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

AMINO- AND GUANIDINO-PHENYLGLUCOSAMINIDES

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Received March 29, 1950

In the foregoing communication (1), several amino- and guanidino-phenyl-β-D-glucosides were described. The present paper deals with analogous glucosaminide derivatives which have been tested for their activity in experimental tuberculosis.

To our knowledge, the first synthesis of a phenylglucosaminide was reported by Irvine and Hynd (2) and consisted in the reaction of a phenol (*e.g.*, salicylaldehyde) with 1-bromo-1-desoxy-3,4,6-triacetyl-D-glucosamine hydrobromide (I),[1] pyridine being used as a hydrogen bromide acceptor. Twenty years later, Helferich and Iloff (3) prepared phenyl N-acetyl-β-D-glucosaminide by heating phenol and pentaacetylglucosamine with *p*-toluenesulfonic acid and deacetylating the product with dilute sodium hydroxide. The following year Helferich, *et al* (4) obtained the completely deacetylated glucosaminide[2] by the interaction of sodium phenoxide and I (phenol as solvent) and subsequent alkaline hydrolysis. Finally, the N-*p*-tosyl derivative of this glycoside was prepared (5) from 1-bromo-1-desoxy-N-*p*-tosyl-3,4,6-triacetyl-D-glucosamine and sodium phenoxide followed by deacetylation with barium methoxide.

In preparing glucosaminides of type VIII from nitrophenols, it appeared necessary to mask the amino group of the sugar moiety with an acyl group capable of facile and selective removal at a desired step in the synthesis. The known N-carbobenzyloxy-1,3,4,6-tetraacetyl-D-glucosamine (6, 7) (II) appeared to be a suitable starting material. However, in the bromination of II with hydrogen bromide in acetic acid,[3] scission of the carbobenzyloxy group resulted giving I, identical with that prepared according to Irvine, *et al* (8) from D-glucosamine hydrochloride and acetyl bromide.

We then found that if potassium *p*-nitro- or 2,4-dinitro-phenoxide, the corresponding free phenol, and I were brought to reaction in dry acetone, 30–45% yields of the nitrophenyl-β-D-glucosaminides (III) were obtained and readily converted to the N-carbobenzyloxy derivatives (IV). Hydrogenation of IV in ethyl acetate with Raney nickel afforded the amino compounds (V) which, as hydrochlorides, were condensed with cyanamide to give the guanidines (VI). Removal of the carbobenzyloxy group of VI was effected with palladized charcoal and hydrogen and the acetyl groups were subsequently cleaved with methanolic sodium methoxide, giving the desired products (VIII).

2,4-Diaminophenyl-3,4,6-triacetyl-β-D-glucosaminide (IX) could be prepared

[1] In view of its high positive rotation, this compound is presumed to have the α-configuration.

[2] As Neuberger and Pitt Rivers (5) observed, designation of the glucosaminides of Helferich and co-workers (3, 4) as β is tentative and based only on their mode of formation and rotation. Similarly, the β-configuration assigned to our compounds is provisional.

[3] N-bromosuccinimide was without effect in a single attempt to brominate II.

either by the hydrogenation (Raney nickel) of III-b or by the hydrogenolysis (palladium-charcoal) of V-b. Sodium methoxide deacetylation of IX yielded 2,4-diaminophenyl-β-D-glucosaminide.

Compounds bearing an NIH number in the experimental part were tested *in vitro*. None of them showed significant inhibition of tubercle bacilli (H37Rv, Dubos-Davis medium).[4]

Acknowledgment: We are indebted to Mr. H. George Latham, Jr., of this Laboratory for the preparation of large amounts of 1-bromo-1-desoxy-3,4,6-triacetyl-D-glucosamine hydrobromide. The microanalyses are from the Institute service analytical laboratory under the direction of Mr. William C. Alford.

EXPERIMENTAL[5]

1-Bromo-1-desoxy-3,4,6-triacetyl-D-glucosamine hydrobromide (I).[1] (a) *From D-glucosamine hydrochloride.* The procedure of Irvine, *et al.* (8) was adapted to larger runs. A well-stirred mixture of 16.8 g. of D-glucosamine hydrochloride and 32 ml. of acetyl bromide was kept at 60–65° until the brown broth had changed to a viscous mass (40–60 minutes). Most of the excess reagent was distilled *in vacuo*. The residue was dried overnight *in vacuo* over potassium hydroxide and calcium chloride and digested with 50 ml. of boiling chloroform. Filtration gave 7.5–10.5 g. of starting material.[6] The filtrate was treated with Norit, then diluted with dry ether (stirring) until crystallization began. After cooling at 3° the precipitate was washed with acetone-ether (1:2) to give 7.5–13.5 g. of I, m.p. 153–155° (dec.), $[\alpha]_D^{20}$ +149° (*c*, 0.34 in acetone) in substantial agreement with the reported values (8).

(b) *From II.*[7] Five grams of II (6, 7), 7 ml. of 30% hydrogen bromide in acetic acid, and 2 ml. of acetic anhydride were shaken for 15 minutes and left at 25° overnight. Addition of 30 ml. of dry toluene and 2 ml. of acetic anhydride, evaporation to dryness *in vacuo*, trituration of the residue with dry ether, and ice-cooling gave 4.3 g. (85%) of I; m.p. 154–155° (dec.), $[\alpha]_D^{20}$ +150° (*c*, 0.49 in acetone) after a recrystallization from acetone ether.

2,4-Dinitrophenyl-3,4,6-triacetyl-β-D-glucosaminide[2] (III-b) *hydrochloride* (NIH 3347). To 10 g. of potassium *2,4*-dinitrophenoxide, 10 g. of *2,4*-dinitrophenol, and 150 ml. of acetone (dried over potassium carbonate) was added during 20–30 minutes (shaking), 9.6 g. of I. The mixture was left at 25° for *ca.* 15 hours. Ether and excess dilute potassium carbonate were added. The aqueous layer[8] was again extracted with ether and the combined extracts were washed once with water, dried, and acidified to Congo Red with alcoholic hydrogen chloride. The oily hydrochloride soon crystallized; yield 3.5 g. (32%), m.p. 198–199° (dec.), needles from methanol-ether, $[\alpha]_D^{20}$ −33.1° (*c*, 0.51 in methanol).

Anal. Calc'd for $C_{18}H_{22}ClN_3O_{12}$: Cl, 7.0. Found: Cl, 7.1.

The *base*, obtained from the hydrochloride with dilute aqueous ammonia, crystallized from ethanol in needles, m.p. 147–148°, $[\alpha]_D^{20}$ +63.1° (*c*, 0.21 in $CHCl_3$), after drying at 77°.

Anal. Calc'd for $C_{18}H_{21}N_3O_{12}$: C, 45.9; H, 4.5.

Found: C, 45.4; H, 4.6.

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[4] 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 and methodological aspects will be given elsewhere.

[5] All melting points were observed in a capillary and are uncorrected. Rotations were made in a 4-dm. tube.

[6] The recovered D-glucosamine salt could be reused in this experiment without purification.

[7] This reaction was carried out by Mr. H. George Latham, Jr., of this laboratory.

[8] Acidification of this aqueous layer gave 15 g. of 2,4-dinitrophenol.

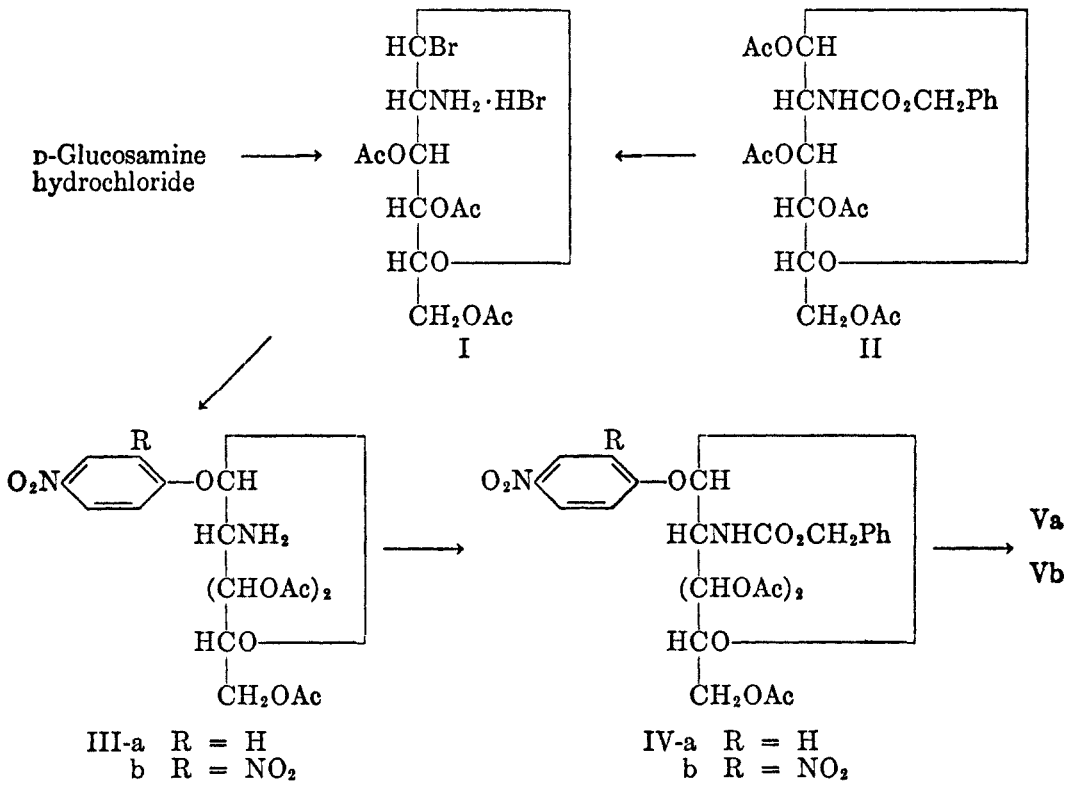
p-Nitrophenyl-3,4,6-triacetyl-β-D-glucosaminide (III-a) *hydrochloride* (NIH 3898). This compound was prepared as described for III-b; yield 44%, fine matted needles from methanol-ether, m.p. 218–220° (dec.), $[\alpha]_D^{20}$ −42.0° (*c*, 0.37 in methanol).

Anal. Calc'd for $C_{18}H_{23}ClN_2O_{10}$: Cl, 7.7. Found: Cl, 7.6.

2,4-Diaminophenyl-3,4,6-triacetyl-β-D-glucosaminide (IX) (NIH 3924). A mixture of 1.6 g. of III-b, 23 ml. of ethyl acetate,[9] and 2 g. of Raney nickel absorbed three moles of hydrogen during 3–5 hours. Rapid filtration at 0° through Filter-Cel into an equal volume of ligroin (30–60°) and ice-cooling gave 1.3 g. (90%) of IX. A quick recrystallization from ethyl acetate gave silvery plates of m.p. 104–107° to a froth; $[\alpha]_D^{20}$ −33.3° (*c*, 0.42 in ethyl acetate).

Anal. Calc'd for $C_{18}H_{27}N_3O_8 \cdot H_2O$: C, 50.3; H, 6.3; N, 9.8; H_2O, 4.2.

Found: C, 50.3; H, 6.4; N, 9.5; Loss (77°, 1 mm.), 4.0.



IX was also prepared in 50–70% yield by the hydrogenation of V-b in ethyl acetate-methanol with 5% palladium-charcoal; reaction time, three hours.

2,4-Diaminophenyl-β-D-glucosaminide (NIH 3913). A mixture of 0.8 g. of IX, 2 ml. of methanol, and 0.2 ml. of methanolic sodium methoxide[10] was shaken to solution and left for one hour at 25° and overnight at 3°; yield of dark brown prisms, m.p. 182–185°, 0.4 g. (75%). They could be obtained white by dissolving them in 10 ml. of boiling methanol, treating the solution with Norit, concentrating the filtrate *in vacuo* under hydrogen to 3 ml., and seeding; m.p. 184–186°, $[\alpha]_D^{20}$ −48.4° (*c*, 0.19 in water).

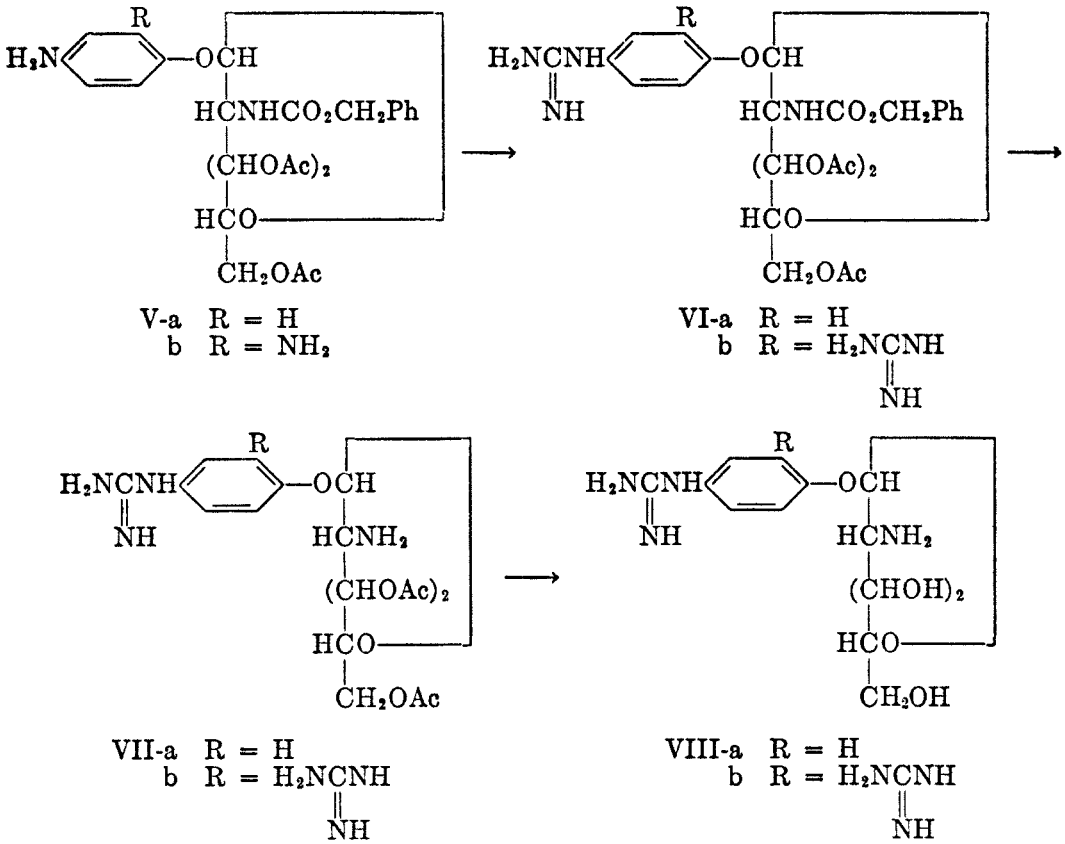
[9] With methanol as the solvent absorption proceeded smoothly, but the resultant filtrate became discolored so rapidly that isolation of IX in a reasonably pure state was practically impossible.

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

Anal. Calc'd for $C_{12}H_{19}N_3O_8$: C, 50.5; H, 6.7.
Found: C, 50.5; H, 7.0.

2,4,-Dinitrophenyl-N-carbobenzyloxy-3,4,6-triacetyl-β-D-glucosaminide (IV-b). To a stirred mixture of 2.0 g. of III-b hydrochloride, 0.8 g. of sodium bicarbonate, and 150 ml. of water was added during 15–20 minutes, 1.2 cc. of benzyl chlorocarbonate. After stirring for 2–3 hours the solid was washed with water and recrystallized from methanol; yield of needles 2.1 g. (88%), m.p. 172–173°, $[\alpha]_D^{20}$ +33.1° (*c*, 0.27 in $CHCl_3$).

Anal. Calc'd for $C_{26}H_{27}N_3O_{14}$: C, 51.6; H, 4.5.
Found: C, 51.6; H, 4.6.



III-b → 2,4-Diaminophenyl-3,4,6-tricetyl-β-D-glucosaminide (IX) ← V-b

p-Nitrophenyl-N-carbobenzyloxy-3,4,6-triacetyl-β-D-glucosaminide (IV-a). As described for IV-b, this product was obtained from aqueous methanol[11] in 90% yield. It crystallized from ethanol[11] as needles, m.p. 176–177°, $[\alpha]_D^{20}$ −8.2° (*c*, 0.46 in $CHCl_3$).

Anal. Calc'd for $C_{26}H_{28}N_2O_{12}$: C, 55.7; H, 5.0.
Found: C, 55.8; H, 5.1.

2,4-Diaminophenyl-N-carbobenzyloxy-3,4,6-triacetyl-β-D-glucosaminide (V-b). Two grams of IV-b and 25 cc. of ethyl acetate absorbed six moles of hydrogen (Raney nickel) during

[11] If the compound was not allowed to crystallize slowly from a warm solution, it precipitated as a gelatinous mass.

4–7 hours. Rapid filtration into two volumes of ligroin (30–60°) gave an oil which soon crystallized; yield 1.7 g. (94%), m.p. 149–150°, needles from propanol-ligroin, $[\alpha]_D^{20}$ ±0.0° (*c*, 0.45 in $CHCl_3$).

Anal. Calc'd for $C_{26}H_{31}N_3O_{10}$: C, 57.2; H, 5.7.

Found: C, 57.2; H, 5.7.

*p-Aminophenyl-N-carbobenzyloxy-3,4,6-triacetyl-β-*D*-glucosaminide* (V-a). Hydrogenation of IV-a as described for IV-b gave a 91% yield of V-a; needles from ethanol, m.p. 167.5–168°, $[\alpha]_D^{20}$ +14.7° (*c*, 0.46 in $CHCl_3$).

Anal. Calc'd for $C_{26}H_{30}N_2O_{10}$: C, 58.9; H, 5.7.

Found: C, 59.0; H, 5.6.

*2,4-Diguanidinophenyl-N-carbobenzyloxy-3,4,6-triacetyl-β-*D*-glucosaminide* (VI-b) *dihydrochloride.* To 1.5 g. of V-b, 0.5 g. of cyanamide, and 75 ml. of ethyl acetate was added 0.45 ml. of conc'd hydrochloric acid and the gelatinous mass was refluxed briskly for 20–24 hrs. During the first eight hours 0.4 g. of cyanamide was added at equal intervals in 0.2-g. portions. After ice-cooling the liquid was decanted from a brown, viscous residue which, in ethanol, was cleared with Norit. Addition of dry ether to the filtrate gave an amorphous hygroscopic solid which was again subjected to this precipitation process. After washing with ether and drying at 77°[12], the sample (1.0 g., 53%) was satisfactory for analysis; $[\alpha]_D^{20}$ +1.2° (*c*, 2.18 in water).

Anal. Calc'd for $C_{28}H_{37}Cl_2N_7O_{10}$: Cl, 10.1; N, 14.0.

Found: Cl, 9.9; N, 13.8.

The *sulfate* was prepared by adding 0.24 g. of silver sulfate in water to 0.54 g. of the dihydrochloride in water, cooling, filtering, and evaporating the filtrate *in vacuo*; oblong plates from water-methanol, m.p. 282–284° (dec.) in a bath preheated to 280°, $[\alpha]_D^{20}$ ±0.0° (*c*, 1.59 in water).

Anal. Calc'd for $C_{28}H_{37}N_7O_{14}S \cdot H_2O$: C, 45.1; H, 5.3; H_2O, 2.4.

Found: C, 44.9; H, 5.4; Loss (160°, 1 mm.), 2.4.[13]

*2,4-Diguanidinophenyl-3,4,6-triacetyl-β-*D*-glucosaminide* (VII-b) *sulfate*[14] Hydrogen was passed through a mixture of 0.5 g. of VI-b sulfate, 0.2 g. of 5% palladium-charcoal, and 10 ml. of water for six hours. The filtered solution was concentrated *in vacuo* to *ca.* 3 ml., ice-cooled, and treated with 0.15 ml. of 5 *N* H_2SO_4. Dilution with a few drops of dioxane gave 0.3 g. (60%) of cubic prisms, m.p. 205° (dec.); $[\alpha]_D^{20}$ −31.8° (*c*, 0.45 in water) after a recrystallization from water. Upon drying at 78° the sample was very hygroscopic but soon became stable as the crystalline pentahydrate.

Anal. Calc'd for $C_{20}H_{32}N_7O_{14}S_{3/2} \cdot 5H_2O$: C, 32.8; H, 5.8; H_2O, 12.3.

Found: C, 32.3; H, 5.9; Loss (97°, 1 mm.), 12.1.[13]

A dried sample was also analyzed.[12]

Anal. Calc'd for $C_{20}H_{32}N_7O_{14}S_{3/2}$: C, 37.4; H, 5.0.

Found: C, 36.8; H, 5.2.

*p-Guanidinophenyl-3,4,6-triacetyl-β-*D*-glucosaminide dihydrochloride* (VII-a). A methanol-ether solution of V-a was acidified to Congo Red with alcoholic hydrogen chloride to give a gelatinous hydrochloride which was filtered, washed with ether, and dried in the desiccator. This hydrochloride (1.5 g.), 0.5 g. of cyanamide, and 20 ml. of 97% ethanol were refluxed for 2–3 hours. Dilution with an equal volume of ether and frequent warming gave 0.9 g. of hygroscopic needles of VI-a hydrochloride. The latter (1.1 g), 0.4 g. of 5% palladium-charcoal, and 25 ml. of methanol were treated with a stream of hydrogen for 6–8 hours. The filtered solution was ice-cooled, acidified to Congo Red with alcoholic hydrogen chloride, diluted with 70 ml. of ether, and cooled in ice to give 0.9 g. of crude VII-a dihydrochloride. For analysis it was recrystallized from methanol-ether, than methanol-ethyl acetate;[11] needles, m.p. 226–227° (dec.), $[\alpha]_D^{20}$ −28.1° (*c*, 0.32 in water).

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[12] The dried compound was very hygroscopic and had to be weighed in a "pig."

[13] On exposure to air the dried sample quickly attained its original weight.

[14] The amorphous trihydrochloride (NIH 3899) was tested.

Anal. Calc'd for $C_{18}H_{28}Cl_2N_4O_8$: C, 44.6; H, 5.5; Cl, 13.9.
Found: C, 44.4; H, 5.6; Cl, 13.8.

The found values are corrected for 1.1% of water, determined by a weight loss at 77°.[13]

2,4-Diguanidinophenyl-β-D-glucosaminide (VIII-b) *sulfate* (NIH 4086). A mixture of 0.6 g. of VII-b sulfate, 6 ml. of methanol, and 3 ml. of methanolic sodium methoxide[10] was shaken for 1–2 hours, cooled to 3°, and filtered. The filtrate, diluted with one volume of ether, gave an amorphous base which, in methanol, was treated with 0.6 ml. of 5 *N* H_2SO_4. The resultant solid was recrystallized from water-dioxane to give 0.4 g. (83%) of sulfate, m.p. 240° (dec.) in a bath preheated to 230°; needles $[\alpha]_D^{20}$ −48.7° (*c*, 0.37 in water). For analysis it was dried at 97° for three hours.[12]

Anal. Calc'd for $C_{14}H_{26}N_7O_{11}S_3/_2$: C, 32.6; H, 5.1; S, 9.3.
Found: C, 32.4; H, 5.2; S, 9.6.

A sample dried to constant weight at 97° gained 11.7% on exposure to air; calc'd gain to $C_{14}H_{26}N_7O_{11}S_3/_2 \cdot 3H_2O$, 10.5%. Thus the air-stable sample appears to be a trihydrate.

p-Guanidinophenyl-β-D-glucosaminide (VIII-a) (NIH 4092). A mixture of 0.6 g. of crude VII-a dihydrochloride, 4 ml. of ethanol, and 4 ml. of sodium methoxide solution[10] gave 0.4 g. of VIII-a, m.p. 224–225° (dec.), containing a little sodium chloride, after two hours at 25° and 1.5 hours at 3°. Recrystallization from water (1 ml.)-methanol (6 ml.) gave heavy prisms or needles, m.p. 225–226° (dec.), $[\alpha]_D^{20}$ −66.7° (*c*, 0.24 in water). The analytical sample was dried at 97°.

Anal. Calc'd for $C_{13}H_{20}N_4O_5$: C, 59.0; H, 6.5.
Found: C, 49.6; H, 6.5.

SUMMARY

Starting from *p*-nitrophenol and 2,4-dinitrophenol, the β-D-glucosaminide derivatives (VIII) of *p*-guanidinophenol and 2,4-diguanidinophenol have been synthesized and found ineffective *in vitro* against tubercule bacilli (H37Rv, Dubos-Davis medium).

2,4-Diaminophenyl-3,4,6-triacetyl-β-D-glucosaminide (IX) was prepared either from 2,4-dinitrophenyl-3,4,6-triacetyl-β-D-glucosaminide (III-b) or from 2,4-diaminophenyl-N-carbobenzyloxy-3,4,6-triacetyl-β-D-glucosaminide (V-b) and deacetylated with methanolic sodium methoxide.

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