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Electronic spectra of 8-azaguanine in solution: Evidence for double-well potential surfaces and effect of dissolved oxygen

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Abstract. Electronic absorption and fluorescence spectra of 8-azaguanine in aqueous solution have been studied. Relative intensities of the two peaks observed in absorption are found to change with time. This result can be interpreted in terms of the double-well potential surfaces of the molecule as has been done earlier for guanine. Like guanine, 8-azaguanine also forms a complex with oxygen dissolved in the solution on irradiation in the absorption region and this makes the $^1\Sigma_g^+ - {}^3\Sigma_g^-$ transition of oxygen in the complex allowed by dipole selection rule. The peak of 8-azaguanine fluorescence when excited by radiations of different wavelengths upto 305 nm lies near 400 nm. This fluorescence differs appreciably from that of guanine and can be interpreted as mainly arising from the second triplet excited state of the molecule which may be populated by intersystem crossing from the first singlet excited state.

Keywords. Excited state; photochemistry; photobiology; mutation.

1. Introduction

8-Azaguanine is a well-known mutagenic agent (Maher *et al* 1980). From the point of view of static electronic structure, it would have only minor differences with respect to guanine. However, the two molecules may show significantly different photophysical and photochemical properties. Guanine is known to possess certain interesting properties which are not shown by the other DNA bases. For example, it has asymmetric double-well potential surfaces in its ground and excited states (Mishra 1986, 1988) and is frequently the site in DNA where several external agents (e.g. carcinogens and anti-carcinogenic drugs) bind (Jeffrey *et al* 1980; Barton and Lippard 1980). In a recent work (Mishra 1988), it has been shown that guanine forms a complex with the dissolved oxygen in aqueous solution on being excited to its first singlet state and subsequent intersystem crossing to the first triplet state. These observations on guanine may be relevant to photodynamic action (Smith and Hanawalt 1969; Dahl *et al* 1987). It is desirable to investigate if these properties of guanine are present in 8-azaguanine or not. A knowledge of these aspects may be useful in understanding the mechanism of the mutagenic action of 8-azaguanine. Further, such a study can throw light on the microscopic physical and chemical aspects in which 8-azaguanine and guanine resemble and differ from each other.

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2. Experimental

Aqueous solutions of 8-azaguanine (from BDH) containing about 1 mg of the compound in 50 ml of distilled water were used for the study. Electronic absorption spectra of the solutions were recorded on several days successively using a Hitachi 320 UV/Vis Spectrophotometer. Fluorescence spectra were studied using an Applied Photophysics flourescence spectrometer (model SP 70B) and a 150 watt xenon lamp.

3. Results and discussion

Electronic absorption spectra of an 8-azaguanine solution were recorded for 25 days regularly every 24 h. Five of these spectra, recorded on different days (numbers 0, 9, 10, 11 and 20) are presented in figure 1. We find that the absorption spectrum of 8-azaguanine recorded on day 0 has a shoulder near 275 nm and a clear peak near 248 nm. On the following days, the shoulder slowly acquires the form of a clear peak whereas the peak converts to a shoulder. It is to be noted that very minor shifts in the positions of the two intensity maxima as well as of the minimum near 260 nm occur in this process. Broadly speaking, the absorption spectrum of 8-azaguanine resembles that of guanine (Mishra 1986, 1988). The corresponding two absorption peaks in the case of guanine have been explained (Mishra 1986, 1988) in terms of asymmetric double-well potential surfaces. Existence of this type of potential surfaces in guanine is supported by different experimental observations on infrared and fluorescence spectroscopy as discussed earlier (Mishra 1988). Since 8-azaguanine is expected to show behaviour similar to guanine, say from the point of view of electronic structure, the absorption spectra of 8-azaguanine presented in figure 1 can also be interpreted in terms of the same type of potential surfaces.

Figure 2 presents certain ultraviolet absorption spectra of a different aqueous solution of 8-azaguanine (second sample) recorded on successive days. In this case, in the beginning itself, the 275 nm peak is stronger than that near 248 nm and the latter peak only appears as a shoulder (spectrum 1). When nitrogen gas was bubbled through this sample for 15 min and the spectrum was recorded immediately after it (spectrum 2), the intensity minimum near 260 nm became clearer than it was before. The absorption spectra recorded on the next two days show the two peaks quite clearly; in the spectrum recorded two days after bubbling nitrogen (spectrum 4), the two peaks are seen to be almost equally intense. On subsequent days, the 275 nm peak became stronger than that near 248 nm and eventually the same situation which existed before nitrogen was bubbled was reproduced. It appears that a change in the relative intensities of the 275 and 248 nm peaks is caused by interaction of the solute with the remaining amount of the dissolved oxygen.

The case of guanine may be compared with that of 8-azaguanine in this respect. A prolonged irradiation of a guanine solution shows appreciable change in relative intensities of the 248 and 275 nm peaks (Mishra 1988) as the shallower well of the ground state gets populated in this process. But an unirradiated fresh solution of guanine always shows the 275 nm peak as less intense than that at 248 nm. Thus it is somewhat surprising that in the case of 8-azaguanine the 275 nm peak can be more intense than that at 248 nm even without any irradiation of the solution. The possible reason for this difference between guanine and 8-azaguanine seems to be a smaller

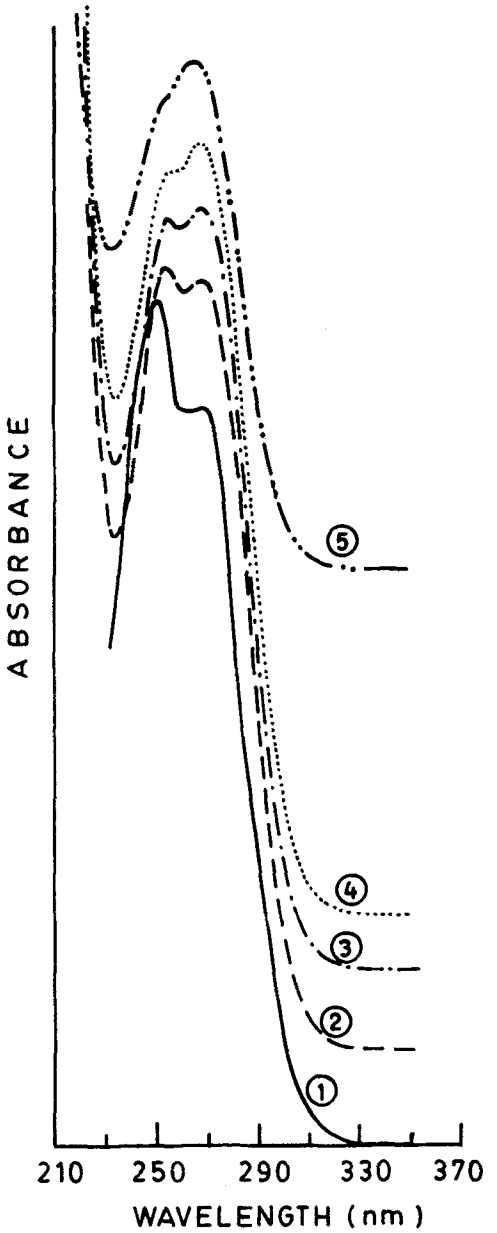

Figure 1. Electronic absorption spectra of an aqueous solution (first sample) of 8-azaguanine. The spectra from 1 to 5 were recorded on different days (taken as 0, 9, 10, 11 and 20) at a fixed time of day. The different spectra are shifted along the absorbance axis with respect to one another for convenience of presentation. In each of the spectra, the absorbance scale used was 0 to 0·50.

potential barrier separating the two minima in the ground state and also possibly in the first singlet excited state of 8-azaguanine than in that of guanine. Further evidence in support of the involvement of oxygen in controlling the behaviour of 8-azaguanine is as follows.

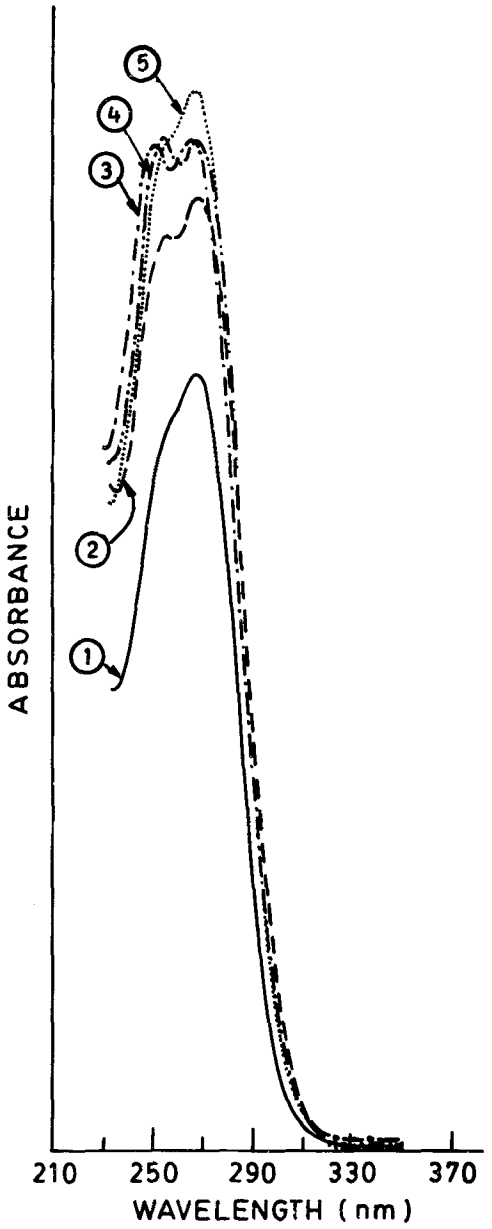

Figure 2. Electronic absorption spectra of an aqueous solution (second sample) of 8-azaguanine. The spectra from 1 to 5 were recorded on days (taken as 0, 0, 1, 2, and 13) at a fixed time of day. It is to be noted that two spectra were recorded on day 0, one before bubbling nitrogen and the other after bubbling nitrogen. The different spectra are shifted along the absorbance axis with respect to one another for convenience of presentation. In each of the spectra, the absorbance scale used was 0 to 1·0.

We studied the absorption spectra of a third solution of 8-azaguanine. It gave an absorption spectra like that of the second sample. Three absorption spectra of this sample are shown in figure 3. The spectrum 3 in this case was recorded 10 days after

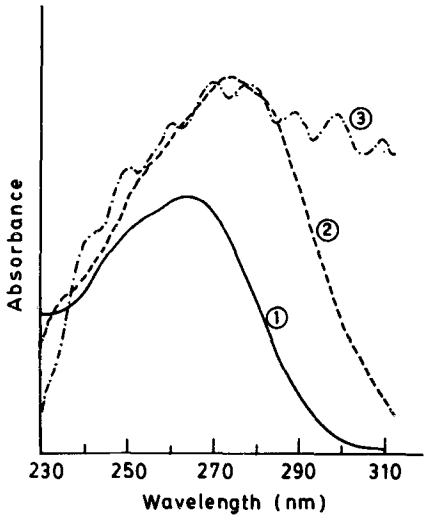

Figure 3. Electronic absorption spectra of an aqueous solution (third sample) of 8-azaguanine. The spectra from 1 to 3 were recorded on days (taken as 0, 8, and 10) at a fixed time of the day. The different spectra are shifted suitably along the absorbance axis with respect to one another for convenience of presentation. In spectra 1, 2 and 3, the absorbance scales used were 0 to 1, 0 to 0·1 and 0 to 0·02 respectively.

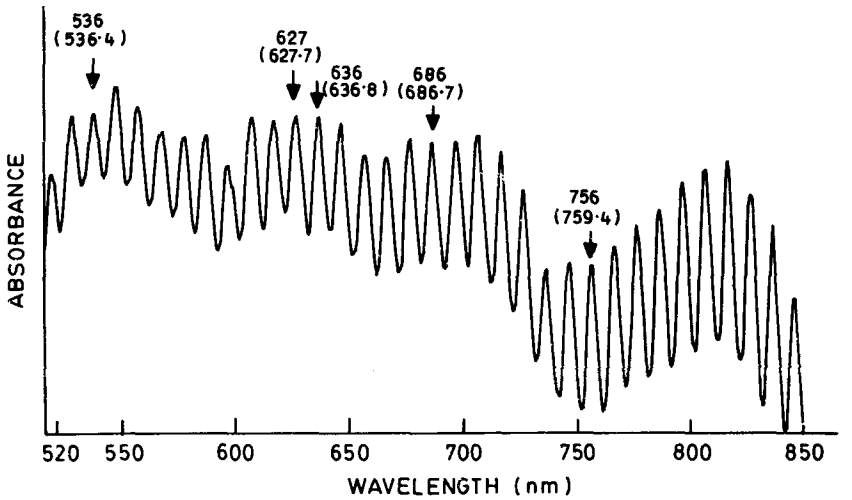

Figure 4. Absorption bands of the $^1\Sigma_g^+ - ^3\Sigma_g^-$ system of oxygen recorded for the third sample on day 10. The absorbance scale used was 0 to 0·02. The bands marked with arrows are the persistent ones. The wavelengths given for these bands are standard values, while the others were obtained by our measurement.

preparing the solution. It has a banded structure. The absorption spectrum of the sample recorded on this day in the region of wavelengths 520 to 850 nm is shown in figure 4. These bands belong to the $^1\Sigma_g^+ - ^3\Sigma_g^-$ system of oxygen (Pearse and Gaydon 1976) and have been earlier observed in association with guanine (Mishra 1988). The persistent bands of this system (Pearse and Gaydon 1976) are known to lie at 759·4,

686·7, 636·8, 627·7 and 536·4 nm. The corresponding bands in the present case were of wavelengths 756, 686, 636, 627 and 536 nm. The agreement between the two sets of wavelengths appears to be satisfactory. Appearance of the oxygen bands is clear evidence of the involvement of oxygen in controlling the behaviour of 8-azaguanine in solution. It may be noted that spectrum 3 of figure 3 cannot be obtained simply by passing excess oxygen into the solution. The main factor in this context is the extent of complexation between the dissolved oxygen and the solute though the amount of oxygen dissolved is important. The process of complexation occurs slowly but it can be accelerated by irradiation.

The above observations can be broadly explained as follows,

$$^1M + h\nu \rightarrow {}^1M^* \rightarrow {}^3M^{**} + {}^3M'^{**}, \tag{1}$$

$$^3M^{**} + {}^3O_2 \rightarrow {}^1M \cdot {}^1O_2^{**} \rightarrow {}^1M \cdot {}^3O_2 + {}^1M' \cdot {}^3O_2, \tag{2}$$

$$^1M \cdot {}^3O_2 + h\nu' \rightarrow {}^3M^{**} \cdot {}^1O_2^{**}. \tag{3}$$

Here M and M′ stand for the deeper and shallower minima of the ground or excited state potential surface of 8-azaguanine respectively. Generally, whatever change occurs to M, a corresponding change would also occur to M′ and vice versa. The absorption spectrum of oxygen shown in figure 4 arises according to (3) above. As explained below, the triplet state of 8-azaguanine involved in (1) to (3) above appears to be mainly the second triplet excited state of the molecule though the first triplet excited state is also involved to some extent and the corresponding steps can be easily added to the above set of processes.

The fluorescence spectra of 8-azaguanine obtained using the first sample are presented in figure 5. Details of these spectra are given in the corresponding caption. One can see that the flourescence peaks in these spectra lie near 400 nm in each case. An aqueous solution of guanine shows three fluorescence peaks (Santhosh and Mishra 1989), one each near 360, 400 and 450 nm. Solutions of guanine which give the bands of oxygen in absorption show strong fluorescence at 400 nm, otherwise, this fluorescence is generally weak; the fluorescence of guanine which is generally prominent (Santhosh and Mishra 1989) is the one having a peak around 360 nm. It appears logical that all the three arise from the same chemical species through appropriate energy transfer which exists in the ground state. A weak S_0 to T_1 absorption in the guanine–oxygen complex has also been observed recently (Mishra 1988). Therefore, the three fluorescence peaks in guanine have been explained (Santhosh and Mishra 1989) as originating from the excited states S_1, T_2 and T_1.

Transitions from T_2 and T_1 would be more or less allowed due to charge transfer interactions of the solute with the dissolved oxygen (Mishra 1988; Birks 1970). If this interaction leads to a weak complexation between the two species, say in accordance with the scheme given above, the allowedness would be enhanced. Therefore, the fluorescence near 400 nm in both guanine and 8-azaguanine can be assigned to the transition from the second triplet excited state (T_2) to the ground state of the solute. It does not appear likely that the excited states of guanine and 8-azaguanine are so differently placed with respect to the corresponding ground states that the transition of 8-azaguanine which would correspond to the 360 nm fluorescence of guanine arising from the $S_1 \rightarrow S_0$ transition, is shifted to 400 nm. It is surprising that the 400 nm fluorescence is so prominent in the case of 8-azaguanine and the one which would be expected to appear with a peak near 360 nm is not clearly seen in figure 5. It is, however,

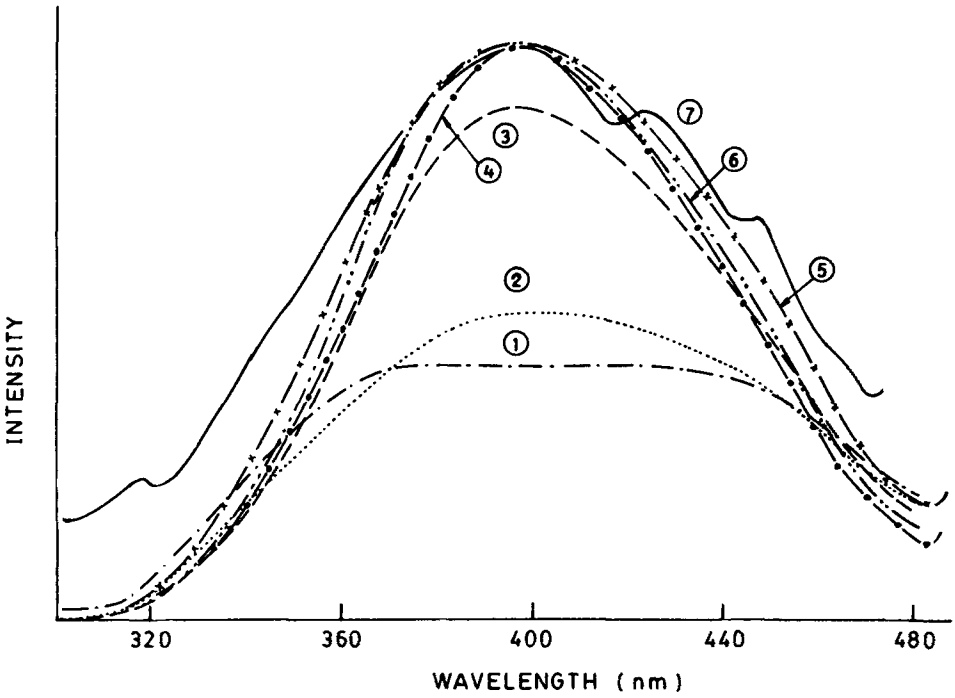

Figure 5. Fluorescence spectra of an aqueous solution of 8-azaguanine (first sample). Spectra 1, 2 and 3 were recorded on day 0 using exciting wavelengths 275, 260 and 248 nm respectively, and correspond to the absorption spectrum 1 of figure 1. The spectra 4 to 7 were recorded using the exciting wavelength 248 nm and correspond to the absorption spectra numbered 2 to 5 in figure 1, respectively.

noted that the fluorescence spectra in figure 5 do have appreciable intensity near 360 nm, though not a peak, and hence the 360 nm fluorescence of 8-azaguanine may be hidden in the envelope of the broad fluorescence having a peak at 400 nm. The 400 nm fluorescence in 8-azaguanine appears to be a consequence of step (3) given earlier, as follows

$$^3M^{**}\cdot{}^1O_2^{**} \rightarrow {}^1M\cdot{}^3O_2 + h\nu''.$$

Fluorescence spectra of 8-azaguanine (not presented) recorded using exciting radiations of wavelengths 280, 285, 290, 295, 300 and 305 nm were found to have a peak each centring around 400 nm and had the same appearance as the one obtained using the 275 nm exciting wavelength (figure 5). However, when the exciting wavelength was increased further, the peak of the resulting fluorescence got red-shifted to near 475 nm. Some of the fluorescence spectra shown in figure 5 also have a shoulder each near 450 nm and it appears that it is this shoulder which takes the form of a well-defined fluorescence when the exciting wavelength is increased to such an extent that the state from where the 400 nm fluorescence originates can no longer be populated consequent to absorption. Three fluorescence spectra obtained using the exciting wavelengths 310, 315 and 320 nm are shown in figure 6. Each of these spectra has a peak near 475 nm. It is likely that when the 310 nm or a higher wavelength exciting radiation is used, a weak absorption occurs from S_0 to higher vibrational levels in T_1 and it eventually causes the fluorescence from T_1 having a peak near 475 nm.

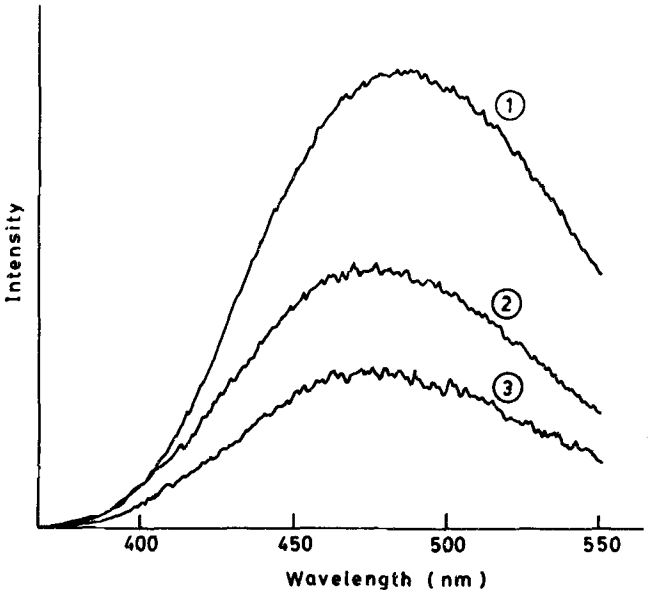

Figure 6. Fluorescence spectra 1, 2 and 3 of 8-azaguanine obtained using exciting wavelengths 310, 315 and 320 nm respectively.

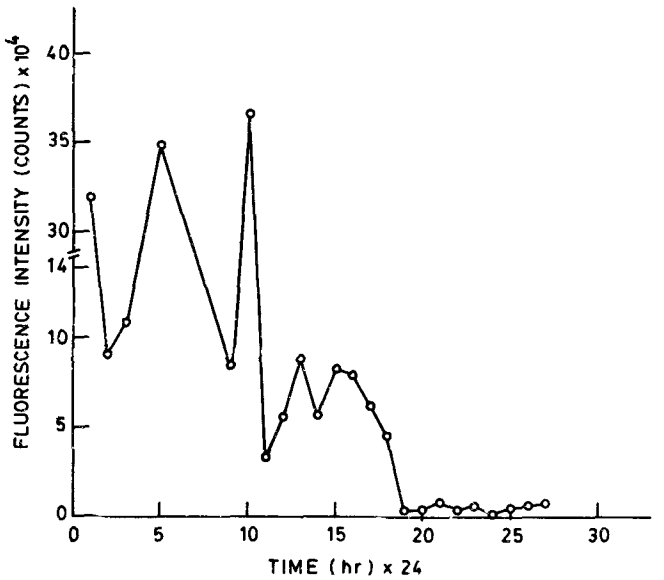

Figure 7. Variation of the intensity of fluorescence (photon counts) of 8-azaguanine at 400 nm with time (first sample). The exciting wavelength was 248 nm.

Variation of the fluorescence intensity of the first sample monitored at 400 nm as a function of time is presented in figure 7. We find that the fluorescence intensity fluctuates strongly for about 2 weeks and then falls drastically. We know that a chemical system undergoing a reaction may show oscillations (Sporns *et al* 1987). The fluctuations seen in figure 7 possibly arise due to the solute undergoing complexation slowly with the dissolved oxygen.

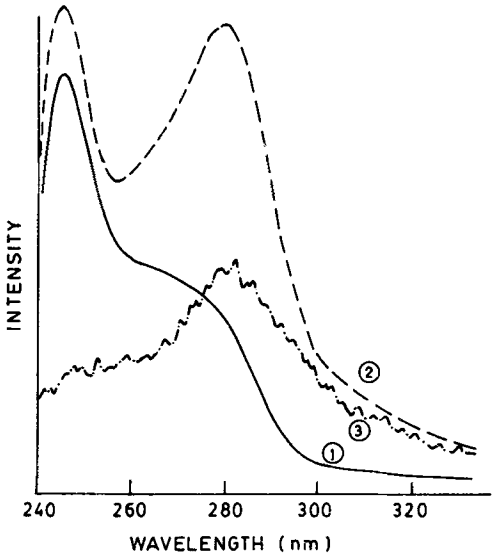

Figure 8. Excitation spectra of fluorescence of 8-azaguanine at 400 nm (first sample). The spectra 1 to 3 correspond to the absorption spectra 1, 2 and 5 of figure 1, respectively.

Excitation spectra of the 400 nm fluorescence of 8-azaguanine are presented in figure 8. The excitation spectra 1, 2 and 3 in this figure correspond to the absorption spectra 1, 2 and 5 in figure 1. We find that the corresponding excitation and absorption spectra have similar appearances. It supports the possibility that absorption from S_0 to S_1 may be followed by an intersystem crossing from S_1 to T_2 and the 400 nm fluorescence originates from T_2. Another possible origin of the 400 nm fluorescence could be given as an excimer of the solute, as has been suggested earlier for certain purine nucleotides (Callis 1983; Vigny and Ballini 1977). But this possibility appears to be remote as the species which gives this fluorescence appears to exist in the ground state also, as discussed above.

4. Conclusions

The following conclusions may be drawn from this study: (i) 8-azaguanine has double-well potential surfaces in its ground and excited states but the molecular geometries corresponding to the two minima on the potential surfaces may not be as different in this case as it is in guanine, (ii) 8-azaguanine is characterised by a much stronger intersystem crossing from S_1 to T_2 than is guanine, and (iii) the dissolved oxygen in a solution of 8-azaguanine strongly influences the behaviour of the solute, as happens in the case of guanine.

Acknowledgements

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