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REACTIVITY OF PYRROLE PIGMENTS. PART XII[1]. ELECTROCHEMICAL REDUCTION OF DIPYRRIN-1(10H)-ONES AND BILIRUBINS

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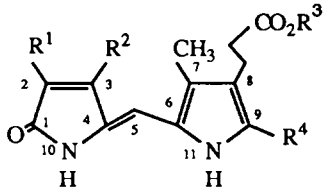
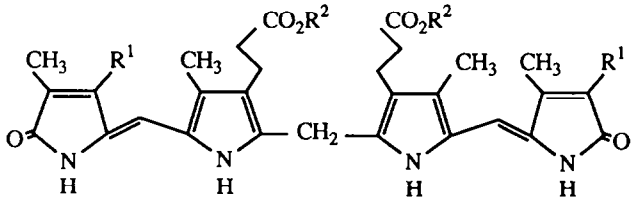
Abstract. Biladiene-ac-diones (bilirubins) in dimethylformamide show a cathodic electrochemical behaviour analogous as that of their partial models the dipyrrin-1(10H)-ones. Exo and endo vinyl substitution results in different reaction products on cathodic reduction, but the specific electroanalytical response depends on many factors and cannot easily be interpreted. The reductive polymers obtained from the bilirubins are suggested to be related to bilifuscin and mesobilifuscin (fecal pigments).

Natural bile pigments could have played a biological role in electron transfer processes, i. e. in the first stages of life evolution[2]. However, the electrochemistry of bile pigments has not been widely studied. Much of the published work is concerned with the redox interconversion between bilirubin and biliverdin structures as well as with the application of electroanalytical techniques in biochemical and clinical analysis of bilirubin[3]. Our goal is to study the effect of the vinyl substituents in the redox properties and electrochemical behaviour of natural bile pigments. Here we present results on cathodic reduction of dipyrrin-1(10H)-ones and bilirubins. The behaviour of these pyrrole pigments in reductive processes is also of interest in the understanding of the bacterial reductive pathway of bilirubin IXα in the intestinal tract of mammals.

We have already reported[4] on the electrochemical behaviour by cathodic reduction of alkyl substituted dipyrrin-1(10H)-ones (formerly: 5(1H)-pyrromethenones[5]). By cyclic voltammetry and polarography aprotic media (dimethylformamide (DMF)/ 0.1 mol·l^{-1} $LiClO_4$) they show two monoelectronic irreversible difussion controlled processes: electrolysis at first and second wave potentials affords reductive dimerization through the bridge carbon atom but at second wave potentials, hydrogenation at the exocyclic double bond also occurs . The presence of proton donors increases the yield of this hydrogenation product. Further, proton donors in the medium (protic solvents and proton acidity) displace the reduction paths -mainly the second one - to more positive potentials and at high proton concentration, the two processes occur together. Owing to this effect of the protic media, previous work on the cathodic reduction of dipirrin-1(10H)-ones[6] was of difficult to interpret. However, the results of the work in DMF/$LiClO_4$ are easier to understand[4] from the point of view of the relationship between structure and



	R^1	R^2	R^3	R^4
1	CH_3	C_2H_5	CH_3	CH_3
2	CH_3	C_2H_5	H	CH_3
3	CH_3	C_2H_5	CH_3	H
4	CH_3	C_2H_3	CH_3	H
5	C_2H_3	CH_3	CH_3	H



	R^1	R^2
6	C_2H_5	H
7	C_2H_3	H
8	C_2H_5	CH_3
9	C_2H_3	CH_3

electrochemical behaviour. The role of $LiClO_4$ as supporting electrolyte is not negligible in determining these results: the anions formed after the electron capture give - through a prototropic process - carboxyamidure anions, which are more stable as their lithium salts.

In this paper, using the same methodological approach as in the references[4a,b], we report on the cathodic reduction of vinyl substituted dipyrrin-1(10H)-ones and of biladiene-ac-diones (bilirubins) with a IX α substitution pattern. Therefore, the electrochemical behaviour of mesobilirubin IX [6], bilirubin IX [7] and their dimethyl esters (8 and 9) has been studied and compared to that of their partial models, i. e. the alkyl substituted dipyrrin-1(10H)-ones 1 - 3 and the so-called endo and exo vinyl substituted dipyrrin-1(10H)-ones 4 and 5 (all of them with a similar substitution pattern: see formula scheme).

It has recently been shown that, in an aqueous medium (pH = 7.4), bilirubins IIIα, IXα, and XIIIα afford a different voltammetric response depending on endo or exo vinyl substitution. As we show here, working in DMF/$LiClO_4$ results in important differences between the electrochemical behaviour of vinyl substituted free acids and of their corresponding dimethyl esters. These differences account for a far from trivial explanation about the effect of vinyl substitution on dipyrrin-1(10H)-ones and bilirubins.

RESULTS AND DISCUSSION

Polarography and Cyclic Voltammetry

Tables 1 - 3 show the electroanalytical results obtained in DMF/$LiClO_4$ with all dipyrrinones and bilirubins (1 - 9). Figures 1 and 2 show polarographic and voltammetric recordings of some representative examples. For more details see experimental part.

Dipyrrin-1(10H)-ones. The results obtained in DMF/$LiClO_4$ with the alkyl substituted dipyrrin-1(10H)-ones 1 and 3 agree with those previously published for dipyrrin-1(10H)-ones with different alkyl substitution patterns [4a,b]: two monoelectronic irreversible diffussion processes, which shift to more negative potentials by increasing the alkyl substitution. This shift is much higher for the first monoelectronic process than for the second one. In fact, the second process appears at similar potentials for all type of non vinyl substituted dipyrrin-1(10H)-ones.

However, alkyl substitution at the terminal alpha position of the pyrrole ring does not affect significantly its reduction behaviour (compare 1 with 3: tables 1 and 2). This last result justifies the use in reduction experiments of 4 and 5 - in spite of not being substituted at the C9 position - as partial models for vinyl substituted bilirubins with IXα, IIIα and XIIIα substitution patterns (bilirubins may conceptually be decomposed in two dipyrrin-1(10H)-one halves but CH_2X substituted at the α carbon atom C9).

The vinyl substituted dipyrrin-1(10H)-ones 4 and 5 show a similar but differentiated behaviour with respect to their non vinyl substituted parents. They also show two irreversible and difussion controlled processes, but the first monoelectronic process appears at a potential $\cong$0.2 V more positive than the corresponding one for dipyrrin-1(10H)-ones 1 and 3 (see Fig. 1). In this case the second process appears at similar potentials than those of the non vinyl substituted dipyrrin-1(10H)-ones, but now this polarographic wave is bielectronic. Comparison between the effects of endo and exo vinyl substitution (4 and 5) shows that for the first electronic process their potential values are very similar. Nevertheless, working under the same experimental conditions allows to observe differences in the order of 10 mV (see Tables 1 and 2). Larger differences can be observed for the second electronic process, which appears at a more negative potential (60 to 90 mV) for the exo vinyl dipyrrin-1(10H)-one (5) than for the endo vinyl dipyrrin-1(10H)-one (4). In case of the free acid 2 compared to its methyl ester 1 both electronic processes are shifted towards more positive potentials ($\cong$ 0.1 V). The role of the acidic groups in this effect upon the reduction potentials is discussed below.

Electrolysis experiments at first wave potential (see below) show, the reductive electrodimerization of the dipyrrin-1(10H)-ones 1, 4 and 5, as we have already reported for some alkyl substituted dipyrrin-1(10H)-ones[4]. Some of the electroanalytical experiments made in order to elucidate the dimerization mechanism are shown in Table 3. In polarography, the plot of potential versus $\log[(I_d - I)/(I_d^{1/3}I^{2/3})]$, I_d being the limiting current and I the current at a given potential[8-10], shows straight lines with slopes about 60 - 70 mV/log (see table 3). In cyclic voltammetry the peak potential varies linearly with the logarithm of the sweep rate, with a dependence of about 20 mV/log (see table 3). These results confirm[4b,8-10] that the reductive dimerization of dipyrrin-1(10H)-ones occurs through an EC (radical-radical) mechanism[8-10]. Although similar results to that of table 3 have been obtained for the mesobilirubin (6) and bilirubin dimethyl ester (9), i. e. suggesting an EC (radical-radical) dimerization

Table 1. Values of polarographic limiting currents and half wave potentials (s.c.e). $5 \cdot 10^{-4}$ mol·l^{-1} substrate in anhydrous DMF, 0.1 mol·l^{-1} $LiClO_4$.

SUBSTANCE	FIRST PROCESS		SECOND PROCESS	
Methyl Esters	$U_{1/2}$(V)	I_d(μA)	$U_{1/2}$(V)	I_d(μA)
1	-1.83	0.9	-2.00	0.6
3	-1.87	0.9	-2.00	0.6
4	-1.62	1.0	-1.93	2.0
5	-1.63	1.1	-2.02	2.1
8	-1.75	1.7	-1.88	1.8
9	-1.60	1.9	-1.81	1.6
Acids				
2	-1.45	0.2	-1.89	0.6
	-1.74	0.9		
6	-1.45	1.7	-1.92	1.9 [a]
7	-1.24	1.0	-1.70	1.7
	-1.37	0.8	-1.90	0.9

a) a second kind maximum is observed at - 1.86 V.

Table 2. Peak potential values (s.c.e) at sweep rate 50 mV s^{-1} (h.m.d.e.). $5 \cdot 10^{-4}$ mol·l^{-1} substrate in anhydrous DMF, 0.1 mol·l^{-1} $LiClO_4$.

SUBSTANCE	FIRST PROCESS		SECOND PROCESS
Methyl esters	U_{p1} (V)	I_{P1} (μA)	U_{p2} (V)
1	-1.89	0.8	-1.99
3	-1.87	0.7	-2.03 [a]
4	-1.66	0.8	-1.99
5	-1.67	0.9	-2.05 [a]
8	-1.81	1.4	-1.96
9	-1.65	1.5	-1.90
Acids			
2	(-1.56)[b] -1.79	1.0	-1.89
8	-1.55 [c]	1.3	(-1.88)[d] -1.97
9	-1.28	0.8	(-1.74) -1.93

a) the discharge of supporting electrolyte overlap the peak.
b) pre-wave shoulder.
c) wide peak.
d) it corresponds to the polarographic second kind maximum.

Table 3. Values of the slopes of semilogarithmic plots of the first wave, the U_p vs. log v (sweep rate) and U_p vs. log f(I) dependences. $5 \cdot 10^{-4}$ mol l^{-1} substrate in anhydrous DMF, 0.1 mol·l^{-1} $LiClO_4$ (other experimental conditions see table 1, 2 and experimental part).

	Slopes of U_{p1} vs. log f(I) [a]	Slopes of U_p vs. log v
1	59	-19
3	65	-19
4	74	-17
5	67	-22

a) $f(I) = (I_d - I)/(I_d^{1/3} \; I^{2/3})$

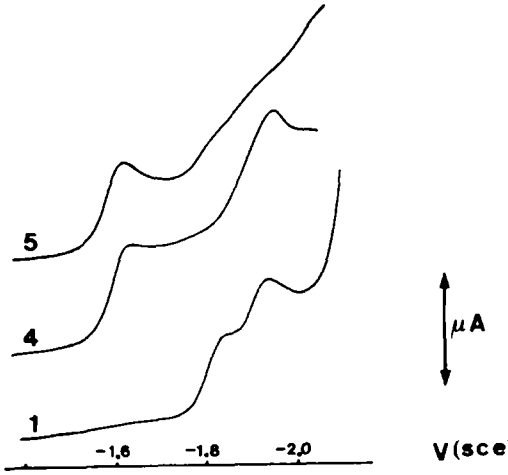


Figure 1. Voltammetry of $5 \cdot 10^{-4}$ mol l^{-1} solutions of the dipyrrinones 1, 4 and 5 in DMF/0.1 mol $\cdot l^{-1}$ $LiClO_4$: sweep rate 10 mV $\cdot s^{-1}$.

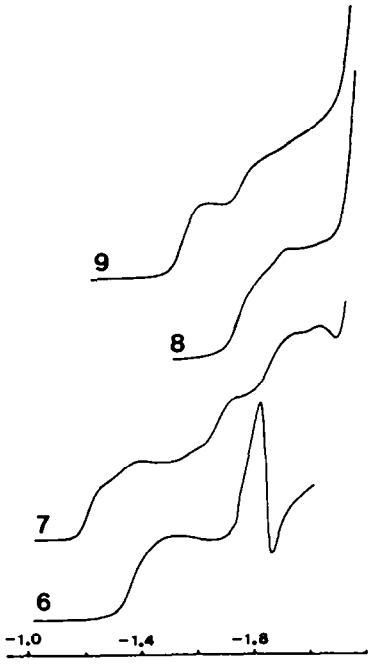


Figure 2. Voltammetry of mesobilirubin (6) bilirubin (7) and their dimethyl esters 8 and 9 in DMF / 0.1 mol l^{-1} $LiClO_4$: $c = 5 \cdot 10^{-4}$ mol $\cdot l^{-1}$, sweep rate 10 mV $\cdot s^{-1}$.

mechanism, the interpretation of these results is not easy owing to the complexity of their first polarographic wave and voltammetric peak. Furthermore, in the theoretical treatment of the dimerization mechanism[9-10], the possibility of polymerization (two dimerization sites in the molecule, is not taken into account).

Biladiene-ac-diones (bilirubins). Mesobilirubin dimethyl ester (8) shows the same polarographic and voltammetric pattern, as its partial model, the alkyl substituted dipyrrinones (e.g. 1 and 3), corresponding to two irreversible electronic processes but both processes appear for 8 at some more positive potentials ($\cong$ 100 mV) and instead of being monoelectronic, as in the case of its model the dipyrrin-1(10H)-ones, they are bielectronic. The first electronic process for 8 probably is not due to a two electron capture by one of the dipyrrin-1(10H)-one halves, because all electroanalytical results obtained for dipyrrin-1(10H)-ones show a first monoelectronic process separated from the next electronic process by more than 0.1 V. More probably, this first bielectronic process of biladiene-ac-diones corresponds to the capture of one electron by each dipyrrin-1(10H)-one unit (see below in electrolysis experiments). EPR experiments agree with this interpretation: galvanostatic electrolysis at low intensity, i. e. at first electronic process potentials, from 1 as well as from mesobilirubin dimethyl ester 8

results in a radical signal, which could be attributed to the corresponding transient or to a more stable free radical formed from the former by exchange (additional work is in progress). According to the potentials of this first electronic process of bilirubins compared to that of their model dipyrrinones (see Table 1 and 2, and Fig. 2), the "double" one electron capture is not affected by destabilizing electronic repulsions.

In the case of the dimethyl ester of natural bilirubin 9, which is composed of the two vinyl substituted partial models 4 and 5, the first electronic process shows the same trend as for 8: i. e. a bielectronic process at similar potentials as its partial models 4 and 5. The second process of 9 is also bielectronic and appears at even more positive potentials ($\tilde{=}$0.12-0,21 V) than the second bielectronic process of the model dipyrrinones 4 and 5.

The results obtained with the free acids 2, 6 and 7 are different from those of their methyl esters. The first process for mesobilirubin 6 - bielectronic and irreversible - appears at more positive potential ($\tilde{=}$0.3 V) than its dimethyl ester 8 and its partial model 2. However, 2 shows a prewave at the same potential as the first process of 6. In contrast with this, the second process of 6 - bielectronic and irreversible - appears at a potential similar to that of 2 and 8. The first electronic process for the vinyl substituted bilirubin 7 also appears at more positive potentials as for its dimethyl ester 9. However, 9 shows a splitting of its first electronic process, which agrees with the results in H_2O (pH = 7.4) reported in the literature[7]. According to ref. [7] the splitting corresponds to a differentiated first monoelectronic process for the endo and exo halves of the molecule [endo half at more positive potential ($\tilde{=}$ 0.15 V) than the exo half].

In conclusion, an effect by the vinyl groups facilitating the reduction is clearly shown both in acids and esters, but a distinct effect on the first electronic process by endo and exo vinyl substitution is clearly observed only in the free acids. The first effect is a direct consequence of the LUMO distribution through the dipyrin-1(10H)-one structure[12]. This distribution allows conjugation with the vinyl group attached to C2 or C3. The different behaviour of acids and their methyl esters is not easily explained. A simple effect of proton donors in the medium does not agree with the effect produced on the voltammograms of the dimethyl esters 8 and 9 by acetic acid addition. Voltammetry of 8 and 9 in the presence of acetic acid (for a molar ratio to substrate between 2 and 4) shows a shift of the second process towards more positive potentials but does affect appreciably the first one.

For the first electronic process and in the absence of proton donors all results point to a mechanistic pathway consisting of a reversible electron capture, which becomes irreversible because of the prototropic processes leading to carboxyamidure anions[4]. An increase in the acidity of the medium can favour reduction - shift towards more positive potentials - by formation of more stable anions than the carboxyamidure one. Nevertheless, even though a possible neighbouring effect by the carboxylic acid group

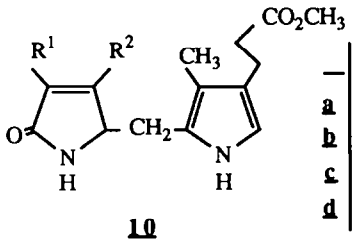
cannot be excluded, we speculate that the important effect by the propionic acid substituents is due to differences on intra- and intermolecular association and to the corresponding changes in the conformational equilibria. It is known, that dipyrrin-1(10H)-ones can exist as free and associated forms (dimers) depending on the solvent[13]: for bilirubins and bilirubin derivatives different types of association exist, giving rise to different conformational equilibria (dimers or other internally associated forms involving the ester or acid groups and the nitrogen-bonded hydrogens)[13]: such associations depend on the media and on wether or not the free-acid carboxylic group is esterified. Moreover, changes in association can determine conformational changes. and we suggest that these different conformers and associated forms afford different electroanalytical responses.

In spite of the different behaviour of the methyl esters compared to the acid forms shown by the present results, these correlate well with those reported in the literature for bilirubin, taking into account the instrumental and experimentals conditions.. However, it has been reported[3h] that bilirubin in 0.02 M aqueous KOH shows a first peak with complex structure at - 0.25 V, which was attributed to adsorption of bilirubin on the electrode. Under our conditions, this peak is also observed, and it much more intense in alkaline solutions. Actually, this peak belongs to the process $2e^- + Hg^{++} \longrightarrow Hg^0$, as we have confirmed in a blank solution, and is the result of a too positive potential start ($\cong$ - 0.1 V),. Nevertheless, its complex structure (at least two maxima), which appears only when bilirubin is present, must be attributed to the presence of several Hg^{++} species corresponding to the complexation of Hg^{++} by bilirubin on the electrode surface.

Electrochemical Reaction Products

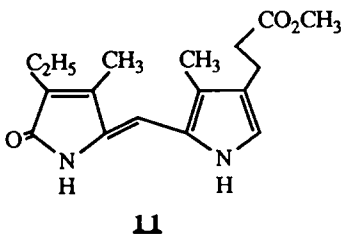
Semi-micro reductive electrolysis experiments on Hg cathode (see experimental part) were performed on some dipyrrin-1(10H)-ones and bilirubin derivatives.

3-Ethyl-2,7,9-trimethyl-8-(2-methoxycarbonylethyl)-dipyrrin-1(10H)-ona (Xanthobilirubinic acid methyl ester) (1): The results obtained confirm the already reported behaviour of dipyrrin-1(10H)-ones on cathodic reduction[2], i.e. in aprotic media at first wave potential reductive dimerization to 14a takes place, and at second wave potential there is also reductive dimerization but this is associated with hydrogenation to the 4,5-dihydro dipyrrin-1(10H)-one 10a. An increase in the proton acidity of the medium, increases the yield of hydrogenated product and decreases the yield of dimers. 4,5-Dihydro dipyprrin-1(10H)-ones are obtained in good yields as the main product: e. g. using sodium dithionit[11]. In this respect, it must be pointed out that the electrochemical preparation (second process potentials and in the presence of proton donors) of 4,5-dihydro-3,7-dimethyl-2-ethyl-8(2-methoxycarbonylethyl)-dipyrrin-1(10H)-one from the corresponding dipyrrinone (Hg cathode: H_2O/THF pH=4.9) had already been reported[6a]. Our present results show that the more complex substitution pattern of the dipyrrin-1(10H)-ones studied here, compared to that of the previously reported ones[2],



	R^1	R^2
a	CH_3	C_2H_5
b	C_2H_5	CH_3
c	CH_3	C_2H_3
d	C_2H_3	CH_3

10



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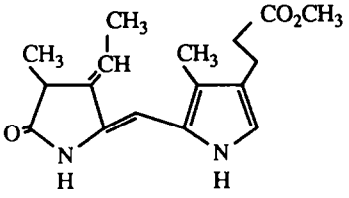

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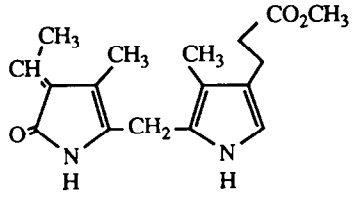

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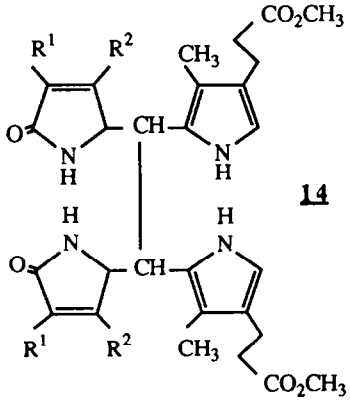


14

	R^1	R^2	R^3
a	CH_3	C_2H_5	CH_3
b	CH_3	C_2H_3	H
c	C_2H_3	CH_3	H

Table 4. Electrolysis reaction products of the vinyl substituted dipyrrin-1(10H)-ones 4 and 5 (≅2 electrons·molecule^{-1}) in the presence of a proton donor; phenol, 0.5 mol·l^{-1}.

Electrolysis Reaction Products[a]		SUBSTRATE: 4 (endo vinyl) (-1.55 V: = 2.1 e)	SUBSTRATE: 5 (exo vinyl) (-1.60 V: = 2.1 e)
vinyl group reduction	3 or 11	20 ± 2 %	55 ± 2 %
exocyclic double bond reduction	10c or 10d	55 ± 2 %	5 ± 2 %
ethylidenic derivatives	12 or 13	25 ± 2 %	35 ± 2 %
tetrahydro derivatives	10a or 10b	---------	5 ± 2 %

a) yields calculated from ^{1}H-NMR spectra and confirmed by semi-preparative HPLC isolation: initial dipyrrinone was not detected.

supresses the stereoselectivity of the reductive dimerization process: for the methyl ester of xanthobilirubinic acid (1), the final reaction mixture consists of a complex mixture of stereoisomers of dimers of constitution formula 14a. The more abundant dimer 14a obtained from 1, which could be isolated by semi-preparative HPLC (see experimental part), does not show by ^{1}H-NMR any symmetry relationship between the two halves of the molecule: i. e., it belongs to the racemic mixture RRSR/SSRS or RRRS/SSSR. These results are clearly in contrast with those reported previously[2] for the reductive dimerization of 2,3-dimethyl dipyrrin-1(10H)-one, where only two dimers (relative ratio 7:3) of type 14 were obtained, both showing ^{1}H-NMR spectra corresponding to dimers with C_{2v}, C_s or C_2 NMR effective symmetry.

Endo and exo vinyl substituted dipyrrinones 4 and 5: Electrochemical reduction of the dipyrrinones 4 and 5 in DMF/$LiClO_4$ at first wave potential confirms a behaviour similar to that of the alkyl substituted dipyrrinones: i.e., reductive dimers bonded through the bridge carbon atoms were obtained. These dimerization processes give complex mixtures of diastereoisomers which are difficult to separate. However, according to the spectroscopic data of the whole reaction mixture and of its fractions separated by preparative HPLC (see experimental part), it is clear that the reaction products are stereoisomers of constitution formula 14. Starting from 4, a principal fraction containing only two diastereoisomers could be isolated by semi-preparative HPLC. In the case of 5, the formation of dimers was only studied in the final reaction mixture.

The molecular mass of the dimers was inferred from MS experiments (FAB analysis). Vinyl groups were present (^{1}H NMR and IR): by ^{1}H-NMR quantitative analysis they do not seem to be affected by the reduction. UV spectra show no conjugation between the lactam and the pyrrole rings. The position of the lactam carbonyl (1700-1660 cm^{-1} compared to 1650-1645 cm^{-1} for 4 and 5) in the IR spectra confirms the absence of exocyclic double bond conjugation). Further, ^{1}H NMR spectra show the presence of N-CH-CH-CH-CH-N spin systems[4a].

Electrolysis experiments were also performed in the presence of a proton donor (phenol 0.5 $mol \cdot l^{-1}$ in DMF/$LiClO_4$) and at potentials corresponding to the beginning of the second polarographic wave (two electrons/molecule). At such experimental conditions the second polarographic wave overlaps with the first one[2]. Moreover, the second wave potentials of vinyl substituted dipyrrinones being very similar to those of the alkyl substituted ones (see tables 1 and 2), in order to avoid the reduction of the first reaction products of 4 and 5, the working electrode must be adjusted to potentials corresponding to the beginning of the wave: more cathodic values result in the formation of the tetrahydro derivatives 10a and 10b, which are also the reaction products - at the same experimental conditions - for the electrolysis of the corresponding non vinyl substituted dipyrrinones. Accurate potential setting allows the obtention of dihydroderivatives as the principal reaction products.

Table 4 shows the differences in the final reaction products obtained by

electrolysis (2 electrons per molecule) of 4 and 5 in the presence of proton donors. A different behaviour towards reduction is clearly observed between 4 and 5:

a) Exocyclic double bond reduction (10c or 10d): for the endo vinyl dipyrrinone 4, the main reaction product is 10c, while for the exo vinyl dipyrrinone 5 only small amounts of 10d are obtained.

b) Vinyl group reduction (1 or 11): occurs for both types of dipyrrinones, with significantly different yields (56 % yield for 5 versus 20% for 4).

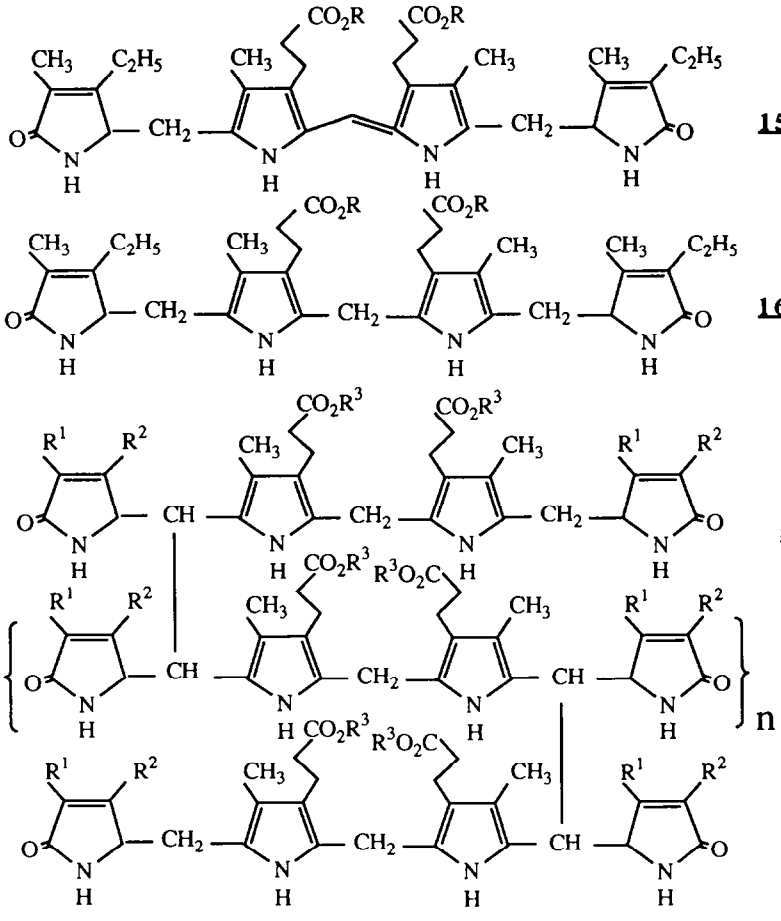
c) Ethylidenic derivatives (12 and 13): are obtained in similar yields for both types of dipyrrinones: in contrast with what occurs with 12, 13 does not involve an exocyclic double bond, i. e. there is no conjugation between the lactam and pyrrole rings.

Dihydro derivatives not involving conjugation between the two remaining C=C double bonds of the lactam ring are not detected neither in case of 4 nor in case of 5. Furthermore, for 4 the structure of 4-vinyl-3-pyrrolin-2-one was not detected either. The main difference between the reduction behaviour of both dipyrrinones consists of the yields of exocyclic doubly reduced derivative obtained from each: while from 4, this is the main reaction product, it is obtained in very low yield from 5. This result agrees with the fact that a 3-vinyl-3-pyrrolin-2-one structure is less easily obtained than the 4-vinyl-3-pyrrolin-2-one, probably because of their relative stabilities. In this respect, the structure suggested for the natural d-urobilin IXα(H_{42}) with the vinyl group in ring A (endo substitution)[13] not reduced, is of interest.

Mesobilirubin and Bilirubin dimethyl esters 8 and 9: The results obtained in the electrolysis of biladiene-ac-diones 8 and 9 confirm the electroanalytical results; i. e., the electrochemical behaviour of bilirubins is that of a chemical structure with two dipyrrin-1(10H)-one units. At potentials corresponding to the first electronic process reductive coupling through the bridge carbon atom of the dipyrrinone units occurs, and this implicates the possibility of polymerization processes. Both charge-to-substrate ratio and amounts of unreacted substrate agree with the presence of a polymerization process. At potentials corresponding to the second electronic process and in the presence of water (8.5 $mol \cdot l^{-1}$), the same type of products as for dipyrrinones are obtained; i. e., besides reductive coupling, hydrogenation of the 5-methylene-3-pyrrolin-2-one structures occurs.

In these experimental conditions, saponification of the methyl ester is a side reaction, which adds difficulties in the chemical separation of the electrolysis reaction products. Saponification occurs because of the alkalinity generated by hydrolysis of the electrolysis reaction products, i. e., the lithium carboxyamidure salts[4a]. Bilirubins, compared to dipyrrinones, require twofold amount of electrons per molecule, which results in a higher alkalinity at the end of the electrolysis.

The structural type of the derivatives obtained (although much of them could not be isolated) was identified by analysis of the final reaction mixture and of its fractions obtained by preparative HPLC (see experimental part).



15

	R
a	H
b	CH_3

16

	R
a	H
b	CH_3

17

a { $R^1 = CH_3$ or C_2H_5; $R^2 = C_2H_5$ or CH_3; $R^3 = CH_3$, H

b { $R^1 = CH_3$ or C_2H_3; $R^2 = C_2H_3$ or CH_3; $R^3 = CH_3$, H

Cathodic electrolysis of 8 at potentials corresponding to the first process (monoelectronic), after a total current passage corresponding to 1 electron per molecule, give very small amounts of reduced derivatives (detected only as spurious peaks by MS): 20 % of the initial bilirubin was present in the mixture. This fact accounts for the occurrence of a polymerization process. The rest of reaction products were only partially soluble in $CHCl_3$. The organic phase does not contain either urobilin IXα (15a) or urobilinogen IXα (16a), the UV/Vis spectra shows that the exocyclic double bond has been reduced (λ_{max} = 225 nm); ^1H NMR spectra confirms this point, and it also shows partial saponification of the methyl esters. The water phase was brown, with colloid aspect: liophilization, dialysis and final liophilization gave a brown powder which was sparingly soluble in DMSO, DMF, etc. This powder did not melt below 300° C: MS (FAB) analysis did not show any significant peak, but elemental analysis indicated its organic nature and

relative ratios between C/H/N corresponding to bile pigments. UV/Vis spectra in DMSO showed a strong absorption under 320 nm and absortion in all visible region up to 600 nm, shoulders were shown about 420 and 520 nm which by HCl addition shifted to 500 nm and 570-605 nm respectively. These absorption bands are characteristic of urobilinoids and biliviolinoids. On the basis of the dimerization of dipyrrin-1(10H)-ones and of the results reported here, we propose structures analogous to 17 for this type of polymers or oligomers. Nevertheless, the central bridges can easily be oxidized to urobilinoid structures and the terminal residues can isomerize to urobilinoid structures and oxidize to biliviolinoid structures, all of which would account for the observed UV/VIS spectra.

In other electrolysis experiments with 8 at second wave potentials, different electron-to-molecule ratio (between 2 and 4) and in the presence of water, bilirubin hydrogenated derivatives [urobilin IXα (15), urobilinogen IXα (16)] and dimers of constitution formula 17 (n = 0) were detected; this indicates for biladien-ac-diones a similar behaviour to that of the dipyrrin-1(10H)-ones. Furthermore, the water phase also contained an insoluble brown polymeric material similar to that described above.

The formation of such polymeric material is important in relationship to the fecal pigment discussion[32]: the so-called bilifuscin and mesobilifuscin are ill-defined fractions isolated from faeces as brown insoluble pigments. In spite of the reducing media of the lumen of the large intestine and of the fate of bilirubin in intestine towards hydrogenated derivatives suggestions on the chemical structure and origin of the fecal pigment have been made involving oxidative coupling of bilirubin and bilirubin derivatives. Bilifuscin and mesobilifuscin could be the pigments generated by oxidation processes from polymers of type 17. These, in turn, will be formed as by-products in the reduction of bilirubin to urobilinogen.

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EXPERIMENTAL

Melting points were determined on a Kofler-Reichert microhot stage apparatus. UV/VIS spectra were recorded on a Perkin-Elmer Lambda 5 instrument: IR spectra on a Perkin-Elmer 681 spectrometer: mass spectra (MS) on a a Hewlett-Packard 5988A instrument eqipped for FAB analysis with a Capillaritron Frasor. ^{1}H-NMR were determined on a Varian XL-200 (200 MHz) or on a Bruker WP 80 SYFT (80 MHz) instrument. EPR experiments were recorded on a Varian E-109E instrument using a planar cell adapted for galvanostatic work (mercury cathode an a platinium sheet as anode): measurements were performed at the beginning of the intensity increase, which was assumed to correspond to the first process potential. High pressure liquid chromatography (HPLC) was carried out on RadialPak silica or C18 columns with a Waters double pump using a variable wavelength detector 5FA 339. Preparative HPLC (PHPLC) at the semimicro scale was carried out through repetitive injection using the same system and conditions as for analytical HPLC or using a silica Porosil 15 cm x 19 mm column (Waters).

Mercury was chemically purified before being doubly distilled. Dimethylformamide (DMF) was anhydrous and p.a. quality. Anhydrous $LiClO_4$ was obtained[16] from p. a. $LiClO_4$

$3H_2O$. Bilirubin IXα was of commercial origin (Sigma) and was tested by HPLC for the presence of isomers IIIα and XIIIα. The preparation and properties of the following compounds are described in the literature: (Z)-3-ethyl-2,7,9-trimethyl-8-(2-methoxycarbonylethyl) dipyrrin-1(10H)-one (xanthobilirubinic acid methyl ester) **1**[17], (Z)-3-ethyl-2,7,9-trimethyl-8-(2-carboxyethyl)dipyrrin-1(10H)-one (xanthobilirubinic acid) **2**[18], (Z)-2,7-dimethyl-3-ethyl-8 (2-metoxycarbonylethyl)-dipyrrin-1(10H)-one (neoxanthobilirubinic acid methyl ester) **3**[19], (Z)-2,7-dimethyl-7-(2-methoxycarbonylethyl)-3-vinyl-dipyrrin-1(10H)-one **4**[20,21], (Z)3,7-dimethyl-7-(2-methoxycarbonylethyl)-2-vinyl-dipyrrin-1(10H)-one **5**[20,21], mesobilirubina IX **7**[22,23]. Mesobilirubin IXα dimethyl ester **8** and bilirubin IXα dimethylester **9** were obtained form the corresponding free acid forms by diazomethane esterification according to ref.[24]

Polarographic and Voltammetric Measurements - Polarograms have been carried out with an Amel 471 multipolarograph with a Tacussel knock (GCMS-MP03). Voltammetric curves have been obtained by means of a Belport HQ 305 sweep generator, a Belport HQ 105 potentiostat with IR compensation and a Houston 2000 X-Y recorder. All measurements were performed in a 25 ml thermostatic cell. As working electrodes we used a Metrohm EA 671 capillary (drop time 2 s: mercury flow 0.798 $mg \cdot s^{-1}$ at 50 cm open circuit) for polarography and a Metrohm E 410 h.m.d.e. with a surface area of 1.56 mm^2 for voltammetry. The reference electrode was a saturated calomel electrode (s.c.e.) separated from the solution by a salt bridge (Luggin capillary) containing the used supporting electrolyte. The auxiliary electrode was a platinium sheet immersed in a solution of supporting electrolyte, and separated from the solution by a sintered glass. The supporting electrolyte was 0.1 $mol \cdot l^{-1}$ $LiClO_4$. Argon was used to deaerate the solutions; during the measurements, solvent-saturated argon was bubbled through the cell.

Electrolysis and Coulometric Measurements - Electrolysis at constant potential was carried out by a Potentiostat Amel 555A. The progress of the reaction was followed by plotting the intensity vs. the reaction time. The coulometric measurements were carried out in the small cells using an integrator Amel 721 to measure the charge. Experiments were performed in 10 ml thermostabilized cells (25 ± 1° C), the anode and the cathode compartments being separated by a double syntered glass. The working electrode was a magnetically stirred mercury pool. The reference and auxiliary electrodes were the same as in polarographic experiments. Argon saturated with solvent vapours was bubbled through the solution 20 minutes before and during the electrolysis. The applied electrolysis potentials were determined from a polarogram of the solution to be electrolyzed, otherwise it is difficult to distinguish between first and second process potentials. At the end of the reaction the normal work up consists on rotoevaporation of the solvent (oil pump vacuum) and extraction of the residue in a $H_2O/CHCl_3$ or H_2O/CH_2Cl_2 mixtures. For full experimental details see ref.[25]

Cathodic electrolysis of **1** at first wave potential. 9ml of a $1 \cdot 10^{-2}$ $mol \cdot l^{-1}$ solution of **1** in DMF/$LiClO_4$ 0.1 $mol \cdot l^{-1}$ were electrolyzed at first process potential (-1.85 V). The current intensity was stabilized after the capture of 0.5 ± 0.05 electron · $molecule^{-1}$: that shows how the substrate acts as proton donor, being the formed anion more difficult to oxidate than the neutral substrate (see results). The residue of the organic phase corresponds to 96 % of the initial weight. Thin layer chromatography and ^{1}H-NMR (200 MHz) show the presence of about 50 % of the initial substrate, in agreement with the coulometric measurement. After column chromatography on silica gel, PHPLC (RadialPak SiO_2: 80 % $CHCl_3$ and 20 % CH_3CN/CH_3OH, 5/1) allows to separate the initial substrate **1** as the more mobile fraction. 4 mg of one of this fractions (R_f twofold that of the substrate) can be identified as one of the estereoisomers of 1,2-bis(3-ethyl-4-methyl-5-oxo-3-pyrrolin-2yl)-1.2-bis[3,5-dimethyl-4-(methoxycarbonylethyl)-2-pyrrolyl]etane (**14a**) according to the following data.

^{1}H-NMR (200 MHZ: $CDCl_3$; δ ppm): 8.9, 8.3, 7.5 and 7.3 (4 broad s, 4H, NH), 4.44 (d, 1H, N-CH, J = 10.6 Hz), 4.04 (d, 1H, N-CH, J = 12.5 Hz), 3.64 (center of multiplet, 1H, bridge CH), 3.63 and 3.55 (2 s, 6H, two $COOCH_3$), 3.32 (d.d, 1H, bridge CH), 2.6-2.2 (m, 12H, CH_2-CH_2-$COOCH_3$ and CH_3-CH_2-), 2.10 and 1.90 (2 s, 6H, pyrrole CH_3-C5), 1.77 (s, 6H, pyrrole CH_3-C3 and lactam CH_3-C4), 1.25 and 0.77 (2 t, 6H, CH_3-CH_2-, J = 7.6 Hz).

IR (KBr; cm^{-1}): 1740, 1685.

UV/VIS (CH_3OH: λ_{max} (nm)): 215

MS [FAB: Xe, DTT; m/z (%)]: 657 (M+23, 2), 634 (M, 4), 633 (M-1, 5), 386 (100), 317 (32). The rest of fractions show similar ^{1}H-NMR spectra.

Cathodic electrolysis of 4 at first wave potential. 9ml of a $1 \cdot 10^{-2}$ mol·l^{-1} solution of 4 in DMF/$LiClO_4$ 0.1 mol·l^{-1} were electrolyzed at first process potential (-1.63 V). The current intensity was stabilized after the capture of 0.6 ± 0.05 electron·molecule^{-1} (see above). The residue of the organic phase corresponds to 96 % of the initial substrate weight. Chromatography and ^{1}H-NMR (200 MHz) show the presence of 40-50 % of the initial substrate. The ^{1}H-NMR spectrum allows to exclude vinyl group reduction (absence of any triplet signal in the 1.3 - 1.1 ppm region). After separation of the initial 4 by column chromatography the rest of the fraction do not show the typical UV/VIS absorption of dipyrrin-1(10H)-one, i.e. no π conjugation occurs between lactam and pyrrole rings. PHPLC three fractions are separated, each showing several peaks by analytical HPLC. All fractions have similar ^{1}H-NMR, IR, and UV/VIS spectra and no differences in the MS(FAB). The second fraction is the most important one (3 mg) and contains two diastereoisomers of 1,2-bis[3-methyl-4-(2-methoxycarbonylethyl)-2-pyrrolyl]-1.2-bis(4-methyl-5-oxo-3-vinyl-3-pyrrolin-2-yl)ethane (14b) in ratio 4:1. The recorded data for the more abundant isomer are:

^{1}H-NMR (200 MHz: $CDCl_3$; δ ppm): 9.0, 8.6, 7.9 and 7.3 (4 s, 4 NH), 6.29 and 6.09 (2 s, 2 pyrrole H-C5), 6.5-4.8 (m, vinyl groups), 4.2-3.6 (m, N-CH and pyrrole-CH), 3.62 (s, 2 $COOCH_3$), 1.93-1.3 (CH_3 groups).
IR (KBr; cm^{-1}): 1740, 1685.
UV/VIS (CH_3OH; λ_{max} nm): 224.
MS [FAB: Xe, DTT; m/z (%)]: 603 (M + 1, 48), 358 (69), 301 (100).

Cathodic electrolysis of 5 at first wave potential. 9ml of a $1 \cdot 10^{-2}$ mol·l^{-1} solution of 4 in DMF/$LiClO_4$ 0.1 mol·l^{-1} were electrolyzed at first process potential (-1.62 V). The current intensity was stabilized after the capture of 0.9 ± 0.1 electron·molecule^{-1} (see above). The residue of the organic phase corresponds to 75 % of the initial substrate weight. By chromatography and ^{1}H-NMR (200 MHz) it is shown that only small amounts of initial substrate are present. By ^{1}H-NMR it is shown that the mixture is composed as in case of the redution of 4, described above, of diastereoisomers of the dimer 1,2-bis[3-methyl-4-(2-methoxycarbonylethyl)-2-pyrrolyl]-1.2-bis(3-methyl-5-oxo-4-vinyl-3-pyrrolin-2-yl)ethane (14b). ^{1}H-NMR (200 MHz, $CDCl_3$, δ ppm): presence of the vinyl groups, presence of the N-CH-CH-CH-CH-N system. IR (KBr; cm^{-1}): 1740-1730, 1710-1670. UV/VIS (CH_3OH; λ_{max} nm): 224. MS [FAB: Xe, DTT; m/z (%)]: 603 (M + 1, 19), 480 (16), 358 (22), 301 (100).

Cathodic electrolysis of 4 in the presence of phenol. 9ml of a 1.4 10^{-2} mol l^{-1} solution of 4 in DMF/$LiClO_4$ 0.1 mol·l^{-1} and phenol 0.5 mol·l^{-1} were electrolyzed at -1.55 V (in this conditions the two electronic processes coincide (see text). The current intensity was stabilized after the capture of 1.7 ± 0.2 electron · molecule^{-1}. The dry residue was solubized in CH_2Cl_2 and the phenol extracted with 1 % aqueous Na_2CO_3. After washing with water and drying with Na_2SO_4 the solution was vacuum evaporated. The residue weight was approximately the same as that of the initial substrate. After column chromatography by PHPLC (Porasil, CH_2Cl_2/EtOH, 100:4, 8 ml·min^{-1} three compounds could be separated. They were identified as 12 (2 mg), 3 (1 mg) and 10c (4 mg) (in order of elution). By HPLC and ^{1}H-NMR analysis of the reaction crude, this was shown to contain 10c, 12 and 3 in ratios 55:25:20 respectively. The physical and spectroscopic data of the obtained 3 agree with those of the literature[19] and of a real sample. For 10c and 12 the recorded data are the following:

3-Ethyliden-2,3-dihydro-2,7-dimethyl-8-(2-methoxycarbonylethyl)dipyrrin-1(10H)-one (12).
^{1}H-NMR (200 MHz, $CDCl_3$; δ ppm): 8.65 and 8.45 (2 broad s, 2H, NH), 6.57 (d?, 1H, H-C9, $\Delta\nu$ = 2.5 Hz), 6.11 (d q, 1H, CH_3-CH=, $|J_{H,CH}|$ = 7.2 Hz, $|J_{H,H-C2}|$ = 2.4 Hz), 5.78 (s, 1H, H-C5), 3.68 (s, 3H, $COOCH_3$), 3.18 (m, 1H, H-C2), 2.8-2.5 (m, 4H, CH_2-CH_2), 2.01 (s, 3H, CH_3-C7), 1.84 (d, 3H, CH_3-CH=, J =7.2 Hz), 1.38 (d, 3H, CH_3-C2, |J| = 7.4 Hz). Double resonance experiments at 6.1, and 3.2.
IR (KBr; cm^{-1}): 1740, 1710.
UV/VIS (CH_3OH; λ_{max}, nm): 282, 340 sh.
MS [m/z (%)]: 302 (M^+, 100), 287 (21), 271 (12), 243 (7), 229 (23).

4,5-Dihydro-2,7-dimethyl-8-(2-methoxycarbonylethyl)-3-vinyldipyrrin-1(10H)-one (10c).
M. p. 120 - 125° C (lit.[26] 123-125° C).
^{1}H-NMR (200 MHz, $CDCl_3$; δ ppm): 8.3 and 6.9 (2 broad s, NH), 6.42 (d?, 1H, H-C9, $\Delta\nu$ = 2.5 Hz), 3.68 (s, 3H, $COOCH_3$), 2.8-2.4 (m, 4H, CH_2-CH_2-), 1.97 (s, 3H, CH_3-C7), 1.90 (s, 3H, CH_3-C2), ABX vinyl spin system: 6.75, 6.70, 6.67 and 6.61 (X part), 5.55-5.45 (m, 2H, AB part). AMX bridge spin system: 4.31 (center of part X), 3.23 (center of part M), 2.44 (center of part A), $|J_{AM}|$ = 15.1 Hz, $|J_{AX}|$ = 9.1 Hz, and $|J_{MX}|$ = 3.1 Hz.
IR (KBr, cm^{-1}): 1740, 1680.
MS [m/z (%)]: 302 (M^+, 1), 271 (1), 229 (1), 180 (100), 122 (11).

Cathodic electrolysis of 5 in the presence of phenol. 7 ml of a $1.4 \cdot 10^{-2}$ mol $\cdot$ l^{-1} solution of 5 in DMF/$LiClO_4$ 0.1 mol$\cdot l^{-1}$ and phenol 0.5 mol$\cdot l^{-1}$ were electrolyzed at -1.60 V (in this conditions the two electronic processes coincide (see text). The current intensity was stabilized after the capture of 1.8 ± 0.2 electron molecule^{-1}. The dry residue was solubized in CH_2Cl_2 and the phenol was extracted with 1 % aqueous Na_2CO_3. After washing with water and drying with Na_2SO_4, the solution was vacuum evaporated. The residue weight was approximately 80 % that of the initial substrate. By column chromatography PHPLC (Porasil, CH_2Cl_2/EtOH, 100:4, 8 ml$\cdot$min^{-1}) two compounds could be separated, which were identified by their spectra as 13 (3 mg), 11 (7 mg) and a mixture 1:1 of 10b and 10d (4 mg) (in order of elution): through HPLC and ^{1}H-NMR analysis of the reaction crude it was shown to be composed by 11, 13 and 10d and 10b in ratios 55:35:5:5 respectively. The spectra of the mixture of 10b and 10d could be interpreted because of the physical data reported in the literature for 10b[26,27] and by comparison with a real sample, which allow to infer the ^{1}H-NMR spectrum of 10d. The physical and spectroscopic data of the obtained 11 agree with those of the literature[28,29] and of a real sample.
(Z)- 2-Ethylidene-2,5-dihydro-3,7-dimethyl-8-(2-methoxycarbonylethyl)dipyrrin-1(10H9-one (13).
M.p. 148-151° C.
^{1}H-NMR (200 MHz, $CDCl_3$; δ ppm): 8.13 and 7.78 (2 broad s, 2H, NH), 6.40 (d?, 1H, $\Delta\nu$ = 2.5 Hz), 6.26 (q, 1H, CH_3-CH=, J = 7.8 Hz), 3.67 (s, 3H, $COOCH_3$), 3.57 (s, 2H, H-C5), 2.8-2.5 (m, 4H, CH_2-CH_2-), 2.32 (d, 3H, CH_3-CH=, J = 7.8 Hz), 1.98 (s, 3H, CH_3-C7), 1.81 (s, 3H, CH_3-C3). Double resonance experiments confirms the structure.
IR (CCl_4, cm^{-1}): 1750, 1700.
UV/VIS (CH_3OH, λ_{max}, nm): 199.
MS [m/z (%)]: 302 (M, 11), 229 (7), 180 (11), 167 (20), 120 (19).
4,5-dihydro-3,7-dimethyl-8-(2-methoxycarbonylethyl)-2-vinyldipyrrin-1(10H)-one (10d)[30,31]
^{1}H-NMR (200 MHz, $CDCl_3$; δ ppm): 8.4 (broad s, 1H, pyrrole NH), 6.8 (broad s, 1H, lactam NH), 6.41 (s, 1H, H-C9), 4.00 (m, 1H, H-C4), 3.67 (s, 3H, $COOCH_3$), 3.2-2.2 (m, 6H, CH_2-CH_2- and H-C5-H), 2.07 (s, 3H, CH_3-C3), 1.98 (s, 3H, CH_3-C7). Vinyl spin system: 6.41 (d d, H_A), 6.23 (d d, H_M), 5.41 (d d, H_X): $|J_{AX}|$ = 11 Hz, $|J_{AM}|$ = 18 Hz, $|J_{MX}|$ = 2 Hz.
MS [m/z (%)]: EI; 302 (M, 6). CI (NH_3); 320 (M + 18), 303 (M+1).

Cathodic electrolysis of 8 and 9 in DMF/Water. 9 ml of a $1.3 \cdot 10^{-2}$ mol$\cdot l^{-1}$ solution of the substrate in DMF/H_2O (85:15), $LiClO_4$ 0.1 mol$\cdot l^{-1}$ were electrolyzed at potential corresponding to the first or second electronic process (determined by polarography in the same solution). The subsequent work-up was the same as described above for the dipyrrin-1(10H)-ones. ^{1}H-NMR of the reaction crude indicates an increase in substrate with saponified propionic acid methyl ester substituents as the number of captured electrons by molecule increase.
8 at first wave potential. At -1.73 V and 1.1 ± 0.1 electron$\cdot$molecule^{-1}. The organic phase (≅60 % of the initial substrate weight) contains (^{1}H-NMR) about 30 % of the initial substrate: this spectrum also shows that the methyl ester group is barely saponified. However, the residue shows UV/VIS and IR spectra with the typical absorptions corresponding to dipyrrin-1(10H)-ones saturated between C4 and C5 (228 nm and 1670 cm^{-1} respectively). MS gives only confident recording on FAB conditions (Xe, DTT/DTE 3:1) with molecular peak cluster centered at 1236 m/z and the corresponding clusters at (M + Na) and (M + K), which as we suggest (see text) corresponds to dimers of type 17a (for n = 0 and R^3 = CH_3) and to dimers with insaturations either exocyclic to the terminal lactam ring or isomerized to form a dipyrrin system: fragmentations are in agreement with a structure of type 17, see below. Smaller clusters corresponding to dimers with one or two saponified carboxy groups are also observed (centered at 1224 and 1208 m/z). The yellow-

brown colloidal water phase was vacuum evaporated, dialyzed (15000-20000) and lyophilyzed. A solid (6 mg, ≅6 % initial weight) was obtained insoluble in non polar organic solvents and partially soluble in H_2O, DMF, DMSO, CH_3CN and AcOH. This solid in water is 80 % retained by a 1 μm "Millipore" filter: no melting point under 300° C: IR (KBr) with a weak band at 1740 cm^{-1} (partial saponification) and a broad band between 1710-1660 cm^{-1}, centered at 1680 cm^{-1}: UV/VIS (DMSO) shows the principal absortion band under 320 nm with shoulders at 420 nm and 580 nm, which shift to 500 nm and 605 nm respectively by HCl addition (reversibility observed by neutralization with tetramethylguanidine). MS (FAB) analysis does not allow to detect any significative peak and the elemental analysis confirms relative ratio of C:H:N corresponding to bile pigments but always a inorganic part was present (salts can be easily formed in the carboxylic acid groups but also in the basic nitrogen of the urobilinoid or biliviolinoid partial structures of the polymer). These results suggest oligomers or polymers of structural form 17, when urobilinoid structures and also biliviolinoid (also called bilipurpurin structures) partial structures are also present in the molecule.

8 at second wave potentials. At -1.85 V and 4.0 ± 0.4 electron · $molecule^{-1}$. Water and organic phase show yellow-brown colour: acidification to pH 3 ($NaHSO_4$) of the water phase allows to increase the yield on organic extract (≅85 % of the initial substrate weight), i.e. carboxylate salts are part of the reaction products. ^{1}H-NMR spectrum shows weak $COOCH_3$ band and absence of ethylenic protons. Two different fractions are obtained by column chromatography on cellulose. The first one contains synthetic urobilinogen IXα (16a) (= 80 %) and small amounts (=15 %) of its dimethyl ester (16b) and of urobilin IXα (15a) (probably formed by air oxidation of 16a). 15a, 16a and 16b were detected by thin layer chromatography (TLC), ^{1}H-NMR (200 MHz), IR and UV/VIS and MS (EI) of the mixture. The molecular peak corresponding to the urobilinogen dimer (17a, n=0, R^3=H) was also detected by FAB analysis. The second fraction contains also a small amount of 15a (TLC, ^{1}H-NMR, UV/VIS) but it is formed principally by dimers and soluble oligomers, as in the former experiment. However, biliviolinoic UV/VIS absorption is not detected in agreement with the higher charge-to-substrate ratio of this experiment. Rather, FAB analysis showed higher proportion peaks in the present experiment than in the former one. These correspond to dimers with free carboxylic acid groups.

9 at first process potential. At -1.60 V and 2.2 ± 0.2 electron·$molecule^{-1}$. Water and organic phase show brown colour: acidification of the water phase to pH 3 ($NaHSO_4$) allows to increase the yield on organic extract (≅ 60 % of the initial substrate weight), i.e. carboxylate salts are part of the reaction products. By ^{1}H-NMR it is shown an important decrease of the signals corresponding to the $COOCH_3$ groups but not of the vinyl groups. These vinyl groups are also shown by IR (KBr) (990 and 925 cm^{-1}), but not the corresponding band (860-790 cm^{-1}) of the exocyclic double bond. UV/VIS spectra show a very low visible region absortion, in spite of its brown colour (showing shoulders at 433 and 565 nm). By column chromatorgaphy on cellulose two fractions were collected. The first one (hexane/$CHCl_3$, 2:1) shows by TLC in silica (CH_2Cl_2/EtOH, 20:1) two principal peaks at R_f 0.7 and 0.5 and small amounts of substances at the origin: ^{1}H-NMR indicates the absence of $COOCH_3$ signals and the presence of the vinyl groups; furthermore, the rest of signals agree with a mixture of dimers of type 17b (n=0, R^3=H): UV/VIS (CH_3OH, λ_{max}); 222 nm. MS(FAB, Xe, DTT/DTE, 3:1, m/z); 1173 (M + 1), 1051 (M - 2-oxopyrrole ring), 927 (M - 2 terminal rings), 586 (M/2). The second column fraction shows by TLC (same conditions as before) only a spot in the origin. The ^{1}H-NMR (200 MHz, $CDCl_3$) spectrum shows broad signals but at similar chemical shifts as far the first fraction. FAB analysis shows peaks as before: peaks corresponding to the dimers with one and two $COOCH_3$ groups could also be detected. All these results suggest that the principal components of this fraction are oligomers of 17b ($n \geqslant 1$). The presence of polymers is also supported by the analysis of a solid interphase obtained in the initial water/CH_2Cl_2 extraction. The water phase was centrifuged, the brown-violet solid washed with water and liophilyzed (5 mg, 7 % of the initial substrate weight). This solid did not melt under 300 C. The ^{1}H-NMR (200 MHz, DMSO-d_6) spectrum showed very broad bands but its signals do not contradict structures of type 17b, although some exocyclic double bonds are present (at the terminal pyrrolinone rings of the oligomer or at the central rings). UV/VIS (DMF; λ_{max}): 324, 420 and 560-580 nm (intensities ratio 5:3:1), the two last absorptions, by HCl addition, shifted to 500 nm and 605 nm respectively (reversibility

was observed by tetramethylguadinine neutralization) showing the presence of urobilinoid and biliviolinoid chromophores. By MS (FAB) analysis no significative peak could be detected and the elemental analysis confirmed relative ratio of C:H:N corresponding to bile pigments, although some inorganic component was always present (salts can easily be formed in the carboxylic acid groups but also in the basic nitrogen of the urobilinoid and biliviolinoid partial structures of the polymer). This results suggest for this solid a structure of type 17b with some of the central pyrrole rings oxidized to pyrromethene partial systems (urobilinoid chromophore) and with the terminal bile pigment units oxidized or isomerized, owing to the presence of the vinyl group to biliviolinoid partial structures. Such type of structure is also confirmed by a negative Ehrlich test (in AcOH), in spite of the presence of urobilinoid and biliviolinoid UV/VIS absorption.

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