Propentdyopents [5-(2-Oxo-2*H*-pyrrol-5-ylmethylene)pyrrol-2(5*H*)-ones] and Related Compounds. Part 2.[1] The *Z* ⇌ *E* Photoisomerisation of Pyrromethenone Systems

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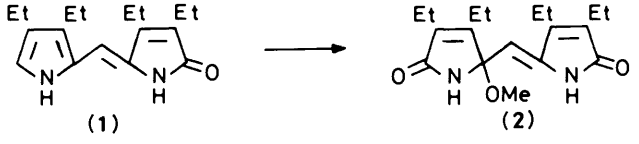
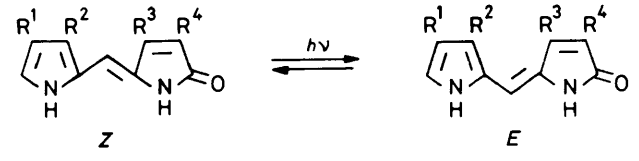
The *Z* ⇌ *E* photoisomerisation of four α-unsubstituted pyrromethenones which are potential precursors of alkanol–propentdyopent adducts is studied using a direct n.m.r. spectroscopic approach in CD_3OD–$CDCl_3$. Chemical-shift assignments are made with the help of n.O.e. and decoupling experiments. For the polyalkylpyrromethenones the photostationary state contains *ca.* 25% of the *E* isomer: a system with a β-ethoxycarbonyl group leads to 43% *E* isomer at photoequilibrium. The *E* isomers can be manipulated in solution without appreciable change, but thermodynamic equilibration to the *Z* isomer (*ca.* 99%) occurs on dry silica. A similar photoisomerisation of (4*Z*,15*Z*)-bilirubin IIIα in ND_3–CD_3OD leads to a photoequilibrated mixture containing 16% of (4*E*,15*Z*)-bilirubin IIIα.

We have recently reported[2] that methanol–propentdyopent adducts can be prepared in satisfactory yields by visible irradiation of α-unsubstituted pyrromethenones (**1**) in oxygenated methanol in the presence of a suitable photosensitiser such as *meso*-tetraphenylporphyrin tetrasulphonic acid tetrasodium salt (**1**) ⟶ (**2**). Autoxidation of α-alkylpyrromethenones had



earlier been reported to give verdins as the main products, the propentdyopents being formed as by-products.[3]

The photo-oxygenation (**1**) ⟶ (**2**) is expected to be accompanied by a presumably (but not necessarily[4]) independent *Z* ⇌ *E* photoequilibration of the starting pyrromethenones, and the purpose of this paper is to investigate this reaction for a series of pyrromethenone systems.



(**3**) $R^1 = R^2 = H$, $R^3 = R^4 = Et$
(**4**) $R^1 = R^2 = Me$, $R^3 = R^4 = Et$
(**5**) $R^1 = R^2 = R^3 = R^4 = Me$
(**6**) $R^1 = CO_2Et$, $R^2 = R^3 = R^4 = Me$
(**7**) $R^1 = R^2 = H$, $R^3 = R^4 = Me$

This reaction has also attracted attention as one of the processes involved in the photoisomerisation of bilirubin IXα, both *in vitro* and *in vivo*,[5] and there is evidence to support the view that it is involved in the $P_r \rightleftharpoons P_{fr}$ photoequilibration in phytochrome.[6] A number of studies related to these problems have been published, generally with pyrromethenones bearing an α-substituent.[7–11] However, the original observation, due to Falk,[12] was made with an α-free system.[13,14]

Table 1. Electronic spectra in MeOH–$CHCl_3$ (2.5:1)

Comp.	λ/nm (ε)
Z-(**3**)	256 (7 100), 382 (37 200)
Z-(**4**)	266 (4 300), 399 (32 500)
Z-(**5**)	264 (5 600), 400 (32 600)
Z-(**6**)	245 (11 00), 256sh (8 200)
	369 (28 400), 380sh (26 300)

The pyrromethenones selected for study [compounds (**3**)—(**6**)] possessed β-substituents (Me, Et, CO_2Et) providing a range of steric and electronic effects, and were synthesized by the condensation of the appropriate formylpyrrole and pyrrol-2(5*H*)-one under basic conditions, with improvements in detail (Experimental section). The condensation leading to the ethoxycarbonylpyrromethenone *Z*-(**6**) did not go to completion and the product was difficult to purify. To avoid the adventitious formation of the *E* isomers, solutions were manipulated rapidly in subdued light.

The photoisomerisations were studied by n.m.r. spectroscopy. The electronic spectra in the solvent system (but protiated) used for this are given in Table 1. Although the spectra are not identical, they are generally similar to one another, with a broad band in the 369—400 nm region (ε 2.8—3.7 × 10^4) extending into the visible. In the present experiments, with visible irradiation through borosilicate glass, only this band will be responsible for excitation.

The n.m.r. spectra, including n.O.e. effects, of nitrogen-flushed solutions of *Z*-(**3**)—(**6**) in CD_3OD–$CDCl_3$ were consistent with the presence of a single *Z* isomer. Irradiation of the n.m.r. tube at 4 °C with a tungsten lamp (60 W) led to the development of a second set of n.m.r. signals due to the *E* isomer, and a photostationary state was established within 5 h. During this time no side reactions were observed provided that oxygen was rigorously excluded, and the *E* isomer (lower R_F than *Z*) was the only product detected on t.l.c. Except in one case (see below) the *E* isomers were not separated, but n.O.e. observations were made on the photoequilibrated mixture. These photoequilibrated mixtures, in contrast to photoequilibrated bilirubin IXα,[15] were stable over the period of the n.O.e. measurements.

The results are summarised in Figure 1. For the *Z* isomers (**3**)—(**6**) irradiation at the *meso*-signal led to nuclear Overhauser enhancement effects at the neighbouring β-positions whether unsubstituted [as in *Z*-(**3**)] or substituted and, together with decoupling experiments, allowed chemical-

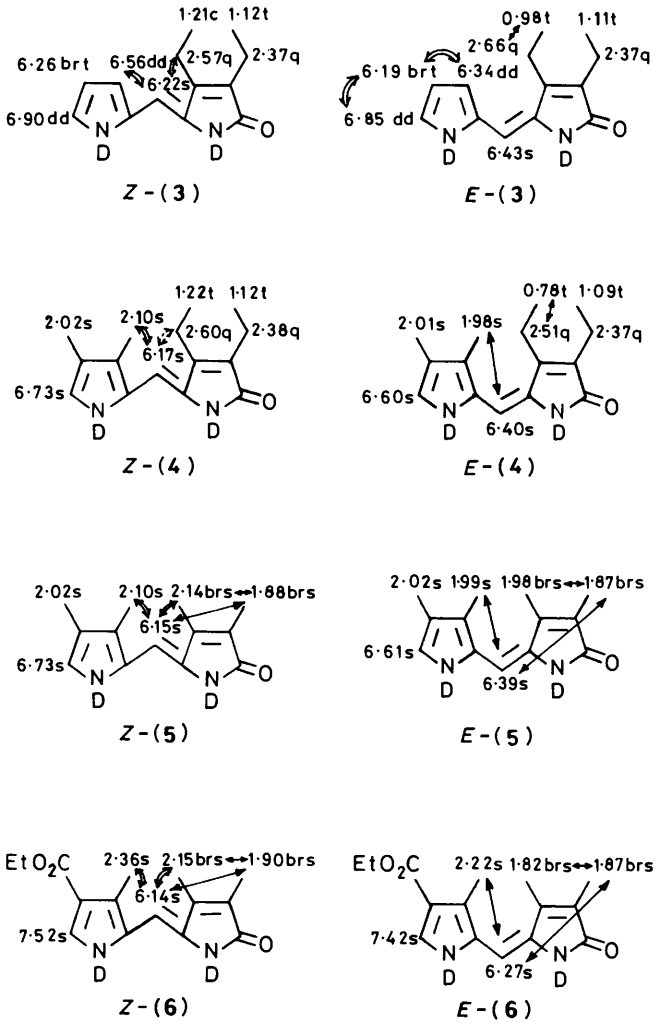


Figure 1. Chemical shifts, n.O.e. effects, and decoupling relationships for the *Z* and *E* isomers (**3**)—(**6**) in CD_3OD–$CDCl_3$ = 2.5:1 measured at 400 MHz and 250 MHz. ⟳ positive n.O.e. effect; ⟳ weakly positive n.O.e. effect; ⟷ coupling established by decoupling experiments.

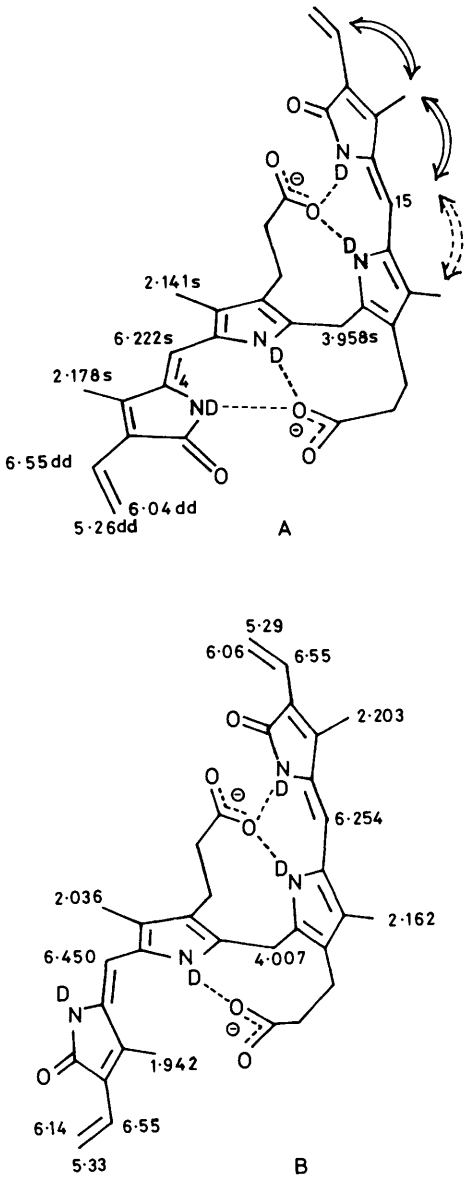


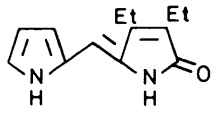
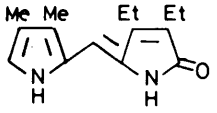
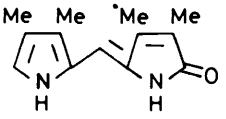
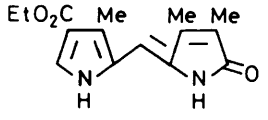
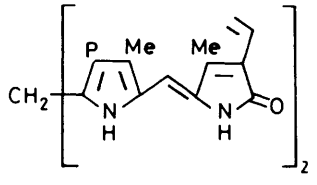
Figure 2. Chemical shift assignments for A (4*Z*,15*Z*)- and B (4*E*,15*Z*)-isomers of bilirubin IIIα (**8**) in the photoequilibrated mixture in ND_3–CD_3OD. The conformations employed are based on *X*-ray results.[16,29]

shift assignments to be made as shown. In addition, the n.O.e. results indicated a roughly planar *syn* conformation at the *meso*-single bond for the *Z* isomers. In contrast, irradiation at the *meso*-signals for the *E* isomers revealed no n.O.e. difference signals in the proton spectra. This is consistent with a non-planar conformation with a considerable twist about the *meso*-single bond in the *E* isomers.[16] Decoupling experiments revealed a number of small long-range effects. For both the *Z* and *E* isomers of (**5**) and (**6**) a small coupling was detected between the *meso*-proton and the methyl group at C-3 in the pyrrolinone ring. Expansion of the spectrum after irradiation at the *meso*-signal revealed a quartet structure for the 3- and 4-methyl groups of the pyrrolinone ring, indicating that there is a weak homoallylic coupling (1.0—1.4 Hz) between these groups. However, in *Z*-(**4**) coupling involving the *meso*-proton could not be detected. Very small couplings between the *meso*-proton and the pyrrole 3-methyl group were detected by peak sharpening in *E*-(**4**)—(**6**) but not in *Z*-(**4**)—(**6**): this surprising (non-zigzag) long-range coupling is presumably associated with the larger degree of twisting about the *meso*-single bond in the *E* isomers.

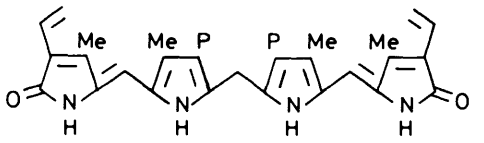
The compositions of the photostationary states under our experimental conditions were estimated from the n.m.r. spectra, and are presented in Table 2. The pyrromethenones (**3**)—(**5**) all show similar *Z*/*E* photoequilibrium constants, representing *ca.* 25% of *E* isomer at photoequilibrium. For the sterically less encumbered system (**7**) a larger proportion of *E* isomer (41% in MeOH) has been reported:[14] it appears, therefore, that, with the introduction of further (or larger) β-alkyl groups as exemplified in (**3**)—(**5**), any steric effect on the photoequilibration is rapidly maximised. However, the ethoxycarbonyl group in (**6**) does appear to exert a small influence on the composition of the photostationary state, increasing the proportion of *E* isomer to 43%. This is in line with Gossauer's work on the photochemistry of pyrromethenones substituted at the α-position with electron-withdrawing groups (CHO, CO_2Bu^t),[9] where $K_{E/Z}$ of *ca.* unity was observed.

The opportunity was taken, using the same experimental approach, to study the photoequilibration of bilirubin. In order to simplify the interpretation of n.m.r. spectra a symmetrical isomer was required, and in order to avoid the formation of

Table 2. *Z*/*E* Photoisomerisation of some pyrromethenones

	Compound	Solvent (CD_3OD–$CDCl_3$ ratio for entries 1—4)	Concentration (mM)	$K_{E/Z}$[a]	% *E* isomer at photoequilibrium (±3%)
1.		5:1 2.5:1	6.3 7.54	0.38 ± 0.04 0.45 ± 0.05[b]	27 31
2.		2.5:1	5.27	0.33 ± 0.06	25
3.		2.5:1	5.36	0.40 ± 0.07	28
4.		5:1 2.5:1	6.2 4.17	0.75 ± 0.07 0.75 ± 0.09	43 43
5.	 Bilirubin IIIα	0.065M ND_3 in CD_3OD	1.7	0.18 ± 0.02[c]	16

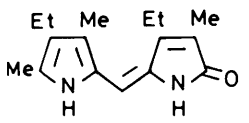
Irradiation conditions. Entries 1—4: 60 W tungsten lamp, 4 °C, standard optical configuration throughout. Sample under nitrogen. Entry 5: 2 × 500 W tungsten lamps, 4 °C, sample sealed *in vacuo*. [a] Composition of photostationary state under the standard conditions based on the mean of the 400 MHz n.m.r. integrations of the α-pyrrole proton and the *meso*-proton signals for *E* and *Z* isomers at photoequilibrium. [b] Measured at 250 MHz. [c] Composition of photostationary state under stated conditions based on mean of 400 MHz n.m.r. integrations of C-5/C-15 and C-10 signals for 4*Z*,15*Z* and 4*E*,15*Z* isomers at photoequilibrium.



(**8**) Bilirubin IIIα (P = $-CH_2CH_2CO_2H$)

analogues of photobilirubin II[17] the IIIα isomer (**8**) was chosen. The solvent system used for the pyrromethenones could not be used here for reasons of low solubility, so ND_3–CD_3OD, which we find can conveniently be prepared from lithium nitride and CD_3OD as required, was used. The degassed solution was irradiated in the n.m.r. tube with a tungsten source (1 000 W, 4 °C) and came to photoequilibrium within 90 min. The n.m.r. spectrum again showed a second set of signals attributed here to the 4*E*,15*Z* isomer. Chemical shift assignments of *meso* and methyl protons were based on those of the four model pyrromethenones above and on n.O.e. experiments on 4*Z*,15*Z*-(**8**). The results are shown in Figure 2. The *Z* ⟶ *E* change is accompanied by an upfield shift for the methyl groups flanking the *meso*-bridge of the pyrromethenone unit (δ 2.178 ⟶ 1.942, δ 2.141 ⟶ 2.036) just as is found in the model compounds [*e.g.* (**5**), δ 2.19 ⟶ 1.98, δ 2.10 ⟶ 2.02], while at the same time the *meso*-proton shifts downfield (δ 6.222 ⟶ 6.450), a change also reflected in the above models [*e.g.* (**5**) δ 6.15 ⟶ 6.39] and in literature precedent,[8,10,13] including bilirubin IIIα in dimethyl sulphoxide.[18] The photostationary state contained 16% of (4*E*,15*Z*)-bilirubin IIIα (Table 1): for bilirubin IXα in methanolic ammonia Lightner and McDonagh[5] give *ca.* 80% (4*Z*,15*Z*), 14% (4*Z*,15*E*), 6% (4*E*,15*Z*), and 1% (4*E*,15*E*), a result presumably based on h.p.l.c. analysis.

After the photoequilibrated mixture of (4*Z*,15*Z*)-bilirubin IIIα and (4*E*,15*Z*)-bilirubin IIIα had been kept at 4 °C in the dark for several days in the sealed n.m.r. tube, a re-determination of the n.m.r. spectrum showed that the 4*E*,15*Z* isomer had completely reverted to the original 4*Z*,15*Z* isomer. The spectrum was identical with the original. This ready thermal *E* ⟶ *Z* reversion is well known in solutions of bilirubin and its isomers.[5] In contrast, the photoequilibrated solutions prepared from the pyrromethenones (**3**)—(**6**) were stable, as determined by n.m.r. spectroscopy, over a period of 17 days (at least) when kept in the dark at room temperature in the absence of oxygen. This contrasts with observations on pyrromethenones in which the pyrrole ring is trialkylated [*e.g.* (**10**)], where the half-life for the thermal *E* ⟶ *Z* conversion at 27 °C in the dark is given as 16 ± 2 h.[11] It has been noted before that if the pyrrole ring contains more than two alkyl groups the *E* isomer cannot be isolated,[13] and electron donor substituents accelerate the *E* ⟶ *Z* reversion in the aryl analogue series.[19]



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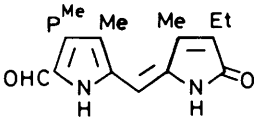

(11)

Electron-withdrawing groups appear to have a profound effect in the opposite sense: for example the *E* isomer of the formylpyrromethenone (**11**) is reported to be stable in trifluoroacetic acid.[9] It thus appears that the relative ease of the biologically important thermal $E \longrightarrow Z$ isomerisation of bilirubins in solution is associated with the trialkylated pyrrole units present as rings B and C.

However, it is possible to achieve thermodynamic equilibration of an α-free *E* pyrromethenone by impregnating it upon silica in a dry state. For example, the *Z*-(**4**)/*E*-(**4**) photoreaction mixture could be separated into its two geometrical isomers by column chromatography. Solutions of the individual isomers showed no appreciable change after 2 days in the dark, but keeping them on dry silica for 45 min established thermodynamic equilibrium in which the *Z* isomer was in such predominance that it was difficult to estimate the *E* isomer accurately (< 1%).

We conclude that in the photochemical reaction (**1**) ⟶ (**2**) the $Z \longrightarrow E$ photoisomerisation of the starting pyrromethenone will occur concomitantly. Whether this isomerisation is a side reaction, or is an obligatory stage in the formation of the methanol–propentdyopent adducts, remains to be determined.

Experimental

General.—N.m.r. spectra were determined on Bruker WP80, A250, or W400 spectrometers with tetramethylsilane as internal reference. Mass spectra were obtained on the MS902 instrument with direct insertion (probe temperature indicated) and an ionizing voltage of 70 eV. I.r. spectra were determined on a Perkin-Elmer 225 spectrophotometer. Electronic spectra were determined in N_2-flushed solvents on a Perkin-Elmer Lambda 5 instrument. In chemical-shift assignments of pyrromethenones, the numbers of carbon atoms of the pyrrole ring are primed, while those of the pyrrolinone ring are unprimed.

Preparation of Pyrromethenones.—The preparation of (*Z*)-3,4-diethyl-5-(pyrrol-2-ylmethylene)pyrrol-2(5*H*)-one [*Z*-(**3**)] has been described.[20] Bilirubin IIIα was prepared from bilirubin IXα by acid-catalysed rearrangement.[21]

(*Z*)-3,4-*Diethyl*-5-(3,4-*dimethylpyrrol*-2-*ylmethylene*)*pyrrol*-2(5H)-*one* *Z*-(**4**).—3,4-Diethylpyrrol-2(5*H*)-one[20] (102 mg) and 2-formyl-3,4-dimethylpyrrole (90 mg) were stirred and heated in 2M NaOH (2 ml) and ethanol (2 ml) for 4.5 h at 95—100 °C. After cooling and adding ice (*ca.* 2 ml), the precipitate was filtered off and washed with water to give the crude pyrromethenone (117 mg). (A further 9 mg was collected on reheating the first filtrate under the same conditions.) The combined product was crystallised from ethanol to give the *pyrromethenone* (121 mg, 68%) as orange needles, m.p. 216—224 °C (sublimes) (Found: C, 72.9; H, 8.4; N, 11.6%; M^+, 244.157. $C_{15}H_{20}N_2O$ requires C, 73.75; H, 8.25; N, 11.45%; *M*, 244.158); λ(EtOH) 264 (6 000), and 400 nm (38 200); ν(KBr) 3 345, 1 655, and 1 625 cm^{-1}; δ($CDCl_3$) 11.13, 10.45 (br s, 2 × NH), 6.83 (d, *J* 3 Hz, pyrrole α-H), 6.17 (s, *meso*-H), 2.58 (q, *J* 7.6 Hz, 4-MeC*H*$_2$), 2.44 (q, *J* 7.6 Hz, 3-MeC*H*$_2$–), 2.13 (s, 3′-Me), 2.05 (br s, 4′-Me), and 1.20 (t, *J* 7.6 Hz, *Me*CH_2); *m/z* (151 °C), 244 (100, *M*), 229 (54, *M* − Me), 215 (20, *M* − Et), and 200 (14, *M* − Me − Et).

(*Z*)-3,4-*Dimethyl*-5-(3,4-*dimethylpyrrol*-2-*ylmethylene*)*pyrrol*-2(5H)-*one* *Z*-(**5**).—3,4-*Dimethylpyrrole*. This was prepared by an adaptation of a published method.[22] To urethane (10.84 g) in dry benzene (60 ml) stirred at ≤ 10 °C was added dropwise freshly distilled thionyl chloride (8.7 ml) and dry pyridine (19.6 ml), each from a pressure equilibrated funnel, in such a way as to neutralise the free acid produced as it formed. The ice-bath was removed, and the mixture was stirred for 1 h. 2,3-Dimethylbuta-1,3-diene (13.8 ml) was added and the mixture was refluxed for 30 min, and then left overnight. The precipitated pyridinium salt was filtered off and washed with benzene. The combined filtrate was evaporated under reduced pressure, and the residue was refluxed under nitrogen with methanolic potassium hydroxide (54 g KOH, 120 ml MeOH) for 2 h. The methanol was distilled off at atmospheric pressure, and the residue was steam distilled. The steam distillate was extracted with ether, and the dried extract (Na_2CO_3) was fractionally distilled under nitrogen to give 3,4-dimethylpyrrole, b.p. 68 °C/18 mmHg (lit.,[22] b.p. 66.5—66 °C at 14 mmHg) as a colourless oil (4.33 g, 41%) which solidified on cooling; δ($CDCl_3$) 7.77 (br s, NH), 6.52 (d, *J* 2.4 Hz, pyrrole α-H), and 2.05 (s, β-Me). The 3,4-dimethylpyrrole was converted into the 2-formyl derivative, m.p. 127—129 °C (lit.,[23] m.p. 129—130 °C) and into 3,4-dimethylpyrrol-2(5*H*)-one, m.p. 120—124 °C (lit.,[24] m.p. 116—118 °C), and these two compounds were allowed to react together under alkaline conditions to give the pyrromethenone *Z*-(**5**), m.p. 254—256 °C (lit.,[25] m.p. 248—250 °C) essentially according to the published methods.

(*Z*)-5-(4-*Ethoxycarbonyl*-3-*methylpyrrol*-2-*ylmethylene*)-3,4-*dimethylpyrrol*-2(5H)-*one* *Z*-(**6**).—N-(2-*Oxopropyl*)*phthalimide*. Potassium phthalimide (1 g, 5.4 mmol) and chloroacetone (0.8 g, 8.65 mmol) were thoroughly mixed and heated at 100 °C for 30 min (oil-bath). The mixture was poured into water (30 ml) in a mortar, ground up, and triturated in the presence of $CHCl_3$–CCl_4 (1:1, 2 × 30 ml). The organic extract was dried (Na_2SO_4), and the filtrate was taken to dryness. Recrystallisation from methanol gave *N*-(2-oxopropyl)phthalimide (0.71 g, 65%) as white prisms, m.p. 117—122 °C (lit.,[26] m.p. 122.9—123.5 °C, from water).

Ethyl 4-*Methylpyrrole*-3-*carboxylate*.—3-*Ethoxycarbonyl*-4-*methylpyrrole*-2-*carboxylic acid*. This was prepared according to the literature;[26] m.p. 195—198.5 °C (lit.,[26] m.p. 195.7—196.8 °C).

The foregoing acid (0.54 g) was heated for 1 h at 230 °C (oil-bath). The reaction mixture was subjected to sublimation (120 °C, 0.2 mmHg) to give a white solid, which crystallised from hexane–ether (3:2) to give ethyl 4-methylpyrrole-3-carboxylate (0.29 g, 69%) as colourless crystals, m.p. 72—74 °C (lit.,[27] m.p. 73—75 °C); δ($CDCl_3$) 8.7 (br s, NH), 7.30 (m, 5-H), 6.45 (m, 2-H), 4.23 (q, *J* 7 Hz, C*H*$_2$Me), 2.27 (s, 3-Me), and 1.32 (t, *J* 7 Hz, CH$_2$*Me*).

Ethyl 2-*Formyl*-3-*methylpyrrole*-4-*carboxylate*.—Phosphorus oxychloride (0.36 ml) was added in portions to dimethylformamide (dry, 3.6 ml) at *ca.* 10 °C (ice-bath). The bath was removed (20 min), and the solution was again cooled and added in several portions to a stirred solution of ethyl 4-methylpyrrole-3-carboxylate (0.60 g) in dimethylformamide (dry, 3 ml) at 10 °C. The cooling bath was removed, and the mixture was refluxed for 15 min, cooled, and poured into 2.5% NaOH–ice (25 ml). The mixture was warmed to 60—70 °C for some minutes until the precipitate was no longer apparent, and then allowed to cool slowly to give long needles, m.p. 124—127 °C (lit.,[28] m.p. 127.5—128.5 °C) of ethyl 2-formyl-3-methylpyrrole-4-carboxylate. A further crop was obtained on acidification of the filtrate; total yield 0.52 g (73%); δ($CDCl_3$) 9.75 (d, *J* 1 Hz,

CHO), 9.48 (br s, NH), 7.65 (dd, *J* 1, 4 Hz, 5-H), 4.32 (q, *J* 7.2 Hz, CH_2Me), 2.61 (s, 3-Me), and 1.36 (t, *J* 7.2 Hz, CH_2Me).

(Z)-3,4-*Dimethyl*-5-(4-*ethoxycarbonyl*-3-*methylpyrrol*-2-*ylmethylene*)*pyrrol*-2(5H)-*one* Z-(**6**).—Ethyl 2-formyl-3-methylpyrrole-4-carboxylate (97.2 mg, 0.537 mmol) and 3,4-dimethylpyrrol-2(5*H*)-one (61.9 mg, 0.558 mmol) in dry ethanol (5 ml) was treated with a few clean scrapings of sodium and refluxed (18 h, argon, stirring). On cooling and adding ice (*ca.* 5 ml) a precipitate formed which was filtered off and washed with water. T.l.c. showed that this product (93 mg) was a mixture of the starting aldehyde and the pyrromethenone product. The aldehyde was largely removed by treatment with dichloromethane (2 ml) and hot chloroform (0.5 ml). The yellow residue was purified by t.l.c. (dark, silica gel, chloroform–methanol = 10:1, R_F 0.6) to give the pyrromethenone (30 mg, 20%). Recrystallisation from chloroform gave bright yellow microneedles, m.p. 297—302 °C (decomp.) (Found: M^+, 274.132. $C_{15}H_{18}N_2O_3$ requires *M*, 274.132); $\lambda_{max.}$(EtOH) 246sh (8 300), 255sh (6 500), 368 (23 700), and 381 nm (21 700); ν(KBr) 3 330, 1 710, 1 652, 1 275, 1 218, 1 160, 1 092, and 1 070 cm^{-1}; δ($CDCl_3$) 10.88 and 10.80 (br s, 2 × NH), 7.61 (d, *J* 3.5 Hz, pyrrole α-H), 6.18 (s, *meso*-H), 4.31 (q, *J* 7.1 Hz, CH_2Me), 2.43 (s, 3′-Me), 2.16 (br s, 4-Me), 1.97 (br s, 3-Me), and 1.37 (t, *J* 7.1 Hz, CH_2Me). On expansion δ 1.97 remained as a br s, but δ 2.16 became an unresolved q, *J* 0.8 Hz; *m/z* (148 °C), 274 (100, *M*), 229 (22, *M* − EtO), 217 (18), 201 (20, *M* − CO_2Et), 200 (18), and 199 (18).

Photoisomerisation and N.m.r. Spectroscopy.—For the pyrromethenones Z-(**3**)—(**6**) solutions were made in CD_3OD–$CDCl_3$ mixtures in a nitrogen atmosphere (see Table 2) and the initial n.m.r. spectrum was taken (400 MHz or, in one case, 250 MHz). Irradiation of the n.m.r. tube at 4 °C was carried out with a 60 W tungsten source 10 cm distant in a standard optical configuration, and n.m.r. spectra were recorded on the same instrument at intervals. Compounds (**3**)—(**6**) came to photoequilibrium with the corresponding *E*-isomer within 5 h, and no appreciable side reactions were observed within this period. When the solutions were kept in the dark for protracted periods, minor side reactions became evident, but thermodynamic equilibrium between *Z* and *E* isomers was not established. Decoupling and n.O.e. experiments were done with the stable photoequilibrated mixtures on the 250 MHz spectrometer. Coupling of the *meso*-proton of Z-(**3**) and E-(**3**) was not studied.

(4*Z*,15*Z*)-Bilirubin IIIα (0.70 mg) was dissolved in ND_3–CD_3OD (0.7 ml, 65mM, prepared from LiN_3 and CD_3OD) and sealed in an n.m.r. tube after degassing (3 freeze–thaw cycles). The sample at *ca.* 4 °C was irradiated (2 × 500 W tungsten lamps, 10 cm) and came to a photostationary state within 90 min. When this sample was kept in the dark at 4 °C for 21 days the signals due to the 4*E*,15*Z*-isomer had disappeared, but were regenerated on re-irradiation. All the n.m.r. spectra, including the n.O.e. experiments, on (4*Z*,15*Z*)-bilirubin IIIα were measured with the 400 MHz spectrometer.

Thermodynamic Equilibration of Z-(**4**)/E-(**4**).—(*a*) The (*Z*)-pyrromethenone *Z*-(**4**) was irradiated under similar conditions to those employed in the n.m.r. experiment, except that ^1H solvents were used, and a 500 W tungsten lamp was employed for a shorter time (15 min). T.l.c. on silica (glass plate, dark, $CHCl_3$: MeOH = 10:1) showed the presence of two yellow bands, a major one of higher R_F (*Z*-isomer) and a minor one of lower R_F (*E*-isomer). The two isomers could be separated on column chromatography on silica eluted with $CHCl_3$–MeOH (100:1) and t.l.c. showed that the *solutions* of the two components were stable and behaved essentially as the single isomers over 2 days at ambient temperature (t.l.c., rapid irrigation, dark). However, the *E*-isomer was very largely converted into the *Z*-isomer when left for 25 min or more on dry silica.

(*b*) After t.l.c. separation on silica as above, of the freshly photoequilibrated *Z*-(**4**)/*E*-(**4**) mixture, the plate was allowed to dry in the dark for 45 min. The two bands were removed, and each was extracted with $CHCl_3$–MeOH. The solvent was removed, and each fraction was dissolved in spectroscopic grade cyclohexane (25 ml). The visible spectra showed the same $\lambda_{max.}$ (393 and 415infl, nm), and the ratio of absorbances led to a value of 18% for the proportion of *E*-isomer present in the original photoequilibrated solution.

The solvent was removed from each fraction, and the residue from each was submitted to t.l.c. (silica, dark). The two yellow bands from each were extracted and estimated comparatively as before except that the two fractions obtained from the initial lower R_F fraction were made up to 5 ml rather than 25 ml. Electronic spectroscopy showed that the proportion of *E*-isomer present after the silica treatment was *ca.* 1%.

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