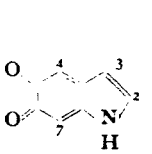
Short Communications

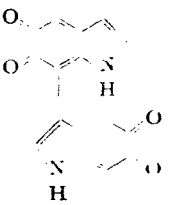
: C 2.04

Sur la biogénèse des mélanines

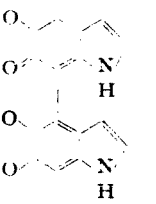
Les études biogénétiques, en particulier celles de RAPER[1,2], ont démontré que la formation du 5,6-dihydroxyindole jouait un rôle central dans la biosynthèse des mélanines. Il est admis, en général, que les étapes suivantes de la biogénèse de ces composés comportent une polymérisation oxidative de l'indole-5,6-quinone (I), mais il existe des divergences de vue en ce qui concerne le mécanisme de cette polymérisation.



I



II



III

Ainsi, bien qu'il soit admis que les quatre carbones 2, 3, 4 et 7 de I sont impliqués dans la polymérisation, BU'LOCK, CROMARTIE ET HARLEY-MASON[3,4] estiment que le processus réactionnel essentiel met en jeu une jonction d'unités successives de I par une liaison C-3–C-7 (dimère du type II) alors que MASON[5], ROBERTSON *et al.*[6] et NICOLAUS ET MANGONI[7] préconisent une réaction principale par l'intermédiaire d'une liaison C-4–C-7 (dimère du type III).

Nous avons fait appel à la méthode des orbitales moléculaires de la chimie quantique (précédemment utilisée pour l'étude de l'existence possible des bandes d'énergie dans les mélanines[8]) pour essayer d'évaluer les probabilités relatives de ces différents mécanismes. Dans ce but nous avons considéré à la fois les aptitudes réactionnelles du monomère I et les caractéristiques de stabilité relatives des dimères II et III.

TABLEAU I

INDICES ÉLECTRONIQUES DES CARBONES DE I

Carbone	*Charge électronique des électrons π*	*Valence libre*	*Energie de localisation pour une attaque (en β ≈ 20 kcal/mole)*		
			électrophile	*nucléophile*	*radicalaire*
2	0.908	0.474	2.20	1.92	2.06
3	1.190	0.461	1.93	2.30	2.12
4	1.091	0.637	1.63	1.62	1.63
7	1.105	0.607	1.71	1.70	1.70

Le Tableau I contient les caractéristiques électroniques essentielles des carbones 2, 3, 4 et 7 de I. On constate que les polarisabilités des carbones du noyau quinonique sont nettement plus grandes que celles des carbones du noyau pyrrolique (comme le laissaient prévoir les valeurs relatives des valences libres correspondantes) et que les énergies de localisation pour n'importe quel type d'attaque sont nettement plus petites pour les carbones 4 et 7 que pour les carbones 2 et 3. Ces énergies mesurant la partie variable essentielle des énergies d'activation impliquées dans les réactions concernant ces carbones, ce résultat permet de prévoir une réactivité générale beaucoup plus grande pour le noyau quinonique que pour le noyau pyrrolique et, partant, suggère fortement un mécanisme de mélanogenèse mettant en jeu une polymérisation principale à travers les carbones 4–7.

Cette conclusion est encore étayée par l'examen des caractéristiques électroniques des dimères II et III. Les énergies de résonance de ces deux composés sont égales, respectivement, à 5.96β et 6.08β. Elles font donc apparaître un incrément de stabilité de $0.12\beta \approx 2$ kcal/mole au profit de la forme III. Corrélativement l'indice mobile de la liaison "simple" réunissant les monomères est de 0.484 dans II et de 0.564 dans III et traduit donc également une délocalisation électronique plus prononcée dans la forme III.

Ainsi, les considérations thermodynamiques et cinétiques sont toutes en faveur d'un chemin principal de polymérisation passant par l'intermédiaire des structures du type III.

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[1] H. S. RAPER, *J. Chem. Soc.*, (1938) 125.
[2] Pour une revue générale voir: R. H. THOMSON, dans M. FLORKIN ET H. S. MASON, *Comparative Biochemistry*, Vol. 3, Academic Press, New York, 1962, p. 727.
[3] J. D. BU'LOCK ET J. HARLEY-MASON, *J. Chem. Soc.*, (1951) 703.
[4] R. I. T. CROMARTIE ET J. HARLEY-MASON, *Biochem. J.*, 66 (1957) 713.
[5] H. S. MASON, dans M. GORDON, *Pigment Cell Biology*, Academic Press, New York, 1959, p. 563.
[6] R. J. S. BEER, T. BROADHURST ET A. ROBERTSON, *J. Chem. Soc.*, (1954) 1937.
[7] R. A. NICOLAUS ET L. MANGONI, *Gazz. Chim. Ital.*, 85 (1955) 1397.
[8] A. PULLMAN ET B. PULLMAN, *Quantum Biochemistry*, Interscience Publishers, New York, sous presse.
[9] A. PULLMAN ET B. PULLMAN, *Biochim. Biophys. Acta*, 54 (1961) 384.

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SC 2189

Magnetic isotropy of the red blood cell

It has been suggested that the shape of the red blood cell is a result of the internal arrangement of the hemoglobin molecules[1]. Many arguments for internal molecular arrangement have been presented[1]. Support for these arguments has been obtained

from polarization optics.[2] An X-ray analysis of the red blood cell indicated random orientation but closest packing of hemoglobin molecules[3] while from electron-diffraction studies, DERVICHIAN[4] has postulated order of molecules at small distances and disorder at large distances in the cell.

From the shape of the hemoglobin molecule as pictured by CULLIS *et al.*[5], magnetic anisotropy would be expected if a preferential non-symmetrical internal [illegible] exists. Magnetic anisotropy of hemoglobin derivatives has been found by BENNET *et al.*[6], as evidenced by a g factor of 2.00–6.00 depending upon the orientation of the heme plane to the magnetic field. We therefore felt that a measurement of magnetic anisotropy or lack of it would give an insight into the internal molecular structure of the hemoglobin within the cell.

The couple and force upon an object in a uniform magnetic field provides a means of measuring its magnetic anisotropy. In vector notation, this couple, G, is given by NYE[7] as:

$$\vec{G} = \vec{M} \times \vec{H} \tag{1}$$

where $\vec{M}$ is the magnetic dipole, and $\vec{H}$ is the magnetic field produced by sources external to the dipole. If $\vec{M}$ is not parallel to $\vec{H}$, the object will experience a couple; while if $\vec{M}$ and $\vec{H}$ are parallel as in the case of an isotropic object, the couple will be zero. In suffix notation Eqn. 1 can be written as:

$$G_{ij} = -M_iH_j + M_jH_i \tag{2}$$

where G_{ij} is an antisymmetrical tensor whose three independent non-zero components form an axial vector. For an object of volume v, where k_{ij} is the component of the susceptibility tensor of the object;

$$M_i = vk_{ij}H_j \tag{3}$$

and

$$G_{ij} = v(-k_{ik}H_kH_j + k_{jk}H_kH_i) \tag{4}$$

One would expect from the red blood cell in its normal biconcave discoid form a maximum of two different principal values for the magnetic susceptibility tensor. The couple produced by the field acting in the plane of k_1 and k_2 can be expressed as:

$$G = {}^1/_2v(k_1 - k_2)H_1H_2 = {}^1/_2v(k_1 - k_2)H^2 \sin 2\phi \tag{5}$$

where ϕ is the angle between the k_1 axis and H.

If an anisotropic cell is suspended in a uniform field, the cell will rotate to the point at which k_1 and H are parallel. The magnitude of the couple causing this rotation can be measured by determining the angular velocity ω, as:

$$G = C\omega \tag{6}$$

where C is the rotational frictional coefficient. This coefficient for the biconcave disc has not been mathematically expressed, but for this discussion can be approximated by the rotational frictional coefficient of the sphere. This is given as[8]:

$$C = 8\pi\eta a^3 \tag{7}$$

where a is the radius of the sphere, and η is the viscosity of the media used for the suspension.

Experimental observations upon the red blood cell were performed in an apparatus as previously described[9], with only slight modification. Flat pole tips with a gap of 0.5 cm were placed upon an electromagnet of 10900 oersteds with a small glass container between the pole tips. The red blood cells were viewed in the media (isotonic saline and sucrose of the same density as the cells) under a magnification of 500 ×. Red blood cells of two individuals and of bovine animals were observed under these conditions, and in one case (human), the blood was treated with $Na_2S_2O_4$. In the other instances, the blood was oxygenated by air at atmospheric pressure. Paracrystalline rat red cells, prepared by the method of PONDER[10], were also observed. The birefringent cells were prepared and viewed at 4° in 3% sodium citrate media during a period of several days.

No specific orientation of the red blood cells was observed within a time period of 60 sec. The Brownian motion of such a cell can give a rotation of as much 20° in this time period so a lower limit must be placed upon the detectable rotation, as $\omega < 1.2 \cdot 10^{-2}$ radians/sec. With these limits, we can estimate a maximum value for the anisotropy. Considering a red blood cell of 4.2 μ radius, 87 μ^3 volume and an angle of 45° to the magnetic vector, the magnetic susceptibility difference can be calculated as less than $4 \cdot 10^{-11}$ from Eqn. 8 as derived from Eqns. 5 and

$$(k_1 - k_2) = \frac{16\pi\eta a^3}{vH^2 \sin 2\phi} \tag{8}$$

This susceptibility difference is the same order of magnitude as might be expected from non-symmetrical shape of an isotropic cell. The effects of orientated paramagnetic molecules such as reduced hemoglobin or ferrihemoglobin should greatly enhance the magnetic anisotropy[11] of the cell unless a cancellation of diamagnetic and paramagnetic contributions occurs. This latter possibility seems remote, and is eliminated by not observing any anisotropy for either reduced or oxygenated cells. In addition, no evidence of orientation on macroscopic samples was found by observations of the magnetic-field effects on scattered or absorbed light intensities, or field-induced birefringence.

From these studies, we conclude that the red blood cell in its normal and birefringent state is magnetically isotropic within the limits of our measurements. This situation would arise for two possibilities. (1) The hemoglobin may be highly oriented within the cell to give cubic symmetry. However, this possibility is ruled out by X-ray-diffraction studies. (2) The hemoglobin may be randomly oriented throughout the cell. It is still possible for localized orientation to exist.

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[1] ERIC PONDER, *Hemolysis and Related Phenomena*, Grune and Stratton, New York, 1948, Ch. 4.
[2] A. TEITEL-BERNARD, *Sang*, 8 (1934) 298.
[3] J. B. BATEMAN, S. S. HSU, J. P. KNUDSON AND K. L. YUDOWITCH, *Arch. Biochem. Biophys.*, 45 (1953) 411.
[4] D. G. DERVICHIAN, *Ann. Chim. Rome*, 40 (1950) 300.
[5] A. F. CULLIS, H. MUIRHEAD, M. F. PERUTZ, M. G. ROSSMAN AND A. C. T. NORTH, *Proc. Roy. Soc. London, Ser. A*, 265 (1962) 161.
[6] J. E. BENNETT, J. F. GIBSON AND D. J. E. INGRAM, *Proc. Roy. Soc. London, Ser. A*, 240 (1957) 67.
[7] J. F. NYE, *Physical Properties of Crystals*, Oxford University Press, New York, 1957, p. 60.
[8] J. J. HERMANS, *Flow Properties of Disperse Systems, Interscience*, New York, 1953, p. 137.
[9] S. J. GILL, C. P. MALONE AND M. DOWNING, *Rev. Sci. Instr.*, 31 (1960) 1299.
[10] E. PONDER, *J. Gen. Physiol.*, 29 (1945) 89.
[11] D. H. WIFFIN, *Free Radicals in Biological Systems*, Academic Press, New York, 1961, p. 227.

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Preliminary Notes

PN 1197

Ring currents in 3:4-benzpyrene, 1:2-benzanthracene, and 1:2;5:6-dibenzanthracene

The work of the PULLMANS and others aimed at relating the electronic configuration of aromatic hydrocarbons to their carcinogenic potency (a theory well summarized by COULSON[1]) has been at least moderately successful. As some of the theory rests on molecular-orbital calculations it is of interest to re-examine the range of validity of molecular-orbital theory as applied to polycyclic hydrocarbons by making theoretical predictions based on the theory which can be checked directly experimentally. In this note, we shall make use of a modification due to MCWEENY[2] of LONDON's[3] theory of diamagnetic anisotropy in aromatic molecules, which is based directly on molecular-orbital theory. We shall obtain theoretical predictions of the π-electron ring currents in several carcinogenic polycyclic hydrocarbons. As these ring currents are responsible not only for diamagnetic anisotropy but also for nuclear-magnetic-resonance chemical shifts the predictions are subject to rather rigorous experimental test. Similar calculations by BERTHIER *et al.*[4], based on LONDON's unmodified theory, predict the total induced moment, but not the individual ring currents.

MCWEENY has shown that the secondary magnetic field, H', at any point, arising from the diamagnetic effect of ring currents in an aromatic hydrocarbon in the presence of an external magnetic field, H, normal to the plane of the molecule, is given by

$$H' = 2\beta\left(\frac{2\pi e}{hc}\right)^2 \frac{S^2 H}{a^3}(\sigma_1 + \sigma_2),$$

where S is the area of a benzene ring, a is the length of a C–C bond, β is the standard resonance integral, and σ_1 and σ_2 are sums involving bond orders and mutual bond polarizabilities obtained from normal (unperturbed) molecular-orbital theory. The