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Note

Separation of some chloramphenicol intermediates by high-pressure liquid chromatography

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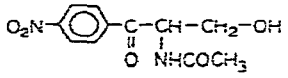
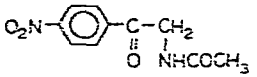
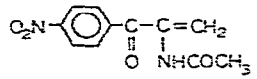
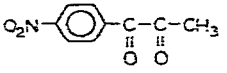
Chloramphenicol is a widely used antibiotic, the purity of which is greatly dependent on the purity of intermediate products in its preparation. At present, several paper chromatographic and thin-layer chromatographic methods are employed in the separation of a number of intermediates or impurities[1–8]. The number of components readily separated is, however, limited and is further restricted by the unsatisfactory quantitation of trace amounts.

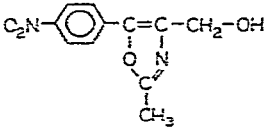
High-pressure ion-exchange chromatography[9] has been previously applied to the separation and quantitation of three of the intermediates. This paper presents the high-pressure liquid chromatographic separation of six intermediates (1–6) on a chemically bonded stationary phase. Compound 1 is the main product of the present synthesis step, 2 is its precursor and 3–6 are contaminants resulting from side reactions.

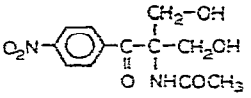
(1) 

(2) 

(3) 

(4) 

(5) 

(6) 

EXPERIMENTAL

Experiments were carried out on a Varian Aerograph LC 4020 UV/RI liquid chromatograph (Varian Aerograph, Walnut Creek, Calif., U.S.A.) equipped with a Model 6000 pulsation-free pump (Waters Assoc., Milford, Mass., U.S.A.) and some custom-made components. The analytical column used (0.25 m × 0.125 in. O.D.) was a MicroPak-CN (Varian Aerograph) thermostatted with a full-length water jacket[10].

Stop-flow injections were made with a 10-μl microsyringe (Scientific Glass Engineering, London, Great Britain) through a modified low-pressure 0.125-in. O.D. injection head (Varian Aerograph) which was leak-free up to 6000 p.s.i. The column temperature was maintained at 40.0 $\pm$ 0.1°.

The eluents were reagent-grade solvents (Reanal, Budapest, Hungary). Compounds 1–6 were prepared by the EGYT Pharmaceutical Factory, Budapest, Hungary.

A liquid chromatography system was sought which would allow fast isocratic separation of at least 10 samples daily and which could be easily maintained in factory environment. Chloroform mixed with different amounts of methanol was chosen as eluent and the samples were dissolved in methanol–chloroform (3:1). Values of the capacity factor, k', obtained are listed in Table I. Carbon tetrachloride was used as unretained compound when determining k', and the procedure was carefully corrected for the delay resulting from the stop-flow injection and build-up of the pump pressure on re-starting.

TABLE I

VALUES OF THE CAPACITY FACTOR, k', FOR COMPOUNDS 1–6

Eluent, methanol–chloroform (3:1); flow-rate, 2.0 ml/min.

Compound	*Methanol (%, v/v)*						
	0.0	*0.50*	*1.0*	*2.0*	*3.0*	*5.0*	*10.0*
1	12.6	6.9	3.8	1.63	1.38	0.41	0
2	2.67	1.67	1.13	0.61	0.54	0.18	0
3	0.44	0.28	0.20	0.17	0.12	0.05	0
4	0	0	0	0	0	0	0
5	5.9	5.22	3.14	1.32	0.95	0.30	0
6	24.8	11.2	8.7	1.96	1.41	0.44	0

RESULTS AND DISCUSSION

Fig. 1 shows the chromatogram of compounds 1–6 with pure chloroform as eluent. All of the compounds were clearly separated, although 4 was eluted together with other non-retained compounds. After the peak of compound 2, another peak was observed due to an unidentified contaminant originating from 6. From the distorted shape of the peak of compound 5, it seems fair to assume that several isomers of 5 are eluted together. A sample of 5 of greater purity could not be obtained in order to test the validity of this assumption. The elution order agrees with that expected from the structure of the compounds. Hydrogen bonding seems to be the governing mechanism. This elution order is quite advantageous as far as the practical aim of the separation is concerned, since compounds 2–6 are contaminants and are frequently present only in trace amounts. However, compound 6 was not easily detected.

The system described above was quite useful for routine separation of impure samples of compound 1 at an average of 12 separations daily. Most of the samples contained only 1–3. Quantitation could be easily carried out down to a level of 0.13%

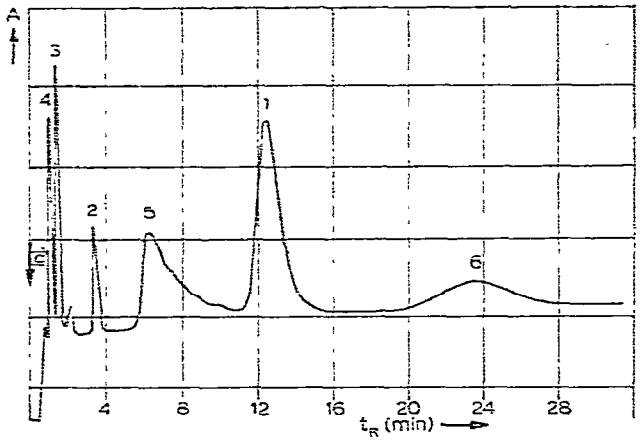


Fig. 1. Separation of compounds 1–6 on a MicroPak-CN column (0.25 m × 0.125 in. O.D.) thermostatted at 40.0°. Eluent (chloroform) flow-rate, 2.0 ml/min.

for 3 and 0.2% for 2, based on the values of the peak heights. True trace-level detection could not be attained because of the strong absorption of the eluent at 254 nm. Studies are now being made in order to find such a system.

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