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CONFORMATIONS OF LUTEOSKYRIN AND RUGULOSIN IN SOLUTION

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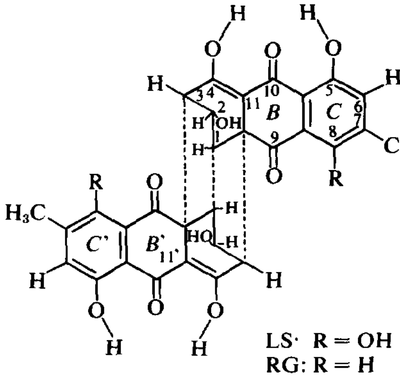
Abstract—The pigments Luteoskyrin (Ls) and Rugulosin (Rg) might adopt *a priori* two extreme conformations, a planar and an angular one. To determine the predominant conformation in solution, the nature of the lowest energy transition of the chromophores and the presence of intramolecular H-bonding have been investigated. The solvent effects on electronic absorption and CD spectra indicated the π–π^* nature of the lowest energy transition. Treatments of the electronic absorption results according to McRae and Kosower relations, IR absorption and PMR spectra suggested the presence of strong intra-molecular H-bonds. From these results it was concluded that Ls and Rg adopted in a variety of solvents the most planar conformation.

INTRODUCTION

Luteoskyrin (Ls) and Rugulosin (Rg) are biologically active pigments[1-8] produced by Penicillium Islandicum Sopp. and Penicillium Rugulosum Thom, respectively. Their structure have been studied by Shibata *et al*[9] and deduced by X-ray diffraction studies on dibromodehydrotetrahydrorugulosin (BrHRg).[10]

The structure of Ls and Rg are reproduced in Fig 1. Rg not only lacks —OH groups in the 8 and 8′ positions but is also a stereoisomer of Ls. Model building[11] indicated that, whereas the BrHRg molecule is rigid due to an additional bond between carbons 11 and 11′, Ls and Rg are more flexible and could adopt different conformations as suggested in Fig 2. Moreover different crystalline forms have been found for Ls and BrHRg.[10,12] As Ls[13,14] and Rg[15] specifically bind to purine residues in single stranded nucleic acids in the presence of divalent ions like magnesium, it seemed of importance to study the conformation of these pigments in solution.



LS· R = OH
RG: R = H

Fig 1. Formular of Ls and Rg. Dash lines represent covalent C—C bonds between the two moieties.

A priori, two limiting conformations (Fig 2) might be envisioned:

(1) The first (AA) (angular–angular conformation) is similar to that in the crystal of BrHRg. It is characterized by an angle of about 150° between the segments joining O_4–O_{10} and O_5–O_{10} in each moiety of the molecule. In other words, the oxygens in the 4 and 4′ positions are, respectively, above and below the surfaces defined by the rings B, C and B′, C′.

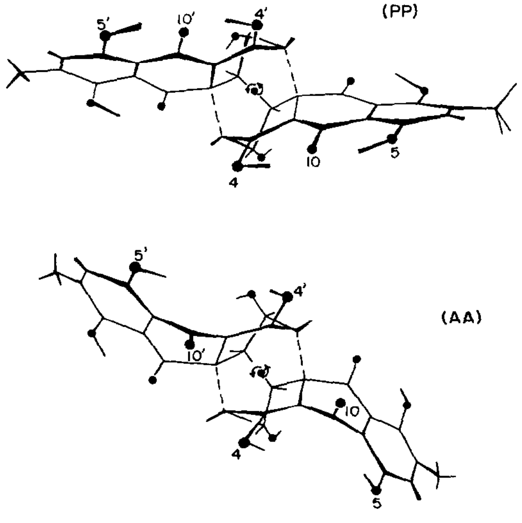


Fig 2. Angular (AA) and planar (PP) conformations of Ls. (·) represents a C_2 symmetry axis.

The B and B′ rings have *boat* conformation with the 9 and 10, 9′ and 10′ carbonyls, respectively pointing out of the plane defined by the aromatic rings C and C′. One might consider that each moiety is a chromophore similar to a substituted acetophenone, presenting either no or very loose internal hydrogen bonds.

(2) In the second conformation (PP) the two moieties are more planar and the segments joining 0_4–0_{10} and 0_5–0_{10} define an angle nearly equal to 180°. The 0_4, 0_{10} and 0_5 oxygens are coplanar. In this case, the chromophores are similar to polyhydroxynaphtoquinones, and strong intramolecular hydrogen bonds are expected.

The aim of the present work was to compare the predominant conformation of Ls and Rg in solution, with the models defined by (AA) and (PP). For that purpose, we investigated the nature of the chromophore and of the H-bonds present. The distinction between acetophenone and hydroxyquinone like chromophore was determined by comparisons of electronic spectra and determination, by solvent effects, of the nature (n–π^* or π–π^*) of the lowest energy electronic transition. Treatments of these solvent effects according to McRae's relation and Kosower's plot, led to the hypothesis of internal hydrogen bonding. Indeed, IR and PMR spectra showed strong intramolecular H-bonds. On this basis it was proposed that, Ls and Rg adopted a planar conformation in most of the solvents investigated.

RESULTS AND DISCUSSION

(1) *Nature of the chromophores in Ls and Rg*

UV—Visible absorption spectra of acetophenone, naphtoquinone anthraquinone and their derivatives have been reported.[16–18] The essential differences between these spectra are the nature and the position of the lowest energy absorption band, which is located around 340 nm for the n–π^* transition in acetophenone derivatives and around 500 nm for the π–π^* transition in polyhydroxynaphto and anthra quinones. The position of the corresponding band, around 390 nm for Rugulosin and 440 nm for the Luteoskyrin, is intermediate between those of the two series mentioned. This situation could result either from the overlapping of n and π orbitals in the angular (AA) conformation, or from the lack of coplaneity of the B,B′ rings in the (PP) conformation.

The relatively high intensity of the Ls and Rg absorption band under discussion ($\epsilon \sim 6500$ per CO group) could argue in favor of the second possibility, but high values of CO n–π^* absorbance have been found for non planar system.[19,20]

Simple comparison with electronic spectra of analogs are thus not conclusive. For this reason we were led to investigate more thoroughly the nature (n–π^* or π–π^*) of the lowest electronic energy transition. The distinction between $n \rightarrow \pi^*$ and $\pi^* \rightarrow \pi^*$ transitions by solvent effects has been proposed by many authors.[21–24]

Electronic absorption and CD spectra of Ls and Rg dissolved in 45 solvents have been recorded. Since the results were similar for the two compounds and by both techniques we only reported results for Ls by electronic absorption spectra.

In Table 1 were listed the frequency values of the lowest energy absorption band of Ls in various solvents.

We observed that the position of this absorption maximum was shifted towards shorter wavelength in the order: methanol > ethanol > n propanol > n butanol > n pentanol > chloroform > carbon tetrachloride. However, the blue shifts observed were small compared to the values reported in the literature.[26] Moreover, blue shifts, by comparison with the spectrum in carbon tetrachloride, were also observed with solutions of Ls and Rg in aprotic solvents such as acetone, acetonitrile, nitromethane. Such displacements were not expected for n–π^* transitions.

The π–π^* nature of the UV band under consideration could be evidenced by computations of the absorption shifts according to the McRae's relation.[27] In this relation, the experimental UV absorption frequency difference $\Delta\bar{\nu}_e = \bar{\nu}_e - \bar{\nu}_o$ between a dissolved and the gaseous states of a molecule was compared to the calculated frequency difference $\Delta\bar{\nu}_c = \bar{\nu}_c - \bar{\nu}_o$ computed from the equation:

$$\Delta\bar{\nu}_c = \bar{\nu}_c - \bar{\nu}_o = (AL_o + B)X + CY + EY^2$$

were A, B, C, E are parameters characteristic of the solute; L_o is the weighted mean wavelength for the solvent:

$$X = \frac{n_D^2 - 1}{2n_D^2 + 1} \quad (n_D = \text{refractive index of the solvent})$$

$$Y = \frac{D-1}{D+2} - \frac{n_D^2 - 1}{n_D^2 + 2} \quad (D = \text{dielectric constant of the solvent})$$

In this treatment, the condition $\Delta\bar{\nu}_e = \Delta\bar{\nu}_c$ only occurs if the main interaction forces are dispersive and electrostatic and if solute–solvent hydrogen bonds are negligible.[27–29]

Results obtained from the computations for Luteoskyrin showed (Fig 3) the same behaviour of this molecule in either protic or non polar solvents (n°1 to 30). This indicated the absence of n–π^* transition in Luteoskyrin. The order of the blue-shift observed in alcohols was just the order of decreasing refractive index values, just as in the case of the (π–π^*) 260 nm transition of benzene.[28]

Other arguments, such as the intensity enhancement of the considered absorption band by acidification of alcoholic solutions of Ls, or the absence of important change in the rotational strength value

Table 1. Experimental and calculated solvent shifts. n_D = refractive index, D = dielectric constant.[53] The meanings of X, Y and $\bar{\nu}$ are explained in the text

No	Solvent	n_D	D	$\bar{\nu}_{exp}$ (cm^{-1})	X	Y	$\bar{\nu}_{exp}-\bar{\nu}_{cal}$ × (cm^{-1})
1	Methanol	1·3256	32·6	22·500	0·168	0·710	0
2	Acetonitrile	1·3440	36·3	22·580	0·174	0·709	10
3	Acetone	1·3585	20·7	22·430	0·180	0·647	0
4	Ethanol	1·3612	24·3	22·400	0·181	0·664	−30
5	Isopropanol	1·3776	18·3	22·380	0·187	0·621	−10
6	1:2-dimethoxyethane	1·3792	6·6	22·380	0·187	0·419	−10
7	Nitromethane	1·3822	38·5	22·400	0·188	0·693	10
8	n-Propanol	1·3850	17·7	22·380	0·189	0·613	0
9	Isobutanol	1·3952	17·2	22·350	0·193	0·603	0
10	n-Butanol	1·3993	17·8	22·350	0·194	0·606	0
11	t-Amylalcohol	1·4045	5·7	22·340	0·197	0·365	0
12	Isopentanol	1·4061	15·6	22·310	0·197	0·584	−20
13	n-Pentanol	1·4072	15·0	22·330	0·197	0·577	0
14	Tetrahydrofuran	1·4075	7·7	22·320	0·197	0·445	−10
15	Dichloromethane	1·4238	9·1	22·280	0·203	0·474	−30
16	2-Octanol	1·4255	7·7	22·300	0·203	0·434	0
17	Chloroform	1·4440	4·8	22·250	0·200	0·293	−20
18	1,2-Dichloro-ethane	1·4442	10·6	22·280	0·209	0·496	10
19	Carbon tetrachloride	1·4589	2·2	22·230	0·214	0·012	−10
20	Cyclohexanol	1·4642	15·0	22·240	0·216	0·547	20
21	Trichloroethylene	1·4772	3·4	22·220	0·220	0·161	−10
22	Benzene	1·4980	2·2	22·180	0·226	0·007	10
23	Tetrachloroethylene	1·5054	2·4	22·150	0·228	0·021	−10
24	Pyridine	1·5120	12·3	22·150	0·230	0·490	0
25	Chlorobenzene	1·5232	5·6	22·140	0·234	0·299	10
26	Carbon disulfide	1·6271	2·6	21·950	0·261	0·006	−30
27	Ethyl ether	1·3524	4·3	22·400	0·177	0·307	−30
28	Triethylamine	1·4003	2·4	22·310	0·195	0·078	−30
29	*p*-Dioxane	1·4240	2·2	22·380	0·203	0·030	70
30	Tri-n-butylphosphate	1·4240	7·9	22·330	0·203	0·441	20
31	Dimethylformamide	1·4302	37·6	22·380	0·205	0·691	80
32	N-Ethylacetamide	1·4331	175·0	22·350	0·206	0·723	50
33	Hexamethylphosphoric triamide	1·4582	30·0	22·310	0·214	0·633	70
34	Formamide	1·4470	109·0	22·390	0·210	0·705	120
35	Dimethylsulfoxide (D M.S.O.)	1·4790	47·0	22·350	0·220	0·655	140
36	Acidic methanol	1·3295	32·6	22·530	0·169	0·709	40
37	Acidic ethanol	1·3629	24·3	22·480	0·181	0·663	50
38	Acetic acid	1·3700	6·2	22·450	0·184	0·407	80
39	Formic acid	1·3714	57·9	22·530	0·184	0·722	120
40	Acidic isopentanol	1·4062	15·2	22·350	0·197	0·579	20
41	Ethyleneglycol no-methyl ether	1·4020	16·0	22·430	0·195	0·589	90
42	Ethylene glycol	1·4290	37·7	22·430	0·204	0·666	130
43	Propylene glycol	1·4324	30·0	22·390	0·206	0·645	110
44	Acidic Propylene glycol	1·4324	30·0	22·420	0·206	0·645	140
45	Glycerol	1·4730	41·1	22·530	0·219	0·649	330

of the corresponding CD band in various solvents, also strongly suggested[27,30] the $\pi-\pi^*$ nature of this band.

On these basis it appeared that the predominant chromophore in Ls and (and Rg) is like a quinonic derivative in the solvents fitting the McRae's plot. This conclusion which means a trend towards planeity, implies a stabilisation of the chromophore by strong intra molecular H-bonds. The H-bonding characteristics of Ls and Rg have been investigated in the following section.

(2) *Hydrogen bonding in luteoskyrin and rugulosin*

In the PP conformation strong intra molecular H-bonding was expected, while solute solvent H-bonds would be predominant in the AA conformation.

Ls and Rg H-bonding properties have been inves-

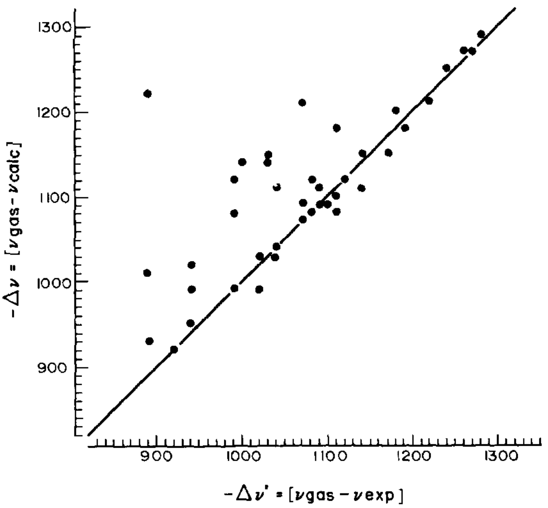


Fig 3. McRae's plot: Calculated *versus* observed frequency shifts (cm^{-1}) in various solvents. Solvent numbers correspond to those listed in Table 1.

tigated by electronic and IR absorption, and by PMR spectroscopies. Deviations from McRae's plot for $n-\pi^*$ bands, are expected when the solute is H-bonded to protic solvents. Similar deviations have also been observed for $\pi-\pi^*$ bands in the case of halogeno-phenols[29] and 2-hydroxy anthraquinone,[31] where H-bonds can be formed between OH groups of the solutes and proton acceptor solvents. Accordingly, the absence of deviation from the least square line fitting non polar solvents, when Ls was dissolved in acetonitrile, acetone, nitromethane, and ethyl ether suggested[32] that all the OH groups of the solute were intramolecularly H-bonded to the vicinal CO groups.

H-bonding between the solute and proton acceptor solvents led to deviations from the McRae's plot, as observed for instance in the case of halogeno-phenols[29] and β-hydroxyanthraquinone.[31] Despite the presence of a number of OH groups, Ls is fitting McRae's plot when dissolved in acetonitrile, acetone, nitromethane or ethyl ether. This behaviour suggested[32] that all the OH groups of the solute were intramolecularly H-bonded to vicinal CO groups. Moreover, the C value ($C_{Ls} = 15$) computed from the McRae's relation[27] indicated a strong internal H-bonding, when compared to the figures found for α-hydroxy or amino-anthraquinones.[31]

However, strong ionizing protogenic (formic acid), protophilic (DMSO), and amphiprotic (formamide, glycols) solvents[25] should then be able to compete successfully with internal H-bonding in Ls and Rg. In this case, disruption of the hydrogen chelation would occur, leading to a blue shift; and new H-bonds would be formed with the solvents, leading to a red shift; the overall shift being the sum of both effects. This could explain the deviations from the McRae's plot for solvents n° 31 to 45 (Table 1, Fig 3).

The latter conclusions might be indirectly checked by Kosower's treatment[33] of solvent effect. Kosower called Z the energy corresponding to the lowest energy absorption band of 1-ethyl-4-carbomethoxypyridinium iodide, dissolved in a given solvent; and E_T the energy of any other compound dissolved in the same solvent. All the interaction forces, including intermolecular H-bonding, being similar in the reference and the studied compounds, Z was found to be linearly related to E_T when changing the solvents.[33]

A similar comparison has been carried out with Ls, using the set of solvents for which the Z values of Kosower reference compound were available. The plot issued from this comparison is given in Fig 4. Solvents 1, 4, 5, 8, 10 and 24, which already fitted the McRae's relation, also fitted Kosower's treatment. The departure of linearity observed in other solvents suggested the interference of additional effects, such as partial or complete loosening of internal H-bonds.

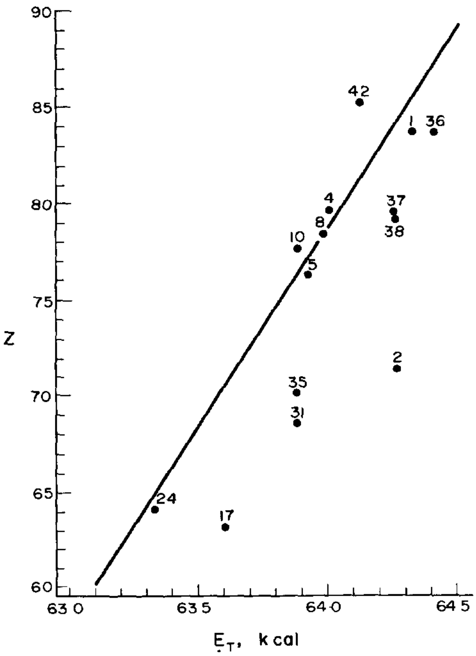


Fig 4. Kosower's plot: Transition energies E_T of Ls *versus* Z. For solvent numbers see Table 1.

At this stage, the localisation in Ls anf Rg of the internal H-bonds has to be considered. Examination of the molecule showed *a priori*, two possibilities:

(a) Hydrogen bonds might* be established between the two moieties, that is between the carbonyls O_{10} and $O_{10'}$ and the hydroxyls $OH_{4'}$ and OH_4 respectively leaving OH_5 and $OH_{5'}$ free. This arrangement would stabilize the (AA) conformation.

(b) The other possibility corresponded to H-bonding located in each moiety, between the CO and the adjacent OH groups, thus stabilizing the (PP) conformation.

Three sets of data were used to choose between these possibilities:

(1) The distances between the oxygens O_{10}---H–$O_{4'}$, and $O_{10'}$---H–O_4 in the (AA) form are of the order of 3·2 A°, whereas they are of the order of 2·4 A° for O_{10}—H–O_4 (or H–O_5). This second type of H-bonding is thought to be stronger than the first one.[34] Furthermore, as the middle "cage" of Ls, and Rg is rigid, no deformation to shorten the distance across the two moieties of the molecule might be expected.

(2) If the hydroxyls in 5 and 5′ were free, the absorption spectra should vary with the solvents as has been described for phenols,[35] effects which had not been observed.

(3) The presence of free OH groups could be directly investigated by IR absorption and PMR.

IR spectra of Ls and Rg dissolved in carbon tetrachloride showed[36] a sharp band at 3620 cm^{-1} and many overlapping broad bands around 3000 cm^{-1}. The band at 3620 cm^{-1}, position corresponding to free OH stretching vibrations,[37] was attributed to the aliphatic 2 and 2′ -OH in Ls and Rg since it disappeared in the acetylated compound 2,2′-diacetylrugulosin (DiAcRg).[38] The broad bands around 3000 cm^{-1} became sharper on a deuteration[36] suggesting the overlapping of C–H and strongly intramolecularly H-bonded O–H stretching vibrations.[40–43] The absence of any absorption band around 3500–3400 cm^{-1} showed neither weak internal H-bonding[39] of the type O_{10}--H–$O_{4'}$ nor intermolecular H-bonds[37] due to the 5,5′ free OH group for example.

Moreover the position of the CO stretching band at 1615 cm^{-1} and 1620 cm^{-1} for Ls and Rg respectively, when dissolved into $CHCl_3$ (instead of 1700 cm^{-1} when free) suggested that the $C=O$ were strongly chelated with the vicinal OH groups, as in the case of peri-hydroxyquinones.[40–44] These data indicated that both -OH group in 4 and 5, and 4′ and 5′ positions respectively are associated to the central ketone group, in Ls and Rg, as well as the 8 and 8′ -OH groups to the vicinal ketones in Ls.

The same conclusion arose from PMR spectra. The protons carried by all the OH groups present in Ls, Rg and DiAcRg were assigned[45] as indicated in Table 2. The chemical shifts of the free OH protons are generally localised around 2·5–5·5 ppm[46] (TMS as standard), as observed for the 2–2′, OH protons in Ls and Rg. By contrast, the protons corresponding to the 4–4′, 5–5′ and 8–8′ OH groups are dramatically shifted downfield, as in the case of strongly chelated OH groups in perihydroxynaphtoquinones.[47]

Table 2. Chemical shifts (δppm/TMS) of hydroxyl protons of solutions in $CDCl_3$.

δppm	OH 4–4′	OH 5–5′	OH 8–8′	OH 2–2′
Ls	14·73	11·55	12·44	4·8
Rg	14·65	11·65		4·7
Di AcRg	14·65	11·55		

In addition, it might be noted that the difference of the downfield shifts of the OH protons could reflect the difference in the strength of the internal bonding.

CONCLUSIONS

All the data collected pointed to the same conclusion that Luteoskyrin and Rugulosin presented strong internal H-bonding in each moiety of the molecule. The planar conformation (PP) appeared thus much favored in the majority of the solvents investigated. This conformation would favor the formation of ordered polychelates between Ls and divalent ions,[48] and might account for binding characteristics of Ls (or Rg) with nucleic acids.[15] [49]

MATERIAL AND METHODS

Ls has been isolated from *Penicillium islandicium* cultivated on Czapeck-Dox medium. The mold mycelium is extracted with acetone and Ls is separated from the other pigments by chromatography on calcium phosphate columns, using benzene as solvent.[50] Ls is crystallized from an acetone–hexane mixture. Crystallized Rg was a gift from Dr Tatsuno, Tokyo. Spectroscopic grade solvents from Merck were used. Basic solvents were additionally purified. Pyridine was distilled from P_2O_5 then from CaH_2 and used immediately. Dioxane was first refluxed over NaOH pellets, distilled, then refluxed over and distilled from sodium.[51] Formamide, dimethylformamide, ethylether and chloroform were purified according to standard procedures[52] and used immediately. Spectra in unpurified protophilic solvents (even in commercial spectroscopic grade solvents) are unreproducible and more or less similar to those obtained in basic methanolic solutions. Hygroscopic solvents such as DMSO were handled in a dry N_2 atmosphere. Acidic alcoholic solutions are obtained by adding micro quantities of concentrated HCl or $HClO_4$ solutions in the spectrophotometer cell in order to have solutions of about 0·1 N.

Spectra have been recorded at various concentrations, using appropriate path lengths (0·1–2 cm). Care has been taken to remain within the range of concentrations where the Beer–Lambert law is valid. The molar extinction coefficients are known with an accuracy better than 95%. The error in wavelength is about 0·5 nm. Absorption spectra were recorded on a Cary 14 and a Cary 15, the former being equipped with a Datex digital recorder. CD spectra were obtained with a Jouan CD II Dichrograph equipped with a digital recorder. Base line corrections and ϵ values were calculated from a set of spectra using P DP 12 or CDC 6600 computers. IR spectra were recorded with a P.E. 225 or 457 apparatus, and proton magnetic resonance spectra with a Varian A 60 or P.E. R 12 apparatus.

The dielectric constants (D) for various solvents used were taken from Landolt-Bornstein Tables.[53] Refractive index were measured with a thermostated Abbe type refractometer.

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