Opitcal Activity of Lactones From Animal and Vegetable Fats

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Abstract

The isolation of optically active lactones from animal and vegetable fats is described. As the optical activity of lactones isolated from butterfat is ascribed to their biological origin, special attention was paid to the optical purity of the lactones. Lactone mixtures from butterfat, goat milk fat and coconut oil were found to be dextrorotatory, those from babassu oil levorotatory. The total lactone mixtures of two out of three samples of palm-kernel oil were slightly dextro-, whereas that of the third one was levorotatory. After isolation of the individual lactones from the mixtures, levo- and dextrorotatory lactones were demonstrated side by side in palm-kernel oil and in coconut oil. The δ-lactones of palm-kernel oil were levo-, the γ-isomers dextrorotatory. In coconut oil only the δ-C_{12} lactone was levorotatory, whereas the other components of the δ-series were dextrorotatory. The specific rotation $[\alpha]_D$ of the chemically pure lactones was considerably lower than that of model lactones, this being an indication of their optical impurity. As it was evident from model experiments that no racemization of the lactones occurred during isolation, it follows that both optical antipodes are formed in the fats in unequal amounts, via different pathways.

Introduction

The occurrence of lactones in milk and milk products has been known for a long time (1–7). The number of lactones in butterfat, isolated and identified by several investigators (6–12), is about 20. With the exception of two unsaturated ones (6,7) and a branched one (11) they can be arranged in two homologous series of saturated aliphatic γ- and δ-isomers.

Lactones are present in the fat partly in the free state and partly bound as precursors in the form of hydroxy acid glyceryl esters (3,6,9,10,13), from which they are split off on ageing or by heating. Their being bound in precursors as well as their occurrence in homologous series were for Boldingh and Taylor (6) indications of their biological formation. This view was supported by the finding that the lactones isolated from butterfat were optically active (6,8).

The presence of series of lactones has also been established in other fats. Dimick et al. (14) found δ-lactones with 10, 12, 14 and 16 C-atoms in the milk and depot fats of both ruminant and monogastric animals, while more recently Watanabe and Sato (15) demonstrated 18 aliphatic γ- and δ-lactones with 6–16 C-atoms in beef fats. Allen (16), investigating the volatile flavor components from coconut oil, found a series of δ-lactones with 6, 8, 10, 12 and 14 C-atoms.

As it was not established whether these lactones were optically active, it seemed to us important to study them in some animal and vegetable fats, paying special attention to their optical activity. This activity was compared with the optimal rotation measured in optically active lactones obtained by reduction of pure keto acids with yeast (Saccharomyces Cerevisiae) (17). The lactones investigated were those from the milk fats of cow and goat, and from the crude fats of coconut, palm kernel, palm fruit and babassu nut.

Experimental Procedures

Isolation and Concentration of the Lactone Mixtures

Free and bound lactones were separated from the fat by steam distillation for 4–5 hr at 180 C and a pressure of 2 mm Hg, the distillate being collected in a trap at −80 C. The trap contents were extracted with ether and the ethereal solution evaporated. The residue, consisting of lactones, fatty acids and unsaponifiable components, was saponified with a 10% aqueous KOH-solution. Unsaponifiable constituents were removed by extration with ether, after which the acids in the soap solution were liberated with sulfuric acid and recovered by ether extraction. After evaporation of the ether, the hydroxy acids present were lactonized by dissolving the acid mixture in benzene and refluxing the solution for ca. 1 hr, using a 60 cm Vigreux column. The water liberated from the hydroxy acids was distilled off with the benzene through the Vigreux column under reduced pressure. The residual mixture of lactones and fatty acids was dissolved in a 10-fold amount of light petroleum and the solution extracted twice with triethanolamine while shaking vigorously, once with an amount equivalent to that of the lactone and fatty acid mixture and once with half that amount; thus the fatty acids precipitated. The triethanolamine soaps were washed with light petroleum and the light petroleum solutions cooled in ice and filtered, using Hyflo as filter aid. The solutions were then collected and after evaporation of the solvent, the residual lactone concentrate was distilled in a cold-finger apparatus (8). The lactone mixtures, isolated in this way, were still contaminated with small amounts of higher fatty acids.

The composition of the overall lactone concentrate was determined by GLC analysis performed on an F & M 400 gas chromatograph, using a column of 5% polyethylene glycol adipate on Diatoport S (122 × 0.3 cm) at 170 C. As reference lactones were used δ-C_6 lactone and the γ- and δ-lactones with 8, 9, 10, 11 and 12 C-atoms.

Separation of Lactone Mixtures by Column Chromatography

The lactone concentrates were separated on a 30 × 1.5 cm column of 15 g silica gel (ex Mallinkrodt), inactivated with 10% water, and mixed with 7.5 g Hyflo. The three successive eluants used were 20% and 40% ether in isooctane (100–150 ml), and pure ether (50–100 ml). The eluates were collected in 10 ml fractions, each of which was investigated by gas chromatography. They contained, successively, the

TABLE I

Racemization of (+)-δ-Decalactone in Alkaline Methanol-Water Mixtures

Methanol, ml	Water, ml	NaOH, (g)	$[\alpha]_D^{20}$ Recovered lactone, degrees
10	90	22	(+)54.0
20	80	11	(+)54.0
20	80	22	(+)54.0
40	60	22	(+) 2.5
70	30	22	0

residual fatty acids, and the γ- and δ-lactones in the order of decreasing molecular weight.

The eluate fractions were combined according to the results of the GLC analysis. After distilling off the solvent, the amount, optical activity and composition of the combined fractions were determined.

Isolation and Identification of the Individual Lactones

To isolate individual lactones, the relevant fractions were subjected to gas chromatography (F & M 810), using a 100 × 1 cm column of 10% silicone oil on Diatoport S at 150–180 C. The lactones were collected in traps cooled with liquid nitrogen.

The chemical purity of the lactones was tested by GLC analysis. Moreover, the lactones from butterfat were purified via TLC on silica gel (Macherey, Nagel & Co., Düren) using ether-isooctane (40:60 v/v) as mobile phase, followed by distillation in a cold-finger apparatus.

The identity of the isolated lactones was established organoleptically by the characteristic odor, which disappeared on saponification and could be regained on acidification, as well as by comparison with model lactones via GLC and IR analysis.

Measurement of Optical Activity

The optical activity of the lactones was determined by measuring the rotation partly in a polarimeter equipped with a sodium lamp (Bellingham and Stanley Ltd. London) at 589 nm (= D line) and partly in a photoelectric precision polarimeter equipped for measurements at 578, 546, 436, 405 and 365 nm (LEP-A2, Carl Zeiss). Measurements were performed on solutions in benzene at room temperature (20–25 C). As a uniform measure of optical activity was adopted the specific rotation at 589 nm $[\alpha]_D$. The rotation at 589 nm was calculated using the Drude equation ($\alpha_\lambda = \frac{A}{\lambda^2-\lambda_0^2}$) and the measured rotation at 546 and 578 nm.

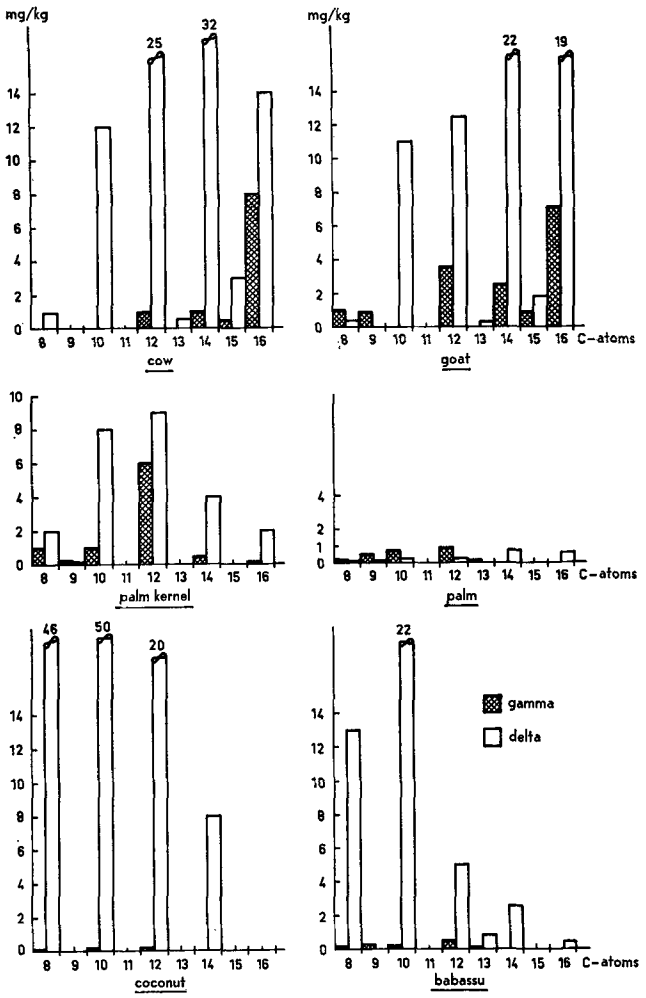

FIG. 1. γ-Lactones (☒) and δ-lactones (□) from cow and goat milk fat and various vegetable fats. The values for butterfat, coconut oil and palm-kernel oil refer to the mean of 4, 4 and 3 samples respectively.

Model Experiments

Possible racemization of the lactones during isolation and analytical manipulations was investigated in the following model experiments.

Experiment 1

Optically active δ-decalactone ($[\alpha]_D^{20}$; undiluted = (+) 57.2°), or δ-dodecalactone ($[\alpha]_D^{20}$ = (+) 48.8°), and glycerol were esterified to hydroxy acid glycerides, using *p*-toluene sulfonic acid as catalyst. The reaction mixture was taken up in ether, and the catalyst and excess glycerol washed out with brine. The ethereal solution was dried with anhydrous sodium sulfate and filtered, and the solvent distilled off. The glycerides were decomposed by heating in a shortpath distilling apparatus, and the lactones and glycerol distilled off. The optical rotation of the recovered lactones was (+) 56.1° and (+) 46.6° respectively.

Experiment 2

Four grams of δ-decalactone ($[\alpha]_D^{20}$, methanol = (+)54.1°) was refluxed for 6½ hr with different mixtures of alkaline methanol and water. After evaporation of the methanol, the lactones were recovered by acidifiction with sulfuric acid, and extraction with ether, followed by lactonization of the hydroxy acids. The optical rotations of the recovered lactones, measured in methanol, are collected in Table I.

Experiment 3

A solution of 120 mg (+)-δ-decalactone ($[\alpha]_D$ = 60.3°; benzene, c = 60 g/1) in 1 kg peanut oil was subjected to steam distillation and the distillate worked up according to the experimental procedure. The lipid fraction extracted from the distillate weighed 682 mg. After removal of unsaponifiable substances (174 mg) and the bulk of fatty acids, the residual fraction (156 mg) was distilled at 130 C; 15 mm Hg. The distillate weighed 99 mg. After separation by column chromatography, 92 mg lactone was recovered, the optical rotation $[\alpha]_D$ being (+)59°.

Influence of Solvent and Concentration on Optical Rotation

The optical rotations of undiluted (+)-δ-decalactone (s.g. = 0.970; $[\alpha]_D$ = 58.0°) and (+)-δ-dodecalactone (s.g. = 0.953; $[\alpha]_D$ = 50.1°) and solutions of these

TABLE 2

Influence of Solvent and Concentration on the Optical Rotation $[\alpha]_D$ (Degrees) of Lactones

Concentration, g/1	(+)-δ-Decalactone		(+)-δ-Dodecalactone	
	Benzene	Methanol	Benzene	Methanol
500	59.0	56.1	51.4	49.0
250	59.5	54.5	52.1	48.4
100	60.1	53.2	53.0	48.0
50	60.2	52.6	53.7	47.8
25	60.3	52.3	54.4	47.5
2.5	60.5	52.1	55.4	47.0

TABLE 3

Composition and Optical Rotation of Lactone Fractions From Coconut Oil Obtained by Column Chromatography

Eluate fractions	Residue on evaporation, mg	Optical rotation $[\alpha]_D$ benzene, degrees	Composition according to GLC analysis: Lactones, %					Other components, %
			δ-C_{14}	δ-C_{12}	δ-C_{10}	δ-C_8	δ-C_6	
1–5	0		....	....	....	....	....	....
6–12	62	0	....	....	....	....	....	100
13,14	0		....	....	....	....	....	....
15	18	(+) 9.5	84	14	....	....	....	2
16	28	(−) 6	23	76	....	....	....	1
17,18	54	(−) 4.5	4	62	33	....	....	1
19–21	147	(+)27	....	5	95	....	....	....
22	18	(+)44	....	....	35	65	....	....
23–29	131	(+)50	....	....	2	98	....	....
Ether total	17	(+)24	6	16	34	40	4	....

lactones were measured in benzene and methanol in decreasing concentrations. The calculated values of $[\alpha]_D$ are summarized in Table II.

Results and Discussion

Lactones Present in Various Fats

According to GLC analysis, the isolated lactone concentrates contained 80–95% lactones. The various amounts of lactones obtained from the different fats are represented in a block diagram (Fig. 1). It appears that both animal and vegetable fats contain series of γ- and δ-lactones, the δ-isomers being the largest components.

A similar relationship as that observed by Boldingh and Taylor (6) and Dimick et al. (14) between the presence of lower fatty acids and the lactone content in animal fats was also found in vegetable fats. Palm oil, obtained from the flesh of the fruit, hardly contains lower fatty acids and has a low lactone content, whereas palm-kernel oil contains a large amount of lower fatty acids and has a considerably higher lactone content.

Optical Activity of the Lactone Mixtures and the Individual Lactones

The optical activity of the isolated lactone concentrates, consisting of lactones and fatty acids, was measured in benzene in a concentration of 30–60 g/liter. From these figures and the lactone contents obtained by GLC analysis, the optical rotation $[\alpha]_D$ of the mixture of lactones was calculated and found to be as follows: Cow milk fat (four samples), (+) 35° ± 3°. Goat milk fat, (+)20°. Palm-kernel oil (three samples), (+)0.5°; (+)8°; (−)10°. Palm oil, not observable. Coconut oil (four samples), (+)30° ± 4°. Babassu oil, (−)12°.

Table III shows the analytical data of partial lactone fractions of the lactone concentrate from 3.34 kg coconut oil. The optical rotation of the lactones was measured in 2 ml benzene.

The lactone concentrates from the other fats were examined in a similar way. The boundary values for the optical rotation $[\alpha]_D$ (benzene) of the various lactone fractions can be summarized as follows: Cow milk fat, all fractions (+)14–40°. Goat milk fat, all fractions (+)11–40°. Palm-kernel oil, all fractions mainly containing γ-lactones (+)2–28°; other fraction (−)2–17°. Palm oil, not measurable. Coconut oil, the fraction that mainly contained δ-C_{12} lactone (−)6°; other fractions (+)5–50°. Babassu oil, all fractions (−)1–17°.

Most of the lactone fractions display a low optical rotation. According to GLC analysis, the fractions were not or were hardly contaminated, so it is unlikely that the low rotation is due to chemical impurity. Further it is shown that in palm-kernel oil and in coconut oil lactones with opposite optical rotation are present side by side.

Some of the lactones from butterfat, palm-kernel oil and coconut oil were isolated individually. The gas liquid chromatograms show only the peaks of the relevant lactones. According to IR analysis they are normal aliphatic saturated γ- and δ-lactones.

Table IV shows the amount of the lactones and their optical rotation $[\alpha]_D$ measured in benzene (2 ml) together with the optical rotation of some model lactones.

The $[\alpha]_D$-values of the lactones from the various fats appear to be different, and all are considerably lower than those of corresponding model lactones. Additional purification of the lactones from butterfat via thin layer chromatography and distillation did not increase the optical rotation, which is a clear indication that the lower $[\alpha]_D$-values are not due to chemical impurity. It must therefore be concluded that the lactones are not optically pure. Since no racemization of the lactones occurs during isolation and analytical manipulations, as was shown in the model experiments, it must be assumed that both optical antipodes of the lactones are formed simultaneously.

According to the rule that whenever optical materials are naturally formed, only one enantiomer is

TABLE IV

Optical Rotation $[\alpha]_D$ (Degrees) of Some Individual Lactones Measured in Benzene

Lactone	Isolated from: Butterfat		Coconut oil		Palm-kernel oil		Model lactones	
	$[\alpha]_D$	Conc. mg/2 ml	$[\alpha]_D$	Conc. mg/2 ml	$[\alpha]_D$	Conc. mg/2 ml	$[\alpha]_D$	Conc. g/liter
δ-C_{16}	(+)36	7.2	....	....	....	....	....	....
δ-C_{14}	(+)37.5	23.6	(+)11	15.0	....	....	....	....
δ-C_{12}	(+)44.5	44.8	(−) 8	39.5	(−)37	9.1	(+)54.4	25
γ-C_{12}	....	....	....	....	(+)34	8.1	(+)41.1[a]	50
δ-C_{10}	(+)44	24.6	(+)28	66.7	(−) 9	4.4	(+)60.3	25
δ-C_8	....		(+)51	81.8	(−)weak	3.4	(+)58.4[a]	22

[a] Measured in methanol (16). For influence of solvent and concentration, see Table II.

produced, it follows from the given data—the occurrence of levo- and dextrorotatory lactones side by side—that they must be formed via different pathways.

ACKNOWLEDGMENT

Miss A. J. Knoops, and K. A. Ploeg, J. H. Recourt and A. A. Memelink assisted in the experiments.

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[Received April 24, 1969]