Registry No.—**5a**, 150-76-5; **5b**, 5307-05-1; **5c**, 2431-91-6; **5d**, 489-01-0; **5e**, 642-71-7; **5f**, 22248-14-2; **5g**, 18113-03-6; **5h**, 17332-11-5; **6a**, 935-50-2; **6b**, 57197-11-2; **6c**, 57197-12-3; **6d**, 33974-39-9; **6e**, 57197-13-4; **6f**, 57197-14-5; **6g**, 57197-15-6; **6h**, 57197-16-7; **7a**, 94-71-3; **7b**, 7495-77-4; **7c**, 122-94-1; **7d**, 13037-86-0; **7e**, 103-16-2; **7f**, 831-82-3; **8**, 52366-32-2; **9a**, 52250-33-6; **9b**, 57197-17-8; **9c**, 57197-18-9; **10**, 3877-67-6; **11**, 57197-19-0; **12a**, 57197-20-3; **12b**, 57197-21-4; **12c**, 57197-22-5; **13**, 732-26-3; **14**, 4971-61-3; **15**, 533-31-3; **16a**, 57197-23-6; **16b**, 57197-24-7; **17**, 57197-25-8; **18**, 20778-61-4; **19**, 5150-42-5; **20**, 57197-26-9; **21a**, 527-60-6; **21b**, 128-37-0; **22a**, 38876-36-7; **22b**, 2411-18-9; **22c**, 15910-49-3; TTN, 13746-98-0.

Supplementary Material Available. Full yield, melting point, analytical, and spectroscopic data (NMR, ir) for compounds **6a–h**, **12a–c**, **14**, and **22a–c** (3 pages) will appear following these pages in the microfilm edition of this volume of the journal.

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A Synthesis of the Pyrazomycins[15]

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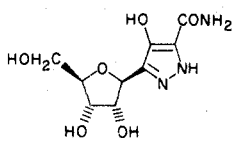
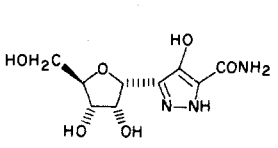
Pyrazomycin (**1**), an antiviral metabolite of *Streptomyces candidus*, and its congener pyrazomycin B (**2**) were synthesized. Reaction of 2,3-*O*-isopropylidene-5-*O*-*p*-nitrobenzoyl-β-D-ribosyl bromide (**6**) with diethyl 1,3-acetonedicarboxylate afforded 3-oxo-2-(2,3-*O*-isopropylidene-5-*O*-*p*-nitrobenzoyl-α-D-ribosyl)glutaric acid diethyl ester (**7**). Diazotization of **7** with *p*-toluenesulfonyl azide gave 5-(2,3-*O*-isopropylidene-5-*O*-*p*-nitrobenzoyl-α-D-ribosyl)-4-oxo-2-pyrazoline-3,5-dicarboxylic acid diethyl ester (**11**). Treatment of **11** with sodium ethoxide accomplished removal of the *p*-nitrobenzoyl group and of the quaternary ethoxycarbonyl function to produce 3-(2,3-*O*-isopropylidene-α-D-ribofuranosyl)-4-hydroxypyrazole-5-carboxylic acid ethyl ester (**12**). Ammonolysis of **12** afforded the corresponding amide **13**, and under slightly different conditions, the epimeric amide **14**. Removal of the isopropylidene group from **13** and **14** completed the synthesis of **1** and **2**, respectively.

In recent years considerable interest has been accorded to *C*-nucleoside antibiotics.[1] In this new class of natural products, pyrazomycin[2] deserves particular attention, owing to reports of its antitumor[3] and broad spectrum antiviral[4] activity. Pyrazomycin, 3-(1′-β-D-ribofuranosyl)-4-hydroxypyrazole 5-carboxamide (**1**), was first isolated from fermentations of a strain of *Streptomyces candidus*.[2,5] This organism has recently yielded a second factor, which was characterized as the 1′-α epimer, pyrazomycin B (**2**).[6] A synthesis of **1** has previously been reported.[7] We now wish to describe a new and shorter synthetic route which allowed the preparation of both **1** and **2**.

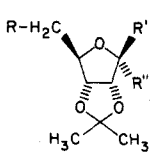


1



2

Our starting material was 2,3-*O*-isopropylidene-D-ribofuranose (**3**),[8] containing both anomers, α and β, in a ratio of 1:9. Reaction of **3** with *p*-nitrobenzoyl chloride in pyridine afforded the two di-*p*-nitrobenzoates **4** and **5** (8:1),

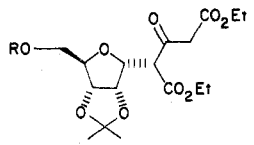


3: R = –OH; R′, R″ = –H, –OH
4: R = R′ = *p*-$NO_2C_6H_4CO_2$–; R″ = –H
5: R = R″ = *p*-$NO_2C_6H_4CO_2$–; R′ = –H
6: R = *p*-$NO_2C_6H_4CO_2$–; R′ = –Br; R″ = –H

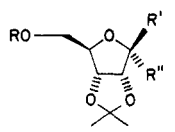
which could be separated by fractional crystallization. The configurations at the anomeric carbon atoms (C-1) in **4** and **5** were assigned with the help of NMR spectral data.

While the conformational motility of five-membered rings ordinarily does not permit configurational assignments to be made on the basis of coupling constants of vicinal protons,[9] it has recently been shown that in *O,O'*-isopropylidene derivatives of furanoses protons in a vicinal cis relationship consistently have coupling constants of 3–6.5 Hz, while those which are trans to each other are coupled by less than 1 Hz.[10] Accordingly, in the NMR spectrum of **4**, the anomeric proton (H-1) gives rise to a singlet at 6.02 ppm (in $CDCl_3$), while H-1 of **5** is seen as a doublet ($J_{1,2}$ = 4.5 Hz) at 6.53 ppm.

When the mixture of **4** and **5** was allowed to react at 0° with a saturated solution of hydrogen bromide in methylene chloride, a single crystalline ribosyl bromide **6** was obtained. In agreement with the β-anomeric structure, the NMR spectrum of **6** (in $CDCl_3$) contains a singlet at 6.49 ppm, assignable to H-1. Alkylation of the potassium salt of diethyl acetonedicarboxylate with **6** was effected in benzene in the presence of a solid–liquid phase transfer catalyst (18-crown-6). The reaction led to a mixture in which the desired C-alkylation product **7** was the major component (43% yield). Chromatographic separation afforded, in addition to **7**, the O-alkylation product **8**, a small amount of its β epimer **9**, and a dialkylated derivative **10**. The configu-

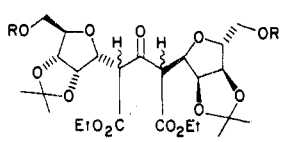


7: R = *p*-$NO_2C_6H_4CO$–



8: R = *p*-$NO_2C_6H_4CO$–
R' = –H
R'' = –O–C(CH_2CO_2Et)=$CHCO_2Et$

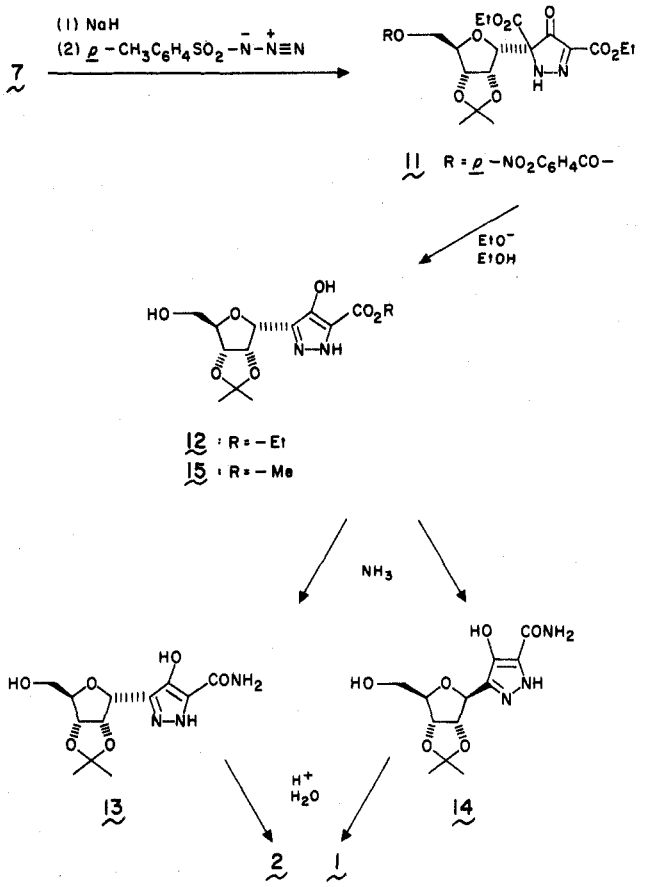
9: R = *p*-$NO_2C_6H_4CO$–
R' = –O–C(CH_2CO_2Et)=$CHCO_2Et$
R'' = –H



10: R = *p*-$NO_2C_6H_4CO$–

rational identities of **8** and **9** were established on the basis of the NMR signals observed for the anomeric proton (H-1). The spectrum of **8** (in $CDCl_3$) contains a doublet for H-1 at 5.55 ppm ($J_{1,2}$ = 4 Hz), while that of **9** possesses two singlets at 5.52 and 5.66 ppm, one of which belongs to H-1 and the other one to the vinylic proton. Lacking usable spectroscopic evidence for the C-1 configuration of **7**, it was assumed that the new carbon–carbon bond had formed with inversion and that it therefore was α, as shown. This contention was confirmed by the subsequent conversion of **7** to **11**. The sodium salt of **7** (prepared with sodium hydride in dimethoxyethane) was subjected to diazotization with *p*-toluenesulfonyl azide,[11] affording directly the cyclic derivative **11**. In agreement with the pyrazolinone structure, the ir spectrum of **11** shows strong N–H stretching at 3445 cm^{-1}. There is no absorption assignable to diazo stretching (ca. 2100–2200 cm^{-1}). The uv spectrum of **11** (in EtOH), arising from *p*-nitrobenzoyl and pyrazolinone absorption,[12] has a maximum at 245 nm (ε 16000), a shoulder at 260 nm (ε 14400), and a second maximum at 330 nm (ε 6800). The presence of a doublet for H-1 (at δ 4.93, $J_{1,2}$ = 3.8 Hz) in the NMR spectrum confirms the α-configuration at C-1 of the ribosyl moiety.

Treatment of **11** with sodium ethoxide in ethanol at room temperature accomplished solvolysis of the *p*-nitrobenzoyl ester function and selective removal of the quaternary ethoxycarbonyl group, resulting in aromatization of



the heterocycle and producing **12**. The uv spectrum of **12** reveals the typical 4-hydroxypyrazole chromophore[13] having absorption maxima at 227 (ε 7300) and 271 nm (5200), when measured in ethanol, and at 235 (5300) and 317 nm (7900) when taken in alkaline solution. The 1'-α configuration is evident from the NMR spectrum (in $CDCl_3$), in which the anomeric proton gives rise to a doublet at 5.26 ppm ($J_{1,2}$ = 2.6 Hz).

When **12** was heated for 3 hr at 100° in methanolic ammonia solution, the major part of the substance was converted to the amide **13**. In addition, the epimeric amide **14** was obtained as a minor by-product, and some material was recovered as the methyl ester **15**. Prolonged exposure of **12** to the above reaction conditions (12 hr) afforded **14** as the sole product. The apparent ease with which **13** epimerized to **14** suggests that intermediate (resonance stabilized) opening of the ribose ring was involved in this transformation. Other *C*-nucleosides are known to exhibit similar behavior.[14] The stereochemical relationship of **13** and **14** is reflected in the differences in their respective NMR spectra. While the spectrum of **13** (in CD_3OD) contains a doublet at 5.27 ppm ($J_{1',2'}$ = 3 Hz) arising from H-1', that of **14** has a singlet at 4.99 ppm.

Removal of the isopropylidene protecting group from **13** and **14**, respectively, with 90% trifluoroacetic acid completed the syntheses of the two antibiotics **2** and **1**. The physical properties of **2** agreed with those reported for pyrazomycin B.[6] Our synthetic **1** was found to be identical with an authentic sample of pyrazomycin.[2,5–7]

Experimental Section

General. Melting points were taken on a Kofler hot stage melting point apparatus (Reichert) and are uncorrected. Thermal analyses were carried out on a Du Pont 900 thermal analyzer. Infrared spectra were obtained on a Perkin-Elmer 621, a Beckman IR-9, or a Digilab FTS 14 spectrometer. Ultraviolet spectra were recorded on a Cary Model 16 spectrophotometer. Rotations were measured on a Perkin-Elmer 141 automatic polarimeter. Proton NMR spec-

tra were obtained on Varian HA-100 and XL-100 instruments and are reported in parts per million downfield from internal tetramethylsilane.

2,3-*O*-Isopropylidene-1,5-di-*O*-*p*-nitrobenzoyl-D-ribofuranoses (4 and 5). To a cold solution of 46.8 g (0.246 mol) of 2,3-*O*-isopropylidene-D-ribofuranose[8] in 650 ml of dry pyridine was added in portions, while vigorously stirring, 115 g (0.62 mol) of *p*-nitrobenzoyl chloride. The reaction mixture was kept stirring for another 1 hr in an ice bath and for 20 hr at room temperature. It was then cooled again and 500 ml of saturated aqueous $NaHCO_3$ was carefully added. Upon dilution with 5 l. of ice–water a precipitate formed, which was collected by filtration, washed with water, and dissolved in 1200 ml of CH_2Cl_2. The organic solution was washed with 600 ml of 0.2 *N* HCl and with water, dried over Na_2SO_4, and evaporated in vacuo. Crystallization of the residue from CH_2Cl_2–Et_2O afforded 97.5 g of 2,3-*O*-isopropylidene-1,5-di-*O*-*p*-nitrobenzoyl-β-D-ribose (4): mp 139–140°; $[\alpha]^{25}D$ 0.00° (*c* 1.673, $CHCl_3$); uv max (EtOH) 257 nm (ε 26000), infl 300 (4000); ir ($CHCl_3$) 1735, 1610, 1535, 1345, 1270 cm^{-1}; NMR ($CDCl_3$) δ 1.39 (s, CH_3), 1.57 (s, CH_3), 4.4–4.9 (3), 4.94 (d, CH_2O), 6.52 (s, H-1), 8.05–8.35 ppm (8, aromatic).

Anal. Calcd for $C_{22}H_{20}N_2O_{11}$: C, 54.10; H, 4.13; N, 5.74. Found: C, 54.10; H, 4.20; N, 5.77.

The mother liquor was evaporated and repeatedly recrystallized from MeOH and CH_2Cl_2–Et_2O. There were obtained 14.5 g of a crystalline mixture of the anomers 4 and 5 and finally 3.8 g of 2,3-*O*-isopropylidene-1,5-di-*O*-*p*-nitrobenzoyl-α-D-ribose (5). Pure 5 had mp 157–158°; $[\alpha]^{25}D$ +12.44° (*c* 1.6322, $CHCl_3$); uv (EtOH) max 258 nm (ε 27100), infl 300 (4200); ir ($CHCl_3$) 1725, 1601, 1535, 1345, 1270 cm^{-1}; NMR ($CDCl_3$) δ 1.37, 1.39 (2 s, CH_3), 4.5–5.1 (5), 6.53 (d, H-1, $J_{1,2}$ = 4.5 Hz), 8.1–8.45 ppm (8, aromatic).

Anal. Calcd for $C_{22}H_{20}N_2O_{11}$: C, 54.10; H, 4.13; N, 5.74. Found: C, 54.13; H, 4.27; N, 5.97.

The combined yield of all fractions was 96.4%.

2,3-*O*-Isopropylidene-5-*O*-*p*-nitrobenzoyl-β-D-ribosyl Bromide (6). 2,3-*O*-Isopropylidene-1,5-di-*O*-*p*-nitrobenzoyl-β-D-ribose (4, or the mixture of anomers) (8.5 g, 17.4 mmol) was added in one portion to 140 ml of dry CH_2Cl_2, previously saturated with anhydrous HBr at 0°. The flask was sealed with a rubber septum and the reaction mixture was stirred in an ice bath for 30 min. It was then allowed to warm to room temperature within 1 hr. The precipitated *p*-nitrobenzoic acid was removed by filtration under a blanket of dry argon. The filtrate was concentrated at 25° (bath temperature) to approximately ¼ of the original volume. The solution was repeatedly diluted with dry Et_2O and petroleum ether (bp 30–60°) and partially concentrated in vacuo until the bromide 6 crystallized spontaneously on the walls of the flask. After cooling, decanting, and washing with petroleum ether, the crystals were dried at room temperature (0.005 mmHg), affording 6.421 g (91.7%) of 6.

An analytical sample was obtained by recrystallization from CH_2Cl_2–Et_2O–petroleum ether: mp 118.5–120.5°; $[\alpha]^{25}D$ −67.45° (*c* 1.478, $CHCl_3$); uv max (Et_2O) 255 nm (ε 13200); ir ($CHCl_3$) 1730, 1530, 1350, 1270 cm^{-1}; NMR ($CDCl_3$) δ 1.39 (s, CH_3), 1.46 (s, CH_3), 4.70 (3, $CHCH_2$), 4.97, 5.29 (2 d, H-2, H-3), 6.49 (s, H-1), 8.28 ppm (4, aromatic).

Anal. Calcd for $C_{15}H_{16}BrNO_7$: C, 44.79; H, 4.01; N, 3.48; Br, 19.86. Found: C, 44.85; H, 4.06; N, 3.40; Br, 19.97.

The bromide 6 was stable for several days when stored in a sealed container under argon at −10°.

Reaction of 2,3-*O*-Isopropylidene-5-*O*-*p*-nitrobenzoyl-β-D-ribosyl Bromide (6) with Diethyl 1,3-Acetonedicarboxylate. To a suspension of 805 mg (20.07 mmol) of KH in 40 ml of dry benzene (stirred under argon) was added dropwise 4 ml of diethyl 1,3-acetonedicarboxylate, followed by a solution of 3.750 g (14.2 mmol) of 18-crown-6 in 30 ml of benzene. After H_2 evolution had ceased, a larger excess (22 ml) of diethyl 1,3-acetonedicarboxylate was added in one portion. Then, a solution of 5.93 g (14.74 mmol) of 6 in 80 ml of dry benzene was added dropwise over 30 min. The reaction mixture was stirred at room temperature for 16 hr (under argon). It was then diluted with 1000 ml of Et_2O. The ether phase was washed with 3 × 300 ml of H_2O, diluted with 300 ml of benzene, and dried (Na_2SO_4). After evaporation of the solvents under reduced pressure, the excess diethyl 1,3-acetonedicarboxylate was distilled off in a Kugelrohr apparatus (bulb to bulb) at 80–85° (0.01 mmHg). The residue was dissolved in 15 ml of toluene–ethyl acetate (10:1) and chromatographed on a column containing 550 g of a mixture of 75% of silica gel 60 and 25% of silica gel PF-254 (both E. Merck). The column was developed with toluene–ethyl acetate, 10:1 (3600 ml, fractions 1–149), 10:1.5 (2300 ml, fractions 150–265), and 10:3 (1300 ml, fractions 266–300). The eluate was monitored by thin layer chromatography in toluene–ethyl acetate (10:1.75) and cyclohexane–ethyl acetate (3:1).

Fractions 80–114, after evaporation and drying in vacuo at 60° (0.01 mmHg), afforded 0.410 g (5.3%) of **3-(2,3-*O*-isopropylidene-5-*O*-*p*-nitrobenzoyl-β-D-ribosyl)oxy-2-pentenedioic acid diethyl ester (9)** as a colorless oil: $[\alpha]^{25}D$ −96.79° (*c* 1.1231, $CHCl_3$); uv (EtOH) max 233 nm (ε 18600), infl 255 nm (14200); ir ($CHCl_3$) 1730, 1705 (sh), 1640, 1605 (sh), 1530, 1270 cm^{-1}; NMR ($CDCl_3$, partial) δ 5.52 and 5.66 ppm (2 s, H-1 and =CH–).

Anal. Calcd for $C_{24}H_{29}NO_{12}$: C, 55.07; H, 5.58; N, 2.68. Found: C, 54.90; H, 5.69; N, 2.53.

Fractions 115–188, upon evaporation and drying, gave 2.850 g of **2-(2,3-*O*-isopropylidene-5-*O*-*p*-nitrobenzoyl-α-D-ribosyl)-3-oxoglutaric acid diethyl ester (7).** An additional 0.450 g of the desired 7 was obtained upon rechromatographing fractions 189–230, giving a total yield of 42.8%: colorless oil; $[\alpha]^{25}D$ +44.8° (*c* 0.9434, $CHCl_3$); uv max (EtOH) 258 nm (ε 11300); uv max (0.1 *N* KOH) 279 nm (ε 28000); ir ($CHCl_3$) 1735, 1605, 1530, 1270 cm^{-1}.

Anal. Calcd for $C_{24}H_{29}NO_{12}$: C, 55.07; H, 5.58; N, 2.68. Found: C, 55.17; H, 5.61; N, 2.62.

Fractions 231–310 (combined with the remainder from fractions 189–230) were rechromatographed on 450 g of silica gel mixture (vide supra). The column was eluted with ethyl acetate–cyclohexane, 25:75 (4200 ml) and 30:70 (3000 ml). From appropriate fractions there was obtained after evaporation 1.625 g (21.1%) of **3-(2,3-*O*-isopropylidene-5-*O*-*p*-nitrobenzoyl-α-D-ribosyl)oxy-2-pentenedioic acid diethyl ester (8):** $[\alpha]^{25}D$ +41.72° (*c* 1.1313, $CHCl_3$); uv (EtOH) max 234 nm (ε 16200), infl 260 (12000); ir 1710, 1685 (sh), 1620, 1590, 1515, 1270 cm^{-1}; NMR ($CDCl_3$, partial) δ 5.49 (s, –CH=), 5.55 ppm (d, H-1, $J_{1,2}$ = 4.0 Hz).

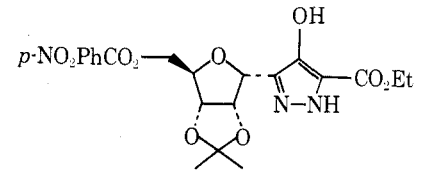
Anal. Calcd for $C_{24}H_{29}NO_{12}$: C, 55.07; H, 5.58; N, 2.68. Found: C, 54.78; H, 5.58; N, 2.59.

Later fractions afforded 1.488 (23.9%) of **2,4-bis(2,3-*O*-isopropylidene-5-*O*-*p*-nitrobenzoyl-α-D-ribosyl)-3-oxoglutaric acid diethyl ester (10):** $[\alpha]^{25}D$ +45.58° (*c* 1.1254, $CHCl_3$); uv max (EtOH) 258 nm (ε 25500); uv max (0.1 *N* KOH) 278 nm (ε 35500); ir ($CHCl_3$) 1730, 1605, 1525, 1270 cm^{-1}.

Anal. Calcd for $C_{39}H_{44}N_2O_{19}$: C, 55.46; H, 5.25; N, 3.32. Found: C, 55.51; H, 5.33; N, 3.25.

5-(2,3-*O*-Isopropylidene-5-*O*-*p*-nitrobenzoyl-α-D-ribosyl)-4-oxo-2-pyrazoline-3,5-dicarboxylic Acid Diethyl Ester (11). A solution of 4.067 g (7.77 mmol) of 7 in 80 ml of dry 1,2-dimethoxyethane (DME) was added during 5 min to a stirred suspension of 200 mg (8.33 mmol) of NaH in 40 ml of dry DME under argon. An excess of tosyl azide (4 ml) was then added dropwise with a syringe. After stirring for 3 hr the reaction mixture was distributed between 1000 ml of cold ethyl acetate and 500 ml of ice–water. The aqueous layer was acidified to pH 3 with 1 *N* HCl and extracted with a second portion of ethyl acetate. The extracts were washed with half-saturated aqueous NaCl, dried (Na_2SO_4), and evaporated in vacuo.

The residual oil was purified by column chromatography on 500 g of silica gel. The column was developed with ethyl acetate–cyclohexane, 40:60 (4000 ml), then 60:40 (1500 ml), and finally ethyl acetate (1000 ml). Excess tosyl azide and tosyl amide (mp 139°, from H_2O) were eluted first. Then fractions were collected, which yielded 3.085 g (84.3%) of 11. Early fractions of 11 were almost pure, while later fractions contained small amounts of a new compound, which had formed during the chromatography, and which was identified as the deethoxycarbonylation product, **3-(2,3-*O*-isopropylidene-5-*O*-*p*-nitrobenzoyl-α-D-ribosyl)-4-hydroxypyrazole-5-carboxylic acid ethyl ester (i).** Analytical samples of 11



i

and i were obtained by preparative thin layer chromatography on silica gel with ethyl acetate–cyclohexane, 60:40.

Pure 11 was obtained as a colorless glass: $[\alpha]^{25}D$ +132.1° (*c* 1.0220, $CHCl_3$); uv (EtOH) max 245 nm (ε 16000), sh 260 (14400), max 330 (6700); ir ($CHCl_3$) 3445, 1760, 1730, 1605, 1535, 1500, 1420, 1390, 1355, 1270 cm^{-1}; NMR ($CDCl_3$) δ 1.27 (t, CH_3CH_2–), 1.36 (t, CH_3CH_2), 1.36 (s, CH_3), 1.61 (s, CH_3), 4.24 (q, CH_3CH_2),

4.34 (q, CH_3CH_2), 4.37 (d, CH_2O), 4.45 (t, H-4), 4.76 (d, H-3, $J_{2,3}$ = 5.8 Hz), 4.93 (d, H-1, $J_{1,2}$ = 3.8 Hz), 5.18 (d of d, H-2), 8.17, 8.32 (aromatic AA'BB'), 8.66 ppm (NH).

Anal. Calcd for $C_{24}H_{27}N_3O_{12}$: C, 52.46; H, 4.95; N, 7.65. Found: C, 52.30; H, 4.98; N, 7.53.

Pure i had $[\alpha]^{25}D$ −31.01° (c 1.0595, $CHCl_3$); uv max (EtOH) 227 nm (ε 12600), 261 (16800); ir ($CHCl_3$) 3450, 1730, 1695, 1610, 1530, 1350, cm^{-1}; NMR ($CDCl_3$) δ 1.37 (s, CH_3), 1.39 (t, CH_3CH_2), 1.59 (s, CH_3), 4.3–4.7 (m, 5), 4.86 (d, H-3, $J_{2,3}$ = 5.7 Hz), 4.97 (d or d, H-2), 5.27 (d, H-1, $J_{1,2}$ = 3.2 Hz), 7.28 (NH), 8.1–8.4 (aromatic AA'BB'), 10.7 ppm (OH).

Anal. Calcd for $C_{21}H_{23}N_3O_{10}$: C, 52.83; H, 4.86; N, 8.80. Found: C, 52.97; H, 4.89; N, 8.41.

3-(2,3-*O*-Isopropylidene-α-D-ribofuranosyl)-4-hydroxypyrazole-5-carboxylic Acid Ethyl Ester (12). To a stirred solution of 5.28 g (9.5 mmol) of 11 in 100 ml of absolute EtOH was added 700 mg of sodium ethoxide. After 45 min at 20° the reaction mixture was neutralized with AG 50W-X4 ion exchange resin (H^+, prewashed with EtOH). The resin was removed by filtration and washed with EtOH. After evaporation of the filtrate in vacuo, the residue was purified by preparative thin layer chromatography on silica gel with ethyl acetate as developing solvent. Elution of the appropriate fractions with ethyl acetate afforded 2.25 g (72%) of 12 (colorless glass): $[\alpha]^{25}D$ −37.94° (c 1.0200, $CHCl_3$); uv max (EtOH) 227 nm (ε 7300), 271 (5200); uv max (0.01 *N* NaOH) 317 nm (ε 7900); ir ($CHCl_3$) 1700, 1540 cm^{-1}; NMR ($CDCl_3$) δ 1.33 (s, CH_3), 1.37 (t, CH_3CH_2), 1.52 (s, CH_3), 3.72 (d, CH_2OH), 4.22 (t, H-4) 4.38 (q, CH_3CH_2), 4.73–4.95 (m, H-2 and H-3), 5.26 ppm (d, H-1, $J_{1,2}$ = 2.6 Hz).

Anal. Calcd for $C_{14}H_{20}N_2O_7$: C, 51.22; H, 6.14; N, 8.53. Found: C, 50.76; H, 6.40; N, 8.25.

3-(2,3-*O*-Isopropylidene-α-D-ribofuranosyl)-4-hydroxypyrazole-5-carboxamide (13). A solution of 600 mg (1.83 mmol) of 12 in 20 ml of dry MeOH was saturated with anhydrous NH_3 at 20° and heated in a sealed tube for 3 hr at 95°. After evaporation to dryness in vacuo, the residue was chromatographed on 125 g of silica gel. The column was developed with AcOEt–Me_2CO–MeOH–H_2O, 70:10:5:2.5. Fractions containing the transesterification product 15 and some β epimer 14 were eluted first. These were saved and resubjected to ammonolysis and chromatography as above. Fractions from both reactions containing the desired α epimer 13 were combined, evaporated, and redissolved in H_2O. The aqueous solution was lyophilized to give 390 mg (69%) of 13 as a white powder: $[\alpha]^{25}D$ −46.22° (c 1.1100, EtOH); uv max (EtOH) 220 nm (ε 7600), 263 (5800); uv max (0.01 *N* KOH) 310 nm (8200); ir (KBr) 1670, 1620, 1590 (sh), 1540, 1380 cm^{-1}; NMR (CD_3OD) δ 1.32 (s, CH_3), 1.49 (s, CH_3), 3.67 (d, CH_2OH), 4.18 (t, H-4), 4.8–5.2 (H-2, H-3), 5.26 ppm (d, H-1, $J_{1,2}$ = 3 Hz).

Anal. Calcd for $C_{12}H_{17}N_3O_6 \cdot 0.5H_2O$: C, 46.75; H, 5.88; N, 13.63. Found: C, 46.95; H, 5.90; N, 13.43.

From the remaining fractions there was obtained 80 mg of 15.

3-(2,3-*O*-Isopropylidene-β-D-ribofuranosyl)-4-hydroxypyrazole-5-carboxamide (14). A solution of 600 mg (1.83 mmol) of 12 in 20 ml of dry MeOH was saturated with anhydrous ammonia at 10–15° and heated in a sealed tube for 12 hr at 95–100°. After removal of the solvents under reduced pressure, the residue was purified by chromatography on 125 g of silica gel, with AcOEt–Me_2CO–MeOH–H_2O, 70:10:5:5, as the eluent. Fractions containing 14 were evaporated in vacuo. The residue was redissolved in H_2O. The aqueous solution was freeze-dried to afford 398 mg (71%) of 14 as a colorless powder: $[\alpha]^{25}D$ −78.45° (c 1.1153, EtOH); uv max (EtOH) 225 nm (ε 9100), 264 (7300); uv max (0.01 *N* KOH) 309 nm (ε 10500); ir (KBr) 1670, 1625, 1590 (sh), 1550, 1390 cm^{-1}; NMR (CD_3OD) δ 1.34 (s, CH_3), 1.55 (s, CH_3), 3.64 (d, CH_2OH), 4.12 (d of t, H-4), 4.7–4.95 (H-2, H-3), 4.99 ppm (s, H-1).

Anal. Calcd for $C_{12}H_{17}N_3O_6 \cdot 0.4H_2O$: C, 47.03; H, 5.85; N, 13.71. Found: C, 47.08; H, 5.86; N, 13.65.

3-(α-D-Ribofuranosyl)-4-hydroxypyrazole-5-carboxamide (Pyrazomycin B, 2). A solution of 335 mg (1.1 mmol) of 13 in 20 ml of 90% CF_3CO_2H was kept under argon at room temperature for 1 hr. Then the solvents were removed at 5° under reduced pressure, at last under high vacuum. The residue consisted mainly (>90% by TLC) of the desired pyrazomycin B (2). It was contaminated with ca. 5% of pyrazomycin (1) which had formed by epimerization during reaction. Purification was accomplished by chromatography on silica gel (125 g) with EtOAc–Me_2CO–MeOH–H_2O, 6:1:1:1. Fractions containing 2 were evaporated at low temperature. The residue was redissolved in H_2O. The aqueous solution was filtered through a millipore filter and freeze-dried. There was obtained 306 mg (90%) of pure 2 as a dihydrate (white powder).

A sample of 2 was recrystallized from H_2O. It had mp 76° (unsharp). Thermal analysis showed a transition from the crystalline form to an amorphous solid at 76°: $[\alpha]^{25}D$ −7.56° (c 0.7278, H_2O); uv max (EtOH) 225 nm (ε 8000), 276 (6700); uv max (0.1 *N* KOH) 304 nm (ε 8400); ir (KBr) 1658, 1622 cm^{-1}; NMR (D_2O) δ 4.35 (m, CH_2OH), 4.67 (m, H-4), 4.9–5.1 (H-2, H-3), 5.83 ppm (d, H-1, $J_{1,2}$ = 3 Hz).

Anal. Calcd for $C_9H_{13}N_3O_6 \cdot 1.5H_2O$: C, 37.77; H, 5.63; N, 14.68. Found: C, 37.91; H, 5.68; N, 14.88.

3-(β-D-Ribofuranosyl)-4-hydroxypyrazole-5-carboxamide (Pyrazomycin, 1). A solution of 406 mg (1.33 mmol) of 14 in 20 ml of 90% CF_3CO_2H was kept at room temperature for 45 min. Then the solvents were removed at 5° under reduced pressure, at last under high vacuum. The residue was chromatographed on silica gel with EtOAc–Me_2CO–MeOH–H_2O, 6:1:1:1. Fractions containing 1 were evaporated at <20°. The residue was redissolved in H_2O. The aqueous solution was filtered through a millipore filter and lyophylized. Recrystallization from water afforded 248 mg (72%) of pyrazomycin (1), mp 112–115°, mmp with an authentic sample 112–115°.

Both the authentic sample and synthetic material formed higher melting polymorphs when stored at room temperature. Thermal analysis of freshly recrystallized material showed a broad endothermic phase transition (melting) at 108°, partial recrystallization at 117° (exotherm), and a second melting range at ca. 170°. When these samples were heated at 135°, cooled, and reheated, they showed only one phase transition at 178°. Thermal analysis of stored samples (3 months) revealed one phase transition (melting point) at 182°: $[\alpha]^{25}D$ −49.6° (c 0.7984, H_2O); uv max (EtOH) 226 nm (ε 7600), 267 (6000); uv max (0.01 *N* KOH) 233 nm (ε 4800), 307 (8300); ir (KBr) 1665, 1610, 1545 cm^{-1}; NMR (D_2O) δ 4.35 (m, CH_2OH), 4.65 (q, H-4, $J_{4,5}$ = 3, $J_{4,5}$ = 5 Hz), 4.79 (t, H-3, $J_{3,2}$ = 5, $J_{3,4}$ = 4 Hz), 4.94 (m, H-2), 5.54 (d, H-1, $J_{1,2}$ = 7 Hz).

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Registry No.—i, 57274-16-5; 1, 30868-30-5; 2, 41897-20-5; 4, 57274-17-6; 5, 57274-18-7; 6, 57274-19-8; 7, 57274-20-1; 8, 57304-90-2; 9, 57274-21-2; 10, 57274-22-3; 11, 57274-23-4; 12, 57274-24-5; 13, 57274-25-6; 14, 57274-26-7; D-ribose-2,3-isopropylidene, 13199-25-2; *p*-nitrobenzoyl chloride, 122-04-3; diethyl 1,3-acetonedicarboxylate, 105-50-0.

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