and 4-acetoxycinnamic acid—substances which do not form strong complexes with caffeine—are not altered significantly by the 12% solution of 7-(2,3-dihydroxypropyl)-theophylline. Furthermore, when 1,3-dimethyluracil—a poor complexing agent for cinnamic acids—is substituted for the theophylline derivative, in the same molar concentration, the peak effluent volume of 3,4-dimethoxycinnamic acid is not significantly different from that obtained with the control columns.

Other applications of this type of complexation to chromatographic separations are being studied. For example, it has been possible to improve the separation of eugenol and isoeugenol on silicic acid columns with the sodium salt of riboflavin-5'-phosphate. Since isoeugenol has a conjugated double bond, its peak effluent volume is more strongly affected by the riboflavin salt than that of eugenol. We have also been able to influence the elution pattern of caffeine and 1,3-dimethyluracil in a predictable manner by using chlorogenic acid or sodium 2,4,6-trihydroxybenzoate solutions as stationary phases of silicic acid columns.

Acknowledgement. This investigation was supported in part by a research grant from the National Institutes of Health, Public Health Service.

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Received April 6th, 1962

J. Chromatog., 8 (1962) 413–414

Notes

Detection of hypoglycaemic sulphonyl-ureas on paper chromatograms

The determination of antidiabetic agents, such as tolbutamide and carbutamide, in biological fluids, blood, urine and drugs has been reported employing spectrophotometric[1–6], colorimetric[7–13], titration[14,15] (indicator), and anodic chronopotentiometric[16] methods. The colour reactions commonly used are the diazotisation of carbutamide and subsequent coupling with a suitable reagent such as 2-naphthol, etc. In the case of tolbutamide or chlorpropamide, the colorimetric measurement of the yellow dinitrophenyl derivative of the resulting amine has been described[9]. It appears

that, except for the spectrophotometric method, no specific reactions are available for a ready identification of these compounds.

The present communication reports the R_F values of these sulphonyl-urea derivatives in various solvent systems, as well as a very convenient method for their detection on a filter paper chromatogram. The developed paper is allowed to react with phenylhydrazine and subsequently sprayed with a solution containing ammoniacal Ni^{2+} to give beautiful pink to violet spots, according to the concentration of the substance. The separation of these substances from a mixture has also been achieved.

The compounds used in these experiments were: tolbutamide [N-(4-methylbenzenesulphonyl)-N'-butyl-urea], carbutamide [N-(4-aminobenzenesulphonyl)-N'-butyl-urea] and chlorpropamide [N-(4-chlorobenzenesulphonyl)-N'-propyl-urea]. These substances were investigated either individually or in a mixture. The paper strip (Whatman No. 1) was developed either by the ascending or the descending technique for 4–6 h at room temperature (30–31°) with the solvent system (given in Table I) and was then dried in a current of hot air. The dry paper was then carefully sprayed with a 2% solution of phenylhydrazine (reagent grade, E. Merck) in benzene and heated in a hot air oven at 190–195° for 2–3 min. This paper, on spraying with a solution containing equal volumes of 10% nickel sulphate solution in water and ammonium hydroxide (sp. gr. 0.88) revealed pink spots against a light brown background.

Ten micrograms of these compounds in a mixture could be separated and easily identified. The small difference between the R_F values of tolbutamide and chlorpropamide suggested that a run for a longer period of time (10–14 h) would be desirable in order to effect a satisfactory separation, particularly from a mixture. *n*-Butanol made alkaline with ammonia, as the mobile phase in descending chromatography produced better resolution. It is interesting to note that in basic systems these compounds are possibly resolved as their salts. In the case of an acid phase (*n*-butanol–acetic acid, 1:1), these substances were found to run almost as fast as the mobile solvent front without any resolution occurring.

While tolbutamide and chlorpropamide showed up as nice pink spots, carbutamide produced a comparatively faint reaction under the prevailing conditions.

TABLE I

R_F VALUES OF SOME HYPOGLYCAEMIC SULPHONYL-UREAS

(Ascending chromatography)

Compound	*EtOH–NH_4OH 6:1*	*i-PrOH–NH_4OH 3:1*	*n-PrOH–NH_4OH 4:1*	*n-BuOH satd. with NH_3*	*i-AmOH satd. with NH_3*
Tolbutamide	0.87	0.90 0.89*	0.82	0.78 0.81*	0.68
Carbutamide	0.71	0.72 0.71*	0.62	0.52 0.47*	0.19
Chlorpropamide	0.88	0.88 0.83*	0.83	0.75 0.73*	0.65

* R_F values in descending chromatography.

The spots were perfectly stable and their areas were found to be fairly consistent with the concentration of the substance applied. Like other sulphanilamides, carbutamide, owing to its 4-aminobenzene group, could also be detected as a yellow spot on white paper by spraying with a 0.5 % solution of *p*-dimethylamino-benzaldehyde in 95 % ethanol; tolbutamide and chlorpropamide, however, failed to respond.

The reaction of phenylhydrazine with a urea to give diphenyl-carbazide[17,18], which subsequently forms a coloured complex[19] with a metal ion such as Ni^{2+}, resistant to redox reactions with phenylhydrazine, has been successfully applied here. This reaction is excellent for a rapid identification of this group of disubstituted sulphonyl-ureas, and also for monosubstituted sulphonyl-ureas, *e.g.* N-(4-methylbenzene-sulphonyl)-urea. Barbiturates, and carbamates, *e.g.* meprobamate (2-methyl-2-*n*-propyl-1,3-propanediol dicarbamate), N,N-diphenyl carbamate, failed to respond to this reaction, while for open chain (straight or branched) ureides, *e.g.* carbromal (2-bromo-2-ethyl-butyryl-carbamide), under the prevailing conditions the reaction proved to be much less sensitive. It appears that cyclic ureides and certain derivatives of carbamic acid are resistant to phenylhydrazine.

This interesting reaction is being further studied with the purpose of applying it to the colorimetric estimation of these drugs and will form the subject of a separate communication. It seems likely that this method will prove most convenient and helpful in forensic work when determining these drugs in biological materials. Work in this direction is in progress.

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Received November 20th, 1961