A New Kind of Fasciolicide: Molecular and Electronic Structures of Some *o*-Hydroxybenzenesulfonanilides

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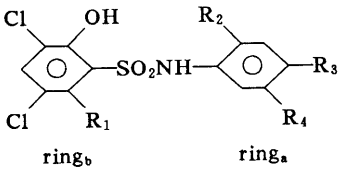
The molecular and electronic structures of some fasciolicidal *o*-hydroxybenzenesulfonanilides (HBSA) have been studied using X-ray diffraction and semiempirical MO calculation. In these compounds, the phenolic hydroxyl forms a strong intramolecular hydrogen bond with an adjacent sulfonyl oxygen atom and the strength of the *d–p* dative S←N bond, which may control the electron delocalization throughout the entire molecule, is affected by substituents on the phenyl rings on both sides. Owing to the poor delocalization, the contribution of the keto-form of the resonance structure is larger for some phenolate anions of HBSA in solution, and this may be a key factor determining the potency of fasciolicidal activity of HBSA.

Keywords molecular, electronic structure; *o*-hydroxybenzenesulfonanilide; fasciolicidal activity; MO calculation

The parasite *Fasciola hepatica* is very damaging to animal husbandry. Through biological experiments, we have discovered that the *o*-hydroxybenzenesulfonanilides (HBSA) possess potent fasciolicidal activity, which is influenced by the nature of the substituents on both phenyl rings of each derivative.[1,2] Compounds with a weak acid-dissociable group such as HBSA are strong uncouplers of oxidative phosphorylation in mitochondria and their fasciolicidal activity is due to this action.[3-5] According to the protonophoric mechanism of uncoupling, the molecule of HBSA, especially the anion produced by acid-dissociation of phenolic hydroxyl, should have a dynamic structure which regulates the hydrophobic and electronic properties of the molecule or anion during proton translocation.[6,7] In order to elucidate the mode of uncoupling (or fasciolicidal activity) of the title compounds, we examined the molecular and electronic structures of some HBSA through X-ray diffraction and semiempirical MO calculation. The structural formula of compounds under investigation are represented as follows:



I : $R_1=R_2=R_3=R_4=Cl$
II : $R_1=H, R_2=R_3=R_4=Cl$
III : $R_1=R_2=R_4=H, R_3=Cl$
IV : $R_1=R_3=Cl$, $R_2=R_4=H$
V : $R_1=R_2=Cl, R_3=R_4=H$
VI : $R_1=Cl, R_2=R_3=R_4=H$

Experimental

X-Ray Crystal Diffraction Analysis for Compound I The intensity data were collected on a NICOLET R_3 diffractometer with graphite-monochromated MoK_α radiation in the ω scan mode over the range of $3.5° \leqslant 2\theta \leqslant 50°$. Finally, 2513 reflections with $I > 3\sigma(I)$ were used in calculations; corrections for Lp factors and for absorption were applied. The crystal belongs to the monoclinic space group $P2_1/a$ with lattice constants: $a=8.029(3)$, $b=22.143(1)$, $c=10.050(1)$ Å; $\beta=108.83(4)°$, $V=169.1(1)$ Å^3, $Z=4$, $D_c=1.79$ g/cm^3, μ (MoK_α) $=11.4$ cm^{-1}. The structure was solved by a combination of direct methods and Fourier synthesis techniques and refined by the full-matrix least-squares method with anisotropic thermal parameters for all non-hydrogen atoms to final $R=0.046$ and $R_w=0.046$. The positions of atom H_1 and H_3 were found by Fourier synthesis and those of other hydrogen atoms by calculation.

Molecular Orbital Calculation All molecular orbital calculations for the studied compounds (including their anions) were performed using the CNDO/M2 program on a Micro-Vax II computer. The initial structural parameters were obtained from crystal structures.

Fasciolicidal Activity Forty-eight fluke-free sheep were each infected with 250 metacercariae of *Fasciola hepatica*. The animals were divided into 6 groups of eight each: 1 control group, and 5 treated groups. An oral dosage of 7.5 mg/kg of a test compound (I—V) was administered to the animals in treated groups at 4 weeks after the infection. The sheep were slaughtered 30 d after the treatment and the numbers of flukes remaining in the livers of treated animals and the unmedicated controls were counted. The activity of each compound was assessed by comparison of the mean fluke burdens in treated and control animals according to the following formula:

$$\text{activity} = \frac{\text{mean control burden} - \text{mean treated burden}}{\text{mean control burden}} \times 100\%$$

The symbols ++++, +++, ++, and + represent 100%, 100—90%, 90—70% and <70% activity, respectively.

Results and Discussion

The structure of compound I is depicted in Fig. 1, and the final positional and thermal parameters with their estimated standard deviations are listed in Table I, with selected bond lengths and bond angles of compound I and compound IV in Table II. Some results of molecular orbital calculation for all the studied molecules are listed in Table III, and for the lowest energy state anions in Table IV.

As shown in Table II, the C_1–S, S–N and N–C_7 bonds of compound I and compound IV are all shorter than single covalent bonds (1.82, 1.67, and 1.47 Å respectively).[8] Owing to the strong inductive effect of the oxygen atoms in the sulfonyl group, the S atom attracts π-electrons of C_1 and the lone pair of N to its unoccupied 3*d* orbitals. As a result, the C_1–S and S–N bonds are shortened by *d–p* dative effects, $C_1 \rightarrow S$ and $S \leftarrow N$, respectively. On the other hand, in *N*-substituted aromatic ring systems, the N atom can adopt an sp^2 hybridization state.[9,10] Therefore,

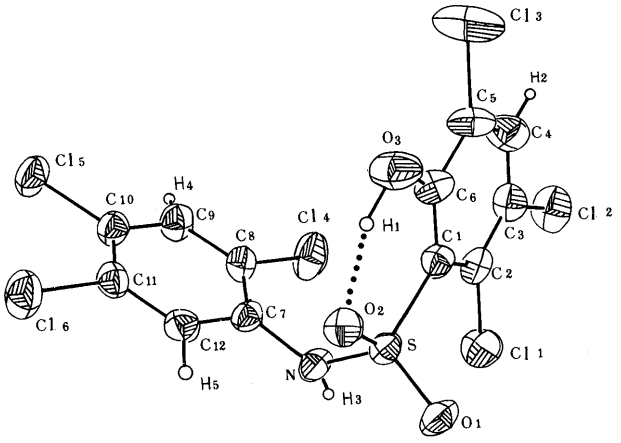


Fig. 1. Molecular Structure of Compound I

TABLE I. Atomic Coordinates ($\times 10^4$) and Thermal Parameters ($Å^3 \times 10^3$) for Atoms of Compound I

Atom	x	y	z	U_{eq}
S	7192 (1)	4579 (1)	4545 (1)	31 (1)
O_1	7802 (4)	5187 (1)	4626 (3)	41 (1)
O_2	6052 (3)	4409 (1)	5336 (3)	36 (1)
O_3	3501 (4)	3983 (2)	3261 (3)	52 (1)
N	8917 (4)	4147 (1)	5086 (3)	35 (1)
Cl_1	8900 (2)	4843 (1)	2140 (1)	53 (1)
Cl_2	6991 (2)	4438 (1)	−944 (1)	62 (1)
Cl_3	1610 (2)	3573 (1)	507 (2)	81 (1)
Cl_4	9406 (2)	3251 (1)	2946 (1)	60 (1)
Cl_5	8372 (2)	1553 (1)	6370 (1)	51 (1)
Cl_6	7954 (2)	2573 (1)	8473 (1)	53 (1)
C_1	6077 (5)	4383 (2)	2760 (4)	33 (1)
C_2	6875 (5)	4492 (2)	1730 (4)	37 (1)
C_3	6051 (5)	4310 (2)	360 (4)	42 (1)
C_4	4429 (6)	4026 (2)	−9 (4)	49 (1)
C_5	3641 (5)	3926 (2)	987 (4)	46 (1)
C_6	4424 (5)	4101 (2)	2391 (4)	38 (1)
C_7	8739 (5)	3521 (2)	5358 (4)	31 (1)
C_8	8923 (5)	3071 (2)	4457 (4)	36 (1)
C_9	8796 (5)	2464 (2)	4772 (4)	41 (1)
C_{10}	8488 (5)	2307 (2)	5998 (4)	36 (1)
C_{11}	8306 (5)	2755 (2)	6913 (4)	35 (1)
C_{12}	8442 (5)	3361 (2)	6600 (4)	35 (1)
H_1	4253 (15)	4098 (14)	4226 (15)	98 (2)
H_2	3913 (15)	3919 (12)	−953 (15)	47 (2)
H_3	10046 (15)	4263 (13)	4891 (15)	76 (2)
H_4	8429 (15)	3696 (12)	7265 (15)	34 (2)
H_5	9200 (15)	2148 (12)	4280 (15)	44 (2)

TABLE II. Selected Bond Lengths (Å) and Bond Angles (deg.) of Compounds I and IV

Bond length			Bond angle		
	I	IV[a]		I	IV[a]
C_1–S	1.780	1.778	C_1–C_6–O_3	126.0	124.9
S–O_1	1.425	1.414	C_1–S–O_2	106.8	108.2
N–C_7	1.428	1.426	C_1–S–O_1	109.9	118.6
S–N	1.626	1.594	S–C_1–C_6	120.0	120.5
S–O_2	1.443	1.429	S–N–C_7	120.5	123.1
C_6–O_3	1.342	1.345	C_1–S–N	106.9	109.7

a) Data cited from reference 2.

TABLE III. Some Bond Orders (P_{r-q})[a] and Atomic Net Charges (NC_s)[b] of Compounds I—VI

No.	NC_s	P_{C_1-S}	P_{S-N}	P_{N-C_7}
I	1.0286	0.8639	0.8560	1.0225
II	1.0116	0.8665	0.8513	1.0233
III	1.0100	0.8675	0.8603	1.0176
IV	1.0377	0.8600	0.8771	1.0144
V	1.0379	0.8605	0.8752	1.0159
VI	1.0353	0.8601	0.8810	1.0111

a) P_{r-q}: WIBERG bond order between atom r and atom q. *b*) NC_s: atomic net charge on atom s.

TABLE IV. Some Bond Orders (P_{r-q}) and Atomic Net Charges (NC_{O_3}) for Anions of Compounds, and Fasciolicidal Activity

No.	NC_{O_3}	P_{C_1-S}	P_{S-N}	P_{N-C_7}	λ_{max}[a]	(ε_{max})[b]	Fasciolicidal activity[c]
I	−0.5348	0.8220	0.4884	1.0666	308	(6.25)	++++
II	−0.5374	0.8223	0.4751	1.0676	300	(4.25)	+++
III	−0.5344	0.8229	0.5012	1.0642	284	(3.13)	+
IV	−0.5170	0.8197	0.5475	1.0642	—	—	++
V	−0.5181	0.8199	0.5445	1.0653	—	—	++
VI	−0.5181	0.8194	0.5595	1.0644	—	—	—[c]

a) λ_{max}: nm, CT band for anions of compounds; electronic absorption spectra were obtained in 0.1 M sodium citrate-sodium hydroxide buffer (pH = 7.4). *b*) ε_{max}: $\times 10^3\ cm^{-1}\ mol^{-1}\ dm^3$. *c*) Fasciolicidal activity of compound VI has not yet been measured.

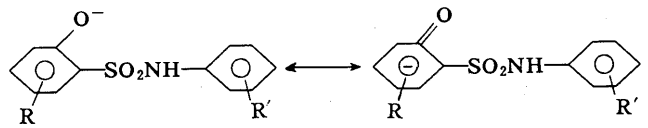
the lone pair of N atoms in these molecules can also effectively conjugate with an adjacent phenyl ring ($ring_a$), strengthening the N–C_7 bond. It can be inferred that through these *d–p* dative bonds the substituted phenyl rings on both sides may be conjugated with each other to some extent.

As depicted in Fig. 1, the phenolic hydroxyl group of compound I and IV can form an intramolecular hydrogen bond with an adjacent sulfonyl oxygen atom (O_3–$H_1\cdots O_2$), and the bond parameters are (1.663 Å, 151.8°) for compound I and (1.798 Å, 156.3°) for compound IV, respectively. These intramolecular hydrogen bonds cause atoms O_2, S, C_1, C_6, O_3 and H_1 to form a six-membered ring which is approximately coplanar with the sulfonyl-substituted phenyl ring ($ring_b$) (dihedral angle 3.5°). All the HBSA may have the same basic structural features as described above.

As indicated above, the dative bonds $C_1 \rightarrow S$, $S \leftarrow N$ and conjugated π-bond N–C_7 in the studied molecules may constitute an important bridge for electron delocalization throughout the entire molecule. Molecular orbital calculations (*cf.* Table III) showed that the strength of these bonds is influenced by the substituents on $ring_a$ and $ring_b$. By comparison with those of compound IV—VI, the dative bonds $S \leftarrow N$ of compounds I—III are much weaker, while the $C_1 \rightarrow S$ bonds and π-bonds N–C_7 are slightly stronger. This is supported by the fact that the bond length of S–N in compound IV is shorter than that in compound I (*cf.* Table II). So, the electron delocalizations of compounds I—III are comparatively poor. In the case of compounds I and II, the $ring_a$ halogen atoms favor sp^2 hybridization of the N atom through strong inductive and conjugative effects. As a result, conjugation between

π-electrons in ring$_a$ and the lone pair of the N atom is further strengthened while the dative bond S←N is weakened. At the same time, the C_1→S bond is somewhat strengthened. In the case of compound III, ring$_a$, like ring$_a$ of compound IV, has only a parahalogen atom and conjugative interaction between the π-electrons in ring$_a$ and the lone pair of the N atom is relatively weakened. However, the S←N bond of compound III is strengthened to a certain extent. This may result from the fact that here the R_1 site is not substituted by a halogen atom.

From the data in Table IV, all anions are similar to their neutral molecules in structural characteristics. Owing to poor delocalization caused by a weaker S←N bond, the phenolic hydroxyl O_3 atom of compounds I—III accumulates more negative charge. In contrast, negative charges on O_3 of compounds IV—VI are dispersed throughout the entire molecule due to the stronger S←N bond. To confirm these results, we observed the electronic absorption spectra of solutions containing anions of all the studied compounds. For compounds I—III, the intramolecular charge transfer transition (CT) bands[11,12] appeared with different intensity (Table IV). This can be explained by the dominant contribution of the phenolate form rather than the keto form, both of which are shown below as the canonical structures.



phenolate form keto form

The intensity of the CT bands of compounds I and II is greater than that of compound III, indicating that in comparison with compounds I and II the electron delocalization into the entire molecule through the S←N bond in compound III is enhanced and in this case the keto form becomes a minor structure, which is consistent with the results obtained by molecular orbital calculation. Moreover, from the data in Table IV, compounds with a greater resonance tendency generally possess more potent fasciolicidal activity. As we will discuss in detail later,[13] this may be rationalized by considering the protonophoric mode of uncoupling in which the anion of the compound as a mediator of proton translocation is stabilized in the nonpolar mitochondrial membrane due to the occurrence of the keto form resonance structure.

Conclusion

The hydroxyl groups of HBSA form intramolecular hydrogen bonds to generate a six-membered ring. The strength of dative bonds between S and C_1 or N, especially S←N, is affected by substituents on the phenyl rings of both sides, and controls the electron delocalization throughout the entire molecule. Accordingly, the contribution of the keto form of the resonance structure is larger for some anions of the title compounds in solution because of the negative charges on phenolic hydroxyl oxygen caused by poor delocalization, and this may be one of the most important factors for potent fasciolicidal activity of HBSA.

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