grant (AI-04965, Institute of Allergy and Infectious Diseases) and a predoctoral fellowship (5-Fl-GM-19,839, Institute of General Medical Sciences, to N. C.). We thank Professor W. S. Johnson and Dr. P. J. Neustaedter for their assistance with the olefinic cyclizations described in this report.

Hypochlorophyll

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Abstract: The photoreduction of chlorophyll and certain other phorbins by ascorbic acid, in pyridine containing ethanol and diazabicyclooctane, gives reduction products, named hypochlorophylls, which are believed to be 1,2-dihydromesochlorophylls. They are oxidized to mesochlorophylls by oxygen in the dark in the presence of weak acid or strong base, and by quinones and phenosafranine in the light. Their spectra closely resemble those of chlorins; in this respect, they are like the tetrahydroporphins from photoreduction of simple metallochlorins.

The photoreduction of simple metallochlorins, such as zinc chlorin[1] or zinc tetraphenylchlorin,[2] gives tetrahydroporphins with chlorin-like spectra, for which structures have been proposed having the β positions of two adjacent pyrrole rings reduced.[3] Chlorophyll or ethyl chlorophyllide a, on the other hand, gives a "tetrahydroporphin" ($ChlH_2$), with an absorption band at 525 mμ in pyridine,[4] the spectrum of which suggests that the closed, conjugated, double bond system characteristic of porphyrins has been interrupted. We wish to report that under certain conditions chlorophyll and other phorbins can be reduced to products that resemble the tetrahydroporphins from simple chlorins in their spectral and photochemical properties. To distinguish this class of reduction products from bacteriochlorins, which are tetrahydroporphins with opposite pyrrole rings reduced, we suggest that they be called *hypochlorins*, and the product from chlorophyll a, hypochlorophyll a. Their properties are still consistent with structures having adjacent pyrrole groups reduced.

The Hypochlorophyll Reaction

We earlier reported that certain aliphatic primary, secondary, and tertiary amines markedly accelerate and direct the course of photoreduction of zinc porphin by ascorbic acid.[1] It is difficult to use primary and secondary amines with chlorophylls, because of aminolytic attack on ring V (see structure Ia in Table I),[5] but tertiary amines are incapable of this reaction. After vain attempts to reduce protochlorophyll to chlorophyll in a manner analogous to the reduction of zinc porphin to zinc chlorin, we examined the effect of tertiary amines on the reduction of chlorophyll a itself.

The sequence of events during a typical reduction of chlorophyll a (Ia) or ethyl chlorophyllide a (Ib, which was used in place of chlorophyll for most of the experiments) in the presence of a rather strong ditertiary base, 1,4-diazabicyclo[2.2.2]octane (DABCO, Houdry Process and Chemical Co.), is shown in Figure 1. In red light, there first appeared the 525-mμ band of $ChlH_2$. In the absence of DABCO, this product accumulates and is the only reduction product of the chlorophyllide with noticeable absorption in the visible—the "Krasnovskii reduction."[4] In the presence of DABCO, this product reached a photostationary state, and the chlorophyllide was converted into another product with an absorption band at 632 mμ. The spectrum of this product, which we shall call ethyl hypochlorophyllide a, has narrow bands in the red and the violet regions, characteristic of porphyrins, and in fact resembles the spectra of certain Mg chlorins. Accompanying the bands of the hypochlorophyllide in Figure 1 is a band at 660 mμ, assigned to ethyl mesochlorophyllide for reasons soon to be evident, and a band, or shoulder, at 610 mμ, belonging to a by-product of unknown nature. There are bands in the violet belonging to these compounds at 434 and 415 mμ, respectively, though these are not prominent in Figure 1.

The conversion to hypochlorophyllide went best when the ethanol content of the solvent was 8–15% by volume. (This quantity is sufficient to "activate" chlorophyll toward the Krasnovskii reduction.[6]) Conversion was somewhat slower in pyridine containing DABCO but not ethanol; the proportion of mesochlorophyllide was larger, but the 610-mμ by-product was almost absent. At 60% ethanol, the yield of hypochlorophyllide was smaller because of Mg loss from $ChlH_2$. In ethanol without pyridine, reduction was very rapid in the presence of DABCO, but the only product was $ChlH_2$ (which rapidly lost Mg). The quantum yield was much larger than when pyridine was the activating base,[6] and probably exceeded 0.1.

DABCO is the best catalyst we have encountered for this reaction, but it is not unique. Reduction in ethanol produced some hypochlorophyllide in the presence of triethylamine, but not in the presence of hexamethylenetetramine or triethylenetetramine. Hypochlorophyllide appears to be a minor product of Krasnovskii re-

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[1] G. R. Seely and K. Talmadge, *Photochem. Photobiol.*, **3**, 195 (1964).

[2] G. R. Seely and M. Calvin, *J. Chem. Phys.*, **23**, 1068 (1955).

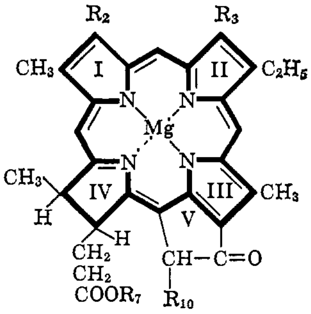
[3] G. R. Seely, *ibid.*, 27, 125 (1957).

[4] A. A. Krasnovskii, *Dokl. Akad. Nauk SSSR*, **60**, 421 (1948).

[5] H. Fischer and S. Goebel, *Ann.*, **524**, 269 (1936).

[6] G. R. Seely and A. Folkmanis, *J. Am. Chem. Soc.*, **86**, 2763 (1964).

Table I. Structures of Chlorophylls and Their Derivatives



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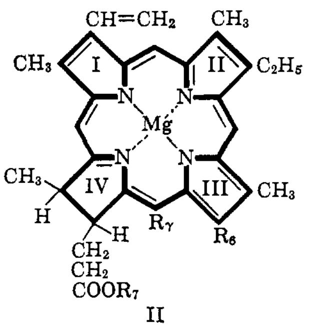

II

Derivative	R$_7$	R$_{10}$	R$_2$	R$_3$
Chlorophyll a (Ia)	Phytyl	COOCH$_3$	CH=CH$_2$	CH$_3$
Ethyl chlorophyllide a (Ib)	C$_2$H$_5$	COOCH$_3$	CH=CH$_2$	CH$_3$
Ethyl mesochlorophyllide a (Ic)	C$_2$H$_5$	COOCH$_3$	C$_2$H$_5$	CH$_3$
Pyrochlorophyll a (Id)	Phytyl	H	CH=CH$_2$	CH$_3$
Chlorophyll b (Ie)	Phytyl	COOCH$_3$	CH=CH$_2$	CHO

	R$_7$	Rγ	R$_6$
Mg chlorin e$_6$ trimethyl ester (IIa)	CH$_3$	CH$_2$COOCH$_3$	COOCH$_3$
Mg isochlorin e$_4$-6-carboxypiperidide ethyl methyl ester (IIb)	C$_2$H$_5$	CH$_2$COOCH$_3$	CONC$_5$H$_{10}$
Mg purpurin 7-lactone methyl ether ethyl methyl ester (IIc)	C$_2$H$_5$	CH$_3$OC—O—C=O COOCH$_3$	

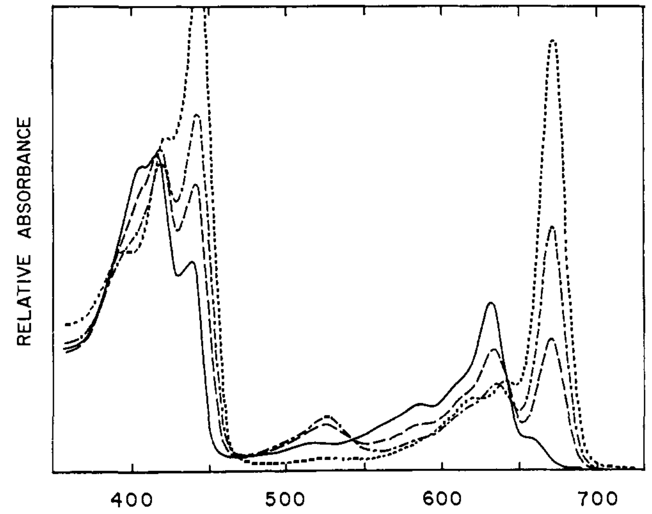

Figure 1. Reduction of ethyl chlorophyllide a to hypochlorophyllide in red light (6600-A Baird atomic interference filter). Composition of solution: 7% (vol.) ethanol, 93% pyridine, 0.125 M DABCO, 4 × 10^{-3} M ascorbic acid, 1.1 × 10^{-5} M chlorophyllide initially; spectra recorded before illumination (---------), and after continuous illumination for 7 min (–·–·–·), 16 min (– – – –), and 71 min (————). In last trace, bands near 660 and 610 mμ belong to by-products.

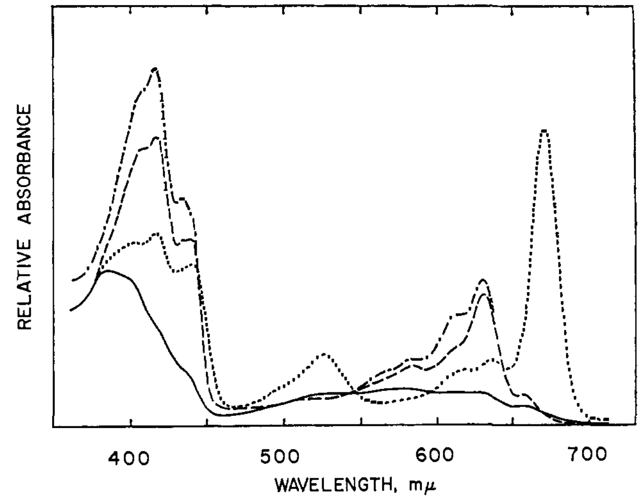

Figure 2. Reduction of ethyl chlorophyllide a in unfiltered light Composition of solution similar to that of Figure 1, but 0.25 M in DABCO and 2 × 10^{-5} M in chlorophyllide. Spectrum initially like the first trace of Figure 1; trace (---------) begun immediately after start of illumination (buildup of ChlH$_2$, 525 mμ, already complete); (————) 6 min later (leucohypochlorophyllide); (– – – –) light off and exposed to air (oxidation to hypochlorophyllide); (–·–·–·) 30 min later, further increase in the by-product absorbing at 610 and 415 mμ.

ductions in pyridine containing only small amounts of water or alcohol, and is identical with the "631 compound" we reported earlier from a wayward Krasnovskii reduction with yellowed ascorbic acid.[6]

If the reaction is run in white light instead of red, a "leucohypochlorophyllide" with a broad absorption band in the visible is formed (Figure 2); this, on exposure to air, is reoxidized rapidly to hypochlorophyllide. Mesochlorophyllide was not produced during reoxidation.

Protected from air, hypochlorophyllide is stable in the solution of its formation, and does not revert to a chlorophyll. It can be transferred intact to benzene or ether by extraction with phosphate buffer (pH 6), but protection from air and light is advisable to minimize oxidation to mesochlorophyllide.

Oxidation of Hypochlorophyll

Quinones. It is characteristic of the simpler hypochlorins that they can be photooxidized by quinones to

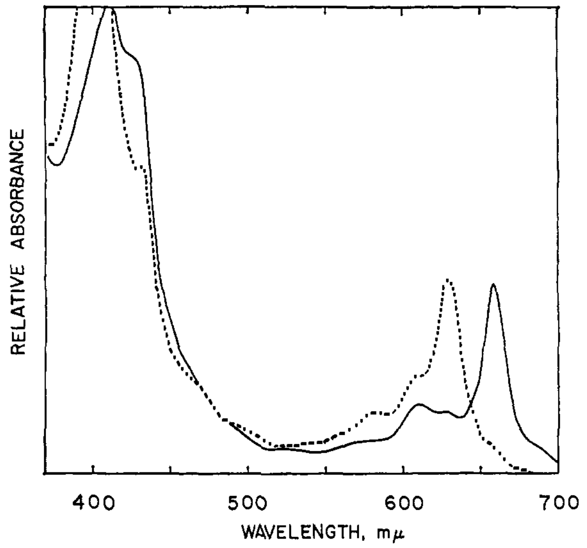

Figure 3. Photooxidation of ethyl hypochlorophyllide to ethyl mesochlorophyllide: (---------) hypochlorophyllide, extracted into benzene, with *ca.* 10^{-2} *M* 2,5-dimethylbenzoquinone; (———) after 30 min of red light (>570 mμ), oxidation to mesochlorophyllide (659 mμ) (the weak band at 685 mμ is probably derived from the 610-mμ by-product).

chlorins or porphins.[2,7] This and the corresponding dark reaction with powerfully oxidizing quinones[8] have established the reduction level of these tetrahydroporphins.

The hypochlorophylls also are photooxidized by benzoquinone, 2,5-dimethylbenzoquinone, and 1,4-naphthoquinone in benzene or ether, in the absence of oyxgen (Figure 3). However, the spectrum of the product of oxidation of ethyl hypochlorophyllide corresponds not to that of ethyl chlorophyllide, as would be expected, but to that of ethyl mesochlorophyllide (Ic). This has been true of most of the hypochlorophylls examined—the spectrum of the chlorophyll derivative recovered by oxidation closely resembles the spectrum of the original chlorophyll, but is shifted to shorter wavelengths by amounts (~10 mμ) commonly encountered on reduction of the 2-vinyl group to ethyl.[9]

Hypochlorophyllide can even be photooxidized by the weak oxidant phenosafranine. By following the simultaneous fall of the bands of phenosafranine and hypochlorophyllide, and rise of the band of mesochlorophyllide (Figure 4), the stoichiometry of the oxidation could be estimated. With ϵ_{538} 6.0 × 10^4 M^{-1} cm^{-1} for phenosafranine, and ϵ_{662} 7.5 × 10^4 M^{-1} cm^{-1} estimated for mesochlorophyllide, the same as for chlorophyllide, it was found that 1.21 moles of mesochlorophyllide was produced per mole of phenosafranine consumed, in best agreement with the formulation of hypochlorophyllide as a dihydromesochlorophyllide. From the absorption changes at 662 and 631 mμ, an extinction coefficient ϵ_{631} 5.8 × 10^4 M^{-1} cm^{-1} was calculated for hypochlorophyllide in 3:8 ethanol–pyridine solution.

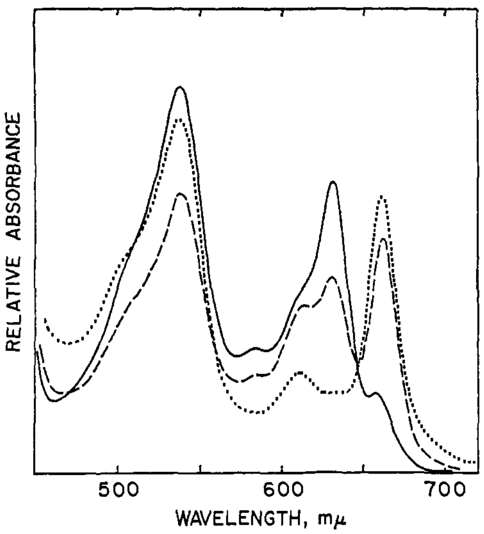

Figure 4. Photooxidation of ethyl hypochlorophyllide by phenosafranine: (———) hypochlorophyllide in 3:8 ethanol–pyridine with 2.2 × 10^{-5} *M* phenosafranine (538 mμ), under N_2; (– – – –) after 25 min of light through Corning filter No. 2424 (>590 mμ), oxidation to mesochlorophyllide (662 mμ); (---------) on aeration, and exposure to white light, phenosafranine is regenerated, and hypochlorophyllide destroyed, part of it being oxidized to mesochlorophyllide.

The position of the band at longest wavelength in the spectrum of the hypochlorophyllide is very near that of 9-deoxo-9-hydroxychlorophyll.[10] As the latter is also a "dihydrochlorophyll," it was thought possible that hypochlorophylls were 9-hydroxy derivatives. However, the spectra of hypochlorophyllide and 9-deoxo-9-hydroxychlorophyll differ in other respects, particularly in the Soret red band intensity ratio; furthermore, 9-deoxo-9-hydroxypyrochlorophyll, prepared by borohydride reduction of pyrochlorophyll, was entirely inert to photooxidation by benzoquinone.

Phase Test. Chlorophyll derivatives, in which the β-keto ester grouping of ring V is preserved, exhibit color changes on treatment with strong base, with the emergence of spectra characteristic of phase test intermediates.[11] The intermediates, which are apparently enolate ions, normally revert to the original chlorophylls if the base is neutralized before hydrolysis occurs.

On addition of tetrabutylammonium hydroxide to a solution of ethyl hypochlorophyllide in pyridine prepared *in situ*, a phase test intermediate spectrum appeared immediately (Figure 5). Neutralization of the base did not restore the spectrum of hypochlorophyllide, but the spectrum of mesochlorophyllide instead. The spectrum of the phase test intermediate from hypochlorophyllide agreed with that from mesochlorophyllide prepared by hydrogenation of chlorophyllide, and with that from "mesochlorophyllide" formed by quinone photooxidation of hypochlorophyllide. The base probably catalyzed rapid oxidation of hypochlorophyllide to mesochlorophyllide by traces of oxidants (*e.g.*, dehydroascorbic acid) present in the solution. Similarly, chlorophyllide was regenerated when ChlH_2, prepared from ethyl chlorophyllide without DABCO, was treated with base. The presence of bases, such as ammonia, in the Krasnovskii reaction is known to pre-

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(7) G. D. Dorough and J. R. Miller, *J. Am. Chem. Soc.*, **74**, 6106 (1952).

(8) U. Eisner, *J. Chem. Soc.*, 3461 (1957).

(9) A. Stern and H. Molvig, *Z. Physik. Chem.*, **178**, 161 (1937). It is true, as a referee has pointed out, that the spectral shift suggests only that the 2-vinyl group has become saturated in some way. As reduction to ethyl seems much the more likely way under the circumstances of the reaction, we have referred to the product as a *meso* derivative for the sake of argument, recognizing that alternatives are conceivable.

(10) A. S. Holt, *Plant Physiol.*, **34**, 310 (1959).

(11) A. S. Holt, *Can. J. Biochem. Physiol.*, **36**, 439 (1958).

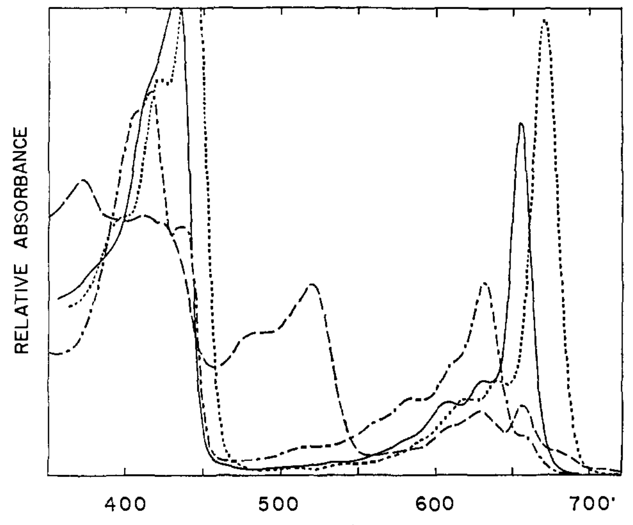

Figure 5. Phase test of hypochlorophyllide and reversion to mesochlorophyllide: (---------) original spectrum of ethyl chlorophyllide in solution of composition like that of Figure 2 (671 and 443 mμ); (-·-·-·) ethyl hypochlorophyllide, prepared by a 30-min exposure to light through Corning filter No. 2030 (>640 mμ); (- - - -) spectrum of phase test intermediate (520 mμ) formed by addition of tetrabutylammonium hydroxide in excess of the ascorbic acid (although formed from hypochlorophyllide, the intermediate appears to be that of mesochlorophyllide); (———) on neutralization of the quaternary base by acetic acid, the spectrum of ethyl mesochlorophyllide appears (657 and 433 mμ).

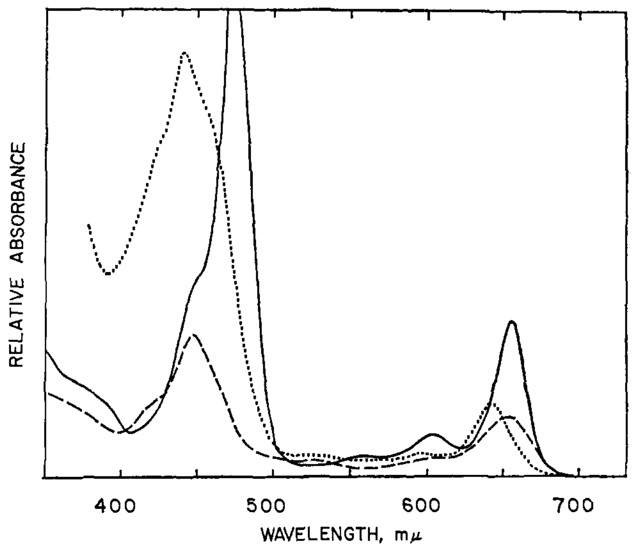

Figure 6. Reduction of chlorophyll b: (———) spectrum before illumination, in pyridine with 8% ethanol and 5 × 10^{-3} *M* ascorbic acid, but without DABCO; (- - - -) after 50 min of light through filter No. 2030 (displacement of Soret band from 472 to 446 mμ suggests reduction of the 3-formyl group; (---------) after transfer to benzene and addition of 2,5-dimethylbenzoquinone; 4 min of red light diminishes long-wave side of red band, leaving spectrum shown. This compound (642 mμ) is then slowly converted to one absorbing *ca.* 655 and 440 mμ.

vent accumulation of dihydrochlorophyll by accelerating its reoxidation to chlorophyll.[12]

Attempted Preparation of Hypopheophorbide. As with bacteriochlorophylls,[13] the Mg is difficult to remove from hypochlorophylls; ethyl hypochlorophyllide, in ether containing acetic acid, was oxidized to ethyl ethyl mesochlorophyllide by air in 1 hr in the dark at 10°, but Mg was not displaced. Addition of excess HCl to an ether–methanol solution of ethyl hypochlorophyllide produced not the expected Mg-free pigment, ethyl hypopheophorbide, but the dihydrochloride of ethyl mesopheophorbide (649 mμ). Neutralization of the acid with pyridine generated the spectrum of the mesopheophorbide, the band positions and relative intensities agreeing with published values.[9] Similarly rapid oxidation in the presence of acid has been reported for octaethylhypochlorin.[8] Hypopheophorbide appears to be too sensitive to oxidation for more than a transitory existence, at least in acid solution. Photoreduction of ethyl pheophorbide by ascorbic acid in the presence of DABCO produced only the dihydropheophorbide familiar from reduction in the absence of DABCO.[6,14,15]

Scope of the Reaction

Ethyl chlorophyllide a, chlorophyll a, pyrochlorophyll a (Id), and ethyl pyrochlorophyllide a all gave hypochlorophylls on reduction in the presence of DABCO, which were reoxidizable to what appeared to be mesochlorophylls. Zinc ethyl pheophorbide a reacted in a similar way. Rather surprisingly, ethyl mesochlorophyllide, whether prepared by H_2–Pd reduction of ethyl chlorophyllide or by oxidation of ethyl hypochlorophyllide, was not photoreduced to the hypochlorophyllide, but instead was very slowly bleached.

In the presence or absence of DABCO, chlorophyll b (Ie) was not reduced to a hypochlorophyll, but to derivatives of unknown structure as shown in Figure 6. Chlorophyll b-3-methanol (666 mμ in pyridine), however, was reduced to a hypochlorophyll (633 mμ) in the presence of DABCO. This was slowly photooxidized by 2,5-dimethylbenzoquinone to a compound (648 mμ in ether) which was probably mesochlorophyll b-3-methanol.

Ethyl 9-deoxo-9-hydroxychlorophyllide a (640 mμ in pyridine) and 9-deoxo-9-hydroxychlorophyll-b-3-methanol (635 mμ) were reduced almost quantitatively to what were probably the meso derivatives (630 and 625 mμ); the former, at least, was not reoxidized by 2,5-dimethylbenzoquinone.

An allomerized chlorophyll derivative, Mg purpurin 7-lactone methyl ether ethyl methyl ester (IIc; 663 mμ in pyridine), was reduced to a hypochlorophyll (620 mμ); this was oxidized by 2,5-dimethylbenzoquinone in benzene to a chlorin which may have been the starting material or its meso derivative (656 mμ in benzene).

Mg chlorin e_6 trimethyl ester (IIa) was slowly bleached, but not reduced to a hypochlorin. Mg isochlorin e_4-6-carboxypiperidide ethyl methyl ester (IIb; 648 mμ in pyridine) was reduced to two compounds (635 and 625 mμ). In ether containing benzoquinone, the 625-mμ band decreased in the light and the 635-mμ band increased. The 625-mμ band perhaps belongs to a hypochlorin, the 635-mμ band to a mesochlorin. Mg isochlorin e_4-6-carboxybutylamide ethyl methyl ester (646 mμ) was similarly reduced to two compounds (636 and 622 mμ); the latter was photooxidized by 2,5-di-

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(12) A. A. Krasnovskii, G. P. Brin, and N. N. Drozdova, *Dokl. Akad. Nauk SSSR*, **150**, 1157 (1963).

(13) A. A. Krasnovskii and E. V. Pakshina, *ibid.*, **148**, 935 (1963).

(14) G. Zieger and H. T. Witt, *Z. Physik. Chem.*, **28**, 286 (1961).

(15) T. T. Bannister in "Photochemistry in the Liquid and Solid States," John Wiley and Sons, Inc., New York, N. Y., 1960, p 110.

methylbenzoquinone, but not to any compound noticeably absorbing in the visible.

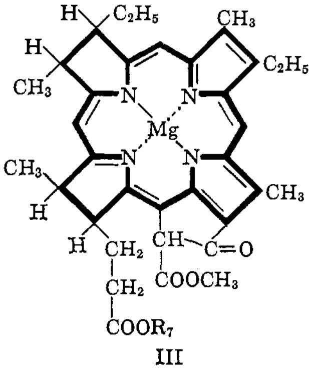
In 95% ethanol containing DABCO, however, Mg isochlorin e_4-6-carboxybutylamide phytyl methyl ester was reduced to a compound absorbing at 615 mμ (and two or three products absorbing in the green region), which might be analogous to the previous 622-mμ compound, if allowance is made for the effect of solvent on the spectrum.

Discussion

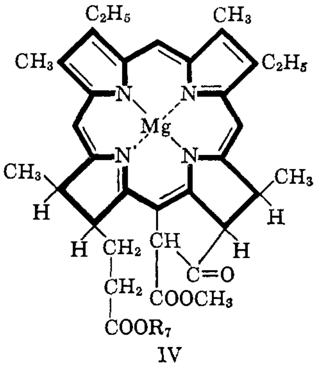
The identification of hypochlorophylls as dihydromesochlorophylls rests upon their facile oxidation to mesochlorophylls by air under acid or alkaline conditions, or photochemically by quinones under anaerobic conditions. The identification of the oxidation products as mesochlorophylls rests on the similarity of their spectra to those reported or readily inferrable for mesochlorophylls.[9] The spectral similarity extends to pheophorbides and to phase-test intermediates. The identification is also supported by the inability to undergo the hypochlorophyll reaction of both authentic mesochlorophyllide, and "mesochlorophyllide" prepared by oxidation of hypochlorophyllide.

As the 9-carbonyl group appears not to become reduced the extra hydrogens of hypochlorophyll must be located somewhere in the conjugated porphyrin ring system. Theoretical reasons have been advanced for expecting that tetrahydroporphins, in which the β positions of two adjacent pyrrole rings are reduced, would have spectra similar to those of chlorins.[3,16,17] The sharpness of the bands in the visible part of the spectrum of hypochlorophylls, a characteristic of porphyrins, argues for retention of planarity and closed conjugation in the ring.

If the hypochlorins are such tetrahydroporphins, the question remains whether ring I or ring III is reduced in hypochlorophylls. Because of the involvement of the 2-vinyl group, we favor ring I (structure III) over ring III (structure IV), but the experimental evidence is inconclusive.



III



IV

If ring III is *not* reduced, reaction of the 9-keto group should produce a considerable change in the spectrum. However, ethyl hypochlorophyllide would not react with piperidine under conditions where ethyl chlorophyllide was aminolyzed; neither would it react with $NaBH_4$ (in the presence of a small amount of phenol, to suppress base-catalyzed oxidation to mesochlorophyllide) while the mesochlorophyllide by-product present with it was reduced to the 9-hydroxy derivative. The 9-keto group does not appear to be enolized in hypochlorophylls, because addition of tetrabutylammonium hydroxide to pyrohypochlorophyll evokes no change in the spectrum. It is not clear whether the lack of reactivity of the 9-keto group is an argument in favor of structure III or structure IV, but it greatly hinders a decision between them.

Ethyl hypochlorophyllide does not appear immediately after onset of illumination, but there is an induction period during which the concentration of $ChlH_2$ builds up. The reaction probably begins with the reduction of $ChlH_2$ by the radical ChlH·, produced by reaction of the triplet excited state chlorophyllide with ascorbic acid. DABCO probably catalyzes redistribution of hydrogens in one of the products of this reduction. Adding DABCO to a solution of $ChlH_2$ in the dark only accelerates its reoxidation to chlorophyllide by available oxidants.

The hypochlorophyll reaction requires electron-attracting groups (vinyl and carbonyl) at the 2 and 6 positions, and is prevented by an electron-attracting group (formyl) at the 3 position. These groups apparently direct the relocation of hydrogen during the base-catalyzed isomerization. The presence of activated hydrogen at C_{10} is not required for the reaction.

The hypochlorophylls are a class of chlorophyll derivatives, the existence of which has not previously been reported. However, a product of reduction of chlorophyll by phenylhydrazine, with a band at 615 mμ in ether–isopentane–alcohol, may belong to this class.[18]

Hypochlorophyll and its derivatives, with what appear to be analogous derivatives of simple porphins, form a class of reduced porphyrins which we believe have the β positions of two adjacent pyrrole rings reduced. Because their spectral and chemical properties are distinct from those of bacteriochlorins having opposite pyrrole rings reduced, we propose that they be designated hypochlorins to emphasize their close spectral relationship with the chlorins from which they are derived by reduction.

Experimental Section

Materials. Chlorophyll a (Ia) and chlorophyll b (Ie) were prepared from spinach by the method of Zscheile and Comar;[19] ethyl chlorophyllide a (Ib) from *Datura stramonium* by the method of Holt and Jacobs.[20] Ascorbic acid was recrystallized from dioxane-water. Diazabicyclooctane, a gift of Houdry Process and Chemical Co., was used as received.

Chlorophyll derivatives were prepared from natural chlorophylls in the small quantities needed for each experiment. Reactions were usually followed spectrophotometrically and terminated when the desired product was in greatest concentration. The pigments were then isolated by extraction, but by-products were not separated chromatographically.

Pyrochlorophyll a and ethyl pyrochlorophyllide a were prepared by heating Ia and Ib in pyridine at 100° for at least 24 hr (the phase test was negative);[21] ethyl mesochlorophyllide a was prepared by H_2-Pd reduction of Ib in acetone;[22] Mg purpurin 7-lactone methyl ether ethyl methyl ester was obtained by treatment of Ib in methanol with $Mg(OCH_3)_2$;[11] chlorophyll b-3-methanol,

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(16) J. R. Barnard and L. M. Jackson, *J. Chem. Soc.*, 1172 (1956).

(17) M. Gouterman, G. H. Wagniere, and L. C. Snyder, *J. Mol. Spectry.*, **11**, 108 (1963).

(18) S. S. Brody, *J. Am. Chem. Soc.*, **82**, 1570 (1960).

(19) F. P. Zscheile and C. L. Comar, *Botan. Gaz.*, **102**, 463 (1941).

(20) A. S. Holt and E. E. Jacobs, *Am. J. Botany*, **41**, 710 (1954).

(21) F. C. Pennington, H. H. Strain, W. A. Svec, and J. J. Katz, *J. Am. Chem. Soc.*, **86**, 1418 (1964).

(22) H. Fischer and E. Lakatos, *Ann.*, **506**, 123 (1933).

Table II. Visible Spectra of Hypochlorophyll Derivatives. Wavelengths of Bands Ascribed to Hypochlorophylls, and Approximate Absorptivities Relative to Absorptivity of Band near 630 mμ

Derivative	Solvent		Long-wave system				Soret system		
Ethyl hypochlorophyllide	Pyridine–ethanol[a]	λ[d]	632	585	560	513	440	418	408
		ρ	1.00	0.37	0.25	0.12	1.27	1.97	1.86
Ethyl hypochlorophyllide	Pyridine	λ	633	585	560	514	437	418	408
		ρ	1.00	0.32	0.17	0.08	1.40	1.70	1.63
Ethyl hypochlorophyllide	Benzene	λ	632	584	560	510	440	415	408
		ρ	1.00	0.37	0.25	0.17	1.40	1.75	1.64
Ethyl hypochlorophyllide	Ether	λ	625	577	536	503	428	405	390
		ρ	1.00	0.30	0.11	0.10	1.55	1.41	1.59
Ethyl hypochlorophyllide	Ethanol	λ	625	579	535	500	...	405	393
		ρ	1.00	0.44	0.25	0.22		2.47	2.25
Hypochlorophyll	Pyridine–ethanol[a]	λ	632	583	560	515	440	416	407
		ρ	1.00	0.39	0.29	0.18	1.35	1.97	1.88
Ethyl pyrohypochlorophyllide	Pyridine–ethanol[a,b] (benzene)	λ	633	585	560	518	440	416	407
		ρ	1.00	0.40	0.29	0.17	1.64	2.39	2.19
Zinc hypopheophorbide	Pyridine–ethanol[a]	λ	625	578	538	503	433	408	394
		ρ	1.00	0.33	0.13	0.11	1.65	1.68	1.97
Hypochlorophyll b-3-methanol	Pyridine–ethanol[a]	λ	633	585	560	518	440	420	408
		ρ	1.00	0.36	0.23	0.13	1.74	2.14	1.82
Hypochlorophyll b-3-methanol	Ether	λ	626	578	535	504	428	405	392
		ρ	1.00	0.33	0.13	0.12	1.87	1.91	1.77
Mg purpurin 7-lactone methyl ether	Pyridine–ethanol[a,b] (benzene)	λ	621[c]	574	550	519	435	418	...
		ρ	1.00	0.36	0.22	0.20	2.45	3.15	...
Zn tetraphenylhypochlorin	Ethanol + pyridine	λ	606	564	525	498	416	407	387
		ρ	1.00	0.35	0.13	0.26	2.93	2.39	1.09
Zn hypochlorin	Ethanol	λ	590	548	513	480	398	383	365
		ρ	1.00	0.19	0.07	0.06	4.0	1.7	1.2

[a] *Ca.* 8% ethanol by volume. [b] Spectrum in benzene almost identical with that in pyridine-ethanol. [c] Shoulder at 640 in pyridine–ethanol, 635 in benzene, probably belonging to a by-product. [d] Wavelengths are given in millimicrons.

9-deoxo-9-hydroxychlorophyllide a (ethyl ester?), and 9-deoxo-9-hydroxychlorophyll b-3-methanol were prepared by $NaBH_4$ reduction in methanol;[10] Mg chlorin e_6 trimethyl ester was obtained by KOH hydrolysis of Ib in boiling methanol for 30 sec under N_2, followed by esterification with diazomethane;[23] Mg isochlorin e_4-6-carboxypiperidide methyl ester and Mg isochlorin e_4-6-carboxybutylamide phytyl methyl ester were prepared by reaction of Ib and Ia with piperidine and butylamine.[5]

Procedure. Except for the variations that have been noted, the hypochlorophyll reaction was run in pyridine containing *ca.* 8% ethanol by volume, 10^{-5} *M* chlorophyll, 10^{-2} *M* ascorbic acid, and 0.25–0.3 *M* DABCO, and flushed with purified N_2. The tube containing the reaction mixture was placed in a Cary Model 14 spectrophotometer, in a special holder through which the contents could be illuminated from the outside while the spectrum was recorded. With light from a 100-w projector bulb through a red filter (*e.g.*, Corning No. 2030, passing λ > 640), conversion to hypochlorophyl was complete within 1 hr. The yield was only about 50% at best. For reoxidation or other experiments, the hypochlorophyll was extracted into benzene or ether with phosphate buffer of pH 6 and washed four times.

Spectra. Positions and relative intensities of bands belonging to hypochlorophylls are compiled in Table II. The relative intensities are probably accurate to about 10% in the long-wave system and 20% in the Soret system, where variable background absorption made estimation less certain. The spectra are all rather similar, the largest variations being in the positions of the weak bands in the green and in the intensity of the first Soret band near 440 mμ, relative to the shorter wave bands. Spectra of Zn hypochlorin[1] and Zn tetraphenylhypochlorin[2] are included for comparison.

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