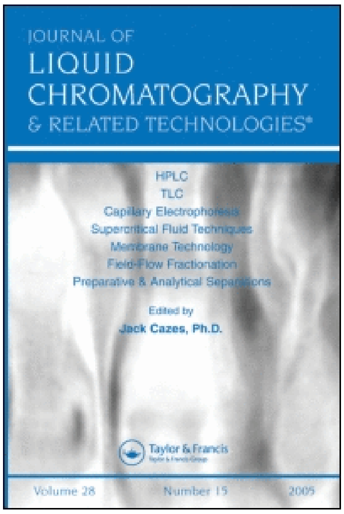

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HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC ISOLATION AND CHARACTERIZATION OF (PHEOPHYTIN)MERCURY(II)

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ABSTRACT

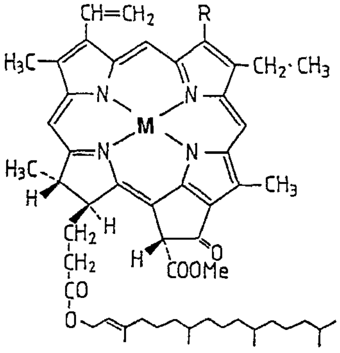
Mercury-substituted chlorophylls, i.e., (pheophytin *a*)mercury(II) and (pheophytin *b*)mercury(II), were separated by reversed-phase HPLC with a C_{18}-bonded silica column and a mobile phase of acetone-methanol. Their spectroscopic and chromatographic characteristics strongly suggest that both (pheophytin *a*)mercury(II) and (pheophytin *b*)mercury(II) form 1 : 1 (metal ion : chlorin ring) complexes.

INTRODUCTION

Metallochlorophylls, in which magnesium of chlorophylls is substituted by other metal ions, are attractive in view of photochemical applications since they have intense visible light absorption bands. Among them, mercury-substituted chlorophylls should have interesting photochemical properties because the large diameter of mercury(II) ion causes distinct electronic interaction between the central metal ion and the chlorin ring. Although several isolation methods by means of HPLC have been studied for iron- (1,2), zinc- (3,4) nickel- (5), copper- (6) and manganese-substituted chlorophylls (7), isolation of mercury-substituted chlorophylls has not been reported. The difficulty in the isolation of mercury-substituted chlorophylls is considered due to their very labile properties such as demetallation (8). In this short communication, a simple and rapid isolation method of (pheophytin a)mercury(II) [(pheo-a)Hg] and (pheophytin b)mercury(II) [(pheo-b)Hg] using reversed-phase HPLC is reported. Furthermore, it is indicated that both [(pheo-a)Hg] and [(pheo-b)Hg] form 1 : 1 (metal ion : chlorin ring) complexes by comparing the spectroscopic and chromatographic characteristics with those of zinc-substituted chlorophylls, i.e., (pheophytin a)zinc(II) [(pheo-a)Zn] and (pheophytin b)zinc(II) [(pheo-b)Zn], which are typical 1 : 1 complexes with no axial ligand. The structure of these complexes is shown in Figure 1.

EXPERIMENTAL

Chlorophylls were extracted from spinach and purified by a literature method (9), followed by their pheophytinization with dilute hydrochloric acid. Pheophytin a [(pheo-a)H_2] and b [(pheo-b)H_2] were seperated by reversed-phase HPLC with a mobile phase of acetone-acetonitrile (1 : 1, v / v). Crude (pheo-a)Hg and (pheo-b)Hg were prepared by stirring a mixture of (pheo-a)H_2 or (pheo-b)H_2 dissolved in acetone and a slight excess of anhydrous mercury(II) acetate dissolved in ethanol for 1 h. Zinc complexes, (pheo-a)Zn and (pheo-b)Zn, were prepared by reaction of zinc acetate with (pheo-a)H_2 or (pheo-b)H_2 (3). The HPLC apparatus was a



M	R	Compd.
Hg	CH_3	(pheo-a)Hg
Hg	CHO	(pheo-b)Hg
Zn	CH_3	(Pheo-a)Zn
Zn	CHO	(Pheo-b)Zn
2H	CH_3	(pheo-a)H_2
2H	CHO	(pheo-b)H_2

FIGURE 1. Structure of (pheo-a)Hg, (pheo-b)Hg, (pheo-a)Zn, (pheo-b)Zn, pheophytin a [(pheo-a)H_2] and pheophytin b [(pheo-b)H_2].

Hitachi 638-80 equipped with an Inertsil ODS-2 column (GL Science). The contour chromatograms were obtained by using a Waters 991J photodiode array detecter. The column temperature was kept at 25 ℃ and the mobile phase flow rate was 1.0 ml / min. Helium gas was bubbled through a reservoir containing the solvent of mobile phase. FTIR spectra were recorded on JEOL JIR-100 spectrophotometer by the polyethylene film method. All operations were carried out in the dark and in argon atmosphere.

RESULTS AND DISCUSSION

HPLC conditions

The separation was carried out just after the crude products were obtained in order to avoid their degradation. Metallochlorophylls have the amphipatic nature due to a lipophilic phytyl group and a relatively hydrophilic matallochlorin. Therefore, a reversed phase C_{18}-bonded silica gel (ODS) column was used. Binary components such as acetone-acetonitrile, acetone-methanol, etc, were examined as the mobile phase. As a result, acetone-methanol was the most suitable for the separation. Figure 2 shows the contour chromatogram for the crude (pheo-a)Hg. In this chromatogram, several unreacted or decomposed metal-free species, i.e., pheophorbide a (pheophytin derivative which has no phytyl group), (pheo-a)H_2, and pheophytin a′ [(pheo-a′)H_2] are detected. Pheophytin a′ is a C-10 epimer of (pheo-a)H_2 (10). Chlorophylls and their derivatives have two strong absorption bands. The exceedingly intense band near 400 nm is assigned to a Soret band and the band near 650 nm to a Q band (11). These bands are shifted upon the replacement of two protons in pheophytin by the metal ion(1-6). From this tendency, the species 2 and 3 in Figure 2 are considered to be mercury complexes. This is supported by the fact that these species have no fluorescence. The presence of two mercury complexes obtained from (pheo-a)H_2 is not surprising because chlorophyll a and its derivatives have a C-10 epimer, i.e., chlorophyll a′, which is formed by epimerization in the polar solution (10,12,13). In fact, the reversed-phase chromatograms for other metallochlorophylls show two species, a and a′ (1,5,6). The retention time of the species a′ is larger than that of a (1,5,6) and the species a′ has the same spectral

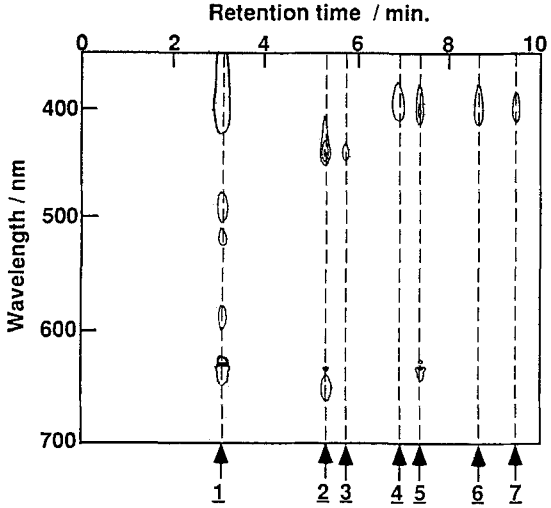


FIGURE 2. Contour chromatogram for crude (pheo-a)Hg.
Mobile phase : acetone/methanol (60/40).
1 = pheophorbide a, **2** = (pheo-a)Hg, **3** = (pheo-a')Hg, **4**, **5** = unknown metal-free species, **6** = (pheo-a)H_2, **7** = (pheo-a')H_2.

characteristics as the species a (1,6,10,12). This is in good agreement with the present case. Hence, it is considered that the species 2 is (pheo-a)Hg and 3 is (pheo-a')Hg. Figure 3 shows the contour chromatogram for the crude (pheo-b)Hg. Based on the same reason as that mentioned above, the species 2 and 3 in this chromatogram are considered to be (pheo-b)Hg and (pheo-b')Hg, respectively. The retention time of (pheo-a)Hg is larger than that of (pheo-b)Hg. This elution order agrees with that for other metallochlorophylls in the reversed-phase HPLC system (1,3-6), consistent with the general tendency that the aldehyde group (-CHO) in (pheo-b)Hg has larger polarity than the methyl group (-CH_3) in (pheo-a)Hg.

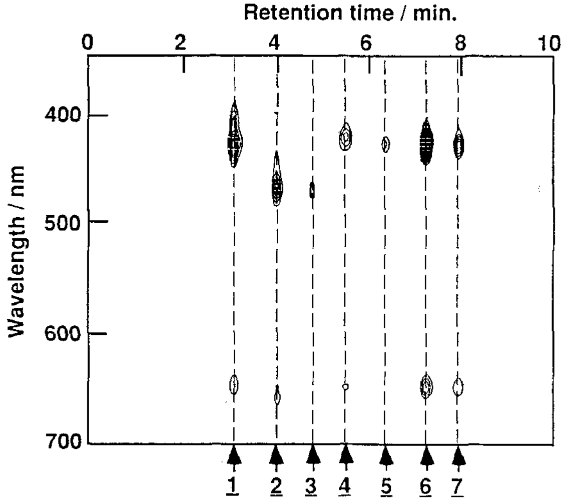


FIGURE 3. Contour chromatogram for crude (pheo-*b*)Hg.
Mobile phase : acetone/methanol (60/40).
1 = pheophorbide *b*, **2** = (pheo-*b*)Hg, **3** = (pheo-*b*′)Hg, **4**, **5** = unknown metal-free species, **6** = (pheo-*b*)H_2, **7** = (pheo-*b*′)H_2.

Identification of (pheo-*a*)Hg and (pheo-*b*)Hg

In the above discussion, it is presumed that (pheo-*a*)Hg and (pheo-*b*)Hg are 1 : 1 (metal ion : chlorin ring) complexes with no axial ligand, similar to other metallochlorophylls, e.g., (pheophytin)zinc complexes. For the mercuryporphyrins, of which the skeletal structure is closely related to that of mercury-substituted chlorophylls, 2 : 1 and 3 : 2 ("double sandwitch type") complexes, which have acetato-mercury bonds, are known, in addition to 1 : 1 complexes (14,15). It is suggested from their spectroscopic and chromatographic characterization that both (pheo-*a*)Hg and (pheo-*b*)Hg are 1 : 1 complexes as follows. Figure 4 shows the FTIR spectrum of (pheo-*a*)Hg, together with those of (pheo-*a*)Zn and (pheo-

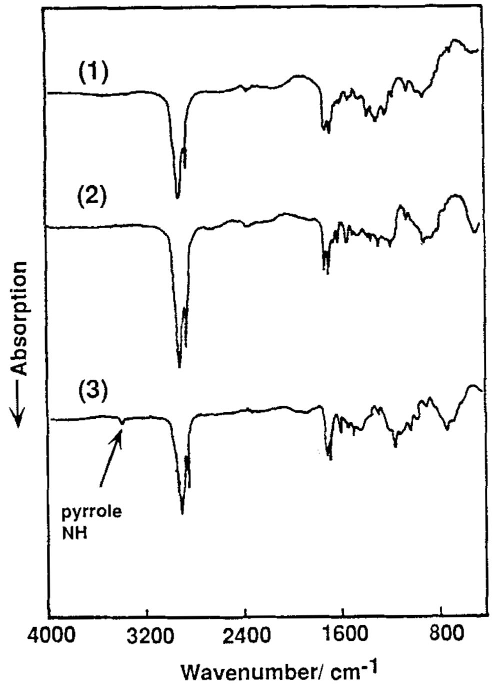


FIGURE 4. FTIR spectra of **(1)** (pheo-a)Hg, **(2)** (pheo-a)Zn and **(3)** (pheo-a)H_2.

a)H_2. The spectral pattern of (pheo-a)Hg is very similar to that of (pheo-a)Zn, the typical 1 : 1 complexes with no axial ligands. The incorporation of the mercury ion is apparent from the absence of the pyrrole N-H stretching absorption, while it is detected at ca. 3400 cm^{-1} in the spectrum of (pheo-a)H_2. More important information is obtained from the CO stretching region. Figure 5 shows the CO stretching region of the spectrum for (pheo-a)Hg, together with those of (pheo-a)Zn and (pheo-a)H_2. The spectral pattern of the CO stretching region is very similar to that of (pheo-a)Zn, in the manner of the numbering in Figure. 5. If (pheo-

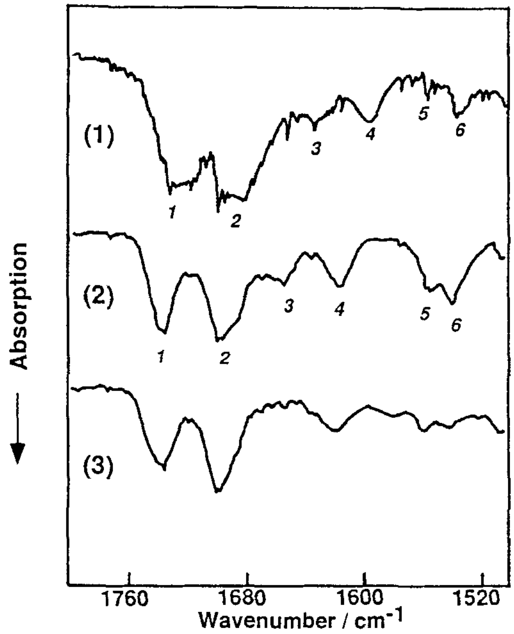


FIGURE 5. FTIR spectra in carbonyl stretching region for **(1)** (pheo-a)Hg, **(2)** (pheo-a)Zn and **(3)** (pheo-a)H_2.

a)Hg is 2 : 1 or 3 : 2 complex, a strong band of the mercury bound acetate should be observed at ca. 1560 - 1580 cm^{-1}, referred to mercury porphyrins (14,15). However, such absorption is not detected here, suggesting that (pheo-a)Hg is 1 : 1 complex. In the same way, (pheo-b)Hg is also considered to be 1 : 1 complex.

For the reversed-phase HPLC of metallochlorophylls using acetone-methanol as a mobile phase, the dependence of the capacity factor (k') on the mixing ratio of acetone-methanol is sensitive to the coordination geometry (5,7). In the case of 1 : 1 complex with no axial ligands, the k' value decreases in proportion to the increasing concentration of acetone

TABLE 1

Absorption Maxima of the Complexes

Complex	λ max of Soret band /nm	Q band /nm
(pheo-*a*)Hg	449[a]	677[a]
(pheo-*b*)Hg	472[a]	664[a]
(pheo-*a*)Zn	425[a]	652[a]
	427[b]	655[b]
(pheo-*b*)Zn	452[a]	637[a]
	455[b]	640[b]

[a] Solvent : acetone/methanol (60/40)

[b] Taken from ref. 3, where the solvent is acetone/methanol (25/75).

(5,7). The relationship between the k' value and the mixing ratio of acetone-methanol was examined for (pheo-*a*)Hg, (pheo-*b*)Hg, and the corresponding zinc complexes. When the acetone concentration increased from 20 to 80 %, the log k' decreased linearly from 0.31 to -0.38, -0.20 to -0.93, 0.59 to -0.33, and 0.41 to -0.44 for (pheo-*a*)Hg, (pheo-*b*)Hg, (pheo-*a*)Zn, and (pheo-*b*)Zn, respectively. Thus, the tendency of the k' value for the mercury complexes is very similar to that for the zinc complexes. This finding is consistent with the 1 : 1 structure of the mercury complexes.

Table 1 summarizes the wavelengths at the absorption maxima of (pheo-*a*)Hg and (pheo-*b*)Hg, together with those of the corresponding zinc complexes. The spectral pattern reflects the π electron symmetry of the macrocycles and the symmetry is strongly affected by the coordination geometry (16). The patterns of (pheo-a)Hg and (pheo-b)Hg were very similar to those of corresponding zinc complexes, indicating these mercury complexes are 1 : 1 complexes with no axial ligand. It is noteworthy that the peak wavelengths of (pheo-a)Hg and (pheo-b)Hg are longer than those of any other corresponding metallochlorophylls. Hence, (pheo-a)Hg and (pheo-b)Hg could be used as the photosensitizers around 450 - 470 nm, if increase their stabilities.

In conclusion, labile complexes (pheo-a)Hg and (pheo-b)Hg are effectively isolated by reversed phase HPLC with a C18-bonded silica column. Their spectroscopic and chromatographic characterization indicate that (pheo-a)Hg and (pheo-b)Hg are 1 : 1 (metal ion : chlorin ring) complexes with no axial ligand.

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REFERENCES

1. K. Furuya, H. Inoue and T. Shirai, Anal. Sci., 3, 353, 1987.
2. H. Hori, K. Kadono, H. Inoue, T. Shirai and E. Fluck, J. Radioanal. Nucl. Chem., 136, 159, 1989.
3. H. Yamashita and H. Inoue, Anal. Sci., 7, 1371, 1991.
4. S. J. Schwartz, J. Liq. Chromatogr., 7, 1673, 1984.
5. K. Furuya, N. Ohki, H. Inoue and T. Shirai, Chromatographia, 25, 319, 1988.
6. H. Inoue, K. Furuya, K. Watanabe, K. Tanaka, T. Shirai and E. Miyoshi, Anal. Sci., 4, 599, 1988.

7. S. Li and H. Inoue, Anal. Sci., 7, 121, 1991.

8. Both (pheophytin *a*)mercury(II) and (pheophytin *b*)mercury(II) are very labile complexes. In spite of their storage at -5 ℃ under argon and in the absence of light, they decomposed completely after 24 h from the isolation.

9. K. Iriyama, M. Shiraki and M. Yoshiura, J. Liq. Chromatogr., 2, 255, 1979.

10. T. Watanabe, A. Hongu, K. Honda, M. Nakazato, M. Konno, and S. Saitoh, Anal. Chem., 56, 251, 1984.

11. C. Weiss, "Electronic absorption spectra of chlorophylls" in The Porphyrins, D. Dolphin ed., Academic Press, 1978, Vol. III, Chap. 3.

12. P. H. Hynninen, M. R. Wasielewski and J. J. Katz, Acta Chem. Scandinavica, B33, 637, 1979.

13. S. Lötjönen and P. H. Hynninen, Org. Magn. Reson., 21, 757, 1983.

14. M. F. Hudson and K. M. Smith, Tetrahedron Lett., 2223, 1974.

15. M. F. Hudson and K. M. Smith, J. Chem. Soc., Chem. Commun., 515, 1973.

16. M. Gouterman, "Optical spectra and electronic structure of porphyrin and related rings" in The Porphyrins, D. Dolphin ed., Academic Press, 1978, Vol. III, Chap. 1.

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