Heterocyclic *N*-Glycosides. VII. Synthesis of 2-Glycosylindazoles

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In continuation of work on the synthesis of nucleoside analogs as potential anticancer agents, preparation of a series of glycosylindazoles was undertaken by the glycosidation procedure developed by Yamaoka, Aso and Matsuda (1). In this procedure a glycosyl halide is reacted with the appropriate heterocyclic compound, in the presence of mercuric cyanide as a hydrogen halide acceptor. This synthetic method which has been previously used in our laboratory for the synthesis of benzotriazole glycosides (2), is simple and gives good yields of the corresponding *N*-glycosides.

In an earlier publication Bräuniger and Koine (3) reported that the condensation of *N*-trimethylsilylindazole with 2,3,4,6-tetra-*O*-acetyl-α-D-glycopyranosyl bromide gave only a N-2 glycoside to which those authors assigned the β-configuration on the basis of the negative optical rotation value.

Recently, Revankar and Townsend (4) have reported the reaction of 2,3,5-tri-*O*-acetyl-D-ribofuranosyl bromide with several *N*-trimethylsilylindazoles to give in excellent yield the corresponding 2-(β-D-ribofuranosyl)-2*H*-indazoles, the exception being the reaction with *N*-trimethylsilylindazole which furnished a mixture of the two nucleosides, 1- and 2-(β-D-ribofuranosyl)indazole.

In view of these facts we deemed it desirable to determine the effect that the method of glycosidation would have on the nature of the *N*-glycosides obtained and also to explore further the utility of the mercuric cyanide glycosidation procedure.

Chart I

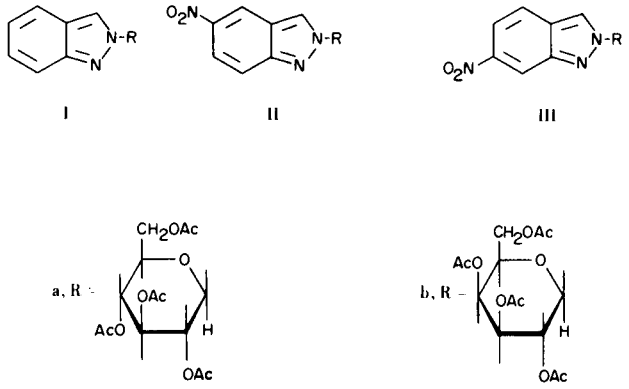
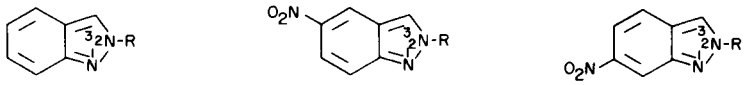


TABLE I

Chemical Shifts for H-3 Indazole Ring Protons
(τ values)



R	$CDCl_3$	DMSO	Δτ(a)	$CDCl_3$	DMSO	Δτ(a)	$CDCl_3$	DMSO	Δτ(a)
Tetra-*O*-acetyl-β-D-glucopyranosyl	1.82	1.45	0.37	1.44	0.91	0.53	1.62	1.09	0.53
β-D-Glucopyranosyl		1.49							
Tetra-*O*-acetyl-β-D-galactopyranosyl				1.41	0.95	0.46	1.55	1.13	0.42
β-D-Galactopyranosyl		1.50			1.05				
CH_3 (6)	2.16	1.69	0.47	1.78	1.27	0.51	1.96	1.44	0.52

(a) $\Delta\tau = \tau_{\text{deuteriochloroform}} - \tau_{\text{DMSO}}$. The Δτ values obtained (6) for 1-methyl-1*H*-indazole, 1-methyl-5-nitro-1*H*-indazole and 1-methyl-6-nitro-1*H*-indazole are 0.1, 0.18, and 0.16, respectively.

In every case studied (Chart I) only the corresponding 2-(β-glycopyranosyl)-2*H*-indazoles were obtained, in yields ranging from 45 to 65%. The position of attachment of the sugar moiety to the indazole ring was established as N-2 on the basis of the significant NMR down-field shift induced by solvent and experienced by the H-3 proton signal on change from chloroform to dimethyl sulfoxide. In fact, as Elguero, Fruchier, and Jacquier have shown (5) in a study of the chemical shifts of the H-3 proton in several 1- and 2-methylindazoles, this proton, in the particular cases of 2-methyl substituted indazoles, is the most sensitive to solvent-induced effects. In Table I are listed the chemical shifts obtained in both deuteriochloroform and DMSO-d_6 for H-3 in the several 2-(β-D-glycopyranosyl)-2*H*-indazoles synthesized and for 2-methylindazoles, along with the $\Delta\tau$ values ($\Delta\tau = \tau_{\text{deuteriochloroform}} - \tau_{\text{DMSO}}$).

On the other hand absorption maxima and extinction coefficients were in the range of those reported (4) for several 2-(β-D-ribofuranosyl)-2*H*-indazoles.

The β configuration of all the compounds was assigned by NMR measurements. The H-1′ signals appeared as doublets (in DMSO-d_6 solution) with coupling constants of 8-9 Hz, which are a clear indication of the diaxial orientation of H-1′ and H-2′.

In passing, it should be noted that the chloroform-insoluble fraction obtained in the isolation of the *N*-glycosides seems to be a complex compound of the corresponding indazole with mercuric chloride or bromide (depending on the glycosyl halide used).

All the compounds obtained were screened in our laboratories for antitumor activity in cell-culture tests against HeLa cells at three dose levels (1, 10, and 100 μg/mg.). No significant activity was found. *N*-glycosides Ia, IIa, and IIIa were also screened against mouse Sarcoma 180 at doses of 400 mg./kg./day, as determined from the toxicity tests. These experiments were carried out according to the criteria established by the CCNSC (6). None of these products showed any significant activity.

EXPERIMENTAL

Melting points are uncorrected. NMR spectra were recorded on a 60 MHz Perkin-Elmer R-12 spectrometer with TMS as an internal standard. UV spectra were measured on a Perkin-Elmer 350 spectrophotometer. Optical rotations were obtained with a Perkin-Elmer 141 polarimeter. T.l.c. was performed on chromatoplates coated with silica gel GF_{254} (Merck) and spots were visualized with ultraviolet light of 254 mμ.

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

To a mixture of 0.015 mole of the glycosyl halide (2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl chloride or 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide), 2.7 g. of mercuric cyanide and 5 g. of anhydrous calcium sulfate (Sikon) in 100 ml. of nitromethane, was added 0.01 mole of the appropriate indazole. The mixture was refluxed for 3-4 hours. After this, it was filtered while still hot in order to remove an insoluble residue which was washed with more hot nitromethane, and the filtrate was evaporated to dryness *in vacuo*. The product obtained was treated with chloroform and filtered to separate a solid (a complex formed by mercuric halide and the corresponding indazole). The chloroform extract was washed with 30% aqueous potassium iodide, water and then dried over anhydrous sodium sulfate. Finally, the product obtained after removing the solvent was purified as specified in each case.

2-(2′,3′,4′,6′-Tetra-*O*-acetyl-β-D-glucopyranosyl)-2*H*-indazole (Ia) and 2-(β-D-glucopyranosyl)-2*H*-indazole.

The crude product was purified by thick-layer chromatography (silica gel PF_{254}, ether-petroleum ether 2:1). After four consecutive developments two fundamental bands were separated. The faster moving band had the same Rf as the indazole. From the slower moving one Ia was obtained as a solid which was recrystallized from ethanol, m.p. 163-164°, $[\alpha]_D$ -37.8° (c 1.0, chloroform); yield, 63%; NMR (DMSO-d_6, τ), 3.69 doublet (H_1', $J_{1',2'}$ 9.3 Hz); UV λ max (ethanol), 277 (ε, 8,176), 293 mμ (ε, 7,008); Lit. (3) m.p. 164-165°, $[\alpha]_D$ -40.3° (c 2.42, chloroform).

This product (1 g.) was treated with 75 ml. of methanolic ammonia (saturated at 0°) and the solution was left overnight at room temperature. After removing the solvent a solid was obtained, 2-(β-D-glycopyranosyl)-2*H*-indazole, m.p. 205-207° (ethanol), $[\alpha]_D$ -33.2° (c 0.56, pyridine); NMR (DMSO-d_6, τ), 4.5 doublet (H_1', $J_{1',2'}$ 8.6 Hz); UV λ max (ethanol), 276 (ε, 7,224), 294 mμ (ε, 6,321).

Anal. Calcd. for $C_{13}H_{16}N_2O_5$: C, 55.71; H, 5.71; N, 10.00. Found: C, 55.62; H, 5.70; N, 10.21.

2-(β-D-Galactopyranosyl)-2*H*-indazole.

The crude product was purified as above by thick-layer chromatography (ethyl acetate-cyclohexane 1:1). The slower moving band afforded a 65% yield of the *N*-glycoside Ib as a syrup which could not be crystallized. The syrup was dissolved in 150 ml. of methanolic ammonia and the solution was allowed to stand overnight at room temperature. The residue obtained after removing the solvent was recrystallized from ethanol to give 2-(β-D-galactopyranosyl)-2*H*-indazole, m.p. 225-227°, $[\alpha]_D$ +3.6° (c 0.49, pyridine); NMR (DMSO-d_6, τ), 4.54 doublet (H_1', $J_{1',2'}$ 8.6 Hz); UV λ max (ethanol), 276 (ε, 7,200), 294 mμ (ε, 6,500).

Anal. Calcd. for $C_{13}H_{16}N_2O_5$: C, 55.71; H, 5.71; N, 10.00. Found: C, 55.77; H, 5.56; N, 9.75.

2-(2′,3′,4′,6′-Tetra-*O*-acetyl-β-D-glucopyranosyl)-5-nitro-2*H*-indazole (IIa).

The crude product was recrystallized three times from ethyl acetate-petroleum ether to give a yellow solid, IIa, m.p. 239-240°; $[\alpha]_D$ -57.4° (c 0.51, chloroform); yield, 45%; NMR (DMSO-d_6, τ), 3.56 doublet (H_1', $J_{1',2'}$ 8 Hz); UV λ max (ethanol), 261 (ε, 23,333), 294 (ε, 8,095), 305 mμ (ε, 8,095).

Anal. Calcd. for $C_{21}H_{23}N_3O_{11}$: C, 51.11; H, 4.66; N, 8.51. Found: C, 51.27; H, 4.61; N, 8.33.

2-(2′,3′,4′,6′-Tetra-*O*-acetyl-β-D-galactopyranosyl)-5-nitro-2*H*-indazole (IIb) and 2-(β-D-galactopyranosyl)-5-nitro-2*H*-indazole.

The syrup obtained after removing the chloroform was chromatographed (thick-layer chromatography, ethyl acetate-petroleum ether 1:1). From the most important band a syrup was separated

(2.7 g.) which was assumed to be the protected *N*-glycoside IIb. Deacetylation of this compound and working up as above furnished a yellow solid 2-(β-D-galactopyranosyl)-5-nitro-2*H*-indazole, m.p. 163-164° (ethanol), $[\alpha]_D$ +4.2° (c 0.52, pyridine); NMR (DMSO-d_6, τ), 4.45 doublet (H_1', $J_{1',2'}$ 8.6 Hz); UV λ max (ethanol), 261 (ε, 22,850), 294 (ε, 7,320), 305 mμ (ε, 7,320).

Anal. Calcd. for $C_{13}H_{15}N_3O_7$: C, 48.00; H, 4.61; N, 12.92. Found: C, 48.25; H, 4.54; N, 12.86.

2-(2',3',4',6'-Tetra-*O*-acetyl-β-D-glucopyranosyl)-6-nitro-2*H*-indazole (IIIa).

The crude reaction residue was directly recrystallized from ethyl acetate to give IIIa, m.p. 228-229°, $[\alpha]_D$ −32.2° (c 0.54, chloroform); yield, 48%; NMR (DMSO-d_6, τ), 3.61 doublet (H_1', $J_{1',2'}$ 8 Hz); UV λ max (ethanol), 264 (ε, 22,300), 284 mμ (ε, 11,827).

Anal. Calcd. for $C_{21}H_{23}N_3O_{11}$: C, 51.11; H, 4.66; N, 8.51. Found: C, 51.28; H, 4.54; N, 8.63.

2-(2',3',4',6'-Tetra-*O*-acetyl-β-D-galactopyranosyl)-6-nitro-2*H*-indazole (IIIb).

The reaction product was recrystallized from ethyl acetate-petroleum ether to give 2.7 g. of IIIb. The analytical sample was recrystallized from ethanol, m.p. 179-180°, $[\alpha]_D$ −3.4° (c 0.55, chloroform); NMR (DMSO-d_6, τ), 3.64 doublet (H_1', $J_{1',2'}$ 8 Hz); UV λ max (ethanol) 263 (ε, 23,989), 283 mμ (ε, 12,814).

Anal. Calcd. for $C_{21}H_{23}N_3O_{11}$: C, 51.11; H, 4.66; N, 8.51. Found: C, 50.91; H, 4.72; N, 8.44.

Acknowledgments.

The authors wish to thank Professor M. Lora-Tamayo for his kind encouragement. Thanks are also due to Dr. V. Gimeno for assistance in the synthetic work.

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

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Received July 31, 1970 Madrid-6 Spain