Interactions between Drugs and Nucleic Acids. Part 1. Dichroic Studies of Doxorubicin, Daunorubicin, and their Basic Chromophore, Quinizarin

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The interpretation of the electronic spectra of complexes between DNA and antitumour drugs must rely on the knowledge of the directions of the transition moments of the drug chromophore. This paper reports the linear and circular dichroism studies (including magnetic c.d.) and semiempirical INDO/S calculations carried out in order to provide evidence for the directions of the transition dipole moments within the quinizarin chromophore present in the antitumour drugs daunorubicin and doxorubicin. The c.d. technique also demonstrated that these two drugs, in spite of the complex substitution pattern of the anthraquinone moiety, have the same sequence of u.v.–visible electronic transitions as the unsubstituted quinizarin. The partial orientation of the investigated molecules required to run their linear dichroism spectra was achieved by using liquid crystalline solvents (both thermotropic and aqueous lyotropic) according the modulated liquid-crystal–linear dichroism technique.

Daunorubicin (**1**) and doxorubicin (**2**) are potent antitumour drugs. Their molecular structures are characterized by the presence of the amino sugar daunosamine residue, linked to an anthracycline chromophore. They are believed to inhibit both DNA replication and RNA transcription by intercalating their planar anthracycline chromophores between the base pairs.[1–5] Structural and configurational changes at the lyxose moiety of the drug can affect the intercalation process and the stability of the DNA–(**1**) or DNA–(**2**) complexes.[6] The binding interactions of (**1**) and (**2**) with nucleic acid have been studied extensively by various optical spectroscopic techniques, especially in the u.v.–visible range.[7–12] Other antitumour drugs having the same anthracycline chromophore have also been studied by the same techniques.[13]

Self-association of (**2**), which plays an important role in this context even at concentrations as low as 10^{-5}M, has been investigated in the same u.v.–visible range.[14–17]

Despite the large number of spectroscopic studies, however, many doubts still persist on the assignment of the bands, and in some cases strongly conflicting conclusions have been reached by different authors (*e.g.* refs. 7 and 13). Also geometries of the (**2**)–DNA intercalation have been suggested[8] on the basis of assignments which are not confirmed by the present study.

We report here a study based on polarization spectroscopic techniques [linear dichroism (l.d.), circular dichroism (c.d.), and magnetic circular dichroism (m.c.d.)] of the basic chromophore of these drugs: quinizarin (**5**). C.d. and l.d. spectra of several derivatives of (**1**) or (**2**) have been reported, indicating that the chromophore (**5**) is a good basis from which to interpret the electronic spectroscopy of these anthraquinone drugs. This study stems from the consideration that all optical spectroscopic investigations (in particular l.d. and c.d.) of the interaction of (**2**) or (**1**) with nucleic acid, or any other biological receptor, must rely on knowledge of the polarizations of their transitions. Stereochemical data available from dichroic studies are always expressed in terms of interaction and relative orientation of transition dipole moments. This information may be interpreted in terms of stereochemical relations of interacting groups or molecules only when the orientations and locations of the transition dipole moments within the single chromophoric framework are known.[18] The l.d. technique is the most direct experimental tool for providing assignments of electronic transitions for the single chromophores of interacting molecules.[19] The uniaxial orientation required to run the l.d. spectrum of the investigated molecules can be provided by oriented solvents, such as liquid crystals,[19–21] or stretched polymer films.[19,22]

In this work we ran l.d. spectra both in thermotropic and in lyotropic aqueous liquid crystals.[23,24] Use of the latter solvents makes it possible to run l.d. spectra even of biological active hydrophilic compounds. In fact their insolubility in thermotropic liquid crystals had heretofore precluded the use of these more effective orienting solvents.

The theoretical support necessary for interpreting the results obtained by the various dichroic techniques was provided by semiempirical CNDO/S[25] and INDO/S[26] calculations. These methods are widely recognised as useful and versatile in assigning the electronic transitions of organic molecules even of relatively large size.[27] The additional possibility of computing within the same framework the m.c.d. spectrum,[28] together with dichroic properties such as transition intensities and polarizations, makes these methods particularly suitable for the present work.

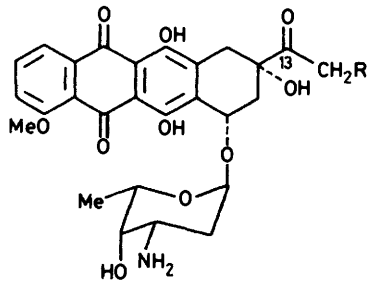
Experimental

Daunorubicin (**1**), doxorubicin (**2**) and its (13*R*)- or (13*S*)-13-hydroxy-13-deoxo-4-demethoxy derivative, and the aglycone (**4**) were obtained by following the procedures described in ref. 2.

The c.d. spectra were recorded with a Mark V Jobin Yvon dichrograph, provided with a Sylex data processing station. Six scans were accumlated for each spectrum and its corresponding baseline. Solutions of concentration *ca.* 3.5×10^{-5}M in methanol and chloroform were used in combination with cylindrical cells of fused silica with pathlength 1 cm.

U.v.–visible spectra were recorded with a Spectracomp 601 spectrophotometer (C. Erba), for the same solutions as used in the c.d. experiments. The aglycone (**4**) was also examined at twice the normal concentration in order to reveal weak absorption in the 320—400 nm range.

The m.c.d., l.d., and average absorption (a.a.) spectra were recorded with a JASCO-J500A dichrograph with l.d. attachment and a 12 kG electromagnet. The l.d. and a.a. spectra were



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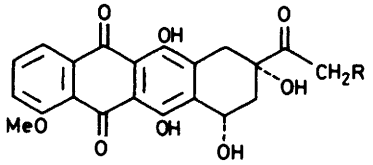
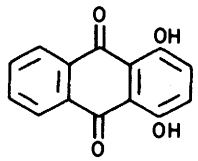
(2) R = OH



(3) R = H
(4) R = OH



(5)

evaluated by following the reduction techniques described in refs. 20 and 21.

The thermotropic liquid crystalline solvent (ZLI 1167; Merck) used to provide the linearly anisotropic orientation required for the l.d. measurements was used without any previous purification. Sample orientation was achieved by rubbing (with a piece of polystyrene foam) the cell inner surfaces previously coated by polyvinyl alcohol[20] or polyimide (ZLI-2650; Merck). The silica cell pathlength was 10—100 μm.

The procedure for preparation of oriented lyotropic solutions is much longer and more delicate; for details see the experimental section of ref. 24.

In the calculations the input geometry of the chromophore (**5**) was taken from the crystallographic co-ordinates reported in ref. 29. Calculations were also performed by modifying the geometry of the carbonyl and hydroxy groups in order to emphasize the role of the hydrogen bonds in the excited state.[30] CNDO calculations[25] were performed by adopting the Mataga-Nishimoto approximation for the Coulomb integrals and a CI limited to 81 monoexcited configurations. The same size of CI was used for INDO/S calculations with the original parametrization proposed by Zerner.[26] In view of the difficulties in interpreting results obtained by using the original parametrization but different sizes of CI,[31] and in view of the small changes observed on introducing doubly excited configurations, in the case of anthracene[32] no multiple excitations were taken into account.

Results and Discussion

All the spectra (c.d., m.c.d., l.d.) were recorded at low solute concentrations such that association processes were minimized.

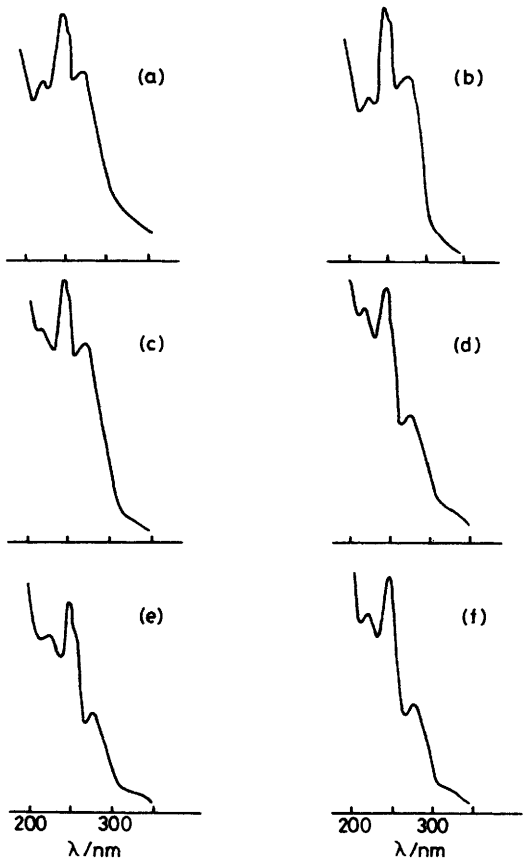


Figure 1. Profiles of the u.v. spectra of quinizarin (**5**) at various concentrations in methanol: (a) 3.4×10^{-4}, (b) 4.5×10^{-4}, (c) 9.5×10^{-4}, (d) 1.1×10^{-3}, (e) 3.7×10^{-3}, (f) 7.5×10^{-3}M

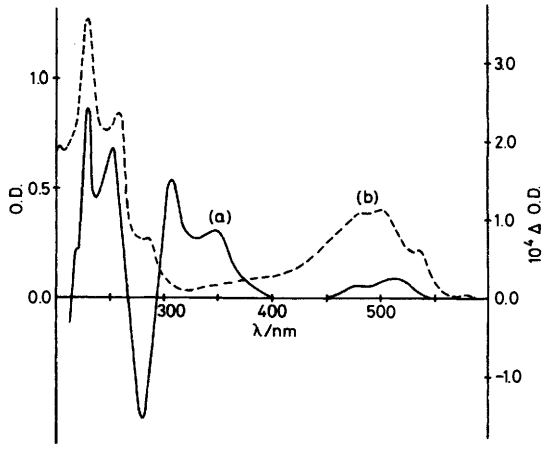


Figure 2. (a) Circular dichroism and (b) absorption spectra of doxorubicin (**2**) and daunorubicin (**1**) in methanol

Like the investigated drugs,[14–17] quinizarin (**5**) is expected to have a tendency to self-associate. The band at 290 nm in its electronic spectrum is particularly sensitive to association processes. Figure 1 shows the gradual decrease of relative intensity of this band in the u.v. profiles of methanolic solutions of (**5**) at concentrations larger than 5×10^{-4}M.

C.d. Spectra of Daunorubicin (**1**), *Doxorubicin* (**2**), *and their Aglycones.*—The spectra (solvent methanol) are given in Figure

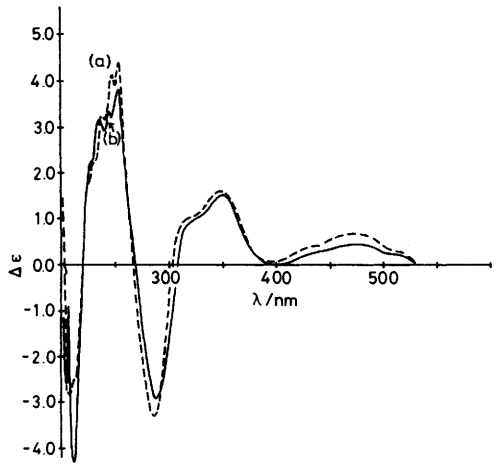


Figure 3. Circular dichroism spectra of (a) 4-demethoxydoxorubicin and (b) (13*R*)- or (13*S*)-13-hydroxy-13-deoxo-4-demethoxydoxorubicin in methanol

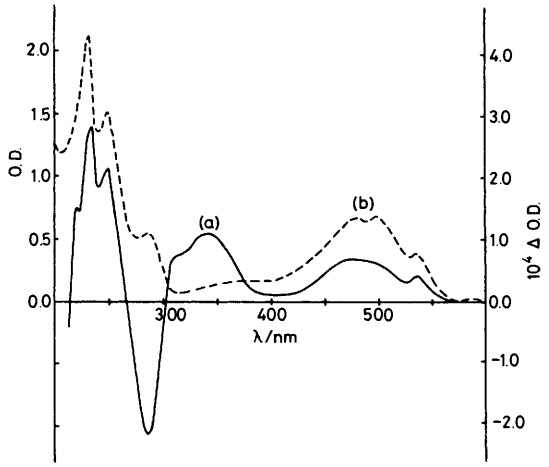


Figure 4. (a) Circular dichroism and (b) absorption spectra of the aglycone (**4**) in methanol

2. The shape is the same for both molecules and changes very little either when the 4-methoxy group is removed (Figure 3) or when the carbonyl chromophore at C-13 is reduced. Furthermore it is striking that the configuration at C-13 does not affect the spectra (Figure 3).

Removal of the daunosamine sugar moiety also does not cause any dramatic spectroscopic change (Figure 4), modifying only the relative intensities of the bands at 305 and 350 nm. This modification may be a useful indicator of hydrolysis, which is a possible degradation pathway for (**1**) or (**2**).

All these c.d. spectra reveal that the pattern of electronic transitions remains basically unchanged for all these derivatives, which are closer to the 'naked' chromophore (**5**). Our decision to tackle the problem on the basis of the assignment of the u.v.–visible transitions of (**5**) is thus strongly supported.

L.d. Spectra of Quinizarin (**5**).—A sample is able to exhibit l.d. only if it has been previously oriented so as to become linearly anisotropic. The l.d. is usually defined as the differential absorption $[E_{\parallel}(\lambda) - E_{\perp}(\lambda)]$ of two plane-polarized components of an electromagnetic radiation, where parallel ($\parallel$) and perpendicular ($\perp$) refer to the optical axis, or director, of the oriented sample. $[E_{\parallel}(\lambda) + E_{\perp}(\lambda)]/2$ is the a.a. of the same oriented sample. L.d. and a.a. spectra may be recorded directly and simultaneously by modulated techniques,[20,21] *e.g.* by using a dichrograph which may be switched from one measurement to the other without touching the sample. This technique can ensure a sensitivity at least two orders of magnitude higher than that provided by the more common static methods.[19,22]

For purely *u*-polarized bands, equation (1) applies, where β

$$\frac{[E_{\parallel}(\lambda) - E_{\perp}(\lambda)]}{[E_{\parallel}(\lambda) + E_{\perp}(\lambda)]} = \frac{3S_{uu}}{2 + S_{uu}} \text{ (where } S_{uu} = \tfrac{1}{2}\langle 3\cos^2\beta - 1\rangle \text{ and } u = x, y, z) \quad (1)$$

stands for the deflections from the sample director of the transition moment *u* directions of all the absorbing guest molecules. The shape and signs of an l.d. spectrum are determined by the distribution of these deflections. The S_{uu} function, averaged ($\langle \cdots \rangle$) over all the transition moment orientations, is an order parameter with values of zero for random orientation or unity for perfect alignment. If the polarization of the investigated transitions is known, information about guest molecule orientation may be obtained and expressed in terms of S_{uu} values. On the other hand, if the molecular orientation inside the sample is known, or may be guessed, the directions of the transition moments within the molecular frame are easily obtained. The latter approach is that used in this investigation.

The l.d. spectrum of (**5**) in thermotropic ZLI-1167 liquid crystal is shown in Figure 5(b); its a.a. spectrum is shown in Figure 5(a). The C_{2v} symmetry of (**5**) greatly simplifies the task of assigning its transitions and makes it possible to apply reduction procedures to the l.d. spectra in order to provide the purely *u*-polarized absorption components $A_u(\lambda)$[19–21] [see Figure 5(c)]. This is because the C_{2v} symmetry sets the *i* directions of the electric transition moments along the *u* axes which define the orientation of the molecule with respect to the director of the sample through diagonal tensor elements S_{uu}.

The preferred orientation of (**5**) within the oriented liquid crystalline thermotropic solvent may be safely guessed on the basis of its lath-like shape or on the basis of measurements made by other techniques, *e.g.* dynamic n.m.r.,[33] capable of providing S_{uu} values.

The tendency of (**5**) to self-associate may cause serious problems when orientational S_{uu} data are quantitatively transferred from one technique to another. Different sensitivities may in fact require very different solute concentrations in the solutions used for the two techniques. The dynamic n.m.r. data of ref. 33, even if used with prudence for this reason, in any case support the reasonably safe guess on the orientational properties of (**5**) which may be made on the basis of its molecular shape: S_{zz} and $(S_{yy} - S_{xx})$ are expected to be positive while both S_{yy} and S_{xx} are negative. Positive l.d. signals are hence due to *z*-polarized transitions only. Thus where overlapping of differently polarized components occurs, the shape of the l.d. must be different from that of the a.a. spectrum.

When only two components overlap and contribute to the l.d. at the same wavelength, they may be resolved by reduction procedures.[19–21] A further complication in (**5**) may in principle occur from the presence of out-of-plane transitions which can overlap with other, in-plane-polarized transitions, rendering these reduction procedures inapplicable. MO calculations were particularly useful in dealing with this problem and rule out this possibility. The results of INDO/S[26] and CNDO/S[25] calculations (Table) show that in the spectral range of interest (from 550 down to 200 nm) no $n\pi^*$ out-of-plane-polarized

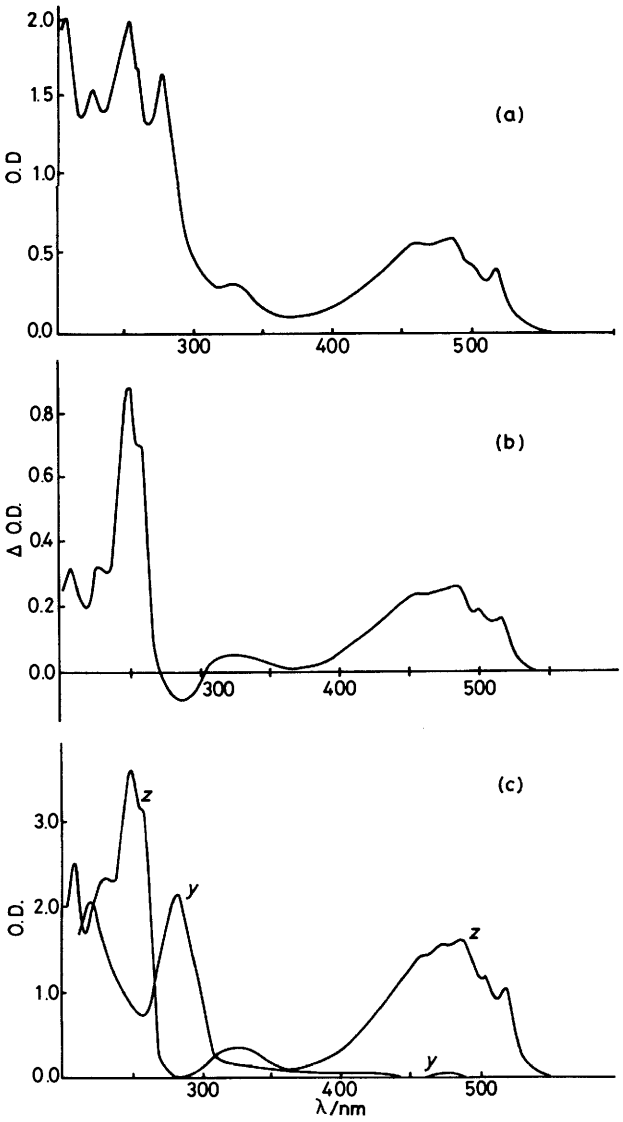


Figure 5. (a) Average absorption, (b) linear dichroism, and (c) absorption components along the molecular *y* and *z* axes of (**5**) oriented by a thermotropic liquid crystalline solvent (OS-35)

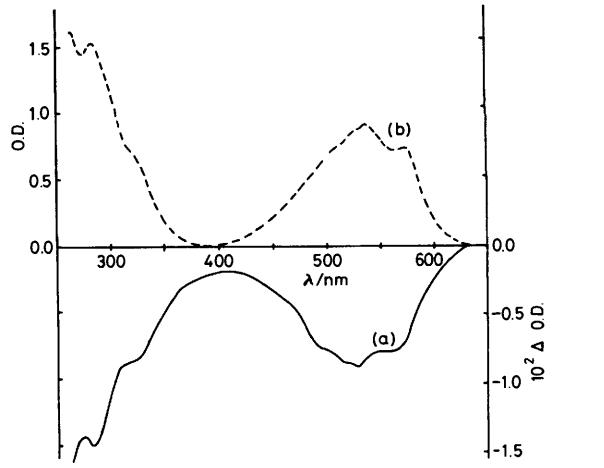


Figure 6. (a) Linear dichroism and (b) average absorption of (**5**) oriented by an aqueous lyotropic liquid crystalline solvent

Table. Transition energies (nm), oscillator strengths (f), and m.c.d. *B* terms ($\times 10^5$ in D^2 cm^{-1}) calculated by (A) CNDO/S, (B) INDO/S, (C) INDO/S with modified geometry (see text)

	(A)		(B)		(C)		
	E	f	*E*	f	*E*	f	−*B*
S_1	459	0.0	459	0.0	523	0.0	0.0
S_2	427	0.003(*x*)	439	0.0	514	0.001(*y*)	−0.021
S_3	313	0.332(*z*)	414	0.003(*y*)	501	0.0	0.0
S_4	331	0.12(*y*)	368	0.233(*z*)	409	0.302(*z*)	36.7
S_5	273	0.009(*z*)	291	0.546(*y*)	319	0.665(*y*)	56.8
S_6	275	0.0	279	0.056(*z*)	306	0.085(*z*)	−45.8
S_7	264	0.006(*x*)	249	0.141(*z*)	264	0.228(*z*)	−23.0
S_8	255	0.077(*y*)	233	0.274(*y*)	238	0.805(*z*)	−644.5
S_9	223	0.001(*x*)	226	0.876(*z*)	236	0.072(*y*)	401.9
S_{10}	219	0.312(*z*)	223	0.075(*y*)	233	0.131(*y*)	184.4
S_{11}	215	0.069(*x*)	213	0.062(*y*)	224	0.233(*y*)	70.2

transitions of sizeable oscillator strength appear. Therefore the l.d. spectrum can be safely examined in terms of in-plane transitions, and a decomposition in terms of the *z* and *y* components appears pertinent to this case.

The reduced spectrum thus obtained [Figure 5(c)] may be divided into three regions: (*a*) a region is dominated by a long-axis-polarized transition between 550 and 380 nm; (*b*) an intermediate region which extends down to 265 nm and shows a *y*-polarized absorption, having a maximum at 290 nm, and a long tail which extends below a *z*-polarized band centred at 325 nm; and (*c*) a region between 265 and 200 nm of strongly allowed transitions with overlapping contributions polarized along the short or long molecular axis.

The INDO/S theoretical results (Table) show a much better agreement with those experimental observations than do results from CNDO/S. The calculated energy location of the lowest-energy *z*-polarized transition is too high. This band has been described as localized on the pseudo-ring parts of the molecule, including the hydroxy and carbonyl groups, with large participation of charge-transfer configurations.[30,34] The poor reproduction of the energy of this band has to be ascribed to the method of calculation, which is aimed at calculating bonded interactions only. In fact, modifications of the geometry of the chromophore (*i.e.* by elongating the C=O and O–H bonds to 1.32 and 1.1 Å, respectively) in order to give a better description of the strong intramolecular hydrogen bonding affords much better results for the energy of this *z*-polarized transition [Table, column (C)].

Two forbidden states (S_1 and S_3) and one (S_2) weak *y*-polarized transition are calculated by the INDO/S method to occur in the region 500—550 nm; the latter can probably be identified with the very small *y*-polarized band at about 470 nm [Figure 5(c)]. It should be noted that, despite the high sensitivity of the experimental technique, the subsequent mathematical manipulations of the l.d. and a.a. spectra in the reduction procedures make the identification of such a weak absorption component very tentative. The decomposition procedure also shows a very weak *y* component with a sigmoid shape below the absorption peak at about 520 nm. This is not shown in Figure 5(c) because of its weakness, and also because it is presumably due to solvent effects only.[35]

The relative intensities of the two bands of region (b) can be well predicted, whereas their sequence in the energy scale is, by contrast, inverted.

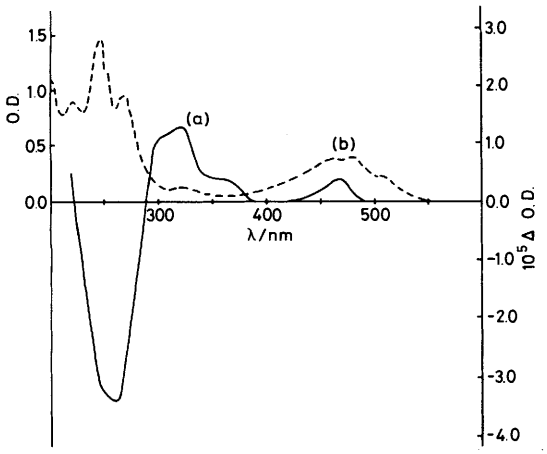


Figure 7. (a) Magnetic circular dichroism and (b) absorption spectra of (**5**) in methanol (H = 13.5 kG)

The sequences of the strongly allowed y- and z-polarized transitions of region (c) are successfully reproduced by the INDO/S calculations.

The l.d. spectrum of (**5**) in lyotropic liquid crystals is given in Figure 6. The components of lyotropic nematic liquid crystals are weakly anisometric micelles. The diamagnetic anisotropy and orientational correlation of these anisometric micelles make possible their macroscopic orientation by a magnetic field. The macroscopic linear anisotropy thus achieved in micellar solutions allows the l.d. of hydrophilic, or even hydrophobic, guest molecules to be recorded. Within their solubilization sites, they are forced by orientation-correlated environments to assume anisotropic orientational distributions. A model of solubilization of guest molecules within the relative framework of l.d. spectra interpretation was recently developed by one of us for these anisometric micelles.[23]

On passing from the thermotropic to the lyotropic solvent the l.d. spectrum changes drastically, reflecting a different orientational distribution for the same solute molecules. This is particularly useful in assignment studies. In the l.d. spectra of (**5**) in lyotropic systems, instead of oppositely signed signals for z- and y-polarized transitions negative contributions are observed for both polarizations. Therefore, the traceless condition of the S_{uu} matrix requires S_{xx} to be positive and greater in its absolute value than both S_{yy} and S_{zz}, in fact $S_{xx} = -(S_{yy} + S_{zz})$. This is a very favourable condition for detecting x-polarizations. They should be revealed by l.d. contributions, which must be positive within an overall negative spectrum and comparatively large relative to the isotropic absorption intensity. This is another example of the flexibility of liquid crystalline solvents in studies of polarization assignments: disc-like or rod-like orientations of the same chromophore may sometimes be obtained simply by changing the mesomorphic solvent without need for the more complicated 'substitution approach'.[20,21]

The strong similarity of the a.a. and l.d. spectra, besides ruling out x-polarized transitions of sizeable intensity, reveals that (**5**) preferentially assumes a disc-like radial intercalation mode within the host micelle. This preferential orientation (labelled mode C in ref. 24) is obtained because the structural anisotropy of the solubilization sites is able to discriminate only between the orientations of the out-of-plane and the in-plane axes. The in-plane y and z directions of (**5**) are not discriminated, thus giving $S_{yy} = S_{zz}$. Their very small experimental value ($S_{zz} = S_{yy} = -0.02$) is due to the almost spherical shape of the host micelles.[24]

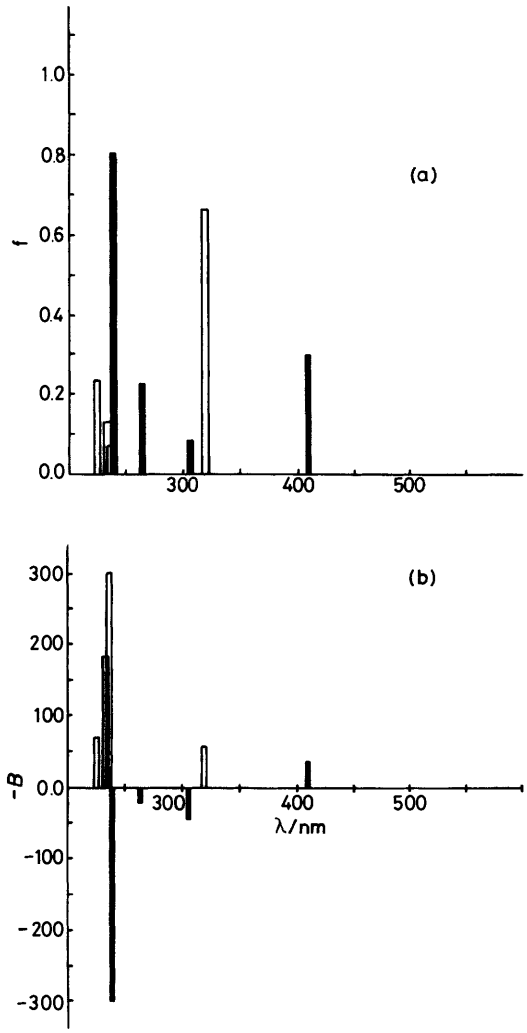


Figure 8. Calculated (a) INDO/S oscillator strength and (b) B terms [see Table, column (C)] for (**5**) (full bars for z-polarized transitions and blank bars for y-polarized)

M.c.d. Spectrum of Quinizarin (**5**).—In the absence of degeneracy of ground or excited states the contribution of the jth transition to m.c.d. of a molecule can be written as equation (2), wherein H is the magnetic field along the direction of

$$\Delta\varepsilon = \gamma\beta HB(j \longleftarrow a)\mathrm{f}(\nu) \qquad (2)$$

propagation of the light, γ a proportionality factor, β the Bohr magneton, ν the absorption frequency, and f(ν) a lineshape function. The $B(j \longleftarrow a)$ term, relative to a transition between the ground state a and the jth excited state, can be written in the form of three contributions[36] [equations (4)—(6)], wherein $\hat{\mu}$ and $\hat{m}$ are the magnetic and electric dipole moment operators,

$$B(j \longleftarrow a) = \sum_{k \neq a,j} B_{jk} + \sum_{k \neq a,j} B_{ka} + B_{ja} \qquad (3)$$

$$B_{jk} = Im\langle j|\hat{\mu}|k\rangle\cdot\langle a|\hat{m}|j\rangle \times \langle k|\hat{m}|a\rangle/(E_k - E_j) \qquad (4)$$

$$B_{ka} = Im\langle k|\hat{\mu}|a\rangle\cdot\langle a|\hat{m}|j\rangle \times \langle j|\hat{m}|k\rangle/(E_k - E_a) \qquad (5)$$

$$B_{ja} = Im\langle j|\hat{\mu}|a\rangle\cdot\langle a|\hat{m}|j\rangle \times (\langle j|\hat{m}|j\rangle - \langle a|\hat{m}|a\rangle)/(E_j - E_a) \qquad (6)$$

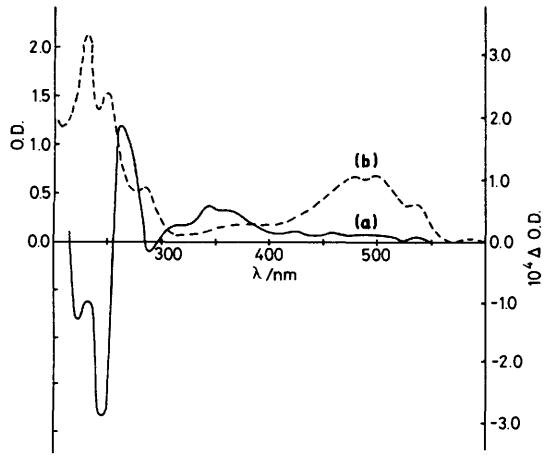


Figure 9. (a) Magnetic circular dichroism and (b) absorption spectra of (**2**) in methanol (H = 13.5 kG)

E_j is the energy of the jth state, B_{jk} represents the magnetic mixing of the electronic excited states j and k, and so forth for the other terms. Calculation details of these molecular constants are reported in ref. 28 and 37. The calculated B terms (Table, last column) show reasonably good agreement with both signs and intensity of the m.c.d. spectra of (**5**) (Figure 7).

The band at lowest energy has a low m.c.d. intensity, in spite of being strongly allowed in the isotropic absorption. This can be explained by the unfavourable mixing of the first absorbing state with the higher energy states, giving rise to small B_{jk} terms. Generally the latter are found to give the most relevant contributions to the final $B(j \longleftarrow a)$ term. However, the observed small m.c.d. intensity may be also due to negative contribution of the underlying y-polarized band detected in this region by l.d. and suggested by INDO/S results (see column 3 of Table).

The positive dichroism around 320 nm must be assigned to the z-polarized band of region (b). The m.c.d. computations forecast a negative sign, on the other hand, for this S_0—S_6 transition. This sign mismatch may just be a consequence of the inversion in energy between this transition and S_0—S_5. The broad negative band at higher energy arises from the S_0—S_5 transition and the contributions of the following z-polarized bands. The inversion of sign at 220 nm is well predicted by the subsequent negative and strong B terms of the S_0—S_9 and S_0—S_{10} transitions. All the calculated B terms are given in Figure 8(b); Figure 8(a) shows the relative calculated oscillator strengths. The m.c.d. shoulder at 360 nm, in correspondance with an absorption minimum, may be considered a manifestation of a weakly allowed or vibronically induced transition enhanced in m.c.d. by a large magnetic moment [term B_{ka} of equation (5)]. This magnetic transition and the z-polarized one, which is revealed by the l.d.s, may be responsible for the double-humped shape shown in this region by the circular dichroism spectra of all the investigated compounds (Figures 2—4 and also Figure 9). The INDO/S description of the states is fairly effective for regions (a) and (c) but is poor and sketchy for region (b).

The long tail which is present in the absorption components of (**5**) from 300 to 380 nm [see Figure 5(c)], and is not z-polarized, could be a strong indication that other transitions, electrically almost forbidden, may be present there. They may borrow intensity in c.d. and m.c.d. by mixing with the strongly allowed states nearby, available at lower and higher energy.

Thus the m.c.d. spectrum of (**5**) basically confirms the conclusions already reached on the basis of the l.d. spectra, but also reveals that the description of the (b) region must further include at least one weakly electrically allowed transition which can be shown only by circular dichroism experiments. This is fully confirmed also by the m.c.d. spectrum of (**2**) (Figure 9). The shapes of the m.c.d. spectra of (**5**) and (**2**) are reasonably close to each other, further evidence that (**5**) is a simplified, but also very effective, model of the chromophore in structures (**1**) and (**2**). The m.c.d. spectrum of (**2**) was obtained by subtracting[38,39] the c.d. spectrum recorded with the magnetic field switched off from the c.d. spectrum obtained when the field is on.

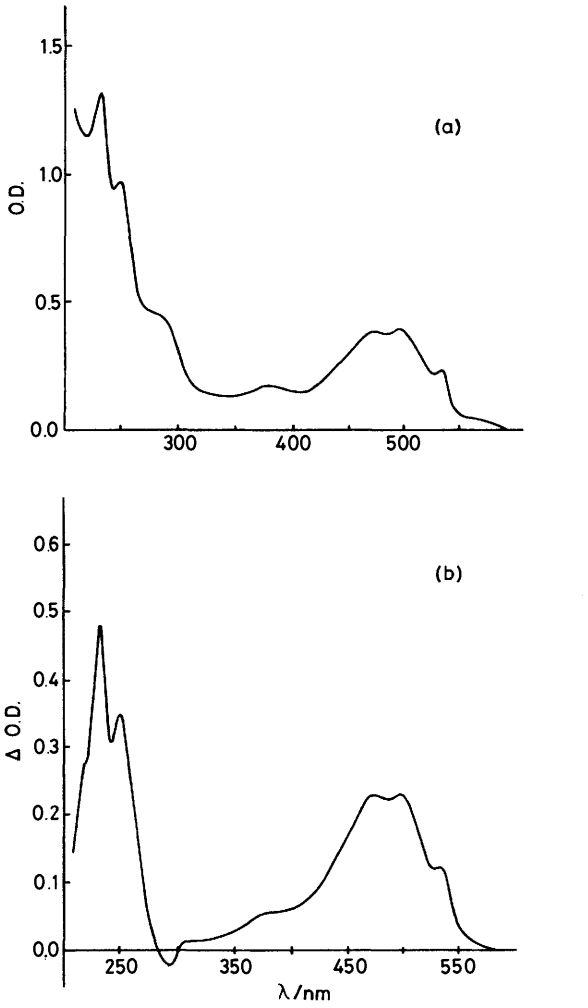


Figure 10. (a) Average absorption and (b) linear dichroism of the aglycone (**4**) oriented by a thermotropic liquid crystalline solvent (OS-35)

L.d. Spectra of the Aglycones.—In Figures 10 and 11 are the a.a. and l.d. spectra of the aglycone (**4**).

The symmetry of the aglycones is lower than that of (**5**). Therefore their transition dipole moments may lie out of the $u = x, y, z$ axes of the orientational system. These deflections of the transition moments, together with the more anisometric shape of the aglycones, may cause differences between the l.d.s of these compounds and those of (**5**). We found that the differences are small when a thermotropic liquid crystal is used as the orienting solvent. The spectral regions (a) 550—380 nm and (c) 265—200 nm of the l.d. in Figure 10(b) are in fact dominated, as in the case of (**5**) [Figure 5(b)], by the polarization components

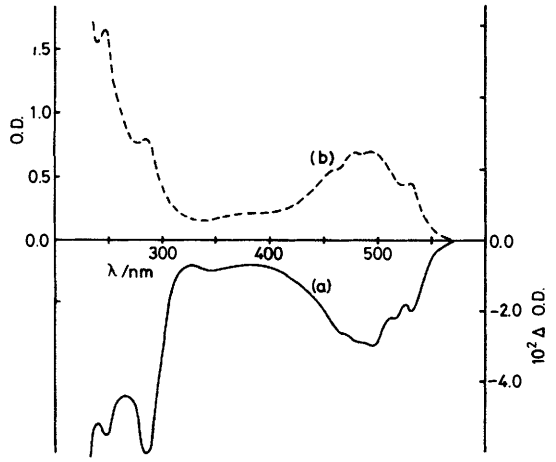


Figure 11. (a) Linear dichroism and (b) average absorption of the aglycone (**4**) oriented by an aqueous lyotropic liquid crystalline solvent

along the long molecular axis. The band at about 290 nm has a negative l.d. here too.

The differences between the l.d. spectra of (**5**) (Figure 6) and the aglycones (Figure 11) in lyotropic liquid crystals are much larger and are particularly interesting. Owing to the more anisometric shape of the aglycones, and possibly to specific interactions within the micellar host [24] as well, the solubilization sites can in this case discriminate between directions in the molecular plane. In fact, the band at 290 is greatly amplified in the l.d. spectrum with respect to the other in-plane polarization. In this case again, the l.d. sign of out-of-plane transitions must be opposite to that in the in-plane polarizations. This solvent therefore provides these aglycones with the best conditions for detecting overlappings of different polarizations. The differences in the shapes of the a.a. and l.d. spectra (Figure 11) clearly display all the strongly allowed long- and short-axis polarizations already identified and assigned in the (**5**) chromophore, but also reveal and confirm the previous yet rather tentative suggestion of two very weak transitions at about 460 and 360 nm. The first is the transition which was detected by the absorption components in Figure 5(c) and also computed for (**5**) as S_0—S_2, y-polarized, in the region of the much more intense z-polarized band. The second is the transition suggested by all the circular dichroism spectra. Their l.d. signals, negative and positive for the first and the second respectively, may thus explain the increasing intensity of the shoulder at about 460 nm and the minimum at about 380 nm, which corresponds to a weak a.a. band at the same energy.

Conclusions

Quinizarin (**5**) provided a good model chromophore for studying the electronic transitions for the drugs (**1**) and (**2**). Semiempirical INDO/S calculations and m.c.d. and l.d. spectra of (**5**) provided reasonable assignments and polarizations of all the strongly allowed transitions displayed by its u.v.–visible spectrum. This information has been transferred to structures (**1**) and (**2**) and their aglycones on the basis of their dichroism spectra.

Two very weakly allowed transitions at about 360 and 460 nm were also suggested by both spectral and computational results. Their detection and assignment must still be considered rather tentative, although this uncertainty does not really affect the substantial purpose of this study, *i.e.* to achieve a reliable basis for further interpretation of l.d. spectra of (**1**) and (**2**) complexes with DNA. Stereochemical interpretations of these l.d. spectra are mainly based on the signals of the well allowed, guest transitions which can strongly interact by means of their large electric dipole moments with the host charge distributions.

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References

1 F. Arcamone, E. Arlandini, M. Menozzi, L. Valentini, and E. Vannini, in 'Cancer Therapy,' ed. F. M. Muggia, C. W. Young, and S. K. Carter, Martins Nijhoff, The Hague/Boston/London, 1982, p. 74.
2 F. Arcamone, Medicinal Chemistry Series, vol. 17, Academic Press, New York, 1981.
3 E. Calendi, A. Di Marco, M. Reggiani, M. B. Scarpinato, and L. Valentini, *Biochim. Biophys. Acta*, 1965, **103**, 25.
4 F. Zunino, R. Gambetta, A. Di Marco, and A. Zaccara, *Biochim. Biophys. Acta*, 1980, **607**, 206.
5 J. B. Chaires, N. Dattagupta, and D. M. Crothers, *Biochemistry*, 1983, **22**, 284.
6 T. W. Plumbridge and J. R. Brown, *Biochim. Biophys. Acta*, 1979, **563**, 181.
7 E. J. Gabbay, D. Grier, R. E. Fingerle, R. Reimer, R. Levy, S. W. Pearce, and W. D. Wilson, *Biochemistry*, 1976, **15**, 2062.
8 H. Fritzsche, H. Triebel, J. B. Chaires, N. Dattagupta, and D. M. Crothers, *Biochemistry*, 1982, **21**, 3940.
9 M. Manfait, A. J. P. Alix, P. Jeannesson, J. C. Jardillier, and T. Theophanides *Nucleic Acid Res.*, 1982, **10**, 3803.
10 M. Manfait, L. Bernard, and T. Theophanides, *J. Raman Spectrosc.*, 1981, **11**, 68.
11 F. Quadrifoglio, A. Ciana, G. Manzini, A. Zaccara, and F. Zunino, *Int. J. Biol. Macromol.*, 1982, **4**, 413.
12 L. Angeloni, G. Smulevich, and M. P. Marzocchi, *Spectrochim. Acta, Part A*, 1982, **38**, 213.
13 M. J. Bell, G. W. Buchanan, B. R. Hollesbone, and E. D. Jones, *Can. J. Chem.*, 1982, **60**, 291.
14 V. Barthelemy-Clavey, J. C. Maurizot, J. L. Dimicoli, and P. Sicard, *FEBS Lett.*, 1974, **46**, 5.
15 J. B. Chaires, N. Dattagupta, and D. M. Crothers, *Biochemistry*, 1982, **21**, 3927.
16 I. J. McLennan, R. E. Lenninski, and Y. Yanuka, *Can. J. Chem.*, 1985, **63**, 1233.
17 M. Menozzi, L. Valentini, E. Vannini, and F. Arcamone, *J. Pharm. Sci.*, 1984, **73**, 766.
18 I. Tinoco, *Adv. Chem. Phys.*, 1963, **4**, 113.
19 J. Michl and E. W. Thulstrup, 'Spectroscopy with Polarized Light. Solute Alignment by Photoselection in Liquid Crystals, Polymers, and Membranes,' Vch., Deerfield Beach, 1986.
20 B. Samori, P. Mariani, and G. P. Spada, *J. Chem. Soc., Perkin Trans. 2*, 1982, 447.
21 B. Samori, *Mol. Cryst. Liq. Cryst.*, 1983, **98**, 385.
22 E. W. Thulstrup and J. Michl, *J. Am. Chem. Soc.*, 1982, **104**, 5594.
23 L. B. A. Johansson, O. Söderman, K. Fontell, and G. Lindblom, *J. Phys. Chem.*, 1981, **85**, 3694.
24 B. Samori and F. Mattivi, *J. Am. Chem. Soc.*, 1986, **108**, 1679; M. Laurent and B. Samori, *ibid.*, in press.
25 J. Del Bene and H. H. Jaffé, *J. Chem. Phys.*, 1968, **48**, 1807.
26 J. E. Ridley and M. C. Zerner, *J. Mol. Spectrosc.*, 1974, **50**, 457.
27 R. L. Ellis and H. H. Jaffé, in 'Semiempirical Methods of Electronic Structure Calculations,' ed. G. A. Segal, Plenum Press, 1977, ch. 2, p. 49.
28 G. Marconi and J. Houben, *J. Chem. Soc., Faraday Trans. 2*, 1985, **81**, 975.

29 R. Angiuli, E. Foresti, L. Riva di Sanseverino, N. W. Isaacs, O. Kennard, W. D. S. Motherwell, D. L. Wampher, and F. Arcamone, *Nature New Biol.*, 1971, **234**, 78.
30 G. Smulevich, A. Amirav, U. Even, and J. Jortner, *Chem. Phys.*, 1982, **73**, 1.
31 A. P. Volosov and V. A. Zubkov, *Theor. Chim. Acta*, 1977, **44**, 375.
32 J. Michl, E. W. Thulstrup, and J. H. Eggers, *Ber. Bunsenges. Phys. Chem.*, 1974, **78**, 575.
33 J. W. Emsley, G. R. Luckhurst, and B. A. Timini, *Chem. Phys. Lett.*, 1985, **114**, 19.
34 T. P. Carter, G. D. Gillespie, and M. A. Connally, *J. Phys. Chem.*, 1982, **86**, 192.
35 F. W. Langkiled, E. W. Thulstrup, and J. Michl, *J. Chem. Phys.*, 1983, **78**, 3372.
36 A. D. Buckingham and P. J. Stephens, *Annu. Rev. Phys. Chem.*, 1966, **17**, 399.
37 J. H. Obbink, J. H. Hezemans, and A. M. F. Hezemans, *Theor. Chim. Acta*, 1976, **43**, 75.
38 G. Wagniere, *Chem. Phys. Lett.*, 1984, **110**, 546.
39 L. D. Barron and J. Vrbancich, *Mol. Phys.*, 1984, **51**, 715.

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