hydrochloride. In the γ-picoline quaternary, this band is apparently shifted to 11.9 μ, and in the quinoline quaternary to 12.0 μ. No standard picoline or quinoline quaternary compounds were available for comparison. The absence of free pyridine is shown by the absence of bands at 13.2–13.3 μ, and the absence of pyridine hydrochloride by the absence of bands at 4.0–4.8, 5.0 μ. The band at 14.4–14.6 μ is common to pyridine, its hydrochloride, as well as the pyridinium quaternary compounds. The C=O of the ester, acid or amide at 5.8–5.9 μ, is evident in all of the quaternary compounds except that from octadecene and the lauryl pyridinium chloride standard.

The γ-picoline product is quite low in all analyses except total chlorine, suggesting contamination with the dichlorostearate. The crude oleic amide product would appear to be contaminated with pyridine hydrochloride. The purified product is in better agreement with theory. The ester fraction from the reaction of methyl oleate plus methanol with pyridine and chlorine is probably contaminated with pyridine (14.6 μ band).

The bactericidal power of the diquaternary from methyl linoleate was no better than that of the monoquaternary from oleate. The quaternary from oleic amide was somewhat better, and the quaternary from octadecene-1 was the best.

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H. M. Boyd and associates performed the chemical analyses; R. Fisher, IR analyses.

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Thin-Layer Chromatography of Aliphatic γ- and δ-Lactones

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Abstract

Homologous aliphatic γ- or δ-lactones are separated by thin-layer chromatography on a mixture of kieselguhr G and silica gel G (1:8). The thin-layer plates are chromatographically impregnated with methanol as the stationary phase and developed 3 times with light petroleum (bp 80–100C) saturated with methanol. γ- And δ-lactones can be separated from each other on the same adsorbent with a 1:1 mixture of light petroleum (bp 30–40C) and isopropyl ether. The systems can be combined 2-dimensionally. The lactones are detected by spraying the chromatoplates either with a 2% solution of iodine in methanol or with alkaline hydroxylamine followed by ferric chloride and acetic acid. These methods were used for the tentative identification of δ-C_{10-16} lactones in commercial Australian butteroil.

Introduction

THE LACTONES CONTRIBUTING to butter flavor have been separated by gas chromatography (2,14) and either as the free compounds or as anilides or hydroxamic acids by paper chromatography (14,2,5,6). The thin-layer chromatography (TLC) of free α,β-unsaturated aliphatic γ-C_{14-26} lactones has been investigated by Kaufmann and Su Ko (4) but their system does not separate lactones below C_{14}. Korte and Vogel (7) separated various C_{4-8} lactones on silica gel G using isopropyl ether as solvent. This and similar systems have been used in the present investigation for the separation of γ- from δ-lactones and for the separation of γ-$C_{4,5\ \&\ 6}$ lactones from each other. Korte's system does not separate higher homologous lactones.

Tharp and Patton (14) separated the free δ-decafrom the δ-dodecalactone by paper chromatography using the upper phase of a 2:1 mixture of heptane: methanol as mobile phase and the lower phase as the equilibrating phase. This formed the basis of the thin-layer partition system described in the present paper for the separation of homologous lactones above C_7. The mobile solvent used in the present investigation was light petroleum (bp 80–100C) saturated with methanol, on plates consisting of 1 part of kieselguhr G to 8 parts of silica gel G. The plates were impregnated with methanol. Methanol impregnation was found essential; without it the lactones remained near the starting point, presumably due to excessive adsorption. Kieselguhr G was added to the silica gel G to reduce adsorption. Any further reduction in adsorptive capacity reduced carrying capacity below detectable limits or caused trailing due to overloading when detectable amounts of material were used.

In a pure partition system, i.e. where $R_f = 1/1 + \alpha\frac{A_S}{A_L}$ (3), reduction in the quantity of stationary phase increases R_f values. However, in TLC on silica gel reduction of stationary phase also bares adsorption sites and so reduces R_f values due to increased adsorption. Therefore, maximum R_f values will be achieved with the minimum amount of stationary phase which still covers all adsorption sites. The optimum amount of stationary phase was determined experimentally.

Since both phases of the partition system are volatile, it was possible to obtain a two-dimensional separation of mixtures of γ- and δ-lactones, with the first direction for homolog separation and the second for class separation.

Experimental

Source of Reference Lactones

γ-Lactones were obtained from the following commercial sources and used without further purification: γ-$C_{4\ \&\ 5}$ lactones from L. Light & Co. Ltd., Colnbrook, Bucks., England; γ-C_6 lactone from Tokyo

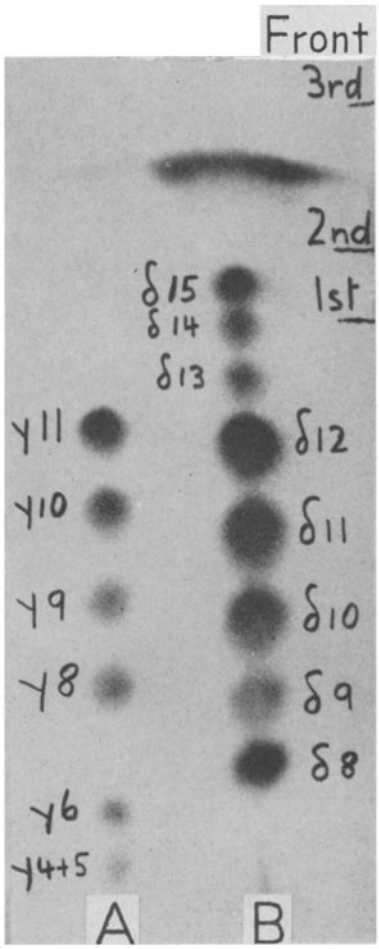

FIG. 1. Partition separation of (A) γ-$C_{4,5,6,8-11}$ lactones and (B) δ-C_{8-15} lactones. Detection—2% I_2 in MeOH. (Impurity between 2nd and 3rd front.)

Kasei Kogyo Co. Ltd., 14–6 Toshima Kita-Ku, Tokyo; γ-$C_{8\ \&\ 10}$ lactones from Aldrich Chemical Co. Inc., Milwaukee, Wis.; γ-$C_{9\ \&\ 11}$ lactones were gifts from W. J. Bush & Co. Ltd., Melbourne, Australia.

δ-Lactones were not commercially available. δ-C_8 Lactone was a gift from Dr. M. F. Ansell of Queen Mary College, University of London. δ-$C_{9\ \&\ 11}$ Lactones were gifts from Dr. L. R. Mattick of Cornell University, Geneva, N. Y. δ-$C_{10\ \&\ 12}$ Lactones were gifts from Dr. J. Boldingh, Unilever Research Laboratory, Vlaardingen, The Netherlands.

δ-Tridecalactone and δ-pentadecalactone were prepared by the $NaBH_4$ reduction of the corresponding ketoacids dissolved in 5% aqueous NaOH and lactonization of the resulting hydroxyacids (10,11,8,9, 13,17).

δ-Tetradecalactone was prepared in very low yield by the aluminum isopropoxide reduction of 5-ketotetradecanoic acid N-methylamide (9).

All δ-lactones were purified by gas chromatography on a 100 × 0.4 cm I.D. column of 20% Apiezon M on acid and alkali washed 60–80 mesh Embacel (14). The column temperature was 210C and the flow rate was adjusted so that the retention time was not less than 3 min. δ-Lactones had a tendency to hydrolyze to the free hydroxyacid on standing but gas chromatography reconverted them to the lactones (1) providing the retention time was at least 3 min.

Solvents

All solvents were commercial Analytical Reagent quality and were used without further purification. Isopropyl ether contains hydroquinone as stabilizer and this causes a band approximately R_f 0.0–0.3 which is stained pale brown with iodine. Although this does not interfere with chromatography clearer photographs were obtained with redistilled isopropyl ether. As a routine procedure the use of redistilled isopropyl ether is not advisable because of the ready formation of explosive peroxides in pure isopropyl ether.

Preparation of Thin-Layer Plates

Desaga (Heidelberg, Germany) equipment was used throughout.

Thin-layer plates were prepared from a slurry of 36 g solid (1 part of kieselguhr G to 8 parts of silica gel G) and 72 ml distilled water, and were air or oven dried. After oven drying, plates were allowed to equilibrate with the atmosphere for about 2 hr before use.

Detection

Lactones were made visible either:

1) by spraying with a 2% solution of iodine in methanol which gave brown to purple spots on a white background, or
2) by using a modified Korte's (7) reagent consisting of
   a) equal volumes of
      i) 5% Hydroxylamine hydrochloride in methanol;
      ii) 12.5 g NaOH dissolved in the minimum volume of water made up to 100 ml with methanol. The solution was decanted from the precipitated NaCl. It was used on the day it was prepared.
   b) 1% $FeCl_3$ in methanol;
   c) glacial acetic acid.

The chromatogram was sprayed heavily with solution (a) and left for at least 10 min. It was then sprayed with the 1% $FeCl_3$ solution (b) till uniformly brown and then with glacial acetic acid (c) till the brown background faded and compounds containing RCOOR′ showed up as orange spots on a white background. The orange changed to yellow as the acetic acid evaporated but could be restored by spraying again with acetic acid.

Iodine spray detects about 4 μg lactones above C_8 and is about 5 times as sensitive as I_2 vapor. I_2 spray is more sensitive than the ferric hydroxamate reagent for lactones above C_8, while the ferric hydroxamate reagent is more sensitive for shorter chain lactones. Routinely, chromatograms were first sprayed with I_2 and after evaporation of the I_2, with the ferric hydroxamate reagent.

Separation of Homologous Series by Partition TLC

For the separation of homologous series, it was necessary to saturate the tank with both mobile (light petroleum bp 80–100C saturated with dry methanol) and stationary (methanol) phases. Saturation with mobile phase was achieved in the conventional manner by lining the tank with filter paper dipping into the light petroleum phase. Saturation with methanol was achieved by placing in the tank a hollow glass tube filled with cotton wool saturated with methanol. The tube, about 18 cm long and about 2 cm in diameter, open at both ends and perforated with a large number of holes 0.75 cm in diameter, was fused to

two 15 cm long vertical rods which supported it in a horizontal position against the top of one of the long walls of the tank.

The TLC plates were impregnated with methanol by ascending chromatography and compounds were applied with the spotting plate covering the TLC plate to minimize evaporation of the methanol. After spotting, the TLC plate was exposed to the atmosphere for 3 min and then developed for 10 min. The plate was removed, the solvent front marked, and solvent evaporated till the plate looked damp. Development and solvent removal were repeated twice. Between developments, the light petroleum should be removed, but not the methanol. If too little solvent has been removed, the solvent front will not be visible in the subsequent development, although a satisfactory chromatogram may still be achieved. Markers, preferably internal, must always be used with this system because R_f values are very variable.

Figure 1 shows an example of the separation of γ-$C_{4,\ 5,\ 6,\ 8\text{–}11}$ lactones (A) and δ-$C_{8\text{–}15}$ lactones (B). All the δ-lactones and γ-$C_{6,\ 8\text{–}11}$ lactones separate clearly from one another. The γ-$C_{4\ \&\ 5}$ lactones form one spot of low R_f, but they separate clearly in the adsorption system described below.

Separation of γ- from δ-Lactones by Adsorption TLC

In adsorption systems δ-lactones have somewhat lower R_f values than the corresponding γ-lactones, due to a more strained ring and hence greater polarity. Isopropyl ether, 2% acetic acid in isopropyl ether, and mixtures of diethyl ether and light petroleum, all on silica gel G have been used successfully in this laboratory for class separation.

The adsorption method was used for the isolation of δ-lactones from a cold finger distillate of commercial Australian butteroil. The cold finger was washed with chloroform and the chloroform solution streaked along the starting line of several 0.5 mm thick silica gel G plates. The plates were developed with 2% acetic acid in isopropyl ether in an unlined tank. The bands were detected with I_2, their outlines marked, and after evaporation of the I_2 the δ-lactone area was scraped off with a "vacuum cleaner" (15) and the lactones eluted with diethyl ether. Some of this solution was evaporated onto KBr for IR spectroscopy. The spectrum was similar to those of δ-$C_{13,\ 14,\ 15}$ lactones recorded in these laboratories with bands at 932, 1053, 1175, 1245, and 1743 cm^{-1} (Beckman IR7).

In order to combine class and homolog separation in a 2-dimensional procedure, a system using the mixed adsorbent layer was developed. A 1:1 mixture of isopropyl ether and light petroleum (bp 30–40C) separates the γ- and δ-lactones which are not separated in the partition system described in the previous section, and separates γ-$C_{4,\ 5\ \&\ 6}$ lactones from each other. For wide separation only one plate at a time should be developed in an unlined tank. Two plates in the tank produce complete saturation in the same way as lining the tank with filter paper. This reduces R_f values and decreases separations. Figure 2 shows the separation of γ-$C_{4,\ 5,\ 6,\ 8,\ 11}$ lactones (A) and of δ-lactones (B), (C) and (D). The plate was developed to the top edge. All the δ-lactones shown have lower R_f values than γ-lactones below C_8.

Two-Dimensional Procedure

Figure 3 shows the two-dimensional separation of γ-$C_{4,\ 5,\ 6,\ 8\ \&\ 10}$ and δ-$C_{8,\ 10,\ 12\ \&\ 14}$ lactones. As in the one-dimensional partition procedure for separating homologs, the plate was first chromatographically impregnated with methanol and the mixture applied with the spotting plate covering most of the TLC

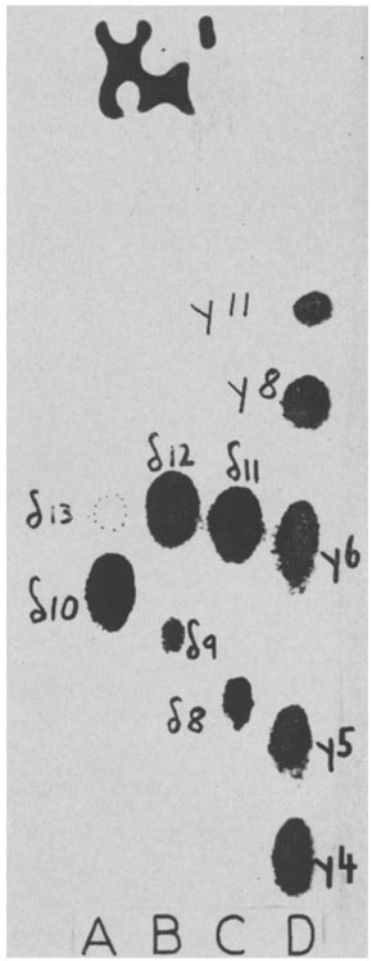

FIG. 2. Class separation by adsorption chromatography (A) δ-C_{10} plus trace of δ-$_{13}$ lactones; (B) δ-$C_{9\ \&\ 12}$ lactones; (C) δ-$C_{8\ \&\ 11}$ lactones; (D) γ-$C_{4,5,6,8,11}$ lactones. Detection—2% I_2 in MeOH. Note: The concentration of γ-$C_{4,5\ \&\ 6}$ lactones is approx. 10 × that of the other lactones.

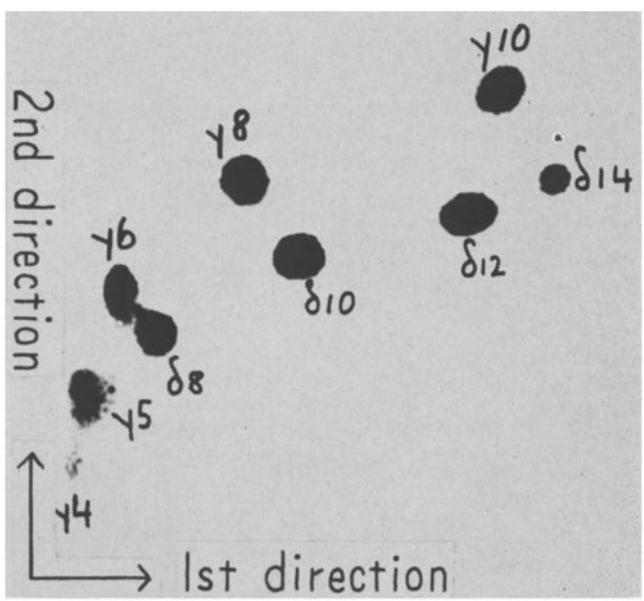

FIG. 3. Two-dimensional separation of γ-$C_{4,5,6,8,10}$ and δ-$C_{8,10,12,14}$ lactones. Detection—2% I_2 in MeOH.

plate. (Markers for the separation of homologs can be placed at the other edge of the plate at this stage.) The plate was then chromatographed according to the partition procedure described above. After the third development, the solvent was evaporated completely and the plate was developed in the second direction in an unlined tank with the 1:1 mixture of light petroleum (bp 30–40C) and isopropyl ether.

Figure 4 shows the partition separation of (A)—an homologous series of δ-C_{8-15} lactones, (B)—a sample of the δ-lactones isolated from commercial top quality Australian butteroil, and a mixture of (A) and (B). (B) shows two strong spots and five fainter spots tentatively identified from their R_f values as δ-$C_{12\ \&\ 14}$ and δ-$C_{10,\ 11,\ 13,\ 15\ \&\ 16}$ lactones, respectively. (In the synthetic mixture an impurity is visible between δ-$C_{12\ \&\ 13}$ lactones.) To the eye the faint spots were far more clearly visible than they appear on the photograph, but at that stage part of the chromatogram still had so much I_2 on it that other sections of the chromatogram could not be clearly photographed. Some of the faint spots are shown more clearly in Figure 5 which is a 2-dimensional chromatogram of the butteroil δ-lactones. The chromatogram has purposely been overloaded. Before chromatography in the second direction, markers for class separation were added at the right hand edge of the chromatogram, (B) butteroil δ-lactones, (C) γ-$C_{4,\ 5,\ 11}$ and δ-C_{12} lactones. This chromatogram provides a further illustration of the use of the two-dimensional procedure and, taken in conjunction with Figure 4, supports the tentative identification in the butteroil of δ-$C_{10,\ 12,\ 14\ \&\ 16}$ lactones. It also shows two fainter spots possibly corresponding to δ-$C_{8\ \&\ 5}$ lactones. Further physico-chemical data are necessary for the complete identification of these compounds.

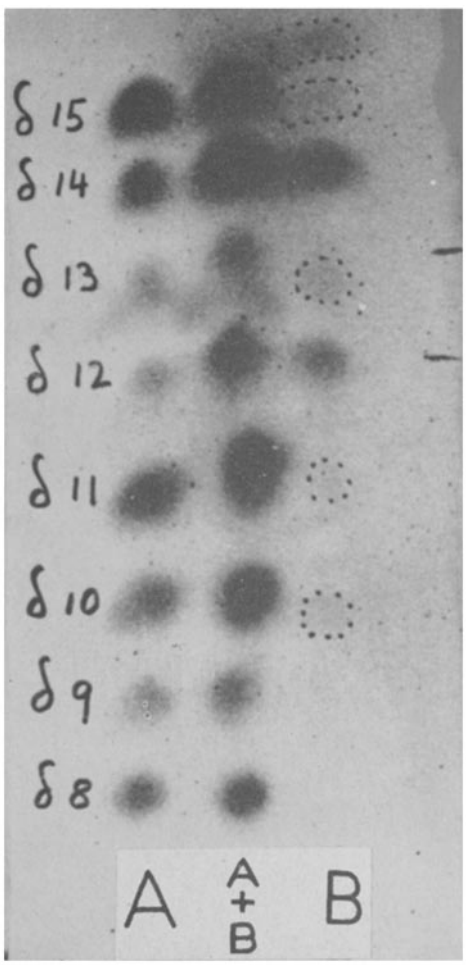

FIG. 4. Homolog separation of (A) δ-C_{8-15} lactones (B) a sample of δ-lactones isolated from top grade Australian butteroil. (Impurity between δ-$C_{12\&13}$ lactones.) Detection—2% I_2 in MeOH.

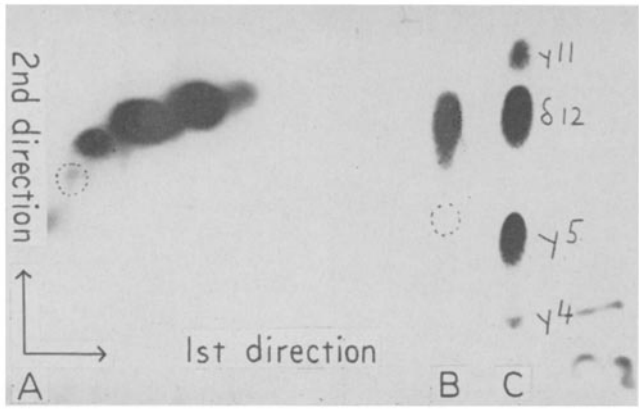

FIG. 5. (A) Two-dimensional chromatogram of butteroil δ-lactones, (B) class separation of butteroil δ-lactones, (C) class separation of γ-$C_{4,5,11}$ and δ-C_{12} lactones.

Separation on Microplates

Good class and homolog separations were also obtained on microplates (12) prepared by dipping two microscope slides back to back into a suspension of 35 g of adsorbent in 100 ml of a 2:1 mixture of chloroform and methanol. When microplates are used, prior impregnation with methanol for the homolog separation is not necessary, partly because the layer still contains some methanol due to the way it was made and partly because, being much thinner than layers on large plates, equilibration with methanol is almost instantaneous. Four-ounce wide-necked reagent bottles were used as development chambers and saturation with methanol was achieved by inserting a methanol saturated cotton wool plug in the lid. Methods of detection are much more sensitive on microplates than on the large plates.

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