[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF THE OHIO STATE UNIVERSITY]

Nitrated Aldonic Acids

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D-Gluconamide and D-galactonamide have been nitrated by the method of Caesar and Goldfrank to yield the pentanitrates which on deamination with nitrosyl chloride produced the pentanitrates of D-gluconic and D-galactonic acids. The methyl esters of the latter were obtained with diazomethane. These nitrated amides and methyl esters were compatible with cellulose nitrate. The compounds were explosive and were unstable toward moisture.

Nitrates of hydroxy acids and their derivatives have been little investigated. The liquid lactic acid nitrate[2,3] and the crystalline L-(+)-tartaric acid dinitrate[4-6] have long been known; they are unstable and undergo interesting and well established transformations. Duval[7] prepared glycolic acid nitrate, and glyceric acid dinitrate in crystalline form. Isoamyl lactate nitrate[8] and the nitrates of methyl,[3] propyl[8] and butyl[8] glycolates have been reported as liquids. It was the objective of the work herein recorded to prepare the pentanitrate of an aldonic acid. It was considered that the carboxylate ester of such a substance might be a suitable explosive plasticizer for cellulose nitrate. In addition, the ease of removal of the nitrate group by reductive hydrolysis[9,10] offered promise for the utilization of this function as a blocking group in carbohydrate syntheses.

The crystalline pentanitrates of D-gluconic and D-galactonic acids were prepared through the general procedure established in the acetate series.[11,12] After preliminary failures with the usual nitration methods, the crystalline pentanitrates of D-gluconamide and D-galactonamide were successfully obtained in high yields through the excellent general nitration procedure of Caesar and Goldfrank,[13] who added sodium fluoride to remove the nitric acid formed in the nitration procedures[14,15] which utilize nitrogen pentoxide in halogenated solvents. A failure to produce a fully nitrated D-gluconamide by nitration with acetic anhydride and nitric acid (98.5%) below 0° has been reported by Filbert.[16]

The crystalline pentanitrates of D-gluconic and D-galactonic acids were formed by deamination of the amides with nitrosyl chloride[12] and from these the crystalline methyl esters were obtained with diazomethane. The preparative directions herein recorded are quite critical and result from an extensive study of the pertinent experimental factors. Thus, an excess of diazomethane leads to undesirable side reactions in the final esterification while a large excess of nitrosyl chloride is requisite in the deamination procedure.

All of these crystalline products were explosive and could be detonated thermally or by percussion. When heated slowly in small quantity they decomposed completely to gaseous products at their melting points. They were stable at room temperature when stored under anhydrous conditions but underwent a slow decomposition when exposed to a moist atmosphere. Strangely, the methyl ester pentanitrates were the least stable. Possibly these were sensitized by the presence of difficultly removable decomposition products introduced by reaction with diazomethane. The amides and methyl esters were compatible with cellulose nitrate and formed clear films which slowly turned yellow, a reaction which was inhibited but not stopped by the incorporation of diphenylamine in the composition. Thus the inherent instability of these substances makes them unsuitable as explosive plasticizers for cellulose nitrate. Likewise, this same property presented their utilization in further chemical reactions. D-Galactonyl chloride pentanitrate crystallized readily but was so unstable toward moisture that it could not be characterized. No conditions could be found for the utilization of D-galactonic acid pentanitrate in direct esterification reactions employing acid catalysis.

Experimental

General.—Experiments were first carried out in small quantity runs with the derivatives of D-galactonic acid because of their excellent crystallizing properties. After the experimental conditions were defined and the nature of the products determined, the results were extended to the D-gluconic acid series. Finally, the quantities of materials handled were increased to 10–20 g.

Preparation of D-Gluconamide and D-Galactonamide.—These substances were prepared by the liquid ammonia ammonolysis of the lactones as described by Glattfeld and Macmillan[17] except that the crude products were recrystallized by solution in water at 25° followed by the addition of absolute ethanol at the same temperature.

D-Galactonamide Pentanitrate.—D-Galactonamide was nitrated by a modification of the procedure of Caesar and Goldfrank.[13] Nitrogen pentoxide was prepared according to these authors except for the inclusion in the three-necked

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(2) L. Henry, *Ber.*, **12**, 1837 (1879).

(3) H. Duval, *Compt. rend.*, **137**, 1262 (1903); *Bull. soc. chim.*, [3] **31**, 243 (1904).

(4) V. Dessaignes, *Compt. rend.*, **34**, 731 (1852).

(5) A. Kekulé, *Ann.*, **221**, 230 (1883).

(6) A. Lachman, THIS JOURNAL, **43**, 577 (1921).

(7) H. Duval, *Compt. rend.*, **137**, 571 (1903); *Bull. soc. chim.*, [3] **29**, 601 (1903).

(8) S. E. Forman, C. J. Carr and J. C. Krantz, Jr., *J. Am. Pharm. Assoc., Scientific Edition*, **30**, 132 (1941).

(9) L. P. Kuhn, THIS JOURNAL, **68**, 1761 (1946); **73**, 1510 (1951).

(10) L. M. Soffer, E. W. Parrotta and J. Di Domenico, *ibid.*, **74**, 5301 (1952).

(11) C. D. Hurd and J. C. Sowden, *ibid.*, **60**, 235 (1938).

(12) M. L. Wolfrom, M. Konigsberg and D. I. Weisblat, *ibid.*, **61**, 574 (1939).

(13) G. V. Caesar and M. Goldfrank, *ibid.*, **63**, 372 (1946).

(14) R. Dalmon, J. Chedin and L. Brissaud, *Compt. rend.*, **201**, 664 (1935).

(15) J. W. H. Oldham, cited by D. J. Bell and R. L. M. Synge, *J. Chem. Soc.*, 1711 (1937).

(16) W. F. Filbert (to E. I. du Pont de Nemours and Co.), U. S. Patent 2,443,903 (1948).

(17) J. W. E. Glattfeld and D. Macmillan, THIS JOURNAL, **56**, 2481 (1934).

reaction flask of a glass stirring rod sealed with heavy walled rubber tubing into a rotatable adapter fitted into the center opening of the flask so that the rod could be manually operated. This device allowed the reaction to be controlled more uniformly.

The nitration solution was freshly prepared to contain 18 g. of nitrogen pentoxide and 2.5 g. of nitrogen tetroxide per 100 ml. of anhydrous ethanol-free chloroform.[18] An amount of 750 g. of this nitrating solution and 12 g. of sodium fluoride were cooled to −5° and D-galactonamide (9 g., previously dried in a desiccator) was added slowly with stirring. The nitration was allowed to continue for 35 min. at 15°. The reaction mixture was filtered by suction through a sintered-glass funnel and the aldonamide nitrate was immediately washed with about 150 ml. of anhydrous ethanol-free chloroform at 0°. To ensure complete removal of sodium fluoride (which causes decomposition of the aldonamide nitrate in later processing) and excess nitrating agent, the nitrate was made into a slurry successively with each of the following: three portions of chloroform at 0°, two portions of ice-cold water, four portions of water at 20°, five portions of water at 40–50°. Before each new portion of liquid was added, suction was applied to remove the preceding one. The crude nitrate was then dried in a desiccator over sodium hydroxide pellets and phosphorus pentoxide; yield 16.6 g. (86%).

The crude nitrate (16 g.) was dissolved in excess methanol (about 600 ml. at 20°) and the solution was then filtered. To the filtrate, which was vigorously stirred, water (also at a temperature of about 20°) was added dropwise until the cloudpoint was reached. The mixture was allowed to stand at room temperature for about 0.5 hr. and then for 3 hr. at 0°. Separation of long, slender crystals usually occurred very rapidly. Subsequent recrystallizations were effected from methanol (50°)–water (60°) in the same manner as the first recrystallization; m.p. 168° (dec., cor.[19]), $[\alpha]^{28}D$ +13° (*c* 1.3, acetone). D-Galactonamide pentanitrate was soluble in ethanol, methanol and dioxane. It was insoluble in ether, light petroleum ether, chloroform and water.

Anal. Calcd. for $C_6H_8O_{16}N_6$: C, 17.15; H, 1.92; N, 20.01. Found: C, 17.23; H, 1.58; N, 19.97.

No isolable product was obtained on nitration with nitric acid (100%) in combination with either concentrated sulfuric or anhydrous acetic acid. Storage of the above nitrating reagent (nitrogen pentoxide) at −10° for more than one day led to low yields and incompletely nitrated products.

D-Gluconamide Pentanitrate.—D-Gluconamide (7.5 g.) was nitrated with nitrogen pentoxide (2.3 moles per mole of hydroxyl of the D-gluconamide) in the presence of sodium fluoride (7.5 g.) as described above for the nitration of D-galactonamide; crude yield 15 g. (93%). Pure material was obtained by recrystallizing the nitrate from methanol–water as described above for D-galactonamide pentanitrate; m.p. 147° (dec., cor.), $[\alpha]^{28}D$ +37° (*c* 3.2, acetone). D-Gluconamide pentanitrate exhibited the same solubility characteristics as D-galactonamide pentanitrate.

Anal. Calcd. for $C_6H_8O_{16}N_6$: C, 17.15; H, 1.92; N, 20.01. Found: C, 17.54; H, 1.84; N, 19.86.

D-Galactonic Acid Pentanitrate.—D-Galactonamide pentanitrate (10.7 g.) was deaminated with nitrosyl chloride (400 g.) in anhydrous acetic acid (800 g.) according to a modification of the procedure of Wolfrom, Konigsberg and Weisblat.[12] The reaction mixture was agitated occasionally and kept at 0° for 1 hr. It was then permitted to warm gradually to room temperature. After the reaction was allowed to proceed for about 20 hr., the temperature of the reactants was raised to 40° and most of the nitrosyl chloride was removed under reduced pressure (water aspirator). The solid residue, obtained on solvent removal below 30° with an oil pump, was immediately dissolved in absolute ether (100 ml.) and the solution filtered. Crystallization was effected by adding light petroleum ether (500 ml.) and leaving the mixture stand overnight at 0°; yield 9.5 g. (88%). Pure material was obtained on recrystallization from ether–(light petroleum ether); m.p. 138° (dec., cor.), $[\alpha]^{30}D$ +21° (*c* 3.7, acetone). D-Galactonic acid pentanitrate was soluble in ether, acetone, ethanol and dioxane. It was slightly soluble in chloroform and was insoluble in benzene and water.

Anal. Calcd. for $C_6H_7O_{17}N_5$: C, 17.12; H, 1.68; N, 16.63; equiv. wt., 421.2. Found: C, 17.31; H, 1.82; N, 16.39; equiv. wt. (electrometric titer), 417.

D-Gluconic Acid Pentanitrate.—D-Gluconamide pentanitrate (10.4 g.) was deaminated with nitrosyl chloride (350 g.) in anhydrous acetic acid (700 g.) in the same manner as described above for the deamination of D-galactonamide pentanitrate and the product was similarly recrystallized from ether–(light petroleum ether added); yield 8.7 g. (83%), m.p. 122° (dec., cor.), $[\alpha]^{28}D$ +35° (*c* 4.4, acetone). D-Gluconic acid pentanitrate displayed the same solubility characteristics as D-galactonic acid pentanitrate.

Anal. Calcd. for $C_6H_7O_{17}N_5$: C, 17.12; H, 1.68; N, 16.63. Found: C, 17.44; H, 1.99; N, 16.55.

Methyl D-Galactonate Pentanitrate.—A solution of D-galactonic acid pentanitrate (1.0 g., 0.0024 mole) in dry ether (10 ml.) was poured slowly with stirring into 6.5 ml. of anhydrous ether at 0° containing diazomethane[20] (0.0024 mole). There was a vigorous evolution of nitrogen gas during the addition of the diazomethane and crystallization took place soon after the completion of the addition. The mixture was kept overnight at −10°; yield of first crop, 0.78 g., m.p. 106–107° (dec., cor.). A second crop was obtained by evaporating the filtrate under reduced pressure to 6 ml. and subsequently adding 20 ml. of light petroleum ether. Crystallization of the product resulted on leaving the mixture stand overnight at −10°; yield of second crop, 0.18 g., total yield 0.96 g. (93%). Pure material was obtained by dissolving the product (0.3 g.) in excess absolute ether (18 ml.) and then slowly adding light petroleum ether (50 ml.). Crystallization of the product resulted when the mixture was maintained overnight at 0°; m.p. 107° (dec., cor.), $[\alpha]^{30}D$ +21° (*c* 3.6, chloroform). Methyl D-galactonate pentanitrate was soluble in chloroform, ethanol, ether and acetone. It was insoluble in light petroleum ether.

Anal. Calcd. for $C_7H_9O_{17}N_5$: C, 19.32; H, 2.08; N, 16.10. Found: C, 19.47; H, 2.04; N, 16.28.

When an excess of diazomethane in ether was used, low yields of the methyl ester were obtained and an oily by-product formed which was difficult to separate from the desired crystalline reaction product.

Methyl D-Gluconate Pentanitrate.—D-Gluconic acid pentanitrate (0.5 g., 0.0012 mole) was esterified with diazomethane (0.0012 mole) in absolute ether (4.7 ml.) and the product was recrystallized from ether–(light petroleum ether) as described above for methyl D-galactonate pentanitrate except that a larger excess of light petroleum ether was required; yield 0.41 g. (79%), m.p. 58° (dec., cor.), $[\alpha]^{30}D$ +34° (*c* 3.6, chloroform).

Anal. Calcd. for $C_7H_9O_{17}N_5$: C, 19.32; H, 2.08; N, 16.10. Found: C, 19.63; H, 2.19; N 16.41.

General Properties of the Nitrates.—All of the above compounds were detonated by a hammer blow on steel and by gently heating a few crystals in an open test-tube behind an explosion screen. All of the purified nitrates showed evidence of rapid decomposition at their melting points where they bubbled violently and oxides of nitrogen were detected by odor. No residue was visible after the nitrate had decomposed. The methyl esters and the amides of the pentanitrates of D-gluconic and D-galactonic acids, in 20% concentration, formed clear films with cellulose nitrate (N, 12.6%), employing ethyl acetate as the solvent. No separation from the film ("blooming") of the nitrated aldonic acid derivatives was noted. The stabilities of the films and of the pure compounds were relatively low. Film stability was not significantly enhanced by the incorporation of 1% diphenylamine. The amides were more stable than the methyl esters. Our preparations of the latter were less stable than the pentanitrates of the acids. In a surveillance period of 8 to 10 months, the purified compounds, maintained at 20–35° in a desiccator, exhibited no visible evidence of decomposition.

D-Galactonic acid pentanitrate reacted with phosphorus pentachloride in anhydrous ether to form a crystalline, halogen-containing substance which was presumbably D-

(18) L. F. Fieser, "Experiments in Organic Chemistry," 2nd ed., D. C. Heath and Co., New York, N. Y., 1941, p. 365.

(19) All melting points were taken on a Fisher–Johns melting point apparatus and are corrected.

(20) F. Arndt, *Org. Syntheses*, **15**, 3, 48 (1935).

galactonyl chloride pentanitrate. It was too unstable for purification and and characterization but was found to decompose in moist air to yield crystalline D-galactonic acid pentanitrate (m.p. 130–133°). Attempts to utilize this substance in esterification reactions were unsuccessful. Likewise, suitable conditions could not be found for the direct esterification of D-galactonic acid pentanitrate with methanol under acid catalysis.

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Constitution of Stachyose[1]

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Partial hydrolysis of stachyose by almond emulsin gives D-galactose and raffinose, sucrose and galactobiose. Acid hydrolysis of stachyose to D-fructose and manninotriose, followed by reduction of the manninotriose to manninotriitol and partial acid hydrolysis gives D-galactose, melibiitol, D-sorbitol and galactobiose. Periodate oxidations of manninotriitol and manninotriose 1-phenylflavazole confirm the presence of a 1,6-linkage between the D-galactose and D-glucose units in stachyose. Stachyose is O-α-D-galactopyranosyl-(1 → 6)-O-α-D-galactopyranosyl-(1 → 6)-O-α-D-glucopyranosyl β-D-fructofuranoside.

Suggested structures[2-7a] for stachyose are at variance on whether there is a 1,4- or a 1,6-linkage between the D-galactose and D-glucose units. In order to distinguish between these possible alternatives, we have subjected stachyose to partial enzymic hydrolysis by almond emulsin, which contains α-galactosidase but which is relatively free from invertase.[8] By interrupting the hydrolysis at an intermediate stage, it has now been possible[9] to identify raffinose as a partial hydrolysis product (others include D-galactose, sucrose and galactobiose). The raffinose was isolated in crystalline form and its identity confirmed by comparison of its X-ray powder pattern with that of an authentic specimen.

In another experiment, stachyose was hydrolyzed by dilute acid to D-fructose and manninotriose,[10] which was reduced to manninotriitol. Manninotriitol, obtained in crystalline form, was subjected to partial acid hydrolysis. Products indicated by paper chromatography were melibiitol, D-galactose, D-sorbitol and galactobiose. The melibiitol was isolated in crystalline form and identified by comparing its properties, including X-ray powder pattern, with those of authentic melibiitol.[11,12]

Periodate oxidations of manninotriitol and of manninotriose-1-phenylflavazole[13] have also indicated that the hexose units in manninotriose are all linked by 1,6-bonds. Further, the large and regular increases in molecular rotation in going from sucrose ($[M]_D$ +22,700) to raffinose ($[M]_D$ +62,400) to stachyose ($[M]_D$ +99,900) indicate that in each case the D-galactose units have an α-configuration.

Experimental

Isolation of Sucrose, Raffinose and Stachyose from *Stachys tuberifera*.—*Stachys tuberifera* (*S. sieboldii*) rhizomes[14] (256 g.), were disintegrated in a Waring Blendor with methanol. Filtration gave 370 ml. of solution (ρ = 57.5° S, 2 dm.) and a pulp which was boiled briefly with water and filtered (400 ml. of filtrate, ρ = 10° S, 1 dm.). The combined filtrates were evaporated to a sirup in a warm air stream. Paper chromatography of the sirup showed small amounts of sucrose and raffinose, much stachyose, small amounts of verbascose and higher molecular weight saccharides.

Sucrose was obtained from a small portion of the sirup by the charcoal column fractionation method.[15] The evaporated 5% ethanol eluate crystallized by addition of glacial acetic acid and butanol. The sucrose was identified by paper chromatography and by comparison of its X-ray diffraction pattern with that of authentic sucrose.[16]

Another portion of the sirup was separated into fractions by large scale paper chromatography. The sirup was placed in a narrow band near one edge of many sheets of filter paper (Eaton and Dikeman 613, 8 × 10″), the papers were stapled in the form of cylinders with the sirup band near the bottom edge and the cylinders were developed in

(1) Journal Paper No. J-2231 of the Iowa Agricultural Experiment Station, Ames, Iowa., Proj. 1116. Supported in part by a grant from the Corn Industries Research Foundation. Presented at the 123rd meeting of the American Chemical Society, Carbohydrate Division, March, 1953.

(2) M. Onuki, *Sci. Papers Inst. Phys. Chem. Research (Tokyo)*, **20**, 201 (1933).

(3) E. F. Armstrong and K. F. Armstrong, *Chemistry and Industry*, **53**, 912 (1934); "The Carbohydrates," fifth ed., Longmans, Green and Co., London, 1934, pp. 162, 188.

(4) M. Adams, N. K. Richtmyer and C. S. Hudson, THIS JOURNAL, **65**, 1369 (1943).

(5a) H. Herissey, A. Wickstrom, J. E. Courtois and P. LeDizet, Intern. Congr. Biochem. Abstracts Communs. 2nd Congr., Paris, 1952, p. 311.

(5b) H. Herissey, A. Wickstrom and J. E. Courtois, *Bull. Soc. Chim. biol.*, **34**, 856 (1952).

(5c) J. E. Courtois, A. Wickstrom and P. LeDizet, *ibid.*, 1121.

(6) D. French and G. M. Wild, THIS JOURNAL, **75**, 2612 (1953); G. M. Wild and D. French, *Proc. Iowa Acad. Sci.*, **59**, 226 (1952).

(7) A. E. Bradfield and A. E. Flood, *Nature*, **166**, 264 (1950).

(7a) R. A. Laidlaw and C. B. Wylam, *J. Chem. Soc.*, 567 (1953).

(8) C. Neuberg, *Biochem. Z.*, **3**, 519 (1907). For this purpose almond emulsin is much to be preferred over yeast melibiase preparations, which also contain invertase.

(9) Previous unsuccessful attempts are recorded by C. Neuberg and S. Lachmann, *ibid.*, **24**, 171 (1910), and J. E. Courtois, C. N. Anagnostopoulos and F. Petek, ref. 5a, p. 240.

(10) C. Tanret, *Bull. soc. chim. (France)*, [3] **27**, 947 (1902).

(11) M. L. Wolfrom and T. S. Gardner, THIS JOURNAL, **62**, 2553 (1940).

(12) D. French, G. M. Wild, B. Young and W. J. James, *ibid.*, **75**, 709 (1953).

(13) H. Ohle and G. A. Melkonian, *Ber.*, **74B**, 279 (1941); G. Neumüller, *Arkiv. Kemi, Mineral. Geol.*, **21A**, No. 19 (1946).

(14) Obtained from Japan through the Bureau of Plant Introduction, U.S.D.A. A second lot was kindly furnished by Prof. Ziro Nikuni, Osaka University, Osaka, Japan.

(15) R. L. Whistler and D. F. Durso, THIS JOURNAL, **72**, 677 (1950).

(16) J. D. Hanawalt, H. W. Rinn and L. K. Frevel, *Ind. Eng. Chem., Anal. Ed.*, **10**, 457 (1938); W. Z. Hassid, M. Doudoroff and H. A. Barker, THIS JOURNAL, **66**, 1416 (1944).