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GENERAL CONTENTS

CONTENTS TO VOLUME III

PHOTOCHEMICAL RING-OPENING IN 20-CHLORO-CHLOROPHYLLS

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1. INTRODUCTION

Chlorophyll (Chl) derivatives are readily and regiospecifically chlorinated at C-20, the free methine-bridge next to the reduced ring D (1-3). The interest in chlorinated chlorophylls has recently been renewed after their isolation from extracts of various green plants, algae and cyanobacteria (4-9) and a suggested relation to photosystem I of oxygenic photosynthesis (4, 5). This relation, and hence the involvement in photosynthesis, has been met with criticism and now been disproved (8, 10-12).

The status of 20-chloro-chlorophylls as natural pigments is still under debate (8, 12). During a systematic investigation of the chemistry of chlorinated chlorophylls, we have now observed a photochemical degradation of these pigments leading eventually to open chain tetrapyrroles, e.g. bile pigments. This raises the possibility that chlorinated chlorophylls may be components of plant tetrapyrrole degradative pathways, on which very little is known to date (13, 14).

2. MATERIALS AND METHODS

Pigments: 20-chloro-pheophorbides of the a-series have been synthesized by the method of (2) from the respective pheophorbides (6). Insertion of magnesium was done according to (15).

Photochemistry: A solution of the pigments in acetone (5-10μM) was irradiated with white light (150 W cold light source, 2 cm distance, ambient temperature). The reaction was followed spectrophotometrically. After conversion to the intermediate ($\lambda_{max} \approx 686$ nm), the solution was kept in the dark for 24 hrs and then worked up. Products were isolated by chromatography on silica gel.

^{1}*H-nmr spectra of the photoproduct:* A solution of 20-Cl-chlorophyll a (I) was irradiated in the nmr tube in acetone-d_6. Spectra were recorded at increasing irradiation times up to a total of 20 min.

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3. RESULTS

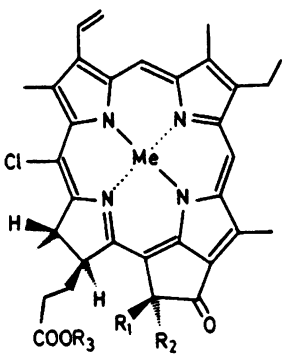
Irradiation of 20-chloro chlorophyll a in acetone results in the progressive formation of a red-shifted pigment. In the difference spectrum, pronounced extrema are observed at 432 and 667 nm (-) and 686 nm (+), with a small net increase in oscillator strength in the red spectral region, and a decrease in the Soret region. If the solution is kept in the dark afterwards, the red-shifted absorption of the photoproduct disappears, with a concomitant broad absorption increase in the 500-600 nm region. The oscillator strength of the red band (Q_Y) is decreased during this reaction. The spectroscopic changes upon treatment of other 20-chloro-chlorophylls were similar. By contrast, demetalated chlorinated pheophorbides, showed only a slow, irreversible bleaching.

Chromatography of the final product mixture yielded a blue pigment showing under neutral conditions a weak, broad absorption in the red spectral region, and more intense band around 320 nm, and no fluorescence. With acid, the red absorption band is reversibly increased, and shifted from 590 to 696 nm, this form is very weakly fluorescent. The pigment is unstable towards prolonged exposure to alcaline conditions.

The formation of the intermediate photoproduct was followed by *in situ* proton nmr spectroscopy. The most informative changes occured in the low-field region, where all signals of the photoproduct are shifted to higher fields.

4. DISCUSSION

Structure of products: From its absorption properties, the photoproduct still appears to be a cyclic-conjugated tetrapyrrole. The nmr spectrum indicates a reduced ring-current. Since only two methine signals appear in both the educt and the photoproduct, the C-20 position lacks a hydrogen substituent in the product, too. The spectra are compatible with a 20-oxonia-chlorophyll (17).



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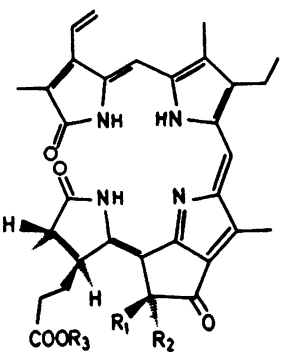

II

R_1 = H, R_2 = $COOCH_3$, R_3 = $C_{20}H_{39}$ (Phytyl)

The final product of the subsequent dark reaction is a bile pigment. The reversible changes upon protonation show the absence of a Mg-ligand, and the wavelength maxima are indicative of a dihydrobilindione (18). The spectral data are compatible with the bilin (II) obtained previously (19) in a non-photochemical reaction from chlorophyll. The ring-opening of oxa-porphyrins to bilins is well documented (20).

The only other photochemical reaction converting a chlorin to an open-chain tetrapyrrole, is the photooxygenation of the bacteriomethyl-pheophorbides *c* and *e*, which are methylated at C-20 (21,22). The common structural feature of the latter and the 20-chloro-chlorophylls is the presence of a bulky substitutent at the C-20 methine bridge, which may be responsible for the facilitated cleavage at this bridge. A noticeable difference is that in the 20-chlorinated pigments, the Mg-complexes are the reactive species, and the metal-free pheophorbides are unreactive; the situation is just opposite in the 20-methylated-pigments.

Significance to chlorophyll breakdown: Several pigments of the cyclic tetrapyrrole-type have been identified in ageing or darkened photosynthetic organisms, including chlorophyllides lacking the esterifying C-17^4 alcohol, pheophorbides lacking the central Mg, pyro-compounds lacking the 13^2 carbomethoxy-group, and pigments showing a combination of the above modifications (see 13, 14). It is unclear at present if any of them are intermediates of natural chlorophyll breakdown, e.g. in leaves before shedding in the fall. There is no chemical evidence which qualifies these pigments as intermediates preparing the macrocycle for degradation, because they are chemically or photochemically at least as stable as the parent chlorophylls, and are highly fluorescent and hence potential sensitizers for photodynamic damage.

Chlorinated chlorophylls would principally offer certain advantages. Their fluorescence yield is lower and we have now demonstrated their easy photodegradation. Chlorination is thus a chemically reasonable, preparatory step for further degradation, and chlorinating enzymes are well known from several sources (23). We, therefore, suggest to keep looking for such pigments *in situ* in spite of their negative correlation with PSI. Since chlorine-containing compounds are abundant in marine plants (24), chlorination may also be involved in the generation of bile pigments involved in bioluminescence (25).

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