Synthesis of 7,10-Dihydroxy-8(*E*)-Octadecenoic Acid[1]

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We report the synthesis of 7,10-dihydroxy-8(*E*)-octadecenoic acid (DOD), which has recently also been reported from bioconversion of oleic acid. One hydroxyl of 1,7-heptanediol was protected as tetrahydropyranyl ether, and the other hydroxyl was used for chain extension by two carbons *via* Wittig reaction to give ethyl 9-tetrahydropyranyloxy-2(*E*)-nonenoate, an α,β-unsaturated ester, which on Dibal-H reduction offered allylic alcohol. The epoxidation at the double bond followed by conversion of the hydroxyl group to chloro gave 9-tetrahydropyranyloxy-2,3-oxirane-1-chlorononane. Treatment of this epoxy nonyl chloride with excess of $LiNH_2$/liquid NH_3; followed by addition of 1-nonanal, gave 18-tetrahydropyranyloxy-9, 12-dihydroxy-10-octadecyne. The lithium aluminum hydride reduction of the conjugated hydroxy acetylenic bond, protection of hydroxyl groups as benzoates and oxidation of the primary ether group, followed by removal of benzoate groups, gave DOD.

KEY WORDS: DOD, epoxidation, 1,7-heptanediol, 1-nonanal, synthesis, Wittig reaction.

In recent years, several microbial and enzymatic approaches for biochemical modification of oils and fats have been reported (1,2). Bioconversions of unsaturated fatty acids by microbes, bacterial strains and yeast have been known for quite a long time (3,4). The transformations of oleic acid involving hydration and isomerization of the double bond and many other transformations with different strains of bacteria are well documented (5–7). In their screening programs for new industrial chemicals from agricultural oils, Hou *et al.* (1) have discovered that a new bacterial strain, PR_3, converts oleic acid to a new fatty acid identified as 7,10-dihydroxy-8(*E*)-octadecenoic acid (DOD). The bioconversion by this bacteria involves geometric isomerization of the double bond from *cis* to *trans* with its shift to the eighth carbon and the introduction of hydroxyl groups at the seventh and tenth carbons, *i.e.*, on either side of the *trans* double bond. We have synthesized this novel fatty acid in the laboratory to make it available for further studies. The synthesis and spectral characterization of this fatty acid form the subject of this communication.

EXPERIMENTAL PROCEDURES

Materials and methods. Analytical-grade reagents were purchased from Aldrich Chemical Co. (Milwaukee, WI). Analytical-grade chemicals and solvents were purchased from Indian Drug and Pharmaceutical Ltd. (Hyderabad, India). Dichloromethane (DCM) was dried by stirring it over calcium hydride overnight and distilling under nitrogen atmosphere. Benzene was dried over sodium metal and distilled. Silica gel (60–120 mesh) was obtained from ACME Synthetic Chemicals (Bombay, India). (Carbethoxymethylene)triphenylphosphorane (8) was prepared by adding triphenylphosphorane (1 eq.) to a solution of ethyl bromoacetate (1 eq.) in benzene and stirring the solution for 1 h. Benzene was removed, and the resulting white mass was dissolved in water. The aqueous solution was neutralized with 10% NaOH solution while using phenolphthalein as an indicator. The Wittig reagent was extracted with benzene and washed free of residual alkali. The benzene solution was dried over anhydrous Na_2SO_4, and the solvent was evaporated.

Infrared (IR) spectra were recorded in chloroform on a Perkin-Elmer 683 Spectrometer (Perkin-Elmer, Norwalk, CT). Proton nuclear magnetic resonance (^1H NMR) spectra were obtained in $CDCl_3$ on a Varian 60-FT (Varian Associates, Palo Alto, CA). Mass spectra were recorded on a V.G. Micromass 7070 H mass spectrometer (V.G. Analytical Ltd., Manchester, England). The synthetic route followed for the synthesis of DOD (A) is shown in Scheme 1 where conditions are a) Dihydropyran, *p*-toluenesulfonic acid (PTSA), dry DCM; b) pyridinium chloromate (PCC), dry DCM, 0°C; c) $Ph_3P{=}CHCOOEt$, dry C_6H_6, reflux; d) DIBAL-H, dry DCM, −78°C; e) $V(acac)_2$, TBHP, dry DCM, 0°C; f) CCl_4, Ph_3P, $NaHC_3$, reflux; g) $LiNH_2$ (3 eq.), liq. NH_3, 1-nonanal; h) LAH, dry THF, 0°C; i) Bz-Cl, triethylamine, dry DCM, 0°C; j) Jones' reagent, acetone, 0°C; k) K_2CO_3, methanol, 0°C.

Synthesis of 7-tetrahydropyranyloxy-1-heptanal (2). 1,7-Heptane-diol (100 mmol) and PTSA (2 mmol) were dissolved in 200 mL DCM and cooled to 0°C. Dihydropyran (90 mmol) was added dropwise over 15 min. After removal of DCM with a rotary evaporator, the crude product was extracted with diethyl ether and purified on a silica gel column to give 75% of 7-tetrahydropyranyloxy-1-heptanol. This compound (75 mmol) was dissolved in dry DCM (150 mL) and cooled to 0°C. PCC (115 mmol) was added in ten portions over 30 min. After stirring for 4 h, ether was added to the reaction mixture, the ethereal extract was filtered through celite to remove sticky residue and the solvent was removed from the filtrate to give 95% of 2. IR 2920, 1720, 1070 cm^{-1}. ^1H NMR δ 9.8 (*t*, 1H, -CHO), 4.55 (*t*, ^1H, O-CH-O), 3.3–3.9 (*m*, 4H, $(O\text{-}CH_2)_2$), 2.2 (*m*, 2H, CH_2-CO), 1.3–1.8 [*s* merged with *m*, 14H, $-(CH_2)_7-$].

Synthesis of 9-tetrahydropyranyloxy-2(E)-nonen-1-ol (3). 2 (50 mmol) was refluxed in dry benzene (100 mL) with (carbethoxymethylene) triphenylphosphorane (55 mmol) for 5 h. The benzene was evaporated, and the product was extracted with hexane. The crude product was purified on a silica gel column to give 96% of ethyl 9-tetrahydropyranyloxy-2(*E*)-nonenoate. This ester (45 mmol) was dissolved in dry DCM (80 mL) and cooled to −78°C. Diisobutylaluminium hydride (DIBAL-H) solution in hexane (18%, 100 mmol) was added slowly under inert atmosphere while stirring (9). After 2 h the reaction was quenched with methanol (1 mL), and the mixture was stirred with an aqueous solution of sodium potassium tartarate (10%, 80 mL). The product was extracted with DCM and purified on a silica gel column to yield 85% of 3. IR 3540, 2950, 1600, 1080, 980 cm^{-1}. ^1H NMR δ 5.8 (*m*, 2H, -CH=CH-), 4.55 (*t*, 1H, O-CH-O), 4.0 (b*d*, 2H, $O\text{-}CH_2\text{-}CH{=}$), 3.3–3.9 [*m*, 4H, $(O\text{-}CH_2\text{-})_2$] 2.0 (*t*, 2H, allylic $-CH_2-$), 1.3–1.8 [*s* merged with *m*, 14H, $-(CH_2)_7-$]. Mass *m/e* (relative intensity): M^+ not seen, 224 (2.0), 140 (3.0), 122 (4.0), 96 (5.0), 101 (20.0), 85 (100.0).

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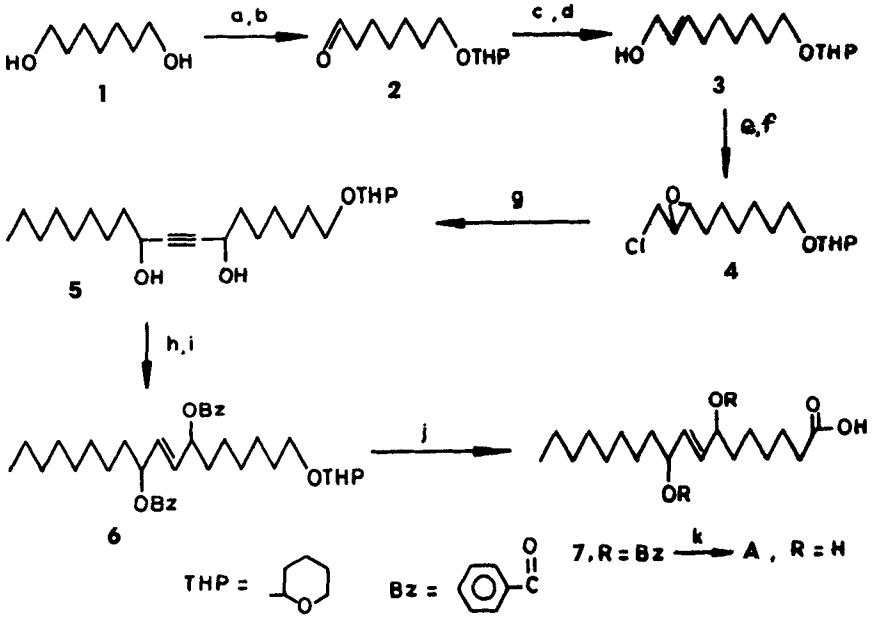

SCHEME 1

Synthesis of 9-tetrahydropyranyloxy-2,3-oxirane-1-chlorononane (*4*). 3 (25 mmol) and catalyst vanadyl acetylacetonate (100 mg) were stirred for 15 min under nitrogen atmosphere. To this mixture, *tert*-butylhydroperoxide (1.2 M in octane, 26 mmol) was added dropwise over 30 min while maintaining the temperature at 0°C. The reaction mixture changed color from green to red. After 10 h, DCM was evaporated, and the crude compound was separated on a silica gel column to give 9-tetrahydropyranyloxy-2, 3-oxirane-1-nonanol in 92% yield. This allylic epoxide (20 mmol) was dissolved in freshly distilled CCl_4 (50 mL). Triphenylphosphine (20 mmol) and $NaHCO_3$ (20 mmol) were added sequentially. The reaction mixture was refluxed for 10 h, and hexane was then added. The precipitated triphenylphosphine oxide was filtered off. The solvent from the filtrate was evaporated, and the product was purified on a silica gel column to yield 97% of 4. IR 3450, 2900, 1250, 1080 cm^{-1}. ^1H NMR δ 4.55 (*t*, 1H, O-CH-O), 3.2-3.9 [*m*, 6H, $(O-CH_2)_2,CH_2-Cl$], 2.8 (*bm*, 2H, protons of oxirane ring), 1.3-1.8 [*s* merged with *m*, 14H, $-(CH_2)_7$]. Mass *m/e* (rel. int.): M^+ 276 (1.0), 191 (2.0), 156 (4.0), 101 (50.0), 85 (100.0).

Synthesis of 18-tetrahydropyranyloxy-9,12-dihydroxy-10-octadecyne (*5*). Ammonia (100 mL) was liquified and collected into a two-necked round-bottom flask connected to a condenser kept at −70°C with dry ice-acetone mixture. The catalyst ferric nitrate followed by freshly cut lithium pieces (45 mmol) was added. The grey color of the solution after 15 min of stirring indicated formation of $LiNH_2$ base in liq. NH_3. Epoxy chloride 4 (15 mmol) in dry tetrahydrofuran (THF) (10 mL) was added dropwise (10). After stirring for 30 min, 1-nonanal (15 mmol) was added dropwise. The reaction mixture was left overnight and quenched with aqueous. NH_4Cl solution. The crude product was extracted with ether, washed twice with water and then purified on a silica gel column to give 5 in 70% yield. IR 3260, 2990, 1080 cm^{-1}, ^1H NMR δ 4.55 (*t*, 1H, O-CH-O), 4.3 (*t*, 2H, CH-OH), 3.2-3.9 [*m*, 4H, $(O-CH_2)_2$], 2.2 (broad *s* exchangeable with D_2O, 2H, -OH), 1.2-1.7 [*s* merged with *m*, 30H, $-(CH_2)_{15}-$], 0.9 (*t*, 3H, $-CH_3$). Mass *m/e* (rel. int.): M^+ not seen, 364 (1.0), 185 (5.1), 157 (2.0), 161 (5.0), 138 (5.0), 149 (7.0), 101 (10.0), 85 (100.0).

Synthesis of 8-tetrahydropyranyloxy-9,12-benzoyloxy-10(E)*-octadecene* (*6*). 5 (10 mmol) was dissolved in dry THF (50 mL), and lithium aluminum hydride (LAH) (20 mmol) was added in portions under inert atmosphere at 0°C. After 3 h of stirring, the reaction mixture was quenched with 30% NaOH solution. The mixture was filtered through celite, and the filtrate was evaporated to give 18-tetrahydropyranyloxy-9,12-dihydroxy-10(*E*)-octadecene in 90% yield. This dihydroxy compound (8 mmol) was dissolved in dry DCM (30 mL) under nitrogen atmosphere, which was cooled to 0°C. Triethylamine (24 mmol) and benzoyl chloride (20 mmol) were added sequentially to the reaction mixture. After stirring for 10 h, DCM was evaporated. The crude product was extracted with ether, washed with water and separated on a silica gel column to give 96% of 6. IR 2990, 1740, 1080, 975 cm^{-1}. ^1H NMR δ 8.0-8.2, 7.4-7.7 (*m*, 5H, aromatic), 5.85 (*dd*, 2H, -CH=CH-), 5.5 (*m*, 2H, -CH-OBz), 4.55 (*t*, 1H, O-CH-O), 3.3-3.9 [*m*, 4H, $(O-CH_2)_2$], 1.2-1.7 [*s* merged with *m*, 30H, $-(CH_2)_{15}-$], 0.9 (*t*, 3H, $-CH_3$).

Synthesis of 7,10-dibenzoyloxy-8(E)*-octadecenoic acid* (*7*). 6 (5 mmol) was dissolved in distilled acetone (20 mL), and Jones' reagent was added till the orange color of the reagent was retained for 15 min. The excess reagent was quenched with isopropanol. Acetone was removed to give

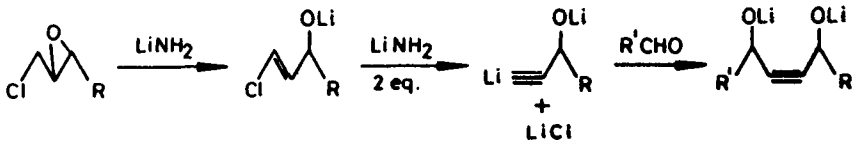

FIG. 1. Opening of 2,3-epoxychloride by $LiHN_2$-liquid NH_3.

acid 7 in 95% yield, which was purified on a silica gel column. IR 2990, 1740, 1712, 975 cm^{-1}. ^1H NMR δ 8.0–8.2, 7.4–7.7 (*m*, 5H, aromatic), 5.85 (*dd*, 2H, -CH=CH-), 5.5 (*m*, 2H, -CH-OBz), 2.3 (*t*, 2H, $-CH_2-CO$), 1.2–1.7 [*s* merged with *m*, 22H, $-(CH_2)_{11}-$], 0.9 (*t*, 3H, $-CH_3$). Mass chemical ionization (CI) *m/e* (rel. int.): 522 (1.0), 400 (20.0), 279 (20.0), 178 (5.0), 105 (60.0), 47 (100.0).

Synthesis of DOD. 7 (2 mmol) was dissolved in methanol and stirred with K_2CO_3 (7 mmol) for 2 h. The reaction mixture was acidified with diluted HCl, and the compound was extracted with solvent ether. Ether extract was washed with water and aqueous NaCl solution, and the compound was separated on a silica gel column to yield 96% of DOD. IR 3450, 2990, 1715, 975 cm^{-1}. ^1H NMR δ 5.6 (*dd*, 2H, -CH=CH-, J = 14.3 Hz), 4.1 (*m*, 2H, -CH-O), 2.3 (*t*, 2H, CH_2-CO), 1.2–1.7 [*s* merged with *m*, 22H, $-(CH_2)_{11}-$], 0.9 (*t*, 3H, $-CH_3$). Mass *m/e* (rel. int.): M^+ 314 (1.0), 278 (3.0), 199 (10.0), 165 (10.0), 175 (15.0), 151 (10.0), 85 (60.0), 43 (100.0).

RESULTS AND DISCUSSION

In retrosynthesis of DOD, the acyl chain can be fragmented at the ninth carbon atom, which offers a C_1-C_9 fragment, namely 7-hydroxy-non-8-enoic acid. This terminal alkyne with an adjacent hydroxyl at the seventh carbon can be obtained from *trans*-2-allylic alcohol (3) by Takano's protocol (10) (Fig. 1). As per Takano's protocol, 3 was epoxidized, and the terminal hydroxyl group was converted to chloro to give 2,3 epoxychloride, which on treatment with excess alkali gives the required terminal acetylene with an adjacent hydroxyl group. The 3 was prepared from 1,7-heptanediol by using the necessary chemical transformations as shown in the scheme.

One hydroxyl group of 1,7-heptanediol was protected as THP-ether, and the other hydroxyl group was oxidized to aldehyde with PCC/DCM to give 72% of 2. The ^1H NMR showed a triplet at 9.8 δ (aldehydic proton) and a broad singlet at 4.55 δ (O-CH-O of tetrahydropyranyl moiety), confirming the presence of both terminal functionalities. The 2 on treatment with stabilized Wittig salt, (Ph_3P=CHCOOEt) in refluxing benzene to create a two-carbon extension, followed by the DIBAL-H reduction of resulting ester, gave allylic alcohol, 9-tetrahydropyranyloxy-2-(*E*)-nonen-1-ol (3), in 80% yield. The ^1H NMR showed a multiplet at 5.8 δ (*trans* double bond) and a broad doublet at 4.0 δ (protons of $O-CH_2-CH=$). The mass spectrum did not show the molecular ion but showed characteristic peaks at 224 (M^+-H_2O) and 122 (M^+-H_2O-OTHP). 3 was epoxidized at the *trans* double bond with vanadyl acetylacetonate (catalyst) and *tert*-butylhydroperoxide in dry DCM under nitrogen atmosphere. The terminal hydroxyl group was converted to chloro by CCl_4/PPh_3 in the presence of $NaHCO_3$ to give 2,3 epoxychloride (4) in 90% yield. The ^1H NMR spectrum showed the shift of peaks from 5.8 δ (*trans* double bond) to 2.8 δ (protons of oxirane ring) and from 4.0 δ ($HO-CH_2$) to 3.5 δ ($Cl-CH_2$). The 4 on treatment with excess (3 eq.) $LiNH_2$ base in liquid NH_3 resulted in the opening of epoxychloride per Takano's protocol to offer terminal alkyne alcohol in the dilithiated form (Fig. 1). On addition of 1-nonanal, the carbanion being more unstable than the oxyanion, it attacks the aldehydic group to give the required chainlength (5) of eighteen carbon atoms. After constructing the basic carbon chain, the reduction of the acetylenic bond and oxidation of the primary alcohol were carried out to get DOD. LAH (11,12) is known to give *trans* double bonds in the reduction of triple bonds that are in conjugation with a hydroxyl group. Compound 5 on LAH reduction gave the *trans* double bond in 90% yield. The J_{AA} value for olefinic protons was 14.3 Hz, confirming the *trans* nature of the double bond. Oxidation of the terminal hydroxyl group, protected as THP-ether, was affected while protecting the secondary hydroxyls at the seventh and tenth carbons as benzoate esters (6) to prevent their oxidation. The peaks observed in ^1H NMR were 8.0–8.2 and 7.4–7.7 δ (aromatic protons), doubled doublet at 5.8 δ (*trans* double bond), 4.55 δ (O-CH-O of ether linkage), multiplet at 3.3–3.9 δ ($O-CH_2$), singlet merged with multiplet at 1.3 δ (methylene protons) and triplet at 0.9 δ (terminal methyl group). The O-THP ether, 6 (being acid-labile) gave a primary hydroxyl group on treatment with Jones' reagent and which was subsequently oxidized to acid 7. The chemical ionization mass analysis showed peaks at 522 (molecular ions), 400 (M^+-BzO), 279 (M^+-2BzO) and typical hydrocarbon fragmentation of fatty acid, confirming the structure of DOD in 7,10-dibenzoate ester form. The hydrolysis of dibenzoate ester 7 by K_2CO_3/methanol at room temperature gave the DOD in 95% yield. The spectral data of the present synthetic DOD matched with the published data of isolated DOD (1). This fatty acid melted at 65°C, and other physical characteristics exhibited were similar to those reported. This enables us to use this novel strategy in the synthesis of other fatty acids having a hydroxyl group conjugated with a double bond or an acetylenic bond in their structure.

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