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SEPARATION AND DETERMINATION OF ALIPHATIC AND AROMATIC AMINES BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH ULTRAVIOLET DETECTION

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SUMMARY

Aliphatic and aromatic primary and secondary amines react with phenyl isocyanate to yield N,N′-disubstituted ureas, very stable and highly UV-absorbing compounds. The reaction is quantitative and is complete in a few minutes.

The derivatives are easily chromatographed on a reversed-phase high-performance liquid chromatographic system. UV-monitoring allows detection down to the 1-ng level. No pH suppression of the eluent is needed.

INTRODUCTION

Underivatized volatile amines can usually be separated by gas–liquid chromatography (GLC) on a polar stationary phase. Derivatization, such as silylation and reaction with aldehydes followed by GLC on a non-polar stationary phase, is also sometimes used. Incomplete derivatization and thermal lability of some of these derivatives can make quantitation difficult.

Amines have also been separated by high performance liquid chromatography (HPLC) using different derivatives, both UV-absorbing and fluorescent, by ion-pair chromatography and cation-exchange chromatography. The HPLC separation of free amines usually requires pH suppression, and this again tends to destroy modern silica-based stationary phases. Poor UV-absorptivity of most aliphatic amines also makes the detection of small amounts difficult[1–3].

Phenyl isocyanate has proved to be an excellent derivatizing reagent for compounds with active hydrogen atoms such as alcohols and water[4–6]. The corresponding urethanes and diphenylurea are easy to chromatograph on a reversed-phase system with UV detection. This method is also well suited for primary and secondary amines, both aliphatic and aromatic. They react with phenyl isocyanate to yield N,N′-disubstituted ureas, as shown in Fig. 1. A single and very stable derivative is obtained even with primary amines. It is easily chromatographed on a reversed-phase system and requires no pH suppression. UV-monitoring at 240–260 nm allows detection of the derivatives down to the 1–10-ng level.

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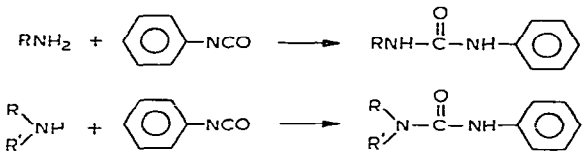

Fig. 1. General reactions of primary and secondary amines with phenyl isocyanate.

EXPERIMENTAL

Apparatus

A Varian 5000 Series liquid chromatograph (Varian Aerograph, Walnut Creek, CA, U.S.A.) equipped with a Perkin-Elmer LC-55 variable-wavelength UV-VIS detector (Perkin-Elmer, Oak Brook, IL, U.S.A.), a Goerz Servogor 541 recorder (Goerz Electro, Vienna, Austria) and a self-packed Spherisorb 10 ODS reversed-phase column (250 × 4.6 mm I.D.) were used for the chromatographic separation and detection of the products. Injections were made by a pressure-tight 10-μl SGE syringe (Scientific Glass Engineering, North Melbourne, Australia) into a Hewlett-Packard septumless on-column injector (Hewlett-Packard, Karlsruhe, G.F.R.).

A Perkin-Elmer 402 scanning spectrophotometer (Perkin-Elmer, Beaconsfield, Great Britain), a Jeol FX-60 Fourier-transform ^{1}H and ^{13}C NMR spectrometer (Jeol, Tokyo, Japan) and a Büchi melting-point apparatus (Büchi, Flavil, Switzerland), together with the HPLC apparatus, were used to check the purity and structure of the amine derivatives.

Reagents

The eluent was a mixture of acetonitrile (ACN; HPLC-grade, Rathburn Chemicals, Walkerburrn, Great Britain) and water, twice distilled. Phenyl isocyanate (PHI) and N,N-dimethylformamide (DMF), distilled under vacuum, as well as all the standard amines used in this study were of analytical grade.

Caution: It is best to handle phenyl isocyanate in a fume cupboard because it is an eye and respiratory irritant.

Standards

N-Phenyl-N′-alkyl(aryl)urea standards were prepared by mixing each amine with PHI in an equivalent ratio of 1:1.1. Because the reaction is very vigorous it is advisable to keep the reaction vessel in an ice-bath and also add the PHI dropwise. After completion of the reaction the precipitate was collected by suction filtration, washed with cold hexane and recrystallized from chloroform until a sharp melting point was obtained.

Note: Avoid water during the first step because it forms N,N′-diphenylurea[5], a product similar to those obtained by the amines. Aniline also forms N,N′-diphenylurea (Fig. 2).

Sample derivatization procedure

It is preferable to dissolve the samples in DMF, because it both seems to catalyze the reaction and also serves as a good solvent for the disubstituted ureas.

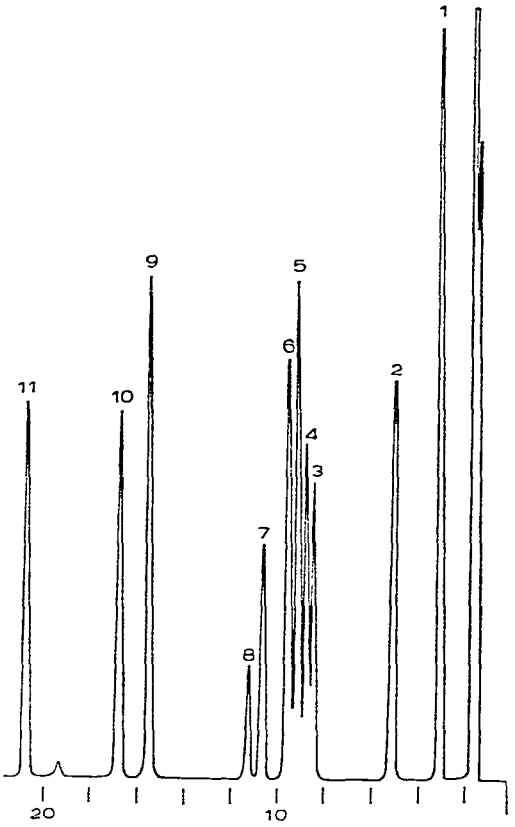

Fig. 2. Gradient run of 10 amines and water derivatized with PHI. Chromatographic conditions: column, Spherisorb 10 ODS, 25 cm × 4.6 mm I.D.; UV monitoring at 240 nm, 1.0 a.u.f.s.; linear gradient from 20% ACN to 70% ACN in 20 min. The peaks correspond to the following amines (*ca.* 1 mg/ml of the phenylurea of each in DMF): 1, ammonia; 2, dimethylamine; 3, diethylamine; 4, 2-butylamine; 5, isobutylamine; 6, *n*-butylamine; 7, benzylamine; 8, water; 9, diphenylamine; 10, dibutylamine; 11, decylamine.

A 0.5-ml volume of the amine (usually less than 10 mg) dissolved in DMF was pipetted into a glass-stoppered test tube containing a short magnetic rod. Then 0.5 ml of PHI was added and the tube placed on a magnetic stirrer for 5 min. In order to destroy the excess of PHI, 0.5 ml of an aliphatic alcohol, *e.g.* methanol, was added. The "destroyer" must be chosen so that it doesn't interfere with the peaks to be determined.

Before injecting the sample, an appropriate amount of internal standard, usually another amine derivative or an O-alkyl-N-phenylurethane[4], was added.

It is advisable to prepare a blank consisting of "derivatized" reagents.

Liquid chromatographic separation and quantitation

Derivatized samples in a DMF solution free of particulate matter were injected (1–10 μl) into the chromatographic system with a pressure-tight syringe. The Spherisorb 10 ODS column was at ambient temperature. The eluent, consisting of

ACN and distilled water, was pumped (2 ml/min) either isocratically or using different gradients depending on the amount or quality of amines to be determined. The peaks were usually monitored at 240 nm.

A 20-cm "plastic integrator", *i.e.* a ruler, was used for both qualitative identification and quantitative determinations. Qualitatively the retention times were compared to those of known standards, and quantitatively the peak height measurement with the internal standard method was used[5].

RESULTS AND DISCUSSION

Amount of phenyl isocyanate, reaction time and temperature. Destruction of excess phenyl isocyanate

As mentioned earlier, alcohols, water, phenols and carboxylic acids, besides amines, react with PHI. Therefore it is advisable to use a large excess of the derivatizing agent. With very diluted samples it is recommended that the water be evaporated or the amines be extracted before derivatization. Otherwise each water molecule consumes two PHI molecules and causes a huge diphenylurea peak to appear in the chromatogram.

Of the compounds mentioned above, amines react the fastest. With simple monoamines the reaction is complete in a few seconds at room temperature, but to ensure complete derivatization in samples, a reaction time of 5–15 min was used.

No thermal treatment is usually needed, at least not elevated temperatures. During the destruction of the excess of PHI the temperature rises. With thermally labile compounds the reaction vessel can be placed in an ice-bath at this step.

If not destroyed before injection, PHI reacts in the column with the water that is part of the eluent.

Aliphatic alcohols are good destroying agents. The chain length must be chosen so that the corresponding alkylphenylurethane doesn't interfere with the peaks to be determined. As a rule, it can be said that methylphenylurethane elutes at *ca.* V_0 with an eluent consisting of equal amounts of ACN and water.

Stability of N,N'-disubstituted ureas

No noticeable changes in the chromatograms of the ureas dissolved in DMF appeared during several days' storage at ambient temperature. This enables samples to be prepared long before the chromatographic run.

Detection and linearity

N-Alkyl-N'-phenylureas show a very strong UV maximum at *ca.* 240 nm. The molar coefficient is *ca.* 20,000 l mol^{-1} cm^{-1}, which allows detection down to the 1–5-ng level.

N-Aryl-N'-phenylureas also show a strong UV maximum at *ca.* 255 nm. The molar coefficient is usually greater than 25,000 l mol^{-1} cm^{-1}, which allows detection even below 1 ng.

The reaction is quantitative, as was shown by letting different amounts of *n*-butylamine react with PHI according to the method described under *Sample derivatization procedure.* The excess of PHI was destroyed with *n*-propanol, which elutes short after N-(*n*-butyl)-N'-phenylurea. N-Dimethyl-N'-phenylurea was used

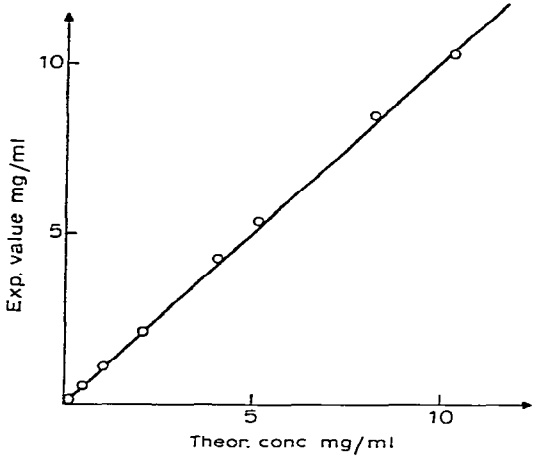

Fig. 3. Linearity curve for N-(*n*-butyl)-N′-phenylurea. For details, see text.

as internal standard. The corresponding purified crystalline ureas were used to calibrate the method. The curve, experimental *vs.* theoretical value, is shown in Fig. 3.

Advantage and disadvantages of the method

Advantages

(1) The derivatives are not pH-sensitive and need no suppression of the eluent.
(2) The derivatives are stable even in solution for days.
(3) Volatile amines can be "caught" and made non-volatile in a few seconds.
(4) Standards are easy to prepare.
(5) The method also works in acidic media.
(6) The method is sensitive.
(7) No catalysts are needed.

Disadvantages

(1) PHI is an eye and respiratory irritant.
(2) Water forms the same derivative as aniline and each water molecule (MW 18) consumes two PHI molecules (MW 120).
(3) Tertiary amines do not form these derivatives.

Applications

To test the usefulness of reversed-phase HPLC together with a gradient system and sensitive UV detection, a series of derivatized amines was chromatographed on the 25-cm 10-μm ODS column using a 20-min linear ACN–water gradient and UV-monitoring at 240 nm. As can be seen in Fig. 2, both aliphatic and aromatic primary and secondary amines form a pure single derivative that is not pH-sensitive and is very stable.

The method was also used to determine a mixture of C_{16}–C_{18} amines used as a coating in fertilizers.

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