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THE SYNTHESIS OF 4,5-DIAMINO-8-β-D-RIBOFURANOSYLPYRAZOLO[3',4'-5,4]-PYRROLO[2,3-d]PYRIMIDINE, A NOVEL TRICYCLIC NUCLEOSIDE[1]

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We wish to report the synthesis of a new tricyclic ring system, pyrazolo[3',4'-5,4]-pyrrolo[2,3-d]pyrimidine, by a unique ring closure procedure. In the course of our investigation of the chemistry of the pyrrolo[2,3-d]pyrimidine nucleoside antibiotics,[3] tubercidin, toyocamycin, and sangivamycin, a preparation of the amidrazone[4] of 4-amino-6-bromo-5-cyano-7-β-D-ribofuranosyl-pyrrolo[2,3-d]pyrimidine (I, 6-bromotoyocamycin)[5] was undertaken.

A suspension of I in absolute ethanol containing excess anhydrous (97%) hydrazine was heated at reflux temperature overnight (18 hr). Removal of the solvent in vacuo yielded an amorphous white solid which recrystallized from a small amount of methanol to afford beautiful colorless crystals (80% yield). This crystalline material (mp 257-258°) was at first presumed to be the amidrazone since a nitrile absorption[6] band in the IR spectrum was not observed. Formation of a tricyclic heterocycle was initially considered improbable, since this would involve a nucleophilic displacement[7] of bromide from a pyrrole derivative. However, the elemental analysis [Anal. Calcd. for $C_{12}H_{15}N_7O_4$: C, 44.86; H, 4.71; N, 30.52. Found: C, 44.68; H, 4.66; N, 30.68.] was found to be consistent for the amidrazone minus hydrogen bromide which indicated that the tricyclic derivative (IV, 4,5-diamino-8-β-D-ribofuranosylpyrazolo[3',4'-5,4]pyrrolo[2,3-d]-pyrimidine) had indeed been formed. This conclusion was further substantiated by the pmr spectrum of IV which showed in addition to the characteristic carbohydrate absorption peaks, a singlet (1 proton) at δ 8.17 (aromatic hydrogen at C_2), and two broad singlets at δ 6.58 (2 protons) and δ 5.89 (2 protons) (the exocyclic amino group of the pyrimidine and pyrazole moieties, respectively).

The pyrazole ring closure could have occurred by either of two alternate routes. Hydrazine could have added first to the nitrile group to form the intermediate amidrazone (III) which could then ring close to the pyrazole via intramolecular nucleophilic displacement of bromide by the terminal nitrogen of the amidrazone. Alternatively, nucleophilic displacement of bromide by hydrazine could occur initially to furnish the intermediate II. 6-Hydrazinotoyocamycin (II)

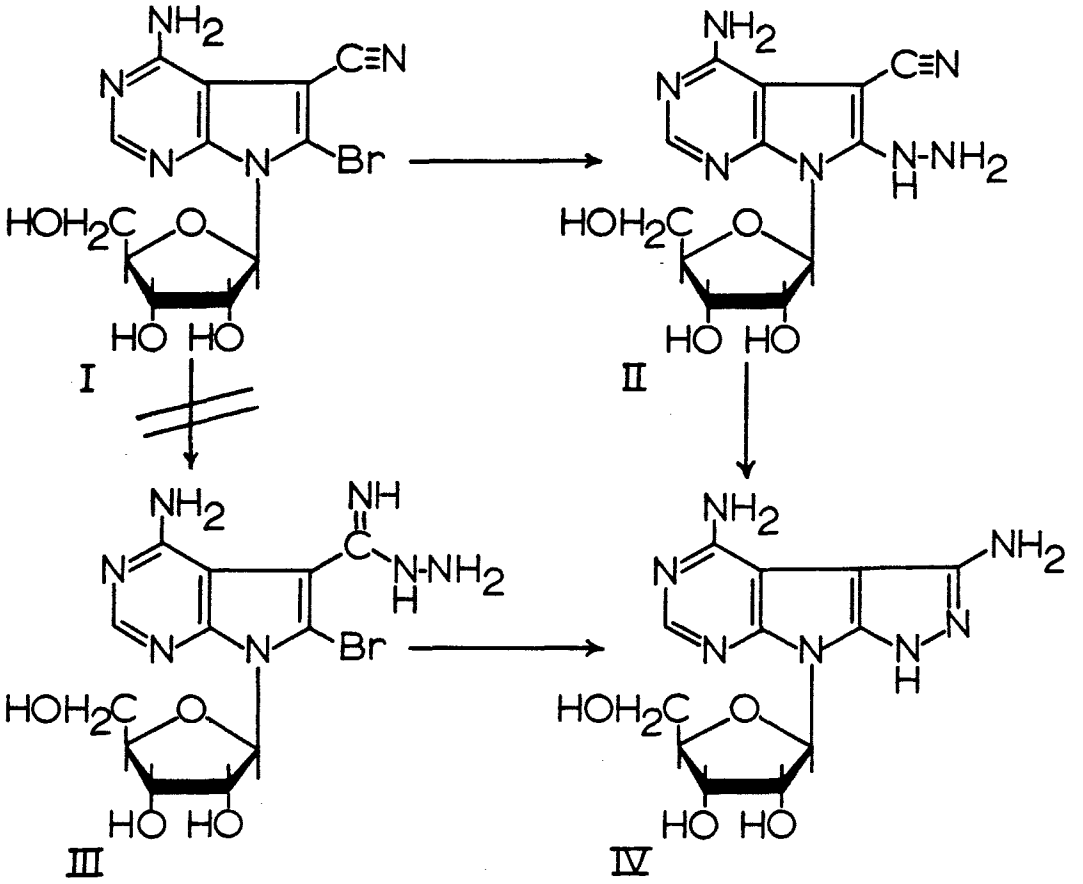

TABLE I

ULTRAVIOLET SPECTRA						
	$\lambda_{max}^{pH\,1}$	ϵ_{max}	λ_{max}^{EtOH}	ϵ_{max}	$\lambda_{max}^{pH\,14}$	ϵ_{max}
I	281 231	12400 16300	284	18300	283	12800
II	289 227	13500 9700	295 222	16800 22200	277	14000
IV	296 252	8400 15800	295 250	8400 17400	293 255	8950 14300

could then form the pyrazole ring *via* nucleophilic attack on the carbon atom of the nitrile group. This prompted a study to determine the actual mechanism of ring closure involved. Careful monitoring (every 5 min) of the reaction mixture (same composition as above) with micro thin layer chromatography [solvent system; EtOAc/*n*PrOH/H_2O (4:1:2)-upper phase, on SilicAR 7GF] showed that as starting material (R_f 0.84) disappeared, a second spot (R_f 0.62) formed, which gradually disappeared to form a third spot (R_f 0.36) corresponding to the isolated product (IV). The experiment was then repeated at 40° and monitored as described above. The reaction mixture was heated at reflux temperature to effect complete solution and then after 45 minutes at 40°, tlc revealed that the spot corresponding to starting material had disappeared and the intermediate spot (R_f 0.62) was the only ultraviolet absorbing component present. The solvent was removed *in vacuo* keeping the temperature below 20° and a methanol/toluene (1:1) mixture (2 x 50 ml) was added and then removed *in vacuo*. The residue was dissolved in an ethanol/ethyl acetate (1:1) mixture at room temperature and the solution was then allowed to stand at 5° for 18 hr. The pale yellow solid which had separated was collected by filtration and air-dried (mp 214-215° dec).

An IR analysis showed a strong nitrile absorption at 2225 cm^{-1}, which eliminated the possibility of an amidrazone intermediate. The ultraviolet spectrum was not identical to the spectrum observed for IV (Table I) thereby establishing that ring annulation had not occurred. Elemental analysis [*Anal.* Calcd. for $C_{12}H_{15}N_7O_4$: C, 44.86; H, 4.71; N, 30.52. Found: C, 44.34; H, 4.83; N, 31.17.] further confirmed the identity of the intermediate as 6-hydrazinotoyocamycin (II) instead of the amidrazone (III). Additional proof that 6-hydrazinotoyocamycin (II) was the actual intermediate in the formation of IV was provided by heating II in absolute ethanol at reflux temperature until solution was complete (*ca.* 4 hr). This furnished a quantitative yield of crystals which exhibited the same ultraviolet spectrum as IV. A mixed melting point with the tricyclic nucleoside (IV) showed no depression.

This conclusively established that the ring closure involves direct nucleophilic attack by hydrazine on the pyrrole ring forming an intermediate hydrazino derivative which subsequently ring closes to the tricyclic nucleoside.

The nucleoside IV represents a novel type of heterocyclic structure with a pyrazole ring condensed at positions 5 and 6 of the tubercidin molecule. Further investigation of this reaction and a study of other nucleophilic displacements on pyrrole is in progress.

REFERENCES

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2. National Aeronautics and Space Administration Fellow, 1965-1968.

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4. The term "amidrazone" as used in this communication refers to the $-\overset{N-NH_2}{\overset{\|}{C}}-NH_2$ moiety, e. g. see A. Giner-Sorolla and A. Bendich, J. Am. Chem. Soc., 80, 3932 (1958).

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6. There was observed an absorption band at 2225 cm^{-1} in the IR spectrum of I.

7. Pyrroles and condensed pyrrole systems are notoriously poor substrates for nucleophilic displacement, K. Schofield, "Hetero-Aromatic Nitrogen Compounds," Plenum Press, New York, 1967, p. 90. A. H. Corwin, "Heterocyclic Compounds," R. C. Elderfield, ed., Wiley, New York, 1950, pp 300-317.