Studies in Isoxazole Chemistry. 5. N-Alkylation and N-Acylation of 5-Amino-3-(5-nitro-2-furyl)isoxazoles[1]

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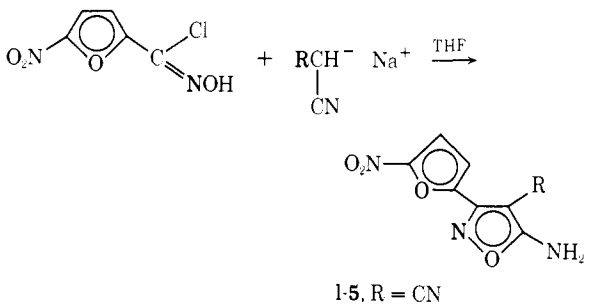
A number of 5-amino-3-(5-nitro-2-furyl)isoxazoles have been synthesized. These compounds reacted with NaH in anhyd THF to form the Na derivatives, which could be alkylated or acylated with appropriate reagents. This process could be repeated to produce the 5-dialkylamino- or 5-alkylacylamino- or 5-diacylamino-3-(5-nitro-2-furyl)isoxazoles. The *tert*-butyl 5-amino- or 5-dimethylamino-3-(5-nitro-2-furyl)isoxazole-4-carboxylates underwent a facile reaction on heating with concd H_2SO_4 to give 5-amino- and 5-dimethylamino-3-(5-nitro-2-furyl)-isoxazoles. These compounds showed antitrichomonal activity comparable to 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole and Gram-negative antibacterial activity comparable to 1-ethyl-7-methyl-1,8-naphthyridin-4-one-3-carboxylic acid.

The presence of an amino or substituted amino group in a 5-membered heterocyclic compound attached to a nitrofuran ring leads to effective antibacterial agents.[2-5] Since our work on 5-methyl-3-(5-nitro-2-furyl)isoxazoles and 3-methyl-5-(3-nitro-2-furyl)isoxazoles gave compounds with excellent antitrichomonal but relatively poor antibacterial activity,[6] it was decided to find out if the introduction of an amino substituent improved the antibacterial effect. Two publications have appeared on the 5-amino-3-(5-nitro-2-furyl)isoxazoles[7,8] after this work was initiated. Although the *in vitro* antibacterial activity of these free amino compounds have been commented upon, no substituted amino derivatives appear to have been prepared.

Chemistry.—The 5-amino-3-(5-nitro-2-furyl)isoxazoles used in this investigation were made in essentially the same way as described in the literature[7,8] (see Scheme I). A detailed preparation of **I-15** is included

SCHEME I



I-5, R = CN
I-12, R = $COOCH_3$
I-15, R = $COOC(CH_3)_3$
I-18, R = $COOCH_2CH_2N(CH_3)_2$

in the Experimental Section, since this was a key compound in many preparations and a modified procedure was used.

Special mention should be made of the dialkylaminoalkyl esters **I-18** to **I-23** which have excellent antibacterial and antitrichomonal activity. The preparation of these compounds has already been described by us.[9]

Attempts to acetylate these amines by the use of AcCl in the presence of Et_3N or Pyr were unsuccessful. Compd **I-12**, on heating under reflux with Ac_2O gave a mixture of **III-1** and **III-10**, which were separated by column chromatog.

4-Cyano- or the 4-carboxylic esters of 5-amino-3-(5-nitro-2-furyl)isoxazole **1** reacted smoothly with NaH in anhyd THF to give dark-colored solutions of **2** (Scheme II, reaction C), which on treatment with alkyl halides, sulfates, or tosylates gave **3** (reaction D), or with acyl halides or better with acid anhydrides formed **4** (reaction E). On subjecting **3** or **4** to the same process, **5**, **6**, or **7** could be obtained by reactions F, G, H, or I. The use of 2 equiv of NaH with excess of the alkylating or acylating reagent (route A or B) resulted in the formation of **5** or **7** in one step.

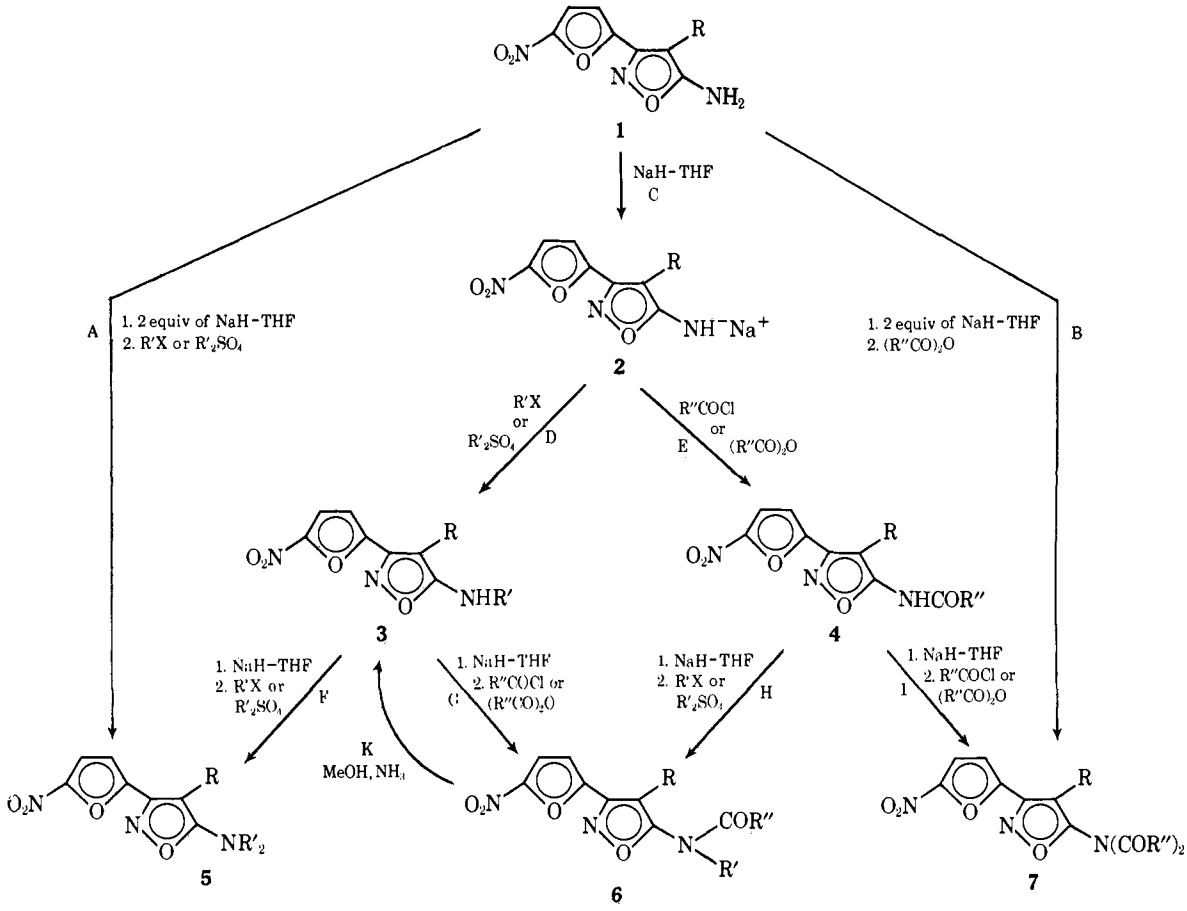
The activity of the 5-amino group was affected by the substituent in the 4 position of the isoxazole ring. Thus **I-1**, did not react with NaH at room temp while **I-5** with 1 equiv of NaH and MeI or Me_2SO_4 (reaction CD), gave **I-7** as the major product with only a trace of **I-6**. Compd **I-6** was however obtained in 90% yield by reaction K. The crude product from reaction CD was analyzed by its nmr spectrum. Essentially the same ratio of products was obtained by using DMSO as solvent, suggesting that the preferential formation of **I-7** is not due to solubility differences between the Na derivatives. The 5-amino-3-(5-nitro-2-furyl)isoxazole-4-carboxylates behave normally to form 5-NHMe and $5\text{-}NMe_2$ derivatives as the main products by reactions CD or A, respectively.

The nature of the alkylating or acylating agent also affects the course of the reaction. Compd **I-12**, for example, with 1 equiv of NaH and BrCN gave **III-11** as the major product. In the case of hindered acyl derivatives, **II-7** for example, only starting material was obtained from the reaction with NaH and MeI. Compds **I-6**, however, reacted with NaH and pivaloyl chloride to give the desired products, **II-12**.

The 5-diacylamino compds, for example **III-12** could be prepared *via* reaction B or CEI. Since the diacylamino compds **7** were found to undergo rapid hydrolysis to the monoacyl derivatives **4**, these compds were not investigated further.

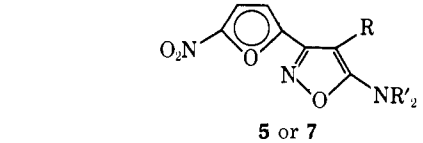
(1) Paper 4 in this seris, R. G. Micetich, *Can. J. Chem.*, **48**, 3753 (1970).
(2) W. R. Sherman and D. E. Dickson, *J. Org. Chem.*, **27**, 1351 (1962).
(3) W. R. Sherman, *ibid.*, **26**, 88 (1961).
(4) K. Skagins, K. Rubinstein, and E. Ifversen, *Acta Chem. Scand.*, **14**, 1054 (1960).
(5) M. Portelli and G. Bartolini, *Ann. Chim. (Rome)*, **53**, 1180 (1963); *Chem. Abstr.*, **60**, 8011*a* (1964).
(6) R. G. Micetich, *J. Med. Chem.*, **12**, 611 (1969).
(7) Geigy Co., Netherlands Patent 66,11584 (Feb 20, 1967); *Chem. Abstr.*, **67**, 64386 (1967).
(8) J. Matsumoto and S. Minami, *Chem. Pharm. Bull.*, **15**, 1806 (1967).
(9) R. U. Lemieux and R. G. Micetich, U. S. Patent 3,522,251 and 3,522,252 (July 28, 1970); *Chem. Abstr.*, **73**, 120609, 130988 (1970).

SCHEME II

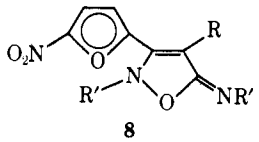


The *tert*-Bu esters **I-15** and **I-17** underwent a smooth reaction on heating in concd H_2SO_4, to give **I-1** and **I-2**, respectively. The reaction of ketene aminals[10] with 5-nitro-2-furyl nitrile oxide, following the procedure of Rajagopalan and Talaty,[11] gave the 5-morpholino derivatives **I-3** and **I-4**.

In reactions where mixtures of products resulted, the components were separated by column chromatog on silica gel using $CHCl_3$–EtOAc mixtures as eluants. In every instance the identity of the compd was established from elemental and spectral (ir and nmr) anal. Nmr spectroscopy was particularly useful for eliminating the possible alternative structures **8** and **9** for the disubstituted derivatives and confirming their structure as **5** or **7**. Thus the two CH_3 signals of the dimethylamino



5 or **7**



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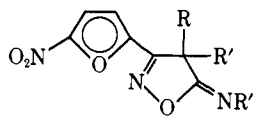

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compd **5** appeared as a sharp singlet at τ 6.98 for **I-2**, 6.79 for **I-7**, 6.78 for **I-14**, and 6.78 for **I-17**, while the two $COCH_3$ signals in **7** appeared as a sharp singlet at τ 7.62 for **III-10** and 7.61 for **III-12**. The two $COOCH_3$ signals in **II-13** also appeared as a sharp singlet at τ 6.08.

Biological Screening Results.—The biological screening of these compds was performed by Dr. Ken E. Price and his associates in the Microbiological Department of Bristol Laboratories, Syracuse, N. Y. Antibacterial and antitrichomonal activity was evaluated using standard procedures. The data are summarized in Tables I–III. The following conclusions can be drawn from the *in vitro* data.

(1) The introduction of an amino or a substituted amino group into the isoxazole ring enhances the Gram-negative antibacterial activity of the nitrofurylisoxazoles while retaining the antitrichomonal activity.[6]

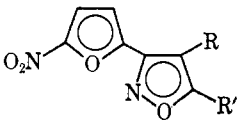
(2) The presence of a CN, CO_2R, or carbodialkylaminoalkoxy group at C_4 of the isoxazole ring is necessary for high levels of activity (see Table I).

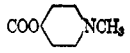
(3) In general the free amino compds are more active than those compds in which the amino group has been alkylated or acylated. However the high activity shown by the diallylamino compds **I-10** and the CF_3CO derivative **II-4** should be noted.

The oral CD_{50} of many of these compds was found to be of the same order as that of naladixic acid.[12] Thus **II-6** had an oral CD_{50} of 15 × 2 mg/kg, **II-11** gave a value of 26 × 2 mg/kg, **II-5** was 11 × 2 mg/kg, and **III-8** was 36 × 2 mg/kg as compared with 35 × 2 mg/kg for naladixic acid, against *Klebsiella pneumoniae*. The dialkylaminoalkyl esters have excellent antibacterial activity.[9]

(10) H. Baganz and L. Domaschke, *Chem. Ber.*, **95**, 2095 (1962).
(11) P. Rajagopalan and C. N. Talaty, *Tetrahedron Lett.*, **38**, 4537 (1966).
(12) Nalidixic acid was supplied by Winthrop Laboratories.

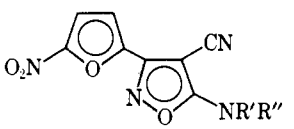
TABLE I

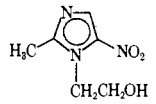


No.	R	R′	Formula	Mp, °C	Yield, %	MIC, μg/ml a	b	c	d	MID50, μg/ml e	f
1	H	NH_2	$C_7H_5N_3O_4$	170–173 dec	62	>50	>50	>50	>50	0.16	0.63
2	H	$N(CH_3)_2$	$C_9H_9N_3O_4$	169–170	77	>12.5	25	6.3	3.1	2.5	1.25
3	H	Morpholinyl	$C_{11}H_{11}N_3O_5$	218–220 dec	35	>12.5	>50	>50	>50	0.63	1.25
4	CH_3	Morpholinyl	$C_{12}H_{13}N_3O_5$	174–176	7.5	>12.5	>50	>50	25	0.32	2.5
5	CN	NH_2	$C_8H_4N_4O_4$	238–240[g]	79	0.1	0.2	0.1	0.2	0.054	0.33
6	CN	$NHCH_3$	$C_9H_6N_4O_4$	228–231	90	3.1	3.1	0.8	0.8	1.25	1.25
7	CN	$N(CH_3)_2$	$C_{10}H_8N_4O_4$	164–166	75	0.8	3.1	1.6	3.1	<0.4	<0.4
8	CN	$NHCH_2CH_2CH_2CH_3$	$C_{12}H_{12}N_4O_4$	123–125	3	3.1	12.5	6.3	3.1	0.16	10
9	CN	$NHCH_2CH{=}CH_2$	$C_{11}H_8N_4O_4$	159–161	3.2[h]	1.6	12.5	1.6	1.6	0.63	0.63
10	CN	$N(CH_2CH{=}CH_2)_2$	$C_{14}H_{12}N_4O_4$	112–113	14.5[h]	12.5	0.8	0.4	0.2	0.32	0.63
11	CN	$N(CH_3)CH_2CH{=}CH_2$	$C_{12}H_{10}N_4O_4$	87–90	94	1.6	>50	12.5	6.3	0.63	0.63
12	$COOCH_3$	NH_2	$C_9H_7N_3O_6$	245–250[i] dec	80	0.4	0.1	0.062	0.031	0.01	0.16
13	$COOCH_3$	$NHCH_3$	$C_{10}H_9N_3O_6$	200–202	53[h]	3.1	6.3	3.1	1.6	0.32	0.32
14	$COOCH_3$	$N(CH_3)_2$	$C_{11}H_{11}N_3O_6$	145–148	12[j]	>12.5	>50	12.5	6.3	5	2.5
15	$COOC(CH_3)_3$	NH_2	$C_{12}H_{13}N_3O_6$	182–183	39	>12.5	>50	>50	>50	2.5	5
16	$COOC(CH_3)_3$	$NHCH_3$	$C_{13}H_{15}N_3O_6$	82–84	56	>12.5	>50	>50	>50	20	>20
17	$COOC(CH_3)_3$	$N(CH_3)_2$	$C_{14}H_{17}N_3O_6$	103–104	71	>12.5	>50	>50	>50	20	>20
18	$COOCH_2CH_2N(CH_3)_2$	NH_2	$C_{12}H_{14}N_4O_6$	174–176 dec	45	12.5	0.4	0.4	0.4	0.63	0.63
19	$COOCH_2CH_2N(C_2H_5)_2$	NH_2	$C_{14}H_{18}N_4O_6$	129–130	30	12.5	1.6	0.4	0.2	0.16	0.63
20	$COOCH_2CH_2$-*N*-piperidyl	NH_2	$C_{15}H_{18}N_3O_6$	172–174 dec	43	12.5	3.1	1.6	0.8	0.32	0.32
21	$COOCH_2CH_2$-*N*-morpholinyl	NH_2	$C_{14}H_{16}N_3O_7$	203–205 dec	47	6.3	12.5	6.3	1.6	<0.4	1.25
22		NH_2	$C_{14}H_{16}N_3O_6$	115–118 dec	38	6.3	3.1	0.8	0.4	5	2.3
23	$COOCH_2CH_2CH_2N(CH_3)_2$	NH_2	$C_{13}H_{16}N_4O_6$	152–154 dec	16	6.3	3.1	1.6	0.4	2.5	2.5

[a] *Staphylococcus aureus.* [b] *Escherichia coli.* [c] *Klebsiella pneumonia.* [d] *Salmonella enteritidis.* [e] *Trichomonas vaginalis.* [f] *Trichomonas foetus.* [g] Reported mp 263°;[7] 250° dec.[8] [h] Obtd in the same reaction; sepd by silica gel chromatog. [i] Reported mp 240 dec;[7] 249–251° dec.[8]

TABLE II



No.	R′	R″	Formula	Mp, °C	Yield, %	MIC, μg/ml a	b	c	d	MID50, μg/ml e	f
1	H	$COCH_3$	$C_{10}H_6N_4O_5$	237–240	77	>12.5	50	>50	100	0.35	3.1
2	H	$COCH_2Cl$	$C_{10}H_5ClN_4O_5$	220–221	24	12.5	3.1	3.1	1.6	1.25	10
3	H	$COCHCl_2$	$C_{10}H_4Cl_2N_4O_5$	150–153	55	3.1	3.1	0.4	0.8	0.31	5
4	H	$COCF_3$	$C_{10}H_3F_3N_4O_5$	201–203	40	0.2	0.2	0.063	0.4	0.06	0.26
5	H	$COCH_2CH_2CH_3$	$C_{12}H_{10}N_4O_5$	189–191	95	12.5	>50	>50	50	5	20
6	H	$COCH(CH_3)_2$	$C_{12}H_{10}N_4O_5$	212–215	86	12.5	>50	>50	50	5	20
7	H	$COC(CH_3)_3$	$C_{13}H_{12}N_4O_5$	240–245 dec	64	>12.5	12.5	6.3	6.3	5	10
8	H	$COOCH_3$	$C_{10}N_6H_4O_6$	202–205 dec	4[g]	12.5	>50	25	12.5	5	>20
9	CH_3	$COCH_3$	$C_{11}H_8N_4O_5$	145–148	82	1.6	12.5	1.6	1.6	1.25	5.0
10	CH_3	$COCH_2CH_2CH_3$	$C_{13}H_{12}N_4O_5$	105–107	70	6.3	50	12.5	12.5	5	10
11	CH_3	$COCH(CH_3)_2$	$C_{13}H_{12}N_4O_5$	114–115	71	12.5	50	12.5	12.5	5	10
12	CH_3	$COC(CH_3)_3$	$C_{14}H_{14}N_4O_5$	118–119	94	6.3	>50	12.5	6.3	5	10
13	$COOCH_3$	$COOCH_3$	$C_{12}H_8N_4O_8$	129–131	55[g]	>12.5	>50	>50	>50	10	20
Metronidazole[h]										0.18	0.17

[a–f] See Table I. [g] See footnote *h*, Table I. [h] Supplied by G. D. Searle and Co.

Experimental Section[13]

Representative examples are described in detail.

***tert*-Butyl 5-Amino-3-(5-nitro-2-furyl)isoxazole-4-carboxylate (I-15).**—The *tert*-butyl cyanoacetate required for this preparation was made by two different routes (see also ref 14). *tert*-BuOH (42.5 g, 0.5 mole) and cyanoacetic acid (37 g, 0.5 mole) were dissolved in EtAcO (1 l.) and cooled in an ice bath. Dicyclohexylcarbodiimide (103 g, 0.5 mole) was added to the cold, stirred soln when an immediate exothermic reaction occurred and urea began to sep. After 3 hr at room temp the ppt was removed by filtration, the filtrate was distd, and 56 g (80%) of *tert*-butyl cyanoacetate, bp 72° (1.8 mm), was obtained as a clear mobile liquid.

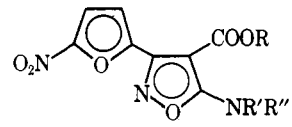
tert-Butyl monochloroacetate[15] (240 g, 1.5 moles) was added slowly to a stirred, ice-cold suspension of well-powdered NaCN (320 g, 8.2 moles) in DMSO (2 l.). The mixt was then stirred for 3 hr at 80°, poured into H_2O (5 l.), and extd with Et_2O (7

(13) All temps are uncorrected. Ir spectra were obtained on a Perkin-Elmer infracord spectrometer Model 137 and nmr spectra with a Varian Associates Model A-60 spectrometer. The nmr and ir spectra of all compounds were in agreement with the assigned structure. All new compds were analyzed for C, H, and N and these results were within ±0.4% of the calcd values. Comp **III-11** was analyzed for H alone since only a small amount was obtained.

(14) R. E. Ireland and M. Chaykovsky, *Org. Syn.*, **41**, 5 (1961).

(15) Available from Raylo Chemicals, Edmonton, Alberta, Canada.

TABLE III



No.	R	R'	R''	Formula	Mp, °C	Yield, %	MIC, μg/ml a	b	c	d	MID50, μg/ml e	f
1	CH_3	H	$COCH_3$	$C_{11}H_9N_3O_7$	152–154	49[g]	3.1		3.1		0.32	0.15
2	CH_3	H	$COCHCl_2$	$C_{11}H_7Cl_2N_3O_7$	149–152	42	12.5	12.5	3.1	3.1	0.16	0.16
3	CH_3	H	$COCF_3$	$C_{11}H_6F_3N_3O_7$	117–119	86	3.1	3.1	0.8	0.4	0.08	2.5
4	CH_3	H	COC_2H_5	$C_{12}H_{11}N_3O_7$	164–166	18.6	3.1	6.3	50	1.6	0.16	1.25
5	CH_3	H	$COCH(CH_3)_2$	$C_{13}H_{13}N_3O_7$	143–146	82	>12.5	>50	50	25	1.25	10
6	CH_3	CH_3	$COCH_3$	$C_{12}H_{11}N_3O_7$	109–113	61	12.5	25	12.5	12.5	1.25	5
7	CH_3	CH_3	$COCH(CH_3)_2$	$C_{14}H_{15}N_3O_7$	91–94	92	>12.5	>50	>50	6.3	20	>20
8	CH_3	CH_3	$COOCH_3$	$C_{12}H_{11}N_3O_8$	145–148	43[h]	>12.5	>50	>50	12.5	10	5
9	CH_3	CH_3	CN	$C_{11}H_8N_4O_6$	135–137	50	12.5	>50	>50	>50	1.25	10
10	CH_3	$COCH_3$	$COCH_3$	$C_{13}H_{11}N_3O_8$	140–141	13.5[g]	3.1	6.3	6.3	0.4	1.25	2.5
11	CH_3	CN	CN	$C_{11}H_5N_5O_6$	280 dec	3	>12.5	25	12.5	12.5	5	10
12	$C(CH_3)_3$	$COCH_3$	$COCH_3$	$C_{16}H_{17}N_3O_8$	115–117	92	>12.5	>50	>50	6.3	10	>20
13	$CH_2CH_2N(C_2H_5)_2$	H	$COCH_3$	$C_{16}H_{20}N_4O_7$	105–107	60	>12.5	25	6.3	3.1	1.25	2.5
14	$CH_2CH_2N(C_2H_5)_2$	H	CO-*n*-Pr	$C_{18}H_{24}N_4O_7$	100–102	62	>12.5	>50	50	6.3	5	20
15	$CH_2CH_2N(C_2H_5)_2$	H	$COCH(CH_3)_2$	$C_{18}H_{24}N_4O_7$	77–79	58	>12.5	>50	>50	25	2.5	20
16	CH_2CH_2-*N*-piperidyl	H	$COCH_3$	$C_{17}H_{20}N_4O_7$	133–135 dec	43	>12.5	6.3	6.3	3.1	0.63	2.5
17	CH_2CH_2-*N*-morpholinyl	H	$COCH_2CH_2CH_3$	$C_{18}H_{22}N_4O_8$	108–110 dec	40	>12.5	50	25	>50	1.25	10
18	4-CH_3-*N*-piperidyl	H	$COCH(CH_3)_2$	$C_{18}H_{22}N_4O_7$	76–79 dec	67	>12.5	50	12.5	6.3	10	20

[a–f] See footnotes *a–f*, Table I. [g] Obtd in the same reaction; sepd by silica gel chromatog. [h] Purified by silica gel chromatog.

× 500 ml). The combined Et_2O exts were dried ($MgSO_4$), filtered, and distd, when 183 g (87%) of *tert*-butyl cyanoacetate, bp 53° (0.77 mm), was obtained.

tert-Butyl cyanoacetate (100 g, 0.71 mole) was added slowly to a well-stirred, ice-cold suspension of NaH (58% in oil, 29 g, 0.71 mole, prewashed with hexane) in dry THF (700 ml) under N_2. When the reaction was complete (no more gas evoln), a soln of 5-nitro-2-furylchloraldoxime[6] (135 g, 0.71 mole) in dry THF (500 ml) was added slowly keeping the temp of the reaction mixt at *ca.* −35°. The mixt was then stirred at this temp for 1 hr and the THF then removed at room temp under reduced pressure. Ice water (1 l.) was added, and the mixt was triturated and filtered. The solid thus obtd was taken up in EtAcO, the soln was dried ($MgSO_4$), and solvent was removed under reduced pressure. The residue was triturated with Et_2O and filtered to give 81.8 g (39%) of a light yellow solid, mp 182–183° dec (Et_2O), reported mp 171°.[7]

***tert*-Butyl 5-Monomethylamino-3-(5-nitro-2-furyl)isoxazole-4-carboxylate (I-16).**—*tert*-Butyl 5-amino-3-(5-nitro-2-furyl)isoxazole-4-carboxylate (**I-15**) (20.65 g, 0.07 mole) was added in portions to a stirred, ice-cold suspension of NaH (58% in oil, 2.9 g, 0.07 mole, prewashed in hexane) in dry THF (200 ml). When the reaction was complete (no more gas evoln), MeI (20 g, 0.14 mole) was added, and the mixt was heated under reflux for 2 hr, after which it was concd under reduced pressure. The dark brown residue was shaken with ice H_2O (50 ml) and extd with EtAcO (4 × 75 ml). The combined org exts were dried ($MgSO_4$) and filtered, and solvent was removed when 20.9 g of a dark brown wax was obtained. The wax was purified by chromatog on Fisher silica gel using $CHCl_3$–EtAcO, 9:1, as eluant, when 11.8 g (56%) of **I-16** was obtained as yellow crystals, mp 90–91° (CCl_4–hexane).

***tert*-Butyl 5-Dimethylamino-3-(5-nitro-2-furyl)isoxazole-4-carboxylate (I-17).**—The same process was repeated on **I-16** (14.6 g, 0.047 mole) using NaH (2.1 g, 0.05 mole) and MeI (13.4 g, 0.094 mole) in dry THF (150 ml). The crude product was a light brown solid weighing 13.4 g. Crystn from CCl_4–hexane gave 10.7 g (71%) of yellow crystals, mp 103–104°.

5-Amino-3-(5-nitro-2-furyl)isoxazole (I-1).—*tert*-Butyl 5-amino-3-(5-nitro-2-furyl)isoxazole-4-carboxylate (**I-15**) (9.7 g, 0.033 mole) and concd H_2SO_4 (50 ml) were heated with stirring at 95° for 2.5 hr, after which the black reaction mixt was poured into ice H_2O (300 ml) and extd with EtAcO (5 × 100 ml). The combined exts were washed with brine, dried ($MgSO_4$), filtered, and concd. The orange-brown residue gave 5.5 g (85%) of yellow crystals, mp 170–173° from THF–hexane.

5-Dimethylamino-3-(5-nitro-2-furyl)isoxazole (I-2) was obtd from **I-17** in 77% yield as yellow microneedles, mp 169–170° (C_6H_6), in a similar manner.

5-Amino-4-cyano-3-(5-nitro-2-furyl)isoxazole (I-5).—Na (34.5 g, 1.5 g-atoms) was dissolved in MeOH (1 l.) in a dry N_2 atm, and the soln was cooled to 0°. Malononitrile (99.0 g, 1.5 moles) was added, and the mixt was stirred for 10 min and then cooled to −40°, after which 5-nitro-2-furylchloraldoxime (283.8 g, 1.48 moles) dissolved in ice-cold MeOH (1 l.) was added slowly. The mixt was allowed to reach ambient temp and filtered. The product was a dark-colored slimy solid which became light grey in color on drying. The prod was washed with ice water, then MeOH and Et_2O, and dried to give 174.4 g (79%) of a grey powder which was sufficiently pure for further reactions. The compd was purified by taking up in THF, treating with alumina, filtering, and concg to a small vol when a light yellow solid, *ca.* 150 g, mp 250° dec, was obtd, reported mp 263°,[7] 250° dec.[8]

4-Cyano-5-dimethylamino-3-(5-nitro-2-furyl)isoxazole (I-7).—Compd **I-5** (9.0 g, 0.04 mole) was added in portions to a stirred, cold (−10°) suspension of NaH (3.8 g, 0.088 mole) in dry THF (150 ml) in an N_2 atm. There was an immediate gas evoln, and the mixt became blood red. After the addn was completed the mixt was stirred for another 15 min at −5°, and Me_2SO_4 (15.1 g, 0.12 mole) was added slowly, maintaining the temp below 0°. It was stirred and heated under reflux on an oil bath at 90° for 2 hr. Initially the mixt became thick (almost a gel) but on heating it became thinner and at the end there was a small amount of solid in a brown liquid. The THF was removed under reduced pressure, ice H_2O (100 ml) was added, and the mixt was extd with EtAcO (700 ml). The org layer was dried ($MgSO_4$ with decolorizing charcoal), filtered, taken to a small vol (*ca.* 50 ml), and cooled, and the yellow crystals (7.5 g, 75%), mp 164–166°, were filtered, washed with hexane, and dried. This compd was quite pure and the color became darker on exposure to light.

4-Cyano-5-monoacetylamino-3-(5-nitro-2-furyl)isoxazole (II-1) was made by treating **I-5** (33.0 g, 0.15 mole) with NaH (6.8 g, 0.165 mole) in dry THF (500 ml) and heating the resulting dark red mixt with Ac_2O (62 g, 0.60 mole) under reflux for 2.5 hr. Concn, trituration, and filtration from ice H_2O gave 30.2 g (77%) of faint yellow crystals, mp 237–240° (AcOH).

4-Cyano-5-methylacetylamino-3-(5-nitro-2-furyl)isoxazole (II-9).—**II-1** (2.1 g, 0.008 mole) was treated with NaH (0.36 g, 0.0088 mole) in dry THF (30 ml), followed by heating under reflux for 2 hr with MeI (5.7 g, 0.04 mole), to give 1.8 g (82%) of yellow crystals, mp 135–138°. Recrystn from EtAcO gave almost white needles, mp 145–148°.

4-Cyano-5-monomethylamino-3-(5-nitro-2-furyl)isoxazole (I-6).—A soln of NH_3 in dry MeOH (25 ml of a 6 *M* soln, 0.15 mole) was added to a soln of **II-9** (4.7 g, 0.017 mole) in dry THF (125 ml). After 2 hr at room temp, the mixt was concd under reduced pressure, the resulting solid was washed with small amts of MeOH and dried, and 3.6 g (90%) of a yellow solid was obtained. Crystn from EtAcO gave light yellow silky needles, mp 228–231°.

Methyl 5-Monoacetylamino-3-(5-nitro-2-furyl)isoxazole-4-carboxylate (III-1) and Methyl 5-Diacetylamino-3-(5-nitro-2-furyl)isoxazole-4-carboxylate (III-10).—Ac_2O (200 ml) and **I-12** (20 g, 0.078 mole) were heated under reflux for 4 hr, after which

the mixt was concd under reduced pressure. The resulting black semisolid was shaken with cold EtAcO (100 ml) and filtered when a grey powder (6.0 g) was obtd. This solid on crystn from EtAcO gave 3.5 g of **III-1**, mp 151–153°.

The combined EtAcO filtrates on concn under reduced pressure gave 22.2 g of a dark brown solid. This solid was eluted from a silica gel (Fisher, 700 ml) column using $CHCl_3$–EtAcO mixts as eluants. Fractions (500 ml) were collected and monitored from the weight, ir spectrum, and mp of the eluted material in each fraction.

III-10 (3.5 g) was collected in the early fractions, as a light yellow solid, mp 143–147°. The structure was established unequivocally from the spectral (ir and nmr) and elemental anal. data.

Later fractions gave 7.8 g of **III-1** as light yellow crystals, mp 151–153°, identical (mmp, ir and nmr spectra) with the sample obtd previously. In this case also the structure was established from the spectral (ir and nmr) and elemental anal. results.

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Synthesis of Indoles from 4-Oxo-4,5,6,7-tetrahydroindoles. 4. Tricyclic Heterocycles

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Condensation of 1-benzoyl-5-bromo-4-oxo-4,5,6,7-tetrahydroindole with substituted thioureas gave 2-amino-4,5-dihydro-6*H*-pyrrolo[3,2-*e*]benzothiazoles, which were dehydrogenated with phenyltrimethylammonium tribromide and debenzoylated. Two of these compds showed good activity in the carrageenin antiinflammatory assay. Examples of the tetrahydropyrrolo[4,3,2-*de*]cinnoline and tetrahydropyridazino[5,4,3-*de*]cinnoline systems were also prepared.

Recently we described a new method of indole synthesis, based upon 4-oxo-4,5,6,7-tetrahydroindoles, which is particularly suitable for fusion of heterocycles to the 4,5-indole positions.[1] The resulting tricyclic systems are intuitively appealing as potential pharmacologically active molecules. When one system of this type, the pyrrolindazole, showed significant activity in standard analgetic and inflammatory assays,[2] we decided to prepare a variety of other tricyclic heterocycles incorporating the indole nucleus.

As previously reported,[1] condensation of 1-benzoyl-5-bromo-4-oxo-4,5,6,7-tetrahydroindole **1a** with substituted thioureas afforded 2-amino-4,5-dihydro-6*H*-pyrrolo[3,2-*e*]benzothiazoles. Preliminary indications of antiinflammatory activity prompted us to prepare a variety of compds of this type (**2a–2e** in Scheme I). For the synthesis of *N*-methylpiperazino derivative **2e** the appropriate thiourea (4-methyl-1-piperazinethiocarboxamide) was required. This compd was conveniently made by condensation of benzoyl isothiocyanate with *N*-methylpiperazine, followed by acid hydrolysis (Experimental Section). Removal of Bz groups from the tricyclic compounds **2a–2e** by alkaline hydrolysis was possible only when there was no H on the 2-amino N (**2d** → **4a** and **2e** → **4b**). Hydrolysis products could not be isolated in the other cases due to their instability.

Dehydrogenation of **4a** with 2,3-dichloro-5,6-dicyanobenzoquinone furnished the fully aromatic derivative **3d** in low yield. However, attempts to dehydrogenate Bz-substituted compounds with this reagent were unsuccessful. After trying some other standard reagents we discovered that phenyltrimethylammonium tribromide was very effective for this type of dehydrogenation. Thus **2b** and **2d** were converted into **3a** and **3b**, resp, in high yields (97% for **3b**). At this time the scope and mechanism of dehydration with $PhN^+Me_3 \cdot Br_3^-$ is not known. However, a bromination–dehydrobromination process seems likely.

In contrast to certain of the dihydro precursors, the fully aromatic compd **3a** was debenzoylated to **3c** which has a H on the 2-amino N. As expected **3b** was readily converted into **3d**.

It was also possible to prepare a 3,4-fused heterocyclic derivative of indole. Thus, treatment of 4-oxo-4,5,6,7-tetrahydroindole-3-carboxamide (**5**) with hydrazine gave pyrrolo[4,3,2-*de*]cinnoline (**7**). A related pyridazino[5,4,3-*de*]cinnoline (**8**) was obtained when methyl 4-oxo-4,5,6,7-tetrahydrobenzofuran-3-carboxylate (**6**) was heated with hydrazine. This reaction gave a second product, $C_8H_{12}N_4O_2$, which corresponds to the reaction of **6** with 2 molecules of hydrazine followed by one cyclization. We are unable to deduce an unambiguous structure for this product from its spectra (Experimental Section).

Biological Activities.—Many of the compds described in this note showed *in vitro* activity against certain bacteria and fungi (Table I). However, this activity was generally at higher test levels. The most active compd was the *N*-benzenesulfonyl analog **1b**.[1] This compd was especially effective against dermatophytes. It was tested topically as a 1% ointment against *Microsproum canis* ATCC 10214 infection on rats, but was inactive in this assay.

The 2 compds most active in the carrageenin antiinflammatory assay in rats were **4a** and **4b**. They gave the following *C/T* ratios for the mean edema volume of paws of 8 control rats divided by that of 2

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