CHROM. 4079

Thin-layer chromatography of some castor-based products

A variety of vinyl monomers derived from castor-based products has been prepared in this laboratory[1,2]. This necessitated the synthesis, as intermediates, of a large number of aliphatic acids and alcohols derivable from castor oil which varied in the nature and/or number of the substituents along the chain. The TLC behaviour of the large number of aliphatic acids and alcohols thus prepared from castor oil was investigated. The TLC behaviour of a few of these products has been reported in the literature[3], but in a different context.

Materials and methods

Methyl esters of castor oil fatty acids, prepared by methanolysis of castor oil[4], were hydrogenated over 1% Raney nickel at 100° and 150 p.s.i.g. Upon crystallisation from large volumes of *n*-hexane, the hydrogenated product furnished lustrous white plates of methyl 12-hydroxystearate, which on chromic acid oxidation gave the corresponding keto esters[5].

Sebacic and 10-undecenoic acids (Riedel, pure) were directly converted to their methyl esters without further purification.

11-Bromoundecanoic acid, obtained by anti-Markownikoff's addition of hydrogen bromide to the double bond of undecenoic acid[6], was converted to the corresponding iodo acid by refluxing the former with sodium iodide in acetone[7].

On treatment with sodium methoxide, the methyl ester of 11-bromoundecanoic acid gave methyl 11-methoxyundecanoate together with small amounts of methyl undecenoate that could be removed easily by fractional distillation[8]. 11-Hydroxyundecanoic acid was obtained in an overall yield of 70% by replacing the bromine of 11-bromoundecanoic acid with an acetoxy group and hydrolysing the acetylated product[9].

10,11-Dibromoundecanoic acid was obtained by addition of bromine to the double bond of undecenoic acid, and upon treatment with sodamide in liquid ammonia, gave 10-undecynoic acid in about a 40% yield[10]. The reaction of undecenoic acid with performic acid followed by hydrolysis of the resulting formoxy-hydroxyundecanoic acid gave 10,11-dihydroxyundecanoic acid in an almost quantitative yield[11].

Methyl esters of 11-hydroxy- and 10,11-dihydroxyundecanoic acids and *n*-amyl ester of 12-hydroxystearic acid were prepared by refluxing, for 8–10 h, a mixture of 0.1 mole of the acid, 0.2 mole of the alcohol, *p*-toluenesulphonic acid (2% by weight) and about 300 ml of benzene, the condensed water being removed azeotropically with benzene using a Dean-Stark trap[12]. This minimises the estolide formation often encountered in the esterification of hydroxy acids.

Undecenol and 1,10-decanediol were prepared by sodium reduction[13] of the corresponding methyl esters, while lithium aluminium hydride was used as reducing agent[14] for the methyl esters of 11-bromo-, 11-methoxy- and 10,11-dihydroxyundecanoic acids. 1,12-Octadecanediol was prepared more conveniently by first reducing the methyl esters of castor oil fatty acids with sodium, then hydrogenating the resulting alcohol and crystallising the hydrogenated product from large volumes

of *n*-hexane. 1,11-Undecanediol was prepared from 11-bromoundecanol via its diacetoxy derivative in a manner similar to that described for the preparation of 11-hydroxyundecanoic acid. 10,11-Dibromoundecanol was obtained by brominating undecenol in a carbon tetrachloride solution at −30° to avoid bromination of the hydroxyl group.

Solids were recrystallised to a constant and sharp melting point, and the liquids were fractionally distilled. The melting points, boiling points and refractive indices of the compounds, as determined by us, are given in Table I.

TABLE I

MELTING POINTS, BOILING POINTS AND REFRACTIVE INDICES OF THE CASTOR-BASED PRODUCTS PREPARED

Compounds	*M.P. (°C)*	*B.P. (°C/mm Hg)*	*Refractive index at (°C)*
Methyl 12-hydroxystearate	56.5–57.0	—	1.4390 (65)
n-Amyl 12-hydroxystearate	44.0–44.5	—	1.4420 (55)
Methyl 12-ketostearate	45.5–46.0	—	1.4356 (55)
Methyl undecenoate	—	122–124/10	1.4318 (30)
Dimethyl sebacate	—	174–176/20	1.4348 (30)
1,11-Diacetoxyundecane	—	162–164/1–2	1.4350 (30)
11-Bromoundecanoic acid[a]	49.5–50.0	—	1.4576 (75)
11-Iodoundecanoic acid[a]	65.5–66.0	—	1.4835 (75)
Methyl 11-methoxyundecanoate	—	122–124/0.5	1.4308 (30)
11-Hydroxyundecanoic acid[a]	65.0–65.5	—	1.4448 (75)
10-Undecynoic acid[a]	41.0–41.5	—	1.4442 (55)
Methyl 10,11-dihydroxyundecanoate	46.5–47.0	—	1.4492 (55)
Undecenol	—	122–124/3–4	1.4438 (30)
11-Bromoundecanol	46.0–46.5	—	1.4652 (55)
11-Methoxyundecanol	—	156–158/15	1.4418 (30)
10,11-Dibromoundecanol	—	—	1.5032 (30)
1,10-Decanediol	72.0–72.5	—	—
1,11-Undecanediol	60.5–61.0	—	1.4444 (75)
1,12-Octadecanediol	77.0–77.5	—	—
1,10,11-Trihydroxyoctadecane	74.0–74.5	—	—

[a] Methyl esters of these acids were prepared for the thin-layer chromatographic study using diazomethane.

Using a thin-layer applicator (Desaga, Heidelberg), glass plates (20 × 20 cm) were coated with a well-stirred suspension of Silica Gel G (E. Merck; 30 g in 60 ml water) to give a layer approx. 270 μ thick. The plates were dried and kept in a dust-free chamber. The compounds were dissolved in chloroform and spotted on the plate. After upward development in a tank, using an appropriate solvent system, the plates were removed, dried, sprayed with chromic acid and kept in an oven at 180–200° until clear dark spots appeared on the plates.

Results and discussion

The compounds were divided into two broad groups for this work, methyl esters and hydroxy compounds. A few products were common to both.

Fig. 1 shows the positions of various esters and that of a synthetic mixture of these on a thin-layer plate.

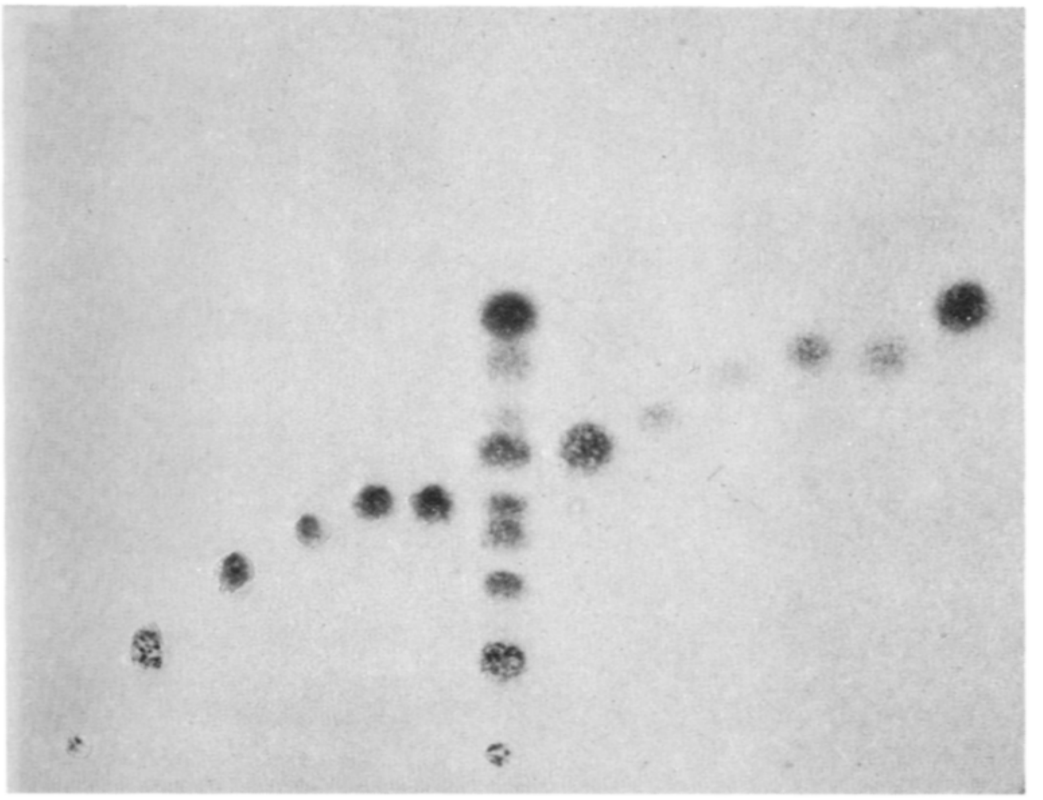

Fig. 1. Chromatogram showing the positions of various methyl esters and a synthetic mixture of these on TLC, where the solvent system was *n*-hexane-ethyl ether (65:35). From left to right the spots represent ($R_F \times 100$ in the parentheses): methyl 10,11-dihydroxyundecanoate (0) methyl 11-hydroxyundecanoate(14), methyl 12-hydroxystearate(24), *n*-amyl 12-hydroxystearate(30), 1,11-diacetoxyundecane(34), dimethyl sebacate (34), the mixture, methyl 11-methoxyundecanoate (41), methyl 12-ketostearate (46), methyl undecynoate (53), methyl 11-bromoundecanoate (55), methyl 11-iodoundecanoate (55) and methyl undecenoate (59).

While most of the compounds were resolved individually, 1,11-diacetoxyundecane and dimethyl sebacate moved together, as did 11-bromo- and 11-iodoundecanoates. Although the spot corresponding to undecynoic acid is found apart from those of the latter two compounds, it could not be identified separately in the mixture. However, a clear separation of undecenoic and undecynoic acids was obtained.

The methyl and *n*-amyl esters of 12-hydroxystearic acid could be separated from each other and from the methyl esters of 11-hydroxy- and 10,11-dihydroxyundecanoic acids; the last compound did not move from the starting point. The effect of various substituents in the chain of undecanoic acid on its mobility on the thin-layer plate can be generalised. The functional groups produce a retarding effect in the order: $-OH > -OCH_3 > I/Br$. The $R_F \times 100$ values for the compounds are given in parentheses in the legend to Fig. 1.

Fig. 2 shows the positions of various alcohols and hydroxy acid esters and of a synthetic mixture of these.

Here also various substituents in the chain of undecanol have the same effect as noted earlier on thin-layer mobility. As expected, 11-methoxyundecanol and methyl 11-hydroxyundecanoate moved together, as did mono- and di-bromoun-

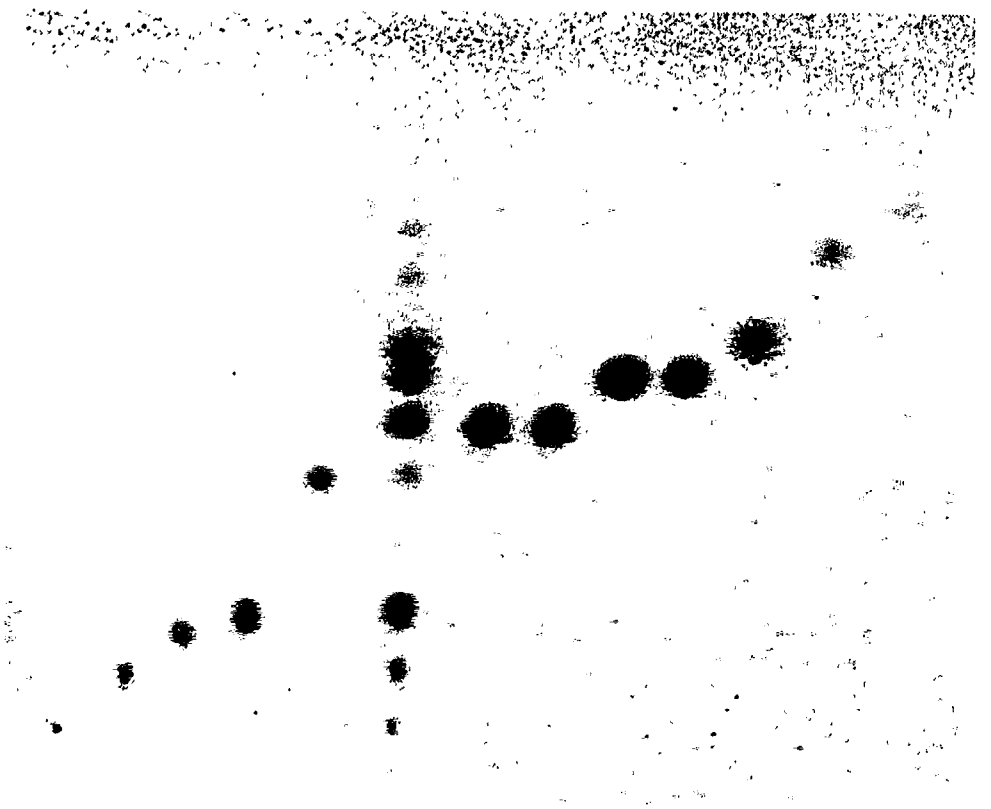

Fig. 2. Chromatogram showing the positions of various alcohols and hydroxy acid esters and of a synthetic mixture of these on TLC, where the solvent system was *n*-hexane–ethyl ether (25:75). From left to right the spots represent ($R_F \times 100$ in the parentheses): 1,10,11-trihydroxyundecane (3), methyl 10,11-dihydroxyundecanoate (11), 1,10-decanediol (18), 1,11-undecanediol (20), 1,12-octadecanediol (38), the mixture, methyl 11-hydroxyundecanoate (46), 11-methoxyundecanol (46), 11-bromoundecanol (53), 10,11-dibromoundecanol (53), undecenol (58), methyl 12-hydroxystearate (67) and *n*-amyl 12-hydroxystearate (74).

decanoates. Moreover, the difference between the mobilities of 1,10-decanediol and 1,11-undecanediol was too slight to enable their resolution. The $R_F \times 100$ values for these compounds are given in parentheses in the legend to Fig. 2.

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Separation of vitamin D from cholesterol by thin-layer chromatography

Vitamin D* which represents the group of physiologically important 9,10-secosterols, otherwise known as open-ring B sterols, exhibits a high degree of biological activity. Since these substances occur in extremely low concentrations in animal tissues, they present a difficult problem[1], generally beyond the scope of conventional physiochemical techniques of analysis. During the course of our studies on this 'trace lipid', we developed in this laboratory highly sensitive gas–liquid chromatographic techniques for its detection in the nanogram range[2–4]. Although several purification techniques have appeared in the literature[5–8], the present paper describes a rapid and simple thin-layer chromatographic system for the prepurification of vitamin D in biological extracts prior to its determination by GLC.

Experimental

Sorbents and reagents. Silica Gel G, Silica Gel HF_{254}, and Aluminium Oxide G were obtained from E. Merck, Darmstadt, Germany. All solvents used in the study were of ACS grade, marketed by Fisher Scientific Company. The marker dye, 2,4-diaminoazobenzene, was purchased from K & K Laboratories, New York. Rhodamine 6G and antimony trichloride were obtained from the Fisher Scientific Company.

General procedure. A mixture of 30 g of sorbent and 66 ml of water was shaken vigorously for about 40 sec, while under partial vacuum created by a water pump. The slurry was immediately spread over five 20 × 20 cm plates at a thickness of 300 μ in a Shandon adjustable spreaderunoplan leveller. The plates were left at room temperature for about 30 min after which they were activated for 2 h at 110°, and stored in Brinkman aluminum carrier racks placed in metal desiccating cabinets (Arthur Thomas & Co., Philadelphia, Pa.).

The plates were generally divided into three vertical lanes, 6 cm wide, terminating on a horizontal line drawn 15 cm from the origin. Samples were spotted on a horizontal line with a 50 μl semi-automatic microsyringe (Hamilton Co., Inc., Whittier, Calif.), and developed in tanks which were previously equilibrated for 1 h with 170 ml of

* The term "vitamin D" refers to both ergocalciferol (vitamin D_2) and cholecalciferol (vitamin D_3).