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Thin-layer and paper chromatography of dinitrophenyl derivatives of amino acids from bacterial cell wall peptidoglycans

Recent studies on the structure of the diaminopimelic acid containing peptidoglycan from *Bacillus stearothermophilus* cell walls[1] have led to improvements in the thin-layer and paper chromatographic separation of 2,4-dinitrophenyl (DNP)-derivatives of the amino acid constituents of these cell walls which are reported here.

Detection and identification of very small quantities of DNP-amino acids on chromatograms is often facilitated by viewing under UV light. This increases the apparent intensity of the spots and shows up certain marked colour differences between the various derivatives, thus improving ease of identification. However, the thin layers of silica gel usually used[2,3] for chromatography of DNP-amino acids give an intense purple fluorescence under UV light which masks the presence of very faint spots and decreases the colour contrasts. We have found that thin layers prepared from a mixture of cellulose and silica gel give a much lower degree of fluorescence and preserve the colour contrasts between the various derivatives. Such layers are also more robust and may easily be marked with a soft pencil without damage to the layer surface.

DNP-derivatives of amino acids from bacterial cell wall peptidoglycans are only poorly resolved by one-dimensional thin-layer chromatography. Dinitrophenol and DNP-alanine migrate close to the solvent front in the system of GHUYSEN *et al.*[3] and are difficult to separate, while DNP-glycine and DNP-serine are invariably only partially resolved. DNP-glutamic acid and di-DNP-diaminopimelic acid move too slowly in most solvents to be absolutely distinguished from each other. The two-dimensional system described here eliminates these difficulties, is relatively rapid, and incorporates the advantages of the mixed silica gel–cellulose layer for the detection of very small quantities of DNP-amino acids.

Thin layers (0.25 mm) of cellulose–silica gel were conventionally prepared from a mixture of 10 g Cellulose MN300 (Macherey, Nagel & Co.) and 4 g Silica Gel H (Merck) homogenised with 80 ml water for 10 min in a Waring Blendor (these quantities are sufficient for five 20 × 20 cm plates). When set (20 min) the plates were dried overnight at 37 °.

Reference markers and unknown mixtures were chromatographed together in the manner depicted in Fig. 1. Development of chromatoplates in the 1st dimension employed two solvent systems successively, *viz.* (I) isopropanol–acetic acid–water (75:10:15)[4] for 15 min and, after drying in a stream of cold air, (II) *n*-butanol–0.15 *N* ammonium hydroxide (1:1, upper phase)[5] until the solvent front approached the top of the plate (approx. 3½ h). The layer was scraped off to a level 2 cm below the front of solvent II and the plate thoroughly dried in air. A second set of reference markers was applied as indicated in Fig. 1 and the plate developed in the second dimension in solvent III, 1.5 *M* sodium phosphate buffer, pH 6.0[6], until the front approached the first set of reference markers (approx. 1½–2 h). All operations were carried out in a darkened room. DNP-alanine values for various DNP-amino acids in this system are shown in Table I. The initial partial development of chromatoplates in solvent I was devised to separate mono-DNP-diaminopimelic acid from DNP-glutamic acid. While,

theoretically, mono-DNP-diaminopimelic acid can be separated from mixtures of other DNP-amino acids by extraction of the latter into ether, in practice some contamination of the ether phase by the aqueous phase is inevitable when working on a micro scale. In such cases traces of mono-DNP-diaminopimelic acid can be erroneously identified as DNP-glutamic acid. Where mono-DNP-diaminopimelic acid is known to be absent the partial development of chromatoplates with solvent I can be omitted with little change in the pattern depicted in Fig. 1.

Preparative separation of larger quantities of DNP-amino acids on paper chromatograms generally requires the inclusion of buffer salts in the developing solvent and paper to prevent tailing[7]. Subsequent elution of separated components from paper

TABLE I

CHROMATOGRAPHY OF DNP-AMINO ACIDS ON THIN LAYERS OF CELLULOSE–SILICA GEL AND WHATMAN NO. 3MM PAPER

	$R_{DNP\text{-}alanine}$		
	Cellulose–silica gel		*Paper*
Solvent system	*I + II*	*III*	*IV*
DNP-glutamic acid	0.45	1.57	0.80
DNP-glycine	0.74	0.83	0.61
DNP-serine	0.76	1.26	0.62
Di-DNP-diaminopimelic acid	0.59	0.52	0.29
Mono-DNP-diaminopimelic acid	0.30	1.61	0.08
Dinitrophenol	1.13	0.82	1.30

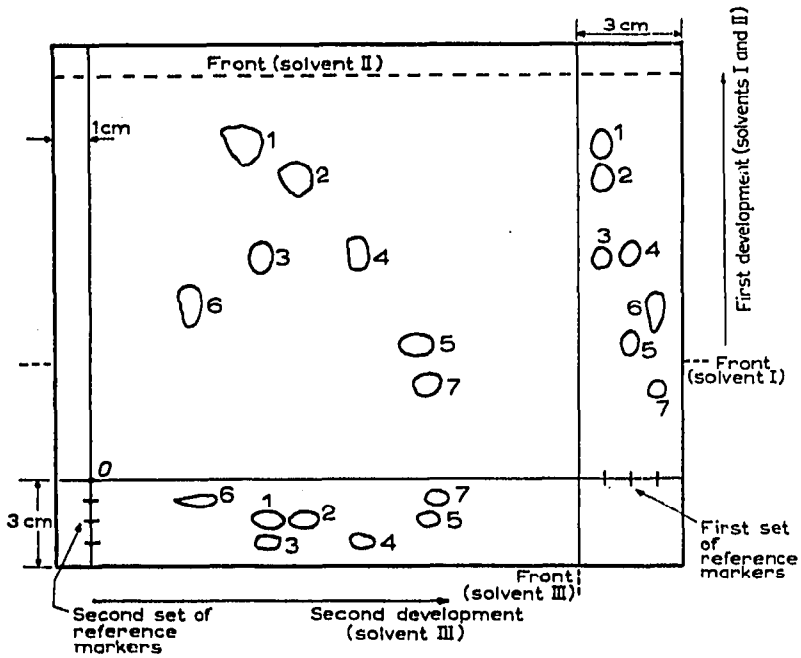

Fig. 1. Tracing of a two-dimensional separation of DNP-amino acids on a cellulose–silica gel thin-layer plate. 1 = Dinitrophenol, 2 = DNP-alanine, 3 = DNP-glycine, 4 = DNP-serine, 5 = DNP-glutamic acid, 6 = di-DNP-diaminopimelic acid, 7 = mono-DNP-diaminopimelic acid, O = origin of mixture of 1–7.

must be followed by a desalting procedure, with consequent loss of material, if the pure compound is required. We have found that this difficulty can be obviated by the use of the completely volatile solvent system IV, *tert.*-amyl alcohol–pyridine–acetic acid–water (110:1.0:0.08:25). Descending development on washed Whatman No. 3MM paper for 30 h gave good resolution, without tailing, of DNP-amino acids with the exception of the DNP-glycine and DNP-serine pair (Table I).

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1 W. D. GRANT AND A. J. WICKEN, *Biochem. J.*, submitted for publication
2 M. BRENNER, A. NIEDERWIESER AND G. PATAKI, in E. STAHL (Editor), *Thin-layer Chromatography—A Laboratory Handbook*, Springer-Verlag, Berlin, Göttingen, Heidelberg, 1965, p. 391.
3 J.-M. GHUYSEN, D. J. TIPPER AND J. L. STROMINGER, *Methods Enzymol.*, 8 (1966) 685.
4 K. H. SCHLEIFER AND O. KANDLER, *Arch. Mikrobiol.*, 57 (1967) 335.
5 J.-M. GHUYSEN, D. J. TIPPER, C. M. BIRGE AND J. L. STROMINGER, *Biochemistry*, 4 (1965) 2245.
6 A. L. LEVY, *Nature*, 174 (1954) 126.
7 S. BLACKBURN AND A. G. LOWTHER, *Biochem. J.*, 48 (1951) 126.

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Spray detection of bile acids on thin-layer chromatograms

The detection of bile acids in thin-layer chromatography (TLC) has been accomplished by means of spray reagents: namely, phosphomolybdic acid[1], antimony trichloride[2] or concentrated sulfuric acid[3]. But the usefulness of color detection in bile acid identification has been demonstrated by KRITCHEVSKY *et al.*[4] and by ANTHONY AND BEHER[5]. The present study expands on the use of color detection, thus aiding in the identification of bile acids.

Materials and methods

The bile acids were obtained from Applied Science Laboratories, Inc., Pa., and isooctane, isopropyl alcohol and acetic acid were all Baker Analysed Reagent grade. A thin-layer plate precoated to a thickness of 0.5 mm with Silica Gel F_{254} (Merck) and a commercial chromatographic chamber (Gelman Instrument Co., Ann Arbor, Mich.) were used. The plates were heated at 100° for 1 h and the chamber was saturated with the solvent isooctane–isopropyl alcohol–acetic acid (40:10:1) prior to the chromatogramphy at room temperature (23–25°). When chromatograms were run, 20 × 20 cm plates were used and the solvent front was permitted to rise 135 mm from the origin. The chromatoplate was then removed from the chamber, air dried for several minutes and then dried thoroughly at 100° for 10 min. After cooling, the plate was exposed to iodine vapor for 15 min, sprayed with tap water and the color was