B. Vigorous Conditions.—A mixture of 105.4 g (0.775 mole) of α-terpinene, 10.45 g (0.349 mole) of paraformaldehyde, and 200 ml of glacial acetic acid was stirred at 111–115° for 72 hr. The usual work-up of the reaction mixture (*supra vide*) gave 59 g of impure α-terpinene (*ca.* 68% pure by glpc analysis) and 50 g of a principal-product fraction, bp 60–108° (0.3–0.4 mm), $n^{24}D$ 1.4882. Glpc analysis[4a] of this fraction showed that it comprised 96% α-terpinene–formaldehyde condensation products, 25% of which was the acetate IIb and 50% the acetate Ib; the remainder consisted of at least four minor products.

Catalytic hydrogenation, in a Parr apparatus, of 25 g of the above mixture of acetates in 75 ml of glacial acetic acid over 1.1 g of Adams catalyst for 70.5 hr at 47–48 psi gave 22 g of the stereoisomeric-saturated acetates VIa, bp 54–87° (1.1 mm), $n^{25}D$ 1.4536. Glpc analysis indicated a purity of 95%.[4a] Saponification of 21 g of the acetates VIa, after a conventional work-up and distillation through a Vigreaux column, afforded two principal-product fractions: (1) 3.5 g, bp 64–72° (0.25 mm), $n^{25}D$ 1.4685; (2) 12 g, bp 72–78° (0.25 mm), $n^{25}D$ 1.4683. Redistillation of fraction 2 gave a center cut which had bp 73–77° (0.3 mm) and $n^{26}D$ 1.4674. Glpc analysis of the latter sample of the isomeric saturated alcohols VIb at 165° on a column containing 15% diethylene glycol sebacate and 5% "Bentone 34" did not resolve the mixture cleanly and gave an unsymmetrical major peak. However, analysis at 165° on a column containing 20% polyphenyl ether (5 ring) partially resolved two major components and indicated the presence of a minor component (slight shoulder in peak with longer retention time).

Anal. Calcd for $C_{11}H_{22}O$: C, 77.58; H, 13.02; mol wt, 170. Found: C, 77.47; H, 12.98; mol wt, 166 (benzene).

The Reaction of α-Terpinene and Formaldehyde in Acetic Acid with Formic Acid as the Added Catalyst.—A mixture of 83.4 g (0.613 mole) of α-terpinene, 8.28 g (0.276 mole) of paraformaldehyde, 174 ml of glacial acetic acid, and 21.0 g of 98–100% formic acid was stirred at 111–114° for 72 hr. A normal work-up of the reaction mixture gave 37.1 g of crude α-terpinene (*ca.* 55% pure by glpc analysis) and 38.8 g of a principal-product fraction containing acetates and formates, bp 61–106° (0.55 mm), $n^{25}D$ 1.4952.

A portion (22.5 g) of the above mixture of esters was added to a solution containing 10.0 g of potassium hydroxide in 100 ml of 95% ethanol and the resulting solution was refluxed for 22.5 hr. After the usual work-up of the reaction mixture the product was distilled to give 15.0 g of impure alcohol Ib, bp 68–80° (0.35 mm), $n^{25}D$ 1.5177. Glpc analysis of the reaction product indicated that compound Ib comprised *ca.* 71%.

Methanolysis of the 1:1 Mixture of the Unsaturated Acetates Ia and IIa.—A mixture of 13 g of a 1:1 mixture of the acetates Ia and IIa (purity 74%), 16 ml of anhydrous methanol, and four drops of concentrated sulfuric acid was refluxed gently for 6.2 hr. Intermittently, 1-ml portions of distillate were removed and 1-ml portions of fresh methanol were added. Following a conventional work-up of the mixture there was obtained 7.5 g of a principal-product fraction that had bp 75–87° (0.7–0.9 mm) and $n^{20}D$ 1.5218. Glpc analysis[4a] of this product showed that it comprised 85% of the single alcohol Ia.

The Boron Trifluoride Etherate Catalyzed Reaction of α-Terpinene with Formaldehyde.—Under a nitrogen atmosphere, a solution of 2 ml of boron trifluoride etherate in 40 ml of methylene chloride was added dropwise over a 1.5-hr period to a stirred mixture of 69.5 g (0.511 mole) of α-terpinene, 7.65 g (0.255 mole) of paraformaldehyde, 100 ml of methylene chloride, and 45 ml of acetic anhydride. This mixture was then stirred at room temperature for 1 more hour, poured into a solution of sodium carbonate, and extracted with ether. Distillation of the washed and dried ($MgSO_4$) ether extract gave 30 g of impure α-terpinene and two principal-product fractions: (1) 30.3 g, bp 67–97° (0.2 mm), $n^{20}D$ 1.4877; (2) 2.2 g, bp 97–110° (0.2 mm), $n^{20}D$ 1.5007. Glpc analysis[4a] showed that (a) the recovered α-terpinene is *ca.* 80% pure, (b) the unsaturated acetates Ia and IIa make up 77% of fraction 1 and fraction 2 is 92% acetate Ia. Presence of the two unsaturated acetates in the two fractions was confirmed by infrared analysis.

Catalytic hydrogenation of the combined fractions 1 and 2 (32 g) in 50 ml of glacial acetic acid over 1 g of Adams catalyst at *ca.* 40 psi for 24 hr at room temperature gave, after a conventional work-up, 21 g of the saturated acetates VIa (93% pure) together with some free alcohols (glpc analysis[4a]), bp 62–70° (0.1 mm), $n^{23}D$ 1.4534. This product (20 g) was heated at 85–86° with 20 ml of acetic anhydride and 20 ml of pyridine for 6.5 hr and then, after a normal work-up, distilled *in vacuo*. Three product fractions were collected: (1) 8.82 g, bp 61–62° (0.2 mm), and $n^{17}D$ 1.4550; (2) 5.92 g, bp 62° (0.15–0.20 mm), and $n^{17}D$ 1.4547; (3) 3.20 g, bp 62° (0.15 mm), and $n^{17}D$ 1.4548. Redistillation of fraction 2 gave a pure sample of the saturated acetates VIa, bp 79–86° (0.60 mm), and $n^{20}D$ 1.4534.

Anal. Calcd for $C_{13}H_{24}O_2$: C, 73.53; H, 11.39. Found: C, 73.75; H, 11.45.

A conventional saponification of 3.86 g of the analytically pure acetates VIa gave, after two careful distillations *in vacuo*, 2.5 g (80%) of the saturated alcohols VIb, bp 83–85° (0.8 mm), $n^{25}D$ 1.4671. Glpc analysis[4a] showed that the product contains only the two stereoisomeric saturated alcohols VIb.

Anal. Calcd for $C_{11}H_{22}O$: C, 77.58; H, 13.02. Found: C, 77.51, 77.39; H, 12.94, 12.75.

The α-Terpinene–Formaldehyde Reaction in Aqueous Formic Acid.—A solution of 15.6 g of 98–100% formic acid in 18 ml of water was added rapidly at room temperature to a mixture of 46.3 g (0.340 mole) of α-terpinene and 10.2 g (0.340 mole) of formaldehyde. This mixture was stirred at 60–61° for 24 hr, cooled, diluted with water, and extracted with ether. From the washed and dried ($MgSO_4$) ether extracts there was obtained, after distillation through a Vigreaux column, 22.8 g of α-terpinene and 19.6 g of a principal-product fraction, bp 90–101° (2.7 mm) and $n^{20}D$ 1.4842, that comprised the 1,3-dioxane VII (74%) together with free alcohols and formates (glpc, infrared, and nmr analysis).

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O-Acylation of *dl*-Carnitine Chloride

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Excellent yields of very pure esters of *dl*-carnitine chloride with fatty acids having 2–18 carbon atoms are obtained by means of a special O-acylation method. Three different experimental procedures are described.

The known biochemical role of O-palmitylcarnitine chloride and the fact that the methods of preparation so far described lead to unsatisfactory yields[1-3] prompted us to study the synthesis of this substance and of its homologs. Previous experiments had shown that acetic acid, as well as acetyl chloride in which carnitine chloride is only slightly soluble, gave unsatisfactory results. The yield was 42% and the end product had to be recrystallized.[4]

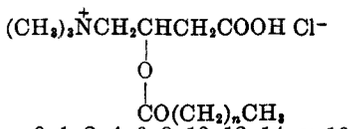
(1) I. B. Fritz and K. T. N. Yue, *Lipid Res.*, **4**, 279 (1963).
(2) I. B. Fritz, E. Kaplan, K. T. N. Yue, *Am. J. Physiol.*, **202**, 117 (1962).
(3) J. Bremer, *J. Biol. Chem.*, **237**, 3628 (1962).
(4) R. Krimberg and W. Wittenandt, *Biochem. Z.*, **251**, 229 (1932); F. Binon, P. Bruckner, and G. Deltour, *Bull. Soc. Chim. Belges.*, **72**, 166 (1963).

TABLE I
EXPERIMENTAL CONDITIONS AND YIELDS

Expt	Method (*n*)	Free acid (*n*)[a]	Acid chloride (*n*)[b]	Solvent	Period of heating at 80°, hr: Before the addn of carnitine	Period of heating at 80°, hr: In the presence of carnitine	Yield, %
1	A	0	0	Free acid	...	3	15
2	A	0	0	Free acid	...	4	80
3	A	0	0	Free acid	3	1	96
4	C	1	$SOCl_2$	Cl_3CCOOH	3	2	97
5	C	2[c]	$SOCl_2$	Cl_3CCOOH	3	3	93
6	A	4[c]	4	Free acid	...	6	62
7	B	4	$SOCl_2$	Free acid	...	5	98
8	B	6	$SOCl_2$	Free acid	3	2	77
9	C	6	$SOCl_2$	Cl_3CCOOH	3	3	90
10	C	8	$SOCl_2$	Cl_3CCOOH	3	3	91
11	C	10	$SOCl_2$	Cl_3CCOOH	3	3	90
12	C	12	$SOCl_2$	Cl_3CCOOH	3	3	90
13	C	14	$SOCl_2$	Cl_3CCOOH	3	3	90
14	C	16	$SOCl_2$	Cl_3CCOOH	3	3	88

[a] $CH_3(CH_2)_nCOOH$. [b] $CH_3(CH_2)_nCOCl$. [c] Under the conditions described by Strack and Lorenz and applied to the betaine of *dl*-carnitine, the yields are in the region of 25–30%.[5]

In a new series of experiments, the following O-acylcarnitine chlorides were prepared.



n = 0, 1, 2, 4, 6, 8, 10, 12, 14, or 16

The procedures devised in the course of these experiments enabled high yields of analytically and electrophoretically pure O-acylcarnitine chlorides to be readily obtained.

In all, three different methods were utilized.

Method A.—*dl*-Carnitine chloride is heated with a mixture comprising an excess of a carboxylic acid and an equimolecular quantity of the same acyl chloride. When the mixture of carboxylic acid and acyl chloride was previously heated for several hours, high yields of pure O-acylcarnitine chloride (n = 0 and 4) were obtained.

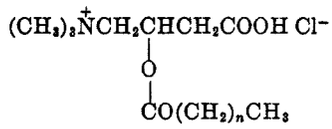
Method B.—Replacement of the acyl chloride by an equimolecular quantity of thionyl chloride gives the same or even better results (compare expt 6 and 7 in Table I). On the other hand, it was found that neither sulfuryl chloride nor *p*-toluene sulfochloride could be employed as substitutes for acyl chloride.

Method C.—Carnitine chloride ceases to be soluble in mixtures comprising either fatty acids of more than six methylene carbons and their chlorides (method A) or fatty acids and thionyl chloride (method B); this difficulty is overcome by using trichloroacetic acid as solvent. A mixture containing the fatty acid and thionyl chloride is heated for 3 hr, as in method B, and then carnitine chloride dissolved in trichloroacetic acid is added. This procedure enabled us to obtain, in yields as high as 88–97%, all the terms in the range of n = 0–16 of acylated derivatives, which could not be synthetized by method A or B (Table II).

Electrophoresis (400 v, Whatman 3MM, pH 4 (acetic acid–pyridine), developed by means of I_2 and NH_3) proves that the acylated derivatives are pure and contain no nonacylated carnitine.

In the infrared spectra (in Nujol) of the acylated derivatives it was observed that the following bands disappeared: the OH stretching band situated at 3260 cm^{-1} and the absorption band of CO, characteristic of the secondary OH function, situated at 1100 cm^{-1} in the case of carnitine; on the other hand, CO stretching bands of the acyl groups are found at 1250 cm^{-1} in the case of the acetate and at about 1200 cm^{-1} in the case of the higher esters. The C=O stretching band of the C(=O)OH function, situated at 1725 cm^{-1} in the case of carnitine, was found to be displaced to about 1715 cm^{-1} and a second C=O stretching band, characteristic of the C(=O)OR functions, appeared at 1735–1740 cm^{-1}.

TABLE II
DATA REGARDING THE ACYLATED DERIVATIVES[a]



Expt[c]	*n* (acyl)	Mp, °C[b]	Anal., %: C	H	N	Cl⁻
3	0	210	45.30	7.54	5.81	14.73
			45.55	7.71	5.79	14.62
4	1	157	47.33	7.94	5.51	13.97
			47.38	8.18	5.50	13.96
5	2	147	49.34	8.28	5.22	13.23
			49.55	8.39	5.22	13.33
7	4	160	52.78	8.85	4.73	11.98
			52.61	8.90	4.76	12.04
9	6	161	55.63	9.33	4.32	10.93
			55.43	9.44	4.34	10.94
10	8	164	58.02	9.73	3.97	10.07
			57.90	9.80	3.98	10.02
11	10	170/1	60.06	10.08	3.68	9.32
			60.17	10.15	3.64	9.21
12	12	164	61.82	10.37	3.43	8.68
			61.70	10.13	3.42	8.73
13	14	161	63.62	10.63	3.21	8.13
			63.29	10.72	3.21	8.01
14	16	152	64.69	10.85	3.01	7.63
			64.40	10.65	3.02	7.43

[a] First figures % calcd; second figures % found. [b] Determined with the Totoli apparatus (capillary tube introduced at 15° below melting point and raised the temperature 5°/min). [c] Registry no.: expt 3, 14919-31-4; expt 4, 14919-32-5; expt 5, 14919-33-6; expt 7, 14919-34-7; expt 9, 14919-35-8; expt 10, 14919-36-9; expt 11, 14919-37-0; expt 12, 14919-38-1; expt 13, 6819-24-5; expt 14, 14919-40-5.

Strack and Lorenz have recently described the acylation of l-, *d*-, and *dl*-carnitine betaines using acyl chloride in the presence of the corresponding carboxylic acid.[5] In the opinion of these authors, the acyl chloride constitutes the acylating agent while the free acid acts solely as solvent. In the case of our acylation experiments with *dl*-carnitine chloride, our observations lead us to conclude that the esterification mechanism is of a special nature. (1) When free acetic acid, acetyl chloride, and carnitine chloride were mixed and the mixture was directly heated to and maintained at 80°, acylation progressed very slowly for the first 3 hr. During the 4th hr, the concentration in acylated derivative rapidly increased from 15 to 80%. Furthermore, when a mixture comprising free acetic acid and acetyl chloride was maintained at 80° for 3 hr and then carnitine chloride was added, the concentration in *O*-acetylcarnitine chloride rose to 96% during the following 60 min. In this latter instance, the process of acylation began immediately, as though the first 3 hr had served to form an activated acylating complex. (2) When carnitine chloride was treated with a previously heated mixture of an equimolecular quantity of acetyl chloride and excess caproic acid, only 4% of O-acetylcarnitine chloride was obtained together with 80% of O-caproylcarnitine chloride. When carnitine chloride was heated with a previously heated mixture of an equimolecular quantity of caproyl chloride and excess acetic acid, acetyl carnitine chloride was obtained in 100% yield. Under these reaction conditions, it was not the acyl chloride but the free acid which provided the acylated carnitine derivative.

However, not all free acids, when employed with an acid chloride, give an acyl group. In the process according to method C (excess free carboxylic acid with equimolecular quantities of thionyl chloride and carnitine chloride, the latter being dissolved in trichloroacetic acid), O-trichloroacetylcarnitine chloride is formed. When carnitine chloride is treated with a mixture of acetyl chloride and trichloroacetic acid, O-acetylcarnitine chloride is obtained with a nearly quantitative yield and no O-trichloroacetylcarnitine is formed. In none of the three acylation methods does the carboxylic function of the carnitine itself react with the hydroxyl group.

(5) E. Strack and I. Lorenz, *Z. Physiol. Chem.*, **343**, 231 (1966).

Experimental Section

Method A. Acetyl *dl*-Carnitine Chloride.—A mixture of 50 ml of acetic acid and 8 g (0.05 mole) of acetyl chloride was stirred for 3 hr at a temperature of 80°. *dl*-Carnitine chloride (9.9 g, 0.05 mole) was added and the solution was stirred at this same temperature for a further 60 min. Excess acetic acid and acetyl chloride were then distilled at reduced pressure on a water bath (50°). The residual viscous mass was dissolved in about 10 ml of hot isopropyl alcohol, the resulting solution was filtered to eliminate traces of unreacted carnitine, and 40 ml of acetone was added to the filtrate. The product crystallized slowly and after a few hours was filtered, washed with acetone, and dried. A yield of 11.6 g was obtained (96% of theory), mp 210°. (For further experiments, see Table I.)

Method B. Caproyl *dl*-Carnitine Chloride.—A mixture of 50 ml of caproic acid and 6 g (0.05 mole) of thionyl chloride was stirred for 3 hr at a temperature of 80°. *dl*-Carnitine chloride (9.9 g, 0.05 mole) was added and the solution was stirred at this same temperature for a further 2 hr. After cooling, the solution was poured into 200 ml of dry ether, and the precipitate was filtered, washed with ether, and redissolved in 50 ml of hot isopropyl alcohol. Dry acetone (400 ml) was added to the isopropyl alcohol solution to enable the unreacted carnitine chloride to be separated. This solution was allowed to stand for 3 hr and a very slight precipitate (*dl*-carnitine chloride) was filtered; 500 ml of dry ether was added to the solution to precipitate the caproylcarnitine chloride which was filtered, washed with ether, and dried. A yield of 28.98 g (98% of theory) was obtained, mp 160°. (See also expt 8 in Table I.)

Method C. Palmityl *dl*-Carnitine Chloride.—A mixture of 50 g of palmitic acid and 6 g (0.05 mole) of thionyl chloride was stirred for 3 hr at a temperature of 80°, after which a solution of 9.9 g of *dl*-carnitine chloride in 50 g of trichloroacetic acid at 40° was added. The solution was maintained at 80°, for 3 hr, while stirring and then poured into 200 ml of dry ether. The precipitate was filtered, washed with ether, and dissolved in 50 ml of hot isopropyl alcohol. The isopropyl alcohol solution, being turbid (traces of *dl*-carnitine chloride), was filtered and 400 ml of dry acetone was added to the filtrate. Palmitylcarnitine chloride was precipitated; the precipitate was filtered, washed with ether, and dried. A yield of 19.6 g (90% of theory) was obtained, mp 161°.

Recovery of Palmitic Acid.—The ethereal washing solution of the raw product was washed with water until neutral and dried on Na_2SO_4. The ether was distilled and the residue was recrystallized in petroleum ether (bp 80–100°). More than 50% of the acid employed was recovered.

Similarly, *dl*-carnitine chloride was acylated by the $CH_3(CH_2)_nCOOH$ acids whose n value = 2, 6, 8, 10, 12, 16, and/or 18.