

Kettering-Meyer Laboratory, Southern Research Institute.

The Ribonucleosides of 4-Aminopyrazolo[3,4-d]pyrimidine (1)

John A. Montgomery, Sarah J. Clayton, and William E. Fitzgibbon, Jr.

4-Amino-1- β -D-ribofuranosylpyrazolo[3,4-d]pyrimidine (I) (2) was desired for biochemical studies of the inhibition of purine biosynthesis by negative pseudo feedbacks. Preparation of I *via* the reaction of the chloromercuri derivative of 4-benzamidopyrazolo[3,4-d]pyrimidine with tri-*O*-benzoyl-D-ribofuranosyl chloride by a published procedure (2) was not satisfactory in our hands. Fusion of 4-benzamidopyrazolo[3,4-d]pyrimidine (2) with tetra-*O*-acetyl- β -D-ribofuranose followed by removal of the acyl groups with sodium methoxide in methanol was found to be a good method for the preparation of I and its 2-isomer (II) (3). Even though the yields are no better than those previously reported, the reaction and isolation procedures are much better.

The ultraviolet spectra of I and II prepared by the

fusion method are in excellent agreement with those reported (2, 5, 6) for the corresponding methyl derivatives of 4-aminopyrazolo[3,4-d]pyrimidine, although the spectra previously reported (2) for I and II prepared by the chloromercuri procedure do not agree with those of the methyl compounds or with those of our samples. A comparison of the melting points and water solubilities (7) with the reported values suggests that our samples may be somewhat purer than those prepared by the chloromercuri procedure. I and II were further characterized by determination of their chromatographic behavior, optical rotations, and infrared and proton magnetic resonance spectra. Although the optical rotation values suggest that these nucleosides have the β configuration no rigorous proof of this point has been obtained.

Ultraviolet Spectra

Compound	0.1 N HCl		pH 7		0.1 N NaOH	
	λ max, m μ	$\epsilon \times 10^{-3}$	λ max, m μ	$\epsilon \times 10^{-3}$	λ max, m μ	$\epsilon \times 10^{-3}$
4-Amino-1-methylpyrazolo[3,4-d]pyrimidine	259	9.4	261	9.0	262	9.7
			277	9.25	275	9.7
4-Amino-1- β -D-ribofuranosylpyrazolo[3,4-d]pyrimidine	258	10.5	260	9.75	260	9.8
			274	11.3	275	11.1
4-Amino-2-methylpyrazolo[3,4-d]pyrimidine	268	9.9	270	8.7	287	10.9
			287	11.0		
4-Amino-2- β -D-ribofuranosylpyrazolo[3,4-d]pyrimidine	268	10.7	268	8.2	268	8.9
			290	10.1	290	10.5

EXPERIMENTAL

The melting points reported were determined on a Meltemp Apparatus and are corrected. The ultraviolet spectra were determined in aqueous solution with a Cary Model 14 spectrophotometer, whereas the infrared spectra were determined in pressed potassium bromide discs with a Perkin Elmer Model 221 spectrophotometer. The p.m.r. spectra were determined in 10% (w./v.) DMSO- d_6 with a Varian A-60 spectrometer.

4-Amino-1- β -D-ribofuranosylpyrazolo[3,4-d]pyrimidine and 4-amino-2- β -D-ribofuranosylpyrazolo[3,4-d]pyrimidine.

A finely ground mixture of 4-benzamidopyrazolo[3,4-d]pyrimidine (762 mg., 3.18 mmoles), tetra-*O*-acetyl- β -D-ribofuranose (1.03 g., 3.18 mmoles), and *p*-toluenesulfonic acid hydrate (64 mg.) was heated at 130° for 5 min. *in vacuo* (25-30 mm. Hg). The vacuum was broken and the mixture heated at 180-190° for 10-15 min. at atmospheric pressure. The resultant dark brown gum was dissolved in 25 ml. of chloroform and the solution washed with two 5-ml. portions of saturated sodium bicarbonate solution followed by one 10-ml. portion of water. The chloroform solution was dried over magnesium sulfate and then evaporated to dryness *in vacuo*. The hard glass (1.3 g.) obtained was dissolved in 11 ml. of methanol containing 25.6 mg. of sodium methoxide, and the solution was refluxed for 45 min. prior to neutralization with glacial acetic acid. The dark solid from *in vacuo* concentration was dissolved in water and the solution extracted with three 50-ml. portions of chloroform. The chloroform was extracted with 25 ml. of water and the aqueous solutions were combined for treatment with charcoal and concentrated *in vacuo* at 35°. The crystalline 4-amino-1- β -D-ribofuranosylpyrazolo[3,4-d]pyrimidine thus obtained was recrystallized from 4.5 ml. of water; yield 110 mg. (12.5%), m.p. 253° (lit. (2) 246°), $[\alpha]_D^{25}$ -81.7 \pm 0.1° (C = 1.99/100 ml. DMF). Thin-layer chromatography on silica gel H using 43 butanol:7 water as eluant showed one ultraviolet absorbing spot which gave a positive periodate-Schiff test. Infrared spectrum (ν max in cm^{-1}): 3440 and 3340 (NH + OH), 3200 and 2900 (CH), 1640 (NH), 1595 and 1565 (C=C and C=N), 1440 (-CH₂-), 1320 (-CH-), 1050 (COC), 785 (ring CH). P.m.r. spectrum (τ): 1.78 and 1.80 (C₃-H and C₆-H), 2.26 (N-H), 3.85 d (C₁'-H), 4.68d and 4.92d (C₂'-OH and C₃'-OH), 5.24 unsym. q (C₂'-H or C₃'-H and C₅'-OH), 5.72 q (C₂'-H or C₃'-H), 6.02 q (C₄'-H), 6.48 unsym. multiplet (C₅'-H).

Anal. Calcd. for C₁₀H₁₃N₅O₄: C, 44.94; H, 4.91; N, 26.18. Found: C, 45.03; H, 4.91; N, 26.22.

In a second run employing 4.0 g. of 4-benzamidopyrazolo[3,4-d]-

pyrimidine, concentration of the mother liquors from the crystallization of the 1-isomer gave 140 mg. of 4-amino-2- β -D-ribofuranosylpyrazolo[3,4-d]pyrimidine, m.p. 154° with softening at 137° (lit. (2) 137° dec.); $[\alpha]_D^{25}$ -98.3 \pm 0.1° (C = 2.028/100 ml. DMF). Thin-layer chromatography on silica gel H (Merck) using 43 butanol:7 water showed one ultraviolet absorbing spot (R_f different from 1-isomer run on same plate) which gave a positive periodate-Schiff test. Infrared spectrum (ν max in cm^{-1}): 3400 broad (NH + OH), 3200 and 2930 (CH), 1645 (NH), 1610 and 1545 (C=C, C=N), 1440 (-CH₂-), 1320 (-CH-), 1050 (COC), 780 (ring CH). P.m.r. spectrum (τ): 1.33 and 1.73 (C₃-H and C₆-H), 2.27 (NH), 4.04d (C₁'-H), 5.49q (C₂'-H or C₃'-H), 5.83q (C₄'-H and C₅'-H or C₅'-H), 6.28 (C₅'-H).

Anal. Calcd. for C₁₀H₁₃N₅O₄: C, 44.94; H, 4.91; N, 26.18. Found: C, 44.71; H, 5.04; N, 26.44.

Acknowledgment.

The authors are indebted to Dr. W. J. Barrett and members of the Analytical Section of Southern Research Institute who performed the spectral, optical rotation, and microanalytical determinations reported herein.

REFERENCES

- (1) This work was supported by funds from the C. F. Kettering Foundation and from the Cancer Chemotherapy National Service Center, National Institutes of Health, Contract No. PH-43-64-51.
- (2) J. Davoll and K. A. Kerridge, *J. Chem. Soc.*, 2589 (1961).
- (3) Previously the fusion procedure has been reported only for the preparation of purine nucleosides (4).
- (4) T. Sato, T. Simadate, and Y. Isleido, *Nippon Kagaku Zasshi*, 81, 1440 (1960); *ibid.*, 81, 1442 (1960); T. Simadate, *ibid.*, 82, 1268 (1961); T. Simadate, Y. Isleido, and T. Sato, *ibid.*, 82, 938 (1961); Y. Isleido and T. Sato, *Bull. Chem. Soc. Japan*, 34, 347, 1374 (1961); J. A. Montgomery and H. J. Thomas, *J. Org. Chem.*, 28, 2304 (1964); W. W. Lee, A. P. Martinez, G. L. Tong and L. Goodman, *Chem. Ind. (London)*, 2007 (1964); M. J. Robins, W. A. Bowles, and R. K. Robins, *J. Am. Chem. Soc.*, 86, 1251 (1964).
- (5) C. C. Cheng and R. K. Robins, *J. Org. Chem.*, 21, 1240 (1956).
- (6) P. Schmidt, K. Eichenberger, M. Wilhelm, and J. Druey, *Helv. Chim. Acta*, 42, 763 (1959).
- (7) Based on the concentrations used to determine optical rotations.

Received June 16, 1964

Birmingham, Alabama