# A FURFURAL REAGENT OF HIGH SPECIFICITY FOR THE DETECTION OF CARBAMATES ON PAPER CHROMATOGRAMS

By M. S. Moss and J. V. Jackson

*From the Metropolitan Police Laboratory, New Scotland Yard, S.W.*1

Received January 25, 1961

A spot reaction with high specificity for the detection of carbamates, and its application to paper chromatograms, is described. Chromatographic separation methods are given, together with results obtained on a number of currently used carbamate drugs. The incorporation of the reagent in a normal screening procedure for acidic and neutral drugs is suggested.

The detection of meprobamate [2,2-di(carbamoyloxymethyl)pentane] on paper chromatograms has been described by both Curry in 1957 and Ludwig and Hoffman in 1959 in personal communications, and also by Bedson (1959) and Walkenstein, Knebel, Macmullen and Seiftner (1958). These workers caused chlorine to react with nitrogen-containing compounds to form a chloramino-derivative which could be located by spraying with a starch-iodide reagent. This reaction was reported by Rydon and Smith (1952) for the detection of peptides, proteins, diketopiperazines, and acylated amino-acids. Modified reagents have been used for the detection of other compounds. Thus, Pan and Dutcher (1956) used the reaction to detect acylated neomycins; Reindel and Hoppe (1954) used it for the detection of proteins, peptides and amino-acids; Jackson and Moss (1960) applied it to the detection of several different kinds of neutral drugs, and Grieg and Leaback (1960) to the detection of several sugar derivatives. It is apparent, therefore, that many different types of compound are detected by this procedure, and it was felt that a more specific reaction for carbamates was required, especially in view of the increased use of this type of drug.

Ludwig and Hoffmann (1957) have described a method for the colorimetric determination of meprobamate in biological fluids. They used the colour reaction which occurs when meprobamate is treated with furfural under the influence of antimony trichloride. They did not establish the chemical nature of the coloured compound, but postulated that it was due to a furylidene coupling through the amido-group of the carbamate, under the influence of a suitable dehydrating agent.

We have investigated this reaction as a spot test for drugs containing the carbamoyloxy group, and find that the procedure described can be simplified to the extent of merely treating with furfural in the presence of concentrated hydrochloric acid.

## *Procedure*

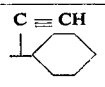
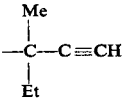
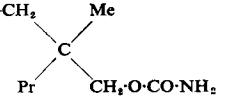
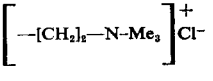
The test solution of the carbamate spotted on to filter paper can be dipped into a mixture of furfural one part and concentrated hydrochloric

acid 4 parts, mixed immediately before use. Because of the very rapid deterioration of this mixture the following procedure is preferable, and gives less background colour.

The paper is very lightly sprayed with furfural, followed by spraying more heavily with concentrated hydrochloric acid. The fumes from this mixture are highly objectionable and should be contained in a fume cupboard. Carbamates appear as purple to intense blue-black spots within a few seconds. The colour intensifies for about half a minute and persists

TABLE I

DETECTION OF CARBAMATES ON PAPER CHROMATOGRAMS

| Drug | R·NH·CO·O·R′ | | Solvent for standard | Acetic and oleic acids | Pentanol and ammonia | Ultraviolet light | Furfural and HCl |
|---|---|---|---|---|---|---|---|
| | R | R′ | | | | | |
| Ethinamate (1-ethynylcyclohexyl carbamate) | H |  | Ethanol | 51 | 93 | − | + |
| Methyl pentynol carbamate (3-carbamoyloxy-3-methylpent-1-yne) | H |  | Ethanol | 72 | 88 | − | + |
| Mephenesin carbamate (1 - carbamoyloxy - methyl - 2 - *o* - tolyloxyethanol) | H | —$CH_2$·CH(OH)·$CH_2$·O·$C_6H_4$·Me | Acetone | 64 | 83 | + | + |
| Meprobamate [2′,2-di(carbamoyloxymethyl)pentane] | H |  | Acetone | 71 | 86 | − | + |
| Styramate (β-hydroxyphenethyl carbamate) | H | —$CH_2$·CH(OH)·$C_6H_5$ | Acetone | 78 | 76 | + | + |
| Carbachol (2 - carbamoyloxyethyltrimethylammonium chloride) | H |  | Methanol | 82 | 0 | − | + |
| Urethane (ethyl carbamate) | H | —Et | Acetone | 89 | 73 | − | +* |
| Phenylurethane (ethylphenylcarbamate) | $C_6H_5$ | —Et | Acetone | 36 | 94 | + | − |
| Urea | | | Methanol | 86 | 16 | − | + |

* Evaporates. Must be spotted immediately before solvent run.

for several months. The background is pale green or grey. Urea is the only commonly occurring interfering substance, which is readily distinguishable from the carbamates by its destruction by urease, by its relative insolubility in ether, and by its $R_F$ value in paper chromatography (see Table I). A positive reaction is also given by phenazone (antipyrine; 2,3-dimethyl-1-phenylpyrazol-5-one) but only after a time lag of about half an hour. The mono- and di-substituted carbamates, phenylurethane and neostigmine, do not react, presumably because of their *N*-substituents. Used as a spot test the reaction will readily detect 5 $\mu$g. of most carbamates.

No reaction was obtained with barbiturates, alkaloids, ureides, diketopiperdines, hydantoins, oxazolidines, purines, sympathomimetic amines, or local anaesthetics of the basic ester type.

*Application to Biological Material*

Spot tests for $\mu$g. quantities of carbamates and other drugs are rarely successful when applied directly to biological material; a systematic extraction scheme is usually essential. Such isolation techniques are time-consuming. For routine testing or preliminary screening, therefore, our aim is to remedy this situation by chromatographic separation and sequential treatment with a series of reagents applied to the same chromatogram. It has been found possible to incorporate the furfural reagent in such an analytical scheme. Table I lists structures, $R_F$ values and reactions of the carbamates examined.

*Acidic Drugs*

For biological samples or pharmaceutical preparations suspected of containing acidic drugs, for example, aspirin or barbiturates together with carbamates, the sample is acidified and extracted with ether. The residue obtained after evaporation of the ether is dissolved in a small quantity of ethanol and spotted on Whatman No. 1 chromatography paper. The chromatogram is developed with a monophasic solvent prepared by shaking 180 ml. of pentanol with 20 ml. of strong ammonia solution. The chromatogram should be developed with this solvent for at least 5 hr., by the ascending method. The paper is then air-dried and the following sequence of reagents used.

Ultra-violet light. (Hanovia Chromatolite) for fluorescent or absorbent drugs.

Cobalt chloride and ammonia (Jackson, 1958) for barbiturates.

Ferric chloride solution for salicylates and phenols.

Furfural and hydrochloric acid for carbamates.

*Neutral Drugs*

For biological samples and pharmaceutical preparations suspected of containing carbamates and neutral drugs only, reversed phase chromatography may be used with advantage. The drug is extracted as for acidic drugs and applied to chromatography paper prepared in the following way.

The paper is dipped in an acetone solution containing 40 per cent oleic acid, and blotted immediately. It is then allowed to dry in air at room temperature. Uniform impregnation of the paper is essential. The solution of the extracted material in ethanol is spotted on the paper and the chromatogram developed for at least 6 hr. by the ascending method with a 50 per cent solution of glacial acetic acid in water which has been equilibrated with a few drops of oleic acid. The paper is air-dried and the following sequence of reagents used.

Ultra-violet light. (Hanovia Chromatolite).

Chlorine and starch with potassium iodide (Jackson and Moss, 1960) for most "neutral" drugs.

Furfural and hydrochloric acid for carbamates.

## REFERENCES

Bedson, H.S. (1959). *Lancet*, **1**, 288–290.
Grieg, C. G. and Leaback, D. H. (1960). *Nature, Lond.*, **188**, 310–311.
Jackson, J. V. (1958). *Chromatographic Techniques*, 1st ed., p. 214. London: Heinemann.
Jackson, J. V. and Moss, M. S. (1960). *Chromatographic and Electrophoretic Techniques*, 2nd ed., Vol. 1, pp. 404–408. London: Heinemann.
Ludwig, B. J. and Hoffmann, A. J. (1957). *Arch. Biochem. Biophys.*, **72**, 234.
Pan, S. C. and Dutcher, J. D. (1956). *Analyt. Chem.*, **28**, 836.
Reindel, F. and Hoppe, W. (1954). *Chem. Ber.*, **87**, 1103–1107.
Rydon, H. M. and Smith, P. W. G. (1952). *Nature, Lond.*, **169**, 922.
Walkenstein, S. S., Knebel, C. M., Macmullen, J. A. and Seifter, J. (1958). *J. Pharmacol.*, **168**, 254–258.