A CHROMATOGRAPHIC METHOD FOR THE SEPARATION OF BRANCHED-CHAIN AND STRAIGHT-CHAIN COMPOUNDS ON COLUMNS CONTAINING UREA

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(Received September 25th, 1967)

INTRODUCTION

An early method used for the separation of branched-chain from straight-chain acids was based upon the sparing solubility of the lead salts derived from straight-chain acids in ether[1] or ethanol[2], but this method was unsatisfactory because of the possible formation of mixed salts derived from both branched- and straight-chain acids.

The formation of inclusion compounds of urea and long-chain material was first described by BENGEN in 1940 (for a review see ref. 3). These compounds are formed more readily by straight-chain than by branched-chain compounds, thus giving a basis for separation. The procedure described by LINSTEAD AND WHALLEY[4] consists of the addition of urea moistened with methanol to a solution of the mixture of straight-chain and branched-chain compounds in light petroleum. After keeping the mixture overnight, the solid is filtered off and yields, on decomposition of the complex with water, mainly straight-chain compounds. The light petroleum filtrate contains the branched-chain fraction.

Various methods involving the use of urea columns have been reported, but do not seem to have found general application in the lipid field. Silica gel impregnated with urea has been used to separate stearic and oleic acids and esters[5], decahydronaphthalene and *n*-hexadecane[6], and a hydrocarbon mixture[7]. Columns of powdered urea have been used by CASON *et al.*[8], and were stated to separate mixtures of straight-chain and branched-chain acids but failed for long-chain esters with more than 18 carbon atoms[9]. In each case the column was eluted with a non-polar solvent to remove the non-clathrated material and with water or methanol to destroy the complex.

While the present work was in progress, chromatography on cellulose paper impregnated with urea and thin-layer chromatography on urea–calcium sulphate layers were reported[10] for long-chain saturated and unsaturated acids.

This paper describes a convenient way of separating branched-chain and straight-chain acids as their methyl esters, using column chromatography. The efficiency of this method (which would also be applicable to other straight-chain and branched-chain compounds) is compared with that of LINSTEAD AND WHALLEY[4]. Some experiments on thin-layer chromatography using plates containing urea are also described.

EXPERIMENTAL

General method for the use of urea columns

Urea and the mixture to be separated, in proportions 5:1, were dissolved in methanol and shaken with Celite 545 (twice the weight of urea). The mixture was then slowly evaporated to dryness on a rotary evaporator. The resulting solid was powdered and packed dry into a chromatographic column. Light petroleum (the fraction b.p. 60–80° was used throughout) was slowly added until the column was saturated; elution with the same solvent yielded branched-chain material. Because of the limited miscibility of light petroleum and methanol, ether was used as an intermediate solvent, and eluted small quantities of mixed compounds. Elution with methanol yielded straight-chain material. However, urea was also eluted by this solvent, and therefore these fractions were concentrated, poured into water, and extracted three times with ether. The combined ethereal layers were washed with water, dried, and evaporated. For straight-chain methyl esters with chain lengths greater than C_{20}, the solubility in cold methanol is low; therefore the columns were extruded and boiled with methanol. After filtration, the hot methanolic solution was treated as above to remove any urea present.

Method of Linstead and Whalley[4]

The test mixture (500 mg) was dissolved in light petroleum (4 ml) and urea (1.5 g) moistened with methanol (15 drops) was added. After standing overnight the solid was filtered off, washed thoroughly with light petroleum, dissolved in water and extracted three times with ether. The ethereal layers were combined, washed with water, dried, and evaporated to give the straight-chain fraction. The light petroleum filtrate and washings were combined, washed with water, dried and evaporated, yielding the branched-chain fraction.

Gas–liquid chromatography

The instrument used was a Perkin-Elmer F11 flame detector gas chromatograph. A 2 m column O.D. 1/8 in., packed with 1.5 % silicone gum rubber E-301 on AW-DMCS Chromosorb W, 80–100 mesh, was used. Air and hydrogen pressures were 15 p.s.i. The carrier gas (nitrogen) pressure was 20 p.s.i. throughout. The relative amounts of branched-chain and straight-chain compounds were estimated from the areas under the chromatographic peaks.

For mixture I at oven temperature 140° the retention times were: methyl myristate, 12 min; methyl 3,5-dimethyltridecanoate, 10 min. For mixture II at oven temperature 240° the retention times were: methyl hexacosanoate, 12.5 min.; methyl 2,4,6-trimethylhexacosanoate, 16 min. For mixture III examination of the tuberculostearic and stearic esters was carried out at 180°, and the retention times were: methyl tuberculostearate, 14 min; methyl stearate, 12 min. For examination of the higher molecular weight components a temperature of 240° was used.

DISCUSSION

Column chromatography

The stationary phase for column chromatography consisted of a mixture of

urea, Celite, and the compounds to be separated, prepared as described in the experimental section and packed dry. Three test mixtures were used; mixture I contained approximately equal amounts of methyl myristate and methyl 3,5-dimethyltridecanoate, mixture II contained methyl hexacosanoate and methyl 2,4,6-trimethylhexacosanoate in the same proportions. Mixture III was a complex mixture of lipids from tubercle bacilli[11] from which unsaturated esters, other than α,β-unsaturated, had been removed, and which contained methyl esters of the following acids: C_{14}–C_{26} straight-chain acids[12a], tuberculostearic acid (10-methyloctadecanoic acid) and lower homologues[12b], and mycolipenic acid (α,β-unsaturated 2,4,6-trimethyltetracosenoic acid) and lower homologues[12c]. The columns were eluted with light petroleum yielding a branched-chain fraction, and then with methanol yielding a straight-chain fraction. For straight-chain esters of higher molecular weight (greater than C_{20}) the solubility in cold methanol is low, so the column was extruded and extracted with hot methanol. The same mixtures were also submitted to the procedure of LINSTEAD AND WHALLEY[4]. In all cases the resulting fractions were examined by gas–liquid chromatography. Results for mixtures I and II are summarised in Table I. The recovery of material in each experiment was almost quantitative.

For mixtures I and II, using the method of LINSTEAD AND WHALLEY the straight-chain fraction was found to contain branched-chain material in small but significant amounts, presumably due either to partial clathrate formation or incomplete removal by washing of branched material adhering to the surface of the solid. The branched-chain fraction was found to contain very little straight-chain material.

For mixture I, using the column method, the branched-chain fraction was found to contain a few percent of straight-chain material, but the straight-chain material was 99 % pure. The thorough elution with petrol must effectively remove all branched-chain material. For mixture II both branched-chain and straight-chain fractions were essentially pure.

For mixture III the initial relative proportions of tuberculostearic and stearic esters were 64:36. While neither method is entirely successful in removing the branched-chain material from the straight-chain fraction in one separation, as the structural difference between the two compounds is slight, the urea column is more efficient (branched-chain: straight-chain, 46:54) than the method of LINSTEAD AND WHALLEY[4] (branched-chain:straight-chain, 58:42). The multibranched mycolipenic esters are removed from the straight-chain fractions effectively by both methods and the branched-chain fractions resulting from both methods contain approximately the same small amount of methyl palmitate, the most abundant straight-chain compound.

It seems from these results that if a pure specimen of straight-chain material is required the urea column is most effective, while for a sample of branched-chain material the procedure of LINSTEAD AND WHALLEY[4] is at least equally efficient. However, an advantage of the urea columns is that by collecting small volumes of eluant the material is obtained in several smaller fractions which can be monitored by gas–liquid chromatography and the purest fractions selected. In the case of mixtures containing a wide range of straight-chain esters the use of the column method also permits separation into fractions of lower and higher molecular weight without the use of fractional distillation.

TABLE I

RESULTS OF COMPARATIVE EXPERIMENTS

Mixture	*Fraction*	*Linstead and Whalley*[4]		*Column*	
		% straight	*% branched*	*% straight*	*% branched*
I	straight	96.5	3.5	99.5	0.5
	branched	1	99	8.5	91.5
II	straight	91	9	99	1
	branched	—	100	—	100

Thin-layer chromatography

Preliminary experiments were carried out using thin-layer chromatography, on plates containing urea. Test compounds used were methyl myristate, methyl 3,5-dimethyltridecanoate, methyl hexacosanoate and methyl 2,4,6-trimethyl hexacosanoate. Plates were made up from Kieselgel G and 15–45 % by weight of urea in water. Samples were applied in solutions of light petroleum or ether. For low percentages of urea some separation of the branched-chain and straight-chain compounds was observed, and the separation increased with the amount of urea present. However, for high percentages of urea, the average R_F value, even with light petroleum as solvent, was 0.62 (ΔR_F 0.15) thus precluding the use of the technique of unidimensional multiple chromatography[13].

When Celite 545 containing 30 % urea was used as the layer, the branched-chain esters moved with the solvent front, while the straight-chain esters did not migrate at all.

Unfortunately preparation of the plates was not reproducible; on some batches all compounds moved with the solvent front. Also the plates were easily overloaded and visualisation of the compounds was difficult; the best method was spraying with a solution of dichlorofluorescein followed by examination under U.V. light (366 mμ).

ACKNOWLEDGEMENT

The author wishes to thank the Medical Research Council for the award of a scholarship.

SUMMARY

This paper describes the separation of branched-chain and straight-chain compounds using a column chromatographic method. The mixture to be separated is incorporated, before the column is packed, into the stationary phase, which consists of urea and Celite. Elution with light petroleum yields branched-chain material; subsequent elution with methanol gives straight-chain material. The efficiency of the method is compared with that of a previously known procedure, also involving urea inclusion compounds. Some experiments using thin layers containing urea are also described.

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