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# Enthalpies and heat capacities of solution of methylpheophorbide, dioxidine and their conjugate in DMF at 298-318 K

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ABSTRACT

This paper focuses on the results of the first accurate determination of enthalpies of solution of 9-ethenyl-14-ethyl-21-(methoxycarbonyl)-3-{(O-methylcarbamoyl)ethyl} -4,8,13,18-tetramethyl-20-oxo-phorbine (Methylpheophorbide *a*, **1**), [3-(hydroxymethyl)-1-oxido-4-oxoquinoxalin-4-ium-2-yl]methanol (Dioxidine, **2**) and their conjugate 9-ethenyl-14-ethyl-3-{[2-(3-hydroxymethyl)]-1-oxido-4-oxoquinoxalin-4-ium-2-methyleneoxy}carbamoyl}ethyl)-4,8,13,18-tetramethyl-20-oxo-phorbine (Conjugate **3**) in liquid N,N-dimethylformamide (DMF) in the temperature range of 298-318 K. Standard enthalpies and heat capacities of solution ($\Delta C^{\circ}_{p}$) have been computed and compared with those for other solid and liquid non-electrolytes to highlight important features of solvation of potential photosensitizers in a simple model of a protein-like environment.

## 1. Introduction

Photodynamic therapy (PDT) with an appropriate photosensitizer (PS) of a porphyrin, phthalocyanine or chlorophyll-type is a clinically approved therapeutic modality for treating many inflammatory and neoplastic processes [1–3]. Various skin diseases including acne and psoriasis, superficially located tumors, complicated wound infections caused by antibiotic resistant bacteria or fungi may be successfully treated with the PDT technique [4]. Most of the second-generation chlorophyll photosensitizers are appropriate derivatives of chlorin $e_6$ or their metal complexes. These are usually synthesized from methylpheophorbide *a* or pheophorbide which are known to be an appropriate versatile synthetic base to develop various PSs [2].

[3-(hydroxymethyl)-1-oxido-4-oxoquinoxalin-4-ium-2-yl]methanol (Dioxidine) is a reserve quinoxaline N-oxide type antimicrobial drug which is important for treating multiresistent pathogenic microflora [5–7]. Dioxidine reveals broad-spectrum activity towards both anaerobic and mixed strains. However, due to its slight teratogenic and mutagenic action it is rarely used in therapeutic doses [5,6]. It is believed that covalent attachment of water-soluble dioxidine to hydrophobic methylpheophorbide *a* increases PS solubility in biological liquids and may enhance its dark toxicity towards different pathogens both before and, especially, after the PDT treatment. Moreover, this conjugation should weaken side effects mentioned above.

In this paper we describe synthesis of such a conjugate and study its thermochemical behavior in liquid DMF which is considered as an appropriate model for a protein-like environment [8]. These results are compared with those for dioxidine and methylpheophorbide *a* as well as with previously reported enthalpies of solution of blood porphyrins and non-electrolytes modeling their side-chains [9–15].

## 2. Experimental

### 2.1. Chemicals

DMF (Panreac, > 99 mass %) was dried with 4 Å molecular sieves for several days and then distilled under reduced pressure at 303 K, the middle fraction being selected. Karl Fisher titration showed that water content in the final product was lower than 0.02 mass %. The standard enthalpy of solution of DMF in water at 298 K was found to be equal to -15.25 kJ $mol^{-1}$, which was in a fair agreement with literature values of -15.27 and -15.31 kJ $mol^{-1}$ [16,17], respectively.

Dioxidine **2** was obtained from its dosage form contained 1 mass % of the drug dissolved in water (Borisovskiy ZMP, Belarus). This solution

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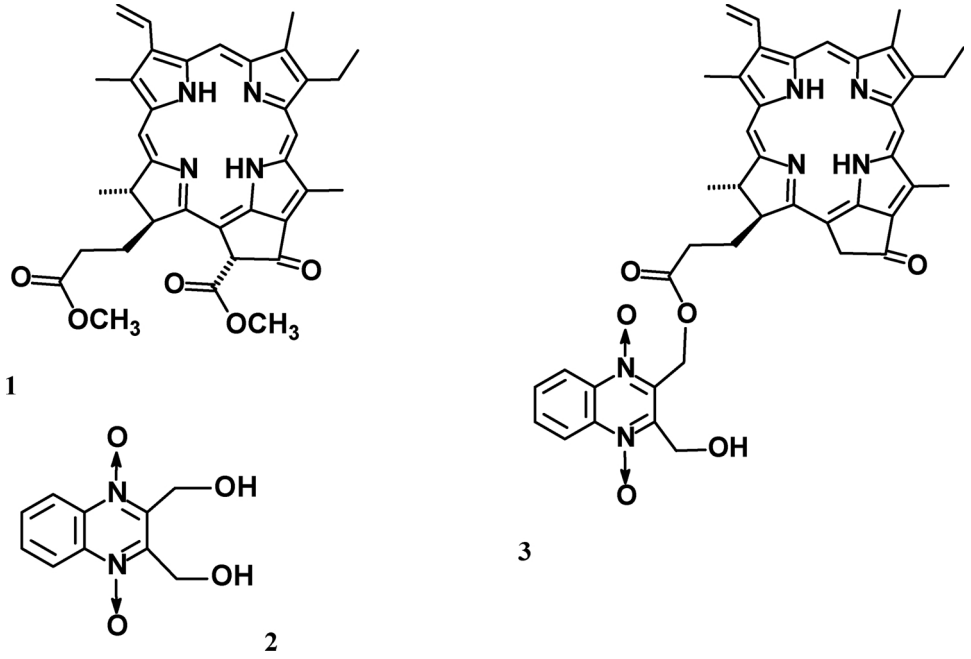


**Fig. 1.** Molecular structures of methylpheophorbide *a* (**1**), dioxidine (**2**), and their conjugate (**3**).

was cooled down to 275 K. Then dioxidine crystals were separated from a solution using Schott filters, washed by cold water several times and dried under reduced pressure at 353 K for several days. Purity of the solute was checked with UV-, visible and $^1$H NMR (see the information given elsewhere [18]).

9-ethenyl-14-ethyl-3-{[2-(3-hydroxymethyl)]-1-oxido-4-oxoquinoxalin-4-ium-2-methyl-eneoxy}carbamoyl}ethyl)-4,8,13,18-tetramethyl-20-oxo-phorbine (Conjugate **3**) was obtained *via* chemical functionalization of methylpheophorbide *a* **1** [19] to attach the dioxidine **2** residue (see Fig. 1). The details of synthesis and purification of the pigment are presented in the Supplementary material file. The final product was identified with $^1$H NMR, MS-, IR- and UV-Vis spectra (see the Supplementary Material file). Both compounds **1, 3** were recrystallized from acetone and dried under reduced pressure at 353 K for several days.

The description of all chemicals with their purity is given in Table 1. The structures of solutes **1-3** are illustrated in Fig. 1.

### 2.2. Apparatus and methods

Enthalpies of solution were measured with the self-built isoperibol automated ampoule calorimeters and described several times before [9–15]. The calorimetric vessel was equipped with a calibration heater, a titanium stirrer and a thermistor. A glass ampoule containing a solute was attached to the stirrer and crushed against the vessel bottom to initiate the dissolution process. Thermistor resistance was directly measured by the Standard Temperature Measuring Instrument (BIC, Minsk). The detection limit of the apparatus was 10 μK. The temperature instability in the «Thermostat A 3» bath (BIC, Minsk) were less than 1 mK in the temperature range from 275 to 350 K. The enthalpies of solution were measured by a comparative method. An electrical calibration was carried out before each experiment. The calorimeter was tested by measuring enthalpies and heat capacities of solution of several non-electrolytes in water including urea, tetramethylurea and 1-

**Table 1**
Chemicals in this study.

| Chemical name | Abbreviation/ phase state | Source | Initial mass fraction | Purification method | Analysis method | Final mass fraction |
|---|---|---|---|---|---|---|
| Water | Liquid | - | - | Bidistillation | - | - |
| 1-propanol | PrOH, liquid | Aldrich | > 0.995 | - | As stated by the supplier | > 0.995 |
| N,N-dimethylformamide | DMF, liquid | Panreac (Spain) | > 0.99 | Drying with 4 Å sieves, vacuum distillation | As stated by the supplier | > 0.99 |
| 9-ethenyl-14-ethyl-21-(methoxycarbonyl)-3-{(O-methylcarbamoyl)ethyl} -4,8,13,18-tetramethyl-20-oxo-phorbine (Methylpheophorbide *a*) | 1, solid | (Chlorin, Russia) | ~0.98 | Recrystallization, vacuum drying | $^1$ H NMR | > 0.95 |
| [3-(hydroxymethyl)-1-oxido-4-oxoquinoxalin-4-ium-2-yl] methanol (Dioxidine) | 2, solid | Borisov. ZMP (Belarus) | 0.01 | Recrystallization, washing, vacuum drying | As stated by the supplier | > 0.99 |
| 9-ethenyl-14-ethyl-3-{[2-(3-hydroxymethyl)]-1-oxido-4-oxoquinoxalin-4-ium-2-methyleneoxy}carbamoyl}ethyl)-4,8,13,18-tetramethyl-20-oxo-phorbine (Conjugate) | 3, solid | Synthesis | - | Column chromatography, recrystallization, vacuum drying | $^1$ H NMR | > 0.95 |

Note. Purity of methylpheophorbide *a* of 98 % was provided by the supplier *via* the HQLC measurements. Our $^1$H NMR study indicates that purity of both macrocycles 1, 3 is of 95 % (see the Supplementary Material File). The value of 0.01 for dioxidine denotes an initial mass fraction of the solute in an ampoule with a sterile solution.