pressure and diluted to 2 ml. with 10 per cent v/v *iso*propanol. Equal amounts of each sample were chromatographed in butanol/acetic acid[2] (ascending) followed by phenol/ethanol[2] on Whatman No. 1 paper and developed with ninhydrin. Two-dimensional chromatograms of the urine desalted for 10 min. gave good discrete spots compared with the original urine. A one-dimensional chromatogram of samples withdrawn at various times during the run is shown in Fig. 2.

It is evident from the results that, provided a 'desalting curve' for the urine is plotted, it is possible to stop the run at a time when most of the salt has been removed but little or no amino-acid has been lost. It is also suggested that a procedure of 'total desalting' might be useful in clinical laboratories to measure the total amount of electrolyte in urine, since this is directly proportional to the average current × time.

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[1] Wood, T., *Biochem. J.*, **62**, 611 (1956).
[2] Smith, I. (ed.), "Chromatographic Techniques, Clinical and Biochemical Applications" (Heinemann, London, 1958).

Biosynthesis of Flavonoids

Several workers have shown that lignin and the '*B* ring' of flavonoids are formed *in vivo* from shikimic acid and certain C_6C_3 compounds. The latter compounds are frequently encountered in plant tissues[1]. The '*A* ring' of flavonoids appears to arise from acetate units[2]. However, the occurrence of the free '*A* ring' or simple derivatives of it have rarely been reported. We have found that on acid treatment kinos yield phloroglucinol, but bark and wood polyphenols do not.

Concentrated hydrochloric acid was added to an aqueous ethanolic (1 : 1) solution (5 ml.) of the kino (0·2 gm.), until the strength was 3 *N*. The open test-tube containing the solution was heated in boiling water for 1 hr., and the reaction products examined by two-way chromatography in butanol/acetic acid/water (6 : 1 : 2) and 6 per cent acetic acid. All the kinos examined produced phloroglucinol, which was identified by co-chromatography, its appearance under ultra-violet light while fuming with ammonia (s.g. 0·88) and its colour reaction to diazotized *p*-nitroaniline[3] and vanillin-hydrochloric acid[4].

Phloroglucinol was isolated from *Pterocarpus marsupium* kino (5 gm.) by treating in a similar manner, adding sodium carbonate to the filtered reaction products until the acid strength was about 1 *N*. The resulting liquor was placed on a polyamide column[5] and, after washing with water, the column was developed with ethanol/water (3 : 7) and the phloroglucinol recovered from the appropriate fractions in 0·5 per cent yield. The crystals did not depress the melting point of authentic phloroglucinol and possessed the same infra-red spectrum.

When the kinos were treated with butanol – hydrochloric acid, the anthocyanidins produced by the leucoanthocyanins present were identified chromatographically using Forestal[6] and formic acid – hydrochloric acid – water[7] solvents, and they all contained a phloroglucinol '*A* ring'. In addition, *Eucalyptus* and *Angophora* kinos gave gallic acid, and some kinos gave methyl gallate and protocatechuic acid which were identified by co-chromatography on 2-way chromatograms.

The kinos examined, and the principal anthocyanidins produced with acid, were as follows: *Eucalyptus calophylla* (pelargonidin and cyanidin), *E. camaldulensis* (cyanidin), *E. consideniana* (delphinidin), *E. corymbosa* (cyanidin), *E. elaeophora* (delphinidin), *E. gigantea* (delphinidin), *E. macrorrhyncha* (delphinidin), *E. obliqua* (delphinidin), *E. polycarpa* (cyanidin), *E. radiata* (delphinidin), *E. regnans* (delphinidin), *E. sideroxylon* (pelargonidin, cyanidin, delphinidin), *E. sieberiana* (delphinidin), *Angophora intermedia* (cyanidin), *Pterocarpus marsupium* (cyanidin), *Butea frondosa* (cyanidin).

The material yielding phloroglucinol with acid treatment was insoluble in ether, and formed unresolved streaks on the solvent axes. When solutions of *E. elaeophora* and *P. marsupium* kinos in aqueous ethanol (1 : 1) were made 2 *N* with sodium hydroxide, kept at room temperature for 2–4 hr., then acidified, phloroglucinol was not detected, so that esters of it are absent. Sugars could not be detected in the acid reaction products.

In contrast, extracts of the wood and phloem tissues of several of the above eucalypts which had formed kinos did not yield phloroglucinol on acid treatment. Extracts from the wood of *E. wandoo*, and the barks of *E. astringens* and *Rhizophora mucronata* which are rich in leucoanthocyanins containing a phloroglucinol '*A* ring' also failed to yield phloroglucinol. Thus, kinos contain phenolic components which are absent in the phloem and woody tissues, and consequently kinos appear to be formed *in situ* from other materials. Further evidence[8] indicates that the polyphenols in kinos and in the tissues of trees are formed from carbohydrates which have been translocated or stored as starch.

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[1] Bate-Smith, E. C., *Sci. Proc. Roy. Dublin Soc.*, **27**, 165 (1956).
[2] Watkin, J. E., Underhill, E. W., and Neish, A. C., *Canad. J. Biochem. Physiol.*, **35**, 229 (1957). Geissman, T. A., and Swain, T., *Chem. and Indust.*, 984 (1957). These contain references to earlier work.
[3] Swain, T., *Biochem. J.*, **53**, 200 (1952).
[4] Bate-Smith, E. C., and Swain, T., *Chem. and Indust.*, 377 (1953).
[5] Hoerhammer, L., Wagner, H., and Leeb, W., *Naturwiss.*, **44**, 513 (1957).
[6] Bate-Smith, E. C., *Biochem. J.*, **58**, 122 (1954).
[7] Harborne, J. B., *J. Chromat.*, **1**, 473 (1958).
[8] Hillis, W. E., and Carle, Ann, *Biochem. J.* (in the press).

PHYSIOLOGY

Œstrogen–Insulin Interaction on the Uterus of the Rat

The physiological activity of a specific hormone may be greatly modified by the presence or absence of other hormones, as evidenced, for example, by the Houssay effect[1]; the inhibitory action of adrenal cortical hormones on the response of the rat uterus to œstrogens[2]; the interaction of the various œstrogens on uterine growth[3]; the synergistic effect of growth hormone and thyroxine on body-growth in hypophysectomized animals[4], etc. Of more immediate