References

Black, J. W., Crowther, A. F., Shanks, R. G., Smith, L. H. & Dornhorst, A. C. (1964). *Lancet*, **1**, 1080–1081.
Burn, J. H. & Hobbs, R. (1957). *Arch. int. Pharmacodyn.*, **113**, 290–295.
Fleury, C. (1957). *Arch. Sci. (Geneva)*, **10**, 107–.
Litchfield, J. T. Jr. & Wilcoxon, F. (1949). *J. Pharmacol.*, **96**, 99–113.

Examination of cinnamon by direct thin-layer chromatography

SIR,—Cassia bark (*Cinnamomum cassia*) is the commonest substitute for cinnamon (*C. zeylanicum*), and whilst it should be possible to distinguish one from the other by macroscopical and microscopical means, analysts have expressed a need for aids to their identification. Dutta (1961) suggested mucilage ash values. It seems logical to attempt to make a distinction by examining the active principles, the essential oils. For this purpose thin-layer chromatography may be used as it has previously been applied to umbelliferous fruits (Betts, 1964). The oils of both barks contain cinnamaldehyde as the principal constituent, but cinnamon oil, unlike cassia oil, contains eugenol. Both of these constituents are not resolved on the usual silicic acid thin-layer plates, and eugenol is not easily detected in the presence of cinnamaldehyde by the spot detection methods previously described.

The modification to the previous technique (Betts, 1964) are as follows: magnesium silicate (TLC, Woelm), after mixing with absolute ethanol (12 g to 50 ml) was spread on plates and left to dry in air. Bark, 500 mg, was extracted with 2·5 ml acetone and spotted on the plates. After the solvent run eugenol was detected as a slate-blue spot, Rf approximately 0·45, by spraying with Folin & Ciocalteu's reagent (Hopkin & Williams Ltd.), as recommended for phenols by Waldi (1965). This was followed by spraying with dinitrophenylhydrazine solution to reveal cinnamaldehyde, Rf approximately 0·55. Folin & Ciocalteu's reagent provides a more sensitive test for eugenol than previous techniques and reveals the presence of eugenol in whole or powdered cinnamon.

An examination was made of various commercial (but not official) specimens of "cinnamon". Three samples of whole bark were obviously not cinnamon B.P. as they consisted of thick pieces, with much cork remaining, in single, double and a few compound quills. Microscopically they corresponded to a specimen of *C. burmanni* bark, kindly supplied by the Museum of the Pharmaceutical Society. This is an adulterant mentioned in the B.P.C. The diameter of the fibres was less than 30 μ, corresponding to true cinnamon, but calcium oxalate was present as small prisms instead of the normal acicular crystals. Chromatographically the samples were devoid of eugenol but contained cinnamaldehyde as did the Museum specimen. Museum samples of true cinnamon readily yielded a eugenol spot.

Seven commercial samples of powdered "cinnamon" were also examined. All the samples when chromatographed showed a cinnamaldehyde spot, but three were devoid of eugenol. These all contained fibres of diameter greater than 40 μ, and were probably samples of *C. cassia*. The large fibres were present in samples containing eugenol. Eugenol was also observed in a specimen which contained small calcium oxalate prisms. The cost of the samples was no guide to their quality. Only one sample appeared to be cinnamon B.P.

Of some Museum specimens of other *Cinnamomum* spp. barks examined chromatographically, *C. loureirii* (another adulterant mentioned in the B.P.C.) contained cinnamaldehyde without eugenol. *C. pedatinervium* and *C. sintok* contained eugenol without cinnamaldehyde, the former source being the

richest. These two eugenol-containing barks both contained acicular calcium oxalate crystals, but their fibres were greater than 40 μ in diameter. Hoppe (1958) gives eugenol as the main constituent of the oil of *C. culilawan*, but the specimen I examined yielded neither eugenol nor cinnamaldehyde.

Department of Pharmacognosy, T. J. BETTS
School of Pharmacy,
29–39 Brunswick Square,
London W.C.1.
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References

Betts, T. J. (1964). *J. Pharm. Pharmacol.*, **16**, *Suppl.*, 131*T*–135*T*.
Dutta, A. B. (1961). *J. Assoc. offic. agr. Chem*, **44**, 639–640.
Hoppe, H. A. (1958). *Drogenkunde*, p. 228, Hamburg: Cram, de Gruyter.
Waldi, D. (1965). *Thin-layer Chromatography*, Editor, Stahl, E., p. 498, New York: Springer-Verlag/Academic Press.

Effects of the amphetamine group on intraneuronal brain amines *in vivo* and *in vitro*

SIR,—Previous studies have shown that (+)-amphetamine in large doses causes a decrease in the brain content of noradrenaline (Smith, 1965). The present investigation was made to study the action of (+)-amphetamine at the cellular level with the help of the histochemical fluorescence method of Hillarp & others. The existence of central dopamine, noradrenaline and 5-hydroxytryptamine (5-HT) neurones has recently been demonstrated by this technique. These neurones have been shown to contain specific mechanisms for uptake and storage of the amines. They have an uptake mechanism, probably localized at the level of the cell membrane and sensitive to, for example, desipramine or cocaine (see review by Hillarp, Fuxe & Dahlström, 1965). Furthermore, they possess a reserpine-sensitive storage mechanism localised in specific granules.

Single injections (i.p.) of (+)-amphetamine (5–60 mg/kg), (±)-amphetamine (15–60 mg/kg), methamphetamine (30 mg/kg) and benzylamphetamine (30 mg/kg) have been given to male, albino rats (Sprague-Dawley, 200–300 g). The animals were killed at 1, 2 or 3 hr after the injection. Pieces from all parts of the brain were dissected, freeze-dried and treated with formaldehyde gas (Dahlström & Fuxe, 1964). Fluorescence microscopic examination showed that (+)-amphetamine, 15–60 mg/kg, caused a fairly marked to marked decrease in number and intensity of the very fine catecholamine (mainly noradrenaline) terminals in, for example, the neocortex, the gyrus cinguli and the formatio reticularis of the lower brain stem. The fine to fairly thick catecholamine terminals of the hypothalamus, on the other hand, remained unaffected even with the higher doses. The dopamine and 5-HT terminals exhibited a normal appearance after all doses except with 60 mg/kg. After this dose, the dopamine terminals showed a distinct decrease in their amine contents. Somewhat less marked changes occurred after (±)-amphetamine and methamphetamine, while benzylamphetamine did not cause any definite decrease in the intraneuronal amine levels of the noradrenaline terminals.

These results are supported by *in vitro* studies on the central monoamine neurone with brain slices (for technical details, see Hamberger & Masuoka, 1965). Noradrenaline terminals in brain slices from neocortex after preincubation with noradrenaline, 10 μg/ml, and rinsing were markedly decreased in