A new method for the detection of diglycerides on a microscale

Work involving the isolation and identification of diglycerides from natural sources can be simplified by the use of paper chromatography. This is well exemplified in the phospholipid field where acid hydrolysis of glycerophosphatides usually yields diglycerides. A new method is described for the detection of diglycerides in microgram quantities by paper chromatography.

It is well known that hydroxylamine reacts with fatty acid esters under alkaline conditions to yield hydroxamates and the free alcohols. When treated in this way, diglycerides give rise to glycerol which can be detected in amounts of ~ 2 μg using the periodate–Schiff's spray reagent as modified for α-glycols by BADDILEY *et al.*[1]. The experimental procedure described allows 5–10 μg of diglyceride (DG) to be detected on paper chromatograms. As such small amounts of DG are detected, reactions liberating if can be followed on a microscale. It is also possible to distinguish between monoglyceride (MG) and DG without the need for standards, since, due to the presence of an α-glycol grouping, MG is detected when sprayed directly with the modified periodate–Schiff's spray and DG is not.

Experimental

Samples of monostearin (MG) and distearin (DG) (*e.g.*, 20 μg in 20 μl) were spotted in duplicate on Whatman No. 1 paper (40 × 15 cm) impregnated with formaldehyde according to HÖRHAMMER *et al.*[2]. Chromatograms were developed for 12 h by the ascending technique in the solvent[2] for phospholipid separation. The solvent front moved about 25 cm at room temperature (22°). Chromatograms were dried and divided lengthwise into two strips (A and B) to obtain spots of MG and DG on each strip. Strip A was sprayed with aqueous alkaline hydroxylamine solution. The spray reagent was prepared by mixing equal volumes of aqueous solutions of 2.5 *M* sodium hydroxide and 2 *M* hydroxylamine hydrochloride. After 30 min, A was sprayed with 3 *M* hydrochloric acid and dipped for 5 sec in water and then allowed to dry. A and B were sprayed with 1 % aqueous sodium metaperiodate solution and after 6 min were treated with sulphur dioxide gas until the liberated iodine was reduced, giving a white background. Immediate spraying with a decolorised, 1 % aqueous solution of pararosaniline hydrochloride, followed by treatment with sulphur dioxide gas gave rise to mauve spots due to DG and MG on A and a spot due only to MG on B. The detection limit for DG was 5–10 μg.

The fact that the glycerol produced by the hydroxylamine treatment was not dissolved out of the paper by the washing indicated that it was probably complexed to unreacted DG or to the paper.

In the system described, the R_F of distearin was 0.95–0.99, spotting 50–5 μg, whereas that of monostearin was 0.75–0.85. In comparison, the R_F values of glycerol and myoinositol were 0.3–0.4 and 0.02 respectively. The myoinositol showed up as a greyish purple spot 15 min after the last sulphur dioxide treatment, whilst the mauve spots appeared in less than 3 min.

When MG and DG were spotted together, the MG R_F rose to $\sim$ 0.86. If $>$ 50 μg of DG were spotted, slight streaking was observed.

Discussion

The described technique identified and distinguished between microgram amounts of DG and MG. It has certain advantages over other published paper chromatographic detection methods. The sensitivity of diglyceride detection is of the same order as that obtained by the use of Rhodamine 6G[3] and is greater than in the pancreatin–α-dextrin–I_2 method[4]. The method is less laborious, it is quicker, and needs less precautions than the system used by MANGOLD *et al.*[4]. Whereas the detection method used by RENKONEN AND RENKONEN[3] depends on the lipid nature of the whole molecule, our method indicates an atomic grouping within the molecule. It can be extended to any fatty acid esters which yield an α-glycol on treatment with alkaline hydroxylamine provided that the appropriate chromatographic paper and solvent system are chosen.

Application

Simple monophosphoinositides, when heated with 99 % acetic acid[5], yield diglycerides and inositol phosphates. This reaction was applied to horse liver monophosphoinositide (1 mg). Evaporation of the acetic acid yielded material, easily identified as DG by the above technique. This enabled the position of the fatty acid groups to be determined, since it excluded the possibility of a fatty acid being esterified to the myoinositol residue. In conjunction with other work[6], this knowledge confirmed the structure of horse liver monophosphoinositide to be a di-O-acyl glycerol-1-(L-myoinositol-1-phosphate). The probable migration of the acyl group in the acetic acid reaction did not influence the R_F of the resultant diglyceride. Distearin was used as the standard DG and after treatment under the conditions of the reaction its R_F had not altered.

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University Chemical Laboratory, B. F. C. CLARK
Lensfield Road, Cambridge (Great Britain)

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