Studies in Isoxazole Chemistry. I. 3- or 5-(5-Nitro-2-furyl)-5- or -3-methylisoxazoles

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Several 5-methyl-3-(5-nitro-2-furyl)isoxazoles and their "flip" isomers, 3-methyl-5-(5-nitro-2-furyl)isoxazoles, have been synthesized and their antibacterial, antitrichomonal, and lysogenic activities have been determined. The antitrichomonal activity of several members of the dialkylaminoalkyl ester series is considerably better than that of 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole and these compounds are characterized by low toxicities. The nmr spectrum is shown to be a convenient method of distinguishing between isomer pairs.

Although nitrofurfural was described in 1930,[1] it was only in 1944 that the antibacterial activity of this system was discovered,[2] and in 1950 the suggestion was made that the substituent -C=NNC= in the 2 position of 5-nitrofuran was necessary for *in vivo* activity.[3] Since then, the literature is full of examples of nitrofurfurylidene derivatives and their vinylogs.[4] More recently attention has been focussed on compounds in which the nitrofuran ring is directly attached to another heterocyclic ring.[5,6] Among the simple heteroaromatic five-membered rings described in the literature are pyrazole,[7,8] isoxazole,[7,9-13] thiazole,[14-16] 1,2,4-oxadiazole,[15,17-19] 1,3,4-oxadiazole,[20,21] 1,2,4-thiadiazole,[15] 1,3,4-thiadiazole,[20,22] and tetrazole.[23] At the time this work was started, the only nitrofurylisoxazole described was methyl 5-methyl-3-(5-nitro-2-furyl)-isoxazole-4-carboxylate and the corresponding acid.[9]

Chemistry.—The various 3-(5-nitro-2-furyl)isoxazole derivatives were made by the reactions illustrated in Scheme I. In many cases, the reaction of 3-(5-nitro-2-furyl)-5-methylisoxazole-4-carbonyl chloride with an alcohol or amine also produced varying amounts of an insoluble by-product, whose identity has not been established.

The 3-methyl-5-(5-nitro-2-furyl)isoxazoles were obtained by the two routes illustrated in Scheme II.

In most cases the compounds were light sensitive, becoming yellow or brown on exposure to sunlight.

Nmr Spectra.—In the case of every compound, the identity was confirmed by its ir and nmr spectrum. In every case the integrated area ratio was found to be an excellent index of the purity of the compound.

Doyle and his coworkers[9] have pointed out the fact that isomeric pairs of isoxazoles have similar melting points and have employed their uv absorption spectra to distinguish between them. The nmr spectra of isomeric pairs of isoxazoles also offers a convenient method of distinguishing between them. Thus the nmr spectrum of methyl 3-furyl-5-methylisoxazole-4-carboxylate ($CDCl_3$) shows the 5-CH_3 signal as a sharp singlet at τ 7.31, while the 3-CH_3 signal of the isomeric methyl 5-furyl-3-methylisoxazole-4-carboxylate is found at τ 7.50. This same trend is also shown by the nitrofurylisoxazoles.

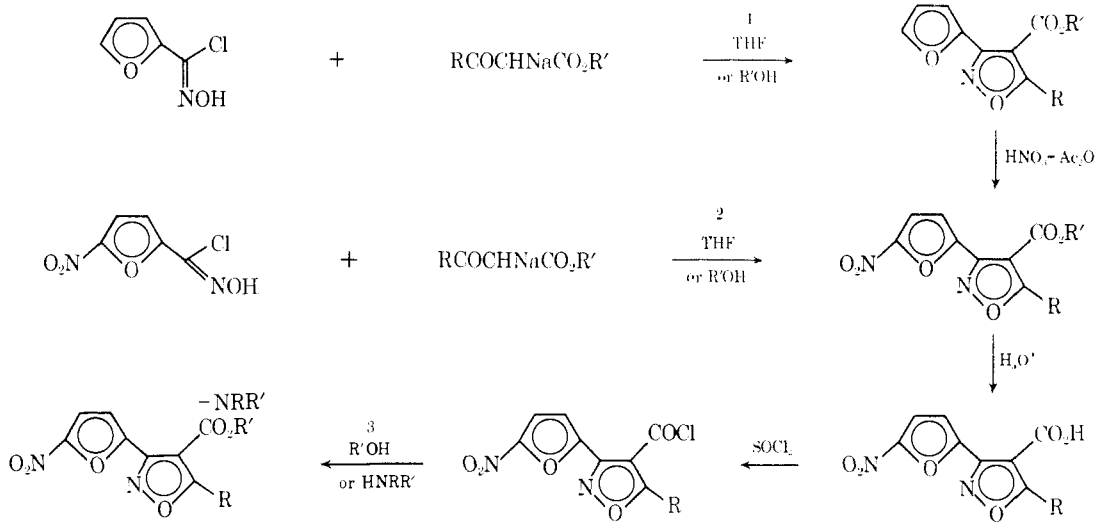
In the reaction of 5-nitro-2-furylchloraldoxime with sodio-1-methoxypentane-2,4-dione, two products are possible, *viz.*, 5-methyl-3-(5-nitro-2-furyl)-4-methoxyacetylisoxazole and 5-methoxymethyl-3-(5-nitro-2-furyl)-4-acetylisoxazole. From the reaction a pale yellow crystalline compound was obtained in 25% yield from MeOH (Table IV, **6**). The nmr spectrum of this compound showed that it was pure and probably 5-methoxymethyl-3-(5-nitro-2-furyl)-4-acetylisoxazole: τ 2.60 (s, nitrofuran 3,4 H's), 5.18 (s, 5-CH_2O–), 6.52 (s, –OCH_3), and 7.42 (s, 4-COCH_3). The residue from the MeOH filtrate consisted of about 70% of the same isomer with 30% of 5-methyl-3-(5-nitro-2-furyl)-4-methoxyacetylisoxazole, from the nmr, since there were additional signals at τ 5.64 (4-COCH_2O), 6.74 (–OCH_3), and 7.30 (5-CH_3). Since the microbiological activity of the mixture was the same as the purified isomer, no further separation was attempted.

Biological Screening Results.—The biological screening of these compounds was performed by Dr. A. Gourovitch and Dr. Ken Price and their associates in the Microbiology Department of Bristol Laboratories, Syracuse, N. Y. Antitrichomonal activity was evaluated using known procedures.[24] The lysogenic activity (minimum inducing dose, MID) was estimated from the ability of the compound to induce phage production in lysogenic bacteria (*E. coli* W1709[λ]). Antibacterial activity was evaluated using standard procedures. The data are summarized in Tables I–IV.

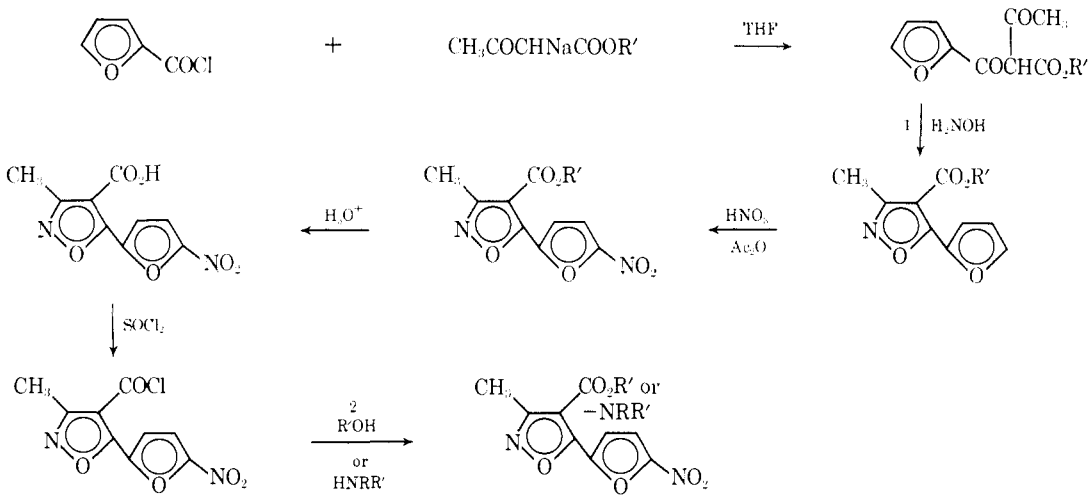
The following general conclusions are drawn from

(1) H. Gilman and G. F. Wright, *J. Am. Chem. Soc.*, **52**, 2550 (1930).
(2) M. C. Dodd and W. B. Stillman, *J. Pharmacol. Exptl. Therap.*, **82**, 11 (1944).
(3) M. C. Dodd, D. L. Cramer, and W. C. Ward, *J. Am. Pharm. Assoc.*, **39**, 313 (1950).
(4) For recent reviews on the nitrofurans see H. E. Paul and M. F. Paul, *Exptl. Chemotherapy*, **2**, 307 (1964); **4**, 521 (1966).
(5) H. A. Burch, *J. Med. Chem.*, **11**, 79 (1968), and previous papers.
(6) P. M. G. Bavin, *ibid.*, **9**, 788 (1966).
(7) R. G. Haber and E. Schoenberger, Netherlands Appl 6,504,329 (Oct 6, 1965); *Chem. Abstr.*, **64**, 12682*h* (1966).
(8) A. A. Ponomarev and L. V. Cherkesova, *Zh. Obshch. Khim.*, **33**, 3946 (1963); *Chem Abstr.*, **60**, 10648*e* (1964).
(9) F. P. Doyle, J. C. Hanson, A. A. W. Long, and J. H. C. Nayler, *J. Chem. Soc.*, 5845 (1963).
(10) Geigy, Netherlands Appl 66,11584 (Aug 18, 1965).
(11) M. Giannella, F. Gualtieri, and M. Pigini, *Farmaco* (Pavia), **22**, 333 (1967).
(12) S. Minami and J. Matsumoto, *Chem. Pharm. Bull.* (Tokyo), **15**, 366 (1967).
(13) J. Matsumoto and S. Minami, *ibid.*, **15**, 1806 (1967).
(14) W. R. Sherman and D. E. Dickson, *J. Org. Chem.*, **27**, 1351 (1962).
(15) W. R. Sherman and A. von Esch, *J. Med. Chem.*, **8**, 25 (1965).
(16) M. Portelli and G. Bartolini, *Ann. Chim.* (Rome), **53**, 1180 (1963); *Chem. Abstr.*, **60**, 8011*a* (1964).
(17) Abbott Laboratories, British Patent 1,025,439 (April 6, 1966); *Chem. Abstr.*, **64**, 19631*b* (1966).
(18) Union Carbide Corp., U. S. Patent 3,218,331 (Nov 16, 1965); *Chem. Abstr.*, **64**, 3552*f* (1966).
(19) R. Lenaers and F. Eloy, *Helv. Chim. Acta*, **118**, 1067 (1963).
(20) W. R. Sherman, *J. Org. Chem.*, **26**, 88 (1961).
(21) A. A. Ponomarev and Z. V. Til, *Zh. Obshch. Khim.*, **33**, 2368 (1963); *Chem. Abstr.*, **59**, 15278*b* (1963).
(22) Eisai Co., Japanese Patent 24,805 (1964); *Chem. Abstr.*, **63**, 9957*g* (1965).
(23) H. R. Snyder, Jr., *J. Med. Chem.*, **10**, 737 (1967).
(24) R. J. Schnitzer, *Exptl. Chemotherapy*, **1**, 289 (1963).

SCHEME I



SCHEME II



the available data. (1) The methyl group in the 5 position of the isoxazole ring is essential for antibacterial and antitrichomonal activity (Table I). (2) The dialkylaminoalkyl esters (Table IIb) have much better antitrichomonal activity than the other esters (Table IIa), and this activity is lost when the amino ester is quaternized (Table IIc). (3) The amides (Table IId), although more active than the esters (Table IIa) against the *Trichomonas* species, were less active than the dialkylaminoalkyl esters (Table IIb). (4) The "flip" compounds (Table III) are, in general, comparable in activity to their isomers. One significant difference is the diethylaminoethyl ester (Table III, **4**) which has an oral ED_{50} of 33 mg/kg compared to 4 mg/kg for its isomer (Table IIb, **2**). It should be noted that the corresponding oral ED_{50} for 1-(2-hydroxyethyl)-2-methyl-5-nitroimidazole[25] (the only systemic trichomonacide described in "New Drugs"[26]) under identical conditions is 36 mg/kg against *Trichomonas foetus*. (5) All the compounds tested showed a high degree of lysogenic activity. (6) Antibacterial activity is limited to the lower alkyl esters. All the other compounds were relatively inactive against *Staphylococcus aureus* Smith, *Proteus vulgaris*, and *Candida albicans* and hence these data are not included in the tables. (7) The only toxic compounds in the entire series were the two monomethylamides (Table IId, **2**, and Table III, **6**) with LD_{50} values of 30 mg/kg, and the N-methylpiperidin-4-yl ester (Table IIb, **12**) with an LD_{50} of 200 mg/kg. All the other compounds tested had LD_{50}'s > 200 mg/kg.

(25) Metronidazole supplied by G. D. Searle and Co.

(26) "New Drugs," Evaluated by the A. M. A. Council on Drugs, American Medical Association, Chicago, Ill., 1967, p 85.

Experimental Section[27]

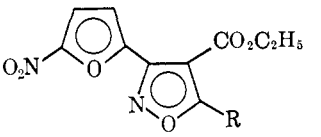
Representative examples are described in detail.

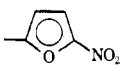
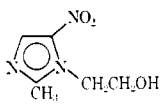
Methyl 3-Furyl-5-methylisoxazole-4-carboxylate.—Nitrosyl chloride (25 ml, *ca.* 0.6 mole) was added slowly to a stirred, cold (−10°) solution of furfuraldoxime (55.5 g, 0.5 mole) in dry Et_2O (1.5 l.). A solid separated immediately and slowly dissolved with gas evolution. The mixture was left at room temperature overnight and then concentrated under reduced pressure, when a red oil was obtained. This oil (the chlorooxime) was used as such without further purification. It became black and tacky on standing at room temperature and exposure to air.

Methyl acetoacetate (58 g, 0.5 mole) was added slowly to an

(27) All temperatures are uncorrected. Ir spectra were obtained on a Perkin-Elmer Infracord spectrophotometer Model 137 and nmr spectra with a Varian Associates Model A-60 spectrometer. The nmr and ir spectra of all compounds were measured and were as expected. Where analyses are indicated by symbols of elements, analytical results obtained for these elements were within ±0.4% of the theoretical values. All new compounds in the tables were analyzed for C, H, and N except where specified.

TABLE I



No.	R	Mp, °C	Method of prepn	Yield, %	Antitrichomonal act. ID50, μg/ml T. foetus	ID50, μg/ml T. vaginalis	ED50, mg/kg, T. foetus Im	ED50, mg/kg, T. foetus Oral	Lysogenic MID, μg/ml	Formula
1	H	54–56	2	20	0.34	0.14	100	200	0.01	$C_{10}H_8N_2O_6$
2	CH_3	81–82[a]	2	65	0.34	0.27	37	62	0.15	$C_{11}H_{10}N_2O_6$
3	C_6H_5	100–102[b]	2	52	2.2	0.15	>200	>200	10	$C_{16}H_{12}N_2O_6$
4		116–117	2	50	0.62	0.24	>200	>200	0.6	$C_{14}H_{10}N_2O_7$
5		131–132	4	40	0.32	0.045	>200	>200	0.01	$C_{14}H_9N_3O_9$
Metronidazole					0.17	0.18	26	36	>80	

[a] Lit.[13] mp 81–82°, 45%. [b] Lit.[13] mp 99–100°, 32%.

ice-cold MeOH solution of NaOMe, made by dissolving Na (11.5 g, 0.5 g-atom) in dry MeOH (600 ml). The chlorooxime obtained above was dissolved in dry MeOH (600 ml) and added slowly to the solution of methyl sodioacetoacetate at −35°. The reaction mixture was allowed to come to room temperature and left overnight, after which time it was concentrated under reduced pressure, ice water (300 ml) was added, and it was extracted with EtOAc (three 300-ml portions). The combined EtOAc layers were dried ($MgSO_4$) and concentrated. The resulting black sticky solid was extracted with hot hexane (four 500-ml portions). The hot extract was treated with decolorizing charcoal, filtered, concentrated, and cooled, when 63 g (60%) of light yellow crystals, mp 85–90°, was obtained, mp 99–101° (hexane). *Anal.* ($C_{10}H_9NO_4$) C, H, N.

***t*-Butyl 5-Methyl-3-(5-nitro-2-furyl)isoxazole-4-carboxylate (Table IIa, 8).**—5-Nitrofurfurylidene diacetate[1,28] (24.3 g, 0.1 mole), $H_2NOH \cdot HCl$ (8.4 g, 0.12 mole), and aqueous HCl (300 ml of a 3 *M* solution) were heated with stirring under reflux until complete solution and for 30 min longer. The resulting yellow solution was cooled with stirring in an ice bath, and the yellow crystals were filtered, washed with ice water (*ca.* 200 ml), and dried in an oven at 40°. The resulting yellow crystals, mp 155–162°,[29,30] weighed 12.6 g (81%). This oxime is soluble in Et_2O, but not appreciably soluble in $CHCl_3$ or CH_2Cl_2. The oxime was used as such without further purification.

An ice-cold solution of nitrosyl chloride (10.0 g, 0.154 mole) in anhydrous Et_2O (100 ml) was added to an ice-cold solution of 5-nitrofurfuraldoxime (21.8 g, 0.14 mole) in Et_2O (*ca.* 300 ml) and the mixture was allowed to come to room temperature. A solid separated and there was a slow evolution of gas. The reaction mixture was left overnight at room temperature and filtered from a small amount of yellow solid. The Et_2O was removed under reduced pressure, hexane (200 ml) was added, and the yellow solid was filtered and dried under vacuum at room temperature. The yellow solid, mp 132–143°,[19] weighed 22.0 g (85%) and was sufficiently pure for the next reaction. It should preferably be used as soon as possible but may be stored under refrigeration for a day or two before use. *t*-Butyl acetoacetate[31] (18.3 g, 0.116 mole) was added to an ice-cold MeOH solution of NaOMe, obtained by dissolving Na (2.7 g, 0.116 g-atom) in dry MeOH (140 ml). The resulting solution was added to a cold (−35°) well-stirred solution of 5-nitro-2-furylchloraldoxime (22 g, 0.115 mole) in MeOH (140 ml). The mixture was allowed to come to room temperature and left overnight, when a yellow solid suspended in a dark red solution was obtained. The MeOH was removed under reduced pressure, water (150 ml) was added, and the mixture was extracted with Et_2O (five 100-ml portions). The combined Et_2O extract was washed with brine, dried ($MgSO_4$), and filtered, and solvent was removed under reduced pressure, when a thick brown wax, 29 g (86%), crystallizing on scratching, was obtained. Recrystallization from hexane gave 16.6 g (49%) of light yellow needles, mp 73–74°. The nmr spectrum indicated no evidence of transesterification.

5-Methyl-3-(5-nitro-2-furyl)isoxazole-4-carboxylic Acid (Table IIa, 1).—*t*-Butyl 5-methyl-3-(5-nitro-2-furyl)isoxazole-4-carboxylate (2.9 g, 0.01 mole) and concentrated H_2SO_4 (10 ml) were mixed and heated on a water bath at 90°, with stirring for 15 min, by which time 39 ml of gas was collected. The mixture was poured into ice–water (150 ml), stirred, and filtered. The solid thus obtained was dissolved in Et_2O (250 ml) and the resulting solution was washed with brine and dried ($MgSO_4$) and solvent was removed under reduced pressure, when 2.1 g (88%) of a faint yellow solid was obtained, mp 200–201°.[9]

5-Methyl-3-(5-nitro-2-furyl)isoxazole-4-carbonyl Chloride.—5-Methyl-3-(5-nitro-2-furyl)isoxazole-4-carboxylic acid (3 g, 0.012 mole) and $SOCl_2$ (20 ml) were heated under reflux for 5 hr and the excess $SOCl_2$ was removed under reduced pressure. The resulting solid crystallized from C_6H_6–hexane as off-white needles, mp 95–98°, 2.1 g (70%).

β-N,N-Diethylaminoethyl 5-Methyl-3-(5-nitro-2-furyl)isoxazole-4-carboxylate (Table IIb, 2).—A solution of β-N,N-diethylaminoethanol (59.0 g, 0.5 mole) in anhydrous Me_2CO (400 ml) was added to a stirred ice-cold solution of 5-methyl-3-(5-nitro-2-furyl)isoxazole-4-carbonyl chloride (65.0 g, 0.25 mole) in Me_2CO (2 l.). The reaction mixture was left at room temperature overnight and the Me_2CO was removed under reduced pressure. Ice–water (1 l.) was added and the mixture was extracted with EtOAc (three 300-ml portions). The combined EtOAc extracts were dried ($MgSO_4$) and concentrated under reduced pressure when an oil resulted. The oil was extracted with hot hexane (four 500-ml portions). Light yellow needles (73.0 g, 87%), mp 40–41°, were obtained from the hexane solution on concentration and cooling.

β-N,N,N-Triethylammoniumethyl 5-Methyl-3-(5-nitro-2-furyl)isoxazole-4-carboxylate (Table IIc, 1).—A mixture of β-N,N-diethylaminoethyl 5-methyl-3-(5-nitro-2-furyl)isoxazole-4-carboxylate (3.4 g, 0.01 mole) and EtI (15.6 g, 0.1 mole) in dry THF (40 ml) was heated with stirring, under reflux for 24 hr, and the resulting solid was filtered, washed with Et_2O, and dried. There was thus obtained 1.7 g (35%) of yellow crystals, mp 155–157°.

Isopropyl 5-Methyl-3-(5-nitro-2-furyl)isoxazole-4-carboxylate (Table IIa, 6).—A mixture of 5-methyl-3-(5-nitro-2-furyl)isoxazole-4-carbonyl chloride (2.05 g, 0.008 mole) and *i*-PrOH (5 ml) was heated on a steam bath for 2 hr and the mixture was con-

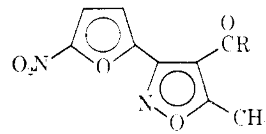
(28) Available commercially from Raylo Chemicals Ltd., Edmonton 82, Alberta, Canada, and Gallard-Schlesinger Chemical Mfg. Corp., Carle Place, L. I., N. Y. 11514.

(29) R. Raffauf, *J. Am. Chem. Soc.*, **68**, 1765 (1946).

(30) G. Gever, *J. Org. Chem.*, **23**, 754 (1958).

(31) Available from Eastman Chemical Products, Inc., Kingsport, Tenn. 37662.

Table II



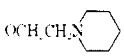
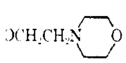
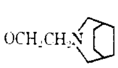
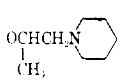
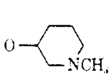
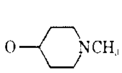
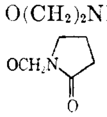
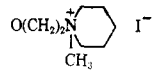
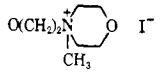
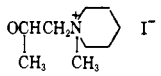
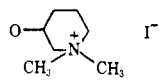
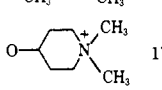
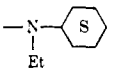
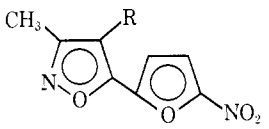
No.	R	Mp, °C	Method of prepn	Yield, %	ID$_{50}$, µg/ml *T. foetus*	ID$_{50}$, µg/ml *T. vaginalis*	Antitrichomonal act. ED$_{50}$, mg/kg, *T. foetus* Im	Antitrichomonal act. ED$_{50}$, mg/kg, *T. foetus* Oral	Lysogenic MID, µg/ml	Formula
	a. Alkyl and Aryl Esters									
1	OH	200–201[a]	2	88	90	18	>400		>40	$C_9H_6N_2O_6$
2	OMe	130–132[b]	1	30	0.45	0.19	7.5	14	0.6	$C_{10}H_8N_2O_6$
			2	65						
3	OEt	81–82[c]	2	65	0.34	0.27	37	62	0.15	$C_{11}H_{10}N_2O_6$
4	O-*n*-Pr	47–49	3	53	0.30	0.07	58		0.15	$C_{12}H_{12}N_2O_6$
5	O-*n*-Bu	44–45	3	35	0.16	4.0	35	60	0.15	$C_{13}H_{14}N_2O_6$
6	O-*i*-Pr	79–80	3	80	3.5	0.5	70	>200		$C_{12}H_{12}N_2O_6$
7	$OCH(CH_3)C_2H_5$	62–63	3	70	3.5	1.8	74	100		$C_{13}H_{14}N_2O_6$
8	O-*t*-Bu	73–74	2	49	1.9	1.5	>200		10	$C_{13}H_{14}N_2O_6$
9	OCH_2CH_2Cl	75–76	3	80	0.9	0.2	38	101		$C_{11}H_9ClN_2O_6$
10	$OCH_2C_6H_5$	95–97	3	41	1.5	0.1		160		$C_{16}H_{12}N_2O_6$
11	OC_6H_5	101–102	3	86	0.7	0.2		>200		$C_{15}H_{10}N_2O_6$
	b. Dialkylaminoalkyl Esters									
1	$O(CH_2)_2N(CH_3)_2$	49–50	3	62	1.7	0.8	2.5	45		$C_{13}H_{15}N_3O_6$
2	$O(CH_2)_2N(C_2H_5)_2$	40–41	3	87	0.5	0.32	3.5	4	0.01	$C_{15}H_{19}N_3O_6$
3	$S(CH_2)_2N(C_2H_5)_2$	46–48	3	28	2.5	2.5	43	>200	0.15	$C_{15}H_{19}N_3O_5S$
4	$OCH(CH_3)CH_2N(CH_3)_2$	46–47	3	38	0.8	0.33	6	6	0.15	$C_{14}H_{17}N_3O_6$
5	$OCH(CH_3)CH_2N(C_2H_5)_2$	47–48	3	36	2.7	0.95	6	3.5	0.15	$C_{16}H_{21}N_3O_6$
6	$OCH_2C(CH_3)_2N(CH_3)_2$	106–107	3	60	0.25	0.44	1.3	3.7	0.04	$C_{15}H_{19}N_3O_6$
7		62–63	3	74	0.9	0.4		35		$C_{16}H_{19}N_3O_6$
8		103–105	3	64	0.38	0.078		45		$C_{15}H_{17}N_3O_7$
9		92–93	3	38	0.05	0.05	3.8	102		$C_{19}H_{23}N_3O_6$
10		86–88	3	56	1.8	0.7		131		$C_{17}H_{21}N_3O_6$
11		88–90	3	64	0.23	0.19		35		$C_{15}H_{17}N_3O_6$
12		75–76	3	46	0.1	0.1		8.2		$C_{15}H_{17}N_3O_6$
13	$O(CH_2)_3N(CH_3)_2$	55–58	3	50	0.14	0.12	2.5	3.5	0.0025	$C_{14}H_{17}N_3O_6$
14	$O(CH_2)_2NHCOCH_3$	146–148	3	23	1.2	0.5		28		$C_{13}H_{13}N_3O_7$
15		134–136	3	51	2.5	3.9	88	>200		$C_{14}H_{13}N_3O_7$
	c. Dialkylammoniumalkyl Esters									
1	$O(CH_2)_2\overset{+}{N}(C_2H_5)_3\ I^-$	155–157	4	35	4.4	6.0		90		$C_{17}H_{24}IN_3O_6$
2	$-O(CH_2)_2\overset{+}{N}(C_2H_5)_2-CH_3\ I^-$	197–199	4	90	12	16		>200		$C_{16}H_{22}IN_3O_6$
3	$-OCH_2C(CH_3)_2\overset{+}{N}(CH_3)_3\ I^-$	180–183 dec	4	54	4.8	12.0		140		$C_{16}H_{22}IN_3O_6$

TABLE II (*Continued*)

No.	R	Mp, °C	Method of prepn	Yield, %	Antitichromonal act.— ID_{50}, μg/ml *T. foetus*	ID_{50}, μg/ml *T. vaginalis*	ED_{50}, mg/kg, *T. foetus* Im	ED_{50}, mg/kg, *T. foetus* Oral	Lysogenic MID, μg/ml	Formula
4		220–222 dec	4	88	12.5	13				$C_{17}H_{22}IN_3O_6$
5		215–217 dec	4	80	3.5	2.5		>200		$C_{16}H_{20}IN_3O_7$
6		209–211 dec	4	91	14	11				$C_{18}H_{24}IN_3O_6$
7		244–245 dec	4	90	>20	>20		>200		$C_{16}H_{20}IN_3O_6$
8		210–213	4	90	14	12		>200		$C_{16}H_{20}IN_3O_6$
					d. AMIDES					
1	NH_2	220–222	3	93	1.05	0.16	20	20	0.15	$C_9H_7N_3O_5$
2	NHMe	156–158	3	60	0.63	0.2	4	6	0.6	$C_{10}H_9N_3O_5$
3	NMe_2	140–141	3	80	1.8	0.5	10	15	10	$C_{11}H_{11}N_3O_5$
4	NH-*t*-Bu	110–111	3	71	3.5	1.1	17	17		$C_{13}H_{15}N_3O_5$
5		164–166	3	82	3.0	0.25	3.5	6.2	20	$C_{13}H_{13}N_3O_6$
6		158–160	3	88	2.4	3.3	41	41		$C_{17}H_{19}N_3O_5$
7	NHC_6H_5	209–211	3	63	3.1	1.0		100		$C_{15}H_{11}N_3O_5$
8	$NHNMe_2$	183–185 dec	3	99	3.2	0.15	28	18	5	$C_{11}H_{12}N_4O_5$
9		110–112	3	91	3.5	1.7	106	>200		$C_{17}H_{21}N_3O_5$

[a] Lit.[9] mp 196–198°, 90%. [b] Lit.[9] mp 125–126°, 64%; lit.[13] mp 121–123°, 51%. [c] Lit.[13] mp 81–82°, 45%.

TABLE III



No.	R	Mp, °C	Method of prepn	Yield, %	Antitrichomonal act.— ID_{50}, μg/ml *T. foetus*	ID_{50}, μg/ml *T. vaginalis*	ED_{50}, mg/kg, *T. foetus* Im	ED_{50}, mg/kg, *T. foetus* Oral	Lysogenic MID, μg/ml	Formula
1	COOH	210–211	1	86	15	16		>200		$C_9H_6N_2O_6$
2	$COOCH_3$	130–133	1	34	0.28	0.045	5	25	0.6	$C_{10}H_8N_2O_6$
3	$COOC_2H_5$	64–66	1	59	0.58	0.035	17	48	0.15	$C_{11}H_{10}N_2O_6$
4	$COO(CH_2)_2NEt_2$	65–67	2	68	0.28	0.15	2.6	33	0.15	$C_{15}H_{19}N_3O_6$
5	$CONH_2$	197–200	2	54	1.7	0.82	5.8	13	0.15	$C_9H_7N_3O_5$
6	$CONHCH_3$	160–163	2	65	0.74	0.3	3.6	13	0.6	$C_{10}H_9N_3O_5$
7	$CON(CH_3)_2$	153–156	2	31	1.1	0.3	3.6	4	10	$C_{11}H_{11}N_3O_5$

centrated under reduced pressure. The residue was extracted with *ca.* 600 ml of hot hexane and the insoluble by-product was rejected. The hexane solution, on concentration and cooling, gave 1.8 g (80%) of light yellow needles, mp 79–80°.

5-Methyl-3-(5-nitro-2-furyl)isoxazole-4-carboxamide (Table IId, 1).—5-Methyl-3-(5-nitro-2-furyl)isoxazole-4-carbonyl chloride (12.8 g, 0.05 mole) was dissolved in dry Me_2CO (500 ml) and concentrated NH_3 (15 ml of 14 *M* solution, 0.20 mole) was added. There was a mild exothermic reaction and a white solid began to separate almost immediately. The mixture was stirred for 2 hr, filtered, washed with ice-water, and dried, giving 11.0 g (95%) of a white solid, mp 220–222° dec.

4-Cyano-5-methyl-3-(5-nitro-2-furyl)isoxazole (Table IV, 1).—A mixture of 5-methyl-3-(5-nitro-2-furyl)isoxazole-4-carboxamide (5.9 g, 0.025 mole) and $SOCl_2$ (50 ml) was heated under reflux for 24 hr (until the ir spectrum showed no amide carbonyl absorption) and concentrated under reduced pressure. The residue crystallized from C_6H_6–hexane as light yellow crystals, 3.6 g (65%), mp 99–101°.

4-Bromoacetyl-5-methyl-3-(5-nitro-2-furyl)isoxazole (Table IV, 3).—Well-powdered phenyltrimethylammonium bromide (10.8 g, 0.05 mole) was dissolved in dry THF (200 ml) and the solution cooled in an ice bath. To this stirred solution, Br_2 (8.0 g, 0.05 mole) was added dropwise[32,33] and 4-acetyl-5-methyl-3-(5-nitro-2-furyl)isoxazole (11.8 g, 0.05 mole) was added immediately after. The reaction mixture was heated in a water bath at 60°. After *ca.* 15 min the color of the solution had changed from brown to light yellow, with the separation of a white solid. After 1 hr at this temperature the mixture was filtered and the solid was washed with Et_2O. The filtrate and washings were concentrated under reduced pressure and the resulting viscous oil was taken up in cold MeOH (50 ml). A light yellow solid separated and was filtered, washed with hexane,

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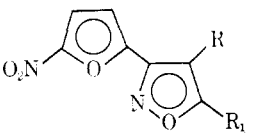
(33) A. Marquet and J. Jacques, *Bull. Soc. Chim. France*, 90 (1962)

TABLE IV



No.	R	R_1	Mp, °C	Method of prepn	Yield, %	Antitrichomonal act. ID_{50}, μg/ml *T. foetus*	*T. vaginalis*	ED_{50}, mg/kg, *T. foetus* Im	Oral	Lysogenic MID, μg/ml	Formula
1	CN	CH_3	99–101	4	65	0.2	0.011	4.5	16	0.01	$C_5H_5N_3O_4$
2	$COCH_3$	CH_3	111–112[a]	2	70	0.52	0.1	8.4	28	0.01	$C_{10}H_8N_2O_5$
3	$COCH_2Br$	CH_3	80–81	4	83						$C_{10}H_7BrN_2O_5$ $C_{11}H_{11}N_5O_4S$
4	$C(CH_3)=NNHCSNH_2$	CH_3	232–234 dec[b]	4	25	2.3	1.3	>200	>200	10	
5	$C(CH_3)=NNHCOOCH_3$	CH_3	224–225	4	17	0.1	0.05		3.5		$C_{11}H_{12}N_4O_6$
6	$COCH_3$	CH_2OCH_3	75–76	2	25	1.5	0.2		18		$C_{10}H_{10}N_2O_6$
7		CH_3	200–205 dec	4	67						$C_{11}H_8N_4O_4S$
8	$COCH_3$	$COOCH_3$	87–88	2	80	>20	7.4				$C_{11}H_8N_2O_7$
9	CN	C_6H_5	176–178[c]	2	36	0.1	<0.05		131		$C_{14}H_7N_3O_4$
10	$COCH_3$	C_6H_5	136–138[d]	2	65	2.8	0.2	>200	>200	0.6	$C_{15}H_{10}N_2O_5$

[a] Lit.[13] mp 111–113°, 58%. [b] Lit.[11] mp 233–237° dec. [c] Lit.[13] mp 178–180°, 56%. [d] Lit.[13] mp 131–132°, 40%.

and dried. Recrystallization from MeOH gave 13.0 g (83%) of yellow crystals, mp 80–81°.

5-Methyl-4-(2-amino-4-thiazolyl)-3-(5-nitro-2-furyl)isoxazole (Table IV, 7).—4-Bromoacetyl-5-methyl-3-(5-nitro-2-furyl)-isoxazole (3.15 g, 0.01 mole) and thiourea (0.85 g, 0.011 mole) were shaken with dry THF (50 ml). A homogeneous solution formed and in *ca.* 5 min a white solid began to separate. The mixture was heated under reflux for 2 hr and concentrated under reduced pressure. The oily residue was triturated with ice–water and filtered to give 2.0 g (67%) of an orange solid which crystallized from EtOAc–hexane as a yellow solid, mp 200–205° dec.

Ethyl 5-Furyl-3-methylisoxazole-4-carboxylate.—Ethyl acetoacetate (130 g, 1 mole) was added slowly to a stirred suspension of NaH (42 g, of 58.5% in oil, 1 mole, prewashed with hexane), in dry C_6H_6 (2 l.). The mixture was then heated with stirring for *ca.* 2 hr until the evolution of H_2 ceased, after which the thick mixture was cooled to *ca.* 15° and furoyl chloride (130.5 g, 1 mole) was added at such a rate as to keep the temperature below 35°. The mixture became yellow and more mobile and was left at room temperature overnight. It was extracted with ice–water (three 200-ml portions) and dried ($MgSO_4$) and the C_6H_6 was removed under reduced pressure. The residue was distilled under vacuum to give 179.2 g (80%) of ethyl α-furoylacetoacetate as a colorless oil, bp 118–120° (0.4 mm). The ethyl α-furoylacetoacetate could be cleaved to ethyl α-furoylacetate, bp 100° (0.4 mm), in 95% yield using the same procedure as for ethyl benzoylacetate.[34]

Ethyl α-furoylacetoacetate (112 g, 0.5 mole) was dissolved in EtOH (500 ml) and to this solution was added $H_2NOH \cdot HCl$ (69 g, 1 mole) in H_2O (80 ml), and the mixture was heated under reflux for 30 min. Ice–water (1.2 l.) was added and the mixture was cooled and filtered. The solid thus obtained was taken up in Et_2O (1 l.) and extracted with 1 *M* NaOH (three 100-ml portions) and H_2O (two 100-ml portions) after which it was dried ($MgSO_4$) and filtered. The filtrate was concentrated to a small volume, cold hexane (50 ml) was added, and the solution was filtered, when 93.5 g (85%) of a white solid was obtained, mp 72–73° (aqueous EtOH). *Anal.* ($C_{11}H_{11}NO_4$) C, H, N.

The methyl ester made in the same way had a melting point of 80–82°. *Anal.* ($C_{10}H_9NO_4$) C, H, N.

Ethyl 3-Methyl-5-(5-nitro-2-furyl)isoxazole-4-carboxylate (Table III, 3).—A solution of $SbCl_3$ (0.5 g) in concentrated HNO_3 (38 ml) was added to ice-cold Ac_2O (350 ml) with stirring, after which ethyl 3-methyl-5-furylisoxazole-4-carboxylate (96.2 g, 0.44 mole) was added in portions. The ester slowly dissolved in the acid mixture. The reaction mixture was warmed to 40–45° for 30 min, then cooled to −10° and NaOH (65 g, in 500 ml of H_2O) was added at such a rate as to keep the temperature below 0°. The solution immediately became dark red and the color slowly became lighter. After the addition was complete the mixture was extracted with Et_2O (four 500-ml portions). The combined Et_2O extracts were washed with 1 *M* aqueous NaOH until the washings were basic, then with ice–water (two 100-ml portions), and dried ($MgSO_4$). On filtration and concentration 86 g of a red viscous oil was obtained. The oil was eluted from a silica column with C_6H_6, when 70 g (59%) of an oil, which crystallized on standing, was obtained. Recrystallization from aqueous EtOH gave light yellow crystals, mp 64–66°.

3-Methyl-5-(5-nitro-2-furyl)isoxazole-4-carboxylic Acid (Table III, 1).—A mixture of 3-methyl-5-(5-nitro-2-furyl)isoxazole-4-carboxylate (26.6 g, 0.1 mole), glacial AcOH (200 ml), and concentrated HCl (200 ml) was heated under reflux for 24 hr, after which time the mixture was concentrated to *ca.* one-fourth its original volume and ice–water (1 l.) was added. The resulting solid was filtered, washed with ice-water, dried, and recrystallized from EtOAc-hexane to give 20 g (86%) of yellow crystals, mp 210–211°.

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(34) R. L. Shriner, A. S. Schmidt, and L. J. Roll, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1963, p 266.