125° for 20 min. The solution was diluted with DMSO (30 ml) and added to DMSO (60 ml) preheated to 130°. This solution was heated at 165° (bath) under N_2 for 4 hr and cooled and $CHCl_3$ was added. The solution was washed successively with H_2O, dilute sodium thiosulfate, and dilute KOH. The organic layer was dried ($MgSO_4$) and concentrated at reduced pressure. The crude aldehyde was dissolved in C_6H_6 (25 ml) and added to sodium bisulfite solution (75 ml). The addition compound was filtered and stirred with saturated aqueous $NaHCO_3$ (650 ml) and Et_2O (850 ml). The Et_2O layer was dried and concentrated under reduced pressure to yield the title aldehyde (7.2 g, 48%), mp 118–120°. Crystallization from acetonitrile gave mp 118–120°

Anal. ($C_{15}H_{10}F_3NO_3$) C, H, N.

4-(Carboxymethyl)-6-(4-trifluoromethylphenyl)-2-pyridyl-(4-trifluoromethylphenyl)carbinol. A slurry of the above aldehyde (6.6 g) in anhydrous Et_2O (150 ml) was treated with a solution of 4-trifluoromethylphenylmagnesium bromide (from 5.4 g of 4-bromobenzotrifluoride and 0.58 g of Mg) in Et_2O. The solution was stirred at room temperature for 1 hr. The mixture was poured into dilute aqueous NH_4OAc (150 ml). The Et_2O layer was dried ($MgSO_4$) and concentrated. The crude carbinol was crystallized from C_6H_6–petroleum ether to yield the title compound (6.9 g, 73%), mp 132–134°.

Anal. ($C_{22}H_{15}F_6NO_3$) C, H, F, N.

Methyl 2-(4-Trifluoromethylbenzoyl)-6-(4-trifluoromethylphenyl)isonicotinate. The title compound was prepared according to the procedure described for 4-carbethoxy-6-trifluoromethyl-2-pyridinecarboxaldehyde. A sample, crystallized from *i*-PrOH, had mp 167–169°.

Anal. ($C_{22}H_{23}F_6NO_3$) C, H, F, N.

2-(4-Trifluoromethylbenzoyl)-6-(4-trifluoromethylphenyl)isonicotinic Acid (1f). A solution of the above ester (5.0 g, 11 mmol) in AcOH (140 ml) and concentrated HCl (27 ml) was refluxed 3.5 hr. Additional HCl (30 ml) was added and the solution was heated an additional 90 min. Filtration gave the title acid (4.4 g, 90%), mp 215–217°. A sample, recrystallized from toluene, had mp 215–217°

Anal. ($C_{21}H_{11}F_6NO_3$) C, H, F, N.

This acid was converted to the bromomethyl ketone **2e** as previously described.

2-(4-Trifluoromethylbenzoyl)-6-(4-trifluoromethylphenyl)-4-pyridylethylene Oxide. The bromo ketone was reduced with $NaBH_4$ as previously described.[1] The resulting epoxycarbinol was oxidized with SeO_2 as described for 4-carbethoxy-6-trifluoromethyl-2-pyridinecarboxaldehyde. Crystallization from *i*-PrOH (10 ml) gave the title compound (2.2 g, 67%), mp 104–106°. A sample, recrystallized from *i*-PrOH, had mp 110–112°.

Anal. ($C_{22}H_{13}F_6NO$) N.

The ethylene oxide was converted to the amino alcohol **3h** by the procedure previously described.[1]

Acknowledgment. The advice and timely suggestions of Drs. T. R. Sweeney and R. E. Strube of the Walter Reed Army Institute of Reserach are gratefully acknowledged.

References

(1) P. Blumbergs, M. P. LaMontagne, A. Markovac, J. G. Moehring, A. B. Ash, and C. L. Stevens, *J. Med. Chem.*, 15, 808 (1972).
(2) A. Markovac, M. P. LaMontagne, P. Blumbergs, A. B. Ash, and C. L. Stevens, *ibid.*, **15**, 918 (1972).
(3) T. S. Osdene, P. B. Russell, and L. Rane, *ibid.*, 10, 431 (1967).
(4) W. E. Feeley and E. M. Beavers, *J. Amer. Chem. Soc.*, 81, 4004 (1959).
(5) R. E. Lutz, P. S. Bailey, M. T. Clark, J. F. Codington, A. J. Deinet, J. A. Freek, G. H. Harnest, N. H. Leake, T. A. Martin, R. J. Rowlett, Jr. J. M. Salsbury, N. H. Shearer, Jr., J. D. Smith, and J. W. Wilson, III, *ibid.*, 68, 1813 (1946).
(6) V. Boekelheide and W. J. Linn, *ibid.*, 76, 1286 (1954).
(7) N. Sperber, M. Sherlock, D. Papa, and D. Kender, *ibid.*, 81, 704 (1959).

Structure and Antischistosomal Activity in the Nitrofuran Series. Requirement for a 5-Nitro-2-furyl–Vinyl Moiety Based on Comparison of 3-(5-Nitro-2-furyl)-Substituted Propionic, Acrylic, and Propiolic Acid Derivatives†

P. B. Hulbert, E. Bueding, and C. H. Robinson*

Department of Pharmacology and Experimental Therapeutics, School of Medicine, and Department of Pathobiology, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Maryland 21205. *Received May 24, 1972*

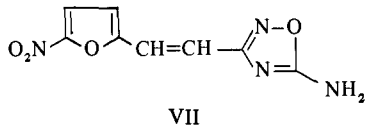
The structural features required for antischistosomal activity in the nitrofuran series have been postulated to include linkage of the nitrofuran *via* a vinyl group to a nitrogen atom of low basicity. This proposal has been examined by the synthesis and testing against *Schistosoma mansoni* of a series of amides of 3-(5-nitro-2-furyl)propionic acid (Ia) and 3-(5-nitro-2-furyl)propiolic acid (IIIa) for comparison with a corresponding series of amides derived from 3-(5-nitro-2-furyl)acrylic acid (IIa). A direct comparison of active amides derived from IIa with exact analogs differing only in the substitution of a C≡C or CH_2CH_2 grouping for a *trans*-CH=CH group has been made. The results show that the vinyl bridge is required for manifestation of antischistosomal activity by such nitrofuran derivatives. Differences in activity among the various amides of IIa are attributed to differences in lipophilicity. Additional studies using *Schistosoma japonicum* revealed that whereas two of the most active amides, IId and IIg, effected few parasitological cures at tolerated doses, the nitrofuran derivative VII was highly effective using large but tolerated doses.

Schistosomiasis, a parasitic disease afflicting approximately 200 million of the world's population, remains a great challenge to the medicinal chemist. (For a recent review, see ref 2.) It has been estimated that over a quarter of a million compounds have been tested for antischistosomal activity;[3] yet no drug has yet emerged which has gained general acceptance and widespread use.

†This investigation was supported in part by Research Grants GM 16492 and AI 08022 from the National Institutes of Health, as well as Research Grant GA BMS 7011 from the Rockefeller Foundation. Presented[1] in part at the Sixth Middle Atlantic Regional Meeting of the American Chemical Society, Baltimore, Md., Feb 1971.

We have already described[4] the general structural and conformational features present in those few nitrofurans and nitrothiazoles which demonstrate good antischistosomal activity and indicated that these features are rather specific. While it is well recognized that nitrofurans may be chemotherapeutically useful against a wide spectrum of organisms,[5] very few of these compounds exhibit antischistosomal activity, suggesting that the structural features necessary for nitrofurans to show this anthelmintic activity are far more precise.

We have reported that *trans*-5-amino-3-[(5-nitro-2-furyl)-vinyl]-1,2,4-oxadiazole (VII)[4] and some close analogs[6]



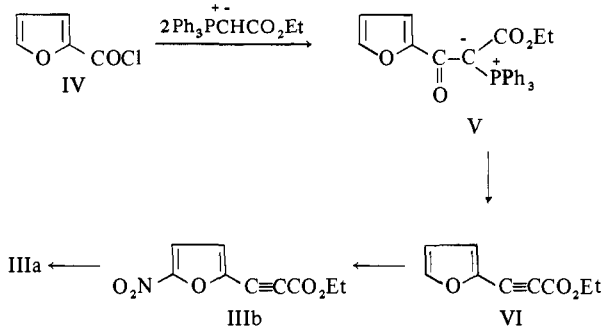
VII

possess excellent antischistosomal activity. As a consequence of this finding, and of reports in the Chinese literature[7] that certain simple amides of nitrofurylacrylic acid possessed clinically useful activity, we have become interested in the particular structural features that confer activity to nitrofurans. As a first step, we have tried to determine the role of the vinyl group, present in both these two series of compounds, and to determine whether the activity of nitrofurans could be improved by modifying this group. In this paper we describe the synthesis of and the results of antischistosomal testing on certain amides of 3-(5-nitro-2-furyl)propionic acid (Ia), 3-(5-nitro-2-furyl)acrylic acid (IIa), and 3-(5-nitro-2-furyl)propiolic acid (IIIa) and demonstrate that the presence of the vinyl group is essential for the antischistosomal activity of such nitrofurans.

Chemistry. 3-(5-Nitro-2-furyl)acrylic acid (IIa), obtained by nitration of furylacrylic acid,[8a] is a readily accessible material, and amides of IIa have been reported.[7b,8b-f] Surprisingly, 3-(5-nitro-2-furyl)propionic acid (Ia) has not been described previously. Because of the acid sensitivity of simple furans, we could not prepare this acid by nitration of furylpropionic acid or its amides. However, by using diimide (HN=NH) (for reviews, see ref 9), generated *in situ* from potassium azodicarboxylate,[10] Ia could be obtained from IIa under mild conditions and in excellent yield.

The acetylenic acid, 3-(5-nitro-2-furyl)propiolic acid (IIIa), has been prepared by alkaline dehydrobromination and concomitant hydrolysis of methyl 3-(5-nitro-2-furyl)-2,3-dibromopropionate,[11] but because of the sensitivity to nitrofurans to bases, this route is not suitable for preparative purposes. We chose the alternative route shown in Scheme I, for which good yields were obtained in each step.

Scheme I



By reaction with 2 molar equiv of carbethoxymethylenetriphenylphosphorane, furoyl chloride (IV) was converted to the keto ylide V, which was then pyrolyzed at 270° for 1 hr *in vacuo*; the acetylenic product,[12] ethyl furylpropiolate (VI), distilled out. This ester (VI) was nitrated with HNO_3 (*d* 1.5) in Ac_2O containing a small amount of concentrated H_2SO_4, at −25°, to yield the nitrofuryl ester IIIb. The known sensitivity of nitrofurans to bases suggested that the parent acid IIIa would be best prepared by acid hydrolysis of IIIb, but attempts to prepare IIIa by this method failed; the acetylenic bond became hydrated. However, it was found that the ester IIIb was extremely readily hydrolyzed in base (*e.g.*, by 0.3 *M* aqueous NaOH in glyme for 3 min at room temperature) to yield the required 3-(5-nitro-2-furyl)propiolic acid (IIIa).

The amides Ic-j, IIc-k, and IIIc-j of these nitrofuryl acids were prepared using the neutral and mild conditions provided by the mixed anhydride procedure developed for peptide synthesis.[13] This method was essential for the preparation of the acetylenic amides IIIc-j, where not only the great susceptibility for amines and hydroxylic solvents to attack conjugated triple bonds in a nucleophilic manner[14] but also the base sensitivity of nitrofurans had to be overcome.

Pharmacology. On administration of active antischistosomal compounds to infected mice, damage to the reproductive system of the female schistosome may be observed as an early event. In addition, a number of specific biochemical changes within the parasite may be used as indicators of compound activity. Two of the changes that may be conveniently measured are (i) the reduction of the glycogen phosphorylase phosphatase activity of the worms and (ii) the consequent decrease in the glycogen content of the worms.

We have shown[4] that all these early events occur before the damaged schistosomes are swept to the liver from the mesenteric veins by the venous blood flow (the "hepatic shift"). Although the schistosomes may gradually recover and return to the mesenteric veins, these early events provide a very useful, and sensitive, assay of biological activity. We believe that an animal should be considered "parasitologically cured" only when *no* live worms may be found after a period of 4-5 weeks.

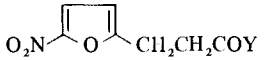
For routine testing, we administered the nitrofuran orally to the infected mice at a dose level of 250 mg/kg twice a day for 5 days. If the nitrofuran proved lethal to some mice during treatment at this dose level, the dose given subsequently to the remaining mice was lowered. To examine the short term effects, a selected number of mice were dissected 8 days after the first dose, and the distribution of live parasites was ascertained. These schistosomes were analyzed biochemically for changes in their glycogen metabolism and assessed by an *intra vitam* staining method[15] for damage to the female reproductive system. To assess longer term effects the surviving mice were examined for live schistosomes 4-5 weeks after the last dose.

Pharmacological Results. The nitrofurans synthesized for this study are shown in Table I, which also reports the antischistosomal activity against *S. mansoni*.

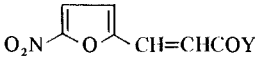
(i) 3-(5-Nitro-2-furyl)propionic Acid Derivatives. 3-(5-Nitro-2-furyl)propionic acid (Ia), its ethyl ester Ib, and its amides Ic-j are all virtually inactive as antischistosomal agents. The first members of the series Ia-c showed appreciable host toxicity, but later members of the series Id-j were well tolerated. The only general property of this series of compounds is that certain members (Ia,c,d,h-j) caused damage to the reproductive system of the parasite. While the isopropylamide Id showed faint antischistosomal activity, this activity was negligible in comparison with its vinyl analog IId.

(ii) 3-(5-Nitro-2-furyl)acrylic Acid Derivatives. This series IIa-k, of which IIa-j were the acrylic acid derivatives corresponding to Ia-j, included compounds showing high antischistosomal activity. Chinese investigators have described[7] a wide range of amides and esters of nitrofurylacrylic acid, of which the amides IId,f-h had the highest activity. Indeed the isopropylamide IId and the *N*-(2-aminoethylpiperidyl)-amide IIg show high activity in our own test procedure, although we observed only a small proportion of parasitological cures at doses below the LD_{50}. We found that the other two reportedly most active compounds, the cyclohexylamide IIf and the amide of ethyl glycinate IIh, were much

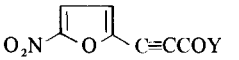
Table I. Short-Term and Long-Term Antischistosomal Effects of 5-Nitro-2-furyl Compounds on *S. mansoni* in Mice



Ia-j



IIa-k



IIIa-j

Compd	Y	Formula[a]	Mp, °C	Lit. mp, °C	Dosage schedule	Mouse mortality, %	Short-term effects 3 days after last dose: Reduction of phosphorylase phosphatase, %	Reduction of glycogen levels, %	Damage to reproductive system, %	Hepatic shift, %	Long-term effects 4-5 weeks after last dose: Reduction in no. of worms, %	Parasitological cures, %
					3-(5-Nitro-2-furyl)propionic Acid Derivatives							
Ia	-OH	$C_7H_7NO_5$	120-122		250 mg/kg b.i.d. 2 doses + 70 mg/kg b.i.d. 4 doses	60	0	0	15	0	0	0
Ib	$-OC_2H_5$	$C_9H_{11}NO_5$	Oil		250 mg/kg b.i.d. 1 dose + 80 mg/kg b.i.d. 1 dose	70	0	0	0	0	0	0
Ic	$-NH_2$	$C_7H_8N_2O_4$	141		250 mg/kg b.i.d. 2 doses + 125 mg/kg b.i.d. 8 doses	10	0	0	32	0	0	0
Id	$-NHCH(CH_3)_2$	$C_{10}H_{14}N_2O_4$	111		250 mg/kg once daily, 5 doses	0	8	12	15	0	15	0
Ie	$-NH(CH_2)_5CH_3$	$C_{13}H_{20}N_2O_4$	81		250 mg/kg b.i.d. 10 doses	20	0	0	0	0	0	0
If	$-NH$-c-C_6H_{11}	$C_{13}H_{18}N_2O_4$	131		250 mg/kg b.i.d. 10 doses	10	0	0	0	0	0	0
Ig	$-NH(CH_2)_2$-c-$NC_5H_{10}\cdot HCl$[e]	$C_{14}H_{22}N_3O_4Cl$	136-138		250 mg/kg b.i.d. 10 doses	10	0	0	0	0	0	0
Ih	$-NHCH_2CO_2C_2H_5$	$C_{11}H_{14}N_2O_6$	81		250 mg/kg b.i.d. 10 doses	0	0	0	13	0	0	0
Ii	$-NHCH_2CONH_2$	$C_9H_{11}N_3O_5$	124		250 mg/kg b.i.d. 10 doses	0	0	0	30	0	0	0
Ij	-c-NC_5H_{11}	$C_{12}H_{16}N_2O_4$	89-90		250 mg/kg b.i.d. 10 doses	40	0	0	20	0	0	0
					3-(5-Nitro-2-furyl)acrylic Acid Derivatives							
IIa	-OH	$C_7H_5NO_5$	238-239 dec	235-236[b]	250 mg/kg b.i.d. 10 doses	0	7	12	0	0	0	0
IIb	$-OC_2H_5$	$C_9H_9NO_5$	126	124-126[c]	250 mg/kg b.i.d. 10 doses	0	0	0	7	0	0	0
IIc	$-NH_2$	$C_7H_6N_2O_4$	233	223[c]	250 mg/kg b.i.d. 8 doses + 125 mg/kg b.i.d. 2 doses	30	72	81	92	100	91	25
IId	$-NHCH(CH_3)_2$	$C_{10}H_{12}N_2O_4$	189-191	187-188[c]	250 mg/kg b.i.d. 10 doses	0	100	100	100	100	83	14
IIe	$-NH(CH_2)_5CH_3$	$C_{13}H_{18}N_2O_4$	111		250 mg/kg b.i.d. 10 doses	50	44	50	15	50	39	0
IIf	$-NH$-c-C_6H_{11}	$C_{13}H_{16}N_2O_4$	180	177-178[c]	250 mg/kg b.i.d. 10 doses	10	52	45	17	20	36	0
IIg	$-NH(CH_2)_2$-c-$NC_5H_{10}\cdot HCl$[e]	$C_{14}H_{20}N_3O_4Cl$	231		150 mg/kg b.i.d. 5 doses	0	55	47	4	0	0	0
					150 mg/kg b.i.d. 10 doses	0	71	61	32	70	73	0
					250 mg/kg b.i.d. 10 doses	10	83	87	38	90	91	28
					400 mg/kg b.i.d. 9 doses	50					100	100
IIh	$-NHCH_2CO_2C_2H_5$	$C_{11}H_{12}N_2O_6$	191-192	190-191[c]	250 mg/kg b.i.d. 10 doses	0	50	50	30	30	40	0
IIi	$-NHCH_2CONH_2$	$C_9H_9N_3O_5$	231		250 mg/kg b.i.d. 10 doses	0	0	0	0	0	0	0
IIj	-c-NC_5H_{11}	$C_{12}H_{14}N_2O_4$	138	136[c]	250 mg/kg b.i.d. 8 doses	10	68	74	17	100	65	0
IIk	$-NHC(CH_3)_3$	$C_{11}H_{14}N_2O_4$	222		250 mg/kg b.i.d. 10 doses	0	23	27	23	0	15	0
VII	Reference compound				150 mg/kg b.i.d. 10 doses	0	100	100	100	100	100	100
					3-(5-Nitro-2-furyl)propiolic Acid Derivatives							
IIIa	-OH	$C_7H_3NO_5$	134-135	129-130[d]	250 mg/kg b.i.d. 7 doses + 125 mg/kg b.i.d. 1 dose	30	0	0	5	0	0	0
IIIb	$-OC_2H_5$	$C_9H_7NO_5$	97.5		250 mg/kg b.i.d. 7 doses	25	0	0	60	0	0	0
IIIc	$-NH_2$	$C_7H_4N_2O_4$	177	160-161[d]	50 mg/kg b.i.d. 6 doses + 20 mg/kg b.i.d. 4 doses	25	0	0	25	20	0	0

IIId	$-NHCH(CH_3)_2$	$C_{10}H_{10}N_2O_4$	126	250 mg/kg b.i.d. 2 doses	75	0	0	50	0	0	0
				60 mg/kg b.i.d. 8 doses	15	0	0	10	0	0	0
IIIe	$-NH(CH_2)_5CH_3$	$C_{13}H_{16}N_2O_4$	93	200 mg/kg b.i.d. 10 doses	35	0	0	25	0	0	0
IIIf	$-NH$-c-C_6H_{11}	$C_{13}H_{14}N_2O_4$	148	140 mg/kg b.i.d. 10 doses	0	0	0	20	0	0	0
IIIg	$-NH(CH_2)_2$-c-$NC_5H_{10} \cdot HCl$[e]	$C_{14}H_{18}N_3O_4Cl$	142 dec	250 mg/kg b.i.d. 4 doses + 150 mg/kg b.i.d. 6 doses	30	0	0	10	0	0	0
IIIh	$-NHCH_2CO_2C_2H_5$	$C_{11}H_{10}N_2O_6$	111–112	140 mg/kg b.i.d. 4 doses + 70 mg/kg b.i.d. 6 doses	40	0	0	35	0	0	0
IIIi	$-NHCH_2CONH_2$	$C_9H_7N_3O_5$	170–171	250 mg/kg b.i.d. 1 dose + 125 mg/kg b.i.d. 9 doses	25	0	0	15	0	0	0
IIIj	-c-NC_5H_{11}	$C_{12}H_{12}N_2O_4$	148	250 mg/kg b.i.d. 10 doses	0	0	0	35	0	0	0

[a] Analyses for C, H, N (and where appropriate Cl) were obtained for all compounds and were within ±0.3% of the theoretical values. [b] Reference 8a. [c] Reference 7b. [d] Reference 11. [e] c-NC_5H_{10} = piperidinyl.

less active than IId and IIg because, although appreciable short-term effects were observed, no parasitological cures were obtained. Of the remaining members of the series IIa-c,-e,i-k, only the rather toxic primary amide IIc showed parasitological cures, although IIe and IIj (the only tertiary amide in the group) showed significant activity.

Dose-response data for the reference compound VII were obtained, Table II, in order to study the relationship between the various biological and biochemical events observed for this series. These data are presented as a log dose-response curve in Figure 1. The values for the reduction of glycogen phosphorylase phosphatase activity and for the consequent loss in glycogen run closely parallel throughout the dose-response curve, indicating their dependence. Substantial effects in these biochemical parameters are noted at doses smaller than those needed to cause the hepatic shift. At doses causing hepatic shift, the percentage of worms shifted (as measured 3–4 days after the last dose) coincides with the percentage reduction in the numbers of live worms (measured 4–5 weeks later). This finding demonstrates that the effect of VII is an irreversible one; *i.e.*, with this compound those worms that are shifted do not recover and return to the mesenteric veins. Instead they die.

The coincident curves for hepatic shift and reduction in the number of worms show that a minimum amount of compound is needed before these effects are seen, but then the curves rise steeply, as required by an "all or nothing" type of toxic response. Similarly, a minimum amount of VII is needed to obtain any parasitological cures, but then a relatively small increase in compound gives rise to complete parasitological cures.

The dose-response data for IIg, Table I, are closely similar to those of VII. Again the reductions in phosphorylase phosphatase activity and in the glycogen levels of the worms are observed at doses of IIg smaller than those necessary to shift the worms. At doses causing hepatic shift, there is an equally good correspondence between the hepatic shift and the final reduction in the numbers of worms again indicating an irreversible effect. The dose-response curve measuring parasitological cures is very steep as is the case with VII.

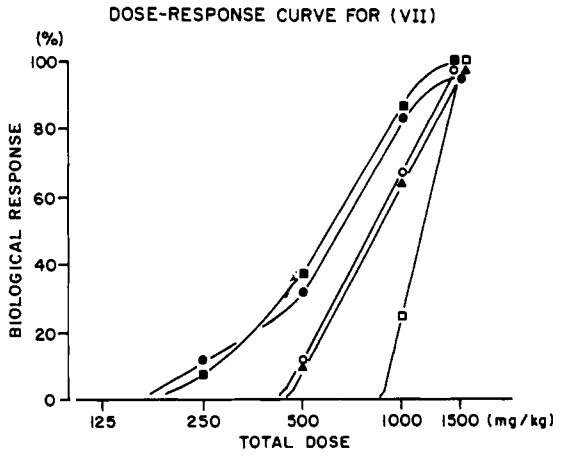


Figure 1. Total dose-response curve for compound VII. Total dose is shown on a logarithmic scale on the abscissa and is the sum of ten equal doses administered twice daily for 5 days. The following biological responses are shown on the ordinate: (–●–) reduction of phosphorylase phosphatase activity; (–■–) reduction of schistosome glycogen levels; (–▲–) the hepatic shift; (–○–) reduction in number of worms; (–□–) parasitological cures.

Table II. Dose Response to Compound VII of Mice Infected with *S. Mansoni*

Unit dose,[a] mg/kg	Mortality, %	Reduction of phosphorylase phosphatase activity, %	Reduction of glycogen levels, %	Damage to female reproductive system, %	Hepatic shift, %	Reduction in no. of worms, %	Parasitological cures, %
12.5	0	0	0	0	0	0	0
25	0	12	8	18	0	0	0
50	0	32	38	44	10	12	0
100	0	84	38	80	65	68	25
150	0	96	100	100	100	100	100

[a] Administered twice daily orally in 25% glycerol for 5 consecutive days.

The effects of IIg, compared with those of VII, indicate that these compounds have the same mode of action. Indeed, at a molar basis, IIg and VII are equipotent. Unit doses of 150 mg/kg of IIg and 100 mg/kg of VII produced respectively 73 and 68% reduction in worms. These unit doses correspond to total doses of 4.55 and 4.50 mmol/kg. Thus, the difference between IIg and VII lies not in their potency as antischistosomal agents but rather in their toxicity to the host. In each compound throughout this series there is close correspondence between the reduction in phosphorylase phosphatase activity and the reduction of glycogen levels. Also, for most compounds the hepatic shift can be correlated with the reduction in the number of worms. These results suggest a common mode of action for the active members of this series.

(iii) 3-(5-Nitro-2-furyl)propiolic Acid Derivatives. The compounds IIIa-j of this series were more toxic than the corresponding members of the other two series. Although they damaged the reproductive system of female worms, no other adverse effects on the parasites were shown.

Discussion

The pharmacological results demonstrate that whereas significant antischistosomal activity is found in derivatives of 3-(5-nitro-2-furyl)acrylic acid (IIa), it is not found in the corresponding derivatives of 3-(5-nitro-2-furyl)propionic acid (Ia) or 3-(5-nitro-2-furyl)propiolic acid (IIIa). This indicates that the vinyl group is essential for the activity of these nitrofurans, and it is presumed that the vinyl group is also an essential feature for the activity of related 5-nitro-2-vinylfurans, such as *trans*-5-amino-3-[(5-nitro-2-furyl)-vinyl]-1,2,4-oxadiazole (VII) (Table I) and some of its congeners.[4] These compounds show the same pattern of early biochemical and morphological changes as derivatives of IIa, which suggests a common mode of action for 5-nitro-2-vinylfuran derivatives.

We believe that the structural features, important for the activity of these compounds, are a furan ring bearing (i) a nitro group at C-5 and (ii) a C-2 side chain containing a vinyl group and a nitrogen atom of low basicity. The necessity for a 5-nitro group is indicated by the lack of activity of the denitro analog of VII[4] and of other 5-substituted 2-furylacrylamides.[16]

The nitro group is known to be required for the antibacterial activity of nitrofurans,[5] and biological reduction of this functional group would appear to be necessary for their action.[17] The nitro group of VII is known to be reduced in mammalian liver systems,[18] and one may postulate that reduction of the nitro group (*e.g.*, to a hydroxylamine or nitroso moiety) in these antischistosomal compounds, either by the host or the parasite, is also a prerequisite for their biological activity. The ease of reduction, as measured by electrochemical methods, of a number of nitrofurans has been correlated with their antibacterial activity,[19] and a similar correlation may become possible with antischistosomal nitro heterocycles.

The requirement for a vinyl group in the C-2 side chain has already been established, and one explanation for its need would be to facilitate reduction of the nitro group. In addition, there appears to be a requirement in these compounds for a nitrogen substituent of low basicity[4] attached by an sp^2 carbon atom to this vinylene group. This further extension of the conjugated system may also contribute to the ease of reduction of the nitrofuran. These suggestions are supported by the observation[17] that the widely studied antibacterial agent, 3-amino-6-[(5-nitro-2-furyl)vinyl]-1,2,4-triazine,[5] which meets all these structural requirements, is over 30 times more easily reduced than nitrofuran itself. Furthermore, this triazine manifests antischistosomal activity, although no consistent parasitological cures were obtained even at the highest tolerated dose levels.‡ This activity was associated with the same biochemical changes as with the 5-nitro-2-vinylfurans described above.

The variation in activity between the various amides of 3-(5-nitro-2-furyl)acrylic acid is striking. We believe this represents the varying ability of these amides to reach their site of action within the parasite. An active antischistosomal agent needs not only to reach the mesenteric veins of the host but must also be capable of penetrating into the schistosome. Therefore, the number of biological membranes the drug must cross will be large, and, in such circumstances, a strong correlation between the lipophilicity of the drug and its antischistosomal activity is likely to exist. According to Hansch[20,21] and to McFarland,[22] when drugs are required to cross a relatively large number of biological membranes to reach the same site of action, the relationship between the logarithm of their biological activity and their lipophilicity approximates to a parabola, in which case there is an optimum value for the lipophilicity of a drug. Indeed, for our series of 5-nitro-2-furylacrylic acid derivatives IIa-k, the π values of the isopropyl substituent (1.3) and the ethylpiperidyl substituent (*ca.* 1.1) are very close, indicating that the active compounds IId and IIg are nearly isolipophilic and may be approaching the optimum lipophilicity for antischistosomal drugs. Furthermore, the $\Sigma\pi$ values of compounds IId and IIg, calculated according to Hansch,[23] are +0.8 and +0.6 which are comparable to the value of +0.9 found[24] for optimum lipophilicity in an unrelated series of antischistosomal compounds. We suggest that the poorer activity of the other amides of 5-nitro-2-furylacrylic acid is a result of a less suitable lipophilic character.

An important aspect of this work is the observation that the three compounds IId, IIg, and IIh, at least two of which have been used in clinical trials in China,[7] produce few parasitological cures in mice at tolerated doses The majority of the treated mice still remain infected although com-

‡E. Bueding and D. A. Henry, unpublished data, 1971.

Table III. Chemotherapeutic Activity of Three Nitrovinylfurans in Mice Infected with *Schistosoma japonicum*

Compd	Dosage	Duration of treatment, days	No. of mice	Mouse mortality, %	Reduction in no. of worms, %	Parasitological cures, %
IId	400 mg/kg once daily	10	15	13	50	13
IIg	400 mg/kg once daily	10	14	14	54	14
VII	500 mg/kg b.i.d.	10	15	0	60	33
	500 mg/kg b.i.d.	15	14	0	89	71
	500 mg/kg b.i.d.	20	15	0	100	100

pounds IId and IIg greatly reduced the numbers of schistosomes. The species of parasite predominant in China is *S. japonicum*; our tests employed *S. mansoni*. To ensure that both species showed similar drug sensitivity, compounds IId and IIg as well as VII were tested against *S. japonicum* (Table III). Although these compounds showed high activity, again it was found that even when IId and IIg were given at doses which were slightly above the maximally tolerated levels, only a few of the mice were parasitologically cured. In contrast, administration of compound VII effected complete parasitological cures, albeit at high but tolerated dose levels.

The limited effectiveness of IId and IIg demonstrates the need in schistosomiasis chemotherapy for careful laboratory tests before clinical trials are attempted. This point is emphasized by our animal experiments with hycanthone in *S. mansoni*. This clinically used drug[25] produces very few parasitological cures in mice.[26] Furthermore, a single dose of hycanthone can give rise to schistosomes resistant to this compound.[26] These results, as well as its reported mutagenic,[27,28] teratogenic,[29] and hepatotoxic[30] effects, must raise doubts about the long-term effectiveness and safety of this drug.

We have examined in this paper structural features required for antischistosomal activity in nitrofurans and have demonstrated the need for a 5-nitro-2-vinylfuran system. A number of amides of 3-(5-nitro-2-furyl)acrylic acid, which exemplify this system, were shown to be highly active but toxic. However, none of these amides showed the low toxicity, and hence good therapeutic properties, of another 5-nitro-2-vinylfuran derivative, compound VII.

Experimental Section

General. Melting points were determined on a Kofler hot-stage melting point apparatus. The procedures followed to obtain mice infected with adult schistosomes have already been described,[6] and the biochemical methods used to determine glycogen levels and the reduction of glycogen phosphorylase phosphatase were those of a previous study.[4] The parameters and staining procedure developed to score damage of the reproductive system of female schistosomes have now been published.[15]

(α-Carbethoxy-α-2-furoylmethylene)triphenylphosphorane (V). To a stirred solution at 25° of (carbethoxymethylene)triphenylphosphorane (104.5 g, 0.30 mol) in dry benzene (1.0 l.), 2-furoyl chloride (IV, 19.6 g, 0.15 mol) in benzene (100 ml) was added slowly over 15 min. The reaction was stirred overnight, and the precipitate (carbethoxy methyltriphenylphosphonium chloride) was then filtered off and the filtrate was evaporated to yield V (66.4 g, 100% yield) as an oil which crystallized on standing, mp 111° (EtOAc–hexane). *Anal.* ($C_{27}H_{23}O_4P$) C, H, P.

Ethyl 2-Furylpropiolate (VI). The keto ylide V (64.0 g) was pyrolyzed under 0.1 mm of vacuum at 270° for 1 hr and the pyrolysis products were collected in a cold trap. This distillate was extracted with petroleum ether (bp 30–60°) (total 500 ml) and the extract filtered through Florisil (200 g). After further elution, the combined petroleum ether fractions gave on evaporation crystalline ethyl 2-furylpropiolate (VI, 16.6 g, 70% yield), mp 39.5–39.8° (hexane). *Anal.* ($C_9H_8O_3$) C, H.

Ethyl 5-Nitro-2-furylpropiolate (IIIb). A nitrating mixture was prepared by adding fuming HNO_3 (*d* 1.52) (19.0 g, 0.3 mol) and concentrated H_2SO_4 (0.5 ml) dropwise to redistilled Ac_2O (70 ml) cooled to −10°; it was then maintained at −25° throughout the reaction period. Ethyl 2-furylpropiolate (VI, 16.4 g, 0.10 mol) in Ac_2O (30 ml) was added and after 30 min, during which time some product crystallized, the whole reaction mixture was poured into ice–water (300 ml). The resulting precipitate, after chromatography on silica gel (200 g) with benzene, gave ethyl 5-nitro-2-furylpropiolate (IIIb) (12.1 g, 0.058 mol, 58%), mp 97.5° (benzene). *Anal.* C, H, N.

5-Nitro-2-furylpropiolic Acid (IIIa). This acid was prepared as required. To a stirred solution, at 25°, of ethyl 5-nitro-2-furylpropiolate (IIIb, 1.045 g, 5 mmol) in $MeOCH_2CH_2OMe$ (50 ml), aqueous 0.33 *N* NaOH (45 ml) was added in one portion. After 3 min, the solution was poured into iced 2 *N* HCl and extracted (three times) with EtOAc. The combined EtOAc extracts were washed (0.1 *N* HCl, a little H_2O, saturated NaCl) and evaporated, with added benzene, to yield 5-nitro-2-furylpropiolic acid (IIIa, 0.902 g, 99%), mp 134–135° (C_6H_6–$CHCl_3$) (lit.[11] mp 129–130°). *Anal.* C, H, N. This compound had ν^{KBr} 2220 cm^{-1}, and all derivatives of this acid showed a similar acetylenic absorption frequency.

3-(5-Nitro-2-furyl)acrylic Acid (IIa). A nitrating mixture was prepared by adding fuming HNO_3 (*d* 1.52) (40 g) and H_3PO_4 (85%, 20 g) dropwise to redistilled Ac_2O (120 ml) and CCl_4 (80 ml) cooled to −10° and stirred mechanically. At −15°, finely powdered furylacrylic acid (40 g) was added in portions (5 g) over 40 min. After further stirring (30 min), the reaction mixture was cooled to −20° and filtered at this temperature. The precipitate, IIa, was thoroughly washed with water and dried *in vacuo* at 50° (35.7 g, 67% yield), mp 238–239° dec (MeOH–EtOAc) (lit.[8a] 235–236°). *Anal.* C, H, N.

3-(5-Nitro-2-furyl)propionic Acid (Ia). To 3-(5-nitro-2-furyl)acrylic acid (IIa, 10.98 g, 60 mmol), dissolved in a mixture of $MeOCH_2CH_2OMe$ (300 ml) and MeOH (250 ml), was added freshly prepared potassium azodicarboxylate (35.0 g, 180 mmol) over 15 min. Glacial AcOH (21.6 g, 360 mmol) in $MeOCH_2CH_2OMe$–MeOH (1:1, 60 ml) was added dropwise over 5 hr. Additional AcOH (21.6 g) was added over a further 5-hr period. The reaction could be followed spectroscopically by periodic removal and work-up of aliquots. In the ir (KBr disk) the CO_2H frequency shifted from 1610 to 1695 cm^{-1}, with disappearance of the C=C absorption at 1660 cm^{-1}, on going from IIa to Ia. In the nmr spectrum (acetone-d_6 with 10% DMSO-d_6) the two doublets (J = 16 Hz) centered on δ 6.55 and 7.52 (*trans*-vinyl hydrogens of IIa) disappeared, and a new multiplet (4 H, CH_2CH_2 of Ia) appeared at δ 2.5–3.0 (DMSO-d_6). The mixture was stirred 12 hr, and the solvents were removed. The residue was partitioned between EtOAc and 2 *N* HCl. The aqueous layer, after ensuring pH <2, was reextracted with EtOAc. The EtOAc layers were combined, washed (2 *N* HCl, water, saturated NaCl), and evaporated with added benzene and toluene to give 3-(5-nitro-2-furyl)propionic acid (Ia, 9.9 g, 89% yield), mp 120–122° (MeOH). *Anal.* C, H, N.

Ethyl 3-(5-Nitro-2-furyl)acrylate (IIb). The acid IIa (1.83 g, 10 mmol) was refluxed in absolute EtOH (50 ml) containing concentrated H_2SO_4 (3 ml) for 5 hr. The solution was allowed to cool overnight, and the precipitated ester IIb was filtered off (1.71 g, 81% yield), mp 126° (lit.[7b] mp 124–126°). *Anal.* C, H, N.

Ethyl 3-(5-Nitro-2-furyl)propionate (Ib). The acid Ia (1.388 g) was dissolved in dry EtOAc (50 ml) and *N*-methylmorpholine (758 mg) added. After cooling to −15°, ethyl chloroformate (742 mg) was added. After 2 min, absolute EtOH (2 ml) was added, and the mixture was stirred overnight at 25°. The filtrate, after chromatography on silica gel, gave the ester Ib (1.257 g, 78% yield) as an oil, which for analysis was crystallized at low temperature from MeOH–pentane–Et_2O. *Anal.* C, H, N.

General Method for Preparation of the Amides Ic–j, IIc–k, and IIIc–j. These were prepared in the following manner (for modifications, see below). To the parent acid (10 mmol) dissolved in dry EtOAc or dry $MeOCH_2CH_2OMe$ (50–100 ml), *N*-methylmorpholine (10 mmol) was added, the solution was cooled to −15° and redistilled ethyl chloroformate (10 mmol) added, and the mixture stirred for 2 min at −15°. The appropriate amine (10 mmol) was added,

and the mixture was allowed to reach 20° while stirring for 30 min and was then filtered. The filtrate (if glyme, this was evaporated and the product taken up in EtOAc) was washed (dilute $NaHCO_3$, H_2O, dilute HCl, H_2O, saturated NaCl) and evaporated. The product was, in most cases, chromatographed on silica gel and was always crystallized, usually from EtOAc–hexane, yields 60–95%. For analytical data, see Table I.

Modifications. (a) For the primary amides Ic, IIc, and IIIc, the mixture was filtered at −15°, 2 min after the addition of ethyl chloroformate, and dry gaseous NH_3 was then introduced. The washing procedure was omitted and the amides were obtained directly by chromatography of the reaction mixture on silica gel. [The amide IIIa could also be obtained directly from ester IIIb by treatment for 10 min at 25° with a solution of 0.88 *N* NH_4OH in EtOH (1:1).]

(b) For the **2-(*N*-piperidino)ethylamides Ig, IIg, and IIIg**, the reaction mixture was filtered at the same stage as (a). After addition of 2-(*N*-piperidino)ethylamine (10 mmol) the reaction mixture was stirred under N_2 for 30 min, and dry HCl gas passed in. After flushing the excess HCl gas away with N_2, the mixture was cooled and filtered to give the required amine hydrochlorides Ig, IIg, and IIIg, which were crystallized from EtOH–C_6H_6.

(c) For the **glycin(ethyl ester)amides Ih, IIh, and IIIh**, 2 equiv (*i.e.*, 20 mmol) of *N*-methylmorpholine was used because glycine (ethyl ester) HCl, rather than the free base, was added.

(d) For the **glycinamide amides Ii, IIi, and IIIi**, procedure c could not be used. The glycinamide had to be generated from its hydrochloride. Thus, glycinamide HCl (10 mmol) in MeOH was treated with NaOMe (10 mmol), the solvent evaporated, and the free amine dissolved in dry DMF. This solution was added to the mixed anhydride reaction mixture, and after 30 min the amide was obtained directly by chromatography of the reaction mixture on silica gel.

Acknowledgment. We thank Miss Jean Fisher and Mrs. Geraldine Peterson for valuable assistance.

References

(1) P. B. Hulbert and C. H. Robinson, Abstracts of the Sixth Middle Atlantic Regional Meeting of the American Chemical Society, Baltimore, Md., Feb 1971, p 31.
(2) L. M. Werbel, "Topics in Medicinal Chemistry," Vol. III, J. L. Rabinowitz and R. M. Myerson, Ed., Wiley-Interscience, New York, N. Y., 1970, p 125.
(3) E. F. Elslager, 12th National Medicinal Chemistry Symposium of the American Chemical Society, Seattle, Wash., 1970.
(4) C. H. Robinson, E. Bueding, and J. Fisher, *Mol. Pharmacol.*, 6, 604 (1970).
(5) K. Miura and H. K. Reckendorf, *Progr. Med. Chem.*, 5, 320 (1967).
(6) E. Bueding, C. Naquira, S. Bouwman, and G. Rose, *J. Pharmacol. Exp. Ther.*, 178, 402 (1971).
(7) (a) H. H. Liu, L. C. Chang, M. L. Hsu, H. P. Chang, K. C. Cheng, and M. Yen, *Sci. Sinica*, **13**, 523 (1964); (b) H. H. Liu, L. C. Chang, M. L. Hsu, H. P. Chang, K. C. Cheng, M. Yen, P. R. Saho, S. H. Hsiao, and C. C. Chan, *Yao Hsueh Hsueh Pao*, 10, 418 (1963); (c) P. J. Shao, S. H. Hsia, C. C. Chan, Y. C. Shang, I. Ho, and Y. S. Hsi, *ibid.*, 12, 642 (1965); (d) J. K. Hsu, C. P. Lu, C. C. Yang, C. S. Lu, and H. J. Chiang, *Chin. Med. J.*, **82**, 92 (1963); (e) T. Y. Huang, K. F. Wang, and T. C. Yu, *ibid.*, **82**, 242 (1963); (f) H. C. Chou, M. Huangfu, and H. L. Chou, *ibid.*, **82**, 250 (1963); (g) H. C. Chou, M. Huangfu, H. L. Chou, and M. H. Wei, *ibid.*, **84**, 591 (1965).
(8) (a) H. Gilman and G. F. Wright, *J. Amer. Chem. Soc.*, **52**, 2550 (1930); (b) T. Takahashi, H. Saikachi, T. Sasaki, K. Suzuki, and E. Moritani, *J. Pharm. Soc. Jap.*, **69**, 286 (1949); *Chem. Abstr.*, **44**, 5373 (1950); (c) M. Ikeda, *Ann. Rep. Pharm. Kanazawa Univ.*, **3**, 27 (1953); *Chem. Abstr.*, 50, 10701 (1956); (d) M. Ikeda, *J. Pharm. Soc. Jap.*, 75, 60 (1955) [*Chem. Abstr.*, 50, 929 (1956)]; 75, 629 (1955) [*Chem. Abstr.*, 50, 3383 (1956)]; 75, 631 (1955) [*Chem. Abstr.*, **50**, 3383 (1956)]; (e) H. Saikachi and K. Suzuki, *Chem. Pharm. Bull.*, 6, 693 (1958); (f) X. Y. Zheng, C. Chen, I. F. Ch'ien, P. C. Kan, H. L. Chin, S. H. Hsiao, and P. J. Shao, *Yao Hsueh Hsueh Pao*, 10, 407 (1963).
(9) (a) S. Hünig, H. R. Müller, and W. Thier, *Angew. Chem., Int. Ed. Engl.*, 4, 271 (1965); (b) C. E. Miller, *J. Chem. Educ.*, 42, 254 (1965).
(10) J. N. Hamersma and E. I. Snyder, *J. Org. Chem.*, 30, 3985 (1965).
(11) F. Kai and H. Ogawa, *Chem. Pharm. Bull.*, 11, 1205 (1963).
(12) G. Märkl, *Chem. Ber.*, 94, 3005 (1961).
(13) (a) M. Bodanszky and M. A. Ondetti, Ed., "Peptide Synthesis," Wiley, New York, N. Y., 1966, p 84; (b) G. W. Anderson, J. E. Zimmerman, and F. M. Callahan, *J. Amer. Chem. Soc.*, 89, 5012 (1967).
(14) E. R. H. Jones and M. C. Whiting, *J. Chem. Soc.*, 1419, 1423 (1949), and earlier references cited therein.
(15) J. G. Bourgeois and E. Bueding, *J. Pharmacol. Exp. Ther.*, 176, 455 (1971).
(16) C. H. Robinson, S. Spengel, and E. Bueding, *J. Med. Chem.*, 16, 79 (1973).
(17) (a) R. E. Asnis, *Arch. Biochem. Biophys.*, 66, 208 (1957); (b) D. R. McCalla, A. Reuvers, and C. Kaiser, *J. Bacteriol.*, 104, 1126 (1970).
(18) M. Morita, D. R. Feller, and J. R. Gillette, *Biochem. Pharmacol.*, **20**, 217 (1971).
(19) K. Hirano, S. Yoshina, K. Okamura, and I. Suzuka, *Bull. Chem. Soc. Jap.*, 40, 2229 (1967).
(20) C. Hansch, *Accounts Chem. Res.*, **2**, 232 (1969).
(21) J. T. Penniston, L. Beckett, D. L. Bentley, and C. Hansch, *Mol. Pharmacol.*, 5, 333 (1969).
(22) J. W. McFarland, *J. Med. Chem.*, 13, 1192 (1970).
(23) A. Leo, C. Hansch, and D. Elkins, *Chem. Rev.*, 71, 525 (1971).
(24) C. A. R. Baxter and H. C. Richards, *J. Med. Chem.*, 14, 1033 (1971).
(25) D. Rosi, G. Peruzzotti, E. W. Dennis, D. A. Berberian, H. Freele, B. F. Tullar, and S. Archer, *ibid.*, 10, 867 (1967).
(26) S. H. Rogers and E. Bueding, *Science*, **172**, 1057 (1971).
(27) P. E. Hartman, K. Levine, Z. Hartman, and H. Berger, *ibid.*, **172**, 1059 (1971).
(28) D. Clive, W. G. Flamm, and M. R. Machesko, *Mutat. Res.*, 14, 262 (1972).
(29) J. A. Moore, *Nature (London)*, in press.
(30) Z. Farid, J. H. Smith, S. Bassily, and H. A. Sparks, *Brit. Med. J.*, **2**, 88 (1972).