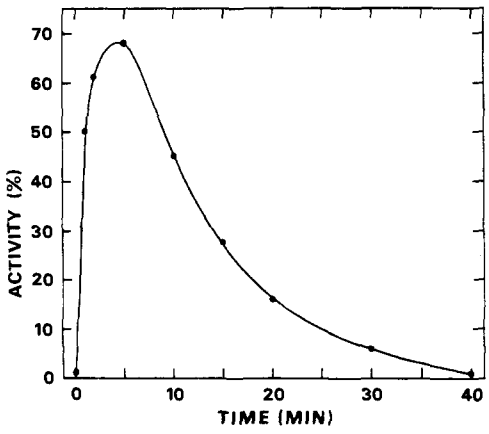

Figure 1. The effect of carboxypeptidase A on the biological activity of 2. The peptide (7×10^{-4} *M*) was incubated with the enzyme (0.34 g/l.) at pH 8.4, 0.1 *M* phosphate buffer, and aliquots were removed, boiled for 5 min, and diluted in Tyrode's solution for the assay on the isolated guinea pig ileum.

increase of the biological activity, reaching a maximum of about 70% the activity of angiotensin II. This was followed by inactivation, as would be expected, since carboxypeptidase removes the C-terminal phenylalanine of angiotensin II with complete inactivation.[9]

Experimental Section

The same procedure was followed in the synthesis of both compounds 1 and 2. Merrifield's solid-phase method,[10] with an automatic peptide synthesizer,[11] was performed as described by Stewart and Young,[12] with some modifications. The synthesis started with 0.4 mmol of *N*-α-Boc-L-leucine-resin. The coupling reaction was performed with a 2.5 *M* excess of Boc-amino acid and DCI. The side-chain protecting groups were the usual ones employed in solid-phase peptide synthesis,[12] with the exception of histidine where the imidazole was protected with the *p*-Ts group[13] recently introduced for this purpose in solid-phase synthesis.[14,15] Because this group was not stable to 4 *N* HCl in dioxane, usually employed in the deprotection step of the solid-phase method, a solution of 25% (v/v) trifluoroacetic acid in $CHCl_3$ was used in this step.

The coupling of the last residue (Boc-β-benzylaspartic acid) was followed by a deprotection step, after which the peptide-resin was washed with EtOH. After washing with CH_2Cl_2 to allow the peptide-resin to swell, the peptides were cleaved from the resin by stirring for 60 min at 0° in anhydrous HF containing 5% (v/v) anisole. The HF was then removed by distillation *in vacuo* and the residue was washed free of anisole with EtOAc. The peptides were then extracted with glacial AcOH and freeze-dried. Purification of the two peptides was accomplished by countercurrent distribution in the system *n*-BuOH:AcOH:H_2O (4:1:5), on a Post Model 3 automatic instrument. In both cases, 200 transfers were sufficient to obtain the pure peptide acetates that were concentrated in a rotary evaporator and freeze-dried.

Asp·Arg·Val·Tyr·Val·His·Pro·Phe·His·Leu·Leu (1) behaved on countercurrent distribution as a homogeneous ninhydrin- and Pauly-positive peptide with a distribution coefficient $K = 0.29$. Only one component was seen by tlc on silica gel with *n*-BuOH:AcOH:H_2O (4:1:1) ($R_f = 0.24$) and with *n*-BuOH:pyridine:AcOH:H_2O (30:20:6:24) ($R_f = 0.55$). High-voltage paper electrophoresis with pyridine acetate buffer, pH 4.9, and with 2 *M* AcOH, pH 2.4, also showed only one ninhydrin- and Pauly-positive spot with the expected mobility. The amino acid analysis, performed on a Beckman Model 120 C amino acid analyzer, gave the following composition: Asp, 1.03; Arg, 1.00; Val, 1.95; Tyr, 0.94, His, 2.10; Pro, 0.98; Phe, 0.97; Leu, 1.99. The yield of the pure peptide was 34% of the theoretical, based on the starting amount of Boc-leucyl-resin.

Asp·Arg·Val·Tyr·Ile·His·Pro·Phe·His·Leu·Leu (2) also was homogeneous on countercurrent distribution ($K = 0.31$), tlc with *n*-BuOH:AcOH:H_2O (4:1:1) ($R_f = 0.27$), and high-voltage paper electrophoresis at pH 4.9 (pyridine acetate) and pH 2.4 (2 *M* AcOH), where it showed the expected mobility. The amino acid composition was found to be: Asp, 1.05; Arg, 0.98; Val, 1.04; Tyr, 0.94; Ile, 0.93; His, 1.96; Pro, 1.01; Phe, 0.97; Leu, 2.07. The yield of the pure peptide was 25% of the theoretical, based on the starting amount of Boc-leucyl-resin.

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Antimicrobials. New Nitrofuran Derivatives

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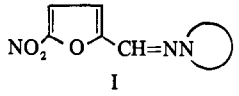
Since the introduction of nitrofurans in human therapeutics, as a result of the investigations of Dodd and Stillman,[1] a tremendous number of compounds of this type have been synthetized. Most of them are derivatives of 5-nitro-2-furfural with an amino compound and possess the general structure I. The linkage -CH=NN< seems to be as essential for the antibacterial activity as the 5-nitro-2-furan residue. Antibacterial compounds derived from the



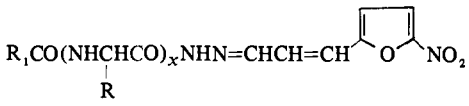
I

vinylogous 3-(5-nitro-2-furyl)acrolein have not been so extensively studied. We hoped that the introduction in the side chain of a vinyl linkage in addition to the imino bond will procure more active compounds. Furthermore, the amine to be condensed with the nitro aldehyde was chosen among the amino acids.

Modified amino acids have already been used as antagonists of natural ones in bacterial cells. For instance,

ethionine, the ethyl analog of methionine, inhibits the methionine supported bacterial growth, and 5,5,5-trifluoronorvaline is an inhibitor of multiplication of *Escherichia coli*.[2] To our knowledge, structures containing simultaneously a nitrofuran and a modified amino acid residue have not been studied.

Chemistry. Compounds relevant to this study are of the general structure II, where R_1 is a furan, thiophene, 5-

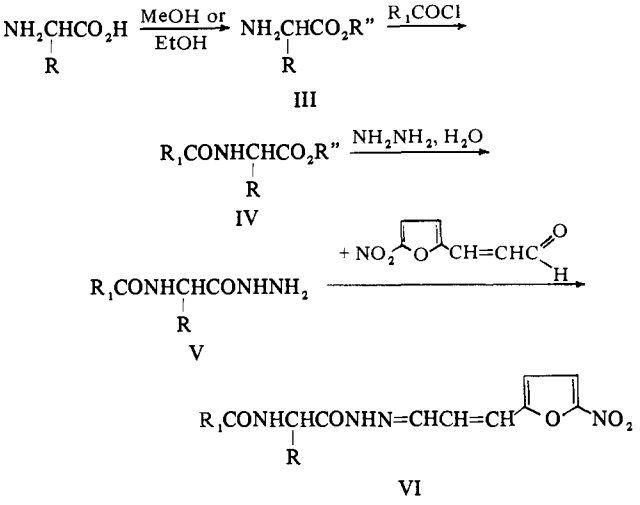


II

nitrothiophene, or a carbobenzoxy residue, and R varies with the nature of the starting racemic amino acid used (these were glycine, alanine, serine, leucine, norleucine, N^{ξ}-(2-furancarbonyl)lysine, phenylalanine, and tyrosine).

The amino acid residue is removed from two of the compounds (**5** and **10**, type II, $x = 0$) in order to determine the contribution of this part of the structure to the biological activity. The synthetic steps leading to II are represented in Scheme I.

Scheme I



The esterification of racemic amino acids was realized following literature methods, with methanol or ethanol in the presence of hydrochloric acid. The amino esters III, either as the free base in benzene solution containing equimolar amounts of triethylamine (method A) or in pyridine solution (method C), or as the hydrochloride salts in aqueous sodium bicarbonate (method B), were allowed to react with the acid chlorides to yield the esters IV. IV was treated with hydrazine hydrate in alcohol solution to afford the desired hydrazides V generally in high yields.

The condensation of hydrazides V with 3-(5-nitro-2-furyl)acrolein took place in methanol, tetrahydrofuran, or, in the case of highly insoluble products, in ethylene glycol as solvent. In most cases, the condensation product crystallized after a short period of heating. If not, the solvent was concentrated and water or ether was added. In many cases, the products were pure and did not need to be recrystallized. However, all analytical samples were purified. Final condensation products VI, hydrazides V, and esters IV are described in Tables I-III, respectively.

Compounds 5 and **10** (general structure II, $x = 0$) were synthetized from the corresponding hydrazides (**44** and **45**) by direct condensation with 3-(5-nitro-2-furyl)acrolein.

Bacteriology. Most of the compounds that were soluble in a mixture of acetone and water and did not precipitate by the addition of the broth culture were tested by a serial dilution method. A solution of the compound under investigation was diluted in a saline peptone broth and was used as the culture medium. In the case of strains growing only in an enriched medium, the broth contained also some drops of ascite serum. The broth was kept at 37° for 24 hr. The inoculum was prepared by diluting the broth 1000-fold to obtain a final concentration of 10^4-10^6 bacteria per milliliter. In the case of slowly growing strains, lower dilution was used to obtain the same concentrations of bacteria. The number of bacteria was determined by measuring the optical transmittance of the inoculum with Meunier's photometer.

Compounds **10** and **14** precipitated from the solvent mixture when broth was added were tested by the plaque method in gelose suspension. Following this method, the solution of the compounds under investigation, in a mixture of acetone and water, was thoroughly mixed with melted gelose, and the hot mixture was poured in petri dishes. In the case of strains needing an enriched broth, some drops of blood were added. The inoculated broth was kept at 37° for 24 hr and was diluted as previously described and laid in strips on the cold surface.

In every case the minimum inhibitory concentration (MIC) was determined after an incubation period of 18 hr at 37°. MIC is the concentration of the compound under study (μg/ml) at which no bacterial growth took place. The range of concentrations varied from 1 to 25 μg/ml. The strains were of clinical origin and were kept in gelose at +4° in our laboratory. The MIC values for these compounds are described in Tables IV-VI and are compared with those for nitrofurantoin and furazolidone, which were used as standards.

Results

All the tested compounds showed high antibacterial activity against the ten strains of bacteria investigated (Tables IV and V). Four compounds (**3-5** and **10**) were equal to or twice as active as nitrofurantoin against *E. coli*. In these compounds, R_1 is a thiophene nucleus, and the amino acid moiety is either glycine (**3** and **4**) or is excluded (**5** and **10**). The replacement of the thiophene group in **3** with a 5-nitrothiophene moiety (**4**) resulted in enhanced antibacterial activity. Similarly, **10** was superior to **5** against most of the strains investigated.

None of these compounds showed significant antibacterial activity *in vivo*, since they failed to protect mice infected with lethal doses of *Staphylococcus aureus* or *E. coli*. This could be attributed to their failure to be absorbed from the gastrointestinal tract after oral administration.

In view of their high antibacterial activity *in vitro*, these compounds were investigated as potential antibacterial agents against gastrointestinal infections. They were tested against a number of specific *E. coli* responsible for gastrointestinal infections in humans. In these studies furazolidine was used as a standard, since it has better activity than nitrofurantoin against these bacteria. **10** was the most active compound in this series and Table VI shows the results of the antibacterial studies with this compound. In preliminary studies in the dog, **10** did not produce vomiting, a side effect commonly encountered with nitrofuran derivatives, even at

dose levels of 350 mg/kg. Clinical investigations with this compound in intestinal infections are underway.

Conclusion. The amino acid residue in compounds of this series did not enhance the biological activity and, if removed, more active compounds were obtained. The high antibacterial activity of N^1-(5′-nitro-2′-thenoyl)-N^2-(5″-nitro-2″-furylacrylidene)hydrazine (**10**) was attributed to the molecule as a whole. The starting N^1-(5′-nitro-2′-thenoyl)hydrazide (**45**) had a good biological activity, but this was widely enhanced in **10** (Tables IV and V).

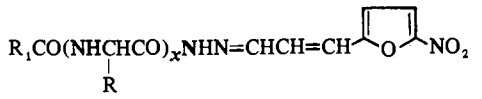
Experimental Section

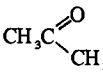
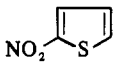
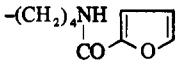
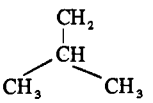
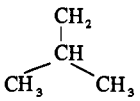
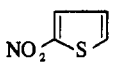
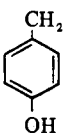
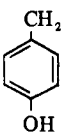
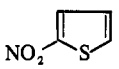
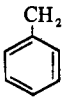
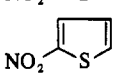
Amino Acid Ester III. Most of them are known products and were made following literature methods. Location quoted hereafter: glycine ethyl ester,[3] (±)-alanine ethyl ester,[4] (±)-serine ethyl ester hydrochloride,[5] (±)-leucine ethyl ester,[6] (±)-lysine ethyl ester,[7] (±)-phenylalanine ethyl ester,[8] and (±)-tyrosine methyl ester.[9] In our hands the hydrochloride had mp 186–188° (MeOH-$(Et)_2O$), and the base had mp 113–115° (*i*-PrOH).

(±)-Norleucine Ethyl Ester. HCl gas was introduced in a slurry of 66 g (0.5 mol) of (±)-norleucine in 375 ml of boiling EtOH during 6 hr. EtOH was evaporated and replaced by the same volume of fresh EtOH. The treatment was repeated. The crude hydrochloride was dissolved in $CHCl_3$, and a saturated solution of NH_3 in $CHCl_3$ was added. NH_4Cl was removed by filtration and the product distilled, bp 102–103° (21 mm). The analytical sample was redistilled: bp 98–99° (18 mm); yield 38.7 g (48.5%). *Anal.* ($C_8H_{17}NO_2$) C, H, N.

Preparation of IV. Method A. *N*-(2-Furancarbonyl)-(±)-alanine Ethyl Ester (31). To a solution of 23.4 g (0.2 mol) of (±)-alanine ethyl ester in 100 ml of benzene containing 20.3 g (0.3 mol) of

Table I

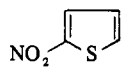
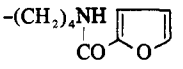
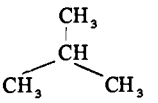
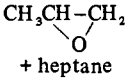
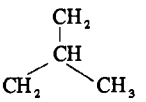
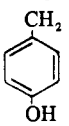
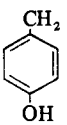
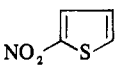
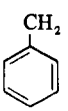
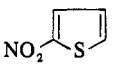
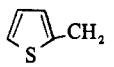
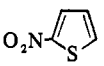


No.	R_1	R^b	x	Formula[c]	Yield,[a] %	Recrystn solvent	Mp, °C
1		CH_3	1	$C_{15}H_{14}N_4O_6$	80		197.5–198.5 dec
2	$C_6H_5CH_2O$-	H	1	$C_{17}H_{16}N_4O_4$	74	MeOH	159–160
3		H	1	$C_{14}H_{12}N_4O_5S$	99	DMF + $(Et)_2O$	233 dec
4		H	1	$C_{14}H_{11}N_5O_7S$	82	AcOEt	222–224 dec
5			0	$C_{12}H_9N_3O_4S$	72	EtOH + DMF	219–220 dec
6			1	$C_{23}H_{23}N_5O_8$	93	DMF + $(Et)_2O$	212–213
7	$C_6H_5CH_2O$-		1	$C_{21}H_{24}N_4O_6$	88	EtOH	145–146
8			1	$C_{18}H_{20}N_4O_5S$	67	MeOH	194.5–195
9	$C_6H_5CH_2O$-	CH_2OH	1	$C_{18}H_{18}N_4O_7$	67	EtOH	151–152
10			0	$C_{12}H_8N_4O_6S$	70	DMF + $(Et)_2O$	235–236
11		-$(CH_2)_3CH_3$	1	$C_{18}H_{20}N_4O_5S$	57	ACOEt	200–201 dec
12	$C_6H_5CH_2O$-		1	$C_{24}H_{22}N_4O_7$	56	MeOH	184–185
13			1	$C_{21}H_{18}N_4O_7$	57	DMF + H_2O	229–230
14			1	$C_{21}H_{17}N_5O_7S$	38	DMF + EtOH + H_2O (traces)	221.5–222
15		-CH_2OH	1	$C_{15}H_{13}N_5O_8S$	85	AcOEt + DMF	217 dec

[a] Yields quoted are of crude products. In many cases the crude products need not to be recrystallized. [b] All amino acid derivatives are racemic ones. [c] All compounds are analyzed for C, H, N, and S, if present. The values are in the limit of ±0.4% of the theoretical ones.

Table II

$R_1CO(NHCHCO)_xNHNH_2$ (R)

No.	R_1	R	x	Formula[d]	Yield,[c] %	Recrystn solvent	Mp, °C
16		CH_3	1	$C_8H_{11}N_3O_3$	100	*i*-PrOH	132-133
17[e]	$C_6H_5CH_2O$-	H	1	$C_{10}H_{13}N_3O_3$	84	*a*	113-115
18		H	1	$C_7H_9N_3O_2S$	97	H_2O	178-179
19		H	1	$C_7H_8N_4O_4S$	80	H_2O	190.5-191
20[f]			0	$C_5H_6N_2OS$	81	H_2O	136-138.5
21			1	$C_{16}H_{20}N_4O_5$	92	EtOH	161-162
22	$C_6H_5CH_2O$-		1	$C_{14}H_{21}N_3O_3$	44	 + heptane	105-108[b]
23			1	$C_{11}H_{17}N_3O_2S$	79	EtOH	146-147
24[g]	$C_6H_5CH_2O$-	$-CH_2OH$	1	$C_{11}H_{15}N_3O_4$	93	EtOH	152-153
25			1	$C_{11}H_{17}N_3O_2S$	90	*i*-PrOH	127-128
26[h]	$C_6H_5CH_2O$-		1	$C_{17}H_{19}N_3O_4$	62	EtOH	194.5-195
27			1	$C_{14}H_{15}N_3O_4$	70	EtOH	175.5-176
28			1	$C_{14}H_{14}N_4O_4S$	40	EtOH	214-216
29		$-CH_2OH$	1	$C_8H_{10}N_4O_5S$	95	H_2O	190 dec
44			0	$C_6H_8N_2SO$	97	AcOEt	96-98
45[i]			0	$C_5H_5N_3O_3S$	70	*a*	153-154

[a]Used as the crude product without further purification. [b]Not analytically pure. This product is used without further purification. [c]See footnote *a*, Table I. [d]Known compounds (17, 20, 24, 26; literature location cited) are not analyzed. The other ones give analytical results for C, H, N, and S, if present, within ±0.4% of theoretical values. [e]S. Simmonds, *Col. J. Biol. Chem.*, 188, 251 (1951); *Chem. Abstr.*, 45, 4305*g* (1951). [f]*Beilstein*, 4th ed, 18, 291 (1934). [g]G. Riley, J. H. Turnbull, and W. Wilson, *J. Chem. Soc.*, 1373 (1957). [h]C. R. Harrington and P. Rivers, *Biochem. J.*, 38, 417 (1944); *Chem. Abstr.*, 39, 3258*g* (1944). [i]G. Carrara, F. M. Chiancone, and V. d'Amato, *Gazz. Chim. Ital.*, 82, 652 (1952); *Chem. Abstr.*, 48, 6424*h* (1954).

$N(Et)_3$ was added slowly 26.1 g (0.2 mol) of 2-furoyl chloride. The temperature raised from 22 to 35° and $HN^+(Et)_3Cl^-$ precipitated. The filtered solution was washed with water and, after drying, the solvent was evaporated: yield 42.2 g (100%); mp 68-71°. After two recrystallizations, it had 72-73° [(*i*-Pr)$_2$O]. *Anal.* ($C_{10}H_{13}NO_4$) C, H, N.

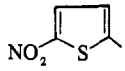
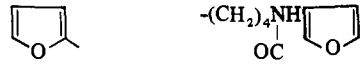
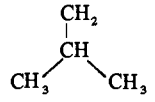
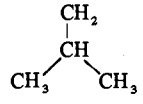
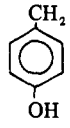
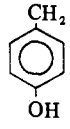
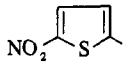
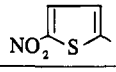
Method B. *N*-(2-Thiophenecarbonyl)glycyl Ethyl Ester (33). To a solution of 21.1 g (0.151 mol) of glycine ethyl ester hydrochloride in 150 ml of water containing 25.3 g (0.3 mol) of $NaHCO_3$ was added simultaneously in 15 min with vigorous stirring 24.1 g (0.165 mol) of 2-thenoyl chloride and 12.7 g (0.151 mol) of $NaHCO_3$ in 38 ml of water. The mixture was heated to 50° during 1 hr, and the precipitated solid was washed with water. After drying, it gave a yield of 25.5 g (79%), mp 88-90°. Recrystallization [(*i*-Pr)$_2$O-AcOEt] gave white crystals, mp 90-90.5°. *Anal.* ($C_9H_{11}NO_3S$) C, H, N, S.

Method C. 2-Thenoyl chloride (20.2 g, 0.137 mol) was added with stirring to a solution of 14.2 g (0.137 mol) of glycine ethyl ester in 70 ml of pyridine. Stirring was continued for 1 hr, and the mixture poured on ice-water. The solid was filtered and dried. The first crop gave 19.4 g of tan crystals, mp 86-88°. The mother liquors extracted with AcOEt furnished a second crop: 6 g; mp 86-88°; yield 25.4 g (86%).

Method B. N^{α},N^{ϵ}-Di(2-furancarbonyl)-(±)-lysine Ethyl Ester

Table III

$R_1CONHCHCO_2R''$ (R)

No.	R_1	R	R''	Formula[a]	Method	Yield, %	Recrystn solvent	Mp or bp (mm), °C
31		$-CH_3$	Et	$C_{10}H_{13}NO_4$	A	100	$(i\text{-Pr})_2O$	72-73
32[b]	$C_6H_5CH_2O-$	H	Et	$C_{12}H_{15}NO_4$	B	75		147-149 (0.7), 29-30
33		H	Et	$C_9H_{11}NO_3S$	C B	86 79	$(i\text{-Pr})_2O$ + AcOEt	90-90.5
34[c]		H	Et	$C_9H_{10}N_2O_5S$	C	82	*d*	119-120
35			Et	$C_{18}H_{22}N_2O_6$	B	63	*i*-PrOH + hexane	101-102
36	$C_6H_5CH_2O-$		Et	$C_{16}H_{23}NO_4$	B	63	*e*	138-139 (0.37-0.4)
37			Et	$C_{13}H_{19}NO_3S$	A B	67 89	Hexane	135-137 (0.3), 66-67
38[f]	$C_6H_5CH_2O-$	$-CH_2OH$	Et	$C_{13}H_{17}NO_5$	B		*g*	
39		$-(CH_2)_3CH_3$	Et	$C_{13}H_{10}NO_3S$	C	80	AcOEt	81-82
40[h]	$C_6H_5CH_2O-$		Me	$C_{18}H_{19}NO_5$	B	82	AcOEt + hexane	110-112[i]
41			Me	$C_{15}H_{15}NO_5$	B	85	AcOEt + hexane (traces)	123-125
42			Et	$C_{16}H_{16}N_2O_5S$	C	83	EtOH	150-151
43		$-CH_2OH$	Et	$C_{10}H_{12}N_2O_6S$	B	68	EtOH	126-127

[a]Known compounds (32, 34, 38; literature location cited) are not analyzed. The other ones give analytical results for C, H, N, and S, if present, within ±0.4% of theoretical values. [b]A. E. Barkdoll and W. F. Ross, *J. Amer. Chem. Soc.*, 66, 951 (1944) [*Chem. Abstr.*, 38, 3965*g* (1945)]; M. Mengelberg, *Chem. Ber.*, 89, 1185 (1956) [*Chem. Abstr.*, 51, 2639*g* (1957)]. [c]W. O. Foyl and J. J. Hefferen, *J. Amer. Pharm. Ass.*, 43, 602 (1954); *Chem. Abstr.*, 49, 12432*a* (1955). [d]Used without further purification. [e]Contains 20% impurities (vpc; used as crude product. [f]See footnote *g*, Table II. [g]The oily product obtained after evaporation of ether is used directly. In our hands it decomposes upon distillation. [h]M. Kinoshita and M. Klostermeyer, *Ann. Chim. (Ger.)*, 696, 226 (1966). [i]Literature gives mp 92-93°. Therefore the product is analyzed for C, H, and N and affords theoretical values in the limit of ±0.4%.

(35). To a solution of 24.7 g (0.1 mol) of (±)-lysine ethyl ester dihydrochloride in 200 ml of water containing 25.4 g (0.3 mol) of $NaHCO_3$ was added simultaneously with vigorous stirring 26 g (0.2 mol) of 2-furoyl chloride and 16.8 g (0.2 mol) of $NaHCO_3$ in 50 ml of water. The mixture was heated to 50° during 1 hr. The reaction mixture did not crystallize. After extraction with AcOEt and evaporation of the solvent, the remaining oil crystallized slowly. The crude waxy product was recrystallized in *i*-PrOH–hexane yielding (63%) white crystals, mp 101-102°. *Anal.* ($C_{18}H_{22}N_2O_6$), C, H, N.

Methods A and B. *N*-(2-Thiophenecarbonyl)-(±)-leucine Ethyl Ester 37. The crude product was distilled in method A, bp 135-137° (0.3 mm). The distillate crystallized mp 62-64°. In method B, the crude product crystallized, mp 66-67°, and could be purified by distillation. An analytical sample was recrystallized, mp 66-67° (hexane). *Anal.* ($C_{13}H_{19}NO_3S$) C, H, N, S.

Method C. *N*-(2-Thiophenecarbonyl)-(±)-norleucine ethyl ester 39: yield 80%; mp 80-81°. An analytical sample was recrystallized, mp 81-82°. *Anal.* ($C_{13}H_{19}NO_3S$) C, H, N, S.

Hydrazides V. Ester IV (0.1 mol) and 0.4 mol of hydrazine hydrate in 30 ml of *i*-PrOH or EtOH or in a mixture of tetrahydrofuran and MeOH (28) and 0.5 ml of AcOH were allowed to react at room temperature (17, 18, 22, 23), at +5° (19), or by heating to reflux 5 hr (16, 21) or 16 hr (44). In some cases the product crystallized on cooling. If not, the reaction mixture was evaporated under reduced pressure and the residue triturated with ether. Crude products were purified in the usual way.

Final Condensation Products VI. Hydrazide V (0.05 mol) was dissolved in 120-200 ml of MeOH or tetrahydrofuran (10). A solution of 0.05 mol of 3-(5-nitro-2-furyl)acrolein[10] in 68 ml of tetrahydrofuran was added. The mixture was boiled 1 hr, concentrated to the half of the original volume, and chilled, and the crystals

Table IV.[a] Serial Dilution Method

No.	*Staphylococcus aureus*	*Escherichia coli*	*Klebsiella pneumoniae*	*Proteus mirabilis*	*Pseudonomas aeruginosa*	*Moraxella glucidolytica*	*Listeria monocytogenes*	*Streptococcus pyogenes*	*Streptococcus faecalis*	*Neisseria sicca*
Nitrofurantoin	20	10	20	25	25	20	20	1	25	5
1	10	15	25	25	25	20	15	1	20	5
2	5	20	25	25	25	20	20	1	15	10
3	5	10	20	25	25	25	20	1	15	5
4	1	5	10	20	25	15	15	1	15	5
5	1	5	20	20	25	25	20	1	10	10
6	25	>25	>25	>25	>25	20	25	1	20	15
7	5	>25	>25	>25	>25	20	20	1	>25	15
8	5	20	25	25	25	20	10	1	20	5
9	10	25	>25	25	25	20	15	1	20	10
11	1	20	25	25	20	20	10	1	15	10
12	5	25	25	20	20	20	10	1	15	15
13	15	>25	>25	>25	20	20	20	1	15	10
15	15	15	25	25	25	20	15	1	20	10
45	25	20	25	25	25	20	25	1	20	5

[a]Activities are given in MIC values (minimum inhibitory concentration) = μg/ml.

Table V.[a] Plaque Method

No.	*Staphylococcus aureus*	*Escherichia coli*	*Klebsiella pneumoniae*	*Proteus mirabilis*	*Pseudonomas aeruginosa*	*Moraxella glucidolytica*	*Listeria monocytogenes*	*Streptococcus pyogenes*	*Streptococcus faecalis*	*Neisseria sicca*
10	10	5	5	15	25	10	10	1	10	5
14	1	>25	>25	>25	>25	20	15	1	1	10

Table VI.[a] Specific Intestinal *E. coli* Strains. Plaque Method

No.	$O_{127}B_8$	$O_{111}B_4$	$O_{126}B_{16}$	$O_{119}B_{14}$	$O_{111}B_4$	$O_{111}B_4$	$O_{128}B_{12}$	$O_{119}B_{14}$	$O_{126}B_{16}$	$O_{55}B_5$
Furazolidone	5	15	10	5	10	10	5	1	1	1
10	5	5	10	5	5	5	5	5	5	5

[a]See nootnote *a*, Table IV.

were filtrated with suction, washed with *i*-PrOH and $(Et)_2O$, dried, and purified by recrystallization.

In the case of highly insoluble products, 0.04 mol of hydrazide V was dissolved at 60° in 200–400 ml of ethylene glycol, 0.04 mol of 3-(5-nitro-2-furyl)acrolein in 68 ml of tetrahydrofuran was added, and the mixture was heated for 30 min at 60°. Crystallization began at this temperature (3). In some cases, the crude products were obtained after addition of $(Et)_2O$ (8) or H_2O (12) to the mother liquors.

Acknowledgment. The authors are indebted to Messrs. Claude Letourneur and Robert Sordet and Mrs. C. S. Sanchez for technical assistance.

References

(1) M. C. Dodd and W. B. Stillman, *J. Pharm. Exp. Ther.*, 82, 11 (1944).
(2) A. Burger, "Medicinal Chemistry," 2nd ed, Interscience, New York, N. Y., 1960, p 96.
(3) *Beilstein*, 4th ed, 4, 780 (1919).
(4) See ref 3, p 819.
(5) D. F. Elliott, *J. Chem. Soc.*, 589 (1949) [*Chem. Abstr.*, 43, 9033*b* (1949)]; F. W. Holly and C. A. Stammers, U. S. Patent 2,772,281 (Nov 27, 1956) [*Chem. Abstr.*, 51, 8145*b* (1957)].
(6) R. W. Chambers and F. H. Carpenter, *J. Amer. Chem. Soc.*, 77, 1522 (1955) [*Chem. Abstr.*, 50, 2426*c* (1956)]; G. Losse and H. Jeschkeit, *Chem. Ber.*, 90, 1275 (1957) [*Chem. Abstr.*, 54, 13008*d* (1960)].
(7) M. Szekerke, M. Kajtar, and V. Bruckner, *Chem. Abstr.*, 64, 17706*d* (1966).
(8) *Beilstein*, 4th ed, 14, 499 (1931).
(9) Organon, Dutch Patent Application 6,509,727 (Jan 30, 1967); Thibaut Gibbs Cie, French Patent 1,121,631 (Aug 22, 1956) [*Chem. Abstr.*, 54, 1345*c* (1960)].
(10) Institut Organischesko Sinteza an Latvisloi, British Patent 1,164, 588 (Feb 22, 1968).

Quaternary Aminooxy Congeners of Acetyl γ-Homocholine

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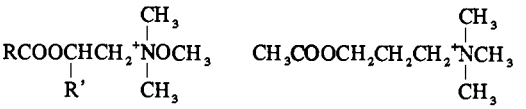
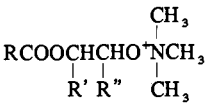
A prior communication[1] described preparation and biological effects of a series of N-methoxylated analogs **1–4** of certain cholinergic agents related to acetylcholine. These methoxyammonium compounds, with one exception, exhibited a low order of cholinergic activity. The unexpected and unexplained dramatically high muscarinic activity of **4**, the *N*-methoxy congener of Bethanechol, suggested further investigation of simple cholinergic agonist molecules possessing an N-O linkage.

Nicolaus, *et al.*,[2] described preparation of **6**, the "reverse" analog of **1**, and a bioisostere of acetyl γ-homocholine (**5**). Compound **6** was described[2] as having "similar activity" to acetylcholine, but quantitative data were not reported. The Nicolaus group also prepared 2-dimethylaminooxyethyl carbamate, the tertiary amine analog of **9**, but this compound was apparently never quaternized for biological testing. Schiatti and Maffii[3] stated that the introduction of an oxygen into the chain of acetylcholine so as to produce **6** has a similar effect as the addition of one more CH_2 group into this position (forming acetyl γ-homocholine) insofar as the affinity of the molecule for acetylcholinesterase is concerned. Both acetyl γ-homocholine and **6** were reported to



1, R = Me; R' = H
2, R = R' = Me
3, R = NH_2; R' = H
4, R = NH_2; R' = Me

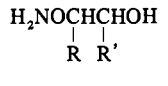
5

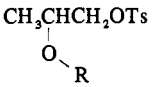


6, R = Me; R' = R" = H
7, R = R' = Me; R" = H
8, R = R" = Me; R' = H
9, R = NH_2; R' = R" = H
10, R = NH_2; R' = Me; R" = H
11, R = NH_2; R' = H; R" = Me

be poorer substrates for the enzyme than was acetylcholine. In the present work, the goal was to prepare the aminooxy systems **6–11** and to evaluate their muscarinic effects.

2-Aminooxyethanol **12** was prepared by a literature method.[2] Alternate methods for **12** were investigated as models for synthesis of the α- and β-methylcholine congeners **13** and **14**. Attempted O-alkylation of *N*-hydroxy-





12, R = R' = H
13, R = H; R' = Me
14, R = Me; R' = H

15a, R = benzyl
b, R = 2-tetrahydropyranyl

urethane with 2-bromoethyl acetate was unsuccessful; Nicolaus, *et al.*,[2] obtained a 10% yield of product in a similar reaction of *N*-hydroxyurethane with 2-chloroethanol.

The monotosylate ester of ethylene glycol was treated with the sodium salt of *N*-hydroxyurethane, according to a procedure of Winternitz and Lachazette;[4] none of the desired O-alkylated product was obtained. When the free OH group of ethylene glycol monotosylate was masked as its benzyl ether, the anion of *N*-hydroxyurethane displaced the tosyl group to form the O-alkylated urethane **16** in adequate yield. However, application of the procedure to two monoetherified tosylate esters (**15a,b**) of propane-1,2-diol either gave very low yields or failed completely. Attempted O-alkylation of *N*-hydroxyurethane with a chloroacetone ketal failed. Compound **13** was obtained in good yield by reaction of acetone oxime with propylene oxide, according to a procedure of Bachman and Hokama,[5] followed by hydrolysis of the O-substituted acetone oxime (Scheme I).

The α-methylcholine analog **14** was prepared by alkylation of *N*-hydroxyurethane with ethyl 2-bromopropionate, followed by $LiAlH_4$ reduction (Scheme II).

The primary alcohols **12–14** were converted directly to their quaternary ammomium salts with methyl iodide-sodium bicarbonate, and these were esterified at room temperature with acetic anhydride. At higher temperatures, acetic anhydride induced cleavage reactions in the quaternary ammonium systems. The carbamate esters were conveniently prepared by reaction with sodium cyanate and trifluoroacetic acid in methylene chloride. All of the carbamates were extremely hygroscopic and were difficult to purify. Spectral (ir, nmr) data on all compounds were consistent with the proposed structures. No attempt was made to resolve those compounds possessing an asymmetric center.

Pharmacology. Compounds **6–11** and the reference com-