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Note

High-performance liquid chromatography of 1-amino-9,10-anthraquinones

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Substituted 1-amino-9,10-anthraquinonesulphonic acids are important as dye intermediates. The quality control of the final products requires both the determination of the purity of these intermediates before condensation with the other components and the determination of their remaining concentrations in the condensation products. The strong polarities and relatively high molecular weights of these substances limit the use of gas–liquid chromatographic methods. On the other hand, high-performance liquid chromatographic (HPLC) methods can be utilized with advantage for these purposes.

Polarography has been applied to the determination of aminoanthraquinone in mixtures with anthraquinone and naphthoquinone[1]. Gas chromatography has been used for the separation of anthraquinone hydroxy and chloro derivatives on silicone stationary phases[2,3] and for the determination of mono- and diamino and nitro derivatives in 1-amino-9,10-anthraquinone[4].

Thin-layer chromatography (TLC) on silica gel has been used for the separation of 9,10-anthraquinone and 1- and 2-amino derivatives using the system toluene–acetone (8:3). The determination was performed by spectrophotometry after extraction into hot dimethylformamide (λ = 475, 435 and 326 nm)[5]. Similarly, anthraquinonedihydroxydiaminodisulphonic acids have been separated by TLC using Silufol with pyridine–25% ammonia solution (1:1) and determined spectrophotometrically at 550–560 nm after extraction into water[6].

Paper chromatography has been used for the separation of 1-amino-9,10-anthraquinone and 1-amino-4-bromo-9,10-anthraquinone-2-sulphonic acid using Whatman No. 1 paper with 2-ethylhexanol–methanol–1.5% ammonia solution (30:17:5). Spectrophotometry after extraction into 0.2 *M* sodium hydrogen carbonate solution was used for the determination (470–485 nm)[7]. Kratochvíl *et al.*[8] determined 1-amino-9,10-anthraquinonesulphonic acids and its condensation products by a spectrophotometric method. Taylor and Gaudio[9] used HPLC for the analysis of substituted bis(aminoalkylamino)anthraquinone (cancer chemotherapeutic agents). Jandera and Churáček[10] used a mobile phase containing a strong inorganic electrolyte for the separation of aromatic polar compounds by a reversed-phase liquid chromatographic method.

In this paper, the separation of 1-amino-9,10-anthraquinones (Ia–If) and their condensation products (If + II → III and IV) is reported in detail.

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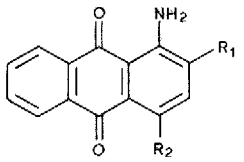
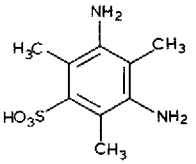


Ia-f



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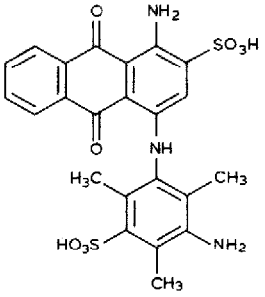
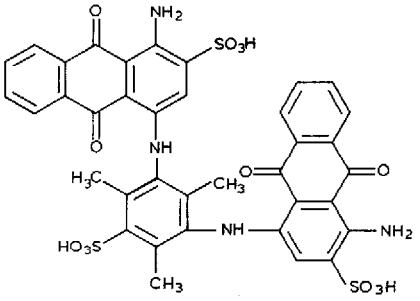


III



IV

	R_1	R_2	Substance
Ia	H	H	1-Amino-9,10-anthraquinone
Ib	Br	H	1-Amino-2-bromo-9,10-anthraquinone
Ic	H	Br	1-Amino-4-bromo-9,10-anthraquinone
Id	Br	Br	1-Amino-2,4-dibromo-9,10-anthraquinone
Ie	SO_3H	H	1-Amino-9,10-anthraquinone-2-sulphonic acid
If	SO_3H	Br	1-Amino-4-bromo-9,10-anthraquinone-2-sulphonic acid
II			2,6-Diamino-1,3,5-trimethylbenzene-4-sulphonic acid
III			Condensation product
IV			Condensation product

EXPERIMENTAL

A Varian Model 5020 liquid chromatograph was used with a Rheodyne 7125 syringe-loading sample injector with a 10-μl, sample loop a Varian UV-50 UV–visible variable-wavelength detector and a CDS-111 L Liquid Chromatography Data System.

A Varian AG Model 8500 liquid chromatograph was used with a septumless seal syringe stop-flow injector, a Variscan UV-visible detector and a CDS-101 Chromatography Data System.

The following chromatographic columns (all stainless steel) were used: (A) 250 × 4 mm I.D., Separon SI C_{18}, 10 μm (Laboratorní přístroje, Prague, Czechoslovakia); (B) 250 × 2 mm I.D., MicroPak CN-10 (Varian, Palo Alto, CA, U.S.A.); (C) 250 × 6 mm I.D., packing as in column A.

Aqueous methanolic and aqueous solutions of sodium sulphate used as the mobile phase were prepared from spectral-grade methanol and anhydrous analytical-reagent grade sodium sulphate (both from Lachema, Brno, Czechoslovakia) and from water doubly distilled in glass with the addition of potassium permanganate and

sodium hydrogen carbonate. The sample substances were obtained from the OTELA Department of this Institute.

RESULTS AND DISCUSSION

Mixtures of substances I–IV were separated by liquid chromatography on bonded reversed-phase columns. A mobile phase containing sodium sulphate as a strong electrolyte was used for this purpose, and also for the quantitative determination of 1-amino-9,10-anthraquinonesulphonic acid derivatives, where complete separation is required. With optimal chromatographic conditions, it is possible to control the composition and purity of dye intermediates as reaction components for subsequent condensation (Fig. 1) or to determine their residual concentrations in the condensation products (Fig. 2a).

The differences in the elution times of the individual components of the sample mixture allow the use of detection at various wavelengths. The sensitivity of the method can be increased 2–2.5-fold (Fig. 2b) using detection of the individual substances at their wavelengths of maximum absorption (Fig. 3).

1-Amino-9,10-anthraquinone and its mono- and dibromo derivatives (undesirable by-products) were separated on a relatively polar CN-bonded phase column (Fig. 4). A disadvantage of this method is irreversible sorption of the strongly polar

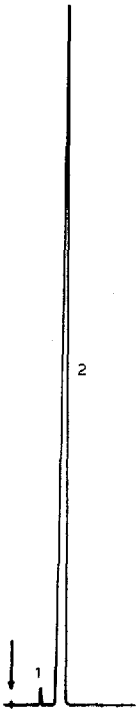


Fig. 1. Separation of amino-9,10-anthraquinonesulphonic acids. LC Varian 5020 column A. Mobile phase: solvent A = 0.2 *M* Na_2SO_4, solvent B = methanol–water (60:40); isocratic elution, 90% B; flow-rate, 1 ml/min. Detection: visible, 480 nm. Compounds: 1 = Ie; 2 = If.

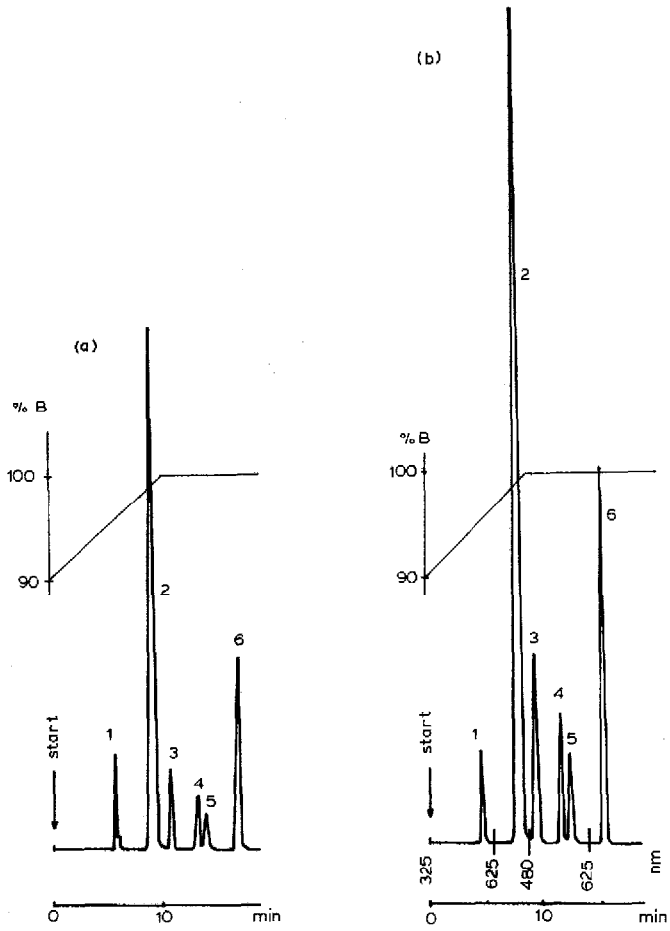


Fig. 2. (a) Separation of condensation products. LC Varian 5020 column C. Mobile phase as in Fig. 1. Elution: linear gradient from 90 to 100% B in 10 min; flow-rate, 1 ml/min. Detection: UV, 325 nm. Compounds: 1 = II; 2 = III; 3 = Ie; 4 = unknown; 5 = If; 6 = IV. (b) Separation of condensation products. Conditions and compounds as in Fig. 2a, except detection, visible 325–625 nm.

sulphonic derivatives on the CN-bonded phase column. The separation of a mixture containing polar and non-polar derivatives of 1-amino-9,10-anthraquinone was achieved by using a reversed-phase system with gradient elution (Fig. 5). Aqueous methanol without a strong electrolyte was used as the mobile phase because the retention of sample substances was too high in the presence of the salt in the mobile phase.

CONCLUSIONS

1-Amino-9,10-anthraquinone derivatives were separated satisfactorily using reversed-phase chromatography for the separation of polar sulphonic derivatives. If both sulphonated and non-sulphonated compounds are present in the sample,

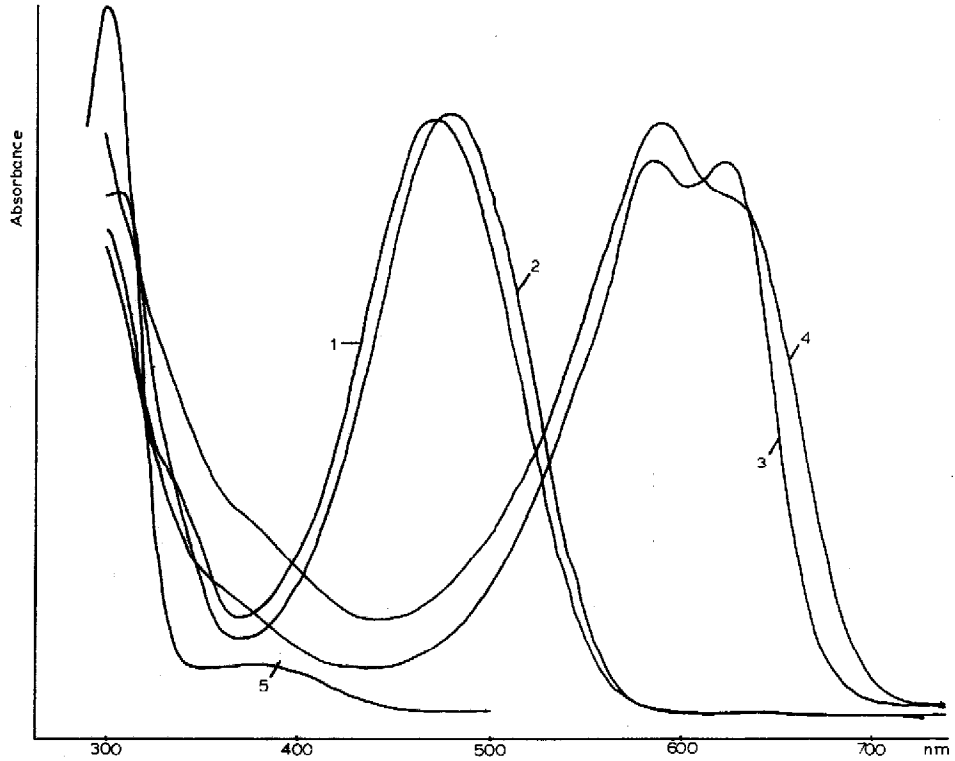
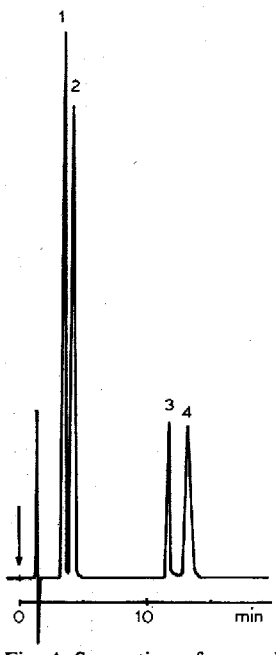


Fig. 3. Absorption curves of various compounds: 1 = Ie; 2 = If; 3 = III; 4 = IV; 5 = II.



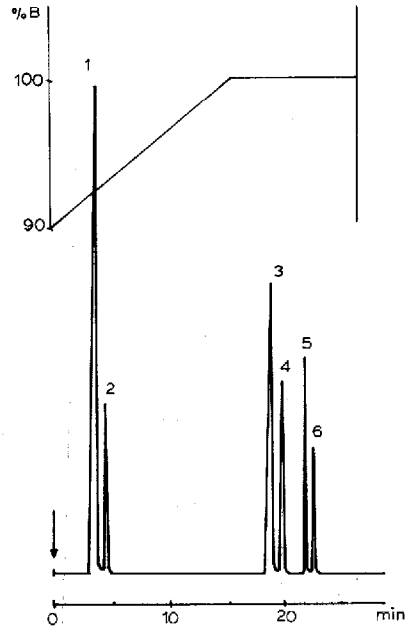


Fig. 4. Separation of non-sulphonic 1-amino-9,10-anthraquinones. LC Varian 8500 column B. Mobile phase: *n*-hexane–diethyl ether (90:10); isocratic elution; flow-rate, 30 ml/h. Detection: visible, 480 nm. Compounds: 1 = Ib; 2 = Id; 3 = Ia; 4 = Ic.

Fig. 5. Reversed-phase separation of 1-amino-9,10-anthraquinones. LC Varian 5020 column C. Mobile phase: solvent A = water, solvent B = methanol; elution, linear gradient from 30 to 100% B in 15 min; flow-rate, 1 ml/min. Detection: visible, 480 nm. Compounds: 1 = Ie; 2 = If; 3 = Ia; 4 = Ic; 5 = Ib; 6 = Id.

the use of gradient elution is necessary. The retention of individual substances depends on the ionic strength of the mobile phase used. The sensitivity and efficiency of the mentioned separations are high, therefore they can be used for quantitative determinations.

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