huge influence of the solvent on the form of the CD bands.[18]

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(18) A. Bacher and H. Harders, unpublished results.

Cyclotetramerization of 2-Dimethylamino-4-*tert*-butylpyrrole. The Tetra-*tert*-butylporphyrins

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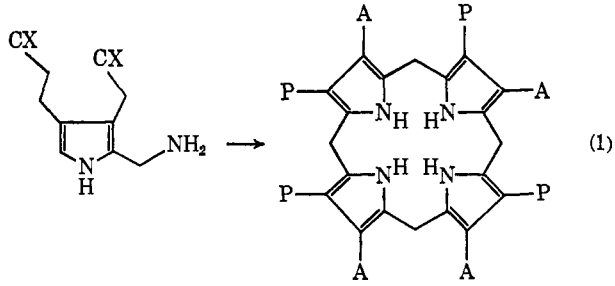
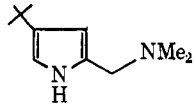
Abstract: The acid-catalyzed cyclotetramerization of 2-dimethylaminomethyl-4-*tert*-butylpyrrole was examined. A mixture of all four isomeric tetra-*tert*-butylporphyrins was produced. Each isomer was isolated in a pure form and characterized spectroscopically. The mechanism of the cyclization was investigated.

Among the questions dealing with the whys and wherefores of porphyrin biosynthesis, certainly foremost is the so-called Type III problem: What is the mechanism by which porphobilinogen (PBG) is enzymatically cyclotetramerized to uroporphyrinogen III (eq 1)?



(1)

The nature of this process, and in particular the stage of the cyclotetramerization at which isomerization occurs, has been the subject of much work.[1-3] As a model of this reaction we have investigated the acid-catalyzed cyclotetramerization of the title compound (**5**). This pyrrole, being unsymmetrically substituted,



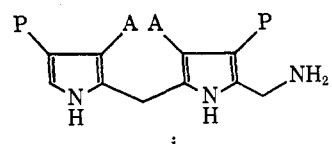
5

allows in principle a distinction between the isomeric tetra-*tert*-butylporphyrins (Figure 1) formed from it. Moreover, as **5** bears only one substituent (*tert*-butyl) other than the dimethylaminomethyl group, it allows a test of the hypothesis that the facile cyclotetramerization of 2-aminomethylpyrroles such as PBG to porphyrinogens (as opposed to simple linear polymerization that is the predominant product when substitution is absent[4]) is at least partially a consequence of the steric bulk of the substituents present on the pyrrole ring.

(1) Reviewed by B. F. Furnham in "Metabolic Pathways," Vol. III, 3rd ed, D. M. Greenberg, Ed., Academic Press, New York, N. Y., 1969.

(2) R. B. Frydman, A. Valasinas, and B. Frydman, *Biochemistry*, **12**, 80 (1973).

(3) R. B. Frydman, A. Valasinas, and H. Rapoport, *FEBS (Fed. Eur. Biochem. Soc.) Lett.*, **25**, 309 (1972), report the role of i as a precursor of uroporphyrinogen III.



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Results

The preparation of **5** was carried out by conventional procedures and is detailed in the Experimental Section. When a solution of **5** in acetic acid was heated to reflux with air or oxygen being bubbled through the solution, there was isolated in 10–25% yield a purple crystalline material which from its uv–visible spectrum was porphyrin in nature. Elemental analysis and mass spectrometry were consistent with this conclusion. In particular, while the mass spectrum exhibited an intense parent ion at *m/e* 534 there was no peak at *m/e* 478 (tri-*tert*-butylporphyrins) or *m/e* 590 (penta-*tert*-butylporphyrins).[5] This makes it unlikely that any processes involving acid-catalyzed isomerization of *tert*-butylporphyrins are involved in the synthesis as this should lead to substantial amounts of de-*tert*-butylation.

The nmr spectrum of the product (Figures 2a and 3a) was clearly inconsistent with any one of the expected products. Although the areas of the high field (*tert*-butyl) and low field (C*H*=C-*t*-Bu and bridging methine) were in the proper ratio of 9 : 1 : 1 the latter consisted of a larger number of singlets than would be possible for any one of the isomers. By integration the multiplet at *ca.* δ 9.1 could be assigned to the C*H*=C-*t*-Bu protons of the pyrrole ring and the region from δ 10 to 11 to the bridging methine ("meso") protons.[6] The con-

(4) (a) U. Eisner and R. P. Linstead, *J. Chem. Soc.*, 3742 (1955); (b) S. Krol, *J. Org. Chem.*, **24**, 2065 (1959).

(5) A small peak at *m/e* 477 $(P - C_4H_9)^+$ was present.

(6) (a) R. J. Abraham, A. H. Jackson, and G. W. Kenner, *J. Chem. Soc.*, 3468 (1961); (b) R. J. Abraham, P. A. Burbridge, A. H. Jackson, and D. B. MacDonald, *J. Chem. Soc. B*, 620 (1966).

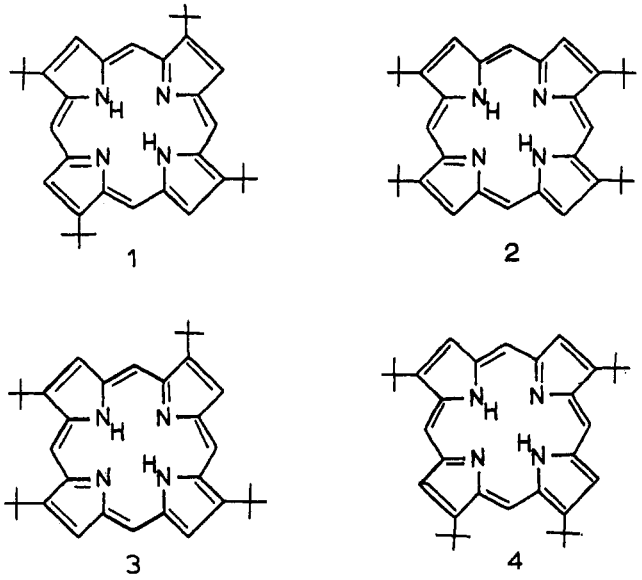


Figure 1.

Table I. Proton Chemical Shifts of Isomeric Tetra-*tert*-butylporphyrins[a]

Porphyrin isomer	Methine hydrogens, no. of adjacent *t*-Bu: 0	1	2	Pyrrole hydrogens	*t*-Bu
I		10.486 (4)		9.202 (4)	2.350 (36)
II	10.030 (2)		10.940 (2)	9.134 (4)	2.327 (36)
		10.527 (1)		9.233 (1)	2.330 (9)
III	10.001 (1)		10.988 (1)	9.190 (1)	2.310 (18)
		10.444 (1)		9.115 (2)	2.284 (9)
IV	9.967 (1)	10.470 (2)	11.033 (1)	9.208 (2)	2.335 (18)
				9.094 (2)	2.293 (18)

[a] Derived from FT nmr spectra ($CDCl_3$, 100 MHz, 32°) of separate isomers. All peaks are singlets with proton counts in parentheses. Maximum errors of $\delta \pm 0.05$ (concentration dependence) and ± 0.003 (chemical shift measurement) are estimated.

Table II. Natural Abundance ^{13}C Chemical Shifts of Tetra-*tert*-butylporphyrins, Isomers I, III, and IV, Plus Related Materials[a]

Porphyrin	$CDCl_3$	*t*-Bu	Meso CH	β-CH	α-C	β-C-*t*-Bu
3	77.7	34.6	103.2	128.54	143.9	155.1
		34.7	103.3	128.47	144.0	154.9
		35.0	103.6	128.1	144.1	154.7
		35.1	103.9	128.0	144.6	154.4
					144.7	
					144.9	
1	77.7	35.0	103.5	128.5	143.0	
		34.5			144.9	154.6
					145.1	154.9
4	77.7	35.0	103.8	128.4	*b*	*b*
		34.6	103.6	127.9		
		34.5	102.9			
3-*tert*-Butylpyrrole	77.7	31.8		106.3	112.7	135.6
		30.6			117.6	

[a] Assignments were by analogy with other cmr spectra of porphyrin derivatives: D. Doddrell and W. S. Caughey, *J. Amer. Chem. Soc.*, **94**, 2510 (1972); A. R. Battersby, J. Moron, E. McDonald, and J. Feeney, *J. Chem. Soc., Chem. Commun.*, 920 (1972). [b] Not observed.

viction that we were dealing with the spectrum of a mixture of isomers was strengthened by the observation that the ratios of singlets in this low-field region

Table III. Effect of Concentration of **5** on Ratio of Isomeric Tetra-*tert*-butylporphyrins[a]

Expt	[5], *M*	Time, hr	Yield, %	I	II	III	IV
1	0.036	2[b]	15	55	3	24	19
2	0.046	2,[b] 22[c]	21	63	5	21	4
3	0.056	2[c]	19	59	5	28	7
4	0.046[d]	2[b]	17	62	2	24	12
5	0.034[e]	2[b]	27	61	4	25	10
6	0.49	2,[b] 3[c]	15	30	5	44	22
7	0.46[f]	2[b]	29	36	5	44	16
8	5.0	2[b]	14	28	10	44	19

[a] In refluxing acetic acid for indicated time with nitrogen or air passed through the reaction mixture. [b] Nitrogen passage. [c] Air passage. [d] Carried out with 4 mol % of [^{14}C]formaldehyde; 0.9% incorporation was observed. [e] Carried out with 4.5 mol % of [^{14}C]formaldehyde; 1.1% incorporation was observed. [f] Carried out with 2.5 mol % of [^{14}C]formaldehyde; 1.2% incorporation was observed.

Table IV. Effect of Added Formaldehyde on Yield of Mixed Tetra-*tert*-butylporphyrin[a]

Expt	[5], *M*	5–Formaldehyde mole ratio	Time, hr	Yield, %
1	0.023	38	2	18
2	0.020	33	0.5	15
3	0.023	23	2	7
4	0.024	19	2[b]	12
5	0.02	6	0.5	7
6	0.02	2.7	1.25	5
7	0.022	3	1.5	1
8	0.024	1.4	1	0
9	0.026	0.7	1	0

[a] In refluxing acetic acid with air bubbling through. [b] Passage of nitrogen rather than air through the reaction mixture.

varied from reaction to reaction. Making the *ad hoc* assumption that moving a *tert*-butyl group on an adjacent pyrrole ring from a distal to proximal position relative to the bridging CH results in a downfield shift of the bridging methine signal of 0.5 ppm and that the same distal to proximal shift on a nonadjacent ring results in a downfield shift of 0.04 ppm results in a remarkably accurate simulation of the observed spectrum on the basis of its being a mixture of all four isomeric tetra-*tert*-butylporphyrins (Figure 2b). Exhaustive (and exhausting) recrystallization of the product did in fact resolve it into four substances, all of which had melting points above 300°, identical uv–visible spectra, identical behavior on thin-layer chromatography, and identical mass spectra, but dramatically different nmr spectra (Figure 3b–e) in close agreement with that predicted on the basis of the above. Final assignments of the proton resonances are presented in Table I. A multiplicity of meso hydrogen signals in the nmr spectra of porphyrins is in itself not novel: Abraham, *et al.*,[6b] have observed multiple singlets for the meso hydrogens of coproporphyrins spanning less than *ca.* 0.1 ppm in chloroform solution. The magnitude of the effect in our compounds is much greater. Ample justification for a sterically originating through space deshielding effect is available in the literature.[7,8] In particular, our results find close analogy in

(7) (a) S. Winstein, F. A. L. Anet, and A. J. R. Bourne, *J. Amer. Chem. Soc.*, **87**, 5249 (1965); (b) W. Nagata, T. Teresawa, and K. Tori, *ibid.*, **86**, 3446 (1964); (c) R. W. Franck and K. Yanagi, *J. Org. Chem.*, **33**, 811 (1968); (d) R. W. Franck and E. G. Lesu, *ibid.*, **35**, 3932 (1970).

(8) B. V. Cheney, *J. Amer. Chem. Soc.*, **90**, 5386 (1968).

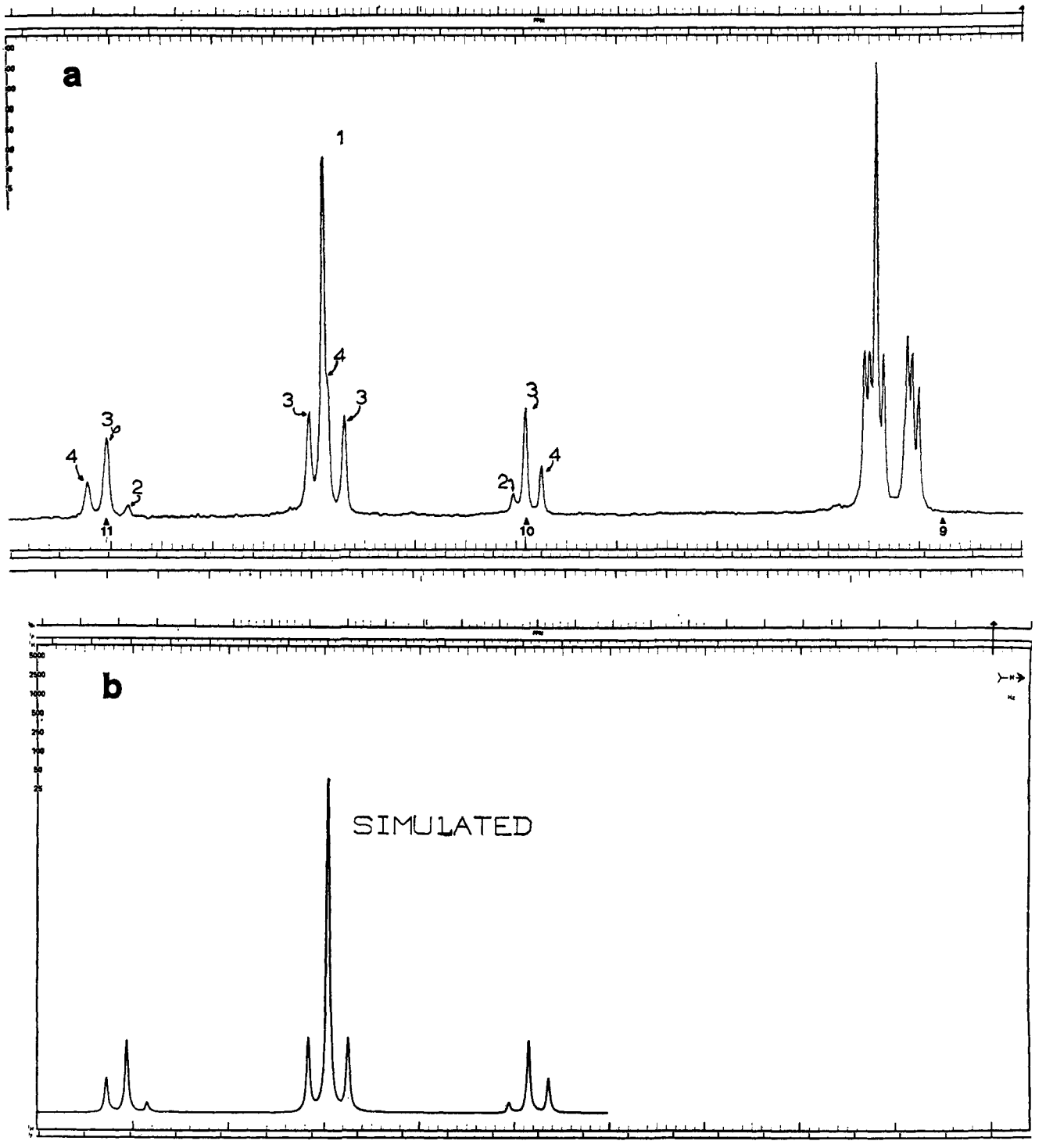


Figure 2. (a) FT nmr spectrum ($CDCl_3$, 100 MHz) of δ 9–11 region of a mixture of **1–4**. Integration of the spectrum affords the composition: 30% **1**, 5% **2**, 44% **3**, 22% **4**. (b) "Simulated" spectrum of a **1–4** mixture of the above composition assuming a chemical shift of δ 9.96 for the highest field singlet. The additive effects of substituents used in deriving this stick spectrum are discussed in the text.

the work of Franck and coworkers on the poly-*tert*-butylnaphthalenes.[7c,d]

Verification of the above assignment is seen in the carbon nmr spectra of these compounds (Table II, Figure 4). Although limitations on the amount of material available restricted us to determination of the spectra of the more abundant isomser I and III (**1** and **3**, respectively), these spectra clearly show the equivalence of related carbons in the former and their complete nonequivalence in the latter. Particularly noteworthy is the presence of four distinct peaks for the bridging methine carbons in the spectrum of **3**: this is the only isomer with all of these carbons nonequivalent.

It is clear then that cyclotetramerization of **5** in refluxing acetic acid affords a mixture of all four isomeric tetra-*tert*-butylporphyrins bearing one *tert*-butyl group per pyrrole ring. With the structural assignments on firm ground one can use the proton nmr spectra to semiquantitatively analyze the reaction mixtures for the four isomeric porphyrins produced as a function of reaction variables (Tables III and IV).

Comparable yields of the four isomeric porphyrins are

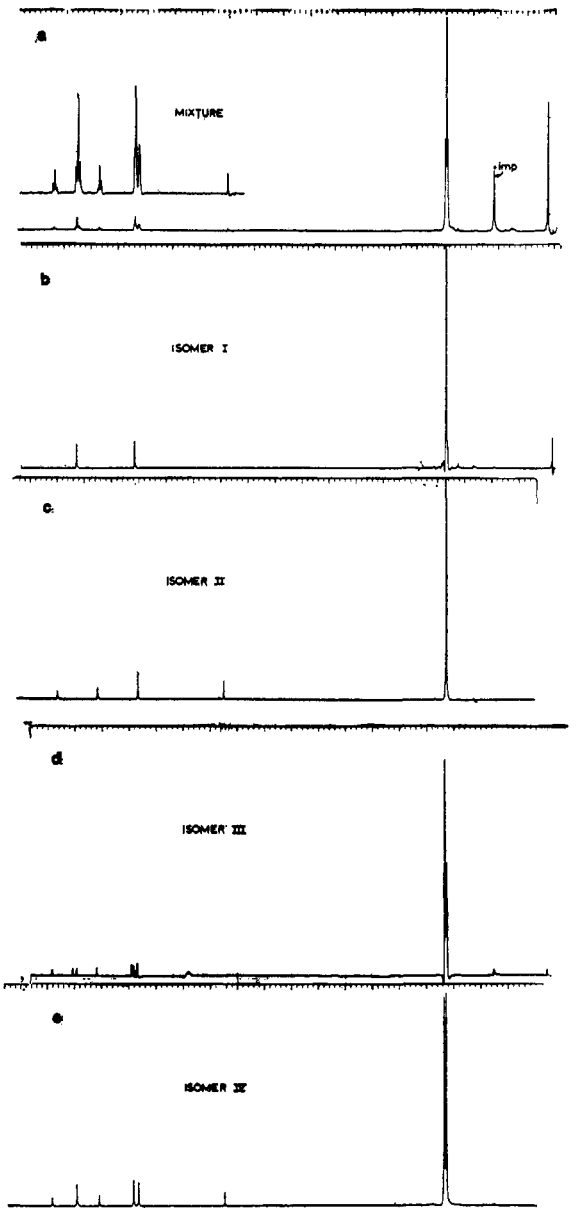


Figure 3. FT nmr spectra ($CDCl_3$, 100 MHz) of a mixture of **1–4** and the pure isomers: (a) mixture; (b) isomer I (**1**); (c) isomer II (**2**); (d) isomer III (**3**); (e) isomer IV (**4**). Tetramethylsilane is at the right margin.

obtained on reaction of 3-*tert*-butylpyrrole with formaldehyde in acetic acid (Table V). The ratio of isomers, however, is markedly different from that arising from reaction of the Mannich base **5** under these conditions.

Table V. Formation of Tetra-*tert*-butylporphyrins from 3-*tert*-Butylpyrrole and Formaldehyde[a]

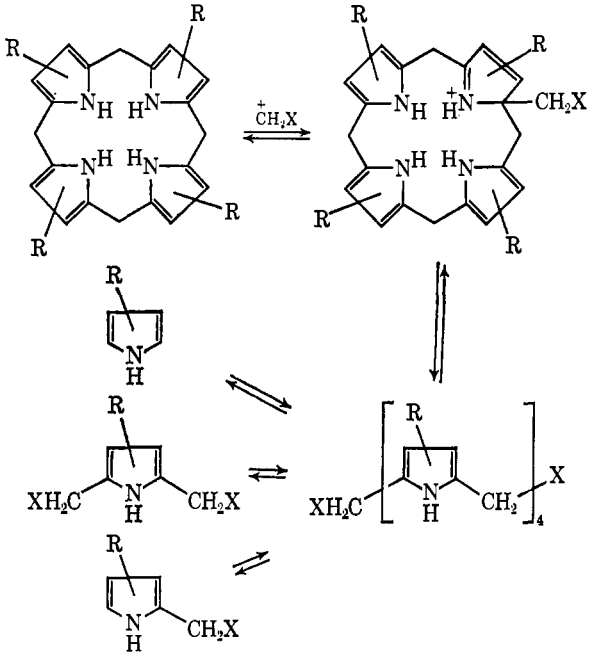
Expt	$[CH_2O]$, *M*	Formaldehyde–3-*tert*-butylpyrrole	Yield, %	Isomer I	Isomer II	Isomer III	Isomer IV
1	0.15	0.5	32		[c]		
2	0.054	0.6	27	16	13	49	22
3	0.024	0.6	37	7	5	57	31
4	0.058	0.8	32		[c]		
5	0.10	0.8	26		[c]		
6	0.024	0.8	8[b]	16	13	47	24
7	4.0	0.6	10	12	8	45	34

[a] In acetic acid at 25°. [b] Allowed to stand at 5° for 2 hr after mixing. [c] Not determined.

Effect of Formaldehyde. Addition of formaldehyde (as formalin) to the reaction mixture of **5** in acetic acid leads to a sharp drop in the yield of porphyrin: the yield is halved at a **5**–formaldehyde molar ratio of 10. At a ratio of approximately 3 the yield of porphyrin approaches 0 (Table IV). Within the accuracy of the analysis, however, the formaldehyde did not affect the isomer ratio produced (Table III, expt 4, 5, and 7). The significance of this observation, however, is obscured by the fact that only by using very low molar ratios of formaldehyde were we able to isolate enough of the porphyrin mixture to analyze.

In order to determine the extent to which added formaldehyde is incorporated into the porphyrins, radioactive formaldehyde was employed. We initially felt that substantial incorporation should be observed by the process involving reversible attack of the conjugate acid of formaldehyde on tetra-*tert*-butylporphyrinogen (Scheme I).

Scheme I



Incorporation of formaldehyde into uroporphyrinogens under acidic conditions with concomitant positional scrambling is well documented.[9] Moreover this would afford a convenient rationalization of the production of all four isomeric porphyrins and of the fact that a product mixture much closer to that expected on statistical grounds was obtained when porphyrins were produced directly by condensation of 3-*tert*-butylpyrrole and formaldehyde (Table V).

However, when **5** was cyclotetramerized in acetic acid in the presence of [^{14}C]formaldehyde, approximately 1% of the formaldehyde was incorporated into the porphyrin (Table IV). The majority of the radioactivity was associated with polymeric material that accompanies porphyrin formation, and some (approximately 5% depending on the run) could be recovered as the dimedone derivative. Under the conditions of the reactions, ratios of **5** to formaldehyde of *ca.* 20–30, equilibration according to Scheme I should lead to almost quantitative incorporation of formaldehyde into porphyrin. One is forced to one of

(9) D. Mauzerall, *J. Amer. Chem. Soc.*, 82, 2601, 2605 (1960).

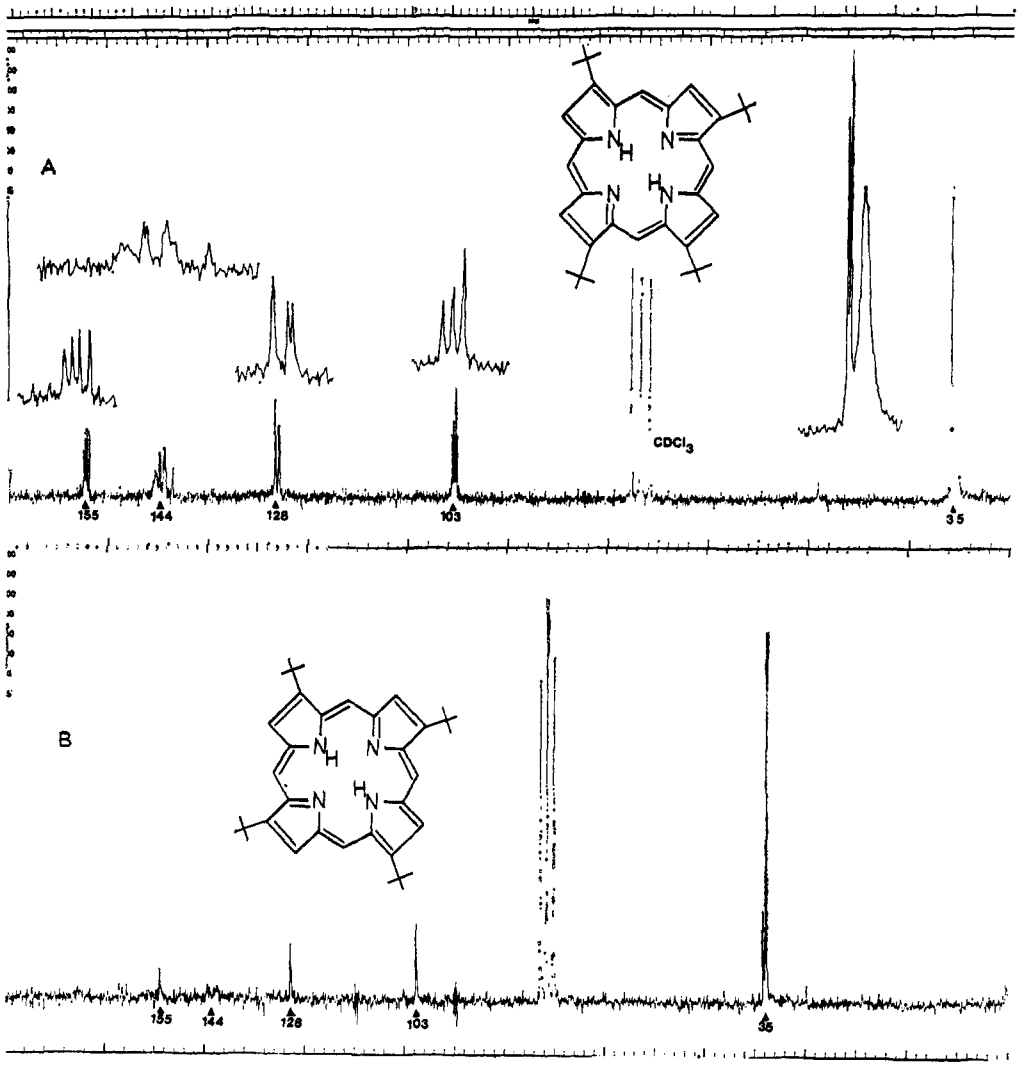
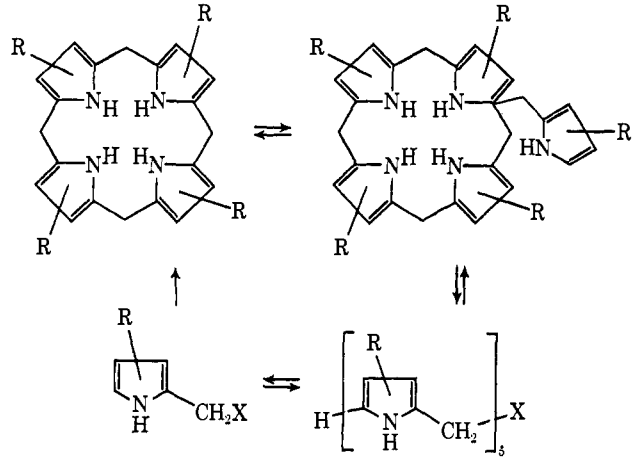


Figure 4. ^{13}C FT nmr ($CDCl_3$, 100 MHz, 12-mm tube) of **1** and **3**: (A) isomer III (**3**), 330 mg, 938 transients, 15-sec pulse delay; (B) isomer I (**1**), 23 mg, 9000 transients, 15-sec pulse delay.

three possible conclusions: (1) there are two temporally separated pools of formaldehyde, the first of which is rapidly consumed and the second of which is associated with interconversion of porphyrinogens according to Scheme I; (2) interconversion of porphyrins at the stage of porphyrinogens proceeds without liberation of one-carbon fragments; (3) neither of the above, *i.e.*, other mechanism are involved. We favor the latter. One can more or less dispose of the second hypothesis with the observation that reduction of **1** to the porphyrinogen stage followed by air dehydrogenation in hot acetic acid does not lead to appreciable rearrangement to the other isomers, while this result together with the absence of incorporation of formaldehyde into porphyrin tend to exclude the first explanation.

What is the explanation of the fact that a mixture of isomers is produced? Examination of Table III indicates a clear trend relating the degree of approach of the isomer ratio to that expected for equilibration of all four isomers with the concentration of **5** employed. Cyclotetramerization of a 0.036 *M* solution of **5** in acetic acid affords a I:III ratio of 2.3. At the highest concentration employed, 5.0 *M*, a I:III ratio of 0.6 is produced. The observed concentration dependence of the degree of isomer equilibration can be most simply

Scheme II



explained by a process akin to Scheme II, wherein one or more of the cationic intermediates involved in the cyclotetramerization is responsible for initiating attack on porphyrinogen. The ring-opened oligopyrrole, by virtue of its presumed equilibration with smaller oligopyrroles, would lead to the observed scrambling of pyrrole units. Other than the observed isomer interconversion the detailed predictions of this scheme

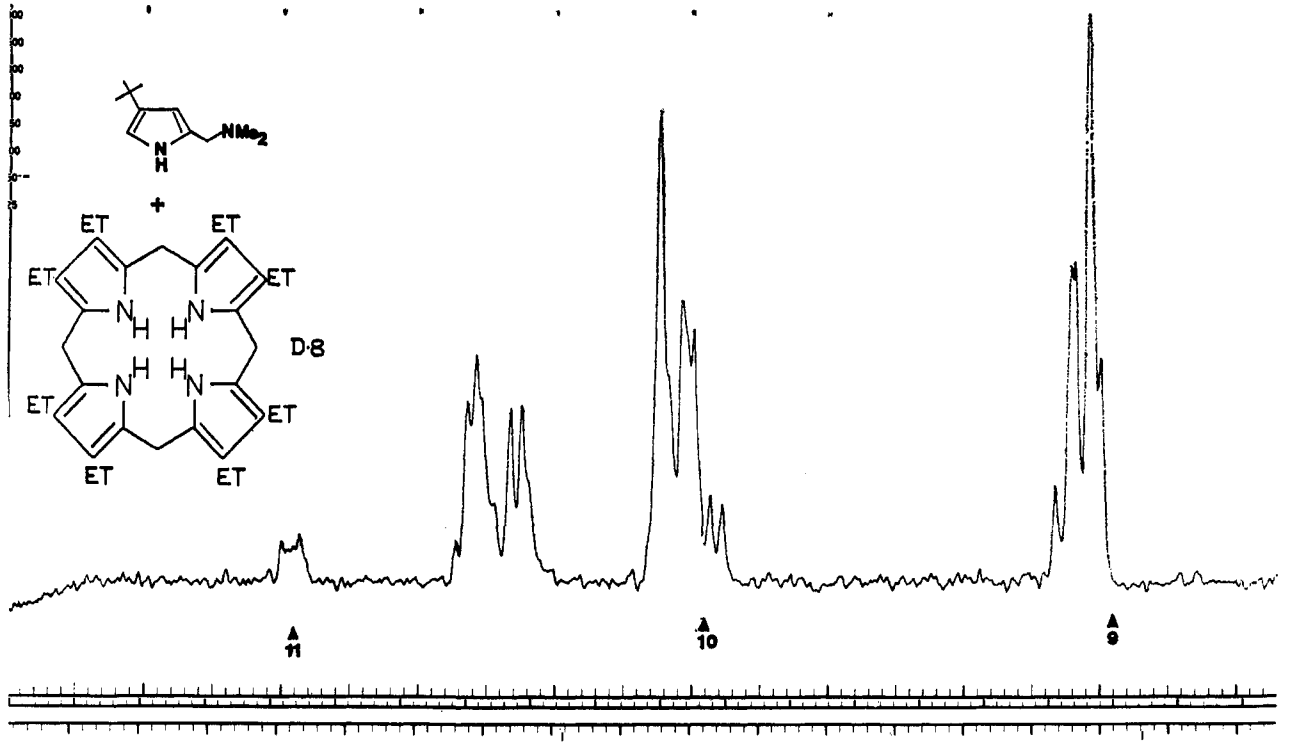


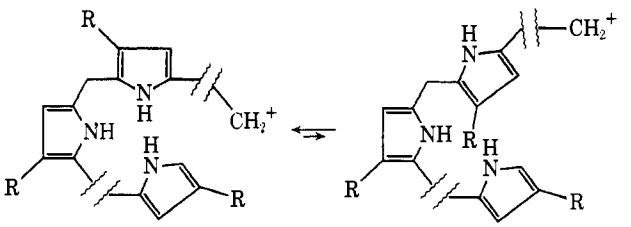
Figure 5. FT nmr spectrum ($CDCl_3$, 100 MHz) of the low-field region of the porphyrin mixture derived from 5 and octaethylporphyrinogen-d_8 in hot acetic acid.

should not be taken seriously. It does, however, make one prediction that is testable in a nonkinetic experiment. One should be able to observe incorporation of pyrrole units (from monomeric Mannich base) into preformed porphyrinogen. This prediction has been verified. When **5** was allowed to cyclotetramerize in acetic acid in the presence of octaethylporphyrinogen-d_8,[10] there was isolated an inseparable porphyrin mixture that by nmr (Figure 5) and mass spectrometry was an essentially random mixture of porphyrins containing x ethyl and $4 - x/2$ *tert*-butyl groups. Under these conditions octaethylporphyrinogen itself does not enter into this type of scrambling process.[12] These results show rather clearly that one or more intermediates in the cyclotetramerization of **5** are capable of attacking octaethylporphyrinogen with ultimate incorporation of the latter's pyrrole rings into mixed ethyl-*tert*-butylporphyrins.

Conclusions

We feel that we have established two points relevant to questions surrounding the cyclotetramerization of 2-aminomethylpyrroles. The efficacy of a *single* bulky alkyl group in directing the cationic oligomerization of **5** toward cyclization rather than linear polymerization must be due not to a blocking of the 3 position (as in, for example, 2-aminomethyl-3,4-diethylpyrrole) but to a population of conformations favoring cyclization, *e.g.*: we suggest that the facility with which porpholibinogen and related materials cyclotetramerize is due to this effect (Scheme III). Secondly, we have demonstrated to our satisfaction that (presumably cationic) intermediates in the **5** to porphyrinogen interconversion are capable of attacking the end product with ultimate formation of all four isomeric porphyrins. Although detailed speculation is unwarranted at the present stage of the relevant enzymology, it seems eminently reasonable to suggest that the crucial isomerization in the biosynthesis of uroporphyrinogen III is mediated by a similar process.

Scheme III



Experimental Section[13]

2-Dimethylaminomethyl-4-*tert*-butylpyrrole (5). A procedure similar to that described for the preparation of the Mannich base of 3,4-diethylpyrrole[14] was followed. To a stirred solution of 6.15 g (50 mmol) of 3-*tert*-butylpyrrole[15] in 100 ml of methanol at −15° was added dropwise over 2 hr a solution containing 4.48 g (55 mmol) of dimethylamine hydrochloride, 5.39 g (55 mg; 1 equiv) of potassium acetate, and 1.65 g (55 mmol) of formaldehyde (4.47 g of 37% formalin) in 25 ml of methanol. Water (25 ml) was then added and the mixture kept at 0° overnight. The product was diluted with 70 ml of 5% hydrochloric acid and extracted with ether. The aqueous layer was made alkaline with 70 ml of 2 *N* sodium hydroxide and extracted with ether. The combined ether extracts

(10) We thank Dr. Y. N. Chuah for this sample prepared by the procedure of Whitlock and Buchanan[11] from 2-dimethylaminomethyl-3,4-diethylpyrrole-d_2.

(11) H. W. Whitlock and D. H. Buchanan, *Tetrahedron Lett.*, 3711 (1969).

(12) Y. N. Chuah, Ph.D. Thesis, University of Wisconsin, 1973.

(13) Nmr spectra were determined at 100 MHz on a Varian XL100-15 instrument. Standard Varian software was used for the natural abundance ^{13}C spectra.

(14) H. W. Whitlock and R. Hanauer, *J. Org. Chem.*, **33**, 2169 (1968).

(15) (a) P. S. Skell and G. P. Bean, *J. Amer. Chem. Soc.*, **84**, 4655 (1962); (b) G. P. Bean, *J. Org. Chem.*, **32**, 228 (1967).

were dried over anhydrous potassium carbonate and concentrated to afford 6.3 g of a gray solid, crystallization of which from hexane afforded 4.0 g (45% yield) of 2-dimethylaminomethyl-4-*tert*-butylpyrrole (**5**) as colorless needles: mp 85.5–86.5°; δ(CCl_4) 1.20 (9 H, s), 2.20 (6 H, s), 3.31 (2, H, s), 5.79 (1 H, d, J = 2 Hz), 6.29 (1 H, d, J = 2 Hz), 9.1 (1 H, br). *Anal.* Calcd for $C_{11}H_{20}N_2$: C, 73.28; H, 11.18; N, 15.54. Found: C, 72.29; H, 11.14; N, 15.43.

In a similar manner 2-dimethylaminomethyl-5-*tert*-butylpyrrole was obtained as a yellow oil in 70% yield from 2-*tert*-butylpyrrole: δ (CCl_4) 1.19 (9 H, s), 2.09 (6 H, s), 3.18 (2 H, s), 5.46 (2 H, m).

Preparation of Tetra-*tert*-butylporphyrin from Mannich Base (5). Using quantities and conditions listed in Table III, a solution of Mannich base **5** in acetic acid was heated at reflux with air or nitrogen bubbling through. At the end of the reaction, the crude yield of porphyrin was measured, using the Soret absorption at 400 mμ ($\epsilon_{C_6H_6}$ 2.1×10^5).

Isolation of the porphyrin was accomplished by the following procedure. A solution of the crude product in chloroform was washed with saturated sodium bicarbonate and water, and concentrated to a dark brown solid. This solid was adsorbed on silica gel and the purple band of the porphyrin eluted with benzene. The visible spectrum of the product showed the principal absorption at 400 mμ with weaker bands at 494, 523, 565, 618, and 642 mμ.

Separation of the four isomers of tetra-*tert*-butylporphyrin (I–IV) was achieved by fractional recrystallization of the eluent from a large-scale preparation (5 mmol of **5** in 5 ml of acetic acid). Three fractions of purple crystals were obtained from methanol. From fraction 1 (474 mg), isomer 1, mp >300°, was isolated in pure form by repeated recrystallization from hexane: δ($CDCl_3$, 100 MHz), 2.35 (36 H), 9.20 (4 H), 10.49 (4 H); $\lambda_{max}^{C_6H_6}$ 400 mμ (ε 251,000), 494 (17,700), 524 (10,000), 565 (7300), 570 (sh 6100), 590 (1400), 619 (4300). Repeated recrystallization of the mother liquors of fraction 1 from hexane afforded isomer **4**: mp >300°; δ($CDCl_3$) 11.03 (1 H), 10.47 (2 H), 9.97 (1 H), 9.21 (2 H), 9.09 (2 H), 2.33 (18 H), 2.29 (18 H); $\lambda_{max}^{benzene}$ 400 mμ (ε 177,000), 495 (13,200), 524 (6500), 565 (5500), 570 (4500), 590 (sh 1100), 619 (2700). Isomer **2** was isolated from fraction 2 (32 mg) by virtue of its low solubility in hexane. Recrystallization of crude **2** from chloroform gave a sample: mp >300°; δ($CDCl_3$), 10.94 (2 H), 10.03 (2 H), 9.13 (4 H), 2.33 (36 H); $\lambda_{max}^{benzene}$ 399 (214,000), 494 (13,800), 526 (7700), 564 (5900), 570 (sh 4900), 590 (1400), 618 (2700). The mother liquors (692 mg) from fraction 3 (255 mg, itself a mixture of all four isomers as indicated by nmr) were adsorbed on silica gel and eluted with benzene. Concentration of the purple eluent and recrystallization of it from methanol afforded isomer **3**: mp >300°; δ($CDCl_3$) 10.99 (1 H), 10.53 (1 H), 10.44 (1 H), 10.00 (1 H), 9.23 (1 H), 9.19 (1 H), 9.12 (2 H), 2.33 (9 H), 2.31 (18 H), 2.28 (9 H); $\lambda_{max}^{benzene}$ 400 (200,000), 494 (14,500), 525 (7800), 527 (sh 7700), 566 (6200), 571 (5100), 590 (sh, 1100), 619 (3200).

An estimate of the amount of each isomer **1–4** formed in large-scale preparations of tetra-*tert*-butylporphyrin was obtained from integration of the nmr spectra (Table I) of the crude chromatographed mixture. Mass spectra (measured at 150–190°) of these mixtures showed strong parent ions at *m/e* 534.

Condensation of 5 in the Presence of Formaldehyde-^{14}C. Formaldehyde-*1*-^{14}C (New England Nuclear, specific activity 2.49×10^7 cpm/mmol as determined by conversion to its dimedone derivative) was employed under conditions described in Table III. In these experiments, an aliquot of the reaction mixture was taken before and after reaction to determine the amount of formaldehyde added and the total number of counts present at the end of the reaction. In addition, the amount of formaldehyde isolated as the dimedone derivative remaining at the end of the reaction was determined by standard isotope dilution techniques.

Condensation of Mannich Base 5 in Presence of Octaethylporphyrinogen-d_8. A solution of 15.6 mg (87 μmol) of **5** and 8.6 mg (16 μmol) of octaethylporphyrinogen-d_8[10] (0% d_0–d_4, 1.8% d_5, 7.4% d_6, 28.8% d_7 52.8% d_8, 7.3% d_9, 1.0% d_{10}; 7.63 D/molecule) in 0.10 ml of glacial acetic acid was freeze–thaw degassed, sealed, and heated at 95° for 2.5 hr. The tube was opened and worked up under air (to oxidize porphyrinogens) and the crude product was chromatographed as above. Low-voltage mass spectrometry (at 260°) of the resulting porphyrin mixture indicated it to have the isotopic composition: 5.5% d_0, 3.4% d_1, 20.7% d_2, 8.0% d_3, 21.9% d_4, 7.7% d_5, 12.7% d_6, 7.4% d_7, 10.0% d_8, 1.7% d_9, 0.9% d_{10}, 0.2% d_{11}; 4.29 deuterium atoms per porphyrin molecule. That calculated for complete randomization of diethylpyrrole (8.8% d_1, 87.2% d_2, 3.8% d_3) and *tert*-butylpyrrole units combining in the ratio 55.2:44.8 is: 4.0% d_0, 1.7% d_1, 17.5% d_2, 6.4% d_3, 28.6% d_4, 8.7% d_5, 20.8% d_6, 5.1% d_7, 5.7% d_8, 1% d_9. The nmr spectrum of this porphyrin mixture exhibited absorptions characteristic of the ethyl group (δ 1.83–2.0 and 3.98–4.21) and *tert*-butyl (δ 2.31–2.32) groups. Comparison of the relative areas of these allows a diethylpyrrole-d_x-*tert*-butylpyrrole ratio of 54:46 to be calculated. The meso hydrogen region exhibited a complexity characteristic of neither octaethylporphyrin *nor* the tetra-*tert*-butylporphyrins, having multiplets at δ 9.95–10.1 and 10.4–10.6 (Figure 5).

Preparation of Tetra-*tert*-butylporphyrin from *tert*-Butylpyrrole. A solution containing 3-*tert*-butylpyrrole and formaldehyde in acetic acid in the porportions described in Table III was stirred for 1 day at 25°. After the usual work-up and purification, the product had the characteristic visible spectrum of tetra-*tert*-butylporphyrin and showed a strong parent ion at *m/e* 534. The composition of the product was determined from the nmr spectrum as described above.

Reduction–Oxidation of Tetra-*tert*-butylporphyrin. To a solution containing 27 mg (0.05 mmol) of **1** (contaminated with approximately 20% of **2**, **3**, and **4**) in 25 ml of warm ethanol under nitrogen was added 0.8 ml (16 mmol) of 40% sodium amalgam. The reaction mixture became colorless after 10 min. After separation of the product from mercury and evaporation of the solvent, the pink residue was dissolved in 10 ml of acetic acid and heated to reflux while a stream of air was bubbled through. An nmr spectrum of the porphyrin, which was obtained in 65% yield after work-up and chromatography, was essentially identical with that of the porphyrin before the reduction.

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