Journal of Chromatography, 138 (1977) 89–96

CHROM. 9998

RECYCLE GEL PERMEATION CHROMATOGRAPHIC SEPARATION OF SOME ACETAL ALCOHOL DIASTEREOISOMERS REPRESENTING PARTIAL POLYGLUCOSIDIC SEQUENCES

JAMES LESEC, FRANÇOISE LAFUMA and CLAUDE QUIVORON

Laboratoire de Physico-Chimie Macromoléculaire, Université Pierre et Marie Curie, Associé au C.N.R.S. No. 24, E.S.P.C.I., 10 rue Vauquelin, 75231 Paris Cedex 05 (France)

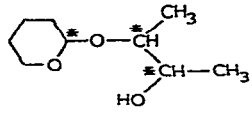
(First received December 2nd, 1976; revised manuscript received February 7th, 1977)

SUMMARY

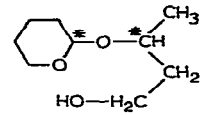
This paper deals with the separation of some acetal alcohol diastereoisomers by recycling gel permeation chromatography; after several trials, this technique appears to be best suited to semi-preparative work. Acetal alcohols have two, three or four asymmetric carbon atoms and are mixtures of two or four diastereoisomers. They participate in hydrogen bonding with the chromatographic solvent (diisopropyl ether), which leads to different average hydrodynamic volumes and, accordingly, different elution volumes for each diastereoisomer. Semi-preparative separations have been performed by recycling 1 to 2 g of solute; we have also shown that separations could be achieved with crude products when preliminary purification was impossible. In these instances, impurities must be collected as they are resolved at the beginning of recycling.

INTRODUCTION

Intramolecular hydrogen bonds are known to stabilize the secondary structure of macromolecules; therefore, in order to precisely determine polyglucosidic conformations, we wished to carry out a spectroscopic study of the chelation systems involved in these compounds. However, it was obvious that working with polysaccharides themselves would be very difficult because of the large number of acceptor and donor groups and of the poor solubility of these compounds in spectroscopic solvents. We therefore prepared model molecules, representing partial polyglucosidic sequences[1]. Such compounds, the formulae of which are indicated below, generally have two, three or four asymmetric carbon atoms and, accordingly, are diastereoisomeric mixtures. Infrared spectroscopy shows that chelation systems are highly dependent on configuration[1–3], and, since each diastereoisomer has its own spectrum, it is impossible to get accurate information from the mixture; therefore, we had to separate the diastereoisomers.



I_A, I_B

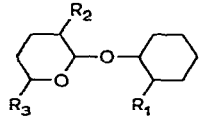


II_A, II_B

EXPERIMENTAL

Materials

Model molecules were 2-alkoxytetrahydropyran derivatives with one or two hydroxyl-functions in differing positions with regard to the acetal group. They were synthesized by the reaction of alcohols or diols with 2,3-dihydropyran, 2-hydroxymethyl-2,3-dihydropyran or their epoxides[1]. Compounds I and II were prepared from *meso* butane-1,3- and -2,3-diols; each is a mixture of two diastereoisomers, although compound I has three asymmetric carbon atoms (the third exists only in one configuration owing to the *meso*-form of butane-2,3-diol). Other compounds used were derivatives of 2-cyclohexyloxytetrahydropyran with the general formula shown below and the parameters listed in Table I.



These model compounds were generally viscous liquids (although some were solid), and were very sensitive to acid hydrolysis and heating. Low yields of purified material could be obtained by vacuum distillation of most (but not of all) the derivatives, so that we had often to separate crude synthesis products. For the spectroscopic

TABLE I

COMPOSITION OF MODEL COMPOUNDS

Compound No.	R_1	R_2	R_3	*Number of asymmetric C atoms*	
				Total	*Responsible for diastereoisomerism*
III_A, III_B	H	OH	H	2	2
IV_A, IV_B	OH (*cis*)	H	H	3	2
V_A, V_B	OH (*trans*)	H	H	3	2
VI_A, VI_B	H	H	CH_2OH	2	2
VII_A, VII_B	CH_2OH (*trans*)	H	H	3	2
$VIII_A$, $VIII_B$, $VIII_C$, $VIII_D$	OH (*cis*)	OH	H	4	3
IX_A, IX_B, IX_C, IX_D	OH (*trans*)	OH	H	4	3
X_A, X_B, X_C, X_D	OH (*cis*)	H	CH_2OH	4	3
XI_A, XI_B, XI_C, XI_D	OH (*trans*)	H	CH_2OH	4	3
XII_A, XII_B, XII_C, XII_D	H	OH	CH_2OH	3	3
$XIII_A$, $XIII_B$, $XIII_C$, $XIII_D$	CH_2OH (*trans*)	OH	H	4	3
XIV_A, XIV_B, XIV_C, XIV_D	CH_2OH (*trans*)	H	CH_2OH	4	3

studies (especially those involving ^{13}C nuclear magnetic resonance spectroscopy), some hundred milligrams of each diastereoisomer were needed; thus, the separation method had to be semi-preparative.

Preliminary results

Initial separations were achieved by gas chromatography. Quantitative separations of the mixtures I_A–I_B and II_A–II_B were performed[2] with Carbowax as stationary phase; unfortunately, higher temperatures were needed to separate the other acetal alcohols and degradation occurred in the injector. Further, the stationary phase was not stable at these higher temperatures. Other column packings, or silylation of the alcohol function, were unsuccessful.

Most of the above-mentioned drawbacks do not exist in liquid–liquid chromatography (LLC). We have performed good analytical separations using a column packed with a Carbowax bonded phase and hexane as solvent. However, on this stationary phase, sample sizes larger than 20 mg cause overloading, and complete resolution of peaks cannot be achieved. Also, crude-product injections cause deterioration of the stationary phase by adsorption of polar secondary products of the mixture, and after a few injections, the column becomes inefficient and difficult to regenerate. Further, the polar phase Carbowax requires a somewhat non-polar solvent such as hexane for good separations, and the solubility of our compounds in such solvents is very low.

All these disadvantages can be avoided by the use of gel permeation chromatography (GPC), which permits resolution of low-molecular-weight compounds with low-porosity polystyrene gels[4–8]; further, the efficiency of this technique can be greatly increased by recycling[9–17]. As in LLC, one can work at room temperature and a good solvent for our compounds can be chosen as mobile phase. Polar compounds are eluted without adsorption, and the polystyrene gel column is very stable under these conditions. Lastly, overload effects in GPC are smaller than with LLC packings, and semi-preparative work is possible. Preliminary trials with the pairs of compounds III_A–III_B, IV_A–IV_B and V_A–V_B [17,18] resulted in quantitative separation of each diastereoisomer using sample sizes of about 1 g. We extended this work to the other molecules in the series.

Apparatus

Separations were performed with a liquid chromatograph (Waters Assoc., Milford, Mass., U.S.A.; Model 301) equipped with an M6000 pump having low internal volume and an R401 differential refractometer as detector. A septum was used for analytical injections (100 μl), and preparative amounts (1–5 ml) were injected by using a 6-port valve equipped with a 2-ml loop, or directly through the pump.

The recycling device consists of a 6-port valve[7–9,16,17] connecting the outlet of the detector to the inlet of the pump; when the valve is in the "recycle" position, the eluate flowing from the detector is continuously reintroduced into the column by the pump. In the "collect" position, the eluate passes directly into a glass siphon counter, which delivers constant volumes (5 ml) into a fraction collector holding 10-ml test-tubes. When siphoning, a mark is recorded on the chromatogram to facilitate identification of each fraction.

Experimental conditions for separations

A polystyrene gel, cross-linked with divinylbenzene (Poragel 60 Å, Waters Assoc.; particle size 37–75 μm), was used as packing; this gel was efficient for compounds of molecular weight lower than 2000.

Preliminary trials have shown that such separations take place only with a proton-acceptor solvent[17,18]. Tetrahydrofuran (THF) was successfully used in analytical studies, but was not suitable for preparative work. After chromatographic separation, each diastereoisomer is collected in very dilute solution and the solvent must be evaporated off; THF is insufficiently, as it generally contains non-volatile impurities that become concentrated in the sample and interfere with subsequent spectroscopic studies.

A volatile ether without the disadvantages of THF and convenient for separations is diisopropyl ether (DIPE); however, DIPE swells the Poragel 60 Å less than does THF (about 15%). Columns (3 ft. × 3/8 in. O.D.) packed with the help of DIPE have a lower efficiency than ones made with THF, probably because of the weak swelling of the gel (250 plates per ft., instead of 450). A set of 10 columns had the necessary efficiency to perform the separations, just as predicted by our equations[16,17]. The flow-rate was adjusted to between 0.5 and 5 ml/min (a low flow-rate was used to carry out experiments overnight).

Under these conditions, the method was very convenient for the preparative separation of our compounds, and, owing to the high capacity of the gel, large samples (1–2 g) could be injected. The elution volumes of all the compounds were between V_0 (the excluded-molecule elution volume) and $V_0 + V_i$ (the smallest-molecule elution volume). There was no damage to the column through adsorption of impurities on the packing, but separations were very slow, elution times being between 8 h and 3 days.

RESULTS AND DISCUSSION

Mixtures of two diastereoisomers

These compounds (I to VII) have only one hydroxyl group; their preliminary purification is possible by vacuum distillation, and the pairs of diastereoisomers are easily resolved. For each of these pairs, we have characterized the ease of separation by the relative difference, $\Delta V/V$, between the corresponding elution volumes[17,18]. The results are shown in Table II, together with the number of cycles needed for complete separation.

TABLE II

SEPARATION OF DIASTEREOISOMERS OF COMPOUNDS I TO VII

Compound No.	*Value of $\Delta V/V$*	*Number of cycles*
I_A, I_B	$3 \cdot 10^{-2}$	10
II_A, II_B	$6 \cdot 10^{-2}$	7
III_A, III_B	$5 \cdot 10^{-2}$	8
IV_A, IV_B	$6 \cdot 10^{-2}$	7
V_A, V_B	$10 \cdot 10^{-2}$	4
VI_A, VI_B	$5 \cdot 10^{-2}$	8
VII_A, VII_B	$4 \cdot 10^{-2}$	9

Fig. 1 shows the recycling GPC chromatogram of a mixture (VI_A–VI_B) previously distilled; since it contained no impurities, the separation could be automatically performed overnight. Eight cycles were necessary for complete separation.

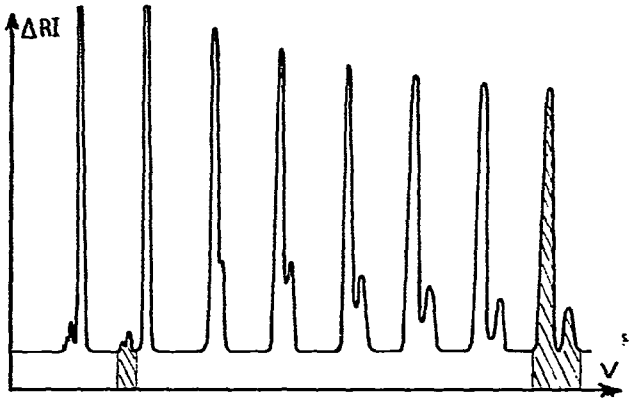


Fig. 1. Recycling GPC of 250 mg of a pure mixture of compounds VI_A and VI_B on a set of 10 columns (each 3 ft. × 3/8 in.) of Poragel 60 Å, with DIPE as mobile phase (flow-rate 3.5 ml/min.). Range: ×32.

Fig. 2 represents the separation of a mixture (VII_A–VII_B) that could not be purified and so contained many impurities. The experiment is more involved, since the impurities must be collected as soon as they separate to avoid re-mixing. The first fraction-collection takes place in the second cycle, for impurities (5) and (6), as well as for high-molecular-weight compounds already separated in the first cycle. Then, in the sixth cycle, impurity peaks (3) and (4) are collected. Diastereoisomers (1) and (2) are well resolved in the ninth cycle.

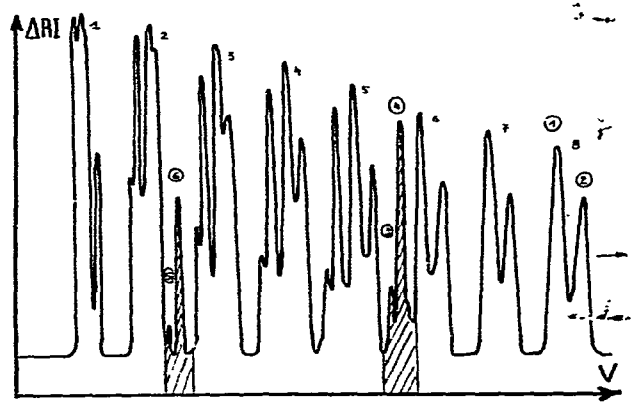


Fig. 2. Recycling GPC of 1.5 ml of a crude mixture of compounds. Range: ×128; other conditions as for Fig. 1. Encircled numbers refer to peaks (see text); uncircled numbers refer to cycles. 1 = VII_A; 2 = VII_B.

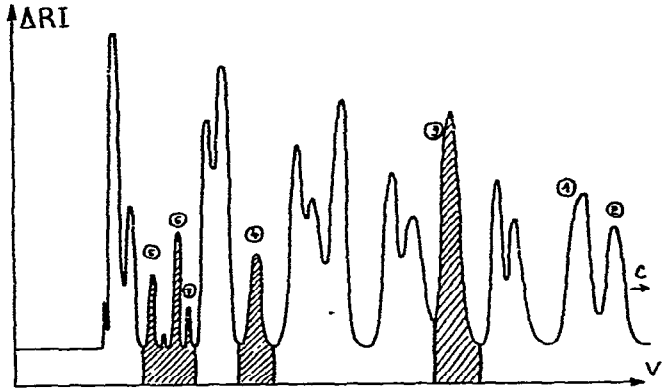


Fig. 3. Recycling GPC of 1.8 g of a crude mixture of compounds $VIII_A$, $VIII_B$, $VIII_C$ and $VIII_D$; conditions as for Fig. 2.

Mixtures of four diastereoisomers

The acetal diols VIII to XIV (in Table I) have three asymmetric carbon atoms presenting two configurations and are, accordingly, mixtures of four diastereoisomers; they are thermally unstable and difficult to purify by vacuum distillation. We have therefore worked with crude products containing up to 10 components, and, as before, impurities had to be collected as soon as they separated. Fig. 3 shows the recycling GPC chromatogram of the mixture $VIII_A$, $VIII_B$, $VIII_C$ and $VIII_D$. During the first and second cycles, impurity peaks (5), (6), (7) and (4) are collected. In the fourth cycle, peak (3), which contains two unresolved diastereoisomers, must be collected so that it does not interfere with the separation of peaks (1) and (2), which are collected (completely resolved) in the seventh cycle and give diastereoisomers $VIII_A$ and $VIII_B$. The mixture corresponding to peak (3) is, in a separate experiment, re-injected; after recycling, it yields the diastereoisomers $VIII_C$ and $VIII_D$ in the same way as the mixture (VI_A–VI_B) shown in Fig. 1.

Table III lists the different fractions obtained with model molecules VIII to XIV and which have been characterized by infrared and nuclear magnetic resonance spectroscopy.

TABLE III

RESOLUTION OF COMPOUNDS VIII TO XIV

Compound No.	*Pure fractions resolved*	*Notes*
VIII	$VIII_A$, $VIII_B$, $VIII_C$, $VIII_D$	—
IX	IX_A, IX_B, IX_C	IX_D very enriched
X	X_A, X_B, X_C	X_D no evidence
XI	XI_A, XI_B, XI_C	XI_D no evidence
XII	XII_A, XII_B	XII_C enriched; XII_D not resolved
XIII	$XIII_A$, $XIII_B$	$XIII_C$, $XIII_D$ not resolved
XIV	XIV_A	XIV_B, XIV_C, XIV_D not resolved

INTERPRETATION OF RESULTS

The results reported here, which are the first example of diastereoisomer separation by recycle GPC, can be interpreted (as we proposed[17,18]), by the occurrence of solute–solvent hydrogen-bond interactions.

For each isomer of a given model molecule in an inert solvent, hydroxyl-groups are involved in intra-molecular hydrogen bonds. The particular oxygen atom of the acetal function with which hydrogen bonding predominates depends on the molecular configuration[1]. In solution in a proton-acceptor solvent (such as DIPE), solvated molecules with larger hydrodynamic volumes are also present, resulting from hydrogen-bond association with the solvent. The three forms (free, chelated and solvated) probably exist in equilibrium, the ratios depending on the diastereoisomer structure and leading to different average hydrodynamic volumes for the two components of each diastereoisomer mixture. This interpretation has been corroborated by complementary recycle-GPC experiments using a non-acceptor or very weak proton-acceptor solvent such as benzene. In this instance, we have detected no evidence of diastereoisomer separation after the same number of cycles that produced effective separation in a proton-acceptor solvent.

The infrared spectra of compounds I to VI in dilute carbon tetrachloride[1] give another experimental verification of this interpretation. For a given compound the isomer with the smaller elution volume (*i.e.*, the larger hydrodynamic volume) is the less chelated one (and consequently more solvated).

CONCLUSIONS

In this paper, we have shown that recycling GPC can be used to separate diastereoisomers of low molecular weight. Semi-preparative amounts (1–2 g) can be injected, and complex mixtures, even of crude products, may be separated. The method is very slow, but could be improved by using microgels such as μStyragel 100 Å. The efficiencies of packed columns of microgels are much better than that of Poragel and will certainly shorten the experiments.

We propose an interpretation of the separation mechanism of these diastereoisomers. Hydrogen bonding between solvent and solutes is necessary to ensure good separation, and, for separation of solvated diastereoisomers, the form (position) and/or strength (equilibrium) of the chelation must differ for the isomers.

REFERENCES

1 F. Lafuma, *Thèse de Doctorat d'Etat*, Paris, 1976.
2 F. Lafuma, R. Audebert and C. Quivoron, *C.R. Acad. Sci.*, *Ser. C*, 276 (1973) 319.
3 F. Lafuma and C. Quivoron, *J. Polym. Sci., Symp.*, 52 (1975) 261.
4 J. G. Hendrikson and J. C. Moore, *J. Polym. Sci., A1*, 4 (1966) 167.
5 W. Heitz, B. Bomer and U. Ullner, *Makromol. Chem.*, 121 (1969) 102.
6 J. Cazes and D. R. Gaskill, *Separ. Sci.*, 2 (1967) 421, and 4 (1969) 15.
7 K. J. Bombaugh, W. A. Dark and R. F. Levangie, *Z. Anal. Chem.*, 236 (1968) 443; *Separ. Sci.*, 3 (1968) 375.
8 M. Duval, B. Bloch and S. Kohn, *J. Appl. Polym. Sci.*, 16 (1972) 1585.
9 K. J. Bombaugh, W. A. Dark and R. F. Levangie, *J. Chromatogr. Sci.*, 7 (1969) 42.
10 K. J. Bombaugh and R. F. Levangie, *J. Chromatogr. Sci.*, 8 (1970) 560.

11 K. J. Bombaugh, *J. Chromatogr.*, 53 (1970) 27.
12 I. Duvdevani, J. A. Biesenberger and M. Tan, *J. Polym. Sci., B*, 9 (1971) 353 and 429; *J. Appl. Polym. Sci.*, 15 (1971) 1549.
13 S. Nakamura, S. Ishiguro, T. Yamada and S. Moriizumi, *J. Chromatogr.*, 83 (1973) 279.
14 H. Kalasz, *J. Chromatogr.*, 78 (1973) 233.
15 R. A. Henry, S. B. Byrne and D. R. Hudson, *J. Chromatogr. Sci.*, 12 (1974) 197.
16 J. Lesec and C. Quivoron, *Analusis*, 4 (1976) 120.
17 J. Lesec, F. Lafuma and C. Quivoron, *J. Chromatogr. Sci.*, 12 (1974) 683.
18 J. Lesec, F. Lafuma and C. Quivoron, *C.R. Acad. Sci., Ser. C*, 278 (1974) 1069.