Notes

Structure and Antischistosomal Activity in the Nitrofuran Series. Requirement for a 5-Nitro Group in 3-(5-Nitro-2-furyl)acrylic Acid Derivatives†

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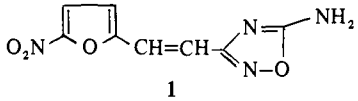
We have recently drawn attention[1,2] to structural and conformational features associated with schistosomicidal action in nitrofuran derivatives. In particular, we observed that the absence of the 5-nitro grouping in *trans*-5-amino-3-[2-(5-nitro-2-furyl)vinyl]-1,2,3-oxadiazole (**1**) is associated with complete loss of antischistosomal activity. The question arises as to whether the nitro group in this and in related nitrofuran derivatives can be replaced by some other electronegative substituent without loss of antiparasitic activity. The precise role played by the nitro group is unknown, but possibilities include reduction by host or parasite to an unstable hydroxylamino or nitroso grouping.

We have attempted to answer the question by synthesizing and testing selected 5-carboxy-, 5-carboxymethyl-, and 5-cyano-2-furyl derivatives for comparison with a corresponding schistosomicidal 5-nitro-2-furyl compound. The similarity in both the type and sequence of biochemical effects and morphological changes induced by both the nitrofuryl derivative **1** and by simple amides of 3-(5-nitro-2-furyl)acrylic acid (*e.g.*, **2**) has suggested[1,2] a similar mode of action. For synthetic reasons we chose to work with the amide **2** rather than the more complex compound **1**, and carboxy, carboxymethyl, and cyano groups were selected for comparison with the nitro grouping for two reasons. Firstly, the Hammett normal substituent constants (para) for CO_2CH_3, CO_2H, and CN are[3] respectively +0.38, +0.41, and +0.67 *vs.* 0.78 for NO_2. If the nitro group's contribution is based primarily on electron withdrawal, then one might expect a graded series of biological activities, with the activity of the cyanofuran derivative somewhat similar to the corresponding nitrofuran. Secondly, the conformational free



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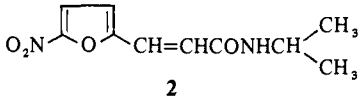

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energies[4] of these groupings suggest that their spatial requirements are similar to or less than that of the nitro group. In the course of the synthetic work we also prepared analogs of **2** in which the nitro group is replaced by CH_3, CH_2OH, and CH=NOH. These were also tested for antischistosomal activity.

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Synthesis. The new *N*-isopropyl-3-(5-substituted 2-furyl)-acrylamides synthesized in this study are collected in Table I. The known[5] 3-(5-methyl-2-furyl)acrylic acid was converted to the isopropyl amide **3** *via* reaction with thionyl chloride to give acid chloride and then treatment with isopropylamine. Related acids were similarly converted to the corresponding isopropyl amide derivatives.

To prepare *N*-isopropyl-3-(5-cyano-2-furyl)acrylamide (**11**), we first oxidized *N*-isopropyl-3-(5-hydroxymethyl-2-furyl)acrylamide (**7**) to the 5-formyl derivative **8** with manganese dioxide. The mixture of syn and anti oximes, **9** and **10**, obtained from **8** was heated with acetic anhydride-sodium acetate to give the required 5-cyano compound **11**. Although the oximes **9** and **10** were separated and characterized, the crude oxime mixture was carried through successfully to the nitrile **11**. If oxime dehydration was effected by acetic anhydride alone, the nitrile **11** was accompanied by its *N*-acetyl derivative as well as by 3-(5-cyano-2-furyl)-acrylic acid. The latter was presumably formed by hydrolytic cleavage of the *N*-acetyl compound. All compounds showed mass spectra and nmr spectra consistent with the postulated structures.

Pharmacological Results. Compounds **3–5**, **7**, **9**, **10**, and **11** were tested for antischistosomal activity in mice infected with *Schistosoma mansoni* and were found to be devoid of antiparasitic activity at dose levels (250 mg/kg b.i.d., 10 doses) which caused significant reduction in the number of worms in the case of the parent 5-nitro compound **2**.

Lower doses of the latter and of other antischistosomal compounds of this group cause a reversible reduction of glycogen phosphorylase phosphatase activity and a glycogen depletion in the worms,[1,2,6] thus providing a sensitive indicator for low antischistosomal activity. Since neither of these two biochemical changes was observed after administration of compounds **3–11**, they were devoid even of borderline activity.

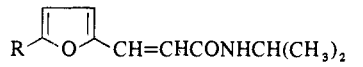
Conclusions

Our experimental results demonstrate that replacement of the nitro group in *N*-isopropyl-3-(5-nitro-2-furyl)acrylamide (**2**) by cyano, carboxy, or carboxymethyl groupings results in complete loss of schistosomicidal activity. We infer that electronegativity alone cannot explain the crucial role played by the nitro group and suggest that *in vivo* reduction to a hydroxylamino or nitroso group may be involved.

Experimental Section

General Procedure for Preparation of *N*-Isopropyl-3-(5-substituted 2-furyl)acrylamides of Table I from the Corresponding Acids, Illustrated by the Synthesis of *N*-Isopropyl-3-(5-methyl-2-furyl)-acrylamide (3). A solution of 3-(5-methyl-2-furyl)acrylic acid[5] (3.04 g, 0.02 mol) in Et_2O (75 ml) and $SOCl_2$ (2.86 g, 0.024 mol) was heated under reflux for 1.5 hr. The solvents were evaporated *in vacuo*, and the crude acid chloride (3.40 g) was dissolved in Et_2O (30 ml) and cooled in a Dry Ice–acetone mixture. Isopropylamine (2.60 g, 0.044 mol) was added; the mixture was kept at −70° for 15 min and was then diluted with H_2O and extracted with $CHCl_3$. The $CHCl_3$ extract was washed with H_2O, dried (Na_2SO_4), and evaporated *in vacuo*. The residue was crystallized from $CHCl_3$–Et_2O to give 1.22 g (32%) of **3**, mp 117–120°, and a second crop of 1.18 g

Table I



Compd no.	R[a]	Mp, °C	Crystn solvents	Formula
3	CH_3	121-122	$CHCl_3$-Et_2O	$C_{11}H_{15}NO_2$
4	$COOCH_3$	164-165	C_6H_6-Et_2O	$C_{12}H_{15}NO_4$
5	COOH	201-203	MeOH-Et_2O	$C_{11}H_{13}NO_4$
6	CH_2OCOCH_3	116-118	EtOAc-ligroin	$C_{13}H_{17}NO_4$
7	CH_2OH	185-186	MeOH	$C_{11}H_{15}NO_3$
8	CHO	157-159	Me_2CO-Et_2O	$C_{11}H_{13}NO_3$
9	CH=NOH	186-191	MeOH-Et_2O	$C_{11}H_{14}N_2O_3$
10	CH=NOH	142-146	Et_2O-C_6H_6	$C_{11}H_{14}N_2O_3$
11	CN	139-140	Et_2O-ligroin	$C_{11}H_{12}N_2O_2$

[a] All compounds were analyzed for C, H, and N, and all gave values within 0.3% of the calculated figures.

(31%), mp 115-118°. Recrystallization raised the melting point to 121-122°.

N-**Isopropyl-3-(5-carboxy-2-furyl)acrylamide (5).** The known[7] 3-(5-carboxymethyl-2-furyl)acrylic acid was converted to the isopropyl amide **4** by the general method described above, and **4** (0.52 g, 2.2 mmol) was dissolved in MeOH (10 ml) and 1% aqueous KOH (16.5 ml) and kept at 25° for 55 min. Acidification and extraction with EtOAc gave crude product (0.48 g) which was crystallized from MeOH-Et_2O to give **5**, mp 201-203° (0.406 g, 83%).

N-**Isopropyl-3-(5-hydroxymethyl-2-furyl)acrylamide (7).** The known[8] 3-(5-acetoxymethyl-2-furyl)acrylic acid was converted to the isopropyl amide **6** by the general method described above, and a solution of **6** (7.80 g, 0.031 mol) in 40 ml of MeOH and 21 ml of 10% aqueous KOH was kept at 25° for 30 min. The solution was acidified, and the precipitate was filtered off, washed with water, and dried *in vacuo*. Crystallization from MeOH-Et_2O gave **7** (4.75 g, 73%), mp 182-185°; second crop (1.20 g, 18%), mp 181-183°. Recrystallization from MeOH-Et_2O raised the melting point to 185-186°.

N-**Isopropyl-3-(5-formyl-2-furyl)acrylamide (8).** The hydroxymethyl compound **7** (1.5 g, 7.2 mmol) in Me_2CO (300 ml) was stirred with activated MnO_2 (5.0 g) at 25° for 2 hr. More MnO_2 (1.0 g) was added, and stirring was continued for 1 hr. Another portion (1.0 g) of MnO_2 was added and stirring was continued for 1.75 hr. The mixture was filtered and the filtrate was evaporated *in vacuo* to give crude aldehyde (1.38 g). Crystallization from Me_2CO-Et_2O gave 1.28 g (85%) of **8**, mp 155-157°. Recrystallization raised the melting point to 157-159°.

Formation of Oximes 9 and 10 from *N*-**Isopropyl-3-(5-formyl-2-furyl)acrylamide (8).** A solution of the aldehyde **8** (3.41 g, 0.016 mol) in MeOH (15 ml) and H_2O (30 ml) containing hydroxylamine sulfate (2.08 g, 0.016 mol) and NaOAc (2.62 g, 0.032 mol) was heated under reflux for 1 hr. Tlc (EtOAc-ligroin, 4:1) showed formation of two products. Removal of MeOH *in vacuo* resulted in the crystallization of the less polar oxime **9** (2.25 g, 61%) which was recrystallized from MeOH-Et_2O to give 0.814 g (22%), mp 187-191°. The aqueous mother liquor was extracted with $CHCl_3$ to give 1.50 g (41%) comprising mainly the more polar oxime **10**. Crystallization from Et_2O-C_6H_6 gave pure **10**, mp 142-146°.

N-**Isopropyl-3-(5-cyano-2-furyl)acrylamide (11).** A solution of the more polar oxime **10** (1.22 g, 5.5 mmol) in Ac_2O (1.5 ml) containing NaOAc (0.675 g) was heated under reflux for 70 min. By dilution with water and extraction with Et_2O-$CHCl_3$ (4:1), crude nitrile (1.04 g) was obtained. Crystallization from C_6H_6-ligroin gave 0.706 g (63%) of **11**, still slightly impure. Dry-column chromatography on silica gel (10 g) with $CHCl_3$ as eluent gave 0.629 g (56%) of **11**, which was crystallized from Et_2O-ligroin to give pure material, mp 139-140°. The nitrile **11** could also be obtained in comparable amount if the crude oxime mixture, **9** and **10**, was used in the reaction.

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Nucleoside Peptides. 5.[1] Synthesis of Certain *N*-(5-Uridineacetyl)amino Acids from 5-Uridineacetic Acid

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Modified nucleosides isolated from transfer ribonucleates have been proposed (for a recent review, see ref 2) to exhibit a regulatory influence on protein biosynthesis as well as influence the tertiary structure of these macromolecules. Inhibition of the growth of certain cancer cell lines by 4-thiouridine[3] and the cytokinin activity of isopentenyl adenosine[4] have been noted and suggest that other minor nucleosides and their derivatives may possess interesting biological activities.

It was, therefore, of interest to study the biological properties of the modified nucleoside 5-uridineacetic acid (**1**) (Scheme I) isolated from yeast transfer ribonucleates[5,6] and several of its analogs. Recently **1** was synthesized[7,8] and the synthetic product found to be identical with the product isolated from natural sources.[8] Since we have investigated the biological properties of a number of nucleoside peptide derivatives and found inhibition of protein biosynthesis[1,9] and antiviral activity[10] in cell culture, we now report the synthesis and biological properties of a number of *N*-(5-uridineacetyl)amino acids prepared from **1**.

The active ester procedure of Bodanszky[11] provided the method of choice for peptide bond formation. Since this technique allows selective bond formation to an amino group in the presence of free hydroxyl groups,[9] it was unnecessary to block the sugar moiety of the nucleoside. A facile preparation of the active ester 5-uridineacetic acid *p*-nitrophenyl ester (**2a**) (Scheme I) was achieved by the action of *p*-nitrophenol and DCC on 5-uridineacetic acid (**1**). This useful intermediate was found to be readily susceptible to nucleophilic attack. Treatment of **2a** with alcoholic ammonia or alcoholic methylamine gave 5-uridineacetamide (**2b**) and 5-uridine-*N*-methylacetamide (**2c**) in yields of 58 and 80%, respectively. Facile peptide bond formation was also achieved by treatment of **2a** with appro-