New Compounds: Nucleosides Derived from D-*Glycero*-D-*gulo*-heptose

By LEON M. LERNER

The preparation of new nucleosides derived from D-*glycero*-D-*gulo*-heptose are described. These nucleosides are distinguished from the naturally occurring nucleosides by their seven carbon chain and pyranose ring. The configurations and anomeric purity of these compounds are unknown.

A NUMBER of nucleosides have been prepared in the last few years, in which the sugar moiety has been altered from that which normally occurs in nature. The reason for this interest was stimulated by the discovery of antibiotics which were identified as nucleosidic substances possessing an altered structure in the carbohydrate. Nucleoside analogs have since been prepared with the intention of obtaining useful agents which would possess antitumor or antimicrobial activity.

The first synthesis of a nucleoside derived from a heptose was described by Kohn *et al.* (1), who prepared 9-β-D-*glycero*-D-*gulo*-heptofuranosyladenine. This compound, as well as several nucleosides derived from hexopyranoses (2), was found to be inhibitory to the multiplication of *Herpes simplex* virus.[1] A natural outcome of these observations was the preparation of some nucleosides derived from a heptopyranose, which is the subject of this communication.

The acetic anhydride–acetic acid reagent described by Montgomery and Hudson (3) was used to acetylate D-*glycero*-D-*gulo*-heptopyranose. This reagent has been used to isomerize sugar acetates to the α anomer. A preponderance of the α hexaacetate was crystallized, but the expected high yield of α anomer did not materialize. Instead, a mixture of α and β anomers was obtained which was separated by fractional crystallization. Such an outcome has been noted previously in the case of D-allose (2), where the β form predominated.

Either anomer could be used in the preparation of the bromide in a hydrogen bromide–acetic acid mixture. Coupling of the bromide with 6-benzamidochloromercuripurine was achieved in refluxing xylene in the usual manner (4). The blocking groups were removed in hot methanolic sodium methoxide and the product, 9-D-*glycero*-D-*gulo*-heptopyranosyladenine, was purified *via* its picrate salt (5). Unfortunately, this procedure resulted in a significant loss of the nucleoside on the resin. When 6-benzamidochloromercuripurine and the hexaacetate were condensed in the presence of titanium tetrachloride, the yield was only about 10% of that obtained by coupling with the bromide. This was in contrast to the results of other workers (6, 7), who have claimed that the yields are increased, often considerably, over that obtained by prior formation of the halogenose. However, Tong *et al.* (8) have had a similar experience to that reported in this paper during their attempt to couple 1,2-di-*O*-acetyl-5-*O*-benzoyl-3-*O*-methyl-D-ribofuranose to a purine.

1-D-*Glycero*-D-*gulo*-heptopyranosyluracil was obtained by the coupling of the bromide with bis(trimethylsilyl)uracil in benzene at room temperature. The intermediate acetate was purified by chromatography on silicic acid and the blocking groups were removed with sodium methoxide. The nucleoside was obtained as a hard, very hygroscopic glass, which migrated as a homogeneous spot in four solvent systems on TLC plates, but did not give a proper analysis. Benzoylation of this substance yielded the pentabenzoate, which served the purpose of being a derivative of the desired product and offered an alternative purification route. However, removal of the benzoate groups yielded a product whose elementary analysis was not much improved over that obtained previously. Application of column and preparative TLC gave further evidence of one component, but the analyses were still relatively poor. The identity of this nucleoside is based upon its ultraviolet spectrum and the preparation of the crystalline benzoate derivative.

The anomeric configuration of 9-D-*glycero*-D-*gulo*-heptopyranosyladenine has been tentatively decided upon as β by reference to the *trans* rule (9). This rule states that a heavy metal salt of a purine or pyrimidine will substitute at the anomeric carbon *trans* to the acyloxy group at C-2 of the sugar. However, the rule is not applicable to the coupling of the halogenose to bis(trimethylsilyl)uracil. Application of nuclear magnetic resonance spectroscopy[2] at 60 Mc. failed to resolve the anomeric configuration of the pentabenzoate derivative of 1-D-*glycero*-D-*gulo*-heptopyranosyluracil.

EXPERIMENTAL

D-*Glycero*-D-*gulo*-heptopyranose was prepared from D-*glycero*-D-*gulo*-heptono-1,4-lactone as described by Wolfrom and Thompson (10). Elementary analyses were performed by the Spang Microanalytical Laboratories, Ann Arbor, Michigan. Thin-layer chromatography (TLC) was performed on Silica Gel HF plates (E. Merck, AG, Darmstadt). Spots were located with an ultraviolet lamp and the plates were routinely exposed to the chromic acid charring method to test for homogeneity. Evaporations were done *in vacuo* at a bath temperature of 40–45°.

Hexa - O - acetyl - D - glycero - D - gulo - heptopyranose—The acetylation of D-*glycero*-D-*gulo*-heptopyranose was carried out directly with the acetylation reagent as described earlier (2, 3). After a reaction time of 5 days followed by the usual workup (2, 3) the α anomer was crystallized from a large

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[1] Paul Kohn, personal communication.

[2] Obtained by Dr. Harry Agahigian of the Baron Consulting Co.

volume of ethyl ether in an open flask, m.p.[3] 155–158°. Recrystallization in the same manner gave the α anomer in 55% yield, m.p. 164–165°, $[\alpha]_D^{25}$° + 85.5° (c 3.10, $CHCl_3$). Lit. (11) m.p. 164°, $[\alpha]_D^{20}$° + 87°.

The mother liquors from these crystallizations were combined and the solvent was removed *in vacuo*. The β anomer was crystallized from 50% aqueous ethanol in an open beaker. The long needles had m.p. 128–130°. Recrystallization in the same manner gave the pure product in a 20% yield, m.p. 133.5–134°, $[\alpha]_D^{22}$° +3.5° (c 6.03, $CHCl_3$). Lit. (11) m.p. 135°, $[\alpha]_D^{20}$° +4.8°.

When a shorter reaction time was used the product distribution was nearly one α to one β.

9-D-Glycero-D-gulo-heptopyranosyladenine—To 25 ml. of a saturated solution of hydrogen bromide in acetic acid was added 2.0 Gm. of hexa-*O*-acetyl-D-*glycero*-D-*gulo*-heptopyranose. After 2 hr. at room temperature the solvent was evaporated *in vacuo* at a bath temperature below 30°. Dry toluene was added and removed 5 times in a similar manner. The orange syrup which remained was dissolved in 25 ml. of dry xylene and added to a well-stirred refluxing mixture containing 6-benzamidochloromercuripurine (2.03 Gm.), diatomaceous earth[4] (1.95 Gm.), cadmium carbonate (1.03 Gm.), and 150 ml. of xylene (4). After the usual workup (4), an orange glass (2.6 Gm.) was obtained by evaporation of absolute alcohol. This material was dissolved in a mixture of sodium methoxide (350 mg.) in 60 ml. of methanol and the solution was refluxed for 1.25 hr. The methanol was removed by evaporation and the residue was dissolved in 35 ml. of water and neutralization was effected with acetic acid. The aqueous solution was washed with 35 ml. of chloroform, treated with activated charcoal (Darco G-60), and concentrated to a light orange syrup. Since the product could not be induced to crystallize at this stage, the picrate was prepared. The syrup was dissolved in 15 ml. of methanol and 25 ml. of 10% methanolic picric acid was added. The flask was chilled in an ice bath for 2 hr., the yellow precipitate was removed by filtration, and it was washed well with cold methanol and cold water. The picrate (1.11 Gm.) started to decompose at 166° and melted at 170–173°.

The picrate was converted to the free nucleoside with the carbonate form of an ion-exchange resin as described previously (2, 5). Crystallization occurred slowly from water in an open flask after 3 days of standing at room temperature. The flask was chilled for several more days in a refrigerator and 142 mg. of white crystals were collected. This material decomposed slowly starting at about 220° and melted completely with decomposition at 273–275°, $[\alpha]_D^{29}$° −7° (c 0.86, 1 *N* HCl); $\lambda_{max.}^{pH\ 1}$ 256 mμ(ϵ 13,800), $\lambda_{max.}^{H_2O}$ 258 mμ(ϵ 14,000), $\lambda_{max.}^{pH13}$ 258 mμ(ϵ 14,200). This substance was homogeneous upon chromatography on Whatman No. 1 paper with 5% aqueous disodium phosphate as the developing solvent, R_{Ad} 1.91 (R_{Ad} of adenine = 1.00).

Anal.—Calcd. for $C_{12}H_{17}N_5O_8$: C, 44.03; H, 5.25; N, 21.40. Found: C, 44.00; H, 5.25; N, 21.45.

1-D-Glycero-D-gulo-heptopyranosyluracil—To 30 ml. of hexamethyldisilazane and 0.6 ml. of trimethylchlorosilane was added 2.3 Gm. of uracil and the stirred mixture was refluxed at an oil bath temperature of 170° for 3 hr., protected from moisture (12). The clear solution was cooled to below 50° and the solvent was distilled under high vacuum at a bath temperature of 50–60°, leaving a slightly turbid syrup. This syrup was dissolved in 100 ml. of dry benzene and added to a solution containing the bromide (prepared from 4.1 Gm. of hexa-*O*-acetyl-D-*glycero*-D-*gulo*-heptopyranose) in 250 ml. of benzene. Mercuric acetate (2.8 Gm.) was added and the mixture was stirred for 5 days, protected from moisture. The solids were removed by filtration, washed with 100 ml. of chloroform, and the solvents were evaporated. The residue was triturated with 150 ml. of chloroform, the insoluble material was removed by filtration, and the filtrate was washed with 30% aqueous potassium iodide, water, and dried over magnesium sulfate. Removal of the chloroform under vacuum gave a syrup weighing 5.6 Gm., $\lambda_{max.}^{EtOH}$ 255 mμ. The syrup was dissolved in a minimum amount of chloroform and applied to the top of a column of silicic acid (100 Gm., Mallinckrodt 100 mesh). The column was eluted with chloroform (750 ml.), chloroform–ethyl acetate (4:1, 500 ml.), and chloroform–ethyl acetate (1:1, 500 ml.). Various sugar derivatives were eluted with all of these solvent mixtures, but no ultraviolet absorbing material. Upon elution with ethyl acetate (500 ml.), 1.0 Gm. of the nucleoside acetate was obtained, $\lambda_{max.}^{EtOH}$ 255 mμ; $\lambda_{max.}^{smear}$ (cm.$^{-1}$) 1750 (acetate ester), 1700 (—NHCO— of pyrimidinone), 1630 (C=C of pyrimidine ring), 1230 (acetate C—O—C), 1070, 1050 (sugar C—O—). This intermediate pentaacetate could not be induced to crystallize and so the acetyl groups were removed by refluxing for 1 hr. in 50 ml. of 0.1 *N* methanolic sodium methoxide. An ion-exchange resin[5] was added until the pH was 6.5. The resin was removed by filtration and the solvent was evaporated. The syrupy material was partitioned between water and chloroform and the aqueous layer was concentrated to dryness. The clear, colorless syrup which remained would not crystallize, but it did form a hard glass upon repeated evaporation with ethanol. The glass was pulverized, yielding 225 mg. of product, which was homogeneous upon TLC in the following four developing systems: 1-butanol–water (86:14 v/v), 5% aqueous disodium phosphate, 1-butanol–acetic acid–water (5:2:3 v/v/v), and 2-propanol–25% aqueous ammonia–water (7:1:2 v/v/v). This substance had $[\alpha]_D^{25}$° −16° (c 0.51, water) and was extremely hygroscopic; ultraviolet spectrum at pH 1: maximum at 258 mμ (ϵ 7430), minimum at 230 mμ(ϵ 1930); at pH 5–7: maximum at 257 mμ(ϵ 7460), minimum at 229 mμ(ϵ 1870); at pH 13: maximum at 258 mμ(ϵ 5400), minimum at 244 mμ(ϵ 4360).

Anal.—Calcd. for $C_{11}H_{16}N_2O_8$: C, 43.42; H, 5.30; N, 9.21. Found: C, 40.74; H, 5.58; N, 7.22.

Since the pentabenzoate was also prepared (see below) as a pure derivative an attempt was made to obtain the nucleoside from this derivative. The benzoate groups were removed as described above for the acetate and the product was worked up in the usual way. The nucleoside was precipitated from methanol by addition of ethyl ether and the

[3] Melting points were obtained on a Fisher-Johns apparatus and are corrected.

[4] Celite 545, Johns-Manville, New York, N. Y.

[5] Dowex 50-(H$^+$), Dow Chemical Co., Midland, Mich.

white solid obtained was dried at 100° *in vacuo* over P_2O_5.

Anal.—Calcd. as for above. Found: C, 42.08; H, 5.59; N, 7.00.

1 - (Penta-O-benzoyl - D - glycero - D - gulo-heptopyranosyl)uracil—To 19 ml. of dry pyridine was added 88.6 mg. of 1-D-*glycero*-D-*gulo*-heptopyranosyluracil and the mixture was stirred in an ice bath. Benzoyl chloride (2 ml.) was added dropwise and after 0.5 hr. at 0° the mixture was stored at room temperature for 21 hr. It was then heated on a steam bath for 20 min., cooled, 50 ml. of benzene was added, followed shortly thereafter by 50 ml. of water. The mixture was stirred for several minutes and the layers were separated. The benzene solution was washed three times with 50-ml. portions of 2 *N* sulfuric acid solution, once with 50 ml. of water, 4 times with 50-ml. portions of saturated sodium bicarbonate solution, and finally with 50 ml. of water. The dried ($MgSO_4$) solution was evaporated to a thin, orange oil. This was triturated with hot petroleum ether and cooled in an ice bath for 1 hr. The petroleum ether was decanted and the procedure was repeated. A hard syrup remained which was dissolved in warm methanol and placed in a freezer. A solid was filtered off in two crops (203 mg.). Recrystallization from chloroform–ethanol in an open flask at room temperature yielded beautiful, clear, star-like clusters of large rods, 117 mg. in two crops; m.p. 123–124.5° (to a very viscous liquid) with prior softening at 119°. $[\alpha]_D^{28}$° −55.9° (c 2.81, $CHCl_3$). λ_{max}^{film} (cm.$^{-1}$) 1730 (benzoate ester), 1680 (—NHCO— of pyrimidinone), 1600 (C=C of phenyl and pyrimidine ring), 1260 (benzoate C—O—C), 1100, 1090, 1065 (sugar C—O—), 706 (monosubstituted phenyl). The product was homogeneous on TLC. The R_f was 0.84 using chloroform–methanol (9:1) as the irrigating solvent.

Anal.—Calcd. for $C_{46}H_{36}N_2O_8$: C, 66.99; H, 4.40; N, 3.40. Found: C, 67.02; H, 4.48; N, 3.37.

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Keyphrases

D-*Glycero*-D-*gulo*-heptose derivatives—synthesis
Nucleosides—D-*glycero*-D-*gulo* heptose derivatives
TLC—separation
Column chromatography—separation
UV spectrophotometry—identity
Optical rotation—identity

New Compounds: Synthesis of Heterocyclic Estrogens

By LARRY GORUM* and W. LEWIS NOBLES

Several nitrogen analogs of diethylstilbestrol have been prepared by a convenient route from pyridyl lithium and the appropriate ketone. These compounds are expected to have estrogenic activity with possible greater selectivity of action than previous known synthetic estrogens.

THE INVESTIGATIONS of Dodds and co-workers (1) on synthetic compounds possessing the physiological action of the female sex hormone estrone reached a climax with the discovery of diethylstilbestrol (1). Since that time much work with molecular modification has been done in an effort to improve the selectivity of pharmacological action in the therapeutic uses of diethylstilbestrol. Endocrine therapy in metastatic prostatic cancer consists initially of estrogen therapy as first demonstrated by the pioneering investigation of Huggins (2). Oral estrogen preparations such as diethylstilbestrol are effective to a limited extent but elicit side effects such as nausea, gynecomastea, and edema. Because of these undesirable side effects it is necessary to find new compounds which possess a more selective estrogenic action.

Many compounds of this type have been prepared and tested and are described in a review by Grundy (3). However, thus far none of the compounds tested offered sufficient selective antineoplastic activity to replace diethylstilbestrol in the clinic. In the hundreds of compounds reported, several contain

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* Current address: Organic Chemicals Department, Research and Development Division, Jackson Laboratory, E. I. du Pont de Nemours and Company, Wilmington, Delaware.