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SYNTHESIS OF CONJUGATES OF ISOBORNYLPHENOLS AND NATURAL CHLORINS

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A series of terpenophenol—chlorin conjugates containing terpenophenol and porphyrin fragments of various structure was synthesized. The terpenophenol fragment was added to the chlorin macrocycle using reactions of the exocyclic ester and the propionate substituent in the 17-position of methylpheophorbide a analogs.

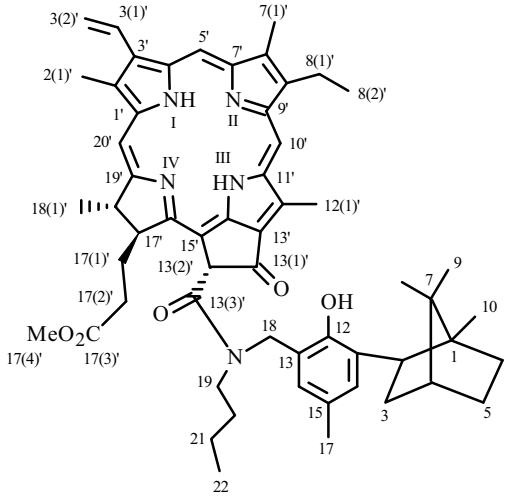
Key words: terpenophenols, chlorins, conjugates, methylpheophorbides.

Compounds with fragments of biologically active molecules might exhibit enhancement of known activity or manifestation of new activity because the biological activity can depend on not only the fragments that are combined in the conjugate but also the way in which they are joined.

Chlorophyll derivatives are the active principles of several antitumor preparations and can be used for therapy of viral diseases [1-3]. Terpenophenol derivatives are well known as biologically active compounds with low toxicity and a broad spectrum of action (radioprotectors, anticancer prophylactic preparations, electron transporters in the respiratory chain, psychotropic compounds, growth inhibitors of pathogenic fungi, exogenic and endogenic antioxidants, hepatoprotectors, etc.) [4-6]. Therefore, the synthesis of chlorophyll derivatives with a terpenophenol substituent from synthons with intrinsic physiological activity is of definite practical interest.

Herein we present results from a study of the potential synthesis of compounds containing terpenophenol and porphyrin fragments using amide and ester bonds.

We recently described the synthesis of conjugate **1** in which the *o*-isobornylcresol and methylpheophorbide *a* fragments were conjugated through an amide bond [7].



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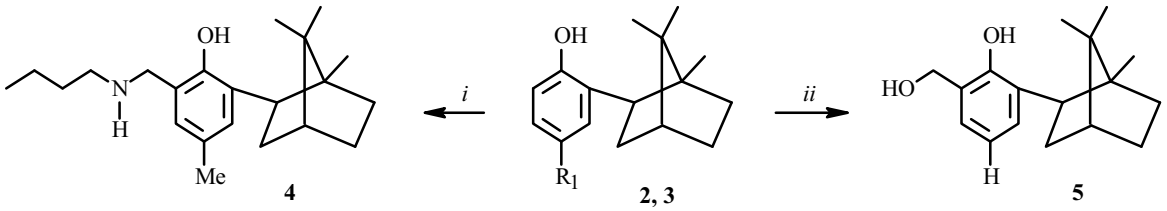
We attempted to modify the terpenophenols by adding an aminomethyl group to cresol **2** and a hydroxymethyl group to phenol **3**. This produced compounds **4** and **5** (Scheme 1) [7, 8].

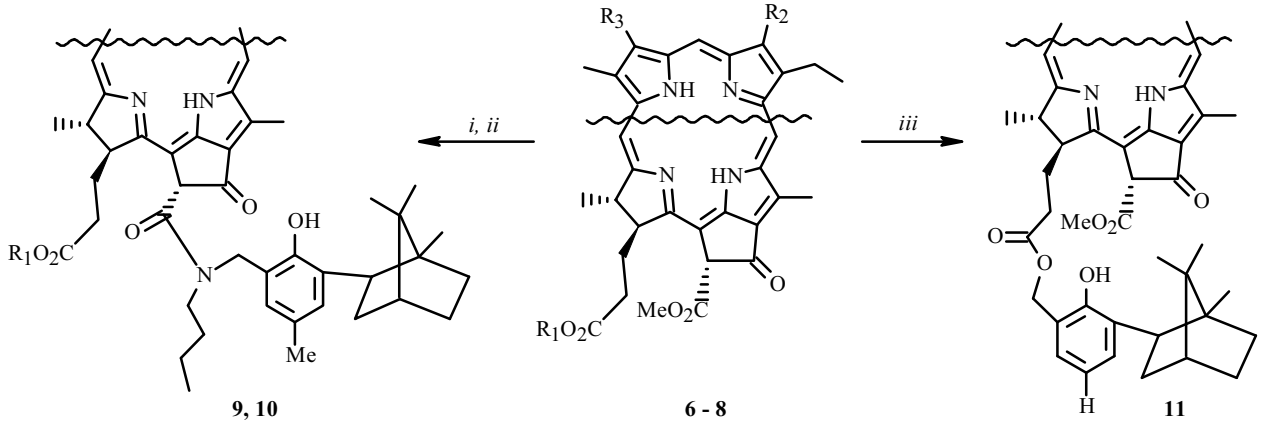


2: R_1 = Me; **3:** R_1 = H

i: a) **2**, Et_2NH, HCHO, benzene, reflux, 6 h; b) $BuNH_2$ (excess), reflux, 48 h, yield 43% [7];
ii: **3**, HCHO, H_3BO_3, toluene, reflux, 60 h, yield 71% [8]

Scheme 1

Methylpheophorbide *b* (**6**), methylpheophorbide *d* (**7**), and pheophorbide *a* (**8**) were used as the second component for synthesizing the conjugates (Scheme 2).



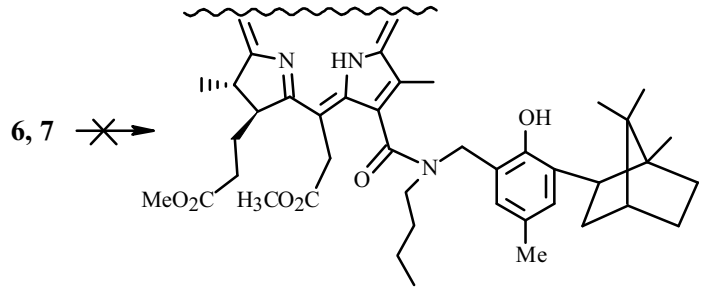
9, 10 **6 - 8** **11**

6, 9: R_1 = Me, R_2 = CHO, R_3 = $CH=CH_2$; **7, 10:** R_1 = R_2 = Me, R_3 = CHO
8: R_1 = H, R_2 = Me, R_3 = $CH=CH_2$; **11:** R_2 = Me, R_3 = $CH=CH_2$

i: **6** → **9**: **4**, toluene, reflux, 24 h, yield 28%; *ii:* **7** → **10**: **4**, toluene, reflux, 12 h, yield 42%;
iii: **8** → **11**: a) $SOCl_2$; b) **5**, Et_3N, CH_2Cl_2, 25°C, 8 h, yield 46%

Scheme 2

It is known that amidation of the ester in the 13(2)-position of methylpheophorbide *a* and its analogs by amines can open the exocycle to form the corresponding chlorin e_6 13-amides [1, 9-15]. The reaction of methylpheophorbide *a* using a five-fold excess of amine **4** and a substantially longer reaction time (up to 48 h) did not open the exocycle. Because this reaction is nucleophilic substitution at the carbonyl C atom, bulky substituents on the N atom in **4** create steric hindrance to the reaction of the components. Furthermore, this reaction may not proceed at all if the partial positive charge on the carbonyl C atom in the 13(1)-position is too small. Introducing into the macrocycle an electron-accepting aldehyde can substantially increase the reactivity of the exocycle toward amines [16, 17]. Therefore, we investigated the reaction of amino derivative **4** with porphyrins **6** and **7** in order to prepare analogs of chlorin e_6 with a terpenophenol fragment. Refluxing in toluene formed the corresponding 13-amides **9** and **10**, analogs to conjugate **1**, without opening the exocycle (Scheme 3).



Scheme 3

As it turned out, the exocyclic ester of methylpheophorbide *a* analogs (**6** and **7**) was less reactive toward amidation than methylpheophorbide *a* itself. This increased the reaction time (from 6 to 12-24 h) and decreased the preparative yield of the products. The reduced reactivity might have been due to a decreased tendency of the formyl analogs of methylpheophorbide *a* toward enolization, which facilitates the amidation [7]. The lower preparative yield was probably due to the greater tendency of the formyl analogs of methylpheophorbide *a* (**6** and **7**) to form tars.

The hydroxymethyl derivative of *o*-isobornylphenol **5** was used to synthesize conjugate **11**, in which the porphyrin and terpenophenol fragments were joined by an ester bond in the 17-position. Because the ester on the periphery of the chlorin macrocycle is less reactive toward nucleophiles, the corresponding acid chloride was used to synthesize **11**. It was prepared *in situ* by reacting pheophorbide *a* (**8**) and thionyl chloride. In contrast with the amide derivatives **9** and **10** described above, the phenol hydroxyl of conjugate **11** apparently was involved in formation of a H-bond. This was consistent with the significant shift of the resonance for the OH proton to weak field in PMR spectra compared with the same resonances in spectra of phenols **4** and **5** and conjugates **9** and **10**.

Because starting terpenophenols **2** and **3** and their derivatives **4** and **5** are mixtures of enantiomers, conjugates **9-11** are mixtures of diastereomers. The doubling of certain proton resonances (terpenophenol fragment in spectra of **9** and **10** and chlorin macrocycle in the spectrum of **11**) indicated that these diastereomers were present.

Thus, reaction of both the carboxyl in the 17′-position and the exocyclic ester can be used to introduce a terpenophenol fragment onto the periphery of the chlorin macrocycle.

EXPERIMENTAL

PMR spectra in $CDCl_3$ were recorded on Bruker Avance II and DRX-400 spectrometers (operating frequency 300 and 400 MHz, respectively); IR spectra, on a Shimadzu IR Prestige 21 IR-Fourier spectrometer in thin layers and KBr disks. Melting points were determined on a Kofler apparatus. The course of reactions was monitored using TLC on Sorbfil and Silufol plates. Toluene was dried over $CaCl_2$ and distilled over metallic sodium. We used petroleum ether (bp 65-70°C). Thionyl chloride, CCl_4, Et_2O, and acetone were freshly distilled. Column chromatography used silica gel (Alfa Aesar 70/230 μ) (packed wet) and triethylamine (Sigma-Aldrich). Compounds **4** and **5** were prepared from *o*-isobornylcresol and *o*-isobornylphenol by the literature methods [7, 8]. Methylpheophorbide *b* (**6**) and pheophorbide *a* (**8**) were obtained from nettles [18, 19]. Methylpheophorbide *d* (**7**) was prepared from methylpheophorbide *a* by the literature method [20]. The spectral properties of **6-8** agreed with those reported [11, 12, 20, 21].

Methylpheophorbide *b* 13(2)-*N*-butyl-*N*-(2-hydroxy-3-(exo-1,7,7-trimethylbicyclo-[2.2.1]hept-2-yl)-5-methylbenzyl)amide (9). Methylpheophorbide *b* (**6**, 0.10 g, 0.16 mmol) was dissolved in dry toluene (5 mL), treated with amine **4** (0.11 g, 0.33 mmol), and refluxed for 24 h. After the reaction was finished, the excess of solvent was distilled at reduced pressure. The reaction mixture was separated by column chromatography over silica gel (eluent CCl_4:acetone with increasing fraction of acetone) to afford **9** (0.04 g, 28%) as dark green crystals, mp 122-124°C. IR spectrum (KBr, cm^{-1}): 2729 (C–H, aldehyde), 1738 (ester C=O), 1699 (C=O, exocycle, aldehyde), 1641 (chlorin band, amide I). $C_{57}H_{67}N_5O_6$. PMR spectrum ($CDCl_3$, 300 MHz, δ, ppm, J/Hz): **aminomethylterpenophenol fragment**: 0.68 (3H, s, Me-10), 0.80 (3H, s, Me-9), 0.92/0.90 (3H, s, Me-8) (two diastereomers, 1:1), 1.44-2.46 (13H, m, CH_2-3, CH_2-5, CH_2-6, CH_2-20, CH_2-21, Me-22), 2.28 (3H, s, Me-17), 3.10 (1H, m, H-2), 3.31-3.40 (2H, m, CH_2-19), 3.54-3.57 (2H, m, CH_2-18), 3.60 (1H, m, H-4), 5.22/5.27 (1H, br.s, OH)

(two diastereomers, 1:1), 7.00 and 7.21 (1H each, both s, H-14, H-16); **chlorin fragment**: 1.73 (1H, br.s, NH-III), 0.29 (1H, br.s, NH-I), 1.55-1.85 [6H, m, Me-18(1′ and Me-8(2′], 2.04-2.47 [4H, m, CH_2-17(1′ and CH_2-17(2′], 3.40 [3H, s, Me-2(1′], 3.61 [3H, s, Me-17(4′], 3.62-3.71 [2H, m, CH_2-8(1′], 3.50 [3H, s, Me-12(1′], 4.46 (1H, m, H-18′), 5.34-5.16 (1H, m, H-17′), 6.24 [1H, d, J = 11.6, H *cis*-3(2′], 6.39 [1H, d, J = 18.0, H *trans*-3(2′], 6.65 [1H, s, H-13(2′], 8.01 [1H, dd, J = 18.0, 12.0, H-3(1′], 8.52 (1H, s, H-20′), 9.35 (1H, s, H-5′), 10.18 (1H, s, H-10′), 10.98 (1H, s, CHO).

Methylpheophorbide *d* 13(2)-*N*-butyl-*N*-(2-hydroxy-3-(exo-1,7,7-trimethylbicyclo-[2.2.1]hept-2-yl)-5-methylbenzyl)amide (10) was prepared from methylpheophorbide *d* (**7**) analogously to **9** by refluxing in toluene for 12 h. Yield 42%, dark green crystals, mp 105-107°C. IR spectrum (KBr, cm^{-1}): 2780 (C–H, aldehyde), 1738 (ester C=O), 1699 (C=O, exocycle), 1678 (C=O, aldehyde), 1639 (chlorin), 1620 (amide-I). $C_{56}H_{67}N_5O_6$. PMR spectrum ($CDCl_3$, 300 MHz, δ, ppm): **aminomethylterpenophenol fragment**: 0.83 (3H, s, Me-10), 0.89 (3H, s, Me-9), 0.90 (3H, s, Me-8), 1.44-2.46 (13H, m, CH_2-3, CH_2-5, CH_2-6, CH_2-20, CH_2-21, Me-22), 2.28 (3H, s, Me-17), 3.12 (1H, m, H-2), 3.26-3.34 (2H, m, CH_2-19), 3.53-3.58 (2H, m, CH_2-18), 3.60 (1H, m, H-4), 5.37/5.39 (1H, br.s, OH) (two diastereomers, 1:1), 6.99 and 7.17 (1H each, both s, H-14, H-16); **chlorin fragment**: 1.90 (1H, br.s, NH-III), 0.56 (1H, br.s, NH-I), 1.55-1.85 [6H, m, Me-18(1′ and Me-8(2′], 2.04-2.47 [4H, m, CH_2-17(1′ and CH_2-17(2′], 3.32 [3H, s, Me-2(1′], 3.76 [3H, s, Me-17(4′], 3.62-3.71 [2H, m, CH_2-8(1′], 3.47 [3H, s, Me-12(1′], 3.73 [3H, s, Me-7(1′], 4.46 (1H, m, H-18′), 5.34-5.16 (1H, m, H-17′), 6.77 [1H, s, H-13(2′], 8.81 (1H, s, H-20′), 9.62 (1H, s, H-5′), 10.28 (1H, s, H-10′), 11.53 (1H, s, CHO).

Pheophorbide *a* 17-(2-hydroxy-3-(exo-1,7,7-trimethylbicyclo[2.2.1]hept-2-yl)benzyl)-benzyl Ester (11). Compound **8** (0.1 g, 0.17 mmol) was dissolved in freshly distilled thionyl chloride (2 mL). The resulting solution was evaporated to dryness at reduced pressure. The solid after evaporation was dissolved in CH_2Cl_2 (3 mL), treated with **5** (0.13 g, 0.51 mmol) dissolved in CH_2Cl_2 (2 mL) and then Et_3N (0.2 mL). The mixture was stirred at room temperature for 8 h and separated by column chromatography over silica gel (eluent petroleum ether:Et_2O, then CCl_4:acetone). Precipitation by pentane from $CHCl_3$ produced ester **11** (0.05 g, 46%) as dark green crystals, mp 80-84°C. IR spectrum (KBr, cm^{-1}): 1624 (chlorin), 1746 (ester C=O), 1706 (C=O, exocycle). PMR spectrum ($CDCl_3$, 400 MHz, δ, ppm, J/Hz): **terpenophenol fragment**: 0.67/0.68 (3H, s, Me-10) (two diastereomers, 1:1), 0.79 (3H, s, Me-9), 0.81 (3H, s, Me-8), 1.41-1.61, 1.81-1.90, 2.00-2.68 (6H, all m, CH_2-3, CH_2-5, CH_2-6), 3.24-3.30 (2H, m, H-2, H-4), 4.89 and 5.07 (1H each, both d, J = 12.2, CH_2-17)/4.94 and 5.16 (1H each, both d, J = 12.4, CH_2-17) (two diastereomers, 1:1), 6.76-6.81, 6.95-6.98 (2H, both m, H-14, H-16), 7.27-7.30 (1H, m, H-15), 7.72/7.73 (1H, s, OH) (two diastereomers, 1:1); **chlorin fragment**: 1.46 (1H, br.s, NH-III), 0.51 (1H, br.s, NH-I), 1.69 [3H, t, J = 7.6, Me-8(2′], 1.74 [3H, d, J = 7.2, Me-18(1′]/1.76 [3H, d, J = 7.2, Me-18(1′] (two diastereomers, 1:1), 2.00-2.68 [4H, m, CH_2-17(1′ and CH_2-17(2′], 3.22 [3H, s, Me-7(1′], 3.39 [3H, s, Me-2(1′], 3.62-3.71 [2H, m, CH_2-8(1′], 3.68 [3H, s, C-12(1′], 3.87/3.88 [3H, s, Me-13(4′] (two diastereomers, 1:1), 4.15-4.50 (2H, m, H-17′, H-18′), 6.17 [1H, d, J = 11.2, H *cis*-3(2′], 6.23 [1H, s, H-13(2′], 6.28 [1H, d, J = 17.2, H *trans*-3(2′], 7.98 [1H, dd, J = 17.2, 11.6, H-3(1′], 8.50/8.52 (1H, s, H-20′) (two diastereomers, 1:1), 9.37 (1H, s, H-5′), 9.51 (1H, s, H-10′).

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