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3-HYDROXY-2-ALKYL CARBOXYLIC ACIDS RELATED TO MYCOLIC ACID

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Abstract—A series of long-chain 3-hydroxy-2-alkyl acids structurally analogous to mycolic acids are reported. The synthesis applied, making use of 3-keto esters as intermediates, permits the insertion of 2-alkyl groups having no structural relationship to the main acid chain.

The mycolic acids (7, with R_1 ranging as high as C_{60} and $R_3 = C_{16}$ to C_{24}) are high molecular weight microbiological products showing, either as such or as derivatives, diverse biochemical and immunological properties.[1] One of the reasons for undertaking the work reported below was to make analogues available for testing, the results of which would help in defining a structure-activity relationship. With this in mind, we have now synthesized a group of long-chain 3-hydroxy-2-alkyl acids 7, all modeled after mycolic acid. Since at least in the first go around, stereochemical individuality was only of secondary importance, we deferred separations and resolutions and focussed instead on the structures.

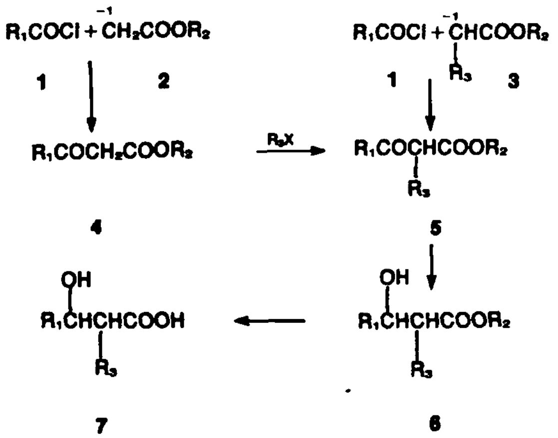
Of the several approaches to β-hydroxy acids, probably the most familiar is the Reformatsky condensation.[2] However, we did not pursue this method, since our target compounds would require reactant pairs—long-chain aldehydes with long-chain α-bromo esters—combinations of which are known to give poor results.[3] Other potentially useful syntheses are known,[4] but because none offered the directness of the process formulated as 1-to-7, we chose to rely on this sequence.[5] This sequence may be regarded as a variation of the Claisen ester condensation, one that enables the R_1 and R_3 groups in 7 to be chosen at will. The results we obtained serve a second purpose in future delineating the scope of this flexible approach, with particular reference to the length of the substituent groups.

Syntheses

The initial stage made use of lithium enolates derived either from acetate or from higher esters (2 or 3).[6] These were acylated with an acid chloride 1[7] to give, respectively, the 3-keto ester 4 or the 2-alkyl-3-keto ester 5. The latter compound could also be reached by alkylation of 4 with alkyl halides, some of which had to be synthesized. Reduction with borohydride followed by saponification of the intermediate 3-hydroxy-2-alkyl esters 6 furnished the desired mycolic analogues 7.

The final products 7 had $R_1 = C_{11}H_{23}$, $C_{17}H_{33}$ and $C_{17}H_{35}$; R_3 ranged from H to $C_{24}H_{49}$.

A troublesom problem encountered in this sequence involved the decreased solubility and lower reactivity of the long-chain compounds, particularly in the acylation step. Juggling with the solvents and operating at somewhat higher temperatures than usual helped here to some extent. Another problem was in the saponification step, 6-to-7, where the yields could not be brought above 70%. Presumably this is the consequence of a side reaction, cleavage of hydroxy ester 6 by a base-catalyzed retrograde aldol process.[8]



An attractive alternate method for synthesizing 3-hydroxy-2-alkyl acid 7 in essentially one step involves adding the dilithium derivative of a fatty acid to an aldehyde carbonyl. This was tried with the dilithium derivative of octadecanoic acid and octadecanal, but was unsuccessful.[9]

EXPERIMENTAL

Temperatures are uncorrected. The NMR curves were determined at 60 MHz, with $CHCl_3$ used at times as internal reference. For tlc, we relied on small commercial plates precoated with silica gel (0.1 mm and 0.25 mm thick). Tlc spots were made visible by exposing the plate to iodine vapors, or by spraying with 5% HSO_4 and then heating at 250–300°, or occasionally by using UV light. The Florisil used for column chromatography was 60–100 mesh. Dry column chromatography[10] was performed preferably with ICN silica gel (Brockmann grade III; 0.2–0.3 mm particle size); Florisil or standard silica gel (0.06–0.2 mm) gave less satisfactory results. Removing low-boiling solvents was accomplished routinely by distillation in a rotating evaporator at water-pump pressures and at external temps maintained at 50° or below.

Alkyl halides. Commercial octadecyl alcohol when treated either with $SOCl_2$ in benzene containing pyridine or with boiling CCl_4 containing triphenylphosphine gave octadecyl chloride. Both methods were acceptable (75–85% yields), but for larger runs the $SOCl_2$ conversion was preferred. Commercial octadecyl

Table 1. Borohydride reduction of keto-esters **5** to hydroxy-esters **6**

R_1	R_2	R_3	mmol of **5**	mmol of $NaBH_4$	solvent (ml)	yield of **6**	mp of **6**
$C_{11}H_{23}$	C_2H_5	C_5H_{11}	3.87	7.27	CH_3OH(25)	91%[a]	oil
$C_{11}H_{23}$	CH_3	$C_{24}H_{49}$	0.42	0.21	CH_3OH(20)	81%[b]	69-70°
$C_{11}H_{23}$	C_2H_5	$C_{24}H_{49}$	0.38	2.3	C_2H_5OH(10)	74%[c]	40-45°
$C_{17}H_{35}$	CH_3	H	3.4	0.85	CH_3OH(60)	89%[d]	56-57°
$C_{17}H_{35}$	CH_3	CH_3	0.85	0.41	CH_3OH(25)	85%[e]	42-45°
$C_{17}H_{35}$	CH_3	$C_{24}H_{49}$	0.25	3.2	CH_3OH(35)	90%[f]	62-66°[f]

[a] Obtained by dry column silica gel chromatography (benzene). The product showed two overlapping spots on a tlc plate with R_f's approx. 0.23 and 0.34 (9:1 hexane–ethyl acetate).

[b] Purified by dry column chromatography ($CHCl_3$). A second pass separated the product into the two diastereomers, one with m.p. 74–75° (R_f 0.8 with $CHCl_3$), and the second with m.p. 67.5–70° (R_f 0.7 with $CHCl_3$).

[c] Obtained by standard chromatography through Florisil using the solvents hexane–benzene (1:1), benzene, and benzene–$CHCl_3$ (1:1). The product showed two overlapping tlc spots, R_f 0.5 and 0.44 with 1:4 ethyl acetate–hexane.

[d] A side product, which was removed conviently as hexane-insoluble material, proved to be the corresponding hydroxy-acid 7 ($R_1 = C_{17}H_{35}$; $R_3 = H$). The hydroxy–ester main product, purified either by dry column chromatography ($CHCl_3$) or by standard chromatography through silica gel ($CHCl_3$), showed R_f 0.35 with $CHCl_3$. When equimolar amount of keto–ester **5** and borohydride were taken, the yield dropped to 61%; with excess borohydride, unidentified polar products were formed.

[e] Chromatography with Florisil and $CHCl_3$ led to product showing a single tlc spot, R_f 0.45 with 85:15 chloroform–ether.

[f] This product could be separated by silica gel chromatography ($CHCl_3$) into two forms, the more polar of which had R_f 0.25 ($CHCl_3$) and the less polar of which, m.p. 79–80°, had R_f 0.3 ($CHCl_3$).

Table 2. Carbon and hydrogen composition for 3-hydroxy-2-alkyl esters **6**

Hydroxy-ester **6**				Calcd.		Found	
R_1	R_2	R_3	Mol. Formula	C	H	C	H
$C_{11}H_{23}$	C_2H_5	C_5H_{11}	$C_{21}H_{42}O_3$	73.69	12.28	78.83	12.34
$C_{11}H_{25}$	C_2H_5	$C_{24}H_{49}$	$C_{40}H_{80}O_3$	78.88	13.16	79.19	13.26
$C_{17}H_{35}$	CH_3	H	$C_{21}H_{42}O_3$	73.63	12.36	73.66	12.35
$C_{17}H_{35}$	CH_3	CH_3	$C_{22}H_{44}O_3$	74.16	12.36	74.00	12.08
$C_{17}H_{35}$	CH_3	$C_{24}H_{49}$	$C_{45}H_{90}O_3$	79.57	13.36	79.58	13.21

Table 3. Hydroxy acids 7 by saponification of hydroxy esters 6

R_1	R_2	mol ratio alkali:ester	saponfication solution (approx.)	Yield of 7	mp of 7
$C_{11}H_{23}$	C_5H_{11}	34[a]	10% KOH (methanol)	74%	72-80°[a]
$C_{11}H_{23}$	$C_{24}H_{49}$	37[b]	10% NaOH (1:19 H_2O-C_2H_5OH)	66[b]	92-94
$C_{17}H_{35}$	H	35[c]	10% NaOH (1:9 H_2O-CH_3OH)	60-69	87-89[c]
$C_{17}H_{35}$	CH_3	40[d]	10% NaOH (CH_3OH plus some H_2O)	60-70	55-58[d]
$C_{17}H_{35}$	$C_{24}H_{49}$	28[e]	10% KOH (1:9 H_2O-CH_3OH)	70	66-68[e]
$C_{17}H_{35}$	$C_{16}H_{33}$	25[f]	10% NaOH (CH_3OH plus some H_2O)	72	79-83[f]
$C_{17}H_{33}$	$C_{16}H_{31}$	11[g]	4% KOH (dioxane, CH_3OH, + some H_2O)	89	liq.

[a] The ethyl ester was used. The product was purified by dry column chromatography through silica gel (9:1 $CHCl_3$–CH_3OH).

[b] This refers to the ethyl ester. The reaction temp. was held at 70°. The crude product was dried, rinsed with hexane in which it was insoluble, and then chromatographed on a dry column (1:1 $CHCl_3$–ether). Treating the corresponding methyl ester in essentially the same way gave the acid, m.p. 92–93°, in 73% yield. The two acids (mixture m.p. 92–93°) gave the same pattern on tlc showing two close-lying spots, R_f approx. 0.36 with 1:1 $CHCl_3$–ether. Furthermore, the acid obtained from the ethyl ester was esterified with diazomethane to yield a methyl ester with the same tlc properties as the first methyl ester. Saponification conditions were important; thus exposing the ethyl ester either to 10% alcoholic KOH or to 10% aqueous KOH, both at room temp. afforded the acid in poor yield mixed with other materials.

[c] Refers to the methyl ester. After washing the hydroxy acid product with cold hexane, it was crystallized from $CHCl_3$ or $CHCl_3$–hexane.

[d] Refers to methyl ester. Product was obtained by standard chromatography through Florisil ($CHCl_3$ and then 1:4 CH_3OH–$CHCl_3$) followed by crystallization from hexane.

[e] The methyl ester was used. Purification was effected by standard chromatography (silica gel, with $CHCl_3$ followed by 1:9 CH_3OH–$CHCl_3$).

[f] The methyl ester was prepared by Claisen condensation (NaH) of two molecules of methyl octadecanoate,[18] and then reduction. The final product was crystallized from hexane.

[g] The molar ratio refers to the methyl ester 6(R_1 = 8-heptadecenyl, R_2 = 7-hexadecenyl, R_3 = CH_3) obtained by the Claisen condensation of methyl oleate[18] followed by reduction. The hydroxy acid was obtained by dry column chromatography through silica gel (20:1 benzene–CH_3OH).

Table 4. Carbon and hydrogen composition for 3-hydroxy acids 7

Hydroxy-acid 7			Calcd.		Found	
R_1	R_3	Mol. Formula	C	H	C	H
$C_{11}H_{23}$	C_5H_{11}	$C_{19}H_{38}O_3$	72.61	12.11	72.83	12.26
$C_{11}H_{23}$	$C_{24}H_{49}$	$C_{38}H_{76}O_3$	78.62	13.10	78.49	12.91
$C_{17}H_{35}$	CH_3	$C_{21}H_{42}O_3$	73.69	12.28	73.60	12.42
$C_{17}H_{35}$	H	$C_{20}H_{40}O_3$	73.11	12.28	73.33	12.43
$C_{17}H_{35}$	$C_{24}H_{49}$	$C_{44}H_{88}O_3$	78.57	13.09	78.86	13.36

chloride contained a large proportion of octadecyl alcohol, and was unsuitable for the Grignard reaction.

Tetracosanyl iodide was available from tetracosanyl bromide or from tetracosanyl alcohol. The bromide was synthesized by extending the carbon chain in octadecyl chloride as follows. With dry argon or nitrogen as an inert atmosphere, octadecylmagnesium chloride was formed by refluxing and stirring a mixture of octadecyl chloride (7.1 g; 25 mmol), MeI (0.03 ml) as a starter, and Mg turnings (0.63 g; 26 mmol) in 15 ml of THF overnight. The Grignard reagent was slowly introduced (1 hr) at 0° to a vigorously stirred soln of 1,6-dibromohexane (17.8 g; 73 mmol) in dry THF (20 ml) containing 1.5 ml of a soln of dilithium tetrachlorocuprate[11] previously prepared from LiCl (1.3 mmol) and copper(II) chloride (0.7 mmol) in 10 ml of THF. Residual Grignard soln was washed in with 10 ml of THF. After 2 hr of stirring, NH_4Cl aq was added, and the mixture was processes as usual to obtain the product, tetracosanyl bromide, which after several crystallizations from acetone weighed 3.1 g (30%) and showed m.p. 51–52.5° (lit.[12] m.p. 52–53.5°), and was homogeneous according to tlc. A less soluble fraction, m.p. 71–73° (or from other runs as high as 84°), which was isolated from the acetone recrystallizations, was taken as the $C_{42}H_{86}$ normal hydrocarbon (lit.[13] m.p. 83–84°). Trials using different molar ratios of reactants gave no better yields of tetracosanyl bromide. This was converted to tetracosanyl iodide by treatment with excess NaI in acetone. Silica gel chromatography (benzene solvent) afforded pure tetracosanyl iodide (90%), m.p. 52–53° (lit.[14] m.p. 54.5–55.5°).

An alternate sequence made use of methyl tetracosanoate and tetracosanyl alcohol as intermediates. The ester was obtained by coupling tridecanylmagnesium bromide with methyl 11 - iodoundecanoate as follows.[15] A bright yellow suspension of methylcopper(I) was formed from ethereal methyllithium (11 ml or 19.8 mmol) and 98% copper(I) iodide (3.90 g; 20.5 mmol) in dry THF. After mixing at −65°, the reaction was stirred at this temp. for 1 hr; it was then slowly warmed to 0° and then returned to −65°. A Grignard soln, which was formed by stirring and refluxing tridecyl bromide (5.42 g; 20.1 mmol), MeI (0.1–0.2 mmol, added initially), and Mg turnings (0.482 g; 20.16 mmol) in 10 ml of THF, was injected (10 min) at −65°, with 5 ml of THF used to complete the transfer. After stirring at −65° for 1 hr, the mixture was allowed to warm to 10°; as soon as a clear soln developed, it was cooled to −65°. Methyl 11-iodoundecanoate (7.0 g; 21.5 mmol) in THF was injected (10 min) to this tridecanyl copper complex, and the mixture was stirred at −65° for 1 hr and then at room temp. for 2 hr before hydrolyzing and processing. Crystallization from acetone afforded white methyl tetracosanoate (4.3 g; 56%, and in other runs up to 75%), m.p. 57–58° (lit.[16] 58.4°). This ester was reduced with LAH in ether to tetracosanyl alcohol, obtained in 86% yield as white, well-formed, homogeneous crystals, m.p. 76–76.5° (lit.[14] 76–77°). Treatment of the alcohol at 110° with iodine and red P (molar ratios, 1:2:0.8) led to the desired homogeneous tetracosanyl iodide (81% after acetone crystallization), m.p. 52.5–53.5°.

Undecyl chloride, tridecyl bromide, and pentadecyl bromide were purchased.

Acyl chlorides. Octadecanoic acid with thionyl chloride or with oxalyl chloride gave octadecanoyl chloride,[17] which was generally distilled (b.p. 165° at 0.1 mm) before use. Dodecanoyl chloride, a commercial product, was redistilled.

Ethyl 3 - ketotetradecanoate (4, $R_1 = CH_3(CH_2)_{10}$– *and* $R_2 = CH_3CH_2$–). The lithio derivative of isopropyl - cyclohexylamine (distilled from calcium hydride) was prepared under N_2 by slowly injecting BuLi (41 ml, 2.40 M in hexane, or 98.4 mmol) into a stirred, −20° solution of freshly distilled amine (20 ml; 110 mmol, in THF (45 ml). Adding pure EtOAc (4.9 ml; 51.9 mmol, followed by 5 ml of THF) at −60° and then holding the mixture at −20° for 30 min allowed the enolate 2 to form.[6] A soln of dodecanoyl chloride (11.6 g; 53.3 mmol) in THF (10 ml plus 5 ml) was added by syringe, and stirring was continued for another 2 hr.

The mixture, acidified at ice-bath temp. with HCl, was extracted with ether. The extract was shaken with portions of dil. HCl, water, $NaHCO_3$aq, and brine before drying and removing all volatiles at room temp. Vacuum distillation of the residue gave 11 g (78%) of colorless 4($R_1 = C_{11}H_{23}$ and R_2 = Et), b.p. 123–125° (0.15 mm). This product, before as well as after distillation, showed a single spot on tlc (R_f 0.5 with 1:4 EtOAc–hexane) (Found: C, 71.30; H, 11.21. Calc. for $C_{16}H_{30}O_3$: C, 71.11; H, 11.11).

Use of methyl instead of EtOAc afforded the corresponding β-keto ester product in about the same yield.

Ethyl 3-keto-2-tetracosanyltetradecanoate (5, $R_1 = C_{11}H_{23}$, $R_2 = C_2H_5$, $R_3 = C_{24}H_{49}$) *by alkylation.* Compound 4($R_1 = C_{11}H_{21}$, R_2 = Et) in THF (0.290 g or 1.07 mmol in 10 ml) was injected gradually into a stirred slurry (−10°) of NaH (49.5 mg or 1.18 mmol in the form of a 57% oil dispersion) with 15 ml of THF. After stirring the soln at 0° for 30 min, tetracosanyl bromide (466.5 mg; 1.12 mmol) in THF (5 ml) plus hexamethylphosphoramide (5 ml) was injected. The alkylation mixture was stirred overnight at room temp. and then refluxed 6–12 hr.

After quenching with ice-cold sat NH_4Claq, and processing in the usual way, the crude product was chromatographed on a Florisil column at first with hexane as solvent and then progressing to 9:1 hexane–benzene, 1:1 hexane–benzene, benzene, 4:1 benzene–chloroform, and lastly chloroform. Unchanged tetracosanyl bromide (95 mg) emerged as an early eluate. The desired product appeared in the 1:1 hexane–benzene and the benzene fractions, followed later by impure starting ester.

Crystallization of the product from hexane afforded pure 5($R_1 = C_{11}H_{23}$, R_2 = Et, $R_3 = C_{24}H_{49}$) as a solid (385 mg; 59%), m.p. 57–58°; R_f 0.61 with 1:4 EtOAc–hexane; IR ($CHCl_3$) 1735, 1710 cm^{-1}; NMR ($CDCl_3$) δ 4.3 (q, J = 7.5 Hz, 2, OCH_2), 3.38 (t, J = 7.5, 1, $\overset{\overset{O}{\|}}{C}\text{–CH–}\overset{\overset{O}{\|}}{C}$), 2.50 (t, J = 6.75, 2, $CH_2\overset{\overset{O}{\|}}{C}$), 1.27 and 0.88 ppm (t overlapped by s and t, 73, remaining H's). (Found: C, 79.45; H, 13.02. Calc. for $C_{40}H_{78}O_3$: C, 79.21; H; 12.87).

No alkylation occurred when THF alone was taken as solvent.

The corresponding 5($R_1 = C_{11}H_{23}$, R_2 = Me, $R_3 = C_{24}H_{49}$), m.p. 62–64°, was prepared in essentially the same way.

Trials making use of lithium isopropylcyclohexylamide in a procedure similar to that described for the synthesis of ethyl 3-keto-2-pentyltetradecanoate failed when applied to the one-step acylation of methyl hexacosanoate with dodecanoyl chloride. At least 80% of the starting ester was recovered. Solubility may have been the problem here, since large amounts of the hexacosanoate failed to dissolve at −60°, and as a result, enolate formation had to be carried out at −30°. Also, during the acylation step (−55°) the reaction mixture persisted as a thick slurry. Using hexamethylphosphoramide with THF did not help. Related attempts to acylate methyl docosanoate with dodecanoyl chloride gave results suggesting that the balky stage was enolate formation.

Ethyl 3-keto-2-pentyltetradecanoate (5, $R_1 = C_{11}H_{23}$, $R_2 = C_2H_5$, $R_3 = C_5H_{11}$). The reactants were ethyl heptanoate (0.87 g; 5.5 mmol), isopropyl-cyclohexylamine (11 mmol), BuLi (11 mmol), and THF solvent (15 ml). The ester in 5 ml of solvent was added at −60° followed, after 1 hr, by dodecanoyl chloride (1.31 g; 6.0 mmol) in 10 ml of THF. The acylation was allowed to proceed for 2 hr at −50° before quenching. The product, ethyl 3-keto - 2 - pentyltetradecanoate was isolated as a homogeneous material (R_f 0.52 with 9:1 hexane-ethyl acetate) by dry-column chromatography[18] (silica gel with benzene) in the form of an oil (1.5 g; 81% based on the ester taken); IR (neat) 1740 and 1710 cm^{-1}; NMR ($CDCl_3$) δ 4.14 (q, J = 7.5 Hz, 2, OCH_2), 3.35 (t, J = 7.5 Hz, 1, $\overset{\overset{O}{\|}}{C}\text{–CH–}\overset{\overset{O}{\|}}{C}$), 2.5 (broad t, 2, $CH_2\overset{\overset{O}{\|}}{C}$), 1.25 and 0.88 ppm (s and t, 35, CH_3's and CH_2's). (Found: C, 74.24; H, 11.60. Calc. for $C_{21}H_{40}O_3$: C, 74.11; H, 11.77).

Methyl 3-keto-2-methyleicosanoate (5, $R_1 = C_{17}H_{35}$, $R_2 = R_3 = CH_3$). The direction for acylating propionate were essentially the same as those used for acylating acetate. The starting materials were methyl propionate (5.95 mmol), BuLi (equimolar amount), and octadecanoyl chloride (1.8 g or 5.95 mmol). Dry-column chromatography through silica gel using 2:1 chloroform–hexane served to purify the product. Zones were detected under UV light. Preparative plate chromatography (1 mm silica gel layer with 1:10 EtOAc–$CHCl_3$ developing solvent) was also effective, although less convenient. Compound 5($R_1 = C_{17}H_{35}$, $R_2 = R_3 =$ Me) was obtained in this way as a homogeneous solid (1.3 g; 61%), m.p. 37–40°; R_f 0.5 with $CHCl_3$ developer; IR ($CHCl_3$) 1725 and 1700 cm^{-1}; NMR ($CDCl_3$ with $CHCl_3$ reference) δ 3.57 and 3.5 (s plus q, J = 4.5 Hz, 4, OCH_3 plus $\overset{O}{\overset{\|}{C}}{-}CH{-}\overset{O}{\overset{\|}{C}}$), 2.55 (t, J = 4 Hz, 2, CH_2CO), 1.32 and 0.95 ppm (m, 36, all other H's). (Found: C, 74.19; H, 11.62. Calc. for $C_{22}H_{42}O_3$: C, 74.52; H, 11.94).

Methyl 3-ketoeicosanoate (4, $R_1 = C_{17}H_{35}$ *and* $R_2 = CH_3$). The procedure here was similar to the one used with the 14-carbon ester. The reactants were isopropyl - cyclohexylamine (39.8 mmol), BuLi (39.6 mmol), THF (100 ml) freshly distilled from LAH, MeOAc (19.8 mmol), and octadecanoyl chloride (6.0 g or 20 mmol in 15 ml of THF). The acylation mixture became homogeneous at −15 to 0°, where it was held for 30 min. Product was purified by column chromatography on silica gel, starting with solvent hexane and then going to hexane containing increasing proportions of $CHCl_3$. Compound 4($R_1 = C_{17}H_{35}$, and R_2 = Me) m.p. 29–31°, was obtained in 63% (4.4 g) yield. Later it was found that purification by dry-column chromatography on silica gel with 2:1 $CHCl_3$–hexane was more convenient. The product showed one spot on tlc (R_f 0.57 with $CHCl_3$ solvent). (Found: C, 73.75; H, 12.20. Calc. for $C_{21}H_{40}O_3$: C, 74.06; H, 11.84).

Methyl 3-keto-2-tetracosanyleicosanoate (5, $R_1 = C_{17}H_{35}$, $R_2 = CH_3$, $R_3 = C_{24}H_{49}$) *by alkylation*. A mixture of 0.345 g (1.00 mmol) of 4($R_1 = C_{17}H_{35}$, R_2 = Me) in THF (27 ml) distilled over LAH was stirred at 0° for 30 min in the presence of commercial BuOK (0.119 g; 1.05 mmol). A soln of tetracosanyl iodide (0.51 g; 1.1 mmol) in 11 ml of THF that had been diluted with hexamethylphosphoramide was injected (5 min), after which stirring was continued for 10 min at 0°, at room temp. for 1.75 hr, and at reflux for 6 hr.

The mixture, concentrated to about half its volume, was cooled, treated with 150 ml of 2.5% HCl, and processed further to isolate the pale yellow crude alkylation product. Chromatography through silica gel (2:1 hexane–$CHCl_3$) gave unchanged tetracosanyl iodide (0.12 g; 24%), m.p. 50–52°, coming out first, followed by the slower moving 5($R_1 = C_{17}H_{35}$, R_2 = Me, $R_3 = C_{24}H_{49}$). This product (0.48 g; 71%), which had m.p. 69–70°, showed a single spot on a tlc plate (R_f 0.5 with $CHCl_3$); IR ($CHCl_3$) 1735, 1705 cm^{-1}; NMR ($CDCl_3$ with $CHCl_3$ reference) δ 3.74 (s, 3, OCH_3), 3.4 (t, J = Hz, 1, $\overset{O}{\overset{\|}{C}}{-}CH{-}\overset{O}{\overset{\|}{C}}$), 2.5 (t, J = 7, $CH_2\overset{O}{\overset{\|}{C}}$), 1.4 and 0.96 ppm (s and t, 82, remaining H's). An additional 40 mg of product, m.p. 69–70°, was obtained as a mixture by processing various chromatography fractions. (Found: C, 80.13; H, 13.24. Calc. for $C_{45}H_{88}O_3$: C, 79.81; H, 13.10).

Isolation of some dialkylation product, which showed no NMR $\overset{O}{\overset{\|}{C}}{-}CH{-}\overset{O}{\overset{\|}{C}}$ signal and a high CH_2 content stressed the importance of avoiding more than equimolar amounts of BuOK. Using tetracosanyl bromide in place of the iodide and t-BuOH instead of THF gave rise to the desired alkylation product, although in lower yield. Neither NaH nor LiH in THF containing hexamethylphosphoramide was effective.

3-Hydroxy esters 6 by borohydride reduction of the 3-keto esters 5. The 3-keto esters 5 were treated with $NaBH_4$ in refluxing MeOH or EtOH for 24 hr. The molar ratios of borohydride-to-ketone varied widely—from 0.25 to 12—generally without affecting the yield drastically. After the reaction period, solvent was removed at room temp. under reduced pressures. The residue was treated with water, NH_4Claq or HClaq, extracted with ether, and processed thereafter in the usual way. Column chromatography or dry-column chromatography furnished pure product, which in several cases showed two spots on tlc and in one case could be separated into the two diastereoisomeric forms. In their IR absorption spectra, generally taken in $CHCl_3$ solution, the hydroxy ester products 6 showed absorptions at 3600–3400 and 1725–1720 cm^{-1}. PMR spectra (generally in $CDCl_3$) for the 2-substituted products showed signals in the regions δ 3.9–3.4 (broad m, CHOH) and 2.5–2.1 ppm (m, CHCO). Compound 6($R_1 = C_{17}H_{35}$, R_2 = Me, R_3 = H) gave PMR signals at δ 4.0–3.8 (broad, CHOH) and 2.45 ppm (distorted d, J = 3 Hz, CH_2CO).

Tables 1 and 2 give details for the individual runs.

Attempts were made to form 6 directly by adding the lithium enolate of methyl docosanoate to the aldehyde carbonyl group of tetradecanal or octadecanal. Runs were made with lithium isopropylcyclohexylamide in tetrahydrofuran-hexamethylphosphoramide at temps ranging from −60 to 10° in 10° intervals and for various reaction periods. In no case was there evidence of condensation. The ester could be recovered in good yield, and tlc showed only the two spots corresponding to the starting materials.

Hydroxy acids 7 from esters 6. The methyl or ethyl ester 6 was refluxed with alkali in aqueous methanol or ethanol for 2–3.5 hr, then cooled and acidified with 5–10% hydrochloric acid. The product was extracted with ether or chloroform, and the extract was washed several times with water and salt solution, dried and stripped of all solvent. The residue was purified by dry or wet column chromatography. Table 2 gives the details for individual runs.

In the hydroxy acid product 7 carrying a 2-alkyl group, thin layer chromatography developed two partially overlapping spots. The tlc solvents found useful included mixtures of chloroform and methanol, 20:3 benzene–methanol, and 1:1 chloroform–ether. The hydroxy acids either neat or in chloroform solution showed IR absorption maxima in the regions 3600–2500 and 1715–1695 cm^{-1}. NMR spectra were taken with the compounds in $CDCl_3$ or CCl_4, with ordinary chloroform as the internal reference. In all cases the hydroxy protons of 7 produced a broad signal at δ 7.2–5, the proton at position-3 came as a multiplet at 4.1–3.4, the proton at position-2 as a multiplet at 2.7–2.45, and the remaining methyl and methylene protons at 1.5–1.3 and 1.05–0.95 ppm. In the single example of the hydroxy acid with methylene at position-2, these methylene protons came out as a distorted doublet. In all cases, the signal associated with the hydroxyl groups was lost when D_2O was added. Experimental integration values were satisfactory. Tables 3 and 4 give further information about the acids 7.

Unsuccessful attempts were made to obtain hydroxy acid 7 ($R_1 = C_{17}H_{35}$; $R_2 = C_{16}H_{33}$) directly by adding the dilithium derivative of octadecanoic acid (formed with lithium di-isopropylamide in tetrahydrofuran-hexamethyl phosphoramide[9] to octadecanal. Tlc of the crude product mixture showed that the only materials present were the unchanged starting acid and aldehyde; further, the acid could be separated and characterized. The possibility that the di-lithium derivative failed to form was excluded by isolating large amounts of hexadecylmalonic acid after bubbling carbon dioxide into the enolate preparation.

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[4] Cf. R. E. Ireland and J. A. Marshall, *J. Am. Chem. Soc.* 81, 2907 (1959); I. Kuwajima, N. Minami and T. Sato, *Tetrahedron Letters* 2253 (1976); C. T. Buse and C. H. Heathcock, *J. Am. Chem. Soc.* 99, 8109 (1977); G. Stork and T. Takahashi, *Ibid.* 99, 1275 (1977).

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