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Estimation of urinary *p*-hydroxyphenyllactic acid by gas chromatography

The excretion of *p*-hydroxyphenyllactic acid (PHPL) is frequently raised in stress and in a wide variety of pathological conditions.[1] Paper chromatography has proved useful for qualitative work and for rough quantitative estimations when excretion is high but inadequate for the determination of normal rates of excretion which appeared, however, to be of the order of 0–5 μg/mg urinary creatinine[1]. Preliminary experiments on the gas chromatography of trimethylsilyl derivatives of phenolic acids were carried out utilising columns containing the liquid phases OV-1, OV-17, SE-30, SE-52, XE-60, QF-1 and polyphenylether (six ring); the most promising separations of PHPL appeared to be obtained with OV-1. An initial study of urinary extracts chromatographed under isothermal conditions indicated minimal interference by other compounds on PHPL peaks when the conditions described below were employed.

Urines were collected from 20 male subjects controlled only to the extent that they appeared healthy and did not deviate from their normal habits with regard to diet and physical activity; the samples were collected at times distributed as evenly as possible throughout the day. Each urine (5 ml) was adjusted to pH 1 (HCl) and shaken with ether (3 × 10 ml) after the addition of $(NH_4)_2SO_4$ (3 g). The dried (Na_2SO_4) evaporated extract was silylated (18 h) with hexamethyldisilazane–pyridine (1:1; 0.5 ml). For quantitative purposes a duplicate extract was prepared after addition of PHPL (20 μg) to the urine (5 ml).

Aliquots (10 μl) were chromatographed on a 2.7 m × 4 mm I.D. column of OV-1 (10% on Diatoport S) at 190° using an argon flow of 50 ml/min and a flame ionisation detector. Typical chromatograms are illustrated in Fig. 1. Calculations of PHPL concentrations utilised the linear relationship between peak height and quantity. Excretion of PHPL was found to be 0.5–3.5 μg/mg urinary creatinine (mean 1.69; S.D. 0.695). This agreement with results indicated by paper chromatography suggested the absence from normal urine of overlapping peaks which might

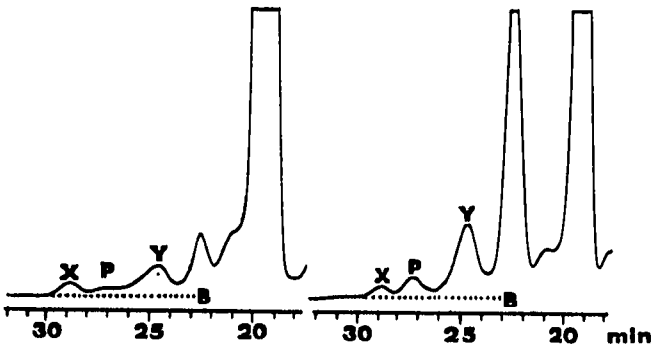

Fig. 1. Details from chromatograms of urine extracts. Extracts were silylated and chromatographed on 10% OV-1 columns, 2.7 m long at 190°. The large peak on the right was due to hippuric acid. The peak P due to PHPL was satisfactorily, though not quite completely, separated from peaks X and Y. Peak heights were measured from a base line B arbitrarily drawn from the trough following peak X. Excretions of PHPL were calculated to be 1.37 and 3.48 μg/mg urinary creatinine in the urines represented by the left-hand and right-hand chromatograms respectively.

interfere with the detection of moderately elevated levels of PHPL. Such levels could be confirmed by chromatography on columns of OV-17 though some caution was required since in some normal urines the PHPL peak was evidently heterogeneous.

In connection with more general studies on the aromatic constituents of urine the behaviour of PHPL under temperature programmed conditions was examined, using columns of OV-1 (10%) of length 1.5 (discarded after preliminary experiments), 2.7, 3.4, 4 and 5.5 m. Results were compatible with the following conclusions. First, overlap between PHPL and peak X (Fig. 1) was considerable on 2.7 m columns but separation between the two peaks became increasingly effective with increasing length of column. Secondly, peak Y (Fig. 1) appeared to contain at least 3 constituents, one of these being the physiologically interesting 4-hydroxy-3-methoxymandelic acid; whilst separation between the main part of this peak and PHPL improved with increasing column length one, usually minor, constituent separated out and overlapped PHPL when 5.5 m columns were used. It thus appeared that optimum separation of PHPL required a 4 m (13 ft.) column; temperature programming from 170° at 1°/min provided suitable experimental conditions.

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Use of silicic acid–glass fiber sheets for bioautography of antimicrobial substances

Bioautography of thin-layer chromatograms is used routinely for detection of antimicrobial substances and is the subject of numerous publications. The general technique is to press the developed thin-layer plate face down onto the surface of agar seeded with a microorganism, remove the plate, and after a suitable incubation period, observe zones of inhibition of the separated antibiotics.

To avoid the adherence of the adsorbent to the agar surface, a number of methods have been used. Probably the most common technique is that of MEYERS AND SMITH[1], who inserted a sheet of filter paper between the plate and the agar surface. Another difficulty which sometimes arises is the lack of contact between the entire plate surface and the agar, resulting in poorly defined spots.

Both of these problems can be avoided by use of a silicic acid–glass fiber sheet (ChromAR® Sheet 500, code 2182, Mallinckrodt Chemical Works, St. Louis, Mo., U.S.A.). This sheet is composed of approximately 70% silicic acid and 30% micro fiber glass, and can be cut to the desired size with a pair of scissors or a paper cutter.