Tetrahedron Letters,Vol.29,No.20,pp 2501-2504,1988 0040-4039/88 $3.00 + .00
Printed in Great Britain Pergamon Press plc

THE SYNTHESIS OF DIHEMATOPORPHYRIN ETHER AND RELATED PORPHYRIN DIMERS

Ian K. Morris and A. David Ward*

Department of Organic Chemistry, University of Adelaide,
P.O. Box 498, Adelaide, South Australia, 5001.

Abstract. Dihematoporphyrin ether (DHE) has been synthesised by two separate procedures; these routes also allow the synthesis of ether linked porphyrin dimers containing one or two vinyl groups rather than hydroxyethyl side chains. The latter dimers, but not DHE, have anti-cancer activity.

Hematoporphyrin derivative (HPD) is a complex mixture of porphyrins that is of considerable interest as a new anti-cancer agent.[1] HPD is administered intravenously and selectively localizes in tumour tissue. Activation of these porphyrins by a 628 nm light source causes cell death via singlet oxygen formation. The treatment is often referred to as photodynamic therapy.

HPD can be separated into two main fractions by gel-filtration chromatography (Sephadex LH-20).[2] The higher molecular weight material (the faster running fraction) has been shown to contain the anti-cancer activity[2,3] and similar material is marketed commercially as Photofrin®. The main component of the active material has been claimed[4] to be dihematoporphyrin ether (DHE), (5) although other workers[5-7] have presented evidence that indicates that the active material is more likely to be a polymer than a dimer. Conflicting evidence has also been obtained concerning the nature of the functional group(s) linking the porphyrin units in the dimer and/or the polymer material, with both an ester and an ether linkage being proposed.[8-12] The situation is even further complicated by the apparent interconversion of ester linked material to ether linked material.[8,9]

In view of the uncertainty regarding the nature of the active material(s) in HPD, the unambiguous synthesis of DHE and other porphyrin dimers and polymers related to DHE is of considerable importance, both for structure-activity relationship studies and also to help in the understanding of the chemistry of these systems. We now report the unambiguous synthesis of DHE by two separate routes.

Hydrogen bromide dissolved in dichloromethane was added to a solution of hematoporphyrin IX dimethyl ester (HP.DME) (1) in dichloromethane and the resulting solution was stirred in the dark at room temperature for twenty minutes. Tlc analysis of the product showed that a complex mixture had formed, although a major product with a slightly higher R_f on silica than that of HP.DME could be clearly distinguished. Careful chromatography of this product, firstly using alumina (Laporte, Grade 1) to isolate the more hydrophobic porphyrins containing dehydrated side chains from the slower running α-hydroxyethyl containing porphyrins, followed by subsequent chromatography of the latter fraction on silica enables

the tetramethyl ester (2) of DHE to be obtained. Although this material runs as a broad single spot on tlc, it must be a mixture of regio- and diastereomers since coupling can occur between either the C2 or C4 hydroxyethyl groups of one porphyrin with the same groups at either the C2' or C4' position of the second porphyrin. As all the hydroxyethyl groups contain a chiral atom a complex mixture of diastereomers will be formed.

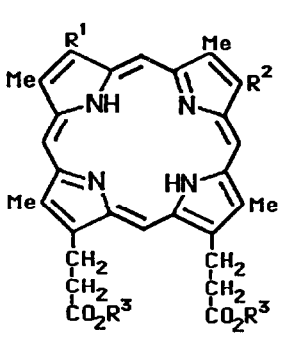
FAB mass spectral analysis shows that the tetramethyl ester (2) of DHE has a strong molecular ion at 1236 (calc. 1235.4) with a peak at 609 being the only major fragmentation.[13] The visible spectrum of (2) in dichloromethane shows a broadened Soret band at 395 nm with other bands at 502, 533, 569 and 622 nm. The proton nmr spectrum shows only the expected signals, although these are rather broad and poorly resolved, as is often the case with porphyrins due to their aggregation in solution as well as in this case to the presence of numerous regio- and diastereomers. In particular, the signals due to the meso protons are complex. The ^{13}C nmr spectrum shows much better resolution and all the expected signals are readily apparent.[14]

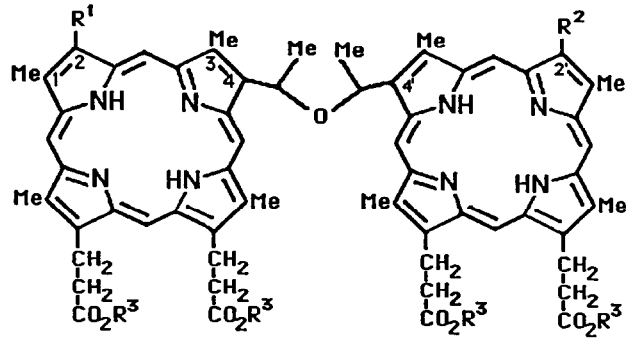
The tetramethyl ester (2) of DHE was also prepared by an alternative route. Partial Jones oxidation of HP.DME (1) gave a mixture of the dimethyl esters of diacetyldeuteroporphyrin, monoacetylmonohydroxyethyldeuteroporphyrin and the starting porphyrin. This mixture was separated into its individual components on alumina (Laporte Grade 1). The monoacetyl product (3) was then dimerised by treatment with hydrogen bromide in dichloromethane as outlined above, however since polymerisation is not a competing process, the reaction time can be extended in order to maximise the dimer yield. The bis acetyl dimer (4) was separated by chromatography into two fractions which we tentatively suggest may correspond to regioisomers. The major difference in the proton nmr of these two fractions is in the chemical shift of the protons of the $CH_3\underline{CH}\text{—}O\text{—}$ ether linking group. The FAB mass spectrum of this dimer (4) shows a strong molecular ion at 1232 and very little fragmentation. Reduction of the bis acetyl dimer with sodium borohydride provided the tetramethyl ester (2) of DHE, identical to the material previously synthesised.

Mild base hydrolysis (NaOH/THF/RT/16 hrs) of the tetramethyl ester (2) of DHE provided DHE (5) itself. Both HPLC and FAB mass spectral data separately indicated that a small amount of elimination may have occured during the hydrolysis leading to vinyl rather than hydroxyethyl side chains. However the major peak in the FAB mass spectrum is that of DHE molecular ion at 1180 with other peaks at 1162 and 582.

DHE is rather labile in aqueous solution and slowly changes to material that has longer retention times on a reverse phase HPLC column. Freshly prepared DHE is only slightly separated from hematoporphyrin on a Sephadex column but an aged solution separates on Sephadex into fast and slow fractions suggesting that a polymeric material is slowly being formed. The major peaks of DHE in the reverse phase HPLC occur in a region of the spectrum where little or no absorption is observed with a Photofrin® sample. This result suggests that DHE is either only a minor component or is totally absent in Photofrin®.

The chromatographic separation of the tetramethyl ester of DHE from the product of the reaction of HP.DME with hydrogen bromide also allows the separation of the tetramethyl ester of the monovinyl monohydroxyethyl ether linked dimer (6) and the tetramethyl ester





(1) $R^1 = R^2 = CH_3CH(OH)-$
$R^3 = CH_3$

(2) $R^1 = R^2 = CH_3CH(OH)-$ $R^3 = CH_3$

(3) $R^1 = CH_3\overset{\overset{O}{\|}}{C}-$
$R^2 = CH_3CH(OH)-$
$R^3 = CH_3$

(4) $R^1 = R^2 = CH_3\overset{\overset{O}{\|}}{C}-$ $R^3 = CH_3$

(5) $R^1 = R^2 = CH_3CH(OH)-$ $R^3 = H$

(6) $R^1 = CH_3CH(OH)-$ $R^2 = CH_2{=}CH-$ $R^3 = CH_3$

(7) $R^1 = R^2 = CH_2{=}CH-$ $R^3 = CH_3$

(8) $R^1 = CH_3CH(OH)-$
$R^2 = CH_2{=}CH-$
$R^3 = CH_3$

(9) $R^1 = CH_3CH(OH)-$ $R^2 = CH_2{=}CH-$ $R^3 = H$

(10) $R^1 = R^2 = CH_2{=}CH-$ $R^3 = H$

[For each structure only one regioisomer is shown.]

of the divinyl ether linked dimer (7). The divinyl dimer (7) can be more conveniently obtained from the reaction of (8) with hydrogen bromide. Provided the reaction time is relatively short, addition of hydrogen bromide to the double bond does not occur to an appreciable extent. Both dimer esters can be hydrolysed using the mild basic conditions to form the corresponding dimer acids (9) and (10). These acids have HPLC retention times that are similar to those of the major portion of Photofrin®. The divinyl dimer (10) readily forms a photoproduct on exposure to diffuse light.

Preliminary *in vivo* testing results,[15] which will be reported in full elsewhere, show that the anti-cancer activity of these dimers varies considerably. DHE is inactive, the monovinyl dimer (9) has moderate activity whereas the divinyl dimer (10) has the same activity as that of Photofrin®.

Acknowledgements. We thank Johnson and Johnson Research (Australia) and the University of Adelaide for financial support of this research; Dr. P. Cowled for the biological testing and Mr. T. Blumenthal for mass spectral measurements.

References and Notes

1. For review articles see Methods in Porphyrin Photosensitization (Ed. D. Kessel) Plenum Press, New York, 1985 and Photochem.Photobiol., 46, Number 5 (1987).

2. Kessel, D. and Chou, T-H., Cancer Res., 43, 1994 (1983) See also reference 7.

3. Dougherty, T.J., Boyle, D.G., Weishaupt, K.R., Henderson, B.A., Potter, W.R., Bellnier, D.A. and Wityk, K.E., in Porphyrin Photosensitization (Ed. D. Kessel and T.J. Dougherty) Plenum Press, New York, p.3, 1983.

4. Dougherty, T.J., Potter, W.R. and Weishaupt, K.R., in Porphyrin Localization and Treatment of Tumors (Ed. D. Doiron and C.J. Gomer) Liss, New York, p.301, 1984 and in Porphyrins in Tumor Phototherapy (Ed. A. Andreoni and R. Cubeddu) Plenum Press, New York, p.23, 1984.

5. Swincer, A.G., Ward, A.D. and Howlett, G.J., Photochem.Photobiol., 41, 47 (1985).

6. Ward, A.D. and Swincer, A.G., in Methods in Porphyrin Photosensitization (Ed. D. Kessel) Plenum Press, New York, p.267, 1985.

7. Kessel, D., Chang, C.K. and Musselman, B., in Methods in Porphyrin Photosensitization (Ed. D. Kessel) Plenum Press, New York, p.213, 1985.

8. Byrne, C.J., Marshallsay, L.V. and Ward, A.D., Photochem.Photobiol., 46, 575 (1987).

9. Kessel, D., Thompson, P., Musselman, B. and Chang, C.K., Photochem.Photobiol., 46, 563 (1987).

10. Dougherty, T.J., Photochem.Photobiol., 46, 569 (1987).

11. Kessel, D., Thompson, P., Musselman, B. and Chang, C.K., Cancer Res., 47, 4642 (1987).

12. Boyle, R.W., Keir, W.F., MacLennan, A.H., Maguire, G. and Truscott, T.G., Cancer Lett., 38, 9 (1987).

13. The major peaks in the mass spectrum are all clusters of peaks with both the M and the M+H ions being prominent.

14. (2) FAB mass spectrum 1236, 609. λ_{max} (CH_2Cl_2) 395, 502, 533, 569, 622. ^{13}C Nmr ($CDCl_3$) 173.7, CO_2R; 135-148, pyrrole carbons; 95.2-99.0, meso carbons; 68.7-73.1, CHO—; 64.0-67.0, CHOH; 51.6, $CO_2\underline{CH_3}$; 35.1-37.0, 20.7-22.0, propionate carbons; 24.8-26.5, side chain methyls; 11.6, ring methyls.

(6) FAB mass spectrum 1218, 609, 592. λ_{max} (CH_2Cl_2) 398, 503, 535, 572, 624.

(7) FAB mass spectrum 1200, 592. λ_{max} (CH_2Cl_2) 399, 503, 537, 574, 626. ^{13}C Nmr ($CDCl_3$) 173.6, CO_2R; 135-147, pyrrole carbons; 119.4, 120.4, 129.3, 130.2, vinyl carbons; 95.2-99.0, meso carbons; 71.0, 71.2, CHO—; 51.5-51.8, $CO_2\underline{CH_3}$; 20.7-21.7, 36.1-37.0, CH_2; 24.1-24.5, side chain methyls; 9.9-12.7, ring methyls.

(5) FAB mass spectrum 1180, 1162, 582. λ_{max} (NaOH/EtOH) 383, 501, 534, 569, 622.

(9) FAB mass spectrum 1160, 564. λ_{max} (NaOH/EtOH) 388, 502, 535, 570, 623.

(10) FAB mass spectrum 1144, 1099, 564. λ_{max} (NaOH/EtOH) 379, 505, 538, 574, 628.

15. The testing procedure has been described previously, (Cowled, P.A. and Forbes, I.J., Cancer Lett., 28, 111 (1985)).

(Received in UK 11 March 1988)