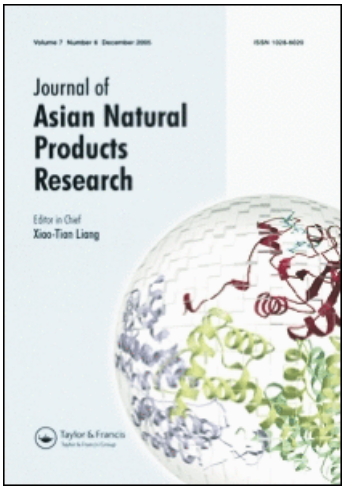

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New tetracyclic diterpenoid and new ceramides from the soft coral Sinularia conferta

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New tetracyclic diterpenoid and new ceramides from the soft coral *Sinularia conferta*

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The new tetracyclic diterpenoid confertdiate (**1**) and two new ceramides, (**2**) and (**3**), have been isolated from the soft coral *Sinularia conferta*, collected from Sanya Bay, Hainan Island, China. Their structures have been elucidated by spectroscopic analysis, and comparison of the ^{13}C NMR data with those of the known diterpenoid isomandapamate confirmed the structure of **1**.

Keywords: Soft coral; *Sinularia conferta*; Tetracyclic diterpenoid; Ceramide

1. Introduction

Soft corals are distributed widely in the South China Sea. During our studies on bioactive natural products from marine organisms, we have examined many species of soft coral, and found various new metabolites [1,2]. Recently, a new sesquiterpene, named confertol, was isolated from the soft coral *Sinularia conferta* [3]. Further separation of the same material led to the isolation of three compounds. Compound **1**, confertdiate, was characterized as a new tetracyclic diterpenoid; compounds **2** and **3**, namely confertamide A and confertamide B, are new ceramides (figure 1).

2. Results and disscussion

A methanolic extract of the soft coral *Sinularia conferta* was subjected to silica gel chromatography and HPLC to afford compounds **1**–**3**.

Confertdiate (**1**) was obtained as colorless viscous oil. Its molecular formula of $C_{25}H_{34}O_8$ was established by HR-EIMS, which showed the $[M]^+$ peak at m/z 462.2270. Its IR spectrum showed a hydroxyl group (3348 cm^{-1}), an α,β-unsaturated ester (1723, 1695 cm^{-1}), and olefinic double bonds (1642, 897 cm^{-1}).

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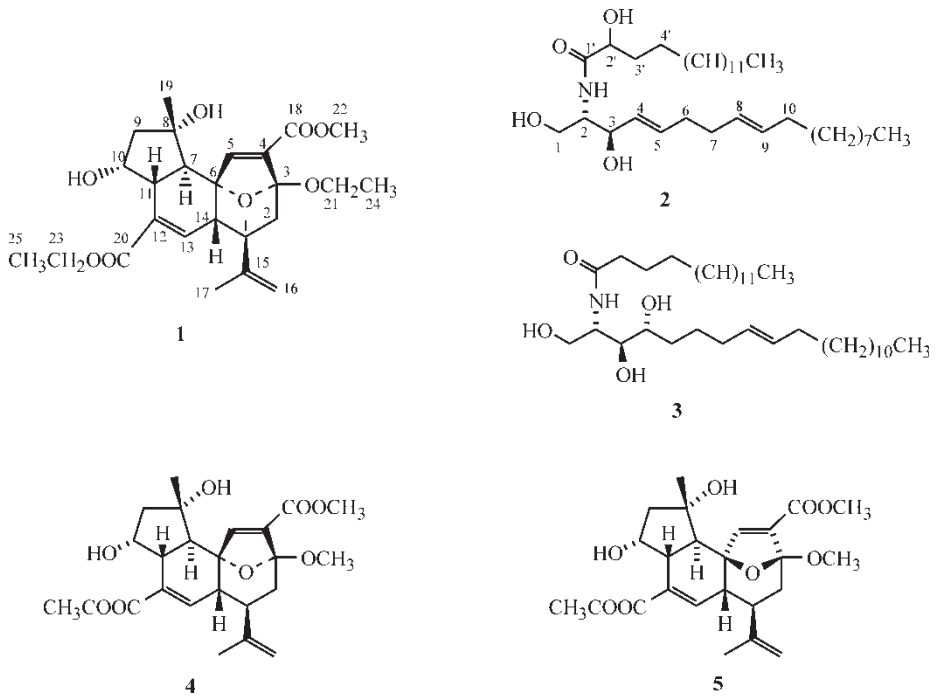

Figure 1. Structures of compounds **1**–**5**.

The ^{1}H NMR spectrum of **1** revealed an isopropenyl group with signals at δ 4.88 and 4.78 (each 1H, br. s) and 1.69 (3H, s). Two olefinic protons at δ 6.68 (s, H-5) and 6.69 (d, $J = 3.0$ Hz, H-13) accounted for the olefinic protons of two α,β-unsaturated ester functionalities. A signal at δ 3.78 (3H s) indicated a methoxyl group and one carbinolic methine proton at δ 4.64 (H-10) demonstrated a secondary hydroxyl.

The ^{13}C NMR data revealed two conjugated ester carbonyls [δ 162.8 (s) and 166.5 (s)], six olefinic carbons [δ 135.4 (s), 150.1 (d), 133.8 (s), 143.4 (d) 144.4 (s) and 113.6 (t)], accounting for two trisubstituted double bonds and one terminal double bond respectively. The two oxygen-bearing carbons at δ 67.7 (d) and 77.5 (s) are attributed to carbons linking a secondary and a tertiary hydroxyl, respectively. Its molecular formula showed nine degrees of unsaturation, two for esters and three for double bonds, indicating the molecule might be a tetracyclic diterpenoid.

The ^{1}H–^{1}H COSY and ^{13}C–^{1}H COSY (HMQC) spectra were used to assign the proton resonance (table 1). In the ^{1}H–^{1}H COSY spectrum the signal at δ 2.25 (H-14) is coupled to δ 6.69 (H-13) and 2.18 (H-1), and, in turn, H-1 correlates to δ 2.00 (H-2). The proton signal at δ 2.51 (H-11) is coupled with δ 2.73 (H-7) and 4.64 (H-10), the latter in turn showing coupling with δ 2.05 (H-9). In addition, two spin systems for 2 × O–CH_2CH_3 have been assigned. The molecule contains eight oxygenated carbons, seven of which are accounted for by two esters, two hydroxyls, and a methoxyl group. The eighth at δ_C 110.9 (s) is considered to be a doubly oxygenated tertiary carbon, strongly suggesting a ketal function [4].

A literature survey revealed that two natural tetracyclic diterpenoids, isomamdapamate (**4**) [4] and mamdapamate (**5**) [5], have been isolated from the same genus (*Sinularia*). They were two diastereomers with regard to the chiral carbons C-3 and C-6. The unique difference in the ^{13}C NMR data was the signal for C-5. On inspection, of the ^{13}C NMR data of **1**, that from C-1 to

Table 1. NMR data of **1**, **4**, and **5**.

	1*		**4**†	**5**†
No.	δ_C	δ_H *(J, Hz)*	δ_C	δ_C
1	43.2 d	2.18 m	43.4 d	43.2 d
2	33.7 t	2.00 dd, 13.0, 5.0 1.69 dd, 11.0, 5.0	33.5 t	33.4 t
3	110.9 s		111.2 s	111.1 s
4	135.4 s		135.0 s	134.8 s
5	150.1 d	6.68 s	150.5 d	154.4 d
6	84.6 s		84.8 s	84.7 s
7	52.7 d	2.73 d 13.0	52.8 d	52.6 d
8	77.5 s		77.5 s	77.5 s
9	51.2 t	2.24 m 2.05 br. d, 14.5	51.2 t	51.3 t
10	67.7 d	4.64 m	67.7 d	67.6 d
11	47.2 d	2.51 br. d, 13.0	47.2 d	46.9 d
12	133.8 s		133.6 s	133.5 s
13	143.4 d	6.69 d, 3.0	143.2 d	143.2 d
14	43.3 d	2.25 m	43.4 d	43.2 d
15	144.4 s		144.4 s	144.2 s
16	113.5 t	4.88 br. s 4.78 br. s	113.6 t	113.4 t
17	19.9 q	1.69 s	19.8 q	19.6 q
18	162.8 s		162.0 s	162.5 s
19	27.0 q	1.59 s	27.0 q	26.8 q
20	166.5 s		166.8 s	166.6 s
21	60.0 t	3.78 m 3.51 m	51.1 q	51.8 q
22	51.8 q	3.78 s	51.9 q	51.8 q
23	61.3 t	4.23 q, 6.0	51.8 q	52.0 q
24	14.1 q	1.24 t, 6.0		
25	15.3 q	1.30 t, 6.0		

* $CDCl_3$, 125 and 500 MHz for ^{13}C for ^{1}H NMR, respectively.
† $CDCl_3$, 22.5 MHz for ^{13}C NMR.

C-20 agrees very well with those of **4** (table 1), indicating that **1** has the same diterpene skeleton as **4**. The significant differences between **1** and **4** are the composition of ester and ether moieties. Compound **1** has an ethoxyl group, one carbomethoxyl and one carboethoxyl groups, whereas **4** has one methoxyl and two carbomethoxyl groups. The HMBC of **1** shows correlation of the methoxy proton at δ3.78 (H_3-22) as well as the olefinic proton at δ6.68 (H-5) to the carbonyl carbon at δ 162.8 (C-18), indicating a carbomethoxy connected to C-4 (figure 2). In addition, the HMBC of **1** shows that the carboethoxy is connected to another olefinic carbon (C-12). The appearance of the C-5 at δ 150.1 suggests that **1** has the same relative stereochemistry as **4**. The structure of confertdiate was thus established as **1**.

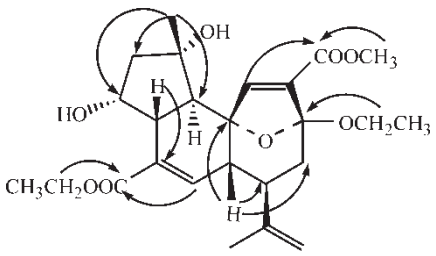

Figure 2. Selected HMBC correlations of **1**.

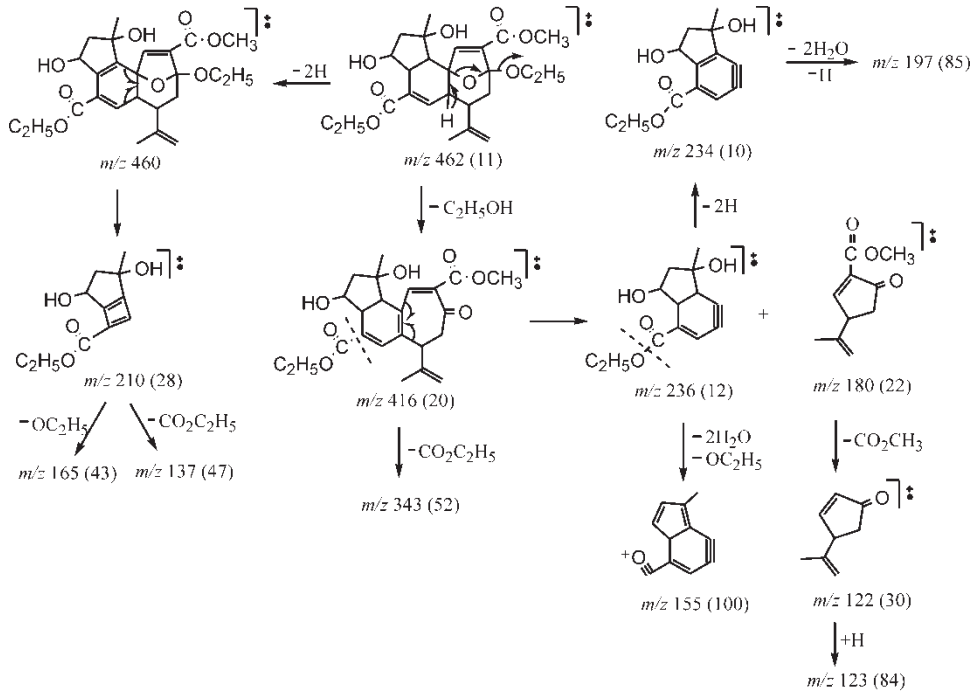

Figure 3. EI-MS fragmentation pattern of **1**.

A careful examination of the MS fragmentation confirmed the structure of **1** as shown in figure 3.

Confertamide A (**2**) was obtained as a white amorphous powder. Its FAB-MS spectrum shows a quasi-molecular ion peak at *m/z* 552 $[M + H]^+$, and the molecular formula of **2** was established as $C_{34}H_{65}NO_4$ in combination with the NMR data. In its ^1H NMR spectrum an intense proton signal at δ 1.20–1.48 and two methyl signals at δ 0.87 ($2 \times CH_3$, t, $J = 7.0$ Hz) reveal the presence of two long-chain aliphatic moieties. A down-field proton signal at δ 7.14 (1H, d, $J = 8.0$ Hz), a carbonyl carbon signal at δ 175.3 (s), and a strong absorption band at 1630, 1534 cm^{-1} in the IR spectrum indicate a –CONH– group. The ^1H, ^{13}C NMR and HMQC spectra suggested the presence of one primary [δ_C 62.1 (t), δ_H 3.90 (1H, m), 3.73 (1H, br. d, $J = 9.5$ Hz)] and two secondary hydroxyl groups [δ_C 74.3 (d), 72.5 (d); δ_H 4.28 (1H, t, $J = 6.5$ Hz), 4.10 (1H, t, $J = 4.0$ Hz)], and two disubstituted double bonds [δ_C 133.8 (d), 131.4 (d), 129.0 (d), 128.9 (d); δ_H 5.79 (1H, dt, $J = 15.0$, 6.5 Hz), 5.37 (1H, dt, $J = 15.0$, 6.0 Hz), 5.52 (1H, dd, $J = 15.0$, 6.5 Hz), 5.41 (1H, dt, $J = 15.0$, 5.5 Hz)]. In the ^1H–^1H COSY spectrum the amide proton at δ 7.14 (NH) is coupled with the methine proton at δ 3.88 (m, H-2), which in turn is coupled with three protons at δ 3.90 (m, H-1a), 3.73 (br. d, $J = 9.5$ Hz, H-1b) and 4.28 (t, $J = 6.5$, H-3). The proton at δ 4.28 (H-3) is coupled with the protons at δ 3.88 (H-2) and 5.52 (H-4). In addition, the ^1H–^1H COSY and HMQC spectra revealed the partial structure: $(OH)CH_2$–CH(NH)–CH═CH–CH_2–CH_2–CH═CH–CH_2–. The large coupling between the olefinic protons ($J = 15.0$ Hz) indicates that both double bonds have *E* configurations. The above spectral data suggest that **2** is a ceramide of the *N*-acyl-sphinga-4(*E*),8(*E*)-dienine class. Methanolysis of **2** gave methyl 2-hydroxyhexadecanoate, detected by GC-MS, as the unique product.

Thus the two long-chain components were unambiguously established as C_{16} and C_{18}. Therefore, **2** should be *N*-2-hydroxyhexadecanoyl-octadecasphinga-4(*E*),8(*E*)-dienine.

The ^{13}C NMR data for C-1, C-2, C-3 and C-4 of **2** are identical with those of *N*-palmitoyl-D-*erythro*-(2*S*,3*R*)-octadecaspinga-4(*E*),8(*E*)-dienine [6], which in combination with the optical rotation suggest that **2** has the same stereochemistry. Therefore, the structure of **2** was determined as *N*-2-hydroxy-hexadecanoyl-D-*erythro*-(2*S*,3*R*)-octadecasphinga-4(*E*),8(*E*)-dienine.

Confertamide B (**3**) was isolated as colorless flakes. A molecular formula of $C_{36}H_{73}NO_4$ was determined by FABMS, ^{13}C NMR and DEPT spectral data. The ^1H and ^{13}C NMR spectra of **3** differ from those of **2** in the absence of signals for two double bonds. Methanolysis of **3** with $MeOH-H_2SO_4$ followed by GC-MS analysis established octadecanoate as the unique product. Therefore, all three hydroxyl groups must be situated in the C_{18} sphingosine moiety. The HMQC and $^1H-^1H$ COSY spectra show correlations between the protons at δ 3.75 (H-2)/3.92 (H-1a) and δ 3.62 (H-1b); δ 3.75 (H-2)/4.13 (H-3); δ 4.13 (H-3)/3.60 (H-4), revealing that these hydroxyl groups should be located at C-1, C-3 and C-4, respectively. The ^{13}C NMR data as well as the optical rotation of **3** agree well with those of a closely related ceramide [7]. Thus, the structure of **3** was determined as *N*-octadecanoyl-D-*erythro*-(2*S*,3*S*,4*R*)-4-hydroxyloctadecsphigosine.

3. Experimental

3.1 General experimental procedures

Melting points were determined using an X-6 micro-melting point apparatus and are uncorrected (Taike Instrumental Company, Beijing). Optical rotations were obtained on a Schmidt-Haensch Polaptronic HNQW 5 polarimeter. IR spectra were recorded on an EQUINOX55 (Bruker) spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on a Varian Unity INOVA spectrophotometer at 500 and 125 MHz, respectively, in $CDCl_3$ using TMS as internal standard. MS spectra were measured on a VG ZAB-HS mass spectrometer with *m*-nitrobenzyl alcohol (NBA) as a matrix. Silica gel 200–300 μm (Qingdao Chemical Industries Co., Ltd.) was used for column chromatography.

GC-MS analysis was performed with a Finnigan-Voyager instrument equipped with a DD-5MS capillary column (30 m long, 0.25 mm i.d., 0.25 μ film thickness). Chromatographic conditions: column oven increased from 80 to 250°C at 15° min^{-1}, then kept at 250°C for 5 min, with helium as carrier gas. Preparative HPLC was performed on a Perkin–Elmer series 200 liquid chromatography with a Perkin–Elmer LC-235 diode array detector operating at 210–280°C, using MeOH as the mobile phase. A Microsorb 7 μm Module C-18 semi-preparative column (∅10 × 300 mm) was used.

3.2 Animal material

The soft coral *Sinularia conferta* (Alcyoniidae) was collected from Sanya Bay, Hainan Island, China. A voucher specimen (No. 98-SY-1) has been deposited in the Research Center of Organic Natural Products, Zhongshan University.

3.3 Extraction and isolation

The soft coral *Sinularia conferta* (660 g, dry wt.) was cut into small slices, and extracted with MeOH (3 L) three times, then twice with MeOH–CH_2Cl_2 (1:1) at room temperature. The solvent was evaporated under reduced pressure to give a dark residue (66 g), which was dissolved in 10% aqueous MeOH, and extracted with n-hexane. The n-hexane extract (A) was concentrated under vacuum and gave a residue (18 g). The resulting aqueous solution was diluted to 30% H_2O in MeOH, and partitioned with CH_2Cl_2 to give extract B (10 g).

Extract B was subjected to silica-gel column chromatography eluting with a gradient of light petroleum (60–80°C)–EtOAc. The fraction that eluted with light petroleum–EtOAc (3:1) was repeatedly chromatographed and further separated by reversed-phase HPLC (MeOH) to afford compound **1** (9 mg).

Extract A was subjected to silica-gel column chromatography, using increasing amounts of EtOAc in light petroleum (60–80°C) as eluent. The fraction that eluted with light petroleum–EtOAc (1:1) was repeatedly chromatographed over silica gel to give compound **2** (18 mg). The fraction that eluted with light petroleum–EtOAc (2:3) was further purified by chromatography over silica gel to afford compound **3** (16 mg).

Confertdiate (**1**): a colorless oil, $[\alpha]_D^{20} + 124.0$ (*c* 0.40, MeOH). IR (KBr) ν_{max} (cm^{-1}): 3348, 1723, 1695, 1642, 1176, 1132, 1070, 1026, 985, 945, 897, 770; EI-MS *m/z* (rel. int.) 462 $[M]^+$ (11), 460 (4), 416 (20), 343 (52), 236 (12), 234 (10), 210 (28), 197 (85), 180 (22), 165 (43), 155 (100), 137 (47), 123 (84), 122 (30). HR-EIMS *m/z* 462.2270 $[M]^+$, (calcd for $C_{25}H_{34}O_8$ 462.2254).

Confertamide A (**2**): $C_{34}H_{65}NO_4$, a white powder, mp 103–104°C, $[\alpha]_D^{20} + 10.1$ (*c* 0.19, $CHCl_3$). IR (KBr) ν_{max} (cm^{-1}): 3355, 3278, 2919, 2841, 1631, 1534, 1466, 1433, 1080, 964, 722. ^{1}H NMR ($CDCl_3$) δ (ppm): 7.14 (d, $J = 9.0$ Hz, NH), 5.79 (dt, $J = 15.0$, 7.0 Hz, H-5), 5.52 (dd, $J = 15.0$, 6.5 Hz, H-4), 5.41 (dt, $J = 15.0$, 5.5 Hz, H-8), 5.37 (dt, $J = 15.0$, 6.0 Hz, H-9), 4.28 (t, $J = 6.5$ Hz, H-3), 4.10 (t, $J = 4.0$ Hz, H-2′), 3.90 (m, H-1a), 3.88 (m, H-2), 3.73 (br. d, $J = 9.5$ Hz, H-1b), 3.23 (br. s, C_2'-OH), 3.12 (br. s, C_1-OH), 2.92 (br. s, C_3-OH), 2.11 (t, $J = 7.5$ Hz, H_2-7), 2.09 (t, $J = 6.0$ Hz, H_2-6), 1.98 (q, $J = 7.0$ Hz, H_2-10), 1.82 (m, H-3′a), 1.63 (m, H-3′b), 1.42 (m, H-4′), 1.20–1.48 (m, 46H), 0.87 (t, $J = 7.0$ Hz, 2 × CH_3); ^{13}C NMR ($CDCl_3$) δ (ppm): 62.1 (CH_2, C-1), 54.6 (CH, C-2), 74.3 (CH, C-3), 129.0 (CH, C-4), 133.8 (CH, C-5), 32.6 (CH_2, C-6), 32.4 (CH_2, C-7), 131.4 (CH, C-8), 128.9 (CH, C-9), 31.9 (CH_2, C-10), 29.7–29.2 (18 × CH_2), 14.1 (CH_3, C-18), 175.3 (C═O, C-1′), 75.2 (CH, C-2′), 34.8 (CH_2, C-3′), 25.1(CH_2C-4′), 14.1 (CH_3, C-16′). FAB-MS *m/z*: 552 $[M + H]^+$, 534 $[M + H - H_2O]^+$.

Confertamide B (**3**): $C_{36}H_{73}NO_4$, colorless flakes, mp 121–123°C, $[\alpha]_D^{20} + 13.5$ (*c* 0.33, MeOH). IR (KBr) ν_{max} (cm^{-1}): 3340, 2920, 2851, 1640, 1547, 1467, 1375, 1278, 1256, 1108, 1046, 721. ^{1}H NMR ($CDCl_3$) δ (ppm): 6.40 (1H, d, $J = 8.0$ *Hz*, NH), 4.13 (1H, br. s, H-3), 3.92 (1H, dd, $J = 10.5$, 2.5 Hz, H-1a), 3.75 (1H, m, H-2), 3.60 (1H, m, H-4), 3.62 (1H, m, H-1b), 2.24 (2H, m, H_2-2′), 1.78 (1H, m, H-5a), 1.62 (1H, m, H-5b), 1.20–1.50 (58H, m,), 0.88 (6H, t, $J = 7.0$ Hz, 18′-CH_3, 18-CH_3); ^{13}C NMR ($CDCl_3$) δ (ppm): 62.1 (CH_2, C-1), 53.2 (CH, C-2),76.5 (CH, C-3), 73.0 (CH, C-4), 33.4 (CH_2, C-5), 29.7–29.4 (24 × CH_2), 22.7 (CH_2, C-17), 14.1 (CH_3, C-18), 174.5 (C═O, C-1′), 36.8 (CH_2, C-2′), 25.8 (CH_2, C-3′), 22.7 (CH_2, C-17′), 14.1 (CH_3, C-18′). FABMS *m/z*: 584 $[M + H]^+$.

3.4 Methanolysis of 2 and 3

The sample (3 mg) was added to a 2 mol L^{-1} H_2SO_4–MeOH solution and boiled under reflux for 6 h. The resultant mixture was then treated by usual procedure. The *n*-hexane extract was analyzed by GC-MS under the general conditions mentioned above. Methyl 2-hydroxyhexadecanoate and methyl octadecanoate were detected, respectively, from the hydrolysis mixtures of **2** and **3**.

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