

Note

Microcrystalline tribenzoylcellulose: a high-performance liquid chromatographic sorbent for the separation of enantiomers

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(Received September 10th, 1985)

Microcrystalline triacetylcellulose was introduced by Hesse and Hagel¹ as a stationary phase for the resolution of racemates. They pointed out that dissolution during the reaction with acetic anhydride at 35°C has to be avoided because the product is then less effective. When the promising results¹ concerning chiral phenyl compounds are considered, it is no surprise that the heterogeneous synthesis of tribenzoylcellulose was intended. Indeed, tribenzoylation of cellulose was accomplished², but, because of the lower reactivity of benzoyl chloride, the reaction was carried out by heating to 80°C for three days. As far as the separation of enantiomers is concerned, this product turned out to be inferior to triacetylcellulose and has not been studied further². Recently, Okamoto *et al.*³, Ichida *et al.*⁴ and Rimböck *et al.*¹¹ coated dissolved tribenzoylcellulose on macroporous silica and obtained several encouraging chiral recognitions. Bearing in mind that non-dissolved materials may be different^{1,3}, we tried to benzoylate cellulose under heterogeneous and mild conditions and compared its separation ability with that of the triacetylated sorbent.

EXPERIMENTAL

Microcrystalline cellulose (Avicel, E. Merck, Darmstadt, F.R.G.) was pretreated with pyridine (40 ml per gram of cellulose) for 20 min at room temperature. Benzoyl chloride (20 ml per gram of cellulose) was added, and the flask was put into an ultrasonic field (Bandelin Sonorex RK 255H, Bandelin Electronic, Berlin, F.R.G.) for 1 h, during which the temperature rose to 45–50°C and the product swelled. It was washed thoroughly with diethyl ether, then with methanol until it was free of chloride anion. After drying at 60°C, a powder sample was subjected to wide-angle X-ray diffraction for 3 h *in vacuo*, using the Cu $K\alpha$ line at 1.54 Å. It gave rise to a pronounced signal at diffraction angle $2\theta = 8.1^\circ$ and a very broad one at 19.3° , which indicated amorphous parts. Another sample was dissolved in dichloromethane, precipitated by methanol and prepared for elemental analysis: calc. for tribenzoylcellulose (TBC), 68.35% C, 4.67% H; calc. for 2.5 benzoyl groups per glucose, 66.82% C, 4.74% H; found, 67.99% C, 4.83% H.

TBC was ground, pre-sieved, and air-separated (Zickzackwindsichter A 100 MRZ, Alpine AG, Augsburg, F.R.G.). The fraction with $d_p = 5\text{--}11\ \mu\text{m}$, measured by the semiautomatic system for picture analysis ASM (Leitz KG, München,

F.R.G.), was swollen in boiling ethanol-4% water for 30 min and slurry-packed at *ca.* 100 bar.

Sorbent and packing of triacetylcellulose (TAC) ($d_p = 8-15 \mu\text{m}$, measured by the above ASM system) corresponds to columns B and C in ref. 5.

The Model 6000 A pump and Model U6K injector were from Waters. Absorbances *A* were measured at 254 nm on the ERC 7210 detector (cell length 10 mm, volume $8 \mu\text{l}$) from ERMA Optical Works. Rotation angles *ALPHA* were measured at 365 (substrates 1-5) or 436 nm (6) on the PE 241 polarimeter (cell length 100 mm, volume *ca.* $100 \mu\text{l}$) from Perkin-Elmer. The $A = f(V)$ and $ALPHA = f(V)$ curves (*e.g.*, Fig. 1) were stored on floppy discs by a microcomputer. Since these detections

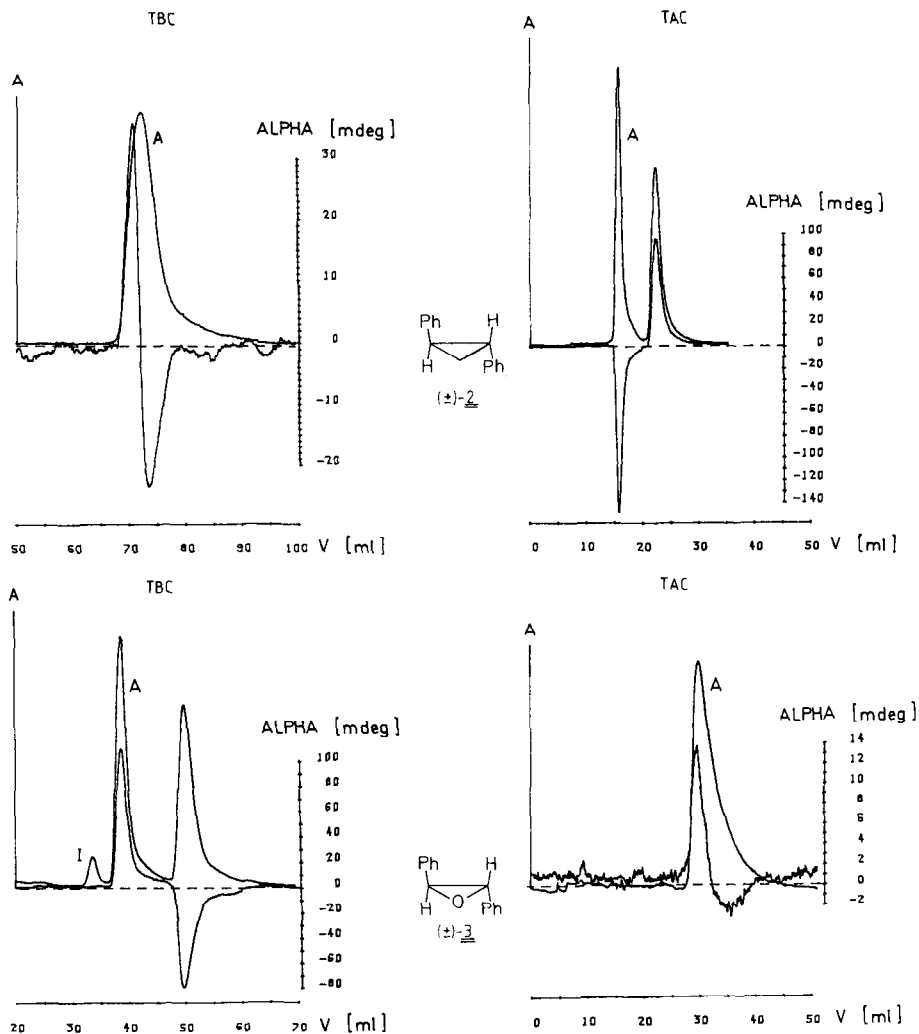


Fig. 1. Chromatograms on tribenzoylcellulose (TBC; left) and triacetylcellulose (TAC; right) in ethanol-4% water at a flow-rate of 1.5 ml/min. Absorbance *A* at 254 nm and rotation angle *ALPHA* at 365 nm plotted vs. *V*, the elution volume after injection of amounts between 100 and $500 \mu\text{g}$. See Table I for parameters *k*, α , *H* and *R_s*. Upper part: (\pm)-2; lower part: (\pm)-3; I = unknown impurity.

TABLE I
HPLC PARAMETERS OF RACEMATES, OBTAINED VIA UV AND POLARIMETRIC DETECTION

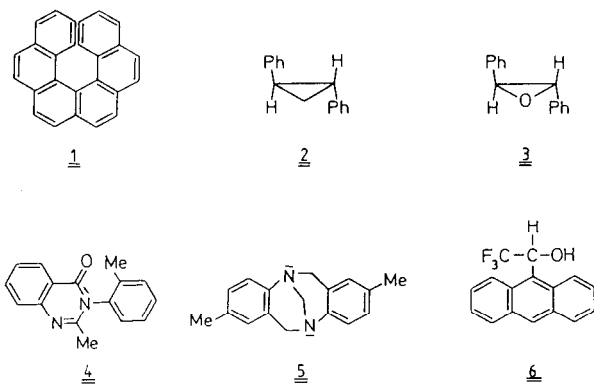
Eluent, ethanol-4% water; flow-rate, 1.5 ml/min. For 6 the sign of rotation refers to 436 nm, for the other substrates to 365 nm.

Sub- strate	Tribenzoylcellulose						Triacetylcellulose					
	k_1	k_2	α	H_1 (μm)	H_2 (μm)	R_s	k_1	k_2	α	H_1 (μm)	H_2 (μm)	R_s
1	ca. 4.5 (-)	ca. 5.6	ca. 1.2*	-	-	<0.6	1.4 (-)	1.9	1.4	ca. 800	ca. 1200	<0.6
2	ca. 6.9 (+)	ca. 7.3	ca. 1.1*	-	-	<0.6	1.2 (-)	2.1	1.8	200	260	2.4
3	3.3 (+)	4.6	1.4	155	170	2.5	ca. 3.0 (+)	ca. 3.8	ca. 1.3*	-	-	<0.6
4	2.0 (-)	3.4	1.7	580	850	1.7	1.0 (+)	1.9	1.9	690	4760	1.0
5	3.5 (-)	6.4	1.8	1100	1830	1.5	1.8 (-)	3.5	1.9	1120	1660	1.5
6	ca. 0.5	ca. 0.5	ca. 1.0**	-	-	-	1.1 (+)	2.4	2.2	820	1400	1.7

* Estimated from polarimetric detection, because the UV curve was unsplit (cf. Fig. 1).

** Estimated from absence of polarimetric detection and from unsplit UV curve.

had to be performed at unequal times, *i.e.* at unequal volumes V , a corresponding V correction was introduced. A Digi-Plot WX 4671 from Watanabe served for graphic display. Separations were carried out in steel columns (250 × 8 mm I.D.) at 22°C and a flow-rate of 1.5 ml/min. The resulting pressures were *ca.* 85 bar on TBC and *ca.* 65 bar on TAC.



1,3,5-Tri-*tert.*-butylbenzene served as a chromatographic reference^{5,6}. Its elution volumes in ethanol–4% water were 8.70 ml for the TBC and 7.11 ml for the TAC column. Hexahelicene (1), *trans*-1,2-diphenyloxirane (3), Troeger's base (5), and 1-(9-anthryl)-2,2,2-trifluoroethanol (6) were commercially available. *trans*-1,2-Diphenylcyclopropane (2)⁷ originated from Professor M. P. Schneider, Wuppertal, F.R.G. 2-Methyl-3-(2'-methylphenyl)-4(3*H*)-quinazolinone (methaqualone) (4) was prepared according to ref. 8. The amounts injected ranged from 10 to 500 μg .

RESULTS AND DISCUSSION

Safanova and Klenkova⁹ described a heterogeneous tribenzoylation of cellulose. Although ultrasound accelerates this reaction, it does not diminish the molecular weight very much⁹. Our product of this synthesis turned out, by X-ray wide-angle diffraction, to be microcrystalline but to contain amorphous parts, too. Packing of 5–11 μm particles was possible by the procedure⁵ used for triacetylcellulose (TAC). Plate heights of retained compounds amounted to 150–2000 μm (Table I) at $u = 0.72$ mm/s and a pressure drop of *ca.* 85 bar. These findings are similar to those for TAC (Table I and refs. 5 and 6), which means that tribenzoylcellulose (TBC) is also a suitable stationary phase for high-performance liquid chromatography (HPLC). When comparing the data of Table I with those obtained from non-identical columns, *e.g.* for 4^{6,10}, it should be kept in mind that the experimental details may influence the numerical values.

As a first step, we compared the behaviour of TBC and TAC towards a few substrates under HPLC conditions, including polarimetric detection. Under equivalent conditions, the retentions (Table I) on the benzoylated sorbent were stronger than on TAC, especially for the aromatic hydrocarbons 1 and 2. The alcohol 6 was an exception because it was scarcely retained at all and therefore not resolved on

TBC. This may be caused by the phenyl groups which, unlike the methyl groups in TAC, would diminish hydrogen bonding between the alcohol 6 and the benzoyl oxygen atom.

The elution order of the enantiomers of 2 is different* on the two sorbents (Table I). The same is true for the enantiomers of 4. Apparently, the mechanisms of chiral recognition by TAC and TBC are different. Two racemates, 3 and 4, show definitely better resolution $R_s = 2(V_2 - V_1)/(w_2 + w_1)$ on TBC ($R_s = 2.5$ and 1.7) than on TAC ($R_s < 0.6$ and $R_s = 1.0$). These improvements are due to larger differences of elution volumes ($V_2 - V_1$; cf. $k_2 - k_1$, Table I) and also to diminished peak widths w_2 of the second enantiomers. Both facts can be seen for the case of 3 in the lower part of Fig. 1. Therefore, TBC may be useful for practical purposes, although more examples must be checked.

We have shown that TBC is about as easily accessible as TAC. In this respect, both stationary phases are superior to many of the silica-based sorbents. Accessibility is important when large-scale separations are considered.

ACKNOWLEDGEMENTS

We are grateful to Miss N. Pustet and Mr. R. Hilz for assistance in the synthesis and air separation of TBC, as well as to Dr. M. A. Cuyegkeng for relevant discussions. Dr. J.-L. Luche (Grenoble, France) kindly informed us about the use of ultrasound. We thank Dr. E. Eibler and Mr. K. H. Ziller for access to the air separator and the Leitz ASM system. We owe the microcomputer for storage and presentation of data to the Rechenzentrum, University of Regensburg, and the programs to Dr. A. Eiglsperger. Support from the Fonds der Chemischen Industrie, Frankfurt, is acknowledged.

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* The different behaviour (Fig. 1 and Table I) of the hydrocarbon 2 and the corresponding ether 3 is not yet understood. One of these differences is the reversal of elution order for the enantiomers of 2 and the absence of such an effect for 3.