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PREPARATION AND EVALUATION OF A CHIRAL DERIVATIVE OF SEPHADEX LH-20

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SUMMARY

A novel lipophilic dextran gel has been prepared by the addition to Sephadex LH-20 of a chiral substituent. The BF_3-catalysed reaction of 23,24-oxido-5β-cholane with LH-20 proceeds readily and yields a gel which swells both in non-polar and in polar organic solvents. The new material has been evaluated as a chromatographic medium with particular reference to the separation of diastereoisomeric compounds. Although there is no apparent selectivity towards chiral molecules, the gel has proved satisfactory for lipid chromatography. Standard elution volumes are quoted for a number of compounds in straight-phase and reversed-phase systems.

INTRODUCTION

One of the more important recent developments in liquid chromatography has been the demonstration of the utility of dextran gels as stationary phases. In particular, advances in lipophilic gel chromatography made by SJÖVALL and co-workers[1] have provided the lipid chemist with a very versatile technique. Methylated Sephadex[2], hydroxypropyl Sephadex[3], hydroxyalkoxypropyl Sephadex[3,4] and trimethylsilyl Sephadex[5] have been prepared for use with organic solvents. Straight-phase, reversed-phase and gel-filtration systems are accessible according to the choice of eluant.

The ease of substitution of dextran gels has promoted work on the design of selective stationary phases. ELLINGBOE *et al.*[6] introduced various specific groups into the sugar ring with this intent. More recently, phosphatidylcholines have been separated according to the length and degree of unsaturation of the side-chain, on a Sephadex-based lipophilic ion-exchange column[7]. Stereoselectivity in liquid chromatography has been known for some time (see, for example, the review by LOSSE AND KUNTZE[8]). In all cases where enantiomeric compounds have been resolved to some extent, an optically active stationary phase has been used. The substitution of Sephadex with one enantiomer of an amino acid yielded a gel capable of resolving certain racemic amino acids in aqueous systems[9]. This parallels developments in gas chromatography (see, for example, NAKAPARKSIN *et al.*[10]).

We considered the extension of stereoselectivity to lipophilic systems. A suitable lipophilic derivative of Sephadex might allow the separation of optical isomers and of closely-similar diastereoisomers. One obvious approach was the attachment of a chiral substituent. Certain requirements are imposed on the choice of substituent groups: (i) it must be possible to introduce them without adversely affecting the gel structure; (ii) they must be stable with respect to physical, chemical and stereochemical properties. These conditions are satisfied, in terms of matrix stability and column performance, by BF_3-catalysed reaction of a terminal epoxide with Sephadex LH-20, a hydroxypropyl derivative of Sephadex G-25 (ref. 11).

We now report the preparation of a hydroxy-5β-cholanyl-substituted derivative of Sephadex LH-20. 23,24-Oxido-5β-cholane, presumed to be a mixture of stereoisomeric oxides, prepared from naturally occurring bile acids, was readily incorporated into LH-20 to give a lipophilic gel which swelled in solvents ranging from hydrocarbons to alcohols, thus allowing it to be used in straight- and reversed-phase systems. Evaluation of the gel as a chromatographic medium in both systems has been carried out for a wide variety of substrates.

EXPERIMENTAL

Materials and methods

Sephadex G-25 was obtained from Pharmacia, Uppsala, Sweden. Bile acids for the preparation of 23,24-oxido-5β-cholane were obtained commercially, as were other samples with the exception of the following. Campesterol and dihydrobrassicasterol were donated by Dr. M. J. THOMPSON and poriferasterol by Dr. G. W. PATTERSON. World Standard pyrethrum extract was obtained from the Pyrethrum Marketing Board of Kenya. A human aortal lipid extract was provided by Dr. A. G. SMITH and Mr. J. D. GILBERT, both of the Chemistry Department, Glasgow University. Nedox 1114, from the Ashland Oil & Refining Co., Minneapolis, Minn., U.S.A., was analysed by gas–liquid chromatography (GLC) and found to contain approximately equimolar amounts of C_{11}, C_{12}, C_{13}, and C_{14} epoxides. An average molecular weight of 191 was used in computing amounts of material for reactions.

Solvents used for chromatography were redistilled before use. Benzene was dried over CaH_2 prior to distillation. Methylene chloride was distilled from CaH_2 and stored over Linde, Type 5A, molecular sieve. All glassware used for reactions and chromatography was "silanized" with a 10% solution of dimethyldichlorosilan in toluene.

GLC – mass spectrometry (MS) was effected using an LKB gas chromatograph – mass spectrometer at electron energy 70 eV. GLC was carried out on a Pye 104 gas chromatograph. Scintillation counting was done on a Philips PW 4510 automatic scintillation analyser. Samples (volume, 15 ml) were prepared in Packard vials using PPO (5.5 g/l) and POPOP (0.1 g/l) in toluene. Optical rotation measurements were taken with a Perkin-Elmer Model 141 polarimeter. A micro-capacity cell was used (volume, 1 ml; path length, 100 mm) with this instrument. Error in the angular rotation was of the order of $\pm$ 0.005°. A Unicam SP800 UV spectrophotometer was used in measurement of the elution profile of racemic usnic acids. Spectra were recorded in ethanol, and the intensity of absorption at 285 nm was used to construct an elution profile curve.

Preparation of the epoxide

23,24-Oxido-5β-cholane was prepared from both deoxycholic acid and dehydrocholic acid. The reaction sequences are summarised in Fig. 1. Brief experimental details follow for reactions not annotated with literature references.

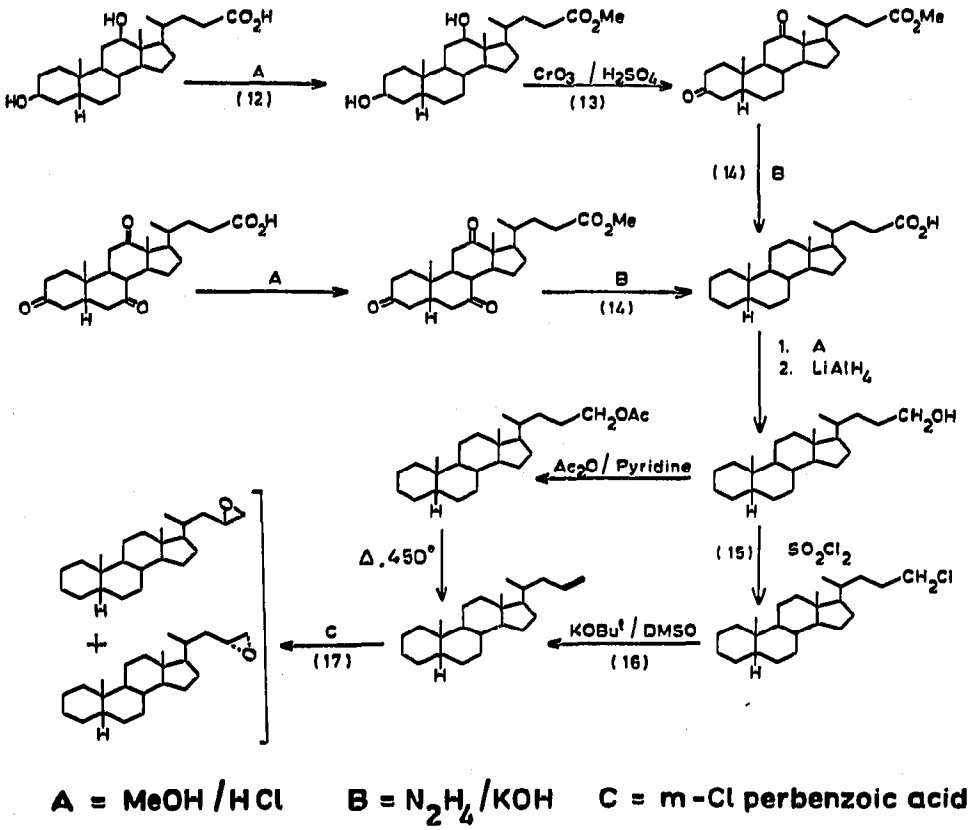

Fig. 1. Reaction scheme for the preparation of 23,24-oxido-5β-cholane. Literature references are indicated in parentheses.

Methylation of bile acids

The acid was dissolved in a 3% methanolic solution of HCl[18] and the mixture boiled under reflux until reaction was complete as indicated by thin-layer chromatography (usually 30 min). Volatiles were removed by vacuum distillation, and the solid product was recrystallised from methanol.

Reduction of methyl 5β-cholanoate to 5β-cholan-24-ol

A suspension of $LiAlH_4$ (2 equiv.) was stirred for 1 h at room temperature in dry ether under a nitrogen atmosphere, and methyl 5β-cholanoate (1 equiv.) was added slowly in ether solution. The reaction was almost complete (> 90%) after a further 1 h. Excess hydride was destroyed by the cautious addition of ethyl acetate. Water was added and 5β-cholan-24-ol was recovered by ether extraction. A sample

recrystallised from ethyl acetate had m.p. range 131–131.5° (recorded[15] m.p. range, 130.5–132.5°).

Preparation of 5β-chol-23-ene

Two methods were used, starting from 5β-cholan-24-ol.

(i) The alcohol was converted to the chloride by reaction with SO_2Cl_2 (ref. 15) and the chloride treated with potassium *tert.*-butoxide in dimethyl sulphoxide (DMSO)[16]. In agreement with the previous work, this gave predominantly the olefin, with some 24-*tert.*-butoxy-5β-cholane (17%). The latter was further characterised by GLC–MS: $I_{SE\text{-}30}$, 2700; $M^{+\cdot}$, 402; major fragment ions at *m/e* 387, 346, 345, 328, 217, 149, 109, 95, 57. The bulk of the olefin ($I_{SE\text{-}30}$, 2320) was not purified before epoxidation as the *tert.*-butyl ether was found not to interfere in any of the subsequent reactions.

(ii) Vapour-phase pyrolysis of 5β-cholan-24-yl acetate gave an oil containing 83% by weight of the olefin (estimated by GLC). A solution of the acetate in light petroleum (b.p., 40–60°) was added in a slow nitrogen stream to a vertical silica tube packed with glass wool and maintained at 450° by an electric oven. The product emerged from the tube as an aerosol which was collected in a cooled trap. Purification of the olefin was effected by column chromatography on alumina, followed by recrystallisation from methanol; the product had m.p. range 87.5–95° (recorded[16] m.p., range 99–100.4°). The presence of the terminal double bond was confirmed by NMR (60 MHz in deuterochloroform): multiplets corresponding to two vinyl protons at 5.0 p.p.m. and one vinyl proton at 5.7 p.p.m. The structure of the olefin was confirmed by oxidative cleavage of the double bond with osmium tetroxide and periodic acid. Analysis of the product and of its methyl oxime by GLC–MS indicated a molecular weight of 330 for the free carbonyl compound and 359 for the oxime. These values correspond to products arising from 5β-chol-23-ene. GLC of the cleavage products indicated that this olefin constituted over 90% of the pyrolysis product.

Preparation of gels

High-resolution columns required gel particles of a narrow size range in the region of 20–40 μ. Sephadex G-25 (superfine grade) was separated into fractions by a continuous-flow sedimentation process[19] and each fraction was converted to an LH-20 type derivative by the method of ELLINGBOE *et al.*[4]. Beads of diameter 24–32 μ were used for the reaction with 23,24-oxido-5β-cholane. Before reaction, the gel was dried overnight in a vacuum oven at 50°. Pre-washing with acetone aided desiccation of the porous material.

A sample of gel (4.3 g) was placed in a flask fitted with a mechanical stirrer and pressure-equilibrating dropping funnel. Dry methylene chloride (40 ml) was added, and the gel was equilibrated by gentle stirring in the solvent overnight. BF_3 etherate (0.43 ml) was added and stirring continued for 10 min. 23,24-Oxido-5β-cholane (3.5 g) in dry methylene chloride (25 ml) was added during 30 min and the suspension stirred for 4 h. After filtering, washing with methylene chloride, methanol, and acetone and drying overnight under vacuum, the gel weighed 7.2 g, representing an uptake of 64% by weight of the oxide. This corresponded to 0.655 cholane residues per sugar residue. The steroidal material in the supernatant was shown by GLC to comprise over 97% of 24-*tert.*-butoxy-5β-cholane.

Column techniques

Analytical-scale columns were (in general) constructed with narrow-bore glass (4–6 mm I.D.) or Teflon (1.6 mm I.D.) tubing (Fig. 2). Glass columns used with gravity flow were fitted with a Glenco No. 3020 Teflon connector at the bottom of the column[20]. The application of low pressure (5–10 p.s.i.) to columns gave convenient flow-rates of 1–2 ml/h. Pressure was applied to the solvent by one of the following methods: (i) from a gas cylinder attached through a regulator to the solvent reservoir bottle; or (ii) using a syringe pump (Type 355, Sage Instruments, Inc., White Plains, N.Y., U.S.A.), in conjunction with a 30-ml Hamilton syringe (No. 1030), which was connected to columns using a Teflon needle. Glass columns were fitted with Pye-Unicam injection heads and column endpieces. The washers, locking nut and ferrule were filed to accommodate the glass tubing used for the column. The ferrule on the endpiece held in place a Teflon net (Pharmacia) used as a bed support. Columns were calibrated to facilitate measurement of bed volumes, and were packed by sedimentation of a slurry of pre-swollen gel under gravity flow together with gentle mechanical vibration. Solvent was allowed to flow through until a constant bed volume was obtained.

Detection of solute in column effluent was achieved almost entirely by the use of liquid chromatographs of the moving wire-flame ionisation detector type. One of these was based upon the design of HAAHTI *et al.*[21] and the other was a Pye-Unicam System 2 liquid chromatograph. The platinum chain[21] was replaced by a

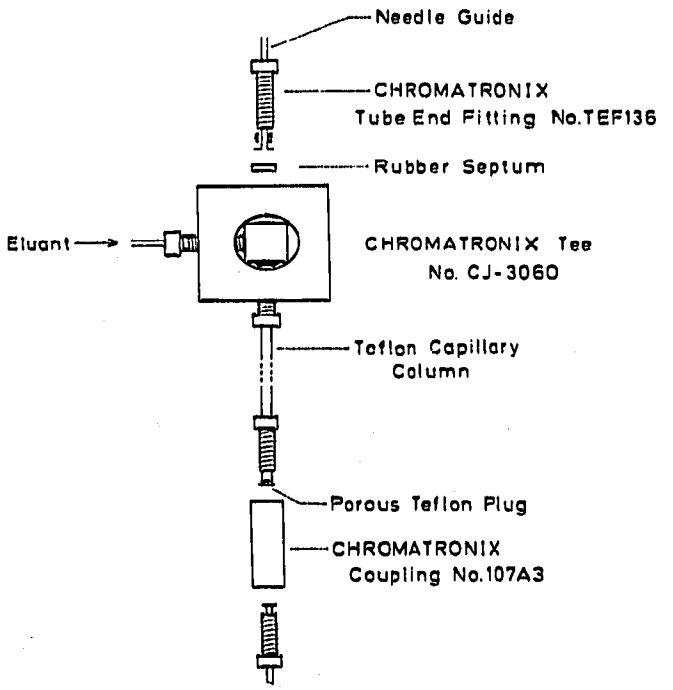

Fig. 2. Construction of a Teflon narrow-bore column for analytical gel chromatography. Teflon connecting tubing was flanged with the Chromatronix flanging tool, FT-1. The bed support was cut from a porous Teflon disc obtained from Pharmacia, Uppsala, Sweden.

wire prepared as follows. Two strands of wire (80/20 Vacrom wire, from the Vactile Wire Co. Ltd., Bootle L20 6AE, Lancs.) were twisted to give 8 turns per cm. The wire was doubled and the process repeated to give a 4-stranded composite wire[22]. This had satisfactory sample capacity,a useful lifetime (1–2 weeks when in continuous use), and a low noise level after rigorous cleaning. Sample size was commonly 25–100 μg, but 1–2 mg could be accomodated on columns of bed volume 10–20 ml without deterioration of peak shape. Elution of 25 μg of material in a peak of width 10 S.E.V.* from an analytical column, bed volume 5 ml, resulted in an average eluate concentration of 50 μg/ml, which was easily detected on the liquid chromatographs. Application of substrates to the Teflon columns was readily achieved by injection from a Hamilton microlitre syringe. The wider glass columns required removal of supernatant solvent prior to sample application, in the normal manner for conventional liquid chromatography.

RESULTS AND DISCUSSION

Hydroxy-cholanyl-substituted LH-20 is a chemically stable gel. Column bleed from analytical-scale columns, in terms of background noise in the liquid chromatograph, has been found to be negligible. This agrees with observations made by ELLINGBOE *et al.*[3,4] and KEATES[22] on the properties of hydroxyalkoxypropyl Sephadex. The gel was evaluated in two solvent systems: benzene, and methanol–heptane (9:1), which gave straight- and reversed-phase separations, respectively. Solvent regain values are quoted in Table I. Maximum solvent uptake is obtained with

TABLE I

SOLVENT REGAIN VALUES FOR HYDROXY-5β-CHOLANYL-SUBSTITUTED SEPHADEX LH-20[a]

Solvent	*Solvent regain value*[b]
Heptane	0.84
Cyclohexane	1.25
Carbon tetrachloride	2.77
Toluene	1.88
Benzene	2.41
Tetrahydrofuran	2.19
Chloroform	3.67
Methylene chloride	2.75
Acetone	1.04
Methanol	1.16

[a] Degree of substitution = 40.3% by weight of hydroxy-5β-cholanyl residues.
[b] Grams of solvent taken up by 1 g of dry gel.

halogenated and with aromatic solvents, but swelling does occur with more polar solvents. The degree of swelling of the gel depends *inter alia* on the degree of substitution and on the nature of the substituent: generally, the solvent regain values increase as the percentage substitution of the hydrophilic gel increases[2].

* See p. 256.

Elution of material from the gel with methanol–heptane (9:1) resulted in reversed-phase separations, in which non-polar compounds were retarded on the gel. One direct consequence of the lower solvent uptake with polar solvents—which also applies to other lipophilic gels—is that the size of the gel beads is smaller in reversed-phase systems than in straight-phase ones. Column flow-rates therefore tend to decrease, whereas resolution is enhanced.

The method of preparation of this gel was developed with the aim of introducing one cholanyl substituent into each sugar residue in the dextran, corresponding to a lipid content of 51% by weight. Trial reactions were carried out with approximately stoichiometric quantities of oxide (Nedox 1114) and various amounts of catalyst. The optimum amount of BF_3 etherate was 0.1–0.2 ml per g of dry gel, agreeing with the findings of ELLINGBOE *et al.*[3]. This method proved satisfactory when used with 23,24-oxido-5β-cholane.

TABLE II

RECOVERY OF MATERIALS OF DIFFERENT POLARITY FROM THE STRAIGHT-PHASE SYSTEM

Compound	*Load (µg)*	*d.p.m. added*	*d.p.m. recovered*	*Recovery (%)*
Cholesteryl palmitate	10	33636	33126	99
Cholesterol	10	4067	4096	101
Palmitic acid	10	43938	43169	98

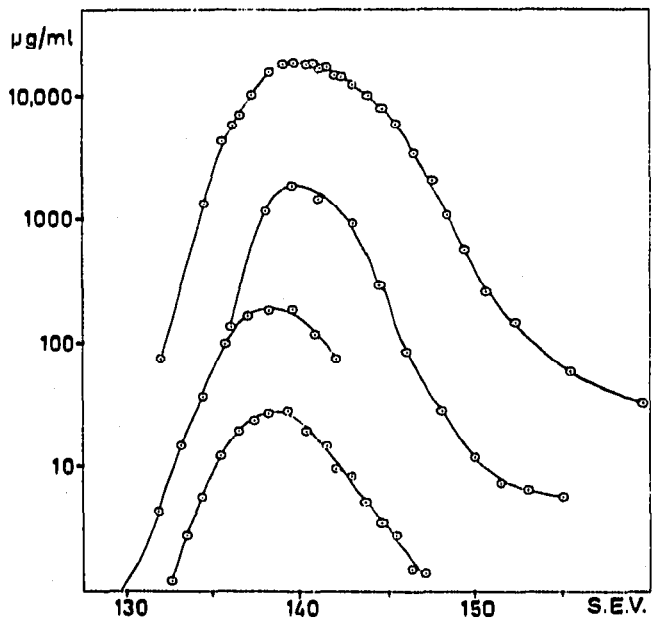

Fig. 3. The effect of increasing the load applied to a gel column (500 × 4 mm I.D.) on the elution profile. The solute, cholesterol, contained 4-^{14}C-labelled material. Concentrations were measured through the activity of collected fractions. The total loads applied were 10 µg, 100 µg, 1,000 µg, 10,000 µg of specific activity 0.1 µCi/mg. Increasing the load broadens the band on the column, but the profile shows that even at high loads, "tailing" is not a serious problem.

Performance of the gel proved satisfactory for a wide range of sample types and loads. Recovery of material from the straight-phase system was assessed by use of radioactively-labelled compounds of diverse polarities (Table II). Virtually quantitative recovery was obtained even at the 10-μg level. Fractions were taken during the elution of cholesterol from the column. Elution profiles are illustrated in Fig. 3. The almost symmetrical peak shape is maintained very well through a 1000-fold increase in the sample load. Generally, non-polar and moderately polar compounds (for example, those with one hydroxyl group) had satisfactory chromatographic profiles in the straight-phase gel system. Serious tailing with more polar compounds was observed and such materials were markedly retarded on the gel. These problems can be circumvented either by derivatisation of polar groups or by the adoption of a reversed-phase system.

TABLE III

S.E.V. DATA FOR STRAIGHT-PHASE AND REVERSED-PHASE SYSTEMS

Compound	*Mol. wt.*	*Cholanyl-gel*		*Nedox-gel*	
		Straight-phase	*Reversed-phase*	*Straight-phase*	*Reversed-phase*
Cholesterol	386	139	309	110	250
Cholestanol	388	132	320		262
Coprostanol	388	118	391		
Epicholestanol	388	132	320		
Epicoprostanol	388	118	391		
Dihydrobrassicasterol	400	133	314		
Campesterol	400	133	314	109.2	270
β-Sitosterol	414	133	324	107.4	294
Stigmasterol	412	129	317	105.1	256
Poriferasterol	412	129	317		
Lanosterol	426		366	82.3	242
5β-Cholan-24-ol	346	147	109		
5α-Androstan-3α-ol	276		159		
Pregn-5-en-3β-ol	302		209		
17α-Ethynylestr-4-en-17β-ol	284	130	143		
Norethisterone	298	133	80		
Mestranol	310		149		
Estr-4-en-17β-ol	260		156		
19-Nortestosterone	274	150	80		
Estradiol 3-methyl ether	286	127	156		
Testosterone	288	140	179		
Androsterone	290	123	84		
Epiandrosterone	290	126	84		
Etiocholanolone	290	133	84		
3β-Hydroxy-5β-androstan-17-one	290	122	84		
Progesterone	314	78		61.3	54
5α-Androstan-3-one	274		171		
Cholestanone	386	70	309	60.0	354
Cholest-4-en-3-one	384	76	248		240
Cholest-5-en-3-one	384	76	311		353
Cholesta-1,4-dien-3-one	382		199		
Cholesta-4,6-dien-3-one	382		199		
Cholest-4-en-3,6-dione	398		149	586	136
5α-Cholestane	372	65	833	62.7	
n-Octacosane	394	62			

TABLE III *(continued)*

Compound	*Mol. wt.*	*Cholanyl-gel*		*Nedox-gel*	
		Straight-phase	*Reversed-phase*	*Straight-phase*	*Reversed-phase*
Cholesteryl acetate	428			56.3	541
Cholesteryl butyrate	456	62		54.5	
Cholesteryl palmitate	624	55	480	49.0	
Cholesteryl benzoate	490	68		54.0	
Cholestanyl acetate	430	65	486		
Epicholestanyl acetate	430	65	486		
Coprostanyl acetate	430	65	434		
Epicoprostanyl acetate	430	65	434		
Dihydrobrassicasteryl acetate	442	62	440		
Campesteryl acetate	442	62	440		
Stigmasteryl acetate	454	67	442	55.2	
Poriferasteryl acetate	454	67	442		
Lanosteryl acetate	470	69			
Lanostenyl acetate	468	69			
Methyl deoxycholate	406	210			
5β-Cholan-24-ol	346	147	109		
5β-Cholan-3,24-diol	362		77		
5β-Cholan-3,12,24-triol	378		63		
5β-Cholan-3,7,12,24-tetrol	394		49		
n-Octan-1-ol	130		104		
n-Octan-2-ol	130		104		
n-Decan-1-ol	158	137	145		
n-Dodecan-1-ol	186	129	153		
n-Tetradecan-1-ol	214	124	167	113.2	
n-Hexadecan-1-ol	242		182	108.2	
n-Octadecan-1-ol	270		201	103.3	
n-Eicosan-1-ol	298	107	224	99.0	
n-Docosan-1-ol	326	104	255	94.6	
n-Tetracosan-1-ol	354	96	293	91.3	
n-Hexacosan-1-ol	382	93	342	88.1	
Trimyristin	812	54	940		
Tripalmitin	848	44	1180	43.2	
Tristearin	884	36	1410	42.2	
D-Usnic acid	344	79			
L-Usnic acid	344	79			
D-Phenylalanine	165		53		
L-Phenylalanine	165		53		
D-Phenylalanine methyl ester	179		81		
L-Phenylalanine methyl ester	179		81		
D-Ephedrine	165	208	63	106	
L-Ephedrine	165	208	63		
L-Norephedrine	151			189	
D-Mandelic acid	152		57		
L-Mandelic acid	152		57		
D-Fenchone oxime	169	87			
L-Fenchone oxime	169	87			
D-Fenchone 2,4-DNPH	334	69			
D-Ephedrine N,O-diacetate	265	70	58		
L-Ephedrine N,O-diacetate	265	70	58		
Pyrethrin I	328		89		
Pyrethrin II	372		80		

Standard elution volumes (S.E.V.)[20] for a number of compounds are quoted in Table III. For comparison, S.E.V. values recorded for hydroxyalkoxypropyl Sephadex gels are included[20,22,23]. It is at once apparent that the order of elution is similar for both types of gel.

In the straight-phase system, hydroxylic, amino, and especially acidic compounds are retarded on the cholanyl-substituted gel. Carbonyl groups do not have a noticeable effect on elution volumes in this system: ketones and esters generally have similar elution volumes to the hydrocarbon analogues: for example, cholesterol, cholesteryl butyrate, 5α-cholestan-3-one, and cholestane have S.E.V. values 139, 62, 71, and 65, respectively. Selective retention of aromatic compounds has been observed in Sephadex G systems[24–28]. No such effect has been found with lipophilic gels studied earlier[20,24,29] or with hydroxy-5β-cholanyl-substituted LH-20. Separation of 5α- and 5β-sterols can readily be obtained but 3-hydroxysteroid epimers have not been resolved, either free or as acetates.

The pattern of elution in the reversed-phase system follows the order: acids, alcohols, hydrocarbons. Lipophilic character is an important factor in determining elution volumes. Thus the presence of a hydrocarbon side-chain on the steroid nucleus causes longer retention of C_{27}, C_{28}, and C_{29} sterols relative to steroids lacking the side-chain. The introduction of one or more double bonds to cholestanone decreases the S.E.V. Methylation of an acid increases its S.E.V. As in the straight-phase system, 5α- and 5β-sterols are separated but 3α- and 3β-hydroxysteroids are not.

The influence of molecular size on elution volumes is illustrated by the data for the *n*-alcohol series. In the straight-phase system, elution is in the order of decreasing molecular weight, while in the reversed-phase system the order is inverted. The separation of *n*-alcohols on a reversed-phase narrow-bore column (750 × 1.6 mm) is illustrated in Fig. 4, representing the liquid chromatograph trace, with background noise removed. The straight-phase system is useful for group separation of mixtures, while reversed-phase systems can, in addition, often resolve similar compounds within a group. These points are illustrated in Figs. 5 and 6.

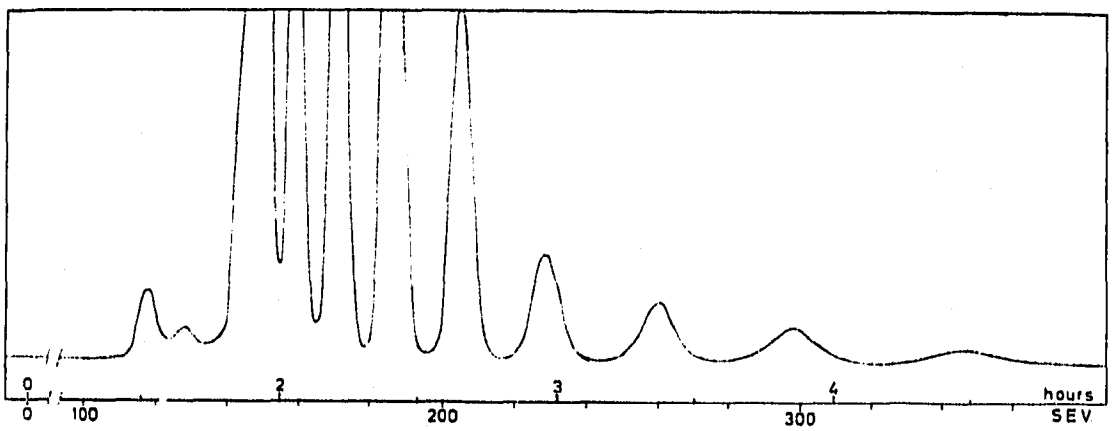

Fig. 4. Chromatography of *n*-alcohols of even carbon number, C_{10}–C_{28}, on a reversed-phase cholanyl LH-20 column (700 × 1.6 mm I.D.) eluted with methanol–heptane (9:1) and detected with a liquid chromatograph by moving wire-FID. The alcohols are eluted in increasing order of molecular weight. The small peaks in the trace below S.E.V. 140 arise from impurities.

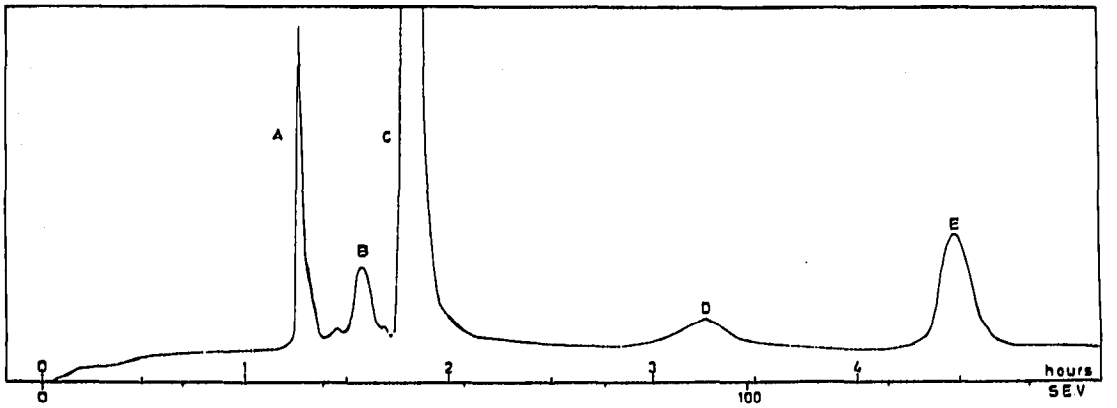

Fig. 5. Group separations achieved on a straight-phase cholanyl LH-20 column (500 × 4 mm I.D.) eluted with benzene, of a total lipid extract derived from a human aorta. A = Hydrocarbons; B = sterol esters; C = triglycerides; D = polar sterol esters; E = sterols.

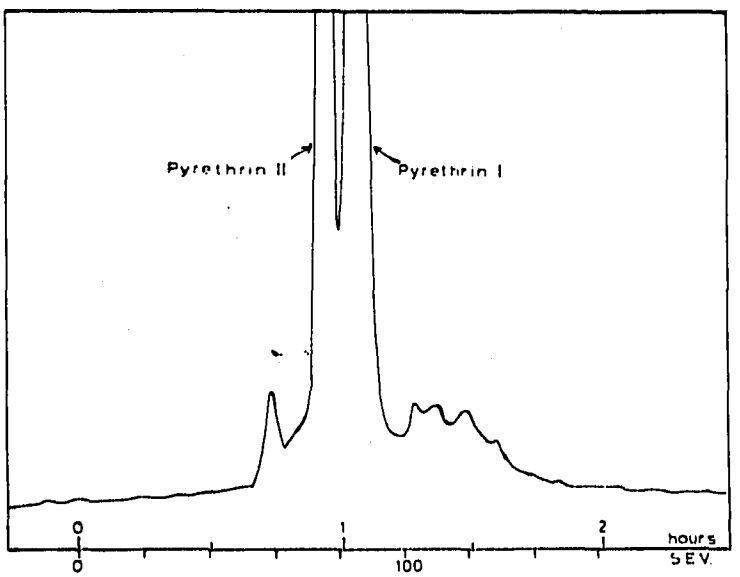

Fig. 6. Chromatography of a World Standard pyrethrum extract on a reversed-phase cholanyl LH-20 column (700 × 1.6 mm I.D.) eluted with methanol–heptane (9:1), showing the partial separation of the similar compounds, pyrethrin I and pyrethrin II. Pyrethrin II contains an additional methoxycarbonyl function which results in its earlier elution.

The gel was examined in both straight- and reversed-phase modes for ability to separate enantiomeric pairs. No success was apparent with any of the sets of compounds used. To confirm that not even partial resolution was obtained, a racemic mixture of usnic acids, with $[\alpha]_D^{20}$ of each isomer, 503° (ref. 30), was chromatographed and fractions were collected during elution. The elution profile, obtained by plotting the UV-absorption intensity *versus* elution volume, was indistinguishable from that obtained for D- or L-usnic acid. The optical rotation of the material in each half of the peak was measured and found to be zero.

The absence of a chiral effect may be explained by a consideration of the solute–gel interaction. Separations on a gel column are based primarily on differences in the partition coefficients of the solute molecules between the mobile phase and the stationary phase. The latter may be considered as a solution of a substituted polysaccharide in the eluting solvent. The composition and properties of the solvent will be influenced by the gel, for example, by preferential adsorption of solvents from a mixed solvent system or by ordering of the solvent around the gel network[1]. There is evidence that the substituent residues provide sites for adsorption in the gel[31] but whether these are on the substituent directly or on an associated solvent layer, must depend on whether the solute can displace the solvent molecules. The absence of a chiral effect indicates that the interaction of solutes with the steroidal hydrocarbon residues is insufficient to lead to differences in the partitioning of enantiomers between the mobile and stationary phases.

CONCLUSION

When Sephadex LH-20 is substituted with hydroxy-5β-cholanyl residues, a gel is obtained which is chemically and mechanically stable. The substitution proceeds readily and is apparently not sterically hindered. As a chromatographic medium, the gel shows the advantages of previously known lipophilic derivatives of Sephadex (*viz.* high sample capacity, good peak shape and good recovery) and can be used to separate mixtures in straight- or reversed-phase systems. No chiral effect has yet been demonstrated, probably because of insufficient solute–gel interaction.

ACKNOWLEDGEMENTS

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