of tetrahydrofuran. Recrystallization from acetone yielded 0.63 g. of the oxido-diol VIa with m.p. 237–239°, $[\alpha]^{20}D$ −66.5°, free hydroxyl band in the infrared. The identical product was obtained when the epoxyketone was reduced with sodium borohydride in methanol solution (30 minutes at 50°, 18 hours at room temperature).

*Anal.* Calcd. for $C_{27}H_{42}O_5$: C, 72.61; H, 9.48. Found: C, 72.26; H, 9.40.

**9α,11α-Oxido-22-isoallospirostan-3,7-dione (VIb).**—One gram of the oxido-diol VIa in 100 cc. of benzene was treated at room temperature dropwise with stirring with a solution of 2.0 g. of sodium dichromate dihydrate[17] in 50 cc. of acetic acid and then left overnight. The usual work-up followed by recrystallization from acetone yielded 0.48 g. of pure oxido-dione VIb with m.p. 271–272.5°, $[\alpha]^{20}D$ −104°, $\lambda_{max.}^{EtOH}$ 286 mμ, log ε 1.85, $\lambda_{max.}^{nujol}$ 1716 cm.$^{-1}$, no free hydroxyl band.

*Anal.* Calcd. for $C_{27}H_{38}O_5$: C, 73.27; H, 8.65. Found: C, 73.55; H, 8.49.

**Δ$^{8(9)}$-22-Isoallospirosten-3,7-dione-11α-ol (VIIa).**—The isomerization of the oxido-dione VIb (0.5 g.) was accomplished by refluxing for one hour with 0.4 g. of potassium hydroxide and 50 cc. of methanol. Recrystallization from chloroform–acetone produced 0.38 g. of colorless crystals with m.p. 262–264°, $[\alpha]^{20}D$ −23.6°, $\lambda_{max}^{EtOH}$ 254 mμ, log ε 4.11, $\lambda_{max}^{nujol}$ 1718 cm.$^{-1}$ (3-ketone), 1660 cm.$^{-1}$ (Δ$^8$-7-ketone) and free hydroxyl band.

*Anal.* Calcd. for $C_{27}H_{38}O_5$: C, 73.27; H, 8.65. Found: C, 73.72; H, 8.60.

**The acetate VIIb** was recrystallized from acetone, m.p. 216–218°, $[\alpha]^{20}D$ +11.7°.

*Anal.* Calcd. for $C_{29}H_{40}O_6$: C, 71.87; H, 8.32. Found: C, 71.71; H, 8.21.

**Δ$^{8(9)}$-22-Isoallospirosten-3,7,11-trione (VIIc) (a) From Δ$^{8(9)}$-22-Isoallospirosten-3,7-dione-11α-ol (VIIa).**—Chromium trioxide (0.25 g.) oxidation at 15° for two hours in 50 cc. of acetic acid of 0.6 g. of the dione VIIa afforded after recrystallization from chloroform–ether 0.31 g. of yellowish crystals of the triketone with m.p. 243–245°, $[\alpha]^{20}D$ −3°, $\lambda_{max.}^{EtOH}$ 268 mμ, log ε 3.95, $\lambda_{max.}^{CHCl_3}$ 1715 and 1682 cm.$^{-1}$, no free hydroxyl band. The ultraviolet absorption maximum at 268 mμ is characteristic[1b,4,17,18] of the Δ$^8$-7,11-dione moiety.

*Anal.* Calcd. for $C_{27}H_{36}O_5$: C, 73.60; H, 8.24. Found: C, 73.53; H, 8.52.

**(b) From Δ$^{8(9)}$-22-Isoallospirosten-3-β,11α-diol-7-one (IIIa).**—When carried out as in (a), 1.0 g. of IIIa yielded 0.47 g. of yellowish trione VIIc, identical in all respects (including infrared spectrum) with the material prepared from VIIa.

**Δ$^{8(9)}$-22-Isoallospirosten-3β-ol-7,11-dione Acetate (VIII) (a) By Sodium Dichromate Oxidation** of **Δ$^{7,9(11)}$-22-Isoallospirostadien-3β-ol Acetate (I).**—Four grams of the diene I[9] in 80 cc. of benzene was oxidized overnight by the general procedure of Fieser, Herz and Huang[17,24] with 7.0 g. of sodium dichromate dihydrate in 80 cc. of acetic acid. The total oxidation product was subjected to a Girard separation and the ketonic fraction (0.8 g.) was chromatographed on 40 g. of ethyl acetate-washed alumina. The eluates possessing an ultraviolet absorption maximum at 266–270 mμ were combined and recrystallized from methanol, affording 0.15 g. (4%) of yellowish crystals with m.p. 212–215°, $[\alpha]^{20}D$ −22°, $\lambda_{max.}^{EtOH}$ 270 mμ, log ε 3.97, $\lambda_{max.}^{CS_2}$ 1736 (acetate) and 1686 cm.$^{-1}$ (Δ$^8$-7,11-dione system).

*Anal.* Calcd. for $C_{29}H_{40}O_6$: C, 71.87; H, 8.32. Found: C, 71.73; H, 8.60.

**(b) By Raney Nickel Reduction of Δ$^{8(9)}$-22-Isoallospirosten-3,7,11-trione (VIIc).**—The selective hydrogenation of 2.0 g. of the unsaturated trione VIIc was carried out exactly as described above for the Raney nickel reduction of Vc to Vd. The yellowish hydrogenation product was acetylated with pyridine–acetic anhydride and chromatographed on ethyl acetate-washed alumina as described under (a). Recrystallization gave 0.94 g. of the yellowish dione acetate VIII with m.p. 213–214°, $[\alpha]^{20}D$ −18°, $\lambda_{max.}^{EtOH}$ 270 mμ, log ε 3.95. The infrared spectrum was identical with that of the sample prepared according to (a).

**22-Isoallospirostan-3β-ol-7,11-dione Acetate (IX).**—A solution of 0.5 g. of the unsaturated dione VIII in 50 cc. of acetic acid was stirred for 3 hours on the steam-bath with 2.5 g. of zinc dust. After filtering, removing the acetic acid *in vacuo*, chromatographing the residue on 20 g. of ethyl acetate-washed alumina and recrystallizing from acetone, there was obtained 0.245 g. of colorless crystals of the saturated dione IX with m.p. 234–236°, $[\alpha]^{20}D$ −76°, $\lambda_{max.}^{CS_2}$ 1736 and 1716 cm.$^{-1}$.

(24) *Cf.* L. F. Fieser, THIS JOURNAL, **73**, 5007 (1951).

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

# Acyl Derivatives of D-Glucosaminic Acid

BY M. L. WOLFROM AND M. J. CRON[1]

Improved preparative directions are recorded for D-glucosamine hydrochloride and for D-glucosaminic acid (I). Schotten–Baumann benzoylation of I gave tetra-O-benzoyl-D-glucosaminic acid (V). Treatment of I successively with alkali and acetic anhydride, liquid ammonia and then acetic anhydride and pyridine, gave 2,3,4,5,6-pentaacetyl-D-glucosaminamide (IV) which on deamination yielded pentaacetyl-D-glucosaminic acid (VI).

Aldonic acids containing a free carboxyl group and having all hydroxyl functions esterified with a simple carboxylic acid are well established derivatives that are useful in synthesis.[2–5] The corresponding substances in the 2-amino-2-desoxyaldose series are unknown and were desired for further synthetic work. We describe herein the preparation in crystalline form of several such acyl derivatives of D-glucosaminic acid.

Improved preparative directions are recorded herein for the depolymerization of chitin to D-glucosamine hydrochloride and for its oxidation to D-glucosaminic acid (I). A Schotten–Baumann benzoylation of the latter led to the formation in low yield (4%) of tetra-O-benzoyl-D-glucosaminic acid (V). Karrer and Mayer[6] had prepared crystalline N-acetyl-D-galactosaminic lactone by acetylation of its sodium salt in aqueous solution followed by treatment with mineral acid. Application of this technique to D-glucosaminic acid yielded an uncharacterized amorphous product (presumably II) that was treated with liquid ammonia according to the general procedure of Glattfeld and MacMillan.[7] The crude amorphous substance

(1) Bristol Laboratories Fellow.

(2) R. T. Major and E. W. Cook, THIS JOURNAL, **58**, 2474, 2477 (1936).

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

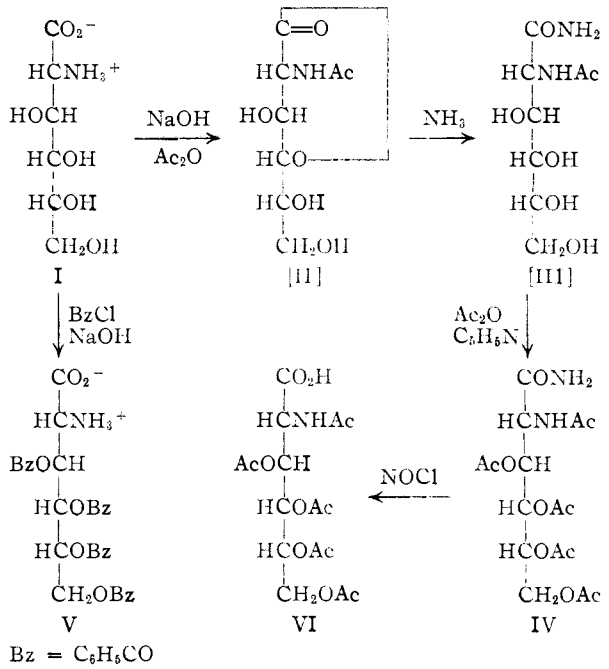
(4) M. L. Wolfrom, M. Konigsberg and D. I. Weisblat, *ibid.*, **61**, 574 (1939).

(5) M. L. Wolfrom, S. W. Waisbrot and R. L. Brown, *ibid.*, **64**, 1701, 2329 (1942).

(6) P. Karrer and J. Mayer, *Helv. Chim. Acta*, **20**, 407 (1937).

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

(presumably III, but uncharacterized) was acetylated to yield crystalline 2,3,4,5,6-pentaacetyl-D-glucosaminamide (IV). The over-all yield of IV was low. Deamination of this substance with nitrosyl chloride[4] led in good yield to the desired crystalline pentaacetyl-D-glucosaminic acid (VI).




## Experimental

**Preparation of D-Glucosamine Hydrochloride.**—Dry crab shells[8] (400 g.) were scraped clean, broken into small pieces and the portions of shell containing the eyes discarded. They were then soaked in 10% HCl for 24 hr., washed well with water by decantation and then the rubbery residues were split open and the inside surfaces scraped clean. The bulk of the water was then removed by squeezing and the mass was essentially saturated with a stream of hydrogen chloride gas.[9] After 24 hr. the material had disintegrated into 400 ml. of a black, somewhat viscous solution. This was refluxed for 3 hr., diluted with an equal volume of water and filtered. The dark amber colored filtrate was stirred with activated carbon and then filtered over a Celite[10] bed. The clear, slightly yellow filtrate was concentrated under reduced pressure to 200 ml. During this process the crystals separated and were removed by decantation. To the combined solids and liquors was added ethanol until its concentration reached 80%. The product was removed by filtration, washed successively with ethanol and ether, and air-dried. Recrystallization of the D-glucosamine hydrochloride was effected by dissolving in a minimum of water and adding ethanol; yield 50 g., $[\alpha]^{25}D$ +72° (*c* 1.0, water, equil.).

**D-Glucosaminic Acid (I).**—The procedure of Pringsheim and Ruschmann[11] was modified by employing one-half the quantity of oxidant added under vigorous mechanical stirring to the mixture heated on a boiling water-bath. After 15 min. the color began to darken rapidly and in a further 10 min. it became dark gray. At this point the hot mixture was filtered and processed as described by Pringsheim and Ruschmann except that crystallization was effected by the addition of methanol; yield 62%, $[\alpha]^{25}D$ −14.8° (*c* 4, 2.5% hydrochloric acid).

**Tetra-O-benzoyl-D-glucosaminic Acid (V).**—To a vigorously stirred, ice-cold solution of 6.4 g. of NaOH in 40 ml. of water, 5 g. of D-glucosaminic acid was added. Over a period of 1 hr., 15 ml. of benzoyl chloride was added dropwise. Stirring was continued for 3 hr. at 0° during which time a mass of heavy, pasty globules formed. The mother liquor was decanted and an excess of ether added to the residue. As the unreacted benzoyl chloride went into solution a mass of white needle-shaped crystals appeared. These were recrystallized from hot abs. ethanol; yield 0.64 g. (4.1%), m.p. 172–173°, $[\alpha]^{22}D$ +42.5° (*c* 1.0, acetone). The presence of a free amine group was indicated by a strongly positive ninhydrin test.

*Anal.* Calcd. for $C_{34}H_{29}O_{10}N$: C, 66.77; H, 4.78; N, 2.29. Found: C, 66.97; H, 4.77; N, 2.04.

**2,3,4,5,6-Pentaacetyl-D-glucosaminamide (IV).**—A solution of 16 g. of NaOH in 40 ml. of water was chilled in an ice-bath. In this cold, vigorously stirred solution, 20 g. of D-glucosaminic acid was then dissolved and to it was added 30 ml. of acetic anhydride at a slow dropwise rate. The solution was stirred for 3 hr. longer in the cold and was then acidified with 20% $H_2SO_4$ to *p*H 3. After distillation to dryness under reduced pressure the residue was heated for 3 hr. in a boiling water-bath under vacuum and was then extracted with four 250-ml. portions of boiling abs. ethanol. After vacuum distillation of the alcohol extract to a volume of 50 ml., the amorphous, very hygroscopic product was precipitated by the addition of 400 ml. of ether; yield 19.5 g.

The amorphous product was dissolved in 100 ml. of liquid ammonia and after evaporation of the excess ammonia the sirupy residue was placed in a desiccator under vacuum for 6 hr. The solid residue was dissolved in 200 ml. of methanol and after treating with activated carbon the volume was reduced to about 20 ml. by distillation under reduced pressure. Addition of ether precipitated an amorphous product and by further reduction in volume of the mother liquor an additional amount of material was obtained; total yield 20.4 g.

A 3.4-g. portion of this product was placed in a flask equipped with a stirrer and a mercury seal. To it was added 50 ml. of a 1:1 acetic anhydride and pyridine solution and the mixture subjected to vigorous stirring for 24 hr. in an ice-bath. The material was all in solution after this time. The solution was then distilled under reduced pressure until a heavy mass of white crystals separated (complex of pyridine and acetic anhydride) at about 15-ml. volume. The mixture was poured into 100 ml. of water and the solution which resulted was extracted with three 75-ml. portions of chloroform. The chloroform was removed by distillation under reduced pressure and the traces of acetic acid were removed by alternately adding and distilling methanol (under reduced pressure). The residue was dissolved in 200 ml. of methanol, treated with activated carbon, and then again reduced in volume. Isoamyl alcohol was then added and distilled under reduced pressure until a total of 200 ml. had been used and a final volume of 60 ml. was reached. After standing for several days the crystalline product was removed, washed successively with isoamyl alcohol and ether and dried under vacuum; yield 0.55 g. Two recrystallizations by the same method yielded a pure product (IV); m.p. 167–168°, $[\alpha]^{29}D$ +9.6° (*c* 1.0, water).

*Anal.* Calcd. for $C_{16}H_{24}O_{10}N_2$: C, 47.52; H, 5.98; N, 6.93. Found: C, 47.63; H, 5.75; N, 7.13.

**Pentaacetyl-D-glucosaminic Acid (VI).**—Nitrosyl chloride was passed into 30 ml. of cold, dry, alcohol-free chloroform until an increase in weight of 1 g. was obtained. A solution of 1.7 g. of 2,3,4,5,6-pentaacetyl-D-glucosaminamide in 50 ml. of dry, alcohol-free chloroform was placed in a moisture-guarded flask and the nitrosyl chloride solution added at 0° under mechanical stirring. The mixture was then allowed to warm to room temperature and maintained there for 20 hr. In a moisture-guarded apparatus, the solution was concentrated to 20 ml. by chloroform removal under reduced pressure. Dilution with 40 ml. of chloroform and the addition of 60 ml. of petroleum ether resulted in a precipitation of the crystalline product; yield 1.31 g. Two recrystallizations from chloroform and petroleum ether yielded a pure product; m.p. 154–156°, m.p. 140–145° on admixture with 2,3,4,5,6-pentaacetyl-D-glucosaminamide, $[\alpha]^{22}D$ +4.6° (*c* 1.1, methanol).

*Anal.* Calcd. for $C_{16}H_{23}O_{11}N$: C, 47.41; H, 5.72; N, 3.46. Found: C, 47.50; H, 5.80; N, 3.94.

(8) Obtainable from Carter-Lanhardt, Inc., Municipal Fish Market, Washington, D. C.

(9) C. S. Hudson, personal communcation.

(10) A siliceous filter-aid manufactured by Johns-Manville Co., New York, N. Y.

(11) H. Pringsheim and G. Ruschmann, *Ber.*, **48**, 680 (1915).

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