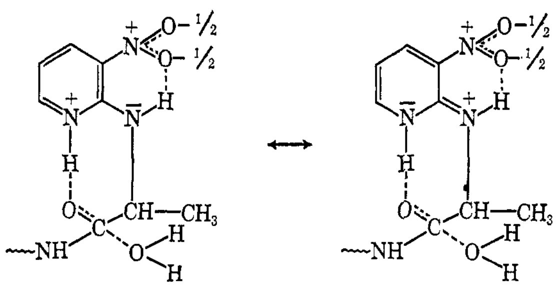
as compared with 5-nitro-2-pyridylalanylglycine. However, a clearer picture of the effect of steric hindrance is presented in Figure 7 in which are reported the ultraviolet spectra of II and III in 1% NaHCO$_3$; the absorption of *ortho* compound II (ϵ_{max} 7100) is less intense than that of the *para* isomer III (ϵ_{max} 12,700). It is well known that the most general consequence of nonplanarity on electronic spectra is a decrease in the absorption intensity of the so-called *K* bands, *i.e.*, absorption bands which owe their existence to the presence of fully extend conjugation. This decrease in absorption intensity may be accompanied, by a shift of the absorption maximum (λ_{max}) either to shorter or to longer wavelengths; in particular the changes in λ_{max} to longer wavelengths, as in the present case, may be expected for molecules in which excitation is attended by a decrease of double-bond character. Studies with *ortho*-substituted anilines and dimethylanilines have shown similar effects;[18,19] the absorption intensity for the 400-mμ band of *o*-nitroaniline is much less intense than for the *para* derivative and there is also some change in absorption maximum.

Therefore it is reasonable to assume that in the ground state there is no possibility of hydrogen bonding in a planar structure for II; on the other hand, geometrical considerations make it probable that in the transition state the *o*-nitro group and the secondary amino nitrogen are coplanar with the pyridine ring. We propose, as a tentative hypothesis, that in this situation hydrogen bond occurs with a contribution to the hybrid from the following canonical structures (eq 4). The transition state is thereby stabilized and the energy barrier is lowered. The energy of formation of the N—H···O bond is $\simeq$2000 cal,[20] so that this factor alone could entirely account for the differences observed in Arrhenius parameters. In the case of the acid hydrolysis of III, it is improbable that steric hindrance is significant and furthermore there is no possibility of intramolecular hydrogen bond; this suggests very strongly that the *o*-nitro group is largely responsible for the steric compression with the two other substrates (II and IV) and for the great enhancement of the rate constants for II. The remarkable stability of IV toward hydrolysis is noteworthy; we would like to suggest that the stability of this compound to acid-catalyzed hydrolysis is probably due to a lack of steric requirements for the formation of the cyclic transition state.


(4)

The foregoing results strongly support the view that the neighboring heterocyclic nitrogen plays an important role in the cleavage of peptide bonds under hydrolytic acid conditions.

Registry No.—β-Alanylglycine, 2672-88-0; 3-nitro-2-pyridyl-*dl*-alanylglycine, 7594-53-8; II, 7594-54-9; IV, 7594-55-0; 5-nitro-2-pyridyl-β-alanylglycine, 7594-56-1; III, 7594-57-2.

Acknowledgments.—The authors wish to thank Professor Ernesto Scoffone for reading the manuscript of this paper and for helpful criticism and Mr. E. De Menego for valuable technical assistance.

(18) G. Glotz, *Bull. Soc. Chim. France*, **1**, 1148 (1934).

(19) W. R. Remington, *J. Am. Chem. Soc.*, **67**, 1838 (1945).

(20) M. Davies, *Ann. Rept. Progr. Chem.*, **43**, 5 (1946).

Synthesis of Pyridazine Derivatives. XIV. Polyaza Heterocycles Derived from Pyrido[2,3-*d*]pyridazine[1]

B. STANOVNIK, A. KRBAVČIČ, AND M. TIŠLER

Department of Chemistry, University of Ljubljana, Ljubljana, Yugoslavia

Received October 28, 1966

The synthesis of polyaza heterocycles starting from pyrido[2,3-*d*]pyridazines is described. Treatment of I or VIII (R = H) with bromoacetaldehyde, bromoacetone, or phenacyl bromide afforded the isomeric imidazo[1,2-*b*]pyridopyridazines (II and IX, R = H, CH$_3$, C$_6$H$_5$). Similarly, the corresponding hydrazino-substituted products (I or VIII, R = NH$_2$) were employed for the preparation of the isomeric fused tetrazolo- or triazoloaza heterocycles (VI and VII and XIII and XIV). Hydrazinolysis of II (R = H) or IX gave the isomeric hydrazino-substituted compounds III (R$_1$ = R$_2$ = H) and X (R$_1$ = R$_2$ = H) from which in two different ways the isomeric tetracyclic imidazo[1,2-*b*]pyrido-*s*-triazolo[3,4-*f*]pyridazines (IV and XI) or their derivatives (V and XII) were obtained.

Continuing our investigations on condensed nitrogen-containing heterocyclic systems,[2-4] it was of interest to prepare some polyaza heterocycles with bridgehead nitrogen or nitrogens as the first step toward further investigations of their reactivity. In the present series, properly substituted pyrido[2,3-*d*]pyridazines were employed as starting materials and through a combination of this ring system with imidazole, triazole, or tetrazole rings, there were obtained several so far unknown parent isomeric polyaza heterocycles.

The chemistry of pyrido[2,3-*d*]pyridazines was developed only recently[5-8] and lately also both isomeric

(1) Paper L on Heterocycles.

(2) A. Pollak and M. Tišler, *Tetrahedron*, **22**, 2073 (1966).

(3) B. Stanovnik and M. Tišler, *Tetrahedron Letters*, 2403 (1966).

(4) B. Stanovnik and M. Tišler, *Tetrahedron*, **23**, 387 (1967).

(5) Y. Nitta, I. Matsuura, and F. Yoneda, *Chem. Pharm. Bull.* (Tokyo), **13**, 586 (1965).

(6) W. L. F. Armarego, *J. Chem. Soc.*, 6073 (1963).

(7) F. Bottari and S. Carboni, *Gazz. Chim. Ital.*, **86**, 990 (1956).

(8) Japanese Patent 22,266 (1965); *Chem. Abstr.*, **64**, 3567 (1966).

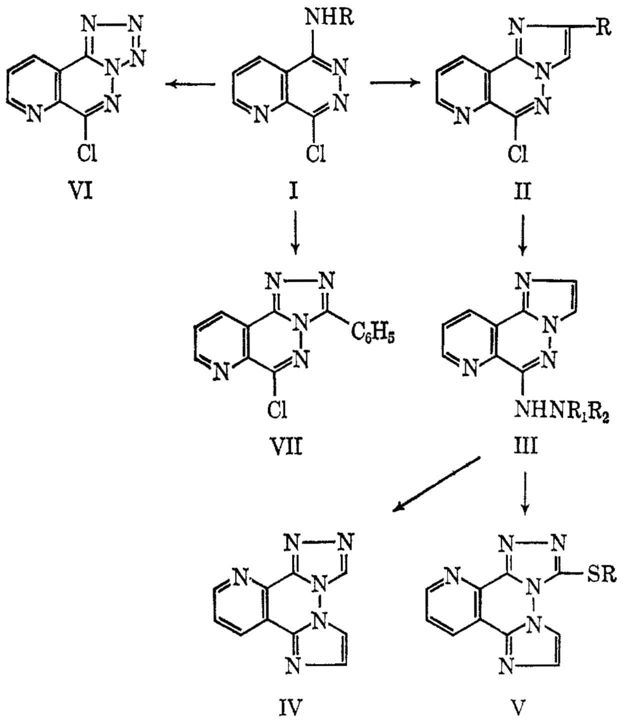
parent compounds, *e.g.*, pyrido[2,3-*d*]pyridazine and pyrido[3,4-*d*]pyridazine were synthesized.[9] The availability of isomeric 5-amino-8-chloropyrido[2,3-*d*]pyridazine (I, R = H) and 5-chloro-8-aminopyrido[2,3-*d*]pyridazine (VIII, R = H) or their corresponding hydrazino analogs (I, R = NH_2, or VIII, R = NH_2)[5] as starting material prompted their conversion into the corresponding isomeric polyaza heterocycles. Thus, I (R = H) afforded with bromoacetaldehyde 6-chloroimidazo[1,2-*b*]pyrido[3,2-*d*]pyridazine (II, R = H) and in a similar way the imidazole ring was formed with bromoacetone or phenacyl bromide giving the corresponding 2-methyl and 2-phenyl analogs (II, R = CH_3 or C_6H_5).

By nucleophilic displacement of the chlorine atom at position 6, which proceeds more readily with II than in the case of the isomeric VIII,[10] the 6-hydrazino compound (III, R_1 = R_2 = H) was obtained. From this compound the parent imidazo[1,2-*b*]pyrido[3,2-*d*]-*s*-triazolo[3,4-*f*]pyridazine (IV) or derivatives thereof could be prepared in two different ways. Compound III (R_1 = R_2 = H) when treated with diethoxymethyl acetate at room temperature afforded only the ethoxymethylene derivative (III, R_1R_2 = $CHOC_2H_5$), but parent tetracycle IV could be obtained upon heating both reactants. On the other hand the corresponding 3-mercapto derivative (V, R = H) was prepared by thermal cyclization of the intermediate-substituted thiosemicarbazide (III, R_1 = H; R_2 = $CSNHC_6H_5$). The last-mentioned reaction could be performed with reasonable yield although it was reported that a similar attempt with simple pyridazines was not successful.[11] Spectroscopic investigation of the high-melting 3-mercapto derivative has shown that the potentially tautomeric thioamide group as a part of the ring system, in reality, exists in the mercapto form. (See Scheme I.)

The intermediate 5-hydrazino-8-chloropyrido[2,3-*d*]pyridazine (I, R = NH_2) referred to above was used for the preparation of 6-chloropyrido[3,2-*d*]tetrazolo[5,1-*b*]pyridazine (VI). This compound, as indicated from the infrared spectral evidence, exists entirely in the tetrazole form (as does the isomeric XIII) and no azidoazomethine–tetrazole equilibrium, observed in some instances of polycyclic tetrazoles[3,4,12-14] could be detected. Furthermore, the 5-hydrazino derivative can be used also for the formation of a new fused triazole ring. For this purpose the benzylidene derivative (I, R = $N{=}CHC_6H_5$) was submitted to dehydrogenative cyclization by means of bromine to give VII.

The synthetic pathways as described above were applied with minor difference for the preparation of the isomeric polyaza heterocycles. Thus, in this series VIII (R = H) was employed for the synthesis of the isomeric imidazo[1,2-*b*]pyrido[2,3-*d*]pyridazines (IX and X) and the obtained 6-hydrazino derivative (X, R_1 = R_2 = H) served as an intermediate for the synthesis of the parent imidazo[1,2-*b*]pyrido[2,3-*d*]-*s*-triazolo[3,4-*f*]pyridazine (XI) and its 3-mercapto derivative (XII). Similarly, the isomeric 8-hydrazinopyrido[2,3-*d*]pyridazine derivative (VIII, R = NH_2) was starting material for the preparation of pyrido[2,3-*d*]tetrazolo[5,1-*b*]pyridazine (XIII) or *via* its benzylidene derivative (VIII, R = $N{=}CHC_6H_5$) for the formation of XIV. It is interesting to note that an orientation of the five-membered ring to be in the proximity of the pyridine ring nitrogen reflects a greater solubility of this series of compounds in water or in common organic solvents. All imidazo[1,2-*b*]pyrido[3,2-*d*]pyridazines or the corresponding [2,3-*d*] isomers formed crystalline monohydrohalide salts with HX acids just as do simple imidazo[1,2-*b*]pyridazines.[4] (See Scheme II.)

SCHEME I



Experimental Section[15]

6-Chloroimidazo[1,2-*b*]pyrido[3,2-*d*]pyridazine (II, R = H).—A mixture of bromoacetaldehyde dimethyl acetal (5 g), HBr (1.2 ml of 48%), and water (1.2 ml) was heated under reflux for 1 hr. The reaction mixture was treated with $NaHCO_3$ until evolution of CO_2 has subsided. After filtration the cake was washed with ethanol to obtain a neutralized solution of bromoacetaldehyde in ethanol (10 ml). To this a suspension of 2.04 g (0.01 mole) of 5-amino-8-chloropyrido[2,3-*d*]pyridazine (I, R = H) in 50 ml of ethanol was added. The mixture was heated under reflux and after about 30 min from the resulting solution the hydrobromide salt of the tricycle began to separate. Heating was continued for 1 hr and upon cooling 2.6 g (91%) of the hydrobromide salt was collected. It can be purified by sublimation *in vacuo* at 250° (0.1 mm) or crystallized from ethanol (melting point over 340°). *Anal.* Calcd for $C_9H_6BrClN_4$: C, 37.86; H, 2.12; N, 19.63. Found: C, 37.62; H, 2.55; N, 19.35.

The free base was obtained in the following manner. The hydrobromide salt was suspended in water (50 ml) and $NaHCO_3$ was added until neutral. The base was filtered and washed

---

[9] G. Queguiner and P. Pastour, *Compt. Rend.*, **262**, 1335 (1966).

[10] A similar difference in reactivity was observed also in the case of 5,8-dichloropyrido[2,3-*d*]pyridazine[5] where calculations of π-electron density and experimental results revealed a greater reactivity of position 5 than 8 toward nucleophilic reagents.

[11] N. K. Basu and F. L. Rose, *J. Chem. Soc.*, 5660 (1963).

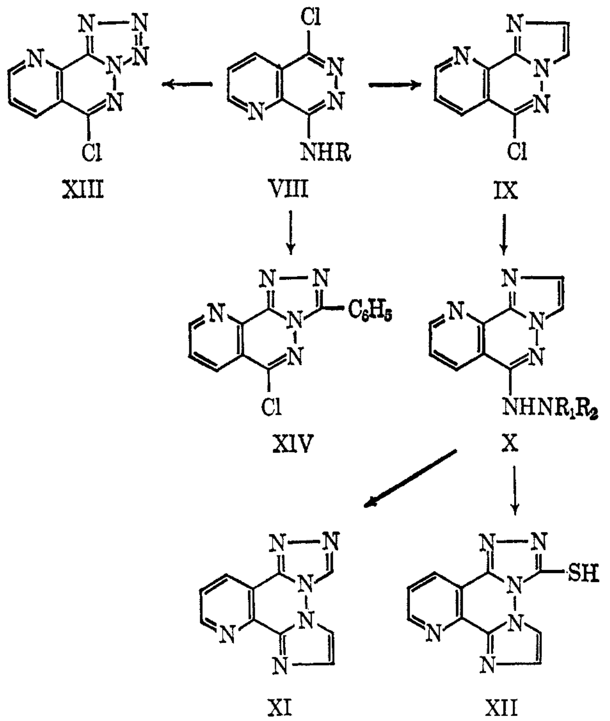
[12] C. Temple and J. A. Montgomery, *J. Am. Chem. Soc.*, **86**, 2946 (1964).

[13] C. Temple, R. L. McKee, and J. A. Montgomery, *J. Org. Chem.*, **27**, 1671 (1962).

[14] C. Temple, M. C. Thorpe, W. C. Coburn, and J. A. Montgomery, *ibid.*, **31**, 935 (1966).

[15] Melting points were taken on a Kofler micro hot stage and are corrected. Infrared spectra were determined on a Perkin-Elmer Model 21 spectrophotometer as mulls in Nujol or hexachlorobutadiene. Ultraviolet spectra were recorded on a Beckman Model DU spectrophotometer.

Scheme II



with water giving 1.6 g (78%) of the pale yellow tricyclic compound which was crystallized from water and had mp 145°; λ_{max}^{EtOH} 226 mμ (ε 15,350) and 264 mμ (ε 22,100). *Anal.* Calcd for $C_9H_5ClN_4$: C, 52.83; H, 2.46; N, 27.38. Found: C, 52.74; H, 2.95; N, 27.31.

The hydrochloride of II (R = H) was obtained from an ethanolic solution of the free base and dry HCl (melting point over 320°, from ethanol). *Anal.* Calcd for $C_9H_6Cl_2N_4$: N, 23.24. Found: N, 23.36.

Similarly the sulfate was prepared (melting point over 300° dec). *Anal.* Calcd for $C_9H_7ClN_4O_4S$: C, 35.72; H, 2.33; N, 18.51. Found: C, 36.05; H, 2.54; N, 18.83.

6-Chloro-2-methylimidazo[1,2-*b*]pyrido[3,2-*d*]pyridazine (II, R = CH_3).— A suspension of I (R = H, 0.6 g, 0.0033 mole) in ethanol (30 ml) was treated with bromoacetone (1 ml) and the mixture was heated under reflux. After about 30 min a clear solution resulted and after additional 15 min of heating under reflux the solution was filtered hot and the filtrate was evaporated to dryness. The crude product separated as the hydrobromide salt and was purified by crystallization from ethanol (yield 90%, mp 312–314°). *Anal.* Calcd for $C_{10}H_8BrClN_4$: C, 40.10; H, 2.69; N, 18.71. Found: C, 39.86; H, 2.94; N, 18.48.

The free base was obtained by dissolving the hydrobromide salt in water and adding $NaHCO_3$ until the solution was neutral. The free base precipitated from the solution and was filtered and crystallized from water yielding 0.35 g of the pure compound, mp 228°. *Anal.* Calcd for $C_{10}H_7ClN_4$: C, 54.93; H, 3.23; N, 25.63. Found: C, 55.06; H, 3.60; N, 25.91.

6-Chloro-2-phenylimidazo[1,2-*b*]pyrido[3,2-*d*]pyridazine (II, R = C_6H_5).—Phenacyl bromide (1 g) was added to a suspension of 0.666 g (0.004 mole) of I (R = H) in 50 ml of ethanol and the mixture was heated under reflux. After about 2 hr the formed product started to separate and after 1 additional hr of heating under reflux the reaction mixture was cooled and filtered. The crude hydrobromide salt (430 mg) was purified by sublimation at 240–250° (0.1 mm), mp over 320°. *Anal.* Calcd for $C_{15}N_{10}BrClN_4$: N, 15.49. Found: N, 15.32.

The free base was obtained by suspending the hydrobromide salt (250 mg) in 5 ml of water, 0.4 g of $NaHCO_3$ was added, and the mixture was heated just to boiling temperature of the solvent. Upon cooling the free base was collected and crystallized from water–DMF (2:1) to give the pure compound (145 mg, mp 195°). *Anal.* Calcd for $C_{15}H_9ClN_4$: C, 64.16; H, 3.23; N, 19.95. Found: C, 63.93; H, 3.61; N, 19.83.

6-Hydrazinoimidazo[1,2-*b*]pyrido[3,2-*d*]pyridazine (III, R_1 = R_2 = H).—A suspension of 204 mg (0.001 mole) of II (R = H) in 2 ml of 80% hydrazineh ydrate was heated under reflux for a few minutes. The reaction mixture was chilled with ice and the separated product was filtered, washed with iced water, and crystallized from water to give the pure compound (175 mg, 82%), mp 234–236°. *Anal.* Calcd for $C_9H_8N_6$: C, 53.99; H, 4.03; N, 41.98. Found: C, 53.68; H, 4.28; N, 41.47.

The dihydrobromide salt of III (R_1 = R_2 = H) had mp 320–322° (from ethanol). *Anal.* Calcd for $C_9H_{10}Br_2N_6$: N, 23.22. Found: N, 23.64.

The dihydrochloride salt of III (R_1 = R_2 = H) had mp 289–290° (from ethanol). *Anal.* Calcd for $C_9H_{10}Cl_2N_6$: C, 39.56; H, 3.69; N, 30.77. Found: C, 39.46; H, 3.98; N, 30.35.

The sulfate salt of III (R_1 = R_2 = H), crystallized from ethanol, showed a melting point over 340°. *Anal.* Calcd for $C_9H_{10}N_6O_4S$: C, 36.25; H, 3.38. Found: C, 36.34; H, 3.54.

The *p*-nitrobenzylidene derivative of III (R_1R_2 = $=CHC_6H_4NO_2$-*p*) was crystallized first from DMF and the product was then washed with hot ethanol, mp 310–311°. *Anal.* Calcd for $C_{16}H_{11}N_7O_2$: C, 57.65; H, 3.33; N, 29.42. Found: C, 57.43; H, 3.18; N, 29.26.

Isopropylidene derivative of III [R_1R_2 = $=C(CH_3)_2$] was recrystallized from acetone to give the pure compound, mp 250°. *Anal.* Calcd for $C_{12}H_{12}N_6$: N, 34.98. Found: N, 34.73.

6-(4′-Phenylthiosemicarbazido)imidazo[1,2-*b*]pyrido[3,2-*d*]pyridazine (III, R_1 = H; R_2 = $CSNHC_6H_5$).—To a solution of 0.7 g (0.0035 mole) of III (R_1 = R_2 = H) in 15 ml of ethanol 0.47 g (0.0035 mole) of phenyl isothiocyanate was added. The mixture was heated to boiling and set aside to cool to about 35°. The precipitate was collected and washed several times with hot ethanol. The crystalline product (0.5 g, 45%) showed mp 216–218°. *Anal.* Calcd for $C_{16}H_{13}N_7S$: C, 57.29; H, 3.90; N, 29.23. Found: C, 57.34; H, 3.84; N, 29.66.

6-Ethoxymethylenehydrazinoimidazo[1,2-*b*]pyrido[3,2-*d*]pyridazine (III, R_1R_2 = $CHOC_2H_5$). A.—Compound III (0.2 g, R_1 = R_2 = H) was suspended in 2 ml of diethoxymethyl acetate[16] and the reaction mixture was left aside at room temperature for 2 days. The filtered product was crystallized from ethanol–water (1:1) to give colorless crystals (0.14 g, 71%) of the pure compound, mp 180°. *Anal.* Calcd for $C_{12}H_{12}N_6O$: C, 56.24; H, 4.72; N, 32.80. Found: C, 56.28; H, 4.71; N, 32.57.

B.—The same product was obtained when heating 50 mg of III (R_1 = R_2 = H) with 0.5 ml of triethyl orthoformate under reflux until a complete dissolution occurred. The hot reaction mixture was filtered and left aside overnight. The separated product (31 mg) was crystallized from a small quantity of triethyl orthoformate. Melting point and mixture melting point with the compound prepared as under A were undepressed. If the compound was heated at a temperature above its melting point (at about 230°), from the melt crystals of the tetracycle began to separate and had mp 321–324°. Mixture melting point with compound IV was undepressed.

Imidazo[1,2-*b*]pyrido[3,2-*d*]-*s*-triazolo[3,4-*f*]pyridazine (IV).—A suspension of 50 mg of III (R_1 = R_2 = H) in 0.7 ml of diethoxymethyl acetate[16] was heated under reflux for 5 min and then kept in the dark at room temperature for 3 days. The separated product was collected (34 mg), crystallized from a small quantity of diethoxymethyl acetate, and washed with ethanol to give an analytical sample of IV, mp 322–324°. *Anal.* Calcd for $C_{10}H_6N_6$: C, 57.14; H, 2.88; N, 39.99. Found: C, 57.02; H, 3.06; N, 40.16.

3-Mercaptoimidazo[1,2-*b*]pyrido[3,2-*d*]-*s*-triazolo[3,4-*f*]pyridazine (V, R = H).—A suspension of 0.3 g of III (R_1 = H; R_2 = $CSNHC_6H_5$) in 2 ml of ethylene glycol was heated under reflux for 5 min. The separated product was filtered from the hot solution and suspended in hot ethanol. This suspension was heated under reflux for a few minutes and filtered, and the residue was washed with boiling ethanol. The obtained product (0.13 g, 58%) had a melting point over 330°; ν_{max} (Nujol or hexachlorobutadiene) at 2564 cm^{-1} (SH group); $\lambda_{max}^{0.1\ N\ NaOH}$ 244 mμ (ε 26,700) and 345 mμ (ε 7920). *Anal.* Calcd for $C_{10}H_6N_6S$: C, 49.59; H, 2.50; N, 34.70. Found: C, 49.84; H, 2.49; N, 34.59.

3-Methylmercaptoimidazo[1,2-*b*]pyrido[3,2-*d*]-*s*-triazolo[3,4-*f*]pyridazine (V, R = CH_3).—A mixture consisting of 74 mg of V (R = H) and 3 ml of 50% ethanol, containing equivalent

---

[16] H. W. Post and E. R. Erickson, *J. Org. Chem.*, **2**, 261 (1938).

quantities of NaOH and MeI was shaken in a sealed flask at room temperature during 1 hr. The crude product was crystallized from ethanol giving 71 mg of the pure compound: mp 255–257°; λ_{max}^{EtOH} 236 mμ (ϵ 18,420), 257 (16,000), and 308 (8800). *Anal.* Calcd for $C_{11}H_8N_6S$: C, 51.56; H, 3.15; N, 32.80. Found: C, 51.77; H, 3.01; N, 33.27.

6-Chloropyrido[3,2-*d*]tetrazolo[5,1-*b*]pyridazine (VI).—A solution of 0.1 g of the hydrazino compound I (R = NH_2) in 3 ml of 30% acetic acid was treated with an aqueous solution of $NaNO_2$ (0.17 g in 2 ml water) dropwise at room temperature. The mixture was then allowed to stand on ice overnight. The separated tetrazolo compound was purified by crystallization from DMF–ethanol (1:2) to give 95 mg of the pure compound: mp 218°; λ_{max}^{EtOH} 220 mμ (ϵ 32,700) and 300 mμ (ϵ 2880). In the infrared spectrum no azide bands could be detected (mull in hexachlorobutadiene). *Anal.* Calcd for $C_7H_3ClN_6$: C, 40.69; H, 1.46; N, 40.68. Found: C, 40.26; H, 1.92; N, 40.68.

6-Chloro-3-phenylpyrido[3,2-*d*]-*s*-triazolo[4,3-*b*]pyridazine (VII). **A.**—The benzylidene derivative of 8-chloro-5-hydrazinopyrido[2,3-*d*]pyridazine (I, R = N=CHC_6H_5) was prepared in the usual way. The crude product was purified by crystallization from ethanol to yield 59% of the brown crystals, mp 210°. *Anal.* Calcd for $C_{14}H_{10}ClN_5$: N, 24.69. Found: N, 24.86.

B.—To a stirred suspension of 1.36 g (0.005 mole) of the above benzylidene derivative (I, R = N=CHC_6H_5) and anhydrous NaOAc (0.7 g) in 30 ml of glacial AcOH a solution of 0.4 g of Br_2 in 10 ml of glacial AcOH was added dropwise at room temperature. After the addition was complete the mixture was heated under reflux for 30 min, the solvent was evaporated *in vacuo* to one-third of the original volume, and the residue was poured into water. The crude product which separated was repeatedly crystallized from DMF–water (1:1) to give 0.8 g (28%) of colorless crystals which had mp 223–224°. A sample for analysis was further sublimed at 200° (10 mm). *Anal.* Calcd for $C_{14}H_8ClN_5$: C, 59.85; H, 2.87; N, 24.95. Found: C, 59.66; H, 3.02; N, 24.99.

6-Chloroimidazo[1,2-*b*]pyrido[2,3-*d*]pyridazine (IX).—To a suspension of 2.04 g (0.01 mole) of 8-amino-5-chloropyrido-[2,3-*d*]pyridazine (VIII, R = H) in 40 ml of ethanol, 10 ml of a neutralized solution of bromoacetaldehyde, prepared from 5 g of bromoacetal, 1.2 ml of 48% HBr, and 1.2 ml of water as described in the case of II (R = H), was added and the mixture was heated under reflux until a clear solution resulted (after about 30 min). Heating was continued for 1.5 hr and the mixture was allowed to stand overnight at room temperature. After evaporating to dryness *in vacuo*, the residue was dissolved in water (30 ml), neutralized with $NaHCO_3$, and kept on ice for 12 hr. The separated product was collected, dried, and crystallized from water to give 1.4 g of the pure compound: mp 171–172°; λ_{max}^{EtOH} 218 mμ (ϵ 16,550) and 262 mμ (ϵ 21,650). *Anal.* Calcd for $C_9H_5ClN_4$: C, 52.83; H, 2.46; N, 27.38. Found: C, 52.62; H, 2.71; N, 27.45.

The compound formed a hydrobromide salt which was purified by sublimation at 250° (0.1 mm), mp over 320°. *Anal.* Calcd for $C_9H_6BrClN_4$: C, 37.86; H, 2.12; N, 19.63. Found: C, 37.70; H, 2.42; N, 19.81.

The sulfate salt of IX was purified by crystallization from ethanol, mp 249–250°. *Anal.* Calcd for $C_9H_7ClN_4O_4S$: N, 18.51. Found: N, 18.59.

6-Hydrazinoimidazo[1,2-*b*]pyrido[2,3-*d*]pyridazine (X, R_1 = R_2 = H).—A suspension of 228 mg (0.001 mole) of IX in 2 ml of 80% hydrazine hydrate was heated under reflux until a clear solution resulted. Upon cooling the separated hydrazino compound was collected, washed with iced water, and dried. The product was then crystallized from water to give 0.18 g of the pure compound, mp 270–271°. *Anal.* Calcd for $C_9H_8N_6$: C, 53.99; H, 4.03; N, 41.98. Found: C, 53.93; H, 4.05; N, 41.49. The compound formed a dihydrochloride salt which was crystallized from ethanol–ether, mp 278°. *Anal.* Calcd for $C_9H_{10}Cl_2N_6$: N, 30.77. Found: N, 30.80.

The dihydrobromide salt of X (R_1 = R_2 = H) had mp 302–303°. *Anal.* Calcd for $C_9H_{10}Br_2N_6$: N, 23.22. Found: N, 23.38.

The *p*-nitrobenzylidene derivative (X, R_1R_2 = =$CHC_6H_4NO_2$-*p*) was prepared in the usual way. The product was washed with hot ethanol, mp 308–309°. *Anal.* Calcd for $C_{16}H_{11}N_7O_2$: N, 29.42. Found: N, 29.66.

6-(4′-Phenylthiosemicarbazido)imidazo[1,2-*b*]pyrido[2,3-*d*]pyridazine (X, R_1 = H; R_2 = $CSNHC_6H_5$).—A solution of 106 mg (0.0005 mole) of X (R_1 = R_2 = H) in 3 ml of ethanol was treated with 68 mg of phenyl isothiocyanate. The mixture wa heated to boiling and set aside to cool slowly to room temperature. The separated product was filtered off and crystallized from ethanol to give 130 mg of the pure compound, mp 175°. If the compound is heated at a temperature of about 190°, from the melt, the corresponding tetracyclic compound XII crystallized and had a melting point over 340°. *Anal.* Calcd for $C_{16}H_{13}N_7S$: C, 57.29; H, 3.90; N, 29.23. Found: C, 57.40; H, 4.22; N, 29.05.

Imidazo[1,2-*b*]pyrido[2,3-*d*]-*s*-triazolo[3,4-*f*]pyridazine (XI).—Compound X (R_1 = R_2 = H; 50 mg) was suspended in 0.7 ml of diethoxymethyl acetate.[16] Some heat was evolved and the hydrazino compound went into solution. Soon after that a product separated, and the reaction mixture was heated under reflux for a few minutes. After standing in dark at room temperature for 3 days the obtained product (42 mg) was crystallized from 1 ml of diethoxymethyl acetate and finally washed with ethanol, mp 334–337°. *Anal.* Calcd for $C_{10}H_6N_6$: C, 57.14; H, 2.98; N, 39.99. Found: C, 57.01; H, 3.06; N, 40.16.

3-Mercaptoimidazo[1,2-*b*]pyrido[2,3-*d*]-*s*-triazolo[3,4-*f*]pyridazine (XII).—The corresponding thiosemicarbazide (X, R_1 = H; R_2 = $CSNHC_6H_5$; 0.1 g) was heated in 2 ml of ethylene glycol under reflux for 15 min. The reaction mixture was left aside at room temperature overnight and the separated product (54 mg) was purified by dissolving it in 5% NaOH and precipitating with concentrated hydrochloric acid. The product was washed with water until free of acid (melting point over 330°). *Anal.* Calcd for $C_{10}H_6N_6S$: C, 49.59; H, 2.50; N, 34.70. Found: C, 49.26; H, 2.82; N, 34.92.

6-Chloropyrido[2,3-*d*]tetrazolo[5,1-*b*]pyridazine (XIII).—This compound was prepared in an analogous manner as the isomeric tetracycle (VI). From 150 mg of the starting VIII (R = NH_2) there was obtained 85 mg of the pure compound, purified by crystallization from ethanol, mp 201°; λ_{max}^{EtOH} 220 mμ (ϵ 29,450). In the infrared spectrum (Nujol or hexachlorobutadiene) no azide bands could be detected. *Anal.* Calcd for $C_7H_3ClN_6$: C, 40.69; H, 1.46; N, 40.68. Found: C, 40.55; H, 1.81; N, 40.29.

6-Chloro-3-phenylpyrido[2,3-*d*]-*s*-triazolo[4,3-*b*]pyridazine (XIV). **A.**—A benzylidene derivative of 5-chloro-8-hydrazinopyrido[2,3-*d*]pyridazine (VIII, R = N = CHC_6H_5) was prepared in the usual way from VIII (R = NH_2). The crude product was purified by crystallization from ethanol and the brownish yellow needles had mp 217°. *Anal.* Calcd for $C_{14}H_{10}ClN_5$: N, 24.69. Found: N, 24.43.

B.—The tricyclic compound (XIV) was prepared from the above benzylidene derivative in a way analogous to isomeric VII. The crude product was extracted with hot dioxane and the residue was crystallized from glacial AcOH to give 0.6 g (21%) of yellow microcrystals, mp 265–267°. *Anal.* Calcd for $C_{14}H_8ClN_5$: C, 59.85; H, 2.87; N, 24.95. Found: C, 59.42; H, 3.22; N, 24.87.

Registry No.—II (R = H), 7589-57-3; II (HBr salt), 7589-58-4; II (HCl salt), 7589-59-5; II (R = CH_3), 7589-60-8; II (R = CH_3, HBr salt), 7589-61-9; II (R = C_6H_5), 7589-62-0; II (R = C_6H_5, HBr salt), 7589-63-1; III (R, = R_2 = H), 7589-64-2; III (R_1 = R_2 = H, 2HBr salt), 7589-65-3; III (R, = R_2 = H, 2HCl salt), 7589-66-4; III (R_1 = R_2 = H, H_2SO_4 salt), 7589-67-5; III (R_1R_2 = $CHC_6H_4NO_2$-*P*) 7589-68-6; III (R_1R_2 = =$C(CH_3)_2$), 7589-69-7; III (R_1 = H; R_2 = $CSNHC_6H_5$), 7589-70-0; III (R_1R_2 = =$CHOC_2H_5$), 7589-71-1; IV, 7589-72-2; V (R = H), 7589-73-3; V (R = CH_3), 7589-74-4; VI, 7589-75-5; VII, 7589-76-6; IX, 7599-33-9; IX (HBr salt), 7589-78-8; IX (H_2SO_4 salt), 7589-79-9; X (R_1 = R_2 = H), 7589-80-2; X (R_1 = R_2 = H, 2HCl salt), 7660-51-7; X (R_1 = R_2 = H, 2HBr salt), 7605-02-9; X (R_1R_2 = =CHC_6H_4-NO_2-*p*), 7589-81-3; X (R_1 = H, R_2 = $CSNHC_6H_5$), 7589-82-4; XII, 7589-83-5; XI, 7589-84-6; XIII, 7589-85-7; XIV, 7589-86-8; I (R = N = CHC_6H_5), 7589-77-7; VIII (R = N=CHC_6H_5), 7589-87-9.

Acknowledgment.—This investigation was supported in part by the Federal Research Fund and in part by Fund "Boris Kidrič." For valuable suggestions in connection with the nomenclature of new systems we wish to thank Mr. K. L. Loening, Director of Nomenclature, Chemical Abstracts Service.

Synthesis of Peptide Derivatives with Actinomycin D Sequence[1]

Johannes Meienhofer

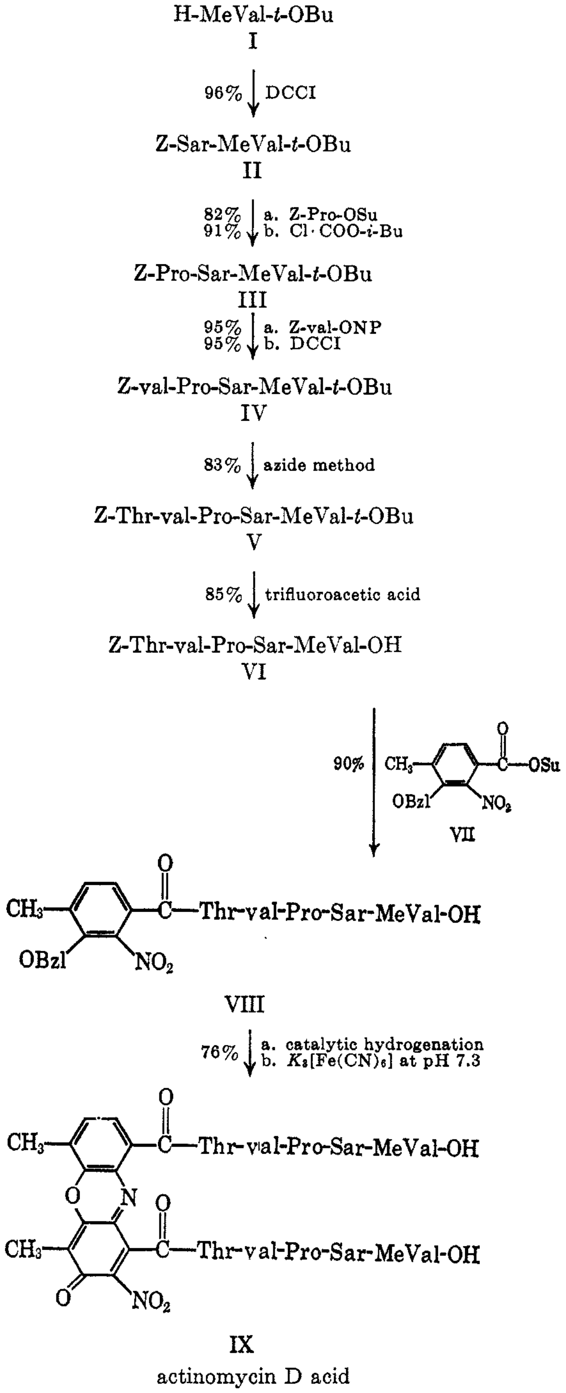
The Children's Cancer Research Foundation, The Children's Hospital Medical Center, and Harvard Medical School, Boston, Massachusetts 02115

Received September 29, 1966

2-Nitro-3-benzyloxy-4-methylbenzoyl-L-threonyl-D-valyl-L-prolylsarcosyl-L-N-methylvaline has been synthesized by stepwise elongation of the peptide chain starting from the C terminal, and has been converted into actinomycin D acid.

Scheme I

Schematic Outline of the Synthetic Pathway



Actinomycin D (C_1)[2] (Figure 1) has been synthesized by Brockmann and Lackner;[3-5] however few experimental details have been published. The protected tetrapeptide For-val-Pro-Sar-MeVal-OBZL[6] prepared starting from For-val-Pro-OH, was after deformylation with 10% HCl in benzylalcohol condensed with 2-nitro-3-benzyloxy-4-methylbenzoylthreonine.[5] The ensuing pentapeptide derivative was hydrogenated and subsequently oxidized to give actinomycin D acid which was lactonized in 28% yield with the use of a mixture of acetylimidazole and acetylchloride to give actinomycin D. This communication describes the synthesis of 2-nitro-3-benzyloxy-4-methylbenzoyl-L-threonyl-D-valyl-L-prolylsarcosyl-L-N-methylvaline (VIII) by a different approach.[7] Starting with C-terminal L-N-methylvaline *t*-butyl ester (I) the peptide chain was synthesized *via* the stepwise elongation procedure[8] (Scheme I). Compound VIII was obtained in an overall yield of 53% based on I. Owing to the extremely weak tendency of N-methylamino acid derivatives to crystallize, all peptide derivatives were obtained as oils or amorphous powders and all purifications had to be carried out by countercurrent distribution (ccd). The purity of a compound was judged by the degree of coincidence of the experimental distribution curve with the theoretical plot, and was verified by elemental analysis of the oils. The purity of benzyloxycarbonyl-L-threonyl-D-valyl-L-prolylsarcosyl-L-N-methylvaline (VI) was demonstrated in addition by a correct amino acid analysis. The optical purity of VI was corroborated by microbiological assays for threonine, valine, and proline.

Actinomycin acid (IX) was prepared by catalytic

---

[1] Supported in part by Public Health Service Research Grant C-6516 from the National Cancer Institute, National Institutes of Health.

[2] L. C. Vining and S. A. Waksman [*Science*, **120**, 389 (1954)] marked one of their actinomycin preparations with the letter D. This was found to be identical with the crystalline actinomycin C_1, isolated by H. Brockmann and H. Gröne, *Naturwiss.*, **41**, 65 (1954).

[3] H. Brockmann and H. Lackner, *ibid.*, **51**, 384 (1964).

[4] H. Brockmann and H. Lackner, *ibid.*, **51**, 435 (1964).

[5] H. Brockmann, H. Lackner, R. Mecke, G. Troemel, and H.-S. Petras, *Chem. Ber.*, **99**, 717 (1966).

[6] Abbreviations used for amino acid residues and protecting groups are standard and described in "tentative rules" of the IUPAC–IUB Commission on Biochemical Nomenclature in Biochemistry **5**, 2485 (1966). For = formyl. Amino acid abbreviations starting with a small letter denote the D configuration.

[7] During the preparation of this manuscript the synthesis of the pentapeptide intermediate V by the same route but using different methods was published by J. P. Marsh, Jr., and L. Goodman, *Can. J. Chem.*, **44**, 799 (1966).

[8] M. Bodanszky and V. du Vigneaud, *J. Am. Chem. Soc.*, **81**, 5688 (1959).