5-(2-Methylphenyl)-4-pentenoic Acid from a Terrestrial Streptomycete

Venugopal J. R. V. Mukku[a], Rajendra P. Maskey[a], Peter Monecke[a], Iris Grün-Wollny[b], and Hartmut Laatsch[a]

[a] Department of Organic Chemistry, University of Goettingen, Tammannstrasse 2, D-37077 Goettingen, Germany
[b] bioLeads, Waldhofer Str. 104, D-69123 Heidelberg, Germany

* Reprint requests to Prof. Dr. H. Laatsch. Fax: +49(0)551-3996660. E-mail: hlaatsc@gwdg.de

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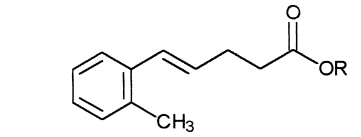
Streptomycete, 5-(2-Methylphenyl)-4-pentenoic Acid

From a terrestrial *Streptomycete,* GW 10/2517, the new 5-(2-methylphenyl)-4-pentenoic acid (**1a**) was isolated. The structure of **1a** was proven by a detailed spectroscopic analysis and by synthesis.

Introduction

ortho-Methyl phenyl alkenoic acids are periodically encountered in nature. Examples include the bronchodilatatoric rubrenoic acids [1] from *Alteromonas rubra*, *o*-methylcinnamide [2] (U-77863, which showed antitumor properties) from *Streptomyces griseoluteus*, and serpentene [3] from *Streptomyces* sp. Tü 3851. In our continuing search for secondary metabolites of bacteria we have now isolated a new member (**1a**) of this class of compounds from a terrestrial *Streptomycete* GW 10/2517. From the same *Streptomycete*, furan-2,5-dimethanol [4] was isolated along with 2-(4-hydroxyphenyl)-ethanol, 2′-deoxythymidine, and 2′-deoxyuridine. We report the taxonomy of the producing strain, isolation, structure determination, and synthesis of compounds **1** and **2** (Fig. 1).



1a: R = H
1b: R = Me

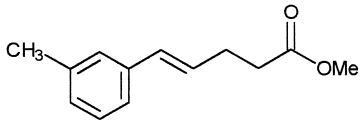


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Fig. 1. Structures of 5-(2-methylphenyl)-4-pentenoic acid (**1a**), its methyl ester **1b**, and 5-(3-methylphenyl)-4-pentenoic acid methyl ester (**2**).

Results and Discussion

Compound **1a** was obtained as a colourless solid with the formula $C_{12}H_{14}O_2$ based on the HR mass spectrum. The ^{1}H NMR spectrum showed multiplets (4 H) for an *ortho*- or *meta*-disubstituted aromatic ring. In the aliphatic region it showed a 3 H singlet (δ 2.32) indicating the presence of a methyl group attached to the aromatic ring, and a broad singlet (δ 2.58, 4 H) due to two methylene groups. Additionally two olefinic proton signals were observed at δ 6.65 (d, J = 15.6 Hz) and 6.08 (dm, J = 15.7 Hz) indicating an E-disubstituted double bond.

The ^{13}C NMR spectrum showed 12 carbon signals, one of them in the carbonyl region (δ 179.2). A free carboxylic acid was inferred based on the fact that there were no ester carbonyl signals in the ^{13}C NMR. This was also confirmed by the formation of a methyl ester on treatment with diazomethane. This implied a 5-arylpentenoic acid where the double bond is in the 4-position, based on the chemical shift and splitting of its proton signals. This structure was confirmed by a H,H COSY spectrum. However, whether the methyl group was in *ortho* or *meta* position of the aromatic ring remained uncertain due to strong signal overlapping. Since HSQC and HMBC spectra and even spectral simulations did not allow us to differentiate, both isomers were synthesized using standard procedures. Direct comparison con-

firmed the isolated compound to be 5-(2-methylphenyl)-4-pentenoic acid (**1a**). Both acids, as well as their methyl esters, were inactive against bacteria and fungi in the usual agar diffusion tests.

The known compounds 3,5-furandimethanol, 4-hydroxyphenyl ethanol, 2′-deoxythymidine, and 2′-deoxyuridine were isolated from the more polar fractions of the *Streptomycete* GW 10/2517 and identified by comparison with AntiBase [5] data.

Experimental Section

NMR spectra were measured on Varian Unity 300 (300.145 MHz) and Varian Inova 500 (499.876 MHz) spectrometers in $CDCl_3$ with TMS as internal standard. CIMS was recorded on a Finnigan MAT 95 A instrument using NH_3 as the collision gas. Preparative HPLC was performed using a RP18 column with a diode array detector. Flash chromatography was carried out on silica gel (230–400 mesh).

Strain GW10/2517

The culture was obtained from the strain collection of bioLeads in Heidelberg, Germany. This organism was Gram-positive, non-acid fast, grew aerobically, and differentiated into substrate and aerial mycelium. The aerial mycelium was monopodially branched with *flexuous* spore chains. Neither aerial hyphae nor substrate mycelium showed fragmentation. Other morphological features such as sporangia, or motile spores, were not observed. The aerial spore mass colour was light pink on yeast extract-malt agar and white on oatmeal and soil extract agar. The substrate mycelium was brown on most media. A light red diffusible pigment was formed on yeast extract-malt extract agar and on soil extract agar. Melanin pigments were not produced on tyrosine agar. The diaminopimelic acid isomer and the sugar composition indicated that the strain had cell walls of type I (L,L-diaminopimelic acid, no characteristic sugars) and belongs to the genus *Streptomyces.*

Fermentation

Three litres of culture medium composed of malt extract (30 g), yeast extract (12 g), and glucose (12 g) in tap water (3 l) were adjusted to pH 7.8 and distributed into 15 1 l-Erlenmeyer flasks. After sterilization, the flasks were inoculated with slant cultures of *Streptomyces* sp. GW 10/2517 and incubated for 72 hours at 28 °C. This culture was used to inoculate a 20-litre jar fermenter containing 18 l of culture medium described above. The medium was adjusted to pH 7.0, incubation was carried out at 28 °C for 72 h with automatic addition of 2 N HCl or 2 N NaOH to maintain the pH between 6.0–7.2, and Niax PPG 2025 (Union Carbide, Belgium N. V. Zwindrecht) to control foaming. Sterile air was supplied (5 l/min) and agitation was at 120 rpm. Active principles were extracted with ethyl acetate from the filtered broth and the cell residue and the extracts combined.

After defatting with cyclohexane, the ethyl acetate extract of the fermentation broth of strain GW 10/2517 was chromatographed on silica gel using a chloroform/methanol gradient (0 – 10% methanol). The fractions were monitored by TLC (UV absorption and anisaldehyde/sulfuric acid spray). Nine fractions were collected which were re-chromatographed on Sephadex LH-20 (methanol) and by HPLC (MeCN / water).

Purification of the second fraction led to the isolation of compound **1a**. Fraction 7 yielded furan-2,5-dimethanol, fraction 8 4-hydroxyphenyl ethanol, and fraction 9 2′-deoxythymidine, and 2′-deoxyuridine.

5-(2-Methylphenyl)-4-pentenoic acid (**1a**)

Colourless wax-like solid, 5 mg from fraction 2, R_f = 0.75 ($CHCl_3$-MeOH 9:1); UV/vis (MeOH): λ_{max} = 210, 249 nm; IR (KBr): ν = 3049, 2923, 1715 (C=O), 1431, 1299, 1208, 970 cm^{-1}. – ^1H NMR (300 MHz, $CDCl_3$): δ = 7.39 (t, J = 4.5 Hz, 1 H), 7.13 (m, 3 H), 6.65 (d, 1 H, J = 15.8 Hz, 5′-H), 6.08 (dm, J = 15.7 Hz, 1 H, 4′-H), 2.58 (m, 4 H, 2′ and 3′-CH_2), 2.32 (s, 3 H, 7′-CH_3). – ^{13}C NMR (50.3 MHz, $CDCl_3$): δ = 179.2 (C-1), 136.4 (C-2′) , 135.1 (C-1′), 130.2 (C-3′), 129.3 (C-5), 129.2 (C-4), 127.1 (C-4′), 126.0 (C-5′), 125.5 (C-6′), 33.9 (C-3), 28.2 (C-2), 19.8 (C-7′). – MS (EI, 70 eV): *m*/z (%) = 190 (73) [M^+], 131 (100) [M – CH_2COOH]$^+$. – HREIMS: m/z = 190.0993 (calcd. for $C_{12}H_{14}O_2$, 190.09938).

2,5-Furandimethanol

Colourless solid (3 mg) from fraction 7, R_f = 0.34 ($CHCl_3$-MeOH 9:1); spectroscopical data were identical with those of a commercial sample.

Syntheses

3-Carboxypropyl-triphenylphosphonium bromide

4-Bromobutyric acid (4.59 g, 27.5 mmol) and triphenyl phosphine (7.20 g, 27.5 mmol) were heated

under N_2 for 3 h at 180 °C. After cooling to r.t., the solid was suspended in dry CH_2Cl_2 (50 ml), dry ether (100 ml) was added, and the precipitate was filtered off under nitrogen and dried; yield 9.48 g (80%). – ^{1}H NMR (300 MHz , CD_3OD): δ = 7.80 (m, 15 H), 3.43 (m, 2 H, P^+-CH_2), 2.59 (t, J = 6.6 Hz, 2 H, α-CH_2-COOH), 1.90 (m, 2 H, β-CH_2).

5-(2-Methylphenyl)-4-pentenoic acid methyl ester (**1b**)

To a solution of hexamethyldisilazane (3.45 ml, 16.5 mmol) in dry THF (20 ml), *n*-BuLi solution in pentane (9.75 ml, 15 mmol) was slowly added under nitrogen at 0 °C. A suspension of the above phosphonium salt (2.15 g, 5 mmol) in dry THF (10 ml) was added and the mixture stirred for 1 h at 20 °C. When the salt was dissolved and the solution turned red, a solution of *o*-tolylaldehyde (600 mg, 5 mmol) in THF (3 ml) was added dropwise. After stirring for 90 min at r.t., water (50 ml) and diethyl ether (20 ml) was added. The ether was discarded after washing with water, the aqueous layers were combined, acidified and extracted several times with ether to obtain **1a**.

The crude acid **1a** was methylated with excess diazomethane and the ester **1b** purified on silica gel (CH_2Cl_2); yield 530 mg (52% for both steps). – ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.38 (t, J = 4.5 Hz, 1 H), 7.15 (m, 3 H), 6.63 (d, J = 15.8 Hz, 1 H), 6.06 (dd, J = 15.8, J' = 6.4 Hz, 1 H), 3.69 (s, 3 H, OMe), 2.53 (m, 4 H, 2 CH_2), 2.32 (s, 3 H, Ar-Me).

The pure acid **1a** was obtained by saponification of **1b** with sodium hydroxide. The NMR data were identical with those of the natural product.

5-(3-Methylphenyl)-4-pentenoic acid methyl ester (**2**)

In the same way as for **1b**, the ester **2** was obtained starting with *m*-tolylaldehyde. Yield 540 mg (53%). – ^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.15 (m, 3 H), 7.02 (d, J = 7.2 Hz, 1 H), 6.40 (d, J = 16.4 Hz, 1 H), 6.18 (dt, J = 16.4, J' = 6.4 Hz, 1 H), 3.69 (s, 3 H, OMe), 2.50 (m, 4 H, 2 CH_2), 2.33 (s, 3 H, Ar-Me).

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