Pergamon

Tetrahedron Letters 39 (1998) 9813–9814

TETRAHEDRON
LETTERS

An Unexpected Allomer of Chlorophyll: $13^2(S)$-Hydroxy-10-Methoxychlorophyll *b*

Kristiina Hyvärinen,[1] Juho Helaja[1,2] and Paavo H. Hynninen[1]*

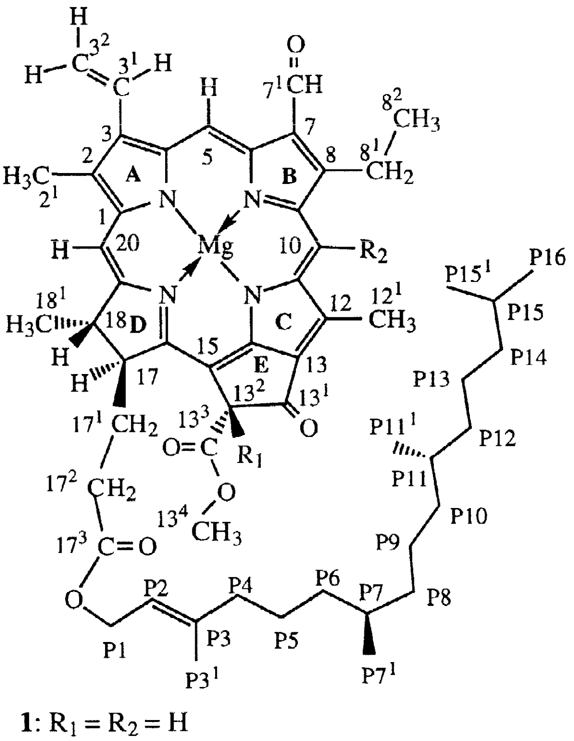
[1]Department of Chemistry, PO Box 55, University of Helsinki, FIN-00014 Helsinki, Finland
[2]Institute of Biotechnology, PO Box 45, University of Helsinki, FIN-00014 Helsinki, Finland

Received 28 September 1998; accepted 12 October 1998

Abstract: The allomerization of chlorophyll *b* in methanol produced $13^2(S)$-hydroxy-10-methoxychlorophyll *b* in a yield of *ca.* 8 %. The formation of this allomer was totally unexpected, as 10-substituted chlorophylls have never been reported before. The structure of the new chlorophyll *b* derivative was determined on the basis of UV/Vis, FAB-MS, ^1H NMR and 2D ROESY NMR spectra. This letter focuses on the NMR analysis.

Keywords: Chlorophyll; Radicals and radical reactions; NMR

The Willstätter's allomerization[1] (oxidation of chlorophyll by molecular triplet oxygen in alcoholic solution) occurs to all chlorophylls (Chls), which have an intact β-ketoester structure at the isocyclic ring. The methanolic allomerization of Chl *b* (**1**) produced a new Chl derivative (yield *ca.* 8 %[2]), which did not have an analog among the Chl allomers described in the literature.[3]



1: $R_1 = R_2 = H$

2: $R_1 = OH$, $R_2 = OCH_3\ (10^1)$

The most prominent differences in the ^1H NMR spectrum of the new derivative (Table 1),[4] as compared with the Chl *b* spectrum,[5] were the presence of two extra singlets (at δ 6.18 and 4.52), as well as the absence of the 13^2-C<u>H</u> and one methine bridge proton signals. On the basis of the correlations (Table 1) seen in the 2D ROESY spectrum of the new derivative,[4] the two methine bridge proton signals in the ^1H NMR spectrum were unambiguously assigned to 5-C<u>H</u> and 20-C<u>H</u> and, hence, it was the 10-C<u>H</u> signal that was absent. The singlet at δ 4.52 was assigned to 10^1-C$\underline{H}_3$ on the basis of the chemical shift, the 3H integral and the ROE correlations. The one-proton singlet at δ 6.18 was ascribed to C-13^2 O<u>H</u>, because the corresponding signal is at δ 6.14 in the ^1H NMR spectrum of 13^2-hydroxychlorophyll *b*.[6] This assignment was confirmed by the ROE correlations. The assignment of the other signals in the ^1H NMR spectrum of the new derivative was straightforward on the basis of the ROE correlations and the comparison with the Chl *b* ^1H NMR spectrum. The ROE correlation between 13^4-C$\underline{H}_3$ and 18^1-

0040-4039/98/$ - see front matter © 1998 Elsevier Science Ltd. All rights reserved.
PII: S0040-4039(98)02180-7

C$\underline{H}_3$ indicated that the absolute configuration at C-13^2 was *S*.[4,7] The NMR results established 13^2(*S*)-hydroxy-10-methoxychlorophyll *b* (**2**) as the structure of the new derivative. The FAB-MS and UV/Vis results[2] provided additional evidence for the structure.

The formation of 13^2(*S*)-HO-10-MeO-Chl *b* was totally unexpected, because there are no publications dealing with C-10 substituted Chls. Further research is needed for understanding how the formation of 13^2(*S*)-HO-10-MeO-Chl *b* adjusts the free radical allomerization mechanism proposed before.[3a,3d]

Table 1. The ^{1}H assignments for 13^2(*S*)-hydroxy-10-methoxychlorophyll *b* in acetone-d_6.

Proton	Chemical shift, δ^{TMS} (ppm)	Multiplicity, $J_{H\text{-}H}$ (Hz)	Protons showing ROE correlation
7^1-CH	11.22	s	5, 8^1, 8^2
5-CH	10.07	s	3^1, 3^2, 7^1
20-CH	8.20	s	2^1, 18, 18^1
3^1-CH (H$_x$)	7.90	dd $^3J_{cis}$ 11.5, $^3J_{trans}$ 17.8	2^1, 3^2, 5
3^2-CH$_2$ (H$_B$, *trans*)	6.24	dd $^2J_{gem}$ 1.5, $^3J_{trans}$ 17.8	2^1, 3^1, 5
3^2-CH$_2$ (H$_A$, *cis*)	5.99	dd $^2J_{gem}$ 1.5, $^3J_{cis}$ 11.5	2^1, 3^1, 5
13^2-COH	6.18	s	17^1/17^2, P2
P2-CH	5.16	't' $^3J_{P1(H,H')\text{-}P2}$ 7.3	13^2, P1, P4
10^1-CH$_3$	4.52	s	8^1, 8^2, 12^1
P1-CH$_2$	4.46	m $^3J_{P1(H,H')\text{-}P2}$ 7.3	P2, P3^1, P4
18-CH	4.32	qd $^3J_{18\text{-}18^1}$ 7.3, $^3J_{17\text{-}18} < 1$	17, 17^1/17^2, 18^1, 20
8^1-CH$_2$	4.20	q $^3J_{8^1\text{-}8^2}$ 7.5	7^1, 8^2, 10^1
17-CH	3.93	m $^3J_{17\text{-}18} < 1$	17^1/17^2, 18, 18^1
13^4-CH$_3$	3.60*	s	18^1
12^1-CH$_3$	3.59*	s	10^1
2^1-CH$_3$	3.18	s	3^1, 3^2, 20
17^1-CH$_2$,17^2-CH$_2$	2.71-2.32	m	17, 18, 13^2
P4-CH$_2$	1.91	m	P1, P2
8^2-CH$_3$	1.68	t $^3J_{8^1\text{-}8^2}$ 7.5	7^1, 8^1, 10^1
P3^1-CH$_3$	1.59	s	P1
18^1-CH$_3$	1.51	d $^3J_{18\text{-}18^1}$ 7.3	13^4, 17, 18, 20

*The assignments of 13^4-CH$_3$ and 12^1-CH$_3$ are interchangeable.

REFERENCES AND NOTES

1. Willstätter, R.; Stoll, A. *Untersuchungen über Chlorophyll*; Springer: Berlin, 1913; pp. 29 and 147.
2. Hyvärinen, K.; Hynninen, P. H. *J. Chromatogr. A*, submitted for publication.
3. (a) Hynninen, P. H. Chemistry of Chlorophylls: Modifications. In *Chlorophylls*; Scheer, H. Ed.; CRC Press, Boca Raton: FL, 1991; pp. 145-209 and the references therein. (b) Kuronen, P.; Hyvärinen, K.; Kilpeläinen, I.; Hynninen, P. H. *J. Chromatogr. A* **1993**, 654, 93-104. (c) Minguez-Mosquera, M. I.; Gandul-Rojas, B. *J. Chromatogr. A* **1995**, 690, 161-176. (d) Woolley, P. S.; Moir, A. J.; Hester, R. E.; Keely, B. J. *J. Chem. Soc., Perkin Trans. 2* **1998**, 1833-1839.
4. The NMR sample was prepared by dissolving 2.7 mg of 13^2(*S*)-HO-10-MeO-Chl *b* in 0.6 ml acetone-d_6 (Fluka, 99.95 %). The ^{1}H NMR and 2D ROESY spectra were recorded as described in Hyvärinen, K.; Helaja, J.; Kuronen, P.; Kilpeläinen; I.; Hynninen P.H. *Magn. Reson. Chem.* **1995**, 33, 646-656, except that the temperature was 285 K (12°C).
5. (a) Sanders, J. K. M.; Waterton, J. C.; Denniss, I. S. *J. Chem. Soc. Perkin I* **1978**, 1150-1157. (b) Hynninen, P. H.; Lötjönen, S. *Synthesis* **1983**, 705-708.
6. Hyvärinen, K.; Helaja, J.; Hynninen, P. H., unpublished data.
7. Helaja, J.; Hyvärinen, K.; Heikkinen, S.; Kilpeläinen, I.; Hynninen, P. H. *J. Mol. Struct.* **1995**, 354, 71-79.