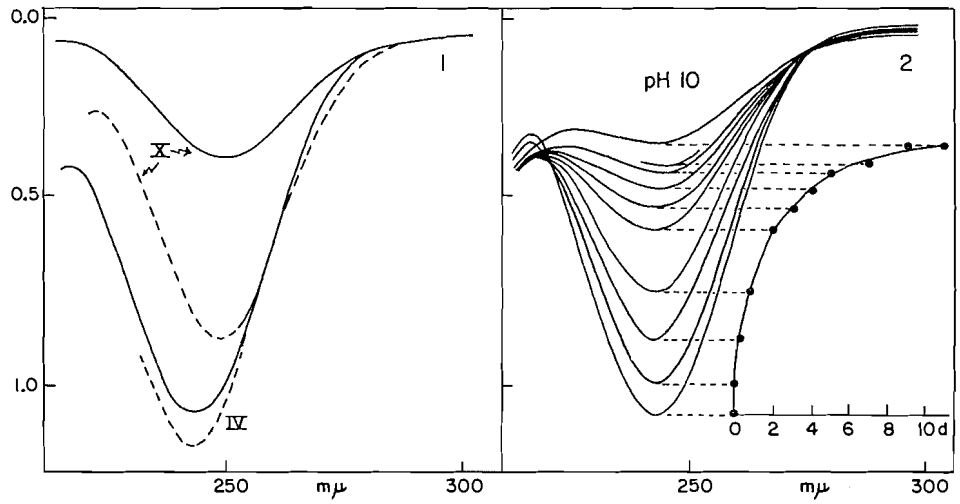


FIG. 1. Ultraviolet spectra of nitroglucoside IV and nitroinositol X at pH 10 (solid lines) and at pH 12 (broken lines); 10^{-4} M solutions in aqueous buffer (cf. Experimental).

FIG. 2. Decrease in absorbance in nitroglucoside IV at pH 10 and room temperature during 11 days. The three lower curves correspond to reaction times of 5 min, 3 h, and 10 h, respectively.

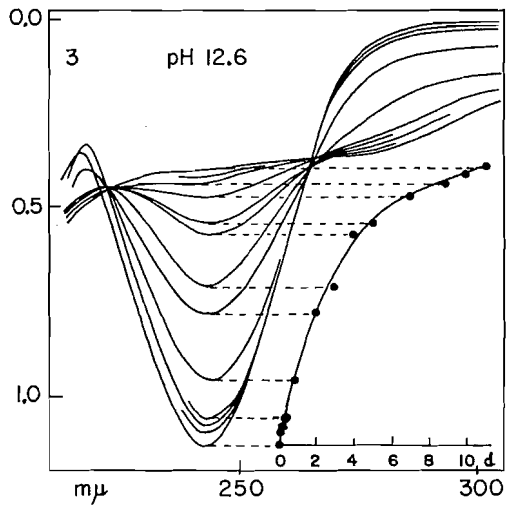


FIG. 3. Decrease in absorbance in nitroglucoside IV at pH 12.6 and room temperature during 11 days.

solution which was allowed to stand at room temperature. The buffer was composed of boric acid, potassium chloride, and sodium hydroxide according to Clark and Lubs (15). For the measurements recorded in Fig. 2, 0.08-ml aliquots of the solution were mixed in the photometer cell with 3 ml of buffer, the concentration in nitro compound thus becoming close to 10^{-4} *M*. The reference cell contained blank buffer. To record also the extinction at pH 12, 0.1 ml of *N* sodium hydroxide was added to the above, 3 ml of buffer. (The pH thus attained was found potentiometrically.)

The data shown in Fig. 3 were obtained analogously, except that IV was dissolved in *N*/10 sodium hydroxide. Again, pH 10 buffer was used as diluent for the measurements.

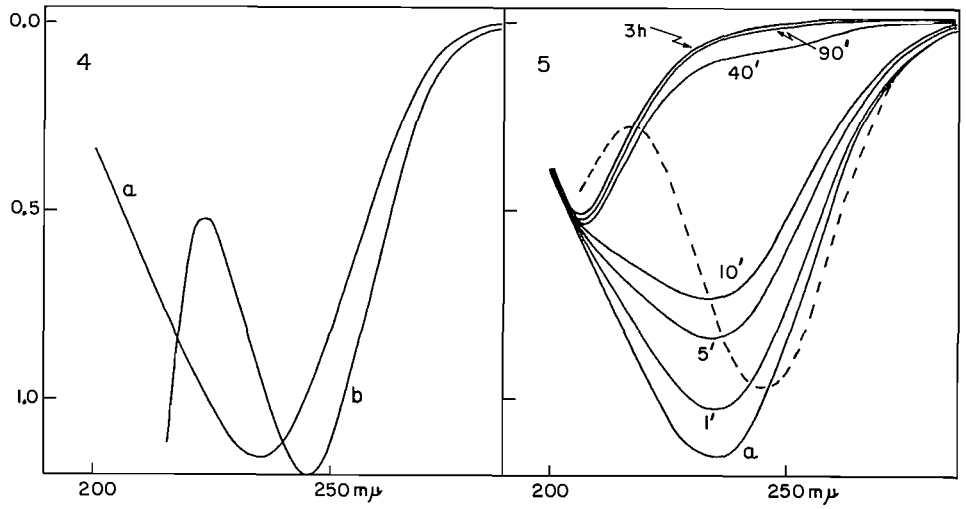


FIG. 4. Nitroolefin IX, 10^{-4} *M* solution in methanol (curve *a*; unchanged upon addition of sulfuric acid); curve *b*, the same after addition of excess sodium hydroxide (0.1 ml *N* NaOH per 3 ml of solution).

FIG. 5. Decrease in absorbance in a 10^{-4} *M* methanolic solution of nitroolefin IX in the presence of 2 equivalents of sodium hydroxide. Curve *a*, IX before the addition of alkali. Broken curve, reaction product at 3 h upon addition of excess alkali (0.1 ml of *N* NaOH per 3 ml of solution).

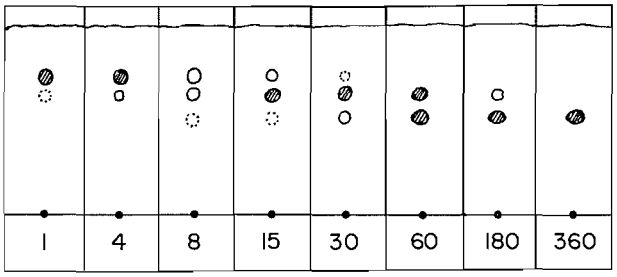


FIG. 6. Thin-layer chromatograms showing the conversion of IX via XVI + XVII into XVIII + XIX (solvent system A). The numbers indicate reaction time in minutes. Strong spots are shaded, medium spots are given as solid circles, trace spots as broken circles.

Action of Base upon Nitroolefin IX

To a 10^{-4} *M* solution of IX in methanol (50 ml) was added 0.1 ml *N*/10 sodium hydroxide solution (2 molar equivalents) and the changes in the ultraviolet spectrum were followed (Fig. 5).

The experiment was repeated with aliquots of 5 ml being withdrawn periodically. The aliquots were deionized by shaking them briefly with a small quantity of Rexyn RG-50 (H^+) that had been washed with methanol and dried in a desiccator. The spectra of the deionized aliquots indicated that the decrease in absorption was arrested. The solutions were then evaporated *in vacuo* and the residues (approximately 0.1 mg) taken up in a few drops of chloroform and applied to thin-layer plates (2.5 × 7.5 cm). Development with solvent A gave the patterns depicted in Fig. 6. The slow-moving spot (R_f 0.53) that occurred as the end product of the reaction was resolved into two spots of R_f 0.46 and 0.39 when larger plates (5 × 20 cm) and solvent B were employed. The following reference compounds were used: XVIII (2) with R_f 0.46 (solvent B)

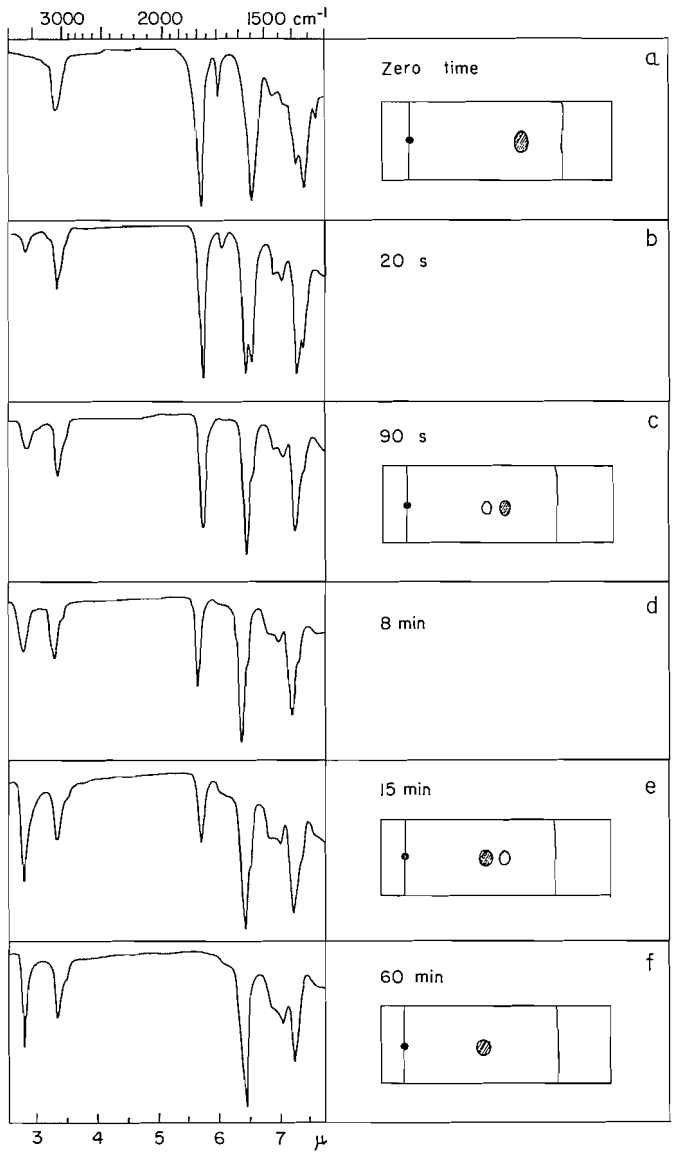


FIG. 7. Infrared spectra and thin-layer chromatograms (solvent A) showing the action of methanolic alkali upon nitroolefin IX.

and 0.53 (solvent A); XIX (2) with R_f 0.39 (solvent B) and 0.53 (solvent A); the product (R_f 0.65 in solvent A) that was obtained by acetylation of XIX (sodium acetate – acetic anhydride; 3 min at 130°).

For the experiments represented by Fig. 7, a solution was made of IX (20 mg) in methanol (20 ml) to which 0.15 ml of *N* NaOH was added. This corresponded to an initial concentration of 3.65×10^{-2} *M* in IX. Aliquots of 2 ml were taken at given intervals and neutralized using *N*/10 hydrochloric acid. From the aliquots withdrawn after 20 s to 60 min ultraviolet spectra were obtained, 0.1 ml of each aliquot having been diluted with 3 ml of methanol. The optical densities at 234 mμ were 55% (20 s), 10% (90 s) and almost 0% (60 min) of that given by a sample of IX of equal concentration but free of alkali. The aliquots were then evaporated to dryness *in vacuo* and the residues were taken up in dry chloroform. Thin-layer chromatograms (solvent A) were obtained (Fig. 7) and infrared spectra were taken of films produced by evaporation of the solvent chloroform on warmed sodium chloride plates (Fig. 7).

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