Journal of Chromatography, 82 (1973) 337–342

CHROM. 6727

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

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(Received April 2nd, 1973)

SUMMARY

Sephadex LH-20 has been substituted with hydroxycyclohexyl residues to give a stable gel for use in lipophilic gel chromatography. The gel can be used for straight-phase and reversed-phase systems. Separations of a model set of steroidal compounds are described. Hydroxysteroids epimeric at C-3, C-7, C-11, C-17 or C-20 have been separated as the free alcohols in a straight-phase system. Five Δ^5-steroids have been separated from the corresponding dihydrosteroids of the 5α-series.

INTRODUCTION

Lipophilic derivatives of Sephadex have already been described in the literature[1–4]. The gels swell in organic solvents and form useful stationary phases for chromatography: they are of particular value for sensitive compounds, by virtue of the mild operating conditions and of the high solute recoveries obtainable. Boron trifluoride-catalysed reaction of a terminal olefin oxide with Sephadex LH-20 (a hydroxypropyl derivative of Sephadex G-25) has been found to proceed readily to yield substituted gels which are chemically stable[2,4,5]. The reaction has now been carried out using cyclohexene oxide, a model compound for the reaction of more complex alicyclic olefin oxides.

EXPERIMENTAL

Sephadex G-25 was obtained from Pharmacia, Uppsala, Sweden. Cyclohexene oxide was obtained from Koch-Light Laboratories Ltd., Colnbrook, Bucks., Great Britain. Other materials used for chromatography were also of commercial origin, except for the following steroids, which were gifts from the persons named: dihydrobrassicasterol (Dr. M. J. Thompson); poriferasterol (Dr. G. W. Patterson); 5β-cholan-7α-ol and 5β-cholan-7β-ol (Prof. M. Martin-Smith); 5-cholestene-3β,24-(RS)-diol 3β-acetate (Dr. G. F. Woods). Solvents were dried and redistilled before use. To facilitate handling of gel materials, all glassware was silanised with a 5% solution of dichlorodimethylsilane in toluene.

Sephadex G-25 was separated into fractions of low-particle-size range[6]. Beads of diameter 20–30 µm were converted to an LH-20-type derivative by reaction with propylene oxide in aqueous sodium hydroxide. This material (2 g) was suspended in dry dichloromethane (6 ml) for 1 h. BF_3 etherate (0.25 ml) was added and the suspension left to equilibrate for 15 min. The reaction flask was transferred to an ultrasonic tank and cyclohexene oxide (5 ml) in dry dichloromethane (5 ml) was added during 10 min with gentle mixing. The flask was removed from the tank and left overnight at room temperature. The gel was filtered, washed with dichloromethane, ethanol, and acetone and dried under vacuum. It then amounted to 3.2 g, the increase of 1.2 g corresponding to 68.5% substitution of free hydroxyl groups, *i.e.* two hydroxycyclohexyl residues per sugar ring.

Chromatographic columns were constructed from glass tubing (100×0.3 cm) following a pattern previously described[7]. The columns were packed by sedimentation under gravity-flow of a suspension of pre-swollen gel in the eluting solvent, until a steady bed volume had been obtained. Materials were applied to the column by injection on to the gel bed from a Hamilton microlitre syringe. The eluate was monitored with a moving-wire flame ionization detector[8]. Flow-rates were measured by collecting eluate in a graduated tube.

RESULTS AND DISCUSSION

The gel absorbed solvents representing a wide polarity range. Solvent regain values are quoted in Table I. Two systems were examined in an evaluation of the gel as a stationary phase for chromatography. Straight-phase and reversed-phase separations were obtained, respectively, with benzene or methanol–heptane (9:1) as the eluant. The ability of the gel to separate materials of varying functionality and structure was examined with a series of steroidal compounds. Values for the standard elution volume (SEV)[7] are quoted in Tables II and III.

The order of elution of materials from the column was found to be similar to that obtained with other lipophilic gels[2,7] in both straight-phase and reversed-phase systems. Eight pairs of steroids containing epimeric hydroxyl substituents

TABLE I

SOLVENT REGAIN VALUES FOR HYDROXYCYCLOHEXYL-SUBSTITUTED LH-20

Solvent	*Solvent regain value**	
	a	*b*
Heptane	0.25	0.38
Cyclohexane	0.44	0.57
Carbon tetrachloride	2.05	1.29
Toluene	1.08	1.25
Benzene	1.11	1.27
Tetrahydrofuran	1.27	0.91
Chloroform	2.44	1.65
Methylene chloride	1.91	1.44
Acetone	0.47	0.59
Methanol	0.63	0.79

* Determined according to Helfferich[10]. *a* denotes g of solvent taken up by 1 g of dry gel, *b* denotes ml of solvent taken up by 1 g of dry gel.

TABLE II

VALUES OF STANDARD ELUTION VOLUME (SEV) FOR THE STRAIGHT-PHASE SYSTEM (BENZENE)

Compound	*Mol. wt.*	*SEV*
5α-Cholestane	372	51.8
5α-Cholestan-3-one	386	55.8
5α-Cholestan-3β-ol	388	88.4
Cholesteryl chloride	404.5	55.6
Cholesteryl acetate	428	52.3
Cholesteryl benzoate	490	50.5
Cholesteryl palmitate	624	44.5
4-Cholesten-3-one	384	56.6
5-Cholesten-3-one	384	55.3
Cholesterol	386	94.4
Coprostanol	388	86.6
Stigmasterol	412	90.8
β-Sitosterol	414	91.6
Dihydrobrassicasterol	400	93.1
Lanosterol	426	79.4
4-Cholestene-3,6-dione	398	56.9
4,6-Cholestadien-3-one	382	58.7
24-Chloro-5β-cholane	364.5	58.0
5β-Cholan-24-ol	346	106.7
7-Ketocholesteryl acetate	442	53.0
Progesterone	314	62.9
5-Pregnen-3β-ol	302	109.6
(±)-Usnic acid	344	64.7
n-Dodecan-1-ol	186	108.2
n-Tetradecan-1-ol	214	101.2
n-Hexadecan-1-ol	242	95.2
n-Octadecan-1-ol	270	89.7
n-Eicosan-1-ol	298	84.9
n-Docosan-1-ol	326	80.2
n-Tetracosan-1-ol	354	76.9
n-Hexacosan-1-ol	382	73.6
Tristearin	890	41.2

at the C-3, C-7, C-11, C-17 or C-20 positions were totally or partially resolved on the straight-phase column. 24(**R**)- and 24(**S**)-hydroxycholesterol were not separable, and, with the exception of 3α- and 3β-hydroxy-5β-androstan-17-one, those epimeric steroids examined which possessed the 5β-configuration (three pairs) were also chromatographically indistinguishable in this system. [For comparison purposes, some of these epimeric pairs were chromatographed on Nedox 1114 gel[2] (Table IV).] Five steroids containing a double bond at the 5-position were found to be separable from the corresponding dihydro (5α) steroids. These separations did not occur on the Nedox gel.

The process underlying the separation of epimeric sterols is presumed to be one of adsorption of the solute on the gel network. Hydrogen bonding provides a probable explanation of such binding and has been implicated by previous workers[7,9-15]

TABLE III

SEV DATA FOR STEROIDS IN THE REVERSED-PHASE SYSTEM METHANOL–HEPTANE (9:1)

Compound	*Mol. wt.*	*SEV*
5α-Cholestane	372	193
Cholesteryl acetate	428	141
5α-Cholestan-3-one	386	118
5α-Cholestan-3β-ol	388	115
Cholesterol	386	118
β-Sitosterol	414	125
Stigmasterol	412	122
n-Tetradecan-1-ol	214	87
n-Hexadecan-1-ol	242	92
n-Octadecan-1-ol	270	97
n-Eicosan-1-ol	298	103
n-Docosan-1-ol	326	109
n-Tetracosan-1-ol	354	116

in connection with retardation of hydroxylic solutes on both lipophilic and hydrophilic gel columns. The expected hydrogen bonding sites are the ether linkages in the gel and there is some evidence for their participation[13,14].

In both 5α- and Δ^5-steroids, equatorial alcohols were retarded relative to their axial epimers. In the 5β-series, three out of four pairs of epimeric alcohols failed to separate, indicating that the ring skeleton must be involved in the solute–gel interaction. This is supported by the observed separations of Δ^5-steroids from the corresponding dihydro (5α) compounds. The olefinic 3-hydroxysteroids were retarded relative to the saturated compounds —probably because of their greater potentiality for hydrogen bonding. Neither cholest-5-ene nor cholesteryl acetate were separable in this system from their dihydro analogues: hydrogen bonding to the 3-position is absent in the hydrocarbons, and would be weak in the acetates.

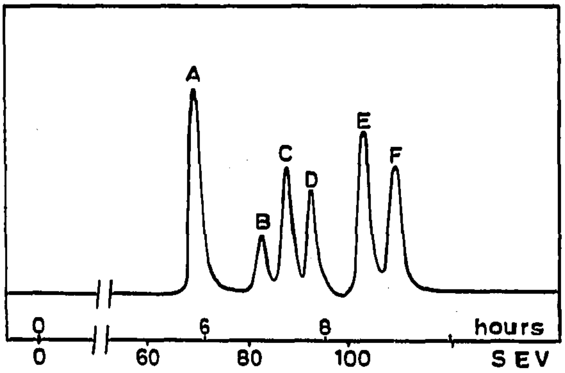

Fig. 1. Separation of epimeric 3-hydroxysteroids on a straight-phase hydroxycyclohexyl LH-20 column (100×0.3 cm I.D.) eluted with benzene. A=Epicholesterol; B=epicholestanol; C=cholestanol; D=cholesterol; E=androsterone; F=epiandrosterone.

TABLE IV

SEV DATA FOR EPIMERIC HYDROXYSTEROIDS AND Δ^5/5α-STEROIDS IN THE STRAIGHT-PHASE SYSTEM (BENZENE)

Compound	*Mol. wt.*	*SEV*		*OH conformation*
		*a**	*b***	
5α-Cholestan-3β-ol	388	88.4	101	equatorial (e)
5α-Cholestan-3α-ol (epicholestanol)	388	82.9	90.5	axial (a)
5β-Cholestan-3α-ol (epicoprostanol)	388	87.0		e
5β-Cholestan-3β-ol (coprostanol)	388	87.0		a
5-Cholesten-3β-ol (cholesterol)	386	94.4	103	e
5-Cholesten-3α-ol (epicholesterol)	386	70.1	76.5	a
3β-Hydroxy-5α-androstan-17-one	290	114	101	e
3α-Hydroxy-5α-androstan-17-one	290	104	93.8	a
3α-Hydroxy-5β-androstan-17-one	290	107	97.2	e
3β-Hydroxy-5β-androstan-17-one	290	104	93.0	a
3α-Hydroxy-5β-pregnan-20-one	318	99.5	93.2	e
3β-Hydroxy-5β-pregnan-20-one	318	99.5	93.2	a
5-Cholestene-3β,7β-diol	402	170	117	e
5-Cholestene-3β,7α-diol	402	162	106	a
5β-Cholan-7β-ol	346	67.0		e
5β-Cholan-7α-ol	346	67.0		a
11α-Hydroxy-pregn-4-ene-3,20-dione	330	115	99.0	e
11β-Hydroxy-pregn-4-ene-3,20-dione	330	108	92.4	a
5α-Androstan-17β-ol	276	103	106	ψ-e
5α-Androstan-17α-ol	276	95.2	97.2	ψ-a
5-Pregnene-3β,20β-diol	318	170	161	more hindered
5-Pregnene-3β,20α-diol	318	183	174	less hindered
5-Cholestene-3β,24-(RS)-diol 3β-acetate	444	69.4	67.3	mobile
3β-Hydroxy-5α-androstan-17-one	290	101		
3β-Hydroxy-5-androsten-17-one	288	108		
17α-Methyl-5α-androstane-3β,17β-diol	306	201		
17α-Methyl-5-androstene-3β,17β-diol	304	221		
3β-Hydroxy-5β-pregnan-20-one	318	97.2		
3β-Hydroxy-5-pregnen-20-one	316	105		
5α-Pregnane-3β,20β-diol	320	193		
5-Pregnene-3β,20β-diol	318	211		
Cholestanyl acetate	430	52.3		
Cholesteryl acetate	428	52.3		
Cholestane	372	51.8		
5-Cholestene	370	51.8		

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* Hydroxycyclohexyl-substituted Sephadex LH-20.

** Nedox 1114-substituted Sephadex LH-20.

The SEV values of the 5β-cholan-7-ols are unusually low, implying that hydrogen bonding is hindered by the molecular geometry. Structural rigidity is an important factor: the markedly different SEV values of C-20 epimers reflect the distinctive steric requirements of their preferred conformations, whereas C-24 epimers failed to separate.

CONCLUSION

Hydroxycyclohexyl LH-20 is a readily prepared, stable gel, with properties similar to those of previously reported Sephadex derivatives. In addition, hydroxylic Δ^5-steroids can be separated on this gel from the corresponding 5α-steroids. The preparation represents the first reported example of the successful reaction of an alicyclic epoxide with a dextran gel. This greatly extends the range of possible reagents for the modification of gels. The incorporation of chiral hydroxycycloalkyl moieties by analogous methods is now under investigation.

ACKNOWLEDGEMENT

This work was partly supported by a grant from the Agricultural Research Council.

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