

[CONTRIBUTION FROM THE INSTITUTE OF EXPERIMENTAL BIOLOGY OF THE UNIVERSITY OF CALIFORNIA]

DISTRIBUTION RATIOS AND ASSOCIATION OF CARBOXYLIC ACIDS*

BY ALBERT E. SMITH AND JOHN W. NORTON

RECEIVED MARCH 28, 1932

PUBLISHED OCTOBER 5, 1932

Probably the best methods for quantitative analysis of mixtures of fatty acids are based on distribution ratios.¹ Distribution also offers a possible method for separating these acids from each other. In certain cases, however, the distribution ratio of a given acid varies greatly with concentration; a theory of this is given below, after listing the experimental results. An analogous theory explaining relative physical properties of certain substituted benzene derivatives has been given elsewhere.²

Experimental

Since different solvents will obviously give different results, it is evidently important to investigate solvents of various types. Solvent pairs studied were first 2,2,4-trimethylpentane ("*i*-octane") and methanol; second, 2,2,4-trimethylpentane and methyl cyanide (acetonitrile). A paper from this Laboratory on distribution ratios between certain other solvent pairs (each pair including water) is now in press.³ Methyl cyanide (acetonitrile) and *i*-octane were very pure samples prepared in this Laboratory, to be described in a later paper. Properties of the *i*-octane did not agree with Edgar's⁴ but checked exactly with later results of Edgar and Calingaert;⁵ 25 cc. of *i*-octane and one drop of 0.1 *N* sodium hydroxide gave a pink color with phenolphthalein; with the methyl cyanide the second drop of the sodium hydroxide gave pink. Like these solvents, the methanol was also distilled in a 6.09-meter fractionating column previously described.⁶ The methanol (measured in equilibrium with *i*-octane at 0°) contained 0.48 milliequivalent of acid per liter. Since the co-existing *i*-octane layer con-

* Aided by grants from the Committee for Research in Problems of Sex of the National Research Council, and from the Rockefeller Foundation. These funds have been generously augmented by the Board of Research and the College of Agriculture of the University of California.

¹ (a) W. U. Behrens, *Z. anal. Chem.*, 69, 97 (1926); (b) C. H. Werkman, *Ind. Eng. Chem., Anal. Ed.*, 2, 302 (1930); (c) O. L. Osburn and C. H. Werkman, *ibid.*, 3, 264 (1931); (d) C. H. Werkman and O. L. Osburn, *ibid.*, 3, 387 (1931).

² N. V. Sidgwick and Elinor K. Ewbank, *J. Chem. Soc. London*, 119, 979 (1921); N. V. Sidgwick and W. M. Aldous, *ibid.*, 119, 1001 (1921); N. V. Sidgwick and H. E. Rubie, *ibid.*, 119, 1013 (1921).

³ R. C. Archibald, *THIS JOURNAL*, 54, 3178 (1932).

⁴ G. Edgar, *Ind. Eng. Chem.*, 19, 145 (1927).

⁵ G. Edgar and G. Calingaert, *THIS JOURNAL*, 51, 1540 (1929).

⁶ Evans, Cornish, Lepkovsky, Archibald and Feskov, *Ind. Eng. Chem., Anal. Ed.*, 2, 339 (1930).

tained no appreciable acid, the acid was probably formic; this was corrected for by subtraction. The formic, acetic and butyric acids were commercial c. p. products (formic being Eastman "99 $\frac{1}{2}$ to 100%") which may contain some water). Caprylic, lauric and palmitic acids were very pure samples prepared in this Laboratory (to be described in another publication). The oleic acid and the 50% oleic-50% linoleic acid were less pure, but gave correct iodine values and titration values. All titrations in determining distribution ratio were with 25-cc. samples, using also phenolphthalein and 0.1 *N* sodium hydroxide. Both layers were titrated. With all but methyl cyanide layers difficulty was found owing to emulsification during titration; this was avoided by making up the 0.1 *N* sodium hydroxide in absolute ethyl alcohol instead of in water.⁷ Values in Tables I and II are means of duplicate titrations. Equilibrium between the layers at 0° was obtained by shaking flasks by hand, these flasks being immersed in ice and water; tests showed that the liquid in the flasks reached 0°. Checks between duplicate samples indicated sufficiency of this procedure for our purposes. The dicarboxylic acids stood for about a week in the solvent mixture at room temperature with occasional shaking. This mixture was filtered and cooled to 0° with shaking. A certain amount of solid acid appeared to separate at the interface, but this was avoided in pipetting out samples. However, the solubilities for dicarboxylic acids in Table II are

TABLE I
DISTRIBUTION RATIOS BETWEEN 2,2,4-TRIMETHYLPENTANE AND METHANOL AT 0°
Concentrations in millimoles per liter

Acid	Hydroc. layer	Methanol layer	Ratio	Av. ratio
Formic	5.05	163.3	0.0309	0.0280
Formic	12.71	508.4	.0250	
Acetic	6.75	144.21	.0468	.0457
Acetic	11.29	252.93	.0446	
Butyric (<i>n</i>)	6.91	81.20	.0840	.0878
Butyric (<i>n</i>)	18.98	206.33	.0916	
Caprylic (<i>n</i>)	6.86	38.01	.180	.190
Caprylic (<i>n</i>)	21.20	105.75	.201	
Lauric (<i>n</i>)	5.25	15.80	.332	.352
Lauric (<i>n</i>)	7.20	20.89	.345	
Lauric (<i>n</i>)	21.53	56.98	.378	
Palmitic (<i>n</i>)	9.91	16.03	.618	.649
Palmitic (<i>n</i>)	29.56	44.18	.669	
Oleic	9.26	15.12	.613	.629
Oleic	18.66	29.76	.627	
Oleic	26.79	41.41	.647	
50% Oleic	7.84	14.21	.552	.5545
50% Linoleic	20.02	35.91	.557	
Linoleic ("Vitamin F") calculated from above data				.487

⁷ See O. Folin and A. H. Wentworth, *J. Biol. Chem.*, 7, 421 (1910), who used sodium ethylate in absolute alcohol for similar purposes.

TABLE II

DISTRIBUTION BETWEEN 2,2,4-TRIMETHYLPENTANE AND METHYL CYANIDE AT 0°

Acid	Concentrations in millimoles per liter			
	Hydroc. layer (c_1)	Cyanide layer (c_2)	c_1/c_2	$\sqrt{c_1/c_2}$
Lauric	15.42	9.34	1.651	0.420
Lauric	45.51	16.57	2.75	.407
Lauric	86.36	22.80	3.79	.408
Oleic	12.73	4.04	3.15	.882
Oleic	65.23	8.21	7.94	.984
Maleic	None (not satd.)	22.9		
Fumaric	None (satd.)	9.1		
<i>o</i> -Phthalic	None (satd.)	17.7		
<i>m</i> -Phthalic	None (satd.)	4.4		
<i>p</i> -Phthalic	None (satd.)	2.5		

probably of value only in the absence of better data. The values of Tables I and II for oleic and linoleic acids (and in fact all data of this paper) were determined because of their relation to the purification of vitamin F,⁸ which has been claimed to be linoleic acid.⁹ For plotting, the simple averages of Table I were used although the values show definite and regular change with concentration. From average values of Table I for oleic and oleic + linoleic acids, one calculates the distribution ratio of linoleic acid between *i*-octane and methanol at 0° as 0.487.

Comparison with Results of Others.—The figure shows the results of the authors for *i*-octane and methanol with "saturated" acids. In the figure are also plotted distribution ratios for acids in isopropyl ether and water at 25°,^{1b,c,d} using weighted means from the three papers cited and neglecting their earlier value for butyric acid. An obvious typographical error by Osburn and Werkman is the interchanging of what they call "partition constants" for the two different proportions of ether and water. The distribution ratios chosen (for a mean concentration of both layers of about 0.05 *M*) are: formic 0.145, acetic 0.184, propionic 0.810, butyric 2.90. Values at 25° for ethyl ether and water are estimated for 0.001 *M* acid in the aqueous layer^{1a,10} (as the ratio changes somewhat with concentration) as: formic 0.30, acetic 0.44, propionic 1.34, butyric 4.6, valeric 17.5, caproic 75. For glycolic and lactic acids in ethyl ether and water at 25°¹¹ one may select round values: glycolic 0.03, lactic 0.08. The α -hydroxy acids apparently give a curve parallel to that for the unsubstituted acids. Values for acetone and glycerol¹² are insensitive to concentration changes;

⁸ H. M. Evans and G. O. Burr, *J. Biol. Chem.*, **74**, lxxii (1927); *Proc. Soc. Exptl. Biol. Med.*, **25**, 41, 390 (1928).

⁹ G. O. Burr and M. M. Burr, *J. Biol. Chem.*, **82**, 345 (1929); **86**, 587 (1930).

¹⁰ H. W. Smith, *J. Phys. Chem.*, **25**, 619 (1922).

¹¹ J. Pinnow, *Z. anal. Chem.*, **54**, 321 (1915); *Z. Untersuch. Nahrungs. Genussm.* **37**, 49 (1919).

¹² H. W. Smith, *J. Phys. Chem.*, **25**, 721 (1921).

one may select for 25°: formic 1.37, acetic 1.78, propionic 3.5, butyric 12.7. From the figure it is evident that for separation of fatty acids from each other, *i*-octane and methanol give by far the poorest separation of the solvent pairs shown.

Distribution Ratio of Stearic Acid between Ethyl Ether and Water; Analysis of Higher Fatty Acid Mixtures.—By extrapolating the curve for ethyl ether and water (assuming a straight line in the figure), one finds for

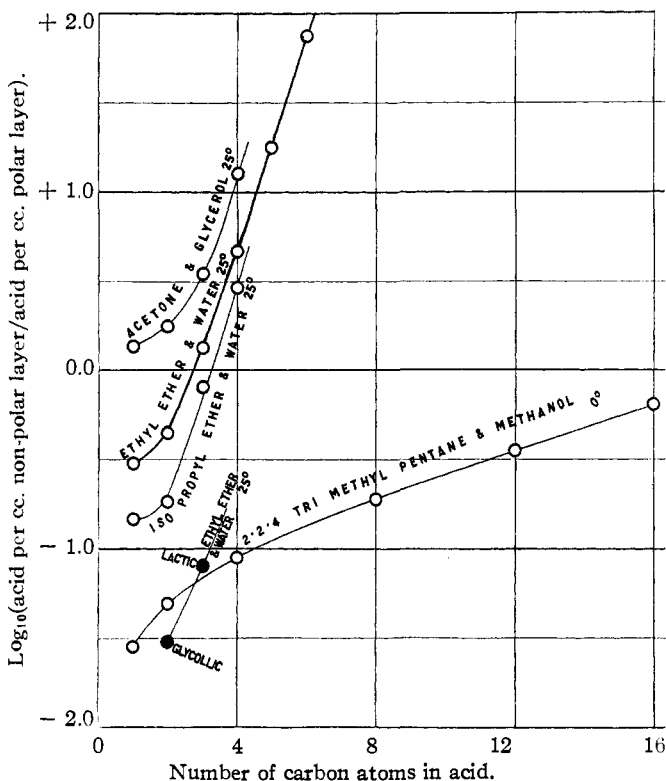


Fig. 1.—Distribution ratios between solvents of normal monocarboxylic fatty acids ($C_nH_{2n}O_2$). These ratios change somewhat with concentration of acid (see text of article). A curve is also shown for α -hydroxy acids.

stearic acid ($C_{18}H_{36}O_2$) a distribution ratio of about 10^9 at 25°; no direct measurement is available. Since the dissociation constants of paraffin fatty acids¹⁸ are around 1.5×10^{-5} it would seem that the analytical method of Behrens^{1a} could be extended to higher fatty acids by keeping the aqueous layer at a definite, fairly high alkalinity during the partition process.

¹⁸ Landolt-Börnstein, "Physikalisch-chemische Tabellen," Julius Springer, Berlin, 1923. 5th ed., p. 1127.

Owing to emulsification a centrifuge might be necessary. It should be possible to modify this method to cover dicarboxylic acids.

Self-Association of Monocarboxylic Acids.—Reference to Table I shows that with *i*-octane and methanol, the distribution ratio changes but slightly with concentration, indicating the acids to have single molecules in both layers. However, Table II shows that the *square root* of the acid concentration in the *i*-octane layer is proportional to the acid concentration in the methyl cyanide; this indicates that here the acid is "self-associated" as double molecules in this *i*-octane layer. By "self-association" is meant combination of carboxyl groups with each other in pairs; this self-association appears to be a strong tendency of the carboxyl group, but may be prevented by the presence of sufficient other molecules which also tend to associate with the carboxyl group. This second type of association (which thus differs from "self-association") *prevents* double acid molecules forming.

Thus self-association evidently is very slight in the methanol or methyl cyanide layers. *i*-Octane is a very non-polar solvent and has little tendency to prevent self-association of the acid, but in Table I a considerable amount of methanol must be dissolved in the co-existing *i*-octane layer. The experiments were at 0°, and one has the "critical solution temperatures" for *i*-octane with methanol at 42.5°. An experiment like Table I at, say, -50°, would undoubtedly show self-association in the *i*-octane layer, due to low solubility of methanol in *i*-octane at that temperature. Similarly, the critical solution temperature of *i*-octane and methyl cyanide is 81°; by conducting the experiments of Table II at, say, +50°, enough methyl cyanide would probably dissolve in the *i*-octane to prevent the self-association of the acid.

Again, when benzoic acid or an aliphatic fatty acid is distributed between water and petroleum ether, the acid is associated as double molecules in the petroleum ether layer.¹⁴

With water and ethyl ether as the two solvents,^{1a} the ordinary "distribution ratio" is reasonably constant (except for a slight trend due partly to ionization in the water layer), indicating no great association in the ether layer. With chloroform and water distribution of these acids,¹⁵ a fair amount of self-association of acid in chloroform layer is found. This all agrees with solubilities of fatty acids in ethyl ether, chloroform and petroleum ether; the solubilities (more particularly with acids having a high ratio of total carboxyl to total carbon) decrease rapidly if solvents are tested, respectively, in the order named. Hence in ethyl ether the affinity of the carboxyl group is satisfied by solvent molecules, the same being true

¹⁴ J. Grossfeld and F. Battay, *Z. Unters. Lebensm.*, **62**, 99 (1931); J. Grossfeld and A. Miermeister, *Z. anal. Chem.*, **85**, 321 (1932).

¹⁵ For complete data see A. Seidell. "Solubilities," D. Van Nostrand Co., New York, Vol. I, 1919, and Vol. II, 1928.

in water, methyl cyanide, methyl alcohol, etc., but only partially so in chloroform, benzene or toluene, and not at all in pure petroleum ether.

Self-Association of Polycarboxylic Acids.—"Self-association" of polycarboxylic acids evidently differs from self-association of monocarboxylic acids, since the latter form only double molecules, while there is hardly any limit to the length of chain possible with the polycarboxylic acid (or with its esters). No definite proof of existence of these chains is presented, but numerous disconnected facts are easily explained by this theory.

Thus, in petroleum ether dicarboxylic acids might form chains of great length, but the dicarboxylic acids tried (Table IV) were almost insoluble in the *i*-octane, even in the presence of the methyl cyanide. This property of low solubility in petroleum ether may be used to separate the dicarboxylic acids from the monocarboxylic of Japan wax.¹⁶ Apparently the chain formation is so effective that the acid crystallizes out of the hydrocarbon. However, the "japanic acids" from the Japan wax, *i. e.*, $C_{20}H_{40}(COOH)_2$ and $C_{21}H_{42}(COOH)_2$ are somewhat soluble in warm petroleum ether and a distribution experiment would probably show a very high power of the acid concentration in the water layer (or in the methyl cyanide layer, whichever is used) proportional to the acid concentration in the petroleum ether layer. The "peculiar coherence and tenacity" of Japan wax fatty acids, found by Tsujimoto¹⁶ to depend on the presence of about 5% of dibasic "japanic acids" is seen to be a mechanical demonstration of affinity between carboxyl groups. Tsujimoto found the same tenacity caused by japanic acid esters of glycerin, which esters he believes cause the "peculiar tenacity" of Japan wax itself. The well-known tenacity of cellulose esters is a further example of "self-association" between ester linkages; tenacity may apparently be produced by esters of either polyhydroxy alcohols or of polycarboxylic acids. Self-association of cellulose esters explains the fact that "the greater the solvent power of a liquid, the lower the viscosity of its solutions," referring to solutions of cellulose ester in pure or mixed solvents.¹⁷ Again, the viscosity of 5 to 25% cellulose nitrate solutions in a given solvent varies as the fourth to fifth power of the cellulose nitrate concentration, according to data of Bogin and Simms,¹⁸ self-association would be expected to increase rapidly with concentration. Their empirical formula is incorrect, however, since, according to them, at a given weight percentage of cellulose ester the viscosity is proportional to the viscosity of the pure solvent mixture. This would give lowest viscosities to cellulose ester solutions with high hydrocarbon content whereas they actually have the highest viscosities.

Evidence of chains is also found in relative melting points of isomers. Comparisons with maleic and fumaric acids (Table III) indicate that the

¹⁶ M. Tsujimoto, *Bull. Chem. Soc. Japan*, **6**, 325, 337 (1931).

¹⁷ T. H. Durrans, "Solvents," D. Van Nostrand Co., New York, 1931, p. 22-29.

¹⁸ C. D. Bogin and C. W. Simms, *Ind. Eng. Chem.*, **20**, 192 (1928).

former acid has its carboxyl groups close together and hence conjugated within the molecule. The series *o-m-p* phthalic acids (Table III) shows the same astonishing gradation in properties here again, *with esters as well as free acids*. Similar melting point series are found with picolinic, nicotinic and isonicotinic acids, with *o-m-p* phthalic dinitrile, etc.¹⁹

TABLE III
PROPERTIES OF DIBASIC ACIDS^a

Acid	<i>o</i> -Phthalic	<i>m</i> -Phthalic	<i>p</i> -Phthalic	Maleic	Fumaric
M. p., acid, °C.	191	347	Sublimes	130.5	287
M. p., dimethyl ester, °C.	Liquid	68	140	Liquid	102
M. p., diethyl ester, °C.	Liquid	11.5	44	Liquid	0.6
G. acid soluble in 100 g. cold water	0.65(13°)	0.013(25°)	0.0015	78.8(25°)	0.70(25°)

^a Beilstein, "Handbuch der organischen Chemie," Julius Springer, Berlin (1918 to 1932).

To illustrate the application of the above ideas to other than carboxyl compounds, consider the polymerization of 1,2-divinylacetylene (C₆H₆). This reaction takes place slowly in the pure substance, even in absence of air and light, but the polymerization is greatly retarded by dissolving the 1,2-divinylacetylene in "aromatic solvents."²⁰ Evidently self-association of the 1,2-divinylacetylene would be largely inhibited by the benzene, and one might reasonably assume self-association to occur preliminary to the irreversible polymerization. One would accordingly predict that the rate of polymerization of the 1,2-divinylacetylene would be many times as fast (perhaps thousands) when highly diluted with a saturated hydrocarbon, as when diluted to the same extent with benzene.

Comparison of Ethyl Ether and Chloroform as Solvents.—"Self-association" of carboxyl groups will be relatively slight, not necessarily in "polar" solvents, but rather in liquids which are good solvents for the carboxyl group. Thus, ethyl ether is a relatively "non-polar" solvent.²¹ Potentially, however, ether is a base and chloroform an acid. One may regard high solubilities of acids in ether, and of bases in chloroform, as incipient salt formation (see Table IV).

One is accordingly not surprised to find the base quinine self-associated as double molecules in the ether layer if distributed between ethyl ether and water.²²

¹⁹ For further discussion of effect of association chains on melting point, see N. V. Sidgwick and others, Ref. 2.

²⁰ O. M. Hayden, *Ind. Eng. Chem.*, **24**, 563 (1932).

²¹ J. H. Hildebrand, *THIS JOURNAL*, **38**, 1452 (1916); **39**, 2297 (1917); **41**, 1067 (1919); also "Solubility," Chemical Catalog Co., New York, 1924.

²² W. E. Treadwell, *Helv. Chim. Acta*, **6**, 744 (1923).

TABLE IV
SOLUBILITIES IN CHLOROFORM AND IN ETHYL ETHER¹⁵

Substance	Soly. per 100 g. of CHCl ₃ , g.	Soly. per 100 g. ether, g.
Atropine C ₁₇ H ₂₃ NO ₃	64.10 (25°)	6.02 (25°)
Quinidine C ₂₀ H ₂₄ N ₂ O ₂ ?H ₂ O	100 (20°)	0.78 (20°)
Fumaric acid C ₄ H ₄ O ₄	0.02 (25°)	0.72 (25°)
Maleic acid C ₄ H ₄ O ₄	0.011 (25°)	8.19 (25°)

Distribution of Mixtures of Fatty Acids in Solvents Allowing Self-Association.—Assume a distribution of mixed monocarboxylic acids between petroleum ether and water. One might reasonably assume all pairs of carboxyl groups to have equal chances of self-association. It accordingly follows that for each acid present its concentration ratio between the two solvents is the same as if that acid were distributed alone with its concentration in the petroleum ether layer, the same as the total acid concentration in the petroleum ether layer of the given mixture. Thus with three mixed acids 0.1 *M*, 0.3 *M* and 0.4 *M*, respectively, in the petroleum ether, each acid has the same distribution ratio as if it were the only acid present, at 0.8 *M*, in the petroleum ether layer. No data are available to test this; some correction would be needed for ionization in the water layer.

Acknowledgment.—For their interest and suggestions the authors are indebted to Drs. Herbert M. Evans and Robert E. Cornish of this Laboratory.

Summary

It is indicated that carboxyl groups have considerable tendency to associate with each other in pairs; this tendency is not destroyed by esterification. This "self-association" may be inhibited by presence of enough of either a somewhat polar or somewhat basic solvent, examples being given. Two carboxyls *close together* in the same molecule self-associate with each other rather than with carboxyls from other molecules. The tenacity of Japan wax and of cellulose esters is explained by these theories. Measurements are given of distribution of fatty acids between *i*-octane and methyl cyanide, and between *i*-octane and methanol; results are compared with those from other solvent pairs. A method is suggested for analyzing mixtures of higher fatty acids. The quantitative influence of fatty acids on each other is discussed for solvents allowing self-association.

BERKELEY, CALIFORNIA