

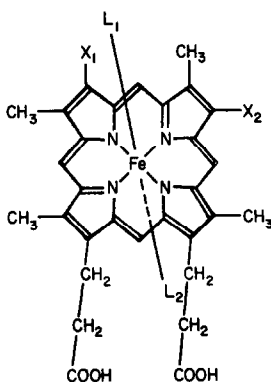
OXYMERCURATION-DEMERCURATION OF PROTOHEMIN IX

PREPARATION OF HEMATOHEMIN IX DIMETHYL ETHER

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Studies concerning the mechanism of oxygenation of hemoglobin have sometimes involved incorporation of synthetic hemins into apohemoglobin (1). It was desirable to obtain synthetic hemins other than those few commonly available. Particularly, it was pertinent to modify the 2- and 4-vinyl groups of protohemin, Ia, to obtain substituent groups with different steric and electronic properties. The oxymercuration-demercuration sequence (2) proved to be selective in its action on the protohemin vinyl groups and the larger part of the molecule remained unaffected. Several attempts at hydroxymercuration-demercuration of protohemin to furnish hematohemin, Ib, were unsuccessful. The use of alcohols instead of water as reactant produced satisfactory yields of hematohemin ethers. The procedure is illustrated in the synthesis of hematohemin IX dimethyl ether, Ic.



- (a) $X_1 = X_2 = -CH = CH_2$, $L_1 = Cl$
- (b)* $X_1 = X_2 = -CH(OH)CH_3$, $L_1 = Cl$
- (c)* $X_1 = X_2 = -CH(OCH_3)CH_3$, $L_1 = Cl$
- (d) $X_1 = X_2 = -CH_2CH_3$, $L_1 = Cl$
- (e)* $X_1 \neq X_2 = -CH = CH_2$ and $-CH(OCH_3)CH_3$,
 $L_1 = Cl$

* Mixtures of diastereomers

Protohemin (500 mg) and mercuric trifluoroacetate (654 mg) were stirred in methanol (100 ml) for one hr while shielded from direct light. To this mixture was added 3N sodium hydroxide (100 ml) and 3N sodium hydroxide (100 ml) containing sodium borohydride (300 mg). The mixture was stirred for one hr, chilled in ice, and slowly acidified with glacial acetic acid (60 ml). Air was bubbled into the mixture for five min to convert iron II to iron III and the mixture was saturated with sodium chloride. Three portions of ethyl acetate (100 ml) extracted most of the color. The combined extract was washed with saturated sodium chloride, water, and then dried over anhydrous sodium sulfate. The ethyl acetate solution was filtered and reduced to dryness on a rotary evaporator at 40°C giving 320 mg of a dark material.

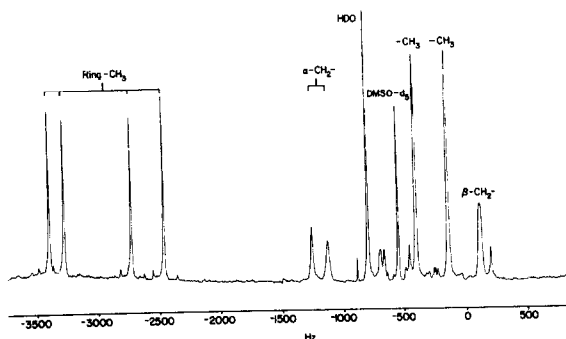
The composition of the crude product was determined by tlc on polyamide and the pure product was isolated by column chromatography on polyamide powder. Procedures for analysis and purification of this and other hemin dicarboxylic acids by chromatography on polyamide have already been reported in detail (3). The fine powder resulting from evaporation of the fractions from column chromatography was obtained in crystalline form by dissolving in minimal methanol and successive extraction with purified hexane until the methanol layer disappeared and the solid was precipitated. The crystalline material was dried under vacuum at room temperature. Calculated for $C_{36}H_{40}ClFeN_4O_6$: C, 60.38; H, 5.63; N, 7.82. Found: C, 60.20; H, 5.50; N, 7.75 (4).

The structure, Ic, is supported by (a) the pyridine-hemochrome spectrum (5), 547, 517, 408 nm, which indicates that the hemin no longer contains the double bond of the vinyl groups of protohemin, (b) the 220 MHz PMR spectrum (Figure 1) which may be compared to the 100 MHz PMR of protohemin (6, 7), (c) a test to determine reaction at any sites other than the vinyl groups of protohemin (8) and (d) spectral and chromatographic comparisons to the same material prepared by a different synthetic route (9, 10).

The use of alcohols other than methanol in the oxymercuration-demercuration sequence on protohemin results in a slower reaction, and products are furnished in the cases of n-butanol and longer straight chain alcohols. Chain branching at the α -carbon greatly slows or prohibits the reaction as in the case of t-butanol. This failure of t-butanol to react is not characteristic of the reaction with simple alkenes (11) and indicates

that the protohemin-mercuric trifluoroacetate adduct is too sterically hindered to allow reaction with t-butanol. This successful application of the oxymercuration reaction to protohemin demonstrates that this versatile reaction can probably be used in wider applications to introduce many functional groups into the porphyrin system (2, 21).

Figure 1



References and Footnotes

- (1) E. Antonini and M. Brunori, "Hemoglobin and Myoglobin and Their Reactions with Ligands," North-Holland Publishing Company, Amsterdam, 1971, p. 286.
- (2) H.C. Brown and P.J. Geoghegan, Jr., *J. Org. Chem.* **35**, 1844 (1970).
- (3) D.W. Lamson, A.F.W. Coulson, and T. Yonetani, *Anal. Chem.* **45**, in press (1973).
- (4) Analyses performed by Midwest Microlab, Ltd., Indianapolis, Indiana.
- (5) J.E. Falk, "Porphyrins and Metalloporphyrins," Elsevier Publishing Co., Amsterdam, 1964, pp. 81 and 240.
- (6) R.J. Kurland, R.G. Little, D.G. Davis, and C. Ho, *Biochemistry* **10**, 2237 (1971).
- (7) Figure 1 shows the 220 MHz pmr spectrum of 0.03M hematohemin dimethyl ether in DMSO-d₅ with ca. 0.12M KCN added in 20µl D₂O. Scale is in Hertz from (CH₃)₄Si. The absorptions for the vinyl protons of protohemin are absent and those for two types of methyl (6H each) are present. Presumably, the one at lower field is that for -O-CH₃ of Ic.

- (8) Mesoheemin, Id, was put through the methoxymercuration-demercuration sequence without change, as indicated by the pyridine-hemochrome spectrum and tlc analysis on polyamide of the recovered material.
- (9) D. W. Lamson, G. V. Woodrow, and T. Yonetani, *Biochemistry*, submitted for publication.
- (10) An oxymercuration reaction on a molecule containing two vinyl groups may produce a mixture of two mono-oxymercured products. A material which may qualify thusly was isolated by chromatography from the preparation described above (3) and partially characterized (3, 9). There is conflicting evidence as to whether the material constitutes the mixed isomers Ie.
- (11) H. C. Brown and M.-H. Rei, *J. Amer. Chem. Soc.* 91, 5646 (1969).
- (12) G. T. Rodeheaver and D. F. Hunt, *Chemical Communications* 1971, 818.