

Synthesis and Vibrational Spectroscopic Study of Selectively $3^1\text{-}^{18}\text{O}$ -Labelled Chlorophyll Derivatives

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Regioselective $3^1\text{-}^{18}\text{O}$ -labelling of chlorophyll derivatives possessing a 3-formyl group such as methyl (pyro) pheophorbide-*d* (**3**, **4**) was carried out efficiently by a simple one-step procedure; by stirring a homogeneous solution of tetrahydrofuran and $\text{H}_2\text{ }^{18}\text{O}$ containing a small amount of trifluoroacetic acid.

Key words: carbonyl group, chlorophyll, IR spectra, isotope label, oxygen-18, regioselective synthesis

INTRODUCTION

Chlorophylls can play various roles in photosynthetic processes and their properties are regulated by local and specific environments [1,2]. The 3^1-oxo group of these chlorophylls are bonded with any other functional groups in natural photosynthetic apparatus and the interaction has been analyzed by vibrational spectroscopies [3]. Therefore, our attention is focused on facile, efficient and specific ^{18}O -labelling of chlorophyll derivatives by organic synthesis. In this work, we report efficient and regioselective ^{18}O -labelling at the 3-CHO of chlorophylls as well as their vibrational spectra.

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MATERIALS AND METHODS

Chlorophyll derivatives such as methyl pyropheophorbide-*a* (**1**) [4], methyl pheophorbide-*a* (**3**) [5], methyl pyropheophorbide-*d* (**4**) [6], methyl pheophorbide-*d* (**5**) [5] (see Figure.1) were prepared, as described previously, from chlorophyll-*a*.

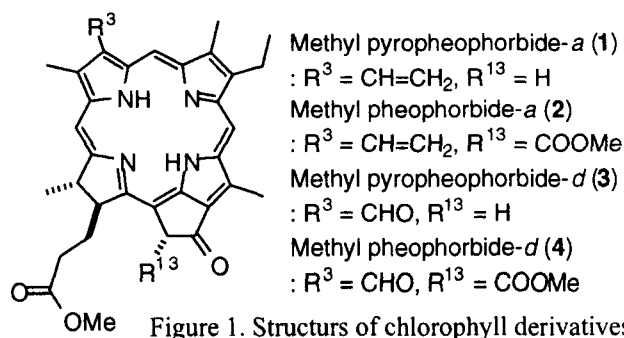


Figure 1. Structures of chlorophyll derivatives

Typical biphasic procedures for ^{18}O -labelling method of chlorophyll derivatives are as follows: trifluoroacetic acid (TFA, 8 μL) and H_2^{18}O (95% ^{18}O atom, 0.1 mL) was added to a distilled CH_2Cl_2 solution (2 mL) of **1** (14 mg). The mixture in a sealed vial was stirred at room temperature for two days. After opening the vial, the reaction mixture was worked up by aqueous 4% NaHCO_3 solution and water, and was purified by flash column chromatography (FCC). The main fraction was recrystallized from CH_2Cl_2 and MeOH to give a 1:4 mixture of **1** and $13^1\text{-}^{18}\text{O-1}$ as a black solid (13 mg, > 90% yield), which was characterized by $^1\text{H-NMR}$, IR and FAB-MS spectra. $^1\text{H-NMR}$ data of the ^{18}O -labelled $13^1\text{-}^{18}\text{O-1}$ showed the same data of the corresponding unlabelled **1**.

Regioselective $3^1\text{-}^{18}\text{O}$ -oxo-labelling was carried out in a homogeneous solution. For example, stirring a THF (2 mL)- H_2^{18}O (0.1 mL) solution of **3** (19.5 mg) and TFA (0.8 μL) for 18 h gave solids after FCC and recrystallization from CH_2Cl_2 and hexane. $^1\text{H-NMR}$ data of the obtained ^{18}O -labelled products showed the same data of the corresponding unlabelled substrate. The products were mainly $3^1\text{-}^{18}\text{O-3}$ and also contained 3^1- and $13^1\text{-doubly}^{18}\text{O}$ -compound and non-labelled compound.

RESULTS AND DISCUSSION

Keto carbonyl oxygens of chlorophyll derivatives reacted with H_2^{18}O under acidic conditions to give the corresponding ^{18}O -labelled compounds. Typically, 3-vinyl-chlorin **1** was examined to give $13^1\text{-}^{18}\text{O}$ -oxo-labelled

$13^1\text{-}^{18}\text{O-1}$ by stirring a biphasic solution (CH_2Cl_2 and acidic H_2^{18}O). The IR spectra of the resulting product and the starting material **1** showed that stretching vibrational band of the 13-carbonyl group of $13^1\text{-}^{18}\text{O-1}$ was down-shifted about 30 cm^{-1} from that of the $13^1\text{-}^{16}\text{O-1}$. The main component of the product was selectively ^{18}O -labelled at the 13-keto group as in $13^1\text{-}^{18}\text{O-1}$ and non-labelled **1** still remained about 20% in the product. Both of 3^1- and $13^1\text{-}^{18}\text{O}$ -oxo-labelling of chlorophyll derivatives were achieved by the above procedures; for example, **3** possessing 3-CHO and 13-CO was doubly labelled.

Then we applied the direct and regioselective ^{18}O -exchange of the 3^1-oxo group in **3** by stirring a homogeneous solution (THF and weak acidic H_2^{18}O). Such mild acidic conditions preferred regioselectively ^{18}O -exchange at the 3-CHO to that at the 13-CO of **3**. The observation is reasonable because the 3-CHO is more reactive than the 13-CO: the regioselective reduction of 3-CHO over 13-CO has been reported [6]. The above reaction gave the products containing mainly $3^1\text{-}^{18}\text{O}$ -oxo-labelled $3^1\text{-}^{18}\text{O-3}$. The stretching vibrational band of the $3^1\text{-}^{18}\text{O}$ -labelled carbonyl group moved to about 30 cm^{-1} lower wavenumber and was well-separated from the unlabelled 13-CO band.

Next we try to prepare $3^1\text{-}^{18}\text{O}$ -labelled **4** possessing a methoxycarbonyl group at the 13^2 -position (see Figure 1). Under the same conditions described above (biphasic and homogeneous), $13^1\text{-oxo-}^{18}\text{O}$ -labelling of methyl pheophorbide-*a* (**2**) was difficult probably due to steric hindrance of the neighboring 13^2 -methoxycarbonyl group. Therefore, the 3-formyl group of **4** was regioselectively

¹⁸O-labelled to give 3¹⁻¹⁸O-oxo-labelled 3¹⁻¹⁸O-4 containing a small amount of non-labelled 4 and a trace amount of 3¹⁻¹³O-4 by both the above acidic conditions.

The present ¹⁸O-labelling procedures are useful for other natural chlorophylls including chlorophyll-*b* possessing 7(3)-CHO and 13²-COOMe and bacteriochlorophyll-*e* possessing 7-CHO and lacking 13²-COOMe.

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