NMR AND QUANTUM CHEMICAL STUDY OF EPIMERIC 2',3'-CYCLIC SULPHITES OF NUCLEOSIDES*

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Quantum chemical calculations were applied to ethylene sulphite regarded as a model for the structures of 2',3'-cyclic sulphites of nucleosides. The structure of epimeric 2',3'-cyclic sulphites was investigated by spectroscopic methods including ^{1}H-NMR, ^{1}H-NMR with benzene-induced shifts and ^{13}C-NMR. Five-membered cyclic sulphites are configurationally stable (barrier to inversion ~180 kJ mol^{-1}). The most stable conformation of the five-membered ring is represented by a twisted envelope. According to ^{1}H- and ^{13}C-NMR- measurements *S* configuration was assigned to those epimers of 5'-chloro-5'-deoxy-2',3'-sulphinyluridine and 5'-chloro-5'-deoxy-2',3'-sulphinyl-6-azauridine, which exhibit positive optical rotation. The both epimeric sulphites differ in the position and integral intensity of bands related to stretching vibrations of the S=O group. The epimer, which is more conformationally flexible (*S* configuration), is preferentially formed.

The reaction of cytidine with thionyl chloride was described several times[1-3], including our contributions[4-9]. Different products were isolated under similar reaction conditions. The 2',3'-O-cyclic sulphite[3] was obtained in acetonitrile at ambient temperature, the 5'-halogeno derivative[2] was formed in hexamethylphosphortriamide. A phosphorus containing intermediate was proposed for the latter reaction[10]. It was proved, by both the chemical reactions and physico-chemical measurements, that the 5'-halogeno-2',3'-cyclic sulphite derivatives are formed in the reaction of unsubstituted nucleosides with thionyl chloride[4-9]. It was also shown[7], that the 5'-halogeno-2',3'-cyclic sulphites are formed as a mixture of two isomers, which belong to a single configuration of the nucleoside with the epimeric centre located on the sulphur atom. The formation of 5'-halogeno-2',3'-cyclic sulphite of cytidine and adenosine in an almost quantitative yield on reaction in acetonitrile (refluxing) also proves[9], that the reaction takes place *via* the primary formation of 2',3'-cyclic sulphite and not *via* the cyclic phosphorus containing intermediate as proposed originally[10]. A proposal was also postulated[7], that *S* configuration corresponds to the (+)-epimer, which is formed in a higher yield and exhibits higher

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mobility on TLC and HPLC chromatography. While the mixture of sulphite epimers in uracil, 6-azauracil and 5-fluorouracil series contained approximately 40% of *R* isomer, the isomeric mixture in the cytosine series was in strong favour of the *S* isomer (90%). Liquid chromatography was used for the determination of the particular isomers in reaction products (Table I). The present paper deals with physico-chemical measurements and quantum chemical calculations on the sulphinyl epimers.

The sulphite group isomerism, which was described for non-symmetrically substituted ethylene sulphites, has been thoroughly investigated by IR and ^{1}H-NMR methods (ref.[11-13]). The isomers are stable and a substitution of ethylene sulphite does not markedly alter the bonding pattern of the sulphite part of the molecule[13]. Planar arrangement of the five-membered cycle was suggested on the basis of electron diffraction data of ethylene sulphite[14].

Quantum chemical calculations of the sulphinyl group structure: Ethylene sulphite was chosen as a model for the quantum chemical study of 2',3'-sulphites of nucleosides. Calculations of the five-membered ring arrangement were performed by *ab initio* (STO-3G basis set) and CNDO/2 methods. Values determined by electron diffraction[14] were used for the bond lengths. The study on ethylene sulphite included the spatial arrangement of the sulphite group, the deviation of the sulphite group from the O—C—C—O plane and the possibility to invert the twisted conformation without a change of configuration on the sulphur atom.

Conformational map computed by the CNDO/2 method, which involves variations of the angles α and β (Fig. 1) is given in Fig. 2. It follows from this map, that a change

TABLE I

Quantitative Composition of the Product of the Reaction of Nucleoside with $SOCl_2$

HPLC was performed on a column filled with LiChrosorb SI 60 (Merck), particle size 10 μm; column length 250 mm; internal diameter 4·2 mm; inlet pressure 2·45 MPa; flow 0·8 ml/min. The detection was performed refractometrically. Mobile phase: benzene–ethyl acetate 80 : 20 (v/v).

Compound	*Ia, IIa*[a]	*Ib, IIb*[b]	*Ic, IIc*[b]	*Id, IId*[c]
S isomer	56·6%	59·0%	62·4%	87·8%
R isomer	43·4%	41·0%	37·6%	12·2%

[a] Mobile phase with 1% of 2-propanol added; [b] mobile phase with 0·2% of 2-propanol added; [c] chromatography was performed on a column filled with LiChrospher SI 100 (Merck), particle size 10 μm; inlet pressure 2·60 MPa; flow 0·95 ml/min. The detection was performed spectrophotometrically at 254 nm. Mobile phase: 70 volume parts of dichloroethane, 27 parts of methanol and 3 parts of 0·5M aqueous solution of ammonium formate (pH 3·0).

of configuration on the sulphur atom is hindered by too high energy barrier ($\sim$180 kJ . . mol^{-1}). Optimal value of the angle α amounts to 116°. Dependence of the energy on the angle β, provided that α = 116°, is given in Fig. 3. Conformational transition from the axial arrangement ($\beta > 0$) to the equatorial one is characterised by an energy increase and for the equatorial arrangement no minimum was found. The optimal β angle value is 13° (CNDO/2 calculation). *Ab initio* calculations in the STO-3G basis set resulted in the optimal α angle value of 112°. Considering energy changes at a variation of this angle we obtain identical conclusions as for the case of the CNDO/2 calculation (Fig. 3). No minimum was found for the equatorial conforma-

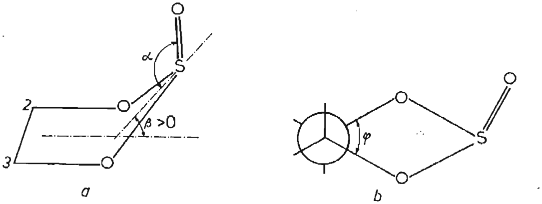


FIG. 1

Geometrical Parameters of Ethylene Sulphite

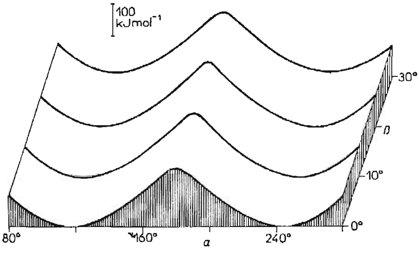


FIG. 2

Dependence of the Energy of Ethylene Sulphite on the Angles α and β (CNDO/2 calculation)

tion. The optimal β angle values is 25°. According to *ab initio* calculations (STO-3G basis set) the twisted conformation is more advantageous for the ethylene sulphite molecule (Fig. 1*b*). The bond lengths were held constant at the examination of the rotation around the C—C bond of ethylene sulphite. Inversion of the twisted conformation exhibits a barrier of 10 kJ mol^{-1} and an energy minimum at $\varphi = 18°$ (Fig. 4).

The results obtained with the use of both methods are in good agreement with ^{1}H-NMR measurements of Green and Hellier[11-13]. The authors found, that the conformation of the sulphite group is axial for the planar arrangement of O—C—C——O atoms. The more accurate measurement resulted in a postulate involving a rapidly inverting twisted envelope conformation without a change of configuration at the sulphur atom. The found extreme values of the angle α amount to 44°.

The charge on the sulphur atoms results markedly positive. CNDO/2 Method affords a value of 0·75, while a value of 0·98 results from the STO-3G calculation. Oxygen atoms are characterised by negative charges (STO-3G: −0·34, −0·34, −0·56;

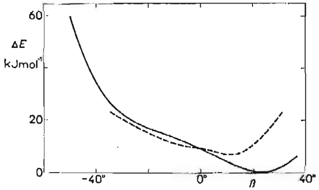


FIG. 3

Dependence of the Energy of Ethylene Sulphite on the Angle β for the Optimal Value of the Angle α
(——— STO-3G calculation; – – – CNDO/2 calculation).

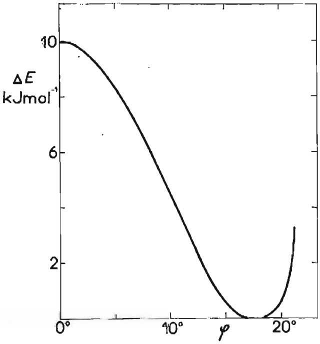


FIG. 4

Energy Changes with the Rotation Around the C—C Bond of Ethylene Sulphite (*ab initio* calculation in the STO-3G basis)

CNDO/2: −0·27, −0·27, −0·46). Dipole moment computed by the CNDO/2 method results as 12·3 . 10^{-30} Cm, the STO-3G result being 13·0 . 10^{-30} Cm. Experimental value of the dipole moment amounts to 12·3. 10^{-30} Cm (ref.[15]).

2′,3′-Sulphites of nucleosides may be regarded as non-symmetrically substituted ethylene sulphite. Hall and coworkers[16] assumed, that the saccharide ring of β-D-ribofuranose derivatives adopts preferentially $_2E$, $^3T_2$ and ^3E conformations. The twisted envelope conformation of the ethylene sulphite five-membered ring of 2′,3′-sulphites of nucleosides combines in a favourable way with the $^3T_2$ conformation of the ribofuranose cycle (Fig. 5).

An experimental method has been suggested in order to verify the configuration of nucleoside derivatives on the basis of quantum chemical calculations of geometrical and electronic structure of ethylene sulphite. The method stems on the NMR measurements utilizing benzene-induced shifts. The benzene molecule is preferentially bound to a site exhibiting electron deficit (formation of a charge-transfer complex), which is represented by the sulphur atom. The benzene six-fold symmetry axis then follows the direction of the dipole vector of the polar group[17,18].

NMR *Spectra*: Firstly, ^{1}H-NMR spectra of both stereoisomers of 5′-chloro-5′-deoxy-2′,3′-O-sulphinyluridine were measured in deuteriochloroform and in a mixture of deuteriochloroform-hexadeuteriobenzene (1 : 1). Chemical shifts of protons of the furanose cycle as well as changes of the chemical shifts induced by the addition of benzene are given in Table II. For various cyclic systems containing the S=O bond the downfield shift of signals related to protons *syn*-axially oriented has been reported in several papers[19−23] dealing with the influence of the S=O bond anisotropy on the chemical shift of neighbouring protons. Thus, it is possible to make a decision on configuration of compounds under study on the basis of com-

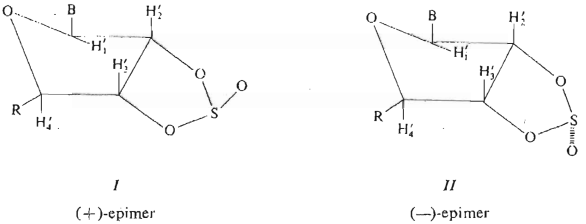
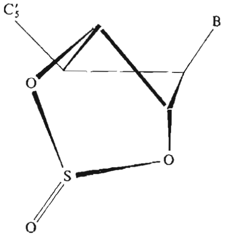


Fig. 5

Suggested Conformations of Sulphinyl Derivatives of Nucleosides paring the chemical shifts of protons of the furanose ring. While the protons $H_{2'}$ and $H_{3'}$ of the (+)-isomer *I* are manifest at lower field (*i.e.* less shielded) than those of the (−)-isomer *II*, the protons $H_{1'}$ and $H_{4'}$ are found at higher field than the corresponding protons of (−)-isomer *II*. Thus, it is possible to assume, that the protons $H_{2'}$ and $H_{3'}$ of the isomer *I* are *syn*-axially oriented with respect to the anisotropic S=O bond and, consequently, the configuration of the (+)-isomer is *S*, while that of the (−)-isomer is *R*. This assignment of configuration is supported by results of the measurement of the solvent effect in benzene. If we assume, that benzene forms a complex preferentially from the positively polarized side of the sulphite



series *a*, B = uracil
b, B = 5-fluorouracil
c, B = 6-arauracil
d, B = cytosine

TABLE II

^{1}H Chemical Shifts of Isomeric 5'-Chloro-5'-deoxy-2,3'-O-sulphinyluridines (δ in $CDCl_3$) Benzene-induced solvent shifts (Δδ in $CDCl_3$ + C_6H_6).

Proton		$H_{1'}$	$H_{2'}$	$H_{3'}$	$H_{4'}$
δ	*I*	5·67	5·82	5·68	4·40
	II	5·89	5·70	5·50	4·88
Δδ	*I*	0·45	0·40	0·22	0·35
	II	0·26	0·27	0·19	0·10

group dipole then for the case of the isomer *I* the protons $H_{1'}$ and $H_{4'}$ should be more shielded, while at the isomer *II* a higher diamagnetic shift should be exhibited by the protons $H_{2'}$ and $H_{3'}$. These suggestions are in approximate agreement with experimental findings. The shift of $H_{1'}$ is not fully significant, because it is probably influenced by a solvation of the nucleoside base.

In the following step ^{13}C-NMR spectra of both isomers of 5'-chloro-5'-deoxy-2',3'-sulphinyl-6-azauridine were measured. The ^{13}C chemical shifts are given in Table III. For the interpretation of these experimental data we used conclusions, which resulted from the works of Buchanan and coworkers[24,25] who investigated conformations of substituted trimethylene sulphites and ethylene sulphites. The γ-C signal of the isomer possessing the axial S=O bond in a *syn*-axial interaction with the pro-

TABLE III

^{13}C Chemical Shifts of Isomeric 5'-Chloro-5'-deoxy-2',3'-sulphinyl-6-azauridines (δ in hexadeuteriodimethyl sulphoxide)

Epimer	C(3)	C(5)	C(6)	C(1')	C(2')	C(3')	C(4')	C(5')
I	157·40	148·69	138·03	90·17	87·09	86·47	86·07	44·62
II	157·42	148·77	138·13	92·64	90·05	88·92	87·64	44·91

TABLE IV

Wavenumbers and Integral Intensities of Bands Related to Stretching Vibrations of the S=O Group

Compound	*Ia*	*IIa*	*Ib*	*IIb*	*Ic*	*IIc*
ν(S=O) (cm^{-1})	1 224[a] 1 222	1 216[a] 1 213	1 223	1 214	1 222	1 217
B ($l\ mol^{-1}\ cm^{-1}$)	1 539[a] 1 384	1 177[a] 994	1 272	950	1 054	888

[a] The spectrum was recorded in $CDCl_3$; the remaining measurements were performed in CH_3CN. IR measurements were carried out on Perkin–Elmer spectrophotometer, model 580 A. Parameters of the single bands were obtained by a band separation technique on a computer[26].

tons at $C_{(2)}$ and $C_{(3)}$ exhibits a diamagnetic shift when compared with isomers in the molecules of which the interaction of this kind is missing. Our isomer with a positive optical rotation exhibits signals of the carbon atoms $C_{2'}$ and $C_{3'}$ at higher field than the isomer with a negative optical rotation. Consequently, in the molecule of (+)-epimer the protons $H_{2'}$ and $H_{3'}$ should be *cis*-oriented with respect to S=O bond. This finding implies *S* configuration.

IR *Spectra*: The ν(S=O) stretching vibrations correspond to 1222 cm^{-1} and 1213–1217 cm^{-1} for *S* and *R* isomers respectively. Integral intensities of these bands are higher for *S* isomers than for *R* isomers (Table IV). It is known from the published reports[11,12,27], that ν(S=O) of cyclic sulphites with a five-membered ring varies in the region 1200–1227 cm^{-1} according to the used solvent and a substitution pattern at $C_{(2)}$ and $C_{(3)}$.

We may state, that results obtained from ^{13}C-NMR spectra of 5'-chloro-5'-deoxy-2',3'-sulphinyl-6-azauridine, as well as results of ^{1}H-NMR spectra, quantum chemical calculations and measurements of the solvent effect with benzene for the isomers of 5'-chloro-5'-deoxy-2',3'-sulphinyluridine lead to identical conclusions on configuration. The stereoisomers exhibiting positive optical rotation possess *S* configuration at the sulphur atom, while isomers with negative optical rotation are of *R* configuration. Moreover, the results of physico-chemical measurements and quantum chemical calculations support the suggestion[7], that the epimer exhibiting greater conformational flexibility (*i.e.* *S* epimer) is formed with some preference.

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