ing, the soln was made basic with 40% NaOH and the product was extracted with Et_2O. The extracts were dried ($MgSO_4$) and concd, and the residue distd (short path) *in vacuo*; the distillate solidified on standing.

(1-Diethylamino-4-pentylamino)-2,3-dihydro-2,2-dimethylbenzofurans (46–49).—A mixture of amino-2,3-dihydro-2,2-dimethylbenzofuran (8.1 g, 0.05 mole) and 4,4-diethoxy-1-diethylaminopentane[7] (11.5 g, 0.05 mole) was heated slowly to 190–200°. Heating was stopped when EtOH no longer was evolved (*ca.* 2.5 hr). The resultant oil was dissolved in EtOH (50 ml) and hydrogenated over PtO_2 at 65° and 3.87 kg/cm². After filtering and evaporation of solvent, the product was distd *in vacuo*.

(1-Amino-4-pentylamino)-2,3-dihydro-2,2-dimethylbenzofurans (50–53).—A soln of amino-2,3-dihydro-2,2-dimethylbenzofuran (32.4 g, 0.2 mole) and 4-bromo-1-phthalimidopentane[8] (29.6 g, 0.1 mole) in EtOH (100 ml) was heated under reflux for 72 hr. Solvent then was removed under reduced pressure, and the residue triturated with Et_2O to separate product from amine·HBr. The Et_2O was evapd and the residue was dissolved in EtOH (200 ml) containing hydrazine hydrate (7.00 g). After heating under reflux for 24 hr, the mixture was filtered and the filter cake triturated with Et_2O. The combined filtrates were concd and the residues distd *in vacuo*. Fumarate salts were prepared by allowing the amino product to react with a calcd amount of fumaric acid in THF. The fumarates were recrystd from MEK.

Ethyl α-Carbethoxy-β-(2,3-dihydro-2,2-dimethylbenzofuranylamino)acrylates.—A mixture of amino-2,3-dihydro-2,2-dimethylbenzofuran (100 g, 0.62 mole) and diethyl ethoxymethylenemalonate (143 g, 0.66 mole) was heated in an open beaker until EtOH evolution ceased (*ca.* 30 min). Products from the 4-amino and 7-amino isomers solidified upon cooling, and were crystallized from 1:1 Et_2O–petr ether (**54** and **2**). Products from the 5-amino (**18**) and 6-amino (**27**) isomers could not be purified.

Ethyl Hydroxy-2,3-dihydro-2,2-dimethylfuroquinolinecarboxylate (3, 10, 19, 28).—The amino acrylates (50 g, 0.15 mole) were added in one portion to boiling Ph_2O (350 g). The soln was heated under reflux for 30 min and then cooled and petr ether added to ppt the product. The ppt was washed thoroughly with petr ether to remove Ph_2O. Additional product could be obtained by recycling the filtrate.

Hydroxy-2,3-dihydro-2,2-dimethylfuroquinolinecarboxylic Acids (4, 11, 20, 29).—A suspension of the furoquinolinecarboxylate ester (31.8 g, 0.11 mole) in 10% NaOH (250 ml) was heated under reflux for 2 hr. The clear soln was acidified with concd HCl, and the resultant slurry was boiled for 40 min to insure complete conversion of Na salt into free acid. After filtering and drying, the product was purified by trituration with hot EtOH.

Hydroxy-2,3-dihydro-2,2-dimethylfuroquinolines (5, 23, 31).—The furoquinolinecarboxylic acids (45 g, 0.17 mole) were decarboxylated by heating to 290–300° in a beaker until effervescence ceased (*ca.* 15 min). The product solidified upon cooling.

Chloro-2,3-dihydro-2,2-dimethylfuroquinolines (6, 12, 24, 32).—A mixture of hydroxyfuroquinoline (15.7 g, 0.073 mole) and $POCl_3$ (53 ml) was heated under reflux for 1 hr. After cooling the mixture was carefully poured over crushed ice (200 g), and the resultant soln made basic with 40% NaOH. The chloroquinolines were recovered as fine needles; **12** pptd as an HCl salt which was converted into the free base by crystallization from H_2O.

Aminoalkylamino-2,3-dihydro-2,2-dimethylfuroquinolines (7–9, 13–17, 25–26, 32–36).—In most cases the chloroquinolines (4.0 g, 0.017 mole) and dialkylaminoalkylamine (0.038 mole) were heated until solid appeared or under reflux for 5 hr. With 3-diethylaminopropylamine the reaction was carried out in a sealed tube at 160° for 15 hr. With 1,4-bis(2-aminopropyl)-piperazine 1 equiv of K_2CO_3 was ground together with the reactants, and the paste was heated in a sealed tube at 160° for 15 hr. The reaction mixtures were partitioned between 20% AcOH and $CHCl_3$; the AcOH solns were made alkaline with 40% NaOH and extracted with $CHCl_3$. The extracts were dried ($MgSO_4$) and concd. Solid products were recrystd; oils were converted to HCl salts.

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Chemistry of Cephalosporin Antibiotics. XX. Synthesis and Biological Properties of 3-Acyloxymethyl-7-[2-(thienyl)acetamido]-3-cephem-4-carboxylic Acid and Related Derivatives[1]

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The title compounds (VII) have been synthesized and evaluated as antimicrobial agents. These new cephalosporins have shown a broad-spectrum antibiotic activity.

Cephalothin (I),[2] a broad-spectrum antibiotic, is hydrolyzed after administration to experimental animals and to man to the less active deacetylcephalothin (II).[3]

It was of interest to determine whether replacement of the acetate by a sterically hindered ester group would render a compound more resistant to hydrolysis. Therefore, the synthesis of these compounds with more bulky ester groups in the 3 position was undertaken.

Several attempts to acylate the 3-hydroxymethyl group in Δ^3-cephalosporins have been reported,[4] but these were not too successful because the molecule is prone to rearrange to the Δ^2 compound or to form the lactone. Since Δ^2 cephalosporins do not lactonize as easily and are more stable to alkaline conditions, Δ^2-deacetyl cephalothin (III), prepared by alkaline hydrolysis of Δ^2-cephalothin (IV),[5] was used as starting material.

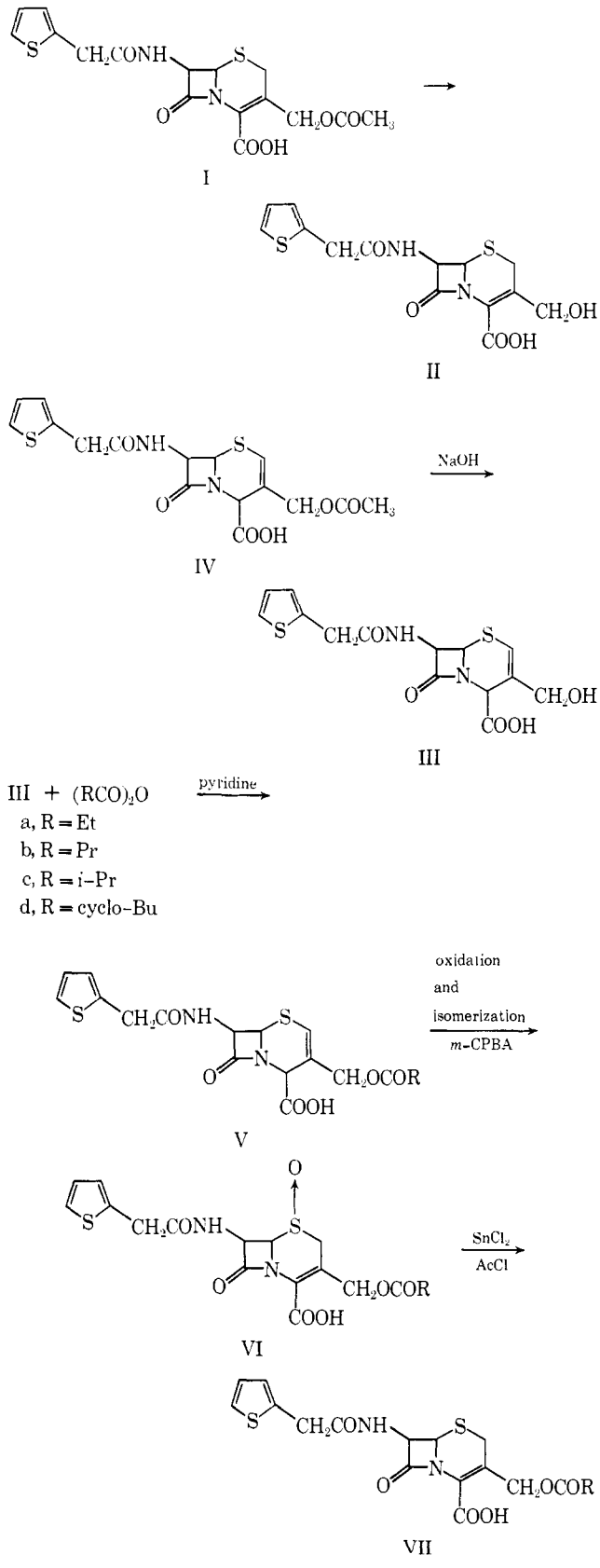
(1) Paper XIX: G. V. Kaiser, R. D. G. Cooper, R. E. Koehler, C. F. Murphy, J. A. Webber, I. G. Wright, and E. M. Van Heyningen, *J. Org. Chem.*, **35**, 2430 (1970).

(2) Cephalothin is the generic name given to 3-acetoxymethyl-7-[2-(thienyl)acetamido]-3-cephem-4-carboxylic acid (KEFLIN, is sodium cephalothin).

(3) (a) C. C. Lee, E. B. Herr, and R. C. Anderson, *Clin. Med.*, **70**, 1123 (1963); (b) W. E. Wick, *Antimicrob. Ag. Chemother.*, **1965**, 870 (1966).

(4) E. M. Van Heyningen, *Advan. Drug Res.*, **4**, 28 (1967).

(5) J. D. Cocker, S. Eardley, G. I. Gregory, M. E. Hall, and A. G. Long, *J. Chem. Soc. C*, 1142 (1966).



Esterification of the 3-CH_2OH in III was carried out by treatment with the appropriate aliphatic acid anhydride in pyridine according to the reported procedure,[5,6] and the esters V were isolated in good yield.

The conversion of these Δ^2 esters, which are biologically less active, to the more potent Δ^3 compounds VII was accomplished by utilizing the oxidative–reductive process for isomerization of the double bond discovered recently in these laboratories.[1,7] The isomerization was accomplished by oxidation of V with 1 equiv of *m*-chloroperbenzoic acid in $CHCl_3$. In this process the heterocyclic sulfide group is oxidized to the sulfoxide, and the double bond isomerizes to the Δ^3 position.

The final problem was the removal of O from S. Although some difficulties in this type of reduction had been reported,[5] an elegant method for the reduction of cephalosporin sulfoxides was discovered by Kaiser, *et al.*[1] Applying this method, we readily reduced the sulfoxides VI with $SnCl_2$ and AcCl in DMF and isolated the desired Δ^3 derivatives VII in good yields.

All compounds were identified and characterized by elemental analyses, uv, ir, nmr spectroscopy, tlc, and by bioautography where applicable (see Tables I and II).

Preliminary *in vitro* antibiotic activities of these compounds against a variety of Gram-positive and Gram-negative bacteria are reported in Table III. For comparison, the activities of sodium cephalothin are included. These new esters VII have essentially broad-spectrum antibiotic activity and they are slightly more active than sodium cephalothin. The butyrate (VII, b) and isobutyrate (VII, c) displayed the highest activities. Compounds V and VI were markedly less active.

The more potent compounds VII in the *in vitro* tests were chosen for *in vivo* assay. Groups of 8 white mice were inoculated with *Streptococcus pyogenes* C203 and then given oral doses of antibiotic 1 and 5 hr after inoculation with the bacterium. Table III also shows that ED_{50} values of the new derivatives are slightly better than that of sodium cephalothin.

Thus, the results of *in vitro* and *in vivo* tests showed that introduction of a sterically more hindered ester function in the 3 position retains good antibiotic activity. A study of enzymatic hydrolysis is in progress and will be reported later.

Experimental Section

Melting points were determined on a Mel-Temp apparatus[5] and were uncorrected. Nmr spectra were obtained on a Varian Associates Model HA-60 spectrometer. Uv spectra were determined on a Cary Model 14 recording spectrometer. Tlc behavior was followed using silica gel plastic sheets with fluorescent indicator (Eastman). The solvent system was AcOH–$CHCl_3$ (15:85). Bioautographs were performed according to techniques described by Jeffery, *et al.*[8]

3-Hydroxymethyl-7-[2-(thienyl)acetamido]-2-cephem-4-carboxylic Acid (III).—Following the procedure of Cocker, *et al.*,[5] 3-acetoxymethyl-7-[2-(thienyl)acetamido]-2-cephem-4-carboxylic acid[5] (30 g, 0.075 mole) was hydrolyzed in a mixture of 25 ml of Me_2CO and 250 ml of H_2O with 1 *N* NaOH (150 ml). The soln was kept at 39° overnight. After cooling and acidification to pH 2 with 2 *N* HCl, it was extracted with EtOAc. After the extract was washed and dried ($MgSO_4$), the solvent was concd to a small vol (*ca.* 100 ml). The pptd hydroxy acid was filtered and dried to give 14.5 g (55%); mp 145–146° dec; R_f 0.145; λ_{max} 232 mμ (ε 15, 700) in H_2O; γ_{max} 5.67 (lactam C=O), 5.76 (COOH), 6.05, and 6.56 μ ($CONH_2$); apparent molecular weight of 360 (calcd 354.4); nmr (D_2O–$NaHCO_3$) peaks at τ 6.18 (side-

(6) R. B. Woodward, K. Heusler, J. Gosteli, P. Naegeli, W. Oppolzer, R. Ramage, R. Ranganthan, and H. Vorbruggen. *J. Amer. Chem. Soc.*, **88**, 852 (1966).

(7) J. A. Webber, E. M. Van Heyningen, and R. T. Vasileff, *ibid.*, **91**, 5674 (1969).

(8) J. D'A. Jeffery, E. P. Abraham, and G. G. F. Newton, *Biochem. J.* **81**, 591 (1961).

TABLE I

Compd	R	Yield, %	Mp, °C dec	Formula[a]	Solvent	Ultraviolet spectral data: λ_{max}, mμ (ε × 10⁻³)
Va	Et	44	125–128	$C_{17}H_{18}N_2O_6S_2$	EtOH	235 (15.4)
Vb	Pr	42	137–140	$C_{18}H_{20}N_2O_6S_2$	EtOH	233 (15.0)
Vc	*i*-Pr	46	150–152	$C_{18}H_{20}N_2O_6S_2$	H_2O	234 (12.4)
Vd	*c*-Bu[b]	35	150–152	$C_{19}H_{20}N_2O_6S_2$	H_2O	233 (13.4)
VIa	Et	47	178–179	$C_{17}H_{18}N_2O_7S_2$	EtOH	237 (11.5); 260 (8.9)
VIb	Pr	45	170–172	$C_{18}H_{20}N_2O_7S_2$	EtOH	237 (12.2); 260 (8.7)
VIc	*i*-Pr	57	173–174	$C_{18}H_{20}N_2O_7S_2$	EtOH	235 (13.6); 260 (9.8)
VId	*c*-Bu	47	170–171	$C_{19}H_{20}N_2O_7S_2$	EtOH	235 (12.8); 260 (9.8)
VIIa	Et	87	151–152	$C_{17}H_{18}N_2O_6S_2$	H_2O	235 (13.2); 260 (8.5)
VIIb	Pr	69	115–119	$C_{18}H_{20}N_2O_6S_2$	H_2O	235 (13.4); 260 (7.4)
VIIc	*i*-Pr	68	115–118	$C_{18}H_{20}N_2O_6S_2$[c]	H_2O	235 (12.3); 260 (7.5)
VIId	*c*-Bu	75	120–122	$C_{19}H_{20}N_2O_6S_2$	H_2O	235 (13.3); 260 (7.8)

[a] All compounds gave elementary analyses for C, H, N, and S within 0.4% of the theoretical values. [b] Cyclobutyl. [c] No S analysis.

TABLE II

NMR SPECTRA DATA, τ, PPM (*J*, Hz)[a]

Compd	Solvent[b]	2-H	3-CH_2	4-H	6-H	7-H	R	AcCH_3	Aromatic protons
Va	A	3.62	5.33	5.03	4.77d (4)	4.42d (4)	8.91t (7)	6.17	3.04d
							7.66q (7)		2.77d
Vb	A	3.62	5.32	5.01	4.72d (4)	4.39d (4)	9.08t (7)	6.13	3.03d
							8.37m		2.72d
							7.68m		
Vc	A	3.55	5.28	5.03	4.71d (4)	4.42d (4)	8.83d (7)	6.16	3.03d
							7.4m		2.77d
Vd	B	3.58	5.29	5.03	4.72d (4)	4.40d (4)	7.87m	6.17	3.03d
									2.77d
VIa	D	6.49d (18)	5.40d (13)		5.12d (4)	4.15q (3; 4)	8.98t (7)	6.12	3.03d
		6.05d (18)	4.75d (13)				7.58q (7)		2.63d
VIb	D	6.48d (18)	5.38d (13)		5.08d (4)	4.16q (3; 4)	9.11t (7)	6.13	3.02d
		6.08d (18)	4.75d (13)				8.4m		2.60d
							7.7m		
VIc	D	6.49d (18)	5.40d (13)		5.09d (4)	4.17q (3; 4)	8.92d (7)	6.13	3.04d
		6.08d (18)	4.76d (13)				7.4m		2.63d
VId	D	6.51d (19)	5.39d (13)		5.09d (4)	4.14q (3; 4)	7.97m	6.12	3.03d
									2.62d
VIIa	C	6.75d (18)	?[c]		4.95d (4)	4.37d (4)	8.91t	6.13	2.98d
		6.38d (18)					7.58q (7)		2.67d
VIIb	C	6.75d (18)	?[c]		4.92d (4)	4.35d (4)	9.11t (7)	6.12	2.95d
		6.35d (18)					8.33m		2.63d
							7.62m		
VIIc	C	6.81d (18)	?[c]		4.95d (4)	4.33d (4)	8.88d (7)	6.13	2.98d
		6.40d (18)					7.4m		2.68d
VIId	D	6.61d (18)	5.08d (13)		4.86d (4)	4.35d (4)	8.92m	6.21	3.04d
		6.28d (18)	4.92d (13)			4.20d (4)			2.62d

[a] Containing Me_4Si as an internal standard. [b] Solvent: A = $CDCl_3$–D_2O; B = $CDCl_3$–DMSO-d_6; C = D_2O–$NaHCO_3$; D = DMSO-d_6. [c] Overlapped with H_2O signal.

TABLE III

BIOLOGICAL ACTIVITIES[a]

Compd	Gram-negative organisms (MIC μg/ml): *Shigella sp.* (N-9)	*Escherichia coli* (N-26)	*Klebsiella sp.* (X-26)	*Aerobacter sp.* (X-68)	Penicillin resistant[b] *Staphylococcus aureus* (V-30)	(V-32)	(V-84)	Keflin® Assay[c]	ED_{50}[d] mg/kg × 2
Sodium cephalothin	12.8	13.4	4.0	6.2	0.5; 1.0	0.5; 1.0	0.4; 0.6	1000	26.7
VIIa	6.7	9.4	5.5	8.1	0.5; 1.0	0.5; 1.0	0.4; 1.0	1150	23.5
VIIb	5.9	9.5	6.4	7.5	0.4; 1.0	0.4; 1.0	0.2; 1.0	1240	23.6
VIIc	14.8	18.0	12.3	12.3	0.5; 1.0	0.5; 1.0	0.4; 1.0	1240	23.6
VIId	8.3	11.8	2.5	12.5	0.4; 1.0	0.5; 1.0	0.1; 1.6	1120	24.1

[a] C. W. Godzeski, G. Brier, and D. E. Pavey, *Appl. Microbiol.*, 11, 122 (1963). The interpretation of these results should be done on a comparative basis only and requires use of an internal standard. [b] The minimum inhibitory concentrations in μg/ml; the first value is without, the second with human serum. [c] The results are reported as μg/ml of Na cephalothin activity against *Bacillus subtilis*. [d] W. S. Boniece, W. E. Wick, D. H. Holmes, and C. E. Redman, *J. Bacteriol.*, **84**, 1242 (1962). Drug administered orally to mice, 1 and 5 hr post injection.

chain CH_2), 5.8 (CH_2OH), 5.12 (H-4), 4.58 (d) and 4.70 (d), *J* = 4.5 (H-6 and H-7), 3.7 (H-2), and 3.0 and 2.63 (aromatic protons). *Anal.* ($C_{14}H_{14}N_2O_5S_2$) C, H, N, S.

3-Acyloxymethyl-7-[2-(thienyl)acetamido]-2-cephem-4-carboxylic Acids (V).—To a soln of 2.12 g (6 mmoles) of 3-hydroxymethyl-7-[2-(thienyl)acetamido]-2-cephem-4-carboxylic acid in 81 ml (1 mole) of pyridine was added 0.018 mole of the appropriate aliphatic acid anhydride. The mixture

was stirred for 40 min at room temp. The soln was then poured slowly into 500 ml of ice-cold 2 *N* HCl (pH 1.0). The mixture was extracted with EtOAc and the acids were extracted from the EtOAc with a soln of $NaHCO_3$ to pH 7.8. The alkaline soln was reacidified with 2 *N* HCl to pH 2.0 and reextracted with EtOAc; this extract was washed with H_2O and dried ($MgSO_4$). The solvent was evapd and the residue was triturated with petr ether to give a powdered product which crystallized from 50% EtOH. The physical data and yields are summarized in Tables I and II. Ir spectra were consistent with the required structures.

3-Acyloxymethyl-7-[2-(thienyl)acetamido]-3-cephem-4-carboxylic Acid 1-Oxides (VI).—3-Acyloxymethyl-7-[2-(thienyl)-acetamido]-2-cephem-4-carboxylic acid (1 mmole) was dissolved in a minimal amount of $CHCl_3$ at room temp and 0.95 mmole of 85% *m*-chloroperbenzoic acid was added. The mixture was warmed slightly and pptn commenced. After being stirred for 15 min at room temp, the solvent was concd to a small vol; the pptd crystals were filtered and recrystd from EtOH. The physical constants, yields, and other pertinent data are given in Tables I and II.

3-Acyloxymethyl-7-[2-(thienyl)acetamido]-3-cephem-4-carboxylic Acids (VII).—The corresponding sulfoxide VI (1 mmole) and $SnCl_2 \cdot 2H_2O$ (2.5 mmoles) were dissolved in 18 ml of DMF. This solution was cooled in an ice bath, and 2.5 ml of AcCl was added. The mixture was stirred at 18° for 15 min, then poured into 40 ml of cold H_2O, and extracted with EtOAc. The extract was washed with H_2O and then dried ($MgSO_4$). The solvent was evapd, and the residue displayed one spot in tlc. Compound VII could be recrystd from EtOH–H_2O. Isolated compounds VII displayed a single biologically active spot on bioautograph. The physical data and yields are listed in Tables I and II. Biological data are shown in Table III.

Acknowledgment.—The author is grateful to G. Maciak and his staff for microanalyses; to D. O. Woolf, L. A. Spangle, and their associates for spectral data; to J. L. Ott, J. Westhead, and W. E. Wick for biological data; to C. T. Pugh for bioautography; and to A. I. Ellis for technical assistance.

Carcinostatic Activity of Thiosemicarbazones of Formyl Heteroaromatic Compounds. VI. 1-Formylisoquinoline Derivatives Bearing Additional Ring Substituents, with Notes on Mechanism of Action[1]

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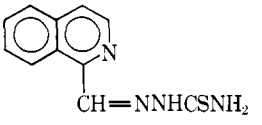
Twenty-two thiosemicarbazones of 1-formylisoquinolines bearing additional substituents in the pyrido or benzo ring were synthesized and tested against a number of mouse tumor systems *in vivo* in an attempt to improve on the parent compound. The major test tumors were L-1210 leukemia, sarcoma 180 (ascites), L-5178Y lymphoma, C-1498 myelogenous leukemia, and the Lewis lung carcinoma. Occasional screening was performed using B-16 melanoma, Ehrlich ascites carcinoma, and sarcoma 180 (solid). The additional substituents studied were: 2-oxide, 3-methyl, 4-acetoxy, 4-hydroxy, 5-cyano, 5-sulfonic acid, 5-sulfonic acid ammonium salt, 5-chloro, 5-fluoro, 5-nitro, 5-acetoxy, 5-hydroxy, 5-trifluoromethyl, 5-*n*-perfluoropropyl, 5-carboxy, 6-methoxy, 7-acetoxy, 7-fluoro, 7-chloro, 7-hydroxy, 7-methoxy, and 8-fluoro. No simple parametric rationale could be found for the effect of additional substituents on activity against a given tumor system. The order of substituent effects changed markedly from one tumor system to another. This negates the validity of any generalized parametric statements. A complicating factor was that 17 of these 22 compounds were more or less poorly absorbed. A number of active compounds in the isoquinoline series and in the corresponding pyridine series have been studied in other laboratories as inhibitors of DNA synthesis in cell-free systems, in cellular systems, and *in vivo*. The powerful inhibition of DNA synthesis ($\sim 10^{-7}$ *M* for 50% inhibition) in all cases studied involves a blockade of mammalian tumor derived ribonucleoside diphosphate reductase (RDR). These compounds are tridentate ligands for transition metals including iron. As a consequence, a detailed model is proposed for the functioning of RDR and for inhibition of this activity by these ligands.

Since the discovery that 1-formylisoquinoline thiosemicarbazone (**1**) is very active against a variety of mouse tumors,[2] there has been considerable interest in this laboratory and in others to find more active derivatives.[3-6]



1

In this study 22 derivatives substituted in either the pyrido or benzo ring have been synthesized and tested on a variety of mouse tumor systems.

Chemistry.—Some of the 5-substituted 1-formylisoquinoline thiosemicarbazones included in the present study (Table IV) have been described previously.[3] The unreported 5- and 8-substituted 1-methylisoquinolines (Table I) were prepared from 1-methyl-5-nitroisoquinoline (I, Scheme I).

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(1) (a) This investigation was supported by Grant CA-03287 from the National Cancer Institute; (b) presented in part at the 169th National Meeting of the American Chemical Society, New York, N. Y., Sept 1969, Abstract MEDI-76.

(2) F. A. French and E. J. Blanz, Jr., *Cancer Res.*, **25**, 1454 (1965).

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