SHORT COMMUNICATION

SULPHAMIC ACID IN ZWITTERION FORM—AN *AB-INITIO* SCF MO STUDY

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INTEREST in the study of sulphamic acid is mainly due to its wide industrial applications[1]. In the crystalline state or in aqueous solution or in various solvents, sulphamic acid has been found to adopt the zwitterion form ($NH_3^+ SO_3^-$)[2-8]. However, the structure and conformation of sulphamic acid in gas phase has not been completely understood either theoretically or experimentally. The previous *ab-initio* studies deal with the neutral form of sulphamic acid[9] and on the formation of zwitterion by bringing SO_3 close to NH_3[10]. In this paper we report a detailed study of the zwitterion form of sulphamic acid and its stability is compared with the neutral one. The studies may be useful in understanding the structure and biological activity of mucopolysaccharides like heparin which contain sulphamic acid groups to a considerable percentage[11].

Ab-initio LCAO-SCF-MO calculations were carried out at the standard RHF/STO-3G level using DEC-10 version of Gaussian 74[12]. The calculations on the zwitterion of sulphamic acid were done on an initial geometry of $R_{NS} = 1.77$ Å, $R_{SO} = 1.685$ Å (The STO-3G optimized values for the neutral form[9]), R_{NH} = 1.01 Å and tetrahedral angles. Then some of the parameters were optimized.

Table 1 shows that using the initial geometry; the molecule prefers the eclipsed arrangement (S-O *cis* to N-H) over the staggered arrangement by 0.7 kcal mol^{-1} and is higher in energy by about 11 kcal mol^{-1} over the neutral form. (The absolute energies of the neutral forms are around −669.743 a.u. for different rotations of N-S and S-O bonds. The values were earlier reported[9]. However, on bond lengths (N-S and S-O) and bond angles (O-S-O and H-N-S) optimization the preference is reversed and the staggered arrangement is preferred by 0.7 kcal mol^{-1}. Such a small difference in the energies of the staggered and the eclipsed conformations indicates almost free rotation around the N-S bond. Perhaps this is the reason for the experimental observation of both the forms (table 2). More interestingly, the optimization has stabilized the zwitterionic form by about 30 kcals/mol over the neutral form.

The optimized N-S (2.62 Å) and S-O (1.62 Å) bond lengths are longer than the average experimental values (table 2) which may be due to overestimation of bond lengths by the STO-3G method. However, the optimized values of O-S-O and H-N-S bond angles agree with the corresponding experimental ones.

The calculated barrier-to-rotation about N-S bond is about 0.7 kcal mol^{-1} (table 1) and is threefold, since the oxygens have been fixed with C_{3v} symmetry with respect to the N-S bond. However, a value of about 3 to 4 kcal mol^{-1} has been reported for the barrier from IR spectral data of polycrystalline sulphamic acid[8]. This

Table 1 *Sulphamic acid[8] Zwitterion form—Bond length and bond angle optimization*

	Initial Geometry[a]		Optimized geometry	
	Eclipsed	Staggered	Eclipsed	Staggered
N-S (A)	1.77	1.77	2.55	2.62
S-O (A)	1 69	1.69	1.64	1.62
O-S-O (deg)	109.47	109.47	118.69	118.62
S-N-H (deg)	109.47	109.47	113.29	113.25
Total energy (hartrees)	−669.73	−669.73	−669.79	−669.79
Relative energy (k cal mol^{-1})	0.00	0.66	0.73	0.00

[a] Using STO-3G optimized N–S and S–O bond lengths of the neutral form[9] and the standard value for N–H bond length.

Table 2 *Observed and calculated bond lengths and angles of sulphamic acid-Zwitterion form.*

N-S (Å)	S-O (Å)	O–S–O (deg)	S-N-H (deg)	Preferred conformation	reference
		Experiment			
1.764	1.421	113.4	115.1	Staggered	
	1.452	114.7	107.9		2
	1.445	117.3	111.0		
1.772	1.438	114.93	—	Staggered	
	1.436	115.91	—		3
	1.435	115.83	—		
1.775	1.446	114.7	111 6	Staggered	
	1.445	116.0	109.3		4
	1.441	115.7	109.5		
1.750	1.430	114 0	—	Eclipsed	
	1.440	115.0	—		5
	1.450	118 0	—		
		Theory			
2.55	1 418	117.0	—	Eclipsed	10
2 617	1.623	118.62	113.26	Staggered	Present study

higher value for the barrier may be due to the strong electrostatic interaction of NH_3^+ and SO_3^- with the neighbouring molecules.

The charge distribution on different atoms of the molecule is given in figures 1a and b. It is seen that the S-O bonds are more polarized in the neutral form (figure 1a) than in the zwitterion form (figure 1b). Along with the theoretically calculated charge densities the values obtained by neutron diffraction studies are also given in figure 1b. The values obtained by three different fitting procedures[4] of the neutron diffraction data differ slightly from each other. Our theoretical values agree with the charge densities obtained by using the valence shell projection method[4] (LS), in which the nitrogen atom carries a net negative charge.

Studies were also carried out on N-methylsulphamic

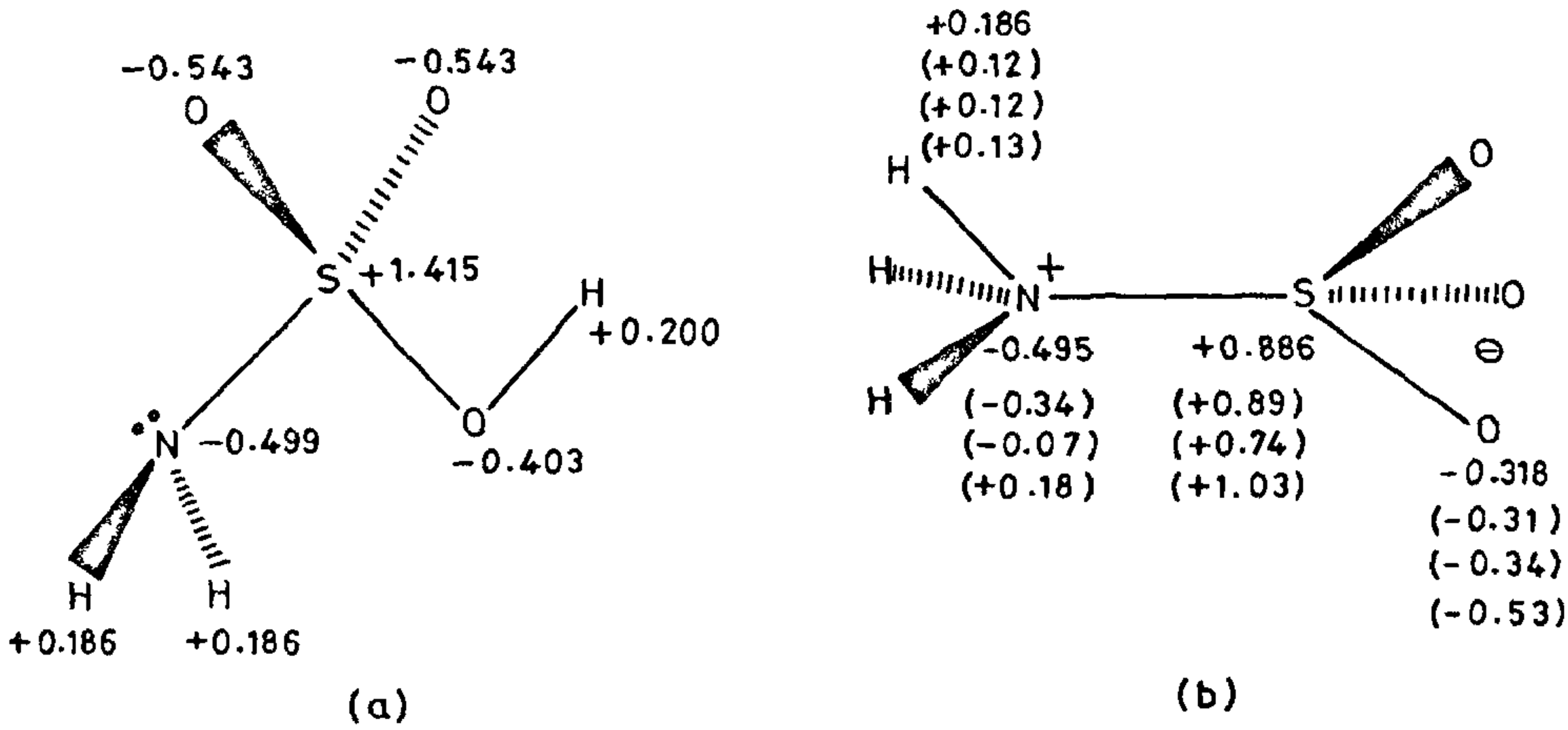

Figure 1. Calculated net atomic charges in (a) neutral form of sulphamic acid, (b) Zwitterion form of sulphamic acid. The values in brackets are the experimental ones[4].

acid ($CH_3NH_2^+SO_3^-$). Using the STO-3G optimized geometry for the N-S and S-O bond lengths of sulphamic acid and standard values[13] for all other parameters, it is found that the eclipsed and staggered conformations of $CH_3NH_2^+SO_3^-$ have almost equal energy (−708.369 hartrees). Further it is found that $CH_3NH_2^+SO_3^-$ is also stable over its neutral analog (CH_3NHSO_3H) by about 30 kcal mol^{-1}.

The stability of the zwitterion form over the neutral form in the isolated molecules of both sulphamic acid and N-methylsulphamic acid suggests that the SO_3^- group is present over a wide pH range and hence is available for calcium (Ca^{2+}) binding in heparin molecule[11].

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ATYPICAL PHYSICOCHEMICAL PROPERTIES OF CYANOGEN BROMIDE FRAGMENTS OF HUMAN SERUM ALBUMIN.

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SEVERAL fragments of serum albumin have been recently used in the study of structural and functional properties of the protein[1-3]. Among others, chemical cleavage with cyanogen bromide (CNBr)* has generally been employed to prepare the fragments. However, very little attention has been paid to the fact that the CNBr cleavage may cause some structural alterations in the resulting fragments. Therefore, the results of such studies are likely to be misinterpreted. In the present report we have shown that CNBr fragments of HSA possess some physicochemical properties which are atypical of other globular proteins. It is probably the first report of its kind and assumes significance as it may affect the inferences made on the basis of the studies involving CNBr fragments of proteins.

All the marker proteins, including albumin and CNBr, were purchased from Sigma Chemical Co., USA. Other chemicals used were of analytical grade. A cystinylated derivative of HSA was prepared[4] and treated with CNBr in 80% (v/v) formic acid for 18 hr in the dark under gentle stirring. Both the reactions *i.e.* cystinylation and CNBr cleavage were completed quantitatively under these experimental conditions. The resulting fragments were first fractionated by a Sephadex G-100 gel chromatography in 0.2 M ammonium formate buffer, (pH 2.8) and subsequently purified by ion exchange chromatography on a DEAE cellulose column equilibrated with 0.005 M sodium phosphate buffer, pH 7.5. The column was eluted using a linear salt-gradient produced by mixing the phosphate buffers containing 0 M and 1 M sodium chloride. The purity of the fragments thus obtained was checked by polyacrylamide gel electrophoresis on 8% gels. The hydrodynamic parameters of CNBr fragments of HSA were determined[5], by calibrating a Sephadex G-200 column having the following characteristics[6]: total volume 316.5 ml; void volume, 126.1 ml and internal volume, 171.7 ml. All light absorption measurements

* Abbreviations: HSA = Human serum albumin
DEAE = Diethylamino ethyl; CNBr = Cyanogen bromide.