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THE SYNTHESIS OF 6-AMINO-4-METHYL-8-(β-D-RIBOFURANOSYL)(4-H,8-H)PYRROLO-[4,3,2-de]PYRIMIDO[4,5-c]PYRIDAZINE, A NEW TRICYCLIC NUCLEOSIDE (1)

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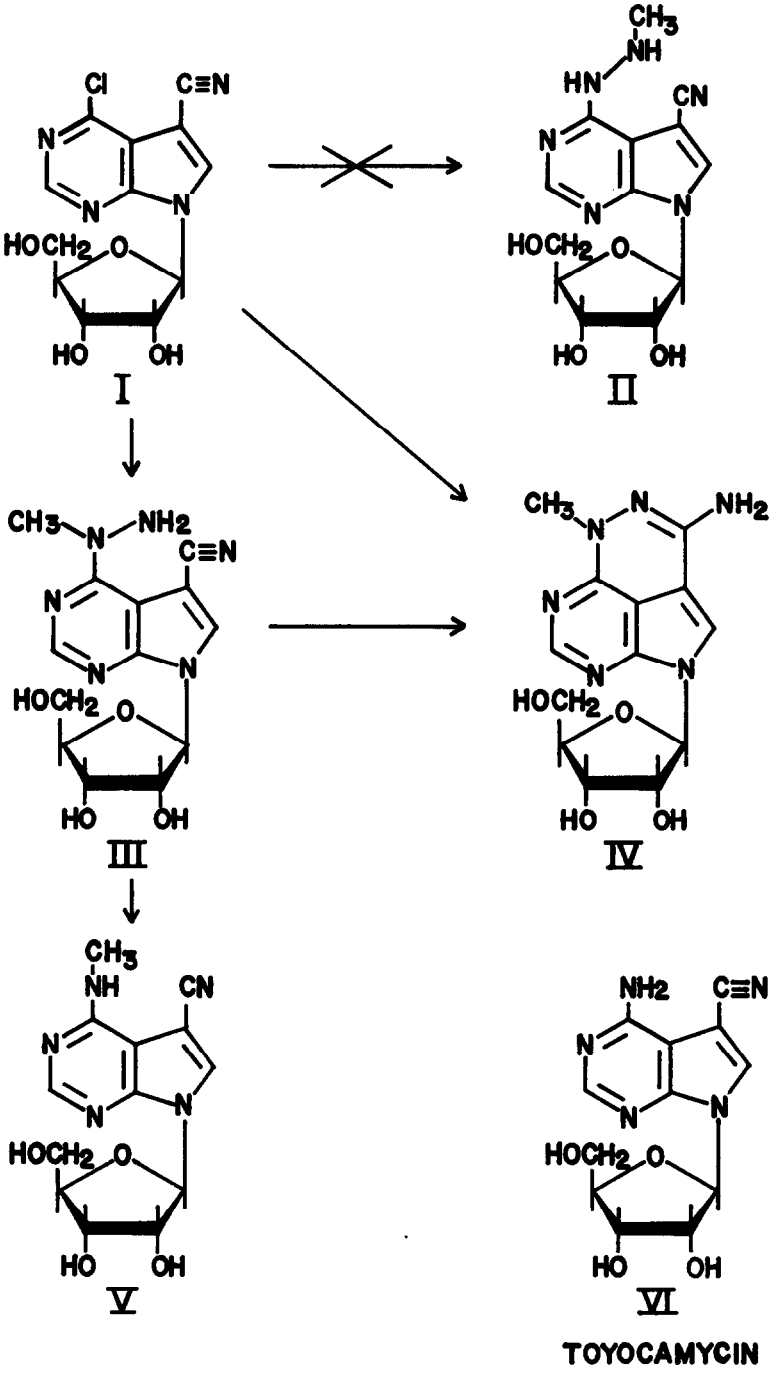
We would like to report the synthesis of a derivative of the new heterocyclic ring system pyrrolo[4,3,2-de]pyrimido[4,5-c]pyridazine. The reaction of 4-chloro-5-cyano-7-(β-D-ribofuranosyl)-pyrrolo[2,3-d]pyrimidine (I) (3) with methylhydrazine should have furnished 5-cyano-4-(N-1-methylhydrazino)-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine (III). However, the absence of a peak in the 2200 cm^{-1} region of the infrared spectrum excluded this possibility. This prompted us to initiate a more detailed study to determine the actual structure of the nucleoside and the pathway involved in the formation of the product.

A molar equivalent of methylhydrazine was added to a solution of I (1 mmole) in 10 ml of absolute ethanol and the mixture was heated at reflux temperature. A yellow solid began to separate from solution after four hours and the heating was continued for a total of 12 hours. The reaction mixture was then cooled at 5° for 6 hours and the solid which had separated from solution was collected by filtration to give 110 mg of product. The solid was air dried and thin layer chromatography (4) revealed a highly fluorescent blue spot with an Rf of 0.3 (I=0.75). The yellow powder had a melting point of 205° with dec. (I=78-80°) (3) and the infrared spectrum (KBr) showed the absence of any cyano in the 2200 cm^{-1} region (5). A pmr spectrum revealed the presence of an N-methyl group at δ3.59 as a 3 proton singlet, a pattern of peaks in the δ3.5-6 region which definitely established the yellow powder as nucleoside material, and a definite upfield chemical shift of the H-6 proton from δ8.38 to δ7.52 which is indicative of a modification of the cyano group in the five position of a pyrrolo[2,3-d]pyrimidine nucleoside (6). That nucleophilic attack by methylhydrazine had not occurred exclusively on the cyano group to furnish 4-chloro-7-(β-D-ribofuranosyl)pyrrolo-[2,3-d]pyrimidine-5-N-methylcarboxamidrazone was established by elemental analysis (7). There were two major possibilities (8) envisaged for the formation of this tricyclic nucleoside material; nucleophilic displacement of the 4-chloro group of I with subsequent ring annulation or initial attack at the 5-cyano group to furnish a N-methylcarboxamidrazone derivative which was then followed by nucleophilic displacement of the 4-chloro group. This prompted us to attempt an isolation and charac-

terization of the intermediate in order to establish which of these two possibilities was actually occurring in this instance.

A solution of I (1 mmole) in absolute ethanol was treated with a molar equivalent of methylhydrazine and stirred at room temperature to afford a white crystalline precipitate after 2-3 min. The crystalline product was collected by filtration, recrystallized from water and air dried to yield 166 mg of compound (mp 173-175°). The infrared spectrum showed a strong absorption band at 2210 cm^{-1} which indicated that the initial reaction had not occurred at the 5-cyano group. Therefore, the initial reaction must be a displacement of the 4-chloro group. The pmr spectrum exhibited an absorption peak at δ3.38 (3 proton singlet) which was assigned to a $N-CH_3$ moiety with no significant upfield chemical shift being observed for the C-6 proton. Elemental analysis for this nucleoside was also consistent with a simple nucleophilic displacement of the 4-chloro group by methylhydrazine. However, a displacement of the 4-chloro group by methylhydrazine could have occurred with the secondary amine, the primary amine, or both moieties. Thin layer chromatography established the presence of only one compound. If the nucleoside 5-cyano-4-(2-methylhydrazino)-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine (II) had been formed by the displacement of the 4-chloro group with the primary amine of methylhydrazine then the pmr spectrum should show the methyl group as a doublet. A peak in the pmr spectrum at δ3.38 (3 protons) for the methyl group was observed as a singlet and on this basis the structure II for the intermediate nucleoside could be tentatively eliminated. On the basis of the above data the intermediate was assigned the structure 5-cyano-4-(N-1-methylhydrazino)-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine (III). However, this structure assignment was rather tenuous and prompted a chemical structure-proof for corroboration.

A facile reduction of heterocyclic hydrazino groups to heterocyclic amines using Raney nickel as a catalyst has been reported (9). If the intermediate nucleoside were III then reduction would afford 4-methylamino-5-cyano-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine (V)(3). However, if the intermediate nucleoside were II, then the reduction product would be 4-amino-5-cyano-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine (VI, toyocamycin) (10). A 10 fold excess (w/w) of W-7 Raney nickel was added to a refluxing solution (EtOH) of the intermediate over a four hour period. After the final addition of Raney nickel the reaction mixture was heated at reflux temperature for 12 hrs. The hot reaction mixture was filtered through a celite bed (7 cm x 1 cm) and the filter cake was washed with hot water. The solvents were removed under reduced pressure and the white powdery residue was recrystallized from water to yield colorless crystals. An infrared spectrum of the product revealed the presence of a cyano group (2210 cm^{-1}) and a mixture melting point with an authentic


Cl
C≡N
HOCH2
HO
OH
I
CH3
NH
HN
CN
II
CH3
NH2
III
NH2
IV
V
NH2
C≡N
VI
TOYOCAMYCIN

sample (3) of 4-methylamino-5-cyano-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine (V) gave no depression. Therefore, the structure of the intermediate was 5-cyano-4-(N-1-methylhydrazino)-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine (III).

A solution of III in water was heated at reflux temperature for 16 hours and then allowed to stand at 5° for 12 hours to afford fine yellow crystals of IV. An infrared spectrum of the product obtained from III showed the absence of a cyano group at 2210 cm^{-1} and the spectrum was superimposable on a spectrum of IV obtained directly from I with no depression on a mixture melting point.[11]

Therefore, we propose that initial attack occurs by the methylamino moiety of methylhydrazine on the 4-chloro group of 4-chloro-5-cyano-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine to yield the intermediate 5-cyano-4-(N-1-methylhydrazino)-7-(β-D-ribofuranosyl)pyrrolo[2,3-d]pyrimidine (III). This intermediate (III) then cyclizes in situ to form the tricyclic nucleoside 6-amino-4-methyl-8-(β-D-ribofuranosyl)(4H, 8H)-pyrrolo[4,3,2-de]pyrimido[4,5-c]pyridazine (IV) as the first derivative of this new ring system.

References

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4. SilicAr 7GF plates 0.25 mm thick with n-BuOH/EtoAc/H_2O (4:1:2, v/v/v) being used as the solvent.
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7. The nucleoside IV analyzed for 3 moles of water which was corroborated by pmr spectroscopy. The addition of D_2O to the sample tube furnished a spectrum which established the presence of five exchangeable protons. Attempts to dehydrate the product by lyophilization and drying in vacuo above 100° were unsuccessful since on exposure to air the compound immediately hydrated.
8. The possibility of a direct displacement of the 4-chloro group by methylhydrazine and attack at the cyano group by another mole of methylhydrazine to furnish 4-methylhydrazino-7-(β-D-ribofuranosylpyrrolo[2,3-d]pyrimidine-5-N-methylcarboxamidrazone was eliminated on the basis of elemental analysis.
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11. U.V. spectral data for the compounds were identical: $\lambda_{max}^{pH\ 1}$ (320), 282; λ_{max}^{MeOH} (311), 292; $\lambda_{max}^{pH\ 11}$ (311), 289; parens = shoulder.