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HYDROXYALICYCLIC DERIVATIVES OF SEPHADEX LH-20 FOR LIPOPHILIC GEL CHROMATOGRAPHY

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SUMMARY

Four hydroxyalicyclic derivatives of Sephadex have been prepared by BF_3-catalysed reaction of LH-20 with 2α,3α-oxido-5α-cholestane, 3β,4β-oxidocyclohexylethyltrimethoxysilane, *exo*-2,3-oxidonorbornane, and *endo*-2,3-oxidobornane, respectively. The gels swelled in solvents of widely different polarities. A preliminary evaluation of the gels as chromatographic stationary phases has been carried out for a straight-phase (benzene) system. Standard elution volumes are quoted for a model set of steroidal compounds.

INTRODUCTION

In our work concerning the evaluation of modified dextrans for use in lipophilic gel chromatography, we have examined a terminal olefin oxide[1] and an alicyclic oxide[2] as reagents for introducing substituents on to the gel network. The BF_3-catalysed reaction of an olefin oxide with Sephadex LH-20 proved satisfactory for this step, in agreement with work by Ellingboe and co-workers[3]. The gels so produced are chemically stable, resulting in low column bleed. Operating conditions are mild, suitable for the chromatography of sensitive compounds. "Quantitative" recovery of solutes from columns[1,4] permits their use with microgram amounts of material. The possibility of preparing selective stationary phases by introducing suitable gel substituents has already been reported[1,5,6]. We now describe the preparation and evaluation of four hydroxyalicyclic derivatives of Sephadex LH-20, for which the preparation of hydroxycyclohexyl LH-20 served as a model reaction[2]. 2α,3α-Oxido-5α-cholestane, 3β,4β-oxidocyclohexyl-ethyltrimethoxysilane, *exo*-2,3-oxidonorbornane and *endo*-2,3-oxidobornane reacted with LH-20 to give lipophilic gels, and a preliminary evaluation of the gels as chromatographic stationary phases has been carried out.

EXPERIMENTAL

Materials

Sephadex G-25 was obtained from Pharmacia, Uppsala, Sweden; *exo*-2,3-epoxynorbornane was obtained from Ralph N. Emanuel Ltd., Wembley, Great

Britain; 3β,4β-epoxycyclohexyl-ethyltrimethoxysilane was obtained from Silar Laboratories Inc., Watervliet, N.Y., U.S.A. Other materials were obtained commercially except for poriferasterol, which was donated by Dr. G. W. Patterson, and 24(RS)-hydroxycholesteryl acetate, which was donated by Dr. G. F. Woods. Solvents were dried and redistilled before use. To facilitate handling of gels, all glassware was silanised with a 5% solution of dichlorodimethylsilane in toluene.

Preparation of epoxides

2α,3α-Oxido-5α-cholestane was prepared from cholesterol by the reaction sequence shown in Fig. 1. All reactions are annotated with literature references except for the epoxidation of 5α-cholest-2-ene, for which brief details are given below. (+)-*Endo*-2,3-oxidobornane was prepared from (+)-α-pinene by the method of Borowiecki and Chrétien-Bessière[10].

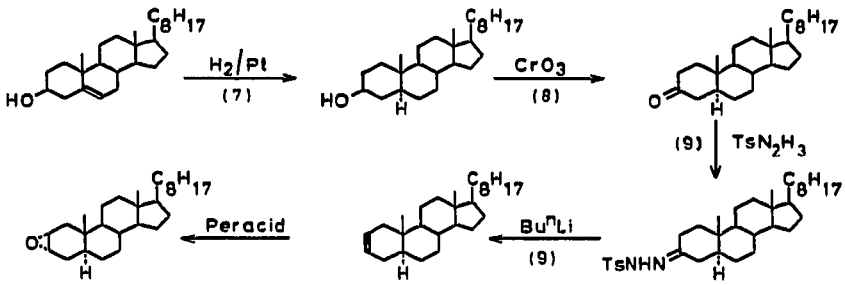

Fig. 1. Reaction scheme for the preparation of 2α,3α-oxido-5α-cholestane. Literature references are indicated in parentheses.

Epoxidation of 5α-cholest-2-ene

5α-Cholest-2-ene (8 g, 0.022 mole) was dissolved in dry dichloromethane (25 ml) and the solution was cooled to 0°. *m*-Chloroperbenzoic acid (4.2 g, 0.024 mole) was added in portions to the stirred solution and the resulting suspension stirred at room temperature for 3 h. The solution was filtered and diluted (100 ml) to facilitate handling. Excess peracid was destroyed with sodium bisulphite (1 g in 20 ml water). The solution was washed with sodium bicarbonate and water, dried and evaporated to yield the oxide (8.2 g, 98%). A sample recrystallised twice from diethyl ether–methanol had m.p. 95–100° (Ref. 11, 2α,3α-oxide, m.p. 104–105°; 2β,3β-oxide, m.p. 91°). The oxide, was presumed to be mostly the α-isomer[11].

Preparation of gels

Sephadex G-25 (superfine grade, 10–40 μ) was separated into low-particle-size range fractions[12], and each fraction converted to an LH-20 type derivative[3]. Beads of diameter 13–17 μm were used for the reaction with 2α,3α-oxido-5α-cholestane, and beads of diameter 17–22 μm were used for all other gels. The experimental method for the preparation of modified gels is described elsewhere[1–3]. Table I summarises the details of the reactions carried out. In some cases, the reaction was repeated using the initial product as starting material, in an attempt to increase the degree of substitution of the gel.

Column techniques

Analytical-scale columns of length 1 m were constructed from narrow-bore (2.5 mm) glass tubing, fitted with Pye-Unicam column end-pieces. Pressurisation of columns using a syringe pump or a gas cylinder followed the method previously described[1]. Solute detection was achieved using moving wire–flame ionisation detector chromatographs. One of these was based on the design of Haahti *et al.*[13] and the other was a Pye-Unicam System 2 liquid chromatograph. Samples (25–100 μg in 1–6 μl solvent) were injected directly on to the gel bed from a Hamilton microlitre syringe.

RESULTS AND DISCUSSION

The gels were chemically stable. Low background noise on the detectors indicated that no column bleed was occurring. Elution data were reproducible over a period of several days, indicating at least short-term stability. The ether linkage between substituent and gel matrix was stable to hydrolysis even in methanol-based solvent systems. The direction of opening of the oxides during the substitution reaction, and therefore the position of the ether linkage on the substituent, is not known. The mode of opening of epoxides is dependent on the catalyst, acid or base, and on the structure of the oxide[14]. The degree of substrate incorporation suggests that substitution of the gel network was not the most favoured reaction. *Endo*-2,3-oxidobornane and *exo*-2,3-oxidonorbornane underwent BF_3-catalysed rearrangement to form carbonyl compounds[10] (infrared absorption bands at 1740 and 1720 cm^{-1}). The other oxides rearranged to ketones and also formed ether-linked polymeric materials whose exact composition is not known. Table I indicates the percentage substitution of the gels; this represents, for the conditions used, a maximum substitution of the (trimethoxysilylethyl)-hydroxycyclohexyl (TMSE-hydroxycyclohexyl) gel and of the hydroxynorbornyl gel.

TABLE I

SUMMARY OF EXPERIMENTAL DETAILS OF GEL REACTIONS

Oxide	*Oxide*	*LH-20*	*No. of repetitions*	*% substitution (by wt.)*
2α,3α-Oxido-5α-cholestane	3.0 g	2.6 g	2	38
3β,4β-Oxidocyclohexyl-ethyltrimethoxysilane	5 ml	2.5 g	1	34
exo-2,3-Oxidonorbornane	2.0 g	1.9 g	1	20
endo-2,3-Oxidobornane	2.1 g	1.5 g	—	7

Solvent regain values are quoted in Table II. The gels swelled in solvents varying widely in polarity. Elution with benzene or with methanol–heptane (9:1) gave straight-phase or reversed-phase systems, respectively. Values for the Standard Elution Volume (S.E.V.)[4] of a number of substances in the benzene system are given in Table III.

TMSE-hydroxycyclohexyl LH-20 appeared to be completely inert with respect to chromatographic properties. No separations were observed of materials varying

TABLE II

SOLVENT REGAIN VALUES FOR HYDROXYALICYCLIC-SUBSTITUTED LH-20

Solvent	*Solvent regain value**			
	a	*b*	*c*	*d*
Cyclohexane	1.04 (1.33)	0.44 (0.56)	0.24 (0.31)	0.40 (0.51)
Benzene	1.38 (1.59)	0.57 (0.66)	0.86 (0.99)	0.84 (0.97)
Chloroform	2.60 (1.76)	0.88 (0.59)	2.41 (1.63)	3.26 (2.20)
Methanol	0.85 (1.08)	0.61 (0.77)	1.12 (1.42)	1.68 (2.13)

* Determined according to Helfferich[15]. Values are for g of solvent taken up by 1 g of dry gel and, in parentheses, ml per g of dry gel. a = Hydroxycholestanyl gel; b = (trimethoxysilylethyl)-hydroxycyclohexyl gel; c = hydroxynorbornyl gel; f = hydroxybornyl gel.

both in size and polarity, in straight-phase or reversed-phase solvent systems: ephedrine, deoxycholic acid, cholesterol and cholesteryl palmitate all had a standard elution volume of 55 in the benzene system.

The order of elution of materials from the other gels follows that observed with previously examined lipophilic Sephadex derivatives[1-4]. A model set of steroidal

TABLE III

STANDARD ELUTION VOLUME (S.E.V.) DATA FOR HYDROXYALICYCLIC-SUBSTITUTED LH-20 GELS IN THE STRAIGHT-PHASE (BENZENE) SYSTEM

Compound	*Mol. wt.*	*S.E.V.*		
		Cholestanyl gel	*Norbornyl gel*	*Bornyl gel*
5α-Cholestane	372	87.2	67.8	57.9
5-Cholestene	370	86.4	71.5	57.9
5α-Cholestan-3-one	386	98.5	74.9	56.5
4-Cholesten-3-one	384	102	78.4	66.8
Progesterone	314	98.5	90.0	83.5
Cholesteryl acetate	428	87.2	67.8	60.1
Cholesteryl butyrate	456	77.9	65.3	59.0
Cholesteryl palmitate	624	75.1	59.2	52.3
Tristearin	890	65.2	58.7	54.1
Cholestanol	388	171	112	88.6
Epicholestanol	388	140	104	80.8
Cholesterol	386	175	117	87.9
Epicholesterol	386	118	88.0	73.5
Stigmasterol	412	167	113	89.0
Poriferasterol	412	167	113	89.0
β-Sitosterol	414	164	108	94.2
Lanosterol	426	118	100	76.1
24(RS)-Hydroxycholesteryl acetate	444	102	90.5	78.3
Epiandrosterone	290	159	176	186
Dehydroepiandrosterone	288	164	177	190
3α-Hydroxy-5β-pregnan-20-one	318	147	98.6	
3β-Hydroxy-5β-pregnan-20-one	318	140	98.6	
(+)-Usnic acid	344	93.5	90.0	74.6
(—)-Usnic acid	344	93.5	90.0	74.6

compounds was chosen for this preliminary evaluation of the gels. Hydrocarbons, esters and ketones had similar elution volumes, while hydroxylic substances were retarded. Three specific types of separation were examined.

(1) The separation of epimeric hydroxylic steroids: 5α-cholestan-3β-ol and cholesterol were separable from their C-3 epimers. 3α- and 3β-hydroxy-5β-pregnan-20-one were separable on hydroxy-5α-cholestanyl LH-20. The separation of epimeric 5β-steroidal alcohols represents an extension of the properties found previously for other lipophilic gels[2]. In addition, the separations of 5α-steroidal epimers were markedly increased on the cholestanyl gel.

(2) The separation of Δ^5/5α-steroids: cholesterol and dehydroepiandrosterone were separable from the corresponding 5α-steroids on all three gels. The degree of separation was less than that achieved on hydroxycyclohexyl LH-20.

(3) The separation of enantiomers and closely similar diastereomers: (+)- and (−)-usnic acids, chosen as test substances because of their high optical activity ($[\alpha]_D^{20} = \pm 503°$) were not separable on either of the chirally substituted gels or on the hydroxynorbornyl gel. Stigmasterol and poriferasterol, and 24(R)- and 24(S)-hydroxycholesteryl-3β-acetates were not separable on any of the gels.

An enhanced separation of epimeric compounds on hydroxy-5α-cholestanyl LH-20 gel compared to the hydroxycyclohexyl LH-20 gel indicates a stronger solute–gel interaction in the former system. This may be due to the larger size of the alicyclic substituent in the steroid-substituted gel. However, the solute–gel interaction is insufficient to allow selective retention of enantiomeric compounds, in agreement with earlier observations on a chiral derivative of LH-20[1].

CONCLUSION

Hydroxyalicyclic derivatives of Sephadex LH-20 are readily prepared from alicyclic oxides, although the efficiency of the substitution process is lower than for reactions involving terminal olefin oxides. The gels are stable and have chromatographic properties comparable with earlier lipophilic Sephadex derivatives. A stronger solute-gel interaction is indicated for hydroxy-5α-cholestanyl-LH-20 than was obtained with other gels. This leads for example to improved separations of epimeric 3-hydroxysteroids but is insufficient to afford separation of enantiomers or of steroids possessing diastereomeric side-chains.

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