Amidines and Guanidines Related to Congocidin. Part IV.[1] Thiophen, Pyridine, and Benzene Analogues

By **D. H. Jones** and **K. R. H. Wooldridge,*** The Research Laboratories, May and Baker Ltd., Dagenham, Essex

Four analogues (Ib)–(Ie) of congocidin (Ia) have been prepared in which the N-methylpyrrole ring has been replaced by other aromatic rings. These compounds, and some related mono- and di-amidines, showed no useful biological activity.

THE synthesis of congocidin [2] (Ia) and some related compounds [1,3,4] prompted us to prepare some analogues in which the *N*-methylpyrrolediyl groups of congocidin

$H_2N{\cdot}(NH{:})C{\cdot}NH{\cdot}CH_2{\cdot}CO{\cdot}NH{\cdot}X{\cdot}CO{\cdot}NH{\cdot}Y{\cdot}CO{\cdot}NH{\cdot}[CH_2]_2{\cdot}C(:NH){\cdot}NH_2$

(I)

	X	Y
a;	*N*-Methylpyrrole-2,4-diyl	*N*-Methylpyrrole-2,4-diyl
b;	*p*-Phenylene	Pyridine-3,5-diyl
c;	*p*-Phenylene	5-Methylthiophen-2,4-diyl
d;	*p*-Phenylene	Thiophen-2,5-diyl
e;	Thiophen-2,5-diyl	*p*-Phenylene

were replaced by pyridinediyl, thiophendiyl, and phenylene groups (Ib)—(Ie). We hoped in this way to preserve the antiviral and trypanocidal activity but to decrease the very high toxicity of congocidin.

To illustrate the general route, which is essentially similar to that employed by Julia *et al.*,[2] the synthesis of one of the congocidin analogues is indicated in the Scheme.

Catalyst poisoning prevented the catalytic reduction of (II) but the nitro-group was reduced in good yield by ferrous sulphate and barium hydroxide. In contrast (V), in which the nitro-group is remote from the thiophen sulphur atom, readily underwent catalytic hydrogenation to the corresponding amino-compound. The final condensation was effected by treating the amino-

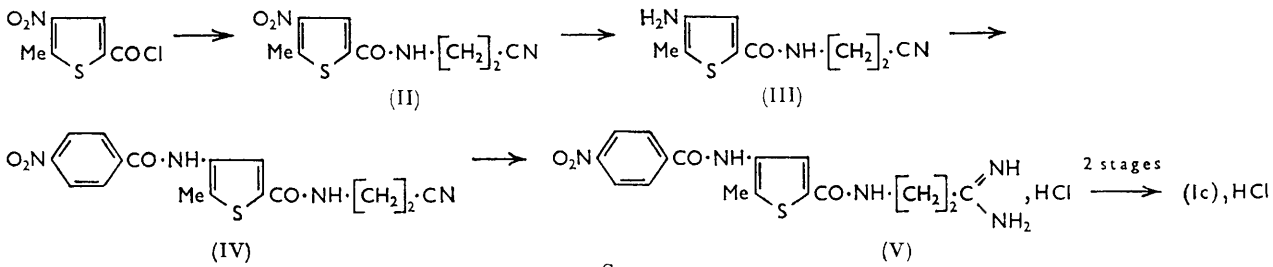
[1] Part III, M. Julia and R. Gombert, *Bull. Soc. chim. France*, 1967, in the press.
[2] M. Julia and N. Préau-Joseph, *Compt. rend.*, 1963, **257**, 1115.
[3] M. Julia and N. Préau-Joseph, *Bull. Soc. chim. France*, 1967, in the press.
[4] M. Julia and R. Gombert, *Bull. Soc. chim. France*, 1967, in the press.

compound and guadinoacetic acid hydrochloride with dicyclohexylcarbodi-imide in dimethylformamide.

A symmetrical diamidine was prepared from the urea (VI) which was obtained from the intermediate (III) and carbonyldi-imidazole.

EXPERIMENTAL

Ethyl 5-Aminopyridine-3-carboxylate.—5-Aminopyridine-3-carboxylic acid [5] (105 g., 0·76 mole) was added to a saturated solution of hydrogen chloride in ethanol (1000 ml.) and the mixture was heated under reflux for 20 hr. Treatment



SCHEME

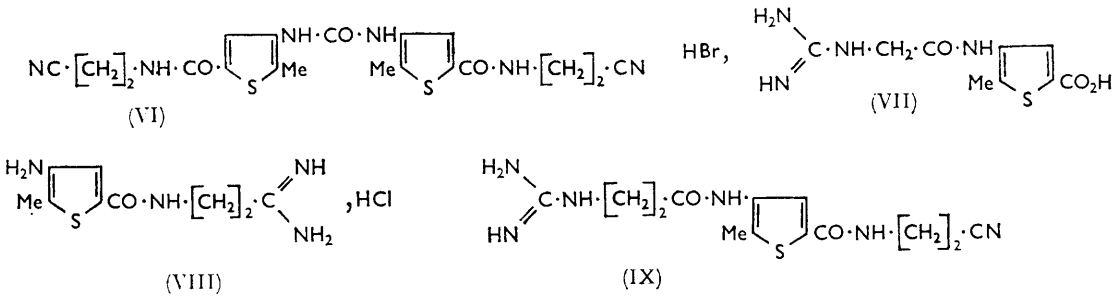


TABLE 1

RCO·NH·Y·CO·NH[CH_2]$_2$·C(:NH)NH_2,HCl

R	Y	M. p.	Yield (%)	Formula	Found (%) C	H	N	S	Required (%) C	H	N	S
p-Nitrophenyl	Pyridine-3,5-diyl	293—294°	58	$C_{16}H_{17}ClN_6O_4$	49·2	4·4	21·3		49·0	4·4	21·4	
p-Nitrophenyl	5-Methylthiophen-2,4-diyl	251—253	48	$C_{16}H_{18}ClN_5O_4S$	46·6	5·0		7·8	46·7	4·4		7·8
p-Nitrophenyl	Thiophen-2,5-diyl	295—300	55	$C_{15}H_{16}ClN_5O_4S,H_2O$	42·9	4·2		8·1	43·4	4·4		7·7
5-Nitro-2-thienyl	*p*-Phenylene	300—304	48	$C_{15}H_{16}ClN_5O_4S$	45·2	4·3		7·5	45·4	4·0		8·0
5-Nitro-2-thienyl	Thiophen-2,5-diyl	284—287 *	62	$C_{13}H_{14}ClN_5O_4S_2,1\frac{1}{2}H_2O$	36·6	4·2		14·7	36·3	4·0		14·9
4-Bromo-3-methyl-5-isothiazolyl	5-Methylthiophen-2,4-diyl	260—270	35	$C_{14}H_{17}BrClN_5O_2S_2,\frac{1}{2}H_2O$	35·4	3·7		13·3	35·4	3·8		13·5
Ureido-	5-Methylthiophen-2,4-diyl	248—252	41	$C_{10}H_{16}ClN_5O_2S$	39·0	5·5		10·5	39·3	5·3		10·5
Phenyl	Thiophen-2,5-diyl	275—277 *	54	$C_{15}H_{17}ClN_4O_2S,H_2O$	48·8	5·0	14·8		48·7	5·2	15·1	
Nitro	5-Methylthiophen-2,4-diyl	180—182	72	$C_9H_{13}ClN_4O_3S$			19·1	10·7			19·1	11·0
Nitro	Thiophen-2,5-diyl	209—210	57	$C_8H_{11}ClN_4O_3S$ †				11·4				11·5
p-(1-Guanidinophenyl)	Thiophen-2,5-diyl	308 *	39	$C_{16}H_{20}ClN_7OS$	47·3	4·7		8·1	46·9	4·9		7·8

* With decomp. † Found: Cl, 12·7. Required: Cl, 12·7%.

An alternative synthetic approach to congocidin analogues would be by the reaction of (VII) and (VIII), the basic guanidine and amidine centres being masked as hydrohalides. Compound (VII) was prepared and condensed with 4-amino-2-cyanoethylcarbamoyl-5-methylthiophen to give (IX), but the synthesis was not further studied.

The congocidin analogues and many related amidines (Table 1) were tested for trypanocidal and antiviral activity but showed no useful biological activity.

[5] H. Meyer and R. Graf, *Ber.*, 1928, **61**, 2202.

with aqueous sodium carbonate afforded the *amino-ester* 90 g., 71·5%), plates from light petroleum (b. p. 60—80°), m. p. 88—89° (Found: C, 57·7; H, 5·9; N, 16·5. $C_8H_{10}N_2O_2$ requires C, 57·85; H, 6·1; N, 16·9%).

Ethyl 5-(4-Nitrobenzamido)pyridine-3-carboxylate.—*p*-Nitrobenzoyl chloride (18·2 g., 0·097 mole) in anhydrous acetone (175 ml.) was added during 30 min. to a mixture of ethyl 5-aminopyridine-3-carboxylate (12·2 g., 0·074 mole) and triethylamine (10·6 g., 0·105 mole) in acetone (100 ml.) at 35—40°, and the mixture was then stirred for 5 hr. at 35—40°. Treatment with saturated sodium hydrogen carbonate solution afforded the *ester* (23·2 g., 100%), prisms from ethanol, m. p. 222—224° (Found: C, 57·1;

H, 4·2; N, 13·4. $C_{15}H_{13}N_3O_5$ requires C, 57·2; H, 4·1; N, 13·3%).

5-(4-Nitrobenzamido)pyridine-3-carboxylic Acid.—Ethyl 5-(4-nitrobenzamido)pyridine-3-carboxylic acid (23·2 g., 0·0736 mole), 2N-sodium hydroxide (40 ml., 0·080 mole), and ethanol (80 ml.) were heated at 100° for 60 min. Acidification with dilute acetic acid afforded the *acid* (17·7 g., 76%), prisms from dimethylformamide, m. p. 321—322° (decomp.) (Found: C, 54·0; H, 4·1; N, 15·0. $C_{13}H_9N_3O_5$ requires C, 54·5; H, 3·2; N, 14·6%).

3-Cyanoethylcarbamoyl-5-(4-nitrobenzamido)pyridine.—5-(4-Nitrobenzamido)pyridine-3-carboxylic acid (12·45 g., 0·0436 mole) was suspended in anhydrous dimethylformamide (120 ml.) at 50° and carbonyldi-imidazole [6] (7·06 g., 0·0436 mole) was added during 10 min.; a vigorous evolution of carbon dioxide ensued. The clear solution was set aside to cool to room temperature and then β-aminopropionitrile [7] (3·15 g., 0·045 mole) was added. The reaction mixture was stirred overnight, and was then concentrated to dryness under reduced pressure. The residue was washed with saturated sodium hydrogen carbonate solution and crystallised from dimethylformamide–ethanol to give the *cyanoethylcarbamoylpyridine hemihydrate* (9·55 g., 63%), m. p. 236—239° (Found: C, 55·2; H, 3·8; N, 20·5. $C_{16}H_{13}N_5O_4,\frac{1}{2}H_2O$ requires C, 55·2; H, 4·1; N, 20·1%).

5-Nitrothiophen-2-carboxylic Acid.—A mixture of 2-formyl-5-nitrothiophen [5] (50·0 g., 0·32 mole) and potassium dichromate (35·0 g., 0·12 mole) in 5N-sulphuric acid (375 ml.) was heated on a stream-bath with stirring for 30 min.[8] The reaction mixture was cooled and the acid (54·4 g., 98%), m. p. 157—158° (lit.,[9] m. p. 157—158°).

2-Cyanoethylcarbamoyl-5-methyl-4-nitrothiophen (II).— A solution of 5-methyl-4-nitrothiophen-2-carbonyl chloride [10] [from 5-methyl-4-nitrothiophen-2-carboxylic acid (50 g., 0·268 mole) and thionyl chloride] in anhydrous methylene dichloride (150 ml.) was added during 45 min. to a stirred mixture of 2-aminopropionitrile (42 g., 0·6 mole) and triethylamine (60 g., 0·6 mole) in methylene dichloride (600 ml.) at 0°. The mixture was stirred at room temperature for 1 hr. and then poured on to water (650 ml.) to give the *nitro-compound* (56 g., 88%), needles from ethyl acetate, m. p. 168—171° (Found: C, 45·3; H, 3·6; N, 17·3; S, 13·7. $C_9H_9N_3O_3S$ requires C, 45·2; H, 3·8; N, 17·6; S, 13·4%).

2-Cyanoethylcarbamoyl-5-nitrothiophen (76%), plates from ethanol, m. p. 133—134° (Found: C, 42·7; H, 2·9; S, 14·3. $C_8H_7N_3O_3S$ requires C, 42·7; H, 3·1; S, 14·2%), was prepared similarly.

4-Amino-2-cyanoethylcarbamoyl-5-methylthiophen (III).—A stirred mixture of ferrous sulphate heptahydrate (800 g., 2·88 mole) and barium hydroxide octahydrate (910 g., 2·88 mole) in water (3300 ml.) was heated to 90° and a suspension of 2-cyanoethylcarbamoyl-5-methyl-4-nitrothiophen (44 g., 0·184 mole) in acetone (200 ml.) was added during 5 min. The mixture was heated at 90° for 2 hr. and then filtered hot; the residue was washed well with boiling water. The pH of the combined filtrates was adjusted to 10 with 10N-sodium hydroxide and then extracted continuously with ether for 48 hr. to give the *amine* (25·5 g., 73%), prisms from ethyl acetate, m. p. 170—171° (Found: N, 20·1; S, 15·1. $C_9H_{11}N_3OS$ requires N, 20·1; S, 15·3%).

5-Amino-2-(cyanoethylcarbamoyl)thiophen (85%), prisms from water, m. p. 149—151° (Found: C, 49·3; H, 4·5; S, 16·4. $C_8H_9N_3OS$ requires C, 49·2; H, 4·6; S, 16·4%) was prepared similarly.

TABLE 2

RCO·NH·X·CO·NH·$[CH_2]_2$·CN

R	X	M. p.	Yield (%)	Formula	Found (%) C	Found (%) H	Found (%) S	Required (%) C	Required (%) H	Required (%) S
p-Nitrophenyl	5-Methylthiophen-2,4-diyl	242—245°	93	$C_{16}H_{14}N_4O_4S$	53·5	3·8	9·3	53·6	3·9	9·0
p-Nitrophenyl	Thiophen-2,5-diyl	265—267	99	$C_{15}H_{12}N_4O_4S$	52·1	3·5	9·5	52·3	3·5	9·3
5-Nitro-2-thienyl	*p*-Phenylene	260—263 *	75	$C_{15}H_{12}N_4O_4S$	52·1	3·6	9·4	52·3	3·5	9·3
5-Nitro-2-thienyl	Thiophen-2,5-diyl	275—276 *	93	$C_{13}H_{10}N_4O_4S_2$	44·6	2·8	18·5	44·6	2·9	18·3
4-Bromo-3-methyl-5-isothiazolyl	5-Methylthiophen-2,4-diyl	218—220	91	$C_{14}H_{13}BrN_4O_2S_2$	40·6	3·1	15·6	40·6	3·1	15·5
Phenyl	Thiophen-2,5-diyl	283—285 *	84	$C_{15}H_{13}N_3O_2S$	60·4	4·4	10·4	60·2	4·4	10·7

* With decomp.

Cyanoethylcarbamoyl Compounds.—The procedure for the preparation of these compounds (see Table 2) is illustrated by the following example.

2-Cyanoethylcarbamoyl-5-methyl-4-(4-nitrobenzamido)-thiophen (IV). *p*-Nitrobenzoyl chloride (3·7 g., 0·02 mole) in anhydrous acetone (10 ml.) was added during 10 min. to a mixture of 4-amino-2-cyanoethylcarbamoyl-5-methylthiophen (4·0 g., 0·019 mole) and triethylamine (3·0 g., 0·03 mole) in anhydrous acetone (200 ml.) at 0°, and then the reaction mixture was stirred for 3 hr. at room temperature. Treatment with saturated sodium hydrogen carbonate solution gave the *cyanoethylcarbamoyl* compound (6·38 g., 93%), m. p. 242—245°.

NN'-Di-(3-ethoxycarbonyl-5-pyridyl)urea.— Ethyl 5-aminopyridine-3-carboxylate (16·6 g., 0·10 mole) was added to a solution of carbonyldi-imidazole (8·1 g., 0·05 mole) in anhydrous benzene (100 ml.) at 50°. The reaction mixture was stirred at 45—50° for 2 hr. and then evaporated to dryness. The residue was crystallised from ethyl acetate–light petroleum (b. p. 60—80°) to give the *diester* (13·9 g., 78%), m. p. 157—159° (Found: C, 56·8; H, 5·2. $C_{17}H_{18}N_4O_5$ requires C, 57·0; H, 5·0%).

NN'-Di-(3-carboxy-5-pyridyl)urea.— Di-(3-ethoxycarbonyl-5-pyridyl)urea (13·9 g., 0·039 mole) and 2N-sodium hydroxide (45 ml., 0·090 mole) were heated on a steam-bath for 15 min. The resulting solution was brought to pH 5 with 2N-sulphuric acid to give the *diacid* (11·2 g.,

[6] R. Paul and G. W. Anderson, *J. Amer. Chem. Soc.*, 1960, **82**, 4596.
[7] S. R. Buc, *Org. Synth.*, Col. Vol. III, 1955, 93.
[8] T. M. Patrick and W. S. Emerson, *J. Amer. Chem. Soc.*, 1952, **74**, 1356.
[9] V. M. Zubarouskii, *Doklady Akad. Nauk S.S.S.R.*, 1952, **83**, 85.
[10] E. C. Campaigne and G. Herschel, *J. Amer. Chem. Soc.*, 1951, **73**, 3812.

95%), m. p. 220—230° (Found: N, 18·2. $C_{13}H_{10}N_4O_5$ requires N, 18·5%).

NN′-*Di*-(5-*cyanoethylcarbamoyl*-5-*pyridyl*)*urea*.— Carbonyldi-imidazole (10·3 g., 0·61 mole) was added to a suspension of di-(3-carboxy-5-pyridyl)urea (9·6 g., 0·318 mole) in dimethylformamide (120 ml.) at 50° followed after 1 min. by β-aminopropionitrile (4·5 g., 0·064 mole). The reaction mixture was stirred overnight at room temperature, and was then evaporated to dryness under reduced pressure. The residue was crystallised from dimethylformamide–methanol to give the *urea* (3·2 g., 41%), m. p. 257—259° (decomp.) (Found: C, 56·0; H, 4·5; N, 27·8. $C_{19}H_{18}N_8O_3$ requires C, 56·2; H, 4·4; N, 27·6%).

NN′-*Di*-(2-*cyanoethylcarbamoyl*-5-*methyl*-4-*thienyl*)*urea* (VI).— 4-Amino-2-cyanoethylcarbamoyl-5-methylthiophen (15·43 g., 0·074 mole) was added to a solution of carbonyldi-imidazole (6·5 g., 0·040 mole) in anhydrous dimethylformamide (200 ml.). The mixture was stirred overnight and the solid was filtered off and crystallised from dimethylformamide–ethanol to give the *urea* (12·5 g., 76%), m. p. 250—254° (Found: C, 51·7; H, 5·2. $C_{19}H_{20}N_6O_3S_2$ requires C, 51·4; H, 4·5%).

NN′-*Di*-(2-*cyanoethylcarbamoyl*-5-*thienyl*)*urea* (29%), m. p. 309—311° (decomp.) (Found: C, 48·5; H, 4·0; S, 14·8. $C_{17}H_{16}N_6O_3S_2$ requires C, 49·1; H, 3·9; S, 15·4%) was prepared similarly.

Amidines.—The procedure used for the preparation of amidines (see Table 1) is illustrated by the following examples.

2-*Amidinoethylcarbamoyl*-5-*methyl*-4-(4-*nitrobenzamido*)-*thiophen hydrochloride* (V). 2-Cyanoethylcarbamoyl-5-methyl-4-(4-nitrobenzamido)thiophen (6·38 g.) was suspended in anhydrous ethanol (125 ml.) and hydrogen chloride was passed through the suspension for 5 hr.; the mixture was kept at 0—5° by cooling. The reaction mixture was set aside to warm to room temperature overnight, and was then poured on to anhydrous ether (2 l.) to give a gum which was washed several times by decantation with ether. The gum was added to ethanol (100 ml.) saturated with ammonia at 0°, and gaseous ammonia was passed into the solution for 1·5 hr. with cooling to 0—5°. The mixture was set aside to warm to room temperature overnight and was then concentrated to dryness under reduced pressure; the residue was crystallised from 2N-hydrochloric acid to give the *amidine hydrochloride* (3·5 g., 48%), m. p. 251—253°.

The following *di-amidines* were prepared similarly: NN′-*di*-(2-*amidinoethylcarbamoyl*-5-*methyl*-4-*thienyl*)*urea dihydrochloride* (57%), m. p. 120° (decomp.) (Found: C, 38·0; H, 5·8; S, 10·4. $C_{19}H_{34}Cl_2N_8O_6S_2$ requires C, 37·7; H, 5·7; S, 10·6%); NN′-*di*-(5-*amidinoethylcarbamoyl*-3-*pyridyl*)*urea dihydrochloride dihydrate* (79%), m. p. 220° (decomp.) (Found: C, 42·0; H, 5·9; Cl, 12·7. $C_{19}H_{30}Cl_2N_{10}O_5$ requires C, 41·5; H, 5·5; Cl, 12·9%); NN′-*di*-(2-*amidinoethylcarbamoyl*-5-*thienyl*)*urea dihydrochloride pentahydrate* (13%), m. p. 180° (decomp.) (Found: Cl, 11·9; S, 10·6. $C_{17}H_{34}Cl_2N_8O_8S_2$ requires Cl, 11·6; S, 10·5%).

2-*Amidinoethylcarbamoyl*-5-(4-*guanidinobenzamido*)*thiophen hydrochloride*. 2-Amidinoethylcarbamoyl-5-(4-nitrobenzamido)thiophen hydrochloride (3·0 g., 0·00755 mole) in dimethylformamide (50 ml.) was hydrogenated at 70 lb./sq. in. over 5% palladium on charcoal. Hydrogen uptake ceased after 92% of theory had been absorbed (1·75 hr.) The filtrate was evaporated under reduced pressure and the residual oil was dissolved in anhydrous ethanol (50 ml.) and heated under reflux with cyanamide (0·686 g., 0·164 mole) for 30 min. The mixture was cooled to give the *guanidino-compound* (1·25 g., 39%), m. p. 308° (decomp.).

Congocidin Analogues.—The procedure used for the preparation of the analogues (see Table 3) is illustrated by the following example.

3-(2-*Amidinoethylcarbamoyl*)-5-(4-*guanidinoacetylamino-benzamido*)*pyridine dihydrochloride* (Ib).—3-(2-Amidinoethylcarbamoyl)-5-(4-nitrobenzamido)pyridine hydrochloride (4·3 g., 0·010 mole) in dimethylformamide (60 ml.) was hydrogenated at 70 lb./sq. in. over 5% platinum on charcoal. Hydrogen uptake ceased after 80% of theory had been absorbed (1 hr.). The filtrate was evaporated under reduced pressure, and the residual oil was added to a solution of guanidinoacetic acid hydrochloride [11] (1·73 g., 0·11 mole) in anhydrous dimethylformamide (30 ml.) at 80°. Dicyclohexylcarbodi-imide (3·10 g., 0·015 mole) was then added and the reaction mixture was heated to 105° for 30 min. On cooling, dicyclohexylurea (2·25 g., 90%) separated. The filtrate was concentrated to dryness and the residue was boiled with methanol. The filtrate was concentrated to an oil which was triturated with acetone to give the *dihydrochloride* (2·35 g., 43%), m. p. 250—255°.

TABLE 3

Compound	M. p.	Yield (%)	Formula	Found (%) C	Found (%) H	Required (%) C	Required (%) H
(Ib)	250—255°	43	$C_{19}H_{23}N_9O_3,2HCl$	45·3	5·4	45·8	5·1
(Ic)	125 *	78	$C_{19}H_{24}N_8O_3S,2HCl$	44·1	5·7	44·1	5·1
(Id)	98—103	67	$C_{18}H_{22}N_8O_3S,2HCl$	42·9	5·0	43·0	4·8
(Ie)	220—225 *	85	$C_{18}H_{22}N_8O_3S,2HCl,H_2O$	41·6	4·8	41·5	5·0

* With decomp.

A solution of the dihydrochloride in water on treatment with saturated sodium sulphate solution afforded the *sulphate trihydrate*, m. p. 220° (decomp.) (Found: C, 40·1; H, 5·0; N, 21·6; S, 5·7. $C_{19}H_{31}N_9O_{10}S$ requires C, 39·6; H, 5·4; N, 21·9; S, 5·6%).

Similarly 2-(amidinoethylcarbamoyl)-5-(4-guanidinoacetylaminobenzamido)thiophen dihydrochloride (Id) afforded the *sulphate trihydrate*, m. p. 220—225° (decomp.) (Found: C, 37·0; H, 5·1; S, 11·0. $C_{18}H_{30}N_8O_{10}S_2$ requires C, 37·2; H, 5·2; S, 11·0%).

Benzyl 5-Methyl-4-nitrothiophen-2-carboxylate.—5-Methyl-4-nitrothiophen-2-carbonyl chloride (22 g., 0·17 mole) was mixed with benzyl alcohol (11·6 g., 0·17 mole) and was heated on a steam-bath for 3 hr. The residue was crystallised from ethyl acetate–light petroleum (b. p. 60—80°) to give needles of the *ester* (16·5 g., 56%), m. p. 48—50° (Found: C, 56·6; H, 4·2; S, 11·6. $C_{13}H_{11}NO_4S$ requires C, 56·3; H, 4·0; S, 11·6%).

[11] E. Brand and F. C. Brand, *Org. Synth.*, Col. Vol. III, 1955, 440.

Benzyl 4-Amino-5-methylthiophen-2-carboxylate.—Benzyl 5-methyl-4-nitrothiophen-2-carboxylate (6·4 g.) was hydrogenated over 5% platinised charcoal at 70 lb./sq. in. (the theoretical volume of hydrogen was taken up in 90 min.) to give the *amino-ester* (5·45 g., 95%), prisms from ethyl acetate–light petroleum (b. p. 40—60°), m. p. 49—51° (Found: C, 63·4; H, 6·3; S, 13·3. $C_{13}H_{13}NO_2S$ requires C, 63·2; H, 5·3; S, 12·9%). [*Hydrochloride*, m. p. 195—200° (Found: C, 55·1; H, 4·9; S, 11·3. $C_{13}H_{14}ClNO_2S$ requires C, 55·1; H, 4·9; S, 11·3%)].

Benzyl 4-Guanidinoacetamido-5-methylthiophen-2-carboxylate.—Benzyl 4-amino-5-methylthiophen-2-carboxylate (5·40 g., 0·022 mole) and guanidinoacetic acid hydrochloride (3·36 g., 0·022 mole) in dimethylformamide (40 ml.) were heated to 90° and dicyclohexylcarbodi-imide (5·4 g., 0·026 mole) was added. The reaction was heated at 90—95° for 30 min., cooled, and filtered. The filtrate was concentrated to dryness under reduced pressure, and the residue was extracted with hot ethanol (100 ml.). The extract was concentrated to dryness, dissolved in water, and treated with sodium hydrogen carbonate solution to give the *ester sesquihydrate* (3·0 g., 36·5%), m. p. 108—111° (Found: C, 51·0; H, 5·4; S, 8·7. $C_{16}H_{18}N_4O_3S,1\frac{1}{2}H_2O$ requires C, 51·4; H, 5·6; S, 8·6%).

4-Guanidinoacetamido-5-methylthiophen-2-carboxylic Acid Hydrobromide (VII).—Benzyl 4-guanidinoacetamido-5-methylthiophen-2-carboxylate (3·0 g.) was dissolved in hydrogen bromide in acetic acid (40 ml.; 37% w/w) and set aside at room temperature for 7 days. Addition of dry ether (200 ml.) afforded a solid (1·9 g.) which was crystallised from ethanol–ether to give the *acid* (1·0 g., 37·5%), m. p. 229° (Found: C, 33·4; H, 4·2; S, 9·9. $C_9H_{13}BrN_4O_3S$ requires C, 33·0; H, 4·0; S, 9·8%).

2-(2-Cyanoethylcarbamoyl)-4-(4-guanidinoacetamido-5-methylthiophen-2-carboxamido)-5-methylthiophen Sulphate (IX).— 4-Guanidinoacetamido-5-methylthiophen-2-carboxylic acid hydrobromide (0·75 g., 0·0023 mole) was dissolved in dry dimethylformamide (10 ml.). Carbonyl di-imidazole (0·45 g., 0·0025 mole) was added and after 30 min., 4-amino-2-cyanoethylcarbamoyl-5-methylthiophen (0·48 g., 0·0023 mole) was added and the mixture was set aside at room temperature for 4 hr. The reaction mixture was then concentrated to an oil which was extracted with hot water (20 ml.). The cooled extract was filtered and treated with 2N-sulphuric acid (2 ml.) to give an oily solid (0·5 g.), m. p. 183—189°. Two crystallisations from water afforded the *sulphate hydrate*, m. p. 200—207° (Found: C, 42·4; H, 4·6; S, 15·6. $C_{18}H_{21}N_7O_3S_2,\frac{1}{2}H_2SO_4,H_2O$ requires C, 42·0; H, 4·7; S, 15·6%).

We thank Mr. S. Squires and Mr. J. Hill for the biological evaluation, Dr. M. Davis for helpful discussions, Mr. S. Bance and his staff for the analyses, and Messrs. J. W. H. Bush, D. C. Mills, and R. Philo for preparative assistance.

[7/1093 *Received, August 18th*, 1967]