(2 mg) for 2 hr at room temperature gave **5** exclusively on the basis of its NMR spectrum.

Methyl 4,6-*O*-Benzylidene-2-*C*-(dibenzoyl)methyl-2,3-dideoxy-3-nitro-α-D-mannopyranoside (9). Treatment of **2** (58.6 mg) with dibenzoylmethane (49 mg, ca. 0.22 mmol) under the conditions used to prepare **8** gave a pure crystalline residue of **9** (88 mg, 85%). The residue (176 mg) was recrystallized from ethanol: yield 149 mg (72%); mp 214° dec; $[\alpha]^{20}D$ −262° (*c* 1, $CHCl_3$); ir (KBr) 1690 (CO), 1550 cm^{-1} (NO_2).

Anal. Calcd for $C_{29}H_{27}NO_8$: C, 67.30; H, 5.26; n, 2.71. Found: C, 67.09; H, 5.19; N, 2.83.

Methyl 2-*C*-(Acetylethoxycarbonyl)methyl-4,6-*O*-benzylidene-2,3-dideoxy-3-nitro-α-D-mannopyranoside (10). Reaction of **2** (58.6 mg) with ethyl acetoacetate (28.6 mg, 0.22 mmol) under the conditions described above for the preparation of **8** gave a mixture (67.7 mg, 80%), which was chromatographed on silica gel (13 × 35 mm) with benzene, and the eluate was evaporated in vacuo to give a syrup of **10** (42.3 mg, 50%), which was crystallized from isopropyl ether: mp 127.5–128.5°; $[\alpha]^{20}D$ −103° (*c* 1, $CHCl_3$); ir (KBr) 1730, 1710 (CO), 1555 cm^{-1} (NO_2).

Anal. Calcd for $C_{20}H_{25}NO_9$: C, 56.73; H, 5.59; N, 3.31. Found: C, 56.78; H, 5.95; N, 3.31.

Methyl 4,6-*O*-Benzylidene-2-*C*-(dicyano)methyl-2,3-dideoxy-3-nitro-α-D-glucopyranoside (11). Treatment of **2** (58.6 mg) with malononitrile (24 mg, 0.36 mmol) under the condition described above for the preparation of **8** gave a syrup (55.3 mg, 77%) consisting of **11** and **12**, in the ratio of 1:1.3 on the basis of NMR spectrum. The syrup (11 mg) was chromatographed on silica gel (17 × 85 mm) developed slowly with benzene. The eluate was collected in 10-ml portions. Fractions 3 and 4 were combined and evaporated in vacuo to give crystals of **11**: yield 36 mg (25%); mp 163.5–164.5°; $[\alpha]^{20}D$ +110° (*c* 1, $CHCl_3$); ir (KBr) 2260 (CN), 1560 cm^{-1} (NO_2).

Anal. Calcd for $C_{17}H_{17}N_3O_6$: C, 56.82; H, 4.77; N, 11.70. Found: C, 56.73; H, 4.92; N, 11.79.

Methyl 4,6-*O*-Benzylidene-2-*C*-(dicyano)methyl-2,3-dideoxy-3-nitro-α-D-mannopyranoside (12). In the above chromatography, fractions 6–12 were combined and evaporated in vacuo to give a syrup (57 mg, 40.1%) of **12**: $[\alpha]^{20}D$ +21.7° (*c* 1, $CHCl_3$); ir (KBr) 2260, 2235 (CN), 1557 cm^{-1} (NO_2).

Anal. Calcd for $C_{17}H_{17}N_3O_6$: C, 56.82; H, 4.77; N, 11.70. Found: C, 56.93; H, 4.85; N, 11.49.

Conversion of 12 into 11. To a solution of benzene (3 ml), 0.2 *N* NaOH (0.8 ml), the catalyst (2 mg), and malononitrile (12 mg) was added a mixture (36 mg) of **12** and **11** (ratio of 1.5:1 by NMR spectroscopy). The reaction mixture was stirred for 15 hr at room temperature, and then washed with water. The benzene layer was evaporated in vacuo to give a residue, which was NMR spectroscopically pure **11**.

Registry No.—**1**, 16697-50-0; **2**, 16697-51-1; **4**, 55853-26-4; **5**, 55853-27-5; **8**, 55853-28-6; **9**, 55853-29-7; **10** isomer a, 55853-30-0; **10** isomer b, 55853-31-1; **11**, 55853-32-2; **12**, 55853-33-3; acetylacetone, 123-54-6; dibenzoylmethane, 120-46-7; ethyl acetoacetate, 141-97-9; malonitrile, 109-77-3; ethyl malonate, 105-53-3.

References and Notes

(1) For example, S. Patai and Z. Rapport, "The Chemistry of Alkenes", S. Patai, Ed., Interscience, New York, N.Y., 1964, Chapter 8.
(2) Some thermodynamically less stable isomers were prepared from nitro olefin derivatives with the following nucleophiles: hydrogen cyanide, hydrazoic acid, theophylline, and 2,6-dichloropurine, T. Sakakibara and R. Sudoh, *Chem. Commun.*, 69 (1974); hydrogen cyanide, H. Paulsen and W. Grewe, *Chem. Ber.*, **107**, 3013 (1974); *N*-bromoacetamide, H. H. Baer and W. Rank, *Can. J. Chem.*, **52**, 2257 (1974); anthranilic acid, H. H. Baer and F. Klenzle, *J. Org. Chem.*, **34**, 3848, 4204 (1969); secondary amines such as morpholine, P. Southwick and J. E. Anderson, *J. Am. Chem. Soc.*, **79**, 6222 (1957).
(3) C. M. Starks, *J. Am. Chem. Soc.*, **93**, 195 (1971); J. Dockx, *Synthesis*, 441 (1973); E. X. Dehmlow, *Angew. Chem.*, **86**, 187 (1974); *Angew. Chem., Int. Ed. Engl.*, **13**, 170 (1974).
(4) For example, K. E. Koenig and W. P. Weker, *Tetrahedron Lett.*, 2275 (1974); M. Makosza and M. Fedorynski, *Synthesis*, 274 (1974).
(5) A phase transfer catalyst has been used in the Michael reaction but the stereochemical studies were not examined; for example, D. A. White and M. M. Baiter, *J. Chem. Soc., Perkin Trans. 1*, 2230 (1973).
(6) The facts that the thermodynamically less stable manno isomers could be isolated exclusively or predominantly by this phase transfer process prompted us to investigate the reactions of **2** with active methylene compounds under homogeneous conditions, and in some cases the manno isomers were formed also as a major product. Details are now in progress.
(7) This reaction mechanism was based on the fact that the reaction of 4-*tert*-butyl-1-cyanocyclohexene with ethyl malonate afforded ethyl 4-*tert*-butyl(e)-2-carbethoxymethyl(a)-1-cyano(a)cyclohexanecarboxylate(e); R. A. Abramovitch and D. L. Struble, *Tetrahedron*, **24**, 357 (1968).
(8) These results are in good agreement with the generalization that manno isomers are thermodynamically less stable than the gluco isomers.
(9) H. H. Baer and F. Klenzle, *Can. J. Chem.*, **45**, 983 (1967).

Synthesis of C Nucleosides. X.[1] Structural Analogs of Formycin B

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A general synthetic route to 2-substituted fused pyrimidones is described. Model reactions of benzylthioacetimidate **8** with different aminocarbethoxypyrazoles give the cyclized structures. The same condensations with glycosyl thioformimidates **10** and **14** lead to pyrazolo[4,3-*d*]pyrimidin-7-ones **11** and **15** and pyrazolol[3,4-*d*]pyrimidin-4-ones **19** and **21**. Removal of the protective ester groups is achieved with methanolic ammonia. The spectroscopic properties of anomers of the ribo and 2′-deoxyribo analogs of formycin B, **3**, **4**, **5**, and **6**, are discussed.

The biological properties of formycins[2] A (**1**) and B (**2**) have stimulated diverse studies on their total synthesis[3] or on the preparation of derivatives with modifications of the heterocycle[4,5] and of the sugar moieties.[6,7]

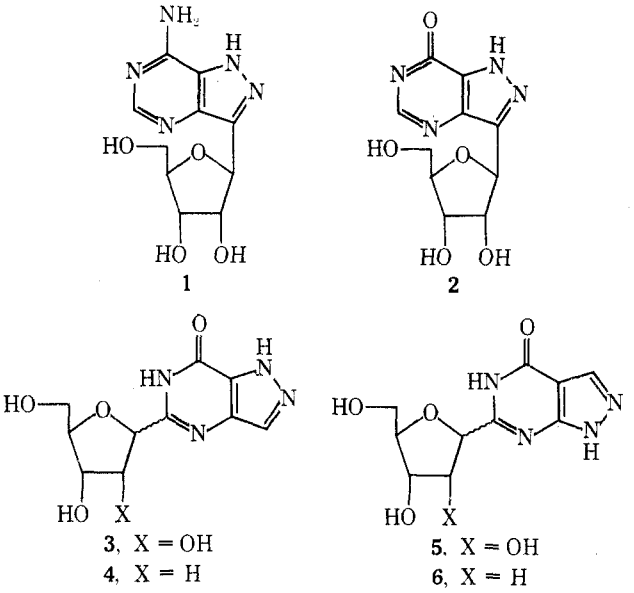
In pursuit of our work on the synthesis of C nucleosides we prepared isomers of formycin B and 2′-deoxyformycin B, whose sugar (ribose and 2-deoxyribose) was linked to carbon 2 of the pyrimidine cycle. We should obtain the 5-glycosylpyrazolo[4,3-*d*]pyrimidin-7-ones **3** and **4**, closely related to formycin B, or the 6-glycosylpyrazolo[3,4-*d*]pyrimidin-4-ones **5** and **6**, related to allopurinol nucleoside (Chart I).

Known methods for the preparation of 2-substituted fused pyrimidines are laborious with poor overall yield[8] or require drastic conditions.[9] A new approach, using much milder conditions, has been developed from the condensation of a thioformimidate[10] with an amino aromatic heterocycle, functionalized in the ortho position by an ester group.

Results

We start with a model reaction, using benzyl thioacetimidate (**8**) and 4-amino-3-carbethoxypyrazole (**7**), prepared by reduction of the nitro ester[11] (Scheme I). By refluxing in

Chart I



pyridine, we quantitatively obtain 5-methylpyrazolo[4,3-*d*]pyrimidin-7-one (**9**), a product not yet described to our knowledge.

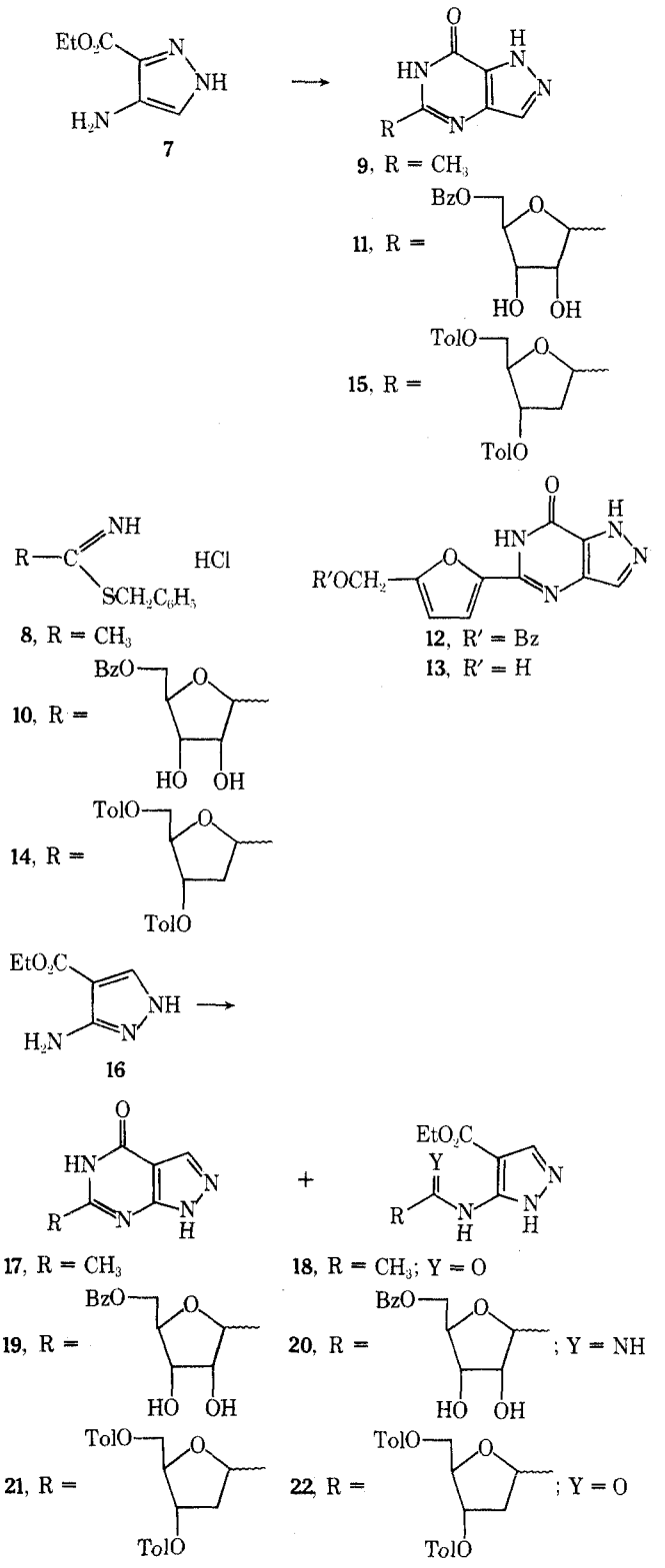
The condensation of **7** with benzyl 5-*O*-benzoyl-D-ribofuranosyl thioformimidate[20] (**10**) under the same conditions leads to 30% of a mixture of pyrazolo[4,3-*d*]pyrimidones **11**α and -β, separated by silica gel column chromatography. The β anomer is predominant (β/α = 70/30). We also isolate from dehydration of the ribose cycle furan **12** as a by-product.

Starting with thioformimidate[1] **14** in the 2-deoxyribose series, the formation of a furan is not observed and the yield of pyrazolopyrimidones **15**α and -β, separated by column chromatography, increases to 75%. The **15**β anomer is slightly predominant (β/α = 60/40). The benzoyl or *p*-toluyl protective groups of **11**α,β, **15**α,β, and **12** are quantitatively removed by methanolic ammonia to give respectively pyrazolo[4,3-*d*]pyrimidones **3**α,β, **4**α,β and furan **13**. The reaction time is shorter for the benzoyl (1 night) than for the *p*-toluyl group (2 weeks).

The same procedure is used in the preparation of substituted pyrazolo[3,4-*d*]pyrimidones: condensation of **8** with commercially available 3-amino-4-carbethoxypyrazole (**16**) gives the expected 6-methylpyrazolo[3,4-*d*]pyrimidin-4-one (**17**), already described.[8b] The yield of **17** is about 60% and we also isolate the amide **18** whose structure is consistent with mass and NMR spectra.

The reaction of benzyl 2-deoxy-3,5-di-*O*-*p*-toluyl-D-*erythro*-pentofuranosyl thioformimidate (**14**) also gives the two anomers (α and β) of amide **22**. The noncyclized amides **18** or **22** could arise from hydrolysis of a N-substituted amidine intermediate; amidine **20** is indeed isolated from condensation of pyrazole **16** with ribofuranosyl thioimidate **10**. The overlap of the sugar protons, even in the 250-MHz spectrum, does not allow the determination of its configuration. Of course, in the ribose, as well as in the 2-deoxyribose series, we isolate the expected pyrazolo[3,4-*d*]pyrimidones **19**α,β and **21**α,β. Treatment of each anomer with methanolic ammonia provides the C nucleosides **5**α,β and **6**α,β.

Scheme I



Discussion

Unlike the previous condensations of thioimidate **10** with *o*-aminocyano heterocycles,[10,20] which gave only the β anomers, the reactions of the same thioimidate with o-aminocarbethoxy heterocycles lead to the formation of α and β anomers of C ribonucleosides. The anomeric mixture of **3** or **5** does not proceed from a thermodynamic equilibrium, since no epimerization is observed from each anomer with the experimental conditions used. Further cyclizations of thioimidate **10** with other bases[25] seem to show that the formation of both anomers is a general rule: the β/α ratio varies with the base, and the β anomer is strongly predominant or exclusive.[20] This fact may be correlated with the steric effect of 2'-OH: in the 2'-deoxy series[1,13] the β anomer is less favored and in the arabinosyl series[10,26] the α anomer is always predominant.

Table II
NMR Chemical Shifts (Parts per Million) at 250 MHz in DMSO-d_6

Compd	Pyrazole H										
		Methyl									
		CH_3									
9	8.0[a]	2.4									
17	8.0[a]	2.3									
		Ribose									
		H-1′	H-2′		H-3′	H-4′	H-5′	H-5″	$J_{1',2'}$, Hz		
3α	8.08	4.87	4.34		4.09[b]	4.09[b]	3.62	3.45	4.9		
3β	8.12	4.65	4.17		4.07	3.98	3.80	3.64	4.0		
5α	8.11	4.90	4.35		4.08[b]	4.08[b]	3.64	3.45	4.5		
5β	8.15	4.65	4.15		4.05	3.97	3.78	3.62	4.2		
		2-Deoxyribose									
		H-1′	H-2′	H-2″	H-3′	H-4′	H-5′	H-5″	$J_{1',2'}$, Hz	$J_{1',2''}$, Hz	$J_{1',2'} + J_{1',2''}$, Hz
4α	8.10	4.90	2.56	2.12	4.25	4.04	3.41	3.41	8.6	4.7	13.3
4β	8.08	4.94	2.21	2.17	4.26	3.90	3.63	3.56	8.4	7.3	15.7
6α	8.13	4.93	2.59	2.12	4.24	4.06	3.43	3.43	8.7	4.5	13.2
6β	8.10	4.96	2.22	2.22	4.26	3.92	3.64	3.58	7.5	7.5	15.0
		Furan									
			H-2′		H-3′		H-5′	H-5″	$J_{2',3'}$, Hz		
13	8.08		7.43		6.52		4.50	4.48	3.2		

[a] Spectra measured at 60 MHz. [b] Not first order spectra: possible error of 0.02 ppm.

Table III
Chemical Shifts of Intermediary Esters in DMSO-d_6 at 250 MHz

Compd	Pyrazole H												
		Methyl											
		CH_3											
18[d]	7.7	2.3											
		Ribose											
		H-1′	H-2′	H-3′	H-4′	H-5′	H-5″	H_o[e]	H_m[f]	H_p[g]	$J_{1',2'}$, Hz		
11α	7.92	4.88	4.37[b]	4.39[b]	4.16	4.52	4.33[b]	8.02	7.55	7.68	3.8		
11β	7.91	4.65	4.38	4.24	4.14	4.53	4.41	7.91	7.42	7.62	3.6		
19α	8.04	5.04	4.40[b]	4.43[b]	4.24	4.62	4.36[b]	8.05	7.58	7.70	3.9		
19β	8.15	4.73	4.42	4.22[b]	4.22[b]	4.56	4.47	7.80	7.42	7.63	3.8		
20[c]	7.73	4.75	4.46	4.75–4.46	4.35	4.75–4.46		7.89	7.21	7.41			
		2-Deoxyribose											
		H-1′	H-2′ H-2″	H-3′	H-4′	H-5′ H-5″		H_o		H_m		ΔH_o	Me		$J_{1'2'} + J_{1'2''}$, Hz
15α[d]	8.0	5.4	2.8–3.1	5.7	5.0	4.6		8.0	7.5	7.2	6.9	0.5	2.2	2.4	11
15β[d]	8.0	5.4	2.6–2.9	5.7	4.8	4.8		8.0	7.9	7.2	7.1	0.1	2.3	2.4	15
21α[a]	8.1	5.3	———	5.4	4.4	4.4		7.8	7.4	7.3	7.0	0.4	2.3	2.4	11
21β[a]	8.1	5.2	———	5.5	4.5	4.5		7.9	7.7	7.3	7.2	0.2	2.3	2.4	15
22α[d]	7.7	4.9–4.4	2.5–2.8	5.5	4.9–4.4	4.9–4.4		7.8	7.5	7.1	6.8	0.3	2.2	2.4	
22β[d]	7.6	4.9	2.5–2.8	5.5	4.6	4.6		7.8	7.6	7.2	7.0	0.2	2.3	2.4	16
		Furan													
		H-2′	H-3′	H-5′	H-5″	H_o		H_m		H_p		$J_{2',3'}$, Hz			
12	8.12	7.53	6.86	5.43		8.01		7.54		7.69		3.5			

[a] Measured at 60 MHz. [b] Not first-order spectra: possible error of 0.02 ppm. [c] Measured in $CDCl_3$. [d] Measured at 60 MHz in $CDCl_3$. [e] Aromatic H in ortho position of C=O. [f] Aromatic H in meta position of C=O. [g] Aromatic H in para position of C=O.

The simultaneous formation of both anomers in each series permits us to suggest some tentative rules for interpretation of the spectroscopic data.

The ultraviolet spectra of C nucleosides **3, 4** and **5, 6** are quite similar to those of their methyl analogs **9** and **17** (Table I, microfilm material). The 5-substituted pyrazolo[3,4-*d*]pyrimidone chromophore absorbs at a higher wavelength than the 6-substituted pyrazolo[3,4-*d*]pyrimidone cycle, whose bathochromic shift from 0.1 *N* hydrochloric acid to 0.1 *N* sodium hydroxide is more important (15 vs. 5 nm).

The isolation of **3, 5** and **4, 6** in each series of C nucleosides greatly facilitates determination of their anomeric configuration by NMR. In the ribose series (Table II) the determination is not based on the coupling constants $J_{1',2'}$, which are practically the same, but on the chemical shifts of $H_{1'}$. The β configuration is attributed to the higher shift of $H_{1'}$, due to the shielding of a cis 2′-OH.[17] The β anomers of **3, 5** show well-resolved sugar protons whereas in the α anomers, $H_{3'}$ and $H_{4'}$ are collapsed. The presence of a benzoyl group moves downfield $H_{5'}$ and $H_{5''}$.

For the 2′-deoxy series (Table II), no significant differ-

Table IV
Relative Intensity (%) of Predominant Ions in Mass Spectra of Substituted Pyrazolopyrimidones

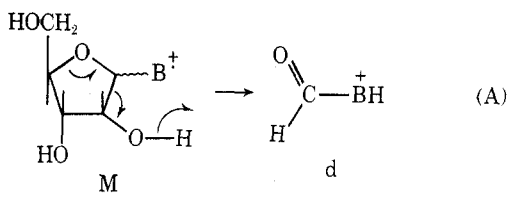
Compd	m/e	232	203 B − 68	192 B + 57	179 B + 44	165 B − 30	163 B − 28	150 B + 15	137 B + 2	136 B + 1	135 B	120 B − 15	110 B − 25	109 B − 26
Methyl														
9								100		<1	9	1	11	2
17								100	2	25			28	7
Ribose	M	M − 17	M − 30 a	M − 31										
						c	d	e						
3α	29	1	<1	1	2	2	7	92	100	2	5	6	4	4	12	8	12
3β	13	25	4	8	1	4	2	100	82	5	13	13	7	7	17	11	21
5α	17	3	4	2	25	22	2	83	1100	10	36	31	19	39	19	56	33
5β	17	6	11	4	15	15	2	100	82	13	39	35	19	44	19	62	37
2-Deoxyribose	M	M − 17	M − 30 a	M − 31										
							d	$(BHCH{=}CH_2)^+$	e					
4α	<1		<1	<1		3	3	11	16	100	5	11	12	6	18	7	19
4β	5		4	2		2	1	4	15	100	5	9	6	2	7	3	9
6α	7	<1	1	2		9	10	2	6	100	3	10	4	9	3	13	6
6β	7	<1	4	2		3	2	4	12	100	6	11	4	9	6	16	7
Furan		M − 17	M − 30		M												
13		22	<1		100	35				1	4	4	13	6	5	3	9

ence on the chemical shift is observed for the anomeric proton and the assignment of the configuration is based on its splitting pattern:[17,18] quartet for the α anomer and triplet for the β, with a larger peak width for the β anomer.[19] We notice a downfield shift in α anomers for $H_{4'}$;[21] this influence of the base on the sugar protons also appears in the chemical shifts of $H_{2'}$ and $H_{2''}$ which are well resolved in α anomers and collapsed in β anomers.[22] Another good criterion for the determination of the configuration for esters **15, 21,** and **22** is based on the difference of chemical shifts of the aromatic protons which are in the ortho position of the carbonyl: ΔH_o is larger in α anomers[13] (Table III) than in β anomers.

The detailed discussion of the NMR spectra of all these nucleosides will be published later.

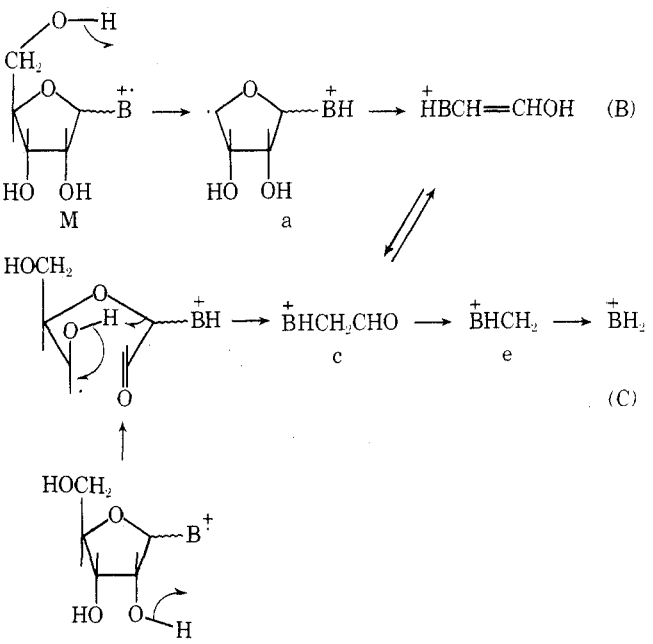
All of the mass spectra (Table IV) of the nucleosides show the molecular ion M. The ribo and 2′-deoxy nucleosides present the same type of fragmentation. The C–C bond between the carbohydrate and the heterocycle is confirmed by the reduced intensity of ions B + H (136) and B + 2H (137).[12a] Peaks at M − 30 and M − 31 demonstrate the presence of an exocyclic hydroxymethyl group and the furanose structure of the sugar. We also observe the characteristic fragmentation of O–$C_{1'}$ and $C_{2'}$–$C_{3'}$ bonds which gives an abundant ion (c) at B + 44 (179) in the ribose series (80–100% rel intensity) and B + 28 (163) in the 2′-deoxy series (major ion); a fragment at B + 28 is observed in all 2′-deoxy C nucleosides synthetized in our laboratory[1,13] and in 2′-deoxyformycin.[6a] Another characteristic ion is found at B + 30 (165) in the ribonucleosides: ion d results from the cleavage of O–$C_{4'}$ and $C_{1'}$–$C_{2'}$ bonds and was proposed as the major fragment of C ribonucleosides;[14] this empirical rule[23] is observed only on α anomers of fused pyrimidine nucleosides **3** and **5.** Ion d is reduced in 2′-deoxynucleosides **4** and **6** and absent in furan **13.** It appears that formation of ion d requires the spatial proximity between the base and 2′-OH (pathway A).[12]

It should be noted that pyrazolo[3,4-*d*]pyrimidones **5** more easily lose 2 mol of water (ion at 232) than pyrazolo[4,3-*d*]pyrimidones **3**; this fragmentation via dehydration without cleavage of the glycosidic bond was mentioned for formycin B[14] and pyrazomycin.[15] Ion M − $2H_2O$ is the major molecular ion of furan **13.**



(A)

As for N nucleosides,[12b] the most significant difference between a couple of anomers is the relative intensity of ion M − 30: the β anomers of **3, 4** and **5, 6** possess a more important ion (a) than the α anomers. The 100% relative intensity of ion c, correlated with the higher intensities of ions a (M − 30), e (B + 15), and B + 2H on β anomers of C



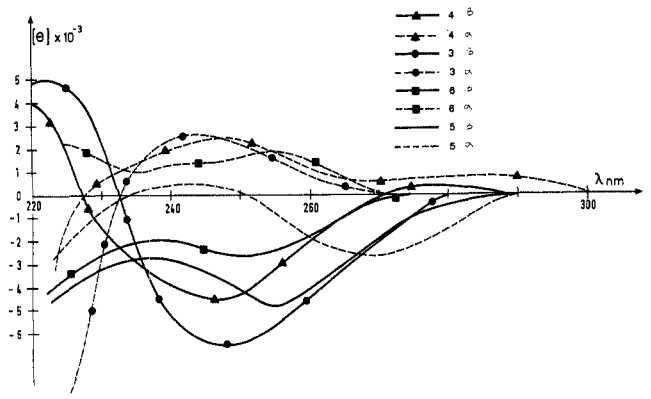


Figure 1. CD spectra of nucleosides in water.

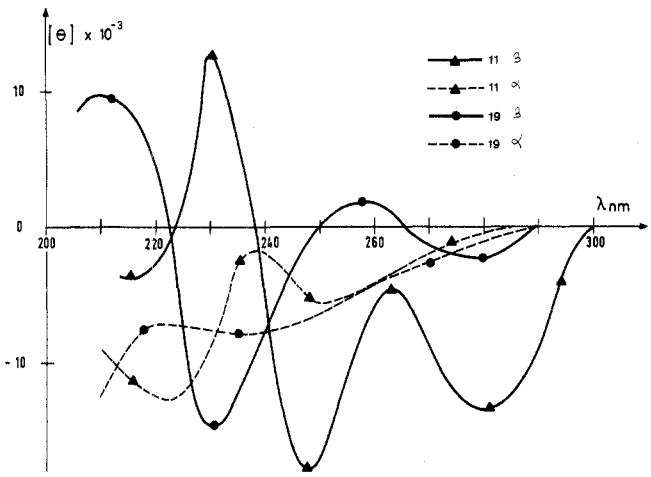
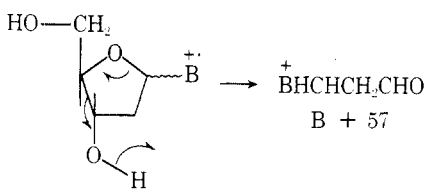


Figure 2. CD spectra of 5′-*O*-benzoyl ribonucleosides in water.

ribonucleosides **3** and **5** may be tentatively depicted by pathway B;[12,16] the same effect was observed on 2-β-D-ribofuranosyladenine.[20] The principal pathway (C), generally admitted for ion c in ribosides,[12] suggests the participation of a 2′-hydroxy group and seems not to predominate in the case of fused pyrimidine nucleosides.

In the 2′-deoxynucleosides, a more significative difference between α and β consists in the higher intensity of peaks at B + 57 for α anomers which could be explained by a fragmentation involving 3′-OH.



Unlike the previous spectroscopic methods, the circular dichroism measurements are less indicative of the configuration. As for N nucleosides,[24] the α anomers of compounds **3, 4,** and **6** present a positive Cotton effect and the β anomers a negative effect. However, the correlation seems not to be general, since **5**α behaves differently, suggesting a difference of conformation (Figure 1).

The most significant effect is observed with 5′-*O*-benzoyl derivatives **11** and **19** of the ribo series: in the β anomers, where the molecular conformation favors a stacking between the benzene nucleus and the heterocycle, the Cotton effects are very important; on the contrary, in the α anomers, where an intramolecular interaction cannot exist, the magnitude of the Cotton effect is well reduced. This difference in the intensities of the Cotton effect on two anomers was observed in all other 5′-benzoylated C ribonucleosides and tentatively used in the determination of the anomeric configuration[20] (Figure 2).

Experimental Section

Melting points were determined with Kofler microscope and were uncorrected. Ultraviolet spectra were determined with a Perkin-Elmer 237 spectrophotometer. NMR spectra were obtained using a 250-MHz Cameca TSN-250 and a 60-MHz Varian EM-360 with tetramethylsilane as internal reference. Mass spectra were obtained with a Varian CH-7 or MS-9. Optical activities were measured with a Perkin-Elmer 241 MC polarimeter and circular dichroism spectra were recorded with a Roussel-Jouan II-185 dichrograph. Chromatographic columns were packed with Silicar 100 mesh Grade I; 0.25 mm thick TLC plates were prepared with Merck Kieselgel $HF_{254+366}$ and visualized with an uv light at 254 nm.

4-Amino-3-carbethoxypyrazole (7). A solution of 660 mg (3.57 mmol) of 3-carbethoxy-4-nitropyrazole in 20 ml of ethanol was hydrogenated at atmospheric pressure with 10% Pd/C for 3 hr. After filtration, the solution was evaporated to dryness and the residue was applied to a silica gel column and developed with ether; 490 mg of **7** (87%) was obtained, mp 90–92°. Anal. Calcd for $C_6H_9O_2N_3$: C, 46.44; H, 5.85; N, 27.02. Found: C, 46.69; H, 5.92; N, 26.98.

General Procedure. The same procedure was used for the condensation of a thioimidate with an aminocarbethoxypyrazole. No attempt was made to optimize the yields obtained.

A solution of 5 mmol of pyrazole and 5 mmol of thioimidate in 15 ml of anhydrous pyridine was refluxed for 16 hr. The mixture was evaporated and dissolved in aqueous ethanol. The solution was neutralized with 1 *N* sodium hydroxide and evaporated to dryness. The residue was recrystallized or applied to a silica gel column (300 g, 75 × 4 cm). Elution with the adequate mixture of solvents gave first the by-products (**12, 18, 20,** and **22**) and then the two anomers. The anomeric separation can also be undertaken after debenzoylation with gel chromatography: 260 mg of compound **3** or **5** was separated on 50 g of Bio-Gel P-2 200–400 mesh (90 × 1.6 cm); 50 mg of compound **6** was separated on 64 g of Sephadex G-10 (120 × 1.2 cm).

Debenzoylation was achieved with saturated methanolic ammonia at room temperature and monitored by TLC; after completion of the reaction, the solution was evaporated to dryness and the residue was washed with benzene and ethyl acetate and recrystallized.

The experimental and physical data are summarized in Tables V and VI (microfilm material).

Registry No.—α-**3,** 55904-41-1; β-**3,** 55904-42-2; α-**4,** 55904-43-3; β-**4,** 55904-44-4; α-**5,** 55904-45-5; β-**5,** 55904-46-6; α-**6,** 55904-47-7; β-**6,** 55904-48-8; **7,** 55904-61-5; **9,** 55904-62-6; α-**11,** 55904-40-9; β-**11,** 55904-50-2; **12,** 55925-84-3; **13,** 55904-63-7; α-**15,** 55904-51-3; β-**15,** 55904-52-4; **17,** 30129-57-8; **18,** 14333-80-3; α-**19,** 55904-53-5; β-**19,** 55904-54-6; α-**20,** 55904-55-7; β-**20,** 55904-56-8; α-**21,** 55904-57-9; β-**21,** 55904-58-1; α-**22,** 55904-59-1; β-**22,** 55904-60-4.

Supplementary Material Available. Tables I, V, and VI will appear following these pages in the microfilm edition of this volume of the journal. Photocopies of the supplementary material from this paper only or microfiche (105 × 148 mm, 24× reduction, negatives) containing all of the supplementary material for the papers in this issue may be obtained from the Business Office, Books and Journals Division, American Chemical Society, 1155 16th St., N.W., Washington, D.C. 20036. Remit check or money order for $4.00 for photocopy or $2.50 for microfiche, referring to code number JOC-75-2825.

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The Gentamicin Antibiotics. 7.[1a] Structures of the Gentamicin Antibiotics A_1, A_3, and A_4

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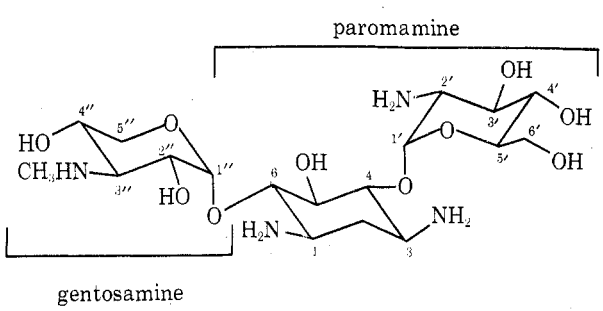
The structures of the gentamicin antibiotics A_1, A_3, and A_4 coproduced with other gentamicins in submerged fermentations of *Micromonospora purpurea* and *Micromonospora echinospora* have been elucidated by proton and carbon-13 magnetic resonance spectroscopy in conjunction with mass spectrometry. Gentamicin A_1 and A_3 are 4-*O*-(2'-amino-2'-deoxy-α-D-glucopyranosyl)-6-*O*-(3''-methylamino-3''-deoxy-β-L-arabinopyranosyl)deoxystreptamine and 4-*O*-(6'-amino-6'-deoxy-α-D-glucopyranosyl)-6-*O*-(3''-methylamino-3''-deoxy-β-L-arabinopyranosyl)deoxystreptamine, respectively. Gentamicin A_4 is 3''-*N*-formylgentamicin A.

Gentamicin A is coproduced with other gentamicins in submerged fermentations of *Micromonospora purpurea* and *Micromonospora echinospora*.[1b,2] Its structure was elucidated by Maehr and Schaffner[3,4] and is shown below.



gentamicin A

Recent investigations in this laboratory have revealed the presence of four new deoxystreptamine-containing antibiotics in crude preparations of gentamicin A which we have designated gentamicins A_1, A_2, A_3, and A_4. The elucidation of the structures of A_1, A_3, and A_4 is the subject of this communication. The structure of gentamicin A_2 is published in the accompanying note.[5a]

Gentamicins A_1, A_3, and A_4 could be separated from A and from each other by thin layer chromatography on silica gel using chloroform–methanol–ammonium hydroxide (3:4:2) as the developer. On a typical chromatogram A_1, A_3, and A_4 had R_A[5b] values of 0.78, 0.40, and 1.62, respectively. Isolation of these compounds in high states of purity was effected by chromatography of the crude mixture on a column of silica gel using the above-mentioned eluent, and in the case of A_1 and A_3 by rechromatography on Dowex 1-X2 ion exchange resin in the hydroxide cycle using water as the eluent.[6,7]

Structures of Gentamicins A_1 and A_3. The proton noise decoupled ^{13}C NMR spectra of A_1 and A_3 were very similar to that of A and indicated the presence of 18 carbon atoms in each compound (Table III). The mass spectra of A_1 and A_3 were also very similar to that of A. Each exhibited a peak at *m/e* 469 attributable to the $(MH)^+$ ion as previously indicated for A.[8] It was apparent, therefore, that A, A_1, and A_3 were isomers. The elemental analyses were consistent with the compositions $C_{18}H_{36}N_4O_{10}\cdot H_2O$ for A_1 and $C_{18}H_{36}N_4O_{10}\cdot 4HCl$ for the hydrochloride salt of A_3, further supporting the above contention.

Hydrolysis of gentamicins A, A_1, and A_3 with 6 *N* hydrochloric acid at 100° for 1 hr followed by paper chromatographic analysis of the hydrolyzate clearly indicated the presence of deoxystreptamine in all of them. Glucosamine and paromamine were present only in the hydrolyzates of A and A_1. The hydrolyzate of A_3 contained 6-amino-6-deoxyglucose. Furthermore, this comparative study indicated the absence of gentosamine (3-methylamino-3-deoxy-D-xylose), one of the hydrolysis products of gentamicin A, in the hydrolyzates of A_1 and A_3, but the presence of another sugar whose R_f was very close to, but not identical with, that of gentosamine. These data strongly suggested, therefore, that A_1 was an isomer of A in the gentosamine moiety, and A_3 was an isomer of A_1 in the glucosamine moiety. Recently, Mallams and coworkers[9a] in our laboratories isolated two new antibiotics named 66-40B and 66-40D from *Micromonospora inyoensis* and showed these to possess the following structures.