(12) T. Kunitake and Y. Okahata, *J. Am. Chem. Soc.*, preceding paper in this issue.
(13) T. Kunitake, Y. Okahata, and S. Hirotsu, *Bull. Chem. Soc. Jpn.*, in press.
(14) W. Tagaki, M. Chigira, T. Amada, and Y. Yano, *Chem. Commun.*, 219-220 (1972).
(15) P. Heitmann, R. Husung-Bubtitz, and H. J. Zunft, *Tetrahedron*, **30**, 4137-4140 (1974).
(16) K. Martinek, A. P. Osipov, A. K. Yatsimirski, V. A. Dadali, and I. V. Berezin, *Tetrahedron Lett.*, 1279-1282 (1975).
(17) K. Martinek, A. P. Osipov, A. K. Yatsimirski, and I. V. Berezin, *Tetrahedron*, **31**, 709-718 (1975).
(18) Y. Okahata, unpublished results in these laboratories.
(19) H. Gutfreund and J. M. Sturtevant, *Biochem. J.*, **63**, 656-661 (1956).
(20) See ref 10, Chapter 5, pp 169-189.
(21) R. A. Moss, R. C. Nahas, S. Ramaswami, and W. J. Sanders, *Tetrahedron Lett.*, 3379-3382 (1975).
(22) I. Tabushi, Y. Kuroda, and S. Kita, *Tetrahedron Lett.*, 643-646 (1974).
(23) T. Kunitake, S. Shinkai, and S. Hirotsu, *J. Polym. Sci, Polym. Lett. Ed.*, **13**, 377-381 (1975).
(24) K. Martinek, A. V. Levashov, and I. V. Berezin, *Tetrahedron Lett.*, 1275-1278 (1975).
(25) S. Shinkai and T. Kunitake, *Chem. Lett.*, 109-112 (1976).

Calculations of Optical Properties of Biliverdin in Various Conformations

G. Wagnière and G. Blauer*

Contribution from the Institute of Physical Chemistry, University of Zurich, 8001 Zurich, Switzerland, and Department of Biological Chemistry, The Hebrew University, Jerusalem, Israel. *Received April 15, 1976*

Abstract: Experimentally observed longer wavelength light-absorption and CD spectra of biliverdin–serum albumin complexes were compared with spectra calculated by an SCF–MO–CI procedure in the frame of an adapted PPP approximation and of the CNDO approximation. A number of selected bile-pigment conformations produced by skewing of bonds at the methene bridges were considered. Both initially "open" and "ring-like" structures of the biliverdin skeleton were taken into account. The variation of spectral changes with conformation indicates improved agreement between theory and experiment in certain intermediate forms between "open" and "ring-like" conformations rather than at either of the extremes. Such intermediate conformations can be produced by skewing a bond at an "outer" methene bridge, in addition to skewing it at the "inner" bridge. The present data give additional weight to the concept that the light absorption of bile pigments and related chromophores, such as those of phytochrome and phycocyanins, depends markedly on their molecular geometry.

In a previously reported work[1] we have tried to compute the absorption and CD spectra of the physiologically important tetrapyrrole bile pigments, bilirubin and biliverdin. The experimentally determined optical properties of the highly specific 1:1 complexes of these bile pigments with serum albumin in aqueous solution[2-5] were compared with those calculated by a suitable SCF–MO–CI procedure using the PPP approximation.[6] Local σ–π separation was assumed in the dipyrromethene chromophores.[1] In order to simplify these calculations, the side chains of both bilirubin and biliverdin were ignored. In the case of bilirubin, the observed energy splitting between the two longest wavelength transitions as well as the sign and magnitude of the corresponding rotatory strengths observed under various conditions was correlated semiquantitatively with the data calculated for various conformations of the bile pigment. These conformations were obtained by changing the magnitude and the sign of the two dihedral angles of rotation around the two single carbon–carbon bonds separating the two dipyrromethenes of bilirubin. An analogous treatment of the biliverdin skeleton at the center methene bridge did not result in satisfactory agreement between experimental and calculated absorption or CD spectra, except for the appearance of the longest wavelength band on the red edge of the spectrum. The latter would be expected for this highly conjugated system in which a methene bridge connects the two dipyrromethene chromophores. Thus, in contrast to bound bilirubin, the two longest wavelength transitions of biliverdin are widely separated and cannot be interpreted in terms of a splitting of the exciton type. The conformations of bound biliverdin considered were produced by skewing the center methene bonds of either "open" or "ring-like" structures. Since the ratio of the oscillator strengths of the two main observed transitions (near 660 and 380-390 nm, respectively) was intermediate between those of the "open" and "ring-like" conformations, it was suggested that some intermediate conformation of biliverdin or a mixture of "open" and "ring-like" structures were present in the material investigated.[1] Such intermediate conformations suggested for bound biliverdin, which should also be energetically reasonable, can be obtained by specific skewing of the chromophores around the dihedral angles assigned in pairs to *all three* methene bridges, while still assuming the individual five-membered rings to remain planar. This procedure is followed in the present work. It can be started from different planar structures and, obviously, it should involve a very large number of conformations. Even with fair agreement between theory and experiment, no selected conformation should be considered to be unique. The cases treated presently allow some limited conclusions on possible conformations of biliverdin.

Computations and Results. The dihedral angles δ_1-δ_6 which characterize the conformations of biliverdin are defined as a twist around the bonds of the methene bridges and are illustrated in Figure 1. The completely planar and closed conformation, which is sterically unlikely, has the values $\delta_1/\delta_2/\delta_3/\delta_4/\delta_5/\delta_6 = 0/0/0/0/0/0$. The sense of rotation for a given angle is determined by looking at the molecule from the corresponding end group toward the central carbon atom C-25. Counterclockwise rotation around a given bond i always corresponds to a positive increase of δ_i. If the nitrogen atoms are taken as pairwise equivalent, then the conformation 0/0/0/0/0/0 shows C_{2v} symmetry. All conformations for which simultaneously $\delta_1 = \delta_2$, $\delta_3 = \delta_4$, $\delta_5 = \delta_6$ then have a twofold axis of symmetry C_2, whereas in all cases with $\delta_1 = -\delta_2$, $\delta_3 = -\delta_4$, $\delta_5 = -\delta_6$ we find a plane of symmetry C_s.

In the choice of conformations to be considered in this investigation both nearly "ring-like" structures and very "open" geometries with δ_1 and δ_2 near 180° were ruled out for the reasons already indicated.[1] Furthermore, deviations of the

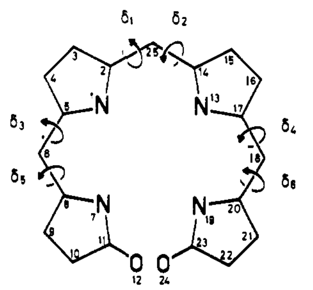

Figure 1. Numbering of atoms and definition of dihedral skewing angles δ_i in the biliverdin tetrapyrrolic skeleton (side chains neglected).

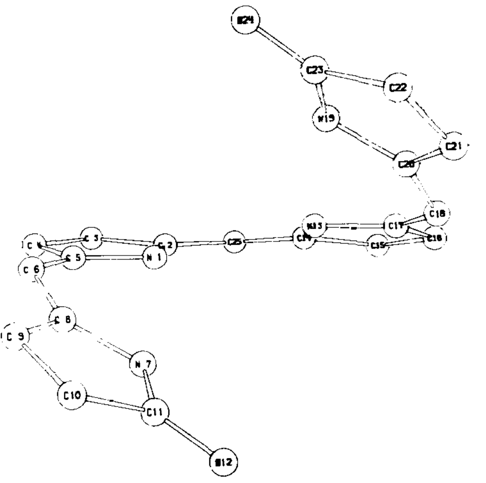

Figure 2. ORTEP[25] plot of the conformation −10/−10/−30/−30/0/0.

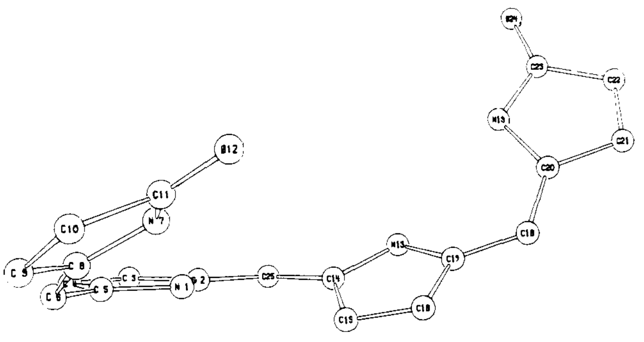

Figure 3. ORTEP plot of the conformation 0/−150/30/30/0/0.

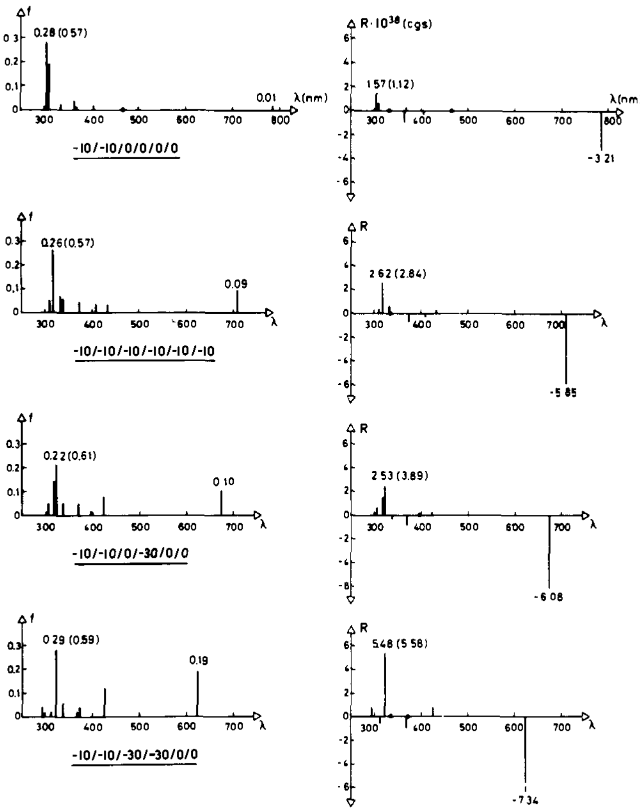

Figure 4. Oscillator strengths, f, and adjacent, corresponding rotatory strengths, R, computed from wave functions of the PPP type for related, more "ring-like" conformations, as indicated. The numbers for f and R in parentheses give the sum of the values for transitions 2–8; transition 1 is the longest wavelength transition.

dihedral angles of more than 30° from coplanarity were considered as unlikely, both on general energetic grounds and because of the predicted concomitant blue shift of the long-wavelength band. Figures 2 and 3 show ORTEP plots of the conformations −10/−10/−30/−30/0/0 and 0/−150/30/30/0/0, respectively. In the last-mentioned case we start from a relatively open geometry with $\delta_2 = -150°$ which by rotation of δ_3 and δ_4 by +30° is then twisted back into an "intermediate" conformation. All geometries considered in this investigation show an essentially left-handed chirality. However, in the case 0/−150/30/30/0/0 the concept of "chirality" is somewhat difficult to apply but may be inferred a posteriori from the sign of the long-wavelength Cotton effect.

The SCF-MO-CI calculations in the frame of the PPP approximation[6] were carried out as described in detail,[1] assuming local σ–π separation. The resonance integrals of the twisted bonds were multiplied by an appropriate cosine factor. The mixing of the 100 lowest singly excited configurations was taken into account. In biliverdin not all nitrogen atoms are electronically equivalent. In the lactam form presently considered, three of them are of the "pyrrole type" and contribute two electrons each to the (pseudo) π-electron system, whereas one nitrogen atom merely contributes one electron and is therefore of the "pyridine type". By hydrogen atom exchange different energetically close-lying tautomeric forms of the molecule are conceivable and may coexist. For simplicity, we have always assumed the same nitrogen atom N-13 to be of the "pyridine type". Although hydrogen atom exchange between N-1 and N-13 is certainly slow compared with the time of an electronic transition, we have nevertheless also performed a calculation for one case in which N-1 and N-13 are taken to be equivalent, with an average effective valence state ionization potential (in eV) of $\frac{1}{2}(19.60 + 13.40) = 16.50$.

The computation of the electric and magnetic transition moments follows the procedure of previous investigations.[7,8] In the frame of the PPP approximation, the axis of the $2p_\pi$ atomic orbital of an atom belonging to a given five-membered ring was taken to be perpendicular to the plane of this ring. The axis of the $2p_\pi$ atomic orbital of the central carbon atom of a methene bridge was assumed to be perpendicular to the plane defined by the three atoms forming the bridge. These $2p_\pi$ orbitals, represented by Slater functions with standard exponents (1.625, 1.950, and 2.275 for C, N, and O, respectively), were then expressed by their components in the reference coordinate system of the molecule. In computing the transition moments, all terms between every component on every atom were taken into account and multiplied by appropriate MO and CI coefficients (see Appendix). From the relatively crude wave functions the oscillator strengths and rotatory strengths were thus calculated without further approximations. The results for the eight lowest excited singlet states are summarized in Figures 4 and 5 and, for selected conformations, in Figure 6, and Tables I and II. As none of the conformations considered

Table I. Computed Results for the Biliverdin Conformation −10/−10/−30/−30/0/0[a]

Method	Excited state	λ, nm	$\langle\nabla\rangle_x$	$\langle\nabla\rangle_y$	$\langle\nabla\rangle_z$	$\langle r \times \nabla\rangle_x$	$\langle r \times \nabla\rangle_y$	$\langle r \times \nabla\rangle_z$	f	$10^{38}R$
PPP	1	622	−0.09509	0.02779	−0.10772	0.6786	0.0462	1.5589	0.195	−7.44
	6	322	0.00827	−0.24628	−0.01081	0.1471	−1.3425	0.2951	0.287	5.48
CNDO	1	571	−0.10645	−0.00851	−0.11197	0.7497	−0.0656	1.6801	0.200	−7.90
	2	331	0.02865	−0.22502	−0.00601	0.0370	−0.8305	0.2067	0.249	3.19
	3	307	−0.00356	−0.09727	−0.00320	0.0762	−0.4085	0.5441	0.043	0.60
	4	276	0.04095	0.01387	0.02797	−0.9723	0.2153	1.1784	0.011	−0.05

[a] The PPP results for excited states 1 and 6 only are given. The components of the dipole velocity transition moments, $\langle\nabla\rangle_x \equiv \langle\psi_a|\nabla|\psi_b\rangle_x \equiv \langle\psi_a|\partial/\partial_x|\psi_b\rangle$, etc. (see Appendix), are in au and the rotatory strengths in cgs units.

Table II. Computed Results for the Biliverdin Conformation 0/−150/30/30/0/0[a]

Method	Excited state	λ, nm	$\langle\nabla\rangle_x$	$\langle\nabla\rangle_y$	$\langle\nabla\rangle_z$	$\langle r \times \nabla\rangle_x$	$\langle r \times \nabla\rangle_y$	$\langle r \times \nabla\rangle_z$	f	$10^{38}R$
PPP	1	637	−0.16430	−0.11842	−0.04631	−0.0592	0.8385	0.2836	0.402	−3.38
	6	324	0.12002	−0.09312	0.16984	−1.0453	−0.8553	2.7541	0.246	7.06
CNDO	1	584	−0.16385	−0.13753	−0.03371	−0.0803	0.8254	0.6199	0.400	−3.66
	2	332	0.11634	−0.11390	0.14720	−0.6235	−0.4789	1.6800	0.234	3.94
	3	312	−0.05568	−0.03153	−0.00946	0.0604	0.3293	−0.4148	0.019	−0.16
	4	279	0.06126	0.11029	0.08246	−0.6569	−0.9195	0.9224	0.093	−0.95

[a] See also footnote *a*, Table I.

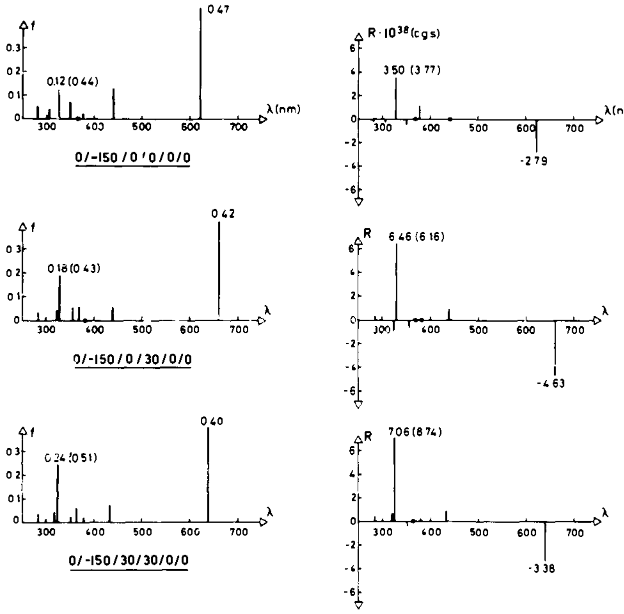

Figure 5. Oscillator strengths and rotatory strengths computed from wave functions of the PPP type for related, more "open" conformations, as indicated (see also legend to Figure 4).

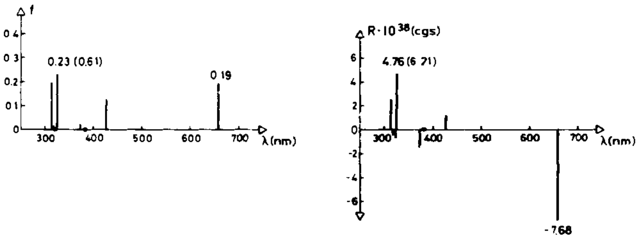

Figure 6. Oscillator strengths and rotatory strengths computed from wave functions of the PPP type for the conformation −10/−10/−30/−30/0/0. The effective valence state ionization potential is here taken to be the same for both nitrogen atoms 1 and 13 (see text; see also legend to Figure 4).

are planar, attention must be given to the question of σ–π interaction. For this reason we have also performed CNDO calculations on the two conformations −10/−10/−30/−30/0/0 and 0/−150/30/30/0/0, considering all 122 valence electrons of the system. We used a CNDO program written by Kuhn.[9] The determination of the one-center and two-center core integrals followed mainly the procedure described by Sichel and Whitehead[10] and Wratten,[11] respectively. However, the absolute value of the resonance integrals was slightly increased and in the formula for β_{AB} the overlap integrals of the π type were multiplied by a factor of 0.7.[12,13a] This has the effect of further lowering the low-lying σ orbitals, which otherwise appear at relatively too high energies. The two-electron γ integrals are determined by the charged-sphere model. The one-center γ integrals are tabulated in ref 12. Subsequent to the SCF part, the mixing of the lowest 120 singly excited electronic configurations was taken into account. The electric and magnetic transition moments were computed without further approximations, as already described.[7] Results are shown in Tables I and II.

Discussion

Both light-absorption and CD spectra of bound biliverdin in aqueous solution have been reported earlier[5] (see also ref 1) and constitute the experimental basis for the comparison with the computed data given below. The observed light absorption of the biliverdin–human serum albumin (HSA) 1:1 complex at pH 7.4 consists mainly of a broad band around 660 nm ($\epsilon_{max} \simeq 2 \times 10^4$ M^{-1} cm^{-1})[13b] and a prominent band at 380–390 nm ($\epsilon_{max} \simeq 4 \times 10^4$ M^{-1} cm^{-1}). The approximate oscillator strengths f_K of these bands are $f_1 \simeq$ 0.2–0.3 and $f_2 \simeq$ 0.9, respectively, and the ratio $f_2/f_1 \simeq 3.3$. The corresponding CD spectrum is characterized by a negative band at the red end (estimated $[\theta]_{max} \simeq -1 \times 10^5$ deg cm^2 dmol^{-1} [13b] at 640–650 nm). The band near 380 nm shows $[\theta]_{max} \simeq 2.7 \times 10^5$ deg cm^2 dmol^{-1}. Resolution of this spectrum into Gaussian curves gives an estimated rotatory strength R_K of about −1.7 DBM for the CD band at the red end and R_K of about +2.9 DBM for the main band near 380 nm; in addition, a smaller band near 535 nm with $R_K \simeq -0.2$ DBM appears to be resolved.[5] [The above θ and R values are corrected by a calibration factor of 1.07 according to ref 3.]

In the previous work,[1] only the central dihedral angles δ_1 and δ_2 were allowed to vary, starting from either "ring-like" ("closed") or "open" structures. For comparison, some of the previous results are reproduced in Figures 4 and 5. The oscillator strength ratio f_2/f_1 between the near-ultraviolet and red bands was too large in the "ring-like" conformations, 30/0/0/0/0/0 or −10/−10/0/0/0/0, and too small in the "open" conformations, 150/0/0/0/0/0 or 150/180/0/0/0/0, as compared with the experimental value $f_2/f_1 \simeq 3$. The presence of either an energetically unlikely conformation $|\delta_1 + \delta_2| \simeq$ 50–70° or of a mixture of "ring-like" and "open" structures in the biliverdin preparations used was considered.[1] However, a more likely possibility of obtaining intermediate conformations, by skewing of additional bonds (δ_3–δ_6), has been suggested.[1] Such cases have now been calculated. Results are summarized in Figures 4 and 5 and Tables I and II.

As in the previous work,[1] the absolute values of the skewing angles, starting from different planar structures, were taken to be either 10° or 30°, although the latter angle may be too large from energetic considerations (see, however, ref 14 where twists of 40° at the methene bridge were taken into account).

The absorption spectrum computed within the PPP approximation is now considered. In order to assess the oscillator strength f_2 of the second, shorter wavelength band, the computed values for the transitions within the range of 280–450 nm are added. It appears from Figures 4 and 5 that the ratio $f_2/f_1 = 3$ is best reproduced by the conformation −10/−10/−30/−30/0/0 if the added value for f_2 (in parentheses) is taken and by the conformation −10/−10/−10/−10/−10/−10 if merely the strongest computed transition in the 350-nm region is being considered. These conformations may be characterized as relatively close to "ring-like" and helically skewed. It may be noticed that the absolute values of the computed oscillator strengths are rather too low as compared with the experiment (Figure 7). The value of f_2/f_1 is very sensitive to conformational changes; it becomes significantly too small in all the cases where $|\delta_2| = 150°$. Qualitatively similar conclusions were reached in recent measurements and calculations performed for planar biliverdin conformations by Chae and Song[15] (see also ref 16). Hückel MO calculations carried out by Burke et al.[17] on various conformations of the planar phytochrome chromophore, the structure of which closely resembles that of biliverdin, are also in qualitative agreement with our calculations pertaining to the long-wavelength absorption.

With regard to the calculated CD spectra of biliverdin (Figures 4–6; Tables I and II), the relatively best agreement of R ratios is obtained in the conformation 0/−150/0/30/0/0, as compared with the experimental value of $R_2/R_1 \simeq -1.7$. Most cases considered give the correct order of magnitude for the rotatory strengths. As with oscillator strengths, R values between about 280 and 450 nm were also added to a value given in parentheses for comparison with the (possibly composite) experimental band in that wavelength region. Unexpectedly, the computed CD spectra appear to be less indicative of conformational changes than the light-absorption spectra (see also ref 1). The most likely conformations suggested by the CD data do not exactly coincide with those derived from light-absorption spectra. However, conformations such as 0/−150/0/30/0/0 or 0/−150/30/30/0/0 may be viewed as being related to a relatively "open" conformation 0/−150/0/0/0/0, which is then twisted back into more "ring-like" structures. A large number of further calculations on additional conformations intermediate between "open" and "ring-like" (e.g., skewing of δ_5 or δ_6, in addition to δ_3 and δ_4, respectively, which would be less likely) might improve the simultaneous agreement of both absorption and CD data with experiment. However, the limitations inherent in the present calculations scarcely justify a large number of additional computations. Moreover, it is difficult to ascertain the uniqueness of the results obtained.

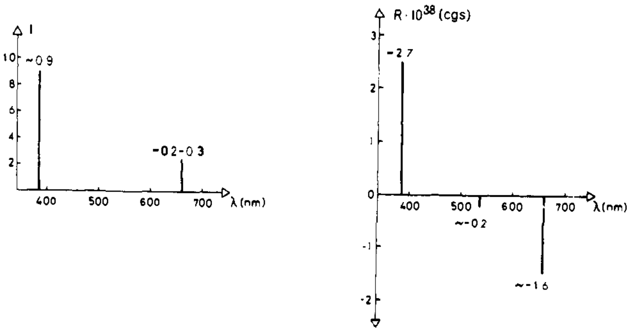

Figure 7. Schematic representation of the experimentally deduced oscillator strengths and rotatory strengths for biliverdin in the HSA complex at pH 7.4 (see Discussion and ref 5).

It may be noted that in all cases calculated, the position of the center of the second main band—experimentally near 380 nm—appears at a shorter wavelength (around 320 nm). Only part of this difference can be attributed to the elimination of the two vinyl side chains for the purpose of the calculation.

If in the computation of the conformation −10/−10/−30/−30/0/0 the effective valence state ionization potentials of N-1 and N-13 are taken to be equal (16.50 eV), the predicted spectrum does not change appreciably (see Figure 6).

The CNDO results (Tables I and II) for the two above-mentioned selected conformations, intermediate between "open" and "ring-like", are in reasonable agreement with the PPP results and do not basically alter our semiquantitative conclusions. There is a surprising similarity in the data obtained for the longest wavelength transition, considering the computational differences. However, the CD data (R_2/R_1 ratio) predicted for the conformation 0/−150/30/30/0/0 agree less well with experiment than in the PPP computation because of the smaller absolute value of the rotatory strength of the second transition. It is worth noting that the results concerning the second CNDO transition are best compared with the sixth PPP transition, which in both molecular conformations considered makes the dominant contribution in the 300–350-nm region. The question may then be raised if some of the other transitions predicted by the PPP method in the 300–400-nm region, with relatively small oscillator and rotatory strengths, are artifacts. In the CNDO method these (possibly virtual) states are conceivably pushed up the energy scale and mix with additional electronic configurations absent in the PPP approximation.

Since free biliverdin in aqueous solution and in the absence of protein does not exhibit significant optical activity in the visible region,[5] it may be assumed that the free bile pigment either has corresponding conformational symmetry or is present in a racemic mixture of two enantiomeric conformers of equal stability. The similarity of the light-absorption spectra of free and HSA-bound biliverdin[5] suggests, in accordance with the present computations, that the biliverdin conformation does not change largely upon binding to the protein. Hence, the large optical activity observed upon binding of the bile pigment to HSA must involve either some additional skewing of the biliverdin chromophore in a left-handed conformation[1] or selective binding of one biliverdin enantiomer by the dissymmetric protein binding site with concomitant rapid racemization and binding of the other enantiomer. In the case of biliverdin complexes with bovine serum albumin, a pH-dependent inversion in the sign of both main CD bands has been observed, showing nearly mirror-image spectra.[5] It may be assumed that a difference in pH at the binding site of the protein inverts the chirality of the bound bile pigment, in a similar way as has been suggested for the bilirubin-HSA complex.[3] Interpretation of CD spectra measured on complexes of bilirubin with albumins from different species[18] also suggests in some cases a difference in chirality of the bound bile pigment caused by a corresponding difference at the binding site.

It should be noted that the optical data obtained experimentally with the biliverdin preparation used[5] may differ from those of biliverdin in vivo, as the conformations of biliverdin may also be different in both cases. The large dependence of the calculated oscillator strengths of biliverdin on its conformation (see also ref 1 and 15) may explain at least part of the differences in absorption characteristics of various biliverdin preparations, since its conformation may depend on the mode of preparation (see, for example, ref 19 and 20). The results obtained presently for biliverdin may also be relevant for a better understanding of the properties of other biliverdin-like pigments such as the chomophores of phycocyanins and of phytochrome.[17,21-24] The effects of skewing bonds in bile pigments other than biliverdin are now under investigation.

Acknowledgments. We are grateful to Mr. M. Iseli for assistance with the computations and to Dr. L. Linowsky for performing the plots with program ORTEP. We thank the Swiss National Foundation for financial support and the computer centers of the University of Zurich and the Federal Institute of Technology for the allotment of computer time.

Appendix

We recall here the formula used to obtain the rotatory strength between the ground state ψ_a and an excited state ψ_b

$$R_{ab} = \frac{e^2\hbar^2}{2m^2c(E_b - E_a)} \operatorname{Im}[\langle\psi_a|\nabla|\psi_b\rangle\langle\psi_b|\mathbf{L}|\psi_a\rangle]$$

where ∇ is the sum of the dipole velocity operators of the individual electrons and $\mathbf{L}$ is the corresponding angular momentum operator. With ψ_a written as a one-determinant function of doubly filled SCF MO's, φ_i, and ψ_b as a linear combination of singly excited configurations, one obtains

$$\langle\psi_a|\nabla|\psi_b\rangle = \sqrt{2}\sum_{ik} B_{ik}\langle\varphi_i|\nabla|\varphi_k\rangle$$

$$\langle\psi_b|\mathbf{L}|\psi_a\rangle = \sqrt{2}\sum_{ik} B_{ik}\langle\varphi_k|\mathbf{L}|\varphi_i\rangle$$

where on the right ∇ and $\mathbf{L}$ are now one-electron operators and the B_{ik} are the configuration interaction (CI) coefficients for the excited state. The rotatory strength then becomes

$$R_{ab} = \frac{e^2\hbar^3}{m^2c(E_b - E_a)}\sum_{ik}\sum_{i'k'} B_{ik}B_{i'k'} \times \langle\varphi_i|\nabla|\varphi_k\rangle\langle\varphi_{i'}|\mathbf{r}\times\nabla|\varphi_{k'}\rangle$$

The oscillator strength f_{ab} is obtained from $\langle\psi_a|\nabla|\psi_b\rangle$ by using the relation

$$\langle\psi_a|\mathbf{r}|\psi_b\rangle = \frac{\hbar^2}{m(E_b - E_a)}\langle\psi_a|\nabla|\psi_b\rangle$$

(although only approximately valid between approximate wave functions) and the well-known formula for f (see, for example, Herzberg, ref 26). Although the semiempirical wave functions used are approximate, the transition moments $\langle\varphi_i|\nabla|\varphi_k\rangle$, $\langle\varphi_i|\mathbf{r}\times\nabla|\varphi_k\rangle$ are computed without further approximations,[7] the φ_i being linear combinations of appropriate Slater orbitals.

References and Notes

(1) G. Blauer and G. Wagniere, *J. Am. Chem. Soc.*, **97**, 1949 (1975).
(2) G. Blauer and T. E. King, *J. Biol. Chem.*, **245**, 372 (1970).
(3) G. Blauer, D. Harmatz, and J. Snir, *Biochim. Biophys. Acta*, **278**, 68 (1972).
(4) G. H. Beaven, A. d'Albis, and W. B. Gratzer, *Eur. J. Biochem.*, **33**, 500 (1973).
(5) G. Blauer and B. Zvilichovsky, *Isr. J. Chem.*, **11**, 435 (1973).
(6) R. G. Parr, "Quantum Theory of Molecular Electronic Structure", W. A. Benjamin, New York, N.Y., 1963, and references cited therein.
(7) W. Hug and G. Wagnière, *Theor. Chim. Acta*, **18**, 57 (1970).
(8) G. Wagnière, *Jerusalem Symp. Quantum Chem. Biochem.*, 127 (1971).
(9) J. Kuhn, Ph.D. Thesis, University of Zurich, 1971.
(10) J. M. Sichel and M. A. Whitehead, *Theor. Chim. Acta*, **7**, 32 (1967).
(11) R. J. Wratten, *Chem. Phys. Lett.*, **1**, 667 (1968).
(12) W. Hug, J. Kuhn, K. Seibold, H. Labhart, and G. Wagniere, *Helv. Chim. Acta*, **54**, 1451 (1971).
(13) (a) J. Kuhn, W. Hug, R. Geiger, and G. Wagniere, *Helv. Chim. Acta*, **54**, 2260 (1971). (b) Both the molar extinction coefficients ϵ and the molar ellipticity $[\theta]$ are based on the total bile pigment.
(14) H. Falk, K. Grubmayr, U. Herzig, and O. Hofer, *Tetrahedron Lett.*, **8**, 559 (1975).
(15) Q. Chae and P.-S. Song, *J. Am. Chem. Soc.*, **97**, 4176 (1975).
(16) J.-H. Fuhrhop, A. Salek, J. Subramanian, C. Mengersen, and S. Besecke, *Justus Liebigs Ann. Chem.*, 1131 (1975).
(17) M. J. Burke, D. C. Pratt, and A. Moscowitz, *Biochemistry*, **11**, 4025 (1972).
(18) D. Harmatz and G. Blauer, *Arch. Biochem. Biophys.*, **170**, 375 (1975).
(19) C. H. Gray, A. Lichtarowicz-Kulczycka, D. C. Nicholson, and Z. Petryka, *J. Chem. Soc.*, 2264 (1961).
(20) J. J. Lee and M. L. Cowger, *Res. Commun. Chem. Pathol. Pharmacol.*, **5**, 505 (1973).
(21) B. L. Schram and H. H. Kroes, *Eur. J. Biochem.*, **19**, 581 (1971).
(22) W. Rüdiger, *Angew. Chem.*, **82**, 527 (1970).
(23) G. Blauer, *Struct. Bonding (Berlin)*, **18**, 69 (1974).
(24) H. Suzuki, T. Sugimoto, and K. Ishikawa, *J. Phys. Soc. Jpn.*, **38**, 1110 (1975).
(25) C. K. Johnson, ORTEP Report ORNL-3794, Oak Ridge National Laboratories, Oak Ridge, Tenn., 1965.
(26) G. Herzberg, "Molecular Spectra and Molecular Structure. I. Spectra of Diatomic Molecules", Van Nostrand, New York, N.Y., 1950, p 383.