was filtered and recrystallized from absolute ethanol acidified with anhydrous HCl gas to give 1.3 g (60%), mp 405–407 °C dec.

**Acknowledgment.** Support of this work by the U.S. Army Medical Research and Development Command under Contract No. DADA17-68-C-8035 is gratefully acknowledged. This is Contribution No. 1449 from the Army Research Program on Malaria. We thank Dr. Edgar A. Steck for calling our attention to the diamidine area and for helpful discussions and advice.

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# Effect of the Solvent-Dependent Conformational System of Hydroxyureas on Predicted vs. Observed Log *P*[1]

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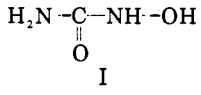
Calculated and observed log *P* values are reported and compared with in vivo and in vitro biological action (L1210 leukemia ILS % and ribonucleotide reductase $ID_{50}$) for hydroxyurea, the 1-N methyl and ethyl, and the 3-N ethyl, *n*-propyl, isopropyl, *n*-butyl, *tert*-butyl, phenyl, and *p*-chlorophenyl analogues. The log *P* values were calculated via the method of Hansch and Leo from literature *f* values and the observed log *P* values were obtained by direct determination after equilibration between octanol and water. Calculations of log *P* for hydroxyurea were found to be appreciably more hydrophilic than the values obtained experimentally. Differences in calculated and observed log *P* ($\Delta$ log *P*) for the substituted analogues were lowest with the 1-N and the bulky 3-N substituents and greatest with the 3-N-substituted straight-chain analogues ($\Delta$ log *P* = 0.70). Different structural species were observed by infrared spectroscopy in dry octanol vs. octanol after water equilibration and drying, and this is proposed as due to changes in conformational equilibrium in the hydroxyurea systems. Differences between calculated and observed log *P* are explained via the stabilization of internally hydrogen-bonded conformers in the case of 1-N or bulky 3-N analogues or destabilization of various conformers allowing maximal interactions with solvent or water which is the case with straight chain 3-N analogues.

Hydroxyurea (I), a clinically effective antileukemia agent,[2] is a unique drug since molecular modification has not produced an analogue with superior biological action as evidenced by the in vivo L1210 activity of substituted analogues (summarized in Table I).[3]



I

The activity of hydroxyurea has been attributed to its ability to inhibit the enzyme ribonucleoside diphosphate reductase[4a] (RDR), and comparison of the in vitro inhibition of this enzyme from Novikoff hepatoma by selected substituted hydroxyurea[4b] (Table I) with the in vivo data indicates inhibitory ability in some cases at concentrations in the general range observed with hydroxyurea by all of the compounds tested ($ID_{50}$ values). The relative importance of drug transportability, metabolizability, availability, and dynamics at the site of action for this drug class at present is not known. In this paper we wish to report discrepancies between calculated and observed log *P* values for hydroxyurea and some of its substituted analogues and an explanation for these differences due to solvent-dependent conformational preferences of various analogues which may have an influence on biological action in vivo.

The transport of hydroxyurea molecules to their site of action involves passage through membranes and the adsorption and desorption to macromolecules in vivo. The in vivo transportability of drug molecules can be evaluated via the partition coefficient in solvents such as octanol–water[5] (log *P*) which measures a drug's relative affinity toward lipophilic and hydrophilic phases. Log *P* values have been measured for many drugs[6] and methods have been developed by Hansch et al.,[7] whereby the log *P* value for a particular drug can be calculated since log *P* has been shown to be an additive–constitutive property of organic compounds.[8] Log *P* can be calculated by adding the fragment values (*f*) of the component functional groups according to eq 1.[9]

$$\log P = \sum_{1}^{\eta} a_\eta f_\eta \qquad (1)$$

In calculating a log *P* for hydroxyurea, the proximity effect of groups which can hydrogen bond must be taken into consideration and generally the *f* value of the most negative fragment is used. Thus calculated log *P* is −2.71 or −2.18 depending on the fragments used to make hydroxyurea (eq 2 and 3).

$$\underset{-2.71}{f(\text{-CONH})} + \underset{-1.54}{f(\text{-NH}_2)} + \underset{-1.64}{f(\text{-OH})} = \log P = -2.71 \qquad (2)$$

$$\underset{-2.18}{f(\text{-CONH}_2)} + \underset{-2.11}{f(\text{-NH-})} + \underset{-1.64}{f(\text{-OH})} = \log P = -2.18 \qquad (3)$$

When these calculated log *P* values are compared to the experimentally obtained log *P* for hydroxyurea (Table I) it is apparent that the actual log *P* is more lipophilic than that obtained by calculation from the component functional groups. In order to calculate log *P* values for

Table I. Comparison of in Vivo L1210 Activity, in Vitro Ribonucleotide Reductase Inhibition ($ID_{50}$), Calculated, Observed, and Δ Log *P*, and Infrared Absorptions in Carbonyl-Nitrogen Region in Dry Octanol and Octanol after Water Equilibration and Drying of Selected Hydroxyureas



| R | R′ | % T/C L1210 (O.D.[b] or M.D.,[c] mg/kg) | RDR $ID_{50}$ conc,[d] mM ± SD (*N*) | Rel RDR $ID_{50}$ concn[e] | Calcd log *P* | Determined log *P* | Δ log *P* (calcd − obsd log *P*) | IR absorptions in carbonyl-nitrogen region (1780–1550 $cm^{-1}$) | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | In dry octanol | In octanol after $H_2O$ equil, drying |
| H | H | 291 (111)[f] | 0.11 ± 0.086 (6)[g] | 1.0 | −2.18 | −1.27 | −0.91 | 1660 | 1650 |
| H | $CH_3$ | 162 (125) | 0.10 ± 0.036 (4) | 0.9 | −0.60 | −0.46 | −0.14 | | |
| H | $C_2H_5$ | 168 (400) | 0.17 ± 0.088 (5) | 1.5 | 0.06 | 0.10 | 0.04 | 1660, 1570 | 1635–1655[j] |
| $C_2H_5$ | H | 137 (400) | | | −0.06 | −0.76 | 0.70 | 1680, 1560 | 1640 |
| *n*-$C_3H_7$ | H | 146 (600) | 0.71 ± 0.20 (2) | 14.4 | 0.48 | 0.22 | 0.70 | 1670, 1550 | 1650, 1670 |
| | | | 0.34 ± 0.10 (3)[h] | 3.8 | | | | | |
| *i*-$C_3H_7$ | H | *i* (400) | 0.80 (1) | 19.0 | 0.35 | 0.20 | 0.15 | 1780, 1720, 1665 | 1700, 1640[k] |
| *n*-$C_4H_9$ | H | (600) | 0.75 ± 0.64 (6)[h] | 6.8 | 1.02 | 0.32 | 0.70 | 1660, 1555 | 1645–1680 |
| *t*-$C_4H_9$ | H | (400) | 0.40 (1)[h] | 9.5 | 0.76 | 0.50 | 0.26 | 1710, 1660 | 1715, 1665, 1645 |
| $C_6H_5$ | H | (400) | | | 0.41 | 0.41 | 0.00 | | |
| *p*-Cl-$C_6H_5$ | H | (400) | | | 1.35 | 1.39 | 0.04 | 1660–1690, 1605 | 1640, 1540 |

[a] According to CCNSC protocol, National Cancer Institute. [b] Intraperitoneal dose given each day for 9 days unless otherwise specified. [c] Maximum nontoxic dose tested. [d] Each determination was performed at four concentrations with two trials at each concentration and $ID_{50}$ determined after graphing and extrapolating. [e] Relative concentration compared to hydroxyurea concentration for each determination and then averaged. [f] q4h dosing every fourth day. [g] Solvent for hydroxyureas is water unless otherwise specified. [h] In 1% $Me_2SO$ ($Me_2SO$ is not inhibitory to enzyme in 1% concentrations). [i] ILS % less than 125%. [j] Absorption range. [k] 1700 is shoulder peak.

substituted hydroxyureas, an *f* value for a –NHCONHOH or $NH_2CONOH$ group must be derived. This can be obtained by subtraction of the *f* value for a hydrogen (0.23) from the derived log *P* value for hydroxyurea.

$$\log P(H_2NCONHOH) - f(H) = f(\text{–NHCONHOH}) \quad (4)$$
$$-1.26 - 0.23 = -1.49$$

Surprisingly, the same *f* value of –1.49 can be obtained for the –NHCONHOH group by subtracting the *f* value for a phenyl group (1.90)[10] from the experimentally determined log *P* value for 3-*N*-phenylhydroxyurea (0.41). This finding appears to be an anomaly due to the unusual behavior in octanol–water as evidenced by the infrared data[4b] since aromatic fragments usually are about +1.0 log unit greater than aliphatic attachments.[10]

$$f(C_6H_5\text{–}) + f(\text{–NHCONHOH}) = \log P(C_6H_5NHCONHOH)$$
$$(1.90) + f(\text{–NHCONHOH}) = 0.41$$
$$f(\text{–NHCONHOH}) = -1.49 \quad (5)$$

The –NHCONHOH group (or the $H_2NCONHOH$ group) is assigned an *f* value of –1.49 and can be used in the calculation of any of the substituted hydroxyureas. Since the effect of substitution at the N-1 vs. N-3 nitrogen for the purpose of calculation is not known, simple addition of methyl or ethyl fragments with *f* of –NHCONHOH is used for the methyl and ethyl analogues according to Leo et al.[10]

methylhydroxyurea (1-N and/or 3-N)

$$f(CH_3) + f(\text{–NHCONHOH}) = \log P \quad (6)$$
$$0.89 + (-1.49) = -0.60$$

ethylhydroxyurea (1-N and/or 3-N)

$$f(CH_3) + f(CH_2) + 1fb + f(\text{–NHCONHOH}) = \log P \quad (7)$$
$$0.89 + 0.66 - 0.12 + (-1.49) = -0.06$$

The calculated log *P*'s for the 3-N *n*-propyl, isopropyl, *n*-butyl, and *tert*-butyl analogues via this method are

3-*N*-*n*-propylhydroxyurea

$$f(CH_3) + 2f(CH_2) + 2fb + f(\text{–NHCONHOH}) = \log P \quad (8)$$
$$0.89 + 1.32 - 0.24 + (-1.49) = 0.48$$

3-*N*-isopropylhydroxyurea

$$2f(CH_3) + f(CH) + 2fb + fcbr + f(\text{–NHCONHOH}) = \log P \quad (9)$$
$$1.78 + 0.43 - 0.24 - 0.13 + (-1.49) = 0.35$$

3-*N*-*n*-butylhydroxyurea

$$f(CH_3) + 3f(CH_2) + 3fb + f(\text{–NHCONHOH}) = \log P \quad (10)$$
$$0.89 + 3(0.66) - 0.36 + (-1.49) = 1.02$$

3-*N*-*tert*-butylhydroxyurea

$$3f(CH_3) + f(C) + 3fb + 2fcbr + f(\text{–NHCONHOH}) = \log P \quad (11)$$
$$3(0.89) + 0.20 - 0.36 - 0.26 + (-1.49) = 0.76$$

Comparing the calculated and observed log *P* values (Table I), it is apparent that the determined log *P*'s are more hydrophilic (with the exception of the 1-*N*-methyl anlogue) than that predicted using Hansch's method. The Δ log *P*'s (calculated – observed log P) for the straight chain 3-N analogues are identical (Δ log *P* = 0.70) while the Δ log *P*'s for the isopropyl and *tert*-butyl are much lower (Δ log *P* = 0.15 and 0.26 for isopropyl and *tert*-butyl, respectively), indicating that the observed log *P*'s are closer to the

calculated values for these compounds. The 1-N-substituted analogues are also found to have lower $\Delta$ log $P$ values than the straight chain 3-N-substituted compounds and it is interesting to note that ethyl substitution of the number 1 nitrogen appears to increase the lipophilic partitioning to a greater extent than substitution of the same group on the number 3 nitrogen (log $P$'s of −0.10 vs. −0.76, respectively).

## Discussion

Hydroxyurea was introduced as a new type of potential antitumor agent[11] in 1963 and some preliminary structure–activity work on hydroxyurea appeared in 1967.[12] The compound is unique in that it is one of the few types of compounds which inhibits the enzyme ribonucleoside diphosphate reductase (RDR) along with guanazole[13] and heteroaromatic aldehyde thiosemicarbazones.[14] The inhibition of this enzyme is claimed to be the mechanism by which hydroxyurea functions as an antitumor agent[4a] and this is supported by some researchers[15] and refuted by others.[16,17]

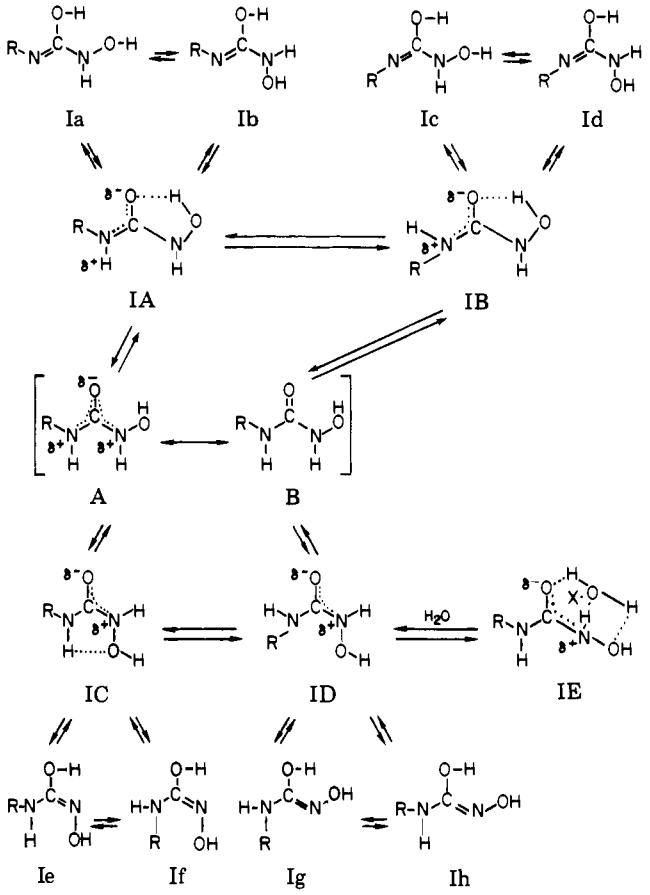
Attempts to optimize and improve the antitumor activity of hydroxyurea by molecular modification have not been successful although selected analogues which are more lipophilic were initially reported to possess superior action.[18] Hydroxyurea thus remains the most potent antitumor agent in the hydroxyurea series but the property possessed by this molecule from which the activity is derived is still not known. The search for the unique property (or properties) of hydroxyurea could be studied by the evaluation of each of the various processes leading to ultimate dynamics at the site of action(s) starting with transportability and availability to the site of action.

The transport, membrane passage, and partitioning capabilities of drugs between hydrophilic and lipophilic tissue have been studied via the concept of the partition coefficient (log $P$) and the octanol–water partitioning system[5] has been selected as the best compromise of an in vitro solution system which most closely mimics the biophase. The study of the partitioning properties of hydroxyureas in this solvent system was undertaken and a solvent-dependent behavior was detected via infrared spectroscopy during the course of the quantitative determinations.[4b] Substituted hydroxyureas were found to possess appreciably different IR absorptions (in the 1760–1550-cm$^{-1}$ region) in dry octanol compared to those which had been equilibrated with water and dried over desiccant. The hydroxyureas were found to be stable under the conditions of the water equilibration and this sensitivity to water inducing spectral changes resembled the behavior of hydroxyureas in various solvents as studied via NMR spectroscopy.[19]

The spectral changes observed in the hydroxyureas were deduced as being due to different structural equilibria in the dry octanol compared to that observed after equilibration with water. In dry octanol it is apparent that one species is present (possessing both amide I and II bands) and that after equilibration with water another form is present which exists even after drying over anhydrous desiccant. All of the spectra of the hydroxyureas in octanol have a strong absorption at 1650 cm$^{-1}$ after water equilibration, indicating a minimal degree of true carbonyl character and a strong C=N absorption. Hydroxyurea appears to be the only member of the series that has essentially the same IR spectral absorptions in dry octanol and after equilibration with water and thus appears to have the same structural form predominating in both of these systems.

The log $P$ values for the aliphatic hydroxyureas and

Scheme I. Conformational and Tautomeric System of Hydroxyureas[a]




[a] Canonical forms A and B represent electronic species where the $\pi$ bond is localized in the C=O bond (B) or distributed across the N-C-N system (A). Conformers IA, IB, and IC are possible due to internal hydrogen bonding and conformer ID can hydrogen bond to solvent or water (IE). Tautomers are possible (1a-h) if bond breaking and bond making occurs. In 1-N-substituted analogues 1-N-H = R'.

hydroxyurea were obtained by direct determination of the aqueous phase after equilibration, separation, and lyophilization. Hydroxyurea is more lipophilic than the calculated log $P$ values and this may be because canonical form B is most representative of its structure due to quick interconversion between conformers IA, IB, and IC because of minimal internal stabilization, and, thus, hydrogen bonding with solvent will predominate. In this system the only conformer which would be stabilized by solvent interactions would be conformer ID which has the least amount of internal hydrogen bonding.

The calculated log $P$ values of the substituted hydroxyureas are obtained from the observed log $P$ values of hydroxyurea and 3-*N*-phenylhydroxyurea after calculation of a fragment value for -NHCONHOH (or $NH_2CONOH$) and all of the calculated values are more lipophilic (except for the 1-*N*-methyl analogue) than the determined values. The substitution of groups on the 3-nitrogen of the hydroxyurea system makes the molecule more hydrophilic than expected, especially straight chains. The structural forms (see Scheme I) which influence the relative hydrophilicity–lipophilicity of substituted hydroxyureas can involve either (a) the conformational isomers IA–ID or (b) tautomers Ia–h which could form if solvent or water acts to catalyze N–H bond breaking and -OH bond making. If conformers are the important

species, then hydrate formation could occur with conformer ID to form a solvate (IE). If tautomerization is catalyzed by water or solvent, then a tautomer or combination of tautomers could be produced to form Ia–h. IR spectra of selected substituted hydroxyureas (3-*N*-*n*-butyl and 1-*N*-ethyl) taken after standing for extended periods in dry solvents having proton affinities closer to water than octanol, such as ethyl ether and tetrahydrofuran (proton affinities in kcal/mol: water 164, octanol 202, ethyl ether and tetrahydrofuran 187),[20] showed no spectral changes in the carbonyl–nitrogen region. When the compounds in these solvents were equilibrated with water and dried, spectral changes were observed which resembled the changes seen in octanol. These data indicated that the spectral changes were not due to tautomeric solvent-induced changes but do not rule out water-catalyzed tautomerization. In an NMR study of substituted hydroxyureas,[19] individual structural entities were found present in dry pyridine and the complex signals of these individual forms coalesced into a single signal on addition of water. Also, the N–H signals exchanged slowly in pyridine in the presence of $D_2O$ over a 1–2-week period in the case of the 1-N-substituted analogues and 3-N isopropyl, *n*-butyl, and *tert*-butyl analogues indicating that tautomerization with water was not occurring or was occurring very slowly (over 1–2 weeks) in comparison to the spectral changes in octanol which occurred after 24 h of equilibration with water. The spectral changes observed in octanol are therefore due to changes in the equilibrium of conformers IA, IB, IC, and ID due to interactions of these conformers with water and octanol.

The differences in the calculated vs. observed log *P* values for the substituted hydroxyureas can be explained via changes in the equilibrium pattern of conformers IA, IB, and/or IC vs. conformer ID. The ethyl, *n*-propyl, and *n*-butyl 3-N analogues all are about equally more hydrophilic than expected. This is probably due to stabilization of the solvate form (IE) of conformer ID pushing the equilibrium in the direction of this conformer due to hydrogen bonding to solvent and/or water. In the case of the 1-N analogues and the 3-N analogues which have bulky groups (isopropyl and *tert*-butyl), the internal hydrogen-bonded conformers are stabilized to a greater extent than the straight chain 3-N compounds. This does not allow for easy interconversion to conformer ID and the equilibrium remains to a greater degree in favor of conformers IA, IB, and/or IC. These compounds would not possess the ability to have dipolar interactions of such strength with water as do those analogues which favor comformer ID, and, thus, these compounds have a greater affinity for the octanol layer than the straight chain homologues.

The significance and implications of the solvent-dependent conformational system of hydroxyureas are that various structural forms might predominate in vivo depending on whether the molecule is in a polar or nonpolar environment and thus the structural preference induced may influence the transportability, membrane transport, partitioning, and even the interactions at the site of action, which may be the case with hydroxyurea vs. an inactive compound such as 3-*N*-*n*-butylhydroxyurea. An apparent solvent dependency involving the in vitro inhibition of ribonucleotide reductase has been observed[4b] which may be due to the interrelationship between solvent and conformational preference influencing the structural species in predominance.

## Experimental Section

**Hydroxyureas.** Hydroxyurea and the 1-N-substituted methyl and ethyl analogues of hydroxyurea were made available for this study by Miss Barbara Stearns of the Squibb Institute of Medical Research. The 3-N-substituted compounds were prepared according to the original procedure of Dresler,[21] melting points corresponded to those reported by Harmon[3c] and Clifton,[22] and compound purity was verified by spectral and elemental analysis.

**Ribonucleotide Reductase Inhibition.** The enzyme ribonucleoside diphosphate reductase was isolated from Novikoff ascites heptoma of rats and purified according to the method of Moore.[23] The assay included 8.3 mM phosphate buffer (pH 7), 2.1 mM ATP, 4.2 mM $MgAc_2$, 0.04 mM $Fe(NH_4)_2(SO_4)_2$, 0.17 mM CDP-$^{32}$P, 6.3 mM dithiothreitol, 1.0 μM rat thioredoxin, and approximately 0.2 mg of partially purified ribonucleotide reductase in a total volume of 0.12 mL. Inhibitors were present in the reaction mixture (in an ice bath) when the enzyme was added; the mixture was then moved to a water bath at 37 °C and incubated 30 min. The reaction was stopped with perchloric acid (1 M); the products were hydrolyzed, separated on Dowex-50, and analyzed according to the method of Reichard.[24] The control activities for the enzyme system in six determinations were 2.28, 0.75, 4.16, 2.20, 1.50, and 2.4 nmol/30 min per tube. In the six enzyme determinations the $ID_{50}$ values were determined by the addition of a hydroxyurea at four concentrations; the enyzme activity was graphed and extrapolated to determine the concentration required to inhibit 50% of enzyme activity. The relative inhibitory concentration in each determination was obtained from the $ID_{50}$ of hydroxyurea used in each determination as a positive control. The solvent for the hydroxyureas was distilled water (pH 7) for hydroxyurea and 1-*N*-methyl- and 1-*N*-ethylhydroxyurea in all determinations. Water was the solvent for the 3-*N*-*n*-propylhydroxyureas in the first (and last) determination and the 3-*N*-*n*-butyl analogue was dissolved in a small amount of 95% ethanol and diluted with water in the first determination. The *n*-propyl analogue in determinations II–IV was dissolved in 6% dimethyl sulfoxide and diluted with $H_2O$ to 1% concentration, and the *n*-butyl analogues were dissolved in 10% dimethyl sulfoxide and diluted with $H_2O$ to 1% concentration in all other determinations.

**Log *P* Determinations.** Partition coefficients for the hydroxyureas were obtained after equilibration of known quantities of compound (100 mg, 500 mg, and 1.0 g) in equal volumes (100 mL) of 1-octanol and water (pH 7.4) for various periods; the optimal equilibration time was 72 h. The solvent layers were separated and the aqueous phase was freeze-dried under vacuum (Virtis lyophilizer) and the hydroxyurea was weighed and analyzed for purity. The hydroxyureas could be obtained unchanged after equilibration with water for 1 week. Since the determined log *P* for hydroxyurea was appeciably more lipophilic than predicted, the possibility of dimer formation in the aqueous phase was investigated. The log *P* determination was carried out in a solution of five parts of octanol to one part of water at varying concentrations to see if log *P* would vary with concentration, but log *P* was not more lipophilic at higher concentration ruling out dimerization. The log *P* values obtained by direct determination were compared to those obtained by colorimetric determination (hydroxyurea) and ultraviolet spectroscopy (3-*N*-phenylhydroxyurea) and found to be essentially the same.

**Infrared Spectra.** The infrared spectra were taken on a Beckman 4250 double-beam infrared spectrometer. The hydroxyureas were dissolved in dry octanol and the spectra of the carbonyl–nitrogen region were taken in liquid plates with dry octanol as the blank. The hydroxyureas were then shaken in equal volumes of octanol and water (pH 7.4) for 24 h, then the octanol layer was separated and dried over anhydrous $Na_2SO_4$, and the spectra were taken as previously described. Solutions of hydroxyureas in octanol were also dried over anhydrous $Na_2SO_4$ prior to taking the infrared spectra and these spectra were identical with those taken in dry octanol.

**Acknowledgment.** This work was supported in part by NIH Grant CAO-4464 of the National Cancer Institute.

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# 4-Amino-6-(trichloroethenyl)-1,3-benzenedisulfonamide, a New, Potent Fasciolicide[1]

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The synthesis and fasciolicidal activity of 4-amino-6-(trichloroethenyl)-1,3-benzenedisulfonamide are reported. A single dose of 15 mg/kg was effective in removing over 90% of immature *Fasciola hepatica* from sheep (6 weeks after infection) and calves (8 weeks after infection). A 2.5 mg/kg dose removed over 90% of mature (16 weeks old) liver fluke from sheep. Single oral doses up to 400 mg/kg were tolerated by sheep without gross toxic symptoms.

The recent discovery of fasciolicidal activities for certain 3,5-disubstituted sulfanilamides and benzenemonosulfonamides[2] prompted the evaluation of related sulfonamides[3] for anthelmintic effects. Activity against mature *Fasciola hepatica* in rats was observed with 4-amino-6-(pentafluoroethyl)-1,3-benzenedisulfonamide[4] after one oral dose of 100 mg/kg. This activity was confirmed against immature, 6-weeks-old liver fluke infections in sheep with a single dose of 100 mg/kg. Extensive structural modifications[5] of this lead culminated in the discovery of 4-amino-6-(trichloroethenyl)-1,3-benzenedisulfonamide (**5**) as a highly active and well-tolerated fasciolicide.[1]

**Chemistry.** The 3-(trichloroethenyl)aniline (**3**) required as starting material was obtained by reduction of 3-(pentachloroethyl)aniline[6] (**2**) with zinc in ethanol or by reduction of 3-(pentachloroethyl)nitrobenzene[6] (1) with iron in aqueous ethanol containing a catalytic amount of hydrochloric acid. Compound **3** was described[6] as a reduction product of 3-(trichloroethenyl)nitrobenzene, which had been obtained by nitration of phenyltrichloroethylene (**6**) with $HNO_3$–$H_2SO_4$. In our hands, however, this procedure furnished after purification by chromatography on silica gel a 57% yield of the isomeric 4-(trichloroethenyl)nitrobenzene (**7**),[10] readily identified by the presence of two AB doublets in the NMR spectrum. This gave on reduction 4-(trichloroethenyl)aniline (**8**) whose NMR spectrum again was in agreement with the proposed para substitution. It was also converted to the disulfonamide **5** by a lengthy route through the monosulfonamide 11. However, bischlorosulfonation of 3-(trichloroethenyl)aniline (**3**), followed by reaction of the disulfonyl chloride **4** with ammonia, gave the desired disulfonamide **5** in 33% yield.

**Biological Data.** In preliminary tests with rats previously infected with *F. hepatica* metacercariae,[7] more than 90% of flukes were eliminated with a single oral dose of 3.1 mg/kg. The minimal effective level of rafoxanide[8] used as a standard in the same test is also 3.1 mg/kg. A single oral dose of 15 mg/kg eliminated over 90% of *F. hepatica* 6 weeks of age from sheep or 8 weeks of age from calves, while a 2.5 mg/kg dose was 90% effective against mature (16 weeks old) infection in sheep.[9] No gross toxic reactions could be observed in one sheep each after a single