The ultraviolet absorption spectrum of filixic acid is in agreement with structure V. In ethanol it has maxima at 228 mμ (ε 41,000) and 288 mμ (ε 29,000) which are typical of enolised 2-acyl*cyclo*hexane-1:3-diones[4]. In connection with studies on the constitution of aspidin and flavaspidic acid AEBI[5] has shown that the extinction values of these compounds may be arrived at by addition of the extinction values of the component nuclei insulated by methylene groups. The addition of the extinction values at the absorption maxima of two butanoylfilicinic acid nuclei (2 × 12,510) and one phlorobutyrophenone (ε 13,000) in the region of 228 mμ gives a value of 38,120 which is in reasonable agreement with the observed value. Similarly, the calculated value at the higher wavelength maximum is 27,210.

Filixic acid does not give a coloration with GIBBS 2:6-dichloroquinonechloroimide reagent[6]. This makes suggest substitution of unlikely alternative structures for filixic acid in which there are unsubstituted positions *para* to phenolic hydroxyl groups.

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Zusammenfassung

Es wird die Vermutung ausgesprochen, dass Filixsäure, ein Inhaltsstoff von Farnwurzeln, die Konstitutionsformel (V) hat.

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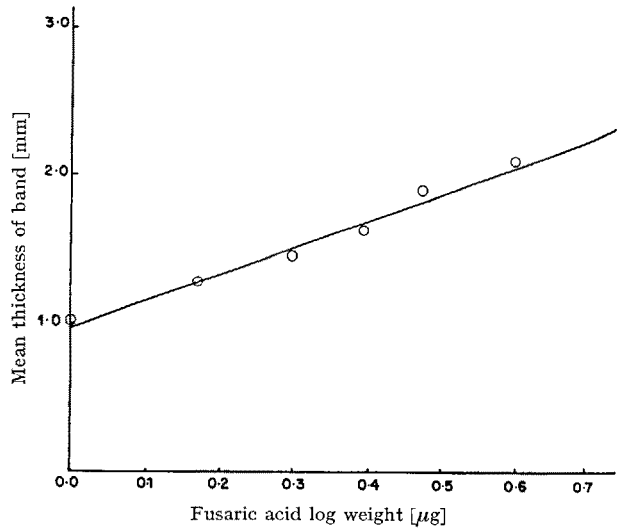
Chromatographic Detection and Estimation of Fusaric Acid

Fusaric acid was first isolated by GÄUMANN and his school of workers[1] from cultures of *Fusarium lycopersici* Sacc. and has since been known to be produced by several Fusaria. The presence of this toxin *in vivo* in wilt-infected cotton plants was reported by us earlier[2] and established to be the *vivo*-toxin in the *Fusarium* wilt of cotton. ZAHNER[3] reported the chromatographic detection and bio-assay of fusaric acid; and KALYANASUNDARAM[4], in this laboratory, developed the agar-cup technique for its assay using a soil bacterium. In view of the considerable difficulty in obtaining sharp demarcation of the fusaric acid band separated chromatographically, possibly owing to the poor ionization of the acid, we have earlier indicated the advantages of separating and identifying this toxin as its copper chelate[2]. In the present communication the detection and quantitative estimation of fusaric acid is described with the technique of paper disk chromatography developed by one of us[5]. It was considered desirable to adapt the area method developed by FISHER *et al.*[6] in preference to elution methods which have practical limitations in the small chromatograms especially when complexes with close Rf values are present.

The following amounts (as aqueous solution) of fusaric acid contained in 0·75 μl were spotted in the centre of circular filter papers (7·4 cm diameter) followed by 4 μg of copper as $CuSO_4$ (5 mg/ml): 1·0; 1·5; 2·0; 2·5; 3·0 and 4 μg. The papers were air-dried and irrigated as indicated elsewhere[5] controlling the period of irrigation at 95 min. The solvent used was *N*-butanol-acetic acid-water (4:1:5) and filter paper-Whatman No. 1. The irrigated chromatograms were sprayed with rubeanic acid (0·1% in acetone) and the colour of the bands intensified by exposure to ammonia vapour[7] (10 s). The developed chromatograms were cut into regular octogens tangentially in the 8 directions (± 45°), mounted between two square glass plates and sealed on two sides with paraffin wax. The thickness of the Cu-fusaric acid complex band was measured on the microscope using an ocular micrometre (low power). The stage readings were recorded between the points where the green colour appeared and disappeared. The mean thickness of the band in the 8 directions was computed (as the mean of 6 chromatograms) for each concentration (varying ± 3%) and plotted against log amount of the toxin on the abscissa. The typical standard curve obtained is shown in the Figure. It would be obvious that the mean thickness bears a linear relationship to the areas of the bands since the Rf values were consistent and reproducible and the bands quite circular. The areas of the complex bands are directly proportional to the logarithm of the amount of fusaric acid and independent of the total copper spotted when it is within reasonable limits. The quantitative estimation of fusaric acid in an unknown sample could easily be carried out by measuring the mean thickness of its Cu-chelate in the range of sensitivity and the amount read as the antilog of the abscissa. It may be added that the curve is not quite sensitive at concentrations of fusaric acid lower than 1 μg or higher than 4 μg.

Log fusaric acid – mean thickness curve *n*-butanol-HAC-water 4:1:5.



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Employing the present technique, the fusaric acid content of the dialysed culture filtrate of *F. vasinfectum* Atk. (grown in Richard's medium in Haffkine flasks – 1·5 l medium/4 l flasks for 8 weeks) was found to be 44·6 mg/l. The dialyzed culture filtrate was concentrated to a small volume under reduced pressure prior to spotting. The fusaric acid content was estimated as indicated earlier. The concentrate was then diluted to 75% and 50% (with distilled water) and spotted again. Pure fusaric acid was added to make up the difference in each case and the total fusaric acid content estimated for recovery. The recoveries obtained at the two levels were 95·5% and 99%, respectively indicating that the method could be employed with advantage for the assay of this toxin.

Rf values of copper and copper-fusaric acid complex in different solvent mixtures.

Solvent mixtures	Rf values	
	Copper	Complex
1. *N*-butanol-HAc-water (4:1:5) .	0·494	0·273
2. *N*-butanol-HAc-water (3·5:1·5:5)	0·666	0·617
3. *N*-butanol-formic acid-water (5:3·5:5)	0·157	0·088
4. Isoamyl alcohol-HAc-water (4:1:5)	0·230	0·090
5. Isopropanol-formic acid-water (4:1:5)	0·460	0·400
6. Aq. Dioxan (1:1)	0·790	0·630
7. Aq.phenol	0·110	Not formed
8. 400 ml of aq. butanol containing 5 g 8-quinolinol to which was added 40 ml glacial acetic acid warmed to solution	0·460	0·180
9. Incorporation of 0·5 g EDTA* in 100 ml aq. butanol	0·100	Not formed
10. Impregnating the paper with 10% alumina prior to irrigation with *N*-butanol-HAC-water (4:1:5)	0·230	Not formed
11. Aq. Collidine	0·182	0·263
12. Aq. Lutidine	0·784	0·299

* Ethylene diamine tetracetate.

The Table gives the Rf values of copper and Cu-fusaric acid complex in the 12 solvent mixtures indicated therein. It may be added that the method could be employed only in the absence of interfering metabolites which would either alter the Rf value of the complex or form chelates having close Rf values. In such cases it would be necessary to use different solvents to eliminate interference and use the corresponding standard curves.

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University Botany Laboratory, Madras (India), April 4, 1957.

Zusammenfassung

Es werden der chromatographische Nachweis und die Bestimmung der Fusarinsäure als Kupferchelat mittels der Rundfiltertechnik beschrieben. Die durchschnittliche Breite des Chelatstreifens steht in einem linearen Verhältnis zu dem Logarithmus der Konzentration von Fusarinsäure. In einem Kulturfiltrat von *F. vasinfectum* Atk. war ein Zusatz von reiner Fusarinsäure quantitativ nachweisbar. Rf-Werte von Cu und Cu-Fusarinsäure-Komplexen in 12 Lösungsmitteln werden angegeben.

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Carbohydrates, Collagen and Elastin of the Normal Aortic Wall and Arteriosclerotic Hyaline Plaques

Reports in the literature on the determination of the carbohydrates and of the collagen and elastin content in the normal aorta and their possible modifications in the course of arteriosclerosis, are relatively few. However studies carried out by means of metachromatic stain reactions indicate that the elastic tissue fibers of the aorta are bathed in a matrix of 'ground substance' constituted mainly of sulphated acid mucopolysaccharides, and that the alterations of this mucinous substance could be followed by modifications of the content in collagenous and elastic fibres of the aorta, which may be of fundamental importance in the evolution of arteriosclerosis[1].

Therefore, in the present research the chemical composition of hyaline plaques from arteriosclerotic aortae was investigated and compared with that of a normal aortic wall.

The samples were severed in three fractions, labelled F. I.–F. II.–F. III.–, according to NEUMAN and LOGAN[2]. We have analyzed samples of 9 different normal aortic walls of men aged from 20 to 40 years and samples collected from hyaline plaques of 9 different cases of arteriosclerosis; the amount of tissue, from each case, which was necessary for chemical analysis, was about 1 g wet weight.

The first fraction contains mainly collagen, the second fraction contains several heterogenous proteins, and the third one contains elastin. The nitrogen was determined in each fraction by Nesslerization[3]; in addition, the hydroxyproline content in F.I., in order to define its true collagen quota, was determined by the method of TROLL and CANNAN[4]. The carbohydrates were determined by the methods described by DISCHE[5]. The presence of the SO_4^- groups was investigated qualitatively by adding $BaCl_2$ 1% to the samples, hydrolized in 5 *N* HCl, dried and made up to ml 1 with water, in order to precipitate $BaSO_4$.

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