

The Synthesis and PMR Study of Certain 3-Substituted 2-(β -D-Ribofuranosyl)indazoles (1a)

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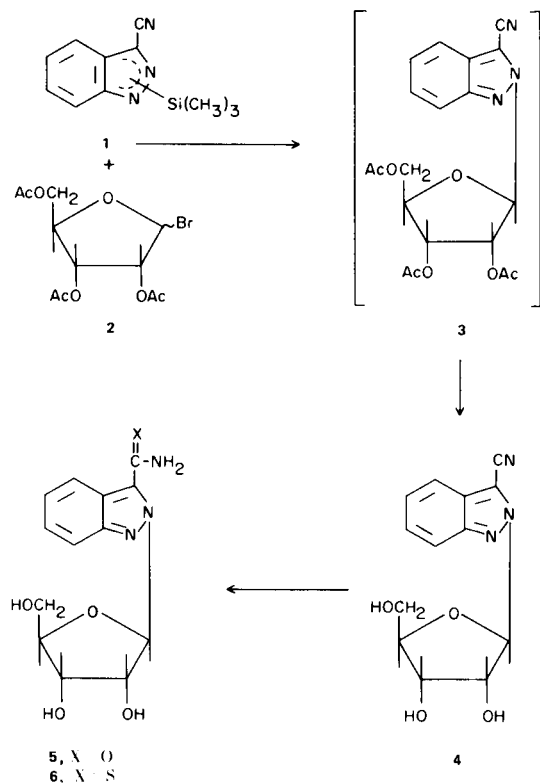
The synthesis of 3-cyano-2-(β -D-ribofuranosyl)indazole (**4**) has been accomplished by a condensation of *N*-trimethylsilyl-3-cyanoindazole (**1**) with 2,3,5-tri-*O*-acetyl-D-ribofuranosyl bromide (**2**) followed by subsequent deacetylation. The reactivity of the 3-cyano group was demonstrated by the conversion of **4** to 2-(β -D-ribofuranosyl)indazole-3-carboxamide (**5**) and 2-(β -D-ribofuranosyl)indazole-3-thiocarboxamide (**6**). The site of ribosylation and the assignment of anomeric configuration for **4** is discussed. The magnetic anisotropy effect of the exocyclic group at C3 on the anomeric proton as determined by pmr spectroscopy is discussed.

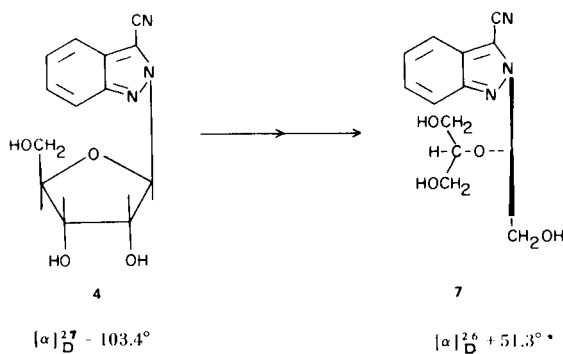
The syntheses of certain indazole nucleosides was recently reported (2) from our laboratory. These nucleosides were prepared in the course of our study on the directive effect which certain groups residing in the benzene moiety of the indazole ring possessed. We now wish to report on the directive effect produced by a cyano group residing in the pyrazole moiety (C3) of the indazole ring.

The silylation of 3-cyanoindazole (3) with hexamethyldisilazane has furnished *N*-trimethylsilyl-3-cyanoindazole

(**1**) in quantitative yield. The condensation of **1** with 2,3,5-tri-*O*-acetyl-D-ribofuranosyl bromide (**2**) in the presence of sodium iodide afforded a 23% yield of nucleoside material (**3**). Deacetylation of the carbohydrate moiety of **3** was accomplished with methanolic ammonia to furnish a nucleoside which was subsequently established as 3-cyano-2-(β -D-ribofuranosyl)indazole (**4**). The low yield of nucleoside by this method prompted us to investigate another method (4) using mercuric bromide and mercuric oxide as catalysts in boiling benzene solution, which afforded an overall 85% yield of **4**. The site of ribosylation was readily determined as N2 by a comparison between the ultraviolet absorption spectra observed for **4** with the ultraviolet absorption spectral data reported (3) and observed (Table I) for the 1- and 2-methyl derivatives of 3-cyanoindazole.

The anomeric configuration of **4** was initially assigned as *beta* on the basis of a large negative specific rotation $[\alpha]_{\text{D}}^{27} - 103.4^\circ$ (pyridine) and the *trans* rule (5). Recent reports on the synthesis of ribofuranosyl benzimidazoles (6), benzotriazoles (7) and indazoles (2) suggested that the silylation procedure will usually afford only the β -anomer. However, it seemed desirable to obtain additional support for the above anomeric assignment. In an effort to assign the anomeric configuration on the basis of the coupling constants ($J_{1,2}$) of the anomeric proton, it was observed that the pmr spectrum of **4** in DMSO- d_6 revealed a doublet centered at 374 Hz with a $J_{1,2}$ of approximately 4 Hz. This precluded the use of this method (8) for unequivocal anomeric assignment of this nucleoside. Therefore, in an effort to obtain additional support for the above anomeric assignment, **4** was subjected to periodate oxidation followed by reduction with sodium borohydride (9). Thus, 3-cyano-2-(β -D-ribofuranosyl)indazole (**4**), $[\alpha]_{\text{D}}^{27} - 103.4^\circ$ produced **7**, $[\alpha]_{\text{D}}^{26} + 51.3^\circ$.





This same change from a large negative to a positive value was observed previously for other indazole nucleosides (2) and this data supports the initial assignment of anomeric configuration as *beta*.

Treatment of **4** with 30% hydrogen peroxide in concentrated ammonium hydroxide solution gave a 95% yield of 2-(β -D-ribofuranosyl)indazole-3-carboxamide (**5**) (infrared spectrum revealed the absence of nitrile absorption in the 2250 cm^{-1} region). The treatment of **4** with hydrogen sulfide and triethylamine in pyridine solution furnished a good yield of 2-(β -D-ribofuranosyl)indazole-3-thiocarboxamide (**6**).

The pmr spectrum of **6** revealed a very significant reduction in the coupling constant ($J_{1,2}$) observed for the anomeric proton, doublet centered at 405 Hz with a $J_{1,2} = 2$ Hz, which lends additional support for the assignment of anomeric configuration as *beta*. Also of considerable interest, was the downfield shift observed for the anomeric proton of **6** in comparison with the anomeric proton of **4**. This significant chemical shift, $\Delta\text{Hz} = 31$ Hz, must be due to the magnetic anisotropy effect of the thiocarbonyl group (10). A comparable shift was still observed on

change of solvent (DMSO- d_6 /deuterium oxide). A large chemical shift, in comparison to **4**, was also observed for the anomeric proton (doubled centered at 407.5 Hz) of **5** ($\Delta\text{Hz} = 33$ Hz) and can be attributed to the magnetic anisotropy effects of the carbonyl group. This is of considerable interest since the anisotropy effect was observed (10) for a number of nucleosides with a C=S group residing in the ring system directly adjacent to the site of glycosidation but the same effect was not observed for the C=O group. This must be due to the difference in proximal effects between the C=O group in the ring and in the present instance where the C=O group is part of an exocyclic group (carboxamide). One other point of interest in the pmr spectra of **5** and **6** was the occurrence of a broad singlet (2 protons) at 484 Hz for the protons of the carboxamide group of **5** and two broad singlets (1 proton each) at 586 Hz and 630 Hz for the protons of the thiocarboxamide group. This difference can presumably be ascribed to a restriction of rotation around the C-N bond of the thiocarboxamide group with free rotation being observed for the carboxamide group. These peaks were firmly established as the NH_2 protons since on the addition of deuterium oxide there was observed a disappearance of these peaks (484 Hz for **5**; 586 Hz and 630 Hz for **6**) with the appearance of a strong HDO peak at approximately 250 Hz.

EXPERIMENTAL (11)

3-Cyano-2-(β -D-ribofuranosyl)indazole (**4**).

Method 1.

To a mixture of dry 3-cyanoindazole (**3**) (7.15 g.) and freshly distilled hexamethyldisilazane (8.0 g.) was added a catalytic amount of ammonium sulfate (approximately 20 mg.) and the

TABLE I

Ultraviolet Absorption Spectra of Ribofuranosylindazoles and Methylindazoles (a)

| Compound | pH 1 | | Methanol | | pH 11 | |
|---|------------------|------------|------------------|------------|------------------|------------|
| | λ max nm | ϵ | λ max nm | ϵ | λ max nm | ϵ |
| 3-Cyano-2-(β -D-ribofuranosyl)indazole, 4 . | 243 (b) | 4950 | 244 (b) | 4125 | 244 (b) | 6325 |
| | 296 | 11800 | 295 | 12100 | 296 | 13500 |
| 1-Methyl-3-cyanoindazole. | 275 | 8640 | 273 | 7225 | 276 | 7065 |
| | 295 | 10050 | 296 | 9100 | 296 | 9420 |
| 2-Methyl-3-cyanoindazole. | 242 (b) | 3915 | 243 (b) | 4550 | 243 (b) | 3915 |
| | 294 | 11300 | 294 | 11300 | 295 | 11000 |
| 2-(β -D-Ribofuranosyl)indazole-3-carboxamide, 5 . | 295 | 9380 | 294 | 10840 | 294 | 9670 |
| 2-(β -D-Ribofuranosyl)indazole-3-thiocarboxamide, 6 . | 302 | 10200 | 302 | 9270 | 300 | 10200 |

(a) Spectra were obtained with a Beckman DK-2 Ultraviolet Spectrophotometer. (b) Shoulder.

reaction mixture heated at 130° with the exclusion of moisture. Within 10 minutes a clear brown solution was obtained which was followed by a profusion of ammonia. The reaction mixture was heated at this temperature for an additional 15 hours with stirring, and then fractionated by distillation *in vacuo* to obtain *N*-trimethylsilyl-3-cyanoindazole (1) as a colorless liquid which solidified on cooling, b.p. 120°/0.5 mm. (10.6 g., quantitative yield). This material was used in the following condensation reaction immediately after distillation. *N*-Trimethylsilyl-3-cyanoindazole (1, 8.6 g.) was mixed with 2,3,5-tri-*O*-acetyl-D-ribofuranosyl bromide (2, from 14.0 g. of 1,2,3,5-tetra-*O*-acetyl-D-ribofuranose) and a few crystals of sodium iodide (approximately 10 mg.). The mixture was heated at 80-85° (oil bath temperature) for 20 minutes *in vacuo* (1.5 mm.) with good stirring. Considerable blackening of the reaction mixture was noticed. The cooled reaction mixture was dissolved in chloroform (250 ml.) and the chloroform phase washed with cold saturated aqueous sodium carbonate solution (3 x 75 ml.) followed by cold water (3 x 75 ml.). After drying over anhydrous sodium sulfate overnight, the chloroform was removed under reduced pressure at 35° to afford a brown syrup. This syrup was dissolved in boiling methanol (200 ml.), treated with Norit and the methanol evaporated to dryness to furnish a syrup (3.7 g., 23%) which was assumed to be 3-cyano-2-(2',3',5'-tri-*O*-acetyl- β -D-ribofuranosyl)indazole (3). This nucleoside material (3) was dissolved in methanolic ammonia (methanol saturated with ammonia at 0°, 100 ml.) and the solution allowed to stand at room temperature for 25 hours with occasional shaking. Methanolic ammonia was removed *in vacuo* on a steam bath to afford a syrup. This syrup was then triturated with cold water (25 ml.) for 2 hours. The solid which had separated was collected, washed with cold water (2 x 20 ml.) and crystallized from aqueous ethanol, with the aid of Norit, to furnish 3-cyano-2-(β -D-ribofuranosyl)indazole (4) as colorless needles, 2.1 g. (82.7%). A small sample was recrystallized from ethanol for analysis, m.p. 161°, $[\alpha]_{\text{D}}^{27} - 103.4^{\circ}$ (C=1, pyridine).

Anal. Calcd. for C₁₃H₁₃N₃O₄: C, 56.72; H, 4.73; N, 15.27. Found: C, 56.79; H, 4.87; N, 15.37.

Method 2.

A suspension of mercuric bromide (10.0 g.) and mercuric oxide (10.0 g.) in anhydrous benzene (250 ml.) was dried azeotropically for 2 hours. A solution of 1 (8.6 g.) in dry benzene (50 ml.) was added to the above suspension followed by 2 (14.0 g.) in dry benzene (50 ml.). The reaction mixture was heated at reflux temperature for 20 hours with good stirring. Considerable frothing was noticed in the beginning. The cooled reaction mixture was filtered and the residual mercuric salt washed with methanol (2 x 50 ml.) and then chloroform (3 x 50 ml.). The combined filtrates were evaporated *in vacuo* and the residual solid was dissolved in chloroform (400 ml.). The chloroform phase was washed with aqueous 30% potassium iodide solution (2 x 50 ml.) followed by cold saturated aqueous sodium carbonate solution (3 x 75 ml.) and water (3 x 75 ml.). After drying over anhydrous sodium sulfate, the chloroform was removed to afford 3 (13.6 g., 85%) as a syrup which was dissolved in methanolic ammonia (400 ml.) and allowed to stand overnight. The methanolic ammonia was removed *in vacuo* to afford a syrup which was triturated with cold water (75 ml.) for 2 hours. The solid which had separated was collected and crystallized from aqueous ethanol to obtain 4 as colorless needles, 8.2 g. (88%), m.p. 161°. $[\alpha]_{\text{D}}^{27} - 103.2^{\circ}$ (C=1, pyridine).

Anal. Calcd. for C₁₃H₁₃N₃O₄: C, 56.72; H, 4.73. Found:

C, 56.75; H, 4.78.

2-(β -D-Ribofuranosyl)indazole-3-carboxamide (5).

To 3-cyano-2-(β -D-ribofuranosyl)indazole (4, 1.5 g.) in 50 ml. of concentrated ammonium hydroxide was added 5 ml. of 30% hydrogen peroxide and the mixture stirred for 3 hours at room temperature. After 10 minutes the starting material had gone into solution. The reaction mixture was allowed to stand at 5° for 18 hours and filtered. The filtrate was evaporated *in vacuo* on a steam bath, the residual solid was collected and washed thoroughly with cold water. The product (1.45 g., 90.7%) was crystallized from aqueous ethanol to yield an analytical sample, m.p. 155° (dec.), $[\alpha]_{\text{D}}^{27} - 92.1^{\circ}$ (C=1, pyridine). The characteristic absorption peak for a cyano group was not observed in the infrared spectrum at 2250 cm⁻¹, but a strong absorption peak was observed at 1650 cm⁻¹ for the -NC=O moiety.

Anal. Calcd. for C₁₃H₁₅N₃O₅·H₂O: C, 50.16; H, 5.46; N, 13.50. Found: C, 50.50; H, 5.30; N, 13.72.

2-(β -D-Ribofuranosyl)indazole-3-thiocarboxamide (6).

To 75 ml. of pyridine containing 1.0 ml. of triethylamine was added 2.0 g. of 4. This solution was stirred at room temperature for 6 hours with continual passage of hydrogen sulfide gas through the solution. The passage of hydrogen sulfide gas was ceased and the dark solution was stirred at room temperature for an additional 18 hours. The pyridine was removed *in vacuo* and the resulting residue was slurried with water several times and evaporated to dryness each time. The solid was washed with a small amount of acetone and the product crystallized from aqueous ethanol to yield 1.2 g. (53.4%) of yellow needles. A small sample was recrystallized from ethanol for analysis, m.p. 182° (dec.), $[\alpha]_{\text{D}}^{27} - 31.4^{\circ}$ (C=1, pyridine). There was an absence of any peak at 2250 cm⁻¹ for the cyano group, but an absorption peak was observed at 1550 cm⁻¹ for the -NC=S moiety in the infrared spectrum.

Anal. Calcd. for C₁₃H₁₅N₃O₄S: C, 50.48; H, 4.86; N, 13.60. Found: C, 50.46; H, 4.85; N, 13.59.

Methylindazoles.

The 1- and 2-methyl derivatives of 3-cyanoindazole have been prepared according to the reported procedure (3). The ultraviolet absorption spectral data for these compounds were obtained and are included in Table I since this data was used to establish the actual site of ribosylation for 4.

Periodate Oxidation and Sodium Borohydride Reduction.

To 40 mg. of 3-cyano-2-(β -D-ribofuranosyl)indazole (4) was added 4.0 ml. of 0.08 *M* sodium periodate solution and the mixture stirred at room temperature for 15 minutes. Sodium borohydride (120 mg.) was then added and the resulting solution allowed to stand at room temperature for another 30 minutes. The excess reducing agent was then destroyed by dropwise addition of 10% acetic acid (1.4 ml.) until gas evolution ceased. The optical rotation was determined on this solution as $[\alpha]_{\text{D}}^{26} + 51.3^{\circ}$ based on the original weight of 4.

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