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Synthesis of podoscyphic acid

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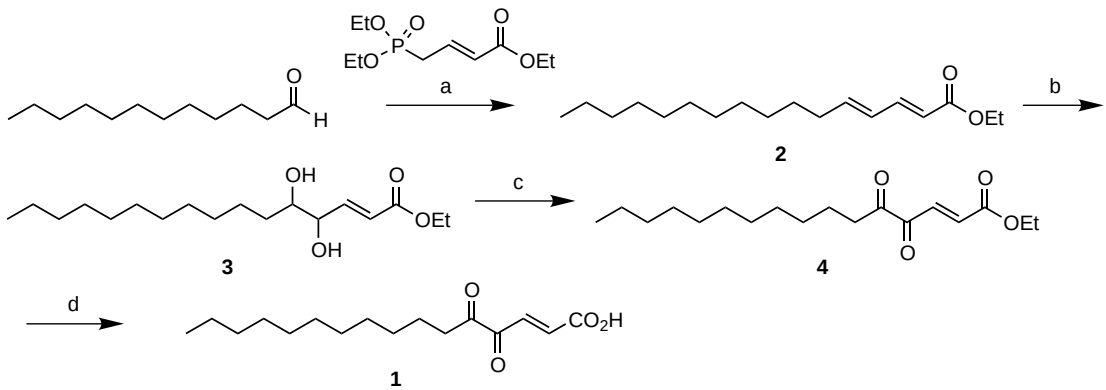
Abstract—Podoscyphic acid **1**, a fungal metabolite inhibiting RNA-directed DNA polymerases, was synthesised in four steps starting from dodecanal. The concluding ester hydrolysis of **4** was not feasible with chemical reagents, only enzymatic hydrolysis was mild enough to release the highly reactive natural product in good yields.

1. Introduction

The fungal metabolite podoscyphic acid **1** was originally isolated from the mycelium of the basidiomycete *Podoscypha petalodes*, during a screening of fungal extracts for new inhibitors of avian myeloblastosis virus and murine leukemia virus.[1] It is an effective and selective inhibitor of reverse transcription, catalysed by RNA-directed DNA polymerase, with an IC_{50} value of 15 μg/ml for avian myeloblastosis virus reverse transcriptase.[1,2] Podoscyphic acid **1** contains a highly unusual γ,δ-dioxoacrylate moiety, and we have recently discovered that the chemical reactivity of the compound is very suitable for the preparation of small libraries of novel compounds of which some have shown interesting biological properties. In order to further survey the biological activities of **1**, as well as study its chemical reactivity and facilitate the preparation of suitable derivatives, a synthetic procedure to obtain the natural product in larger amounts was required.

2. Results and discussion

The synthetic route developed, depicted in Scheme 1, starts from the commercially available aldehyde dodecanal. The coupling of dodecanal with triethyl 4-phosphonocrotonate using Wadsworth–Emmons conditions[3,4] proceeded smoothly, and the diene **2** was obtained in 72% yield (as a 1:9 mixture of the *cis* and *trans* isomers). The diene **2** was thereafter subjected to catalytic dihydroxylation with osmium tetroxide (according to Sharpless' dihydroxylation protocol) for



Scheme 1. *Reagents and conditions*: (a) LDA, THF, −78 to 20°C (72 %); (b) OsO_4, $K_3Fe(CN)_6$, K_2CO_3, quinuclidine, *t*-BuOH/H_2O, stirred for 4 days (51 %); (c) TEMPO, CH_2Cl_2, 0°C (100%); (d) Novozyme 435, isopropyl ether, 2 h (70%).

Keywords: podoscyphic acid; synthesis lipase; enzymatic hydrolysis.

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four days, and the 4,5-dihydroxylated product **3** was obtained in 51% yield.[6,7] The 1,2-diketo functionality was then introduced by oxidation of **3** with TEMPO, (2,2,6,6)-tetramethyl-1-piperidinyloxy, resulting in the ethyl ester of podoscyphic acid **4** in quantitative yield.[5] Surprisingly, other mild oxidation procedures, e.g. the Swern oxidation, did not give a satisfactory result. The final step, the hydrolysis of the ester **4** to give free podoscyphic acid **1**, was anticipated to be the easiest but turned out to be difficult. All commonly employed chemical procedures for ester hydrolysis, both acidic and basic, were tried, but none gave a satisfactory result. The reason for this is the chemical reactivity of the γ,δ-dioxoacrylate moiety in podoschyphic acid, as soon as **1** is formed by chemical hydrolysis it is affected by the hydrolytic conditions to yield, at best, modest amounts of **1**. An attempt to reverse the order of transformations and first hydrolyse the ester **3** to the corresponding acid and then oxidise the diol to podoscyphic acid **1** failed, as the oxidation was not possible to perform even with TEMPO. However, with a lipase,[8] a 70% conversion of the ester **4** to podoscyphic acid **1** could be achieved in a few hours at room temperature. The spectroscopic data of the synthetic podoscyphic acid were identical with those reported for the natural product.[1]

3. Experimental

3.1. General procedures

Unless otherwise noted, chemicals were of p.a. quality and obtained from commercial suppliers and used without further purification. TLC analyses were made on Merck DC–Alufolien Kiselgel 60 F254 SiO_2 plates, visualised by spraying with anisaldehyde/sulphuric acid and warming to 120°C. The MS spectra (FAB ionisation) was recorded with a JEOL SX102 spectrometer, and NMR spectra (in $CDCl_3$) with a Bruker ARX 500 spectrometer at 500 MHz (^1H) and 125 MHz (^{13}C). The chemical shifts are reported in ppm with the solvent signals ($\delta_H = 7.26$ and $\delta_C = 77.0$) as reference. COSY, HMQC and HMBC experiments were recorded with gradient enhancements using sine shaped gradient pulses.

3.1.1. Hexadeca-2,4-dienoic acid ethyl ester 2. To a solution of diisopropylamine (1.99 mmol, 1.22 equiv.) in dry THF (3 mL) at −78°C under a nitrogen atmosphere, BuLi (1.63 mmol, 1.11 equiv.) was added gently to generate LDA. After 10 min, triethyl 4-phosphonocrotonate (2.17 mmol, 1.11 equiv.) was added dropwise, and after an additional 15 min, dodecanal (1.63 mmol, 1.0 equiv.) dissolved in dry THF (3 mL) was cannulated into the reaction mixture. The reaction was allowed to reach 0°C and stirred for 1 h. The reaction was then stirred at room temperature for an additional hour, whereafter the reaction mixture was diluted with saturated NH_4Cl (10 mL) and extracted with diethyl ether (3×20 mL). The combined ether layers was dried with $MgSO_4$ and the solvent removed under reduced pressure. The remaining oily extract was purified by SiO_2 chromatography, EtOAc/heptane (1:20), resulting in 330 mg (72%) of a 9:1 mixture of the (2*E*,4*E*) and (2*E*,4*Z*) isomers of hexadeca-2,4-dienoic acid ethyl ester (**2**). The NMR data for the major isomer is given: ^1H NMR (400 MHz, $CDCl_3$): δ 0.87 (t, $J=7.5$ Hz, 3H), 1.23–1.33 (m, 16H), 1.29 (t, $J=7.1$ Hz, 3H); 1.42 (m, 2H), 2.16 (t, $J=7.5$ Hz, 2H), 4.28 (q, $J=7.0$ Hz, 2H), 5.78 (d, $J=15.4$ Hz, 1H), 6.12 (dt, $J=15.3$ and 6.4 Hz, 1H), 6.16 (dd, $J=10.0$ and 15.3 Hz, 1H), 7.26 (dd, $J=10.0$ and 15.4 Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.5, 14.7, 23.1, 29.1, 29.5, 29.6, 29.7, 29.8, 29.95, 30.1, 32.3, 33.4, 60.5, 119.6, 128.8, 145.1, 145.6, 167.7. HRFABMS: 281.2480 ($M+H^+$, $C_{18}H_{33}O_2$ requires 281.2480).

3.1.2. (2*E*)-4,5-Dihydroxy-2-hexadecenoic acid ethyl ester 3. $K_3Fe(CN)_6$ (2.14 mmol, 3.0 equiv.), K_2CO_3 (2.14 mmol, 3.0 equiv.) and quinuclidine (0.035 mmol, 5 wt%) were added to a 1:1 solution of water: *t*-BuOH (4 mL) under stirring. OsO_4 in *t*-BuOH (0.021 mmol, 3 wt.%) was then added to the solution and the mixture was cooled to 0°C. Methanesulfone amide (0.71 mmol, 1.0 equiv.) was then added to the cooled solution followed by hexadeca-2,4-dienoic acid ethyl ester (**2**) (0.71 mmol, 1.0 equiv.). The reaction was stirred for 4 days under a nitrogen atmosphere and followed with TLC. Sodium metabisulphite (1.1 g) was then added to the reaction mixture and the suspension was stirred for 30 min. The reaction was diluted with brine (10 mL) and extracted with CH_2Cl_2 (4×20 mL), the organic phase was dried with $MgSO_4$ and concentrated under reduced pressure. Chromatography on SiO_2 with EtOAc/heptane (1:8) resulted in 115 mg (51%) of (2*E*)-4,5-dihydroxy-2-hexadecenoic acid ethyl ester **3** and 33 mg (16%) recovery of the starting material **2**. ^1H NMR (400 MHz, $CDCl_3$): δ 0.89 (t, $J=7.0$ Hz, 3H), 1.15–1.33 (m, 18H), 1.29 (t, $J=7.0$ Hz, 3H), 1.62 (m, 2H), 2.03 (d, $J=3.8$ Hz, 1H), 2.40 (d, $J=5.2$ Hz, 1H), 3.57 (brs, 1H), 4.15 (d, $J=3.6$ Hz, 1H), 4.28 (q, $J=7.0$ Hz, 2H), 6.16 (d, $J=15.9$ Hz, 1H), 6.94 (dd, $J=15.6$ Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1, 14.3, 22.6, 22.7, 25.5, 29.3, 29.4, 29.5, 29.6, 29.6, 31.9, 33.0, 60.6, 74.0, 74.1, 122.3, 147.0, 166.4. HRFABMS: 315.2549 ($M+H^+$, $C_{18}H_{35}O_4$ requires 315.2535).

3.1.3. (2*E*)-4,5-Dioxo-2-hexadecenoic acid ethyl ester 4. (2*E*)-4,5-Dihydroxy-2-hexadecenoic acid ethyl ester (0.3 mmol, 1 equiv.) was dissolved in CH_2Cl_2 (5.0 mL) and cooled to 0°C. To the solution, TEMPO (0.015 mmol, 0.05 equiv.), KBr (0.15 mmol, 0.5 equiv.) and water (0.3 mL) was added, followed by saturated $NaHCO_3$ (4 mL) and saturated NaOCl (4 mL). After stirring for 10 min the phases were separated and the water phase was extracted with CH_2Cl_2 (2×20 mL). The combined organic phase was dried with $MgSO_4$ and concentrated resulting in a quantitative yield of (2*E*)-4,5-dioxo-2-hexadecenoic acid ethyl ester (100%). ^1H NMR (400 MHz, $CDCl_3$): δ 0.87 (t, $J=7.5$ Hz, 3H), 1.15–1.42 (m, 16H), 1.29 (t, $J=7.1$ Hz, 3H), 1.63 (m, 2H), 2.81 (t, $J=7.5$ Hz, 2H), 4.28 (q, $J=7.0$ Hz, 2H), 6.92 (d, $J=16.4$ Hz, 1H), 7.78 (d, $J=16.4$ Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1, 14.3, 22.7, 22.9, 29.1, 29.3, 29.4, 29.5, 29.6, 29.7, 31.9, 36.4, 62.0, 132.9, 135.6, 165.3, 187.4, 199.9. HRFABMS: 311.2227 ($M+H^+$, $C_{18}H_{31}O_4$ requires 311.2222).

3.1.4. (2*E*)-4,5-Dioxo-2-hexadecenoic acid 1, podoscyphic acid. (2*E*)-4,5-Dioxo-2-hexadecenoic acid ethyl ester (0.3 mmol, 1 equiv.) was dissolved in methyl *t*-butyl ether saturated with water (10 mL) and immobilised *Candida antarctica* Lipase B (Novozyme 435) (20 mg) was added. The slurry was stirred at room temperature for 2 h whereafter the Novozyme 435 pellets were removed by filtration and the organic solvent evaporated under reduced pressure. Purification by chromatography on silica gel with EtOAc/heptane (1:6) containing 1% HOAc yielded 60 mg (70%) of (2*E*)-4,5-dioxo-2-hexadecenoic acid and 17 mg (18%) recovery of the starting material **3**. The spectroscopic data were identical to those reported for the natural product[1]. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.87 (t, $J=7.5$ Hz, 3H), 1.15–1.42 (m, 16H), 1.63 (m, 2H), 2.81 (t, $J=7.5$ Hz, 2H), 6.92 (d, $J=16.4$ Hz, 1H), 7.78 (d, $J=16.4$ Hz, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 14.1, 22.7, 22.9, 29.1, 29.3, 29.4, 29.5, 29.6, 29.7, 31.9, 36.4, 133.7, 134.3, 167.4, 186.8, 199.3. HRFABMS: 283.1904 ($M+H^+$, $C_{16}H_{27}O_4$ requires 283.1909). TLC EtOAc/heptane (1:6) containing 1% HOAc, $R_f=0.66$

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