aryl α -ketones have strong light-absorption in the region of 310 $m\mu$ and thus would interfere with the intended spectrophotometric determination of the arylcoumarone systems (VII). The acid-treated lignin solution was therefore made alkaline and treated with sodium borohydride to eliminate any carbonyl groups present⁷. From the absorption curve of the reacidified solution a $\Delta\epsilon$ curve was obtained by "subtraction" of the absorption curve of a similar lignin solution which had been reduced with borohydride and then reacidified but which had not been subjected to "acidolysis". This $\Delta\epsilon$ curve showed a distinct band with a maximum at 310 $m\mu$ and a shape very similar to that of the main absorption band of the arylcoumarones III and IV. This indicates that arylcoumarone systems (VIIa + VIIb) were actually formed during the hot acid treatment of the lignin and, consequently, that arylcoumaran elements (VI) were present in the original lignin. Comparison of the $\Delta\epsilon$ value at 310 $m\mu$ with the corresponding value for the model conversion I $\rightarrow$ III indicated that in the Björkman lignin sample used, about 18 % of the phenylpropane units were present in dimeric elements of the phenylcoumaran type (VIa + VIb).

To investigate the proportion of phenolic coumarone elements (VIIa) formed, "ionisation $\Delta\epsilon$ " curves were obtained for the carbonyl-free acidolysis lignin, and for carbonyl-free lignin which had not been subjected to acidolysis. From these two curves a difference curve was prepared which was expected to be the "ionisation $\Delta\epsilon$ " curve for the phenolic guaiacylcoumarone systems (VIIa) formed in the acidolysis. The position of the main band of this difference curve (max 338 $m\mu$) was in fact identical with that of the corresponding band obtained in a model experiment (I $\rightarrow$ III). Comparison of the $\Delta\epsilon$ values at 338 $m\mu$ for both experiments indicated that about 16 % of the phenylpropane units in the Björkman lignin used had given a phenolic coumarone system (VIIa). The fact that the major part of the coumarone was of the phenolic type (VIIa) rather than the ether type (VIIb) is explained by the previous finding⁶ that acidolysis of lignin liberated a considerable amount of phenolic hydroxyl groups.

Preceding work⁶ has shown that about 25 % of the phenylpropane units in spruce Björkman lignin are of the phenylglycerol type linked by a β -aryl ether bridge to the adjacent monomer, or in other words, that

up to 50 % of the lignin may consist of dimeric elements of the guaiacylglycerol- β -dihydroconiferyl ether type. The present study indicates that nearly 20 % of the lignin is made up of phenylcoumaran dimers (VIa + VIb). Although the various dimeric elements may "overlap" to some extent, one or both of the two phenylpropane moieties of a dimeric element also forming part of a following one, the results obtained account for the structural elements of an appreciable part of the total average lignin molecule.

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Determination of ϵ -Aminocaproic Acid in Urine by Means of High-Voltage Paper Electrophoresis

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It has been reported that ϵ -amino-*n*-caproic acid (ϵ -ACA) is a potent inhibitor of plasminogen activation *in vitro*^{1,2}. Preliminary studies in this hospital by Nilsson *et al.*³ suggest that the compound may also be effective *in vivo* in the treatment of fibrinolysis associated with various diseases. It was therefore desirable to obtain information about the fate of ϵ -ACA in the body and to determine whether it is absorbed efficiently from the intestinal tract. To accomplish this a method for the estimation of ϵ -ACA in urine was needed.

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However, there seemed to be no specific reaction available allowing a direct assay of this amino acid and we regarded the conventional two-dimensional paper chromatography too difficult and time-consuming for our purpose. We therefore developed a quicker and simpler method for the determination of ϵ -ACA, principally similar to methods already developed in this laboratory for the determination of some other amino acids, e.g. γ -aminobutyric and glutamic acids⁴. The method consists of isolation of ϵ -ACA by high-voltage paper electrophoresis, development of color with a ninhydrin and copper spray, elution of the spots from paper and measurement of the color in a spectrophotometer.

Method. The following reagents were used: 1) Buffer for electrophoresis, a mixture of formic acid, acetic acid, acetone and water (15:10:15:65 v/v) with a final pH of 1.2. 2) Spray reagents, a) Ninhydrin, 0.5 % in *n*-butanol previously saturated with a mixture of pyridine, acetic acid and water (10:1:95) with a pH of 6, and b) Copper reagent⁵, prepared by mixing a saturated solution of cupric nitrate, 10 % nitric acid, water and methanol (1:0.2:4:95). 3) Methanol, as eluent, 4) ϵ -ACA, reference standard solution containing 1 mg per ml of water. The ϵ -ACA used here was obtained from L. Light & Co. Ltd. Colnbrook, England. The method for high-voltage paper electrophoresis was that of Wieland and Pfeleiderer⁶ and Wieland and Dose⁷ using Whatman No. 3 filter paper.

Preliminary experiments indicated excellent separation of ϵ -ACA from normally-occurring amino acids in urine except alanine. Separation from the latter could be achieved with paper chromatography in the second dimension in a quick-running solvent mixture of methanol, water and pyridine (80:20:4). However, during the first 24 h after administration of ϵ -ACA in the usual clinical dosage (0.0 g), the excretion of the compound was of such a magnitude that accurate assays could be performed on diluted urine in which interference from alanine was insignificant. Thus in the procedure to be described, a volume of urine equivalent to 1 minute's excretion was diluted to 10 ml with water prior to analysis. 0.005 to 0.04 ml samples of diluted urine and reference standards (5 to 40 μ g) were applied to the paper with micropipettes at a distance of 8 cm from the anode end. With an operating voltage of 2 000 V and a current of 60 mA — resulting in a potential gradient of 50 V/cm — the ϵ -ACA

migrated about 10 cm towards the cathode in an experimental period of 1 h. On removal from the apparatus the paper was dried in an oven at 80°C for at least 1 h. It was then sprayed with the ninhydrin reagent and the color developed by heating in an oven at 80°C for 30 min. The purple color obtained was then converted to a more stable light-red color by spraying with the copper-complexing reagent and the paper was allowed to dry in a cool current of air. Each colored spot was then cut out, placed in a small glass-stoppered test-tube and eluted for 30 min with 4 ml of methanol. A clear portion of the paper of corresponding size was eluted also and used as a blank. The measurements of the color intensity were made at 508 m μ in a Beckman spectrophotometer model B. The extinction values of reference spots were proportionate to concentration over a range 0 to 30 μ g, with 1 μ g giving an average value of 0.02 to 0.025. From the extinction values obtained with the reference standards a graph was constructed and from this the concentration of the urine sample was read. Recovery of ϵ -ACA added to diluted urine was satisfactory (95 $\pm$ 10 %). In three patients who received on different days single 6.0 g doses of ϵ -ACA orally and intravenously the compound was found to be excreted rapidly in the urine, 40 to 65 % of the dose being recovered in the first 12 h. Since the percentage excreted after oral and intravenous administration was similar, the compound is thought to be completely absorbed from the intestinal tract.

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