Circular Dichroism of Rifamycin S

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Abstract: The UV-vis absorption and circular dichroism spectra of rifamycin S are reported in the range of 600-200 nm. The comparison of the above spectra with those of "ansa" hydrogenated derivatives of rifamycin S indicates that the main source of optical activity in the 300-200 nm region is the interaction of the "ansa" and naphthoquinone polarizabilities. This interpretation is supported by DeVoe calculations of the CD in the same range. Two possible conformational situations can describe the solution stereochemistry of rifamycin S.

The rifamycins are a family of antibiotics obtained by fermentation and chemical modification and are widely used in the treatment of a large number of bacterial infections. The structure of rifamycins was investigated by chemical, spectroscopic, and X-ray methods.[1] The basic structure of the rifamycins is reported in Figure 1; they belong to the class of ansamycins,[2] which are characterized by the common feature of an aromatic ring spanned by an aliphatic bridge called "ansa".

Chiroptical properties of rifamycins have not yet been reported; however, they could provide additional information on the structure of rifamycins in solution and be of value in studying the mechanism of action of related antibiotics.

In fact, the interaction between rifamycins and bacterial DNA-dependent RNA polymerase[3] involves stereochemical modifications which can be, in principle, detected by chiroptical techniques.

In the present paper the electronic absorption (UV-vis) and circular dichroism (CD) spectra of the rifamycin S are discussed in the range of 600-200 nm in order to identify the contributions coming from different chromophoric systems of the molecule and from their possible interaction.

Experimental Section

Preparation. Pure samples of rifamycin S (I), 16,17,18,19-tetrahydro-I (II), 16,17,18,19,28,29-hexahydro-I (III), and 1,2-dihydro-2,5-dihydroxy-2,4-dimethyl-7-aminonaphtho[2,1-*b*] furan-1,6,9-trione (IV) (model compound) were prepared according to known procedures.[4]

Spectroscopic Measurements. UV-vis spectra, in the range of 600-200 nm, were obtained by means of a Cary 14 spectrophotometer and CD spectra, in the same spectral range, were carried out with a Jobin Yvon mark III dichrograph. Unless otherwise specified, all spectra were measured at room temperature, using freshly prepared methanol acidic solutions (0.4-0.6 g/L and 1-0.05 cm path length cells).

All CD spectra were carried out with spectral bandwidth of 0.2 nm in the range of 600-210 nm and 0.5 nm below 210 nm.

Rifamycin S (I). UV-vis, λ_{max} (ϵ) 390 (4900), 334 (7900), 300 (sh) (12 900), 276 (27 100), 240 (sh) (25 400), 217 nm (30 900); CD, λ_{max} ($\Delta\epsilon$) 437 (+7.06), 353 (-4.89), 320 (sh) (+1.5), 300 (+12.4), 278 (-24.6), 240 (sh) (+14.8), 225 (+41.4), 202 nm (-55.3).

Rifamycin S (I). Solvent MeOH/NaOH (pH 10); UV-vis, λ_{max} (ϵ) 525 (4500), 440 (sh) (2800), 316 (30 400), 260 (21 700), 226 nm (31 500); CD, λ_{max} ($\Delta\epsilon$) 515 (+2.81), 426 (+6.38), 338 (+22.9), 314 (-22.7), 270 (-9.83), 250 (sh) (+7.9), 227 (+25.7), 207 nm (-49.1).

Tetrahydrorifamycin S (II). UV-vis, λ_{max} (ϵ) 395 (4200), 332 (8800), 300 (sh) (13 400), 277 (22 200), 235 (24 700), 210 nm (21 800); CD, λ_{max} ($\Delta\epsilon$) 420 (+4.81), 346 (-2.29), 315 (sh) (+5.8), 301 (+12.0), 274 (-5.13), 239 (+7.38), 215 (sh) (-12.8), 202 nm (-37.2).

Hexahydrorifamycin S (III). UV-vis, λ_{max} (ϵ), 393 (4300), 333 (9000), 300 (sh) (13 800), 276 (22 500), 234 (25 900), 215 nm (21 600); CD, λ_{max} ($\Delta\epsilon$) 433 (+3.93), 333 (+3.99), 300 (+8.62), 274 (-3.55), 252 (+3.02), 235 (+2.37), 222 (-2.15), 200 nm (-12.2).

Model Compound (IV). UV-vis, λ_{max} (ϵ) 390 (sh) (6100), 347 (12 600), 314 (10 200), 272 (19 200), 227 (26 200).

Calculations. Programs based on the DeVoe model[5] were used for the CD calculations, using an IBM 360/158 computer.

The oscillators, which are the subjects of DeVoe treatment,[5] are described in terms of their locations, polarization directions, and their frequency-dependent complex polarizabilities.

The imaginary parts of the latter are directly obtained from the experimental absorption spectra and the real ones are calculated by means of Kronig-Kramers transformations, according to the following formulas:[5]

$$\mathrm{Im}\alpha(\nu) = \frac{6909c\epsilon(\tilde{\nu})}{8\pi^2 N_0 \tilde{\nu}}$$

$$\mathrm{Re}\alpha(\tilde{\nu}) = \frac{2}{\pi}\int \frac{\mathrm{Im}\alpha(\tilde{\nu}')\tilde{\nu}'}{\tilde{\nu}'^2 - \tilde{\nu}^2}\,\mathrm{d}\tilde{\nu}'$$

A series of preliminary calculations was carried out starting with only couples of interacting dipoles in order to single out rapidly the main sources of optical activity. Successive additional oscillators were taken into account, gradually getting the final picture of six, all-order interacting, polarizabilities. Polarization directions and locations will be discussed later.

Results and Discussion

Electronic Absorption Spectra (UV-vis). In the elucidation of the structure of rifamycins, the UV-vis spectra were reported by Prelog and co-workers.[4] As the OH at C_8 of rifamycin S (I) (Figure 1) is ionizable,[6] the UV-vis spectra of I were carried out in both acidic and basic solution. The spectra show a similar shape with a general red shift of the bands in the ionized form with respect to the unionized one. All the data hereafter reported were obtained in methanol-acid solutions in order to have the molecules mostly in the unionized form.

The absorption spectra between 600 and 200 nm of rifamycin S (I), 16,17,18,19-tetrahydro-I (II), and 16,17,18,19,28,29-hexahydro-I (III) derivatives and of the model compound of the naphthoquinone moiety (IV) are reported in Figure 2. (IV is actually in Me_2SO a 70:30 mixture of hemiketalic and α-diketonic forms as derived from ^1H NMR spectroscopy.[7]) By examining the spectra and considering also the data reported for 5-hydroxy-1,4-naphthoquinone and related compounds,[8,9] it appears

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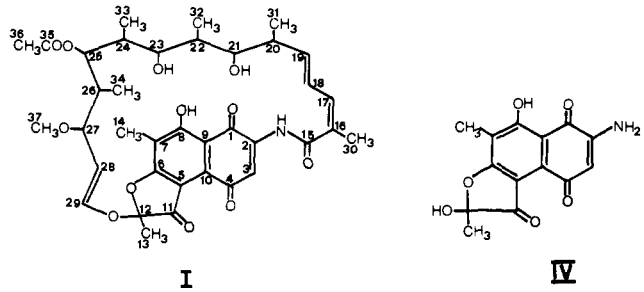

I IV

Figure 1. Structure of the rifamycin S I and of the model compound IV.

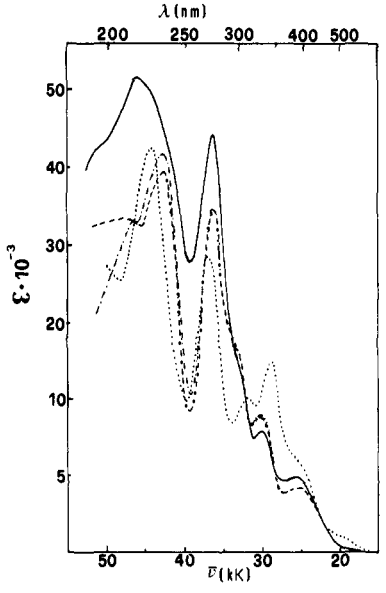

Figure 2. UV spectra of I (–), II (---), III (–·–), and IV (···) in methanol–acid solution.

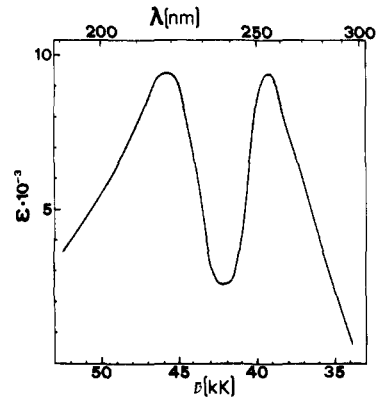

Figure 3. Differential UV spectrum between I and II.

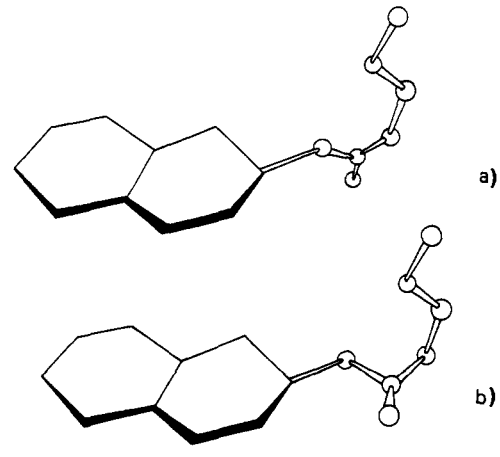

Figure 4. C_{15} carbonyl group limiting geometries in I: (a) partially conjugated with the two dienic double bonds; (b) nonconjugated with the two dienic double bonds.

that in the range of 600–290 nm the absorption observed is dominated by the transitions of the naphthoquinone chromophore. At higher energy (290–200 nm) the absorption spectrum becomes more complex because several other absorptions contribute in this spectral range in addition to the two bands at 275 and 233 nm, which are associated with the aforementioned chromophore.

The calculated difference spectra between rifamycin S and its structurally related compounds II and III in the range of 290–210 nm show two maxima at about 260 and 220 nm, due to two distinct absorption bands present in I but lacking in II and III. In particular, with comparison of rifamycin S with its tetrahydro derivative II (Figure 3), the maxima are at 218 nm ($\epsilon \sim 10\,000$, $\mu^2 \sim 12.5$ debye^2) and at 256 nm ($\epsilon \sim 10\,000$, $\mu^2 \sim 10.2$ debye^2). These contributions to the absorption were reported to be related to an extended chromophore involving the two dienic double bonds partially conjugated with the carbonyl group of the amide.[10] The hypothesis of a partial conjugation is consistent with the value of the dihedral angle (167.5°) between the planes defined by the atoms (Figure 1) C_{19}–C_{18}–C_{17} and C_{18}–C_{17}–C_{16}, respectively, and by that (136.4°) between the planes containing C_{17}–C_{16}–C_{15} and C_{16}–C_{15}–O, as measured in the solid state.[11]

In addition, other related unsaturated carbonyl compounds are reported, that exhibit two well-detectable absorption bands in this spectral range[12] and at least two bands are also predictable on theoretical grounds.[13]

However, it must be noted that in the spectral range of 300–200 nm the *trans*, *trans*-3,8-dimethyl-4,6-diene,[14] an entirely conjugated diene that could be related to the diene moiety in I, absorbs at 230 nm (ϵ_{max} 30 000) and the *N*-isopropylsorbamide,[15] a trans, trans-$\alpha,\beta,\gamma,\delta$-conjugated amide that could be related to the diene–amide moiety in I, exhibits a single band at 256 nm (ϵ_{max} 32 400).

Thus an alternative explanation of the presence in I of the two absorptions at 218 and 256 nm could be given by admitting the existence in solution of an equilibrium between two "ansa" conformations, each of them being characterized by one of the chromophoric systems contained in the aforementioned diene and conjugated amide derivatives.

In other words, the amide group could assume in rifamycin S two different arrangements, giving rise to the local geometries reported in Figure 4, i.e., conformation a (dieneamide chromophore, 256 nm) and conformation b (diene chromophore, 218 nm).

A similar interpretation has been reported already for the dienone system of β-ionone, which shows two bands at 223 and 296 nm.[12] Indeed, the arrangement of conformation a for the amide group is represented by rifamycin B[11], a hydroquinonic rifamycin, while that of conformation b is represented by tolipomicinone, a quinonic ansamycin.[16]

Circular Dichroism Spectra (CD). The CD spectrum of rifamycin S has not yet been described, and it is reported in Figure 5 together with those of its tetrahydro II and hexahydro III

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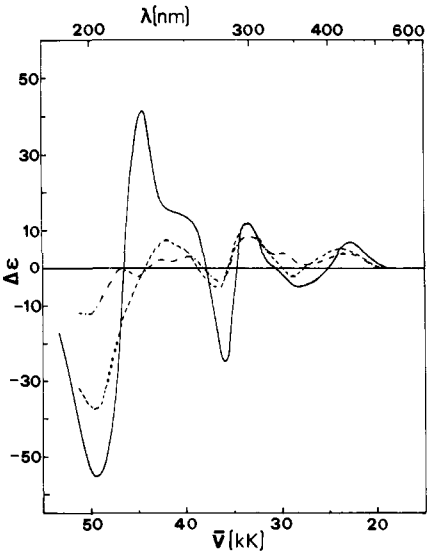

Figure 5. CD spectra of I (—), II (- - -), and III (—·—) in methanol–acid solution.

derivatives in the same experimental conditions adopted for the corresponding electronic absorption spectra.

With respect to the absorption, the CD shows a greater number of bands, and this can provide additional information both on the electronic assignments and on the mutual interaction between different chromophores of the molecule.

From the comparison of CD spectra of I, II, and III between 600 and 290 nm, at least five bands can be identified.

The good correspondence in the shape and the similar intensity of the CD spectrum of I to those of its hydrogenated derivatives indicate that the CD in this spectral range is dominated by the chromophore connected with the naphthoquinone moiety. The region at shorter wavelengths (290–200 nm) of the CD spectrum of I is characterized by a negative band at 280 nm, by a well-detectable positive shoulder at 245 nm, and by the couplet centred at 215 nm, which has a positive peak at 222 nm and a negative peak at 202 nm.

In the spectral range in question, the CD intensity of the hydrogenated derivatives II and III is remarkably lower with respect to I, expecially in the correspondence of the 280-nm band and of the positive extremum of the couplet.

The exciton form of the couplet[17] and the high intensity of the CD in the range in question reasonably suggest that the mutual interaction of the naphthoquinone and the "ansa" chromophores is the main source of optical activity. Calculations were carried out to confirm the above hypothesis and to explain the CD difference betweeen the rifamycin S and its hydrogenated derivatives.

Circular Dichroism Computations. As it is well-known, a Cotton effect can arise from different mechanisms.[18,19] When one of these mechanisms is dominant we have specialized treatments, as, for example, the sector rules for $n \rightarrow \pi^*$ transition in ketones[20] and carboxylic derivatives[21] ("static coupling mechanism"[19]), or polarizability treatments, as the DeVoe one[5] (a "dynamic coupling mechanism"[19]). This last treatment examines the optical activity arising from at least two chromophoric groups polarized by an external electromagnetic radiation field and coupled to each other by their own dipolar oscillating fields. This mechanism is generally the most important one in the case of allowed transitions located in different chromophores that assume a chiral disposition.

In the present case, the DeVoe polarizability treatment can be foreseen as appropriate in the high-energy region, 290–200 nm, where the most intense absorptions, that is, the electrically allowed ones, of the naphthoquinone and of the "ansa" moieties are located.

In order to carry out computations using the DeVoe treatement, it is necessary to establish the frequency-dependent polarizabilities (in practice the absorption band due to the single transiton considered), the polarization direction of the transitions themselves, and their location.

It is quite clear that structural differences affect the above parameters. Therefore, following the hypothesis that only the conformation of I present in the solid state (Figure 4a) is responsible for both the electronic transitions at 218 and 256 nm, we carried out the calculations of set a. On the contrary, assuming that the above conformation is allied to the 256-nm transition only and the conformation having the local geometry reported in figure 4b is responsible for the transition at 218 nm, set b and c calculations were performed, respectively. The final CD spectrum will be, of course, a weighted average of the set b and set c calculations.

The most important sources of UV absorption, which have been taken into account, are:

Set a. 1. Benzenoid[22] and quinoid[22] bands centered at 233 and 275 nm, ϵ_{max} 26 000, and 22 000, respectively. Despite their different nature,[8] the former is almost $\pi \rightarrow \pi^*$ aromatic and the latter is charge-transfer type; these two transitions are both polarized into the plane of the chromophore.

Recent semiempirical SCF–CI calculations[9] on electronic states of 1,4-naphthoquinone indicate a long-axis polarization of the benzenoid transition and a short-axis polarization of the quinoid one.

In the absence of any quantitative indications and to simplify the treatment, both the dipoles of the above transitions were located in the middle of the junction of the benzene and quinone moieties.

2. The two bands at 218 and at 256 nm, obtained from the difference $\epsilon(I) - \epsilon(II)$ (Figure 3) and connected with the diene–amide system. The band at 218 nm was assumed to be polarized in the C_{16}–C_{19} direction. This assumption can be justified by taking the molecular excited state of the diene as essentially made by in-phase excitations of the two individual double bonds. The middle point of the C_{16}–C_{17} bond was assumed as the location of the above oscillator.

As far as the second band is concerned, we considered as variational parameters both the polarization and the location along the diene–amide skeleton of the corresponding transition dipole, lacking any indication from the literature.

3. Amide $\pi \rightarrow \pi^*$ band centered at 193 nm (ϵ_{max} 7000), obtained as an average peptide contribution from spectral data of oligopeptides containing aromatic residues.[23] Polarization direction was taken from experimental data,[23] and the carbon atom of the amide group was chosen as the location of the related dipole.

4. Olefin $\pi \rightarrow \pi^*$ band centered at 193 nm, ϵ_{max} 10 000, polarized along the C_{28}–C_{29} bond of the olefinic chromophore and located in the middle of the bond itself.

Set b. Same as in set a except now only one absorption band at 255 nm ($\mu^2 = 21$ D^2) is considered to represent the conjugated "dienone" chromophore instead of the two bands at 218 and 256 nm. The corresponding oscillator was located on C_{16}.

Set c. Same as in set a with the sole exception that a pure dienic band at 218 nm ($\mu^2 = 25$ D^2) is considered instead of the two bands of the partially conjugated "dienone" chromophore. The corresponding oscillator was located in the middle of the C_{17}–C_{18} bond.

It is to be noted that other oscillators connected to transitions at higher energies (e.g. alkyl polarizabilities) should be taken into

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Table I. Interacting Bands (x), Main CD Characteristics Obtained by DeVoe Treatment (Lines 1-9) in Set a Computations, and Experimental Values (Line 10)

	bands						Δε			
line	benzenoid 4.30 μm^{-1} (233 nm)	quinoid 3.64 μm^{-1} (275 nm)	dienic 4.44 μm^{-1} (218 nm)	dienonic 3.91 μm^{-1} (256 nm)	amidic 5.2 μm^{-1} (193 nm)	olefinic 5.2 μm^{-1} (193 nm)	3.6 μm^{-1}	3.9 μm^{-1}	4.2 μm^{-1}	4.8 μm^{-1}
1	x				x				-0.02	-0.03
2	x					x		0.4	-2.8	-4.4
3	x		x				0.0	1.2	28	-19
4	x	x	x				-10	2	37	-18
5		x		x			-17	21	3	0.3
6	x	x	x	x			-20.6	19.2	36.4	-19
7	x	x	x	x		x	-20.8	18	31.4	-24.3
8	x	x	x	x	x	x	-20.7	17.6	34.6	-44.7
9	x	x	x	x	x	x	-16.1	15.3	32.9	-45.3
10	exptl						-24	15	41.5	-54

Table II. Interacting Bands (x) and Main CD Characteristics Obtained in Set b Computations

	bands				Δε			
line	benzenoid 4.30 μm^{-1} (233 nm)	quinoid 3.64 μm^{-1} (275 nm)	dienonic 3.91 μm^{-1} (256 nm)	olefin 5.2 μm^{-1} (193 nm)	3.6 μm^{-1}	3.9 μm^{-1}	4.2 μm^{-1}	4.8 μm^{-1}
1	x	x	x		-25.3	23.1	7.9	2
2	x	x	x	x	-25.6	22.2	2	-9

Table III. Interacting Bands (x) and Main CD Characteristics Obtained in Set c Computations

	bands					Δε			
line	benzenoid 4.3 μm^{-1} (233 nm)	quinoid 3.64 μm^{-1} (275 nm)	diene 4.44 μm^{-1} (218 nm)	amide 5.2 μm^{-1} (193 nm)	olefin 5.2 μm^{-1} (193 nm)	3.6 μm^{-1}	3.9 μm^{-1}	4.2 μm^{-1}	4.8 μm^{-1}
1	x	x	x			-13.8	7.8	37.6	-25
2	x	x	x	x		-13.7	10.6	45.7	-36
3	x	x	x	x	x	-14.3	12	37	-43

account for a more complete treatment because the coupling of these transitions with those of the chromophores considered can affect the higher energy side of the spectrum observed.[24] In addition, static contributions[19] have been completely disregarded owing to the dynamic character of the treatment used.

The main CD characteristics obtained by the DeVoe treatment of calculations in sets a, b, and c are reported in Tables I, II, and III, respectively. Table I contains also the prominent experimental values (line 10).

Taking the results shown in Table I as a reference, we can make the following general comments. The calculations with couples of dipoles (Table I, lines 1-3, 5) show the shape of the experimental CD spectrum can be reproduced only when the quinoid transition at 275 nm interacts with the dienoic one at 256 nm (line 5) and when the benzenoid transition (233 nm) interacts with the diene transition (218 nm) (line 3).

The calculated spectral features of the different partners in the couplet are in complete disagreement (lines 1 and 2) with that experimentaly found (line 10).

The Δε values that more closely approach the experimental ones have been obtained, taking into account four polarizabilities all together (line 6); the introduction of additional polarizabilities does not affect, at least in a relevant extent, the results (line 7 and 8).

The results are qualitatively unaffected also by varying the location of the dienone dipole, and line 9 shows the largest variation observed by changing the dipole location along the diene-amide backbone.

Only the CNDO-SCF-CI polarization directions of the naphtoquinoid transitions[9] give agreement between calculated and experimental CD values.

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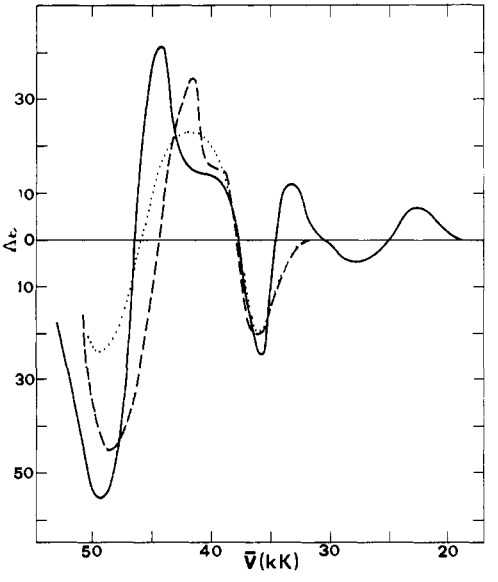

Figure 6. Experimental (-) and calculated [(- - -) set a, (···) sets b and c)] CD spectra of I.

The best calculated CD spectrum is compared with the experimentally detected one in Figure 6.

Tables II and III show the best results obtained when the "dienone" transition at 256 nm and the diene transition at 218 nm interact separately with the two naphthoquinoid absorptions at 275 and 233 nm.

Figure 6 reports the CD spectrum obtained from the weighted average of set b and set c calculations, assuming an equilibrium constant of 1 for the interconversion reaction between the two conformations. The spectrum is compared with the CD spectrum

calculated from set a and the CD spectrum measured experimentally.

Different geometries could be used in order to obtain a better fitting of the experimental data if more precise information on the conformation of the molecule in solution were available.

Conclusions

Down to 290 nm the CD of the rifamycin S is mainly due to the transitions allied to the naphthoquinone chromophore as indicated by its similarity to the CD of the hydrogenated derivatives (Figure 5).

The 290–200-nm spectral range is dominated by the contribution from the dynamic coupling between the allowed transitions of the above chromophore at 233 and 275 nm and the "ansa" transitions at 218 and 256 nm.

The main features of the CD spectrum of rifamycin S can be accounted for by two exciton couplets centered at about 3.8 μm^{-1} (263 nm) and at about 4.7 μm^{-1} (213 nm), the former being connected with the coupling of the 275 and 256 nm transitions and the latter with the coupling of the 218 and 233 nm transitions.

We carried out the calculations considering a single geometry for the rifamycin S molecule and two electronic transitions connected with a diene–amide chromophore as well as assuming the existence in solution of an equilibrium between two different conformations, each characterized by a single "ansa" transition.

In both cases we obtained $\Delta\epsilon$ values in agreement with those experimentally detected; so that, at the present, the stereochemistry in solution of rifamycin S remains undefined with respect to the optical activity.

However, the prevailing exciton origin of the CD in the 290–200 nm spectral range makes stimulating the CD investigation of the rifamycin-like antibiotics in connection with their biological activity being the chiroptical properties that are certainly affected by interactions with other molecules.