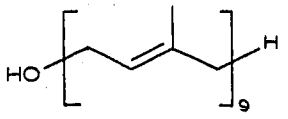
CHROM. 5482

Analytical methods for tobacco lipids

I. A rapid method for the estimation of solanesol by thin-layer densitometry

The present communication describes a convenient and rapid method for the estimation of solanesol in flue-cured tobacco on a micro-scale.

Solanesol (3,7,11,15,19,23,27,31,35-nonamethyl-2,6,10,14,18,22,26,30,34-hexatriacontanonaen-1-ol) is a naturally occurring acyclic trisesquiterpenoid alcohol, the most abundant known source being tobacco.



When first isolated from flue-cured tobacco by ROWLAND *et al.*[1] in 1956, it was reported that solanesol constituted 0.4% of the dry weight. More recently, work in these laboratories and elsewhere[2,3] has indicated that the solanesol content of tobacco is, in fact, considerably higher (*ca.* 1–2%).

The gravimetric estimation of solanesol after column chromatography on silicic acid or alumina has been reported[3,4] and we attempted a similar procedure using Florisil. It was found that such a method led to low and variable values for solanesol. One possible explanation has been offered by STEDMAN *et al.*[4] who suggested that such discrepancies might arise from incomplete extraction or from the difficulties of isolating solanesol. REID AND HELLIER[5] have suggested that solanesol and other lipids in a hexane extract polymerise on standing to form insoluble resins. Our observation that the free solanesol concentration in a hexane extract of tobacco decreases on standing with the attendant formation of an insoluble material could be explained in terms of the formation of such resins. It would appear, therefore, that the time which elapses between the extraction and subsequent estimation should be kept to a minimum. Thus methods involving lengthy extraction procedures and column chromatography are unsatisfactory.

A semi-micro analytical method for the estimation of solanesol in tobacco using IR techniques after Soxhlet extraction and column chromatography has been reported[2] but for the reasons mentioned above we considered this unsuitable.

The development of a method for the quantitative estimation of solanesol presented several problems. Since it is not possible to separate it completely from other tobacco lipids by column chromatography, colorimetric or gravimetric methods are unreliable. The low volatility and poor flame ionisation detector response of solanesol and its derivatives in gas–liquid chromatography render this technique unsuitable, although such a method has been described for the separation of de ves of solanesol from those of other isoprenoid alcohols[6,7]. Since solanesol exhi o selective absorption[1], UV spectroscopy cannot be used. However, submici quantities of solanesol can be detected by thin-layer chromatography (TL d by visualisation with mild oxidising or charring reagents.

ver densitometry provides a rapid and sensitive quantitative metho

A major difficulty encountered is the distribution of the visualising agent uniformly over the TLC plate, which is not easy to achieve by the widely used method of spraying after development. Recently, a method was described[8] where phosphomolybdic acid (PMA) was incorporated in the adsorbent before the preparation of the plates giving a uniform distribution of the reagent within the layer. We have found it more convenient to use commercially available, pre-coated plates and to impregnate them with PMA by immersion in an alcoholic solution of the reagent.

The plates were developed in benzene which facilitated the separation of solanesol (R_F = 0.07) from the major interfering lipids, *viz.* diglycerides (R_F = 0.01), fatty acids (R_F = 0.02), sterols (R_F = 0.03) and triglycerides (R_F = 0.16). Although its R_F is low in this solvent, linear calibration curves were obtained for solanesol within the range 0.5–2.5 μg.

Visualisation was effected by heating the plates when solanesol appeared as dark blue spots on a yellow background. PMA was more sensitive to solanesol than to the other lipids which were not usually visualised under the experimental conditions used. Densitometry was normally carried out immediately, but the blue colour is stable for at least 2–3 h provided the plates are kept in the dark.

Using the present method the amount of solanesol in two tobacco samples can be estimated in four hours; the coefficient of variation is ± 3 % (estimated from the standard error of the mean for duplicate determinations).

Experimental

Apparatus. "Chromoscan" recording densitometer with thin-layer attachment (Joyce Loebl & Co., Ltd.): operation mode, reflectance; wedge, 0–1.0 *D*; cam, C; gear ratio, 1:2; filters, 1 1.0 *D* before sample photomultiplier, sodium light filter in sample beam; slit dimensions, 3 × 0.5 mm (variable slit).

Drummond Microcap micropipettes of 1 μl capacity (Shandon Scientific Ltd.).

Reference standard. A sample of solanesol, m.p. 35–36°, was obtained by column chromatography of tobacco lipids on Florisil (Floridin Co., U.S.A.) followed by repeated preparative-layer chromatography on Silica Gel G. Standard solutions in benzene containing 0.5, 1.0, 1.5, 2.0 and 2.5 μg/μl were prepared.

Preparation of TLC plates. Pre-coated Silica Gel G 20 × 20 cm plates (E. Merck) were immersed in a solution of PMA (Analar grade, Hopkin and Williams Ltd.) in ethanol (1.25 % w/v) for 1 min, dried at 120° for 2 min, and allowed to cool.

Extraction procedure. Finely-ground tobacco (1 g) was shaken mechanically with *n*-hexane (50 ml) for 30 min. The mixture was filtered under suction and the procedure was repeated with the residue. The combined extracts were concentrated at 35° *in vacuo* and made up to 10 ml with *n*-hexane (contains *ca.* 0.5–1.0 μg/μl solanesol).

Analysis. Aliquots (2 × 1 μl) of the solutions of two tobacco extracts were applied in duplicate in the middle of the TLC plate. The five solanesol standards were spotted on each side of the test samples. The plate was developed in a lined tank containing benzene until the solvent front had travelled *ca.* 16 cm. After drying, the plate was heated in a forced draught oven at 120° for 20 min. The plate was then [illegible] at right angles to the direction of development so that all the solanesol spots [illegible] in a single run (R_F = 0.07). The test spots were scanned [illegible] The [illegible] peaks produced on the recorder chart was calculated by [illegible] the

peak height by the width at half-height, and average values were determined. The amount of solanesol in the standard solutions was plotted against the peak area and from the resulting calibration curve (linear within the range 0.5–2.5 μg) the weight of solanesol in each tobacco extract was determined.

The authors wish to thank Mr. W. J. IRVINE for helpful suggestions and the Directors of Carreras Limited for permission to publish this work.

Carreras Research Division, Nevendon Road,
Basildon, Essex (Great Britain)

B. H. WOOLLEN
D. H. JONES

1 R. L. ROWLAND, P. H. LATIMER AND J. A. GILES, *J. Amer. Chem. Soc.*, 78 (1956) 4680.
2 W. R. BILINSKY AND R. L. STEDMAN, *J. Ass. Offic. Agr. Chem.*, 45 (1962) 532.
3 S. MCDONALD AND R. G. NICHOLLS, *17th Tob. Chem. Res. Conf., Montreal, Quebec, Sept. 22–25th 1963.*
4 R. L. STEDMAN, W. R. BILINSKY AND A. P. SWAIN, *U.S. Dep., Agr., ARS*, 73-38 (1962) 12 pp.
5 W. W. REID AND D. N. HELLIER, *Chem. Ind.*, (1961) 1489.
6 B. O. LINDGREN, *Acta Chem. Scand.*, 19 (1965) 1317
7 A. R. WELLBURN AND F. W. HEMMING, *J. Chromatogr.*, 23 (1966) 51.
8 J. C. TOUCHSTONE, A. BALIN, T. MURAWEC AND M. KASPAROW, *J. Chromatogr. Sci.*, 8 (1970) 443.

First received April 6th, 1971; revised manuscript received June 1st, 1971