The Addition of Glycosyl Azides to Benzyne

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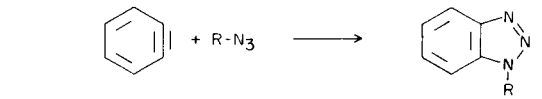
Due to our interest in the preparation of *N*-glycosides of benzotriazole for further testing as potential anticancer agents, we turned our attention to the reaction of organic azides with benzyne. This is a well known method for the preparation of *N*-1 substituted benzotriazoles. This reaction has found numerous applications (1). However this is the first time that a glycosyl azide has been employed; glycosyl azides are easily accessible *via* glycosyl halides and sodium azide.

In the synthetic method here described the configuration of the *N*-glycosides obtained must be the same as in the starting material, for it is not reasonable to assume that addition to benzyne could take place with inversion of configuration at the anomeric carbon. In all the cases studied by us, β-glycosyl azides were used [2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl azide (I_a); 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl azide (I_b) and 2-acetamido-2-deoxy-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl azide (I_c)].

The structures of the products obtained were established beyond reasonable doubt by means of analysis, U.V. and N.M.R. spectroscopy. The U.V. spectra of these derivatives are similar to those of 1-alkylsubstituted benzotriazoles (2). In the N.M.R. spectra the anomeric protons appear as a doublet. The value of their coupling constants are in the usual range for diaxial couplings. This implies that the benzotriazole group must occupy an equatorial position and also confirms the expected β-configuration of the *N*-glycosides, assuming there is not conformational equilibria and that the sugar is in the *C*-1 form.

The benzyne was generated in the presence of the azides following the experimental procedure of Reynolds (1c). The corresponding *N*-glycosides (II) were isolated by column chromatography. In the case of the reaction of 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl azide (I_b), the isolation of the *N*-galactoside (II_b) was accomplished by column chromatography on neutral alumina.

In the last reaction, in addition to the 1-(2′,3′,4′,6′-tetra-*O*-acetyl-β-D-galactopyranosyl)benzotriazole (II_b) a complex mixture of products, with similar R_f, was isolated. This mixture was further purified by repeated t.l.c. on a preparative scale. In this way another *N*-galactoside (II_d) was isolated. The U.V. spectra of this compound shows that we are dealing with a benzotriazole substituted at *N*-1. The analysis of the N.M.R. spectrum indicates that it is a tri-*O*-acetyl-β-D-galactopyranosyl benzotriazole (II_d). Comparison of its N.M.R. spectra with the one corresponding to the tetraacetyl-*N*-galactoside (II_b) suggests that the *C*-3′ acetyl group is the one that has been removed. On the other hand examination of the acetyl region seems to indicate that the acetyl group which was lost occupied an equatorial position. This is further confirmed as follows: the well defined quartet at 4.60 τ ($J_{3'2'}$ = 9.4 cps; $J_{3'4'}$ = 3.1 cps) that is present in the spectrum of the tetraacetyl-*N*-galactoside, and assigned by us to the proton at *C*-3′, has moved upfield in the case of the triacetyl-*N*-galactoside, appearing near the proton attached to *C*-5′ and *C*-6′.



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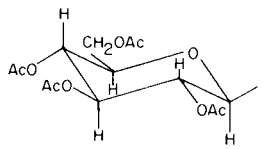

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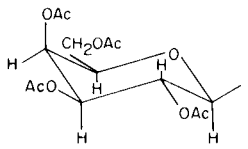

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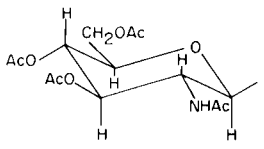

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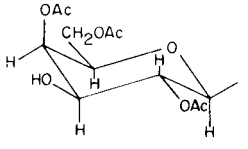

d: R =

The chemical literature reports some cases of deacetylations of sugar derivatives (3) that have taken place on basic alumina. Presently we are studying the possibility of carrying out partial selective deacetylations of *N*-glycosides making use of chromatographic columns with various types of adsorbents.

EXPERIMENTAL

Melting points were taken on a Kofler apparatus. U.V. spectra were determined with a Perkin-Elmer 350 spectrophotometer and N.M.R. spectra with a Perkin-Elmer-R-10 spectrometer. Finally, specific rotations were obtained with a Perkin-Elmer 141 polarimeter.

Column chromatography was performed using silica gel of particle size 0.05-0.2 mm or neutral alumina activity I (Merck). T.l.c. was performed with 0.25 mm chromatoplates of silica gel PF_{254} (Merck) and spots were developed with U.V. light of 254 mμ and sulfuric acid in ethanol, 30%.

General Procedure for the Preparation of the *N*-Glycosides.

To a refluxing solution of 0.01 mole of the glycosyl azide and 0.025 mole of recently distilled *n*-butyl nitrite in methylene chloride (40 ml.) was added in the course of two hours and while stirring a solution of 0.02 mole of anthranilic acid in 25 ml. of acetone. The whole operation was performed under anhydrous conditions. Once the addition had been completed, the dark colored mixture was heated an additional thirty minutes.

The syrupy product that was obtained by evaporation of the solvents under reduced pressure was purified by column chromatography using the conditions specified in each case.

1-(2′,3′,4′,6′-Tetra-*O*-acetyl-β-D-glucopyranosyl)benzotriazole (II_a).

The crude reaction product showed on t.l.c. some starting material (azide Ia), strongly fluorescent products, and the expected *N*-glycoside (R_f 0.36; cyclohexane: ethyl acetate 1:1).

Column chromatography on neutral alumina in petroleum ether (b.p. 50-70°) and eluting with mixtures of petroleum ether: methylene chloride (3:1, 2:1 and 3:2) yielded 3.3 g. of slightly colored solid. Crystallization from alcohol afforded II_a, m.p. 118-119°; $[\alpha]_D$ -61.5° (c 1.04, chloroform). Lit (4) m.p. 118-119°; $[\alpha]_D$ -61.7° (c 0.8, chloroform); U. V. λ max (ethanol); 253 (ε, 6,870); 261 (ε, 6,080) (sh); 283 mμ (ε, 3,900); N.M.R. (deuteriochloroform, τ): 3.82 doublet (H_1', $J_1'_{,2'}$ 8,9 cps); methyl singlets at 7.93 (6H), 7.99 (3H) and 8.25 (3H).

Anal. Calcd. for $C_{20}H_{23}N_3O_9$: C, 53.45; H, 5.12; N, 9.35. Found: C, 53.48; H, 5.30; N, 9.35.

1-β-D-Glucopyranosylbenzotriazole.

This product was obtained by deacetylation of II_a with methanolic ammonia, yield, after recrystallization from methanol-ether, 80%; m.p. 197°; $[\alpha]_D$ -20.4 (c 1.00, ethanol); U.V. λ max (ethanol); 254 (ε, 6,840); 260 (ε, 6,400) (sh); 282 mμ (ε, 4,200); N.M.R. (DMSO, τ); 4.03 doublet ($H_1'_{,2'}$, 9.1 cps).

Anal. Calcd. for $C_{12}H_{15}N_3O_5$: C, 51.24; H, 5.33; N, 14.94. Found: C, 51.18; H, 5.60; N, 14.80.

1-(2′-Acetamido-2′-deoxy-3′,4′,6′-tri-*O*-acetyl-β-D-glucopyranosyl)-benzotriazole (II_c).

Thin layer chromatography of the reaction product showed a spot (R_f 0.35, ethyl acetate) corresponding to the *N*-glucoside plus several others. By column chromatography on silica gel in ethyl acetate and eluting with the same solvent II_c was isolated, m.p. 188° (ethanol-water); $[\alpha]_D$ -69.1° (c 1.11, chloroform); U.V. λ max (ethanol), 254 (ε, 6,860); 260.5 (ε, 6,200) (sh); 283 mμ (ε, 3,910); N.M.R. (deuteriochloroform, τ); 3.32 doublet (H_1', $J_1'_{,2'}$ 9.6 cps); methyl singlets at 7.92 (9H) and 8.35 (3H).

Anal. Calcd. for $C_{20}H_{24}N_4O_8$: C, 53.57; H, 5.35; N, 12.50. Found: C, 53.81; H, 5.67; N, 12.68.

1-(2′-Acetamido-2′-deoxy-β-D-glucopyranosyl)benzotriazoles.

This compound was prepared by deacetylation of II_c with methanolic ammonia; white solid, m.p. 210° dec; (methanol-ether); $[\alpha]_D$ -48.8° (c 1.11, ethanol); yield, 83%; U. V. λ max (ethanol); 255 (ε, 6,615); 260 (ε, 6,270) (sh); 281 (ε, 4,150); 286 mμ (ε, 3,960) (sh); N.M.R. (deuterium oxide, τ); 3.92 doublet (H_1', $J_1'_{,2'}$ 9.2 cps); methyl singlet at 8.52 (3H).

Anal. Calcd. for $C_{14}H_{18}N_4O_5$: C, 52.17; H, 5.66; N, 17.36. Found: C, 52.43; H, 5.69; N, 17.53.

1-(2′,3′,4′,6′-Tetra-*O*-acetyl-β-D-galactopyranosyl)benzotriazole (II_b).

The crude product resulting from the reaction was chromatographed on neutral alumina (200 g.) in petroleum ether. The elution was carried out with various mixtures of petroleum ether: methylene chloride (4:1) (250 ml.); (3:1) (1,000 ml.); (3:2) (500 ml.); (1:1) (400 ml.) and (1:3) (200 ml.) taking fractions of 50 ml. each.

From fractions 1-4 were eluted 0.062 g. of a yellowish oil that was not identified. Fractions 5-9 yielded 0.9 g. of a mixture of II_b plus some of the yellow oil. Purification of the *N*-galactoside was accomplished by preparative t.l.c. on silica gel PF_{254} plates of 2 mm thickness, using as a solvent a mixture of ethyl acetate:petroleum ether (1:2). Each plate was allowed to run three times. In this way there was isolated 0.5 g. of a white product that crystallized from methanol-water, m.p. 98-99°; $[\alpha]_D$ -53.2° (c 1.06, chloroform); U.V. λ max (ethanol); 253 (ε, 7,000); 258 (ε, 6,380) (sh); 281 mμ (ε, 4,040); N.M.R. (deuteriochloroform,τ); 3.74 doublet (H_1', $J_1'_{,2'}$, 9.4 cps); 4.10 triplet (H_2', $J_1'_{,2'}$, $J_2'_{,3'}$, 9.4 cps); 4.34 triplet (H_4', $J_3'_{,4'}$, $J_4'_{,5'}$, 3.1 cps); 4.60 quartet (H_3', $J_3'_{,2'}$, 9.4 cps; $J_3'_{,4'}$, 3.1 cps); 5.7 multiplet (H_5', $2H_6'$); methyl singlets at 7.72 (3H), 8.00 (6H) and 8.25 (3H).

Anal. Calcd. for $C_{20}H_{23}N_3O_9$: C, 53.45; H, 5.12; N, 9.35. Found: C, 53.24; H, 4.97; N, 9.40.

Finally, 2 g. of a white solid was collected from fraction 9-40. This solid consisted of four products of very close R_f values (t.l.c., ethylacetate-petroleum ether, 1:1). As in the previous case purification was achieved using preparative t.l.c. with a mixture of ethyl acetate:petroleum ether (2:1). Allowing several runs for each plate there was isolated 0.38 g. of the least mobile product, 1-(2′,4′,6′-tri-*O*-acetyl-β-D-galactopyranosyl)benzotriazole (II_d), m.p. 185° (ethyl acetate:petroleum ether); U.V. λ max (ethanol); 253; 259; 281 mμ; N.M.R. (DMSO-d_6, τ); 3.61 doublet (H_1', $J_1'_{,2'}$ 9.4 cps); 4.43 (triplet (H_2', $J_{ax\text{-}ax}$ 9.4 cps); 4.61 broad band (H_4'); multiplet between 5.4-6.0 (H_3', H_5' $2H_6'$); methyl singlets at 7.73 (3H), 8.00 (3H) and 8.22 (3H).

Anal. Calcd. for $C_{18}H_{21}N_3O_8$: C, 53.07; H, 5.15; N, 10.32. Found: C, 53.28; H, 5.22; N, 11.10.

1-β-D-Galactopyranosylbenzotriazole.

The deacetylation of II_b was accomplished using the conditions already described. The deacylated triazole was obtained as a white solid, m.p. 172-173° (methanol); $[\alpha]_D$ -3.3° (c 0.99, ethanol). The yield was quantitative; U.V. λ max (ethanol); 254 (ε, 6,000); 260 (ε, 5,000); 280 mμ (ε, 3,680); N.M.R (DMSO-d_6, τ); 4.10 doublet (H_1', $J_1'_{,2'}$ 9.3 cps).

Anal. Calcd. for $C_{12}H_{15}N_3O_5$: C, 51.24; H, 5.33; N, 14.94. Found: C, 50.96; H, 5.22; N, 14.70.

Acknowledgments.

The authors thank Dr. M. Rico for a discussion of the NMR spectra.

This work was supported by a grant from the Fondo Nacional para el Fomento de la Investigación.

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Received August 5, 1968 Madrid, Spain