Synthesis of Indazole *C*-Nucleosides and Analogues

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1-Deoxy-1-diazo-3,6-anhydro-4,5,7-tri-*O*-benzoyl-D-*allo*-heptulose (III) has been prepared from 2,5-anhydro-3,4,6-tri-*O*-benzoyl-D-allonic acid. 1,3-Dipolar cycloaddition of III to benzyne afforded the indazole *C*-nucleoside analog V. Cycloaddition of methyl 6-deoxy-6-diazo-2,3-*O*-isopropylidene-β-D-ribohexofuranosid-5-ulose (IV) to the benzyne generated from 5-methylanthranilic acid gave a mixture of the β-isomeric *C*-glycosylindazoles VI and VII along with traces of the corresponding α-anomers VIa and VIIa. Finally, a multistep transformation of the acyclic carbohydrate moiety of 2,3,4,5-tetra-*O*-acetyl-1-(indazol-3-yl)-keto-D-ribopentulose (I, R = H, n = 3, D-ribo) led to the *C*-nucleoside indazole, 3-(2,3-*O*-isopropylidene-β-D-ribofuranosyl)-indazol (X), as the major product.

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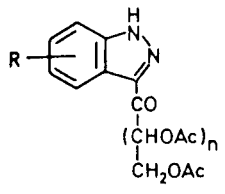
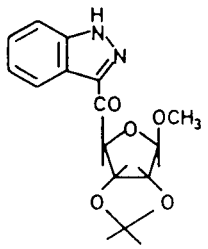
In a previous paper (1) we described the 1,3-dipolar cycloaddition of acyclic 1-diazo-1-deoxy-2-ketosugars to benzynes to give *C*-glycosylindazoles of type I. We also reported the synthesis of indazole *C*-nucleoside analog II by cycloaddition of methyl 6-deoxy-6-diazo-2,3-*O*-isopropylidene-β-D-ribohexafuranosid-5-ulose (2) to benzyne.



I

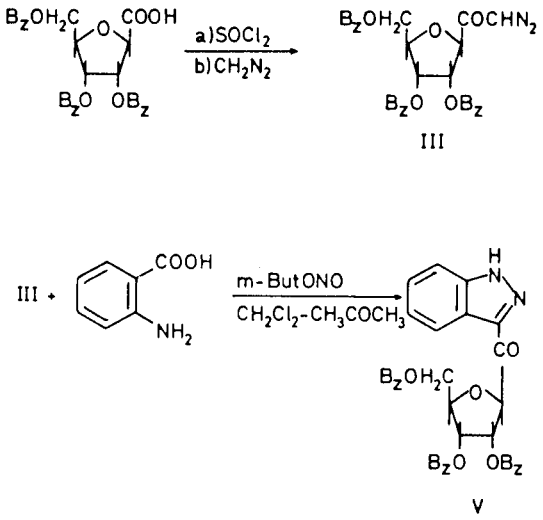


II

All of these *C*-glycosylindazoles were tested in our laboratories for antitumor activity against HeLa cells and only compound II showed a significant activity (DI_{50} = 9 μg./ml.). This fact and our interest in the preparation of *C*-nucleosides and analogous compounds has prompted us to prepare other *C*-glycosylindazoles in which the carbohydrate moiety is in the cyclic form. The synthesis of these compounds has been accomplished by two different routes depending on the type of linkage between the sugar and the heterocyclic base. Thus, the nucleoside analogues V, VI, VII, VIa and VIIa in which the ribofuranose moiety is separated from the indazole ring by a keto group, were prepared by 1,3-dipolar cycloaddition of diazoketoses to benzynes. On the other hand, the indazole *C*-nucleoside X was obtained, along with traces of XI, from the previously described 2,3,4,5-tetra-*O*-acetyl-1-(indazol-3-yl)-keto-D-ribopentulose (I, R = H, n = 3, D-ribo) (1) by a multistep transformation of its acyclic carbohydrate chain.

The diazoketoses employed in the cycloaddition reactions were methyl 6-deoxy-6-diazo-2,3-*O*-isopropylidene β-D-ribohexafuranosid-5-ulose (IV) (2) and the hitherto unknown 1-deoxy-1-diazo-3,6-anhydro-4,5,7-tri-*O*-benzoyl-D-*allo*-heptulose (III). This latter was obtained in 53% yield as a syrup from 2,5-anhydro-3,4,6-tri-*O*-benzoyl-D-allonic acid (3) *via* its acid chloride (Scheme I). Its structure was clear from its elemental analysis and its strong absorption in the diazo region (2130 cm^{-1}) shown in its ir spectrum. It should be pointed out that this furanosyl diazoketone can provide a versatile starting material for the synthesis of β-D-ribofuranosyl-*C*-nucleosides by elaboration of the heterocyclic system from the suitably functionalized *C*-glycosyl derivative, obtained by conversion of the reactive diazoketone group.

SCHEME I



III

V

Cycloaddition of III to benzyne, following the experimental procedure (1) used for the preparation of I (R = H) and II led to the expected homonucleoside V in 41% yield (Scheme I) which was identified from its analytical and spectroscopic data (Tables I and II).

Reaction of IV with 5-methylanthranilic acid under identical conditions to those used in the cycloaddition

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Table I

Chemical Shifts for Compounds V, VI, VII, VIa and VIIa at 100 MHz with TMS as Internal Standard
τ Values

Compound	Solvent	H_1'	H_2'	H_3'	H_4'	H_5'	$H_6'a$	$H_6'b$	CH_3 / CH_3	Others
V	Deuteriochloroform	--	4.03 (d)	3.82 (dd)	4.15 (dd)	⟵ 5.20 (m) ⟶			---	1.82 (m, 1, H_4), 2.10 and 2.70 (m, 18, Ph)
VI + VII	Deuteriochloroform	4.86 (s)	5.35 (d)	4.42 (dd)	4.35 (d)	--	--	--	8.38, 8.63 (s) ($\Delta\delta$ = 0.25)	1.84 (d, 1, H_4, VII), 1.90 (d, 1, H_4, VI), 6.86 (s, 6, OCH_3, VI + VII), 7.54 (s, 6, CH_3, VI + VII)
VIa + VIIa	Deuteriochloroform	4.80 (s)	5.28 (d)	⟵ 4.47 (m) ⟶		--	--	--	8.76, 8.59 (s) ($\Delta\delta$ = 0.17)	1.84 (d, 1, H_4, VIIa), 1.90 (d, 1, H_4, VIa), 6.62 (s, 6, OCH_3, VIa + VIIa), 7.54 (s, 3, CH_3, VIIa), 7.60 (s, 3, CH_3, VIa)

Table II

Coupling Constants for Compounds V, VI, VII, VIa and VIIa (Hertz)

Compound	$J_{1',2'}$	$J_{2',3'}$	$J_{3',4'}$	$J_{4',5'}$	$J_{5',6'a}$	$J_{5',6'b}$	$J_{6'a,6'b}$	Others
V	--	2.5	6	5	(a)	(a)	(a)	
VI + VII	0	6	<1	--	--	--	--	VI: $J_{4,6}$ = 2 VII: $J_{4,5}$ = 9.5
VIa + VIIa	0	6	(a)	--	--	--	--	VIa: $J_{4,6}$ = 2 VIIa: $J_{4,5}$ = 9.5

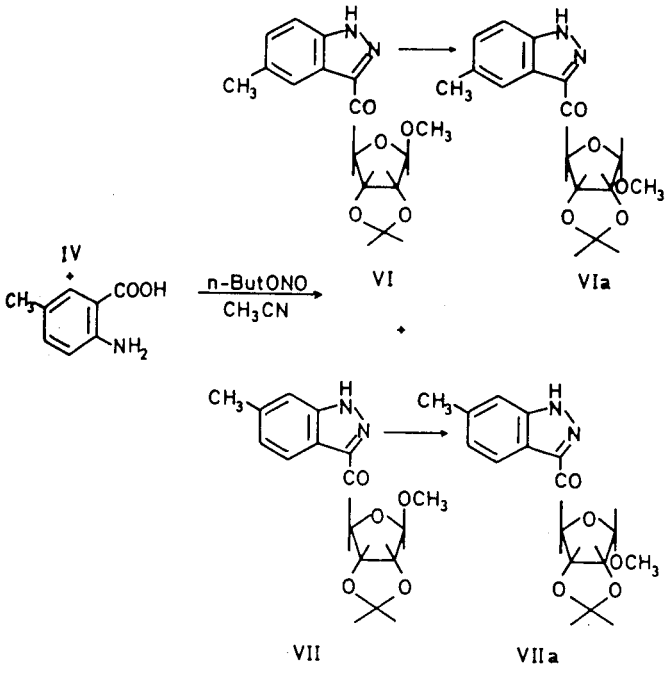
(a) Unresolved.

Table III

Chemical Shifts for Compounds X and XI at 100 MHz with TMS as Internal Standard
τ Values

Compound	Solvent	H_1'	H_2'	H_3'	H_4'	$H_5'a$	$H_5'b$	CH_3 / CH_3	Others
X	Deuteriochloroform	4.52 (d)	⟵ 5.07 (m) ⟶		5.62 (m)	6.08 (dd)	6.36 (dd)	8.36, 8.46 (s) ($\Delta\delta$ = 0.28)	2.22 (m, 1, H_4), 2.80 (m, 3, H_5, H_6, H_7)
X	DMSO	4.84 (s)	4.94 (d)	5.22 (dd)	5.96 (m)	⟵ 6.57 (d) ⟶		8.47, 8.68 (s) ($\Delta\delta$ = 0.21)	2.20 (m, 1, H_4), 2.74 (m, 3, H_5, H_6, H_7) 5.18 (s, 1, OH)
XI	DMSO	4.57 (d)	⟵ 5.13 (m) ⟶		5.84 (t)	⟵ 6.43 (d) ⟶		8.67, 8.80 (s) ($\Delta\delta$ = 0.13)	2.08 (m, 1, H_4), 2.80 (m, 3, H_5, H_6, H_7) 5.15 (b, s, 1, OH)

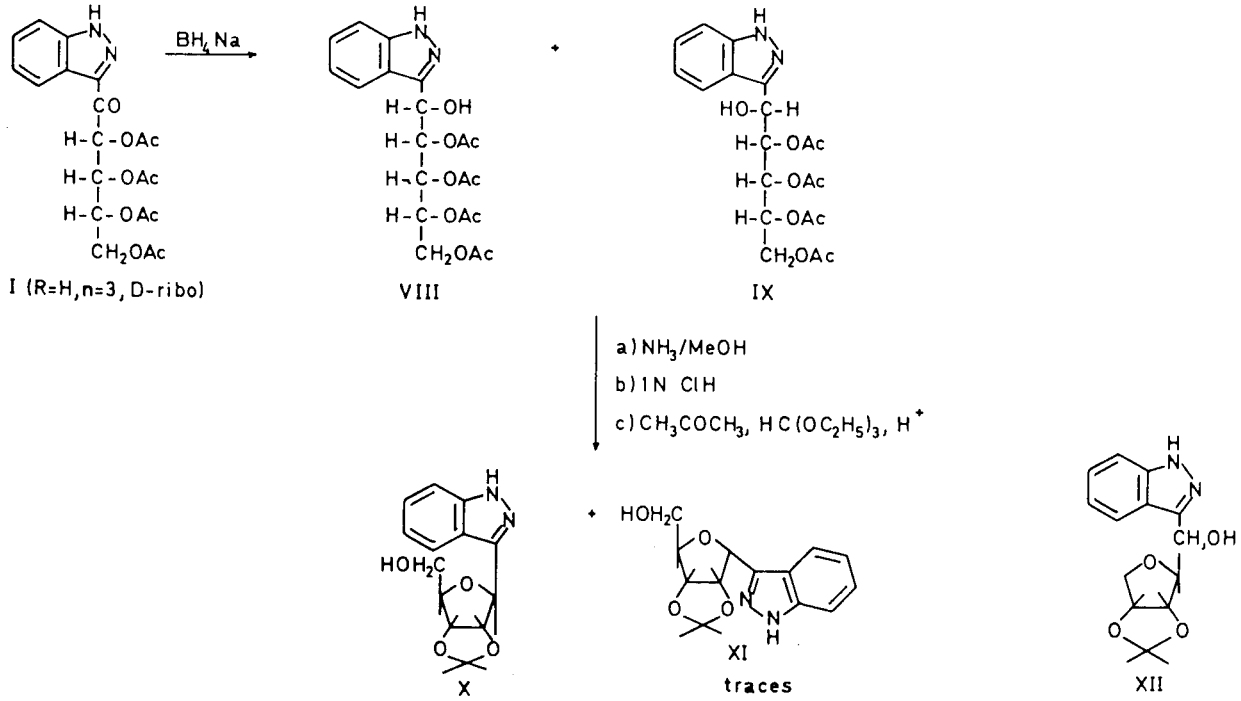
SCHEME II



of acyclic α-diazoketoses to substituted benzynes (1) (Scheme II) followed by preparative tlc led to the separation of two components. The minor component, obtained in 1.6% yield, was assigned as a chromatographically homogeneous mixture of α-anomers VIa and VIIa, according to its ^{1}H nmr spectroscopic data. The major component, obtained in 16% yield, was found to be an analytically pure, but inseparable mixture of β-anomers VI and VII, along with traces of the α-anomers VIa and VIIa. The fact that, in spite of consecutive preparative thick layer chromatographies, the mixture of VI and VII could not be isolated in anomeric purity, revealed that the anomerization took place in the nucleoside stage and not in the starting material. The existence in both components of a preparatively inseparable isomeric mixture resulting from the two possible modes of cycloaddition was apparent from the ^{1}H nmr spectra (Tables I and II). Thus, in the region corresponding to H_4 of the indazole ring appeared two superimposed doublets, one of them at 1.90 τ with a *meta* coupling and the other at 1.84 τ with an *ortho* coupling. This indicates the presence of 5- and 6-substituted indazoles. It should be noted that this proton is easily assignable, since its chemical shift is the lowest of those of the benzenic ring of the indazole (4), as well as of most of the compounds I and II (1). The ratio of positional isomers could be deduced from this signal in both cases and turned to be VI:VII ~ 5:6.

The second type of reactions was directed towards the preparation of 3-D-ribofuranosylindazoles by transformation of the acyclic sugar chain of 2,3,4,5-tetra-*O*-acetyl-1-(indazol-3-yl)keto-D-ribopentulose (I, R = H, n = 3, D-ribo) (Scheme III). This multistep transformation consisted on reduction of the keto group, deacetylation,

SCHEME III



cyclization and final conversion into the isopropylidene derivatives. Reduction of the keto group with sodium borohydride in 1,2-dimethoxyethane gave a mixture of *allo* and *altro* isomers VIII and IX which could neither be resolved by tlc nor could its ratio be determined by ^{1}H nmr. In an attempt to separate these epimeric alcohols, the mixture was acetylated but the corresponding *N*-acetyl pentaacetate derivatives were also chromatographically undistinguishable. Deacetylation of the mixture of VIII and IX with methanolic ammonia afforded the mixture of pentitols which were not isolated as such but were directly cyclized in 1*N* hydrochloric acid at room temperature for 24 hours. Finally, the crude mixture of anhydro compounds was converted into the isopropylidene derivatives. Preparative tlc led to the detection of a complex mixture. The predominating component, obtained in 18% yield from I (R = H, n = 3, D-ribo), was found to be X. A second minor constituent was also isolated and tentatively identified as XI by comparison of its ^{1}H nmr spectrum with that of X (Tables III and IV).

Table IV

Coupling Constants for Compounds X and XI (Hertz)

Compound	$J_{1',2'}$	$J_{2',3'}$	$J_{3',4'}$	$J_{4',5'a}$	$J_{4',5'b}$	$J_{5'a,5'b}$
X	0 (b)	6	4	3 (b)	4 (b)	12 (b)
	3 (c)			5 (c)	5 (c)	0 (c)
XI	3	(a)	0	5	5	0

(a) Unresolved. (b) In DMSO-d_6. (c) In deuteriochloroform.

The structural assignment of the carbohydrate moiety of X was made on the basis of its ^{1}H nmr spectral values and nuclear magnetic double resonance technique. Thus, when the spectrum was registered in dimethyl sulfoxide, the signal corresponding to H_1' appeared at 4.84 τ with a $J_{1',2'}$ of 0 Hz. This coupling constant value indicates unequivocally that X is a furanose with a β-configuration. The structure of 1',4'-anhydro was demonstrated by nuclear magnetic double resonance. Thus, when the spectrum was run in chloroform, the signal of H_1' appeared as a doublet at 4.52 τ with a $J_{1',2'}$ of 3 Hz (5). This doublet collapsed into a singlet by irradiation of the two protons corresponding to the 1,3-dioxolane ring which in this solvent appeared included in a multiplet centered at 5.07 τ. This irradiation also caused a simplification of the splitting pattern of H_4'. However, the signals for $H_5'_a$ and $H_5'_b$ remained unchanged. These facts clearly confirmed that the structure of X is that proposed and not the alternative 2',5'-anhydro derivatives, namely 3-(3,4-*O*-isopropylidene-2,5-anhydro-D-*allo*(or D-altro)-pentahydroxypentyl)indazole (XII).

The tentative assignment of XI as the corresponding α-anomer of X was based on the comparison of ^{1}H nmr data for these compounds. The difference between the chemical shifts of the two isopropylidene methyl signals for XI is smaller than that for X. The chemical shift of H_1' for XI occurs at lower field than that for X. These two criteria have been utilized previously to assign the configuration at C_1' to a pair of anomeric *C*-glycosyl derivatives (6). Still further support for this assignment comes from the observed value of $J_{3',4'}$ of 0 Hz in compound XI ($J_{3',4'}$ of 4 Hz in X). This is in agreement with the correlation which Moffat, *et al.* (6a), have found for the values of $J_{3',4'}$ in a series of *C*-glycosydes derived from 2,3-*O*-isopropylidene-D-ribose. These authors have observed that those derivatives with a β-configuration have values of $J_{3',4'}$ of 4-5 Hz, while in the corresponding α-anomers they have a value of 0 Hz.

All of the *C*-glycosylindazoles described have been tested for antitumor activity against HeLa cells. Only the isomeric mixture of VI, VII, VIa and VIIa did show a significant activity (DI_{50} = 14 μg./ml.).

EXPERIMENTAL

Melting points were determined on a Kofler apparatus and are uncorrected. Proton nuclear magnetic resonance spectra were recorded at 100 MHz on a Varian XL-spectrometer using TMS as internal standard, ultraviolet spectra with a Perkin-Elmer 350 spectrophotometer. Optical rotations were measured with a Perkin-Elmer 141 polarimeter. Analytical thin layer chromatography was performed with 0.25 mm chromatoplates of silica gel GF_{254} (Merck) and preparative layer chromatography on 20 x 20 cm glass plates coated with a 2 mm layer of silica gel PF_{254} (Merck). The compounds were detected with uv light of 254 nm or by spraying with sulfuric acid in ethanol, 30%.

1-Deoxy-1-diazo-3,6-anhydro-4,5,7-tri-*O*-benzoyl-D-*allo*-heptulose (III).

A solution of 1.0 g. (2.04 mmoles) of 2,5-anhydro-tri-*O*-benzoyl-D-allonic acid (3) and 4 ml. of freshly distilled thionyl chloride was heated under reflux for 0.5 hour. The excess thionyl chloride was evaporated *in vacuo* and the resulting syrup was treated with an ethereal solution of diazomethane (about 14 mmoles). The reaction mixture was stirred at room temperature for 0.5 hours and then evaporated leaving a syrup that was chromatographed by tlc using ethyl acetate-petroleum ether (1:1). Elution of the major moving band gave 0.552 g. (53%) of III as a syrup; ir: 2130 (N_2), 1650 cm^{-1} (CO); pmr (deuteriochloroform): τ 4.21 (m, 3, H_1, H_4, H_5), 5.34 (m, 4, H_3, H_6, $2H_7$).

Anal. Calcd. for $C_{28}H_{22}N_2O_8$: C, 65.36; H, 4.31; N, 5.44. Found: C, 65.58; H, 4.50; N, 5.31.

2,5-Anhydro-3,4,6-tri-*O*-benzoyl-1-(indazol-3-yl)-keto-D-*allo*-hexulose (V).

To a stirred, refluxing solution of 0.514 g. (1 mmole) of the α-diazoketose III and 2.575 g. (2.5 mmoles) of distilled *n*-butyl nitrite in methylene chloride (5 ml.), a solution of 0.274 g. (2 mmoles) of anthranilic acid in acetone (5 ml.) was added in the course of 20 min. Once the addition was completed, the dark coloured mixture was refluxed for two additional hours. The solvents were removed and the residue was chromatographed

by preparative tlc using ethyl acetate-petroleum ether (1:1). The major band afforded a syrup which was treated with active charcoal to give 0.265 g. (41%) of V as a foam; $[\alpha]_D^{20}$ -57° (c, 0.90 chloroform); uv (ethanol): λ max 206 (ε 22,400), 230 (ε 41,100), 272 (ε 13,800) (sh), 283 (ε 16,700), 304 nm (ε 20,700).

Anal. Calcd. for $C_{34}H_{26}N_2O_8$: C, 69.14; H, 4.43; N, 4.74. Found: C, 69.03; H, 4.56; N, 4.71.

Methyl 5-Deoxy-5-keto-2,3-*O*-isopropylidene-5-(5- and 6-methylindazol-3-yl)-β-D-ribotetrafuranoside (VI and VII) and Methyl 5-deoxy-5-keto-2,3-*O*-isopropylidene-5-(5- and 6-methylindazol-3-yl)-α-D-ribotetrafuranoside (VIa and VIIa).

To a mechanically stirred solution of 1 g. (4.13 mmoles) of the α-diazoketose IV in acetonitrile, contained in a three-necked flask, 1.25 g. (8.26 mmoles) of 5-methylanthranilic acid in acetonitrile (60 ml.) and 1 g. (10.3 mmoles) of freshly distilled *n*-butyl nitrite were added dropwise over a period of one hour. Once the addition was completed, the dark coloured mixture was refluxed for three hours. Then, the solvent was removed and the residue was chromatographed by preparative tlc using five developments with ethyl acetate-petroleum ether (1:2). The slowest moving band afforded 0.03 g. (1.6%) of a mixture of VIa and VIIa (5:6 by ^{1}H nmr) as a solid that could not be resolved by tlc. The fastest moving band gave 0.30 g. (16%) of a foam which was shown by ^{1}H nmr to consist of a 5:6 mixture of VI and VII along with about 10% of a 5:6 mixture of VIa and VIIa.

Anal. Calcd. for $C_{17}H_{20}N_2O_5$: C, 61.43; H, 6.06; N, 8.42. Found: C, 61.70; H, 6.12; N, 8.14.

This mixture of four components was repeatedly rechromatographed. Each time two bands were separated and eluted. The fastest and major band led always to a mixture of VI, VII, VIa and VIIa in identical ratio to that above described. The slowest and minor band afforded a 5:6 mixture of VIa and VIIa.

3-(2,3-*O*-isopropylidene-β- and α-D-ribofuranosyl)indazole (X and XI).

A stirred solution of 1.64 g. (3.77 mmoles) of I (R = H, n = 3, D-ribo) in 1,2-dimethoxyethane (12 ml.) was cooled to 0° and to it was slowly added 0.120 g. (3.15 mmoles) of sodium borohydride. The mixture was then allowed to warm to room temperature for 4 hours while stirring. The solvent was evaporated and the residue was partitioned between chloroform and water. Evaporation of the chloroform left a syrup which was chromatographed by preparative tlc using three developments with ethyl acetate-petroleum ether (1:1). The major band afforded 1.35 g. of a mixture of VIII and IX as a colorless syrup that could not be resolved by tlc and was then treated with methanolic ammonia (60 ml.) at room temperature for 24 hours. Evaporation of the solvent left an oily residue which was treated with hydrochloric acid (1*N*, 24 ml.) at room temperature for 24 hours. The residue obtained after evaporation of the solvent was dissolved in dry acetone (30 ml.) and to this solution, ethyl orthoformate (5 ml.) and a 1*M* solution of hydrogen chloride in ether (1 ml.) were added. The mixture was stirred at room temperature for 17 hours and then the solution was neutralized with concentrated ammonium hydroxide and evaporated to dryness. The residue was dissolved in a small amount of water and the solution extracted several times with ethyl acetate. The organic phase was dried over sodium sulfate and evaporated leaving a complex mixture which was chromatographed by preparative tlc using four developments with chloroform-ethyl acetate (4:1). The major band gave a solid which was recrystallized from ethyl acetate-petroleum ether to afford 0.175 g. (18%) of X with m.p. 161°; $[\alpha]_D^{20}$ -44° (c, 0.45 chloroform); uv (ethanol): λ max 213 (ε 14,500), 254 (ε 3,000), 261 (ε 2,900), 287 (ε 4,350), 292 (ε 4,250), 300 nm (ε 3,700).

Anal. Calcd. for $C_{15}H_{18}N_2O_4$: C, 62.05; H, 6.24; N, 9.65. Found: C, 61.73; H, 6.04; N, 9.38.

The slowest moving band yielded 0.05 g. of a syrup that was rechromatographed using three developments with chloroform-methanol (9:1) to give 0.019 g. (2%) of a compound which was tentatively assigned as XI on the basis of its ^{1}H nmr spectrum.

REFERENCES AND NOTES

(1) E. García-Abbad, M. T. García-López, G. García-Muñoz and M. Stud, *J. Heterocyclic Chem.*, 13, 1241 (1976).

(2) A. Hampton, F. Perini and P. J. Harper, *Carbohydr. Res.*, 37, 359 (1974).

(3) M. Bobek and J. Farkas, *Collect. Czech. Chem. Commun.*, 34, 247 (1969).

(4) P. J. Black and M. L. Heffernan, *Aust. J. Chem.*, 16, 105 (1963).

(5) L. B. Townsend in "Synthetic Procedures in Nucleic Acid Chemistry", Vol. 2, W. W. Zorbach and R. S. Tipson, Eds., Wiley-Interscience, New York, N. Y., 1973, p. 331.

(6a) H. Ohrui, G. H. Jones, J. G. Moffatt, M. L. Maddox, A. T. Christensen and S. K. Bryam, *J. Am. Chem. Soc.*, 97, 4602 (1975); (b) F. G. de las Heras, S. Y.-K. Tam, R. S. Klein and J. J. Fox, *J. Org. Chem.*, 41, 84 (1976).