Circular Dichroism of Rifamycin Antibiotics. 2.[1] Circular Dichroism and Stereochemistry in Solution of 8-*O*-Methylrifamycin SV

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Abstract: The circular dichroism spectrum of the rifamycin derivative 8-*O*-methylrifamycin SV has been recorded between 600 and 190 nm: an intense exciton-like couplet is present between 250 and 190 nm. This feature can be fit in an excellent way by means of all-order polarizability calculations, using the structure found in the solid state for rifamycin B *p*-iodoanilide. The stereochemistry in solution of the present compound is then discussed on the basis of this result.

Rifamycins constitute a well-known class of natural and semisynthetic antibiotics that have acquired a great importance in therapy. Their structure has been investigated in the past years by means of chemical, spectroscopic, and X-ray methods.[2] In particular, recent papers by Vaciago et al.[3,4] attempted to provide a relationship between the biological activity of rifamycin derivatives and the molecular conformation in solution, determined by NMR spectroscopy. Only recently a circular dichroism (CD) approach to the solution structure of a molecule of this class has been described:[1] the CD spectrum of rifamycin S has been recorded and an analysis of the main spectral features carried out by means of all-order coupled oscillator calculations.[5] A definitive structure in solution for this molecule has not been obtained, because a satisfactory fitting of the experimental data has been obtained for two different conformations.[1]

With the aim to unambiguously correlate CD features with the structure in solution, the CD spectrum of 8-*O*-methylrifamycin SV, **1**, has been investigated, and the results are discussed in the present paper. This molecule (Figure 1) has been chosen for several reasons: (i) it possesses a hydroquinone structure, i.e., the same structure of the antibiotic rifampicin used in therapy; (ii) the UV and the CD spectral dependence on the pH of the solution is avoided because the acidic proton in the 8-position has been substituted by a methyl group, and (iii) the CD is very strong below 300 nm with evident exciton-like features,[6] suggesting that dynamic contributions should dominate the CD spectrum in this range.

Results and Discussion

a. Absorption and CD Spectra. The UV-vis spectrum of **1**, in methanol, between 600 and 190 nm, shows three main absorptions at 470 (ϵ_{max} 4200), 314 (ϵ_{max} 21 500), and 222 nm (ϵ_{max} 44 000) (Figure 2). In the same spectral range, several bands are observable in the CD spectrum (Figure 3): at 470 ($\Delta\epsilon_{max}$ −2), 342 ($\Delta\epsilon_{max}$ +5), 310 ($\Delta\epsilon_{max}$ +28), 285 ($\Delta\epsilon_{max}$ −10), 254 ($\Delta\epsilon_{max}$ −15), 233 ($\Delta\epsilon_{max}$ +81), and 209 nm ($\Delta\epsilon_{max}$ −74). The shortest wavelength (320–200 nm) Cotton effects correspond to electrically allowed transitions and are quite intense. In particular a strong couplet[7] is present between 200 and 250 nm; this feature indicates a coupled oscillator mechanism as the main source of optical activity at least below 250 nm. Therefore, the same approach based on the De Voe model and followed in the case of rifamycin S[1] can be used to get information on the stereochemistry of **1** in solution. In the coupled oscillator treatments for optical activity,[8] the transitions on a chromophoric group are represented by one or more electric dipoles, and they are coupled together owing to their own dipolar oscillating fields. The optical activity comes from the interaction of dipoles centered on groups chirally disposed with respect to each other. It is then clear that these calculations require a knowledge of the transitions of the various chromophoric groups (i.e., polarization direction and polarizability allied to each transition) and of the structure of the molecule to define the relative disposition of the interacting chromophores.

b. Characterization of the Main Absorption and Structural Hypothesis. Owing to the complex structure of **1** (Figure 1), a detailed assignment of the above absorptions is not a simple matter. Actually, several chromophoric systems can be responsible for the absorptions observable in the UV-vis range, the most important being the substituted hydroquinone, the carbonyl, the dienonic, and the olefinic chromophores. To single out the absorptions of the aromatic system of **1**, it is useful to consider the absorption spectrum of 16,17,18,19,28,29-hexahydro-8-*O*-methylrifamycin SV, **2**, obtained by catalytic hydrogenation of **1**. The spectrum of **2** (Figure 2) shows two bands at 230 (ϵ_{max} 30 000) and 310 nm (ϵ_{max} 20 000), indicating that the substituted aromatic system strongly contributes to the overall absorption in the range studied. As a matter of fact, the UV spectrum of 1,4,5-trihydroxynaphthalene[9] shows absorptions at 222 nm (ϵ_{max} 50 000) and between 315 and 350 nm (ϵ_{max} 8500).

In order to provide information on the polarization direction of the above absorption bands, MO-CI calculations, using a standard CNDO/S-CI program, were carried out on some substituted hydroquinones, which can be assumed as good models of the aromatic part of **1**. This choise was due to the limitations of the program which does not allow treatment of molecule **1**, having more than 50 nonhydrogenic atoms. The molecules chosen were 1,4,5-trihydroxynaphthalene, 1,4-dihydroxy-3-methoxynaphthalene, and 1,4,8-trihydroxy-5-formylnaphthalene. The calculations show that in the range 200–350 nm, two groups of transitions are present at about 215–230 and 300–330 nm, respectively. They give rise to the most intense absorptions and are

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(4) Cellai, L.; Cerrini, S.; Segre, A.; Brufani, M.; Fedeli, W.; Vaciago, A. *J. Org. Chem.* **1982**, *47*, 2652.
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(6) See note 17 in ref 1.
(7) Haas, G.; Hulbert, P. B.; Klyne, W.; Prelog, V.; Snatzke, G. *Helv. Chim. Acta* **1971**, *54*, 491.
(8) See, for instance: Mason, S. F. "Molecular Optical Activity & the Chiral Discriminations"; Cambridge University Press: New York, 1982; pp 42–47.
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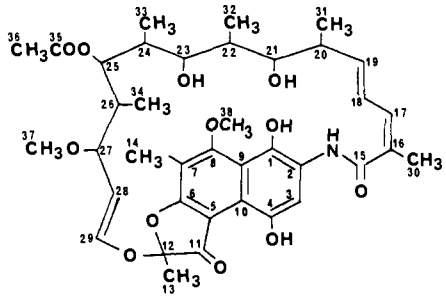

Figure 1. Chemical structure of 8-*O*-methylrifamycin SV, **1**.

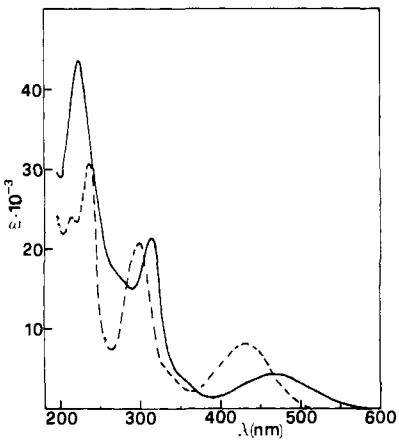

Figure 2. Absorption spectra in the UV–vis region of **1** (—) and **2** (---), in methanol solution.

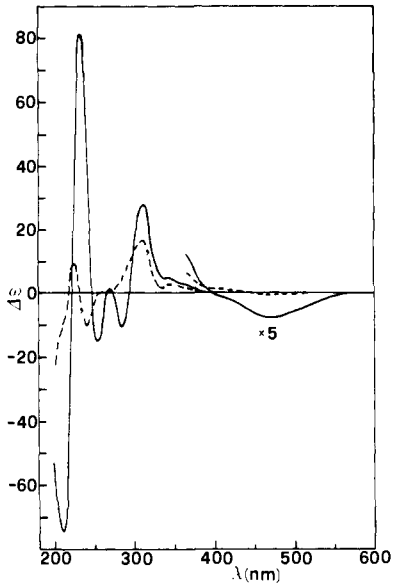

Figure 3. CD spectra of **1** (—) and **2** (---) in methanol solution.

polarized along the long and short axis of the naphthalene nucleus, respectively, and, moreover, their characteristics are practically unaffected by number, nature, and position of the substituents.[10]

Information on the absorption of the chromophoric systems on the ansa of the molecule can be provided by the difference spectrum of **1** and the corresponding ansa-hydrogenated compound **2**, (ϵ_1–ϵ_2) which is reported in Figure 4. A couple of bands are observed at 220 (ϵ_{max} 17 000) and 262 nm (ϵ_{max} 13 000). These

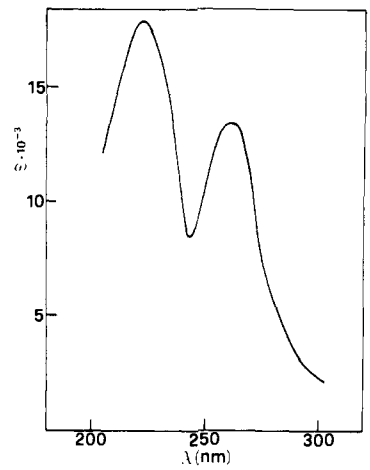

Figure 4. Difference spectrum (ϵ_1 – ϵ_2) between **1** and **2**.

values can be compared with the corresponding figures obtained for the ansa absorptions in the case of rifamycin S at 218 (ϵ_{max} 10 000) and 256 nm (ϵ_{max} 10 000); i.e., the frequency positions of the two couples of bands are very similar, whilst some difference can be observed in the intensities. The origin of these two absorptions has been already discussed for rifamycin S,[1] and two possible interpretations were provided: (i) the two bands are both due to a distorted dienone chromophore and (ii) in solution, two different molecular conformations are present, corresponding to two different situations of the ansa. A detailed NMR and X-ray study of several rifamycins, recently carried out by Vaciago et al.,[3,4] shows that the conformational situation of the dienamide fragment should be the same in the solid state and in solution for several derivatives of rifamycin SV, giving support to hypothesis (i). In order to have a better insight on the nature of these transitions, CNDO/S-CI calculations have been carried in the present work also for the dieneamide chromophore having the same structure found[11] for the corresponding fragment of rifamycin B, **3**, in the solid state. These calculations provide two electrically allowed transitions at 260 and 215 nm, in good agreement with those experimentally found at 262 and 220 nm. In addition, both of the bands show similar polarization directions, which are near to the C_{16}–C_{19} direction. The above results point out that in the molecule **1** in solution, a distorted dienone chromophore is present. According to the above discussion, the structure used in the calculations of the CD spectrum of **1** was that found in the solid state[11] for rifamycin B *p*-iodoanilide, **3**.

c. De Voe Computation. With the aim to rapidly individuate the origin of the most important contributions to the CD spectrum, a set of preliminary computations was carried out, using only couples of oscillators, one located on the aromatic ring, the other on the ansa moiety. The most significant results of these calculations are reported in Table I. An intense positive couplet, like that experimentally found, is obtained only when the oscillators (osc.) corresponding to the most intense absorptions (the 220-nm ansa band, osc. I, and the hydroquinone 230-nm band, osc. III) are polarized along the directions provided by the CNDO/S-CI calculations (line 1). Changes of one of these directions strongly reduce the intensity of the couplet (lines 2 and 3). The interaction of osc. II (the 260-nm absorption band of the ansa) with osc. III provides another couplet. A negative couplet is obtained when the polarization direction of osc. II is nearly perpendicular to that of osc. I (lines 4 and 5); on the contrary, a positive couplet, giving rise to a negative interference with the first one (i.e., reducing the overall CD intensity around 240 nm), is obtained when the polarization direction of osc. II is similar to that of osc. I. The last oscillator to be taken into account is osc. IV, which represents the aromatic absorption at about 330 nm. It interacts in the strongest way with the nearest in frequency oscillator, i.e., osc.

(10) Other weaker transitions result in different spectral ranges, but they are quite dependent on the substitution pattern, as far as the energy, intensity, and polarization are concerned. The intrinsic limitations of the CNDO/S method employed in these calculations heavily affect the results of the computations; whilst the experimental absorptions below 200 nm is strong, the calculated spectra do not show intense transitions below 210 nm down to 170 nm.

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Table I. Exciton-like Effects Calculated Using Couples of Interacting Oscillators

line	ansa[a] osc. *n* (polarization direction)[c]	aromatic nucleus[b] osc. *n* (polarization direction)	$\Delta\epsilon$ (λ nm)
1	I[d] (15–19)	III[e] (long axis)	−60 (211); +57 (239)
2	I (17–19)	III (long axis)	−23 (210); +23 (239)
3	I (16–19)	III (long axis)	−4 (209); +4 (239)
4	II[f] ($\perp$ to 15–19 through 17)	III (long axis)	+25 (239); −39 (260)
5	II (17–18)	III (long axis)	+16 (239); −24 (260)
6	II (15–19)	III (long axis)	−27 (239); +41 (260)
7	II ($\perp$ to 15–19 through 17)	IV[g] (long axis)	+14 (267); −12 (300)
8	II ($\perp$ to 15–19 through 17)	IV (short axis)	−6 (267); + 5 (299)
exptl			−15 (254); −10 (285) −74 (209); +81 (233)

[a] Oscillators located on the dienamide fragment, centered in the middle of 17–18. [b] Oscillators located on the aromatic nucleus, centered in the middle of the naphthalene ring. [c] The numbers in parentheses refer to two atoms of **1** which define the dipole direction. [d] Dienamide 220-nm transition. [e] Aromatic 225-nm transition. [f] Dienamide 260-nm transition. [g] Aromatic 310-nm transition.

Table II. Main Features of the Calculated CD Spectra, Using more than Two Oscillators[a,b]

line	osc. I	osc. II	osc. IIII	osc. IV	$\Delta\epsilon$ (λ, nm)
1	*	*	*		−56 (211), +82 (239), −38 (260)
2	*	*	*	*	−51 (211), +84 (238), −46 (260), +2 (292), −1 (319)
3	*	*[c]	*	*	−50 (211), +88 (238), −48 (260), −4 (310)
4	*	*[d]	*	*	−53 (211), +78 (236), −39 (260), +4 (310)
exptl					−74 (209), +81 (233), −15 (254), −10 (285)

[a] The number of the oscillators are the same as in the Table I. [b] The polarization direction of these oscillators are those found in the exploratory step, unless otherwise specified. [c] The polarization direction of this oscillator is the following: perpendicular to 15–19 but forming an angle θ = 15° with the plane defined by the atoms 15–16–17. [d] The polarization direction of this oscillator is the following: perpendicular to 15–19 but forming an angle θ = −15°.

II. However, this interaction provides only relatively small effects; the results of calculations obtained for two different polarization directions of osc. IV, along the long and short axes of the naphthalene nucleus, are reported in lines 7 and 8, respectively.

In summary, the results obtained in this first set of calculations provide the polarization directions of the oscillators corresponding to the main absorptions. Of course, the above directions will be used in the next step to reproduce, at best, the experimental spectrum.

The results of the calculations where more than two oscillators are involved are reported in Table II. Line 1 shows results with the three most important oscillators, having the polarization directions previously found, and in lines 2–4, results are reported including all four oscillators used in the calculations.

In Figure 5 a comparison between the experimental and calculated CD spectrum is shown. The difference is very small for the negative maximum at 210 nm and the positive one at 240 nm, whilst it becomes significant at 270 and 310 nm. This fact suggests the existence of at least two transitions carrying a positive CD and centered at about 270 and 310 nm. Actually only two bands on the aromatic moiety and another two on the ansa have been employed to reproduce the overall CD spectrum between 200 and 350 nm. Certainly many other transitions (as suggested also by the CNDO calculations) are present in this range and they have been disregarded.

In summary, the CD of **1** is dominated by two main exciton-like effects: a negative band at 210 nm and a positive one at 230 nm.

Actually, the first step of calculations was already able to provide these results, no significant improvement of the calculated spectrum being provided by the more complex computations, even varying the parameters employed.

Conclusions. A good fit of the CD spectrum of **1** between 250 and 190 nm has been obtained by means of coupled oscillator

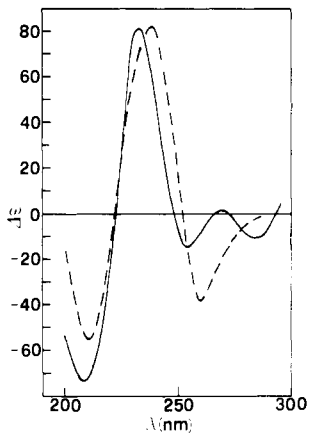

Figure 5. Experimental (—) and calculated (---) CD of **1**, between 300 and 200 nm.

calculations, following the De Voe scheme. This result shows that the largest part of the observed optical activity in the range 250–190 nm is accounted for by the coupling of the long-axis polarized aromatic band with the dienone bands.

From a structural point of view, it has to be noticed that the above good fit of the CD spectrum of **1** has been obtained by using the geometry found in the solid state for rifamycin B *p*-iodoanilide, **3**. This indicates that the structure in the solid state of **3** can be considered as a good model of the structure of **1** in solution.

The results obtained show that CD spectroscopy can be used to assign the absolute stereochemistry of large, chiral molecules, as ansamycine derivatives, in a nonempirical way, when a dynamic coupling mechanism dominates the optical activity, at least in a certain spectral region. This fact points out that CD spectroscopy is becoming more and more a powerful and reliable method in structural assignments.

Experimental Section

Preparation. A sample of pure 8-methoxyrifamycin SV, **1**, was kindly supplied by G. G. Gallo, Laboratori di Ricerca Lepetit, Milano, Italy. The 16,17,18,19,28,29-hexahydro-8-*O*-methylrifamycin SV, **2**, was prepared by catalytic reduction of **1**, following a known procedure for rifamycin derivatives.[12]

Spectroscopic Measurements. UV–vis spectra, in the range 600–190 nm, were obtained by means of a Jasco J 710 spectrophotometer, and c.d. spectra, in the same spectral region, were carried out with a Jasco J 500 C spectrometer. All spectra were measured at room temperature, using freshly prepared methanol solutions (0.5/0.1 g/L) and 1/0.1 cm path length quarz cells.

Calculations. All the calculations reported in the present paper were carried out, by means of programs derived from the De Voe treatment, following the procedures described in a previous paper.[1]

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