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# **Advances in Photosynthesis Research**

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## **Volume II**

*edited by*

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## PREFACE

The Sixth International Congress on Photosynthesis took place from 1 to 6 August 1983, on the Campus of the "Vrije Universiteit Brussel", in Brussels, Belgium. These Proceedings contain most of the scientific contributions offered during the Congress.

The Brussels Congress was the largest thus far held in the series of International Congresses on Photosynthesis. It counted over 1100 active participants. The organizers tried to minimize the disadvantages of such a large size by making maximum use of the facilities available on a university campus. Most contributions were offered in the form of posters which were displayed in a substantial number of classrooms. The discussion sessions, twice a day, four or five in parallel, took place in lecture rooms in the very vicinity of these classrooms. In this way it was attempted to generate the atmosphere of a small meeting. The unity of the subject Photosynthesis was preserved in the ten plenary lectures, organised in such a way that a general overview of two diverse topics was given every day. In addition, there were the five times four parallel symposia dealing with some sixteen general topics.

Every editor of proceedings of a congress is faced with the problem of editing and arranging the contributions, a problem compounded by the wide diversity and the large number of the 753 manuscripts. This editor did very little in the way of editing the papers: all papers were prepared, camera-ready, by the authors themselves and there was no proof-reading. The main reason for this was the need to ensure speedy publication. The contributions are arranged in four volumes but the Proceedings form one set. Although some attempts were made to bring related topics together in one volume, the volumes I to IV should be seen as a succession of chapters, rather than as volumes in their own right. Thus, artificial and arbitrary subdivisions were avoided. A page limit was imposed in order to prevent oversized volumes.

The contributions are arranged in chapters which have no direct relation to the sessions or symposia in which they were presented. The sole criterium for putting a contribution into a certain chapter was its contents. The contributions offered during the Round Table Discussion on Light-Controlled Development of the Photosynthetic Apparatus, July 29 to 30, 1983 in Antwerp, are also included in these Proceedings. They comprise most of the contents of Chapter 7 of Volume IV.

## XXIV

The early publication date of these Proceedings could not have been realised without the efforts of, and the pleasant cooperation with, Mr. Ad Plaizier of Martinus Nijhoff Publishing House. Thanks are due to all Congress members, whose active participation made the Congress a success and these volumes an important document on the state of photosynthesis research. The very much needed assistance of the Local Organizing Committee is gratefully acknowledged. The Photosynthetic Community is indebted to the "Vrije Universiteit Brussel" for making available its premises, facilities and staff. Thanks are also due to the administrative staff of the Congress: secretaries, hostesses, technicians and the two diligent computer programmers, Mr. W. Dierickx and Mr. B. Philips. Special appreciation goes to Ms Blanche van den Haute for her dedicated work in the preparation and the management of the Congress and her help in editing these volumes.

Brussels, March 1984

C. Sybesma, Editor

STRUCTURE OF A CHLOROPHYLL-RC1

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INTRODUCTION

The primary donor in the reaction center of photosystem I (P 700) has a red-shifted absorption maximum as compared to the bulk of the antenna pigments. P700 is generally believed to be one or a pair of chlorophyll a molecule(s). The recent isolation of a new chlorophyll (chlorophyll-RC1) which has been related quantitatively to the content of P700 in a variety of preparations (1) has raised the possibility that a structurally different molecule may be responsible for this function. Chlorophyll-RC1 had originally been isolated from Scenedesmus, and subsequently also from a cyanobacterium and from spinach (2). Following a gift of a chlorophyll-RC1 sample from Scenedesmus to us by Doernemann and Senger, we have also isolated a similar pigment from a different cyanobacterium, Spirulina geitleri and converted it to its methylphosphoride(s). Here, we wish to report its molecular structure as 13<sup>2</sup>(R)-Hydroxy-20-chloro-17(S), 18(S)-Methylphosphoride a (structure 1), which corresponds structure 2 for chlorophyll-RC1.

Methylphosphoride-RC1 Preparation

Spirulina geitleri (100 gms) was extracted with cold methanol, and the extract demetalated with dilute hydrochloric acid under nitrogen. The crude pheophytins were chromatographed on silica 60 (Merck) with carbon tetrachloride/acetone = 96:4. The fractions containing pheophytin a were transesterified with methanol/sulfuric acid under nitrogen. The resulting methylphosphorides were chromatographed first on a silica 60 (Merck) column with methylene chloride/acetone = 96:4, then on silica (Merck) thin layer plates with carbon tetrachloride/acetone = 90:10, and finally by reverse-phase HPLC on Lichrosorb-RP 18 (Merck) with methanol. Two pure fractions (FI, FII) with essentially identical absorption spectra (Fig. 1) were obtained in a yield of 36 and 5 µg, respectively. Both are unstable in light and convert to two well defined products with higher and lower R<sub>f</sub> values, respectively, than the parent compound. The structural data given below were obtained with FI of methylphosphoride - RC1.

ABSORPTION AND CIRCULAR DICHROISM

The absorption spectrum of methylphosphoride -RC1 (FI) is red-shifted with respect to that of methylphosphoride a. The only other significant difference is the inverted intensity ratio of the two minor absorptions in the wavelength range between 500 and 550 nm. The only group of chlorophyllous pigments showing these spectral features all have a substituent at the

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C-20 methine bridge. The Cd-spectrum of FI is red-shifted as well but otherwise similar to that of methylpheophorbide a (structure 3) with respect to the sign of the major bands (fig. 2). This indicates the same absolute S-configuration at the asymmetric centers, C-17 and C-18. Also present is the intense negative band characteristic for the  $13^2$  - carbomethoxy group in the  $13^2$  (R) configuration (3). A further significant difference is the relatively large ellipticity of the red as compared to the Soret-band, which is typical for steric hindrance due to methine substituents (3).

#### MASS SPECTRA

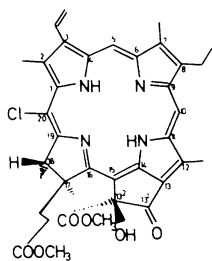
The mass spectrum of methylpheophorbide -RC1 (FI) has a molecular ion at 656 mass units. This corresponds to an increase of 50 mass units as compared to the molecular ion of methylpheophorbide a. The fragmentation of the two pigments in the electron impact spectrum is similar. The (M+2) ion is relatively large. Since hydrogenation-dehydrogenation processes are well known for tetrapyrrole mass spectra (4), a field-desorption spectrum was taken of the same pigments. The molecular ion of FI at 656 as well as the intense ion at 658 mass units are observed in these spectra, too (fig. 3). The observed peak pattern fits well to the structural formula  $C_{36}H_{37}N_4O_6Cl$ . (0.7 % error), corresponding to the addition of one atom of oxygen and one atom of chlorine to methylpheophorbide a.

#### PROTON NMR SPECTRA

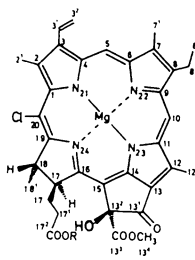
The proton nmr spectrum of methylpheophorbide -RC1 (FI) was also quite similar to that of methylpheophorbide a (fig. 4). There is, however, no signal in the range of  $\delta = 8-9$  ppm, where the C-20 proton of pheophorbides is generally observed (5). Since there is no additional signal in the low field range, there must be an nmr inactive substituent at C-20. This is supported by smaller but distinct shifts of one aromatic methyl signal (probably C-2) and the signals of the C-18 substituents, which are neighbors to C-20. The only other distinct difference is the high field shift of the singlet assigned to the proton at C-13<sup>2</sup>. A similar shift has been reported for an allomer of bacteriochlorophyll a bearing a hydroxy group rather than a proton at the  $13^2$  position (6).

#### DISCUSSION

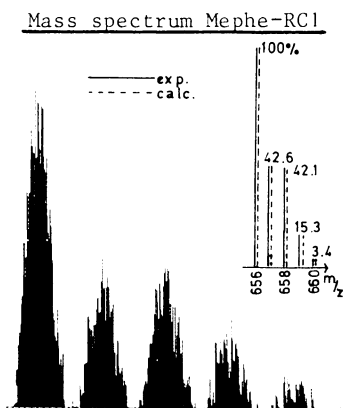
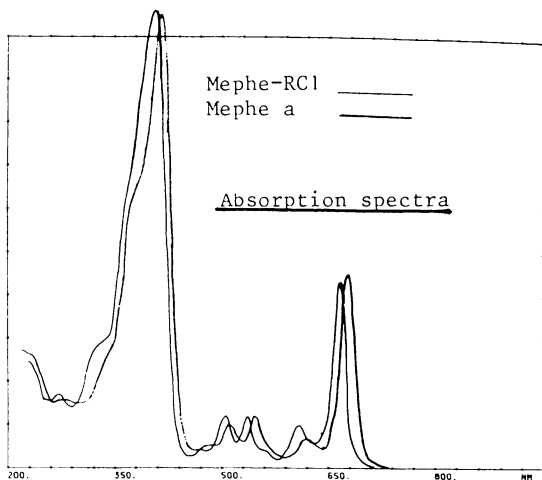
Based on the spectroscopic data presented above, the methylpheophorbide -RC1 (FI) is  $13^2$ -hydroxy-20-chloro-17(S), 18(S) methylpheophorbide a (structure 1), which corresponds to structure 2 for chlorophyll -RC1. Absorption and circular dichroism reflect the new substituent at C-20, which is proved by the mass spectra to be a chlorine atom. Sign and magnitude of the nmr shifts in the neighborhood of C-20 are comparable to those reported by Hynninen et al. for 20-chloro-methylpheophorbide a (7). The heavy-atom effect of the chlorine would also explain the decreased fluorescence observed by Doernemann and Senger for chlorophyll -RC1 (2) and by us for its methylpheophor-



1: METHYLPEOPHORBIIDE - RC1 (F1)

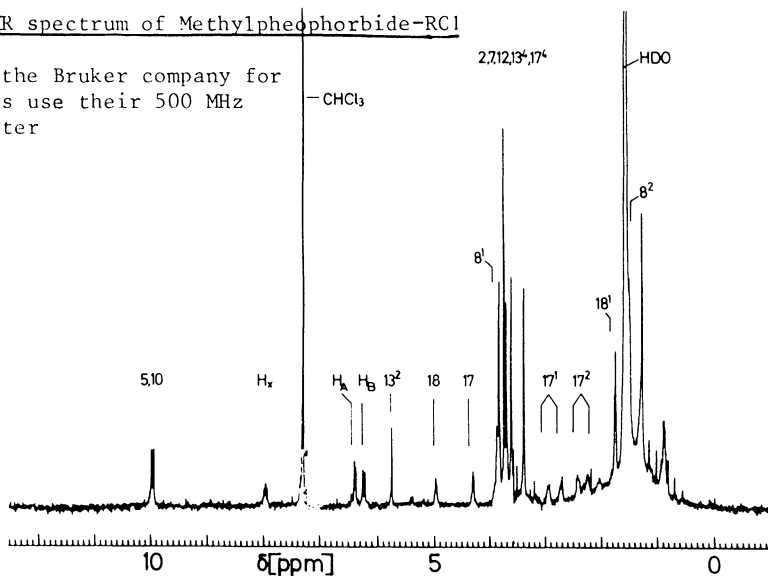


2: CHLOROPHYLL - RC1 (F1)



Proton NMR spectrum of Methylpheophorbide-RC1

We thank the Bruker company for letting us use their 500 MHz spectrometer



bide. The oxygen must then be located at the isocyclic ring, most likely as a hydroxy-substituent at C-13<sup>2</sup>. An exchange of the two groups (OH at C-20, Cl at C-13<sup>2</sup>) is unlikely, since meso-hydroxyporphyrins are generally present as the oxophlorin-isomers which have quite different absorption and NMR spectra (8). This structure would also explain the second pigment (FII) with an absorption similar to that of FI as the 13<sup>2</sup> (S)-isomer.

Both the substitution with chlorine at C-20 and the oxidation at C-13<sup>2</sup> are well known artifacts in chlorophyll chemistry (6, 7,9). The introduction of a chlorine atom during the conversion of chlorophyll -RC1 to its methylpheophorbide can be ruled out, because the product was obtained as well when any chlorine containing chemicals (chlorinated hydrocarbons, hydrochloric acid, sodium chloride, etc) were omitted during the entire procedure. It is thus very likely that this substituent is already present in chlorophyll -RC1 in vivo and is responsible for the red-shifted absorption. It should be noted, however, that chloride ions are present in the photosynthetic membrane. Oxidation at C-13<sup>2</sup> is principally possible, too, but unlikely with the precautions taken by us. The <sup>252</sup>Cf plasma desorption mass spectrum of chlorophyll -RC1 (2) has a molecular ion indicative of one additional oxygen being present together with one chlorine atom already in the original pigment, too.

The structure 2 suggested for chlorophyll -RC1 is not readily compatible with its function as the primary donor of photosystem 1 (e.g. chemical reactivity, redox behavior (2)). There are at least two possible explanations for this apparent contradiction: firstly, this pigment could have another function e.g. as an electron acceptor or a special antenna pigment. Since all P700 preparations contain rather large amounts of additional (antenna ?) chlorophylls, this possibility cannot be ruled out. Secondly, chlorophyll -RC1 may indeed be an artifact produced from an unusually reactive chlorophyll (a) species (e.g. P700), which is formed even during the extraction under very mild conditions (cold methanol, nitrogen atmosphere). Since the primary photo-reactants are expected to show an unusual chemistry, this possibility cannot be ruled out either. Further work is now in progress to try and clarify these questions.

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