

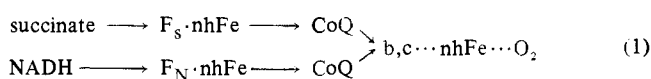
Antimetabolites of Coenzyme Q. 14.† Quinolinequinone Analogs Which Inhibit Mitochondrial DPNH-Oxidase and Succinoxidase‡

Christine M. Bowman, Frederick S. Skelton, Thomas H. Porter, and Karl Folkers*

Institute for Biomedical Research, The University of Texas at Austin, Austin, Texas 78712. Received May 19, 1972

6-Hydroxy-5,8-quinolinequinone analogs of coenzyme Q have been tested for inhibition of mitochondrial DPNH-oxidase and succinoxidase systems. In succinoxidase, the greatest inhibitions were by analogs with tetradecyl, pentadecyl, hexadecyl, ω -cyclohexylpentyl, and ω -cyclohexyloctyl side chains. DPNH-oxidase was less sensitive than succinoxidase to these analogs; and the pentadecyl, hexadecyl, and ω -cyclohexylpentyl analogs were the most inhibitory. For both enzymes, inhibition was lower for side chains of less than ten carbon atoms. The optimum length of the lipoidal side chain for inhibition was about 15 carbon atoms. Reduction of the pyrido moiety of a quinolinequinone analog greatly reduced inhibitory activity. Four 7-hydroxy-5,8-quinolinequinones inhibited only succinoxidase. The inhibition of CoQ enzymes by the 6-hydroxy-5,8-quinolinequinones correlated reasonably well with antimalarial activity of these analogs against *Plasmodium berghei* in mice. The 6- ω -cyclohexyloctyl-7-hydroxy analog exhibited the highest antimalarial activity and highest inhibition of succinoxidase.

There are two sites¹³ for the function of coenzyme Q in mitochondrial electron transport as exemplified by eq 1



where nhFe = nonheme iron, F_s = succinate dehydrogenase (flavoprotein), F_N = NAD dehydrogenase (flavo-protein); and b, c = cytochromes. This knowledge is essentially based upon the organic structural specificity of CoQ for activity in the succinoxidase and NADH-oxidase systems of beef heart^{13,14} and yeast mitochondria.^{14,15} Increasing isoprenoid side-chain lengths of members of the CoQ group enhances activity of NADH-oxidase in beef heart mitochondria, but shorter side-chain lengths appear to increase activity of NADH-oxidase in yeast mitochondria.¹⁴ There appears to be little specificity for the side-chain length in the succinoxidase system. Ernster, *et al.*,¹⁵ indicated that CoQ may act as a regulator of the interaction of NADH and succinate dehydrogenase with the cytochrome system.

Coenzyme Q has been found in the malarial parasite (*i.e.*, *Plasmodium knowlesi*, *P. cynomolgi*, *P. lophurae*, *P. berghei*, and *P. falciparum*). Coenzyme Q₈, I, is the dominant form of CoQ in the parasite as determined both by a differential analysis of normal and infected blood^{16,17} and by a ¹⁴C-labeling technique using *in vitro* cultures of erythrocytes infected with *P. knowlesi*¹⁸ and *P. falciparum*.¹⁹

Since CoQ is indispensably involved in oxidative metabolism and is biosynthesized by *Plasmodium*, the design of specific CoQ inhibitors of either the function and/or the biosynthesis of CoQ in *Plasmodium* is a new approach to the chemotherapy of malaria.

New 5,8-quinolinequinones have been synthesized, and a number of these new heterocyclic quinones were found to possess marked antimalarial activity against *P. berghei* in the mouse.^{9,10} Of these quinones, 7- ω -cyclohexyloctyl-6-hydroxy-5,8-quinolinequinone was found to inhibit the succinate-cytochrome c reductase and the succinate-coenzyme Q reductase systems in beef heart submitochondrial systems.⁸ These inhibitions could be reversed by coenzyme Q₆. Similarly, a number of 6-alkylamino-5,8-quinoline-

quinones were highly inhibitory to both the NADH-oxidase and the succinoxidase enzyme systems, and inhibition of the NADH-oxidase system could be completely reversed by CoQ₁₀.¹⁰

We now describe the inhibition of mitochondrial DPNH-oxidase and succinoxidase CoQ enzyme systems by 7-alkyl-6-hydroxy-5,8-quinolinequinones and their isomeric 6-alkyl-7-hydroxy-5,8-quinolinequinones. The former derivatives were found to be potent inhibitors of both these *in vitro* mitochondrial systems; the latter compounds were inhibitory only in succinoxidase.

Experimental Section

Protein was determined by the method of Lowry, *et al.*,²⁰ using the Folin phenol reagent. Cytochrome c from horse heart and NADH were obtained from the Sigma Chemical Co., St. Louis, Mo.

Preparation of Beef Heart Mitochondria. Heavy beef heart mitochondria (HBHM) were prepared by a modified method of Löw and Vallin.²¹ The final mitochondrial pellet was suspended in a medium which was 0.25 M in sucrose and 0.01 M in Tris HCl, pH 7.5. The suspension was kept cold in an ice bath, homogenized by means of a power-driven Potter-Elvehjem homogenizer, and centrifuged at 18,800 rpm (25,000 g) in a Spinco ultracentrifuge (60-Ti rotor) for 10 min. The heavy layer was then resuspended in 0.25 M sucrose, homogenized, and adjusted to 30 mg of protein per milliliter of buffer.

In Vitro Assay Procedure. The methodology of Szarkowska²² was utilized. Each flask contained 0.2 ml of KOH (20%) in the center well and 0.2 ml of enzyme in the side arm. The order of addition of reagents and quantities used were as follows: Tris HCl (0.1 M, pH 7.5), 1 ml; sucrose (1 M), 0.5 ml; mitochondrial phospholipids (12.8 mg/ml), 0.05 ml; inhibitor, 0.05 ml (in absolute ethanol); EDTA (0.8 μ mol/ml) 0.1 ml; cytochrome c (3 μ g/ml of H₂O), 0.05 ml; absolute ethanol (total volume not to exceed 0.1 ml).

Results and Discussion

The inhibition of DPNH-oxidase and succinoxidase by 12 7-alkyl-6-hydroxy-5,8-quinolinequinones was assayed in heavy beef heart mitochondrial systems (Tables I and II, respectively). In the succinoxidase system, the greatest inhibitions were given by the quinone analogs in which the 7-alkyl groups were tetradecyl, pentadecyl, hexadecyl, ω -cyclohexylpentyl, and ω -cyclohexyloctyl. In the DPNH-oxidase system, which appeared to be less sensitive than the succinoxidase system, the pentadecyl, hexadecyl, and ω -cyclohexylpentyl analogs were the most inhibitory. In both enzyme systems, the inhibitory activity was lower for the shorter (less than ten carbon atoms) alkyl chains.

†Several previous papers¹⁻¹² (see also R. S. Pardini, T. H. Porter, and K. Folkers, unpublished results) have provided knowledge on the chemistry and assay of analogs of coenzyme Q which demonstrate antimetabolite activities. For convenience, these papers are now being numbered.

‡Coenzyme Q. 151.

Table I. Inhibitory Activity of 7-Alkyl-6-hydroxy-5,8-quinolinequinones in the CoQ Enzyme System DPNH-Oxidase

R	Concn ^a	S.A. ^b	Inhibition, % found	Estd A.I. ₅₀ ^c
-(CH ₂) ₄ CH ₃	190	0.50		>100
-(CH ₂) ₇ CH ₃	19	0.37	26	
-	32	0.25	50	16
-(CH ₂) ₉ CH ₃	2.5	0.41	17	
	4.2	0.28	44	
	5.1	0.17	66	2.2
-(CH ₂) ₁₂ CH ₃	4.2	0.25	50	
	5.1	0.14	71	2.1
-(CH ₂) ₁₅ CH ₃	3.2	0.24	51	1.6
-(CH ₂) ₁₄ CH ₃	2.5	0.22	56	1.3 ^d
-(CH ₂) ₁₅ CH ₃	1.9	0.31	38	
	3.2	0.19	58	
	6.4	0.085	83	1.4
-(CH ₂) ₁₈ CH ₃	2.9	0.32	35	
	3.6	0.20	60	1.7
-CH ₂ -c-C ₆ H ₁₁	190	0.33	33	>100
-(CH ₂) ₅ -c-C ₆ H ₁₁	38	0.31	38	
	45	0.28	43	
	64	0.20	60	27
-(CH ₂) ₈ -c-C ₆ H ₁₁	2.5	0.27	46	1.3
-(CH ₂) ₈ -c-C ₆ H ₁₁	3.8	0.24	51	
	4.5	0.22	56	
	5.1	0.16	77	1.9

^aConcentration of inhibitor in nmol/mg of mitochondrial protein. ^bSpecific activity (S.A.) defined as oxygen specific activity in μ atoms/min/mg of protein. ^cAntimetabolite index (A.I.) defined as nmoles of antimetabolite per nmoles of mitochondrial coenzyme Q₁₀ for 50% inhibition. The average concentration of CoQ in mitochondria was determined from several preparations of mitochondria and was found to be approximately 2 nmol/mg of mitochondrial protein. This value was assumed for the calculation of antimetabolite indices. ^dFor 56% inhibition. ^eS.A. = 0.49.

Table III lists certain 5,8-quinolinequinones tested for *in vitro* inhibition of coenzyme Q enzyme systems containing exogenous CoQ₁₀ in the standard reaction mixture. These *in vitro* inhibitions could be completely reversed by addition of further coenzyme Q₁₀ (CoQ₁₀) to the system. In comparing the *in vitro* inhibitory activities of these 5,8-quinolinequinones, there appeared to be an optimum length for the hydrocarbon side chain of about 15 carbon atoms for maximum activity [i.e., 7-*n*-pentadecyl-6-hydroxy-5,8-quinolinequinone (5)]. A longer side-chain unit, exemplified by 7-*n*-nonadecyl-6-hydroxy-5,8-quinolinequinone (6), adversely affected activity.

Reduction⁹ of the pyridine moiety of 7-*n*-tetradecyl-6-hydroxy-5,8-quinolinequinone (4) gave the tetrahydro analog 7 with reduced inhibitory activity (Table III). 1,2,3,4-Tetrahydro-7-*n*-tetradecyl-6-hydroxy-5,8-quinolinequinone (7) was one-tenth as active in the DPNH-oxidase system and one-fourth to one-third as active in the succinoxidase enzyme system as the corresponding unsaturated analog. Apparently, changes in the conformation of the pyridine ring and/or alterations in the oxidation-reduction potential of the quinone upon reduction of the pyridine moiety resulted in the reduction of *in vitro* activity.

Four isomeric compounds in the 6-alkyl-7-hydroxy-5,8-quinolinequinone series have been tested in both *in vitro* enzyme assays, DPNH-oxidase and succinoxidase, using beef heart mitochondrial (Table IV). These compounds inhibited only the succinoxidase system at the levels tested. One

Table II. Activity of 7-Alkyl-6-hydroxy-5,8-quinolinequinones in the CoQ Enzyme System Succinoxidase

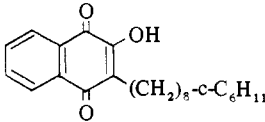
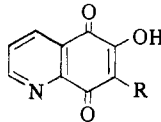
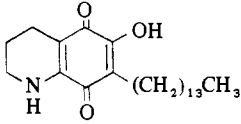
R	Concn ^a	S.A. ^b	Inhibition, % found	Estd A.I. ₅₀ ^c
-(CH ₂) ₄ CH ₃	230	0.39	15	>100
-(CH ₂) ₇ CH ₃	16	0.28	40	
	20	0.25	48	
	22	0.19	60	
	27	0.070	80	9.5
-(CH ₂) ₉ CH ₃	2.2	0.31	34	
	2.7	0.22	53	
	5.5	0.047	90	1.3
-(CH ₂) ₁₂ CH ₃	2.7	0.34	26	
	3.6	0.22	54	
	3.8	0.27	41	
	4.4	0.092	80	
	5.7	0.027	90	1.7
-(CH ₂) ₁₃ CH ₃	2.2	0.24	40	1.1 ^d
-(CH ₂) ₁₄ CH ₃	0.82	0.34	28	
	1.4	0.29	40	
	1.6	0.171	63	0.75
-(CH ₂) ₁₅ CH ₃	1.1	0.29	40	
	~1.4	0.20	58	
	1.6	0.17	64	0.65
-(CH ₂) ₁₈ CH ₃	2.7	0.23	50	1.4
-CH ₂ -c-C ₆ H ₁₁	160	0.34	28	>80
-(CH ₂) ₃ -c-C ₆ H ₁₁	22	0.25	47	
	27	0.091	80	1.1
-(CH ₂) ₅ -c-C ₆ H ₁₁	1.6	0.29	38	
	2.1	0.17	64	0.9
-(CH ₂) ₈ -c-C ₆ H ₁₁	2.2	0.25	46	
	4.4	0.012	97	1.2

^aConcentration of inhibitor in nmol/mg of mitochondrial protein. ^bSpecific activity (S.A.) defined as oxygen specific activity in μ atoms/min/mg of protein. ^cAntimetabolite index (A.I.) defined as nmoles of antimetabolite per nmoles of mitochondrial coenzyme Q₁₀ for 50% inhibition. The average concentration of CoQ₁₀ in mitochondria was determined from several preparations of mitochondria and was found to be approximately 2 nmol/mg of mitochondrial protein. This value was assumed for the calculation of antimetabolite indices. ^dFor 40% inhibition. ^eS.A. = 0.47.

of the compounds, 6- ω -cyclohexylpentyl-7-hydroxy-5,8-quinolinequinone, was considerably less active in the succinoxidase system than the other three derivatives. A concentration of 157 nmol/mg of mitochondrial protein of 6-*n*-tetradecyl-7-hydroxy-5,8-quinolinequinone (Table IV) gave ~50% inhibition of the succinate system but did not inhibit DPNH-oxidase. In comparison, the more potent isomer, 7-*n*-tetradecyl-6-hydroxy-5,8-quinolinequinone (Tables I and II), gave 50% inhibition at concentrations of ~2-3 nmol in both the DPNH- and succinoxidase enzyme systems. The selective inhibition exhibited by these 6-alkyl-7-hydroxy-5,8-quinolinequinones may prove therapeutically advantageous.

The *in vitro* activity of the 7-alkyl-6-hydroxy-5,8-quinolinequinones correlates reasonably well with the *in vivo* antimalarial activity against *P. berghei* in mice.⁹ Maximum antimalarial activity was exhibited for 7-*n*-alkyl side chains of approximately 15 carbon atoms; a marked decrease in *in vivo* activity was noted for the shorter 7-*n*-octyl analog and for the longer 7-*n*-nonadecyl derivative.⁹ A notable exception to the correlation occurred in the 7- ω -cyclohexylalkyl series. 7- ω -Cyclohexyloctyl-6-hydroxy-5,8-quinolinequinone exhibited the highest antimalarial activity.⁹ Contrary to expectations, the 7- ω -cyclohexylpentyl derivative showed maximum *in vitro* activity in

Table III. Inhibition of Coenzyme Q Enzyme Systems by 7-Alkyl-6-hydroxy- and 1,2,3,4-Tetrahydro-7-alkyl-6-hydroxy-5,8-quinolinequinones^f

No.	<i>In vitro</i> additions ^a	<i>In vitro</i> assay systems ^g					
		Specific activity ^b	Inhibitor concn, ^c nmol	% reversal ^d	Specific activity ^b	Inhibitor concn, ^c nmol	% reversal ^d
	CoQ ₁₀	0.553			0.524		
1	 (reference compound)	0.278	7	95	0.258	14	95
							
2	R = (CH ₂) ₈ -c-C ₆ H ₁₁	0.274	4	100	0.248	6	98
3	R = (CH ₂) ₁₂ CH ₃	0.276	4	100	0.250	5	100
4	R = (CH ₂) ₁₃ CH ₃	0.284	3	100	0.252	5	100
5	R = (CH ₂) ₁₄ CH ₃	0.282	2	100	0.251	5	98
6	R = (CH ₂) ₁₈ CH ₃ ^e	0.282	12	100	0.255	18	96
7		0.281	30	85	0.252	18	98

^a100 nmol of CoQ₁₀ was added in each case. ^bMicroatoms of oxygen per minute per milligram of protein. ^cFor 50% inhibition. ^dAfter addition of a further 200 nmol of CoQ₁₀. ^eMp 90–93°, test sample; mp 97–99°, analytical sample. ^f*In vitro* assays were conducted by the Warburg method using intact heavy beef heart mitochondria. ^gNo. A.I.₅₀ was calculated because of the addition of exogenous CoQ₁₀.

Table IV. Inhibitory Activity of 6-Alkyl-7-hydroxy-5,8-quinolinequinones in the CoQ Enzyme Systems DPNH-Oxidase and Succinoxidase

R	DPNH-Oxidase ^c				Succinoxidase ^c			
	Concn ^a	S.A. ^b	Inhibition, % found	A.I. ₅₀	Concn ^a	S.A. ^b	Inhibition, % found	A.I. ₅₀
-(CH ₂) ₇ CH ₃		0.553				0.446		
	230	0.690		>100	115	0.220	50	58
-(CH ₂) ₁₃ CH ₃		0.259				0.235		
	157	0.281		>80	153	0.118	50	77
-(CH ₂) ₈ -c-C ₆ H ₁₁		0.565				0.537		
	194	0.681		>100	78	0.217	60	
-(CH ₂) ₅ -c-C ₆ H ₁₁		0.226				0.115	80	29
	130	0.217		>65	170	0.198	23	>85

^aMicroatoms of oxygen per minute per mg of protein. ^bNanomoles of inhibitor per milligram of protein. ^cReaction mixture contained no exogenous coenzyme Q.

DPNH-oxidase and succinoxidase enzyme systems (Tables I and II). In the 6-alkyl series, 6- ω -cyclohexyloctyl-7-hydroxy exhibited the highest activity of all compounds tested in both the *P. berghei* mouse test and the succinoxidase system.

Certain analogs^{9,10,23} of CoQ have already been demonstrated to have antimetabolite activity *in vitro* and to show antimalarial activity *in vivo* without toxicity to the host. These data^{9,10,23} constitute the evidence that selective toxicity for an antimetabolite of CoQ has been achieved and allow the prediction that new analogs can be synthesized which could have even more effective selective toxicity in chemotherapy. Such selective toxicity can be based on differential inhibition of pathways.

Acknowledgments. Appreciation is expressed to Mrs. Alice Ma for excellent technical assistance. This work was supported by the U. S. Army Medical Research and Development Command Contract No. DADA 17-69-C-9067 and is Contribution No. 1068 from the Army Research Program on Malaria.

References

- (1) C. Hall, M. Wu, F. L. Crane, N. Takahashi, S. Tamura, and K. Folkers, *Fed. Amer. Soc. Exp. Biol.*, **25** (2), 530 (1966).
- (2) C. Hall, M. Wu, F. L. Crane, H. Takahashi, S. Tamura, and K. Folkers, *Biochem. Biophys. Res. Commun.*, **25**, 373 (1966).
- (3) M. Jeng, C. Hall, F. L. Crane, N. Takahashi, S. Tamura, and K. Folkers, *Biochemistry*, **7**, 1311 (1968).

- (4) F. S. Skelton, R. S. Pardini, J. C. Heidker, and K. Folkers, *J. Amer. Chem. Soc.*, **90**, 5334 (1968).
 (5) J. C. Catlin, R. S. Pardini, G. D. Daves, Jr., J. C. Heidker, and K. Folkers, *ibid.*, **90**, 3572 (1968).
 (6) J. C. Catlin, G. D. Daves, Jr., and K. Folkers, *J. Med. Chem.*, **14**, 45 (1971).
 (7) A. Castelli, E. Bertoli, G. P. Littarru, G. Lenaz, and K. Folkers, *Biochem. Biophys. Res. Commun.*, **42**, 806 (1971).
 (8) F. S. Skelton, C. M. Bowman, T. H. Porter, K. Folkers, and R. S. Pardini, *ibid.*, **43**, 102 (1971).
 (9) T. H. Porter, F. S. Skelton, and K. Folkers, *J. Med. Chem.*, **14**, 1029 (1971).
 (10) T. H. Porter, F. S. Skelton, and K. Folkers, *ibid.*, **15**, 34 (1972).
 (11) R. S. Pardini, J. C. Catlin, J. C. Heidker, and K. Folkers, *ibid.*, **15**, 195 (1972).
 (12) T. H. Porter, F. S. Skelton, C. M. Bowman, and K. Folkers, *ibid.*, **15**, 504 (1972).
 (13) G. Lenaz and G. D. Daves, Jr., and K. Folkers, *Arch. Biochem. Biophys.*, **123**, 539 (1968).
 (14) G. Lenaz, A. Castelli, G. P. Littarru, E. Bertoli, and K. Folkers, *ibid.*, **142**, 407 (1971).
 (15) L. Ernster, I. Lee, B. Norling, and B. Persson, *FEBS Lett.*, **3**, 21 (1969).
 (16) P. J. Rietz, F. S. Skelton, and K. Folkers, *Int. Z. Vitaminforsch.*, **37**, 405 (1967).
 (17) F. S. Skelton, P. J. Rietz, and K. Folkers, *J. Med. Chem.*, **13**, 602 (1970).
 (18) F. S. Skelton, K. D. Lunan, K. Folkers, J. V. Schnell, W. A. Siddiqui, and Q. M. Geiman, *Biochemistry*, **8**, 1284 (1969).
 (19) J. V. Schnell, W. A. Siddiqui, Q. M. Geiman, and F. S. Skelton, K. D. Lunan, and K. Folkers, *J. Med. Chem.*, **14**, 1026 (1971).
 (20) O. H. Lowry, N. J. Rosebrough, A. L. Farr, and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).
 (21) H. Löw and I. Vallin, *Biochem. Biophys. Acta*, **69**, 361 (1963).
 (22) L. Szarkowska, *Arch. Biochem. Biophys.*, **113**, 519 (1966).
 (23) T. H. Porter, C. M. Bowman, and K. Folkers, *J. Med. Chem.*, in press.

Irreversible Enzyme Inhibitors. 197.^{†,‡} Water-Soluble Reversible Inhibitors of Dihydrofolate Reductase with Potent Antitumor Activity Derived from 4,6-Diamino-1,2-dihydro-2,2-dimethyl-1-phenyl-s-triazine

B. R. Baker[§] and Wallace T. Ashton*

Department of Chemistry, University of California at Santa Barbara, Santa Barbara, California 93106. Received September 26, 1972

A series of 39 derivatives of the title compound was prepared with the object of optimizing reversible inhibition of dihydrofolate reductase and cell membrane transport while achieving sufficient water solubility for effective intravenous administration. Four compounds which met all the criteria were selected for antitumor testing *in vivo*. Of these, three showed excellent activity against Walker 256 ascites, intramuscular Walker 256, and Dunning leukemia ascites in the rat. Overall, the most active compound *in vivo* was 1-[3-chloro-4-(*m*-dimethylcarbamoylbenzyloxy)]phenyl-4,6-diamino-1,2-dihydro-2,2-dimethyl-*s*-triazine ethanesulfonate (**36**).

An earlier report from this laboratory demonstrated that several potent reversible as well as irreversible inhibitors of dihydrofolate reductase are highly effective in promoting cures[#] of the Walker carcinosarcoma 256 and Dunning leukemia tumor systems in the rat when given by intraperitoneal injection.² From a clinical standpoint it would be desirable to administer such a compound by intravenous infusion, so that administration could be discontinued at once should toxicity develop. Unfortunately, the inhibitors which have shown the greatest effectiveness *in vivo*² lack sufficient aqueous solubility. Solubilities of representative compounds **1-4** are given in Table I. Even the most soluble of these (**3**), a potent agent against Walker 256 ascites when administered intraperitoneally, was not soluble enough to achieve a toxic dose intravenously.³ It was proposed that insufficient solubility in body fluids hindered the distribution of **3**, thus accounting for its substantially lower activity against intramuscular Walker 256 when given intraperitoneally.³

Consequently, a study was undertaken to prepare a series of reversible inhibitors of dihydrofolate reductase with the

object of achieving a solubility in water of at least 25 mg/ml (50 mg/ml being preferred), while at the same time maintaining sufficient binding to the enzyme and cell membrane transport. The combination of the last two factors is reflected in the ED₅₀ against L1210 leukemic cell culture.^{4,5} The solubilization study was limited exclusively to reversible inhibitors on the basis of (a) their demonstrated equal or superior effectiveness *in vivo* compared to the corresponding irreversible inhibitors (sulfonyl fluorides),² (b) the apparent metabolic degradation of the sulfonyl fluoride group,^{6,7} and (c) the desolubilizing effect of the fluoro-sulfonyl moiety, as seen by comparing **3** and **4** (Table I).

Because inhibitors **2** and **3**, which contain an amide bridge, were considerably more soluble than **1**, the investigation of other amide-containing side chains appeared promising. Certain obvious structural modifications expected to increase water solubility, such as the introduction of

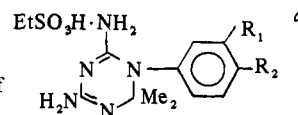


Table I. Solubility of

No.	R ₁	R ₂	Solubility, mg/ml (H ₂ O)
1	Cl	O(CH ₂) ₃ OC ₆ H ₅	<0.5
2	Cl	OCH ₂ CONHC ₆ H ₅	5
3	H	(CH ₂) ₂ CONHC ₆ H ₄ - <i>m</i> -CH ₃	10 ^b
4	H	(CH ₂) ₂ CONHC ₆ H ₃ -3-CH ₃ -4-SO ₂ F	1.1 ^c

^aSee ref 2 for biological data for these compounds. ^bData from ref 3. ^cF. R. White, Drug Research and Development, NCI, unpublished data.

[†]This work was generously supported by Grant CA-08695 from the National Cancer Institute, U. S. Public Health Service.

[‡]For the previous paper in this series see Baker and Ashton.¹

[§]Deceased Oct 1971.

*To whom correspondence should be addressed at the Department of Pharmaceutical Chemistry, College of Pharmacy, Medical University of South Carolina, Charleston, S. C. 29401.

[#]The term "cure" is used here throughout to mean an *apparent* cure; all surviving treated animals were sacrificed at the end of the fixed evaluation period.