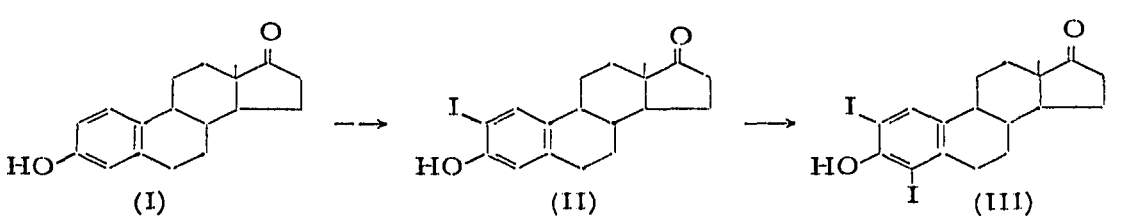
Iodine as a location reagent in thin-layer chromatography
Iodination of phenols

Iodine vapour is a convenient, non-specific reagent for locating colourless compounds in both paper and thin-layer chromatography[1–5]. A wide variety of common functional groups are unaffected by it, and it can be used for the location, prior to quantitative analysis, of steroids which do not absorb ultraviolet light[6]. A recent comparison[4] of methods for the detection of organic compounds, including phenols, on thin-layer chromatograms has shown that the use of iodine, either alone or in combination with a fluorescent dye, is more sensitive than is the alternative method involving ultraviolet illumination of silica gel plates impregnated with a fluorescent dye. (In the case of phenols, the relatively low extinction coefficient of the chromophore may account for the reduced sensitivity of the latter method.) We have been interested in the estimation of trace amounts of phenolic steroids separated on thin-layer chromatograms, and, in order to decide if iodine vapour can be safely used to locate the phenolic zones required for subsequent quantitative analysis, we have studied its action upon oestrone (I) as a model compound.

When an oestrone zone adsorbed at the starting line of a silica gel (Merck GF_{254}) plate was exposed to iodine vapour, an irreversible reaction occurred to give two products. These were revealed when the plate was developed using a mixture of chloroform and ether (9:1), after the excess iodine had disappeared. The products were more mobile than oestrone and were readily located on the chromatogram under ultraviolet light ($\lambda = 254$ mμ), or by re-iodination. The experiment was repeated on a preparative scale and the products were isolated by extraction of the zones with chloroform. The major product was identified as 2-iodo-oestrone (II) by comparison (melting point, mixed melting point, and infrared spectrum) with an authentic sample prepared from oestrone in acetic acid solution by the action of iodine in the presence of mercuric acetate[7]. Analytical and spectral data showed that the minor component was 2,4-di-iodo-oestrone (III). This compound was also obtained both by the action of iodine vapour on 2-iodo-oestrone adsorbed on silica gel and by conventional mercuric acetate-catalysed iodination of oestrone in acetic acid solution.

Acetylation of the phenolic hydroxyl group reduces the reactivity of the aromatic ring, and both oestrone acetate and its 2-iodo-derivative on silica gel were unattacked after prolonged exposure to iodine vapour.



(I) (II) (III)

Investigation of the rate of the reaction in preparative runs (100 μg of steroid/cm^2) showed that iodination of oestrone is virtually complete after 6 h at room temperature, the ratio of the resulting mono- and di-iodo derivatives then being approximately 3:1. The yields of 2-iodo-oestrone (II) after 20 and 60 min were 10 % and 32 %, respectively, as determined spectrophotometrically after isolation by thin-

layer chromatography. These results indicate that some reaction will occur even during the time required (1–2 min) for location of zones on a thin-layer chromatogram, with resulting contamination of the phenolic material subsequently isolated.

Qualitative work has shown that neither the calcium sulphate present as binder in the silica gel, nor the ultraviolet sensitizer, affect the rate of iodination. Furthermore, the results are unchanged when the plates are dried at 80° prior to iodination, to ensure that no traces of organic solvent remain adsorbed on the silica gel to act as a reaction medium. It is clear, however, that the silica gel itself plays a part in the reaction, as we were unable to detect any chemical reaction between iodine vapour and oestrone, when the latter was spread over the surface of a glass plate. It appears, then, that the efficiency of the iodination process may be traced to the catalytic effect of the silica gel. In agreement with this, the rate of formation of the two iodo-derivatives increased as the amount of oestrone adsorbed on a given area of silica gel was diminished. We conclude that iodine vapour does not constitute a non-destructive reagent for the location of phenols on silica gel chromatograms, particularly at low concentrations where the ultraviolet method is inapplicable. The reagent has also been found to attack cortisone, although the product was not identified[6].

We believe that the simple technique described above will be of value in the preparation of iodo-derivatives of phenols on a micro scale, and may be useful for the synthesis of phenols labelled with radioactive iodine.

Department of Chemistry, University of Aberdeen (*Great Britain*)
WM. BROWN
A. B. TURNER

1 G. BRANTE, *Nature*, 163 (1949) 651.
2 G. C. BARRETT, *Nature*, 194 (1962) 1171.
3 I. E. BUSH, *Biochem. J.*, 50 (1952) 370.
4 B. V. MILBORROW, *J. Chromatog.*, 19 (1965) 194.
5 D. WALDI, in E. STAHL (Editor), *Thin-layer Chromatography*, Springer, Berlin, 1965, p. 493.
6 J. S. MATTHEWS, A. L. PEREDA V. AND A. AGUILERA P., *J. Chromatog.*, 9 (1962) 331.
7 A. HILLMANN-ELIES, G. HILLMANN AND U. SCHIEDT, *Z. Naturforsch.*, 8b (1953) 436.

Received June 27th, 1966