[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, WASHINGTON UNIVERSITY]

D-*arabino*-L-*galacto*-Nonose and D-*arabino*-L-*talo*-Nonose

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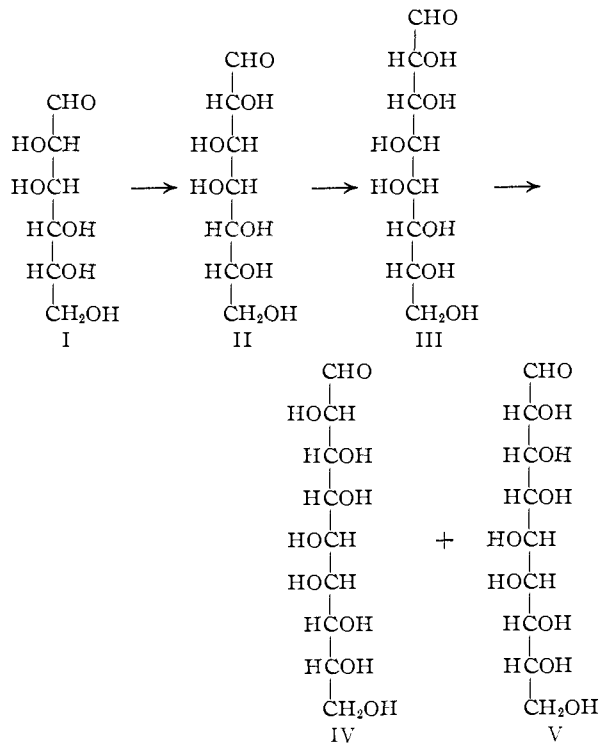
The condensation of nitromethane with D-*erythro*-L-*manno*-octose in alkaline methanol provides a mixture of 1-deoxy-1-nitro-D-*arabino*-L-*galacto*-nonitol and 1-deoxy-1-nitro-D-*arabino*-L-*talo*-nonitol in 90% yield. Application of the Nef reaction to the two deoxynitrononitols gives the corresponding, amorphous aldononoses, D-*arabino*-L-*galacto*-nonose and D-*arabino*-L-*talo*-nonose. Each of the two sugars yields a crystalline phenylhydrazone on treatment with phenylhydrazine and a crystalline lactone on oxidation with bromine. Configurations have been assigned to the two nonoses by application of qualitative optical rotatory rules. Neither of the aldononoses is fermentable by *Saccharomyces cerevisiae*.

The report[1] by Fischer and Passmore, in 1890, that they had synthesized a crystalline "*d*-mannononose" which was readily fermented by fresh brewers' yeast, with evolution of carbon dioxide and presumably with the production of ethanol, has aroused interest and speculation among carbohydrate chemists and biochemists over the intervening years.[2] Particularly intriguing is the fact that later attempts by Fischer, in collaboration with R. Hagenbach, to prepare the fermentable nonose led instead to a non-crystalline, non-fermentable product.[3]

The fermentable nonose of Fischer and Passmore was obtained by successive Kiliani–Fischer cyanohydrin syntheses from D-mannose, with a "*d*-mannoheptose" and a "*d*-mannooctose" as intermediates. It is now known that the latter two sugars are, respectively, D-*glycero*-D-*galacto*-heptose[4] and D-*erythro*-L-*manno*-octose.[4,5] Thus the fermentable nonose would appear to be limited in structure to that of either D-*arabino*-L-*galacto*-nonose or D-*arabino*-L-*talo*-nonose.

In more recent work,[6] Wolfrom and Wood have prepared D-*erythro*-L-*manno*-nonulose and D-*erythro*-L-*gluco*-nonulose by the Arndt–Eistert synthesis from the corresponding octonic acids and found that neither ketose is fermentable by *Saccharomyces*. They point out that should the biochemical behavior of these nonuloses parallel that of D-fructose, then one should not expect to find fermentability in any of the aldononoses derivable from D-*glycero*-D-*galacto*-heptose by the cyanohydrin synthesis. However, it must be recalled that whereas D-galactose is fermentable by adapted yeasts, including *Saccharomyces*,[7] the related ketose D-tagatose is reported to be inert to the action of several species of *Saccharomyces*.[8] Accordingly, it seemed desirable to examine directly the fermentability of D-*arabino*-L-*galacto*-nonose and D-*arabino*-L-*talo*-nonose.

In the present work successive nitromethane syntheses were applied, first to D-*mannose* (I) to prepare D-*glycero*-D-*galacto*-heptose[9] (II) and then,



from it, D-*erythro*-L-*manno*-octose[10] (III). Condensation of the latter with nitromethane in alkaline methanol produced in 90% yield a mixture of the related, epimeric 1-deoxy-1-nitro-nonitols. One of the latter is virtually insoluble in water and was thus separated readily from its more soluble epimer. That the two products are indeed epimeric was established by converting their octaacetates individually, through treatment with sodium bicarbonate,[11] to the same acetylated nitroölefin (D-*erythro*-L-*manno*-3,4,5,6,7,8.9-heptaacetoxy-1-nitro-1-nonene).

The less soluble of the deoxynitrononitols has been assigned the structure 1-deoxy-1-nitro-D-*arabino*-L-*galacto*-nonitol in view of the following evidence. Application of the Nef reaction produced an amorphous aldononose (phenylhydrazone, m.p. 205–206°) showing a molecular optical rotation of −12,100. This value is in reasonably good agreement with the values for D - galactose (+14,400), D-*glycero*-D-*galacto*-heptose (+13,600) and D-*glycero*-L-*galacto*-heptose (−13,700), indicating

(1) E. Fischer and F. Passmore, *Ber.*, **23**, 2226 (1890).

(2) More recently, L. Sternfeld and F. Saunders, THIS JOURNAL, **59**, 2653 (1937), have reported briefly that a synthetic "*d*-*β*-gluconononose" is fermentable by *Torula cremoris* with the production of acid but no evolution of gas.

(3) E. Fischer, "Untersuchungen über Kohlenhydrate und Fermente," Julius Springer, Berlin, 1909, p. 582; C. S. Hudson, *Adv. in Carbohydrate Chem.*, **1**, 1 (1945).

(4) G. Peirce, *J. Biol. Chem.*, **23**, 327 (1915).

(5) R. M. Hann, W. D. Maclay, A. E. Knauf and C. S. Hudson, THIS JOURNAL, **61**, 1268 (1939).

(6) M. L. Wolfrom and H. B. Wood, Jr., *ibid.*, **77**, 3096 (1955).

(7) E. Fischer and H. Thierfelder, *Ber.*, **27**, 2031 (1894).

(8) C. M. McCloskey and J. R. Porter, *Proc. Soc. Exptl. Biol. Med.*, **60**, 269 (1945).

(9) J. C. Sowden and R. Schaffer, THIS JOURNAL, **73**, 4662 (1951).

(10) J. V. Karabinos and C. S. Hudson, *ibid.*, **75**, 4324 (1953).

(11) E. Schmidt and G. Rutz, *Ber.*, **61**, 2142 (1928); J. C. Sowden and H. O. L. Fischer, THIS JOURNAL, **69**, 1048 (1947).

that the nonose has the L-galacto configuration at the top four asymmetric centers[12,13] and is D-*arabino*-L-*galacto*-nonose (IV). Further confirmation of this configurational assignment was obtained from the related nononic acid. Oxidation of the nonose with bromine yielded a crystalline nononic lactone. Titration of the latter with sodium hydroxide yielded a solution of the sodium salt showing $[\alpha]^{20}D$ — 4.38°. Removal of sodium from this salt by ion-exchange yielded the free, crystalline nononic acid with $[\alpha]^{20}D$ + 3.70°. Thus, again, the hydroxyl group on carbon two of the nononic acid is to the left in the Fischer projection according to the alkali salt rule of Levene,[14] and hence the acid is D-*arabino*-L-*galacto*-nononic acid.

The more soluble of the epimeric deoxynitrononitols gave, upon application of the Nef reaction, an amorphous nonose (phenylhydrazone, m.p. 209–210°) with molecular optical rotation of −3,100. This value is intermediate between those of D-talose (+3,800), D-*glycero*-D-*talo*-heptose (+2,600) and D-*glycero*-L-*talo*-heptose (−3,500), and hence the sugar is assigned the D-*arabino*-L-*talo*-nonose structure (V). Oxidation of the nonose with bromine yielded a crystalline nononic lactone.

Neither of the two epimeric aldononoses showed evidence of being fermented when tested with five different strains of *Saccharomyces cerevisiae*.[15]

A comparison of our epimeric nonoses and their derivatives with the Fischer–Passmore and Fischer–Hagenbach products is shown in Table I. From

TABLE I

	Fischer–Hagenbach nonose	D-*arabino*-L-*galacto*-nonose	Fischer–Passmore nonose	D-*arabino*-L-*talo*-nonose
Nonose				
M.p., °C.	Sirup	Sirup	*ca.* 130	Sirup
$[\alpha]D$	−35°	−44.6°	+50° (init.)	−11.9°
Nonose phenylhydrazone				
M.p., °C.	209	205–206	223	209–210
Nononic lactone				
M.p., °C.	199.5	205–206	175–177	184–185
$[\alpha]D$	+60°	+58.5°	−41°	+23.5°
Nononic phenylhydrazide				
M.p., °C.	254	251	254	....

these data it seems probable that Fischer and Hagenbach were dealing with D-*arabino*-L-*galacto*-nonose. However, our D-*arabino*-L-*talo*-nonose and its derivatives differ in all important respects from the products of Fischer and Passmore. The nature of the crystalline, fermentable product obtained by the latter authors remains a mystery. We can only conclude from the present work that their compound was neither of the two aldononoses to be expected from D-*erythro*-L-*manno*-octose through application of the cyanohydrin synthesis.

(12) R. M. Hann, Alice T. Merrill and C. S. Hudson, *ibid.*, **57**, 2100 (1935).

(13) M. L. Wolfrom and P. W. Cooper, *ibid.*, **72**, 1345 (1950).

(14) P. A. Levene, *J. Biol. Chem.*, **23**, 145 (1915); P. A. Levene and G. M. Meyer, *ibid.*, **31**, 623 (1917).

(15) The authors are indebted to Dr. Carl C. Lindegren and Mr. David Pittman, Biological Research Laboratory, Southern Illinois University, Carbondale, Ill., for the fermentation tests.

It is noteworthy that the condensation of nitromethane proceeds more efficiently with D-*erythro*-L-*manno*-octose than with any sugar hitherto reported.

Experimental

1-Deoxy-1-nitro-D-*arabino*-L-*galacto*-nonitol and 1-Deoxy-1-nitro-D-*arabino*-L-*talo*-nonitol.—To a stirred suspension of 4.8 g. of D-*erythro*-L-*manno*-octose[10] in 19 ml. of methanol and 10.8 ml. of nitromethane (distilled from activated alumina) was added a solution containing 0.7 g. of sodium in 24 ml. of methanol and the mixture was stirred for 48 hours. After cooling to −20°, the precipitated sodium acinitrononitols were filtered and washed with cold methanol. Without drying, the sodium salts were suspended with stirring in 250 ml. of water and 2.0 ml. of acetic acid was added slowly. The resulting precipitate was filtered and washed with cold water to yield 4.44 g. (73.6%) of crude 1-deoxy-1-nitro-D-*arabino*-L-*galacto*-nonitol. Recrystallization was effected with only minor loss by dissolving the product in hot water (*ca.* 1 g./l.), decolorizing with carbon and concentrating. The pure deoxynitrononitol showed m.p. 243–244°. Due to the very low solubility of this product, its optical rotation was not observed.

Anal. Calcd. for $C_9H_{19}O_{10}N$: C, 35.9; H, 6.31; N, 4.65. Found: C, 36.4; H, 6.60; N, 4.53.

Concentration of the filtrate from the crude product above yielded crude 1-deoxy-1-nitro-D-*arabino*-L-*talo*-nonitol. Recrystallization of the latter from a small volume of water, with the aid of decolorizing carbon, yielded the pure deoxynitrononitol, m.p. 208–209° and $[\alpha]^{20}D$ −3.2° in water, *c* 0.8.

Anal. Calcd. for $C_9H_{19}O_{10}N$: C, 35.9; H, 6.31; N, 4.65. Found: C, 36.1; H, 6.33; N, 4.92.

D-*erythro*-L-*manno*-3,4,5,6,7,8,9-Heptaacetoxy-1-nitro-1-nonene.—Acetylation of 1-deoxy-1-nitro-D-*arabino*-L-*galacto*-nonitol, for 48 hours at room temperature, with acetic anhydride containing a trace of sulfuric acid gave the crystalline octaacetate in 90% yield. Recrystallized from ethanol, this product showed m.p. 140–141° and $[\alpha]^{20}D$ +23° in chloroform, *c* 4.6.

Anal. Calcd. for $C_{25}H_{35}O_{18}N$: C, 47.1; H, 5.53. Found: C, 46.9; H, 5.70.

Similar acetylation of 1-deoxy-1-nitro-D-*arabino*-L-*talo*-nonitol yielded an amorphous octaacetate.

The above octaacetates (0.2 g.) were separately refluxed for 2 hours with sodium bicarbonate (0.2 g.) suspended in benzene (3 ml.). Filtration and concentration then provided in each instance D-*erythro*-L-*manno*-3,4,5,6,7,8,9-heptaacetoxy-1-nitro-1-nonene in 45% yield. After recrystallization from ethanol, the acetylated nitroölefin showed m.p. and m.m.p. 123–124° and $[\alpha]^{20}D$ −9° in chloroform, *c* 3.

Anal. Calcd. for $C_{23}H_{31}O_{16}N$: C, 47.8; H, 5.41; N, 2.43. Found: C, 47.6; H, 5.45; N, 2.77.

D-*arabino*-L-*galacto*-Nonose.—1-Deoxy-1-nitro-D-*arabino*-L-*galacto*-nonitol (0.8 g.) was dissolved by stirring (20 min.) in 3.0 ml. of 2 *N* sodium hydroxide. The resulting solution was added dropwise to a stirred solution containing 1.5 ml. of sulfuric acid and 1.6 ml. of water at room temperature. After several minutes of stirring, unreacted deoxynitrononitol (40 mg.) was filtered off and the solution was deionized over columns of Amberlite IR-100[16] and Duolite A-4.[17] The effluent was concentrated at reduced pressure to a small volume and treated with 0.33 ml. of phenylhydrazine in 1 ml. of 25% acetic acid. The D-*arabino*-L-*galacto*-nonose phenylhydrazone, which began to separate after a few minutes, was collected the next day and washed with each of several portions of water, ethanol and ether; yield 0.74 g. (82%), m.p. 205–206° dec. No satisfactory recrystallization medium was found for the phenylhydrazone, and this is reflected in the following analytical data which are averages of four determinations on different samples.

Anal. Calcd. for $C_{15}H_{24}O_9N_2$: C, 50.0; H, 6.71; N, 7.77. Found: C, 49.2; H, 6.76; N, 7.88.

Cleavage of the phenylhydrazone with benzaldehyde in the usual manner yielded amorphous D-*arabino*-L-*galacto*-nonose,

(16) A product of Rohm and Haas Co., Philadelphia, Pa.

(17) A product of Chemical Process Co., Redwood City, Cal.

$[\alpha]^{20}D$ −44.6° in water, *c* 1. Using the Kline–Acree[18] modification of the Willstätter–Schudel hypoiodite titration, 0.117 g. of the nonose (assuming quantitative cleavage of 0.156 g. of the phenylhydrazone) consumed 0.879 meq. of iodine; equiv. wt. calcd. 270, found 266 ± 2.

Oxidation of D-*arabino*-L-*galacto*-Nonose.—To a solution of 0.90 g. of D-*arabino*-L-*galacto*-nonose in 50 ml. of water was added 1.12 g. of barium carbonate and 1.5 ml. of bromine. After standing in the dark for 30 hours, the excess bromine was removed by aeration and barium was removed by the addition of a slight excess of dilute sulfuric acid and filtration. The solution then was passed over a small column of Duolite A-4 in the acetate form and concentrated to dryness at reduced pressure. Extraction of the resulting residue with two 100-ml. portions of boiling ethanol, followed by concentration of the extracts, produced 0.42 g. (47%) of nearly pure lactone, m.p. 204–206°. Recrystallization from ethanol yielded pure D-*arabino*-L-*galacto*-nononic γ-lactone, m.p. 205–206°, $[\alpha]^{20}D$ +58.5° in water, *c* 0.5.

Anal. Calcd. for $C_9H_{16}O_9$: C, 40.3; H, 6.01. Found: C, 40.4; H, 6.13.

A solution of 0.154 g. of the above lactone in 3.5 ml. of 0.2 *N* sodium hydroxide was diluted to a volume of 10.0 ml. with water. After 1 hour, the resulting solution of sodium salt (0.177 g.) showed constant $[\alpha]^{20}D$ −4.38°. Rapid passage of the salt solution over Amberlite IR-100, *or* the addition of acetic acid, followed by cooling for several hours, produced crystalline D-*arabino*-L-*galacto*-nononic acid. After recrystallization from a small volume of water, with rapid cooling, the acid showed m.p. 223° and $[\alpha]^{20}D$ +3.7° (constant for 30 minutes) in water, *c* 0.3.

Anal. Calcd. for $C_9H_{18}O_{10}$: C, 37.8; H, 6.34; neut. equiv., 286. Found: C, 37.1; H, 6.51; neut. equiv., 288 ± 2.

Treatment of the above lactone with phenylhydrazine and acetic acid in the usual manner yielded D-*arabino*-L-*galacto*-nononic phenylhydrazide,[3] m.p. 251°.

D-*arabino*-L-*talo*-**Nonose.**—1-Deoxy-1-nitro-D-*arabino*-L-*talo*-nonitol was converted to the corresponding sugar as described above for its epimer, except that in this instance any unchanged deoxynitrononitol remained in solution in the hydrolysis reaction mixture. From 0.32 g. of the deoxynitrononitol there was obtained 0.32 g. (84%) of D-*arabino*-L-*talo*-nonose phenylhydrazone, m.p., without recrystallization, 209–210°.

Anal. Calcd. for $C_{15}H_{24}O_8N_2$: C, 50.0; H, 6.71; N, 7.77. Found: C, 49.8; H, 6.74; N, 7.78.

Cleavage of the phenylhydrazone with benzaldehyde gave the amorphous nonose, $[\alpha]^{20}D$ −11.9° in water, *c* 2.

Quantitative oxidation of the nonose with hypoiodite[18] showed an equivalent weight of 265 ± 2; calculated, 270.

D-*arabino*-L-*talo*-**Nononic** γ-**Lactone.**—Oxidation of 210 mg. of D-*arabino*-L-*talo*-nonose with bromine, as described above for its epimer, yielded 45 mg. of D-*arabino*-L-*talo*-nononic γ-lactone. After recrystallization from ethanol, the product showed m.p. 184–185° and $[\alpha]^{20}D$ +23.5° in water, *c* 0.4.

Anal. Calcd. for $C_9H_{16}O_9$: C, 40.3; H, 6.01. Found: C, 40.2; H, 6.03.

Fermentation Tests.[15]—The two nonoses were tested separately for fermentability with five different strains of *Saccharomyces cerevisiae* by both the capillary tube method[19] and a manometric method. In the capillary tube tests, neither nonose showed evidence of being fermented when held for as long as 8 days, whereas D-glucose used as a control gave a positive result in 6 hours. In two manometric tests, the following R.Q. values were observed: D-glucose, 2.8 and 2.1; endogenous, 0.62 and 0.59; D-*arabino*-L-*galacto*-nonose, 0.55 and 0.80; D-*arabino*-L-*talo*-nonose, 0.71 and 0.68. It was concluded that neither of the two nonoses is fermentable by *Saccharomyces cerevisiae*.

(18) G. M. Kline and S. F. Acree, *J. Research Natl. Bur. Standards*, **5**, 1063 (1930); *Ind. Eng. Chem., Anal. Ed.*, **2**, 413 (1930).

(19) C. C. Lindegren, *Wallerstein Lab. Communs.*, **19**, 49 (1956).

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, CLARK UNIVERSITY, AND THE WORCESTER FOUNDATION FOR EXPERIMENTAL BIOLOGY]

D-Homosteroids. II. Derivatives of 3β-Hydroxy-17a,17a-dimethyl-D-homoandrostan-17-one[1]

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3β-Hydroxy-17a,17a-dimethyl-D-homoandrostan-17-one derivatives with halide and with methoxy at carbon 16 were prepared. Proof of the position of the halide is given and some isomerizations and substitutions of these halides are described.

A description of the synthesis of 3β-hydroxy-17a,17a-dimethyl-D-homoandrostan-17-one has been given in the preceding paper.[2] We wish now to report the preparation of some α-halo and α-methoxy keto derivatives of that compound.

Treatment of 3β-acetoxy-17a,17a-dimethyl-D-homoandrostan-17-one (I) with one mole of bromine in acetic acid–ether solution gave 3β-acetoxy-16α-bromo-17a,17a-dimethyl-D-homoandrostan-17-one (II). The axial position of the halide was confirmed by its single ultraviolet absorption peak at 322 mμ[3] and also by the absence of a shift of the ketonic absorption in the infrared spectrum.[4] An attempt was made to dehydrobrominate the bromoketone II by refluxing it for 1 hour in collidine or by keeping its dimethylformamide solution for 2 hours at 100°. Instead of the expected α,β-unsaturated ketone the isomeric 16β-bromoketone IV (λ_{max} 281 mμ) was obtained, and the original ketone absorption wave number was shifted from 1705 to 1715 cm.$^{-1}$. The absence of elimination is due to the steric effects of neighboring groups: the 15β (axial) hydrogen is hindered by the angular 18-methyl group and is therefore not available for attack by a base with high steric requirements such as collidine or dimethylformamide, which have to bring about the transelimination of the elements of hydrogen bromide. On the other hand, attack by a base on the sterically available

(1) Taken in part from a dissertation by Milan Uskoković in partial fulfillment of the requirements for the Ph.D. degree in Organic Chemistry, Clark University. Presented, in part, before the Division of Organic Chemistry, 134th National A.C.S. Meeting, Chicago, Ill., Sept., 1958. This investigation was supported, in part, by grants PHS-CY-2193 and PHS-C-321.

(2) M. Uskoković, M. Gut and R. I. Dorfman, THIS JOURNAL, **81**, 4561 (1959).

(3) R. C. Cookson, *J. Chem. Soc.*, 282 (1954); R. C. Cookson and S. H. Dandegaonker, *ibid.*, 352 (1955).

(4) R. N. Jones, D. A. Ramsay, F. Herling and K. Dobriner, THIS JOURNAL, **74**, 2828 (1954); E. G. Gumnis and J. E. Page, *J. Chem. Soc.*, 3847 (1957).