TABLE I

2-AMINO-4,6-SUBSTITUTED-5-ARYLAZOPYRIMIDINES

No.	Substituent 4	Substituent 6	Substituent 5	Mp, °C	Formula	Analysis	([I]/[S]$_{0.5}$)
1	NH_2	NH_2	C_6H_5	261[a]		[c]	0.31
2	NH_2	H	C_6H_5	275[a]	$C_{10}H_{10}N_6$	C, H, N	1.00
3	NH_2	CH_3	C_6H_5	216[a,b]	$C_{11}H_{12}N_6$	C, H, N[d]	0.20
4	NH_2	OH	C_6H_5	>320[c]	$C_{10}H_{10}N_6O$	*c*, *e*	0.063
5	NH_2	OH	2-$C_2H_5C_6H_5$	>360[b]	$C_{12}H_{14}N_6O$	C, H, N	0.010
6	OH	OH	C_6H_5	>360[b]	$C_{10}H_9N_5O_2$	C, H, N	>4.0
7	OH	OH	2-$C_2H_5C_6H_5$	>360[b]	$C_{12}H_{13}N_5O_2$	C, H, N	>4.0

[a] Recrystd from *i*-PrOH. [b] Recrystd from DMF. [c] Standard compd, ref 1. [d] Lit. [K. Tanaka, E. Omura, T. Sugawa, Y. Sanno, Y. Ando, K. Imai, and M. Kawashima, *Chem. Pharm. Bull.*, **7**, 1 (1959)] mp 224–226°. [e] Lit. mp >300° (F. R. Benson, L. W. Hartzel, and W. L. Savell, *J. Amer. Chem. Soc.*, **72**, 1816 (1950).

with those of Baker, *et al.*,[5] comparing 2,4,6-triamino- and 2,4-diamino-6-hydroxy-5-alkylpyrimidines on pigeon liver dihydrofolate reductase. Elimination of the 6-amino function (**2**) reduces its activity while its replacement by Me (**3**) increases activity but not to the same extent as does the 6-OH group (**4**). Of particular interest is the finding that introduction of the *o*-Et group into 2,4-diamino-6-hydroxy-5-phenylazopyrimidine produces a 6-fold increase in activity, thus paralleling the effects of the same substitution into 2,4,6-triamino-5-phenylazopyrimidine. The similarity of these substituent effects suggests quite strongly that the binding orientations of the 2,4,6-triamino- and 2,4-diamino-6 hydroxy-5-phenylazopyrimidines on dihydrofolate reductase are identical or very closely similar.

Experimental Section[6]

Synthetic Procedure.—The compounds listed in Table I were prepd by coupling diazotized $PhNH_2$ and *o*-ethylaniline with the appropriate pyrimidine according to the methods previously described.[1,2]

Enzyme Procedure.—The inhibitory activities of the compds were detd with dihydrofolate reductase from chicken liver[7] using the procedure previously described.[2]

(5) B. R. Baker, B.-T. Ho, and D. V. Santi, *J. Pharm. Sci.*, **54**, 1415 (1965).

(6) Melting points were recorded on a Thomas-Kofler hot stage and are corrected. Analyses were performed by Dr. A. E. Bernhardt and, where indicated only by symbols of the elements, were within 0.4% of the theoretical values.

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Antigenic Polypeptides. Synthesis and Immunochemical Studies of Poly(L-phenylalanyl-L-glutamyl-L-alanylglycyl)-glycine-*1*-^{14}C Ethyl Ester

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A recent investigation of the immunochemical properties of poly(Tyr-Glu-Ala-Gly)Gly-*1*-^{14}C Et ester,[1,2] has shown that the polypeptide is antigenic, eliciting antibodies in rabbits.[3] A desire to ascertain the role of the phenolic OH group of the tyrosyl residue of this antigen on its immunochemical properties prompted the synthesis of poly(Phe-Glu-Ala-Gly)Gly-*1*-^{14}C Et ester (**1**).

Chemistry. The polymerizing unit Phe-γ-*tert*-Bu-Glu-Ala-Gly pentachlorophenyl ester·HCl (**4**) and the necessary intermediates for its preparation were synthesized as detailed in the Experimental Section. The polymerization was performed at a reagent concn of 100 mmoles/l. in the presence of a preformed monomer since this has been shown to produce linear high molecular weight polypeptides.[1,2,4-8] Following this established procedure the insoluble polymer, poly(Phe-γ-*tert*-Bu-Glu-Ala-Gly)Gly-*1*-^{14}C Et ester was prepared; from which the protecting *tert*-Bu groups were removed by the use of 90% F_3CCO_2H to yield poly(Phe-Glu-Ala-Gly)Gly-*1*-^{14}C Et ester (**1**). After extensive dialysis, the polymer was purified and fractionated by passage through a calibrated column of Sephadex G-50.[9] By this means the mol wt of the polypeptide was found to be 1×10^4.

Immunochemistry.—Two rabbits were immunized with **1** using the same protocol as that previously described.[3] To aliquots of the sera obtained from each rabbit were added incremental amounts of the synthetic polypeptide **1**. A precipitin reaction was observed for one of the rabbits, as shown in Figure 1.

Conclusions.—It has been reported that tyrosine and phenylalanine are equally effective in enhancing antibody formation in random copolymers.[10] Previously, we have cast some doubt on this finding being applicable for linear sequential polypeptides.[11] However, from subsequent work it has now been found that replacement of the tyrosyl residue with the phenylalanyl moiety in the antigen, poly(Tyr-Glu-Ala-Gly)Gly-*1*-^{14}C Et ester, still affords an antigenic polypeptide. Thus it has been concluded that the phenolic OH group is not a necessity in order to confer antigenicity to a molecule.

† Ph.D. Thesis, Tufts University, Medford, Mass. 02155.

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(3) B. J. Johnson and E. G. Trask, *ibid.*, **59**, 724 (1970).

(4) B. J. Johnson, *J. Med. Chem.*, **14**, 488 (1971).

(5) B. J. Johnson and E. G. Trask, *ibid.*, **14**, 251 (1971).

(6) B. J. Johnson and D. S. Rea, *Can. J. Chem.*, **48**, 2509 (1970).

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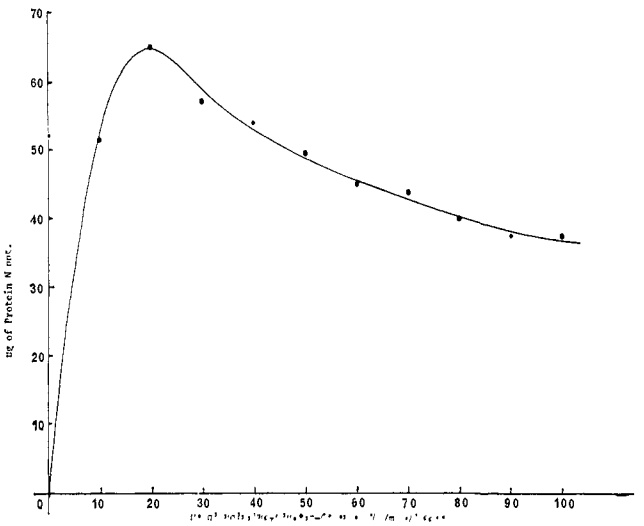

Figure 1.

Experimental Section

Melting points were taken with a Mel-Temp apparatus and are uncorrected.

***Z*-Phe-γ-*tert*-Bu-Glu-Ala-Gly-OMe (2).‡**—To a soln of 15.1 g (27.6 mmole) of Z-Phe pentachlorophenyl ester in 200 ml of CH_2Cl_2 was added 10.5 g (27.6 mmoles) of γ-*tert*-Bu-Glu-Ala-Gly Me ester·HCl and 3.0 g (30 mmoles) of Et_3N. The mixt was stirred overnight at room temp and concd, and the product was dissolved in EtOAc, washed with 10% citric acid soln and H_2O, and then dried (Na_2SO_4) and concd *in vacuo* to give the product as an oil. This material was chromatog on a column of Silicar CC-7 using $CHCl_3$–EtOAc (1:1) as eluent, to give the fully blocked tetrapeptide; crystn from EtOAc–hexane yielded 13.5 g (78.5%): mp 183–185°, $[\alpha]^{24}D$ −12.5° (*c* 2.69, DMF). *Anal.* ($C_{32}H_{42}N_4O_9$) C, H, N.

***Z*-Phe-γ-*tert*-Bu-Glu-Ala-Gly Pentachlorophenyl Ester (3).**—To a soln of 12.9 g (20.6 mmoles) of the fully blocked tetrapeptide **2** in 150 ml of MeOH was added 21 ml of 1 *N* NaOH and the soln was stirred for 90 min and then concd under reduced pressure. The residue was flooded with H_2O, acidified with 10% citric acid soln, and extd into EtOAc. The EtOAc soln was dried (Na_2SO_4) and concd under reduced pressure to give the tetrapeptide free acid as a solid; yield 12.6 g (100%). To this material in 90 ml of DMF was added 5.5 g (20.6 mmoles) of pentachlorophenol and 9.6 g (22.6 mmoles) of 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate. The mixt was stirred overnight at room temp. The reaction mixt was added to 400 ml of H_2O and the solid material was collected, washed with H_2O, and crystd from MeOH to yield 7.2 g (40.5%): mp 202–203°, $[\alpha]^{26}D$ −14.5° (*c* 5.59, DMF). *Anal.* ($C_{37}H_{39}Cl_5N_4O_9$) C, H, N.

Phe-γ-*tert*-Bu-Glu-Ala-Gly Pentachlorophenyl Ester·HCl (4).—A soln of 7.1 g (8.24 mmoles) of the tetrapeptide active ester **3** in 100 ml of MeOH was added to 0.8 g of 10% Pd/C. To this was added 8.25 ml of MeOH contg 0.30 g (8.24 mmoles) of dry HCl, and the mixt was hydrogenated for 2 hr. The reaction mixt was filtered, and the filtrate was concd to give a solid which was washed with Et_2O to yield 5.5 g (87.5%): mp 220°. $[\alpha]^{26}D$ 4.25° (*c* 4.7, DMF). *Anal.* ($C_{29}H_{34}Cl_6N_4O_7$) C, H, N.

Poly(Phe-Glu-Ala-Gly)Gly-*1*-^{14}C Et Ester (1).—To a soln of 1.0 mg of glycine-*1*-^{14}C Et ester·HCl (spec activity nCi/mmole) and 1.39 g (13.7 mmoles) of Et_3N in 5 ml of DMSO was added slowly a soln of 3.0 g (4.06 mmoles) of the polymerizing unit **4** in 34 ml of DMSO. The reaction mixt was shaken for 3 days at room temp and then centrifuged to yield the polymer which was washed with three 35-ml portions of H_2O and three 35-ml portions of Et_2O and dried to give the fully blocked polymer. This material was dissolved in 50 ml of 90% F_3CCO_2H and stirred for 50 min, and then concd under reduced pressure to yield the crude polypeptide **1**. This material was suspended in 40 ml of H_2O and dissolved by the addn of 4 *N* NaOH to pH 7.5. The soln was dialyzed against distd H_2O for 15 hr and then lyophilized to yield the Na salt of the polymer. This material was acidified to pH 2.5 with 6 *N* HCl in order to convert it to the free acid and dialyzed, with frequent changes of H_2O for 2 days. The free polypeptide **1** was obtained by lyophilization to yield 0.7 g (41%). *Anal.* ($C_{19}H_{24}N_4O_6 \cdot H_2O$) C, H, N.

Molecular Weight Determination.—A calibrated column of Sephadex G-50 (2.5 × 38.0 cm) was employed for the mol wt detn. Using 0.15 *M* NaCl as eluent, 4 mg of the Na salt of poly(Phe-Glu-Ala-Gly)Gly-*1*-^{14}C Et ester was passed through it and the polypeptide was eluted in a vol equiv to that corresponding to a mol wt of 1 × 10^4.

Immunochemical Results.—Two rabbits were treated at weekly intervals with 500 μg of poly(Phe-Glu-Ala-Gly)Gly-*1*-^{14}C Et ester **1**. The first 2 weeks they were injected intradermally using complete Freunds adjuvant as suspension medium and the 3rd week they were injected sc. The injection on the 4th week was done iv using buffered saline. Bleedings were conducted on the following week and the serum from one animal gave a precipitin reaction with polymer **1** as shown in Figure 1.

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‡ Z = benzyloxycarbonyl.

Synthesis and Reactions of Some Pyrrolidinediones

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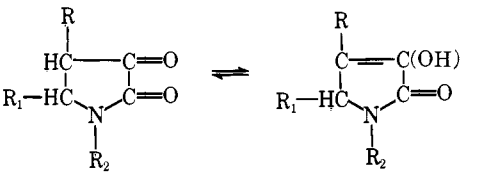
The present paper describes the synthesis and reactions of some new 2,3-, 2,4-, and 3,4-pyrrolidinediones. A number of 2,3-pyrrolidinediones are known,[1,2] and some of the new derivatives were found to possess an antimicrobial activity in preliminary screening tests. Their synthesis was carried out by the condensation of oxalacetic ester and phenylpyruvic acid and derivatives, as well as ethyl ethoxalylpropionate with different aldehydes and amines (Table I). The yields ranged from 30 to 60%.

SCHEME I



2,3-Pyrrolidinediones derived from phenylpyruvic acid failed to give a phenylhydrazone, an oxime, or an anil derivative and were recovered unchanged.

Attempts to prepare some 4-benzyl-2,3-pyrrolidinediones by the condensation of benzylpyruvic acid with different aldehydes and amines were unsuccessful. However, a 4-benzyl-2,3-pyrrolidinedione was prepared by condensing **2** with PhCHO in dil HCl soln to give the corresponding benzylidene derivative which was reduced with $NaBH_4$ to the required 4-benzyl-2,3-pyrrolidinedione.

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