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Dedicated to professor Gh. Marcu at his 80th anniversary

MOLECULAR PACKING OF SOME CAROTENOIDS IN LANGMUIR MONOLAYERS AT THE AIR/WATER INTERFACE

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ABSTRACT. The characteristic molecular areas were determined from surface pressure versus molecular area isotherms of three carotenoids: β,β-carotene-4-one (echinenone, ECH), β,β-carotene-4,4'-dione (canthaxanthin, CAN) and 4,4'-diapo-ψ,ψ-carotene-4,4'-dial (APO), spread and compressed as Langmuir monolayers at the air/water interface. Quantum chemical semi-empirical SCF MO calculations (AM1 and PM3) are performed for the optimized geometries of carotenoid molecules and similar theoretical results are obtained by both methods. The characteristic surface molecular areas are discussed in terms of molecular packing in Langmuir monolayers and intermolecular interactions, respectively. The orientation of these carotenoid molecules in the Langmuir monolayers is discussed by using a rotating rigid plate model and optimized molecular geometries.

Keywords: *carotenoids, Langmuir monolayers, molecular packing, molecular structure, semi-empirical MO calculations.*

INTRODUCTION

Langmuir monolayers are obtained by spreading amphiphilic organic molecules using an appropriate solvent or a mixture of organic solvents at the air/water interface [1-3]. Generally, these monolayers are compressed up to their collapse and they are employed as model systems in studying surface phenomena, associated with various applications in science and technology [4]. In particular, carotenoids can be potential key elements for molecular electronic devices. Also, carotenoids are essential biological active molecules, which can influence or modulate the properties of cellular and subcellular structures [5, 6]. Therefore, the behavior of Langmuir monolayers of carotenoids is of interest and is investigated at the air/water interface both in pure state [7-10] and in mixtures with lipids [11-14] or electrolytes [15-18]. The obtained data show that the local ordering and molecular packing of carotenoids in Langmuir monolayers at the air/water interface depend primarily on the carotenoid molecular structure for the same aqueous phase and at similar conditions for compression rate and spreading rate.

The objective of this paper is to investigate experimentally and theoretically how the equilibrium intermolecular interactions and hydrophilic and hydrophobic forces affect the molecular packing of some carotenoids in Langmuir monolayers at the air/water interface. In the following the rotating rigid plate model previously proposed by us [19] for carotenoid molecules is reconsidered, and used to explain the molecular

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packing of the three chosen carotenoids for this study. Optimized geometries of the molecules are used, as resulted from semi-empirical MO computations. Finally, the obtained results are summarized in the conclusion section of this paper.

THE ROTATING RIGID PLATE (RRP) MODEL OF CAROTENOIDS

In order to correlate the structural and geometrical characteristics of carotenoid molecules with their surface properties as monolayers at the air-water interface, the rotating rigid-plate model of carotenoid molecules was previously proposed [19-21]. In the framework of this model, the molecule (all-*trans* isomer) is considered to be rigid (due to its delocalized π-bond system), but free rotation about the simple σ-bonds has also been taken into account, the most stable conformation being assumed. For the bond lengths and bond angles, experimental data (from X-ray analysis) have been used, when available (for canthaxanthin [22]); otherwise they have been approximated from the bond lengths and covalent radii given in chemical tables and the bond angles from the hybridization types.

The hydration of the polar headgroup of the molecule, immersed in water, was modeled by the inclusion of hydrogen-bridged water molecules. Two H_2O molecules are considered to be linked to the C=O group, thus anchored in the water phase.

The carotenoid molecule is seen as a parallelepiped; its length, *c*, is taken as the length of the chain axis (*c*'), including the hydration water molecules (see Fig.1 for canthaxanthin molecule). The length of the headgroup axis (*a*'), and also including hydration water, *a*, is the width of the molecular plate, while *b* represents its thickness – given mainly from the out-of-plane hydrogen and carbon atoms [21].

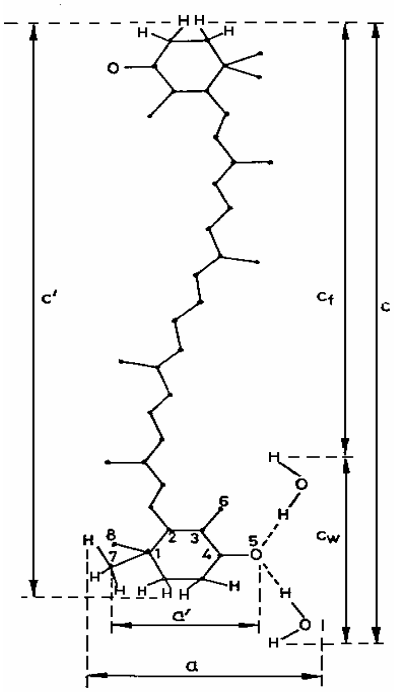


Fig.1. Rigid plate model of the canthaxanthin molecule [1]

In the estimation of the molecular areas in the monolayer at the air/water interface, arguments were given for a calculation in which the molecule is assumed to perform a free rotation about the vertical chain axis, and the resulting vertical cylinders adopt a tetragonal close packing [19, 21]. This does not necessarily imply a real rotation, but also a random orientation of the head group axis in the interface plane. Therefore, since $b < a$, the mean area occupied by a molecule in the interface layer should be $A_4 = a^2$, instead of $A_p = ab$, corresponding to a close packing of vertical rigid plates with parallel orientation.

Another estimated characteristic of the monolayer was the immersed fraction α of the molecules, defined as the ratio between the length of the part of the chain immersed in water, as determined by the hydration, c_w (Fig.1) and the total chain length, *c*:

$$\alpha = c_w/c \quad (1)$$

The free part of the chain, c_f is therefore c-c_w For echinenone and canthaxanthin, the values obtained from this model [19] were: a = 0.78 nm, b = 0.49 nm, c = 3.2 nm, c_w = 0.7 nm, α = 0.22 and A_4 = 0.61 nm^2, A_p = 0.38 nm^2.

The present paper deals with the application of the RRP model to three carotenoid pigments, namely β,β-carotene-4-one (echinenone, ECH), β,β-carotene-4,4'-dione (canthaxanthin, CAN) and 4,4'-diapo-ψ,ψ-carotene-4,4'-dial (4,4'-diapolycopenedial, APO) (Fig. 2), using their molecular structure, as resulted from semi-empirical MO calculations (AM1 and PM3).

EXPERIMENTAL PART

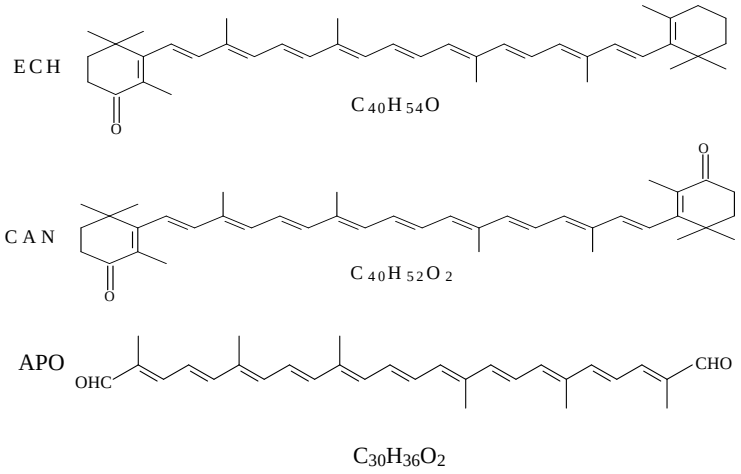


Fig.2. Molecular structure of carotenoids: β,β-carotene-4-one: ECH, β,β-carotene-4,4'-dione: CAN and 4,4'-diapo-ψ,ψ-carotene-4,4'-dial: APO.

The three natural carotenoid pigments (ECH, CAN and APO) used here were supplied by Hoffmann-La Roche and present *all-trans* configuration. The high purity spreading solvents, benzene pro-analysis or a mixture of benzene and 2-4% absolute ethanol, were purchased from Merck. Known amounts of the compounds dissolved in the spreading solvents were placed by means of a Hamilton syringe or a micropipette on the air/water interface. The water subphase was double distilled water with a resistivity of 18 Mohm cm^{-1}. Compression of the monolayer was started after about 10 minutes, allowed for solvent evaporation. Then, the spread monolayer of each carotenoid compound was compressed at a chosen rate of compression in the interval from 0.01 to 0.03 nm^2/(molecule·min), by using the Langmuir equipment KSV 5000. The reproducible compression isotherms, in terms of surface pressures versus molecular areas, were recorded at 20 ^{o}C as described elsewhere [5]. The surface pressure was measured by the Wilhelmy method within the error of ± 0.2 mN/m. The compression curves were reproducible within 0.02 – 0.04 nm^2/molecule. The results do not depend on the compression rate in the region used. Each isotherm given in this paper represents the mean of at least 10 different recordings.

COMPUTATIONAL PART

MO calculations were performed, regarding σ and π electrons, on the three carotenoids (ECH, CAN and APO) in their all-trans forms [23]. Computations were made at the restricted Hartree-Fock (RHF) level using two semi-empirical SCF MO methods: Austin Model 1, AM1 [24] and the Parametric Model number 3, PM3 [25], by means of HyperChem 7.5 software package [26]. The two methods are based on the Neglect of Differential Diatomic Overlap (NDDO) integral approximation, and differ by their parameterization. The computation options were: total charge: 0; spin multiplicity: 1 (singlet); state: lowest. The geometries of the molecules were optimized by the Polak-Ribiere (conjugate gradient) algorithm approach. The SCF convergence limit was of 0.042 kJ/mol, and RMS gradient was of $4.18 \cdot 10^9$ kJ/(m×mol).

For the optimized geometry, energetical parameters (total electronic energies, enthalpies of formation), electron distributions (charge densities and bond orders) and geometrical parameters (bond lengths, bond angles, torsion angles) were obtained [23]. Both computational methods gave quite similar results for the geometry of the molecules. The first inertial axis of the molecule is roughly going along the conjugated chain of the molecules, while the second axis is perpendicular to it in the molecular plane.

RESULTS AND DISCUSSION

The ***compression isotherms***, in terms of surface pressure (π, mN/m) versus mean molecular area (A, nm^2) curves for three carotenoids, viz. β,β-carotene-4-one (ECH), β,β-carotene-4,4'-dione (CAN) and 4,4'-diapo-ψ,ψ-carotene-4,4'-dial (APO), spread at the air/water interface, are presented in Fig. 3.

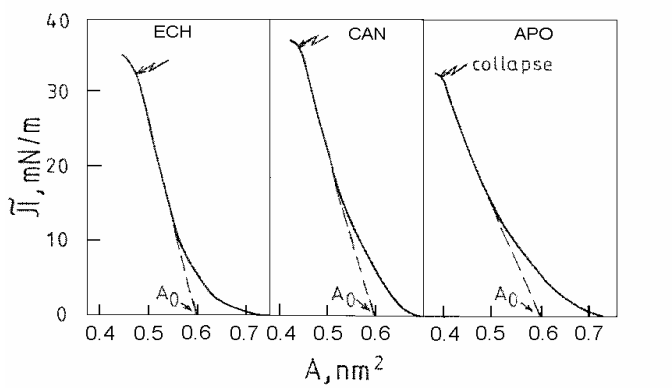


Fig.3. Compression isotherms: surface pressure versus mean molecular area for carotenoids: β,β-carotene-4-one (ECH), β,β-carotene-4,4'-dione (CAN) and 4,4'-diapo-ψ,ψ-carotene-4,4'-dial (APO) spread as monolayers at the air/water interface, at 20 ^oC.

From these compression isotherms, surface properties were determined, namely: the limiting molecular area A_0 (see Fig. 3), obtained by extrapolating to π= 0 the high pressure linear portion of the compression isotherm; A_c, is the collapse area and the corresponding surface pressure noted π_c, the collapse pressure (Table 1).

Table 1.

Surface characteristics of the three carotenoids studied.

Carotenoid	π_c (mN m^{-1})	A_0 (nm^2)	A_c (nm^2)
ECH	32	0.60	0.48
CAN	36	0.60	0.44
APO	32	0.60	0.40

The ***optimized molecular geometry***, as resulted from the PM3 calculations, is visualized for the three molecules in the plane of the first two inertial axes in Fig.4.

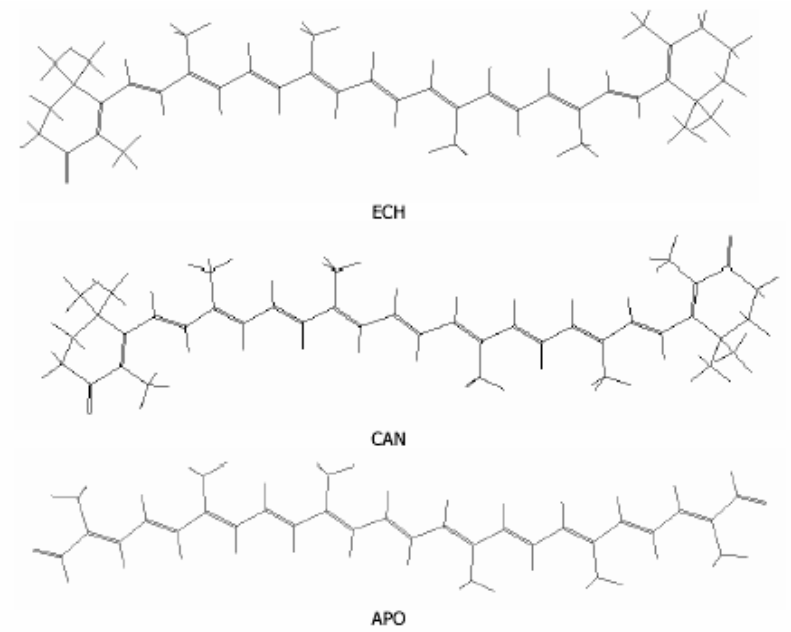
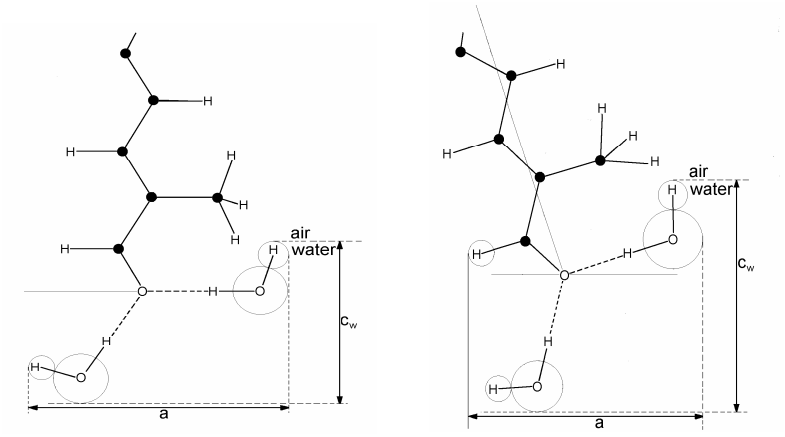


Fig. 4. Optimized geometries (PM3 calculation) for the three carotenoid molecules, represented in the plane of the first and second inertial axes.

For a discussion of the **molecular orientation and packing at the air/water interface,** on these models the length of the chain was measured, as the maximum size in the direction of the first axis: 2.93 nm (ECH), 2.94 nm (CAN), and 3.02 nm (APO). Since in these representations, distances between atomic nuclei are obtained, the covalent radii of the end atoms (H for ECH and CAN, O for APO) should be added, what gives for the lengths *c*' (Fig. 1) the values: 2.99 nm (ECH), 3.00 nm (CAN), and 3.15 nm (APO).

Following the considerations given in the earlier treatment [19], about 0.1 nm have to be added to *c'* for the lower water molecule bonded to the C=O group in ECH and CAN, thus obtaining a *c*-value of about 3.1 nm for these two molecules. As for the immersed part of these chains, given by the vertical projection of the two O···H-O bridges and of the other two O-H bonds, it was estimated to be c_w = 0.7 nm, the immersed fraction (eq. 1) being α = 0.23.



(A) **(B)**

Fig.5. The shape of the lower part of the APO-molecule and the size of its hydrated polar head group for a vertical orientation of the molecular axis – 1st inertial axis (A) and for an angle of 20° to the vertical (B)

For the APO molecule, a similar model was sketched in Figure 5A, for the lower part of the molecular chain. An angle of 60° of the C=O bond to the air-water interface was assumed, corresponding to a vertical orientation of the conjugated chain, as in earlier MO studies on this compound [27, 28]. Adding 0.23 nm for the vertical projection of the lower O···H-O bridge, we obtain for the total (hydrated) chain length *c* = 3.38 nm. As for the immersed part of the chain, besides the contribution of the lower water molecule (0.23 nm + oxygen radius), we have to consider the vertical projection of the O-H bond of the second water molecule and the H radius. This gives a c_w value of about 0.43 nm and an immersed fraction α = 0.13, less than for the other two molecules.

A previous investigation on these three carotenoid molecules [23], correlating surface properties with MO calculated quantities, viz. dipole moments, has suggested that the APO molecules should deviate from the vertical, i.e. to be inclined in order to decrease the angle of the C=O bond to the air/water interface. This orientation would allow for an increased interaction with the water molecules. The angle should be about 40°, i.e the deviation of the molecule axis from the vertical, of about 20° (Fig. 5B). For this orientation, the depth of the hydration layer, given by the two hydrogen-bonded water molecules, would be c_w = 0.56 nm and the length of the immersed part of the molecular chain about 0.6 nm. The total length of the hydrated chain is given by adding to *c'* the contribution of the lower water molecule

to the hydration, about 0.29 nm, and is therefore c = 3.44 nm. The immersed fraction α becomes now 0.17. Thus the inclination allows for a better hydration of the APO molecule.

The areas occupied by the molecules in the monolayer can be estimated from the molecular volumes, *V*, calculated as QSAR properties by the HyperChem software, divided by the calculated length of the molecule *c*'. These values are given in Table 2 and are in quite good agreement with the experimental values of extrapolated molecular areas, A_o [19], obtained by extrapolating the high pressure linear portion of the surface pressure – area curves to pressure π = 0.

Table 2.

Calculated and experimental molecular areas in monolayers

Molecule	*c*' (nm)	*V* (nm^3)	*V/c'* (nm^2)	*a* (nm)	*b* (nm)	A_4 (nm^2)	A_p (nm^2)	A_o (nm^2)	A_c (nm^2)
ECH (1)	2.99	1.8334	0.613	0.74	0.56	0.548	0.414	0.60	0.48
(2)	3.10			0.87		0.757	0.487		
(3)	3.2			0.78	0.49	0.608	0.38		
CAN (1)	3.00	1.8292	0.610	0.75	0.52	0.562	0.39	0.60	0.44
(2)	3.10			0.88		0.774	0.458		
(3)	3.2			0.78	0.49	0.608	0.38		
APO (1)	3.15	1.4923	0.474	0.60	0.24	0.36	0.144	0.60	0.40
(2)	3.38			0.585		0.342	0.14		

(1) without hydration; (2) with hydration; (3) estimated in [1]

On the assumption that the dimensions of the "molecular plate", are in first approximation colinear with the inertial axes: *c* with the 1st, *a* with the 2nd and *b* with the 3rd axis, we can evaluate these dimensions. As above for *c*, from the representations of the optimized geometries of the molecules in the planes of the first and second inertial axes (Fig.4), in the plane of the first and third axes or of the second and third inertial axes (Fig. 6) the other two dimensions of the molecules, *a* and *b*, can be measured. In Table 2, these values are given as read from the diagrams with addition of the radii of the end atoms (1), and with addition of the hydration water molecules (2). The values estimated in [19] are also given (3). For the APO molecule, the value (2) was obtained from the model (Fig.5A) of the hydrated head group.

The molecular areas have been calculated from these 3 kinds of molecular dimensions:

(i) from the model of the freely rotating molecular rigid plates, with tetragonal close packing, $A_4 = a^2$;

(ii) from the model of close packing of parallel oriented rigid plates, $A_p = ab$

These values are also given in Table 1. The A_c values are the molecular areas at the collapse of the carotenoid monolayer.

The A_4 values estimated in [19] for ECH and CAN are in good agreement with the experimental A_o values. The A_4 values calculated form the values (2) are larger than the values reported in [19] (3). The explanation is that the *a*-values (3) were estimated for the head group only, while the *a*-values (2) refer to the "width" of the entire molecule, in its real geometry. The same does apply to the "thickness" *b*-values (1) and (3).

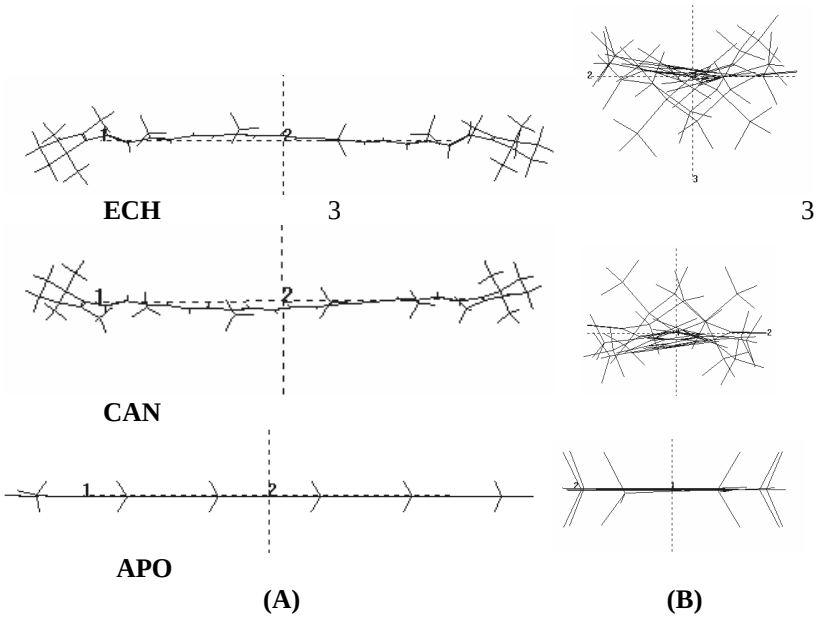


Fig.6. The carotenoid molecules, represented in the plane of the first and third inertial axes (A) and of the second and third inertial axes (B). The scales of the representations are different.

The A_p values (2), calculated from the dimensions of the hydrated ECH and CAN molecules, are in much better agreement with the molecular collapse areas than the estimated values (3); moreover, they give also the right order (ECH > CAN), while the values estimated in [19] make no difference between the two molecules. It can be assumed that near the collapse pressure, the free rotation of the molecules is hindered and the head group axes tend to assume a parallel orientation.

The APO molecule represents a different case. The lower immersed fraction for this substance brings about that higher energies are necessary to perform a vertical orientation of the chain axes. The molecules seem to keep their inclined orientation even at the collapse, due to the weak intermolecular interactions, as suggested in [23]. Therefore, the molecular areas calculated for a vertical orientation are much lower than the experimental A_o and A_c values.

CONCLUSIONS

Three carotenoid molecules, ECH, CAN, and APO, have been studied in Langmuir monolayers at the air/water interface. The surface characteristics, like limiting molecular areas, collapse areas and collapse pressures are well correlated with the carotenoid molecular structures and the molecular orientation at the air/water interface. The obtained data are explained by the interplay of the intermolecular hydrophilic and hydrophobic forces which lead to stable Langmuir monolayers, with

the rotating molecules tetragonally packed in the liquid state of the monolayers (rotating rigid plate model of the carotenoid molecules). At collapse, the non-rotating carotenoid molecules are close packed in Langmuir monolayers.

Quantum chemical semi-empirical SCF MO calculations (AM1 and PM3) are performed for the optimized geometries of carotenoid molecules. From the obtained geometry of the molecules, the parameters for the rotating rigid plate model of the molecules could be estimated, in good agreement with the experimental values for the limiting molecular area A_0 and the collapse area A_c. As regards the orientation of these carotenoid molecules in the monolayer, for the echinenone and canthaxanthin molecules a perpendicular direction of the molecular axis to the air/water interface is found, while for the 4,4'-diapo-ψ,ψ-carotene-4,4'-dial (APO) molecule a deviation from the vertical is suggested.

It would be of interest to study other molecules also having the poly-ene chain attached to beta–ionone rings or to a flexible moiety like an alkyl chain.

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