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Total Synthesis of a Reported Fluorometabolite from *Streptomyces* sp. TC1 Indicates an Incorrect Assignment. The Isolated Compound Did Not Contain Fluorine

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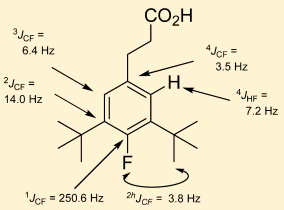
Supporting Information

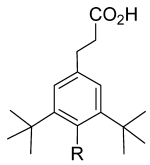
ABSTRACT: 3,5-Di-*tert*-butyl-4-fluorophenylpropionic acid (**1**) was recently reported as a natural product from *Streptomyces* sp. TC1. This was a notable disclosure because fluorinated natural products are exceedingly rare, and in this case it suggested that the bacterium had the capacity to mediate an enzymatic aryl fluorination reaction. However, a synthesis of the putative metabolite **1** demonstrates that the spectroscopic data are inconsistent with the proposed structure. There is no evidence that the isolated compound contained a fluorine atom.



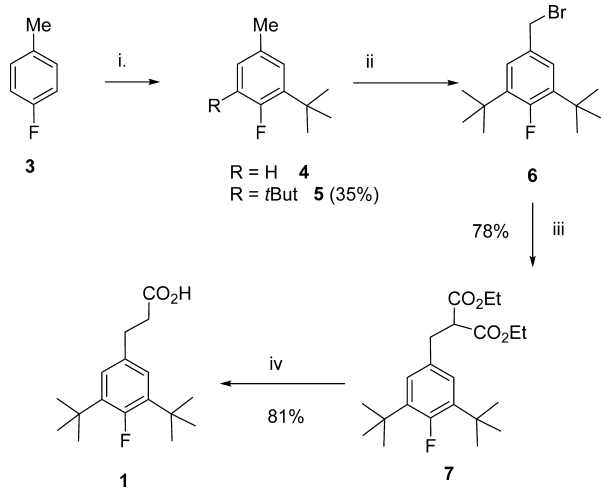
Di-*tert*-butylfluorophenylpropionic acid (**1**) was recently reported in this journal as a novel fluorometabolite, isolated from the microorganism *Streptomyces* sp. TC1.[1] The isolation immediately attracted attention[2] because the identification of fluorine-containing metabolites is extremely rare.[3,4] Also this would be a particularly intriguing compound, as it suggests that the microorganism has an enzyme that can accomplish an aryl fluorination, a class of enzyme reaction without precedent. Such an enzyme would have exciting biotechnological potential in view of the large-scale utility of aryl fluorides in the fine chemicals industry. However, the ^{19}F NMR signal in the Supporting Information (SI)[1] is extremely broad (4000 Hz) and is not consistent with a soluble low molecular weight organo-fluorine compound, which would be expected to have a sharp, highly resolved signal. Additionally the ^{1}H and ^{13}C NMR spectra do not appear to have any proton to fluorine ($^{4}J_{HF}$) or carbon to fluorine ($^{1}J_{CF}$) coupling constants. None are visible or reported. Thus, there is no evidence from the submitted NMR data that the compound actually contains a fluorine atom. The structure of **1** is claimed based primarily on X-ray structure analysis. However, it is well known that it is difficult for a crystallographer to distinguish hydroxyl from fluorine, as these substituents have a similar number of electrons and the hydrogen of the OH is not easily resolvable. On the face of it, the X-ray structure could actually be that of phenol **2**. Thus, we decided to prepare compound **1** by synthesis to compare its spectroscopic properties with the reported data of the compound isolated from *Streptomyces* sp. TC1.

RESULTS AND DISCUSSION

The synthesis route to **1** is shown in Scheme 1. 4-Fluorotoluene (**3**) was treated with 2-chloro-2-methylpropane and aluminum trichloride, following a Friedel-Craft's protocol described for a similar reaction with toluene.[5] A complete conversion of fluorotoluene (**3**) to the mono- (**4**) and di-alkylated (**5**) products was achieved in a ratio of 2:5 as determined by ^{19}F NMR. These products could be separated by



Scheme 1. Synthesis of 1[a]



[a]Reagents and conditions: (i) $AlCl_3$, *t*-BuCl; (ii) NBS, $(PhCO)_2O_2$, CCl_4; (iii) NaH, THF, $CH_2(COOEt)_2$; (iv) (a) LiOH, THF, H_2O; (b) HCl; (c) aq H_2SO_4, reflux.

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fractional distillation, and the desired disubstituted product **5** was isolated in 35% yield. Compound **5** was a crystalline solid, and X-ray analysis supported its structure as shown in Figure 1a. In the ^{1}H NMR spectrum of **5** there was a very clear $^{4}J_{HF}$

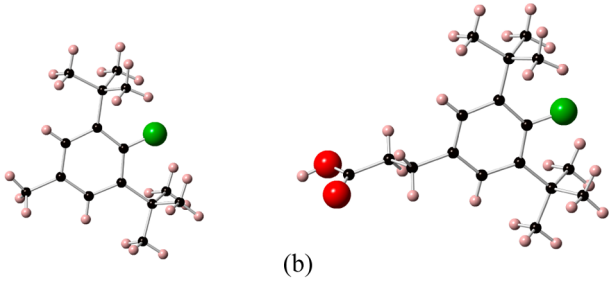

Figure 1. X-ray structures of (a) Friedel–Crafts alkylation product **5** (CCDC 992852) and (b) a synthetic sample of **1** (CCDC 992853).

coupling constant (7.6 Hz) between the fluorine and the 2/6 aryl protons. Bromination of **5** was efficiently achieved by treatment with elemental bromine (Br_2) and benzoylperoxide in CCl_4 as the solvent[5] to generate benzyl bromide **6** in 78% yield. Benzyl bromide **6** was then treated with diethyl malonate and sodium hydride,[6] to give alkylated malonate **7**, followed by treatment in aqueous base to mediate a hydrolysis.[7] Carboxylic acid **1** was then prepared by acid (aq H_2SO_4)-catalyzed decarboxylation of the intermediate malonic acid.

The ^{1}H, ^{19}F, and ^{13}C NMR spectra all provide signatures for the presence of fluorine. A summary of coupling constants is shown in Figure 2. Most obviously the $^{4}J_{HF}$ coupling (7.2 Hz)

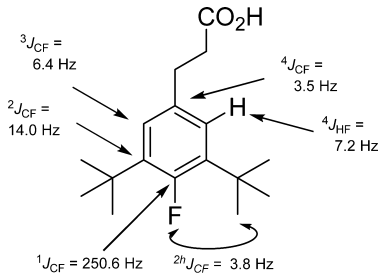

Figure 2. Significant J_{FH} and J_{CF} coupling constants determined by NMR analysis of synthetic **1**. Coupling constants to carbon around the ring identify the presence of fluorine. These are not reported or obvious in the data presented for the compound isolated from *Streptomyces* sp. TC1.[1]

observed here between fluorine and the 2/6 aryl hydrogens is not apparent in the reported data for structure **1** in the isolation paper.[1] Also there are obvious fluorine to carbon couplings to all of the aryl ring carbons in the data for synthetic **1**. A notable observation is a through-hydrogen $^{2h}J_{CF}$ coupling of 3.8 Hz between the fluorine atom and the methyl hydrogens of the *tert*-butyl groups. Carbon–fluorine scalar couplings can be transmitted through short noncovalent hydrogen to fluorine contacts.[8] The shortest contact distance between the fluorine atom and the closest *tert*-butyl methyl hydrogens is 2.25 Å (from the X-ray structure), a distance significantly shorter than the van der Waal contact distance (2.47 Å).[9] Thus, the observed $^{2h}J_{CF}$ coupling is consistent with this short contact. Electrospray ionization mass spectrometry (EIMS) of **1** was conducted in both +ve and −ve ion modes and gave characteristic ions of $[M + Na]^+$ = 303.1 amu and $[M - H]^-$ = 279.1 amu. We have also carried out a FAB mass spectrometry analysis, to compare to the FAB spectrum of the compound isolated in the original paper. This gave as expected $[M + H]^+$ = 281.1 amu for **1**; however the original FAB reported a signal at 280.18 amu in positive ion mode, which suggests a molecular ion of 279.1 amu. This does not fit with the organofluorine compound **1**, or with phenol **2**, which would be expected to report a FAB $[M + H]^+$ of 279.1.

It is clear that the NMR data for synthetic **1** do not match the compound reported from *Streptomyces* sp. TC1.[1] The very broad peak observed in the originally reported ^{19}F NMR (δ_F −168.01 ppm) most probably arises from the Teflon lining in the NMR probe, with can give rise to a featureless and broad baseline signal over a long acquisition and contrasts with the very sharp signal (δ_F −110.1 ppm) for the synthetic sample of **1**. This may have led to the conclusion that the X-ray structure contained fluorine; however OH and F can be difficult to distinguish by X-ray analysis. The isolate was shown to have antioxidant activity.[1] We tentatively suggest that the compound that was isolated, or contaminated the isolation procedure, was the phenolpropionic acid **2** and that there was a misinterpretation of the X-ray, NMR,[10] and possibly mass spec data. This structural motif is found widely in commercial antioxidants. For example the methyl ester of **2**, that is compound **8**, is a relatively well-known industrial antioxidant (called "stabiliff") used in fragrance mixtures and perfumes,[11] and Irganox 1010 (**9**)[12] is used in the plastics industry to protect products against thermo-oxidative degradation.

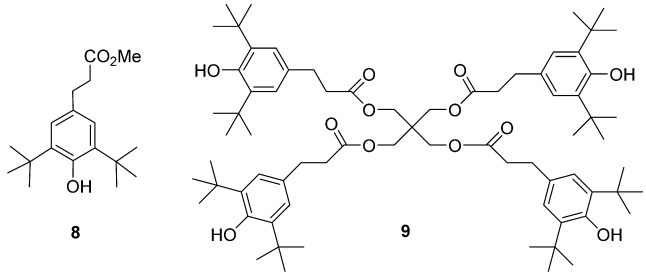

Figure 3. Representative industrial antioxidants (stabiliff, **8**, and Irganox 1010, **9**) carrying the 3,5-di-*tert*-butyl-4-fluorophenolpropionate motif.

EXPERIMENTAL SECTION

General Experimental Procedures. All chemicals were purchased from Sigma-Aldrich, Alfa Aesar, Fisher Scientific, and Fluorochem. All reactions were carried out in oven-dried glassware under an argon atmosphere using a double-vacuum manifold with the inert gas passing through a bed of silica gel and molecular sieves. Anhydrous CH_2Cl_2 and THF were obtained from an MBraun MB SPS-800 solvent purification system, where the solvent was dried by passage through activated filter columns and dispensed under an atmosphere of argon. Petroleum ether refers to the fraction with a boiling point between 40 and 60 °C. All chemicals were used as supplied. All NMR spectra were recorded using Bruker Avance III 500 and Bruker Avance 300 or 500 spectrometers. ^{1}H NMR spectra were recorded at either 300 or 500 MHz. ^{13}C NMR spectra were recorded using the DEPTQ or UDEFT pulse sequence and broadband proton decoupling at either 75, 100, or 125 MHz. ^{19}F NMR spectra were recorded at 282, 376, or 470 MHz. All chemical shifts, δ, are stated in units of parts per million (ppm), relative to a standard. For ^{1}H NMR and ^{13}C NMR the reference point is TMS (δ_H and δ_C: 0.00 ppm). For

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^{19}F NMR the reference point is CCl_3F (δ_F: 0.00 ppm). Melting points were determined using a Griffin MPA350 or an Electrothermal 9100 melting point apparatus and are uncorrected. High- and low-resolution mass spectra were obtained by electospray ionization (ESI) using either an LTQ Orbitrap XL spectrometer or a Waters Micromass LCT spectrometer in positive or negative mode.

*3,5-Di-tert-butyl-4-fluorotoluene, **5**.* 2-Chloro-2-methylpropane (8.4 g, 91 mmol) was added in two portions over a period 30 min to a rapidly stirred solution of $AlCl_3$ (1.5 g, 11.3 mmol) in *p*-fluorotoluene (5.0 g, 45.4 mmol) at 0 °C for 6 h. The reaction was continued for a further 6 h at room temperature. Water was added, the product was extracted into CH_2Cl_2, then the solvent was removed under reduced pressure, and the product was purified by Vigreux distillation. The product **5** crystallized on standing (3.5 g, 35%). Mp 82−83 °C; ^{1}H NMR (CDCl3, 300 MHz) δ 6.88 (2H, dd, J_{HF} = 7.23, 0.5 Hz, H-2, H-6), 2.30 (3H, s, CH_3), 1.30(18H, d, J_{HF} = 1.1 Hz, 6 CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 159.6 (d, $^1J_{CF}$ = 249.4 Hz, C-4), 137.3 (d, $^2J_{CF}$ = 14 Hz, C-3), 131.7 (d, $^4J_{CF}$ = 3.4 Hz, C-1), 125.6 (d, $^3J_{CF}$ = 6 Hz, C-2), 34.4 (CH_3), 30.1 (d, $^{2h}J_{CF}$ = 4 Hz, 6 × CH_3), 21.3 (C, $C(CH_3)_3$); ^{19}F NMR ($CDCl_3$, 282 MHz) δ −112.2 (s, 1F); HR-CIMS, *m/z* 222.1779 (calcd for $C_{15}H_{23}F$, 222.1784).

*3,5-Di-tert-butyl-4-fluoro(bromomethyl)benzene, **6**.* A solution of 3,5-di-*tert*-butyl-4-fluorotoluene (**5**; 2 g, 9.0 mmol), *N*-bromosuccinimide (1.6 g, 9.0 mmol), and benzoylperoxide (0.05 g, 0.2 mmol) in CCl_4 (120 mL) was heated under reflux for 3 h. The solution was cooled, filtered, and concentrated under reduced pressure, and the product was purified by flash column chromatography (petrol) to afford a colorless oil, **5** (2.1 g, 78%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.23 (2H, d, $^4J_{HF}$ = 7.0, H-2, H-6), 4.52 (2H, s, CH_2Br), 1.42 (18H, d, J_{HF} = 1.1 Hz, 6 × CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 162.3 (d, $^1J_{CF}$ = 250 Hz, C-4), 138.4 (d, $^2J_{CF}$ = 14 Hz, C-3), 132.4 (d, $^4J_{CF}$ = 3.6 Hz, C-1), 126.5 (d, $^3J_{CF}$ = 7.3 Hz, C-2), 41.9 (CH_2Br), 35.4 (C, $C(CH_3)_3$), 30.4 (d, $^4J_{CF}$ = 4 Hz, 6 × CH_3); ^{19}F NMR ($CDCl_3$, 282 MHz) δ −106.7 (s, 1F); HR-EIMS, *m/z* 221.1700 (calcd for $C_{15}H_{22}F$, 221.1706), which corresponds to $[M - Br]^+$.

*Diethyl (3,5-Di-tert-butyl-4-fluorobenzyl)malonate, **7**.* Diethyl malonate (1.38 g, 8.6 mmol) was added dropwise to a stirred suspension of sodium hydride (60% dispersion in mineral oil, 0.21 g, 8.6 mmol) in THF (10 mL) at 0 °C. A solution of 3,5-di-*tert*-butyl-4-fluoro(bromomethyl)benzene (**6**; 2 g, 6.66 mmol) in anhydrous THF (10 mL) was added after 30 min over a period of 10 min, followed by stirring for 2 h at room temperature. The reaction mixture was then diluted with H_2O (20 mL), and the product extracted into EtOAc, washed, and concentrated under reduced pressure. The residue was then purified by flash column chromatography (petrol/Et_2O, 9:1) to afford diester **7** as a viscous oil (1.5 g, 60%). ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.90 (2H, d, $^4J_{HF}$ = 7.1 Hz, H-2, H-6), 4.10 (4H, q, *J* = 7 Hz, 2CH_2CH_3), 3.54 (1H, t, *J* = 8 Hz, CH), 3.07 (2H, d, *J* = 8 Hz, $PhCH_2$), 1.28 (18H, d, J_{HF} = 1.1 Hz, 6 × CH_3), 1.15 (6H, q, *J* = 7 Hz, 2CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 169.0 (2 × COOEt), 160.3 (d, $^1J_{CF}$ = 250 Hz, C-4), 137.3 (d, $^2J_{CF}$ = 14 Hz, C-3), 131.9 (d, $^4J_{CF}$ = 3.6 Hz, C-1), 125.5 (d, $^3J_{CF}$ = 6.5 Hz, C-2), 61.5 (2 × OCH_2), 54.1 (CH), 34.6 (2 × $C(CH_3)_3$), 34.5 ($PhCH_2$), 30.1 (d, $^4J_{CF}$ = 3.6 Hz, 6 × CH_3), 14.1 (2 × CH_2CH_3); ^{19}F NMR ($CDCl_3$, 282 MHz) δ −110.3 (s, 1F); HR-CIMS *m/z* 380.2368 (calcd for $C_{22}H_{33}FO_4$, 380.2363).

*3-(3,5-Di-tert-butyl-4-fluoropheny)propanoic acid, **1**.* A solution of LiOH/H_2O (30 mg 0.7 mmol) in H_2O (2 mL) was added to a stirred solution of diethyl (3,5-di-*tert*-butyl-4-fluorobenzyl)malonate (**7**; 100 mg 0.27 mmol) in THF (2 mL), and stirring was continued for 2 h. The reaction was then neutralized with cHCl to pH = 2, and the product extracted into EtOAc. The organic extracts were concentrated under reduced pressure. The product was heated under reflux in aqueous sulfuric acid for 6 h. On cooling, the product crystallized and was purified by flash chromatography (EtOAc/petrol ether, 2:1) to afford **1** (60 mg, 81%) as colorless needles. Mp 165−166 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.92 (2H, d, *J* = 7.20 Hz, H-2, H-6), 2.85 (2H, t, *J* = 7.2 Hz, $PhCH_2$), 2.60 (2H, t, *J* = 7.2 Hz, CH_2COOH), 1.30 (18H, d, $^5J_{HF}$ = 1.1 Hz, 6 × CH_3); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.2 (COOH), 160.1 (d, $^1J_{CF}$ = 250.6 Hz, C-4), 137.4 (d, $^2J_{CF}$ = 14 Hz, C-3), 134.1 (d, $^4J_{CF}$ = 3.50 Hz, C-1), 124.8 (d, $^3J_{CF}$ = 6.4 Hz, C-2), 35.8 (C, CH_2COOH), 34.5 (C, $PhCH_2$), 30.6 ($C(CH_3)_3$), 30.1 (d, $^{2h}J_{CF}$ = 3.8 Hz, 6 × CH_3); ^{19}F NMR ($CDCl_3$, 282 MHz) δ −110.1 (s, 1F); HR-EIMS (−ve ion mode) *m/z* 279.1763 (calcd for $C_{17}H_{24}FO_2$, 279.1760), which corresponds to $[M - H]^-$; (+ve ion mode) *m/z* 303.1727 (calcd for $C_{17}H_{25}FNaO_2$, 303.1736), which corresponds to $[M + Na]^+$; FAB *m/z* 281.1, which corresponds to $[M + H]^+$.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Analytical data including ^{1}H, ^{13}C, and ^{19}F NMR spectra and selected mass spectra for compounds **5**, **6**, **7**, and **1** are illustrated. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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