

Chlorophyll and Related Substances. Part II. The Dehydrogenation of Chlorin to Porphin and the Number of Extra Hydrogen Atoms in the Chlorins.*

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The dehydrogenation of chlorin by various agencies has been studied. The use of quinones of high potential brings about a rapid conversion of chlorin into porphin. The stoichiometry of this reaction has been studied, and it has been shown that chlorin is a dihydroporphin.

It is well established, mainly through the work of Conant, Hans Fischer, and Stoll and their collaborators, that the chlorins derived from natural sources are hydroporphyrins. This is true, for example, of the important phorbides of the chlorophyll series, which contain the isocyclic ring connecting the δ - and γ -carbon atoms. It is generally held that the chlorins and phorbides are at the dihydro-level as first postulated for chlorophyll by Stoll and Wiedemann (*Naturwiss.*, 1932, **20**, 706; *Helv. Chim. Acta*, 1933, **16**, 183) but, whilst there is an impressive body of testimony in support of this, it is difficult to find decisive experimental evidence. (For summaries see Linstead, *Ann. Reports*, 1935, **32**, 362; 1937, **34**, 375;

• Part I, preceding paper.

H. Fischer, *FIAT Rev. Biochem.*, **1**, 142; Stoll and Wiedemann, *Fortschr. chem. Forsch.*, 1952, **2**, 538.) Most of the facts could, for example, be reconciled with the interpretation that the chlorins were tetrahydrides, although this would create a consequential problem of structure in the higher hydrides of the bacteriochlorophyll series.

Elementary analysis in compounds of this type is not easy and the results are hardly conclusive. Table I summarises the data found for porphin, chlorin, and ætiochlorin.

TABLE I.

		C, %	H, %	N, %	Total, %	C/H	
Porphin	Found :	Fischer and Gleim ¹	77.5	4.8	17.95	100.3	16.08
		Rothemund ²	77.2	4.8	17.9	99.9	16.08
		Eisner and Linstead ³	(a) 76.7	4.9	—	—	15.53
	Calc. for C ₂₀ H ₁₄ N ₄	(b) 76.8	4.8	—	—	—	15.87
		77.4	4.55	18.06	—	—	17.03
Chlorin	Found :	Eisner and Linstead ³	(a) 76.9	5.47	17.5	99.9	14.08
		(b) 77.2	5.52	—	—	—	13.95
	Calc. for dihydride, C ₂₀ H ₁₆ N ₄	76.9	5.2	17.9	—	—	14.90
	Calc. for tetrahydride, C ₂₀ H ₁₈ N ₄	76.4	5.8	17.8	—	—	13.24
Ætiochlorin	Found :	Fischer <i>et al.</i> ⁴	79.4	8.4	11.9	99.7	9.42
		Schlesinger, Corwin, and Sargent ⁵	79.6	8.4	—	—	—
		79.5	8.4	—	—	—	9.53
		79.7	8.3	—	—	—	(Mean)
		79.7	8.3	—	—	—	—
	Calc. for ætioporphyrin, C ₂₂ H ₂₂ N ₄	80.3	8.0	11.7	—	—	10.04
	Calc. for dihydride, C ₂₂ H ₂₄ N ₄	79.95	8.4	11.7	—	—	9.52
Calc. for tetrahydride, C ₂₂ H ₂₆ N ₄	79.6	8.8	11.6	—	—	9.07	

¹ *Annalen*, 1936, **521**, 157. ² *J. Amer. Chem. Soc.*, 1936, **58**, 625. ³ Present work. ⁴ *Annalen*, 1930, **479**, 27. ⁵ *J. Amer. Chem. Soc.*, 1950, **72**, 2867.

It will be seen that our chlorin and (particularly) the ætiochlorin of previous workers give figures agreeing better for a dihydride than a tetrahydride. Nevertheless the differences in the theoretical figures are not great, particularly in the ætio-series, and the experimental values tend to run a little high in hydrogen and low in carbon, owing, no doubt, to the obstinate retention of water. (The same trend can be seen in the figures obtained for the three synthetic samples of porphin.) There is therefore some lowering of the experimental C/H ratio (last column); and the results are less precise than could be desired, and are certainly not conclusive. Unequivocal evidence on the hydrogenation level was therefore highly desirable, and we have accordingly studied the dehydrogenation of chlorin.

Early work on the dehydrogenation of more complex chlorins of natural origin involved rather drastic conditions (see Fischer and his co-workers, *Annalen*, 1931, **490**, 84; 1937, **527**, 138; Fischer-Orth, "Die Chemie des Pyrrols," Leipzig, 1937, Vol. II, p. 2). Conant and his co-workers (*J. Amer. Chem. Soc.*, 1931, **53**, 359, 1615, 2382) were the first to study the process quantitatively, for which purpose potassium molybdicyanide was used. Calvin and his associates have recently examined the photo-oxidation of zinc *ms*-tetraphenylchlorin in presence of molecular oxygen or quinones (*ibid.*, 1948, **70**, 699; 1949, **71**, 4024, 4031). Schlesinger, Corwin, and Sargent (*loc. cit.*) have shown that the oxidation of metal-free ætiochlorin with molybdicyanide or ceric sulphate was incomplete even after long periods.

Our work on chlorin showed considerable differences between the ease of dehydrogenation of the metal-free compound and its metallic derivatives, comparable with some of those reported in the literature for more complex chlorins. The ready dehydrogenation of copper chlorin to copper porphin under acid conditions has been mentioned in Part I. The conversion of magnesium chlorin into magnesium porphin is complete within 5 minutes at room temperature on irradiation in the presence of quinones of quite low potential. On the other hand, metal-free chlorin is a relatively stable substance. Its solutions in benzene can be kept indefinitely in the dark and are not affected when molecular oxygen is passed through them for several hours on irradiation. Quinones, however, bring about a smooth dehydrogenation which we have investigated in some detail.

Braude, Jackman, and Linstead (*J.*, 1954, 3548) have shown that the ease of dehydrogenation of 1 : 4-dihydronaphthalene by quinonoid acceptors increases with the potential of the quinone. Braude, Brook, and Linstead (*ibid.*, p. 3569) showed that tetrachloro-1 : 2-benzoquinone and, particularly, 2 : 3-dichloro-5 : 6-dicyano-1 : 4-benzoquinone, were very powerful dehydrogenating agents for various hydrocarbon donors. Similar effects have now been observed for hydrogen transfers in which chlorin acts as donor. Chlorin was completely dehydrogenated by excess of the following quinones : dichlorodicyano-1 : 4-benzoquinone, 2 minutes at 80°; tetrachloro-1 : 2-benzoquinone, 10 minutes at 130°; but only partially by phenanthraquinone (E° 0.47 v) in 10 hours at 140°.

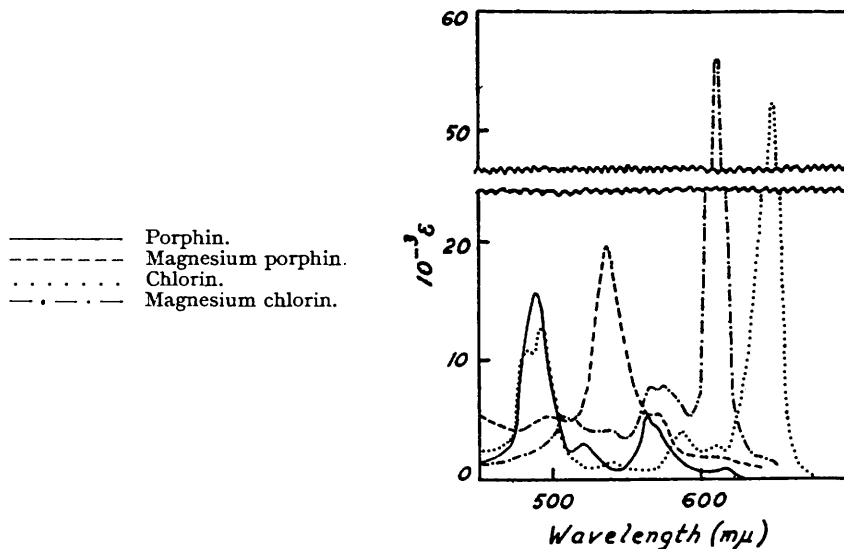


TABLE 2. Light-absorption data.

λ_{\max} (m μ)	ϵ	Solvent	Ref.	λ_{\max} (m μ)	ϵ	Solvent	Ref.
Porphin				Magnesium porphin			
395 *	261,000	Benzene	a	402 *	487,000	Benzene	a
489.5	16,000			497	5,200		
520	3,000			537	19,600		
563	5,200			572	5,400		
568.5 †	4,400			491	—	Pyridine-ether	c
616	890			535.3			
489	14,800	Benzene	b	566			
519	2,500			572.2			
562	4,700			602.5			
615	800						
634	160						

* Soret band.

† Inflection.

References : a, This work. b, Stern, *Z. phys. Chem.*, 1936, **177**, A, 58. c, Rothemund, *J. Amer. Chem. Soc.*, 1936, **58**, 625.

The reactions between the two powerful quinones and chlorin have been studied quantitatively. It was first established that the reaction product was porphin. When chlorin was heated in concentrations of about 10^{-4} molar for an hour at 130° in boiling chlorobenzene with an excess (2.5 mols.) of tetrachloro-1 : 2-benzoquinone, dehydrogenation was complete and a yield of 61% of crystalline porphin could be isolated. It was characterised by analysis and light absorption and by conversion into the magnesium derivative. Our data (Table 2 and the Figure) for metal-free porphin and its magnesium derivative are in good agreement with those of Stern *et al.* (*Z. phys. Chem.*, 1936, **177**, A, 58) and of Rothemund (*J. Amer. Chem. Soc.*, 1936, **58**, 627), respectively.

The stoichiometry of the dehydrogenation was next examined. For the purpose of estimating the content of chlorin and of porphin in a solution the light absorption was measured at 637.5, 563, and 490 $m\mu$. The first wavelength corresponds with the most important maximum of chlorin in the visible region. Porphin is transparent in this region, and the intensity of absorption accordingly is directly related to the concentration of unchanged chlorin. The absorption at the other two wavelengths, after an appropriate correction for the unchanged chlorin, give values for the porphin formed. In control experiments it was shown that tetrachloro-1 : 2-benzoquinone was decomposed thermally to the extent of about 10% after 2 hours at 130° in boiling chlorobenzene solution and that this figure was not appreciably changed by the presence of the corresponding quinol of or porphin. For the purpose of these experiments the quinone was estimated by making use of its light absorption at 460 $m\mu$. It was also shown that porphin was not appreciably affected by treatment with the quinone and that the reaction was irreversible under the conditions used. Chlorin was found to be thermally stable in chlorobenzene solution at 130°. Although it was shown that solutions of chlorin did not strictly obey Beer's law, the deviation was not great enough to have a significant effect on the results.

The actual transfer dehydrogenations were carried out in boiling chlorobenzene solutions, approximately 10^{-4} molar. The extent of reaction in replicate experiments showed an appreciable variation which was overcome by carrying out the dehydrogenation in sealed ampoules. Under these conditions reproducible results were obtained. Exactly equivalent (1 : 1) amounts of the reagents were used. If chlorin is a dihydride, PH_2 , then the reaction should be :



Hence 1 mol. of quinone could give a maximum conversion into porphin of 100%. If, on the other hand, chlorin is a tetrahydroporphin, the reaction producing porphin becomes :



1 mol. of quinone can then only give rise to a maximum conversion into porphin of 50%, provided that there is no thermal or catalysed dehydrogenation not involving transfer.

The results are given in detail in the experimental section. In a reaction period of 1.5 hours about 50% of the chlorin had been decomposed and the mean conversion of this into porphin was 95%. After longer periods dehydrogenation increased, reaching a limiting value of about 70% after 4.5 hours. The mean conversion of chlorin into porphin was slightly lower, being 94% in 3 hours and 92% in 4.5 hours. The departure from the theoretical value is attributed to side reactions involving the decomposition of both the quinone and the porphin as indicated in the blank experiments.

Experiments using a slight (17%) excess of quinone led to an increase in the rate of production of porphin and the ratio, porphin produced/chlorin consumed, nearly approached unity. As was to be expected, a higher proportion of chlorin was dehydrogenated, yielding about 80% of porphin in 3 hours.

Attention was next turned to 2 : 3-dichloro-5 : 6-dicyano-1 : 4-benzoquinone. The quinone was very unstable, its benzene solution being decomposed to the extent of 30% in 24 hours at room temperature, but since its hydrogenation is much faster than this decomposition, it was possible, by using freshly prepared solutions, to obtain good results for the dehydrogenation of chlorin.

With a 1 : 1 ratio of quinone to chlorin, 80% dehydrogenation was obtained after 3 hours at room temperature or 1 hour at 55°. Conversion of chlorin into porphin was 100%. Longer reaction periods gave a 90% dehydrogenation, which is probably the limiting value in view of the instability of the quinone. We also found that with a 1 : 2 ratio of quinone to chlorin, 50% of the chlorin was converted into porphin, thus confirming the stoichiometry of dehydrogenation.

All the results were consistent with equation (1) and a dihydride structure for chlorin and quite incompatible with its being a tetrahydride. The close spectroscopic resemblance referred to in Part I provides strong evidence that the natural chlorins are at the same level of hydrogenation, in agreement with current views.

EXPERIMENTAL

Dehydrogenation of Magnesium Chlorin.—Benzene solutions of magnesium chlorin containing 1 : 2-naphtha-, 2-methyl-1 : 4-naphtha-, and 1 : 4-benzoquinone severally were irradiated with light from a 300-watt bulb. Within 5 min. the magnesium chlorin band at 610 m μ was replaced by a weak diffuse band at 537 m μ due to magnesium porphin.

Preparation of Porphin.—Chlorin (15.3 mg.) and tetrachloro-1 : 2-benzoquinone (30.3 mg., ~2.5 mol.) in chlorobenzene (100 c.c.) were boiled under reflux in an atmosphere of nitrogen for 1 hr.; no more chlorin could then be detected. The cooled solution was extracted with 2N-hydrochloric acid (8 times), and the extracts washed with benzene, basified with ammonia, and extracted with benzene. Concentration of the benzene solution afforded porphin (9.4 mg., 61%) which crystallised from benzene in deep reddish-brown platelets (Found : C, 76.7, 76.8; H, 4.9, 4.8%; C/H, 15.5, 15.9. C₂₀H₁₄N₄ requires C, 77.4; H, 4.55%; C/H, 17.0). For light absorption, see Table 2.

Magnesium Porphin.—This was prepared in the same way as magnesium chlorin (see Part I) and formed a red solution with a red fluorescence in ultraviolet light. For light absorption, see Table 2.

Dehydrogenation of Chlorin.—(a) *With tetrachloro-1 : 2-benzoquinone.* In the following blank experiments, solutions of the quinone (~10⁻⁴M) were boiled in chlorobenzene (i) alone, (ii) in presence of the quinol (1 mol.), and (iii) with porphin (0.5 mol.) and the quinol (1 mol.). The decomposition of the quinone was followed spectroscopically by measurement of the band at 460 m μ . In presence of porphin it was necessary to apply the appropriate corrections for the absorption of the latter.

Percentage of quinone recovered after treatment with boiling chlorobenzene.

Time (min.)	Quinone alone	Quinone + quinol	Quinone + quinol + porphin
25	96.5	99.3	98.0
60	93.6	98.0	94.9
120	89.3	91.3	90.8

It was established that under these conditions porphin remained essentially unchanged and that chlorin was thermally stable.

The following procedure was adopted for the experiments tabulated below. Solutions containing respectively chlorin (6.24 mg./100 c.c.; 1 mol.) and the quinone (4.92 mg./100 c.c.; 1 mol.) in dry chlorobenzene were prepared. Glass ampoules were filled with 1 c.c. of each solution, sealed, and placed in a bath at 130°. After a suitable reaction period the contents of the ampoules were quantitatively transferred to volumetric flasks, the volume made up to 10 c.c. with benzene and the absorptions at 637.5, 563, and 490 m μ were measured. From the first value the percentage of unchanged chlorin was determined; from the last two figures, after the appropriate corrections for the absorption of chlorin, the percentage of porphin formed. It should be pointed out that owing to the relatively low absorption of porphin at 563 m μ the accuracy of results calculated from this wavelength is not very high.

Time of reaction :	1.5 hr.		3 hr.		4.5 hr.	
	Unchanged chlorin, %	47.2	47.7	28.4	28.5	26.85
Porphin formed, % { from 490 m μ band ...	50.3	50.3	67.2	65.0	66.25	65.95
{ from 563 m μ band ...	49.05	49.05	67.5	68.3	68.3	68.3
% conversion of chlorin { (490 m μ band) ...	95.3	92.9	93.9	94.3	90.6	90.0
{ (563 m μ band) ...	96.2	93.8	90.9	95.5	93.4	93.3
Mean conversion	94.55%		93.65%		91.8%	

In the following experiments a 17% excess of quinone was used. The reaction was carried out in 10-c.c. flasks fitted with air condensers, and the time of reaction was 2 hr.

					Mean
Unchanged chlorin, %	18.7	27.7	11.2	23.2	20.2
Porphin formed, % { from 490 m μ band	74.0	67.7	84.9	75.7	75.6
{ from 563 m μ band	82.6	74.5	84.7	75.5	79.3
% conversion of chlorin { (490 m μ band)	91.0	93.5	95.6	98.6	94.7
{ (563 m μ band)	101.6	103.1	95.4	98.3	99.6

Similarly, mean values (6 experiments) for a reaction period of 1 hr. were 35.6% unchanged chlorin and 65.9% and 68.3% for porphin formed, representing conversions of 102.3% and 106%, respectively. For a reaction period of 3 hr. (mean of 4 experiments), 17.6% chlorin was unchanged and 79.2% and 82.6% porphin was formed, representing conversions of 97.5% and 100.3%, respectively.

(b) *With 2 : 3-dichloro-5 : 6-dicyano-1 : 4-benzoquinone.* Solutions of chlorin (6.24 mg./100 c.c.; 1 mol.) and the quinone (4.54 mg./100 c.c.; 1 mol.) in dried benzene were prepared and used immediately. 1 c.c. portions of both solutions were sealed into ampoules as described above and kept either in a thermostat at 55° or at room temperature with the exclusion of light.

Temp.	Time, hr.	Unchanged chlorin, %	Porphin formed, %		% Conversion	
			(490 m μ)	(563 m μ)	(490 m μ)	(563 m μ)
55°	0.5	24.85	75.0	71.9	99.8	95.7
55°	0.5	24.3	74.35	71.9	98.2	95.0
Room	2 } 1 }	15.7	83.55	86.0	99.1	102.0
Room	2 } 1 }	14.6	84.9	91.6	99.4	107.2
Room	2 } 2 }	12.6	89.2	95.3	102.1	109.0
Room	3	21.6	80.9	85.0	103.2	108.4
Room	20	10.45	91.15	92	101.8	103.8

The following results were obtained from the reaction of 0.5 mol. of quinone with 1 mol. of chlorin.

Temp.	Time, hr.	Unchanged chlorin (%)	Porphin formed (%)		Conversion (%)	
			(490 m μ)	(563 m μ)	(490 m μ)	(563 m μ)
55°	1	56.1	41.1	40.4	93.6	92.0
55°	1	54.75	42.5	39.4	93.9	87.1
Room	24	52.0	45.6	45.2	95.0	94.2
Room	24	53.2	43.1	41.35	92.1	88.4

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