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Note

Thin-layer chromatographic method for the separation and determination of the products of the reaction of amides with formaldehyde

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Amides (*e.g.*, urea, acetamide and benzamide) react with formaldehyde under alkaline and acidic conditions to yield polymeric products. In earlier papers[1–6] on the kinetics and mechanism of these reactions, the quantitative determination of the individual low-molecular-weight compounds formed in the aqueous reaction mixture in the initial stages was not reported. Quantitative thin-layer chromatographic methods developed for the kinetic investigation of these reactions are reported in this paper.

EXPERIMENTAL

Materials

Urea. BDH (Poole, Great Britain), analytical-reagent grade, recrystallized from water (m.p. 133°C).

Formaldehyde. BDH, analytical-reagent grade; an aqueous solution (38%) containing less than 2% of methanol was used.

Monomethylolurea (MMU) (m.p. 110–111°C), dimethylolurea (DMU) (m.p. 132°C) and methylenediurea (MeDU) (m.p. 207–208°C). These were prepared by standard methods[7].

Dimethylenetriurea (DMeTU). Urea (80 g) and formaldehyde (13 ml) are stirred well in the presence of 0.5 ml of phosphoric acid. The mixture becomes homogeneous and a white solid separates after 1 h. The precipitate is filtered, washed with cold water and crystallized repeatedly from water until completely free from higher homologues. The m.p. is 243°C.

Trimethylenetetraurea (TMeTeU). This is prepared by the method reported by Kadowaki[8] with modifications. Formaldehyde (78 ml) and urea (100 g) are added to 300 ml of water and the pH is adjusted to 2.5 by adding hydrochloric acid. The mixture is kept for 1 day, then the solidified mass is stirred with hot water and filtered. The filtrate on cooling yields a white solid, which is crystallized repeatedly until the TMeTeU is free from higher homologues. The m.p. is 248°C.

Acetamide. BDH, analytical-reagent grade (m.p. 81°C).

Monomethylolacetamide (MMA). Acetamide (12 g) and formaldehyde 15.3 ml are mixed with potassium carbonate (1 g), the mixture is warmed gently and the viscous liquid is kept aside in a vacuum desiccator. The solid mass is extracted with methanol. The filtrate on evaporation yields a hygroscopic solid (m.p. 51°C).

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Methylenebisacetamide(MeBA). Acetamide (23 g) is weighed into a 100-ml conical flask and stirred with formaldehyde (15.3 ml) and concentrated hydrochloric acid (4 ml) for 15 h. The precipitated compound is filtered and crystallized repeatedly from ethanol, giving crystalline needles (m.p. 199–201°C).

Benzamide. BDH, analytical-reagent grade (m.p. 130°C).

Monomethylol benzamide (MMB). Benzamide (12 g) is dissolved in the minimum volume of methanol and stirred with formaldehyde (15.3 ml) in the presence of potassium carbonate (1 g) for 24 h. The reaction mixture is cooled in ice. The crystals separated are filtered and recrystallized from ethanol, giving plates of m.p. 108–110°C.

Methylenebisbenzamide (MeBB). Benzamide (12 g) is dissolved in the minimum volume of methanol and stirred with formaldehyde (3.8 ml) and concentrated hydrochloric acid (1 ml) for 24 h. The solvent is evaporated and the precipitated compound is filtered and recrystallized from hot ethanol (m.p. 222–224°C).

The identities of the above compounds were confirmed from their nuclear magnetic resonance spectra, measured on a Varian A60 instrument operating at at 100 MHz at 25°C in deuterated dimethyl sulphoxide in the presence of anhydrous calcium chloride.

Chromatographic technique

Silica gel G plates (0.5-mm layer) (BDH) were used for separation, except for the methylol derivatives of urea, which were separated on cellulose plates (0.5-mm layer) (Loba, Bombay, India). After air drying, the plates were heated at 100–105°C (for 10 min for cellulose and 30 min for silica gel).

Aqueous solutions (0.02 *M*) of the compounds (10–20 μl) were spotted on the plates and developed using a suitable solvent system (see below). The dried plates were sprayed with a colour reagent or viewed under UV light. The spots were marked, scraped off and, after suitable treatment, measured quantitatively using a Systronics spectrophotometer.

Procedure A. The methylols of urea were separated on cellulose plates using pyridine–chloroform–water (40:16:5) and methylene ureas on silica gel plates using isopropanol–toluene–water–acetic acid (70:20:3:2). The spots corresponding to these compounds were made visible by spraying with a 1% solution of phenylhydrazine in 30% sulphuric acid followed by 0.1% iron(III) chloride solution. The reddish pink spots were scraped off separately and centrifuged after the addition of 5 ml of reagent I (see below). The clear solution was transferred completely into a boiling-tube. Reagent II (see below) (5 ml) was added and the mixture heated on a boiling water-bath for 20 min using an air condenser, preferably in the dark. It was then rapidly cooled in ice–water and diluted to 25 ml and the absorbance of the pink solution was measured at 520 nm.

Reagent I was prepared by dissolving 1.5 g of iron (III) chloride hexahydrate in 30 ml of 85% phosphoric acid and diluting with 15 ml water. This solution (0.5 ml) was made up to 500 ml using 10% (v/v) sulphuric acid.

Reagent II was prepared by dissolving 0.17 g of thiosemicarbazide in 100 ml water and mixing with a solution of diacetyl monoxime (0.85 g) in 100 ml of warm water. The mixture was made up to 500 ml and kept in an amber-glass bottles.

Procedure B. The products of the acetamide–formaldehyde reaction were

separated on silica gel plates using methanol–chloroform (3:2) and the products of the benzamide–formaldehyde reaction on silica gel plates using diethyl ether as the solvent. The spots corresponding to the benzamide derivatives could be qualitatively detected by iodination. The acetamide derivatives could be detected by spraying with the reagent described in procedure A (with a sulphuric acid concentration of nearly 80%).

TABLE I

R_F VALUES, λ_{max} AND MOLAR ABSORPTIVITIES (ε) OF THE COLOURED EXTRACTS OF THE VARIOUS COMPOUNDS ANALYSED

Compound	R_F	λ_{max} *(nm)*	$\varepsilon(l \cdot mol^{-1} \cdot cm^{-1})$
MMU*	0.48	620	8359
DMU*	0.64	520	7782
MeDU*	0.41	520	13040
DMeTU*	0.21	520	23060
TMeTeU*	0.053	520	21100
MMA**	0.64	570	14701
MeBA**	0.82	570	11034
MMB**	0.40	570	14695
MeBB**	0.72	570	12867
Urea*,***	0.37	—	—
Benzamide**,‡	0.473	—	—
Acetamide**,‡‡	0.432	—	—

* Developed using procedure A.
** Developed using procedure B.
*** Detected using Erlich's reagent.
‡ Detected by iodination.
‡‡ Detected by chlorination followed by potassium iodide–starch solution[9].

However, for quantitative determination the spots were located and marked in a UV chamber, scraped off separately, extracted with water and the silica gel removed by centrifuging. The clear solution was made up to 25 ml, mixed with 0.3 ml of 0.1 *M* chromotropic acid and carefully made up to 50 ml with concentrated sulphuric acid. It was then heated on a boiling water-bath for 10 min, cooled to room temperature and the absorbance of the violet solution measured at 570 nm.

RESULTS AND DISCUSSION

Quantitative determinations were made from Beer's law plots of absorbance *versus* concentration. The R_F values of the compounds and λ_{max} values and molar absorptivities of the coloured extracts are given in Table I. The accuracy of the method is $\pm$ 2%.

The reaction between amides and formaldehyde is complex. However, the products can be limited to methylols and low-molecular-weight methylene compounds by adjusting the reaction conditions. The initial products, in both acidic and alkaline media, are the methylol derivatives. These condense with amides to yield methylenebisamides. In an acidic medium the methylenebisamides predominate over the

methylol derivatives. The reaction can be arrested for kinetic analysis by adding saturated bisulphite solution.

MMU, DMU, MeDU, DMeTU and TMeTeU are hydrolysed by sulphuric acid and the formaldehyde liberated reacts with phenylhydrazine to give pink spots in the presence of iron (III) chloride. The liberated urea is determined by spectrophotometry. The phenylhydrazone colour is discharged on heating and does not interfere in the determination.

With MMA, MeBA, MMB and MeBB, the formaldehyde liberated on hydrolysis is determined by its colour reaction with chromotropic acid.

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