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Oxygen-mediated spectral oscillations in biomolecules and importance of coexisting tautomers: Case of guanine†

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The result obtained by spectroscopic and quantum chemical studies on the electronic structure, spectra and oxygen-mediated spectral oscillations in guanine have been discussed. The origin of the asymmetric double-well nature of the ground state potential surface of the molecule, suggested by the experimental results, has been shown theoretically to lie in the simultaneous occurrence of certain tautomers. The spectral oscillations observed in this case have been explained in terms of complexation of guanine with the dissolved oxygen, electronic excitation and deexcitation of the complex, and accompanying variation in relative populations of the tautomers. Though the specific case of guanine has been discussed, the arguments are also applicable to other systems which occur in more than one tautomeric forms and which complex with oxygen.

1. Introduction

Though carcinogenesis is a complex biological phenomenon, yet there is good evidence that in certain respects it can be treated as a chemical problem. The observed facts that several events relating to carcinogenesis (e.g. attack of carcinogens, binding of anti-cancer drugs and photodynamic actino) mostly occur at the guanines[1-4] suggest that this base of DNA possesses certain special chemical properties which make it prone to involvement in carcinogenesis. The earlier studies on guanine and the other purines in which attention was focussed on the equilibrium properties of the molecules[5-7] have not been able to offer any specific clue in this regard. Photodimerisation of thymine and certain similar photoreactions of cytosine have usually been regarded as the main cause of light-induced carcinogenesis[8-13]. But it has become clear that the role fo the purines, particularly guanine in this context cannot be ignored. Formation of a recently found oxidation product of guanine, namely 8-hydroxyguanine, has been suggested to be related to carcinogenesis[14-16]. The role of oxygen in causing several harmful effects on biological systems including carcinogenesis has been recognised[17-21]. The ultraviolet B region (290-320 nm) which is not prominently absorbed by the primidines (and hence would not cause their dimersation efficiently) is known to be carcinogenic[22,23]. The UV radiation in this region is also not preferentially absorbed by the purines or by DNA. The carcinogenesis caused by the UV B region may, therefore, be related to some other species present in the biochemical environment. Tautomerisations of biomolecules may also produce lethal biological effects since recognitions and functions of biomolecules are structure-specific. Knowledge available in these contexts is scanty, particularly from the point of view of microscopic molecular mechanisms. We have attempted to study here some of these aspects relating to guanine.

2. Computational Methods

Ground state molecular geometries were optimised using the MNDO and AM1 molecular orbital methods[24,25]. The possible modes of binding of guanine with oxygen and with oxygen and water together were studied using the MNDO method. Electronic spectra were computed using the CNDO/s-CI method[26]. Such semi-empirical calculations can provide a satisfactory comparative picture of the electronic spectra of a class of molecules (including the different tautomers) which at the *ab initio* level would be a prohibitively difficult job.

3. Results and Discussion

The results obtained in our experimental study of guanine would be briefly described and the spectra would not be presented here. The theoretical results obtained in order to explain the experimental observations would subsequently be given.

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3.1 *Electronic absorption and fluorescence spectra of guanine*

The UV absorption spectrum of guanine has two peaks, one near 250 nm and the other near 275 nm (refs 27, 28). When the *p*H of the solution is made alkaline (~12), the 275 nm peak shifts to ~290 nm and it also becomes much stronger than that near 250 nm which acquires the form of a shoulder. The reverse is observed under acidic conditions (*p*H ~2) (ref. 29). When an oxygen-rich solution of guanine is UV-irradiated, the intensity of the 275 nm peak rises while that of the 250 nm falls for some time (over a few days); then the reverse process occurs. Finally, the 275 nm peak takes the form of a weak shoulder and the shorter wavelength absorption maximum shifts to near 210 nm[28]. These observations may be classified into two categories: (i) oscillation of intensities near 275 and 250 nm which would be related to guanine, and (ii) strong eventual modification in the absorption spectrum which must be ascribed to the formation of a reaction product of guanine with other molecules present in the environment, e.g., oxygen and water.

The manner in which the relative intensities of the two absorption peaks change on change in *p*H or due to aeration and UV irradiation of the solution, particularly increase in intensity of one at the cost of that of the other, and vice versa, supports the asymmetric double-well model for guanine suggested earlier[27]. The fluorescence spectrum of guanine excited near 250 nm shows a peak near 350 nm and a shoulder near 450 nm[30]. When the guanine solution is flushed with nitrogen, the intensity of the 450 nm shoulder is decreased. But when the solution is flushed with oxygen, the fluorescence peak becomes very broad and the intensity near 450 nm is strongly increased[30]. A clear meaning of these results and of those discussed above is that guanine interacts thoroughly with the dissolved oxygen in the solution. The emissions near 350 and 450 nm have been interpreted as fluorescence from the lowest singlet state and phosphorescence from the lowest triplet state respectively[29,30].

3.2 *Theoretical explanation of experimental results*

We have to find answers to the following three questions: (i) what is the origin of the asymmetric double-well nature of the potential surfaces, (ii) how do spectral oscillations occur, and (iii) how does oxygen interact or bind with guanine. When a single species of guanine [e.g. the normal keto from shown in Fig. 1(a)] was considered, the following result was obtained by molecular orbital calculations[31]. The ring structure of the molecule is almost planar while the amino group is non-planar (pyramidal). Geometries of guanine-keto (N9-H) (G1), guanine-enol (N9-H) (G2), and guanine-keto (N7-H) (G3) forms were optimised using AM1 and MNDO methods and the results (for G1) are compared with those obtained by X-ray crystallography[32] in Table 1. The calculated and observed geometries of G1 are in satisfactory agreement.

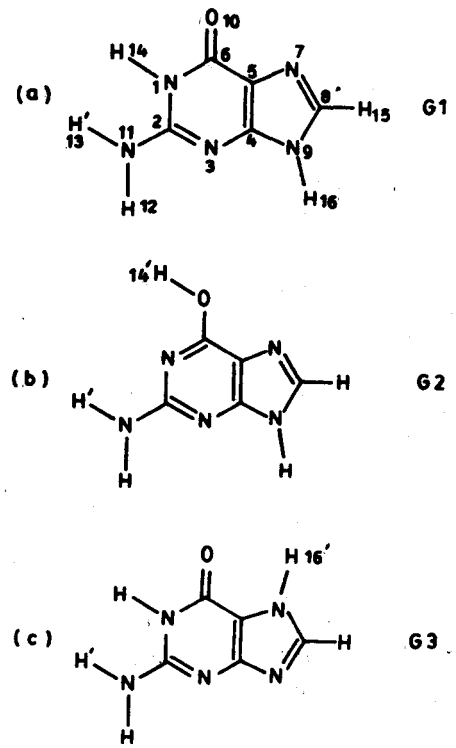


Fig. 1—Structures of three tautomeric species of guanine: (a) keto (N9-H) form (G1), (b) enol (N9-H) form (G2) and (c) keto (N7-H) form (G3). Except where indicated, the numbering of atoms in G2 and G3 is the same as in G1.

Reflection of the amino group in the ring plane of G1 produces two equivalent structures and thus the potential surface in this respect is of symmetric double-well type. Qualitatively, similar results in this respect were obtained when the other tautomeric structures of guanine were considered. Therefore, the asymmetric double-well nature of guanine could arise due to some other possible reasons, e.g., occurrence of more than one tautomeric species. Relative stabilities of several tautomers of guanine have been studied earlier[33-35]. The three most stable structures (Fig. 1a-c, out of the various possible ones are: (i) keto (N9-H)

Table 1—Optimised and experimental geometries of guanine and its tautomers (Å and degrees)

Parameter*	G 1			G 2**		G 3**	
	AM1	MNDO	X-ray	AM1	MNDO	AM1	MNDO
1-2	1.410	1.395	1.395	1.399	1.366	1.418	1.400
2-3	1.360	1.336	1.331	1.386	1.370	1.348	1.330
3-4	1.382	1.383	1.359	1.360	1.356	1.386	1.383
4-5	1.444	1.417	1.375	1.465	1.436	1.442	1.412
5-6	1.447	1.455	1.419	1.417	1.421	1.447	1.457
1-6	1.421	1.451	1.402	1.351	1.369	1.405	1.443
5-7	1.396	1.397	1.394	1.402	1.400	1.390	1.387
7-8	1.346	1.340	1.311	1.340	1.336	1.396	1.406
8-9	1.415	1.406	1.378	1.417	1.416	1.356	1.349
4-9	1.394	1.383	1.378	1.397	1.386	1.408	1.394
6-10	1.239	1.220	1.228	1.366	1.336	1.244	1.224
2-11	1.390	1.411	1.335	1.396	1.400	1.429	1.411
11-12	0.984	1.006	—	0.992	1.004	1.000	1.007
12-13	0.991	1.005	—	0.992	1.005	0.997	1.005
1-14	0.996	1.005	—	—	—	0.997	1.005
10-14'	—	—	—	1.001	0.952	—	—
8-15	1.095	1.086	—	1.096	1.086	1.097	1.870
9-16	0.985	0.995	—	0.985	0.995	—	—
7-16'	—	—	—	—	—	0.986	0.994
1-2-3	124.5	125.2	123.3	126.6	128.8	124.8	125.1
2-3-4	114.2	112.4	112.2	113.4	111.3	115.6	112.5
3-4-5	125.4	127.7	128.4	125.1	126.1	122.3	127.5
4-5-6	119.1	119.6	119.2	115.1	116.9	121.6	119.9
4-5-7	110.3	110.9	110.6	110.0	110.6	106.9	108.7
5-7-8	104.9	104.3	103.8	104.9	104.9	105.8	104.6
7-8-9	113.1	112.8	114.0	113.8	112.6	113.6	112.4
5-6-10	128.7	132.0	128.8	117.8	123.5	127.0	130.8
3-2-11	117.8	117.7	120.6	116.3	115.9	116.3	117.9
2-11-12	117.3	113.4	—	117.2	114.7	113.7	113.2
2-11-13	119.2	114.1	—	117.0	114.7	114.3	114.1
2-1-14	120.4	118.0	—	—	—	120.0	118.4
6-10-14'	—	—	—	109.6	114.2	—	—
7-8-15	125.6	125.4	—	125.3	126.6	122.4	125.2
8-9-16	127.5	126.8	—	127.4	125.9	—	—
5-7-16'	—	—	—	—	—	125.7	127.2
1-2-3-4	−2.3	0.0	—	−2.3	0.0	2.7	−1.7
2-3-4-5	1.6	0.0	—	1.1	0.0	−0.3	0.8
3-4-5-6	−0.8	0.0	—	0.1	0.0	−0.2	−0.4
3-4-5-7	179.7	180.0	—	179.8	180.0	179.6	180.0
4-5-7-8	−0.2	0.0	—	0.0	0.0	0.1	0.0
5-7-8-9	0.0	0.0	—	0.0	0.0	0.0	0.0
4-5-6-10	−179.9	180.0	—	179.2	180.0	−180.9	180.0
4-3-2-11	−178.1	185.4	—	−174.9	185.8	172.2	173.4
3-2-11-12	187.0	−12.4	—	195.2	−29.2	177.3	13.8
3-2-11-13	157.9	222.3	—	164.5	202.6	222.5	137.1

(contd)

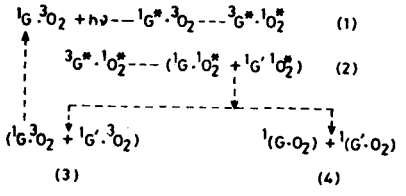
Table 1—Optimised and experimental geometries of guanine and its tautomers (Å and degrees)—*Contd*

Parameter*	G 1			G 2**		G 3**	
	AM1	MNDO	X-ray	AM1	MNDO	AM1	MNDO
3-2-1-14	178.2	184.8	—	—	—	183.1	175.5
5-7-8-15	180.1	180.0	—	180.0	180.0	180.0	180.0
5-6-10-14'†	—	—	—	179.6	180.0	—	—
15-8-9-16	−0.5	0.0	—	179.3	0.0	—	—
4-5-7-16'‡	—	—	—	—	—	179.6	179.5

*For Atomic numbering, see Fig. 1(a-c).
**X-ray data not available.
†Corresponds to guanine enol (N9-H) form (G2).
‡Corresponds to guanine keto (N7-H) form (G3).

form (G1), (ii) enol (N9-H) form (G2) and (iii) keto (N7-H) form (G3). According the MNDO method, the relative stabilities of these structures are[33] G2 > G1 > G3. AM1 calculations, however, predict[34] the relative stabilities of the three tautomers as G1 > G3 > G2. According to an experimental study, G1 has an abundance of 84% while G2 has an abundance of 16% near the neutral *p*H. According to another study, the relative populations of G1 and G2 depend on the environment: in a non-polar environment, the populations of the two species are nearly equal while in a polar environment, the population of G1 dominates[5] over that of G2. The highest stability of G1 among the various tautomers is supported by an *ab-initio* calculation also[35]. In view of these theoretical and experimental results, the minima of the asymmetric double-well model can be assigned to G1 and G2. According to the calculations mentioned above, one more minimum (G3) near those corresponding to G1 or G2 can also occur. However, it is likely that two of the wells are almost merged with each other and the overall appearance is one of a double-well type of potential surface.

The second question relating to the interaction of oxygen with guanine is quite complex from the computational point of view. The main complexity is that we do not known whether the oxygen molecule attacks guanine as such or its interaction with guanine initiates through some other species (e.g. O_2^-, HO_2 radical, O_4 etc.) when the solution is flushed with oxygen and UV irradiated[36-38]. The spectral oscillation can, however, be explained assuming that the interaction mainly occurs between the oxygen molecule and guanine as in Scheme 1.



Scheme 1

Here G and G′ stand for two tautomeric structures of guanine (e.g. G1 and G2). At step (1), the triplet (excited) of guanine is produced by intersystem crossing. At step (2), more G′ is produced than G due to favourability of the non-vertical transition[39]. In step (2), either the stable reaction products (4) are formed or the complex shown at step (3), in which G′ would be in a significant abundance, is produced though we assumed its population to be zero at the initial stage. As shown in the above scheme, the cycle can repeat and thus more and more G′ would be produced. When G′ is in larger abundance than G, their roles would be interchanged and thus as oscillation would be set up. Formation of the product (4) would dampen the oscillation which would be slow since its crucial cause, namely the complexation of guanine with oxygen, is itself slow.

It should be pointed out that in the scheme given above, we have considered only two tautomeric species for the sake of simplicity but actually we expect three species (G1, G2 and G3) to be involved in it, as discussed earlier. Oscillations in chemical systems are well known[40]. We recognise that in the above scheme of processes, two aspects can be easily studied by quantum chemical computations. These are: (i) electronic trans-

itions in guanine and its tautomers, and (ii) nature of the product (4) and its electronic absorption spectrum. The theoretical results obtained in this context are discussed below. The phototautomerisation shown in step (2), which is crucial for oscillations, cannot be studied reliably with the available molecular orbital methodologies due to non-vertical and transition probability controlled nature of this event.

The following points may also be noted with regard to the above scheme: (i) It would be valid for other compounds also which occur in more than one tautomeric forms. Besides guanine, oscillation has also been observed in adenine[41]. (ii) Formation of a guanine-oxygen complex (e.g. one of charge transfer type) is required to initiate the set of processes shown in the above scheme. (iii) The phototautomerisation and oscillation involved in the above scheme are facilitated by the fact that O_2 has triplet ground state and long-lived singlet excited states.

Electronic transitions in the different tautomers of guanine were studied usng the CNDO/s-CI method[27]. Further, these calculations were done using the molecular geometries obtained by both the MNDO and AM1 methods. The calculated UV absorption wavelengths for π-π^* transitions in the different tautomers of guanine are given in Table 2. As mentioned earlier, two absorption peaks are observed experimentally in guanine solutions, one around 275 nm and the other near 250 nm[27,28]. We find that there is quite appreciable difference between the calculated and observed excitation wavelengths of guanine. The spectral results obtained by the MNDO optimised geometries are somewhat better than those obtained by the AM1 optimised geometries. The differences between the calculated and observed excitation energies when the experimental geometry for the keto form of guanine is used in the calculation are found to be similar to those obtained with the MNDO optimised geometries[42]. Thus it is found that the calculated excitation wavelengths in guanine are appreciably larger than those oberved experimentally[29]. This fact seems to point to some inherent problem in treating the cases like that of guanine theoretically since the same approach has been found to be quite good in a number of other cases. Coexistence of more than one species may provide them additional stability which may increase the excitation energies with respect to those obtained for them individually. This aspect is not included in the present theory.

The possible mode of binding of oxygen to guanine producing a stable product was studied theoretically. Suitability fo the AM1 and MNDO (SCF) methods was tested for this calculation by studying the ground state geometries of O_2 and O_3. The AM1 method predicted a too short internuclear distance in O_2 (1.084 Å) while the corresponding MNDO value (1.134 Å) was closer to the experimental value (1.2 Å) (ref. 43). The MNDO method yielded a better geometry than the AM1 method for O_3 also. Hence the MNDO method was preferred over the AM1 method to study the binding of O_2 with guanine. The overall ground state multiplicity in these calculations was taken to be singlet. There are three double bonds (one C=C and two C=N) in guanine ring. At each of these bonds, stable binding of an oxygen molecule was predicted by the calculations. However, the most stable binding of O_2 occurred to the C=C double bond at which the five- and six-membered rings of guanine join. The binding of O_2 and water (as H+OH) to guanine was also studied. This calculation showed that OOH and OH groups would simultaneously bind to the C4 and C8 sites of guanine respectively. This predicted reaction product of guanine, O_2 and water should only be taken as a model system and the above result suggests that the sites C4 and C8 of guanine can simultaneously participate in such reactions[31].

Table 2—Calculated excitation wavelengths and oscillator strengths in three tautomers of guanine using AM1 and MNDO optimised geometries. Only allowed transitions are included here

Tautomer*	Excitation wavelength (nm)†	Oscillator strength†
G 1	336.2 (302.8)	0.39 (0.35)
	264.5 (259.9)	0.21 (0.30)
	253.4 (243.4)	0.10 (0.10)
G 2	297.3 (291.1)	0.09 (0.1)
	279.4 (270.1)	0.49 (0.46)
	233.0 (227.1)	0.02 (0.01)
G 3	294.4 (286.0)	0.21 (0.17)
	290.7 (284.3)	0.01 (0.06)
	273.2 (264.9)	0.26 (0.24)
	232.2 (227.1)	0.13 (0.13)

*Structures of the three tautomers are given in Fig. 1.
†The results obtained using the MNDO optimised geometries are given in parentheses and the other values were obtained using the geometries obtained by the AM1 method. Experimentally observed peaks lie at 275 and 250 nm.

We can generalise the above result in the sense that polymeric reaction products of the type R1-(G-O-O-G)$_n$-R2, (n = an integer; R1, R2 = H, OH) may be formed under appropriate conditions. Some evidence in favour of this type of reaction products is available as folows: (i) UV-irradiated oxygen-rich solutions of guanine, when studied for several successive days, eventually show absorption spectra having a peak (or shoulder) near 210 nm and a shoulder near 275 nm. These spectra are quite different from those of a fresh guanine solution. Theoretically computed spectra of the guanine-O_2 and guanine-O_2-water reaction products mentioned above are in agreement with this change in the spectrum[28-31]. (ii) We have isolated a reaction product of guanine and oxygen from an aqueous solution which is polymeric and in which the binding pattern appears to be in accordance with the above suggested pattern[44].

As mentioned earlier, 8-hydroxyguanine is formed under UV irradiation of biological systems and it is considered to be relevant to carcinogenesis[14-16]. The theoretically computed spectrum of 8-hydroxyguanine shows that this molecule would absorb at higher wavelengths than those where guanine absorbs. Thus the change in the UV absorption spectrum observed by us in UV-irradated oxygen-rich solutions of guanine, discussed earlier, cannot be explained by invoking the formation of 8-hydroxyguanine. Therefore, the reaction product of guanine and oxygen formed in our experiments[27,28] would be different from 8-hydroxyguanine. The oxygen molecule has certain electronic transitions near the UV B region[23,43]. The carcinogenecity of the UV B region may be due to the fact that oxygen complexed with biomolecules may absorb the radiation in this region with enhanced intensity, and it may eventually cause the biological damage via oxidation of the important constituents.

4. Conclusion

Two tautomers of guanine are present in aqueous solutions near the neutral *p*H in appreciable abundance; there can also be a third one in small amounts. Guanine reacts with the dissolved oxygen in aqueous solutions under ultraviolet irradiation. In this situation, relative populations of the tautomers of guanine can change, which causes oscillations in its spectrum. Eventually guanine-O_2 and guanine-O_2-water reaction products may be formed which may be polymeric. Such reactions may be important from the point of view of biological systems and they may be related to carcinogenesis. Other systems having more than one coexisting tautomeric species and ability to complex with oxygen may also behave in a way similar to that of guanine.

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References

1 Jeffrey A M, Kinoshita T, Santella R M, Grunberger D, Katz L & Weinstein I B, in *Carcinogenesis: Fundamental mechanisms and environmental effects*, edited by B Pullman, P O P Ts'o & H Gelboin (Reidel, Boston, MA) 1980, p. 565.
2 Barton J K & Lippard S J, in *Nucleic acid-metal ion interactions*, edited by T G Spiro (Wiley, New York) 1980, p. 31.
3 Smith K C & Hanawalt P C, *Molecular photobiology, inactivation and recovery* (Academic Press, New York) 1969, p. 179.
4 Dahl T A, Midden W R & Hartman P E, *Photochem Photobiol*, 46 (1987) 345.
5 Person W B, Szczepaniak K, Szczesniak M, Kwiatkoswki J S, Hernandez L & Czerminski R, *J Molec Struct*, 194 (1989) 239.
6 Lee G C Y & Chan S I, *J Am chem Soc*, 94 (1972) 3218.
7 Chenon M T, Pugmire R J, Grant D M, Panzica R P & Townsend L B, *J Am chem Soc*, 97 (1975) 4627.
8 Moan J & Peak M J, *J Photochem Photobiol B: Biol*, 4 (1989) 21.
9 Patrick M H, *Photochem Photobiol*, 25 (1977) 357.
10 Kittler L & Lober G, in *Photochem Photobiol Rev*, edited by K C Smith (Plenum, New York) 1977, p. 39.
11 Temin H M, *Cancer Res*, 34 (1974) 2835.
12 Brash D E & Haseltine W A, *Nature*, 298 (1982) 189.
13 Bohr V A, Phillips D H & Hanawalt P C, *Cancer Res*, 47 (1987) 6426.
14 Kuchino Y, Mori F, Kasai H, Inoue H, Iwai S, Miura K, Ohtsuka E & Nishimura S, *Nature*, 327 (1987) 77.
15 Floyd R A, West M S, Eneff K L & Schneider J E, *Free Radical Biol Med*, 8 (1990) 327.
16 Floyd R A, West M S, Eneff K L & Schneider J E, *Arch Biochem Biophys*, 273 (1989) 106.
17 Biaglow J E, Varnes M E, Jacobson B & Suit H D, *Adv Exp Med Biol*, 180 (1984) 323.
18 Duplaa A M & Teoule R, *Int J Red Biol Relat Stud Phys Chem Med*, 48 (1985) 19.
19 Ling C C & Robinson E, *Int J Red Oncol*, 15 (1988) 1179.
20 Parker J B, *Photochem Photobiol*, 48 (1988) 225.
21 Laval F, *Mut Res*, 201 (1988) 65.
22 Tyrrel R M & Pidoux M, *Photochem Photobiol*, 47 (1988) 405.
23 Chatterjee S N, Agarwal S & Bose B, *Indian J Biochem Biophys*, 27 (1990) 254.
24 Dewar M J S & Thiel W, *J Am chem Soc*, 99 (1977) 4899.
25 Dewar M J S, Zoebisch E G, Healy E F & Stewart J J P, *J Am chem Soc*, 107 (1985) 3902.
26 Del Bene J & Jaffé H H, *J chem Phys*, 47 (1968) 1221; 48 (1968) 1807.

27 Mishra P C, *J molec Struct*, 144 (1986) 309.
28 Mishra P C, *J molec Struct*, 195 (1989) 201.
29 Santhosh C & Mishra P C, *Proc Ind Acad Sci (Chem Sci)* (submitted).
30 Santhosh C & Mishra P C, *J molec Struct*, 198 (1989) 327.
31 Santhosh C & Mishra P C, *Int J Quant Chem* (in press).
32 Voet D & Rich A, *Prog Nucl Acid Res Mol Biol*, 10 (1970) 183.
33 Sygula A & Buda A, *J molec Struct (Theochem)*, 92 (1983) 267.
34 Norinder U, *J molec Struct (Theochem)*, 151 (1987) 259.
35 Sabio M, Topiol S & Lumma (Jr) W C, *J phys Chem*, 94 (1990) 1366.
36 Bielski B H J & Cabelli D E, *Int J Radiat Biol*, 59 (1991) 291.
37 Long C A & Ewing G E, *J chem Phys*, 58 (1973) 4824.
38 Goodman J & Brus L E, *J chem Phys*, 67 (1977) 4398, 4408.
39 Lamola A A, in *Energy transfer and organic photochemistry*, edited by A A Lamola, N J Turro (*Interscience Publishers*, New York), 1969.
40 Glansdroff P & Prigogine I, *Thermodynamic theory of structure, stability and fluctuations* (Wiley-Interscience, New York), 1971.
41 Santhosh C & Mishra P C, *J molec Struct*, 220 (1990) 25.
42 Srivastava S K & Mishra P C, *Int J Quant Chem*, 16 (1979) 1051.
43 Huber K P & Herzberg G, *Molecular spectra and molecular structure, IV, Constants of diatomic molecules*, (Van Nostrand Reinhold, New York), 1979, 496.
44 Santhosh C & Mishra P C, *J Photochem Photobiol*, 61A (1991) 281.