ISOMERISATIONS AND CYCLISATIONS IN BILE PIGMENTS

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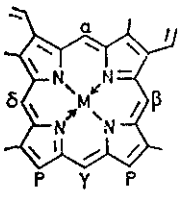
This article provides a survey of the isomerisations and cyclisations occuring in bile pigments. Three types of cyclisation have been established, leading to new condensed heterocyclic systems. The neoverdins, isopterobilin, isophorcabilin and sarpedobilin are formed from such transformations.

Contents

[+]This review is dedicated to Professor Edgar Lederer on the occasion of his 70th Birthday, June 5th 1978, in recognition of his pioneering research on natural pigments.

1. INTRODUCTION

Naturally occuring bile pigments or bilins, are tetrapyrroles resulting from the oxidation of protoporphyrin IX. Two series are found, depending on the specific opening of the ring at the methine bridge α or γ (fig.1). The Vertebrate pigments biliverdin and bilirubin, the plant phytochrome[1-3] and the algal phycocyanin or phycoerythrin chromophores[4-8] belong to the IXα series. The Lepidopter pigments pterobilin, phorcabilin and sarpedobilin[9] belong to the IXγ series. These three γ bilins are isomers and are related by biochemical and photochemical relationships[10,11,17]. These reactions consist in isomerisations followed by cyclisations. The interest in the biological properties of phytochromes recently focussed the research on the conformation and on the isomerisation of such molecules.



P = $CH_2 \cdot CH_2 \cdot CO_2H$

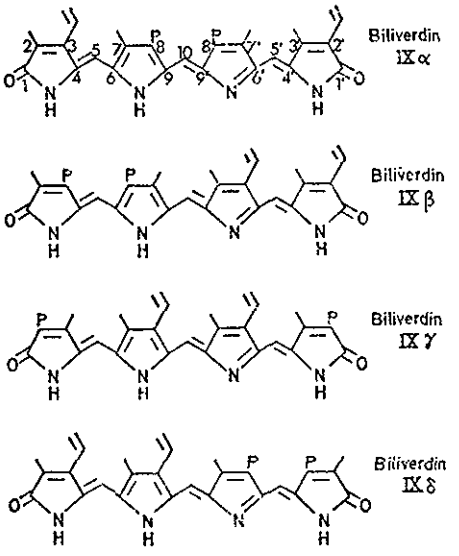


Fig.1

Bilirubin[12] and biliverdin[13] have been recently analysed by X-rays. In the crystal structure of bilirubin (fig.2) the bridges a and c still have the configuration syn-Z as found in the porphyrins (fig.3). The plane of the cycles A-B forms an angle of 98° with the plane of cycles C-D, the whole being maintained by hydrogen bonds. A cisoid arrangement with the Z configuration of the two conjugated pyrrole rings is not dependant on intra-molecular hydrogen bondings; the oxodipyrromethenes also have the same preferential structure[14].

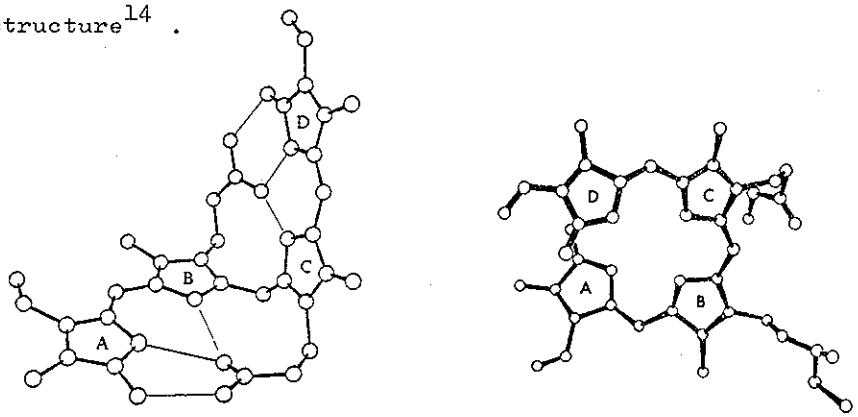


Fig.2 X-ray crystal structures of bilirubin[12] (left) and biliverdin[13] (right)

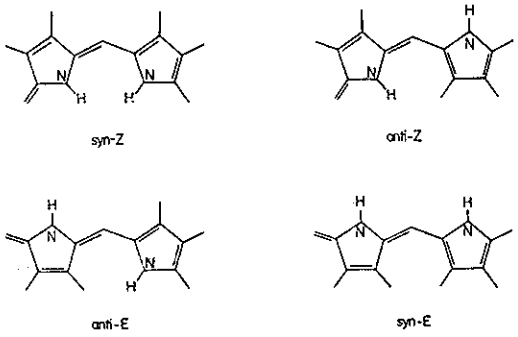


Fig.3 Configuration of dipyrromethenes

The X-ray study of Zn-octaethylformylbiliverdinate[15] has shown the possibility for a bilatriene to deviate from the plane and enter into different types of helical arrangements.A near planar helical conformation with an intramolecular distance of 3,34 Å between the two pyrrolone oxygen atoms has been found for biliverdin dimethylester[13] .A high degree of bond fixation within the tetrapyrrole rings is found;the chromophore is in the lactam form with three pyrrole N-H protons corresponding to the A,B and D rings,without evidence for any disorder.

The plant phytochrome which regulate many of the light responses in plants,occurs in two forms distinguished by their different absorption in the visible range.The red form P_R (660nm) is converted to the far red form P_{FR} (730nm) by irradiation with red light.Burke et al.[16] suggested that this spectral shift may be accounted for in terms of cis-trans geometric isomerisation around the methine bridges.On the other hand,Struckmeier et al.[15] and Sheldrick[13] believe that the spectroscopic shift observed for the phytochrome chromophore may well be produced by a simple stretching of the molecule,perpendicular to its plane and that the two forms may have very similar "cyclic" conformations. Following Scheer et al.[25],the transformation of P_R into P_{FR} would be a photochemical dimerisation.

We have established the possibility of isomerisations and cyclisations occuring with some bile pigments.These reactions leading to novel polycyclic derivatives show the easy isomerisation of methine bridges and the particular reactivity of the vinyl groups in the 7' and 8 positions.

2. ISOMERISATIONS OF VERDINS INTO NEOVERDINS

The irradiation (600-700nm) of verdins having a vinyl group in the 8 (central) position leads to the formation of new isomeric pigments, the neoverdins. Thermal treatment produces the same results[9,17,18]. Biliverdin IX γ (pterobilin) is transformed into a neobiliverdin IX γ which is identical with the natural phorcabilin. 7'-methoxy-hydropterobilin is converted into a methoxy-hydrophorcabilin and the biliverdin IX δ into a neobiliverdin IX δ (fig.4).

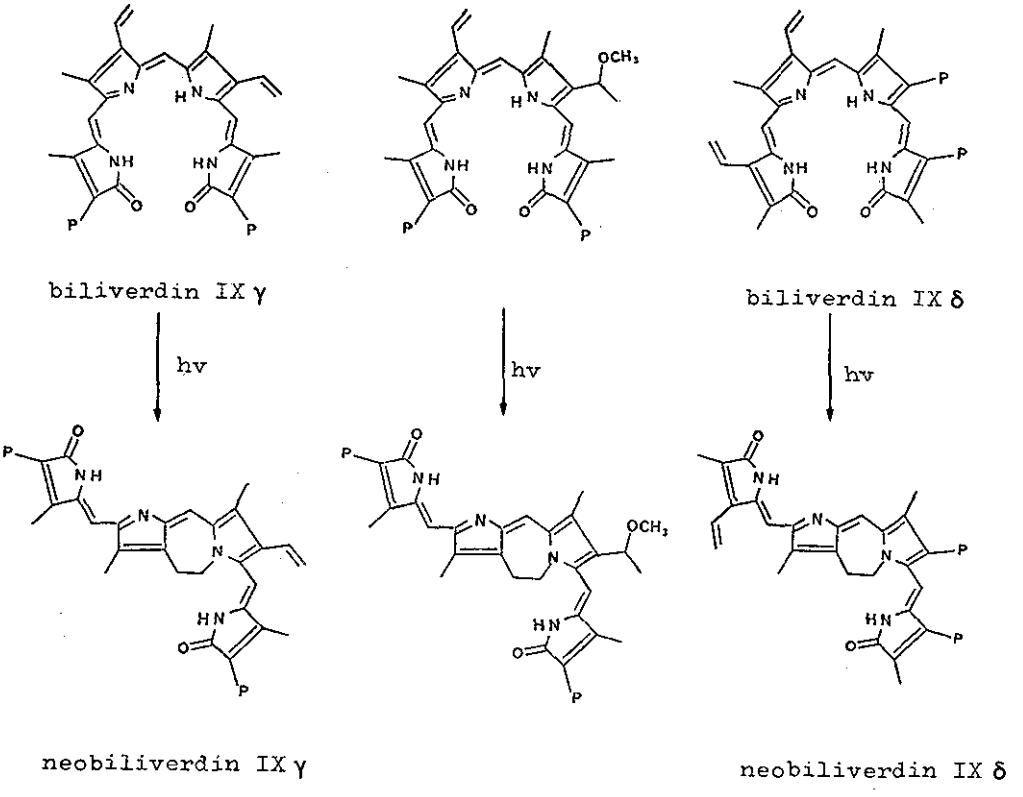


Fig.4 Isomerisations of verdins into neoverdins (P=CH_2CH_2COOR; R=H or CH_3)

These neoverdins are pentacyclic substances with a central hetero-heptagonal cycle and an extended conformation.

The formation of these compounds may be understood in the following way (fig.5).As the verdins probably havea closed conformation in solution,the first step of the reaction must be a geometrical isomerisation of the central methine bridge.This is the first report of a syn-Z --→ anti-E photochemical or thermo-isomerisation of a methine bridge in the bile pigments.The second step is a Michael like intramolecular addition of the pyrrolic amine on the vinyl group activated by conjugation with electrophilic groups such as C=N or C=O.Polar solvents favour the reaction because of the possibility of stabilisation of the intermediate diionic form.

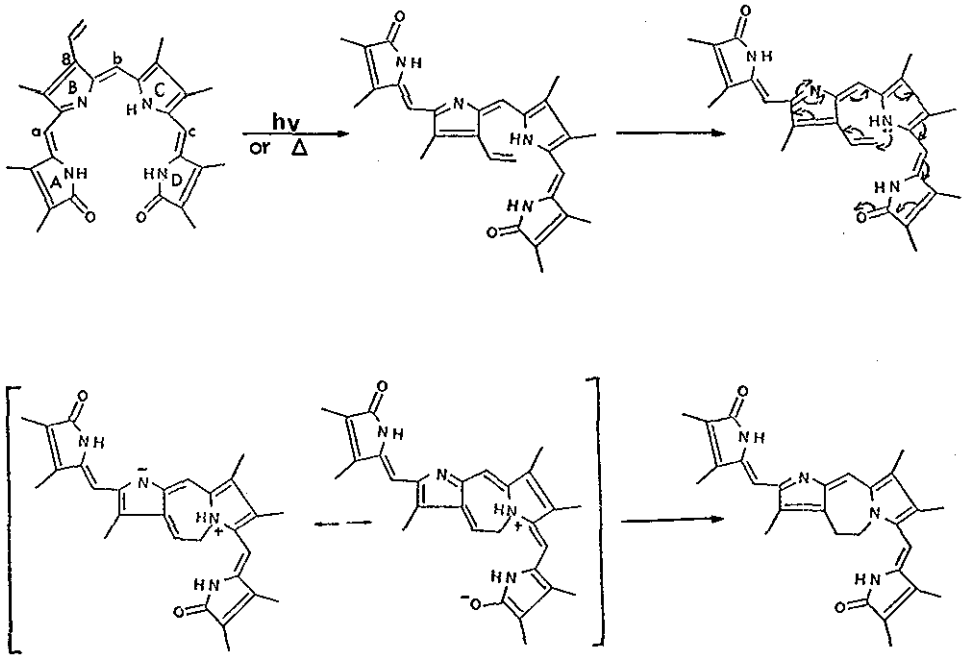


Fig.5 Proposed mechanism for the cyclisation of biliverdins IXδ or IXδ

Intramolecular photocyclisations through the addition of an amine on vinyl groups have been reported in the series of substituted anilines[23,24].

The main spectral features corresponding to this molecular modification at the central methine bridge are the following. In the NMR spectra, the central methine bridge proton singlet is shifted downfield ($\Delta\delta$ =-0,5ppm). In the absorption spectra, a hypsochromic shift of the absorption maximum in the visible region is observed (biliverdin:650nm, neobiliverdin:550nm) also a hyperchromic effect on the corresponding extinction coefficient (biliverdin: ε =15000; neobiliverdin: ε = 45000). There are no more major electronic transitions in the 300-400nm range (fig.6). Neobiliverdins are the first models of tetrapyrroles with an open extended conformation;

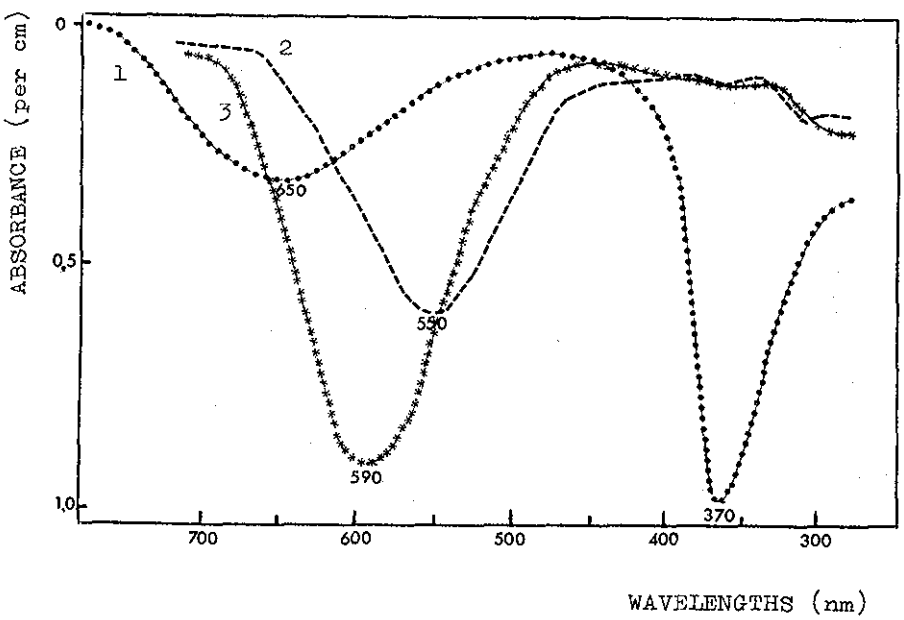


Fig.6 Absorption spectra in MeOH of:(1) pterobilin ester (2)phorcabilin ester (3) sarpedobilin ester

it is of interest to check if these optical properties fit with the theoretical calculations made by the authors.It was concluded that "in the closed conformations,the calculated oscillator strength of the 300-400nm band system are greater by an order of magnitude than the one for the long-wavelength 600-700nm band. In the open conformations,the situation is reversed and the long wavelength transition gains in intensity at the expense of the transitions at shorter wavelengths"[19,16].The absence of the 300-400nm band would seem to be a valuable criterion for an open conformation.

3.ISOMERISATION OF PHORCABILIN INTO SARPEDOBILIN

The structure of sarpedobilin,proposed on the basis of spectrometric data and chemical considerations,is hexacyclic and has an enol group[20] .This pigment may be obtained by irradiation of phorcabilin (500-700nm) in protic solvents.It has not been found after thermal treatment.

The first step of the reaction could be the photoisomerisation syn Z --→ syn E of the methine bridge c (fig.7).Recently,Falk et al.[21] have published a photoisomerisation of a synthetic verdin which they also suppose to be a syn Z --→ syn E isomerisation of one of the exo methine bridges.

The second step of the transformation must be an addition of the 7'-pyrrolic vinyl group to the C-3' carbon atom which acts as an electrophilic center,due to the enolisation of the 1'-carbonyl group.The process is followed by a prototropic rearrangement probably assisted by the protic solvent.

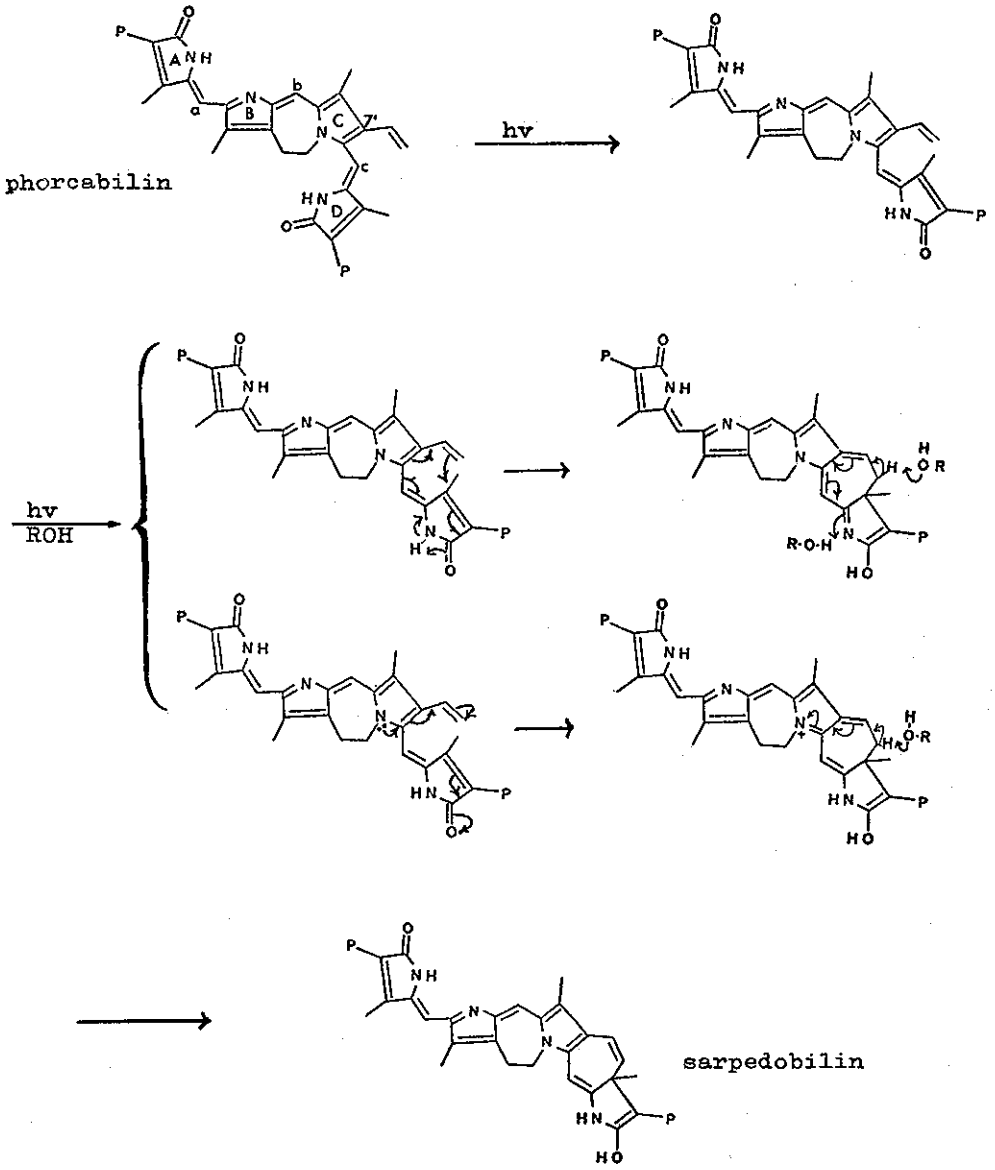


Fig.7 Proposed mechanism for the isomerisation of phorcabilin into sarpedobilin (P=CH_2CH_2COOR;R=H or CH_3)

4. REACTIONS IN MeOH/H^+-ISOMERISATION OF VERDINS INTO ISOVERDINS

Bile pigments with a vinyl group in 7'-position (biliverdin IX β, biliverdin IX γ and phorcabilin) easily undergo acid catalysed methanol addition (table). Biliverdin IX γ having vinyl groups in the 7' and 8 positions first form the 7'-methanol adduct[9]. This, and the previously described reactions, underline the more nucleophilic behaviour of the C-7'α carbon atom and the electrophilic behaviour of the C-8 α carbon atom. This may be a good indication that the more favourable tautomeric form for this pigment is the one with ring B as a pyrrolenine ring and ring C as a pyrrole ring.

A more energetic treatment of biliverdin IX γ and phorcabilin in MeOH/H^+ leads to the formation of new pigments which are a methoxyhydro-isopterobilin methyl ester and an isophorcabilin methyl ester (fig.8). These substances result from the substitution of the 7'β $-OCH_3$ by the pyrrolone N-H. This cyclisation involves the rotation around the C-5' C-6' bond. Chae and Song[22], however, suggested that this conformation may predominate for biliverdin IX α in solution.

Table: reactivity of bile pigments dimethylesters in MeOH-20% H_2SO_4 during 15h at 20°C.

Methyl esters of pigments	IX α	IX β	IX γ	IX δ	phorcabilin
Unchanged	95%	24%	20%	93%	8%
$HOCH_3$ adduct	2%	54%	75%	3%	72%
Other derivatives	3%	22%	5%	4%	20%

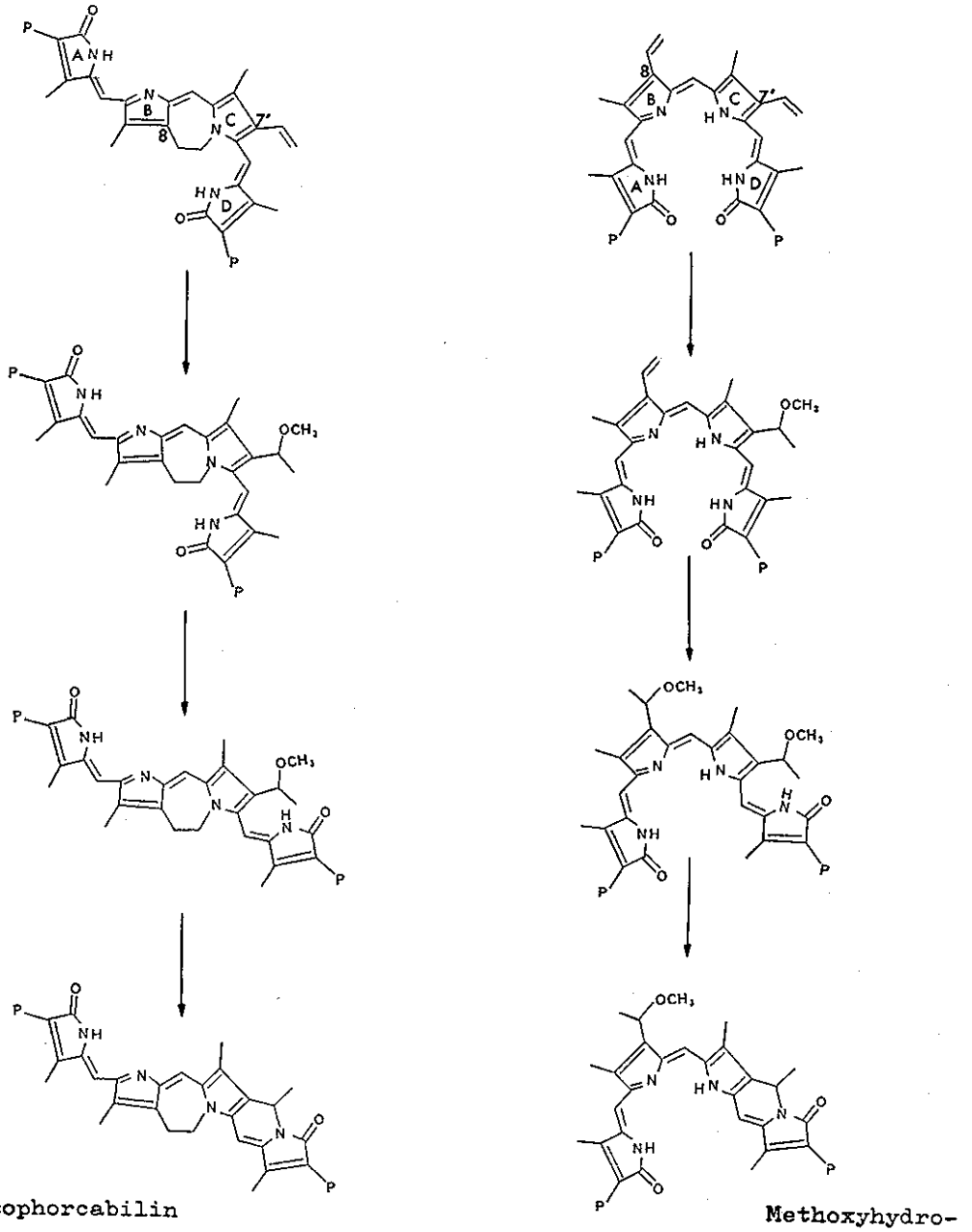


Fig.8 Reactions of phorcabilin and pterobilin in MeOH/H^+ (P=CH_2CH_2COOR R=H or CH_3)

5. CHROMIC ACID DEGRADATIONS

CrO_3 degradations[2] of normal tetrapyrrole pigments lead to the formation of 4 maleimide units which are valuable for structure elucidations. CrO_3 oxidations of the new polycyclic pigments produce maleimides and particular compounds in which the heterocycles are bound through a N-ethylene (neoverdins) or ethylidene (isoverdins) bridge (di- and tri-imides; fig.9)[9,18,20].

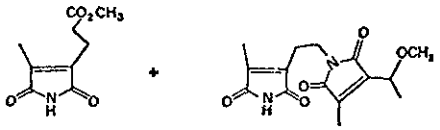
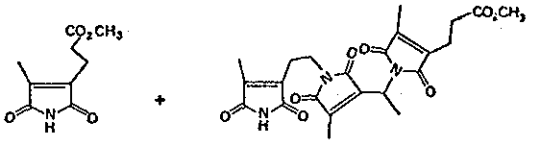
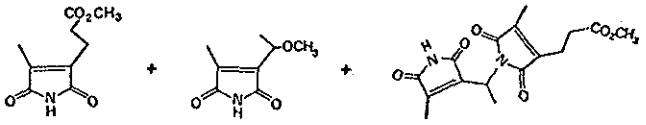
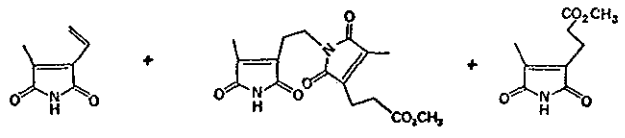
Compound oxidised	Products identified
Methoxyhydro *phorcabilin*	
Isophorcabilin	
Methoxyhydro isopterobilin	
Neobiliverdin IXδ	

Fig.9 CrO_3 oxidation of neo and iso-verdin esters

CONCLUSION

A number of new original bile pigments with polycyclic structures have been obtained by partial synthesis[18]. Three possibilities of cyclisation have been established in the series of the tetrapyrrole pigments. The interest of this study is to demonstrate the reality of the isomerisations of methine bridges under the action of light, temperature or solvents and to define the particular reactivity of the vinyl groups in the 7' and 8 positions, which are responsible for the observed cyclisations. The resulting polycyclic molecules are new condensed heterocyclic systems. The neobiliverdins, isopterobilin, isophorcabilin and sarpedobilin may also represent valuable models of tetrapyrroles, with various degrees of rigidity, necessary for further conformational studies in the field of bile pigments.

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