[EXPERIMENTAL BIOLOGY AND MEDICINE INSTITUTE, NATIONAL INSTITUTES OF HEALTH]

AMINO- AND GUANIDINO-PHENYLGLUCOSIDES

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The amino- and guanidino-phenylglucosides described in this paper were synthesized for chemotherapeutic studies in experimental tuberculosis. The commercially accessible *o*-, *m*-, and *p*-nitrophenols and 2,4-dinitrophenol served as starting materials.

Pigman (1) prepared 2,4-dinitrophenyltetraacetyl-β-D-glucoside (I) by utilizing the Michael synthesis as modified by Mannich (2) (aqueous sodium hydroxide, acetone, acetobromoglucose). On repeating this procedure we were able to obtain I in only 9% yield in agreement with Pigman. By carrying out the reaction in dry acetone with potassium carbonate, the yield of I could be increased to 55%. This variation has also been applied successfully to the gluco-

TABLE I

EFFECT OF ACIDITY OF THE PHENOL

PHENOL	K_a at 25°C. (3)	YIELD OF GLUCOSIDE, %	
		K_2CO_3-Me_2CO (A)	$NaOH$-H_2O-Me_2CO (B)
2,4-Dinitro-	1.0×10^{-4}	55	9
o-Nitro-	6.8×10^{-8}	46	40
p-Nitro-	6.5×10^{-8}	35	24
m-Nitro-	1.0×10^{-8}	14	36

sidation of *o*- and *p*-nitrophenols. On the other hand, with *m*-nitrophenol the procedure developed by Mannich (2) was found preferable.

The data in Table I show that within this limited series the yield of glucoside varies directly with the acidity of the phenol in procedure A and inversely in procedure B with the exception of *o*-nitrophenol.

Hydrogenation of the nitrophenyltetraacetylglucosides with Raney nickel led readily to the amino compounds whose hydrochlorides condensed with cyanamide in ethyl acetate[1] to give good yields of the corresponding guanidino derivatives. In ethanol, the solvent usually employed, the cyanamide reaction failed to give the desired results. Further, no tractable products could be isolated when the deacetylated aminophenylglucoside hydrochlorides and cyanamide were brought to reaction in ethanol.

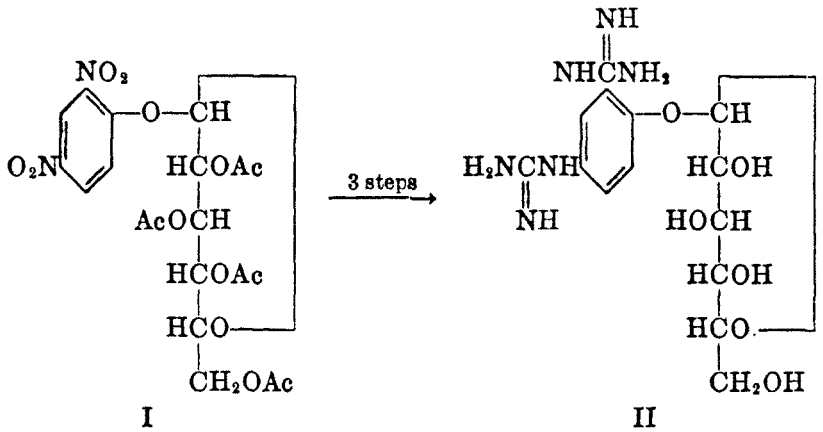
The amino- and guanidino-phenylglucosides were catalytically deacetylated with sodium methoxide in methanol.

Many of the basic glucosides or their salts were found to contain solvate water or ethanol. The water or ethanol content was determined by drying the com-

[1] Ethyl acetate as solvent was previously used to advantage in preparing 9-phenanthrylguanidine derivatives (4).

pounds to constant weight *in vacuo* at a temperature which would leave the molecule intact otherwise. However, in removing ethanol from *m*-aminophenyltetraacetyl-β-D-glucoside hydrochloride at 77°, hydrogen chloride was eliminated simultaneously, and pure *m*-aminophenyltetraacetyl-β-D-glucoside (base) resulted.

All compounds designated in the experimental part with NIH numbers were tested *in vitro*. None of these compounds showed significant inhibition of tubercle bacilli (H37Rv, Dubos-Davis medium).[2]



I II

Acknowledgment: We express thanks to Dr. Nelson K. Richtmyer of this Institute for helpful suggestions. The microanalyses are from the Institute service analytical laboratory under the direction of Mr. William C. Alford.

EXPERIMENTAL[3]

Acetobromoglucose.[4] β-Pentaacetylglucose, (30 g.), 40 ml. of 30–32% hydrogen bromide in acetic acid, and 10 ml. of acetic anhydride were allowed to stand overnight. Addition of 180 ml. of toluene and 10 ml. of acetic anhydride, evaporation of the solution to a sirup *in vacuo* (bath temperature 50–60°), and treatment of the sirup with 50 ml. of dry ether and and 50 ml. of ligroin (30–60°) gave, after two hours at room temperature and ice-cooling, 26 g. (82%) of product, m.p. 89–91°; lit. (6), m.p. 88–89°.

2,4-Dinitrophenyltetraacetyl-β-D-glucoside (I).[5] Five grams of 2,4-dinitrophenol, 5 g. of anhydrous potassium carbonate, 10 g. of acetobromoglucose, and 100 ml. of acetone (dried over potassium carbonate) were refluxed for 20 hours, diluted gradually (shaking) with an equal volume of water and ice-cooled. The precipitate was recrystallized from ethanol to give 6.8 g. (55%) of I, m.p. 176–177°, $[\alpha]_D^{20}$ + 34.9° (*c*, 1.09 in $CHCl_3$); lit. (1), m.p. 173–177° (cor.), $[\alpha]_D^{20}$ + 34.5° (*c*, 4 in $CHCl_3$).

[2] The compounds have been tested in the Tuberculosis Research Laboratory, U. S. Public Health Service, Cornell University Medical College, New York, N. Y., under the direction of Dr. Bernard D. Davis. An outline of the over-all plan of the cooperative project and methodological aspects will be given elsewhere; *cf.* "Cyclitol Derivatives. I." (5).

[3] All melting points were observed in a capillary and are uncorrected.

[4] We are indebted to Dr. H. G. Fletcher, Jr., of this Institute for the procedure used in this preparation.

[5] The β-configuration has been assigned to these glucosides on the basis of their rotation and mode of synthesis.

Anal. Calc'd for $C_{23}H_{22}N_2O_{14}$: C, 46.7; H, 4.3.
Found: C, 46.8; H, 4.4.

o-Nitrophenyltetraacetyl-β-D-glucoside was prepared as described for I (reflux time 2–6 hours, yield 46%); m.p. 160.5–161.5°, $[\alpha]_D^{20}$ + 43.0° (*c*, 1.20 in $CHCl_3$); lit. (7), m.p. 160–162°, $[\alpha]_D^{2'}$ + 45° ($CHCl_3$) and (8), m.p. 158–159°, $[\alpha]_D^{20}$ + 53.2° ($CHCl_3$).

The *p-isomer* (reflux time 15 hours, yield 35%) melted at 174–175° (7a, 8).

The *m-isomer* was more satisfactorily prepared by the Mannich procedure and had m.p. 136–137°, $[\alpha]_D^{20}$ − 34.8° (*c*, 0.5 in $CHCl_3$); lit. (1), $[\alpha]_D^{20}$ — 37° and (8), $[\alpha]_D^{20}$ − 26.8°.

2,4-Diaminophenyltetraacetyl-β-D-glucoside. Three grams of I, 3.0 g. of Raney nickel, and 75 ml. of methanol absorbed six moles of hydrogen during three hours. The mixture was filtered quickly through Filter-Cel and evaporated to dryness at reduced pressure (hydrogen atmosphere). Recrystallization of the residue from ethanol (under hydrogen) gave 2.3 g. (87%) of the diamine, m.p. 149–150°, $[\alpha]_D^{20}$ − 22.6° (*c*, 0.93 in methanol); glittering plates.

Anal. Calc'd for $C_{20}H_{26}N_2O_{10}$: C, 52.9; H, 5.8.
Found: C, 53.1; H, 6.1.

The *dihydrochloride* (NIH 3631) crystallized from methanol-ether in needles, m.p. 176–177.5° (dec.), $(\alpha)_D^{20}$ − 26.6° (*c*, 1.02 in methanol).

Anal. Calc'd for $C_{20}H_{26}Cl_2N_2O_{10}\cdot H_2O$: C, 44.0; H, 5.6; H_2O, 3.3.
Found: C, 43.8; H, 5.5; Loss (77°, 1 mm.), 3.8.

o-Aminophenyltetraacetyl-β-D-glucoside. The procedure used in this and in subsequent reductions was identical to the one above except that it was unnecessary to isolate and purify the products under hydrogen; yield 90%, m.p. 132–133°, $[\alpha]_D^{20}$ − 33.9° (*c*, 1.06 in methanol).

Anal. Calc'd for $C_{20}H_{25}NO_{10}$: C, 54.7; H, 5.7.
Found: C, 54.8; H, 5.7.

p-Aminophenyltetraacetyl-β-D-glucoside was obtained in a yield of 72%, m.p. 132–133.5°, $[\alpha]_D^{20}$ − 14.7° (*c*, 1.21 in $CHCl_3$). Helferich and Peters (9) used palladium-barium sulfate in this hydrogenation and reported a yield of 60%, m.p. 127–130°, $[\alpha]_D^{20}$ − 15.5°.

m-Aminophenyltetraacetyl-β-D-glucoside crystallized in glittering blades, m.p. 150–150.5°, $[\alpha]_D^{20}$ − 22.9° (*c*, 0.20 in methanol); yield 90%.

Anal. Calc'd for $C_{20}H_{25}NO_{10}$: C, 54.7; H, 5.7.
Found: C, 54.6; H, 5.9.

The *hydrochloride*, prepared with ethanolic hydrogen chloride, crystallized from methanol-ether in broad needles, m.p. below 125° (froth), $[\alpha]_D^{20}$ − 30.6° (*c*, 0.24 in methanol).

Anal. Calc'd for $C_{20}H_{26}ClNO_{10}\cdot C_2H_5OH$: C, 50.6; H, 6.2; Cl, 6.8; HCl + C_2H_5OH, 15.8.
Found: C, 51.0; H, 6.1; Cl, 7.1; Loss (77°, 1 mm.), 15.7.

The residue from the loss-in-weight determination proved to be the original, pure base.

2,4-Diaminophenyl-β-D-glucoside (NIH 3640). One gram of 2,4-diaminophenyltetraacetyl-β-D-glucoside, 10 ml. of methanol, and 0.5 ml. of methanolic sodium methoxide[6] gave, after one hour at 25° and one hour at 3°, 0.6 g. (95%) of glucoside; needles from water-ethanol, m.p. 189–191° (dec.), $(\alpha)_D^{20}$ − 47.8° (*c*, 0.90 in water).

Anal. Calc'd for $C_{12}H_{18}N_2O_6\cdot C_2H_5OH$: C, 50.6; H, 7.3; C_2H_5OH, 13.8.
Found: C, 50.7; H, 7.3; Loss (130°, 1 mm.), 12.6.

The *monohydrochloride* was obtained from the unstable dihydrochloride, either on allowing the latter to stand in air or by boiling it in methanol; needles from water-methanol which char at 190–200°, but do not melt below 300°.

Anal. Calc'd for $C_{12}H_{19}ClN_2O_6$: Cl, 11.0. Found: Cl, 11.3

o-Aminophenyl-β-D-glucoside (NIH 3634). This deacetylation was effected similarly to the one above. After 15 hours at 3° the yield was 75%, m.p. 183.5–184.5°, $[\alpha]_D^{20}$ − 71.2°. (*c*, 0.55 in water).

Anal. Calc'd for $C_{12}H_{17}NO_6\cdot\frac{1}{2}H_2O$: C, 50.6; H, 6.5; H_2O, 4.7.
Found: C, 50.4; H, 6.4; Loss (140°, 1 mm.), 4.8.

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[6] Three grams of sodium in 100 ml. of methanol.

A sample dried at 130° gave the following analysis:

Anal. Calc'd for $C_{12}H_{17}NO_6$: C, 53.1; H, 6.3.

Found: C, 52.8; H, 6.4.

p-Aminophenyl-β-D-glucoside (NIH 3646) was obtained in a yield of 65%, m.p. 156–158°, $[\alpha]_D^{20}$ − 55.5° (water); lit. (9), m.p. 157–160°, $[\alpha]_D^{20}$ − 65°.

m-Aminophenyl-β-D-glucoside (NIH 3773) crystallized from methanol in needles of m.p. 138–140°[7], $[\alpha]_D^{20}$ − 66.0° (*c*, 0.25 in water).

Anal. Calc'd for $C_{12}H_{17}NO_6 \cdot \frac{3}{4} H_2O$: C, 50.6; H, 6.6; H_2O, 4.7.

Found: C, 50.5; H, 6.9; Loss (77°, 1 mm.), 4.5.

On recrystallization from ethanol and drying for one hour at 77° the compound gave the following analysis:

Anal. Calc'd for $C_{12}H_{17}NO_6 \cdot C_2H_5OH$: C, 53.0; H, 7.3; C_2H_5OH, 14.5.

Found: C, 53.2; H, 6.8; Loss (135°, 1 mm.); 12.2.[8]

2,4-Diguanidinophenyltetraacetyl-β-D-glucoside dihydrochloride. One gram of 2,4-diaminophenyltetraacetyl-β-D-glucoside dihydrochloride, 0.5 g. of cyanamide,[9] and 50 ml. of ethyl acetate, was refluxed for one-half hour, cooled, decanted and the residue dried. The 1.1 g. of tan solid was dissolved in ethanol and the solution shaken with Norit. Addition of ether and cooling gave a solid which was filtered and ground in a mortar under ether. By repeating this purification process 0.8 g. (67%) of an amorphous, hygroscopic solid was obtained which was suitable for analysis after drying at 75°; m.p. 180–225° (dec.), $[\alpha]_D^{20}$ − 8.3° (*c*, 0.54 in ethanol).

Anal. Calc'd for $C_{22}H_{32}Cl_2N_6O_{10}$: Cl, 11.6; N, 13.7.

Found: Cl, 11.9; N, 13.5.

The *sulfate* (NIH 3752) was prepared by addition of 0.9 g. of the dihydrochloride in water to 0.4 g. of silver sulfate in 50–60 ml. of water, ice-cooling, filtering, evaporating the filtrate to dryness *in vacuo*, and triturating the residue with ethanol; 0.8 g. of plates from water-methanol, m.p. 296° (dec., evac. tube)[10], $[\alpha]_D^{20}$ − 17.9° (*c*, 0.43 in water).

Anal. Calc'd for $C_{22}H_{32}N_6O_{14}S$: C, 41.5; H, 5.1; N, 13.2.

Found: C, 41.5; H, 5.2; N, 13.1.

o-Guanidinophenyltetraacetyl-β-D-glucoside hydrochloride (NIH 3789). A mixture of 4.0 g. of *o*-aminophenyltetraacetyl-β-D-glucoside, 1.0 g. of cyanamide, 55 ml. of ethyl acetate, 5 ml. of ethanol, and 1.4 ml. of 20% ethanolic hydrogen chloride, refluxed 40 minutes and cooled gave 2.2 g. (50%) of hydrochloride, m.p. 182–187°; needles from 97% ethanol, m.p. 192–194°, $[\alpha]_D^{20}$ − 8.6° (*c*, 0.48 in water).

Anal. Calc'd for $C_{21}H_{28}ClN_3O_{10} \cdot \frac{1}{2}H_2O$: C, 47.9; H, 5.6; H_2O, 1.7.

Found: C, 47.7; H, 5.7; Loss (140°, 1 mm.), 1.7.[11]

p-Guanidinophenyltetraacetyl-β-D-glucoside hydrochloride (NIH 3786). Two grams of *p*-aminophenyltetraacetyl-β-D-glucoside, 0.5 g. of cyanamide, 25 ml. of ethyl acetate, and 0.8 ml. of 20% ethanolic hydrogen chloride, refluxed two hours and ice-cooled, gave 1.4 g. (60%) of product, m.p. 218–221.5°. It crystallized from water in large prisms which effloresce[12] immediately after filtration to a crystalline powder, m.p. 220–223°, $[\alpha]_D^{20}$ − 27.4° (*c*, 0.36 in water).

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[7] In a preheated bath this compound would melt as low as 115° with bubbling.

[8] The low loss-in-weight and hydrogen values are probably due to some ethanol loss when the sample was dried at 77° prior to analysis.

[9] The cyanamide used was a gift from the American Cyanamid Company. It was freed of about 20% of dicyandiamide by digestion with ether, filtration, and addition of ligroin (30–60°) to the filtrate.

[10] The substance did not melt in an open capillary.

[11] The dried sample was very hygroscopic and quickly attained its original weight.

[12] A recrystallization from methanol-ether gave a mixture of prisms and blades which, after filtration, yielded the same powder of m.p. 220–223°.

Anal. Calc'd for $C_{21}H_{28}ClN_3O_{10}$: C, 48.7; H, 5.5.
Found: C, 48.4; H, 5.5.

m-Guanidinophenyltetraacetyl-β-D-glucoside hydrochloride (NIH 3776). Two grams of *m*-aminophenyltetraacetyl-β-D-glucoside hydrochloride, 0.4 g. of cyanamide, and 25 ml. of ethyl acetate, refluxed 0.5 hour, gave 1.8 g. (80%) of product, m.p. 236–237°, $[\alpha]_D^{20}$ − 22.4° (*c*, 0.39 in water); large needles from methanol-ether.

Anal. Calc'd for $C_{21}H_{28}ClN_3O_{10}$: C, 48.7; H, 5.5.
Found: C, 48.5; H, 5.4.

2,4-Diguanidinophenyl-β-D-glucoside sulfate (NIH 3769) (II). A mixture of 0.7 g. of 2,4-diguanidinophenyltetraacetyl-β-D-glucoside sulfate, 5 ml. of methanol, and 3 ml. (over two moles) of methanolic sodium methoxide[6] was shaken for one hour and let stand for four hours at 25–30° and overnight at 3°. The mixture was filtered (Filter-Cel) and the filtrate diluted with ethanol-ether. Cooling gave 0.35 g. of an amorphous solid which, in a little ice-cold water, was treated with 0.4 ml. of 5 *N* H_2SO_4. Dilution with methanol and cooling gave 0.35 g. (70%) of amorphous sulfate.[13] It was dissolved in a little water and precipitated with methanol (cooling), then ground in a mortar with methanol and filtered. Finally, for analysis it was digested 0.5 hr. in boiling methanol and dried at 77° *in vacuo*; m.p. 220–225° (brown froth), $[\alpha]_D^{20}$ − 37.2° (*c*, 0.41 in water).

Anal. Calc'd for $C_{14}H_{24}N_6O_{10}S$: C, 35.9; H, 5.2; N, 17.9.
Found: C, 35.7; H, 5.3; N, 17.8.

o-Guanidinophenyl-β-D-glucoside (NIH 3842). One gram of *o*-guanidinophenyltetraacetyl-β-D-glucoside hydrochloride, 4 ml. of methanol, and 2 ml. of methanolic sodium methoxide,[6] were let stand seven hours, cooled at 2° and filtered. Dilution with dry ether gave an amorphous solid which crystallized on trituration with hot absolute ethanol; yield 0.5 g. (80%), m.p. 206–207° (dec.), $[\alpha]_D^{20}$ − 88.3° (*c*, 0.41 in water), needles from aqueous methanol-ether or 70% ethanol.

Anal. Calc'd for $C_{13}H_{19}N_3O_6$: C, 49.8; H, 6.1.
Found: C, 49.6; H, 6.1.

The *picrate* crystallized from methanol-ligroin (30–60°) or water in yellow needles, m.p. 208–210°.

Anal. Calc'd for $C_{19}H_{22}N_6O_{13}$: C, 42.1; H, 4.1.
Found: C, 41.9; H, 4.2.

p-Guanidinophenyl-β-D-glucoside picrate. This deacetylation was effected similarly to the previous one. The amorphous base in a little water was added to a hot solution of 0.5 g. of picric acid in 15 ml. of water to give 0.9 g. (80%) of picrate, m.p. 185–190°; yellow needles from water, m.p. 195–196.5°.

Anal. Calc'd for $C_{19}H_{22}N_6O_{13}\cdot 2H_2O$: C, 39.5; H, 4.5; H_2O, 6.2.
Found: C, 39.5; H, 4.7; Loss (117°, 1 mm.), 6.6.

The *hydrochloride* (NIH 3787), prepared by addition of a slight excess of 20% ethanolic hydrogen chloride to an ice-cold dioxane solution of the picrate and dilution with an equal volume of ether, crystallized from methanol-ether (Norit) in prisms of m.p. 223° (dec.), $[\alpha]_D^{20}$ − 53.0° (*c*, 0.33 in water).

Anal. Calc'd for $C_{13}H_{20}ClN_3O_6$: C, 44.6; H, 5.8.
Found: C, 44.6; H, 5.9.

m-Guanidinophenyl-β-D-glucoside (NIH 3783). This deacetylation was effected as described in the two previous experiments. From the methanol filtrate, diluted with 0.5 ml. of dry ether, 0.5 g. (80%) of small prisms separated. Recrystallized from water-ethanol, then methanol-ether, they melted at 200–202° (dec.), $[\alpha]_D^{20}$ −57.0° (*c*, 0.37 in water). A sample was dried at 97° for analysis.

Anal. Calc'd for $C_{13}H_{19}N_3O_6$: C, 49.8; H, 6.1.
Found: C, 49.5; H, 6.6.

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[13] Repeated efforts to crystallize this and several other salts failed.

SUMMARY

1. The β-D-glucosides of *o*-, *m*-, and *p*-aminophenol, 2,4-diaminophenol, and their guanidino analogs are described.

2. A variation of the Michael synthesis has given improved yields of certain nitrophenylglucoside tetraacetates.

3. Hydrogenation of the nitrophenylglucoside tetraacetates with Raney nickel yielded the corresponding amino compounds.

4. The latter, as their hydrochlorides, were converted to the guanidino derivatives in good yield with cyanamide in ethyl acetate.

5. Deacetylation of the tetraacetates of the basic glucosides has been effected with sodium methoxide in methanol.

BETHESDA 14, MD.

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