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SYNTHESIS OF THE ω6 (5*Z*,8*Z*)-TETRADECADIENOIC AND (7*Z*,10*Z*)-HEXADECADIENOIC POLYENE FATTY ACIDS

A. B. Golovanov,* K. D. Ganina, N. V. Groza, S. V. Eremin, and G. I. Myagkova

An approach using acetylenes was used and optimized to synthesize rare natural ω6 polyene fatty acids. The key synthetic step was cross-coupling of a propargyl derivative with a terminal acetylene in the presence of equimolar amounts of Cu(I).

Keywords: polyunsaturated fatty acids, cross-coupling, cytotoxicity, anti-inflammatory activity.

ω6 Unsaturated fatty acids for research on their biological activity must be synthesized because they occur in insufficient quantities in natural sources. C14:2 ω6 acid is known to occur in plant root vacuoles, e.g., those of larch, where its content was <0.4% of the total fatty acids [1].

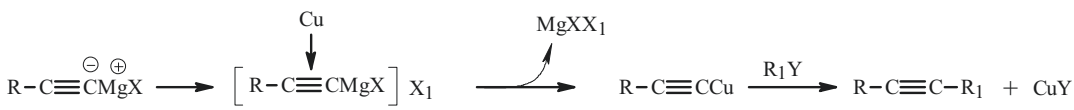
The goal of the present work was to develop a universal approach to the synthesis pure natural ω6 polyunsaturated fatty acids and their synthetic analogs. These could be used to design more complicated lipids for biological research on the processes underlying pathological conditions and to act as tools for studying physiological reactions.

Two main approaches to the chemical synthesis of polyunsaturated compounds have appeared. The first is the Wittig reaction and its modifications. In particular, deuterium-labeled (5*Z*,8*Z*)-tetradecadienoic acid was synthesized using a Wittig reaction involving condensation of the appropriate phosphonium bromide and aldehyde [2]. However, as a rule, this method produced a mixture of methylene-separated unsaturated moieties with the *Z*- and *E*-configurations [3].

Use of acetylene and its derivatives to form C–C bonds (cross-coupling) was most reliable because the task of synthesizing polyunsaturated compounds included the design of a certain hydrocarbon chain length and the introduction of unsaturation and the required functional groups.

Previously, alkylation by, e.g., propargyl halides of an ethynyl atom of acetylenic precursors was widely used. For this, acetylenic precursors were alkylated as their magnesium halides (Iotsich reagent). The reaction occurs through an S_N2 mechanism and is conducted with catalytic amounts of Cu(I) [4].

The reaction mechanism can be represented as:



X = Hal, X_1 = Hal, CN, Y = Hal, OTs

The alkylating agents were propargyl halides. Rather forcing conditions (17 h, 60°C) were required for the reactions. The method had one drawback, i.e., prototropic isomerization and, as a result, the formation of side products [4]. Previously, we developed a strategy with acetylenes in which a different type of cross-coupling reaction was employed.

The key step of this method was the reaction of propargyl halide and a terminal acetylene to form a polyacetylene, hydrogenation of which produced a polyene with double bonds primarily of the *Z*-configuration (Scheme 1).

M. V. Lomonosov Moscow State University of Fine Chemical Technologies, 119571, Moscow B-571, Prosp. Vernadskogo, 86, e-mail: a.golovanov@chemist.com. Translated from *Khimiya Prirodnykh Soedinenii*, No. 1, January–February, 2015, pp. 28–31. Original article submitted September 17, 2014.

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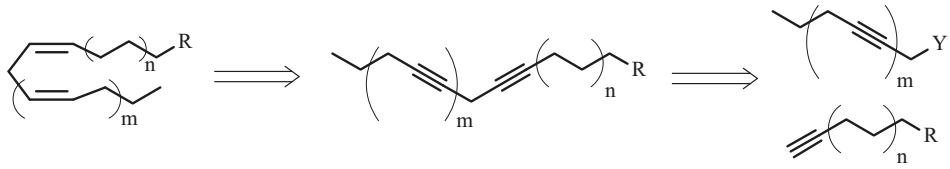
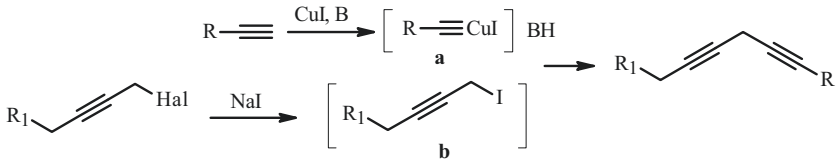


R = COOH, COOMe; Y = Hal, OTs
m = 1, 2, 3 … ; n = 1, 2, 3 …

Scheme 1

Iodides are the most reactive of the propargyl halides. However, their acetylene groups are rather unstable. Their preparation *in situ* using the appropriate base and solvent could form the Cu(I) complex of the terminal acetylene (**a**) [5] without preliminary metalation of the acetylene:



B = K_2CO_3, Na_2CO_3
Hal = Cl, Br

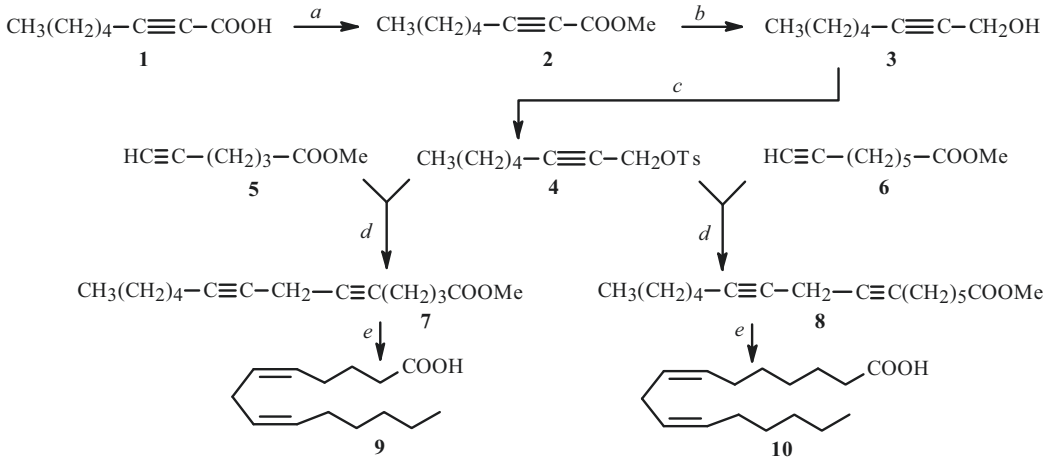
The reaction yield and rate were found to depend on the nature of the base and the solvent polarity. The yield was quantitative after only 40 min for the reaction in DMF with K_2CO_3 at room temperature. However, trace quantities of condensation products were observed only after 3 h in Me_2CO or with Na_2CO_3. Therefore, the reaction required two equivalents of I^-, one of which was consumed by forming complex **a** and the other, formation of propargyl iodide (**b**). Therefore, the reaction involved one equivalent of NaI and CuI and two equivalents of K_2CO_3.

Condensations using this methodology produced high yields (75–81%) under rather mild conditions. Propargyl halides and tosylates could be used.

The high-purity short-chain propargyl bromides that were required for our approach necessitated labor-intensive processes. In order to obviate this obstacle, we synthesized propargyl tosylates, which were rather easily identified and separated by column chromatography.

The acetylenic precursors for the natural polyene fatty acids and their derivatives that were synthesized in the present work were obtained by us using the polyacetylene approach and organocopper reagents in the key propargyl cross-coupling with a terminal acetylene [6]. Use of Brown catalyst [7] was proposed in the present work for preparing the polyenes.

The synthetic strategy was based on the methyl esters of tetradeca-5,8-diynic (**8**) and hexadeca-7,10-diynic (**7**) acids, which were obtained via cross-coupling of 1-(tosyloxy)-2-octyne (**4**) with the methyl ester of 5-hexynic acid **5** and the methyl ester of 7-octynic acid **6**, respectively. The methyl ester of 2-octynic acid **2** was prepared using diazomethane according to the standard method and commercially available 2-octynic acid **1**. The methyl ester of 2-octynic acid was reduced to 2-octynol **3** using $LiAlH_4$ followed by tosylation under basic conditions (Scheme 2).



a. CH_2N_2; *b*. $LiAlH_4$, THF; *c*. TsCl, KOH; *d*. CuI, NaI, K_2CO_3, DMF; *e*. 1. P-2 Ni, EtOH, 2. NaOH, $MeOH/H_2O$

Scheme 2

The course of the reaction was monitored by TLC and PMR (hydrogenation products). The structures of the synthesized compounds were confirmed using PMR and ^{13}C NMR spectral data. Target compounds **9** and **10** were purified by flash-chromatography over silica gel and were additionally purified using preparative reversed-phase HPLC. HPLC analysis of **9** and **10** showed isolated peaks for the target compounds of purity 97.5 and 98%, respectively.

EXPERIMENTAL

PMR and ^{13}C NMR spectra were recorded in $CDCl_3$ with TMS internal standard for PMR and the $CDCl_3$ resonance (δ_C 77.19 ppm) as the standard for ^{13}C NMR on a Bruker MSL 300 MHz spectrometer. Chemical shifts are given in ppm; spin–spin coupling constants, in Hz. The final products were purified by preparative HPLC over a Lichrospher 100 RP18 column (250 × 22.5 mm, 10 μm, Knauer, Berlin, Germany) at eluent flow rate 5 mL/min (MeCN–H_2O–AcOH, 60:40:0.05). Flash-chromatography used Silica gel 60 (Acros, 60–200 and 40–63 μm). TLC of the synthesized compounds used Silica gel 60 F254 plates (Merck, Germany). Solvents were dried if they were not high-purity reagents (Merck, Acros Org). Glassware and syringes were dried at 140°C before use. Cross-couplings were carried out under a dry Ar atmosphere.

Synthesis of Starting Compounds.

Methyl Ester of 2-Octynic Acid (2). A KOH solution (25 mL, 40%) was treated with Et_2O (10 mL) to form two layers. The Et_2O layer was treated with *N*-nitrosomethylurea (7.00 g, 178.50 mmol), which reacted at the interface. The Et_2O layer became yellow (saturation by diazomethane). A solution of **1** (10 g, 71.00 mmol) in Et_2O was purged with the excess of diazomethane (Ar carrier). The appearance of a yellow color in the solution of **1** and the TLC results indicated when the reaction was finished. The solvent was removed *in vacuo*. The residue was chromatographed using Et_2O–petroleum ether (1:2). Yield of **2**, 10.03 g (91.2%), R_f 0.56 (Et_2O–hexane, 1:1). ^1H NMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 3.72 (3H, s, OCH_3), 2.25 (2H, t, J = 6.8, H-4), 1.55 (2H, m, H-5), 1.2–1.4 (4H, m, H-6, 7), 0.87 (1H, t, J = 7, CH_3). ^{13}C NMR spectrum (75 MHz, $CDCl_3$, δ, ppm): 170.99 (s, C-1), 89.90 (s, C-3), 72.85 (s, C-2), 53.54 (s, OCH_3), 31.49 (s, C-6), 27.18 (s, C-5), 22.56 (s, C-7), 18.65 (s, C-4), 14.21(s, C-8).

2-Octynol (3). A suspension of $LiAlH_4$ (3.00 g, 78.70 mmol) in anhydrous THF (40 mL) was cooled to –5°C, stirred, treated with a solution of **2** (10.03 g, 65.00 mmol) in anhydrous THF (5 mL), stirred at –5°C for 3 h, decomposed by cold H_2O (100 mL), acidified by H_2SO_4 solution (1 M) to pH 2–3, and extracted by Et_2O (3 × 50 mL). The extract was washed with saturated NaCl solution. The combined organic fraction was dried over Na_2SO_4 and decanted. The solvent was removed *in vacuo*. The residue was chromatographed over a column of silica gel using a gradient of Et_2O–petroleum ether (1:3, 1:2, 1:1). The yield of **3** was 6.25 g (76%), R_f 0.37 (Et_2O–hexane, 1:1). ^1H NMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 4.25 (2H, m, H-1), 2.25 (2H, t, J = 6.8, H-4), 1.55 (2H, m, H-5), 1.2–1.4 (4H, m, H-6, 7), 0.87 (1H, t, J = 7, H-8). ^{13}C NMR spectrum (75 MHz, $CDCl_3$, δ, ppm): 87.13 (s, C-3), 78.52 (s, C-2), 51.01 (s, C-1), 31.29 (s, C-6), 28.11 (s, C-5), 21.78 (s, C-7), 19.15 (s, C-4), 14.11 (s, C-8).

1-(Tosyloxy)-2-octyne (4). A solution of **3** (3.00 g, 23.79 mmol) and TsCl (6.30 g, 33.30 mmol) in anhydrous Me_2CO (4 mL) was treated with KOH (1.98 g, 35.70 mmol) and K_2CO_3 (1.64 g, 11.88 mmol) in H_2O (4 mL) to form a suspension that was purged with Ar, stirred at 0°C for 30 min, warmed, stirred for 1 h, and decomposed with H_2O (100 mL). The mixture was extracted with Et_2O (4 × 50 mL). The combined organic fraction was dried over Na_2SO_4 and decanted. The solvent was removed *in vacuo*. The residue was chromatographed over a column of silica gel using a gradient of Et_2O–petroleum ether (1:3 to 1:1). The yield of **4** was 3.69 g (56%), R_f 0.54 (Et_2O–hexane, 1:1). ^1H NMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 7.95 (2H, d, H-2′, 6′), 7.41 (2H, d, H-3′, 5′), 4.70 (2H, t, H-1), 2.42 (3H, s, Ar-CH_3), 2.05 (2H, m, H-4), 1.2–1.4 (6H, m, H-5, 6, 7), 0.87 (3H, t, J = 7, H-8). ^{13}C NMR spectrum (75 MHz, $CDCl_3$, δ, ppm): 148.24 (s, C-1′), 134.75 (s, C-4′), 129.97 (s, C-3′, 5′), 127.83 (s, C-2′, 6′), 89.76 (s, C-3), 70.84 (s, C-2), 53.21 (s, C-1), 31.23 (s, C-6), 28.15 (s, C-5), 23.18 (s, C-7), 20.54 (s, Ar-CH_3), 19.07 (s, C-4), 14.23 (s, C-8).

Synthesis of Polyyne Precursors and Target Compounds.

Methyl Ester of Tetradeca-5,8-diynic Acid (8). A suspension of previously dried CuI (3.15 g, 16.50 mmol), NaI (2.47 g, 16.50 mmol), and K_2CO_3 (2.28 g, 14.20 mmol) in anhydrous DMF (3 mL) was treated with **4** (1.85 g, 6.45 mmol) and the methyl ester of 5-hexynoic acid (**5**, 1.0 g, 7.80 mmol) dissolved in anhydrous DMF (3 mL). The mixture was purged with Ar, stirred at 20°C for 12 h, decomposed with saturated NH_4Cl solution (150 mL), and extracted with Et_2O (5 × 40 mL). The organic extract was dried over Na_2SO_4. The solvent was evaporated. The residue was chromatographed using a gradient of Et_2O–petroleum ether (1:3 to1:1). The yield of **8** was 1.15 g (75.9%), R_f 0.54 (Et_2O–hexane, 1:1). ^1H NMR spectrum

(300 MHz, $CDCl_3$, δ, ppm, J/Hz): 3.68 (3H, s, OCH_3), 3.11 (2H, m, H-7), 2.44 (2H, t, J = 7.4, H-2), 2.17–2.25 (2H, m, H-4), 2.12–2.15 (2H, m, H-10), 1.81 (2H, t, H-3), 1.48 (2H, t, H-11), 1.32–1.37 (4H, m, H-12, 13), 0.87 (3H, t, J = 6.8, H-14). ^{13}C NMR spectrum (75 MHz, $CDCl_3$, δ, ppm): 173.21 (s, C-1), 80.50 (s, C-5), 77.32 (s, C-9), 76.67 (s, C-6), 74.50 (s, C-8), 51.36 (s, OCH_3), 32.95 (s, C-2), 30.94 (s, C-12), 28.47 (s, C-11), 26.25 (s, C-3), 24.65 (s, C-13), 22.20 (s, C-4), 18.61 (s, C-10), 13.98 (s, C-14), 12.04 (s, C-7).

(5*Z*,8*Z*)-Tetradecadienoic Acid (9). Nickel acetate tetrahydrate (1.39 g, 10.23 mmol) was dissolved in EtOH (95%, 40 mL), stirred, treated with $NaBH_4$ (0.38 g, 10.23 mmol), purged with H_2, treated with ethylenediamine (1.08 mL, 20.46 mmol) and the methyl ester of **8** (1.15 g, 4.45 mmol), and stirred at 10°C for 0.5 h. The amount of H_2 was measured using a gas burette. When H_2 absorption (10 mL) was finished, the catalyst was filtered off. The filtrate was acidified with H_2SO_4 (1 M) and extracted with Et_2O (4 × 50 mL). The extract was dried over Na_2SO_4 and chromatographed using a gradient of Et_2O–petroleum ether (1:3 to 1:1). The yield of the methyl ester of (5*Z*,8*Z*)-tetradecadienoic acid was 0.84 g (73%), R_f 0.56 (Et_2O–hexane, 1:1).

Aqueous NaOH solution (0.3 M, 4 mL) under Ar was treated with a solution of the methyl ester of (5*Z*,8*Z*)-tetradecadienoic acid (0.84 g, 3.50 mmol) in MeOH (3 mL) and stirred at room temperature for 8 h. The MeOH was evaporated. The residue was acidified with aqueous HCl (1 M) to pH 5.0. The products were extracted by Et_2O (3 × 50 mL). The Et_2O extracts were dried over Na_2SO_4. The solvent was evaporated. The product was purified over a column of silica gel (Et_2O–hexane, 3:1) and reversed-phase HPLC. The yield of **9** was 0.45 g (62%), R_f 0.57 (Et_2O–hexane, 2:1), t_R 9 min (MeCN–H_2O–AcOH, 60:40:0.05). ^1H NMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 5.37 (4H, m, H-5, 6, 8, 9), 2.76 (2H, m, H-7), 2.36 (2H, t, J = 7, H-2), 2.09–2.17 (2H, m, H-4), 2.00–2.09 (2H, m, H-10), 1.71 (2H, m, H-3), 1.27–1.37 (6H, m, H-(11–13)), 0.87 (3H, t, J = 6.8, H-14). ^{13}C NMR spectrum (75 MHz, $CDCl_3$, δ, ppm): 179.99 (s, C-1), 130.43 (s, C-9), 129.41 (s, C-5), 128.41 (s, C-6), 127.52 (s, C-8), 33.38 (s, C-2), 31.49 (s, C-12), 29.30 (s, C-11), 27.18 (s, C-10), 26.41 (s, C-4), 25.58 (s, C-7), 24.47 (s, C-3), 22.56 (s, C-13), 14.21 (s, C-14).

Methyl Ester of Hexadeca-7,10-diynic Acid (7). A suspension of previously dried CuI (2.68 g, 14.13 mmol), NaI (2.12 g, 14.13 mmol), and K_2CO_3 (1.30 g, 13.05 mmol) in anhydrous DMF (3 mL) was treated with **4** (1.80 g, 6.30 mmol) and the methyl ester of 7-octynic acid (**6**, 1.15 g, 7.56 mmol) dissolved in anhydrous DMF (3 mL). The mixture was purged with Ar, stirred at 20°C for 12 h, decomposed by saturated NH_4Cl solution (150 mL), and extracted with Et_2O (5 × 40 mL). The organic extract was dried over Na_2SO_4. The solvent was evaporated. The residue was chromatographed using a gradient of Et_2O–petroleum ether (1:3 to 1:1). The yield of **7** was 1.19 g (70.9%), R_f 0.54 (Et_2O–hexane, 1:1). ^1H NMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 3.66 (3H, s, OCH_3), 3.12 (2H, t, J = 6.8, H-9), 2.31 (2H, t, H-2), 2.23–2.14 (4H, m, H-6, 12), 1.64–1.35 (12H, m, H-(3–5, 13–15)), 0.87 (3H, t, J = 7, H-14). ^{13}C NMR spectrum (75 MHz, $CDCl_3$, δ, ppm): 173.31 (s, C-1), 80.56 (s, C-7), 77.36 (s, C-11), 76.77 (s, C-8), 74.43 (s, C-10), 51.32 (s, OCH_3), 34.05 (s, C-2), 32.67 (s, C-14), 30.83 (s, C-5), 29.07 (s, C-13), 28.60 (s, C-4), 25.65 (s, C-3), 22.23 (s, C-15), 18.65 (s, C-6,12), 13.98 (s, C-16), 10.67 (s, C-9).

(7*Z*,10*Z*)-Hexadecadienoic Acid 10. Nickel acetate tetrahydrate (1.48 g, 10.90 mmol) was dissolved in EtOH (95%, 40 mL), stirred, treated with $NaBH_4$ (0.41 g, 10.90 mmol), purged with H_2, treated with ethylenediamine (1.48 mL, 22.00 mmol) and methyl ester **9** (1.18 g, 4.78 mmol), and stirred at 10°C for 1 h. The amount of H_2 was measured using a gas burette. When H_2 absorption (10 mL) was finished, the catalyst was filtered off. The filtrate was acidified by H_2SO_4 (1 M) and extracted with Et_2O (4 × 50 mL). The extract was dried over Na_2SO_4 and chromatographed using a gradient of Et_2O–petroleum ether (1:3 to 1:1). The yield of the methyl ester of (7*Z*,10*Z*)-hexadecadienoic acid was 0.90 mg (75%), R_f 0.55 (Et_2O–hexane, 1:1).

Aqueous NaOH solution (0.3 M, 4 mL) under Ar was treated with a solution of the methyl ester of (7*Z*,10*Z*)-hexadecadienoic acid (0.90 g, 3.58 mmol) in MeOH (5 mL) and stirred at room temperature for 8 h. The MeOH was evaporated. The residue was acidified with aqueous HCl (1 M) to pH 5.0. The products were extracted with Et_2O (3 × 50 mL). The Et_2O extracts were dried over Na_2SO_4. The solvent was evaporated. The product was purified over a column of silica gel (Et_2O–hexane, 3:1) and by reversed-phase HPLC. The yield of **10** was 0.55 g (64%), R_f 0.57 (Et_2O–hexane, 2:1), t_R 4 min (MeCN–H_2O–AcOH, 60:40:0.05). ^1H NMR spectrum (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 5.39 (4H, m, H-7, 8, 10, 11), 2.82 (2H, t, J = 6.8, H-9), 2.35 (2H, m, H-2), 2.10 (4H, m, H-6, 12), 1.45–1.28 (12H, m, H-(3–5, 13–15)), 0.87 (3H, t, J = 7, H-16). ^{13}C NMR spectrum (75 MHz, $CDCl_3$, δ, ppm): 173.21 (s, C-1), 131.10 (s, C-7), 130.24 (s, C-11), 128.80 (s, C-8), 128.20 (s, C-10), 32.95 (s, C-2), 30.94 (s, C-14), 29.47 (s, C-13,5), 28.25 (s, C-6,12), 27.56 (s, C-4), 25.65 (s, C-9), 23.20 (s, C-3), 22.61 (s, C-15), 13.98 (s, C-16).

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