A thin-layer and an improved paper-chromatographic method for the separation of cucurbitacin B and 23,24-dihydrocucurbitacin B*

While studying the enzyme cucurbitacin B Δ^{23}-reductase[1] [NAD(P)H: cucurbitacin BΔ^{23}-oxidoreductase] it became apparent that the existing method[2] for the chromatographic separation of cucurbitacin B (B) and 23,24-dihydrocucurbitacin B (B_1) was unsatisfactory for assaying enzyme activity. The principal disadvantages of all existing methods[3,4] for the separation and detection of various plant bitter principles are that the chromatograms cannot be preserved for any length of time and that the detection of the spots is often made difficult by the background colour. Development of the chromatograms in a mobile phase of carbon tetrachloride–benzene (1:1, v/v), followed by successive spraying with a 2 % solution of vanillin in chloroform and a 5 % solution of bromine in carbon tetrachloride resulted in good separation and well defined coloured spots on an almost colourless background. These chromatograms could be preserved for a considerable length of time.

Experimental

Chromatographically pure B_1 and B were kindly supplied by Dr. P. R. ENSLIN of the National Chemical Research Laboratories, C.S.I.R., Pretoria, and Dr. S. REHM of the Horticultural Research Institute, Roodeplaat, Pretoria, respectively. All other chemicals were of analytical grade quality.

Paper chromatography. Whatman No. 1 paper (18 cm × 56 cm) was impregnated with 50 % formamide in absolute ethanol as described by ENSLIN *et al.*[3,4]. The excess ethanol was allowed to evaporate at room temperature over a period of 30 min.

Quantities of 150–200 μg of B and 100–150 μg of B_1 in chloroform or methanol were applied in the usual manner. After equilibration for 2 h, the chromatogram was developed for about 6 h in a mobile phase of carbon tetrachloride–benzene (1:1, v/v), after which the paper was dried at 90° for 10 min.

Thin-layer chromatography. A suspension of 20 g of cellulose powder (200 mesh) in 200 ml of distilled water, was plated on a 24 cm × 9 cm plate in a thickness of 0.025 cm. The plate was dried at 105° for 1 h. After cooling at room temperature, the chromatoplate was impregnated with formamide by spraying with about 15 ml of a 50 % solution of formamide in absolute ethanol. Excess ethanol was evaporated in a stream of cold air for 30 min.

Quantities of 150 μg of B_1 and 200 μg of B in chloroform or methanol were applied in the usual manner. After equilibration for 1 h the chromatoplate was developed for 2.75 h at room temperature in a mobile phase similar to the one described above. In all cases development was completed within 3 h after which the chromatoplate was dried at 105° for 30 min.

Detection of bitter principles. The dried paper chromatostrips and chromatoplates were sprayed successively with a 2 % solution of vanillin in chloroform and a 5 % solution of bromine in carbon tetrachloride. After spraying the paper chromatostrips were heated in an oven at 70–75° for 3–5 min and the chromatoplates at 90° for 5–10 min. Under these conditions B and B_1 gave rise to a dark purple and yellow coloured spot, respectively, on a colourless background.

* Taken in part from a thesis submitted by J. C. SCHABORT to the Faculty of Agriculture of the University of Pretoria, in partial fulfilment of the requirements of the M.Sc. (Agric.) degree.

Results and discussion

The R_F values of cucurbitacin B and 23,24-dihydrocucurbitacin B are given in Table I.

The temperature and period of drying after development as well as after spraying were found to be critical for colour development and elimination of background colour. The colours produced were very stable and the chromatoplates and chromatostrips could be preserved for several months. In the case of paper chromatography separation could be improved by allowing the solvent front to run off. Thin-layer chromatography yielded much better resolution in considerably shorter periods. The effective separation of B and B_1 by thin-layer chromatography is illustrated in Fig. 1. Normal variations in R_F values were observed. However, separation was always satisfactory.

TABLE I

R_F VALUES OF CUCURBITACIN B AND 23,24-DIHYDROCUCURBITACIN B

Method	*R_F value*	
	Cucurbitacin B	*23,24-Dihydrocucurbitacin B*
Paper chromatography	0.36	0.41
Thin-layer chromatography	0.27	0.35

At higher concentrations of B, isocucurbitacin B was detected as a brown spot at an R_F value slightly higher than that of B. Isocucurbitacin B usually occurs in small quantities in all preparations of B (ref. 2).

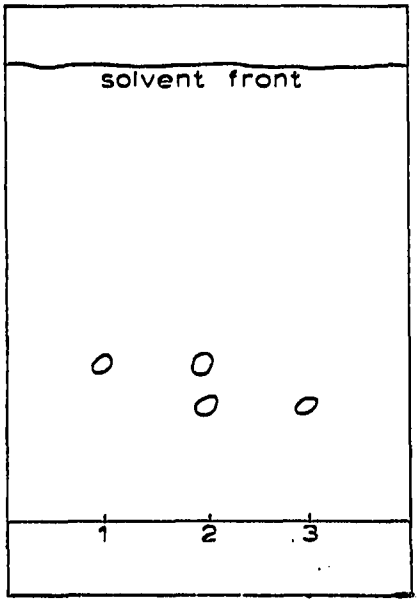

Fig. 1. Thin-layer chromatography of B and B_1. Numbers 1, 2 and 3 correspond to application of B_1, a mixture of B and B_1, and B respectively.

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The detection of diethylstilboestrol (DES) in urine by thin-layer chromatography

The use of synthetic hormones, with both an anabolic and an oestrogenic action, such as diethylstilboestrol (*trans*-α,α'-diethyl-4,4'-stilbenediol) has gained in popularity during the last years, especially in connection with the fattening of calves and poultry. The fact that these compounds belong to the so-called biologically highly active compounds and the presumptions expressed in literature that they possess carcinogenic or cancer-stimulating activity made several governments decide to prohibit the use of compounds with a hormonal activity for other than therapeutic purposes. Hence the detection of DES is of special interest.

In The Netherlands DRYER's[1] bromimetric method and BANES AND UMBERGER's[2,3] U.V.-irradiation method, both in a modification of HUIS IN 'T VELD*, are used for quantitative determination of DES. Besides these, need was felt for a simple specific identification method. Thin-layer chromatography turned out to be suitable for this purpose.

The use of paper chromatography for the detection of DES has been described a few times. In accordance with the chemical character of DES a method based on so-called "reversed-phase" chromatography[3–6] had to be developed. In view of the time-consuming character of this technique it is surprising that more attention has not

* L. G. HUIS IN 'T VELD, Laboratory of Endocrinology, National Institute of Public Health, Utrecht, personal communication.