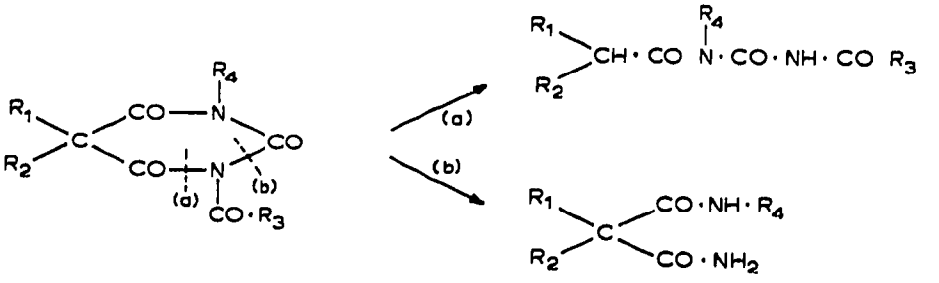
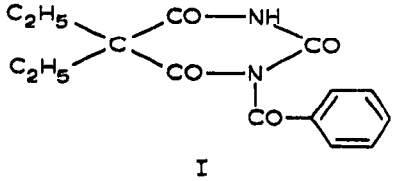
CHROM. 4731

Separation of the degradation products of 1-benzoyl-5,5-diethylbarbituric acid by means of vapour programmed thin-layer chromatography

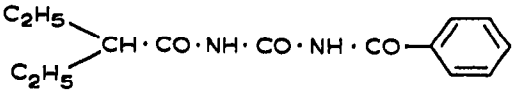
It has previously been established that during the alkaline hydrolysis of N-acyl derivatives of barbituric acid the splitting of the ring occurs near to the acyl radical[1,2], as a result of which the corresponding derivatives of acetylurea (a) and the diamide of malonic acid (b) are formed



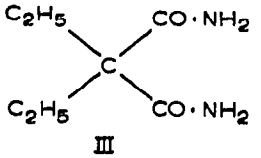
Investigation of the alkaline hydrolysis of 1-benzoyl-5,5-diethylbarbituric acid (I) showed that the degradation products included N-benzoyl diethylacetylurea (II), diethylmalonic acid diamide (III), diethylmalonic acid monoamide (IV) and diethylmalonic acid (V).



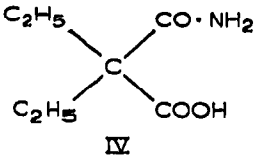
I



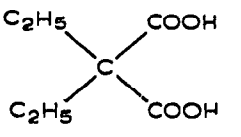
II



III



IV

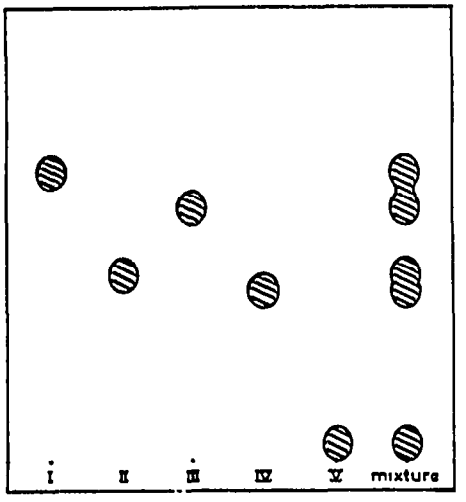


V

Attempts were made to separate and identify these compounds by means of TLC but in spite of many variations in the chromatographic systems it was not possible to resolve compounds I and III and II and IV. The best separation possible was that using silica gel and ethanol–isopropanol (75:25) as solvent system with a development distance of 15 cm. The results are shown in Fig. 1.

Recent work by DE ZEEUW[3,4] has shown that the solvent vapour in contact with the adsorbent has considerable influence on the separation process and he designed a special development chamber whereby the method known as vapour programmed TLC can be carried out under reproducible conditions to give a more efficient resolution of compounds than can be achieved with the conventional saturated or unsaturated chambers.

Application of vapour programmed TLC to the five compounds under discussion gave very good separations, as shown in Fig. 2. Reproduction of the separation was extremely good, the R_X values (1-benzoyl-5,5-diethylbarbituric acid = 1) being



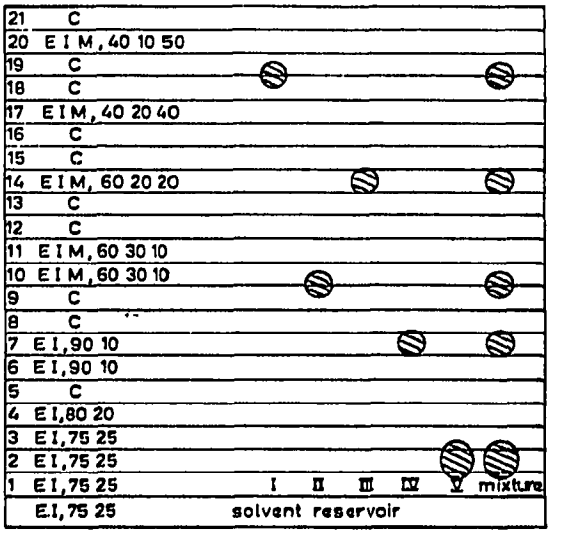


Fig. 1. Chromatogram of a mixture of compounds separated by conventional TLC. Development over 15 cm, solvent system, ethanol–isopropanol (75 25) I = 1-Benzoyl-5,5-diethylbarbituric acid; II = N-benzoyl diethylacetylurea, III = diethylmalonic acid diamide, IV = diethylmalonic acid monoamide, V = diethylmalonic acid.

Fig. 2. Chromatogram of a mixture of compounds separated by vapour programmed TLC. C = Chloroform, E = ethanol, I = isopropanol, M = methanol. I = 1-Benzoyl-5,5-diethylbarbituric acid; II = N-benzoyl diethylacetylurea, III = diethylmalonic acid diamide, IV = diethylmalonic acid monoamide, V = diethylmalonic acid

(II) 0.50, (III) 0.77, (IV) 0.35, and (V) 0.07. Moreover, the spots were round and compact without tailing and this was important having regard to the need for their quantitative determination by means of densitometry[5].

Experimental

Layer: MN Silica Gel G (Macherey, Nagel & Co.).

Plate size: 20 × 20 cm.

Layer thickness: 250 mμ.

Activation: Air dried for 15 min, heated at 110° for 1 h, stored on a desiccator over silica gel and reheated at 110° for 15 min prior to use.

Solvents: ethanol, isopropanol, methanol, chloroform (all redistilled).

Running distance: 20 cm (time, approx. 60 min).

Temperature: 20–22°; temperature of evaporating unit 27–28°.

Load: 4 μl 0.2% solution in methanol.

Spray reagent: 0.05% dithizone in carbon tetrachloride.

The troughs of the vapour phase TLC chamber were partially filled (5 ml) with mixtures of the solvents as indicated in Fig. 2. The running solvent was ethanol–isopropanol (75:25) and this was introduced into the solvent reservoir after equilibration had been allowed to proceed for 10 min.

We would like to thank Mr. R. BROWN for constructing the vapour programme TLC chamber (from the description given by DE ZEEUW[3,4]). One of us (M.M.) would also like to thank the Chelsea College for awarding her a post doctoral research scholarship enabling her to work in the pharmacognosy research laboratories of the College.

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CHROM. 4698

Thin-layer chromatography of substituted methyl β-maltosides

During the synthesis[1] of substituted methyl β-maltosides, extensive recourse was made to chromatography. This note records the chromatographic behavior of these maltoside derivatives; both thin-layer (TLC) and dry-column[2] chromatography are reported.

Experimental

Thin-layer plates (0.25 mm thickness) were prepared with a Quickfit Instrument* apparatus from a slurry of Silica Gel G (25 g) and water (50 ml). Before use, the plates were air dried, horizontally, for 16 h. Solvents were purified by distillation. Plates were developed until the solvent front had ascended 13 cm above the spotting site. Benzene–absolute ethanol (2:1) was used to develop the unacylated maltosides, and toluene–methanol (50:1) for the acylated maltosides as well as for multiple ascents. Spots were detected by spraying with a solution of ethanol–water–concentrated sulfuric acid (10:5:1) and heating until charred. All spots turned black upon charring except the deoxy-maltosides, which charred dark brown after changing from various shades of yellow. Iodine vapor also located the *p*-tolylsulfonyl (tosyl) maltosides when a nondestructive method was needed.

Dry-column chromatography was used to isolate the tosylated maltosides (compounds 3, 7, and 11, Table I). A 3-g sample of the partially tosylated reaction mixture of methyl β-maltoside was introduced onto the dry column by first dissolving the mixture in a slurry consisting of 15 g of Davison Grade 12 silica gel and 50 ml 95% ethanol. After the ethanol had evaporated, the residue was introduced on top of a dry Silica Gel G column (200 g, 4 × 40 cm) and the column was developed with

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