A Study of the Bile Pigments and Related Compounds in Solution by ^{13}C Nuclear Magnetic Resonance Spectroscopy

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The natural abundance ^{13}C n.m.r. spectra of six bile pigments and several related compounds are reported. Two of the former, namely mesobiliverdin-IXα dimethyl ester (11) and mesobilirubin-IXα dimethyl ester (16), were specifically ^{13}C-enriched at several positions in the molecule, which allowed the complete assignment of their quaternary pyrrolic carbon signals and thus aided in the assignment of the latter in the other bile pigments. Substituent changes in the pyrromethen-5(1*H*)-one model compounds give rise to ^{13}C chemical shift changes indicative of considerable delocalisation over the entire conjugative system. The shifts for the bile pigments indicate that the lactam is the preferred tautomeric form in all cases. The magnitudes of the large number of one-bond carbon–carbon spin–spin coupling constants for the enriched compounds correlate with bond lengths derived from crystallographic studies of similar compounds and with the relative percentage of *s*-character of the carbon–carbon bond involved in the coupling. Consequently the extent of bond delocalisation in (11) and (16) was deduced, and for (11) it is shown to be higher over rings II and III than over rings I and II, or over rings III and IV.

THE open-chain tetrapyrrolic compounds, the bile pigments, are derived in nature from the oxidation and ring opening of the prosthetic groups of haemoproteins. They are found in the waste materials excreted by mammals and as such have no biological function. In lower animals and plants, however, they play vital roles. In green plants and algae they are found bound to protein to give chromoproteins (so-called biliproteins) which take part in photosynthesis. The most important of these is phytochrome which acts as a photoreceptor for the photoregulation of growth in all higher plants.

In mammals, breakdown of haemoglobin initially gives protoporphyrin-related products which on oxidation lead to biliverdin. Its reduction in the spleen and liver yields bilirubin which undergoes further reactions by intestinal bacteria to give the complex mixture of bile pigments excreted in the waste products.

Although the constitution of many of the bile pigments has been established for many years,[1] mainly from the pioneering work of Fischer and his co-workers, several stereochemical problems remained. In particular there were ambiguities as to the *Z*- or *E*-configuration at the methine bridges, the possibility of tautomerism in the I and IV rings, the overall conformation of the chromophore and the bonding pattern, and hence the degree of bond delocalisation within the tetrapyrrole skeleton. Although spectroscopic data[2] and molecular orbital calculations[3] have suggested possible solutions of these problems, it is only recently that light has been shed on them through the established crystal structures of bilirubin,[4] mesobilirubin,[5] and biliverdin dimethyl ester.[6]

The main findings from the crystal structures are that the exocyclic double bonds are in *Z*-configurations and that the I and IV rings exist in the lactam form.[5] For the two pigments with central methylene groups, the most stable conformation has sets of hydrogen bonds between the carboxylic acid functions and the pyrrole hydrogens and terminal lactam groups. Although the generality of the above has yet to be established, we may venture applying many of these findings to the structure of the compounds in solution.

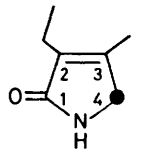
Proton n.m.r. in this area of research has been particularly useful in establishing the molecular constitution of natural and synthetic pigments. Although ^{13}C n.m.r. is now being extensively used in porphyrin research[7] to establish structure and elucidate biosynthetic mechanisms, only a limited amount of data exists for the bile pigments. We report here the ^{13}C n.m.r. spectra of several naturally occurring bile pigments and various model compounds used in order to establish, as far as possible, signal assignments. It soon became clear, however, that the assignment of the quaternary pyrrolic carbons was difficult and presented the same problems that occur in their assignment for the porphyrins[8] and vitamin B_{12} derivatives.[9] In order to overcome this, several ^{13}C-labelled derivatives of mesobiliverdin dimethyl ester and mesobilirubin dimethyl ester have been synthesised and have led to an unequivocal assignment of the quaternary carbon signals in these compounds, which by comparison allows many of these signals in the remaining bile pigments to be assigned.

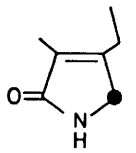
The model compounds and bile pigments are shown in Schemes 1 and 2, respectively. The numbering system for the bile pigments follows the corrin system nomenclature and is that used by Chemical Abstracts.

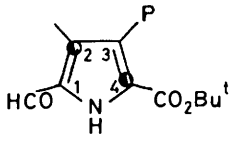
EXPERIMENTAL

All spectra were recorded on a Varian XL-100-12 spectrometer operating in the Fourier transform mode at 25.16 MHz and locked to the deuterium resonance (15.40 MHz) of the solvent, except for (3) and the comparison spectra noted below which were run on a Varian CFT-20 spectrometer. The XL-100 was controlled with a Varian 620-L computer equipped with a moving-head disc together with complementary software. Instrumental parameters were usually chosen to give a 12 K transform which yielded, through a centroid interpolation routine,[10] a resolution of better than 0.8 Hz for sweep widths of 5 000 Hz.

Samples of each compound were made up in CD_2Cl_2, except for (1b), (2b), (9b), and (10a) which were dissolved in $CDCl_3$, at concentrations between 1.8 and 4×10^{-4}M. Noise-decoupled and single frequency off-resonance proton decoupled spectra[11] were obtained at 36 ± 1 °C with internal tetramethylsilane as standard in 10-mm sample


(1a,b)[b]


(2a,b)[b]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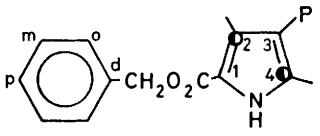
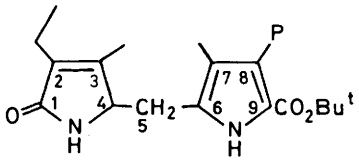
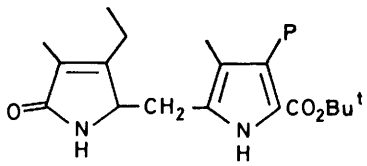
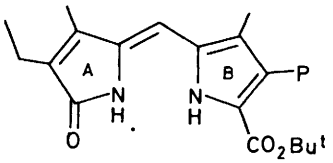
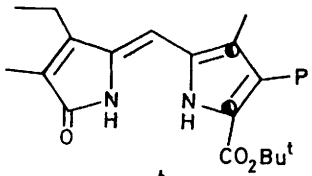

(4a,b)[b]


(5)


(6)


(7)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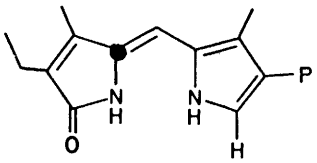
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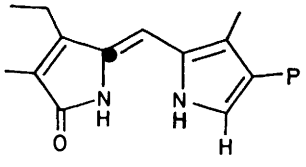
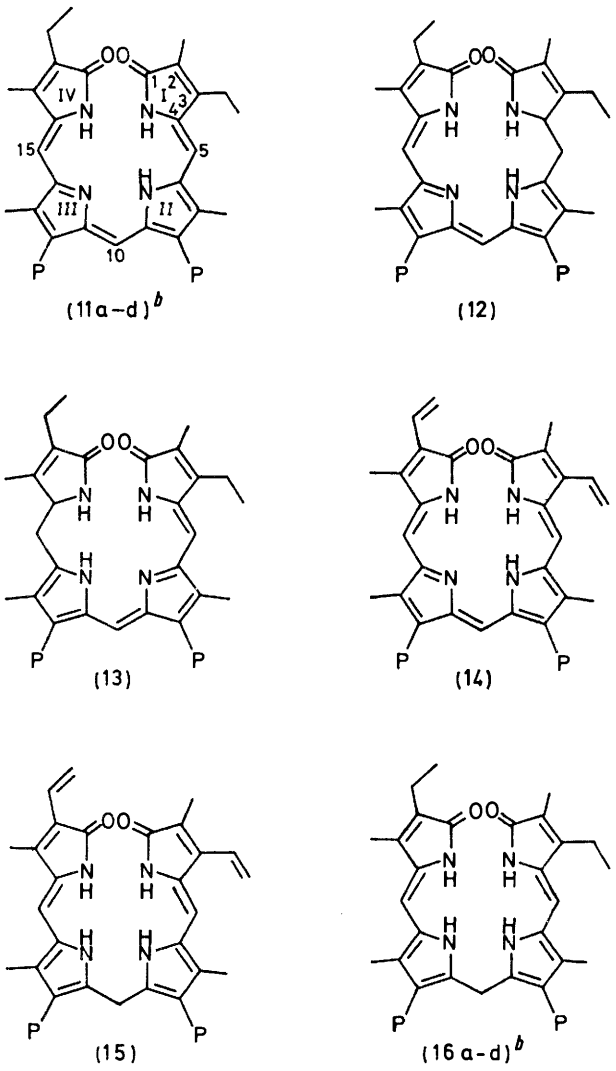

(10a,b)[b]

P = $[CH_2]_2CO_2Me$

SCHEME 1 Compounds (1)—(10) related to the bile pigments [a]

[a] The systematic names of compounds (1)—(10) are shown below. However, for convenience in the comparison of ^{13}C data for these compounds with those of the bile pigments the carbon numbering systems shown in the Scheme has been adopted. (1) 3-Ethyl-4-methyl-Δ^3-pyrrolin-2-one; (2) 4-ethyl-3-methyl-Δ^3-pyrrolin-2-one; (3) t-butyl 5-formyl-4-methyl-3-(2-methoxycarbonylethyl)pyrrole-2-carboxylate; (4) benzyl 4-(2-methoxycarbonylethyl)-3,5-dimethylpyrrole-2-carboxylate; (5) t-butyl 4-ethyl-4′-(2-methoxycarbonylethyl)-3,3′-dimethyl-5-oxo-2,4-dihydro-2,2′-dipyrrylmethane-5′-carboxylate; (6) t-butyl 3-ethyl-4′-(2-methoxycarbonylethyl)-4,3′-dimethyl-5-oxo-2,4-dihydro-2,2′-dipyrrylmethane-5′-carboxylate; (7) t-butyl 4-ethyl-4′-(2-methoxycarbonylethyl)-3,3′-dimethyl-5-oxo-2,4-dihydro-2,2′-pyrromethene-5′-carboxylate; (8) t-butyl 3-ethyl-4′-(methoxycarbonylethyl)-4,3′-dimethgl-5-oxo-2,4-dihydro-2,2′-pyrromethene-5′-carboxylate; (9) 4-ethyl-4′-(2-methoxycarbonylethyl)-3,3′-dimethyl-5-oxo-2,4-dihydro-2,2′-pyrromethenone (methyl isoneoxanthobilirubinate); (10) 3-ethyl-4′-(2-methoxycarbonylethyl)-4,3′-5-oxo-2,4-dihydro-2,2′-pyrro-methene (methyl neoxanthobilirubinate). [b] The enriched carbon atoms in (1b). (2b), (9b), and (10b) are indicated as (●), whil ethose of (3b), (4b), and (8b) are indicated as (◑). Compounds (3b) and (4b) consisted of a 50 : 50 mixture of the ^{13}C-enriched com-pounds at C-2 and C-4, (8b) of a 50 : 50 mixture of the ^{13}C-enriched compounds at C-9 and C-13, and (1b), (2b), (9b). and (10b) were enriched at C-4.



SCHEME 2 Bile pigments (11)—(16)[1]

[a] Compounds (11)—(16) are: (11) mesobiliverdin-IXα dimethyl ester; (12) isomesobiliviolin-IXα dimethyl ester; (13) mesobiliviolin-IXα dimethyl ester; (14) biliverdin-IXα dimethyl ester; (15) bilirubin-IXα dimethyl ester; (16) mesobilirubin-IXα dimethyl ester. [b] Compounds (11b) and (16b) were ^{13}C-enriched at C-4, (11c) and (16c) at C-16 and (11d) and (16d) were 50 : 50 mixtures of the ^{13}C-enriched compounds at C-7 and C-9.

tubes. Spectra of the ^{13}C-enriched compounds (1b), (2b), (3b), (4b), (8b), (9b), (10b), (11b), (11c), and (11d) were also run on a Varian CFT-20 spectrometer with 8 K data points to enable identification of splittings arising from ^{13}C–^{13}C coupling. The AB spectra arising from the ^{13}C–^{13}C interactions were analysed with the aid of a program based upon LAOCOON III.[12] From these analyses it was found that in all cases the ^{13}C-isotope effect upon the carbon chemical shift was smaller than the experimental error.

Two-bond deuterium isotope effects across nitrogen upon ^{13}C chemical shifts have been observed in various amino-acids and peptides,[13] and were shown to be larger than longer range effects. Similar effects have not been reported for pyrrolic systems. In order to test the possibility of using such effects for assignment purposes the ^{13}C spectra of pyrrole and indole were recorded in hexadeuteriodimethyl sulphoxide [$(CD_3)_2SO$] before and after D_2O exchange. It was found that all carbons α to the nitrogens undergo upfield shifts of 0.15 p.p.m. upon exchange of N–H for N–D while the effects upon carbons further away were smaller than 0.05 p.p.m. Thus in order to facilitate the ^{13}C assignments in the bile pigment precursors the ^{13}C spectra of compounds (5), (6), (7), and (8a) were recorded in $(CD_3)_2SO$ on the CFT-20 before and after D_2O exchange.

^{13}C-Labelled pyrrolin-2-ones (1b) and (2b) were prepared from the corresponding ethyl 3-oxocarboxylates according to the procedures given in the literature for the synthesis of the unenriched compounds, (1a) [14] and (2a) [15] respectively, using $Na^{13}CN$ (Merck Sharp & Dohme GmbH, Munich) for the cyanohydrin formation. The pyrroles (3a) and (3b) were prepared from the corresponding benzyl 4-(2-methoxycarbonylethyl)-3,5-dimethylpyrrole-2-carboxylates [(4a) [16] and (4b) respectively] by the method given in ref. 19. The ^{13}C-labelled pyrrole (4b) was synthesised as follows. Magnesium turnings (317 mg) were dissolved under nitrogen in dry methanol (9.1 ml) containing a few drops of carbon tetrachloride. To the stirred clear solution benzyl α-(2-methoxycarbonylethyl)acetoacetate [17] (2.02 g) was added dropwise, and the mixture refluxed for 2 min before the removal of the solvent. The residue was treated with dry diethyl ether (20 ml), and the suspension was heated to reflux temperature before an ethereal solution of [*carbonyl*-^{13}C]acetyl chloride (1 g in 10 ml) was added dropwise. The mixture was stirred overnight at room temperature, acidified with 20% aqueous sulphuric acid, and extracted repeatedly with diethyl ether. The organic phases were dried (Na_2SO_4), and the crude residue (3.2 g), which was obtained after evaporation of the solvent under reduced pressure, was dissolved in dry tetrahydrofuran (25 ml) and hydrogenated on palladised charcoal (10%, 400 mg) at room temperature and atmospheric pressure. The catalyst was filtered off and the solvent evaporated under reduced pressure. The crude methyl 4-[*carbonyl*-^{13}C]acetyl-5-oxohexanoate was condensed with benzyl acetoacetate according to the procedure given for the synthesis of (4a) in ref. 16 yielding (4b), in 32% overall yield [referred to benzyl α-(2-methoxycarbonylethyl)acetoacetate]. The ^{13}C spectrum of (4b) reveals the presence of ^{13}C-labels at both C-2 and C-4 thus confirming earlier results [18] obtained from experiments using ^{14}C-labelled ethyl acetoacetate which demonstrated that cyclisation to the pyrrole ring takes place under preferential cleavage of the acetyl group of the acetoacetic ester moiety.

Pyrromethen-5(1*H*)-ones (7),[19] (8),[20] (9a),[21] (9b), (10a),[21] and (10b), as well as the dipyrrylmethan-5(2*H*)-ones (5) [19] and (6) [20] were prepared from the above building blocks, and used as intermediates for the synthesis of mesobiliverdin-IXα dimethyl ester (11),[22] isomesobiliviolin-IXα dimethyl ester (12),[23] and mesobiliviolin-IXα dimethyl ester (13) [23] according to procedures described elsewhere. Bilirubin-IXα dimethyl ester (15) [24] and biliverdin-IXα dimethyl ester (14) [25] were obtained from commercial bilirubin-IXα (E. Merck, Darmstadt), according to known procedures, by esterification and by oxidation and subsequent esterification respectively. Mesobilirubin-IXα dimethyl ester (16a), as well as the ^{13}C-enriched derivatives (16b—d), were prepared by reduction of (11a) and (11b—d) respectively with sodium borohydride.[26]

RESULTS AND ASSIGNMENTS

The ^{13}C chemical shifts of all the compounds are reported in Table 1 and the ^{13}C–^{13}C spin–spin coupling constants in the enriched compounds in Table 2.

A detailed description of the assignment for all the compounds is contained in a Supplementary Publication (SUP No. 22537, 9 pp.).* In essence the results of off-resonance experiments, consideration of their chemical shifts and ^{13}C–^{13}C coupling constants, and careful internal comparisons allowed unambiguous assignment of the signals of compounds (1)—(10) and the hydrogen-bearing carbons of compounds (11)—(16). The assignments of the quaternary carbon signals of the pyrrole rings of compounds (11) and (16) followed directly from the enriched compounds while those of the remaining compounds were from comparisons with these and with compounds (1)—(10).

DISCUSSION

In the introduction we have summarised the main structural problems in this area of chemistry, many of which have been solved for specific compounds by crystallographic studies. Here we will be concerned with the generality and validity of these conclusions for the diverse collection of bile pigments and model compounds studied in solution.

^{13}C *Chemical Shifts.*—Comparison of the shifts of (7) and (9), and of (8) and (10) shows large downfield shifts for the two former compounds for C-2, C-4, C-5, C-6, C-8, and C-9, while the remaining resonances stay relatively constant. The pronounced shifts in ring I are a consequence of conjugative interaction of the substituent at C-9, as shown below in Scheme 3, with the additional latter two canonical structures for the C-9 substituted compounds contributing significantly to the resonance hybrid. Thus the shifts are indicative of substantial delocalisation between the two pyrrolic rings. The slightly larger changes for (8) and (10) than for (7) and (9) indicate a more planar system for the former two compounds.

In contrast the shift changes seen from the comparison of (1) and (5), and of (2) and (6) are in accord with the

* For details of the Supplementary Publication scheme, see Notice to Authors No. 7, *J.C.S. Perkin II*, 1978, Index issue.

TABLE 1

^{13}C chemical shifts of compounds (1)—(16) [a]

Carbon/ Compound	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
1	176.47	177.00	130.21	116.81	174.63	175.03	174.26	174.82
2	134.14	127.63	130.21	127.60	134.94	128.64	133.82	127.43
3	148.68	154.72	129.26	120.29	151.68	157.54	142.25	148.52
4	50.20	48.55	126.29	130.32	60.15	58.48	135.85	134.58
5					29.86	29.99	97.88	97.94
6					129.18	129.39	128.19	128.17
7					117.65	117.64	123.43 [b]	123.48
8					129.07	129.16	129.21	129.20
9					119.61	119.68	123.52 [b]	123.55
10								
11								
12								
13								
14								
15								
16								
17								
18								
19								
1^{I}			179.45	161.43				
2^{I}	16.65	8.18	8.49	10.68	16.94	8.42	17.03	8.47
2^{II}	13.17				13.18		13.57	
3^{I}	12.99	21.08	20.32	19.68	12.14	20.25	9.79	18.24
3^{II}		12.72	34.97	34.97		13.12		14.75
3^{III}			173.51	173.52				
3^{IV}			51.76	51.52				
4^{I}			160.38	11.43				
4^{II}			82.87					
4^{III}			28.44					
7^{I}			CH_2	65.53	9.04	9.01	9.34	9.34
8^{I}			d	136.74	21.32	21.34	21.19	21.19
8^{II}			o	128.13	35.46	35.50	35.46	35.40
8^{III}			m	128.59	173.88	173.90	173.77	173.79
8^{IV}			p	128.13	51.62	51.62	51.66	51.67
9^{I}					161.41	161.44	160.99	160.97
9^{II}					80.91	80.92	81.48	81.49
9^{III}					28.60	28.62	28.55	28.55
12^{I}								
12^{II}								
12^{III}								
12^{IV}								
13^{I}								
17^{I}								
18^{I}								
18^{II}								

TABLE 1 (*Continued*)

Carbon/ Compound	(9)	(10)	(11)	(12)	(13)	(14)
1	174.60	174.36	173.61	175.44	173.37	171.67 *
2	130.41 *	123.36	129.09	129.17	129.27	127.60 (2), 128.65 (7), 129.24 (13), 129.69 (18)
3	142.71	148.48	146.86	157.43	147.38	
4	130.26 *	128.60	140.50	57.19	142.11	
5	101.65	101.14	96.22	29.54	98.16	97.78
6	124.89	124.51	149.69	120.37	120.12	149.09
7	123.96	123.36	128.17	130.19	129.81	
8	123.39	122.70	137.73	131.62	131.80	137.73
9	120.99	120.75	140.95	134.31	134.09	140.03 (3), 140.42 (4), 140.42 (16), 141.07 (9), 141.16 (17)
10			114.96	116.91	116.89	115.05
11			141.84	139.27	138.51	142.40
12			138.23	143.12	142.11	138.53
13			128.56	146.97	142.65	
14			151.11	163.32	162.85	151.60
15			96.22	97.96	29.27	97.91 †
16			141.88	142.41	58.83	
17			140.50	141.06	151.52	
18			134.95	135.42	135.70	
19			172.84	172.86	175.11	172.59 *
1I						
2I	17.34	8.00	8.30	8.35	8.57	9.54 ‡
2II	13.65					
3I	9.81	17.97	18.06	20.32 *	18.14	126.24 §
3II		14.89	14.58	13.41	14.61	120.02 ‖
3III						
3IV						
4I						
4II						
4III						
7I	9.47	9.37	9.57	9.41†	9.47	9.66 ‡
8I	21.18	20.80	20.15	20.19 §	20.30	20.17
8II	35.24	34.91	35.55	35.62 ‡	35.60 ‡	35.46
8III	173.86	173.68	173.41	173.36 ‖	173.45	173.39
8IV	51.71	51.51	51.88	51.85	51.85	51.90
9I						
9II						
9III						
12I			20.15	20.32 §	20.30	20.17
12II			35.55	35.72 ‡	35.73 ‡	35.46
12III			173.41	173.41 ‖	173.46	173.39
12IV			51.88	51.85	51.85	51.90
13I			9.57	9.61 †	9.81	9.66 ‡
17I			9.57	9.76 †	12.09	9.66 ‡
18I			17.17	17.36	16.72	126.98 §
18II			12.77	13.02	13.04	122.36 ‖

Carbon/ Compound	(15)		(16)
1	172.94 *		174.58
2		119.80 (8), 119.90 (12)	123.87
3			147.78
4			129.15
5	101.88 †	123.89 (2)	100.31
6		124.07 (6)	124.47
7		124.50 (7)	123.33
8		124.74 (13)	119.29
9		124.91 (14)	131.23
10	22.90	125.26 (18)	22.66
11			131.87
12		128.74 (4)	119.40
13		129.85 (9)	123.92
14		131.91 (11)	124.57
15	104.04 †	132.39 (16)	101.56
16			130.44
17		141.27 (3)	142.16
18		142.11 (17)	130.44
19	174.82 *		174.79
1I			
2I	9.63 ‡		8.15
2II			
3I	125.98 §		18.14
3II	117.96 ¶		15.00
3I			
4I			
4II			
4II			
7I	9.88 ‡		9.74
8I	20.42		20.47
8II	35.87		35.87
8II	173.73		173.75
8IV	51.77		51.72
9I			
9II			
9II			
12I	20.42		20.47
12II	35.87		35.87
12II	173.73		173.75
12I	51.77		51.72
13I	9.88		9.70 *
17I	9.88		9.78 *
18I	127.55 §		16.99
18II	122.11 ¶		13.71

[a] Only the chemical shifts, in p.p.m. are given for the unenriched compounds. The following indicate assignments that may be interchanged *, †, ‡, §, ¶ and ‖. [b] These signals overlap in $(CD_3)_2SO$ and hence cannot be unambiguously assigned.

introduction of a CH_2 group into (1) or (2) at C-4 without any inter-ring interaction. The upfield shift of C-3^{I} is similar to the upfield shift for the methyl group found on going from toluene to *o*-xylene.[27] Thus only those rotamers are significantly populated where steric interactions of the substituents at C-3 and C-7 are absent.

These results are in agreement with crystal structure

TABLE 2

^{13}C–^{13}C spin–spin coupling constants in the enriched compounds (Hz)

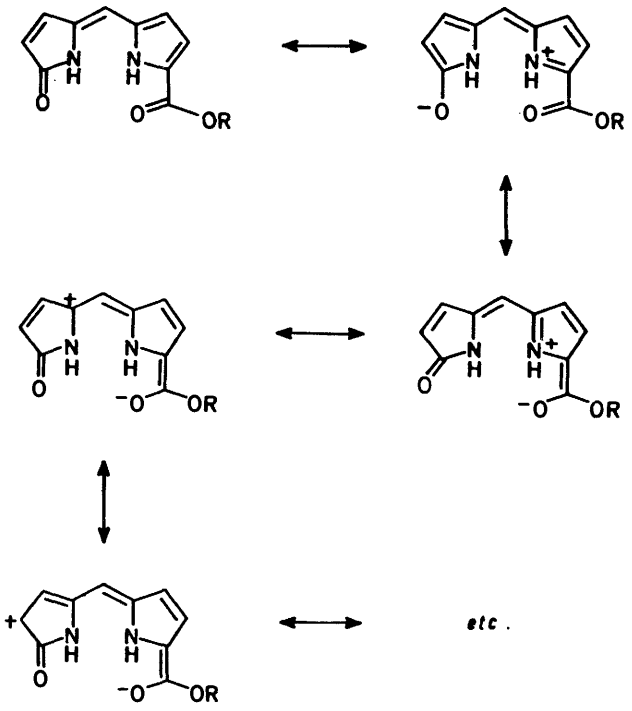
Compound	^1J	^2J	^3J	^4J
(1b)	37.5 (3,4)	6.4 (1,4)	3.5 (2^{I},4)	<1 (2^{II},4)
		<1 (2,4)		
		3 to 5 (3^{I},4)		
(2b)	37.6 (3,4)	6.8 (1,4)	3.8 (2^{I},4)	
		1.1 (2,4)	<1 (3,^{II}4)	
		4.4 (3^{I},4)		
(3b)	46.7 (2,2^{I})			
(4b)	69.4 (1,2)	7.1 (1,4)		
	54.1 (2,3)			
	66.4 (3,4)			
	47.0 (2,2^{I})			
	50.0 (4,4^{I})			
(8b)	48.6 (7,7^{I})		<6 (4,7) [a]	
(9b)	55.2 (3,4)	7.8 (1,4)	6.0 (4,7)	<1 (2^{II},4)
	81.9 (4,5)	2.8 (4,6)	4.1 (2^{I},4)	
		3.1 (3^{I},4)		
(10b)	54.9 (3,4)	8.2 (1,4)	5.5 (4,7)	
	81.8 (4,5)	2.5 (4,6)	4.8 (2^{I},4)	
		3.1 (3^{I},4)	<1 (3^{II},4)	
(11b)	53.1 (3,4)	7.0 (1,4)	4.2 (4,7)	
	79.4 (4,5)	2.5 (4,6)	4.3 (2^{I},4)	
		2.4 (3^{I},4)	<1 (3^{II},4)	
(11c)	54.0 (16,17)	8.5 (16,19)	4.0 (13,16)	<1 (16,18^{II})
	78.4 (15,16)	2.4 (14,16)	4.1 (16,18^{I})	
		0 to 3 (16,17^{I})		
(11d)	57.5 (6,7)	5.0 (5,7)	4.9 (9,12)	
	62.4 (7,8)	*b*	*b*	
	58.7 (8,9)			
	70.5 (9,10)			
	47.5 (7,7^{I})			
(16b)	54.9 (3,4)	7.7 (1,4)	5.7 (4,7)	
	81.0 (4,5)	2.5 (4,6) [c]	4.5 (2^{I},4)	
		3.4 (3^{I},4)	<2 (3^{II},4) [a]	
(16c)	~54.7 (16,17)	2.8 (14,16)	~6.3 (13,16)	
	81.2 (15,16)	~3 (16,17^{I})	3.7 /(16,18^{I})	
		d (16,18)		
		d (16,19)		
(16d)	*e* (6,7)	(5,7)	6.1 (4,7)	
	55.1 (7,8)	(7,10)	<4.3 (9,12)	
	68.2 (8,9)	(7,8^{I})		
	50.6 (9,10)	(8^{I},9)		
	48.2 (7,7^{I})	4.7 (9,11) [c]		

[a] Broadened peak. [b] Couplings $J_{4,7}$ and $J_{9,11}$ could not be determined because of overlapping lines and the proximity of C-9 and C-11, respectively. [c] Uncertain, see text. [d] Not observed. [e] Only inner lines of AB system observable and these were overlapped with other lines.

data where the pyrromethen-5(1*H*)-one system is practically planar with an inter-ring angle of only 3.9° and bond lengths that indicate a considerable degree of delocalisation over the conjugated system,[28] while for the dipyrrylmethan-5(2*H*)-one system [29,30] the inter-ring angle is large (72—100°), thus precluding steric interactions between substituents on the two rings.

In (12) and (13) the introduction of a methylene group into the bridge positions causes one pyrrolic carbon signal to move downfield to 162.6 ± 0.3 p.p.m., 11 p.p.m. to low field of any signal in (11). This shift is quite characteristic of those of conjugated imino-carbons.[9b,31,32,*] This strongly suggests that bond delocalisation is minimal within the three conjugative pyrrolic rings [II, III, and IV in (12), and I, II, and III in (13)]. The absence of such signals in (11) indicates that delocalisation occurs over the II and III rings (see below). The most favourable forms for (12) and (13) are shown in Scheme 2 with the central of the three rings possessing the imino-carbon [at C-14 for (12) and C-6 for (13)]. Such a structure would be stabilised by the maximum number of intramolecular hydrogen bonds between protons on the amino-nitrogens and the imino-nitrogen of the three-ring fragment.

According to ^1H n.m.r. studies [33] (15) exists as two distinct molecular species in non-polar solvents. This finding was compatible with an intramolecular hydrogen-bonded species, that has bonding between the ester



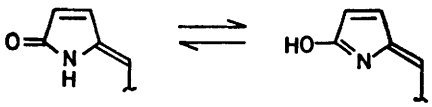
SCHEME 3

carbonyl oxygen atoms and the lactam hydrogen atoms, giving rise to restricted rotation about the central methylene bridge and a freely rotating, non-bonded species.[2] The same phenomenon was concluded from the ^1H spectrum of (15) in the deuteriomethylene chloride solutions used here. However, the second molecular species was present at ⩽10% and thus this went undetected in the ^{13}C spectra because of the signal-to-noise ratio. Similarly, only one molecular species was detected for each of the compounds (11)—(16).

The possibility of lactam–lactim tautomerism (Scheme 4) in the bile pigments has received considerable attention. In the solid state it has been shown that for

* When the β-effects of substituents are taken into account imino-carbon signals in vitamin B_{12} derivatives are found in the region 160 ± 5 p.p.m.

(14) [6] and for the corresponding free dicarboxylic acid of (16) [5] the bis-lactam form is the more stable. It is important to establish that this is also true for the solution structure. Manitto *et al.*[34] have established a ^{13}C criterion based upon the chemical shift of the C-1 (C-19) signal. They established from model compounds that in deuteriochloroform solution the lactam form signal was in the region 172—176 p.p.m. while the lactim form was in the region from 166.6 to 166.9 p.p.m. Thus bilirubin and its dimethyl ester and thioacetic acid adduct were all shown to be predominantly in the bis-lactam form.[34] Comparison with the present data indicated that all the compounds (11)—(16) occur also predominantly in the lactam form. However, this does not exclude either a fast or slow equilibrium between the lactim and lactam tautomer with a predominance of the latter. In the latter case however, for all the compounds studied, only one set of ^{13}C signals was observed. The signal-to-noise ratios were such as to set an upper limit of $\leqslant 10\%$ for the lactim isomer. These results agree with the crystal structures, ^1H n.m.r. data,[33] and the



SCHEME 4

recent photometric pK measurements of Falk *et al.*,[35] who demonstrated that the lactam form was preferred by at least four to ten orders of magnitude over the lactim form for the bile pigments in solution.

Carbon–Carbon Spin–Spin Coupling Constants.—From the above it is clear that one of the fundamental problems with the bile pigments is the determination of the bonding pattern and hence the degree of bond delocalisation within the tetrapyrrole skeleton. Work along these lines is being actively pursued with investigations both in the solid state [4-6] and in solution by a combination of ^1H n.m.r. spectroscopy and the use of lanthanide shift reagents to deduce the molecular conformation of model compounds.[36] In the present work the considerable number of carbon–carbon spin–spin coupling constants for mesobiliverdin dimethyl ester, mesobilirubin dimethyl ester, and various model compounds permits us to probe the bonding situation of these molecules in solution, since the one-bond coupling constants ($^1J_{CC}$) are known to reflect the nature of the bond between the coupled nuclei.[27]

Extending the early work [37] on $^1J_{CH}$, Frei and Bernstein [38] showed that $^1J_{CC}$ is proportional to the product of the s-characters of the carbon atoms forming the carbon–carbon bond. From their and more recent studies,[39-42] it emerges that such a proportionality can only be expected provided there are no changes in electronegative substituents attached to the carbon atoms. Similarly, a relationship between $^1J_{CC}$ and the C–C bond distance would be expected only for a closely related series of compounds.[38]

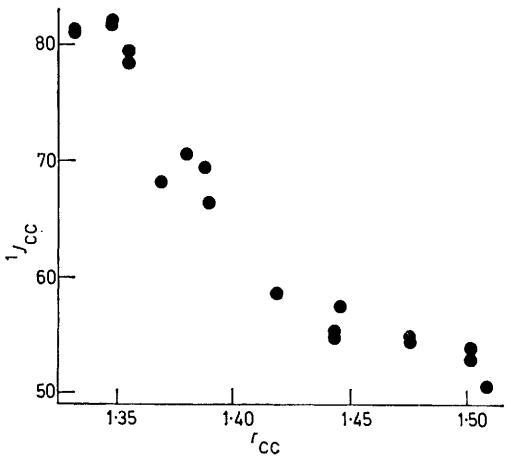
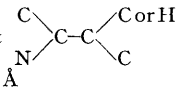
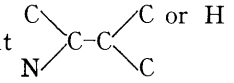


FIGURE 1 Plot of $^1J_{CC}$ (in Hz) for the fragment  against the C–C length (r_{CC}) in Å

In Table 3 there are 19 $^1J_{CC}$ values related to the fragment  where the only change in substituent is a carbon for a hydrogen atom. Bond lengths, available for biliverdin dimethyl ester, bilirubin and related compounds, are shown in Table 3. A plot of the $^1J_{CC}$ values against these, given in Figure 1, shows a smooth non-linear dependency. Thus $^1J_{CC}$ is clearly

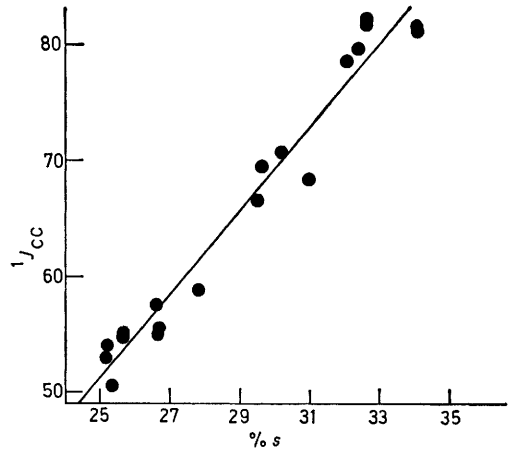


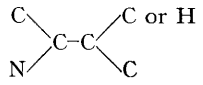
FIGURE 2 Plot of $^1J_{CC}$ (in Hz) against the relative percentage s-character for the bond between the coupled carbons (slope = 3.611 and intercept = 39.051)

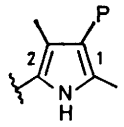
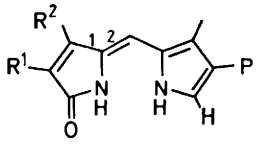
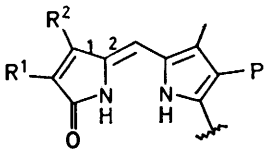
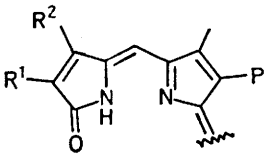
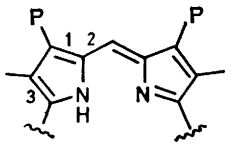
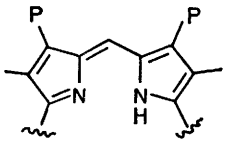
a function of the bond length. The relative percentage of s-character of these bonds was deduced from the bond length; * a plot of these against the $^1J_{CC}$ values is given in Figure 2. Both plots indicate that the $^1J_{CC}$ values reflect the bonding situation in solution, which

* The relative percentage s-character was evaluated from the smooth non-linear plot of s-character against bond length for the four compounds ethane, ethylene, acetylene, and benzene. The values are not absolute, as the effects of electronegative substituents have not been taken into account, but their relative values are correct for a particular fragment.

TABLE 3

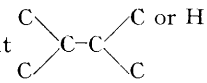
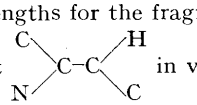
Relationship between $^1J_{CC}$ values and the bond lengths and relative percentage *s*-characters for the fragment



Compound			$^1J_{CC}$ (Hz)	Bond length (Å) [a]	Relative % *s*-character [b]	Ref. [a]
 (4b)		1	66.4	1.389	29.47	30
		2	69.4	1.387	29.60	30
 (9b)	R^1 = Et, R^2 = Me	1	55.2	1.443	26.67	28
		2	81.9	1.347	32.59	28
(10b)	R^1 = Me, R^2 = Et	1	54.9	1.443	26.67	28
		2	81.8	1.347	32.59	28
 (11b)	R^1 = Me, R^2 = Et	1	53.1	1.501	25.17	6
		2	79.4	1.354	32.01	6
(16b)	R^1 = Me, R^2 = Et	1	54.9	1.475	25.67	5
		2	81.0	1.331	34.04	5
 (11c)	R^1 = Et, R^2 = Me	1	54.0	1.501	25.17	6
		2	78.4	1.354	32.01	6
(16c)	R^1 = Et, R^2 = Me	1	~54.7	1.475	25.67	5
		2	81.2	1.331	34.04	5
 (11d)		1	58.7	1.418	27.80	6
		2	70.5	1.379	30.14	6
		3	57.5	1.445	26.59	6
 (16d)		1	68.2	1.368	30.95	5
		2	50.6	1.508	25.33	5

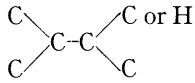
[a] Bond lengths were taken from the references shown and are averages for compounds (11b), (11c), (11d), (16b), (16c), and (16d).
[b] The relative percentage *s*-characters were calculated as indicated in the text.

TABLE 4

Further relationships between $^1J_{CC}$ values and the bond lengths for the fragment  in the present compound (A) and for the fragment  in vitamin B_{12} (B).

	(A)				(B)		
Compound	Bond	$^1J_{CC}$ (Hz)	Bond length [a] (Å)	Ref.	Bond	$^1J_{CC}$ (Hz)	Bond length (Å)
(3b)	2–2[1]	46.7	1.499	30	4–5	100	1.46
(4b)	2–2[1]	47.0	1.502	30	15–16	100	1.46
(11d)	7–7[1]	47.5	1.483 [b]	6	9–10	111	1.39
(16d)	7–7[1]	48.2	1.522 [b]	5	14–15	123	1.34
(8b)	7–7[1]	48.6	1.502	28			
(4b)	2–3	54.1	1.412	30			
(16c)	7–8	55.1	1.436 [b]	5			
(11d)	7–8	62.4	1.365 [b]	6			

[a] Bond lengths commonly have standard deviations between 0.01 and 0.02 Å. [b] Average value.

turns out to be similar to that expected in the solid. A similar situation is seen to hold for the limited number of values for the fragment  (Table 4), where a steady increase in the value of $^1J_{CC}$ is associated with a decrease in the bond length for the coupled carbon atoms. There is, also, a close correspondence of the published $^1J_{CC}$ values for ^{13}C-enriched vitamin B_{12} [9b] and typical values of the bond lengths for the corrin system [43] (Table 4).

For (11) bond delocalisation between rings II and III is indicated by the magnitude of $^1J_{9,10}$ which is identical with the value of similar couplings (72 ± 2 Hz) for the same coupled fragment in various porphyrin derivatives [44] where there is delocalisation over the entire porphyrin system in each case. The fact that the value of $^1J_{9,10}$ is less than $^1J_{4,5}$, and that $^1J_{3,4}$ is less than $^1J_{6,7}$ and $^1J_{8,9}$ in (11) clearly indicates that the bond delocalisation occurs more between rings II and III than over rings I and II, or over rings III and IV. Such a situation supports the view that these bilindiones may best be regarded as substituted pyrromethenes [6,45] rather than two condensed pyrromethen-5(1*H*)-ones. Support for this view is afforded by the decrease of the value of the $^3J_{CC}$ associated with the I and II rings ($^3J_{4,7}$), and III and IV rings ($^3J_{13,16}$) of (11b) and (11c) compared with those of (10b) and (9b) ($^3J_{4,7}$) respectively, which reflect the changed bond lengths and interplanar angles. Further evidence of delocalisation within the II ring of (11) is found in the increased value of $^1J_{7,8}$ compared to that of $^1J_{2,3}$ in (4), suggesting a shortening of the corresponding bond.

For (16) the bonding pattern is quite different and this is reflected in the coupling constants. The increase in the value of $^1J_{8,9}$ of 10 Hz and decrease in $^1J_{7,8}$ of 7 Hz for (16c) compared with those in (11d), and their similarity to the couplings in (4b), indicates a much smaller degree of bond delocalisation in (16), with the C(7)–C(8) bond being essentially a single bond and the C(8)–C(9) bond a double bond.

The values of $^1J_{CC}$ to the ring methyl groups fall in the range 46.7—50.0 Hz which is larger than the value for toluene (44.2 Hz) and indicates bond shortening in these compounds.

Thus, in conclusion, the combination of the evidence from ^{13}C chemical shift and carbon–carbon spin–spin coupling constant data strongly supports the generalisations of the conclusions reached from crystallographic studies regarding the stereochemistry of the bile pigments and shows its validity for a range of these in solution.

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REFERENCES

[1] A. Gossauer in 'Methodicum Chimicum,' ed. F. Korte, Academic Press, London, 1978, vol. 11, part 3, p. 142.

[2] (*a*) C. C. Kuenzle, M. H. Weibel, R. R. Pelloni, and P. Hemmerich, *Biochem. J.*, 1973, **133**, 364 and references therein; (*b*) H. Lehner, S. E. Braslavsky, and K. Schaffner, *Annalen*, 1978, 1990.

[3] (*a*) M. J. Burke, D. C. Pratt, and A. Moscowitz, *Biochemistry*, 1972, **11**, 4025; (*b*) Q. Chae and P-S. Song, *J. Amer. Chem. Soc.*, 1975, **97**, 4176; (*c*) G. Wagnière and G. Blauer, *J. Amer. Chem. Soc.*, 1976, **98**, 7806.

[4] R. Bonnett, J. E. Davies, and M. B. Hursthouse, *Nature*, 1976, **262**, 326.

[5] W. Becker and W. S. Sheldrick, *Acta Cryst.*, 1978, **B34**, 1298.

[6] (*a*) W. S. Sheldrick, *J.C.S. Perkin II*, 1976, 1457; (*b*) H. Lehner, S. E. Braslavsky, and K. Schaffner, *Angew. Chem.*, 1978, **90**, 1012.

[7] H. Scheer and J. J. Katz in 'Porphyrins and Metalloporphyrins,' ed. K. M. Smith, Elsevier, Amsterdam, 1975, p. 399.

[8] S. G. Boxer, G. L. Closs, and J. J. Katz, *J. Amer. Chem. Soc.*, 1974, **96**, 7058; A. R. Battersby, G. L. Hodgson, M. Ihara, E. McDonald, and J. Saunders, *J.C.S. Perkin I*, 1973, 2933; D. N. Lincoln, V. Wray, H. Brockmann, and W. Trowitzsch, *J.C.S. Perkin II*, 1974, 1920.

[9] (*a*) A. R. Battersby, M. Ihara, E. McDonald, J. R. Redfern, and B. T. Golding, *J.C.S. Perkin I*, 1977, 158; (*b*) A. I. Scott, C. A. Townsend, K. Okada, M. Kajiwara, R. J. Cushley, and P. J. Whitman, *J. Amer. Chem. Soc.*, 1974, **96**, 8069.

[10] L. Ernst and D. N. Lincoln, *J. Magnetic Resonance*, 1974, **16**, 190; 1975, **17**, 272.

[11] R. R. Ernst, *J. Chem. Phys.*, 1966, **45**, 3845; K. G. R. Pachler, *J. Magnetic Resonance*, 1972, **7**, 442.

[12] V. Wray and D. N. Lincoln, *J. Magnetic Resonance*, 1975, **18**, 374.

[13] H. K. Ladner, J. J. Led, and D. M. Grant, *J. Magnetic Resonance*, 1975, **20**, 530.

[14] H. Plieninger and M. Decker, *Annalen*, 1956, **598**, 198.

[15] H. Plieninger and J. Kurze, *Annalen*, 1964, **680**, 60.

[16] A. W. Johnson, E. Markham, R. Price, and K. B. Shaw, *J. Chem. Soc.*, 1958, 4254.

[17] N. F. Albertson, *J. Amer. Chem. Soc.*, 1952, **74**, 249.
[18] J. W. Harbuck and H. Rapoport, *J. Org. Chem.*, 1971, **36**, 853.
[19] A. Gossauer and D. Miehe, *Annalen*, 1974, 352.
[20] A. Gossauer and G. Kühne, *Annalen*, 1977, 664.
[21] H. Plieninger and U. Lerch, *Annalen*, 1966, **698**, 196.
[22] W. Siedel, *Z. physiol. Chem.*, 1935, **237**, 8.
[23] H. Plieninger, K. Ehl, and K. Klinga, *Annalen*, 1971, **743**, 112.
[24] D. W. Hutchinson, B. Johnson, and A. K. Knell, *Biochem. J.*, 1973, **133**, 493.
[25] W. J. Cole, D. J. Chapman, and H. W. Siegelman, *Biochemistry*, 1968, **7**, 2929.
[26] M. S. Stoll and C. H. Gray, *Biochem. J.*, 1977, **163**, 59.
[27] J. B. Stothers, 'Carbon-13 NMR Spectroscopy,' Academic Press, London, 1972.
[28] D. L. Cullen, P. S. Black, E. F. Meyer, D. A. Lightner, G. B. Quistad, and C. S. Pak, *Tetrahedron*, 1977, **33**, 477.
[29] R. Bonnett, M. B. Hursthouse, and S. Neidle, *J.C.S. Perkin II*, 1972, 1335.
[30] W. S. Sheldrick, W. Becker, and J. Engel, *Acta Cryst.*, 1977, **B33**, 2669.
[31] N. Naulet, M. L. Filleux, G. J. Martin, and J. Pornet, *Org. Magnetic Resonance*, 1975, **7**, 326.
[32] A. Gossauer, B. Grüning, L. Ernst, W. Becker, and W. S. Sheldrick, *Angew. Chem. Internat. Edn.*, 1977, **16**, 481.
[33] C. C. Kuenzle, *Biochem. J.*, 1970, **119**, 395.
[34] G. Severini-Ricca, P. Manitto, D. Monti, and E. W. Randall, *Gazzetta*, 1975, **105**, 1273.
[35] H. Falk, S. Gergely, K. Grubmayr, and O. Hofer, *Annalen*, 1977, 565.
[36] H. Falk, S. Gergely, and O. Hofer, *Monatsh.*, 1974, **105**, 1004.
[37] N. Muller and D. E. Pritchard, *J. Chem. Phys.*, 1959, **31**, 768 and 1471.
[38] K. Frei and H. J. Bernstein, *J. Chem. Phys.*, 1963, **38**, 1216.
[39] W. M. Litchman and D. M. Grant, *J. Amer. Chem. Soc.*, 1967, **89**, 6775.
[40] G. A. Gray, P. D. Ellis, D. D. Traficante, and G. E. Maciel, *J. Magnetic Resonance*, 1969, **1**, 41.
[41] G. A. Gray, G. E. Macial, and P. D. Ellis, *J. Magnetic Resonance*, 1969, **1**, 407.
[42] G. E. Maciel, J. W. McIver, N. S. Ostlund, and J. A. Pople, *J. Amer. Chem. Soc.*, 1970, **92**, 11.
[43] K. Venkatesan, D. Dale, D. C. Hodgkin, C. E. Nockolds, F. H. Moore, and B. H. O'Connor, *Proc. Roy. Soc.* 1971, **A323**, 455.
[44] A. R. Battersby, E. Hunt, and E. McDonald, *J.C.S. Chem. Comm.*, 1973, 442; A. R. Battersby, E. Hunt, M. Ihara, E. McDonald, J. B. Paine, F. Satoh, and J. Saunders, *ibid.*, 1974, 994; A. I. Scott, A. J. Irwin, L. M. Siegel, and J. N. Shoolery, *J. Amer. Chem. Soc.*, 1978, **100**, 316.
[45] W. S. Sheldrick, A. Borkenstein, M. Blacha-Puller, and A. Gossauer, *Acta Cryst.*, 1977, **B33**, 3625.