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Note

Thin-layer chromatography of chloramphenicol and thiamphenicol

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The gas chromatographic determination of chloramphenicol and thiamphenicol without derivatization has been reported[1,2], but under certain circumstances chloramphenicol is completely degraded[3]. However, the gas–liquid chromatography (GLC) of the bis-trimethylsilyl (TMS) ether of chloramphenicol can be readily performed[2–7] and is the most suitable method for the quantitative analysis of this compound. Some problems related to the preparation of this derivative have been described[6,7]. Similarly, the bis-TMS ether of thiamphenicol can be chromatographed[1,4,8,9]. During our investigation of the GLC of the bis-TMS ether of thiamphenicol and its *erythro* isomer, we found that both diastereoisomers contained some monochloro analogue[9]. However, thin-layer chromatography (TLC) proved that this impurity is an artifact formed during the silylation.

Numerous TLC systems have been reported for the analysis of chloramphenicol and have been used to check its purity during its synthesis[10], to investigate its degradation products[11–15] and to separate it from the latter for quantitative determination[13,16] and to identify it in complex mixtures[17–28], possibly followed by quantitative determination[29–31]. Inorganic adsorbents, such as silica gel[11–17,20–25,29,30], silanized silica gel[31], a silica gel and Kieselguhr mixture[18], aluminium oxide[10] and organic adsorbents, such as cellulose[17,26,27] and polyamide[19], in combination with a great variety of solvent mixtures, have been used. Only three reports on the TLC analysis of thiamphenicol have been published[22,23,32].

In this paper, we report the separation of thiamphenicol, chloramphenicol and their monochloro analogues by TLC on silica gel using ethyl acetate and ethyl acetate–methanol as the mobile phase.

EXPERIMENTAL

TLC was carried out on pre-coated plates of silica gel 60 F_{254}, layer thickness 0.25 mm, height 20 cm (Merck, Darmstadt, G.F.R.). Detection was accomplished by quenching of 254 nm-induced fluorescence. The substances were applied as 1% solutions in acetone, usually in amounts of 10–25 μg, and ethyl acetate and ethyl acetate–methanol mixtures were used as the mobile phase.

Thiamphenicol (Zambon, Milan, Italy) and chloramphenicol (Lepetit, Milan, Italy) were commercial samples. The DL-*erythro* isomer of thiamphenicol and 1-(*p*-methylsuphinylphenyl)-2-dichloroacetamido-1,3-propanediol were supplied by Prof.

D. Della Bella (Zambon). The D-*erythro* isomer of chloramphenicol was prepared from chloramphenicol, which is the D-*threo* isomer[6]. D-*threo*-1-(*p*-Methylsulphonylphenyl)-2-monochloroacetamido-1,3-propanediol and D-*threo*-1-(*p*-nitrophenyl)-2-monochloroacetamido-1,3-propanediol were obtained by acylation of the corresponding amines.

D-threo-1-(p-Methylsulphonylphenyl)-2-monochloroacetamido-1,3-propanediol

To a solution of 490 mg of D-*threo*-1-(*p*-methylsulphonylphenyl)-2-amino-1,3-propanediol, prepared from thiamphenicol[33], and 0.28 ml of triethylamine in 80 ml of acetone, was added 1.6 ml of a 10% solution of monochloroacetyl chloride in acetone, and the resulting mixture was stirred for 1 h at room temperature. After evaporation of the solvent, recrystallization of the residue from ethylene dichloride yielded 400 mg of D-*threo*-1-(*p*-methylsulphonylphenyl)-2-monochloroacetamido-1,3-propanediol, m.p. 137.5–138.5°; $[\alpha]_D^{20} = -12°$ ($c = 1$, ethanol); mass spectrum, very weak molecular ion at *m/e* 321, ($M - H_2O$) ion at *m/e* 303, with a fragmentation pattern similar to that of thiamphenicol.

D-threo-1-(p-Nitrophenyl)-2-monochloroacetamido-1,3-propanediol

The acylation of 1 g of D-*threo*-1-(*p*-nitrophenyl)-2-amino-1,3-propanediol (Gist-Brocades, Delft, The Netherlands) was carried out as described above. After completion of the reaction, the solvent was evaporated and the residue was partitioned between water and ethyl acetate. The ethyl acetate solution was washed with water and dried. Evaporation of the solvent and recrystallization of the residue from a mixture of ethyl acetate and ligroine gave D-*threo*-1-(*p*-nitrophenyl)-2-monochloroacetamido-1,3-propanediol (0.8 g), m.p. 90.5–92° (a m.p. of 83–86° for the crude compound has been reported[34]); $[\alpha]_D^{20} = -12°$ ($c = 1$, ethanol); mass spectrum, very weak ($M + 1$) ion at *m/e* 289, ($M - H_2O$) ion at *m/e* 270, with a fragmentation pattern resembling that of chloramphenicol[3,35].

RESULTS

The compounds were readily separated and the R_F values are indicated in Table I. Amounts of 10–25 μg were usually applied, but amounts up to 250 μg can be used. A thin-layer chromatogram of 100 and 250 μg of thiamphenicol did not show any 1-(*p*-methylsulphonylphenyl)-2-monochloroacetamido-1,3-propanediol, although 1 μg of the latter can be detected. Similarly, the *erythro* isomer of thiamphenicol contained no monochloro analogue.

TLC revealed the presence of a small amount of 1-(*p*-methylsulphinylphenyl)-2-dichloroacetamido-1,3-propanediol in the *erythro* isomer of thiamphenicol. This impurity was also detected by GLC[9].

Although the R_F values of thiamphenicol and its *erythro* isomer differ slightly, a mixture of these diastereoisomers was not separated even with ethyl acetate, which gives the greatest resolution. Amounts of 10% and more of the *erythro* isomer in chloramphenicol, or *vice versa*, were easily detected on a thin-layer chromatogram of 10-μg samples developed with ethyl acetate. The limit of detection was increased when ethyl acetate–methanol mixtures were used and upon increasing the amount of sample applied; only the presence of at least 25% of each isomer could be detected

TABLE I

R_F VALUES OF CHLORAMPHENICOL, THIAMPHENICOL AND SOME RELATED COMPOUNDS

Compound	*Solvent system*				
	Ethyl acetate	*Ethyl acetate–methanol (99:1)*	*Ethyl acetate–methanol (98:2)*	*Ethyl acetate–methanol (95:5)*	*Ethyl acetate–methanol (90:10)*
Chloramphenicol	0.50	0.54	0.58	0.67	0.74
erythro-Chloramphenicol	0.54	0.57	0.61	0.69	0.75
D-*threo*-1-(*p*-Nitrophenyl)-2-monochloroacetamido-1,3-propanediol	0.28	0.33	0.39	0.53	0.66
Thiamphenicol	0.35	0.39	0.41	0.55	0.66
erythro-Thiamphenicol	0.36	0.40	0.42	0.56	0.67
D-*threo*-1-(*p*-Methylsulphonylmethyl)-2-Monochloroacetamido-1,3-propanediol	0.16	0.19	0.23	0.37	0.54
DL-*erythro*-1-(*p*-Methylsulphinylphenyl)-2-dichloroacetamido-1,3-propanediol	0.05	0.06	0.09	0.16	0.32

on a 10-μg sample in ethyl acetate–methanol (98:2) and of about 50% on a 25-μg sample in ethyl acetate alone. GLC separated chloramphenicol and thiamphenicol from their *erythro* isomers, and the limits of detection were lower[6,9].

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