Experimental Section

Materials. Picrylimidazole was synthesized from picryl chloride (1 mmol) and imidazole (2 mmol) in 2 mL of *N,N*-dimethylformamide. After 30 min at room temperature, in the dark, the solution was poured into ice-cooled water, filtered, and washed with water and ethanol. The light yellow crystals decompose at 187–188 °C (lit.[36] 205 °C). The NMR spectrum agrees with that of the literature.[37] Attempts to purify the product by recrystallization or column chromatography resulted in decomposition of picrylimidazole; hence, we use the compound freshly synthesized without further purification. The purity of S was checked by UV-visible spectra under acidic and basic conditions. Under basic conditions (NaOH, 0.01 M) it rapidly hydrolyzes, giving a spectrum coincident with that of picric acid (λ_{max} = 358 nm (ϵ = 15 000 M^{-1} cm^{-1})). In acid solution there was no absorption at λ = 358 nm.

Dabco was twice recrystallized from petroleum ether (bp 60–80 °C), mp 155–156 °C. Imidazole was recrystallized from benzene, mp 89–90 °C. Acetic acid was distilled, bp 118 °C. Dioxane was purified by the method of Fieser[38] and was stored over $LiAlH_4$ from which it was distilled as needed. Water twice distilled in a glass apparatus was used throughout.

All the inorganic salts were reagent grade commercial reagents and were used without further purification.

The buffer solutions were prepared from solutions of one of the buffer species of known concentration. After the required amount of acid or base and compensating electrolyte was added, the pH of the solutions were determined. If the pH of the set with constant buffer ratio differ by more than 5×10^{-3} pH unit, a drop of diluted acid or base was added to adjust the pH to that of the more concentrated solution. Four or more buffer concentrations were used at each pH.

UV spectra were recorded on a Beckman 24 spectrophotometer, and the change in optical density during a kinetic run was measured on the same instrument at the maximum absorption of picric acid (λ = 358 nm). pH measurements were carried out on a Seybold digital pH meter at 25 °C. Standard buffers prepared according to literature[39] were used to calibrate the electrode.

Kinetic Procedures. Reactions were initiated by adding the substrate dissolved in dioxane to a solution containing all other constituents. Rate constants were determined by following the appearance of picric acid at 25 °C and μ = 1 M. All kinetic runs were carried out under pseudo-first-order conditions with substrate concentrations of about 5×10^{-5}–4×10^{-4} M. Rate constants are accurate to ±3% and were computed from plots of ln ($OD_\infty - OD_t$) vs. time. Most reactions were followed to 80–90% conversion, but for the slowest runs the reaction was followed up to 10–50% conversion. The infinity value for these reactions was determined by hydrolysis of a portion of the substrate solution at high pH. The hydrolyzed substrate was diluted to a concentration suitable for reading the absorption (OD ≈ 0.8) with a buffer of the same pH as the reaction solution. Throughout the paper $(H^+) = 10^{-pH}$.

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1,5-Dithia-2,4,6,8-tetrazocine: A Novel Heterocycle of Unusual Properties

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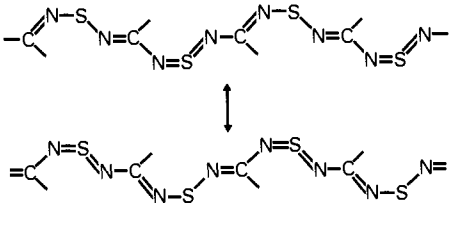
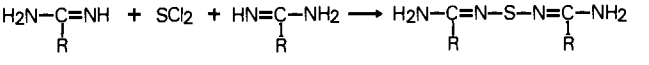
Abstract: Four representatives of a novel heterocycle—1,5-dithia-2,4,6,8-tetrazocine—were prepared. In the 3,7-diphenyl (**3**) and 3,7-bis(*p*-methoxyphenyl) (**5**) derivatives, the eight-membered ring was found to be planar by X-ray diffraction analysis; this and the bond lengths as well as the electronic spectra suggest a delocalized, aromatic system of 10 π electrons. On the other hand, 3,7-bis(dimethylamino)-1,5-dithia-2,4,6,8-tetrazocine (**4**) is folded along an axis drawn through the sulfur atoms; the short distance between the latter atoms is suggestive of their partial bonding in this compound.

In a research program pursued some time ago at the Woodward Research Institute and directed toward the synthesis of organic conductors, linear polymers of type **1** were considered, among other structures, as promising candidates for conductivity.[2]



1

One of the ways considered for the construction of such polymeric compounds involved the intermediates **2**—the expected products from the reaction of two molecules of an amidine and one molecule of sulfur dichloride.



2

† Deceased July 8, 1979.

(1) (a) Woodward Research Institute; (b) Harvard University; (c) CIBA-Geigy Ltd. (d) Part of this work was taken from the Ph.D. thesis of D. Wenkert, Harvard University, 1979.
(2) For a theoretical treatment of the problem of conductivity in this and other types of one- and two-dimensional polymers, see M.-H. Whangbo, R. Hoffmann, and R. B. Woodward, *Proc. R. Soc. London, Ser. A* **366**, 23 (1979).

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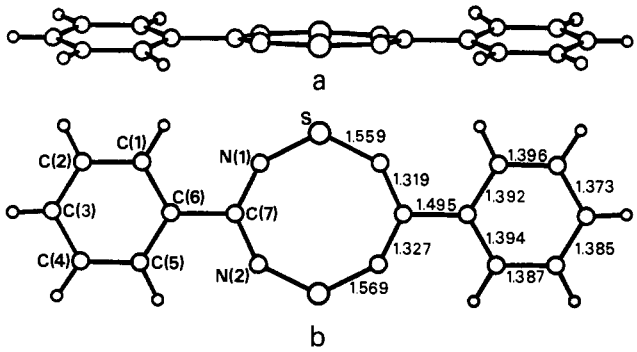
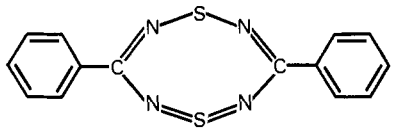


Figure 1. Structure of 3,7-diphenyl-1,5-dithia-2,4,6,8-tetrazocine (**3**) with atomic numbering scheme and bond distances.

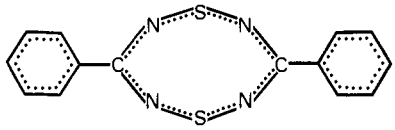
However, the reaction of amidines with sulfur dichloride (in the presence of diazabicycloundecene) proved very complicated in our hands and no compounds of structure **2** could be isolated from the complex product mixtures.

On the other hand, with benzamidine as starting material, a minor product of unusual properties could be obtained from such a reaction in yields ranging between 5% and 10%. It formed yellow, transparent, shiny plates, mp 225–226 °C (from benzene–ethyl acetate), only slightly soluble in most organic solvents, with an elemental analysis and a mass spectrum fitting the empirical formula $C_{14}H_{10}N_4S_2$. This and other evidence (cf. Experimental Section) pointed to structure **3** with the novel 1,5-dithia-2,4,6,8-tetrazocine ring system.



3

A crystal structure analysis not only confirmed the general correctness of the aforementioned structure assignment but disclosed still further interesting structural features of the new compound. Within the limits of the accuracy of the measurements, the eight-membered ring was found to be perfectly planar, with the two phenyl rings only slightly (by 9.7°) distorted out of its plane (Figure 1). All S–N bond lengths in the heterocycle were found to be equal as were those of its C–N bonds. The average S–N distance (1.564 Å) in **3** is shorter than the mean S–N bond length in *s*-trithiazyl chloride[3] (1.605 Å) and is slightly longer than the S–N bond in various sulfodiimide compounds[4] (1.54 Å). Making use of the S–N bond order–bond length correlation of Nyburg,[4a] we ascribe a bond order of between 1.7 and 1.8 to the S–N bonds. As the average C–N distance (1.323 Å) is practically equal to the mean bond length of *s*-triazine[5] (1.319 Å), we can assume a C–N bond order of 1.5. All these findings suggest the presence of a delocalized, aromatic 10π-electron system as represented by the structure **3a**.



3a

In this connection, the characteristic ultraviolet spectrum of **3** with its absorption maxima at 409, 318, 306.5, 294.5, 281, and

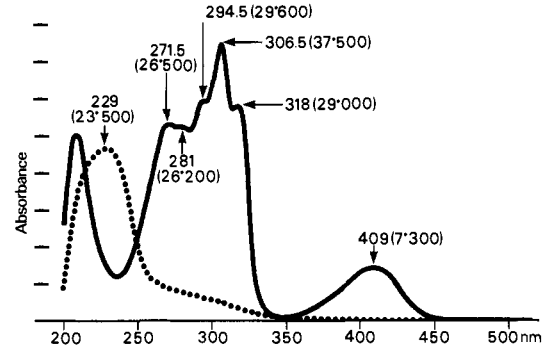


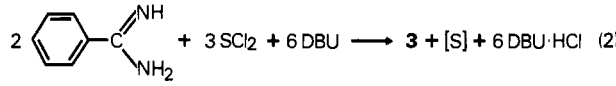
Figure 2. Ultraviolet absorption spectra (in 96% ethanol) of 3,7-diphenyl-1,5-dithia-2,4,6,8-tetrazocine (**3**) (full line) and of 3,7-bis(dimethylamino)-1,5-dithia-2,4,6,8-tetrazocine (**4**) (dotted line).

271.5 nm (Figure 2) is of special interest.

The manifold chemistry of sulfur dichloride leaves enough room for speculation on how the new cyclic system could have been formed. An oxidoreductive disproportionation (reaction 1) would

$$S^{II} + S^{II} \longrightarrow S^{0} + S^{IV} \qquad (1)$$

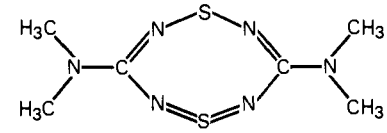
not only account for the tetravalent sulfur atom in the "classical" representation **3** but also explain the observed presence of elemental sulfur in the crude reaction product. Accordingly a 3:2 stoichiometry of SCl_2 to benzamidine would be required for the formation of **3**, as shown in eq 2.



It was difficult to prove this stoichiometry in a reaction of such a low yield. However, using SCl_2 in a ratio to benzamidine lower than shown in (2) led to still lower yields of **3**. Besides **3** and elemental sulfur, no other individual products could be isolated.

As shown by titration with $HClO_4$ in AcOH–C_6HCl, the new compound does not possess basic properties. It proved remarkably stable to heat, not changing even on prolonged refluxing in xylene and only slowly decomposing to benzonitrile when heated neat at 220–240 °C. With KOH or HCl in aqueous dioxane, **3** was easily hydrolyzed to benzamidine; however, in nonpolar solvents (benzene, toluene, xylene), it proved unreactive toward substoichiometric amounts of various nucleophiles (benzylamine, lithium benzothiazolyl-2-mercaptide, sodium hexamethyldisilazane, butyllithium).[6] It was not oxidized by *m*-chloroperbenzoic acid in boiling methylene chloride.

All our attempts to prepare, by a similar reaction of formamidine and SCl_2 in the presence of DBU, the parent 1,5-dithia-2,4,6,8-tetrazocine were unsuccessful. On the other hand, *N,N*-dimethylquanidine, liberated in situ from its hydrochloride with an excess of DBU, reacted in CH_2Cl_2 with sulfur dichloride (in a 2:3 molecular ratio) to give, in a good yield of 54%, a yellow crystalline compound, mp 212 °C, analyzing for the expected 3,7-bis(dimethylamino)-1,5-dithia-2,4,6,8-tetrazocine (**4**).



4

Surprisingly, however, the ultraviolet spectrum of the bis(dimethylamino) compound was very different from that of the phenyl-substituted compound **3**, lacking the intense long-wavelength absorption pattern of the latter (Figure 2).

(3) G. A. Weigers and A. Vos, *Acta Crystallogr.*, **20**, 192 (1966).
(4) (a) S. C. Nyburg, *J. Cryst. Mol. Struct.*, **3**, 331 (1973); (b) J. Leitch and S. C. Nyburg, *ibid.*, **3**, 337 (1973); (c) G. Leandri, V. Busetti, G. Valle, and M. Mammi, *J. Chem. Soc. D*, 413 (1970).
(5) B. J. Wheatley, *Acta Crystallogr.*, **8**, 224 (1955).

(6) The aim of the latter experiments was to release, by initial ring opening of a few molecules, a "polymerization" of **3** to the aforementioned linear polymer **1**. These efforts remained fruitless.

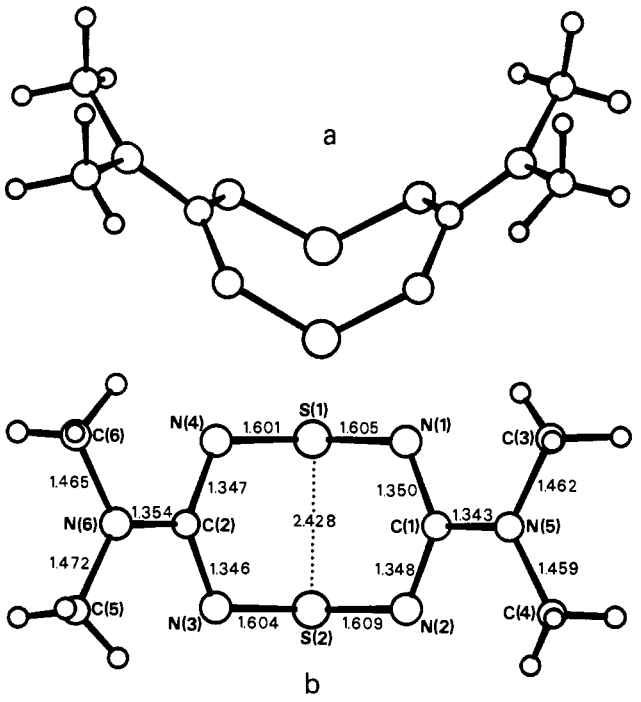
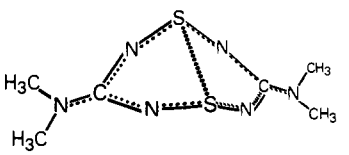


Figure 3. Structure of 3,7-bis(dimethylamino)-1,5-dithia-2,4,6,8-tetrazocine (4) with atomic numbering scheme and bond distances.

A crystal structure analysis showed that this different UV behavior was paralleled by a remarkable difference in the molecular shape of both compounds. In contrast to the planar compound **3**, the bis(dimethylamino) derivative was folded along an axis drawn through the two sulfur atoms of the eight-membered ring; all atoms of the molecule (excepts the hydrogens of the methyl groups) are situated in two planes which intersect in the above-mentioned axis at an angle of 101° (Figure 3). As before with **3**, all S–N bonds are of the same length and so are all C–N bonds, including even those between the ring C atoms and the dimethylamino substituents. The average S–N distance (1.605 Å) is considerably longer than the corresponding average value in **3** (1.564 Å) and is equal to the mean S–N bond length in *s*-trithiazyl chloride.[3] The average C–N distance is 1.348 Å. This again is longer than the mean C–N distance in **3** (1.323 Å). Both the S–N and the C–N bond lengths lie between the typical values for the corresponding single and double bonds.

The short distance between the sulfur atoms S(1) and S(5) is another remarkable feature of the molecule of **4**. Contrary to the situation in the planar ring of **3**, where these atoms are 3.79 Å apart, their distance in **4** is only 2.428 Å;[7] it is longer than a disulfide bond (2.06 Å in S_6) but substantially shorter than the sum of two sulfur van der Waals radii (3.6 Å) and thus suggests a partial bonding.

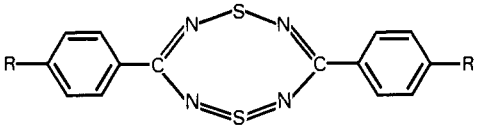
This and other abovementioned structural evidence point to an electronic delocalization for which the following schematic representation **4a** is suggested:[8]



4a

Partial delocalization of the free electron pairs of the dimethylamino nitrogens into the ring system, as suggested in structure **4a**, explains the lack of basic properties of the compound in titration with $HClO_4$ in $AcOH–C_6H_5Cl$.

To further study the observed, remarkable effect of ring substituents on the shape and electronic spectra of 1,5-dithia-2,4,6,8-tetrazocines, two more representatives, namely, compounds **5** and **6** with *p*-methoxyphenyl and *p*-(ethoxycarbonyl)phenyl substituents, respectively, in positions 3 and 7, were prepared. However, the ultraviolet spectra of both compounds were similar to that of the 3,7-diphenyl derivative **3**, thus suggesting that the different electronic contributions (opposite in character) of the parasubstituents did not, in these two cases, substantially affect the electronic system of the eight-membered ring. Indeed, in an X-ray diffraction analysis of the bis(*p*-methoxyphenyl) compound **5**, the heterocyclic ring was found planar and the general shape of the whole molecule was very similar to that of **3**.



5: R= CH_3O—
6: R= C_2H_5O—C(=O)—

The novel 1,5-dithia-2,4,6,8-tetrazocines described in this paper represent a further, interesting contribution to the colorful family of cyclic NSN compounds[9,10] and it is hoped that they evoke the interest of theoreticians for a thorough, quantitative analysis of their unique electronic system.

Experimental Section

A. Preparative Part. Mps are uncorrected. NMR spectra were recorded on a Varian XL-100A spectrometer. Mass spectra were obtained with a Varian CH7 spectrometer. R_f values were determined on Merck silica gel 60 F_{254} TLC plates.

Benzamidine, mp 79 °C, was liberated from its hydrochloride (Fluka AG, Buchs, Switzerland) with aqueous NaOH and extracted into CH_2Cl_2. ***p*-Methoxybenzamidine**, mp 212 °C, and ***p*-(ethoxycarbonyl)-benzamidine**, mp 110–112 °C, were prepared from the corresponding nitriles by the Pinner procedure.[11,12] ***N,N*-Dimethylguanidine hydrochloride**, mp 145–146 °C, was purchased from Fluka AG, Buchs, Switzerland.

3,7-Diphenyl-1,5-dithia-2,4,6,8-tetrazocine(3). To a solution of 2.40 g (20 mmol) of benzamidine and 9.12 g (60 mmol) of diazabicycloundecene in 45 mL of absolute CH_2Cl_2, a solution of 1.9 mL (3.09 g, 30 mmol) of freshly distilled sulfur dichloride (Fluka) in 20 mL of CH_2Cl_2 was added dropwise over 10 min while the reaction mixture was stirred under nitrogen in an ice–water bath. After 2 h, the cooling bath was removed and stirring was continued for an additional 1 h at room temperature. The resulting dark red-brown reaction mixture was diluted with more CH_2Cl_2 and successively washed with water (two 100-mL portions) and saturated brine (50 mL). Drying over Na_2SO_4 of the now orange-yellow organic phase and evaporation under vacuum afforded 1.7 g of a crude product consisting, according to TLC (1:1 ethyl acetate–toluene), of the yellow, most mobile **3** and of several other components. A chromatographic filtration through a column of 100 g of Merck silica gel (the crude product was preadsorbed on another 15 g of the sorbent) in 1:1 ethyl acetate–toluene (350 mL) and repeated crystallization of the eluate from ethyl acetate and ethyl acetate–benzene finally afforded 220 mg (7.4%) of **3** as bright yellow, shiny plates, mp 225–226 °C; at temperatures close to the melting poing (~220 °C), a reshaping of the crystals accompanied by sublimation was observed. R_f 0.69 (1:1 toluene–ethyl acetate); UV λ_{max} (96% EtOH) 409 nm (ϵ 7300), 318 (29 000), 306.5 (37 500), 294.5 (29 600), 281 (26 200), 271.5 (26 500); IR (KBr) 6.27,

(7) A similar short distance (2.58 Å) between two "nonbonded" sulfur atoms was found in the molecule of S_4N_4. B. D. Sharma and J. Donohue, *Acta Crystallogr.*, **16**, 891 (1963).

(8) A similar dependence of the shape and electronic delocalization in an eight-membered ring on the character of substituents has been recently observed in 1,4-dihydro-1,4-diazocines: H.-J. Altenbach, H. Stegelmeier, M. Wilhelm, B. Voss, J. Lex, and E. Vogel, *Angew. Chem.*, **91**, 1028 (1979); M. Breuninger, B. Gallenkamp, K.-H. Müller, H. Fritz, H. Prinzbach, J. J. Daly, and P. Schönholzer, *ibid.*, **91**, 1030 (1979).

(9) Cf. the review by H. W. Roesky, *Angew. Chem.*, **91**, 112 (1979).

(10) A nonaromatic, eight-membered NSN heterocycle formally resembling our compounds, but with two silicon instead of two carbon atoms in the ring, was described some time ago by H. W. Roesky and H. Wiezer, Chem.-Ztg., **97**, 661 (1973). See also the crystal structure analysis by G. Ertl and J. Weiss, *Z. Naturforsch., B*, **29B**, 803 (1974).

(11) P. Oxley and W. F. Short, *J. Chem. Soc.*, 147 (1946).

(12) F. E. Di Gagi and O. Gisvold, *J. Am. Pharm. Assoc., Sci. Ed.*, **38**, 154 (1949).

6.72, 6.91, 7.32, 7.60, 7.77, 8.11, 8.62, 9.20, 9.99, 10.27, 10.66, 10.80, 12.82, 14.47 μm; MS (90 °C) *m/e* 298 (M*), 253, 252, 195, 181, 167, 149, 135, 103, 77, 64, 46. Anal. Calcd for $C_{14}H_{10}N_4S_2$ (298.38): C, 56.36; H, 3.38; N, 18.78; S, 21.49. Found: C, 56.33; H, 3.17; N, 18.61; S, 21.29.

Hydrolysis of 3. A solution of 15 mg of **3** in 4 mL of dioxane and 1 mL of 1 N aqueous HCl was heated at 50 °C for 2 h. The resulting reaction mixture was partitioned between water and CH_2Cl_2, and the aqueous phase was made alkaline (3 mL of 1 N NaOH) and extracted with fresh CH_2Cl_2 to give, after evaporation of CH_2Cl_2, 7 mg of benzamidine, mp 78 °C. A similar result was obtained when **3** (15 mg) was heated at 50 °C in 4 mL of dioxane and 1 mL of 1 N KOH for 80 min.

3,7-Bis(dimethylamino)-1,5-dithia-2,4,6,8-tetrazocine(4). To a stirred suspension of 1.98 g (16 mmol) of *N,N*-dimethylguanidine hydrochloride in 20 mL of absolute CH_2Cl_2, 9.52 mL (64 mmol) of diazabicycloundecene followed by 1.52 mL (2.46 g, 24 mmol) of freshly distilled SCl_2 in 10 mL of absolute CH_2Cl_2 were slowly added at 0 °C. The dark reaction mixture was stirred 1 h at 0 °C and 3 h at room temperature. CH_2Cl_2 (100 mL) was added, and the resulting soltuion was washed several times with water and dried over Na_2SO_4. Evaporation of the solvent under vacuum afforded a complex crude product, which was repeatedly extracted with hot ethyl acetate. The combined extracts were evaporated under vacuum and the resulting yellow powder was chromatographed on a Merck silica gel column (100 g) with 4:1 toluene–ethyl acetate. After a final recrystallization from ethyl acetate, 1.0 g (54%) of **4** was obtained as yellow plates, mp 212 °C (with sublimation). R_f 0.47 (1:1 toluene–ethyl acetate); UV λ_{max} (96% EtOH) 280 nm (inflection; ε 3850), 229 (23 500), 219 (inflection; 22 000); IR (KBr) 6.47, 7.20, 7.43, 8.25, 9.30, 11.27, 11.36, 12.90, 14.00 μm; ^{1}H NMR (100 MHz; in $CDCl_3$) δ 3.15 (s, 12); ^{13}C NMR (25.2 MHz; in $CDCl_3$) δ 179.951, 38.921; MS (20 °C) *m/e* 232 (M*), 188, 162, 148, 116, 102, 46. Anal. Calcd for $C_6H_{12}N_6S_2$ (232.32): C, 31.02; H, 5.21; N, 36.18; S, 27.60. Found: C, 31.10; H, 5.26; N, 36.10; S, 27.43.

3,7-Bis(*p*-Methoxyphenyl)-1,5-dithia-2,4,6,8-tetrazocine (5). A solution of 6.23 g (41.5 mmol) of *p*-methoxybenzamidine, 3.95 mL (62 mmol) of SCl_2, and 18.95 g (124.5 mmol) of DBU in 350 mL of CH_2Cl_2, prepared at 0 °C, was stirred at room temperature for 24 h. Washing with water and with brine, evaporation of the organic phase under vacuum, and chromatography of the crude product on 90 g of silica gel (the product was preadsorbed on 10 g of the sorbent) gave, after eluting several fractions with 1:49 and 1:9 ethyl acetate–hexanes (discarded), a total of 520 mg of a yellow material eluted with 1:9 ethyl acetate–hexanes and with ethyl acetate alone. Rechromatography of the latter material on 40 g of silica gel with chloroform eluant finally provided 282 mg (3.8%) of **5** as yellow crystals, mp 265 °C. For analysis, a sample was recrystallized twice from acetonitrile: mp 268 °C; UV λ_{max} (dioxane) 433 nm (ε 4700), 334 (35 300), 322 (48 700), 311 (39 200), 293 (27 800), 285 (26 200); IR (KBr) 6.30, 6.71, 6.88, 7.30, 7.70, 7.76, 8.04, 8.21, 8.61, 9.78, 11.96 μm. Anal. Calcd for $C_{16}H_{14}N_4O_2S_2$ (358.43): C, 53.61; H, 3.94; N, 15.63; S, 17.89. Found: C, 53.62; H, 3.96; N, 15.78; S, 17.93.

3,7-Bis[*p*-(ethoxycarbonyl)phenyl]-1,5-dithia-2,4,6,8-tetrazocine (6). In a way described for **5**, 4.42 g of a crude product was prepared from 4.0 g (20.8 mmol) of *p*-(ethoxycarbonyl)benzamidine, 1.98 mL (31.2 mmol) of SCl_2, and 9.5 g (62.4 mmol) of DBU in 250 mL of CH_2Cl_2. Upon chromatography on 90 g of silica gel (the product was again preadsorbed on 9 g of the sorbent), some impurities were first removed on elution with 1:49 and 1:9 ethyl acetate–hexanes. Compound **6** was eluted with a 1:1 mixture of the above-mentioned solvents and was recrystallized twice from acetonitrile: 108 mg, mp 305 °C. A further amount (197 mg) of **6** was obtained by combining the mother liquors of crystallization with an AcOEt eluate from the original column and rechromatography of the material thus obtained on silica gel with chloroform (total yield 6.6%). UV λ_{max} (dioxane) 402 nm (ε 7930), 338 (38 600), 326 (48 600), 276 (22 900); IR (KBr) 5.86, 7.13, 7.35, 7.61, 7.86, 8.01 (sh), 8.58, 8.90, 9.09, 9.74, 9.90, 10.60, 11.52, 11.74, 12.83 μm. Anal. Calcd for $C_{20}H_{18}N_4O_4S_2$ (442.50): C, 54.28; H, 4.10; N, 12.66; S, 14.49. Found: C, 54.35; H, 4.13; N, 12.76; S, 14.26.

B. Crystal Structure Analyses. 3,7-Diphenyl-1,5-dithia-2,4,6,8-tetrazocine (3). Crystal data: a = 10.901 (3) Å, b = 5.694 (2) Å, c = 12.200 (3) Å, β = 117.56 (3)°, monoclinic, space group $P2_1/c$, Z = 2, molecular symmetry c_i, d_{calcd} = 1.476 g cm^{-3}, d_{obsd} = 1.473 g cm^{-3}.

Data were collected on a Picker FACS I diffractometer using graphite–monochromated Mo Kα radiation (λ 0.709 26 Å) in the θ–2θ mode in the range $1° \le 2\theta \le 56°$. After the data reduction 1441 unique reflections ($I \ge 2.0\sigma(I)$) were retained for the refinement of the structure. The structure was solved by direct methods, using the program MULTAN 71.[13] The hydrogen atoms were located in difference maps and included in the refinement with isotropic temperature factors. For all other atoms anisotropic temperature factors were introduced. After several cycles the refinement converged to a final value of R = 0.037.

Table I. Bond Angles (Deg) of 3,7-Diphenyl-1,5-dithia-2,4,6,8-tetrazocine (3)

N(1)–S–N(2)′	127.0 (2)	C(4)–C(5)–C(6)	119.2 (3)
S–N(1)–C(7)	140.9 (2)	C(1)–C(6)–C(7)	120.6 (3)
C(7)–N(2)–S′	142.9 (2)	C(1)–C(6)–C(5)	119.8 (3)
C(2)–C(1)–C(6)	120.1 (3)	C(5)–C(6)–C(7)	119.7 (3)
C(1)–C(2)–C(3)	120.2 (3)	N(1)–C(7)–N(2)	129.2 (3)
C(2)–C(3)–C(4)	119.8 (3)	N(1)–C(7)–C(6)	115.1 (3)
C(3)–C(4)–C(5)	121.0 (4)	N(2)–C(7)–C(6)	115.7 (3)

Table II. Bond Angles (Deg) of 3,7-Bis(dimethylamino)-1,5-dithia-2,4,6,8-tetrazocine (4)

N(1)–S(1)–N(4)	114.3 (2)	C(2)–N(6)–C(5)	121.4 (4)
N(2)–S(2)–N(3)	113.3 (2)	C(2)–N(6)–C(6)	121.5 (4)
S(1)–N(1)–C(1)	117.7 (3)	C(5)–N(6)–C(6)	117.0 (4)
S(2)–N(2)–C(1)	117.7 (3)	N(1)–C(1)–N(2)	124.0 (4)
S(2)–N(3)–C(2)	117.7 (3)	N(1)–C(1)–N(5)	117.8 (4)
S(1)–N(4)–C(2)	117.3 (3)	N(2)–C(1)–N(5)	117.9 (4)
C(1)–N(5)–C(3)	121.7 (4)	N(3)–C(2)–N(4)	124.8 (4)
C(1)–N(5)–C(4)	120.9 (4)	N(3)–C(2)–N(6)	117.2 (4)
C(3)–N(5)–C(4)	117.4 (4)	N(4)–C(2)–N(6)	117.7 (4)

Table III. Bond Distances (Å) of 3,7-Bis(*p*-methoxyphenyl)-1,5-dithia-2,4,6,8-tetrazocine (5)

S–N(1)	1.559 (2)	C(3)–C(4)	1.387 (4)
S–N(2)′	1.564 (2)	C(4)–C(5)	1.381 (4)
N(1)–C(7)	1.330 (3)	C(5)–C(6)	1.398 (4)
N(2)–C(7)	1.328 (3)	C(6)–C(1)	1.380 (4)
C(6)–C(7)	1.497 (3)	C(3)–O(1)	1.365 (3)
C(1)–C(2)	1.401 (4)	O(1)–C(8)	1.433 (6)
C(2)–C(3)	1.391 (4)		

Table IV. Bond Angles (Deg) of 3,7-Bis(*p*-methoxyphenyl)-1,5-dithia-2,4,6,8-tetrazocine (5)

N(2)′–S–N(1)	127.2 (1)	C(1)–C(2)–C(3)	118.4 (3)
S–N(1)–C(7)	142.2 (2)	C(2)–C(3)–C(4)	120.1 (3)
S–N(2)′–C(7)′	142.2 (2)	C(2)–C(3)–O(1)	124.2 (3)
C(6)–C(7)–N(2)	115.7 (2)	C(4)–C(3)–O(1)	115.6 (2)
N(1)–C(7)–N(2)	128.5 (2)	C(3)–C(4)–C(5)	120.6 (3)
C(6)–C(7)–N(1)	115.9 (2)	C(4)–C(5)–C(6)	120.4 (3)
C(7)–C(6)–C(1)	120.9 (2)	C(5)–C(6)–C(1)	118.5 (2)
C(7)–C(6)–C(5)	120.6 (2)	C(3)–O(1)–C(8)	117.8 (3)
C(6)–C(1)–C(2)	121.9 (3)		

Figure 1; bond angles are given in Table I. The standard deviations are 0.004–0.006 Å in the distances and 0.2–0.4° in the angles between nonhydrogen atoms.[14]

3,7-Bis(dimethylamino)-1,5-dithia-2,4,6,8-tetrazocine (4). Crystal data: a = 8.469 (3) Å, b = 8.453 (3) Å, c = 8.075 (3) Å, α = 105.64 (3)°, β = 113.00 (3)°, γ = 82.94 (3)°, triclinic, space group P_1, Z = 2, d_{calcd} = 1.506 g cm^{-3}, d_{obsd} = 1.48 g cm^{-3}.

Data were collected on a Picker FACS I diffractometer using graphite-monochromated Mo Kα radiation (λ 0.709 26 Å) in the θ–2θ mode in the range $1° \le 2\theta \le 50°$. After the data reduction 1527 unique reflections ($I > 2.0\sigma(I)$) were retained for the refinement of the structure. The structure was solved by direct methods, using the program MULTAN 71.[13] The hydrogen atoms were located in difference maps and included in the refinement with isotropic temperature factors. For all other atoms anisotropic temperature factors were introduced. After several cycles the refinement converged to a final value of R = 0.067.

Figure 3a shows a perspective view of the molecule. The atomic numbering scheme and bond lengths are given in Figure 3b; bond angles are listed in Table II. The standard deviations are 0.004–0.006 Å in the distances and 0.2–0.4° in the angles between nonhydrogen atoms.[14]

Due to the nonplanarity of the eight-membered ring, all bond angles in the heterocycle are smaller in **4** (N–S–N = 113.8°, S–N–C = 117.6°, N–C–N = 124.4°) than in **3** (N–S–N = 127.0°, S–N–C = 141.0°, N–C–N = 129.2°). The molecule nearly adopts C_{2v} symmetry. The

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(14) Tables of atomic coordinates with their standard deviations are available as supplementary material.

nitrogen atoms N(9) and N(12) lie within experimental error in the plane of their three substituents.

3,7-Bis(*p*-methoxyphenyl)-1,5-dithia-2,4,6,8-tetrazocine (5). Crystal data: a = 6.3967 (4) Å, b = 34.486 (5) Å, c = 7.3826 (4) Å, orthorhombic, space group *Pbca*, Z = 4, molecular symmetry C_i. Data were collected on a Syntex $P2_1$ diffractometer using graphite-monochromated Cu Kα radiation (λ 1.541 78 Å) in the θ-2θ mode in the range $3° \leq 2\theta \leq 135°$ at scan speeds of 2.93-29.30°/min, depending on the intensity of the reflection. Lorentz-polarization and empirical absorption corrections (μ = 29.71 cm^{-1} for Cu Kα) were applied. After the data reduction 951 unique reflections ($I \geq 3.0\sigma(I)$) were retained for the refinement of the structure.

The position of the sulfur atom was found by interpretation of a Patterson map; the residual atoms could be located in difference maps. The compound displays crystallographic C_i symmetry. The hydrogen atoms which were also located in difference maps were included in the refinement together with isotropic temperature factors. For all other atoms anisotropic temperature factors were introduced. After several cycles the refinement converged to a final value of R = 0.041.

The atomic numbering scheme is the same as that of Figure 1. Bond lengths and bond angles are listed in Tables III and IV. There are no significant differences in the geometry between the molecules **3** and **5**; the bonding parameters in the *p*-methoxyphenyl groups are well in the expected range. The phenyl rings are distorted by 9.1° out of the plane of the eight-membered ring; the carbon atoms of the methoxy substituents are only 0.05 Å off the phenyl plane.[14]

Supplementary Material Available: Final atomic coordinates of **3-5** (3 pages). Ordering information is given on any current masthead page.

The Active Site Electrostatic Potential of Human Carbonic Anhydrase

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Abstract: The electrostatic potential fields in the active sites of the B and C isozymes of carbonic anhydrase are examined in vacuo. Both isozymes catalyze the reversible reaction $CO_2 \rightleftharpoons HCO_3^- + H^+$, but they have different activities and sensitivities to inhibitors. The proteins are modeled by point charges derived from ab initio molecular orbital calculations on amino acid residues. Hydration of ionized residues is simulated by the use of limiting-case models, and a discussion of solvent effects is included. The active site potentials of the two isozymes are considerably different and may be used to rationalize the functional differences.

Introduction

Molecular electrostatic potential is one method to correlate structure and activity of biomolecules.[1] The potential at a point in space near a molecule is the work needed to bring a unit positive charge from infinity to that point, assuming that the unit charge has no perturbing effect on the molecule. Maps of the electrostatic potential have recently been employed to determine sites of protonation and H-bond formation in small molecules.[2] Similar maps for enzymes can be useful in predicting qualitative features of enzyme-substrate and enzyme-inhibitor interactions. In this paper we construct and analyze the electrostatic potential of two isozymes of carbonic anhydrase in vacuo and take a first step in considering solvent effects.

Very few electrostatic potential maps of proteins exist in the literature. They are carboxypeptidase A,[3,4] lysozyme,[5,6] and cytochrome *c*.[7] (In the case of lysozyme, both the electrostatic potential and the energy of interaction with a single water molecule was studied.) In each case the protein has been modeled as a collection of point charges. For the first two proteins, one point charge was assigned per protein atom with the point charges derived from Mulliken population analysis of molecular orbital calculations on isolated residues. We[8] and others[1,2,9,10] have demonstrated that this model is able to mimic the more exact electrostatic potential calculated directly from molecular wave functions. It was also shown[8,10] that electrostatic potentials derived from wave functions are relatively insensitive to the presence of normal and strong hydrogen bonds or to most perturbing effects of nearby molecules. In addition, the qualitative features of the maps do not appear strongly dependent on the molecular orbital basis set.[1-3] Thus point charge libraries derived for isolated residues should be applicable to the study of polypeptides and proteins in which a large number of residues are in spatial proximity. Examples and extended discussion of electrostatic potential representation by point charges, its sensitivity to the presence of hydrogen bonds and to the choice of atomic orbital basis, and construction of the point charge library are given in the supplementary material.

The influence of solvent, substrate, or counterions has not been explicitly included in this investigation. We have, however, simulated a particularly important solvent effect, the screening of ionized residues near the protein surface, by use of limiting-case models. We also give a detailed discussion of how microscopic solvent effects may be taken into account and how they might influence the electrostatic potential. Furthermore it should be noted that the in vacuo treatment itself is useful for several purposes: (i) to obtain a first approximation of those active site regions where substrates and water molecules are most likely to

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