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BRANCHED-CHAIN FATTY ACIDS. XXI. INVESTIGATION OF METHODS OF PURIFICATION AND SEPARATION[1]

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As an introduction to the separation of branched-chain fatty acids from the lipids of the tubercle bacillus, it has proved expedient to make a study of the behavior of pure synthetic branched-chain fatty acids. In the present paper are described studies of oil-diluted distillation of esters of fatty acids, and the solubilities of the lead salts of various branched-chain fatty acids.

OIL-DILUTED DISTILLATION

A. W. Weitkamp (1, 2) has made very effective use of oil-diluted ("amplified") distillation for separation of small amounts of esters. Addition of a neutral oil keeps an efficient column full and operating properly so that amounts of ester may be used which are not sufficient to keep the column properly full. Saponification gives the acids which may be separated from the neutral oil. The application of this method for separation of acids from bacteria has obvious advantages. Unfortunately, we have found that the method is complicated by two factors: (a) the boiling point of the ester is changed appreciably by the presence of the oil, (b) the efficiency of the column for separation of the esters is greatly reduced by the presence of the oil. Although these conclusions are in contrast to those reached by Weitkamp (2) the differences seem explicable. The shift in boiling point is small and observable only with elaborate control of pressure. It is of interest, however, that the boiling points reported by Weitkamp (2) for methyl stearate and palmitate, on oil-diluted distillation, are in excellent agreement with those obtained by us on oil-diluted distillation. The different conclusions concerning the efficiency of the column probably arise from the fact that Weitkamp's column is so much more efficient than ours that he could lose a large part of his efficiency and still quantitatively separate such things as esters of fatty acids differing by two carbon atoms. Our conclusions do not necessarily qualify Weitkamp's conclusions concerning the usefulness of oil-diluted distillation, but rather place a limit on the applications of the method.

In Figure 1 are two plots for distillation of a mixture of methyl palmitate and methyl stearate. The dashed line is that for an oil-diluted distillation (4.05 g. of esters; 25 ml. of an appropriate fraction of Eureka White Oil[2]), while the solid line is that for distillation of the ester mixture alone. Quantities have been adjusted so that milliequivalents under the two curves are the same. The major

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[2] In order to use an oil with properties as close as possible to those of the oil which has been thoroughly explored by Weitkamp (1, 2), Eureka White Oil was purchased from the Standard Oil Co. of Indiana.

difference in the effectiveness of separation is apparent. The discontinuity in the line for the undiluted esters resulted from the fact that there was a region of essentially dry column between the two esters. It will be noted that the boiling point of methyl palmitate is lowered by about three degrees, in the oil-diluted distillation, whereas the boiling point of methyl stearate is essentially unaffected.

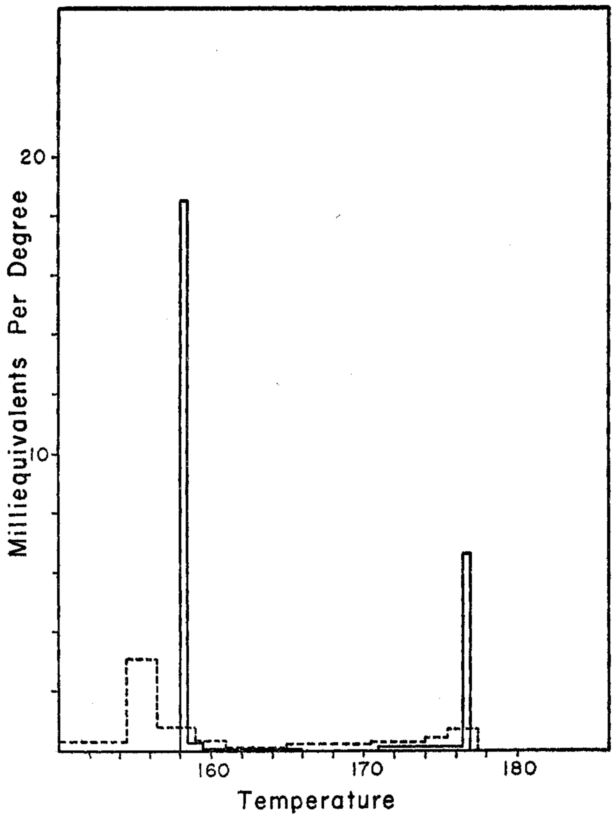

Figure 1. Distillation of a Mixture of Methyl Palmitate and Methyl Stearate. Dashed line, oil-diluted ester distillation; solid line, undiluted ester distillation. The width of each step indicates the boiling point range of that fraction. For the fraction of methyl palmitate of b.p. 158–158.5°, m.p. 28.2–29.2° [literature (3), m.p. 30.55°]. For methyl stearate of b.p. 176.5–177°, m.p. 33.5–37.5° [literature (3), m.p. 39.1°].

This resulted from the fact that in this run the oil was nearly exhausted[3] by the time the stearate fraction was reached. It follows that for things normally boil-

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[3] The boiling range of a mixture such as paraffin oil depends on the mixture from which the given fraction is being distilled. Thus, a fraction collected in a given range does not re-distil through the same column entirely in the same range. Some art is required to select a fraction which will yield a suitable amount of oil distilling over the range covering the boiling points of the ester mixture. It is especially difficult to estimate the distribution of oil over the range.

ing 3–4° apart, an unfortunate distribution of oil could make the boiling points nearly overlap, especially since esters of different structure may have their boiling points shifted by different amounts. Also, the boiling point of the ester would change as the concentration of oil changed during fractionation, and this appears to be the reason that the boiling point is "spread" by addition of oil, as shown in Figure 1.

TABLE I

DISTILLATION OF A MIXTURE OF ETHYL NONADECANOATE AND ETHYL 4-METHYLOCTADECANOATE

PROPERTIES OF FRACTION	B.P., °C. (3.75 MM.)	WT., G.
Fore-run	192 –194.5	0.35
Ethyl 4-methyloctadecanoate, freezing pt. 15°	194.5–195.5	3.55
Intermediate, liquid	195.5–196	1.85
Intermediate, semi-solid	196 –197.5	1.30
Intermediate, semi-solid	197.5–198	0.80
Ethyl nonadecanoate, m.p. 31–35.3°[a]	198 –198.5	3.20

[a] Pure ethyl nonadecanoate melts at 36.1° (3).

TABLE II

OIL-DILUTED DISTILLATION OF A MIXTURE OF ETHYL NONADECANOATE AND ETHYL 4-METHYLOCTADECANOATE

B.P., °C. (3.75 MM.)	DISTILLATE (Ester plus Oil)			
	wt., g.	milliequiv. of ester	milliequiv. per gram	milliequiv. per degree
184–191[a]	5.2	2.441	0.469	0.787
191–193	3.8	2.487	.655	1.244
193–194	1.5	1.020	.680	1.020
194–195	1.8	1.258	.700	1.258
195–196	2.8	2.005	.716	2.005
196–198	2.2	1.597	.725	0.799
198–199	2.55	0.874	.342	.874
199–202	0.95	.180	.190	.060
Total	20.8	11.862 (94% of original ester)		

[a] The acid recovered from this fraction melted at 46.5–49.0°. After one crystallization from acetone, wt. 0.1 g., m.p. 48.3–49.4°. Pure 4-methyloctadecanoic acid (4) melts at 52.6–52.8°.

One or both of the above-mentioned effects resulted in no observable separation in an oil-diluted distillation of ethyl nonadecanoate and ethyl 4-methyloctadecanoate, whereas undiluted distillation gave considerable separation. In Table I are the data on distillation of about 11 g. of a mixture of these esters. About one third of the total was collected in intermediate fractions, the remainder being collected in fractions having a high concentration of each ester. The three intermediate fractions (3.55 g. used) from this distillation were mixed with 25 ml.

of oil of b.p. 184–202° (3.75 mm.) and redistilled through the same column at the same pressure. Data assembled in Table II show: (a) the boiling points of the esters have been lowered, (b) no appreciable separation has been accomplished, (c) the lowest-boiling fraction contains a rather impure sample of methyl 4-methyloctadecanoate.

SOLUBILITIES OF LEAD SALTS

The solubility in ether of the lead salts of branched-chain fatty acids has been used (5) for separation of such acids from straight-chain acids, but no data on the actual solubilities of the lead salts of branched-chain acids of known structure have been obtained. In Table III are assembled the data for the solubilities at 20–25°, in absolute ether and absolute ethanol, of the lead salts of branched-chain acids of various structures. Although the normal acid with as few as eleven carbons gives a lead salt which is nearly insoluble in either ether or ethanol, most of the branched-chain acids give lead salts which are very soluble in ether and very sparingly soluble in ethanol. A reliable general separation of branched-chain acids cannot be secured in this way, however, for some of the branched-chain acids give lead salts which are sparingly soluble in ether. The solubilities of the lead salts in ether are not entirely a function of melting point of the acid, for 14-methyloctadecanoic acid (sparingly soluble lead salt) melts considerably lower (6) than 4-methyloctadecanoic acid (soluble lead salt). Also, 6-methyl- and 10-methyl-tetracosanoic acids (7), whose melting points differ by only 5°, give lead salts with very different solubilities in ether. No simple correlation which will permit prediction of the solubilities of the lead salts is apparent.

TABLE III

SOLUBILITIES OF LEAD SALTS

ACID	SOLUBILITY OF LEAD SALT, g./100 ml.,	
	In Ether	In Ethanol
Hendecanoic	0.004	0.018
2-*n*-Butyl-2-ethylnonanoic	>36[a]	>36
4-Methyloctadecanoic	>15	0.09
8-Methyloctadecanoic	>17	.34
11-Methyloctadecanoic	>25	.30
14-Methyloctadecanoic	0.40	.02
16-Methyloctadecanoic	0.10	—
15-Ethylheptadecanoic	>28	.12
3,3-Dimethyloctadecanoic	>19	.20
12-*n*-Hexyloctadecanoic	>20	.16
6-Methyltetracosanoic	0.34	.04
10-Methyltetracosanoic	>22	.09

[a] A sample of 0.36 g. dissolved readily in 1 ml. of solvent; similarly for other very soluble salts.

EXPERIMENTAL

Distillations were carried out in the column which has been described in detail (8), and the boiling points are corrected. Esters were pure samples whose preparation has been described in earlier papers of this series or were purified samples from commercially available acids.

Lead salts were prepared from pure samples of the acids previously described, as follows. A solution of about 0.5 g. of acid in 10 ml. of 95% ethanol was titrated in a 50-ml. centrifuge tube to the Phenolphthalein end-point with 1 *N* potassium hydroxide. After addition of 35 ml. of water, 1.1 equivalent of neutral lead acetate in 5 ml. of water was added dropwise with swirling. The lead salt (solid or oily) was removed by centrifugation, washed several times with water, dried, and weighed. Yields were 90–100%.

Representative salts were analyzed, with the following results. *Lead 15-ethylheptadecanoate,* Pb, 25.88 (calc'd, 25.81); *Lead 10-methyltetracosanoate,* Pb, 21.70 (calc'd, 21.35); *Lead 16-methyloctadecanoate,* Pb, 27.40; C, 55.43; H, 9.10 (calc'd, 25.81, 56.89, 9.32). The latter sample probably contains about 2.4% lead oxide. Several of the ether-soluble lead salts left a trace of insoluble material which was readily soluble in dilute nitric acid. In order to avoid error from traces of insoluble material, solubilities in Table III were determined by evaporating an aliquot of a saturated solution. Saturated solutions were obtained by heating the solutions in contact with excess lead salt under reflux for 1–2 hours, then allowing the mixture to stand at room temperature overnight.

SUMMARY

There are presented data on difficulties encountered in the use of oil-diluted distillation. These are change of boiling point of the component being diluted and a decrease in the efficiency of the column.

Data are also presented on the solubilities in ether and ethanol of the lead salts of branched-chain saturated acids.

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