## Thin-layer chromatographic identification of thirteen medicinally important carbamates

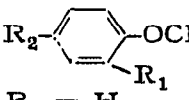
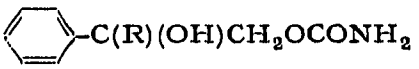
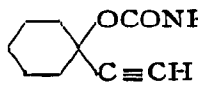
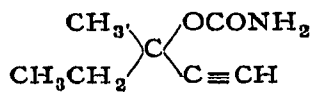
Certain carbamates are used in medicine as minor tranquillizers, skeletal muscle relaxants, anti-hypertensive agents and anti-neoplastic drugs. Most have some hypnotic effect. Since many are subject to misuse, specific tests for their identification are of value to enforcement agencies.

Identification by derivative formation is difficult if only a small amount of drug is available. Melting point determination is of little value since many of the drugs have low melting points and therefore require extensive purification in order to obtain decisive results. Although the use of paper chromatography has been reported for the separation of certain N-unsubstituted carbamates[1,2], the advantages of thin-layer chromatography have not been fully utilized. Thus carisoprodol has been separated from meprobamate and mebutamate[3] by thin-layer chromatography but the latter two carbamates were not resolved satisfactorily. Using two solvent systems, LINDFORS[4] separated ethinamate, hexapropymate and meprobamate, while HEYNDRICKX *et al.*[5] resolved hexapropymate, urethane, and meprobamate in one system. MOSS AND JACKSON[1] showed that a furfural–hydrochloric acid spray was a sensitive detecting agent for the N-unsubstituted carbamates on paper chromatograms. The use of a modified furfural spray reagent has been reported[5].

This report will show that thirteen carbamates (Table I) may be distinguished from each other by the use of three chromatographic systems and three spray reagents.

TABLE I

NOMENCLATURE AND STRUCTURAL FORMULAE OF CARBAMATES EXAMINED

| *Compound* | *Structural formulae* | |
|---|---|---|
| |  | |
| Chlorphenesin carbamate | $R_1 = H$ | $R_2 = Cl$ |
| Mephenesin carbamate | $R_1 = CH_3$ | $R_2 = H$ |
| Methocarbamol | $R_1 = OCH_3$ | $R_2 = H$ |
| |  | |
| Hydroxyphenamate | $R = C_2H_5$ | |
| Styramate | $R = H$ | |
| | $R_1CH_2C(R_2)(CH_3)CH_2OCONH_2$ | |
| Carisoprodol | $R_1 = OCONHC_3H_7$(iso) | $R_2 = C_3H_7(n)$ |
| Mebutamate | $R_1 = OCONH_2$ | $R_2 = C_4H_9(sec)$ |
| Meprobamate | $R_1 = OCONH_2$ | $R_2 = C_3H_7(n)$ |
| Tybamate | $R_1 = OCONHC_4H_9(n)$ | $R_2 = C_3H_7(n)$ |
| Emylcamate | $CH_3CH_2C(CH_3)(C_2H_5)OCONH_2$ | |
| Ethinamate |  | |
| Methylpentynol carbamate |  | |
| Urethane | $CH_3CH_2OCONH_2$ | |

*Materials and methods*

The carbamates were obtained from pharmaceutical manufacturers or commercial suppliers. In some instances they were isolated from solid dosage forms and recrystallized. Alcohol or acetone solutions were used for chromatography. For routine analysis, tablets were finely ground in a mortar and the powder shaken with 95 % ethanol. The volume of alcohol used was that which would give an estimated concentration of 10 mg of drug per ml of alcohol. Capsule powders and liquids (*e.g.* tybamate) were treated similarly.

Glass plates (200 × 200 mm) were coated with 0.25 mm layers of absorbent, by means of the Shandon Unoplan apparatus. Two types of plates were prepared, one from Silica Gel G (Merck, Darmstadt: 30 g in 60 ml of water) and the other type from Kieselguhr G (Merck, Darmstadt: 30 g in 55 ml of water). For visualization by ultraviolet absorbance, Fluorescent Indicator Green (Woelm, Eschwege) was added to give 2 % by weight based on the dry adsorbent.

The plates were air dried for 8 h and stored in air. If urgently required the plates may be air dried for one half-hour, then heated for one half-hour at 110° without affecting the results.

The chromatograms were developed in glass tanks (21 × 20.5 × 8 cm), lined with filter paper. Fifty ml of the solvent was used to wet the filter paper, the excess poured off, and 100 ml of solvent added.

*Chromatography systems*

*System A*

Plates: Silica Gel G.

Solvent: The lower layer obtained by shaking together acetic acid–carbon tetrachloride–chloroform–water (100:60:90:50).

*System B*

Plates: Kieselguhr G, impregnated with formamide. The plates were dipped in a solution of 5 % formamide in methanol, and allowed to dry in air for 12 min before application of the samples.

Solvent: benzene–chloroform (30:120) saturated with formamide.

*System C*

Plates: Kieselguhr G impregnated as in System B.

Solvent: carbon tetrachloride saturated with formamide.

The spots on formamide impregnated plates diffuse on standing. Such plates must be developed immediately after the sample is applied, and sprayed or observed under ultraviolet light immediately after development.

To obtain accurate $R_F$ values a small mark was made beside the spot, opposite its centre as soon as the spot was made visible by spraying or ultraviolet absorption. A short wavelength (254 mμ) ultraviolet source was used to observe absorption.

*Spray reagents*

(1) *Furfural–hydrochloric acid.* Furfural, if discoloured, was distilled at atmospheric pressure in a stream of nitrogen This reagent kept well if stored in a dark bottle in a refrigerator, and flooded with nitrogen whenever a portion was removed.

The plate was sprayed well, but not soaked with furfural, then immediately sprayed with concentrated hydrochloric acid.

(2) *Furfural–sulphuric acid.* This was applied as for (1) except that concentrated sulfuric acid was used, instead of hydrochloric acid.

(3) *Vanillin–sulphuric acid*[6]. Five grams of vanillin were dissolved in 100 ml of concentrated sulphuric acid. This reagent could be stored for several weeks. The plate was sprayed well, but not soaked with the reagent. Yellow spots appeared and the plate was heated in an oven at 110° until a standard spot of meprobamate, applied at the same time as the other spots, turned blue. The plate was removed from the oven, the colour of the spots noted immediately, and again 15 min after removal from the oven.

*Results and discussion*

The $R_F$ values observed are shown in Table II, and the colour reactions with the various sprays in Table III. Except for the pairs carisoprodol–tybamate and methylpentynol carbamate–ethinamate, the compounds are satisfactorily resolved by means of systems A or B, with $R_F$ differences greater than 0.05. However carisoprodol–tybamate and methylpentynol carbamate–ethinamate are well resolved by means of system C.

The characteristic colours of many of the carbamates with the vanillin–sulphuric acid spray permit distinctions between many compounds with similar $R_F$ values. Methocarbamol and chlorphenesin carbamate are just resolved with system A and the distinctive colour reaction of methocarbamol with the vanillin reagent is ample confirmation of its difference from chlorphenesin carbamate. Where a mixture is present the upper and lower halves of the spot are usually sufficiently different in colour to show that it is not homogeneous. This is also useful for confirmation of identity, since it is only necessary to apply the unknown substance and the known substance to the same place on the starting line, and check the homogeneity of the colour developed with the vanillin reagent.

The furfural–hydrochloric acid spray is the most sensitive detection reagent for all the carbamates examined producing blue-black spots with as little as 2 to 5 $\mu$g. Emylcamate is an exception and requires approximately 12.5 $\mu$g for visualization with this spray. However, when treated with the furfural spray followed by sulphuric acid, emylcamate gives a magenta spot with as little as 1 $\mu$g of material. Recently a modified furfural spray reagent has been proposed[5]. The reagent takes more time to prepare than the simple furfural–hydrochloric acid spray, and the spots develop more slowly, but the background is better with the modified spray reagent, with a resulting increase of about four times the sensitivity of the furfural–hydrochloric acid spray.

MOSS AND JACKSON[1] reporting on the specificity of the furfural–hydrochloric acid reagent, observed that of a large number of drugs examined, only phenazone and urea gave blue-black colourations. The reaction with phenazone was reported to be very slow, but urea is well distinguished by its low $R_F$ value in our systems. In the present investigation salicylamide in addition to phenazone was also found to give a blue-black colour with the furfural–hydrochloric acid and the HEYNDRICKX modification. It is included in Tables II and III, and is distinguished from the carbamates by its $R_F$ value in system A and the blue-white fluorescence appearing in the centre of an absorbing spot when the plate is irradiated with ultraviolet light of 254 m$\mu$ wavelength. Of the carbamates listed in Table I only those containing a phenyl group absorb at 254 m$\mu$.

TABLE II

$R_F$ VALUES OF MEDICINAL CARBAMATES, UREA AND SALICYLAMIDE

| *Compound* | *Chromatographic system* | | | | |
|---|---|---|---|---|---|
| | *A* | *A* | *B* | *B* | *C* |
| Urea | 0.13 | 0.12 | 0.00 | 0.00 | 0.03 |
| Methocarbamol | 0.22 | 0.22 | 0.31 | 0.33 | 0.00 |
| Chlorphenesin carbamate | 0.27 | 0.26 | 0.31 | 0.29 | 0.00 |
| Styramate | 0.29 | 0.28 | 0.19 | 0.21 | 0.00 |
| Mephenesin carbamate | 0.34 | 0.32 | 0.44 | 0.43 | 0.00 |
| Meprobamate | 0.37 | 0.37 | 0.35 | 0.34 | 0.00 |
| Mebutamate | 0.39 | 0.38 | 0.41 | 0.40 | 0.00 |
| Hydroxyphenamate | 0.42 | 0.41 | 0.50 | 0.50 | 0.04 |
| Salicylamide | 0.51 | 0.54 | 0.40 | 0.41 | 0.10 |
| Urethane | 0.60 | 0.61 | 0.57 | 0.61 | 0.11 |
| Carisoprodol | 0.64 | 0.65 | 0.79 | 0.81 | 0.25 |
| Tybamate | 0.65 | 0.70 | 0.78 | 0.84 | 0.37 |
| Methylpentynol carbamate | 0.70 | 0.72 | 0.70 | 0.71 | 0.25 |
| Ethinamate | 0.72 | 0.75 | 0.74 | 0.75 | 0.37 |
| Emylcamate | 0.80 | 0.82 | 0.77 | 0.81 | 0.66 |
| Solvent front | 10 cm | 15 cm | 10 cm | 15 cm | 10 cm |
| Running time | 26 min | 78 min | 12 min | 21 min | 16 min |
| Drying time | — | — | 12 min | 15 min | 12 min |

TABLE III

VISUALIZATION OF CHROMATOGRAPHIC SPOTS OF MEDICINAL CARBAMATES, SALICYLAMIDE AND UREA

| *Compound* | *Ultraviolet light,* | *Spray reagents*[a] | | | | |
|---|---|---|---|---|---|---|
| | *254 mμ*[b] | *1*[c] | *2*[d] | *3*[e] | *3*[f] | *3*[g] |
| Urea | — | + | + | R | NC | NC |
| Methocarbamol | + | + | + | Y | Pi | R-P |
| Chlorphenesin carbamate | + | + | + | Y | NC | NC |
| Styramate | + | + | + | Y | Gr-Br | Gr-Br |
| Mephenesin carbamate | + | + | O | Y | P | Pi-Br |
| Meprobamate | — | + | + | Y | B | B |
| Mebutamate | — | + | + | Y | B | B-Gr |
| Hydroxyphenamate | + | + | + | Y | Gr-P | P-Gr |
| Salicylamide | + Fl | + | — | — | F-Br | F-Br |
| Urethane | — | + | + | Y | F-Y | F-Y |
| Carisoprodol | — | + | + | Y | P | B |
| Tybamate | — | + | + | Y | B | D-B |
| Methylpentynol carbamate | — | + | O | P | P | B-Gr |
| Ethinamate | — | + | O | Y | B | Br-Gr |
| Emylcamate | — | + | P-R | P | P | P |

[a] Designation of colours: B = blue; Br = brown; Gr = grey; O = orange; P = purple; Pi = pink; R = red; Y = yellow; D = dark; F = faint; NC = no colour.

[b] + Indicates absorption, + Fl, absorption, blue fluorescence at centre of spot.

[c] + Indicates blue-black spots.

[d] + Indicates blue-black spots, except as noted. The background darkens rapidly and blue-black spots merge into it.

[e] Colour immediately after spraying.

[f] Colour on heating at 110°. (See text.)

[g] Colour on standing in air for 15 min, after heating.

Bethanecol and carbachol, carbamates which lack N-substituents, react with the furfural–hydrochloric acid spray, but they have zero $R_F$ values in all the systems included in this study. The compounds may be separated in a system reported by TAYLOR[7].

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## Zur Standardisierung der Dünnschichtchromatographie

## II. Die $R_F$-Werte eines spezifischen Nachweises für Phenacetin und chemisch verwandte Verbindungen

In einer vorausgegangenen Untersuchung[1] wurden für die Dünnschichtchromatographie (DC) in tubes folgende Vorteile gegenüber den üblichen Methoden festgestellt: (1) durch die exakte Einstellbarkeit des Wassergehaltes der Kieselgelschicht sind die $R_F$-Werte besser als mit bisher üblichen Methoden reproduzierbar; (2) die beschichteten inaktiven tubes ermöglichen, bei verschiedenen Temperaturen aktiviert, die Auswahl der jeweils günstigen Trennungsbedingungen; (3) die standardisierten Bedingungen erlauben den direkten Vergleich der Ergebnisse mit denen anderer Untersuchungen.

Diese Untersuchungen wurden fortgesetzt. Dabei wurde eine bisher anscheinend unbekannte Reaktion von Phenacetin gefunden. Die bisher übliche Nachweismethode mit Salpetersäure[2] ist ziemlich unspezifisch, da ganze Stoffgruppen gelbe Nitrierungsprodukte ergeben können. Auch der Nachweis mittels des Eisen(III)chlorid–Kaliumhexacyanoferrat(III)-Reagenzes[3] kann nicht als spezifisch angesehen werden. Die Spezifität der Phenacetin-Nachweismethode wurde an 18 Substanzen geprüft, gleichzeitig wurde die Reproduzierbarkeit der $R_F$-Werte der Tube-DC mit der Plattenmethode bei Anwendung von zwei Kammern (KS und S) verglichen.