[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, WASHINGTON UNIVERSITY]

Anhydridization of 1-Deoxy-1-nitrohexitols

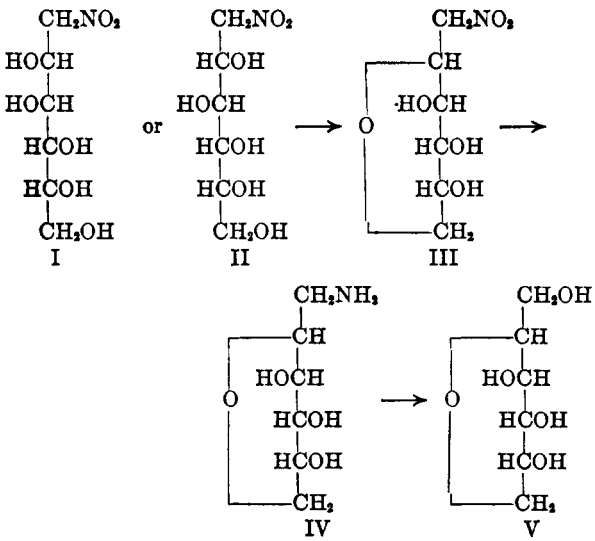
JOHN C. SOWDEN AND MARVIN L. OFTEDAHL

Received October 12, 1960

Heating aqueous solutions of 1-deoxy-1-nitro-D-mannitol *or* 1-deoxy-1-nitro-D-glucitol results in the formation, in about 65% yield, of 2,6-anhydro-1-deoxy-1-nitro-D-mannitol. The latter is also obtained, but in lower yield, by heating 1-deoxy-1-nitro-D-mannitol above its melting point or in aqueous acid. The 2,6-anhydro-1-deoxy-1-nitro-D-mannitol was converted, by reduction to the amine followed by treatment with nitrous acid, to 1,5-anhydro-D-mannitol (styracitol). Similarly, the sirupy mixture of epimeric deoxynitrohexitols obtained by condensing nitromethane with D-xylose yields, upon boiling in aqueous solution, 2,6-anhydro-1-deoxy-1-nitro-D-gulitol. By reduction followed by treatment with nitrous acid, the latter yields 1,5-anhydro-L-glucitol (the enantiomorph of polygalitol). It is considered likely that the α-nitroolefin is an intermediate in the anhydridization reaction.

In an early application[1] of the aldose-nitromethane condensation reaction[2] it was observed that 4,6-*O*-benzylidene-D-glucose and nitromethane in alkaline methanol formed not only the expected 5,7-*O*-benzylidene-1-deoxy-1-nitro-D-*glycero*-D-*gulo*-heptitol but also, in small yield, a 2,6-anhydro-5,7-*O*-benzylidene-1-deoxy-1-nitroheptitol. Subsequently,[3] it was observed that certain sirupy 1-deoxy-1-nitrohexitols, on long standing at room temperature, deposited crystals whose analyses and behavior with periodate revealed them to be 2,6-anhydro-1-deoxy-1-nitrohexitols. This anhydridization of the deoxynitroalditols has now been examined further.

Heating 1-deoxy-1-nitro-D-mannitol (I) above its melting point (133–134°), or in acidic aqueous solution, or simply in water, yields 2,6-anhydro-1-deoxy-1-nitro-D-mannitol (III). The latter is obtained, for example, in 63% yield by refluxing a 10% aqueous solution of I for forty-eight hours. Lower yields of III, 19% and 39% respectively, are obtained by heating I directly at 150° or in 1% aqueous sulfuric acid. The epimer of I, 1-deoxy-1-nitro-D-glucitol (II) also produces III (65%) when heated in 10% aqueous solution. Thus it seems probable that D-*arabino*-3,4,5,6-tetrahydroxy-1-nitro-1-hexene is an intermediate in the anhydridization reaction and that III is formed by addition of the C_6 hydroxyl group across the double bond of the intermediate nitroolefin. Alternatively, the carbonium ion formed by abstraction of hydroxyl from C_2 could be a common intermediate in the anhydridization of the epimeric deoxynitrohexitols, leading to the anhydro compound either directly or by way of the nitroolefin.



The ring structure of III was revealed by the observation that it rapidly consumes two molecular equivalents of periodate with the concomitant formation of one molecular equivalent of formic acid. An initial attempt to determine the configuration of III was made by subjecting it to the conditions of the Nef reaction[4] in the expectation that the product could be converted by reduction to a 1,5-anhydrohexitol. It was found, however, that III is inert to the conditions of the Nef reaction in the temperature range 25° to 100°. This behavior presumably is attributable to steric hindrance, to which the Nef reaction is sensitive.[5] Configuration for III eventually was established through reduction to 1-amino-2,6-anhydro-1-deoxy-D-mannitol (IV) and treatment of the latter with nitrous acid to obtain the known 1,5-anhydro D-mannitol (styracitol) (V).

The side-products of the anhydridization of I are of some interest. D-Mannonic γ-lactone was isolated in 5% yield from the mother liquors of the acid-catalyzed reaction and D-arabinose was isolated similarly in 5% yield from the uncatalyzed, aqueous reaction. In addition, both reaction conditions produced minor amounts of a sirupy substance, isolated chromatographically, which was

(1) J. C. Sowden and H. O. L. Fischer, *J. Am. Chem. Soc.*, **68**, 1511 (1946).

(2) *Cf.* J. C. Sowden, *Advances in Carbohydrate Chem.*, **6**, 291 (1951).

(3) J. C. Sowden, unpublished results.

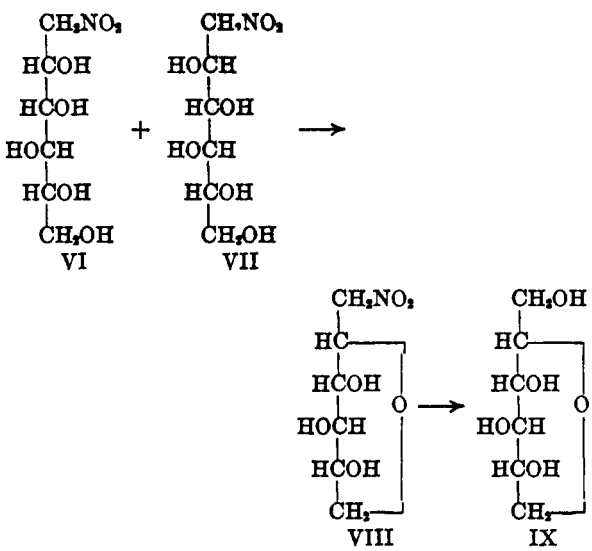
(4) J. U. Nef, *Ann.*, **280**, 263 (1894); J. C. Sowden and H. O. L. Fischer, *J. Am. Chem. Soc.*, **66**, 1312 (1944); W. E. Noland, *Chem. Revs.*, **55**, 137 (1955).

(5) E. E. van Tamelen and R. J. Thiede, *J. Am. Chem. Soc.*, **74**, 2615 (1952).

presumably the epimer of III. The acid-catalyzed hydrolysis of nitromethylene compounds to hydroxylamine and carboxylic acids is well-known,[6] so the above formation of D-mannonic γ-lactone is not unexpected. The formation of D-arabinose can be explained by assuming a partial reversal of the nitromethane-aldose condensation in neutral, aqueous solution. Such a reversal apparently occurs even at room temperature, since aqueous solutions of 1-deoxy-1-nitro-D-mannitol show chromatographic evidence for the production of D-arabinose on long standing.

Since epimeric deoxynitroalditols apparently anhydridize to the same major product with equal ease, our attention was turned next to the sirupy mixture of 1-deoxy-1-nitro-D-gulitol (VI) and 1-deoxy-1-nitro-D-iditol (VII) obtained by the condensation of D-xylose with nitromethane.[7]

Refluxing a 10% aqueous solution of this mixture for forty-eight hours produced 2,6-anhydro-1-deoxy-1-nitro-D-gulitol (VIII), isolated in 46% yield based on D-xylose. The structure and configuration of VIII were established by converting it, through reduction to the amine (not isolated) and treatment of the latter with nitrous acid to give the enantiomorph (IX) of the known 1,5-anhydro-D-glucitol (polygalitol).



The anhydridization of VI + VII also gave a crystalline isomer of VIII, isolated in 1% yield based on D-xylose. Attempts to prove the structure and configuration of this minor product through periodate oxidation did not yield conclusive results. Both III and VIII rapidly consume two molecular equivalents of periodate (ten minutes) with the production of one molecular equivalent of formic acid and no formaldehyde. The observed specific optical rotations of the enantiomorphic dialdehydes thus formed (but not isolated) were +34° and −33.5° respectively. A slow, additional oxidation, presumably initiated at the original C_2, was observed for both compounds, consuming a further two molecular equivalents of periodate and producing an additional molecular equivalent of formic acid in about five days. Optical activity disappeared during this slow, secondary oxidation. With the minor anhydro product from D-xylose, consumption of the first two molecular equivalents of periodate was slow and erratic, requiring from one to six hours. During this time, no measurable formaldehyde was produced, but the observed production of acid also was very low and the original specific optical rotation of −38° (based on the starting material) changed only moderately in a less-negative direction. Final periodate consumption again was near four molecular equivalents with the production of two molecular equivalents of acid. It was not possible to decide from these data between a 2,6-anhydro structure (e.g. 2,6-anhydro-1-deoxy-1-nitro-D-iditol) and a 2,5-anhydro structure for this product.

It is noteworthy that the anhydridization of the 1-deoxy-1-nitrohexitols described above leads preferentially to that configuration at C_2 in the 2,6-anhydro product which allows the least hindered chair conformation. Thus, 2,6-anhydro-1-deoxy-1-nitro-D-mannitol, which can assume a chair conformation with only one bulky group (hydroxyl) axial, is formed to a much larger extent than is the epimeric D-glucitol derivative, which must have either two hydroxyls or one hydroxyl and the nitromethylene group axial in the chair conformation. Similarly, 2,6-anhydro-1-deoxy-1-nitro-D-gulitol, which can have all bulky groups equatorial, apparently is formed in great excess over the epimeric D-iditol derivative, which must have either three hydroxyls or the nitromethylene group axial in the chair conformation.

The sugar nitroalcohols, anhydronitroalcohols and α-nitroolefins show interesting similarities in structure and behavior to the sugar disulfones and their related anhydro and α-olefin derivatives.[8]

EXPERIMENTAL

2,6-Anhydro-1-deoxy-1-nitro-D-mannitol. (a) 1-Deoxy-1-nitro-D-mannitol[9] (5.68 g., m.p. 133–134°) was heated at 150° for 70 min. The melt then weighed 5.16 g., the weight loss corresponding approximately to one molecular equivalent of water. Crystallization from a small volume of ethanol yielded 0.97 g. (18.6%) of 2,6-anhydro-1-deoxy-1-nitro-D-mannitol, m.p. 170–171° and $[\alpha]_D^{25}$ −52.5° in water, *c* 4.

Anal. Calcd. for $C_6H_{11}O_6N$: C, 37.3; H, 5.74; N, 7.25. Found: C, 37.2; H, 5.77; N, 6.88.

(6) V. Meyer and C. Wurster, *Ber.*, **6**, 1168 (1873); H. B. Hass and Elizabeth F. Riley, *Chem. Revs.*, **32**, 373 (1943).

(7) J. C. Sowden and H. O. L. Fischer, *J. Am. Chem. Soc.*, **69**, 1048 (1947).

(8) *Cf.* D. L. MacDonald and H. O. L. Fischer, *J. Am. Chem. Soc.*, **74**, 2087 (1952); R. Barker and D. L. MacDonald, *J. Am. Chem. Soc.*, **82**, 2297 (1960).

(9) J. C. Sowden and H. O. L. Fischer, *J. Am. Chem. Soc.*, **69**, 1963 (1947).

Acetylation with acetic anhydride containing a trace of sulfuric acid gave the triacetate in 75% yield. After recrystallization from ether-petroleum ether (b.p. 33–58°), this product showed m.p. 77–78° and $[\alpha]_D^{25}$ −69° in chloroform, *c* 6.

Anal. Calcd. for $C_{12}H_{17}O_9N$: C, 45.1; H, 5.37; N, 4.39. Found: C, 45.3,H, 5.51; N, 4.27.

(b) A solution containing 10 g. of 1-deoxy-1-nitro-D-mannitol in 100 ml. of 1% sulfuric acid was refluxed until the optical rotation became constant (48 hr.). After ion-exchange to remove sulfuric acid, concentration and recrystallization from ethanol yielded 3.55 g. (39%) of 2,6-anhydro-1-deoxy-1-nitro-D-mannitol, m.p. 170–171°.

Descending paper chromatography of a sample of the mother liquors on Whatman no. 1 paper, using the upper phase of 1-butanol-ethanol-water (4:1:5) and periodate-benzidine spray,[10] showed the presence of 1-deoxy-1-nitro-D-mannitol (R_f 0.33), the 2,6-anhydro product (R_f 0.49), D-mannonic γ-lactone (R_f 0.21) and a fourth component (R_f 0.59) which presumably was 2,6-anhydro-1-deoxy-1-nitro-D-glucitol. A sample of this latter component isolated chromatographically, failed to crystallize or to yield a crystalline acetylation product.

Seeding the sirupy mother liquors with D-mannonic γ-lactone produced, after recrystallization from aqueous ethanol, 0.44 g. (5.2%) of this product,[11] m.p. and mixed m.p. 149–151° and $[\alpha]_D^{25}$ +52.8° initial in water, *c* 4.

(c) A solution of 20 g. of 1-deoxy-1-nitro-D-mannitol in 200 ml. of water was refluxed until the optical rotation became constant (48 hr.). Concentration and recrystallization then yielded 11.5 g. (63%) of 2,6-anhydro-1-deoxy-1-nitro-D-mannitol, m.p. 170–171°. Paper chromatography of a sample of the mother liquors, as described in (b), showed the presence of starting material, the major anhydro product, the presumed 2,6-anhydro-1-deoxy-1-nitro-D-glucitol and D-arabinose (R_f 0.19). The latter (m.p. 157–159°; $[\alpha]_D^{25}$ −102° equil. in water, *c* 1) was isolated in approximately 5% yield by seeding the sirupy mother liquors.

When 1-deoxy-1-nitro-D-glucitol[9] was heated in water as above, the major product (65%) again was 2,6-anhydro-1-deoxy-1-nitro-D-mannitol, m.p. and mixed m.p. 170–171° and $[\alpha]_D^{25}$ −52.6° in water, *c* 4.5.

Oxidation of 2,6-anhydro-1-deoxy-1-nitro-D-mannitol in aqueous solution with periodate showed the consumption of two molecular equivalents of oxidant after 10 min. with the production of one molecular equivalent of formic acid and no formaldehyde. At this stage, the specific optical rotation (based on the dialdehyde) was +34°. After 120 hr., a total consumption of four molecular equivalents of periodate was observed, with the production of two molecular equivalents of formic acid, to give an optically-inactive solution.

Subjecting 2,6-anhydro-1-deoxy-1-nitro-D-mannitol to the conditions of the Nef reaction,[4] using either hydrochloric or sulfuric acid and reaction temperatures from 25° to 100°, resulted in all instances in nearly-quantitative recovery of the starting material.

1-Amino-2,6-anhydro-1-deoxy-D-mannitol. A solution containing 1 g. of 2,6-anhydro-1-deoxy-1-nitro-D-mannitol in 30 ml. of water was treated with 200 mg. of Adams catalyst and hydrogen at atmospheric pressure. Hydrogenation was complete in about 10 hr. Filtration onto a slight excess of oxalic acid dihydrate followed by concentration at reduced pressure, finally with ethanol, yielded 1.0 g. (85%) of 1-amino-2,6-anhydro-1-deoxy-D-mannitol oxalate monohydrate, m.p. 128–131° and $[\alpha]_D^{25}$ −39.5 in water, *c* 4. The same product was obtained in 70% yield when Raney nickel was employed as the hydrogenation catalyst.

Anal. Calcd. for $C_7H_{16}O_7N$: C, 37.2; H, 7.13; N, 6.19. Found: C, 37.0; H, 7.07; N, 5.95.

Heating the monohydrate at 100° and 0.1 mm. for 72 hr. gave the anhydrous oxalate salt, m.p. 124–125° and $[\alpha]_D^{25}$ −42.6° in water, *c* 5.

1,5-Anhydro-D-mannitol (Styracitol). An amount of 2.1 g. of 2,6-anhydro-1-deoxy-1-nitro-D-mannitol was reduced as above with hydrogen and Adams catalyst, with the addition of 1 ml. of glacial acetic acid to the solution. The amine solution was filtered and treated with 0.95 g. of sodium nitrite and an additional 1 ml. of glacial acetic acid. After 24 hr., the solution was de-ionized over Dowex-50 and Duolite A-4 resins and concentrated at reduced pressure to a sirup. The latter was crystallized from a small volume of ethanol to give 0.74 g. (41.5%) of crude 1,5-anhydro-D-mannitol, m.p. 147–151°. Recrystallization from ethanol gave the pure anhydroalditol,[12] m.p. and mixed m.p 154–155° and $[\alpha]_D^{25}$ −50.6° in water, *c* 4.4.

2,6-Anhydro-1-deoxy-1-nitro-D-gulitol. A suspension of 50 g. of D-xylose in 100 ml. of methanol and 180 ml. of nitromethane was stirred with a solution containing 10.8 g. of sodium in 350 ml. of methanol for 24 hr. The precipitated sodium deoxynitroalditols then were collected by filtration, washed with cold methanol, dissolved in 500 ml. of ice water and de-ionized over Dowex-50 resin. The effluent was partially concentrated at reduced pressure and the weight of the residual solution then adjusted to 660 g. with water. This solution was refluxed for 48 hr., decolorized with carbon, and concentrated to a sirup. Further concentration from ethanol then yielded 32.2 g. (50%) of crude, crystalline anhydrodeoxynitroalditols, m.p. 125–133°. Recrystallization from ethanol provided 29.6 g. (46%) of 2,6-anhydro-1-deoxy-1-nitro-D-gulitol, m.p. 135–136° and $[\alpha]_D^{25}$ −15.2° in water, *c* 4. In initial experiments, this product was obtained in a dimorphic form, m.p. 115–116°, which readily gave the higher-melting form on seeding.

Anal. Calcd. for $C_6H_{11}O_6N$: C, 37.3; H, 5.74; N, 7.25. Found: C, 37.5; H, 6.00; N, 7.19.

From the mother liquors of the above separation was obtained 0.65 g. (1%) of a second isomer, m.p. 169–170° and $[\alpha]_D^{25}$ −38.3° in water, *c* 3.3.

Anal. Calcd. for $C_6H_{11}O_6N$: C, 37.3; H, 5.74. Found: C, 37.5; H, 5.74.

Periodate oxidation of the 2,6-anhydro-1-deoxy-1-nitro-D-gulitol paralleled that of 2,6-anhydro-1-deoxy-1-nitro-D-mannitol described above, except that the specific optical rotation of the intermediate dialdehyde in this instance was −33.5°. Successive, small-scale periodate oxidations of the minor anhydro isomer gave erratic results with regard to rate, acid produced, and change in optical rotation. These have been discussed in general terms in the introduction.

1,5-Anhydro-L-glucitol ("L-Polygalitol"). A solution containing 5.0 g. of 2,6-anhydro-1-deoxy-1-nitro-D-gulitol and 6 ml. of glacial acetic acid in 65 ml. of water was reduced with hydrogen at atmospheric pressure in the presence of 1.0 g. of Adams catalyst. Hydrogenation was complete in 24 hr. Following filtration to remove catalyst, the solution was treated with an additional 2 ml. of glacial acetic acid and 2.57 g. of sodium nitrite and allowed to stand for 24 hr. It then was de-ionized over Dowex-50 and Duolite A-4 resins and concentrated to a thin sirup. The latter, on seeding with 1,5-anhydro-D-glucitol,[13] slowly crystallized to give 2.28 g. (51%) of crude, 1,5-anhydro-L-glucitol, m.p. 112–17°. Recrystallization from ethanol gave the pure anhydroalditol, m.p. 141–142° and $[\alpha]_D^{25}$ −40.4° in water, *c* 2.6.

Anal. Calcd. for $C_6H_{12}O_5$: C, 43.9; H, 7.36. Found: C, 44.0; H, 7.42.

Richtmyer and Hudson[13] report m.p. 141–142° and $[\alpha]_D^{25}$ +42.5° in water for 1,5-anhydro-D-glucitol (polygalitol).

(10) M. Viscontini, D. Hoch, and P. Karrer, *Helv. Chim. Acta*, **38**, 642 (1955).

(11) E. Fischer and J. Hirschberger, *Ber.*, **22**, 3222 (1889).

(12) L. Zervas, *Ber.*, **63**, 1689 (1930).

(13) N. K. Richtmyer and C. S. Hudson, *J. Am. Chem. Soc.*, **65**, 64 (1943).

The enantiomorphs showed identical x-ray powder diffraction patterns.[14]

(14) We are indebted to Dr. A. V. Guzzo of this laboratory for the x-ray comparison.

Acknowledgment. The authors wish to acknowledge the generous support of the Corn Industries Research Foundation, Washington, D. C., during the course of this work.

St. Louis, Mo.

[Contribution from the Warner-Lambert Research Institute]

Reaction of 4-Hydroxy-3,5-diiodophenylpyruvic Acid with 3,5-Diiodotyrosine

ROBERT I. MELTZER and R. JOHN STANABACK

Received September 2, 1960

4-Hydroxy-3,5-diiodophenylpyruvic acid readily reacts with 3,5-diiodotyrosine and with *N*-acetyl-3,5-diiodotyrosine to give thyroxine and *N*-acetylthyroxine, respectively. The presence of an oxidizing agent is required.

In 1939 von Mutzenbecher[1] reported that incubation of weakly basic solutions of 3,5-diiodotyrosine at 38° gave rise after a few days to small amounts of thyroxine. Part of the interest in this reaction has been based on the reaction's potential for the synthesis of thyroxine and its analogs; part of the interest has been based on the speculation that knowledge of the *in vivo* formation of thyroxine might be gained by an understanding of this *in vitro* reaction.

Among the postulates on the course of the reaction has been the suggestion that a molecule of 3,5-diiodotyrosine is first converted to 4-hydroxy-3,5-diiodophenylpyruvic acid. This is then followed by reaction of such a molecule with another molecule of 3,5-diiodotyrosine to form thyroxine.

Hillmann[2] investigated the possibility of such a coupling and reported that under his unspecified conditions, a yield of about 3% was obtained when 4-hydroxy-3,5-diiodophenylpyruvic acid was allowed to react with either 3,5-diiodotyrosine or *N*-acetyl-3,5-diiodotyrosine. Interestingly, Hillmann reported that oxygen was detrimental to the reaction. Since it had already been shown[3] that oxygen was necessary for the conversion of 3,5-diiodotyrosine to thyroxine, Hillmann's work implied that the conversion of 3,5-diiodotyrosine to 4-hydroxy-3,5-diiodophenylpyruvic acid occurred by oxidation, but that the subsequent coupling did not entail oxidation. The subsequent report by Pitt-Rivers and James[4] on the isolation of ϵ-*N*-hydroxypyruvyl-α-acetyllysine from an incubation of ϵ-*N*-(N-acetyl-3,5-diiodotyrosyl)-α-*N*-acetyllysine, strengthened our opinion that the coupling step was an oxidative process. We, therefore, decided to carry out a coupling of 3,5-diiodotyrosine with 4-hydroxy-3,5-diiodophenylpyruvic acid.

When we added 4-hydroxy-3,5-diiodophenylpyruvic acid to 3,5-diiodotyrosine in a borate buffered (*p*H 7.8) solution kept saturated with oxygen, within a very short time, we obtained 10% yields of crude thyroxine. If either the pyruvic acid or the oxygen was omitted, no product was obtained. When the addition of the 4-hydroxy-3,5-diiodophenylpyruvic acid was made in small portions or dropwise, as a 1-butanol solution, and when a second phase such as 1-butanol, chloroform, carbon tetrachloride, or toluene was present, yields of crude thyroxine well in excess of 20% could be obtained. The instability of 4-hydroxy-3,5-diiodophenylpyruvic acid under the conditions of the reaction, and the need for sufficient oxygen to be present for the occurrence of the desired reaction, are probably the reasons for the increased yield on the portionwise addition of the pyruvic acid. Because of the instability of the 4-hydroxy-3,5-diiodophenylpyruvic acid in solution and the resulting losses, an excess was desirable. The maximum yield was obtained when approximately 40% excess of 4-hydroxy-3,5-diiodophenylpyruvic acid was used.

Some oxidants other than oxygen were also tried. Potassium ferricyanide in the place of oxygen, or in addition to oxygen, was unsatisfactory. Iodic acid at *p*H 6.5–7, however, could take the place of oxygen, although the yield was decreased. Similarly, hydrogen peroxide or *t*-butyl peroxide could be used in place of oxygen, but again at the expense of some of the yield. When, however, *t*-butyl peroxide was used in catalytic amounts in addition to oxygen, yields of almost 30% were obtainable.

The literature reports[5] that incubations in which *N*-acetyl-3,5-diiodotyrosine alone is used, usually

(1) P. von Mutzenbecher, *J. physiol. Chem.*, **261**, 253 (1939).

(2) G. Hillmann, *J. Naturforschung*, **11b**, 474 (1956).

(3) C. R. Harington and R. V. Pitt-Rivers, *Biochem. J.*, **39**, 157 (1945).

(4) R. V. Pitt-Rivers and A. T. James, *Biochem. J.*, **70**, 173 (1958).

(5) R. V. Pitt-Rivers, *Biochem. J.*, **43**, 223 (1948).