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Note

Use of routine preparative high-performance liquid chromatography in the separation of isomers

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Recent advances in techniques[1–10] and in the efficiency of the apparatus available for preparative high-performance liquid chromatography (HPLC)[11,12] has made the separation of multigram quantities possible. A major problem encountered in the use of these machines, however, is the correct choice of eluent. Although some authors[13–15] recommend direct scale-up from thin-layer chromatography (TLC), in our experience this is not always a good guide and often leads to either a failed separation or waste of time and solvent with mid-course adjustments to eluent composition and flow rate.

Many reactions performed in the synthetic laboratory yield one or more products which can be readily isolated and purified by gravity column chromatography or by non-chromatographic techniques such as distillation or crystallisation. However, it frequently occurs that a reaction will give a mixture of two components with very similar chemical, physical and chromatographic properties, this usually being the case when the products are isomeric. In this case the laboratory chromatography techniques are inadequate and our work has been concerned with the development of an HPLC system using commercially available equipment for the routine separation of such isomeric mixtures.

To simplify the procedure for choosing the eluent system, a mixture of two solvents was used as eluent. In practice either ethyl acetate–pentane or methanol–dichloromethane in various ratios have been found to cover most situations adequately. Water–methanol mixtures were used for reversed-phase separations. Initially several thin-layer chromatograms of the sample mixure are run and the results used as a guide for the choice of absorbent and eluent. The system which results in R_F values in the range 0.2 to 0.5 is considered appropriate. A glass column (250 × 15 mm) is then packed using the chosen absorbent and eluent and a 50-mg sample of the mixture is run repeatedly, the eluent being adjusted until an acceptable separation is obtained. The pure components separated by this means are usually sufficient for structural determination by physical methods. If it is then desired to carry out a large-scale separation, a large column may be set up in the Jobin-Yvon apparatus using the same absorbent and eluent system.

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EXPERIMENTAL

Materials

The mixtures of isomers for separation were submitted by chemists in our research laboratories and were the products of chemical synthesis. Large-scale separations and some small-scale separations were carried out using Merck silica gel K60 (40–63 μm). Other small-scale separations were carried out using Merck LiChroprep Si 60 (15–25 μm). The reversed-phase material was prepared in our own laboratories from Merck silica gel K60 (40–63 μm) and octadecyl trichlorosilane supplied by Magnus. The material was capped with trimethyl silyl chloride.

Equipment

Large-scale separations were carried out using a Jobin-Yvon Chromatospac Prep 100. Small-scale separations were carried out using a Kontron pump (10 ml/min maximum), Jobling glass columns, an LDC sample injector, an Altex single-wavelength UV detector and an LDC refractive index detector. A Gilson CPR was used for fraction collection.

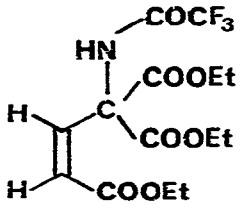
RESULTS AND DISCUSSION

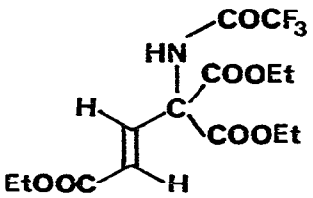
The criteria governing the use of HPLC in the organic synthesis laboratory include cost, versatility, safety and efficiency relative to other forms of chromatographic and classical methods of compound isolation. One variable factor which relates directly to the efficiency of this technique is the choice of the eluent and absorbent used for a particular separation. We have described here a systematic procedure which enables the correct absorbent and eluent for a large-scale separation to be determined quickly and efficiently using a small glass column. This enables larger-scale chromatography to be carried out efficiently with minimum cost in time and money.

The basis of the system we chose was the Jobin-Yvon Chromatospac Prep 100, descriptions of which can be found elsewhere[10,11]. Initial results suggested that progression directly from TLC to the Jobin-Yvon was feasible. In practice, however, this was only possible in the case of relatively simple separations and this led us to experiment with an intermediate system.

The system chosen for an intermediate separation was based on the 250 × 15 mm and 450 × 15 mm Jobling glass columns. These columns are easy to dry-pack with a simple 10 ml/min flow-rate pump and are inexpensive. The 450 × 15 mm column holds about 30 g of packing which will separate 50–100 mg of a two-component mixture. This gives enough sample for nuclear magnetic resonance and infrared spectra. Thus, components may be identified at an early stage and a decision can then be taken on whether a large-scale separation is required.

Figs. 1–5 are given to illustrate the utilisation of this technique. In Fig. 1 a mixture of *cis*- and *trans*-triesters was produced by the addition of diethyl N-trifluoroacetylaminomalonate to ethyl propiolate[16]. Clean separation of the two isomers initially resulted from the use of pentane–ethyl acetate (9:1), but it was found that the ratio could be reduced to 4:1 with no loss of resolution, but significant decrease in time. Accordingly, these conditions were used on a large scale and pure samples of both isomers readily isolated.





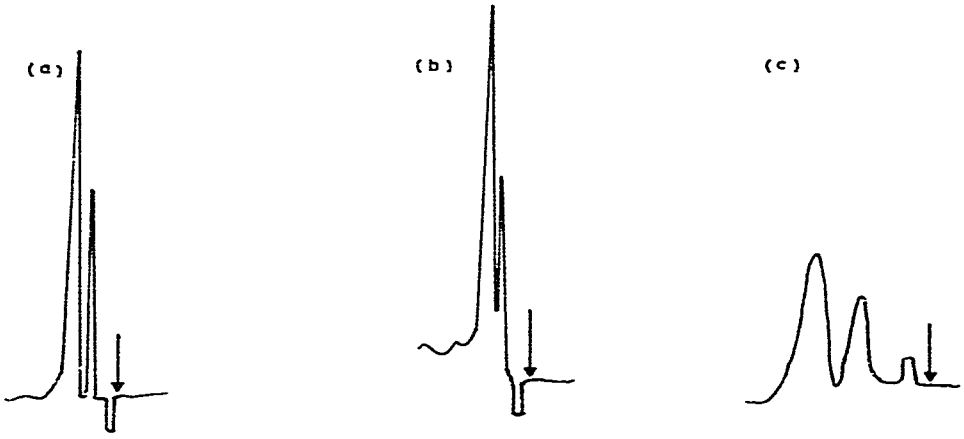


Fig. 1. Separation of *cis*- (left structure, less polar) and *trans*- (right structure, more polar) triesters on silica using refractive index detection. Et = Ethyl.

Separation	*Eluent: n-pentane–ethyl acetate*	*Flow-rate (ml/min)*	*Packing (g)*	*Injected*	*Retention time (min)*		*Yield (g)*	
					cis	*trans*	*cis*	*trans*
(a) Small scale 1	9:1	4	12	50 mg	5	8	–	–
(b) Small scale 2	4:1	4	12	50 mg	2	4.5	–	–
(c) Large scale	4:1	30	1500	6.5 g	120	190	1.2	2.7

Note: Although the 9:1 solvent ratio gives a better separation than 4:1 on the small scale, the 4:1 ratio was chosen for the large-scale separation because this is just enough to give a complete separation. Also, the relatively lower flow-rate used on the preparative scale tends to improve the separation of the two components provided that diffusion does not increase.

The separation in Fig. 2 was more complicated in that a four-component mixture was involved but again it was found that the most polar solvent mixture which was capable of separating the components on the small scale gave a good separation on a large scale.

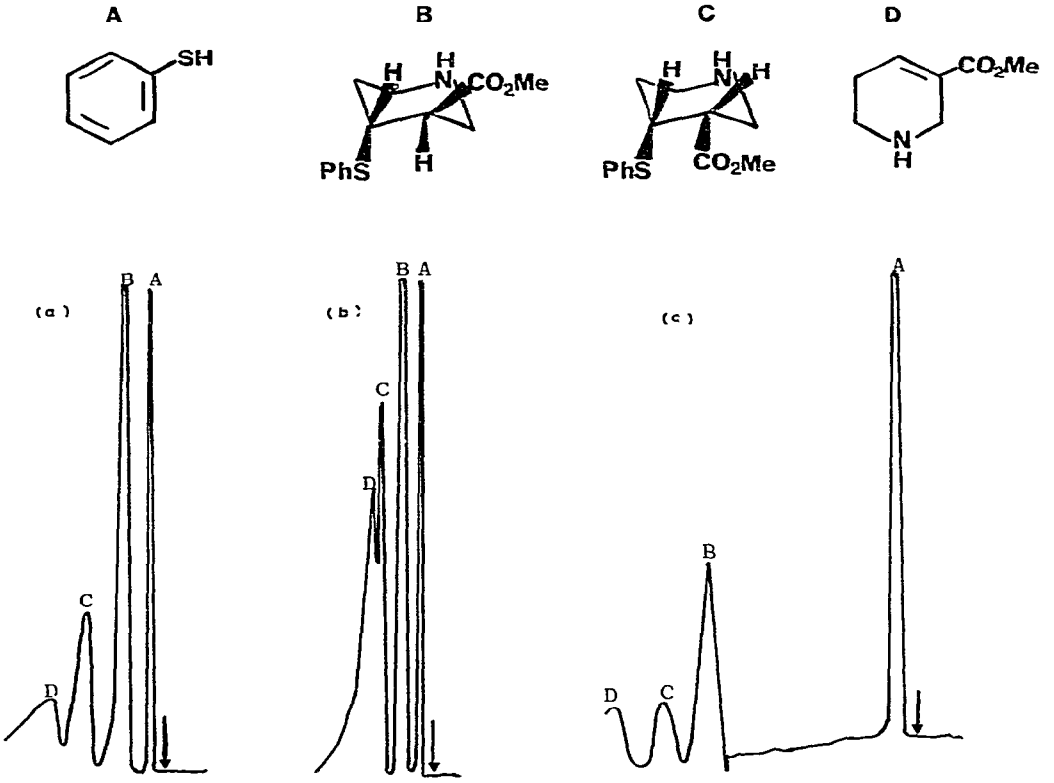


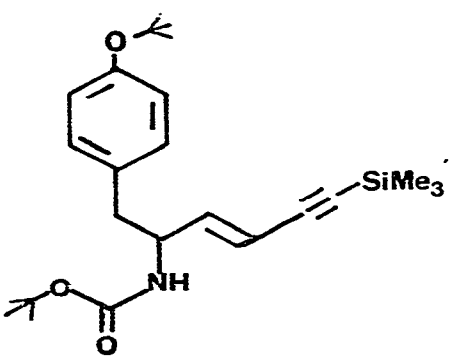
Fig. 2. Separation of four components [structures A–D, where B (*trans*) and C (*cis*) are isomers] on silica using UV detection. Me = Methyl; Ph = phenyl.

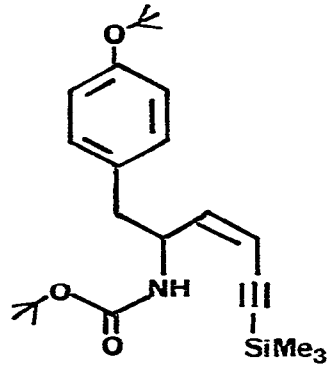
Separation	*Eluent: dichloro-methane–methanol*	*Flow-rate (ml/min)*	*Packing (g)*	*Amount injected*	*Retention time (min)*		*Yield (g)*	
					trans	*cis*	*trans*	*cis*
(a) Small scale 1	99.5:0.5	1	12	50 mg	11	22.5	–	–
(b) Small scale 2	99:1	1	12	50 mg	6.5	12.5	–	–
(c) Large scale	99:1	30	500	0.9 g	400	480	0.2	0.04

The conditions which were just enough to give adequate separation of the components were chosen. The relatively lower flow-rate and the smaller injection mixture-to-packing ratio tends to improve the large-scale separation.

In Fig. 3 another *cis–trans* mixture[17] was readily separated. In this case the same eluent mixture was used on a large scale but the flow-rate could be increased tenfold with no loss of resolution.

The use of the intermediate column to give sufficient material for spectroscopic analysis is shown in Fig. 4. Despite a very difficult separation, sufficient material was obtained of each isomer for their structures to be defined.





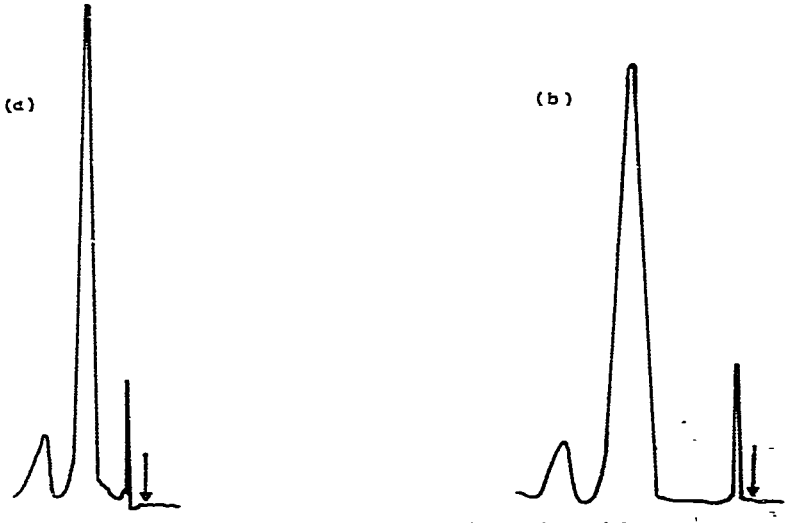
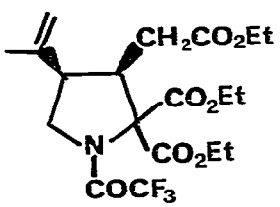
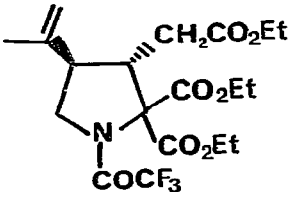


Fig. 3. Separation of *trans* (left structure, less polar) and *cis* (right structure, more polar) isomers on silica using UV detection.

Separation	*Eluent: n-pentane-ethyl acetate*	*Flow-rate (ml/min)*	*Packing (g)*	*Amount injected*	*Retention time (min)*		*Yield (g)*	
					cis	*trans*	*cis*	*trans*
(a) Small scale	95:5	4	32	100 mg	16	33	—	—
(b) Large scale	95:5	40	1000	9.0 g	400	640	5.7	1.1

In all the previous separations silica was used as the support and the final example shows that the method could be extended to the reversed-phase technique. No separation occurred on ordinary phase under the conditions used. Accordingly, a





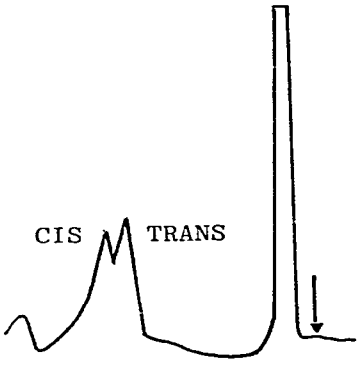


Fig. 4. Small-scale separation of *cis* (left structure, more polar) and *trans* (right structure, less polar) isomers on silica using refractive index detection. Conditions: Eluent, *n*-pentane–ethyl acetate (9:1); flow-rate, 3 ml/min; packing, 30 g; amount injected, 50 mg. Retention times: *cis*, 30 min; *trans*, 33 min.

column was set up which was capable of separating the test mixture shown and then was injected with successive 50-mg samples of the mixture. Fractions were collected as shown (Fig. 5) and pooled. Analytical HPLC confirmed that one isomer was recovered in fraction 1 (f1), the other in fractions 3 (f3) and 4 (f4) and the purity of the fractions can be clearly seen.

It should be emphasised that in most of these cases the components were either inseparable by TLC and were discovered to be mixtures only by anomalies in the nuclear magnetic resonance spectra or showed incomplete separation.

We consider that the system developed allows the routine separation of difficult-to-separate mixtures, as illustrated here in the case of isomeric mixtures, efficiently and routinely by preparative HPLC.

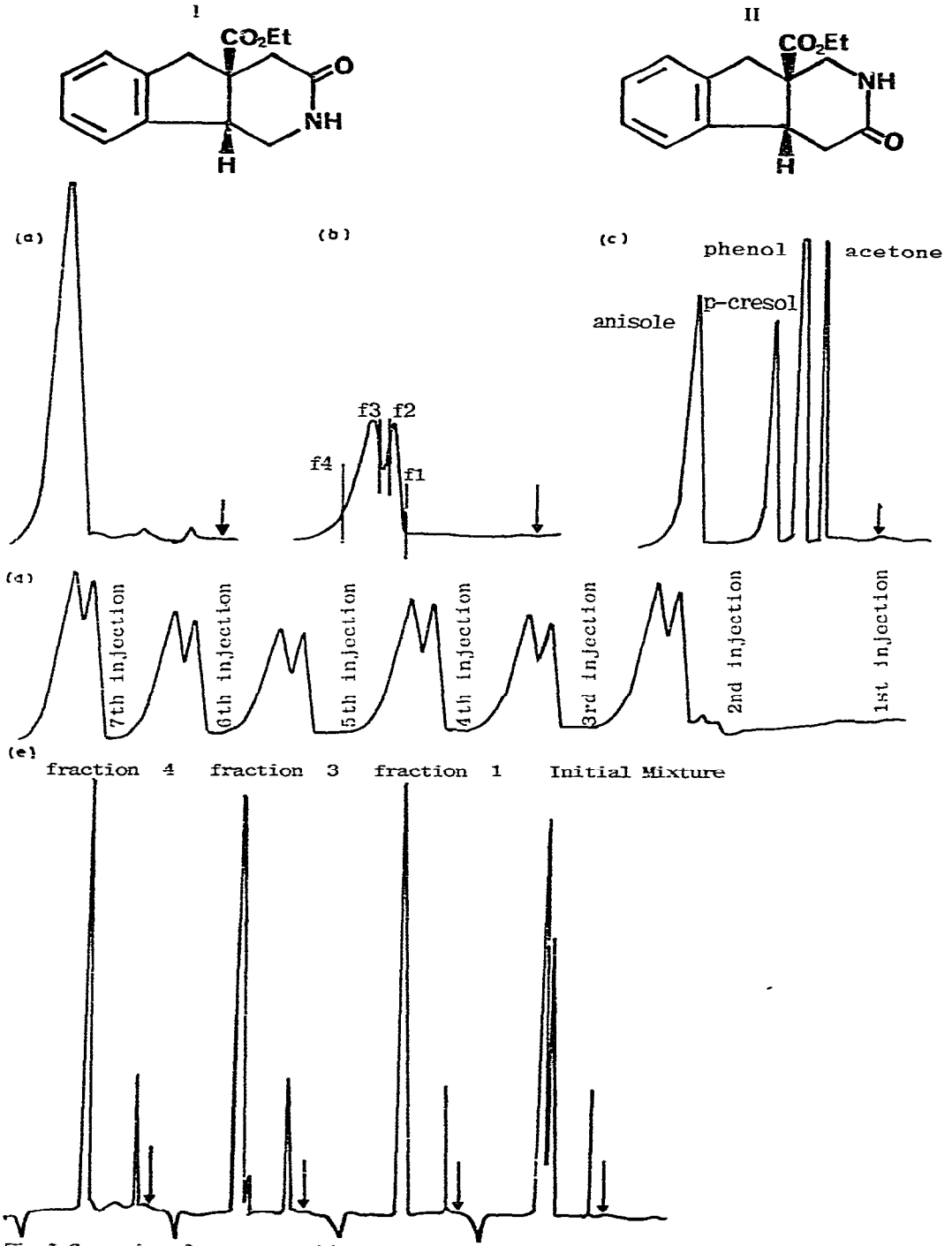


Fig. 5. Separation of two structural isomers (see structures) using UV detection. (a), Normal phase (no separation); conditions: eluent, dichloromethane–methanol (98:2); flow-rate, 3.5 ml/min; packing, 31 g; amount injected, 25 mg. (b) and (c), Reversed phase (b: research mixture; c: test mixture); condition eluent, methanol–water (40:60); flow-rate, 5 ml/min; packing, 30 g; amount injected, 50 mg; retention times: I, 94 min; II, 106 min. (d), Separation of 800 mg of mixture by repeated injections of 50 mg on a small column; fractions 1–4 (f1–f4) were taken as indicated in (b); yields (total injected 800 mg): f1, 230 mg; f2, 150 mg; f3 + f4, 350 mg; total, 730 mg; useful recovery: I, 29%; II, 44%. (e), Comparison of fractions by analytical HPLC; conditions: column, Spherisorb ODS (5 μm); eluent, methanol–water (65:35); pump, # DC constametric; detector, LDC spectromonitor.

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