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SYNTHESIS OF METHYL [2-^{13}C-2,2-d_2]- AND [3-^{13}C-3,3-d_2]TETRACOSANOATE.

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SUMMARY

The synthesis of methyl [2-^{13}C-2,2-d_2]- and methyl [3-^{13}C-3,3-d_2]tetracosanoate is reported. The carbon-13 was introduced by cyanation of 1-bromodocosane and 1-bromoheneicosane using 18-crown-6 ether and potassium [^{13}C]cyanide in hexamethylphosphoramide. Cyanation reactions with carbon-13 proceeded at better than 90% yield by this method. Triphasic catalysis was used to introduce the CN group in successive homologations of the labeled compounds. This method was found to be label-sparing with typical yields being 95%. No scrambling of deuterium was observed.

Keywords: Methyl [2-^{13}-2,2-d_2]tetracosanoate; methyl [3-^{13}C-3,3-d_2]tetracosanoate; cyanation; 18-crown-6 ether; triphasic catalysis

Isotope labeled malonic, 2-methylmalonic, propionic, and succinic acids have been used as precursors to determine

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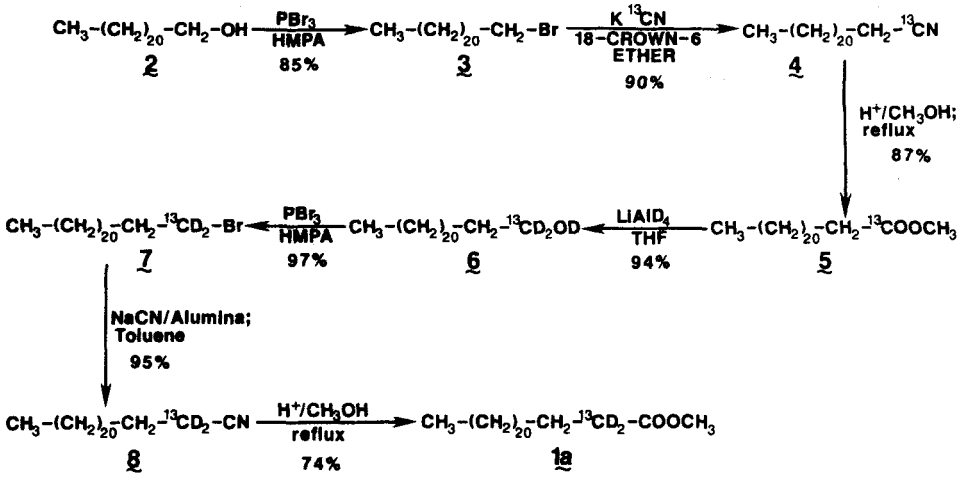
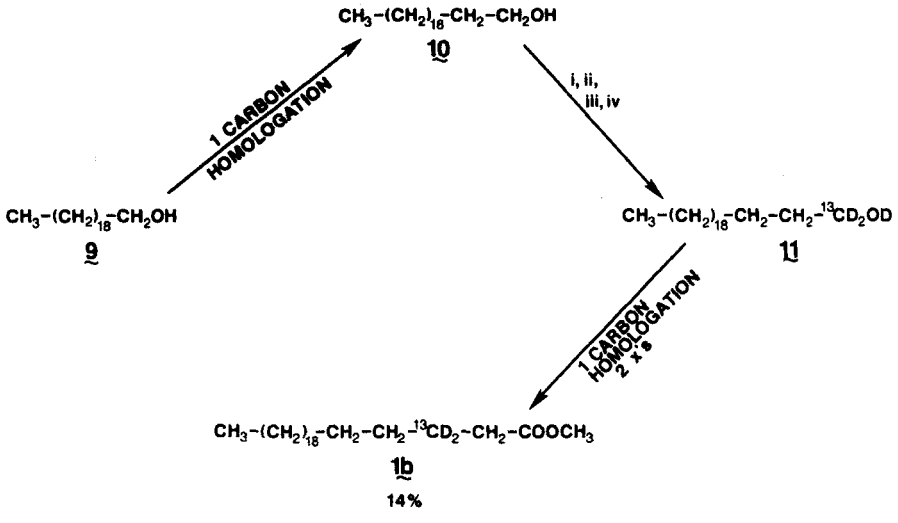
biosynthetic pathways to normal- and branched-alkanes which are present in cuticular waxes of insects [1,2,3]. When carbon-14 (^{14}C) is biochemically incorporated, the position of the label in the molecule is determined by tedious and meticulous oxidative degradation. Alkanes are particularly resistant to oxidation and the products of oxidation may lead to ambiguity as to the position of the label because the severe conditions required to degrade the molecule result in distribution of the label in the randomized fragments. However, carbon-13 (^{13}C) labeling techniques allow the investigator to use non-destructive measurements (^{13}C NMR) to observe the position of the incorporated label. For example, ^{13}C NMR analysis of the isolated hydrocarbon fractions from housefly surface lipids showed that the 2-methyl group in 2-[^{13}C]methylmalonic acid was the origin of the methyl branch in internally branched methyl alkanes [3]. In the course of further insect studies to determine the participation of alpha and/or beta carbons and hydrogens in the biosynthetic mechanisms for the production of the alkanes by either decarboxylation or decarbonylation [4] of the corresponding fatty acids, it became necessary to synthesize two doubly labeled isomers of tetracosanoic acid with deuterium and carbon-13 in the number 2 or number 3 carbon position. It is the synthesis of both isomers (compounds **1a** and **1b**) that we are reporting here.

The synthetic routes are shown in **Schemes** 1 and 2. The 1-docosanol (**2**) was brominated with phosphorous tribromide in hexamethylphosphoramide (HMPA), to form **3** (85%), the mass spectrum corroborated the proposed structure with peaks at m/z 388 and 390 for the molecular ion which had the 1.00:0.98 ratio for the two isotopes of bromine. Peaks for the cyclic bromonium ion, $C_4H_8Br^+$, at m/z 135 and 137 were characteristic fragments for alkyl bromides. Compound 3 was converted to the labeled

SCHEME 1



nitrile, **4**, in 90% yield by reaction with $K^{13}CN$ using 18-crown-6 ether in hexamethylphosphoramide (HMPA) [5,6]. Hydrolysis of the nitrile in methanolic-HCl and chloroform as co-solvent (3:2, V:V) gave the corresponding methyl [1-^{13}C]tricosanoate, **5**, in 87% yield. In experiments with unlabeled material we found that hydrolysis of nitrile groups with methanolic-HCL alone resulted in low yields of the corresponding acid methyl ester. These results were probably due to poor solubility of the long chain compounds in methanol. The yield was increased by using chloroform as co-solvent. The reduction of **5** with $LiAlD_4$ produced the doubly labeled compound, tricosanol, **6** (94% yield). The homologation sequence was again repeated except that NaCN was used to introduce the final carbon. The nitrile, **8**, was synthesized in a label-sparing synthesis by a method using triphasic catalysis [7,8]. In this reaction the nitrile was introduced via NaCN coated alumina which was stirred with the bromide, **7**, in toluene at 100^{0} C for five days. The last cyanation reaction provided **8** in high yield (96%) because of the



SCHEME 2

high ratio of impregnated cyanide to alkyl bromide. Hydrolysis of the nitrile, **8**, to **1a** with the co-solvents $CHCl_3$-methanol/HCl was preferred over that of basic hydrolysis because of the danger of exchange and possible scrambling of the incorporated deuteriums. The ^{13}C NMR spectrum of **1a** showed the expected quintuplet for the coupled $-^{13}CD_2-$ in the alpha carbon position.

The synthesis of **1b** is outlined in the **scheme 2**. It follows the same procedure for homologation and labeling as for the synthesis of **1a** except that the starting material was n-eicosanol, **9**, and homologation with ^{13}C occurred at the heneicosanol, **10**, stage with subsequent reduction with $LiAlD_4$ to give **11**. Two subsequent 1-carbon homologations gave **1b**.

EXPERIMENTAL

The $K^{13}CN$ was purchased from Amersham International, U.K., $LiAlD_4$ was purchased from Aldrich Chemicals. Melting points are

uncorrected. Infrared spectra (IR) were recorded on a Perkin Elmer 337 spectrometer. The NMR spectra of the compounds were determined on a JEOL FX90Q Fourier transform spectrometer with proton at 89.55 MHz and carbon at 22.5 MHz. The mass spectra (MS) were determined on a Finnigan MAT-112S in the electron impact mode using the solid sample probe. Data acquisition and storage were made with a SS200 data system dedicated to the mass spectrometer.

1-Bromodocosane (3). To a well stirred solution of 25.4 g. (0.078 moles) of 1-docosanol ,2, in 110 ml of HMPA at 50 ^{o}C was added dropwise 24 g (0.088 moles) of PBr_3 over 60 min. After the addition was complete the mixture was heated at 80^{o} for 4 h. The reaction was stirred at room temperature for an additional 60 h and then poured over 2 liters of crushed ice. When the ice had melted the solid was collected by filtration and dried at ambient conditions. The solid was triturated with methanol, the solvent decanted and treated with charcoal, filtered, and 3 allowed to crystallize. Recrystallization of the product from methanol gave 25.8 g of 3 in 85% yield; mp.46-48 ^{o}C; MS, m/z (rel int) 388 (1.8; M^{+}.), 309 (6.4, M - Br), 151 (18, $C_5H_{10}Br^{+}$), 135 (36, $C_4H_8Br^{+}$).

[1-^{13}C]Tricosanenitrile (4). A solution of 1.0 g (15 mmol) of K^{13}CN, 5.8 g (15 mmol) of 3, 3.9 g (15 mmol) of 18-crown-6 ether [5,6] in 100 mL of HMPA was stirred for two days at 62 ^{o}C with moisture protection. The reaction mixture was poured onto 1 L of ice and the precipitate collected by filtration. The solid was recrystallized from methanol to yield 4.5 g (90%) of 4; mp 51.5-52.5 ^{o}C; IR (KBr pellet) 2190 (-^{13}CN), 2950, 2880, 1455, 1380 (-CH_3), 2940, 2845, 1475, 720 cm^{-1} (-CH_2-); MS, m/z (rel int) 336 (5, M^{+}.), 307 (8, M - ^{13}CN), 98 (48, $C_5{}^{13}C_1H_{11}N^{+}$·), 97 (42, $C_5{}^{13}C_1H_{10}N^{+}$).

Methyl [1-^{13}C]tricosanoate, (5). A solution of 3.0 g (8.9

mmoles) **4** in 150 mL of 30% $HCl_{(g)}$/MeOH and 100 mL of $CHCl_3$ was stirred with gentle reflux for 16 h and then cooled. Gas chromatography (GC) was used to insure complete reaction by analyzing aliquots during that time. Water (250 mL) was added and the solution extracted with three-100 mL volumes of Et_2O, and the organic layer dried over $MgSO_4$. After filtering from the drying agent the organic solvent was removed in vacuo. The residue was dissolved in a small volume of hexane and applied to a column (1x10 cm) of silicic acid. The ester was eluded from the column with hexane: ethyl acetate (8:2,V:V). The yield was 2.8 g of **5** (87%); mp 53.5-54.5 °C; IR (KBr pellet) 1690 ($^{13}C{=}O$), 1160 ($^{13}C{-}O$), 2950, 2885, 1475, 1375 ($-CH_3$), 2920, 2845, 1455, 717, 727 ($-CH_2-$) cm^{-1}; MS, m/z (rel int) 369 (37, M^+.), 338 (5, M - OCH_3), 340 (3, M - CH_2CH_3), 326 (12, M - $CH_2CH_2CH_3$), 144 (23, $(CH_2)_6{}^{13}CO_2CH_3^+$), 88 (75, $(CH_2)_2{}^{13}CO_2CH_3^+$), 75 (100, $CH_3O(^{13}C{=}OH)CH_2^+$).

[1-^{13}C-1,1-d_2]1-Tricosanol, (**6**). Compound **5** (2.5 g, 6.8 mmoles) was reduced to **6** with $LiAlD_4$ (2.5 g) in dry THF (100 mL) at gentle reflux for 3 h. After hydrolysis followed by extraction with ether, the compound was recrystallized from EtOH to yield 1.95 g (94%) of **6**; mp. 71-73 °C; IR (KBr pellet) 3310, 960 (-OH, $-{}^{13}CD_2OH$), 2950, 2885, 1475, 1375 ($-CH_3$), 2920, 2845, 1455, 719, 730 cm^{-1}($-CH_2-$); MS, m/z (rel int) 343 (0.5, M^+.), 325 (3, M - 18), 294(1, M - 49: [M-(H_2O+$CH_2{}^{13}CD_2$]), alkene series: 55, 69, 83, 97, 111, 125...; Alkane series: 43(100), 57, 71, 85, 99.... TMS derivative . 415 (12, M^+), 400(100, M-15).

[1-^{13}C-1,1-d_2]1-Bromotricosane, (**7**). The compound was synthesized using the methods described for bromination of **3**. From 1.9 g (5.5 mmol) of **6** and 1.5 g (5.5 mmol) of PBr_3 in 50 mL of HMPA was obtained 2.1 g (97%) of **7**; mp 50-51 °C; IR (KBr pellet) 590 cm^{-1} ($-{}^{13}CD_2Br$); MS, m/z (rel int) 405 (1.0, M^+.), 326 (4.5, M - Br), 138 (23.5)

Impregnation of alumina with NaCN [7,8]. A 100 mL flask was charged with 5.0 g (100 mmoles) of NaCN in 12 mL of distilled H_2O and 10 g of neutral alumina were added in one portion. The water was removed in vacuo on a rotary evaporator at a bath temperature <65 °C. The impregnated alumina was then dried in an oven at 110 °C. The reagent was stored in a desiccator.

[2-^{13}C-2,2-d_2]1-tetracosanenitrile, (8). Into a round-bottom flask equipped with a magnetic stir-bar, condenser and $CaCl_2$ drying tube was added a solution of 1.3 g (3.2 mmoles) of 7 in dry toluene followed by 4.8 g of the NaCN impregnated alumina (32 mmol with respect to NaCN). The mixture was heated with stirring in an oil bath at 100 °C for 6 days. The progress of the reaction was followed by GC. The contents of the flask were poured into a buchner funnel and the alumina washed with three 50 mL portions of hexane. The filtrate was concentrated in vacuo. The product was recrystallized from MeOH to yield 1.1 g (95%) of 8; mp. 51.5-53.5 °C; IR (KBr pellet) 2240 cm^{-1} (-CN); MS m/z (rel int) 352 (24, M^+.), 326 (0.5, M - CN), $-(CH_2)_n{}^{13}CD_2CN^+$ series: 43(100), 57(96), 71(37), 85, 99, 113, 127,...323.

Methyl [2-^{13}C-2,2-d_2]tetracosanoate, (1a). A solution of 500 mg (1.4 mmol) of 8 in 100mL of $CHCl_3$ and 150 mL of 30% (weight) $HCl_{(g)}$ in MeOH was heated for 8 h under gentle reflux with $CaCl_2$ protection. GC was used to monitor the course of the reaction. The mixture was poured onto 500 mL of ice water and then extracted with ether. The dried organic layer yielded a solid which was applied to a 1 x 10 cm silica gel column. The compound was eluded with hexane:ethyl acetate (80:20, V:V) to yield 400 mg (74%) of 1a; mp 58-58.5 °C; IR (KBr pellet) 2950, 1470, 1370 ($-CH_3$), 2910, 2800, 1460, 718, 728 ($-CH_2-$), 1740, 1265, 1110 cm^{-1} (C=O); ^{13}C NMR ($CDCl_3$) 36.0 (pentuplet, J=1.0 Hz, CD_2 ^{13}C-enriched), 32.0 (s, ^{13}C-natural abund.); MS, m/z (rel int) 385 (100), 355 (21, M - 31), 340(58), 283 (22), 202 (12),

146 (9), 145 (11), 88 (29), 77(20, $-^{13}CD_2=C(OH)-OCH_3^+$).

The synthesis of **1b** involved the same homologation reactions as for **1a** except that the sequence of insertion of label was made at a different step and the starting material was of two carbon units less.

[1-^{13}-1,1,-d_2]1-Docosanol, (11). Compound 11 was synthesized by a one carbon homologation performed twice from 9 using the sequence: bromination, cyanation, hydrolysis, reduction, bromination, C-13 cyanation with 18-crown-6 and $K^{13}CN$, hydrolysis and reduction with $LiAlD_4$. The mp of 11 was 69-69.5 °C; IR (KBr pellet) 3330, 2950, 2910, 2840, 1450, 960, 720 cm^{-1}; MS, m/z (rel int) M^+. at 329 not found; m/z 311(1.6%, M - 18); alkene series m/z 69(100%), 83, 97, 111, 125.... MS for trimethylsilyl ether derivative: m/z 401 not found; m/z 386(100%, m - 15, m - CH_3).

Methyl [3-^{13}C-3,3-d_2]tetracosanoate, (**1b**). The compound was synthesized by single carbon homologations performed twice on 11; the yield from 9 was 14%; mp 60-61 °C; IR (KBr pellet) 2960 , 2910, 2850, 1740, 1460, 1440, 1350, 1200, 1180, 720 cm^{-1}; ^{13}C NMR ($CHCl_3$) 31.3 (pentuplet, J=0.96 Hz, CD_2 ^{13}C-enriched), 28.9 (s, CH_2 ^{13}C-natural abundance); MS, m/z (rel int) 385 (64), 354 (8, M-31), 339 (17), 283 (12), 202 (13), 146 (41), 90 (90), 74 (100, $-CH_2=C(OH)-OCH_3$).

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