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Tetrahedron Letters
Tetrahedron Letters 46 (2005) 1145–1147

Electronic structure of the enolate anion of chlorophyll *b*

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Received 29 November 2004; accepted 16 December 2004
Available online 8 January 2005

Abstract—The enolate anion of chlorophyll *b* (Chl *b*) has been synthesized under deoxygenated conditions and its electronic structure characterized for the first time by ^{1}H NMR and electronic absorption spectroscopy. The formation of the enolate anion caused a marked perturbation to the 18 π-electron [18]diazaannulene aromatic pathway of Chl *b*. This perturbation appeared as noticeable upfield shifts, exceeding 1 ppm, for the *meso*-CH protons of the Chl *b* enolate anion. Nevertheless, the enolate anion remained diatropic, maintaining aromaticity in its delocalized macrocycle.

The isocyclic ring, also called exocyclic ring or cyclopentanone ring, E of all chlorophylls (except the chlorosome chlorophylls[1]) is a substituted β-keto ester and therefore has a strong aptitude to enolize (Scheme 1).[2,3] The enol derivatives of chlorophylls, such as the free enol, enolate anion, enol ether and enol ester, are of considerable interest for several reasons. Firstly, the enol derivatives are important in relation to the theory of electron delocalization and aromaticity/antiaromaticity in large macrocyclic π-systems.[4] Secondly, the enolate anion of Chl is the first intermediate in the Willstätter allomerization reaction of Chl, which results in several oxidized Chl derivatives, called allomers.[2,3,5–9] Thirdly, it has been suggested that the enol derivatives of Chl may be involved in the primary events of photosynthetic reaction centres.[1,10–12]

The ^{1}H NMR chemical shifts are widely regarded as a sensitive criterion for the aromaticity or antiaromaticity of organic π-systems.[13] In our previous paper, we described the ^{1}H NMR spectrum and chemical shift differences (enolization increments) for the enolate anion of Chl *a* (**3**).[12] The macrocyclic *meso*-CH protons of Chl *a* (**1**) were found to experience ca. 1 ppm upfield shifts on enolization. These upfield shifts were interpreted in terms of two parallel effects: (1) electron density is transferred from the isocyclic ring E to the macrocycle, implying increased shielding; (2) the macrocyclic ring current is reduced. The other chemical shift changes were attributed to conformational alterations, induced by the enolization.

As none of the ^{1}H signals exhibited extensive line broadening, the diradical hypothesis of Weller[14] was ruled out in the ground state of the Chl *a* enolate anion. However, the situation might be different for the enolate anion of Chl *b* (**4**), because its strongly electron withdrawing C-7 formyl group induces differences in the electron densities and chemical reactivity of the molecule as compared with Chl *a*. Our observations have verified that Chl *b* (**2**) clearly behaves differently from Chl *a* (**1**) in the Willstätter allomerization reaction, which produced from Chl *b* an entirely new type of allomer, the 13^2(*S*)-hydroxy-10-methoxy-Chl *b*.[7] A bimolecular aromatic radical substitution (S_R 2 Ar), involving the 13^2-substituted 11-radical intermediate, was proposed as the mechanism of formation of this allomer.[8,9]

To learn about the electronic structure of the Chl *b* enolate anion (**4**), we prepared it under deoxygenated conditions comparable to those previously used for the preparation of the Chl *a* enolate anion (**3**).[12] After the addition of the HO^- base to the deoxygenated pyridine solution of Chl *b*,[15] the red Chl *b* enolate anion was

Keywords: Electron delocalization; Aromaticity; Chlorophyll enolization; Photosynthesis; NMR; Spectroscopy.
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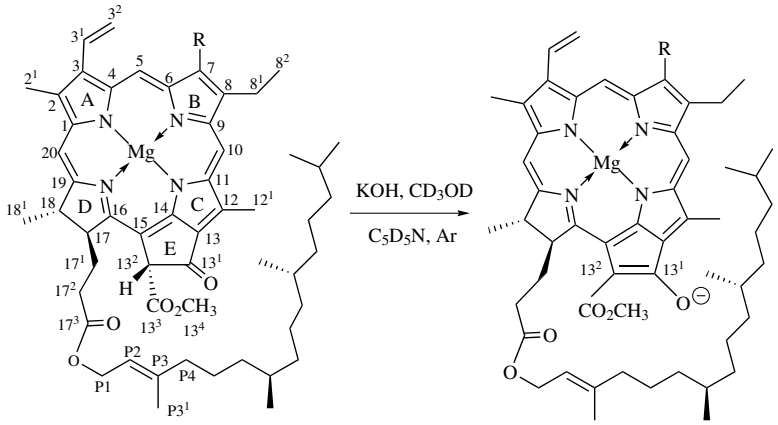
doi:10.1016/j.tetlet.2004.12.075



1 R = CH_3, Chl *a*
2 R = CHO, Chl *b*

3 R = CH_3, Chl *a* enolate anion
4 R = CHO, Chl *b* enolate anion

Scheme 1. Base promoted conversion of Chl **1** or **2** to the Chl enolate anion **3** or **4**.

formed immediately. It was stable long enough to enable the measurement of its ^{1}H NMR spectrum with clearly assignable signals. As can be seen from Table 1, the ^{1}H NMR chemical shifts of the Chl *b* enolate anion resemble those of the Chl *a* enolate anion, except that the 7^1-formyl proton resonance at δ 11.20 of the former replaces the 7^1-methyl proton resonance at δ 2.87 of the latter. Another difference between the two spectra is that the Chl *b* enolate anion shows more prominent chemical shift changes (upfield shifts) for the *meso*-CH protons: $\Delta\delta_H = -1.18$, -1.25 and -1.07 for the 5-, 10- and 20-CH protons, respectively. These $\Delta\delta_H$ values of the Chl *b* enolate ion (**4**) are ca. 0.2 ppm more upfield shifted than the corresponding values for the Chl *a* enolate ion (**3**). This result indicates that the formyl group has an additional weakening effect on the macrocyclic ring current. We find such an effect of the formyl group unexpected, considering the electron withdrawing property of the formyl group, which should cause deshielding, not shielding, for the 5-CH, 10-CH and 8^1-CH_2 protons. However, in the present case, the conjugated π-system of the enolate anion **4** mediates the electron withdrawing effect of the formyl group down to the isocyclic ring E, from which electron density is transferred over the delocalized π-system.

Interestingly, the 8^1-CH_2 protons of the Chl *b* enolate anion appeared as a deceptively simple quartet[16,17] showing further splitting, which we interpret as an indication of the slow rotation of the group due to its bonding to the sp^2-hybridized C-8 and the possible steric hindrance exerted by the vicinal formyl group. The observed chemical shift changes for the 13^4-CH_3, 17-CH, 18^1-CH_3 and 18-CH protons of the Chl *b* enolate ion are, $\Delta\delta_H = 0.07$, 0.48, 0.15 and -0.78, respectively (Table 1). These $\Delta\delta_H$ values can be attributed to the

Table 1. ^{1}H NMR chemical shifts (δ_H, ppm, relative to Me_4Si in pyridine-d_5) for Chl *a*, Chl *a* enolate anion, Chl *b* and Chl *b* enolate anion

Atom no.	$\delta_{H,\,Chl\,a}$ [a]	$\delta_{H,\,Chl\,a\ enolate}$ [a]	$\Delta\delta_H$ [c]	$\delta_{H,\,Chl\,b}$ [b]	$\delta_{H,\,Chl\,b\ enolate}$ [b]	$\Delta\delta_H$ [c]
2^1	3.28	3.02	−0.26	3.24	2.90	−0.34
$3^1(H_X)$	8.23	7.94	−0.29	8.20	7.84	−0.36
$3^2(H_{cis})$	5.96	5.75	−0.21	6.01	5.77	−0.24
$3^2(H_{trans})$	6.27	6.06	−0.21	6.45	6.18	−0.27
5	9.70	8.78	−0.92	10.82	9.64	−1.18
7^1	3.18	2.87	−0.31	11.54	11.20	−0.34
8^1	3.67	3.34	−0.33	4.16	3.75	−0.41
8^2	1.56	1.41	−0.15	1.73	1.55	−0.15
10	9.85	8.79	−1.06	10.00	8.75	−1.25
12^1	3.62	3.20	−0.42	3.63	3.15	−0.48
13^2	6.64	—	—	6.65	—	—
13^4	3.72	3.82	0.10	3.85	3.92	0.07
17	4.29	4.87	0.58	4.33	4.81	0.48
17^1, 17^2	2.78–2.07	2.42–2.17	−0.36–0.10	2.85–2.20	2.52–1.93	−0.33–0.27
18	4.43	3.75	−0.68	4.46	3.68	−0.78
18^1	1.50	1.71	0.21	1.58	1.73	0.15
20	8.52	7.68	−0.84	8.46	7.39	−1.07
P1	4.67	4.39	−0.28	4.79	4.48	−0.31
P2	5.36	5.68	0.32	5.48	5.77	0.29
$P3^1$	1.56	1.58	0.02	1.68	1.68	0.00
P4	1.86	1.94	0.08	1.97	2.04	0.07

[a] Values taken from Ref. 12.
[b] Values measured on a Bruker Avance spectrometer, ν (^{1}H) = 500 MHz.
[c] $\Delta\delta_H = \delta_H$(enolate) − δ_H(Chl).

conformational alterations occurring in rings E and D on enolization. In the enolate, the exocyclic ring E and its 13^2-methoxycarbonyl group become more co-planar with the delocalized macrocycle. In consequence, steric crowding and strain are increased in the lower periphery of the molecule. This steric strain causes conformational alterations in ring D, that is, the 17-C is lifted above the average macrocyclic plane to such an extent that its proton becomes located inside the deshielding cone of the double bond between carbons 13^2 and 13^1 in the exocyclic ring E of the enolate ion. Another consequence from the steric crowding in the lower periphery of the molecule seems to be that the orientation of the 17-propionate phytyl ester group changes in such a fashion that the C-18 proton becomes located inside the shielding region of the 17^3-C=O group.

Electronic absorption spectroscopy (UV–vis) is another sensitive method to study electronic states of organic molecules, that contain many π-electrons, enabling low-energy π–π* transitions. In its pure form, the enolate anion of Chl *b* exhibits the colour of red wine and an electronic absorption spectrum with marked changes in the locations and intensities of the absorption bands as compared with the spectrum of Chl *b* (Fig. 1; see also Fig. 2 in Ref. 2). The intensity of the Soret band (λ_{max} at 472.5 nm for Chl *b* and at 448.5 nm for its enolate ion) is markedly reduced and a relatively intense, broad band appears between 500 and 600 nm. In addition, the chlorin band (λ_{max} at 655.5 nm for Chl *b*) seems to vanish completely. How can we explain these striking differences between the electronic absorption spectra of Chl *b* and its enolate anion in terms of electronic structure?

The correct answer to this question was given long ago by Kuhn et al.[18] (see also Seely[2]), who ascribed the formation of the red colour of the Chl phase-test intermediate to the delocalization of the negative charge of the enolate ion into the macrocyclic π-system. We have to envisage the Chl enolate anion as a resonance hybrid, for which we can write the 13^2-carbanion structure and the 13^1-oxide anion structure as the main limiting structures.[3,8,9] Though these are the principal contributors to the electronic structure of the Chl enolate ion, comparable with those usually written for organic enolate ions, they are not adequate to describe the precise electronic structure of the Chl enolate ion, because the negative charge is partially delocalized over the macrocyclic π-system. Therefore, in addition to those two, we should write many other limiting resonance structures to the Chl enolate ion. The structure with a negative charge on the nitrogen atom of the 'pyrrolenine' ring B was considered by Seely[2] to have a substantial contribution in the resonance hybrid on the basis of Woodward's assumption that the pyrrolenine ring B tends to attract electrons more strongly than the sub-rings A, C or D.[19] This structure shows an interrupted delocalized π-system, which seems to explain the more profound alterations in the electronic spectrum of the enolate ion.

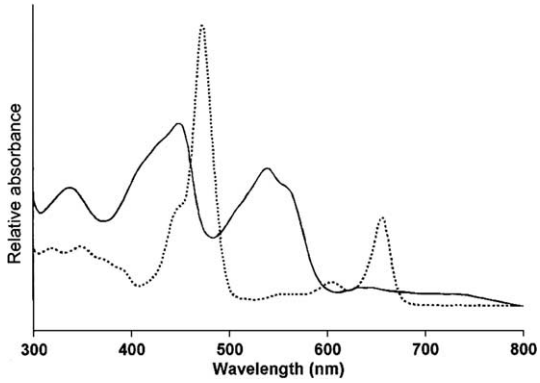


Figure 1. Electronic absorption spectra of Chl *b*, **2** (···) and its enolate anion, **4** (—) in pyridine. An amount of 0.5 mg of potassium *tert*-butoxide was added to a pyridine solution (3.0 mL) of Chl *b*, $c = 1.6 \times 10^{-5}$ mol L^{-1}, in a quartz cuvette (light path = 10 mm). The solution was deoxygenated by bubbling argon gas through it before and during the addition of the base and the cuvette was closed air-tight with a Teflon stopper.

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