Centrifugal chromatography*

III. Chromatography of some substances of the vitamin B_6 group

A study undertaken to investigate some reactions of pyridoxine derivatives in biological material called for a rapid method of separating sulphur derivatives derived from pyridoxine. BERNHART *et al.*[1] have proved that these substances are formed when condensed milk is sterilized. In this case bis-4-pyridoxyl disulphide is formed as a result of the reaction of pyridoxal with cysteine. The chromatographic methods that we have elaborated appeared to be the most suitable for studying the reduction products of bis-4-pyridoxyl disulphide. Recently much effort has been spent in studying the polarographic behaviour of these substances, which can also be used for analytical purposes[2]. We have succeeded in separating bis-4-pyridoxyl disulphide by combining thin-layer chromatography with paper chromatography. In order to accelerate the chromatographic separation on paper we have taken full advantage of the centrifugal technique[3]. The substances mentioned were prepared according to procedures proposed by WENDT AND BERNHART[4].

Centrifugal paper chromatography

For this purpose the apparatus described by PAVLÍČEK *et al.*[3] was used. The chromatographic systems were isopropyl alcohol–29 % ammonia–water (8:1:1) and *n*-butanol–acetic acid–water (4:1:1). Both systems ensure a good separation of bis-4-pyridoxyl disulphide from the two other derivatives (*cf.* Table I). Chromatography was performed on Whatman No. 3 paper, the developing time with the first system being 25 min, with the second system 45 min; detection was carried out by observation in U.V. light.

TABLE I

CENTRIFUGAL SEPARATION OF SOME SUBSTANCES OF THE VITAMIN B_6 GROUP

Solvent system: isopropyl alcohol–29 % ammonia–water (8:1:1)

Compound	R_F
Pyridoxol	0.69
Bis-4-pyridoxyl disulphide	0.83
4-Pyridoxthiol	0.69

Thin-layer chromatography

The separation of pyridoxol from 4-pyridoxthiol was achieved by chromatographing the compounds in the form of their azo dyes with diazotized sulphanilic acid. These derivatives were prepared according to HAIS AND MACEK[5]. In the technique employed, use was made of an unstiffened layer of aluminium oxide as proposed by MOTTIER

* Two previous communications (*J. Chromatog.*, 6 (1961) 187; *ibid.*, 7 (1962) 19) are considered as Parts I and II of this series.

AND POTTERAT[6]; the developing solvent was ethanol–amyl alcohol–water (1:1:1), and the developing time 25 min. The results are given in Table II.

TABLE II

THIN-LAYER CHROMATOGRAPHY OF SOME SUBSTANCES OF THE VITAMIN B_6 GROUP

Azo dye of	R_F
Pyridoxol	0.85
Bis-4-pyridoxyl disulphide	0.80
4-Pyridoxthiol	0.52

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A note on the use of partially-overloaded β-ray ionization detectors in gas chromatography*

One of the characteristics of the LOVELOCK β-ionization detector is that its response to a fraction becomes less than linear when, at a given instant, the concentration of that fraction in the detector exceeds a critical value[1]. The instantaneous concentration of component in the detector can be reduced by diluting the sample or by lowering column temperature or gas flow rate but changes of this nature tend to prolong the analysis or cause fractions with early emergence times to be hidden in the solvent peak. Moreover, it is not always possible to anticipate the situation and, occasionally, repetition of the analysis with a diluted sample is not possible.

Studies of the fatty acid composition of single seeds often involve a minute quan-

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