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SYNTHESIS OF [5-^{14}C]-DODECANE AND [8-^{14}C]-HEXADECANE

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Dedicated to the memory of Dr. Michael Binder.

SUMMARY

[5-^{14}C]-Dodecane and [8-^{14}C]-hexadecane were synthesized starting with [1-^{14}C]-octanoic acid. The carboxylic acid was reduced to 1-octanol, which was esterified to n-octyl p-toluenesulfonate. Following a Corey-House procedure, the sulfonate was either reacted with Li[Cu(butyl)$_2$] to [5-^{14}C]-dodecane (40 % overall yield), or with Li[Cu(octyl)$_2$] to [8-^{14}C]-hexadecane (24 % overall yield). The lithium di-alkyl cuprates were prepared from butyl lithium and n-octyl bromide, respectively. Preliminary experiments with non-labeled compounds using a Wittig reaction as chain extension step, turned out to be less favourable. The Corey-House route provides a simple method for the synthesis of specifically ^{14}C-labeled alkanes from commercially available [1-^{14}C]-carboxylic acids.

Key Words: [5-^{14}C]-dodecane, [8-^{14}C]-hexadecane, alkanes.

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INTRODUCTION

Industrial progress has resulted in a contamination of the environment with a multitude of organic xenobiotics. This holds also true for mineral oil and and its products, e. g. gasoline, kerosene, or gas oil, which are spilled into the oceans and soil in huge quantities. Remediation of these contaminated sites is often performed using microbiological methods; these take advantage of microorganisms capable of utilizing different aliphatic and aromatic hydrocarbons as carbon source[1]. Open chain, aliphatic hydrocarbons are thus degraded by numerous organisms[2], often with the aid of excreted or membrane-bound biosurfactants[3], but usually with some preference for C_9 - C_{18} straight-chain, saturated alkanes[4]. At present the reason for this, the mechanisms of uptake and metabolism, the biosynthesis of biosurfactants, etc. are only understood to some extent. Further fundamental research in this area is required, and would provide means for a better application of bioremediation.

Uptake or metabolism studies concerning alkanes are at best performed using ^{14}C-labeled model compounds. Some of these are commercially available, but without exeption these alkanes are labeled at C(1) of the carbon chain. Upon degradative attack by microorganisms this label would be rapidly lost, thus preventing a further tracing of the xenobiotic. In addition, the majority of interesting alkanes, especially the long-chain ones, are not available.

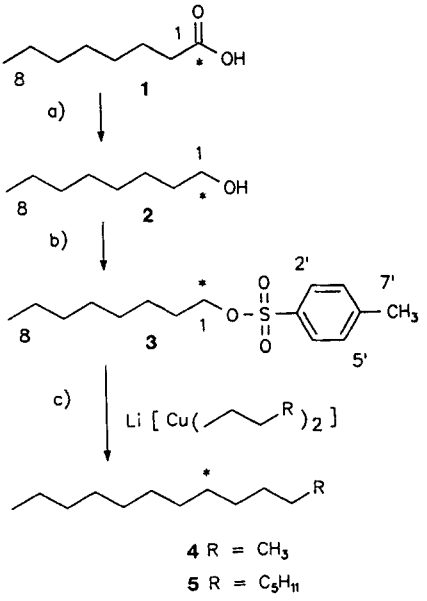
The present investigation deals with a simple and practicable synthesis of two alkane model compounds labeled amidst the aliphatic carbon chain, namely [5-^{14}C]-dodecane and [8-^{14}C]-hexadecane, starting with commercially available [1-^{14}C]-octanoic acid. However, the methods outlined below may also be used for the synthesis of various other specifically ^{14}C-labeled straight-chain

alkanes and possibly branched-chain aliphatic hydrocarbons. To our knowledge, the synthesis of alkanes ^{14}C-labeled in this manner has not been reported.

RESULTS AND DISCUSSION

Preliminary studies were performed along two synthetic pathways, which both starting with non-labeled octanoic acid on the one hand and an n-octyl halide on the other, ultimately resulted in the formation of a carbon-carbon bond between these two straight-chain compounds. The final carbon chain extension was accomplished either by a Wittig reaction or by a Corey-House synthesis. Both reaction sequences were examined with regard to yield and practicability, and using as precorsor [1-^{14}C]-octanoic acid, would lead to a defined ^{14}C-label at position C(8) in hexadecane. In addition, the Corey-House synthetic route was performed with octanoic acid and butyl lithium as precursors, thus affording dodecane which would be ^{14}C-labeled at position C(5) by use of the [1-^{14}C]-labeled carboxylic acid.

Concerning the Wittig route, non-labeled octanoic acid was esterified with BF_3·MeOH in 95 % yield[5], followed by reduction of the methylester to octanal (55 % yield) using diisobutylaluminium hydride[6]. The product was reacted with n-octyltriphenylphosphonium iodide in THF to 8-hexadecene (50 % yield) which again was converted to hexadecane by catalytic hydrogenation in cyclohexane (95 % yield). Overall yield of this reaction sequence was 24 % in relation to octanoic acid. Correspondingly, the Corey-House route outlined in Scheme 1 and to be discussed below, was performed with non-labeled octanoic acid, and turned out to be more advantageous; with regard to the carboxylic acid, the overall yields amounted to 49 % and 45 % for dodecane and hexadecane, respectively. Consequently, this reaction sequence was applied for the synthesis of the ^{14}C-labeled straight-chain alkanes.



Scheme 1. a) $LiAlH_4$, Et_2O; b) p-TosCl, pyridine/$CHCl_3$, 0°C, 3 h, subsequently 22°C, 16 h; c) Et_2O, -20°C to -30°C, 2 h, subsequently 22°C, 16 h; * = position of ^{14}C-label.

For that purpose, commercially available [1-^{14}C]-octanoic (**1**) was reduced to the alcohol **2** with $LiAlH_4$ in 68 % yield (75 % for the non-labeled sequence). **2** was converted to the tosylate[7] **3**; using a molar ratio 1-octanol:tosylchloride:pyridine of 1:1.5:2 in $CHCl_3$, the product was obtained in 82 % yield (after preparative TLC; non-labeled sequence: 89 %) without pyridine derived side-products[8]. On account of ethanol used as stabilizer a purification of $CHCl_3$ on Al_2O_3 was necessary; thus, the formation of ethyl p-toluenesulfonate was avoided.

For the Corey-House synthesis of [5-^{14}C]-dodecane (**4**), butyl lithium and purified[9] CuI were transformed to lithium dibutyl cuprate which in turn was in situ reacted[10] with **3** to the hydrocarbon **4**; after purification by preparative TLC a pure product was obtained in 72 % yield (73 % for the non-labeled

sequence). **4** was identified by cochromatography with non-labeled dodecane (TLC, GLC), and by its mass spectrum.

For the preparation of [8-^{14}C]-hexadecane (**5**), octyl lithium was synthesized[11] from n-octyl bromide and lithium in Et_2O; the concentration of the resulting solution was evaluated[12]. Octyl lithium and CuI in Et_2O were converted to lithium dioctyl cuprate which in turn was reacted with the tosylate **3** to crude **5** as described above. Preparative TLC afforded the pure hydrocarbon in 43 % yield (non-labeled sequence: 68 %); the product cochromatographed with non-labeled hexdecane (TLC, GLC) and was proven by its mass spectrum.

The overall yields of [5-^{14}C]-dodecane and [8-^{14}C]-hexadecane amounted to 40 % and 24 %, respectively. Compared to the corresponding non-labeled compounds the low yield of [8-^{14}C]-hexadecane can be refered to the extremely moisture sensitive reaction of lithium dioctyl cuprate with the tosylate **3**. It is to be expected that alkane preparations on a larger scale (approx. 500 mg; 1 - 10 mCi) will result in yields comparable to the non-labeled reaction sequences.

EXPERIMENTAL

General. All reactions with air or moisture sensitive reagents were carried out in oven- and flame-dried glassware under a positive pressure of dry Ar or N_2 using standard Schlenk techniques. TLC: Machery & Nagel and Merck precoated plates SIL-G 25 UV_{254}, 0.25 mm and silica gel 60 F_{254}, 1.00 mm, respectively. GLC: Hewlett-Packard HP 5890, Series II, equipped with a SE 54 capillary column (25 m x 0.2 mm x 0.58 µm film thickness). IR spectra: Beckman Acculab 4 or Perkin-Elmer FTIR 1750; in cm^{-1}. ^{1}H- and ^{13}C-NMR spectra: Varian VXR 300 at 300 and 75 MHz, resp.; chemical shifts in ppm rel. to TMS, coupling constants J in Hz.

EI-MS: Hewlett-Packard MSD HP5971 A; in m/z (rel. intensity in %). IR, NMR and MS measurements were performed with non-labeled reference compounds, unless otherwise noted.

[1-^{14}C]-1-Octanol (2). $LiAlH_4$ (70 mg, 1.75 mmol) was added to a solution of [1-^{14}C]-octanoic acid (**1**; 144 mg, 1 mmol, 102 μCi; Sigma Chemical Company) in Et_2O (15 ml). The reaction mixture was refluxed for 4 h, and stirred for 5 d (TLC control) at 22°C. H_2O and H_2SO_4 were added, the precipitate was filtered off, and the aqueous phase was extracted with Et_2O. The organic phase was dried, and the solvent was evaporated yielding **2** (87 mg, 0.67 mmol, 68 μCi). TLC analysis showed cochromatography with non-labeled 1-octanol and a radiochemical purity of 95 %; **2** was used without further purification. IR (liq. film): 3500-3000s, 2920s, 2830s, 1450m, 1040s. ^{1}H-NMR ($CDCl_3$): 0.88 (t, J = 7.0, 3 H-C(8)); 1.29 (m, 10 H-C(3 - 7)); 1.55 (m, 2 H-C(2)); 3.12 (br. s, OH); 3.60 (t, J = 7.0, 3 H-C(1)). ^{13}C-NMR ($CDCl_3$): 14.12 (C(8)), 22.74 (C(7)), 25.88 (C(3)), 29.40 (C(5)), 29.53 (C(4)), 31.93 (C(6)), 32.74 (C(2)), 62.79 (C(1)). MS: 84 (44, $M^+ - H_2O - CH_2{=}CH_2$), 70 (53), 56 (85), 41 (100, $C_3H_5^+$), 31 (69, $H_2C{=}OH^+$), 27 (70).

[1-^{14}C]-n-Octyl p-toluenesulfonate (3). Pyridine (108 μl, 1.34 mmol) and subsequently, p-toluenesulfonic acid chloride (191 mg, 1 mmol) were added slowly to a solution of **2** (87 mg, 0.67 mmol, 68 μCi) in $CHCl_3$ (10 ml, purified on a column of Al_2O_3) at 0°C. The reaction mixture was stirred for 3 h at 0°C and then for 16 h at 22°C. Et_2O and H_2O were added, the organic phase was separated, washed with 2 M HCl, 5 % Na_2CO_3, H_2O, and dried over Na_2SO_4. After evaporation of the solvent the product showed a radiochemical purity of 92 % (TLC). Purification by preparative TLC (solvent system: CH_2Cl_2:Et_2O, 4:1) afforded radiochemically pure **3** (156 mg, 0.55 mmol, 55.6 μCi) cochromatographing (TLC) with non-labeled n-octyl p-toluenesulfonate. IR (liq. film): 3100-3000w, 2930s,

2850s, 1600m, 1500m, 1460m, 1360s, 1180s, 1100s, 900s, 810s, 660m. ^{1}H-NMR ($CDCl_3$): 0.87 (t, J = 7.0, 3 H-C(8)); 1.23 (br. m, 10 H-C(3 - 7)); 1.64 (m, 2 H-C(2)); 2.45 (s, 3 H-C(7')); 4.03 (t, J = 7.0, 2 H-C(1)); 7.78 (d, J = 8.4, H-C(2'), H-C(6')); 7.37 (d, J = 8.4, H-C(3'), H-C(5')). ^{13}C-NMR ($CDCl_3$): 14.57, 21.55, 22.59, 25.33, 28.81, 28.87, 29.05, 31.69 (C(2 - 8), C(7')), 70.72 (C(1)), 127.82, 129.82, 133.27, 144.67 (C(1' - 6')). MS: 284 (2, M^+), 173 (100, SO_3-$C_7H_6^+$), 155 (29, SO_2-$C_7H_7^+$), 112 (39, $C_8H_{16}^+$), 91 (56, $C_7H_7^+$), 84 (37, $C_6H_{12}^+$), 70 (26), 65 (17, $C_5H_5^+$), 56 (23, $C_4H_8^+$), 41 (27, H_2C=CH-CH_2^+), 39 (8, $C_3H_3^+$).

[5-^{14}C]-Dodecane (4). For purification charcoal (0.5 g) was added to a solution of CuI (6.57 g) and KI (65 g) in H_2O (50 ml); the mixture was stirred for 10 min, and filtered through Celite. After dilution of the clear solution with H_2O pure CuI precipitated which was filtered off and dried under high vacuum in the dark. Lithium dibutyl cuprate was prepared by adding slowly 0.7 ml butyl lithium (1.15 mmol; 1.6 M in hexane; Merck) to a stirred suspension of freshly purified CuI (110 mg, 0.58 mmol) in Et_2O (5 ml) at -30°C. Subsequently, 3 (71 mg, 0.25 mmol, 25 μCi) in Et_2O (5 ml) was added slowly; the slurry was stirred for 2 h at -20°C to -30°C, and for 16 h at 22°C. After addition of H_2O and Et_2O the reaction mixture was filtered; the organic phase was separated, washed with saturated NaCl solution and dried over Na_2SO_4. The crude product (79 % radiochemical purity) was subjected to preparative TLC (solvent: cyclohexane) yielding pure 4 (34 mg, 0.20 mmol, 19.9 μCi) which cochromatographed (TLC, GLC) with non-labeled reference compound dodecane. IR (liq. film): 2930s, 2850s, 1460m, 1380w, 720w. ^{1}H-NMR ($CDCl_3$): 0.88 (t, J = 7, 3 H-C(1), 3 H-C(12)); 1.27 (m, 20 H-C(2 - 11)). ^{13}C-NMR ($CDCl_3$): 14.16 (C(1), C(12)), 22.84 (C(2), C(11)), 29.50, 29.55, 29.84, 29.89 (C(4 - 9)), 32.11 (C(3), C(10)). MS (labeled compound): 170 (5, M^+), 141

(1, $C_{10}H_{21}^+$), 127 (2), 113 (3), 99 (5), 85 (26), 71 (48), 57 (100).

[8-^{14}C]-Hexadecane (5). For the preparation of octyl lithium n-octylbromid (50.21 g, 0.26 mol) was added to lithium (3.6 g, 0.52 mol) in Et_2O (200 ml) within 20 min at 0°C. The mixture was stirred at 22°C for 16 h, and filtered through Celite. The concentration of octyl lithium (1.55 M) was evaluated using diphenylacetic acid. Lithium dioctyl cuprate was prepared by reaction of CuI (110 mg, 0.58 mmol) with octyl lithium (0.75 ml, 1.16 mmol) as described for the corresponding butyl compound; subsequently, **3** (71 mg, 0.25 mmol, 25 μCi) was added, reacted, and worked up as described above. The crude product was purifued by preparative TLC (solvent: cyclohexane) yielding pure **5** (26 mg, 0.12 mmol, 11.7 μCi) which cochromatographed (TLC, GLC) with non-labeled hexadecane. IR (liq. film): 2960s, 2930s, 2850s, 1460m, 1380w, 720w. ^{1}H-NMR ($CDCl_3$): 0.88 (t, J = 7, 3 H-C(1), 3 H-C(16)); 1.26 (m, 28 H-C(2 - 15)). ^{13}C-NMR ($CDCl_3$): 14.16 (C(1), C(16)), 22.82 (C(2), C(15)), 29.53, 29.83, 29.87 (C(4 - 13)), 32.08 (C(3), C(14)). MS (labeled compound): 226 (31, M^+), 197 (1, $C_{14}H_{29}^+$), 183 (2), 169 (3), 141 (4, $C_{10}H_{21}^+$), 127 (5), 113 (7), 99 (11), 85 (44), 71 (64), 57 (100).

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