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Статья

X−Ray Single−Crystal Structures and NMR Characterization of Three Vinyl Substituted Methylpyropheophorbide *a* Derivatives

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*Three recently synthesized tetrapyrrole compounds: (E)-3²-bromopyropheophorbide a methyl ester (**1**) and its derivatives with π-conjugated acrylate (**2**) and phenyl (**3**) substituents are structurally characterized using X-ray diffraction along with 1D and 2D NMR spectroscopy. All compounds are crystallized in chiral space groups. The planar molecules form stacks extended in one direction. The ¹H and ¹³C signals were completely assigned using 2D {¹H,¹H} correlation spectroscopy (COSY), nuclear Overhauser effect spectroscopy (NOESY), {¹H,¹³C} heteronuclear single quantum coherence (HSQC), and heteronuclear multiple bond correlation (HMBC) experiments. The details of structure elucidation and signal assignment process are given using compound **2** as an example.*

Keywords: Chlorophyll, X-ray structure, natural chlorin, π-extended pheophorbide, NMR, photosensitizer, photodynamic therapy, solar energy.

Изучение строения трех винил−замещенных производных метилпирофеофорбида *a* методами РСА и ЯМР

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*Методами РСА и спектроскопии ЯМР изучены структуры трех новых модифицированных природных хлоринов: метилового эфира (Е)-3²-бромпирофеофорбида а (**1**) и его производных с π-сопряженными метилакрилатным (**2**) и фенильным (**3**) заместителями. Все соединения кристаллизуются в хиральных пространственных группах. Плоские молекулы формируют стопки, расположенные в одном направлении. Сигналы в спектрах ¹H и ¹³C ЯМР были полностью отнесены с помощью двумерной {¹H,¹H} гомоядерной корреляционной спектроскопии (COSY), спектроскопии ядерного эффекта Оверхаузера (NOESY), {¹H,¹³C} эксперимента по гетероядерной однаквантовой когерентности (HSQC) и гетероядерной корреляции через множественные связи (HMBC). Процесс отнесения сигналов в спектрах подробно приведен на примере соединения **2**.*

Ключевые слова: Хлорофилл, РСА, природные хлорины, феофорбид с расширенной π-системой, ЯМР, фотосенсибилизатор, фотодинамическая терапия, солнечная энергия.

Introduction

Chlorophylls have been studied for over two centuries and, while much of their basic chemistry was established almost 100 years ago, real breakthroughs in their exact bio-chemical function were only made in the last decades by X-ray crystallographic analysis of both reaction center and antenna complexes. However, no isolated chlorophyll molecule has ever been characterized by X-ray crystallography. About 55 single crystal X-ray structures the most closely related to chlorophylls and pheophorbides (Pheo) are available.[1] Among them only few metal-free pheophorbides are characterized by single-crystal X-ray diffraction. The first structure of a Pheo *a* derivative, methyl pheophorbide *a*, was reported by Calvin and coworkers in 1972.[2] Overall, the structure has exhibited a planar macrocycle with only minimal deviations from planarity. The structure has identified the basic characteristics of the chlorophyll derivative, *i.e.* the characteristic zig-zag conformation of the cyclopentanone ring, differences between the reduced pyrrole ring and the others, and conjugation of the vinyl group with the aromatic system. Likewise, methyl mesopyropheophorbide *a* (methyl phytochlorin)[3] exhibits a planar macrocycle.

Synthetic hydroporphyrins are studied better. There are about 100 "nonphytochlorin" or "non-chlorin e_6" type crystal structures.[1] One of the most recent papers describes crystal structure of the (B)Chl derivative with a β-diketonate moiety at the C3 position.[4] However, the problem of single crystal growing still limits the possibility of X-ray structural study. Therefore, any progress in this field is of significant importance.

Recently, we have found a new approach towards chlorophyll *a* modification using (E)-3²-bromopyropheophorbide *a* methyl ester as an initial reagent. New derivatives, (E)-3²-(2-methoxycarbonylvinyl)pyropheophorbide *a* methyl ester and (E)-3²-phenylpyropheophorbide *a* methyl ester, were synthesized.[5] These compounds are of special interest for development of new photosensitizers for various applications including photodynamic therapy and solar energy conversion. In this work, we report X-ray single-crystal structures and NMR characterization of these three methyl pyropheophorbide *a* derivatives (Figure 1).

Experimental

(E)-3²-Bromopyropheophorbide a methyl ester (1), (E)-3²-(2-methoxycarbonylvinyl)pyropheophorbide a methyl ester (2) and *(E)-3²-phenylpyropheophorbide a methyl ester (3)* were synthesized using a known procedure.[4] Small single crystals of compounds **1–3** were grown by slow isothermal evaporation of their solutions in dichloromethane to which ethanol had been added (in the dark).

The single-crystal X-ray diffraction experiments for compounds **1–3** were carried out on a STOE Stadi-Vari diffractometer equipped with Pilatus100K detector, at room temperature using focusing mirror collimated CuK_α radiation. The structures were solved and refined with *SHELXS97*.[6] In compounds **2** and **3**, all non-H atoms were refined with anisotropic displacement parameters. For compound **1**, which contains six independent molecules in the asymmetric unit, in view of extremely small and poorly diffracting single crystal, some of non-H atoms were isotropically refined to minimize the number of variables. All hydrogen atoms were placed at idealized positions (N–H 0.86 Å, C–H 0.93–0.97 Å),
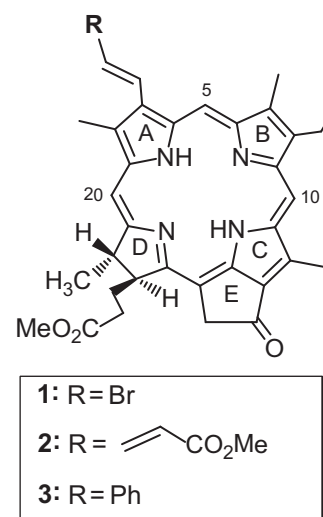

Figure 1. Chemical structures of pyropheophorbides **1–3**.

and refined as riding with U_{iso}(H)=1.2–1.5U_{eq}(C, N). The crystallographic data for **1–3** are summarized in Table 1, and their molecular structures drawn with *PLATON*[7] and *Mercury*[8] are shown in Figures 2–4. The visualization of the corresponding crystal packings are provided as a supplementary data.

NMR spectra were recorded at 298 K on Bruker Avance III 600 spectrometer. The signals of carbon atoms and those of residual protons in deuterated solvents were used to calibrate the ^{13}C and ^1H scales, respectively.[9] All experiments were based on Bruker's standard techniques. NOESY spectra were recorded using 600 ms mixing time and a recovery delay of 1.0 s. $^1H–^{13}C$ HMBC spectra were recorded with 60 ms delay for evolution of long-range couplings.

Results and Discussion

Single crystals of **1–3** for X-ray structural experiment were prepared by slow evaporation of their solutions in dichloromethane-ethanol mixtures. Structures **1–3** are built of six, one and two independent heterocyclic molecules, respectively (Figures 2–4).

The geometric parameters of compounds **1–3** are normal and well-corresponding to those observed in the related compounds found in the Cambridge Structural Database (CSD, Version 5.35 with updates).[10] Heterocyclic rings in **1–3** are essentially planar; maximal outlets of the N and C atoms are 0.022, 0.007, 0.019 Å and 0.089, 0.024, 0.096 Å, respectively. The four five-membered pyrrole rings are nearly coplanar. Cyclopentanone rings also are planar. The C–C bonds in the reduced pyrrole rings are slightly longer than in the others. For the two N–H units, the formation of intramolecular N–H···N hydrogen bonds [N···N=2.722–3.052 Å] was found in structures **1–3**. Interestingly, all compounds are crystallized at chiral space groups.
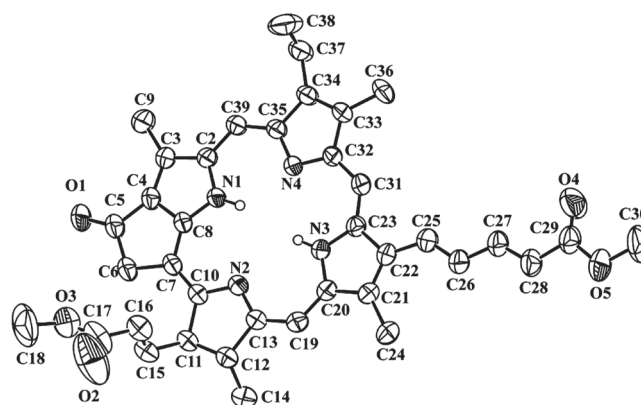
The crystal packings (see Supplementary Data) also exhibit one common feature for all compounds – the molecules form stacks extended in one direction, namely, in [100] for **2** and **3**, and in [010] for **1**. The 3-D framework of structure **1** is formed by numerous weak contacts between macromolecules, including C–H···C, C–H···Br, C–H···N, C–H···O interactions. Molecules in structure **1** are almost coplanar;

Vinyl Substituted Methylpyropheophorbide *a* Derivatives

Table 1. Crystallographic data for structures **1–3**.

Compound	1	2	3
Empirical formula	$C_{34}H_{35}BrN_4O_3$	$C_{38}H_{40}N_4O_5$	$C_{40}H_{40}N_4O_3$
Formula weight	627.57	632.74	624.76
Crystal system	Monoclinic	Orthorhombic	Triclinic
Space group	$P2_1$	$P2_12_12_1$	$P1$
a (Å)	14.6861(3)	6.91350(10)	7.2411(3)
b (Å)	21.3480(5)	14.6490(2)	13.4347(6)
c (Å)	29.9300(10)	32.9371(4)	18.7166(8)
α (°)	90	90	109.785(3)
β (°)	91.101(3)	90	100.407(3)
γ (°)	90	90	90.300(4)
V, Å³	9381.9(4)	3335.73(8)	1680.78(13)
Z	12	4	2
Dc	1.333	1.260	1.234
μ, mm⁻¹	2.108	0.678	0.622
$F(000)$	3912	1344	664
Radiation type	CuK_α	CuK_α	CuK_α
Θmax (°)	70.00	70.00	70.00
Reflections collected	26110	6263	7780
Reflections with $I \geq 2\sigma(I)$	9377	3907	3222
Number of parameters	1490	440	845
Goodness-of-fit	1.070	0.836	0.821
Flack parameter	0.05(2)	0.5(3)	-0.5(7)
Refinement on	F^2	F^2	F^2
$R[F^2>2\sigma(F^2)]$, $wR(F^2)$, S	0.097, 0.265, 1.070	0.044, 0.107, 0.836	0.085, 0.228, 0.821
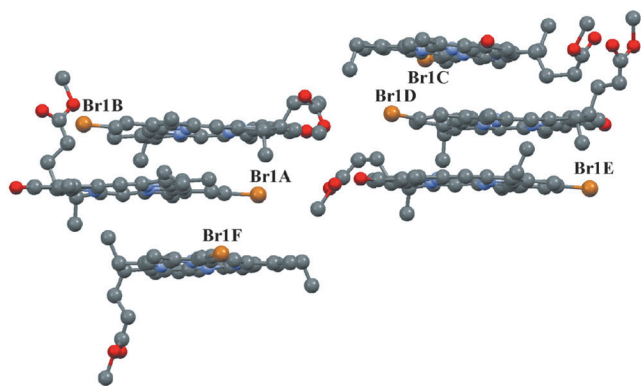
Figure 2. A content of asymmetric unit of **1** showing six independent molecules with the labeled bromine atoms. Non-H atoms are shown as spheres with arbitrary radii. H atoms were omitted for clarity.

Figure 3. Molecular structure of **2** showing the atomic numbering and 50 % probability displacement ellipsoids. C-bound H atoms were omitted for clarity.

maximal angle between mean planes does not exceed 5°. In structure **2**, stacks are stabilized by weak intermolecular C–H···C bonds [C(24)···C(34)=3.684 Å, C(24)···C(35)=3.688 and 3.702 Å]. The alternating molecules are additionally bonded through weak interactions between CH group of the reduced pyrrole ring and carboxylic oxygen of the $CH_2CH_2COOCH_3$ fragment [C(11)···O(2)=3.546 Å]. Neighboring

molecules in stacks are coplanar and separated by ~3.43 Å. There are only Van-der-Waals contacts between adjacent stacks. In structure **3** molecules are combined in stacks by weak intermolecular C–H···C bonds [C(4)···C(37) =3.630 Å, C(4A)···C(38)=3.652 Å, C(12)···C(40)=3.712 Å]. The molecules in the stacks are not coplanar; the angle between mean planes is 6.23°. The adjacent stacks are united by weak C–H···O contacts [O(1)···C(24A)=3.286 Å, O(1)···C(25A)= 3.193 Å, O(1A)···C(4)=3.476 Å, O(1A)···C(27)=3.309 Å,
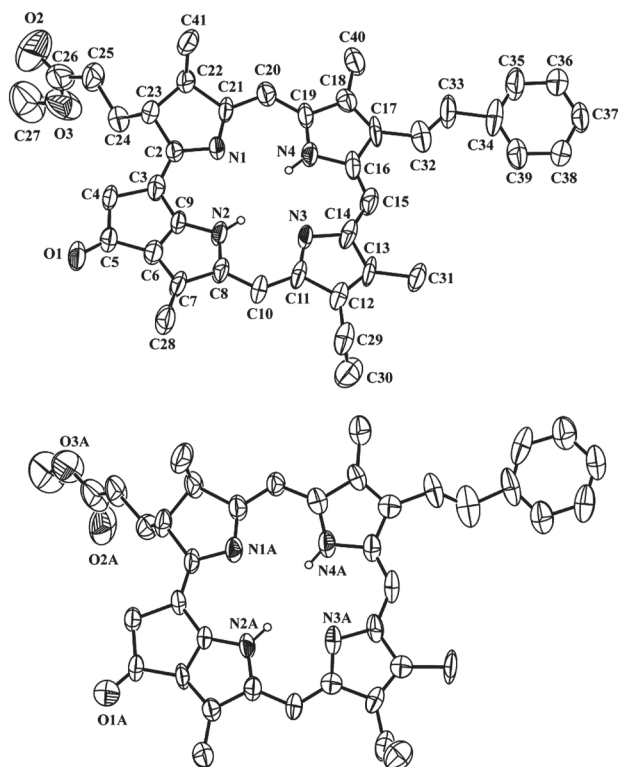
Figure 4. Two separately drawn independent molecules of **3** showing the atomic numbering and 30 % probability displacement ellipsoids. In the second independent molecule (at the bottom); labeling of carbon atoms corresponds to that in the first (top) molecule. C-bound H atoms were omitted for clarity.

O(3A)···C(25)=3.606 Å]. In the earlier reported structures,[11] π–π interactions of pyrrole rings of the pheophorbides adjacent molecules were found. In structure **1–3** this effect is not seen.

Compounds **1–3** were also studied by 1D and 2D NMR spectroscopy. The ^{1}H and ^{13}C NMR spectra were assigned using 2D {[1]H,[1]H} correlation spectroscopy (COSY), nuclear Overhauser effect spectroscopy (NOESY), {[1]H,[13]C} heteronuclear single quantum coherence (HSQC), and heteronuclear multiple bond correlation (HMBC) experiments.

The NMR characteristics for compounds **1–3** are provided as a supplementary material. The details of structure elucidation and signal assignment process are given below using (E)-3²-(2-methoxycarbonylvinyl)pyropheophorbide *a* methyl ester (**2**) as a representative example.

The assignment of the chlorin macrocycle signals was started from H5, H10 and H20. H20 was distinguished by the characteristic chemical shift, and H5 was distinguished from H5–H3¹ correlation in NOESY. Other observed spatial correlations (H20–H18, H20–H2¹, H5–H7¹, H7¹–H8², H8²–H10, H10–H12¹) have allowed assignment of H2¹, H7¹, H8¹, H8² (with COSY data), H12¹. Together with signals of the *meso*-protons, these signals were the basis for ^{13}C NMR spectrum assignment from distant heteronuclear correlations in HMBC. HSQC experiment has revealed all the one-bond correlation peaks thereby providing the ^{13}C NMR spectral data of the hydrogenated carbons. Selected {^{1}H,^{13}C} HMBC correlation are shown in Figure 5. It was found that the signals of the quaternary carbon atoms at the α-positions of the pyrrole rings of the macrocycle are situated in a lower
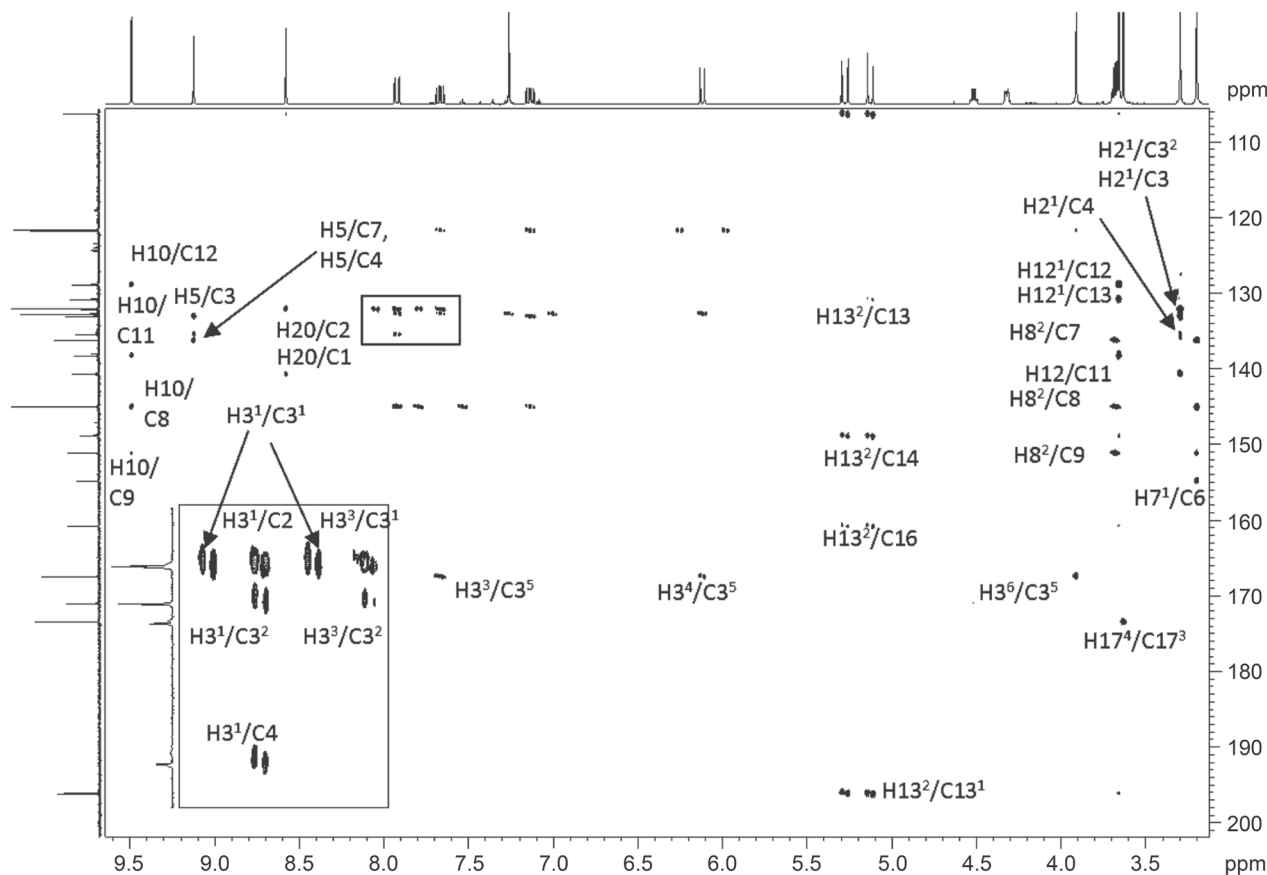

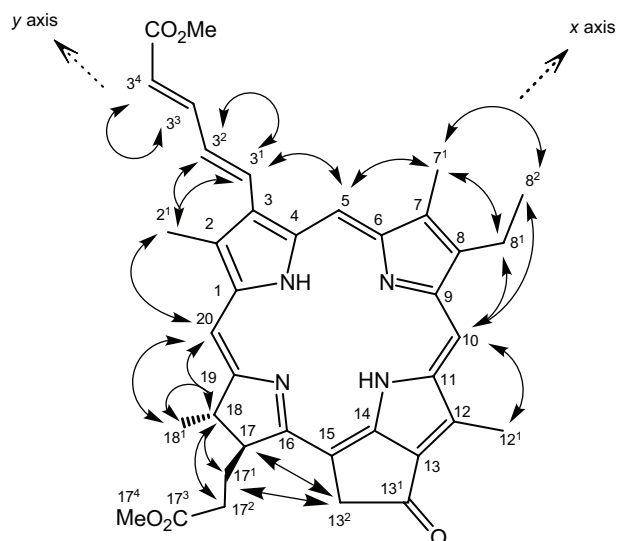**Figure 5.** ^{1}H–^{13}C HMBC spectrum of compound **2**. The quaternary carbon region.

Figure 6. Structure of compound **2**. Observed ^1H–^1H NOEs are marked with bi-headed arrows, including those not mentioned in the text.

field region compared to the corresponding β-carbons within the same pyrrole ring system apparently due to the deshielding effect of the nitrogen atom.

The side chains at positions 17 and 18 were separated using HMBC cross-peaks with ring carbons and assigned from COSY (H17–H17^1–H17^2) and HSQC which has revealed the chemical shifts of the diastereotopic protons at positions 17^1 and 17^2. The NOESY spectrum has shown the following correlations which confirmed the conformation of sp^3-nodes at positions 13^2, 17 and 18: H13^{2a}–H17, H17^{1a,b}–H18, H17^{2a}–H18, H18–H18^1.

The proton spin system of the pyrrole ring A unsaturated side chain was assigned by the data of COSY from H3^1 to H3^4. The *EE* configuration of the double bonds followed by the observed $^3J_{H3^1–H3^2}$ and $^3J_{H3^3–H3^4}$ of 15 Hz. The NOESY spectrum has also revealed the orientation of the C3-side chain. Observed H2^1–H3^1, H2^1–H3^2 NOE correlations are consistent with the X-ray data and sustain the represented conformation of the C3-substituent which aligned along the *y* axis of the macrocycle (Figure 6).

Conclusions

In summary, isothermal evaporation technique applied to solutions in the dichloromethane-ethanol mixture has allowed to grow single crystals of three methyl pyropheophorbide *a* derivatives. Combination of the X-

ray diffraction with 1D and 2D NMR spectroscopy has revealed details of molecular and crystal structures for (E)-3^2-bromopyropheophorbide *a* methyl ester (**1**), (E)-3^2-(2-methoxycarbonylvinyl)pyropheophorbide *a* methyl ester (**2**), and (E)-3^2-phenylpyropheophorbide *a* methyl ester (**3**). The results of two methods well correlate. Thus, conformation of the C3-side chain found by NMR is consistent with the X-ray data, showing that the C3-unsaturated substituent is aligned along the *y* axis of the macrocycle. The reported X-ray diffraction and NMR spectral data may be useful as a reference for characterization of the analogous compounds.

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