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Synthesis of 11-Oxo[1,11-^{14}C]eicosanoic and 13-Oxo[1,13-^{14}C]docosanoic Acids by the Acylation of Cyclic Enamines*

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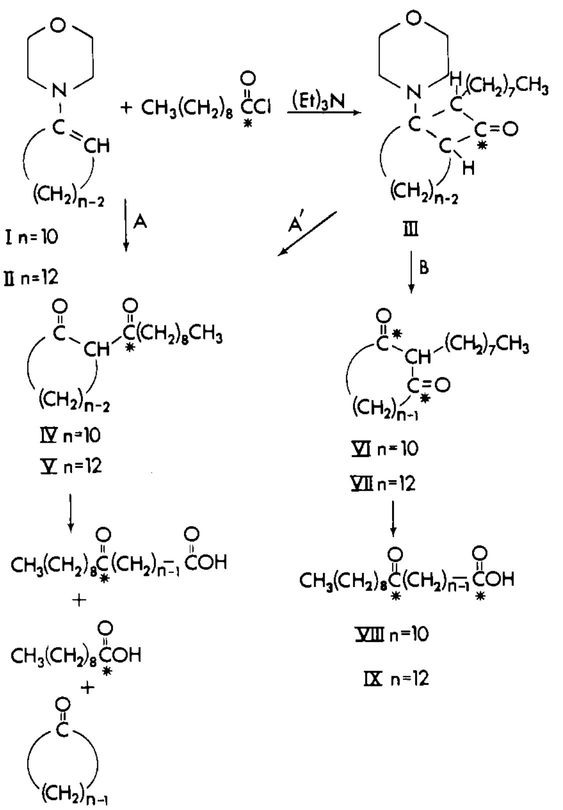
ABSTRACT: The acylation of 1-morpholino-1-cyclodecene (I) and 1-morpholino-1-cyclododecene (II) with decanoyl chloride produces predominantly the symmetrical β-diketones 2-octylcyclododecane-1,3-dione (VI) and 2-octylcyclotetradecane-1,3-dione (VII). When [1-^{14}C]decanoyl chloride is employed in the synthesis, and the β-diketones are hydrolyzed, the products are 11-oxo[1,11-^{14}C]eicosanoic acid (VIII) and 13-oxo[1,13-^{14}C]docosanoic acid (IX). The oxo acids can be reduced to the corresponding saturated fatty acids, providing a method of synthesis for long-chain fatty acid containing ^{14}C in two positions. Distribution of radioactivity in the product fatty acids and mass spectra of the intermediate β-diketones confirm the structure of the β-diketones formed in the acylation.

The enamine synthesis (Hünig *et al.*, 1967; Hünig and Buysch, 1967a) provides an attractive method for synthesis of specifically labeled long-chain oxo, hydroxy, and saturated fatty acids using commercially available [1-^{14}C]fatty acids of shorter chain length. For example, the chain length of the starting acid can be extended by n carbon atoms by acylating the enamine of a cyclic ketone containing n carbon atoms with the chloride of the starting acid (Scheme I). The intermediate keto acid produced contains an oxygen at position $n + 1$ which can be reduced to a hydroxyl group or to a methylene group. According to Optiz *et al.* (1962), acylation in the presence of triethylamine proceeds by way of the ketene which reacts with enamines to form cyclobutanones such as III. In a study of the effect of enamine ring size on reaction pathway with acetyl chloride and triethylamine, Hünig and Hoch (1966) found that the symmetrical β-diketone was produced in the case of large ring enamines where n = 11–15, (similar to path B, Scheme I) and hence must have involved a cyclobutanone intermediate. For smaller ring enamines where n = 5–8, the acetylcyclanone was the major product, and it could be formed by either path A or A′. When n = 9 the yield was low though both mechanisms appeared to be operative.



SCHEME I

Both reaction pathways give the same oxo acid. The mechanism becomes important, however, if the method is to be used for increasing the chain length of commercially available

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[1-^{14}C]fatty acids. Should the results with acetyl chloride be general for higher acid chlorides, then extension of a [1-^{14}C]-fatty acid chain by $n = 5$–8 additional carbon atoms would place the radioactivity specifically in the $n + 1$ carbon atom. On the other hand, extension of the chain by $n = 11$–15 carbon atoms would produce a fatty acid labeled equally in the $n + 1$ carbon atom and the carboxyl carbon atom.

In this report we describe the synthesis of 11-oxo[1,11-^{14}C]-eicosanoic acid (VIII) and 13-oxo[1,13-^{14}C]docosanoic acid (IX) which confirms the expected labeling pattern for $n = 12$ and demonstrates that path B is also the principle mechanism when $n = 10$.

Experimental Section

Gas–liquid chromatography was carried out on an F & M Model 700 instrument with dual 4 ft × 0.25 in. glass columns and dual hydrogen flame detectors. Columns were packed with 10% diethylene glycol succinate on 100–120 mesh Gas Chrom Q (Applied Science Laboratories). The β-diketones were analyzed at 180° and a helium carrier gas flow of 60 cc/min. BF_3–methanol reagent (14%) was obtained from Applied Science Laboratories. Thin-layer chromatography was carried out on plates prepared from Adsorbosil-1 (Applied Science Laboratories) and silicic acid column chromatography was performed on Mallinckrodt Silicar CC-4, 100–200 mesh. Mass spectra were obtained on a Nuclide low-resolution mass spectrometer at 70 eV. Nuclear magnetic resonance spectra were obtained on a 90-mHz Bruker nuclear magnetic resonance spectrometer. Elemental analyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Mich.

Radioactive samples were assayed in a Tri-Carb Model 3214 liquid scintillation spectrometer at 78.8% efficiency by dissolving aliquots in 15 ml of scintillator solution (4 g of 2,5-diphenyloxazole, 50 mg of 1,4-[bis(2,5-phenyloxazolyl)]benzene/l. of toluene). Samples of CO_2 from the Schmidt degradations were collected in 0.5 ml of Hyamine hydroxide, added to the scintillator solution, and counted at an efficiency of 69%. Scintillator materials and Hyamine hydroxide (1 M in methanol) were obtained from the Packard Instrument Co.

Decanoic acid was obtained from the Hormel Institute, Austin, Minn. Sodium [1-^{14}C]decanoate was obtained from New England Nuclear. Decanoyl chloride and [1-^{14}C]decanoyl chloride were prepared by reaction of the acid or sodium salt with oxyalyl chloride (Distillation Products Industries, Rochester, N. Y.) in benzene according to the method of Engel and Just (1955). Benzene and excess oxalyl chloride were removed either *in vacuo* or under a stream of nitrogen, and the decanoyl chloride was used immediately for the acylation reaction without further purification.

Cyclodecanone and cyclododecanone were obtained from Aldrich Chemical Co. 1-Morpholinocyclodecene (I) and 1-morpholinocyclododecene (II) were prepared by the method of Hünig *et al.* (1961). The crude enamines, purified by vacuum distillation, were stored in a desiccator in the cold room without extensive decomposition, and aliquots were utilized at various times for the acylation reactions.

2-Octylcyclododecane-1,3-dione and 2-Octyl[1,3-^{14}C]cyclododecane-1,3-dione (VI). Acylation of 2.8 g of I (12 mmoles) in the presence of 1.8 ml of triethylamine (13 mmoles) with 2.5 g of decanoyl chloride (13 mmoles) was carried out in 10 ml of chloroform as described in the procedure of Hünig *et al.* (1967). After subsequent hydrolysis for 30 hr with 4.5 ml of 6 N HCl and 8 ml of additional chloroform, the chloroform was evaporated, water was added, and the products were extracted with hexane. The hexane extract was washed with aqueous $NaHCO_3$ and the residue (500 mg) was added to methanol saturated with copper acetate. The solution was warmed to 50° for 5 min and cooled in an ice bath. The copper chelate which crystallized was harvested by filtration and then shaken with hexane and dilute HCl to regenerate the β-diketones.

Gas–liquid chromatographic analysis of the recovered oil showed that it consisted of 96% 2-octyl-1,3-cyclododecanedione (VI) (retention time 8.5 min or 8.2 relative to methyl stearate) and 4% of 2-decanoylcyclodecanone (IV) (retention time 9.6 min or 9.2 relative to methyl stearate). The symmetrical isomer VI was freed of IV by six recrystallizations from cold methanol to give white needle crystals: mp 51.0–51.5°; ultraviolet maximum (C_2H_5OH) 306 mμ (ϵ 187); infrared spectrum ($CHCl_3$) 1675 cm^{-1} with shoulder at 1700 (C=O); nuclear magnetic resonance spectra at (2% in $CDCl_3$) δ 3.62 (t, 1, J = 7 Hz, COC*H*(CH_2)CO), 2.50 (t, 4, J = 6 Hz, *CH*$_2$CO), 1.64 (broad m, about 6, CH_2), 1.27 (s, broad methylene envelope with shoulder at 1.22, about 22), and 0.90 (t, 3, J = 5.5 Hz, CH_3); mass spectrum (70 ev) *m/e* (relative intensity) 308 (11), 290 (4), 265 (4), 252 (6), 238 (5), 234 (11), 211 (4), 209 (7), 196 (32), 195 (4), 181 (14), 178 (12), 155 (9), 154 (7), 153 (6), 138 (7), 136 (5), 135 (12), 126 (4), 125 (8), 121 (11), 112 (12), 111 (14), 110 (14), 109 (7), 107 (6), 100 (4), 99 (6), 98 (36), 97 (22), 96 (11), 95 (18), 94 (6), 93 (9), 85 (7), 84 (22), 83 (30), 82 (8), 81 (25), 80 (5), 71 (18), 70 (18), 69 (45), 68 (11), 67 (24), 58 (9), 57 (22), 56 (15), 55 (100), 54 (9), 53 (9), 43 (57), 42 (26), and 41 (93).

Anal. Calcd for $C_{20}H_{36}O_2$: C, 77.87; H, 11.76. Found: C, 77.83; H, 11.94.

The procedure was modified to a smaller scale for synthesis of 2-octyl[1,3-^{14}C]cyclododecane-1,3-dione. [1-^{14}C]Decanoyl chloride was prepared from 0.01 mmole (0.24 mCi) of sodium [1-^{14}C]decanoate, 0.5 ml of oxalyl chloride, and 1 ml of benzene, followed, after 1 hr at room temperature, by addition of 10.7 mg (0.062 mmole) of unlabeled decanoic acid in 2 ml of benzene and further reaction for 1 hr. After removal of benzene and excess oxalyl chloride under a stream of nitrogen, the residue in 5 ml of chloroform was added slowly to a mixture of 32 mg of I (0.14 mmole) and 50 μl of triethylamine (0.36 mmole) in 3 ml of chloroform cooled in an ice bath. The reaction proceeded for 8 hr at room temperature, the mixture was hydrolyzed for 24 hr after addition of 0.65 ml of 1 N HCl, and the product was isolated by extraction into hexane. The hexane extract was washed with aqueous $NaHCO_3$ and evaporated leaving 29 mg of oil, which was placed on a 2-g silicic acid column packed in hexane. The column was eluted with 2% ether in hexane and the fractions were assayed for radioactivity. Fractions containing the β-diketones (27% yield) were pooled and consisted of 93% VI and 7% IV. After two recrystallizations from methanol, 3.6 mg of crystals consisting of 98% VI and 2% IV was obtained. This product, which had a specific activity of 2.1 mCi/mmole, was utilized below in the preparation of [^{14}C]oxo acid VIII.

2-Octylcyclotetradecane-1,3-dione and 2-Octyl[1,3-^{14}C]cyclotetradecane-1,3-dione (VII). Acylation of II (2.7 g, 10 mmoles) with 1.26 g of decanoyl chloride (6.6 mmoles) in the presence of 1.2 ml of (8.0 mmoles) of triethylamine was carried out as described above for I. The crude product was distilled *in vacuo* giving recovered cyclododecanone (bp 85° (1.5) and 0.88 g (43% yield) of β-diketone mixture (bp 160° (1.5)). According to gas–liquid chromatography analysis the mixture consisted of 90% 2-octylcyclotetradecane-1,3-dione (VII) (retention time 15.6 min or 15.0 relative to methyl stearate) and 10% 2-decanoylcyclododecanone (V) (retention time 18.3 min or 17.6 relative to methyl stearate). The symmetrical isomer VII was freed of V by five recrystallizations from cold methanol to give white needle crystals, mp 45.5–46.5°; ultraviolet maximum (C_2H_5OH) 302 mμ (ε 187); ultraviolet spectrum ($CHCl_3$) 1675 cm^{-1} with shoulder at 1700 (C=O); nuclear magnetic resonance spectra at (2% in $CDCl_3$) δ 3.62 (t, 1, *J* = 7 Hz, COC*H*(CH_2)CO), 2.47 (t, 4, *J* = 7 Hz, C*H*$_2$CO), 1.68 (broad m, about 6, CH_2), 1.28 (s, broad methylene envelope, about 26), and 0.90 (t, 3, CH_3); mass spectrum (70 ev) *m/e* (relative intensity) 336 (6), 335 (18), 317 (7), 292 (5), 279 (9), 262 (9), 239 (5), 238 (6), 237 (10), 225 (21), 224 (70), 223 (5), 209 (15), 206 (9), 197 (4), 196 (5), 195 (4), 183 (4), 182 (8), 181 (8), 168 (4), 167 (11), 166 (8), 164 (4), 163 (6), 156 (4), 155 (8), 154 (5), 153 (6), 152 (4), 151 (4), 150 (5), 149 (11), 148 (6), 142 (4), 140 (5), 139 (7), 138 (8), 137 (6), 136 (5), 135 (8), 128 (7), 127 (4), 126 (9), 125 (12), 124 (7), 123 (7), 122 (5), 121 (9), 113 (5), 112 (15), 111 (21), 110 (19), 109 (13), 108 (8), 107 (9), 100 (10), 99 (8), 98 (41), 97 (21), 96 (17), 95 (27), 94 (6), 93 (9), 85 (12), 84 (18), 83 (32), 82 (15), 81 (29), 80 (6), 79 (10), 71 (19), 70 (11), 69 (32), 68 (10), 67 (21), 59 (5), 58 (14), 57 (24), 56 (13), 55 (100), 54 (8), 53 (5), 43 (57), 42 (14), 41 (78), 39 (6), 32 (17), 29 (21), 28 (67), and 27 (7).

Anal. Calcd for $C_{22}H_{40}O_2$: C, 78.51; H, 11.98. Found: C, 78.79; H, 11.85.

Preparation of 2-octyl[1,3-^{14}C]cyclotetradecane-1,3-dione was carried out by the small-scale acylation procedure described above employing 28 mg (0.11 mmole) of II, 50 μl (0.36 mmole) of triethylamine, and 14.8 mg (0.078 mmole) of [1-^{14}C]decanoyl chloride (about 3.2 mCi/mmole). Chromatography of the crude product on a 2-g column of silicic acid packed in hexane produced 12 mg (46% yield) of β-diketone mixture when the major radioactive fractions (eluting solvent 2% ether in hexane) were pooled. The mixture consisted of 96% VII and 4% V by gas–liquid chromatography analysis. Two recrystallizations from methanol gave 8.3 mg of solid consisting of 98.3% VII and 1.7% V. This product, which had a specific activity of 2.6 mCi/mmole, was utilized below in the preparation of [^{14}C]oxo acid IX.

2-Decanoylcyclododecanone (*V*). Sodium hydride was substituted for sodium amide in an attempt to prepare V by acylation of cyclododecanone according to the procedure of Hauser *et al.* (1954) as modified by Hünig *et al.* (1967). Gas–liquid chromatography analysis of the crude product showed that a yield of only 2% of V was obtained. The crude mixture was added to methanolic copper acetate, and the small amount of solid formed on cooling was recrystallized three times from methanol before regenerating the β-diketone. This method produced only 1.5 mg (0.05% yield) of purified V, which had the same retention time on gas–liquid chromatography as the minor β-diketone product from the acylation of enamine **II** above. Mass spectrum showed (70 eV) *m/e* (relative intensity) 337 (4), 336 (12), 318 (4), 224 (5), 208 (11), 191 (4), 183 (12), 182 (53), 181 (4), 167 (8), 166 (5), 164 (10), 163 (15), 156 (4), 155 (28), 149 (5), 148 (5), 139 (6), 138 (4), 136 (5), 135 (4), 127 (5), 126 (22), 125 (8), 124 (4), 123 (4), 122 (4), 121 (7), 112 (24), 111 (19), 110 (5), 109 (7), 108 (5), 107 (5), 100 (7), 99 (8), 98 (47), 97 (19), 96 (12), 95 (21), 93 (6), 85 (22), 84 (15), 83 (24), 82 (14), 81 (35), 80 (6), 79 (6), 72 (6), 71 (52), 70 (10), 69 (38), 68 (13), 67 (29), 59 (6), 58 (16), 57 (57), 56 (11), 55 (87), 54 (7), 53 (7), 44 (5), 43 (100), 42 (16), 41 (67), 39 (8), 29 (31), 28 (4), and 27 (17).

2-([1-^{14}C]Decanoyl)cyclodecanone (*IV*). The trailing fractions from the silicic acid column chromatography of 2-octyl-[1,3-^{14}C]cyclododecane-1,3-dione (VI) described above contained 3.2 mg of an oil consisting of 85% 2-([1-^{14}C]decanoyl)-cyclodecanone (IV) and 15% of VI. After three recrystallizations from methanol, the material contained 98% IV and 2% VI. Mass spectrum showed (70 eV) *m/e* (relative intensity) 308 (4), 290 (6), 196 (6), 181 (10), 178 (6), 170 (4), 169 (11), 163 (10), 155 (22), 154 (18), 153 (10), 152 (4), 139 (8), 138 (8), 137 (12), 136 (41), 135 (31), 134 (8), 126 (4), 125 (11), 124 (4), 123 (7), 122 (4), 121 (9), 112 (17), 111 (27), 110 (8), 109 (9), 108 (7), 107 (11), 100 (4), 99 (7), 98 (42), 97 (24), 96 (8), 95 (23), 94 (14), 93 (14), 91 (7), 85 (24), 84 (21), 83 (22), 82 (13), 81 (33), 80 (9), 79 (13), 77 (6), 73 (6), 72 (4), 71 (43), 70 (15), 69 (46), 68 (13), 67 (32), 60 (4), 59 (4), 58 (10), 57 (66), 56 (18), 55 (86), 54 (9), 53 (11), 45 (4), 44 (4), 43 (93), 42 (23), 41 (100), 40 (4), 39 (12), 38 (12), 36 (31), 29 (32), 28 (10), and 27 (20).

11-Oxoeicosanoic Acid and 11-Oxo-[1-11-^{14}C]eicosanoic Acid (*VIII*). A 284-mg (0.92 mmole) sample of crude β-diketone mixture (96% VI and 4% IV) was added to 10 ml of absolute ethanol and 0.45 ml of 40% aqueous NaOH and refluxed for 2 hr. The slurry was acidified, water was added, and the mixture was extracted with hexane to give 273 mg (96% yield) of 11-oxoeicosanoic acid (VIII). Three recrystallizations from methanol gave a sample with mp 85.5–86.0°; lit. (Breusch and Kirkali, 1965) mp 86.5–87.3°.

Anal. Calcd for $C_{20}H_{38}O_3$: C, 73.57; H, 11.73. Found: C, 73.83; H, 11.51.

The methyl ester of VIII was prepared by heating 20 mg of VIII with 1 ml of BF_3–methanol reagent for 5 min at 75° in a closed tube. Recrystallization of the methyl ester three times from methanol gave a sample with mp 53.0–54.0°. Gas–liquid chromatography analysis showed a single peak with a retention time of 26.4 min or 25.2 relative to methyl stearate. Mass spectrum showed (70 eV) *m/e* (relative intensity) 341 (5), 340 (8), 309 (15), 267 (4), 241 (5), 229 (14), 228 (76), 213 (23), 210 (7), 209 (6), 197 (4), 196 (13), 186 (4), 183 (7), 181 (9), 178 (6), 172 (7), 171 (46), 170 (100), 158 (4), 156 (5), 155 (31), 154 (14), 153 (14), 152 (6), 140 (11), 139 (45), 138 (37), 137 (5), 136 (4), 135 (17), 129 (5), 128 (5), 127 (8), 125 (6), 121 (8), 115 (7), 114 (6), 113 (7), 112 (16), 111 (17), 110 (22), 109 (9), 100 (7), 99 (11), 98 (62), 97 (25), 96 (24), 95 (22), 94 (6), 93 (6), 87 (12), 86 (4), 85 (31), 84 (22), 83 (23), 82 (17), 81 (25), 79 (7), 74 (13), 73 (5), 72 (8), 71 (57), 70 (8), 69 (62), 68 (8), 67 (23), 60 (13), 59 (22), 58 (36), 57 (51), 56 (9), 55 (66), 54 (6), 53 (4), 45 (4), 43 (89), 42 (12), 41 (49), 39 (5), 29 (25), and 27 (10).

Hydrolysis of 3.6 mg of the purified radioactive β-diketone mixture (98% VI and 2% IV) together with 3 mg of added VI as carrier was carried out in 0.7 ml of absolute ethanol and 0.1 ml of 1 N KOH. The isolated 11-oxo[1,11-^{14}C]eicosanoic acid had a specific activity of 1.1 mCi/mmole.

13-Oxodocosanoic Acid and 13-Oxo[1,13-^{14}C]docosanoic Acid (*IX*). A 565-mg (1.7 mmoles) sample of the crude β-diketone mixture (90% VII and 10% V) was hydrolyzed as described above in the preparation of VIII to give 565 mg (95% yield) of 13-oxodocosanoic acid (XI). Three recrystallizations from methanol gave a sample with mp 90–91°, lit. (Breusch and Kirkali, 1965) mp 91–92°.

Methyl 13-oxodocosanoate was prepared with BF_3–methanol as described above for methyl 11-oxoeicosanoate. The product had a mp 60.5–61.5°, lit. (Tulloch *et al.*, 1968) mp 60–61°. Gas–liquid chromatography analysis showed a single peak with a retention time of 34.0 min or 32.4 relative to methyl stearate. Mass spectrum showed (70 eV) *m/e* (relative intensity) 369 (4), 368 (10), 338 (4), 337 (17), 295 (7), 269 (6), 257 (17), 256 (86), 243 (7), 242 (4), 241 (27), 238 (8), 237 (5), 225 (7), 224 (20), 200 (6), 199 (27), 198 (31), 196 (4), 186 (4), 183 (9), 182 (6), 181 (12), 173 (4), 171 (28), 170 (86), 168 (6), 167 (30), 166 (37), 163 (13), 157 (6), 156 (5), 155 (43), 154 (9), 152 (6), 149 (20), 148 (10), 143 (12), 141 (6), 140 (6), 139 (8), 137 (5), 135 (4), 129 (5), 128 (7), 127 (19), 126 (54), 124 (6), 123 (8), 122 (5), 121 (6), 115 (5), 114 (10), 113 (11), 112 (37), 111 (17), 110 (22), 109 (15), 108 (4), 107 (8), 101 (6), 100 (14), 99 (11), 98 (51), 97 (42), 96 (21), 95 (42), 93 (10), 87 (23), 86 (5), 85 (40), 84 (24), 83 (46), 82 (27), 81 (42), 79 (5), 75 (5), 74 (37), 73 (7), 72 (9), 71 (76), 70 (11), 69 (69), 68 (12), 67 (32), 59 (34), 58 (48), 57 (56), 56 (14), 55 (100), 54 (6), 53 (6), 45 (4), 44 (5), 43 (98), 42 (18), 41 (51), 39 (8), 29 (30), 28 (5), and 27 (11).

Hydrolysis of 8.2 mg of the purified radioactive β-diketone mixture (98.3% VII and 1.7% V) in 0.7 ml of absolute methanol and 0.1 ml of 1 N KOH produced 7.4 mg of 13-oxo[1,13-^{14}*C*]docosanoic acid (IX) which was purified by chromatography on a 1-g column of silicic acid (eluting solvent 15% ether in hexane). Specific activity of the purified product was 2.6 mCi/mmole.

[1,13-^{14}C]Docosanoic Acid. Modification of the Wolff–Kishner reduction described by Hünig *et al.* (1967) was further modified for the reduction of 6.2 mg of 13-oxo[1,13-^{14}*C*]docosanoic acid (IX). The oxo acid was dissolved in 1 ml of triethanolamine containing 70 mg of KOH in a 150-mm test tube. The test tube was warmed in an oil bath to 110° and 1 ml of 95+% hydrazine (Eastman Chemicals) was added. The temperature was boosted first to 140° and held constant for 24 hr and then to 190° and held constant for 6 hr, by which time the vigorous bubbling had subsided. The mixture was cooled and acidified and the product was extracted with hexane. Thin-layer chromatography of the product (developing solvent, hexane–ether–acetic acid, 85:15:2, v/v) showed approximately equal amounts of docosanoic acid and 13-oxodocosanoic acid. The mixture was applied to a 2-g silicic acid column in hexane and eluted with 5% ether in hexane. Fractions of 1 ml were collected and assayed for radioactivity, and the most active fractions were pooled to give 2.6 mg (44% yield) of [1,13-^{14}*C*]docosanoic acid. Thin-layer chromatography of the purified acid showed a single iodine-staining spot with the same R_F as authentic docosanoic acid (Applied Science Laboratories) and with 98% of the radioactivity on the thin-layer chromatographic plate located in this spot.

Beckmann Rearrangement of the Oximes of [1,i-^{14}C]Oxo Acids VIII and IX. Aliquots of the [^{14}*C*]oxo acids were diluted with carrier acid and recrystallized. The procedure for IX is described and was similar for both acids. A 93-mg sample of IX was dissolved in 4 ml of 80% ethanol in a 50-ml round-bottom flask along with 210 mg of sodium acetate and 210 mg of hydroxylamine hydrochloride. The solution was refluxed for 4 hr, 4 ml of water was added, and the product was extracted with ether. The residue from evaporation of the ether was heated with 2 ml of concentrated H_2SO_4 at 100° for 1 hr, 2 ml of water was added, and chloroform extraction yielded 71 mg of crude amide mixture. Thin-layer chromatography of the amide product (developing solvent, hexane–ether–acetic acid, 80:20:2, v/v) showed a single iodine-staining spot with R_F about 0.1 and no oxime or unreacted oxo acid. The amide mixture, in a 50-ml round-bottom flask fitted with a condenser, was heated with 4 ml of 95% H_2SO_4 at 120° for 6 hr. Water was added and the hydrolysis products were extracted with ether, the ether was filtered over anhydrous Na_2SO_4, and the ether was evaporated to give 29 mg of a mixture of mono- and dicarboxylic acids. The product acids were separated by preparative thin-layer chromatography (developing solvent, hexane–ether–acetic acid, 85:15:2, v/v) and further purified by elution from a 2-g silicic acid column. Dicarboxylic acids were eluted with 4% methanol in chloroform, and specific activities were determined on adjacent peak fractions from this column. Decanoic acid was eluted from a 1-g silicic acid column with 5% ether in hexane and specific activities were determined on neighboring peak fractions. No decanoic acid was isolated in the degradation of VIII and the yield was low in the degradation of IX, probably because of losses from volatility during the hydrolysis and subsequent work-up.

Schmidt Degradations. The procedure described by Goldfine and Bloch (1961) for decarboxylation of fatty acids was modified by extending the reaction time at 70° to 3 hr and the equilibration time at room temperature to 1 hr. Recoveries of [^{14}*C*]CO_2 trapped in the Hyamine hydroxide from decarboxylation of standard [1-^{14}*C*]fatty acids was typically 95%.

Results

Acylation of 1-morpholinocyclodecene and 1-morpholinocyclododecene with decanoyl chloride in the presence of triethylamine, followed by hydrolysis of the enamines, produced predominantly the symmetrical cyclic β-diketones VI and VII (path B, Scheme I). These diketones both gave a triplet nuclear magnetic resonance signal for the methylene proton at 3.6 ppm (J = 7 Hz), and hence can be correlated with 2-methyl-1,3-cyclotetradecanedione (Hünig *et al.*, 1967) and 1,4-bis(cyclotetradecan-1,3-dione-2-yl)butane (Hünig and Buysch, 1967b). The symmetrical structures of VI and VII were confirmed by subsequent degradation of radioactive samples and by mass spectrometry.

Gas–liquid chromatography analysis of the crude β-diketones showed in each case a minor second peak with a retention time of 1.15 relative to the major product. Acylation of cyclodecanone with decanoyl chloride by a modification of the procedure of Hauser *et al.* (1954) produced a very low yield of a β-diketone with the same retention time on gas–liquid chromatography as the minor peak produced in the synthesis of VI. The mass spectrum of this compound, and the mass spectrum of the minor product accompanying VII, provide supporting evidence that these minor products are the unsymmetrical β-diketones IV and V.

Degradation of [1,i-^{14}C]Oxo Acids. Oxo acids VIII and IX were produced in good yield by hydrolysis of the β-diketones. Decarboxylation of the synthetic oxo acids was accomplished by a modification of the Schmidt degradation (Goldfine and Bloch, 1961). The data shown in Table I indicate that 50% of the radioactivity was present in the carboxyl group of these oxo acids as well as of the docosanoic acid produced by re-

TABLE I: Decarboxylation of Synthetic and Standard Fatty Acids.[a]

[^{14}C]Fatty Acid	Amt (cpm)[b]	CO$_2$ Collected[c] cpm	CO$_2$ Collected[c] % of Total
Synthetic acids			
[1,11-^{14}C]VIII	636	253	49
[1,13-^{14}C]IX	1837	818	47
[1,13-^{14}C]-Docosanoic	1672	761	48
Standard acids			
[1-^{14}C]-Decanoic	3572	3380	95
[1-^{14}C]-Eicosanoic	2412	2260	94

[a] Procedure for Schmidt degradation is described in the Experimental Section. [b] Average of four replicate samples containing approximately 250 μg of [^{14}C]fatty acid each. [c] Average of four samples each, corrected for decreased efficiency of counting in Hyamine solution. In all cases the four replicate samples agreed within ±5%.

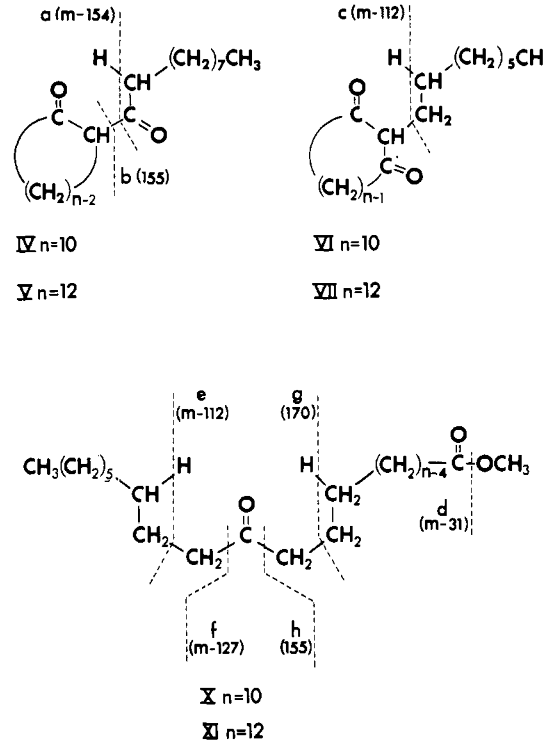

FIGURE 1: Some diagnostic fragment ions in the mass spectra of the synthetic β-diketones and oxo acid methyl esters.

duction of IX. This is the distribution expected from the symmetrical intermediate diketones VI and VII (path B, scheme I), whereas path A or A′ would yield oxo acids with no radioactivity in the carboxyl group.

Further support for the presence of radioactivity in both the carboxyl and carbonyl carbons of the oxo acids was obtained by Beckmann rearrangement of their oximes. Hydrolysis of the resulting amides produces decanoic acid, which contains the keto carbon atom from the parent oxo acid, and a dicarboxylic acid which contains both the keto and carboxyl carbons of the parent oxo acid. The specific activities of these degradation products are compared with the parent oxo acids in Table II. The dicarboxylic acids have a specific activity almost equivalent to the oxo acid. In one experiment the volatile decanoic acid was lost during the degradation and work-up procedure. In the second experiment enough decanoic acid was recovered to determine its specific activity as one-half that of the oxo acid. The precision of these determinations was not as good as that in the Schmidt degradations, nevertheless the data support the radioactive distribution predicted from path B rather than that from path A or A′ of Scheme I.

Mass Spectrometry. The mass spectra of the β-diketones synthesized in this study show great similarity between the isomeric structures. Table III lists a few ions which could be considered diagnostic in distinguishing between the isomers. The ions can be rationalized as shown in Figure 1. Ions of type a and b have been found to be important in the studies of acyclic, β-diketones (Schamp and Vandewalle, 1966; Bowie *et al.*, 1966). Abundant ions of type c, formed by H rearrange-

TABLE II: Degradation of [1,i-^{14}C]Oxo Acids VIII and IX.[a]

Oxo Acid Degraded	Specific Activity (cpm/mmole)[b] Oxo Acid	Dicarboxylic Acid	Decanoic Acid[c]
VIII	8.6×10^4	8.3×10^4	
	9.1×10^4	7.7×10^4	
IX	1.6×10^6	1.3×10^6	0.68×10^6
	1.8×10^6		0.79×10^6

[a] Acids were degraded by Beckmann rearrangement of their oximes as described in the Experimental Section. [b] Duplicate determinations are shown in all but one case. [c] Not isolated in the degradation of VIII.

TABLE III: Comparison of Several Fragment Ions Found in the Mass Spectra of the Isomeric β-Diketones.

Ion[a]	m/e	Rel Intensity IV	Rel Intensity VI	m/e	Rel Intensity V	Rel Intensity VII
		% of base peak			% of base peak	
M (parent)	308	4	11	336	12	6
a	154	18	7	182	53	8
b	155	22	9	155	28	8
c	196	6	32	224	5	70

[a] See Figure 1.

TABLE IV: Intensities of Fragment Ions Found in the Mass Spectra of Oxo Acid Methyl Esters X and XI.

	X		XI	
Ion[a]	m/e	Rel Intensity (% of Base Peak)	m/e	Rel Intensity (% of Base Peak)
M (parent)	340	8	368	10
d	309	15	337	17
e	228	76	256	86
f	213	23	241	27
g	170	100	170	86
h	155	31	155	43

[a] See Figure 1.

ment and loss of the side chain, were found in the mass spectra of 2-alkyl-1,3-cyclohexanediones (Vandewalle *et al.*, 1967). Ions produced by γ and δ bond cleavage are also important in the spectra of 2-alkyl-1,3-cyclohexanediones (Vandewalle *et al.*, 1967) but these ions are of low intensity in these larger ring diketones (at M − 99 and M − 113). They are more intense in the symmetrical isomers VI and VII than in IV and V.

A few other differences between the isomers appear. For example, the unsymmetrical diketones IV and V contain more intense ions at m/e 43, 57, and 71 than their symmetrical isomers VI and VII. Diketones VI and VII contain ions of low intensity at m/e (M − 74) which are, nevertheless, considerably more intense than the same ions in diketones IV and V. Ions at m/e 41, 43, and 55 are strong in the spectra of all four diketones.

Mass spectra of methyl 11-oxoeicosanoate (X) and methyl 13-oxodocosanoate (XI) showed strong ions corresponding to cleavages α and β (with H rearrangement) to each side of the keto group (see Figure 1 and Table IV). These are the fragmentations expected from a study of a series of methyl oxooctadecanoates reported by Ryhage and Stenhagen (1960).

Discussion

The enamine synthesis provides a method for lengthening a fatty acid chain by n carbon atoms as well as a method for synthesis of particular oxo and hydroxy fatty acids. Since carboxyl labeled fatty acids of short and intermediate chain length are readily available commercially, this procedure also provides a method of synthesis of longer chain radioactive fatty acids. When n = 10–15, the mechanism of the acylation with [1-^{14}C]fatty acid chlorides in the presence of triethylamine provides a product fatty acid labeled in two positions, the carboxyl group and carbon $n + 1$. If a singly labeled fatty acid product is desired, this procedure will have a disadvantage. In our case the products were used for incorporation studies (Hunter and Light, 1970) and the disadvantage was minimized by knowing that both positions were equally labeled. The procedure offers a distinct advantage when radioactivity in the carboxyl group is desired. The overall procedure is simple to adapt to a small scale while retaining good overall yield from readily available radioactive starting material.

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