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[Received October 30, 1964—Accepted January 29, 1965]

Determination of the Length of Polymethylene Chains in Salts of Saturated and Unsaturated Fatty Acids by Infrared Spectroscopy[1]

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Abstract

The length of polymethylene chains is determined by counting the number of, or measuring the position of, methylene vibration peaks in the 1070–710 cm^{-1} and/or the 1380–1170cm^{-1} regions of the IR spectrum of salts of fatty acids. Plotting this peak position against the phase relationship of the vibration in adjacent methylenes gives a curve which is independent of the chain length. (The *phase relationship,* $\Phi/\pi = k/(m+1)$; where ϕ is the phase difference in radians between adjacent methylenes in a chain; m is the number of methylenes in the chain; $k = 1,2,3,\ldots\ldots m$, with $k = 1$ generally assigned to the in-phase vibration.) Separate curves are obtained for methylene wagging and for two arrays of coupled twisting-rocking vibrations.

Coupled twisting-rocking vibrations give as many as one peak per methylene group in the 1070–710 cm^{-1} region with silver, sodium, potassium and barium salts of saturated acids. Lead salt peaks split. These peaks show the total length of salts of both saturated and *trans*-unsaturated acids, but only the length of the carboxylate segment in salts of *cis*-unsaturated acids. (The carboxylate segment comprises the carbons from the carboxylate carbon to the first unsaturated carbon, inclusive.)

Wagging vibrations in the 1380–1170 cm^{-1} region show the total chain length of saturated salts and the length of the carboxylate segment in unsaturated salts, both *cis* and *trans.* This region also has peaks for twisting-rocking vibrations, and they are most conspicuous in the spectra of silver and barium salts.

Introduction

THE POTENTIAL OF IR spectra for the identification of fatty materials has not been fully realized, since many fatty acids are liquid at room temp and randomly oriented. Differences in the spectra related to the length of methylene chains are mainly quantitative. When free rotation about the single bonds is eliminated by examining the materials in the solid state, qualitative variations have been found in the spectra. Examples in the literature include saturated fatty acids, esters, salts and ketones, and even *trans*-mono-unsaturated acids (1,4–7,12,14,17,18,24,26,27,29). Generally, recognition of all the useful peaks has been quite incomplete, and the assignment of peaks to vibrations in the molecule has been disputed (8,9,11,13,16,20,22,28).

We have obtained the spectra of a number of metal salts of a series of saturated fatty acids. The salts are more easily handled and have fewer interfering peaks than the crystallized acids. The salt spectra are compared with those of saturated hydrocarbons which have recently been studied in some detail (19, 23). We have discussed only the regions where peaks due to methylene wagging, twisting and rocking vibrations are found, and in hydrocarbons this is 1413–1170 cm^{-1} and 1061–721 cm^{-1} (19,20,23). We also investigated the spectra of salts of mono-unsaturated acids for comparable behaviour.

When the carbon skeleton is an extended zigzag chain, as in crystals, the methylenes may vibrate in-phase (phase difference 0°), out-of-phase (phase difference 180°), and with phase differences between 0° and 180°. For m methylenes in a chain there are m possible phase relationships between adjacent groups. As a result, the chain may have m vibration frequencies which are seen as m absorption peaks in the IR spectrum. Each peak is not assigned to a particular methylene group, but to the vibration of the groups relative to each other as a whole.

In this paper we are concerned with three ways in which the methylenes may vibrate; wagging, twisting and rocking. Assuming the absence of coupling between the vibrations, there are m possible phase relationships with m absorption peaks for each way in any chain. Thus a C_6 acid or salt with four methylene groups could show $3 \times 4 = 12$ absorption peaks that might be assigned to the vibrations of interest. The number of peaks predicted by theory was found in the hydrocarbon spectra, although this is less than predicted for the salts because of symmetry in the hydrocarbons. The assignments for hydrocarbons were complicated by coupling of the twisting and rocking vibrations which also overlapped the wagging vibrations (19,23).

Experimental

Acids

Commercially available fatty acids (C_3–C_{22}, except C_{21}) were used for preparation of the salts. Subsequently the saturated acids, C_4 and C_7–C_{20}, were analysed as the methyl esters by gas liquid chroma-

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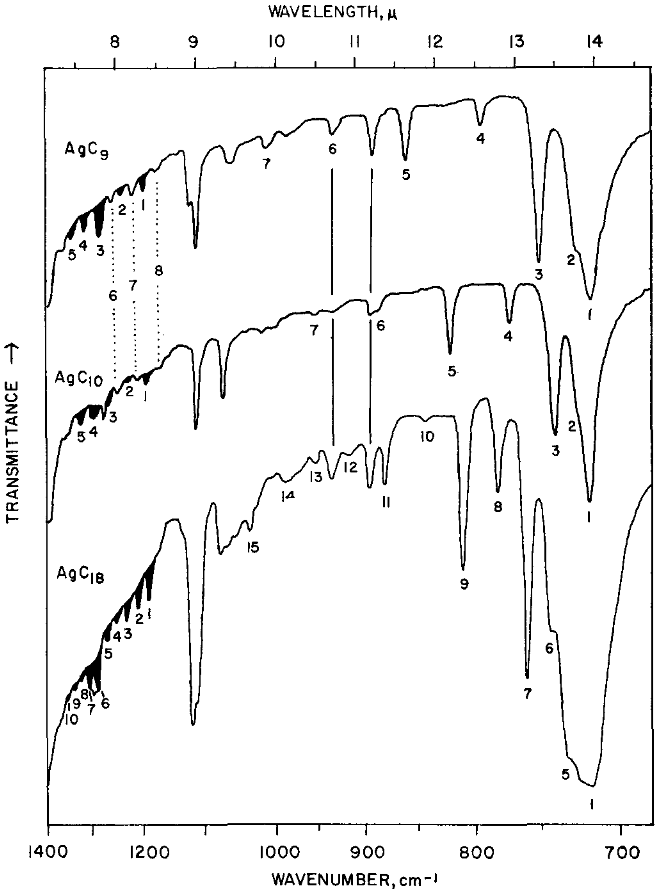

FIG. 1. IR absorption spectra of the silver salts of saturated C_9, C_{10} and C_{18} fatty acids, from 1400–680 cm^{-1}. Peaks assigned to methylene vibrations are numbered: black peaks—wagging; peaks joined by dotted lines—high frequency twisting-rocking; the remainder—low frequency twisting-rocking. Peaks joined by solid lines are assigned to end group vibrations.

tography (GLC) (15). Instead of the separated ester in petroleum ether, the methanol solution was injected directly into a column of 10% diethylene glycol-succinate on Chromosorb W (80–100 mesh) in a Barber-Colman, model 10 chromatograph, with flame ionization detector. The purities were 96% or better, except for C_{16}, C_{18} and C_{20} as shown in Table I.

The unsaturated acids; elaidic (*trans*-9-C_{18}), oleic (*cis*-9-C_{18}), petroselenic (*cis*-6-C_{18}), palmitoleic (*cis*-9-C_{16}), 11-eicosenoic (*cis*-11-C_{20}), and erucic (*cis*-13-C_{22}) were obtained from the Hormel Institute.

TABLE I
Purity of Acids by GLC of Solutions of the Methyl Ester[a]

Acid		Impurity	
No. of carbons	%	No. of carbons	%
4	100		
7	97	6	2
8	99		
9	97	8	3
10	100		
11	100		
12	96	10	1
		14	2
13	97	11	1
14	97	12	1
		16	1
15	99		
16	85	18	14
		17	1
17	96	15	1
		19	1
18	90	14	1
		16	6
		17	1
		20	1
19	99		
20	94[b]	18	4[b]
22	100		

[a] In methanol except where noted.
[b] In petroleum ether.

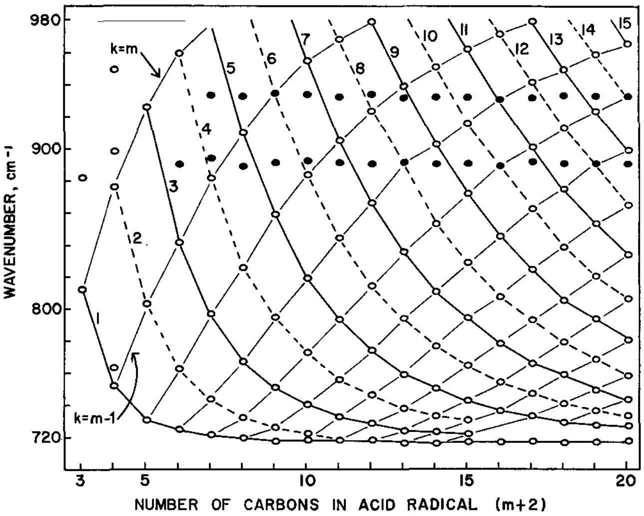

FIG. 2. Distribution pattern above 710 cm^{-1}, for methylene twisting-rocking vibration peaks in saturated silver salt spectra.
O——O stronger peaks, *k* odd
O- - -O weaker peaks, *k* even
● end group peaks

They were rated > 99% pure with no impurities by GLC examination of the methyl esters.

Silver Salts

The saturated acids (0.1–0.2 g) from C_8 up, were each dissolved in aqueous acetone (5–10 ml of 1:2 by volume) with a little steam heat for the longer chain acids. The C_6 and C_7 acids were dissolved in water only and the shorter chain acids were treated directly. The theoretical amount of 0.1 M completely aqueous silver ammonium nitrate (14) was added to the acid solution and the resulting precipitate centrifuged out. The solid was washed with water and dried under high vacuum at room temp in the dark.

The silver ammonium nitrate solution was added directly to the unsaturated acids. Acetone (one-tenth the volume of silver solution) was also added to the solid unsaturated acids. The centrifuge tubes used for the unsaturated salt preparation were flushed with nitrogen, covered with foil and stored in the dark at room temperature for 5 to 6 days. The resulting precipitate was centrifuged out, washed with water, dried under vacuum, washed with acetone and again dried under high vacuum in the dark. This gave about a 90% yield of pure salt.

Sodium Salts

The saturated acids were dissolved in 95% ethanol and a tiny drop of phenolphthalein solution added. Aqueous sodium hydroxide (1 *N*) was added dropwise until a pink colour persisted while heating on a steam bath. The solution was evaporated to dryness, the solids dissolved in hot absolute ethanol, and the solution filtered and redried.

Sodium salts of the C_{10} and C_{18} saturated acids and of the unsaturated acids were also prepared by grinding 5 mg of the appropriate silver salt with 500 mg of sodium bromide for 5 min in a stainless steel tube in a Wig-L-Bug. The resulting powder was pressed under vacuum into a pellet about 0.04 in. thick.

Potassium, Barium and Lead Salts

Potassium salts of the C_4, C_{10}, C_{11} and C_{19} saturated acids were prepared by neutralization with potassium hydroxide, as above. Potassium tridecanoate was also

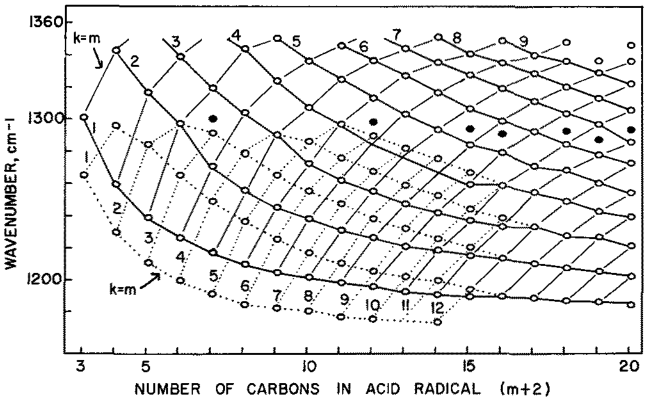

Fig. 3. Distribution patterns above 1170 cm^{-1} for methylene vibration peaks in saturated silver salt spectra.

O———O methylene wagging
O......O methylene twisting-rocking
● see text

made by grinding and pelleting the silver salt with potassium chloride.

Barium salts of the C_4, C_{10}, C_{11} and C_{19} saturated acids were prepared according to the method of Meiklejohn et al. (14). Lead salts of the C_4, C_6, C_{10-13} and C_{19} acids were prepared according to the AOCS method (2). The soluble (short chain) lead salts were isolated by vacuum drying the ethanol solution and washing the solids with acetone.

Infrared Spectra

The dry salts were smeared directly on a window, or, in an area of low humidity they were ground in a mortar with hexane and the resulting paste spread on a window and dried in air. The salt films were relatively thick, and coarse enough to cause loss of energy by scattering in the short wavelength region of the spectrum. The window materials used were NaCl, KBr and AgCl, the more active short chain silver salts being spread on AgCl. The spectra of these films and of the pellets (see *Sodium* and *Potassium Salts*) were recorded on a Perkin-Elmer 112 Infrared Spectrometer with NaCl prism calibrated against gases, from 1622 to 667 cm^{-1}, and on a Perkin-Elmer 137 (Infracord) from 4000 to 670 cm^{-1}.

All spectra were checked for purity of the salts, thus eliminating any contributions or interference from the reagents etc., e.g. unsaponified acid (1710 cm^{-1}), unreacted silver ammonium nitrate (830 cm^{-1}), by-product sodium carbonate (885 cm^{-1}), reaction of the salt with the window (Na and K salt 1560 cm^{-1}, Ag salt 1515 cm^{-1}), hydrate formation (3400 and 1650 cm^{-1}), etc. Infracord spectra were quite satisfactory for comparing the salts in the low wavenumber region, but the 112 spectrometer was preferable for the high wavenumber region, and for measuring all the peak positions.

Results

Figures 1 and 6 reproduce the spectra of three saturated and three unsaturated salts, respectively, for the region 1400–680 cm^{-1}. Methylene vibration peaks are numbered and were identified by comparison with hydrocarbon data (19,23). These data include distribution patterns (peak position versus the number of carbons in each chain), and phase relationship curves (peak position versus the phase relationship ϕ/π in all chains) for the methylene wagging, twisting and rocking vibrations. The spectra, regions 1070–710 cm^{-1} and 1380–1170 cm^{-1} are discussed separately.

Figures 2 and 3 show the distribution patterns for the methylene vibrations in saturated silver salts. Figures 7A and 7B are the same for the *cis*-unsaturated sodium salts. The assigned phase relationships are listed in the following tables of peak position: Table II, saturated silver salts; Table III, various caprates; Table IV, some saturated lead salts; Table V, *cis*-unsaturated silver and sodium salts; and Table VIa,b, silver and sodium elaidate. Figures 4 and 5 are the phase relationship curves for the saturated silver salts, and Figures 8, 9 and 11 the curves for the *cis*- and *trans*-unsaturated salts.

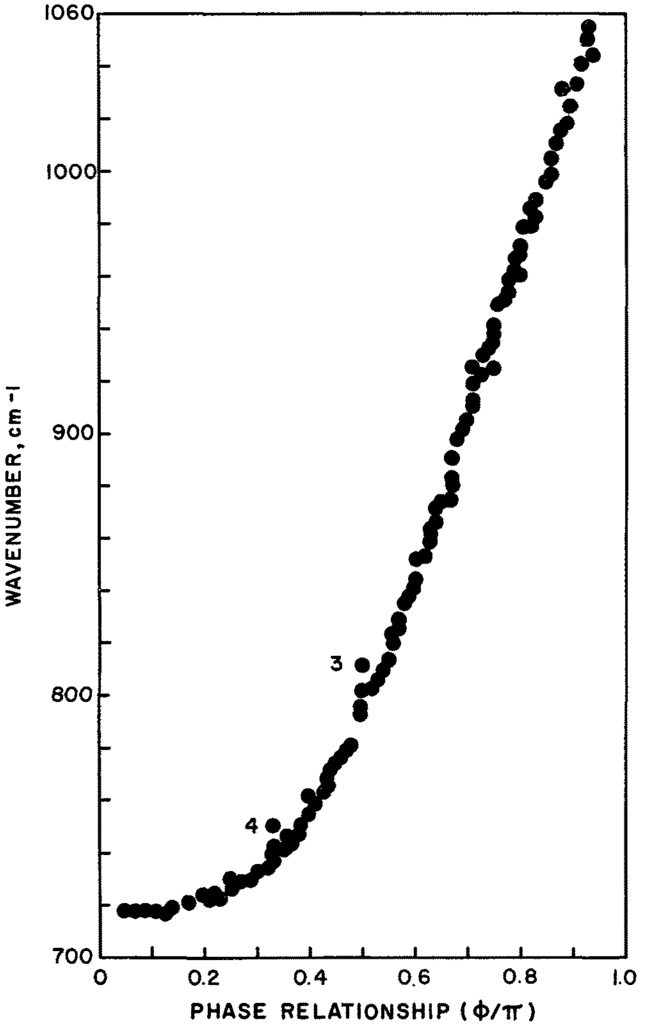

Fig. 4. Phase relationship curve above 710 cm^{-1}, for methylene twisting-rocking vibrations in saturated silver salts, C_3 through C_{22}, except C_{21}. Numbered points belong to propionate and butyrate.

It appears quite practical to determine the length of fatty acid chains by counting the number of, or measuring the position of methylene peaks in the 1070–710 cm^{-1} and/or the 1380–1170 cm^{-1} regions of their salt spectra. Both the number of peaks and their position depend on the presence or absence of a double bond, and on the configuration (*cis* or *trans*) around the double bond, but are relatively independent of the cation. The two regions separately indicate the total chain length for all the saturated salts, and separately indicate the length of the carboxylate segment for the *cis*-unsaturated salts. The 1070–710 cm^{-1} region gives the total chain length, and the 1380–1170 cm^{-1} region the carboxylate segment length for the *trans*-unsaturated salt.

The intensity of the different methylene vibration peaks depends on the cation. While the 1380–1170

TABLE II

Absorption Peaks in the Spectra of Saturated Silver Salts

Phase relationship		1380–1170 cm^{-1}		1070–710 cm^{-1}
k	ϕ/π	Methylene wagging cm^{-1}	Methylene twisting-rocking cm^{-1}	Methylene twisting-rocking cm^{-1}
Acetate ($m = 0$)				
		1342[c]		930[b]
				1034[c]
				1053[c]
Propionate ($m = 1$)				
1	0.50	1301	1264	812
		1373[c]		881[c]
				1015[c]
Butyrate ($m = 2$)				
1	0.33	1259	1296	751
				762[c]
2	0.67	1343	1229	875
				897[c]
				949[c]
				1040[c]
Valerate ($m = 3$)				
1	0.25	1238		730
2	0.50	1317	1284[e]	802
3	0.75	1361[e]	1210[e]	925
Caproate ($m = 4$)				
1	0.20	1226		724
2	0.40	1297[e]	1297[e]	762
3	0.60	1340[e]	1266	841
				890[b]
4	0.80		1199	961
				1008[c]
				1049[c]
Enanthate ($m = 5$)				
1	0.17	1217		721
2	0.33	1270		743
		1301[c]		
3	0.50	1320	1290[e]	796
4	0.67	1353	1248	881
				893[b]
				933[b]
				975[c]
5	0.83	1374[d,e]	1191	985
				1006[c]
				1036[c]
				1052[c]
Caprylate ($m = 6$)				
1	0.14	1209		719
2	0.29	1255		732
3	0.43	1303		768
4	0.57	1339[e]	1278	826
				889[b]
5	0.71	1372[d,e]	1236	911
				933[b]
6	0.86		1185	999[d]
				1027[c]
				1043[c]
				1064[c]
Pelargonate ($m = 7$)				
1	0.13	1205		717
2	0.25	1245		726
3	0.38	1290[e]		751
4	0.50	1323	1290[e]	795
5	0.63	1350	1265	859
				891[b]
6	0.75	1373[d,e]	1225	935[a]
				988[c]
7	0.88		1183	
				1016[c]
				1037[c]
				1059[c]
				1066[c]
Caprate ($m = 8$)				
1	0.11	1201		718
2	0.22	1238		725
3	0.33	1272		740
4	0.44	1309		772
5	0.56	1337[e]	1285	819
6	0.67	1368[e]	1255	883
				892[b]
				933[b]
7	0.78		1217	954
				1003[c]
8	0.89		1179	1019[d]
				1029[c]
				1056[c]
Hendecanoate ($m = 9$)				
1	0.10	1198		718
2	0.20	1231		
3	0.30	1262		733
4	0.40	1297[e]		756
5	0.50	1325	1297[e]	794
6	0.60	1346	1275	844
				891[b]
7	0.70	1361[e]	1246	905
				933[b]
8	0.80	1374[d,e]	1210	968
				1001
				1020
9	0.90		1177	1025[e]
Laurate ($m = 10$)				
1	0.09	1196		718
2	0.18	1226		
3	0.27	1255		729
4	0.36			746
		1298[c]		
Laurate (m =10) cont.				
5	0.45	1313		774
6	0.55	1337	1289	814
7	0.64	1353	1267	866
				891[b]
8	0.73	1372[d,e]	1238	923
				933[b]
9	0.82		1205	979
				996[c]
				1016[c]
10	0.91		1175	1033[d]
				1046[c]
				1059[c]
Tridecanoate ($m = 11$)				
1	0.08	1193		717
2	0.17	1221		
3	0.25	1247		726
4	0.33			738
5	0.42	1302		760
6	0.50	1327		793
7	0.58	1344	1280	835
8	0.67	1360[e]	1258	891[a]
				931[b]
9	0.75	1374[d,e]	1231	938
10	0.83		1201	989
				1030[c]
11	0.92			1040
				1055[c]
Myristate ($m = 12$)				
1	0.08	1191		717
2	0.15	1218		
3	0.23	1242		723
4	0.31			733
5	0.38	1293		750
6	0.46	1316		776
7	0.54	1335		810
8	0.62	1351	1275	853
				891[b]
9	0.69	1371[d,e]	1252	902
				932[b]
10	0.77		1225	951
				987[c]
11	0.85		1199	997
				1005[c]
				1023[c]
				1037[c]
				1052[c]
12	0.92		1173	
Pentadecanoate ($m = 13$)				
1	0.07	1189[e]		717
2	0.14	1215		
3	0.21	1237		722
4	0.29	1260[e]		730
5	0.36	1284		743
		1294[c]		
6	0.43	1306		764
7	0.50	1327		794
8	0.57	1341[e]		829[e]
9	0.64	1362[e]	1267	872
				892[b]
10	0.71	1374[d,e]	1244	916[e]
				934[b]
11	0.79		1220	962
12	0.86		1194[e]	1005
13	0.93			1050
Palmitate ($m = 14$)				
1	0.07	1189		717
2	0.13	1213[e]		
3	0.20	1233		
4	0.27	1259[e]		
5	0.33	1279		737
		1291[c]		
6	0.40	1299		754
7	0.47	1319		778
8	0.53	1335		807
9	0.60	1349		845
10	0.67		1259	889[a]
11	0.73	1374[d,e]	1238	930[a]
12	0.80		1213[e]	972
13	0.87		1189	1011
				1025[c]
				1042[c]
14	0.93			1058[e]
				1064[c]
Margarate ($m = 15$)				
1	0.06	1189		717
2	0.13	1210		
3	0.19	1233[e]		
4	0.25	1254		
5	0.31	1271		733
6	0.38	1292		747
7	0.44	1310		766
8	0.50	1328		793
9	0.56	1340[e]		824
10	0.63	1354[e]		862
				892[b]
11	0.69	1374[d,e]		901
				932[b]
12	0.75		1233[e]	941
13	0.81			979
				993[c]
14	0.88			1016[e]
Mangarate ($m = 15$) cont.				
				1022[c]
				1044[c]
				1069[c]
15	0.94			
Stearate ($m = 16$)				
1	0.06	1187		717
2	0.12	1207		
3	0.18	1227		
4	0.24	1248		
5	0.29	1267		729
6	0.35	1284		741
		1292[c]		
7	0.41	1303		759
8	0.47	1319		779
9	0.53	1336		806
10	0.59	1348		838
11	0.65			874
		1362[c]		891[b]
12	0.71	1373[d,e]		913
				933[b]
13	0.76			950
14	0.82			986
				1031[c]
				1050[c]
15	0.88			
16	0.94			1061[e]
Nonadecanoate ($m = 17$)				
1	0.06	1186		717
2	0.11	1205		
3	0.17	1224		
4	0.22	1242		
5	0.28	1260		728
6	0.33	1277		737
		1287[c]		
7	0.39	1296		750
8	0.44	1312		768
9	0.50	1328		793
10	0.56	1340[e]		821
11	0.61	1351		853
12	0.67			891[a]
13	0.72	1373[d,e]		923
				932[b]
14	0.78			959
15	0.83			993
				999[c]
16	0.89			1023[e]
				1035[c]
				1046[c]
17	0.94			
Arachidate ($m = 18$)				
1	0.05	1185		718
2	0.11	1202		
3	0.16	1221		
4	0.21	1239		
5	0.26	1254		727
6	0.32	1272		734
7	0.37	1285		744
		1293[c]		
8	0.42	1305		759
9	0.47	1321		780
10	0.53	1335		805
11	0.58	1346		833
12	0.63			864
				891[b]
13	0.68			898
14	0.74	1372[d,e]		932[a]
15	0.79			966
				986[c]
16	0.84			998[e]
				1009[c]
17	0.89			1025[e]
				1046[c]
				1057[c]
18	0.95			1062[e]
Behenate ($m = 20$)				
1	0.05	1184		718
2	0.10	1199		
3	0.14	1216		
4	0.19	1232		
5	0.24	1248		
6	0.29	1263		
7	0.33	1278		737
8	0.38	1286		747
		1296[c]		
9	0.43	1308		762
10	0.48	1322		781
11	0.52	1332		803
12	0.57			830
13	0.62			857
14	0.67			891[a]
15	0.71	1371[d,e]		919
				933[b]
16	0.76			949
17	0.81			978
				991[c]
18	0.86			1006[e]
				1023[c]
				1054[c]
				1062[c]
19	0.90			
20	0.95			

[a] Also assigned to end group peak. [b] End group peak only. [c] Not assigned to methylene vibration. [d] Tentative assignment. [e] cm^{-1} less accurate.

cm^{-1} region indicates the chain length twice for the silver or barium salts, the same region gives the chain length only once for the sodium, potassium or lead salts. Most of the peaks in this region are assigned to methylene wagging vibrations (1380–1180 cm^{-1}), or methylene twisting-rocking vibrations (1300–1170 cm^{-1}). It is the peaks for these latter vibrations that are too weak to be useful with the sodium, potassium and lead salts. Peaks in the 1070–710 cm^{-1} region are assigned to methylene twisting-rocking vibrations. These are easy to identify because of their alternating intensity, and are the most valuable for analytical purposes, providing they do not split as with lead salts.

Discussion

1070-710 cm^{-1} Region

Saturated. Figure 1 and Table II show that the number of peaks in this region of the silver salt spectra increases with the number of methylenes in the chain. The peaks also alternate in intensity, strong and weak, and have an over-all gradient from strongest about 720 cm^{-1} to weakest at the high wavenumber end. Thus the 720 cm^{-1} peak represents an in-phase vibration. Since we have assigned $k = 1$ to the in-phase vibration, this peak is identified by this number in the illustrations. For short chain salts the remaining peaks are identified by consecutive numbers (k values).

Figure 2 is the graph of peak position versus the number of carbons in each silver salt. Peaks of comparable relative intensity in chains of increasing length are joined in one direction, i.e. strong peaks with $k = 1$ and weaker peaks with $k = 2$, etc. Peaks with $k = m$ or $k = m-1$, etc. are joined in the other direction. This gives a distribution pattern with converging lines at the low wavenumber end. While no difficulty is experienced in assigning k values to short chain compounds which have one peak per methylene group, the pattern assists in assignments for the longer chain spectra, especially near the low wavenumber end.

This increase in the number of peaks with chain length and the general shape of the distribution pattern corresponds closely to hydrocarbon behaviour. The actual number of peaks and their wavenumber values, however, are not exactly the same for symmetry reasons, etc. Forbidden peaks in the hydrocarbons are the weaker ones in the salt spectra. Silver, sodium, potassium and barium salts all give similar distribution patterns, so that it is reasonable to assume that hydrocarbon assignments may be used for salt spectra. In hydrocarbons, peaks near 721 cm^{-1} are assigned to almost pure in-phase rocking, and peaks above this are due to coupled twisting and rocking, with almost pure out-of-phase twisting near the high end of the range (19,23).

Two peaks consistently appear about 890 and 930 cm^{-1} in the silver salt distribution pattern. These are marked by solid lines and solid spheres in Figures 1 and 2, respectively. They are included in our distribution pattern for the methylene vibrations when the overlapping peaks would not be resolved by our instrument. The peak at 891 cm^{-1} occurs in salts with different cations, and is undoubtedly a methyl rocking vibration (13,19,23).

The second peak, at 933 cm^{-1} in the silver salt spectra, is the weaker of the two. Table III shows that the position depends on the cation, and Figure 6 shows that it is the stronger of the two for salts other than silver. It must be produced by a vibration involving the carboxylate group, $CO_2(-)$, and may be associated with the cation sensitive peaks observed about 700, 1300 and 1430 cm^{-1}. In acids, peaks at these four positions are assigned to various vibrations of the carboxyl group, COOH, (3,10,25). Further study is required for their complete assignment in the salts. In most spectra all four peaks are least obvious with the silver salts.

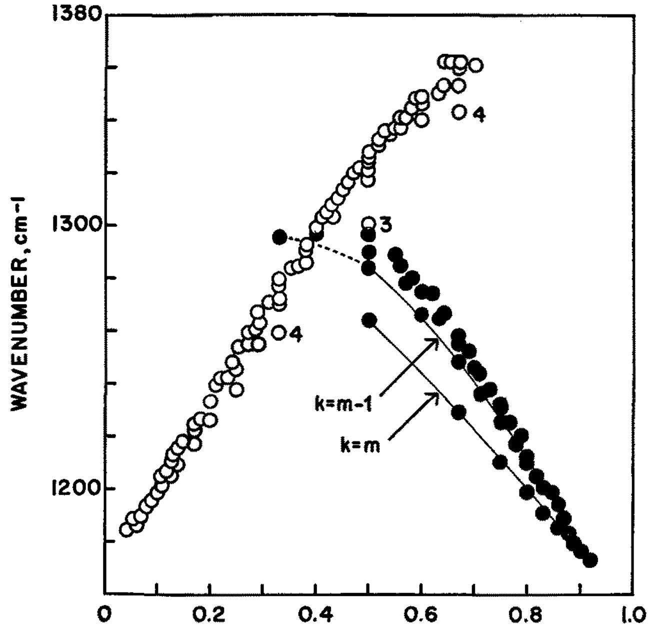

Fig. 5. Phase relationship curve above 1170 cm^{-1}, for methylene wagging (○) and twisting-rocking (●) vibrations in saturated silver salts, C_3 through C_{22}, except C_{21}. Numbered points belong to propionate and butyrate.

Table II lists the assignments for the silver salts. Methylene vibrations are identified by their intensity and their ability to fit a smooth extension of the phase relationship curve. Values of the phase relationship ϕ/π are calculated from known values of m and assigned values of k. Figure 4 shows the methylene phase relationship curve which is quite smooth, with

TABLE III
Assigned Absorption Peaks in the Spectra of Metallic Caprates (C_{10}; $m = 8$)

Phase relationship		Methylene peaks, cm^{-1}				
k	Φ/π	Ag	Ba	K	Na	Pb
Wagging 1380–1170 cm^{-1}						
1	0.11	1201	1201	1201	1200	1201
2	0.22	1238	1238	1239	1237	1236
3	0.33	1272	1274	1275	1275	1270
4	0.44	1309	1311	1313	1311	1303
5	0.56	1337	1336	1339	1340	1335
6	0.67	1368				1357
7	0.78					
8	0.89					
Twisting-rocking 1380–1170 cm^{-1}						
1	0.11					
2	0.22					
3	0.33					
4	0.44					
5	0.56	1285	1284			1282
6	0.67	1255	1255		1257	1253
7	0.78	1217	1216		1216	1217
8	0.89	1179	1179			
Twisting-rocking 1070–710 cm^{-1}						
1	0.11	718	719	721	721	719
2	0.22	725				
3	0.33	740	740	738	738	739
4	0.44	772	774	775	773	776
5	0.56	819	823	825	824	828
6	0.67	883	886	888	888	889
		892[b]	892[b]			
		933[b]	932[b]	925[b]	925[b]	931[b]
7	0.78	954	955	954	954	955
8	0.89	1019			1015	

[b] End group peak only.

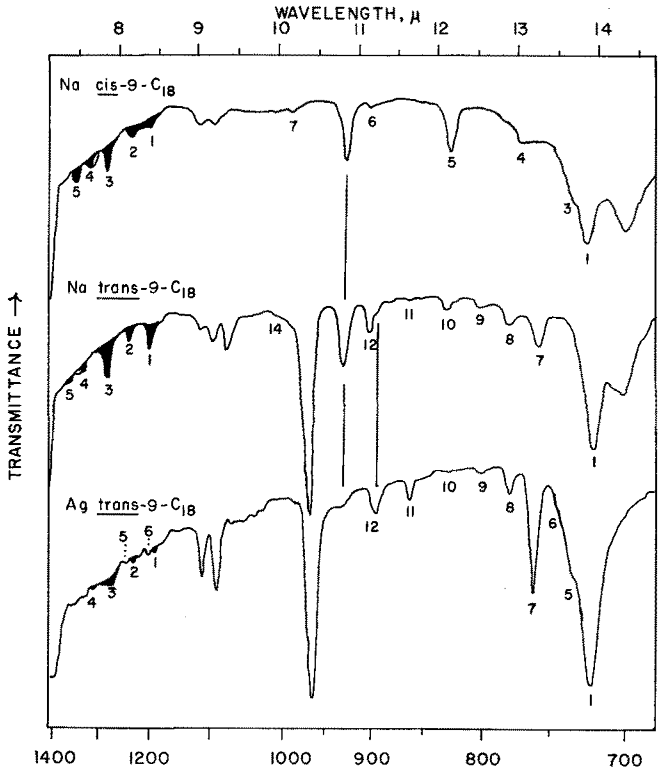

FIG. 6. IR absorption spectra of sodium and silver salts of *cis-* and *trans*-9-C_{18} acids, from 1400–680 cm^{-1}. Peaks assigned to methylene vibrations are numbered: black peaks—wagging; peaks with dotted lines—high frequency twisting-rocking; the remainder—low frequency twisting-rocking. Peaks joined by solid lines are assigned to end group vibrations.

only the points for short chains off the curve. Weak peaks of reasonable but uncertain position are included in the curve. From 980 cm^{-1} up, assignments are more difficult because of overlapping C-C stretching peaks.

Silver, sodium, potassium, barium and lead salt spectra give practically identical phase relationship curves. Thus the number of methylenes in any salt of unknown chain length may be determined from the distribution pattern for several reference salts, or from the phase relationship curve for a single reference salt. Table III lists the peak positions for the different caprates. Since wavenumber values for individual assignments vary slightly, it is preferable to use standards as close to the unknown as possible.

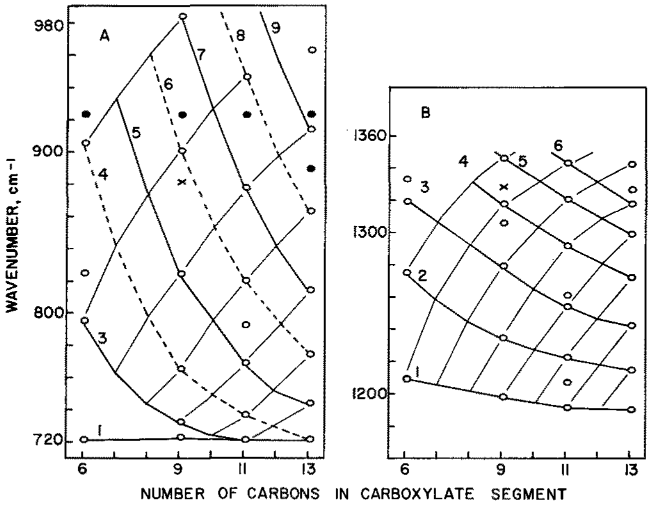

FIG. 7. Distribution patterns for methylene vibrations in the carboxylate segment of *cis*-unsaturated sodium salts.

○——○ stronger peaks
○- - -○ weaker peaks
● end group peak
× peak in C_{16} and not in C_{18}

A. Twisting-rocking above 710 cm^{-1}.
B. Wagging above 1180 cm^{-1}.

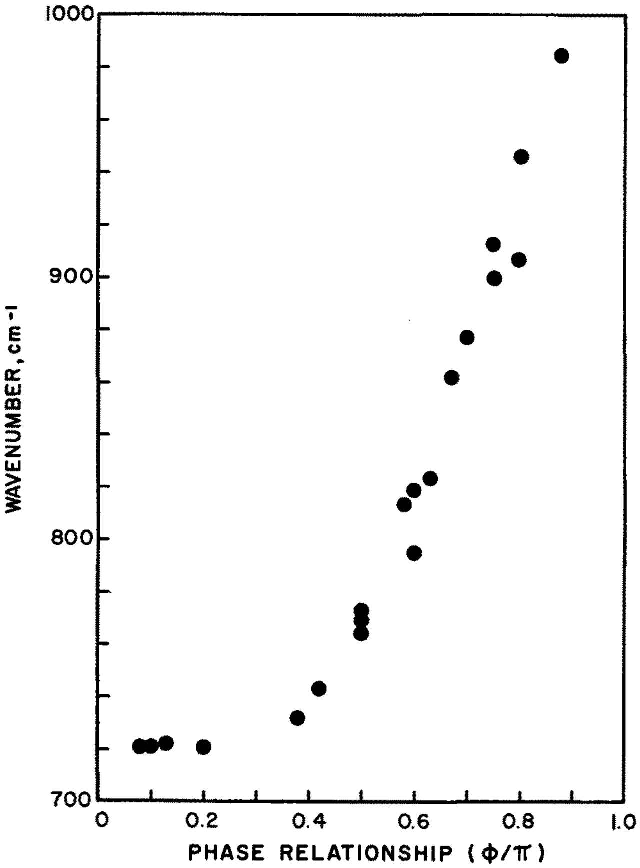

FIG. 8. Phase relationship curve above 710 cm^{-1}, for methylene twisting-rocking vibrations in the carboxylate segment of *cis*-unsaturated sodium salts, including *cis*-6-C_{18}; *cis*-9-C_{16}; *cis*-9-C_{18}; *cis*-11-C_{20}; *cis*-13-C_{22}.

The distribution pattern for the lead salts is not immediately apparent from a graph of peak position versus the number of carbons. Bands at the ends of the pattern split and overlap, giving more peaks than the salts of other carbons and changing relative intensities. Splitting has also been observed with some hydrocarbons (21). Table IV lists the assignments of *k* for the lead salts. They are chosen to give the best fit on the phase relationship curve found for the salts of other cations. In the table, bracketed peaks are assigned to the second component of the split. They are chosen by their intensity or position.

Unsaturated, Cis. Comparison of Figures 1 and 6, and Tables II and V show that there are fewer peaks relative to the total chain length in the unsaturated than in the saturated salt spectra. However, both types have an intensity gradient and an alternation of peak intensity in this region. The peaks in oleate (*cis*-9-C_{18}) and in palmitoleate (*cis*-9-C_{16}) spectra have the same positions, thus they are produced by the methylenes in the carboxylate segment. The *cis* double bond must disrupt the vibrations of the fatty radical as a unit, and peaks for methylene vibrations beyond the unsaturation are weaker than those adjacent to the carboxylate group.

The unsaturated salt peak positions, relative intensity and separation do not exactly match those of any saturated salt. The limited number of salts exam-

TABLE IV
Absorption Peaks in 1070–710 cm^{-1} Region of the Spectra of Saturated Lead Salts

Phase relationship		Methylene twisting-rocking^{d} cm^{-1}
k	ϕ/π	
Butyrate (m=2)		
		713^{c}
1	0.33	757 (793)
		845^{c}
		872^{c}
2	0.67	881
		895^{c}
		932^{c}
		949^{c}
		1018^{c}
		1050^{c}
Caprate (m=8)		
1	0.11	719 (731)
2	0.22	
3	0.33	739 (747)
4	0.44	776
5	0.56	828
6	0.67	889^{a}
		931^{b}
7	0.78	955
		1002^{c}
8	0.89	
		1058^{c}
Hendecanoate (m=9)		
1	0.10	719 (732)
2	0.20	
3	0.30	732 (742)
4	0.40	757 (762)
5	0.50	800
6	0.60	852
		892^{b}
Hendecanoate (m = 9) cont.		
7	0.70	907 (911)
		931^{b}
8	0.80	968
		983^{c}
		1001^{c}
		1022^{c}
9	0.90	
		1045^{c}
		1054^{c}
Nonadecanoate (m=17)		
1	0.06	719 (731)
2	0.11	
3	0.17	
4	0.22	
5	0.28	731
6	0.33	737
7	0.39	751 (756)
8	0.44	772
9	0.50	797
10	0.56	826
11	0.61	858
12	0.67	891^{a}
13	0.72	924
		930^{b}
14	0.78	
15	0.83	991
		1000^{c}
16	0.89	
		1036^{c}
17	0.94	

[a] Also assigned to end group peak.
[b] End group peak only.
[c] Not assigned to methylene vibration.
[d] Bracketed peaks are the second component of the split.

TABLE V
Absorption Peaks in the Spectra of *cis*-Unsaturated Silver and Sodium Salts

Phase relationship		1380–1170 cm^{-1}				1070–710 cm^{-1}	
k	ϕ/π	Methylene wagging cm^{-1}		Methylene twisting-rocking cm^{-1}		Methylene twisting-rocking cm^{-1}	
		Ag	Na	Ag	Na	Ag	Na
Petroselenate (*cis*-6-C_{18}; m=4)							
1	0.20	1211	1209			722	721
		1226^{c}					
2	0.40	1276	1275	1290		734	
						743^{c}	
3	0.60	1315	1318	1243		789	795
		1330^{c}	1333^{c}			817^{c}	825^{c}
						887^{b}	
4	0.80					922	907
						931^{b}	923^{b}
						991^{e}	
						1011^{c}	
						1024^{e}	
						1036^{e}	
Palmitoleate (*cis*-9-C_{16}; m=7)							
1	0.13	1198	1197			719	722
2	0.25	1234	1234				
		1271^{c}					
3	0.38	1277	1278			733	732
		1301^{c}	1305^{c}				
4	0.50	1313	1317	1283		763	764
		1327^{c}				781^{c}	
5	0.63	1345	1346	1252		823	824
						884^{e}	881^{c}
6	0.75						900
						931^{b}	923^{b}
7	0.88						985
							1042^{c}
Oleate (*cis*-9-C_{18}; m=7)							
1	0.13	1197	1197			719	722
2	0.25	1235	1234				
3	0.38	1276	1278			733	732
			1305^{c}				
4	0.50	1313	1317	1283		763	765
						783$^{c, e}$	
5	0.63	1345	1346	1252		823	823
6	0.75			1209			899
						931^{b}	923^{b}
7	0.88						984
11-Eicosenoate (*cis*-11-C_{20}; m=9)							
1	0.10	1194	1191			717	721
2	0.20	1222	1222				
3	0.30	1252	1254				
			1261^{c}				
4	0.40	1288	1292			740	736
5	0.50	1315	1320			768	769
							792^{c}
6	0.60	1339	1342	1264			819
7	0.70			1233		873	877
						931^{b}	923^{b}
8	0.80				1207	962^{d}	946
							983^{c}
							1005^{c}
9	0.90						1028^{d}
							1053^{c}
							1067^{c}
Erucate (*cis*-13-C_{22}; m=11)							
1	0.08	1190	1190			718	721
2	0.17	1218	1214				
3	0.25	1243	1241				
4	0.33	1274	1271				
5	0.42	1295	1298			745	743
6	0.50	1317	1317			770	773
			1326^{c}				
7	0.58	1339	1343			813	813
8	0.67						862
							889^{b}
9	0.75						913
						931^{b}	923^{b}
						962^{f}	964^{f}
10	0.83						
11	0.92						
							1020^{c}
							1060^{c}

[b] End group peak only.
[c] Not assigned to methylene vibration.
[d] Tentative assignment.
[e] cm^{-1} less accurate.
[f] *Trans* peak.

ined also makes drawing of a distribution pattern uncertain. Assignments of k are chosen to give a phase relationship curve with the same shape as that of the saturated salts. Peaks observed with both sodium and silver salts are included, and the number of methylenes in the carboxylate segment is used for m.

Figures 8 and 7A are the phase relationship curve and the resulting distribution pattern, respectively, for the unsaturated sodium salts. The curve is shifted on both the wavenumber and phase scales as compared to the saturated salt curve, but it is assumed that these peaks also represent coupled methylene twisting-rocking vibrations. Silver salts give similar results, although some of the weaker peaks were not detected.

The unsaturated salt spectra have end group peaks near 890 and 930 cm^{-1}, and their behaviour with different cations parallels that of the saturated salts. In spite of duplication in carboxylate segment methylene peaks, oleate and palmitoleate spectra may be distinguished by the presence or absence of unassigned weak peaks. These may represent vibrations of the methyl group segment.

Unsaturated, Trans. The *trans* salt spectra in Figure 6 have many more peaks in this region than the *cis* salt spectra. See also Tables V and VIa. The *trans* salt peaks do have an intensity gradient, but do not alternate in intensity as consistently as the saturated and *cis*-unsaturated salt peaks. The positions of the *trans* salt peaks are also different from the others. However, their spacing suggests that a correlation with methylene vibrations may be obtained. The two strongest elaidate peaks are at 717 and 755 cm^{-1}. Only spectra for the C_9, C_{14}, C_{18} and C_{19} saturated salts have strong peaks near these.

The elaidate salt spectra show many more peaks than the saturated C_9 salt spectra. It is unlikely that the extra peaks are due to splitting, since this has not been reported for sodium soaps. Thus the elaidate peaks do not appear to indicate the length of the carboxylate or methyl group segments, both of which have nine carbons. The C_{14} pattern matches only the two strongest elaidate peak positions and not the weaker ones, so no correlation is obtained with this length.

The position of all the elaidate peaks, both strong and weak, is bracketed by peaks of corresponding strength in the saturated C_{18} and C_{19} salt spectra. For example, the k = 7 peak (strong) occurs at 758 and 751 cm^{-1} in sodium stearate and nonadecanoate, respectively, while sodium elaidate shows a strong peak at 755 cm^{-1}. Similarly, the k = 8 peak (weaker) occurs at 781 and 768 cm^{-1} in sodium stearate and

TABLE VIa

Absorption Peaks in the 1070–710 cm^{-1} Region of the Spectra of Silver and Sodium Elaidate (*trans*-9-C_{18}; $m=16$)

Phase relationship		Methylene twisting-rocking cm^{-1}	
k	ϕ/π	Ag	Na
1	0.06	719	717
2	0.12		
3	0.18		
4	0.24		
5	0.29	731	
6	0.35	741	
7	0.41	757	755
8	0.47	775	776
9	0.53	798	799
10	0.59	824	827
11	0.65	859	859
		892[b]	
12	0.71	896	898
			926[b]
13	0.76	930[a, d]	
		962[f]	963[f]
14	0.82	968[d]	969[d]
		984[c]	995[c]
15	0.88		
16	0.94	1037	
		1051[c]	

[a] Also assigned to end group peak.
[b] End group peak only.
[c] Not assigned to methylene vibration.
[d] Tentative assignment.
[f] *Trans* peak.

nonadecanoate, respectively, while sodium elaidate displays a weaker peak at 776 cm^{-1}.

Thus, considering the position, relative intensity and separation of the peaks, it seems that this region gives the vibrations of methylenes in the entire length of the *trans*-unsaturated salt. The vibrations are probably of the twisting-rocking type. Apparently the slight distortion in the carbon skeleton does not prevent coupling of the vibrations of methylenes on opposite sides of the double bond. The hydrogens on the unsaturated carbons may also be involved. It is interesting to speculate that the irregularity in alternating intensities (for elaidate at $k = 9$) may be related to the position of the double bond in the chain.

Values of k are assigned to the elaidate peaks by comparison with saturated C_{18} and C_{19} spectra, and ϕ/π calculated on the assumption that m represents the total number of carbons minus two. Figure 9 is the phase relationship curve for sodium and silver elaidate. It is shifted very little from the saturated salt curve (Fig. 4). Like the saturated salts, silver elaidate shows more low wavenumber methylene peaks than the sodium elaidate. See also Table VIa, $k = 5,6$.

The elaidate end group peak behaviour (890 and 930 cm^{-1}) is similar to that of the saturated and *cis*-unsaturated salts. Although both saturated and *trans*-unsaturated spectra give peaks for the total chain length, there is never any possibility of confusing them since isolated *trans* bonds have a strong peak about 962 cm^{-1}. Hydration of sodium elaidate causes some shifting of the peaks, but more change in their relative intensity.

TABLE VIb

Absorption Peaks in the 1380–1170 cm^{-1} Region of the Spectra of Silver and Sodium Elaidate (*trans*-9-C_{18}; $m=7$)

Phase relationship		Methylene wagging cm^{-1}		Methylene twisting-rocking cm^{-1}
k	ϕ/π	Ag	Na	Ag
1	0.13	1196	1197	
		1225[c]		
2	0.25	1236	1235	
		1262[c]		
3	0.38	1278	1275	
		1301[c]		
4	0.50	1310	1313	
		1321[c]	1328[c]	
5	0.63	1336[d]	1340[d]	1253
6	0.75			1210
7	0.88			

[c] Not assigned to methylene vibration.
[d] Tentative assignment.

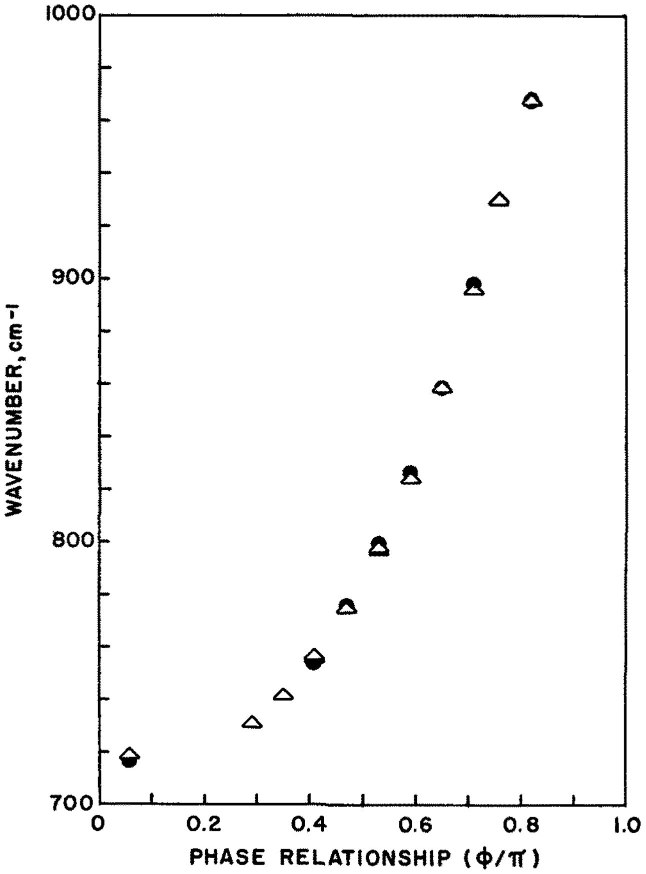

FIG. 9. Phase relationship curve above 710 cm^{-1}, for methylene twisting-rocking vibrations in the total length of the *trans*-unsaturated C_{18} salt.

△	silver salt
●	sodium salt

1380-1170 cm^{-1} Region

Saturated. Figure 1 shows that the number of peaks in this region is not constant, and that they are weaker than those in the 1070–710 cm^{-1} region. The relative intensity of peaks in the two regions varies with salts of different cations. Also the total number of peaks in the 1380–1170 cm^{-1} region depends on the cation, although there is still a correlation between the number of peaks and methylenes. It is assumed that this confusion is due to overlapping of the methylene wagging and high-frequency twisting-rocking vibration peaks as observed with the hydrocarbons (19,23).

In salts of corresponding length, certain peak positions shift very little with different cations. These peaks are assigned to methylene wagging vibrations. Table III lists their position for the sodium, silver, potassium, barium and lead salts of capric acid. Meiklejohn et al. also noted them in the spectra of sodium and barium salts (14).

Solid lines in Figure 3 mark the distribution pattern of the wagging vibrations of the silver salts. Parallelling hydrocarbon spectra, $k = 1$ indicates the low wavenumber peaks. Atmospheric water vapour peaks from our single beam equipment mask part of the wagging pattern from 1340 cm^{-1} up. A $k = 4$ wagging peak was not recognized for the $C_{12\text{-}14}$ silver salts, probably because of their crystal structure and the presence of impurities.

Certain other peaks in this region are seen only with some cations, and these peaks are assigned to coupled twisting-rocking vibrations of the methylenes. Silver and barium salt spectra, particularly, show

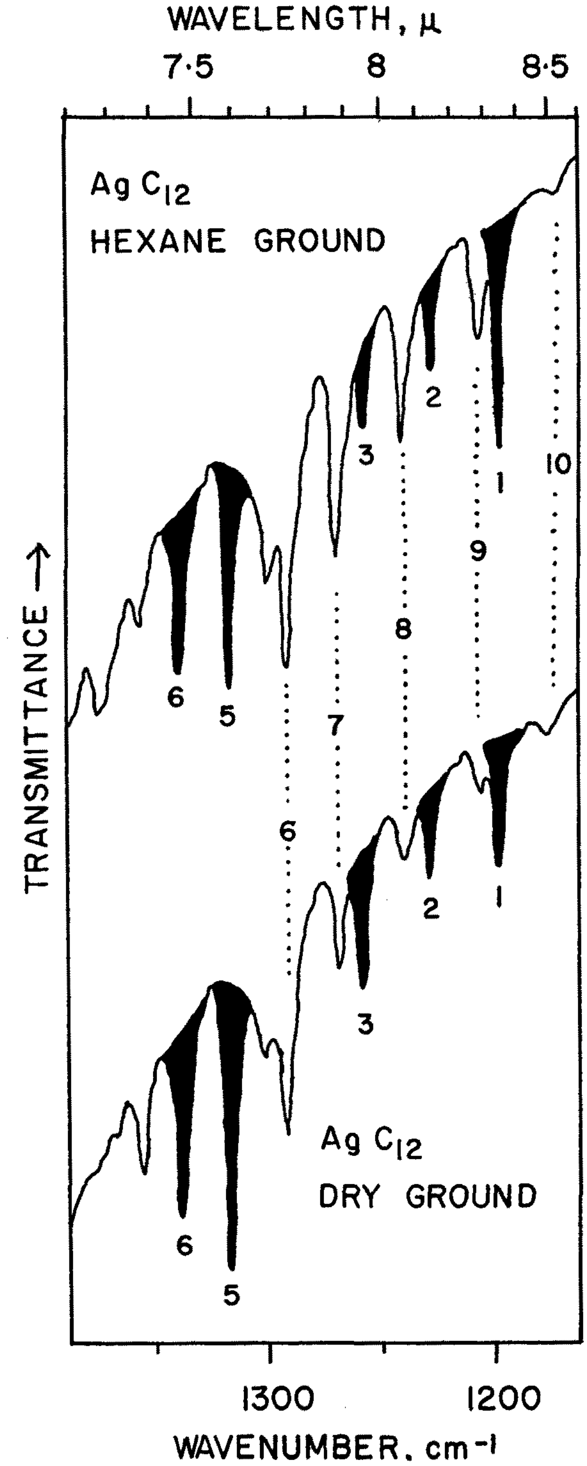

FIG. 10. IR absorption spectra of silver laurate after dry grinding and after grinding in hexane. Peaks assigned to methylene vibrations are numbered: black peaks—wagging; peaks joined by dotted lines—twisting-rocking.

these peaks. Dotted lines in Figure 3 mark their distribution in the silver salts. Peak positions which are uncertain because they are so weak are included in the pattern when they seem reasonable. The twisting-rocking peaks are not resolved from the wagging ones in the longer chain compounds, and they are less obvious with impure salts. Values of k are obtained by comparison with hydrocarbon results. In hydrocarbons, peaks at the high end (1295 cm^{-1}) represent almost pure in-phase twisting and those near the low end (1170 cm^{-1}) almost pure out-of-phase rocking.

The method of sample preparation affects the relative intensity of wagging and twisting-rocking peaks in this region of the silver salt spectra. See Figure 10. When silver laurate is ground dry, many of the peaks assigned to a methylene wagging vibration are stronger than the nearest peak assigned to a twisting-rocking vibration. When it is ground in hexane and dried, the relative intensity of some of these peaks changes, but not consistently with the assignment. Frequently, complete reversal is obtained, or adjacent peaks from the two patterns are of equal intensity and not resolved. The variations are not connected with chain length, but are assumed to indicate structural changes in the crystal. They were observed on silver salts of C_{10-13} and C_{15} acids, and not on sodium or barium caprate. Salts of other acids were not treated both ways.

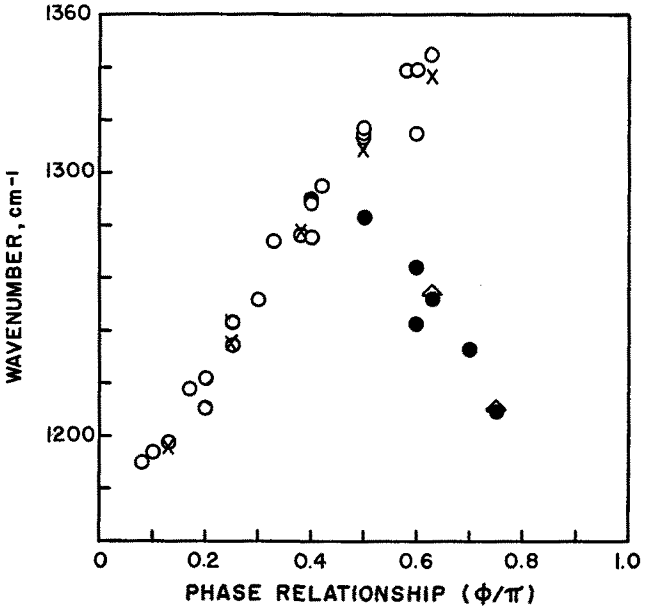

FIG. 11. Phase relationship curves above 1170 cm^{-1}, for methylene vibrations in the carboxylate segment of *cis*- and *trans*-unsaturated silver salts.

○ wagging, and ● twisting-rocking in *cis*-6-C_{18}; *cis*-9-C_{16}; *cis*-9-C_{18}; *cis*-11-C_{20}; *cis*-13-C_{22}.

× wagging, and △ twisting-rocking in *trans*-9-C_{18}.

Figure 5 shows the phase relationship curves for the silver salts. These parallel the results with hydrocarbons (23), and confirm the assignment of peaks in this region to the wagging and twisting-rocking distribution patterns. The wagging curve is quite neat except for short chain peaks. The complete curve, however, is not available because peak positions for the higher k values were not accurately measured on our single beam instrument.

The twisting-rocking curve has an interaction effect, possibly with the methyl group, and it is especially noticeable in the $k = m$ peaks. Also the $k = 1$ peak (1296 cm^{-1}) for the C_4 silver salt does not fit the curve too well, but comparison with salts of other cations indicates that the assignment is valid. Generally, the position of peaks with low values of k approaches a limit about 1300 cm^{-1}. Here the two curves meet and details are not very clear. However, salts of all cations have comparable phase relationship curves for methylene wagging vibrations, and those that give a twisting-rocking distribution pattern have comparable curves for these vibrations.

The confusion around 1300 cm^{-1} is not detailed in full. A few of the silver salt peaks close to 1300 cm^{-1} consist of two or more unresolved bands, although they are marked as single peaks. Solid spheres in Figure 3 represent peaks which are not included in either pattern. Some or all of this unassigned absorption may be due to methylene vibrations, or it may be carboxylate vibrations as discussed at the end of the 1070–710 cm^{-1} section on saturated salts.

Unsaturated, Cis. Unsaturated salts have fewer peaks in this region than saturated salts of the same total chain length. See Figures 1 and 6, and Tables II and V. The similarity of oleate and palmitoleate spectra suggests that the number and the position of the peaks is related to the length of the carboxylate segment. The double bond must prevent coupling of the methylene vibrations in the two segments. The wagging vibrations which are parallel to the chain naturally will be more sensitive to bending of the backbone than the rocking vibrations which are perpendicular. The twisting vibrations will also be affected.

Figure 7B outlines the distribution pattern for the methylene wagging vibrations in sodium salts of the *cis*-unsaturated acids. The silver salts have peaks at all the same positions, plus others whose positions and strengths are satisfactory for assignment to twisting-rocking vibrations. This is additional support for considering all of these peaks as caused by vibrations of the carboxylate methylenes. Some of the unassigned peaks may represent twisting-rocking vibrations of the carboxylate segment, or wagging vibrations of the methyl segment, but they are only weak peaks.

Assignments for the *cis*-6-C_{18} salts are the most uncertain, especially the peaks about 1320 and 1330 cm^{-1}. The former is the stronger in the sodium salt and the latter the stronger in the silver salt; thus the 1320 cm^{-1} peak is assigned to a wagging vibration although it does not fit the phase relationship curve so well as the other point. No assignment is made for the 1330 cm^{-1} peak.

Figure 11 shows the phase relationship curves for the *cis*-unsaturated silver salts. Values of m are calculated from the number of carbons in the carboxylate segment minus two, as in the low wavenumber region. Values of k are assigned according to the distribution pattern for saturated salts, since peak separations are the same for corresponding values of m. Table V lists the resultant values of ϕ/π. The phase relationship curves for the unsaturated salts agree quite well with those of the saturated salts, except for a shift of about 10 cm^{-1} on the wavenumber scale. Thus the saturated phase relationship curves may be used for the determination of unsaturated carboxylate segment chain lengths if the shift is taken into account.

Unsaturated, Trans. The *trans* salt spectrum in Figure 6 has the same number of peaks as the *cis* salt spectrum, so this region gives vibrations of the carboxylate segment methylenes. See also Tables V and VIb. In all respects in this region, elaidate spectra agree with those of oleate salts. Silver elaidate has peaks assigned to twisting-rocking and wagging vibrations, while the sodium salt has only the wagging peaks, etc. Figure 11 shows the close correspondence of the phase relationship curves for the silver salts.

ACKNOWLEDGMENTS

H. W. Lemon provided continued encouragement and assistance. This research was carried out under a grant to the Ontario Research Foundation from the Province of Ontario, received through the Department of Economics and Development.

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[Received September 18, 1964—Accepted November 24, 1964]

Glyceride Distribution in Adipose and Liver Glycerides of Animals[1]

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Abstract

The glyceride distribution in depot fats from a series of animals was determined by pancreatic lipase hydrolysis, isolation of hydrolytic products by thin-layer chromatography (TLC), and fatty acid analysis by gas-liquid chromatography (GLC).

Distribution of the principal types of glycerides (S_3, S_2U, SU_2 U_3) in the internal and external adipose tissue fats from the same pig was nonrandom. The percentages of palmitic acid at the 2-position in these adipose fats were comparable. However, liver glycerides from this same animal differed strikingly from adipose glycerides, having, for example, only ca. 15% of its palmitic acid in the 2-position compared with > 80% for adipose fats. The liver glycerides of lamb, rabbit, and dog also differed considerably from adipose glycerides in glyceride distribution and in percentages of individual fatty

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[1] Presented at the AOCS meeting, Chicago, October 11–14, 1964.
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