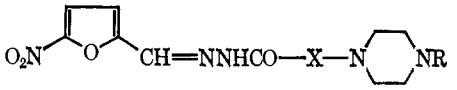
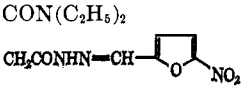
TABLE V

5-NITRO-2-FURALDEHYDE PIPERAZINOACYLHYDRAZONES



No.	X	R	Recrystn solvent	Mp, °C	Yield, %	Formula[f]
1	CH_2	H	70% EtOH	269–271	85[a]	$C_{11}H_{15}N_5O_4 \cdot 2HCl$[g]
2	CH_2CH_2	Me	EtOH	135–137	60	$C_{13}H_{19}N_5O_4 \cdot H_2O$
3	$CH(CH_3)$	Me	*i*-PrOH–H_2O	117–119	80	$C_{13}H_{19}N_5O_4 \cdot 2H_2O$[i]
			i-PrOH–H_2O	212–214		$C_{13}H_{19}N_5O_4 \cdot 2HCl \cdot H_2O$[h]
4	CH_2	Et	EtOAc	152	85	$C_{13}H_{19}N_5O_4$
			EtOAc	145–147		$C_{13}H_{19}N_5O_4 \cdot CH_3COOH$
5	CH_2	*n*-Pr		164–166	65	$C_{14}H_{21}N_5O_4$
			EtOH	98–101		$C_{14}H_{21}N_5O_4 \cdot 2CH_3COOH$
			90% EtOH	223–224		$C_{14}H_{21}N_5O_4 \cdot 2HCl \cdot 3H_2O$[h]
6	CH_2	*i*-Pr	Me_2CO	156–158	50	$C_{14}H_{21}N_5O_4$
			80% EtOH	237–239		$C_{14}H_{21}N_5O_4 \cdot 2HCl \cdot H_2O$[h]
7	CH_2	*n*-Bu	*i*-PrOH	158–159	90	$C_{15}H_{23}N_5O_4$
			95% EtOH	226–227		$C_{15}H_{23}N_5O_4 \cdot 2HCl$[h]
8	CH_2	n-$C_{12}H_{25}$	EtOAc	144–145	65[b]	$C_{23}H_{39}N_5O_4$
			MeOH	211–212		$C_{23}H_{39}N_5O_4 \cdot 2HCl$[h]
9	CH_2	Citronellyl	70% EtOH	139–141	50	$C_{21}H_{33}N_5O_4$
			EtOH	140 dec		$C_{21}H_{33}N_5O_4 \cdot 2HNO_3$[e]
10	CH_2	Geranyl	70% EtOH	110–112	65[c]	$C_{21}H_{31}N_5O_4$
			MeOH	138–140		$C_{21}H_{31}N_5O_4 \cdot 2HNO_3$
11	CH_2	CH_2CH_2OH	EtOAc	181–183	37	$C_{13}H_{19}N_5O_5$
12	CH_2	$CH_2C_6H_5$	EtOAc	200–201	50	$C_{18}H_{21}N_5O_4$
			95% EtOH	185–187		$C_{18}H_{21}N_5O_4 \cdot 2HCl \cdot 2H_2O$[h]
13	CH_2	$CH_2CH_2C_6H_5$	*i*-PrOH	166–168	80	$C_{19}H_{23}N_5O_4$
			60% EtOH	239–240		$C_{19}H_{23}N_5O_4 \cdot 2HCl$[j]
14	CH_2	C_6H_5	EtOH	200–201	80	$C_{17}H_{19}N_5O_4$
			70% EtOH	228–230		$C_{17}H_{19}N_5O_4 \cdot HCl$[h]
15	CH_2	C_6H_4-4-NO_2	Dioxane–H_2O	229–230	90	$C_{17}H_{18}N_6O_6$
16	CH_2	$COCH_3$	MeOH	193–195	60	$C_{13}H_{17}N_5O_5$
17	CH_2	$CON(C_2H_5)_2$	EtOAc	171–172	65	$C_{16}H_{24}N_6O_5$
18	CH_2			260–261	94[d]	$C_{18}H_{20}N_8O_8$

[a] The reaction mixt was treated with Et_2O and the oil was dissolved in MeOH and acidified with anhyd HCl to give the HCl salt. [b] The base was pptd with H_2O from the reaction mixt. [c] The pptd base must be washed quickly to avoid decompn. [d] The reaction was carried out with 0.02 mole of 5-nitro-2-furaldehyde and 7.5 ml of AcOH. The reaction mixt was treated with hot EtOAc and the ppt was washed with hot aq dioxane. [e] The HNO_3 salt was prepd by acidifying of the MeOH soln of the base with dil HNO_3. [f] See Table II, footnote *c*. [g] O anal. also. [h] See Table II, footnote *d*. [i] H: calcd, 6.71; found, 7.20. [j] Cl: calcd, 15.47; found 14.94.

azinoacetates (or -*N*-propionates) in 2 ml of EtOH was added 0.02 mole of hydrazine hydrate. The mixt was refluxed for 2–12 hr. The reaction time was detd by tlc on silica gel G (developed in MeOH–C_6H_6, 95:5, and sprayed with Dragendorff's reagent). The esters showed R_f values higher than the acylhydrazines. Then EtOH was evapd and the residue was distd or crystd (Table IV).

5-Nitro-2-furaldehyde *N*-Piperazinoacylhydrazones. General Procedure.—To a soln of 0.01 mole of *N*-piperazinoacylhydrazine in 4 ml of AcOH was added a soln of 0.01 mole of 5-nitro-2-furaldehyde in 1 ml of AcOH. The reaction was exothermic. The mixt was stirred for 1 hr below 40°, poured into Et_2O, and stirred until a solid, which was filtered and crystd, pptd. Some products pptd as acetates (**4**, **5**), other as bases (**12**–**17**). When an oil was obtd, it was dissolved in H_2O and the base was pptd by making the soln alkaline with Na_2CO_3 (**2**, **3**, **5**, **6**, **7**, **9**, **10**, 11). The HCl salts were obtd by conventional ways in EtOH. (Table V).

Pharmacological Methods. For acute toxicity NMRI albino mice (18–20 g) and for urinary excretion Wistar albino rats (200–250 g) were used. Acute toxicity, antimicrobial and antifungal activity *in vitro*, and urinary excretion were determined as previously described.[5,10]

(10) E. Massarani, D. Nardi, L. Degen, and M. J. Magistretti, *J. Med. Chem.*, **9**, 617 (1966).

Synthetic Antibacterials. 3.[1] Nitrofurylvinyl-1,8-naphthyridine Derivatives

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Our interest in nitrofuran derivatives,[2,3] bolstered by the finding that certain nitrofurylvinyl-1,8-naphthyridines[3] possess outstanding activity against *Pseudomonas aeruginosa*, led us to investigate the synthesis

(1) Paper 2: S. Nishigaki, K. Ogiwara, K. Senga, S. Fukazawa, K. Aida, Y. Machida, and F. Yoneda, *Chem. Pharm. Bull.*, **18**, 1385 (1970).

(2) S. Nishigaki, F. Yoneda, H. Matsumoto, and K. Morinaga, *J. Med. Chem.*, **12**, 39 (1969).

(3) S. Nishigaki, F. Yoneda, K. Ogiwara, T. Naito, R. Dohmori, S. Kadoya, Y. Tanaka, and I. Takamura, *Chem. Pharm. Bull.*, **17**, 1827 (1969).

TABLE I

In Vitro ANTIBACTERIAL ACTIVITY

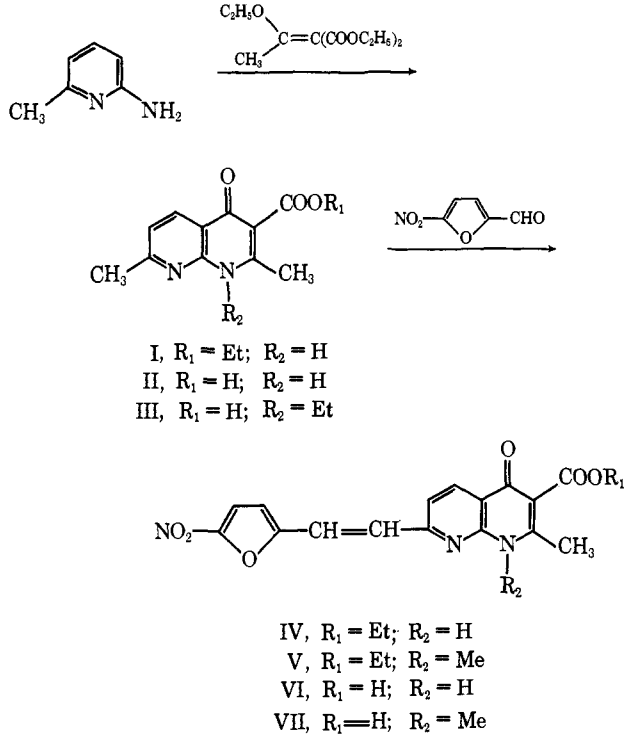
	Min inhib concn, μg/ml[a]									
No.	*Deplococcus pneumoniae* Dp-1	*Streptococcus hemolyticus* A 089	*Staphylococcus aureus* 209 P	*Bacillus subtilis* PCI 219	*Staph. enteritidis* 1891	*Staph. pullorum* Chuyu 114	*Escherichia coli* 0-55	*Klebsiella pneumoniae* 101	*Proteus vulgaris* HX-19	*Ps. aeruginosa* 347
I	>25	>25	>25	>25	>25	>25	>25	>25	>25	>25
II	>25	>25	>25	>25	>25	>25	>25	>25	>25	>25
III	>25	>25	>25	>25	>25	>25	>25	>25	>25	>25
IV	1.5	0.2	0.4	3	3	>12	>12	6	3	>12
V	0.78	0.39	1.56	3.13	3.13	12.5	6.25	6.25	3.13	12.5
VI	0.78	0.19	0.39	1.56	1.56	6.25	6.25	6.25	3.13	12.5
VII	0.78	0.39	1.56	6.25	6.25	12.5	6.25	12.5	3.13	12.5
Nalidixic acid	6.2	>25	>25	12	1.5	6	3	6	3	>25

[a] Min inhibitory concn is the lowest concn of compd that prevents visible growth after 48 hr of incubation at 37°.

and antibacterial activity of some of the corresponding derivatives of the homologs, 4-hydroxy-2-methyl-7-[2-(5-nitro-2-furyl)vinyl]-1,8-naphthyridine-3-carboxylic acid derivatives.

Reaction of 6-amino-2-picoline with diethyl ethoxyethylidenemalonate[4] gave ethyl 2,7-dimethyl-4-hydroxy-1,8-naphthyridine-3-carboxylate (I) in 22% yield. Hydrolysis of I by boiling in ethanolic NaOH gave the corresponding carboxylic acid (II), which was converted to 1-ethyl-2,7-dimethyl-4-oxo-3-carboxylic acid III EtI. Treatment of I with 5-nitrofurfural in a

SCHEME I



I, R_1 = Et; R_2 = H
II, R_1 = H; R_2 = H
III, R_1 = H; R_2 = Et

IV, R_1 = Et; R_2 = H
V, R_1 = Et; R_2 = Me
VI, R_1 = H; R_2 = H
VII, R_1 = H; R_2 = Me

mixture of AcOH and Ac_2O led to the formation of ethyl 4-hydroxy-2-methyl-7-[2-(5-nitro-2-furyl)vinyl]-1,8-naphthyridine-3-carboxylate (IV). The latter was methylated with MeI to yield ethyl 1,2-dimethyl-7-[2-(5-nitro-2-furyl)vinyl]-4-oxo-1,8-naphthyridine-3-carboxylate (V). Hydrolysis of IV and V by boiling in a mixture of AcOH and HCl gave the respective carboxylic acids (VI and VII).

The test results showed that compounds IV–VII possess antibacterial activity against both Gram-negative and Gram-positive organisms (see Table I).

(4) L. A. Williams, U. S. Patent 3,202,512, 1965.

Experimental Section[5]

Ethyl 2,7-Dimethyl-4-hydroxy-1,8-naphthyridine-3-carboxylate (I).—A mixt of 10.8 g (0.1 mole) of 6-amino-2-picoline and 23 g (0.1 mole) of diethyl ethoxyethylidinemalonate was heated in 400 ml of Dowtherm A for 1 hr at 240–250°. After cooling, the reaction mixt was dild with petr ether, and the ppts were collected by filtration, washed with C_6H_6, dried, and recrystd from H_2O to give 5.4 g (21.9%) of pale yellow needles: mp 223°; nmr (DMSO-d_6) 1.25 (t, 3, J = 7.5 Hz, CH_2CH_3), 2.34 (s, 3, CH_3-7), 2.54 (s, 3, CH_3-2), 4.15 (q, 2, J = 7.5 Hz, CH_2CH_3), 7.31 (d, 1, J = 8.0 Hz, H-6), 8.21 (d, 1, J = 8.0 Hz, H-5). *Anal.* ($C_{13}H_{14}N_2O_3$) C, H, N.

4-Hydroxy-2,7-dimethyl-1,8-naphthyridine-3-carboxylic Acid (II).—One gram (0.004 mole) of I was refluxed in 40 ml of 10% NaOH in 50% EtOH for 1 hr. After cooling, the reaction mixt was neutralized with 10% HCl. The ppts were collected by filtration, washed with H_2O, dried, and recrystd from EtOH to give 0.8 g (88.6%) of pale yellow powder, mp 288°. *Anal.* ($C_{11}H_{10}N_2O_3$) C, H, N.

1-Ethyl-2,7-dimethyl-4-oxo-1,8-naphthyridine-3-carboxylic Acid (III).—To a soln of 0.44 g (0.002 mole) of II in 10 ml of EtOH including 0.48 g of KOH and 3.5 ml of H_2O was added 1.87 g (0.012 mole) of EtI, and the mixt was refluxed for 17 hr. After cooling, the yellow powder which sepd was filtered and recrystd from EtOH to give 0.2 g (58.8%) of pale yellow powder, mp 201–203°. *Anal.* ($C_{13}H_{14}N_2O_3$) C, H, N.

Ethyl 4-Hydroxy-2-methyl-7-[2-(5-nitro-2-furyl)vinyl]-1,8-naphthyridine-3-carboxylate (IV).—A mixt of 0.5 g (0.002 mole) of I and 0.3 g (0.002 mole) of 5-nitrofurfural in 4 ml of AcOH–Ac_2O (1:1) was heated under reflux for 3 hr. After cooling, the product was removed by filtration, washed with AcOH, dried, and recrystd from EtOH to give 0.2 g (26.7%) of yellow powder, mp 221–223° dec. *Anal.* ($C_{18}H_{15}N_3O_6$) C, H, N.

Ethyl 1,2-Dimethyl-7-[2-(5-nitro-2-furyl)vinyl]-4-oxo-1,8-naphthyridine-3-carboxylate (V).—To a mixt of 0.22 g (0.0006 mole) of IV and 0.29 g of K_2CO_3 in 10 ml of DMF was added 0.26 g (0.0018 mole) of MeI and refluxed for 4 hr. The reaction mixt was dild with H_2O, and the ppts which separated were filtered. Recrystn from Me_2CO gave 0.13 g (57.0%) of brown powder, mp 210° dec. *Anal.* ($C_{19}H_{17}N_3O_6$) C, H, N.

4-Hydroxy-2-methyl-7-[2-(5-nitro-2-furyl)vinyl]-1,8-naphthyridine-3-carboxylate (VI).—To a mixt of 30 ml of AcOH and 2.7 ml of concd HCl was added 1 g (0.0027 mole) of IV and heated for 2 hr at 130°. After evapn of the reaction mixt, the residue was washed with H_2O, filtered, and dried. Recrystn from AcOH gave pale brown powder, mp >300°. *Anal.* ($C_{16}H_{11}N_3O_6$) C, H, N.

1,2-Dimethyl-7-[2-(5-nitro-2-furyl)vinyl]-4-oxo-1,8-naphthyridine-3-carboxylic Acid (VII).—To a mixt of 20 ml of AcOH and 1.8 ml of concd HCl was added 0.6 g (0.0016 mole) of V, and the mixt was heated for 2 hr at 130°. After cooling, the reaction

(5) Melting points are uncorrected. Nmr spectra were obtained with a JNM-C-60 spectrometer. Where analyses are indicated only by the symbols of the elements, analytical values are within 0.4% of the theoretical values.

mixt was dild with EtOH, and the ppts were collected by filtration and dried. Recrystn from Me_2CO gave brown powder, mp 220° dec. *Anal.* ($C_{17}N_{13}N_3O_6$) C, H, N.

Linear Polypeptides of a Known Primary Structure. Synthesis and Immunochemical Studies of Poly(*O*-methyl-L-tyrosyl-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-OEt,[1,2] has shown that the polypeptide is antigenic, eliciting antibodies in rabbits.[3] In order to ascertain the role of the phenolic OH on the antigenicity of the polypeptide we wish to report the synthesis and immunochemical properties of poly(*O*-Me-Tyr-Glu-Ala-Gly)Gly-*1*-^{14}C-OEt (1).

Chemistry.—The polymerizing unit *O*-Me-Tyr-γ-*tert*-Bu-Glu-Ala-Gly-OC_6Cl_5·HCl (**4**) and the necessary intermediates for its preparation were synthesized as detailed in the Experimental Section. The polymerization was performed at a reagent concentration of 100 mmoles/l. in the presence of a preformed monomer since this has been shown to produce linear high molecular weight polypeptides.[1,2,4-9] Following the established procedure the insol polymer, poly(*O*-Me-Tyr-γ-*tert*-Bu-Glu-Ala-Gly)Gly-*1*-^{14}C-OEt was prepared; from which the protecting *tert*-Bu groups were removed by the use of 90% F_3CCO_2H to yield poly(*O*-Me-Tyr-Glu-Ala-Gly)Gly-*1*-^{14}C-OEt (1). After extensive dialysis, the polymer was purified and fractionated by passage through calibrated columns of Sephadex G-100[10] and Corning CPG 10-240 glass granules. By this means the mol wt of the polypeptide was found to be at least 1×10^5.

Immunochemistry.—Two rabbits were immunized with poly(*O*-Me-Tyr-Glu-Ala-Gly)Gly-*1*-^{14}C-OEt (1), using the same protocol as that previously described.[3] To aliquots of the pooled sera were added incremental amounts of the polypeptide 1. A precipitin reaction was observed. The total amount of protein pptd was estimated by analysis for N (Kjeldahl). From these results the precipitin curve shown in Figure 1 was obtained. For comparative purposes the precipitin curve for poly(Tyr-Glu-Ala-Gly)Gly-*1*-^{14}C-OEt[3] is also shown.

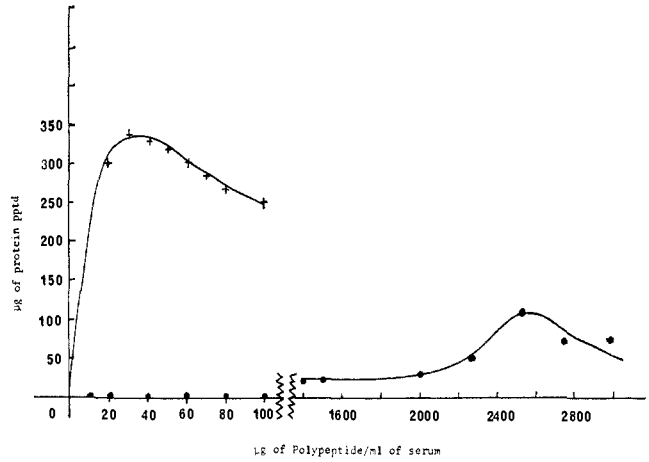

Figure 1.—Precipitin curve: +, poly(Tyr-Glu-Ala-Gly) *vs.* its antisera;[2] ●, poly(*O*-Me-Tyr-Glu-Ala-Gly) *vs.* its antisera.

Conclusions

Conversion of the phenolic OH groups of the tyrosyl residues in the parent antigen, poly(Tyr-Glu-Ala-Gly)-Gly-*1*-^{14}C Et, to their Me ethers still gave a molecule that was antigenic. Thus, it has been concluded that the presence of the phenolic OH groups is not a necessary prerequisite for antibody formation in this system. However, the equivalence points of the precipitin curves of the two polymers are not the same. Thus it would appear that the antigenic determinates of the polypeptide 1 are different from those of the parent antigen, poly(Tyr-Glu-Ala-Gly)Gly-*1*-^{14}C-OEt.

Experimental Section

Melting points were taken with a Mel-Temp apparatus and are uncorrected. Optical rotations were taken with a Carl Zeiss precision polarimeter.

Z-*O*-Me-Tyr-γ-*tert*-Bu-Glu-Ala-Gly Me† (**2**).—To a mixt of 17 g (58 mmoles) of Z-*O*-methyltyrosine in 400 ml of CH_2Cl_2 and 16 g (58 mmoles) of pentachlorophenol was added 13.2 g (64 mmoles) of *N,N*-dicyclohexylcarbodiimide. The soln was stirred at room temp for 12 hr, filtered, and then concd *in vacuo*. The residue was dissolved in EtOAc and washed with H_2O, $NaHCO_3$ soln, and H_2O, then dried (Na_2SO_4) and concd *in vacuo* to give the activated ester, 15 g (45%). To 10.5 g (18.2 mmoles) of this pentachlorophenyl ester in 250 ml of CH_2Cl_2 was added 6.5 g (17 mmoles) of γ-*tert*-Bu-Glu-Ala-Gly-OMe·HCl and 1.75 g (17 mmoles) of Et_3N. The reaction mixt was stirred overnight at room temp and then concd *in vacuo*. The residue was dissolved in EtOAc and washed with H_2O, 10% citric acid soln, and H_2O, then dried (Na_2SO_4), and concd *in vacuo*. The crude product was chromatographed on Silicar-CC-7, eluted with $CHCl_3$ and crystd from EtOAc–hexane to yield 7 g (63%): mp 152–154°; $[\alpha]^{31}D$ −24.4° (*c* 1.04, DMF). *Anal.* ($C_{33}H_{44}N_4O_{10}$) C, H, N.

Z-*O*-Me-Tyr-γ-*tert*-Bu-Glu-Ala-Gly-OC_6Cl_5·HCl (**3**).—A soln of 7 g (10.7 mmoles) of **2** in 250 ml of MeOH was treated with 10.7 ml of 1 *N* NaOH with stirring for 2 hr and then concd under reduced pressure. The residue was flooded with H_2O, acidified with 1 *N* HCl, and extd into EtOAc. The EtOAc soln was dried (Na_2SO_4) and concd under reduced pressure to give the tetrapeptide free acid as an oil. To this material in 200 ml of CH_2Cl_2 was added 2.85 g (10.7 mmoles) of pentachlorophenol and 5.0 g (11.8 mmoles) of 1-cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-*p*-toluenesulfonate. The mixt was shaken for 48 hr at room temp. The solvent was removed *in vacuo* and the residue was washed with H_2O and crystd from MeOH to yield 3.6 g (37%): mp 202–205°; $[\alpha]^{31}D$ −21.2° (*c* 1.09 DMF). *Anal.* ($C_{38}H_{41}Cl_5N_4O_{10}$) C, H, N.

***O*-Me-Tyr-γ-*tert*-Bu-Glu-Ala-Gly-OC_6Cl_5·HCl** (**4**).—To a fine suspension of 3.5 g (3.93 mmoles) of the tetrapeptide pentachlorophenyl ester **3** and 0.5 g of 10% Pd/C in 500 ml of MeOH was added 0.144 g (3.93 mmoles) of dry HCl in MeOH, and the suspension was hydrogenated for 2 hr. The reaction mixt was filtered and the filtrate was concd. The residue was crystd from MeOH–

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