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TETRAHEDRON: *ASYMMETRY*

Enantioselective synthesis of natural biologically active ivaide A: 1,3-di-(*R*)-β-hydroxy-glyceride glycerol

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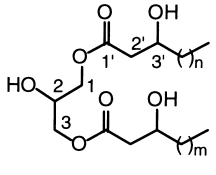
Abstract

The natural 1,3-di-β-hydroxy-glyceride glycerol ivaide A **1** from *Ajuga iva* has been synthesised by diacylation of dihydroxyacetone with 3-hydroxyhexadecanoic acid, followed by reduction to the corresponding glycerol derivative. The enantiomerically pure (*R*)-β-hydroxyhexadecanoic ester intermediate **6** was obtained by a coupling reaction of an ethylacetoacetate dianion and the corresponding bromoalkyl, followed by the known reduction of the resulting β-ketoester **5** by fermenting baker's yeast.

1. Introduction

Ivaides A, B and C are novel homologous compounds isolated from the leaves of *Ajuga iva* (Labiatae) as a mixture of 1,3-diacyl-glycerol derivatives.[1] The unseparated ivaides A **1**, B **2** and C **3** differ only by having different lengths of the fatty acid residues: 3-hydroxytetradecanoic and 3-hydroxyhexadecanoic acids (Fig. 1).

Preliminary biological tests show interesting antifeedant activity of the mixture of compounds **1**, **2** and **3** towards *Spodoptera littoralis* larvae[1] and antibacterial activities against *Escherichia coli*, *Staphylococcus aureus*, *Salmonella typhimurium* and *Pseudomonas aeruginosa*. Moreover, these compounds show a very high chelating power toward lithium cations. All their fragments on LSIMS and MS/MS



1 : Ivaide A : n = m = 12
2 : Ivaide B : n = 12, m = 10
3 : Ivaide C : n = m = 10

Figure 1.

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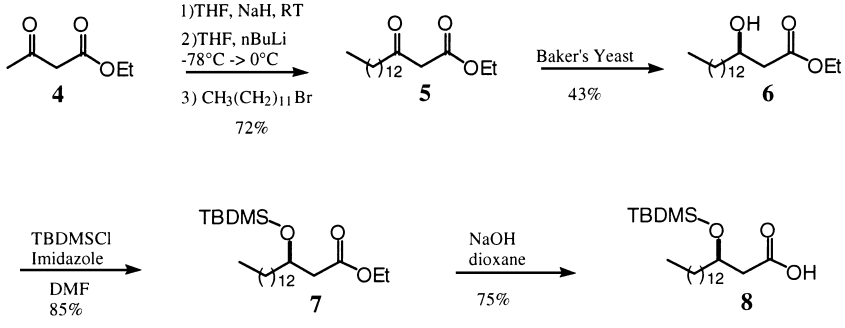
spectra retain the lithium cation. These properties motivated us to synthesise one of them in order to confirm its structure and to continue biological and chemical studies on pure specimens. Optically active 3-hydroxyalkanoic esters exist widely in biological systems.[2] Free 3′-(*R*)-hydroxypalmitic acid which corresponds to the fatty acid part of ivaide A **1** is also produced in high yield by the yeast NRRL Y-6954.[3] In this paper, we describe a total synthesis of glycerol 1,3-((*R*)-3′-hydroxy)hexadecanoate corresponding to the ivaide A **1** ($C_{35}H_{68}O_7$). Among the steps investigated for the preparation of this diglyceride was access to selectively 1,3-diacylated glycerol via the *O*-protected β-hydroxyhexadecanoic acid. Diacylation of dihydroxyacetone followed by its reduction to the glycerol derivative was successful. The β-hydroxyhexadecanoic acid was prepared by baker's yeast reduction of the corresponding 3-oxopalmitate as described by others.[4]

2. Results and discussion

3-Oxopalmitate **5** was prepared in one step as reported by Beijer[5] and coworkers using Weiler's efficient alkylation of the dianion of a β-ketoester.[6] Generation of a dianion of ethylacetoacetate and treatment with 1-bromododecane give the monoalkylated precursor **5** in 72% yield after purification by flash chromatography. The reduction of the β-ketoester by baker's yeast at room temperature resulted in the formation of the (*R*)-β-hydroxyester **6** in 43% (>97% ee).[2] In order to avoid any secondary reaction and/or *trans*-esterification in the rest of our synthesis steps, the β-hydroxyl function was protected as *tert*-butyldimethylsilylether **7** in 85% yield. Base hydrolysis of **7** in dioxane afforded the free acid **8** in 75% yield after precipitation from heptane (Scheme 1).

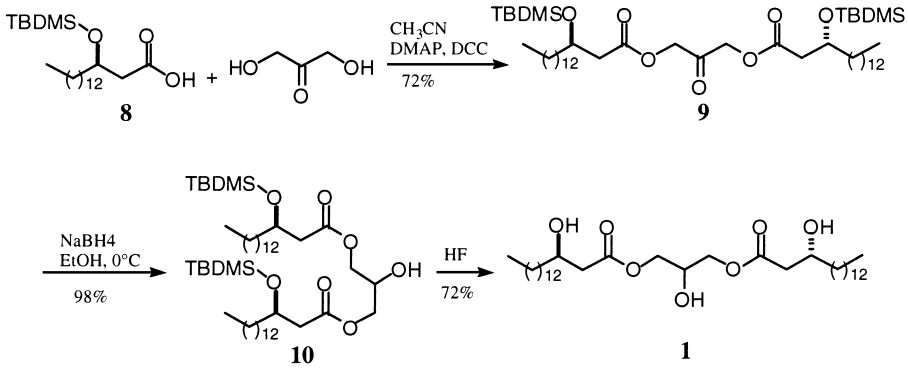


Scheme 1.

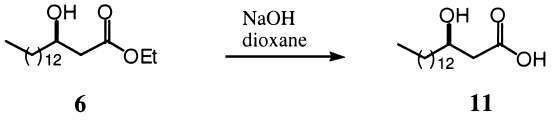
Diacylation of 1,3-dihydroxypropan-2-one[7,8] with **8** activated by 1,3-dicyclo-hexylcarbodiimide in the presence of *N*,*N*-dimethylaminopyridine provides the C_2-symmetric 1,3-((*R*)-3′-protected hydroxy)hexadecanoate **9** in 72% yield. Compound **9** was reduced[8] with $NaBH_4$ in ethanol at 0°C to **10** in 98% yield. Deprotection of hydroxyl was carried out in 40% aqueous HF and gave the desired glycerol 1,3-((*R*)-3′-hydroxy)hexadecanoate **1** in 72% yield (Scheme 2). NMR and MS data of synthetic ivaide A were in agreement with those reported for the natural product.[1]

The enantiomeric purity was examined by comparison of compound **11** obtained by hydrolysis of β-hydroxyester **6** (Scheme 3). The specific rotation determined for **11** $[\alpha]_D^{20}$ −14 (*c* 1, $CHCl_3$) corresponding to the (*R*) configuration was in agreement with the one reported in the literature.[4a]

The synthesised compound **1** also showed interesting antibacterial activities as indicated above. Further chemical investigation and biological assays are currently in progress.



Scheme 2.



Scheme 3.

3. Experimental

3.1. General methods

Chemicals were purchased from commercial sources and used without further treatment except when specified. THF was freshly distilled from sodium benzophenone under nitrogen. ^{1}H and ^{13}C NMR spectra were recorded using Bruker AC-200 (200 MHz) and AC-300 (300 MHz) spectrometers with tetramethylsilane or solvent ($CDCl_3$, CD_3OD) as internal standard (δ ppm). A ZabSpect/T mass spectrometer was used in the LSIMS experiment. MS 9 (CI) was used for HRMS. IR spectra were recorded on a Bio-Rad win-IR PRO spectrometer. Melting points were determined on a Büchi 510 apparatus using capillary tubes.

3.2. 3-Oxo-hexadecanoic acid ethyl ester **5**

To a solution of sodium hydride (1.2 g, 50 mmol) in THF, distilled ethylacetoacetate **4** (4.16 g, 32 mmol) was added dropwise. The resulting mixture was stirred for 30 min at room temperature and then cooled at −78°C. A solution of *n*-butyllithium in hexane (16.1 mL, 35.2 mmol) was added dropwise. After stirring for an additional 1 h at 0°C, 1-bromododecane (19.1 mmol) in THF (8 mL) was added and the mixture was stirred for 12 h. Ethanol (15 mL) was added slowly at room temperature. The resulting solution was filtered through a Celite pad, concentrated in vacuo and purified by chromatography on silica gel (heptane:EtOAc, 9:1) to give the β-ketoester **5** (4.1 g, 72%) as a solid: mp 41–42°C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.87 (t, *J*=6.5 Hz, 3H, -$(CH_2)_{12}$-C*H*$_3$), 1.25 (m, 21H, -$(CH_2)_9$-CH_3 and O-CH_2-C*H*$_3$), 1.55 (m, 4H, -CO-CH_2-C*H*$_2$-C*H*$_2$-), 2.50 (t, *J*=7.6 Hz, 3H, -CO-C*H*$_2$-CH_2-), 3.42 (s, 2H, -CO-C*H*$_2$-CO_2-), 4.20 (q, *J*=6.5 Hz, 2H, CH_3-C*H*$_2$-CO_2-); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.3, 29.4, 43.1, 49.4, 167.3, 203.0. LSIMS *m/z* (rel. intensity) 321 $[M+Na]^+$ (48), 299 $[M+H]^+$ (100), 211 (67), 115 (31); HRMS calcd for $C_{18}H_{34}O_3$ (M^++1): 299.2587; found: 299.2587.

3.3. 3-Hydroxyhexadecanoic acid ethyl ester **6**

To a mixture of distilled water (950 mL), baker's yeast (84 g) and saccharose (105 g), a solution of β-ketoester **5** (4.0 g, 13.4 mmol) in ethanol (120 mL) was added at 30°C. After 24 h stirring at room temperature, baker's yeast was removed by filtration and the filtrate was extracted with ethyl acetate (250 mL×5). The combined organic layers were dried over anhydrous $MgSO_4$ and concentrated in vacuo. Purification by silica gel column chromatography (heptane:EtOAc, 8:2) gave (*R*)-β-hydroxyester **6** (1.73 g, 43%, >97% ee) as a solid: mp 47–48°C; $[\alpha]_D^{20}$ −19 (*c* 1, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 0.85 (t, *J*=6.4 Hz, 3H, -$(CH_2)_{12}$-C*H*$_3$), 1.25 (m, 21H, -(C*H*$_2$)$_9$-CH_3 and O-CH_2-C*H*$_3$), 1.43 (m, 4H, -CO-CH_2-C*H*$_2$-C*H*$_2$-), 2.35 (dd, J_{AB}=17.1 Hz, J_{AX}=8.5 Hz, 1H, H_{2a}), 2.48 (dd, J_{AB}=17.5 Hz, J_{BX}=4.3 Hz, 1H, H_{2b}), 2.93 (br s, 1H, OH), 3.96 (m, 1H, -C*H*OH-), 4.15 (q, *J*=7.7 Hz, 2H, CH_3-C*H*$_2$-CO_2-); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.9, 14.0, 29.4, 36.4, 41.2, 60.5, 68.0, 173.0. LSIMS *m/z* (rel. intensity) 323 $[M+Na]^+$ (31), 301 $[M+H]^+$ (100), 283 (66); HRMS calcd for $C_{18}H_{36}O_3$ (M^++1): 301.2729; found: 301.2728.

3.4. 3-(tert-Butyldimethylsilanyloxy)hexadecanoic acid ethyl ester **7**

β-Hydroxyester **6** (1.5 g, 5 mmol) was added to a mixture of *tert*-butyldimethylchlorosilane (1.13 g, 7.5 mmol) and imidazole (1.02 g, 15 mmol) in dry DMF (7 mL) at room temperature. After 15 h stirring, the solvent was removed in vacuo. Chromatography of the residue on silica gel (heptane:EtOAc, 9:1) gave (*R*)-β-hydroxyester **7** (1.76 g, 85%) as an oil: $[\alpha]_D^{20}$ −18 (*c* 1, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 0.01 (s, 3H, CH_{3a}-Si-), 0.03 (s, 3H, CH_{3b}-Si-), 0.83 (m, 12H, *t*Bu-Si- and -$(CH_2)_{12}$-C*H*$_3$), 4.1 (m, 3H, -O-C*H*$_2$-CH_3 and -C*H*OH-). LSIMS *m/z* (rel. intensity) 415 $[M+H]^+$ (10), 357 (100); HRMS calcd for $C_{24}H_{51}O_3Si$ (M^++1): 415.3636; found: 415.3607.

3.5. 3-(tert-Butyldimethylsilanyloxy)hexadecanoic acid **8**

To the protected (*R*)-β-hydroxyester **7** (1.6 g, 3.86 mmol) dissolved in dioxane (45 mL), NaOH 1N (45 mL) was added at room temperature. The mixture was stirred for 24 h, concentrated under reduced pressure and the residue was precipitated from heptane to give (*R*)-β-hydroxyhexadecanoic acid **8** (1.1 g, 75%) as a semisolid: $[\alpha]_D^{20}$ −16 (*c* 1, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 0.06 (s, 3H, CH_{3a}-Si-), 0.07 (s, 3H, CH_{3b}-Si-), 0.89 (m, 12H, *t*Bu-Si- and -$(CH_2)_{12}$-C*H*$_3$), 1.27 (m, 18H, -(C*H*$_2$)$_9$-CH_3), 1.54 (m, 4H, -Si-O-CH-CH_2-C*H*$_2$-C*H*$_2$-), 2.51 (m, 2H, -Si-O-CH-C*H*$_2$-CO_2H), 4.11 (m, 1H, -C*H*O-Si-); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 18.0, 22.7, 25.2, 25.7, 29.3, 37.2, 41.7, 69.5, 176.3. LSIMS *m/z* (rel. intensity) 393 $[M+Li]^+$ (45), 387 $[M+H]^+$ (91), 369 (18), 329 (36); HRMS calcd for $C_{22}H_{47}O_3Si$ (M^++1): 387.3294; found: 387.3276.

3.6. 3-(tert-Butyldimethylsilanyloxy)hexadecanoic acid 3-[3-(tert-butyldimethylsilanyloxy)hexadecanoyloxy]-2-oxo-propyl ester **9**

To a solution of **8** (1.0 g, 2.6 mmol) in dry CH_3CN (9 mL), was added 1,3-dihydroxypropan-2-one (126 mg, 1.4 mmol), DCC (535 mg, 2.6 mmol) and DMAP (32 mg, 2.6 mmol) at room temperature and the mixture was stirred for 18 h. The reaction mixture was then filtered and concentrated in vacuo. Chromatography of the residue on silica gel (heptane:EtOAc, 6:4) gave **9** (833 mg, 72%) as an oil: $[\alpha]_D^{20}$ −20 (*c* 1, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$) δ 0.05 (s, 3H, CH_{3a}-Si-), 0.08 (s, 3H, CH_{3b}-Si-), 0.87 (m, 12H, *t*Bu-Si- and -$(CH_2)_{12}$-C*H*$_3$), 1.25 (m, 18H, -(C*H*$_2$)$_9$-CH_3), 1.53 (m, 2H, -C*H*$_2$-$(CH_2)_9$-CH_3),

1.65 (m, 2H, -C*H*$_2$-(CH$_2$)$_{10}$-CH$_3$), 1.90 (m, 2H, -C*H*$_2$-(CH$_2$)$_{11}$-CH$_3$), 2.55 (br s, 1H, H$_{2a}$), 2.59 (br s, 1H, H$_{2b}$), 4.15 (m, 1H, -C*H*O-Si-), 4.74 (s, 2H, -O-CH$_2$-CO-); ^{13}C NMR (75 MHz, CDCl$_3$) δ 14.2, 29.5, 32.0, 37.5, 42.1, 66.3, 69.4, 171.0, 198.0. LSIMS *m/z* (rel. intensity) 827 [M+H]$^+$ (14), 811 (50), 769 (42), 327 (100); HRMS calcd for $C_{47}H_{95}O_7Si_2$ (M$^+$+1): 827.6579; found: 827.6616.

*3.7. 3-(*tert*-Butyldimethylsilanyloxy)hexadecanoic acid 3-[3-(*tert*-butyldimethylsilanyloxy)hexadecanoyloxy]-2-hydroxypropyl ester **10***

To a solution of **9** (780 mg, 9.4 mmol) in ethanol, NaBH$_4$ (35.7 mg, 0.94 mmol) was added at 0°C for 10 min. After removal of ethanol under reduced pressure and addition of water, the mixture was extracted with CH$_2$Cl$_2$. The organic layer was washed and dried over MgSO$_4$. Dichloromethane was removed in vacuo and the resulting product precipitated from heptane to give **10** (763 mg, 98%) as a semisolid: $[\alpha]_D^{20}$ −16 (*c* 1, CHCl$_3$); ^{1}H NMR (200 MHz, CDCl$_3$) δ 0.03 (s, 3H, CH$_{3a}$-Si-), 0.08 (s, 3H, CH$_{3b}$-Si-), 0.88 (m, 12H, *t*Bu-Si- and -(CH$_2$)$_{12}$-C*H*$_3$), 1.25 (m, 18H, -(C*H*$_2$)$_9$-CH$_3$), 1.50 (m, 2H, -C*H*$_2$-(CH$_2$)$_9$-CH$_3$), 1.75 (m, 2H, -C*H*$_2$-(CH$_2$)$_{10}$-CH$_3$), 1.90 (m, 2H, -C*H*$_2$-(CH$_2$)$_{11}$-CH$_3$), 2.49 (br s, 1H, H$_{2a}$), 2.51 (br s, 1H, H$_{2b}$), 4.15 (m, 3H, -C*H*O-Si- and -CH$_2$-O-CO-); ^{13}C NMR (75 MHz, CDCl$_3$) δ 14.2, 22.7, 29.7, 37.6, 42.4, 65.2, 68.1, 69.5, 172.0. LSIMS *m/z* (rel. intensity) 835 [M+Li]$^+$ (21), 771 (13), 327 (100); HRMS calcd for $C_{47}H_{97}O_7Si_2$ (M$^+$+1): 829.6744; found: 829.6772.

*3.8. 3-Hydroxyhexadecanoic acid 2-hydroxy-3-(3-hydroxyhexadecanoyloxy)propyl ester **1***

To a solution of **10** (700 mg, 0.84 mmol) in CH$_3$CN (3 mL), was added 40% aqueous HF (0.08 mL, 2 equiv.) and the mixture was stirred for 2 h. To the reaction mixture, an excess of 10% NaHCO$_3$ was added. The resulting compound was extracted twice with CH$_2$Cl$_2$. Purification by chromatography on silica gel (heptane:EtOAc, 7:3 and then 6:4) gave **1** (363 mg, 72%) as a solid: mp 75°C; $[\alpha]_D^{20}$ −8 (*c* 2, CHCl$_3$); IR ν_{max} cm^{-1} 3386, 2956, 2915, 2848, 1690, 1471, 1311, 1176; ^{1}H NMR (200 MHz, CDCl$_3$) δ 0.88 (t, *J*=5.2 Hz, 3H, -(CH$_2$)$_{12}$-C*H*$_3$), 1.25 (m, 22H, -(C*H*$_2$)$_{11}$-CH$_3$), 1.45 (m, 2H, -CO-C*H*$_2$-(CH$_2$)$_{11}$-CH$_3$), 2.45 (dd, J_{AB}=11.6 Hz, J_{AX}=5.8 Hz, 1H, H$_{2'a}$), 2.56 (dd, J_{AB}=11.6 Hz, J_{BX}=2.9 Hz, 1H, H$_{2'b}$), 4.03 (m, 1H, -C*H*OH-CH$_2$-CO-), 4.10 (m, 1H, -C*H*OH-CH$_2$-O-CO-), 4.22 (m, 2H, -CH$_2$-O-CO-); ^{13}C NMR (75 MHz, CDCl$_3$) δ 14.1, 22.7, 29.4, 36.9, 42.6, 65.3, 68.7, 171.5. LSIMS *m/z* (rel. intensity) 607 [M+Li]$^+$ (30), 579 (26), 551 (7), 537 (4), 313 (40); HRMS calcd for $C_{35}H_{69}O_7$ (M$^+$+1): 601.5035; found: 601.5027.

*3.9. 3-Hydroxyhexadecanoic acid **11***

(*R*)-β-Hydroxyhexadecanoic acid **11** (29.6 mg, 70%) was prepared as indicated above (see compound **8**). Mp 84°C (lit.[4a] 76.8°C); $[\alpha]_D^{20}$ −14 (*c* 1, CHCl$_3$, lit.[4a] $[\alpha]_D^{20}$ −13.8); ^{1}H NMR (200 MHz, CD$_3$OD) δ 0.88 (t, *J*=4.6 Hz, 3H, -(CH$_2$)$_{12}$-C*H*$_3$), 1.30 (m, 20H, -(C*H*$_2$)$_{10}$-CH$_3$), 1.47 (m, 4H, -CO-C*H*$_2$-C*H*$_2$-(CH$_2$)$_{10}$-CH$_3$), 2.35 (dd, J_{AB}=14.8 Hz, J_{AX}=5.1 Hz, 1H, H$_{2a}$), 2.43 (dd, J_{AB}=14.8 Hz, J_{BX}=3.7 Hz, 1H, H$_{2b}$), 3.96 (m, 1H, -C*H*OH-); ^{13}C NMR (75 MHz, CD$_3$OD) δ 13.0, 22.3, 29.3, 36.7, 41.8, 67.9, 174.2. LSIMS *m/z* (rel. intensity) 273 [M+H]$^+$ (97), 255 (84), 237 (25), 214 (100), 197 (88); HRMS calcd for $C_{16}H_{33}O_3$ (M$^+$+1): 273.2447; found: 273.2430.

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