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MOLECULAR STRUCTURE AND MONOLAYER PROPERTIES OF SOME CAROTENOIDS

MARIA TOMOAIA-COTISEL[a], CSABA RACZ[a], GHEORGHE TOMOAIA[b], CALIN FLOARE[c], ROBERT TOTOS[d], LIVIU BOBOS[a], OANA PASCU[a] and ANCA DUMITRU[a]

[a]"Babes-Bolyai" University of Cluj-Napoca, Department of Physical Chemistry, 400028 Cluj-Napoca, Romania
[b]"Iuliu Hatieganu" University of Medicine, Department of Orthopaedic Surgery, 400015 Cluj-Napoca, Romania
[c]National Institute for Research and Development of Isotopic and Molecular Technologies, P.O. Box 700, Cluj-Napoca, Romania
[d]"Raluca Ripan" Institute of Chemistry,400294 Cluj-Napoca, Romania

ABSTRACT. Compression isotherms of some carotenoids: 4-oxo-β-carotene, 4,4'-dioxo- β-carotene and the di-apo-4, 4'-carotenedial, spread at the air / water interface, are reported. The high molecular area portions of the compression isotherms are described by means of van der Waals – type state equation and interaction parameters are derived. Simple HMO calculations are performed without and with induction parameters and dipole moments are calculated. Surface properties viz. collapse pressure and surface compressibility modulus and interaction parameters are discussed in terms of dipole-dipole interactions, as well as of conformational analysis of the carotenoid molecules in monolayers in good agreement with the experimental results.

Keywords: *carotenoids, monolayer properties, molecular structure, dipole moment, state equation*

INTRODUCTION

Carotenoids are important biological molecules that play vital roles in cellular differentiation, growth control, photooxidative protection, cell membrane stability, photosynthesis, vision process and nutrition [1]. Recently, more studies attempting to relate carotenoids to therapy of cancer, coronary heart disease, HIV infections or light-sensitive diseases are undertaken. These are exciting areas for the study of functions and actions of carotenoids in biological systems. Nevertheless, it is clear that the varied biological activities of carotenoids in living organisms are determined by the biophysical and chemical properties of these molecules. These properties and particularly the surface activity of carotenoids are determined by their molecular structure and geometry [2]. Further, carotenoids and their metabolites may influence or modulate the properties of cellular and subcellular structures [1].

In this paper we describe the monolayer behaviour of three carotenoid pigments, like 4-oxo-β-carotene (OC), 4,4'-dioxo- β-carotene (DOC) and the di-apo-4, 4'-carotenedial (DAC), in single-component monolayers. Then, we present the correlation between the monolayer properties and molecular structure of the three carotenoids spread at the air/water interface.

EXPERIMENTAL PART

The three carotenoids (OC, DOC and DAC) examined are all-trans isomers of high purity established by chromatography and spectral analysis. The spreading solvent for every carotenoid compound was benzene pro-analysis or a mixture of benzene and 2-4% absolute ethanol at the air/water interface. After 10 minutes allowed for solvent evaporation the spread monolayer of carotenoid compound was compressed at a chosen rate of compression and reproducible compression isotherms were recorded at 20 ± 2 ^{o}C as described elsewhere [1].

RESULTS AND DISCUSSION

Compression isotherms, in terms of surface pressure (π, mN/m) versus mean molecular area (A, nm^2) curves are recorded for three carotenoids, viz. 4-oxo-β-carotene (OC), 4, 4'-dioxo- β-carotene (DOC) and di-apo- 4, 4'-carotenedial (DAC), spread at the air/water interface.

The molecular structures and formulae of the studied carotenoids are given in Fig.1.

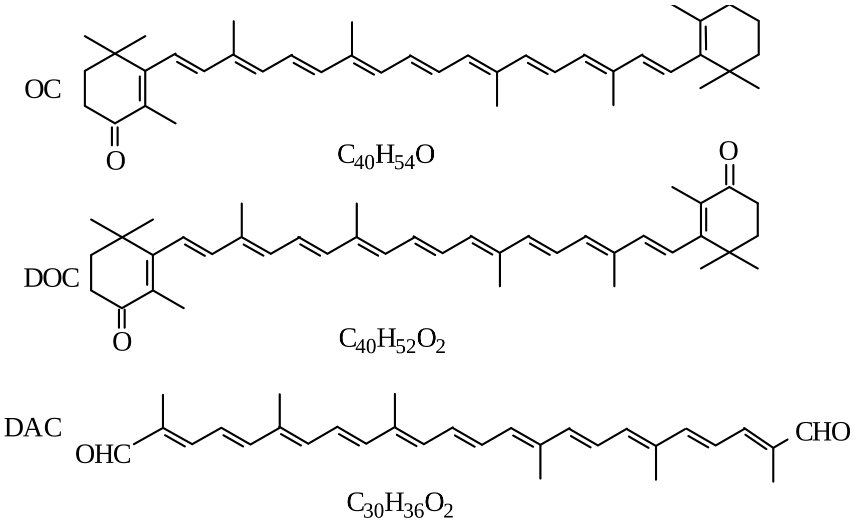


Fig. 1. Molecular structure of the three carotenoids: OC: 4-oxo-β-carotene, DOC: 4,4'-dioxo- β-carotene and DAC: di-apo- 4,4'-carotenedial.

The compression isotherms of the three carotenoids are presented in Fig. 2.

At high molecular area, the compression isotherms are described by means of the van der Waals type state equation:

$$(\pi + \alpha / A^{3/2}) (A - A_0^{'}) = kT \qquad (1)$$

where α and A_0' are empirical parameters to be derived from the experimental curves, α being a measure of the intermolecular attraction and A_0' is coarea. These parameters have been derived from the experimental π-A pairs, obtained at low π values (up to about 15 mN/m). They are presented in Table 1, together with surface characteristics derived from the high π portion of the compression isotherms, viz. collapse pressure (π_c) and surface compressional modulus (C_s^{-1}), defined as :

$$C_s^{-1} = - A_0 \left(\partial \pi / \partial A \right)_T \qquad (2)$$

where A_0 stands for the limiting molecular area (see Fig. 2) obtained by extrapolating to π = 0, the high pressure linear portion of the compression isotherm, having the slope $(\partial \pi / \partial A)_T$.

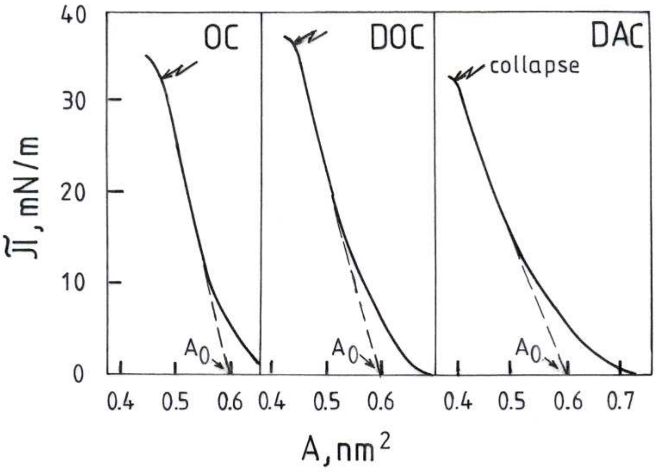


Fig. 2. Compression isotherms of the three carotenoids: 4-oxo-β-carotene (OC), 4,4'-dioxo- β-carotene (DOC) and di-apo-4,4'-carotenedial (DAC) spread as monolayers at the air/water interface.

From compression isotherms the surface characteristics are determined, namely π_c, C_s^{-1}, A_0' and α value, and given in Table 1.

Table 1.

Surface characteristics of the three carotenoids studied.

Carotenoid	π_c (mN m^{-1})	C_s^{-1} (mN m^{-1})	α 10^{30} (N m^2)	A_0' (nm^2)
OC	32	151	9.02	0.438
DOC	36	145	8.74	0.432
DAC	32	95	6.71	0.392

In order to correlate $\pi_{c,}$ α and C_s^{-1} values with the electronic structure of the molecules studied, HMO calculations have been performed in two approximation [3], by taking into account only the delocalized π-bond system, viz.:

- *approach 1* corresponds to the parametrization : $\alpha_C = 0$, $\alpha_O = 1$, $\beta_{CC} = \beta_{CO} = 1$;
- *approach 2* for the C - atoms carrying a CH_3 – group, an induction parameter of $\alpha_C = -0.5$ was used and for those linked to C - atoms of the ionone ring, not included into the conjugated system, $\alpha_C = -0.3$ was taken.

The π – electron density, q_r , and bond order, p_{rs}, values obtained from the HMO coefficients were used to calculate the dipole moment of each bond. For this purpose a semiempirical method was used, based upon the following hypotheses:

1. The dipole moment of CO in the sum of the dipole moment of the C-O σ-bond and of that of the C-O π-bond, both are directly proportional to the internuclear distance, the latter one being a linear function of the π-bond order**,** p_{rs}.
2. The dipole moment of the C-C σ-bond is equal to zero.
3. The dipole moment of the π-bonds, both of C-O and C-C ones, is directly proportional to the difference between the π-electron densities of the atoms linked to each other $\Delta q_{rs} = q_r - q_s$.

By using literature data concerning the length of C-O and C-C single and double bonds, dipole moments of ketones and ethers, as well as Δq_{rs} and p_{rs} values calculated in the HMO approximation for a C-O localized π-bond, the above hypotheses allowed us to derive the following semiempirical formulae:

$$\mu_{CO} = (0.5221 + 1.2614\,\Delta q_{rs})\,(6.3950 - p_{rs})\,10^{-30}\ \mathrm{C\,m}$$
$$\mu_{CC} = (8.6876 - 1.1283\,p_{rs})\,\Delta q_{rs}\,10^{-30}\ \mathrm{C\,m} \qquad (3)$$

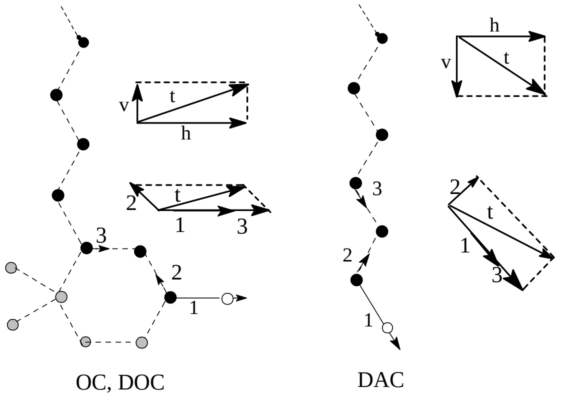


Fig. 3. Composition of the dipole moments of the first 3 bonds, and the decomposition of the total dipole moment in horizontal and vertical components.
Symbols : ◍ C - atom (sp^3); ● C - atom (sp^2); ○ O - atom (sp^2).

Table 2.

Dipole moments of the first n bonds (μ_t), their horizontal (μ_h) and vertical (μ_v) components, angles (β) formed with the air/water interface and correlations with surface characteristics (b)

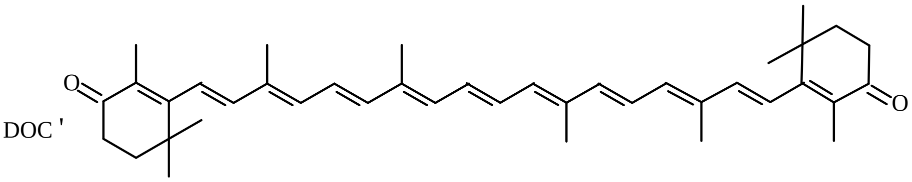
Surfactant	n	Approach 1							Approach 2						
		μ_t	μ_h	μ_v	β	$\frac{\alpha}{\mu_h}$	$\frac{C_S^{-1}}{\mu_h}$	$\frac{\pi_c}{\mu_h}$	μ_t	μ_h	μ_v	β	$\frac{\alpha}{\mu_h}$	$\frac{C_S^{-1}}{\mu_h}$	$\frac{\pi_c}{\mu_h}$
OC	1	9.14	9.14	0.00	0.00	-	-	-	9.94	9.94	0.00	0.00	-	-	-
	3	9.82	9.53	2.36	13.90	0.95	1.59	-	9.79	9.63	1.73	10.18	0.94	1.57	-
	12	6.94	6.15	3.21	25.07	-	-	5.20	13.36	13.01	3.05	13.19	-	-	2.46
DOC	1	9.09	9.09	0.00	0.00	-	-	-	9.93	9.93	0.00	0.00	-	-	-
	3	9.69	9.41	2.31	13.81	0.93	1.54	-	9.58	9.43	1.68	11.12	0.93	1.54	-
	12	7.72	7.14	2.94	20.85	-	-	5.05	14.52	14.26	2.73	10.83	-	-	2.53
DAC (DOC’)	1	9.09	4.55	-7.87	-60.0	-	-	-	9.93	4.96	-8.60	-60.00	-	-	-
	3	9.69	6.71	-6.99	-46.19	1.00	1.42	-	9.58	6.18	-7.33	-49.89	-	-	-
	12	11.01	8.98	-6.37	-35.33	-	-	3.56	6.72	1.35	-6.59	-78.39	-	-	-

Units: μ_t / 10^{-30} C m; μ_h / 10^{-30} C m; μ_v / 10^{-30} C m; β / ^{o}; (α / μ_h) / N m C^{-1}; (C_s^{-1} / μ_h) /10^{28}N $C^{-1}m^{-2}$; (π_c / μ_h) / 10^{27} N C^{-1} m^{-2}

The dipole moment of the individual bonds have been composed vectorially, by taking into account the geometric configuration of the molecules [4-6] and by considering the latters to adopt a vertical orientation at compression of the monolayer [5]. The composition of the dipole moments of the first three bonds is visualized in Fig. 3.

The resulting total dipole moment, μ_t, characterizes the polarity of the head –group, anchored into the aqueous subphase. It was decomposed into a horizontal component, μ_h, parallel with the air / water interface, and a vertical one, μ_v perpendicular to the interface. Also, the angle β, formed by μ_t with the interface has been computed.

Results are presented in Table 2, where n stands for the number of bonds taken into account at the calculation of μ, beginning from the C = O group. We mention that in Table 2 the values computed in approach 2 and given in the line of DAC (DOC**'**) do not refer to DAC but to an isomer of DOC in which the ionone ring has the configuration given in Fig.4 and having for the conjugated system the same configuration as DAC.



$C_{40}H_{52}O_2$

Fig. 4. Molecular structure of DOC'.

As seen from Table 2, the dipole moment of the head-group (μ_{h3}) decreases in the order OC > DOC > DAC and in the same order also varies α values, the dependence being almost linear, as seen from the constancy of the ratio α/μ_{h3}. The C_S^{-1} values vary in the same order and the ratio C_S^{-1}/μ_{h3} is also almost constant. This constancy is better in approach 2 than in approach 1, with respect to OC and DOC. This suggests the idea that both α and C_S^{-1} are determined mainly by the horizontal component of the head group dipole moment. The contribution of the hydrocarbon chain to μ_h is quite different with the substances studied and is reflected differently in approaches 1 and 2. In approach 1 one has $\mu_{h3} > \mu_{h12}$ with OC and DOC, but in approach 2 the reverse is observed. Since this overall dipole moment must play an important role near the collapse, the ratio π_C/μ_{h12} was calculated giving an almost constant value in the case of OC and DOC, the constancy being better in approach 2.

Calculations performed in approach 2 for the isomer DOC**'** reveal the correctness of the configuration of DOC given in Fig.1, which is consistent also with X-ray evidence [4]. According to our results the vertical component of the dipole moment of DAC differs fundamentally from those of OC and DOC, the negative pole being oriented downwards in the case of the former, and upwards with the latter ones. The correctness of this theoretical result would be worth to verify by means of surface potential measurements.

In future studies we will extend our theoretical and experimental investigations on various carotenoids to determine the correlation between the surface properties and actions of carotenoids in biological systems. Undoubtedly, it is clear that the varied biological activities of carotenoids in living organisms are determined by the structural and chemical properties of these molecules. The actual research on the relations between the dipole moments and surface potentials for different biosurfactants is in progress in our laboratories.

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