Synthesis of 4-Amino-3-(β-D-ribofuranosyl)pyrazole-5-carboxamide *via* Ring Fission of Formycin Derivatives and Purification Using High Pressure Liquid Chromatography (1)

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Two methods have been established for the synthesis of 4-amino-3-(β-D-ribofuranosyl)-pyrazole-5-carboxamide. This nucleoside can be viewed as a potentially versatile intermediate for the synthesis of various bicyclic *C*-nucleosides. The use of HPLC for the final purification of "pure" samples on the basis of elemental analysis is described.

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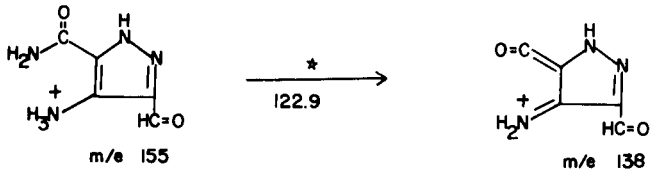
Sir:

The isolation and structural elucidation of pyrazomycin (pyrazofurin) (2) and bredinin (3) as 4-hydroxy-3-(β-D-ribofuranosyl)pyrazole-5-carboxamide (I) and 5-hydroxy-1-(β-D-ribofuranosyl)imidazole-4-carboxamide (II), respectively, have generated considerable interest due to their biological and chemotherapeutic activity (4). The amino analog of bredinin [5-amino-1-(β-D-ribofuranosyl)imidazole-4-carboxamide, AICA-riboside, IV] has also been the subject of numerous chemical and biological investigations (5). In fact, the 5′-phosphate derivative of IV (AICAR) has been firmly established (5) as a very important intermediate in the *de novo* pathway of purine biosynthesis. We now wish to report on two methods for the synthesis of the nucleoside 4-amino-3-(β-D-ribofuranosyl)pyrazole-5-carboxamide (III) which can be viewed as a *C*-nucleoside analog of AICA riboside and should be a key intermediate for the synthesis of bicyclic *C*-nucleosides.

Method A for the synthesis of III involved the treatment of 3-(β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidin-7-one (4) (formycin B, VI) for 4 hours with 85% hydrazine hydrate at reflux temperature. An 85% yield of 4-amino-3-(β-D-ribofuranosyl)pyrazole-5-carboxhydrazide (VIII) (m.p. foams 83°, liquifies 110°) was obtained following dry column chromatography (6) on silica gel. This structural assignment for VIII was supported by a comparison of the uv spectral data obtained for VIII [uv (λ max in nm, $\epsilon \times 10^{-3}$) methanol, 279 (2.6), 231 (2.5); *p*H 11, 276 (2.7), 240 sh (2.3)] with the uv spectral data reported (7) for the model compound 4-amino-3-methylpyrazole-5-carboxhydrazide. The nucleoside VIII was recrystallized from methanol to furnish an analytical sample. *Anal.* Calcd. for $C_9H_{15}N_5O_5$: C, 39.55; H, 5.53; N, 25.67. Found: C, 39.51; H, 5.90; N, 25.70. Reduction of the hydrazide moiety of VIII with Raney nickel in boiling 50% aqueous ethanol furnished (1) the nucleoside III [isolated in only 6.6% yield by using thick layer chromatography (9)] (m.p. sinters 75-77°): uv (λ max in nm, $\epsilon \times 10^{-3}$) methanol, 282.5 (3.9); *p*H 11, 283 (3.4). *Anal.* Calcd. for $C_9H_{14}N_4O_5 \cdot 0.5\ H_2O$: C, 40.45; H, 5.62; N, 20.95. Found: C, 39.99; H, 5.86; N, 20.78. However, this sample (1) of III has subsequently been estimated to be only approximately 80% pure as judged by high pressure liquid chromatography (8).

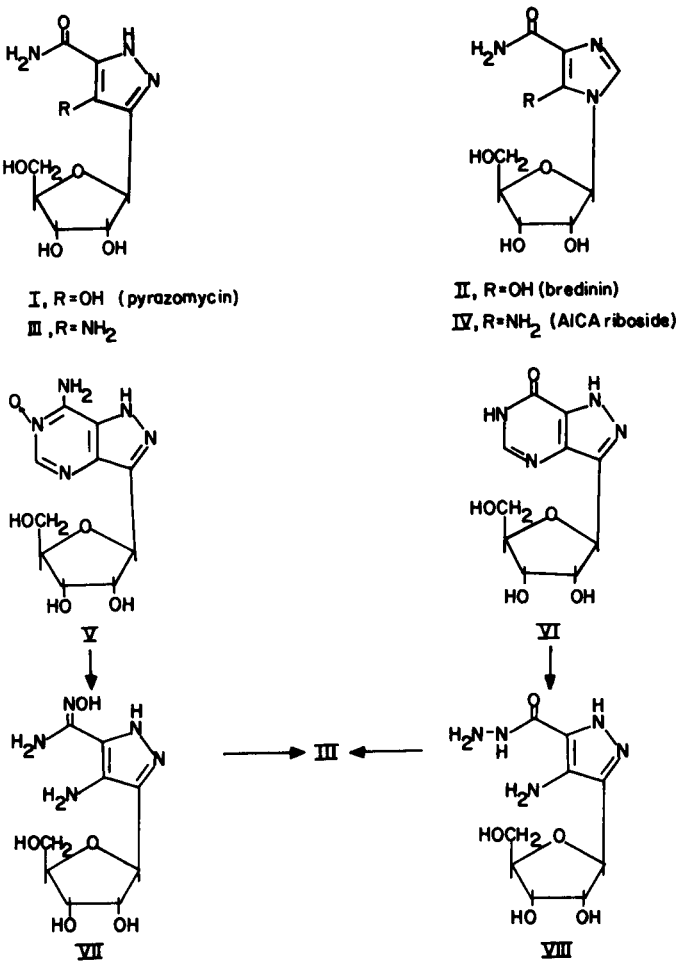
This low yield prompted us to initiate an alternate route (Method B) for the synthesis of III. 7-Amino-3-(β-D-ribofuranosyl)pyrazolo[4,3-*d*]pyrimidine N^6-oxide (1) (V) was dissolved in water and the solution stirred with Dowex 50 x 4 (H^+) at room temperature for 15 hours. 4-Amino-3-(β-D-ribofuranosyl)pyrazole-5-carboxamidoxime (VII) (42%, m.p. 180-184° dec.) was eluted from the resin with a 7% aqueous ammonium hydroxide solution. The uv spectrum of VII was found to be very similar to the uv spectral data reported (7) for the known 4-amino-3-methylpyrazole-5-carboxamidoxime. Further purification of VII was accomplished by recrystallization from methanol (m.p. 190-192° dec.). *Anal.* Calcd. for $C_9H_{15}N_5O_5$: C, 39.55; H, 5.53; N, 25.67. Found: C, 39.59; H, 5.56; N, 25.77; uv (λ max in nm, $\epsilon \times 10^{-3}$) *p*H 11, 270 (6.2), 237 (6.3). A catalytic of VII in dilute sodium hydroxide solution was followed by dry column chromatography (6) to furnish a 16%

yield of III from V. However, this nucleoside material was only approximately 90% pure as determined by HPLC (8). The nucleoside III was finally obtained in a pure state by reverse phase HPLC (8) (m.p. wide range > 85°). *Anal.* Calcd. for $C_9H_{14}N_4O_5$: C, 41.86; H, 5.46; N, 21.70. Found: C, 41.64; H, 5.52; N, 21.63. M^+: m/e 258; $[\alpha]_D^{24.5}$ = -56.5° (C = 1.0, water); uv (λ max in nm, $\epsilon \times 10^{-3}$) water, 282 (4.8), 232.5 sh (4.5); *p*H 11, 282 (4.9) 232.5 sh (4.9). The uv spectral data obtained for III was very similar to the uv spectral data reported (7) for the known 4-amino-3-methylpyrazole-5-carboxamide and the presence of an *o*-aminocarboxamide structure was further (10) supported by an abundant m/e 138 in the mass spectra of III. The pmr spectra (DMSO-d_6, δ 4.71, d, H-1′, $J_{1',2} \simeq 6.5$ Hz) also supported our structural assignment for III.



HPLC was required (*vide supra*) for the final purification of what we had initially assumed were pure samples of III on the basis of elemental analysis. For example, elemental analysis for the impure sample of III was in very close agreement with the calculated values for a hemi-hydrate of III. However, HPLC analysis did not support this conclusion and very clearly emphasizes the danger in assuming that compounds are pure when moles or fractional moles of solvents must be added to make the experimental analysis fit the calculated values. This illustrates the tremendous utility of HPLC in determining the purity of compounds.

The *in vitro* cytotoxicity of I (11), III and IV has been evaluated in our laboratory using L1210 cells in culture (12). It was of interest that III and IV, at a concentration of 10^{-4} *M*, had no effect on cell growth. On the other hand, I at a concentration of 10^{-4} *M* caused a total inhibition of cell growth after a delay of approximately 24 hours, with an ID_{50} of 2 x 10^{-7} *M*. Thus, a replacement of the 4-OH group of pyrazofurin (I) by an $-NH_2$ group (III) has resulted in an apparent loss of cytotoxicity. These data provide some support for the proposal (13) that the 4-OH group of pyrazofurin may be essential for its activity. The nucleoside III can be viewed as a *C*-nucleoside analog of AICA riboside with the distinction that it will most likely be resistant towards an enzymatic cleavage by a nucleoside phosphorylase, but may still function as a substrate for an appropriate kinase to form the analog of AICAR. It is tempting to postulate that the



5′-phosphate derivative of III, *vide supra*, may subsequently be established as a key intermediate in the biosynthetic pathway of certain *C*-nucleoside antibiotics, *e.g.*, formycin and formycin B.

Acknowledgment.

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(6) J. T. Baker chromatographic grade silica gel containing 0.5% by weight Dupont # 609 Phosphor was deactivated with water to parallel the activity of our tlc plates; see B. Leov and M. Goodman, *Chem. Ind. (London)*, 2026 (1967).

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(8) HPLC was conducted on a Waters Associates instrument. Analytical results were obtained on a 4 x 300 mm μ Bondapak C_{18} (Waters) column and the preparative separation was performed on an 8 x 1800 mm Porasil B-C_{18} (Waters) column with water as the eluting solvent. Compound III has a k' of 0.52 on the μ C_{18} column.

(9) Twenty by forty cm plates spread with 2 mm of SilicAR-7GF (Mallinckrodt Chemical Co.).

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(11) The pyrazofurin A was a generous gift from Dr. K. Gerzon of Eli Lilly and Co., Indianapolis, Indiana.

(12) Cultured L1210 cells were grown in Fischer's Medium for Leukemic Cells of Mice supplemented with 10% horse serum, 100 units of penicillin and 100 μg. streptomycin/ml. (Grand Island Biological Company, Santa Clara, California). Test compounds were dissolved in phosphate buffered saline, and the solutions sterilized by filtration (0.22 μ average pore diameter Millipore filter, Millipore Corp., Bedford, Massachusetts) before addition to the medium. Growth was evaluated using sets of replicate tubes, each containing 5 ml. of cell suspension at 1.5-2.5 x 10^4 cells/ml. in medium with the desired concentration of the test compound. Cells were counted using a Coulter counter (Coulter Electronics, Hialeah, Florida), two tubes per time point, over a period of 3 days, and the average values for cell number per ml. were plotted as a semilogarithmic function of time. The relative logarithmic growth rate (RLGR) was the ratio of the slope of the test curve to the slope of the control, or, in terms of the population doubling time (T_d): RLGR = T_d of control/-T_d of test. The ID_{50} was defined as the minimal concentration required to reduce the RLGR to 50% of control.

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