chromatography of steroids

E. Heftmann

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CHROMATOGRAPHY OF STEROIDS

（内部交流）

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Preface

Chromatography has become one of the most important methods in steroid research. Yet, since the appearance of Neher's book *Steroid Chromatography* in 1964 [769], no comprehensive review of this subject has been published. Having accumulated over 2000 reprints on the chromatography of steroids since 1964, I can well understand the reluctance of my colleagues to write such a review, but I do feel a need to organize this information for my own benefit as well as theirs.

In citing the literature, I obviously had to be selective in order to keep this monograph within a reasonable size. Even after eliminating all the material presented by Neher, I found myself with more information than I could possibly use. I could have solved my problem by referring the reader to the numerous review articles, chapters, and books on individual aspects of steroid chromatography. However, I feel that the reader is entitled to enough detail so that he can at least decide which articles to look up and, preferably, so that he can repeat experiments without referring to the original papers. I therefore decided to omit that part of the literature which I found less original and more difficult to obtain. I am fully aware of the risk of incurring the wrath of some colleagues who may feel slighted by such omissions, but I hope that more unbiased readers will appreciate my effort at simplifying their literature search.

The literature citations have been handled by the methods currently used by *Chemical Abstracts* and other publications of the American Chemical Society. For the more common steroids, I have used the trivial names. The systematic names (IUPAC–IUB 1967 revised tentative rules) [507] are shown in the subject index. It is assumed that the readers are familiar with the general terminology, theory, and techniques of chromatography. These aspects are only covered as they relate to steroids. Readers requiring further information on chromatography are referred to my book *Chromatography* [422]. Some background material on steroids and a relatively recent guide to the steroid literature will be found in my book *Steroid Biochemistry* [420].

Extensive bibliographies on chromatography are being published regularly in the *Journal of Chromatography* and other analytical journals, biennially in *Analytical Chemistry*, in several Elsevier books [233, 234, 681, 682], as well as by various manufacturers of chromatographic equipment and supplies. Many other aspects of steroid analysis are covered in my recent book *Modern Methods of Steroid Analysis* [421].

Most of this book was written while I was at the Federal Institute for Lipid Research of the German Federal Republic in Münster under the terms of a U.S. Senior Scientist Award by the Humboldt Foundation. I am deeply grateful to the Director of the H.P. Kaufmann Institute, Professor H.K. Mangold, and its staff as well as to the staff of the Humboldt Foundation for their most generous support and cordial welcome.

Münster, October 1975 ERICH HEFTMANN

Commercial Products

Trade designation	Chemical nature	Source
Adsorbosil-CABN	$AgNO_3$ on SiO_2	Applied Science Laboratories, Stage College, Pa., U.S.A.
Alpha-8-Metricel	membrane filter	Gelman Instruments, Ann Arbor, Mich., U.S.A.
Amberlite IRC-50	polyacrylic acid	Rohm & Haas, Philadelphia, Pa., U.S.A.
Amberlite XAD-2	macroporous styrene–divinylbenzene resin	Rohm & Haas, Philadelphia, Pa., U.S.A.
Amberlyst XN-1006	anion-exchange resin	Rohm & Haas, Philadelphia, Pa., U.S.A.
Amberlyst A-6	anion-exchange resin	Rohm & Haas, Philadelphia, Pa., U.S.A.
AN-600	50% cyanoethyl methyl silicone	Analabs, North Haven, Conn., U.S.A.
Anakrom ABS	silanized diatomaceous earth	Analabs, North Haven, Conn., U.S.A.
ANH	cyanoethyl silicone	DuPont de Nemours, Wilmington, Del., U.S.A.
BDSA	bis(dimethylsilyl)acetamide	Supelco, Bellefonte, Pa., U.S.A.
BSA	N,O-bis(trimethylsilyl)acetamide	Supelco, Bellefonte, Pa., U.S.A.
BSTFA	N,O-bis(trimethylsilyl)trifluoroacetamide	Supelco, Bellefonte, Pa., U.S.A.
Celite	diatomaceous earth	Alltech Associates, Arlington Heights, Ill., U.S.A.
Cellex E	ECTEOLA-cellulose	Bio-Rad, Richmond, Calif., U.S.A.
Centri-Chrom	centrifugally accelerated LC system	Ivan Sörvall, Norwalk, Conn., U.S.A.
Chromosorb	styrene–divinylbenzene copolymer	Analabs, North Haven, Conn., U.S.A.
CMDMCS	chloromethyldimethylchlorosilane	Pierce Chemical Co., Rockford, Ill., U.S.A.
CMDMTMDS	1,3-bis(chloromethyldimethyl)-1,1,3,3-tetramethyldisilazane	Pierce Chemical Co., Rockford, Ill., U.S.A.
CNSi	cyanoethyl methyl silicone	General Electric, Schenectady, N.Y., U.S.A.
Corasil	porous silica	Waters Associates, Milford, Mass., U.S.A.
Corasil C_{18}	permanently bonded octadecylsilane	Waters Associates, Milford, Mass., U.S.A.
CTpA	Carbowax 20M–terephthalic acid	Applied Science Laboratories, State College, Pa., U.S.A.
DC-200	silicone	Regis Chemical Co., Morton Grove, Ill., U.S.A.
DCTFA	*sym*-dichlorotetrafluoroacetone	Applied Chemical Corp., Morristown, N.J., U.S.A.
DEGS	diethylene glycol succinate	Regis Chemical Co., Morton Grove, Ill., U.S.A.

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Dexsil-300	polycarboranesiloxane	Analabs, North Haven, Conn., U.S.A.
Diatoport S	diatomaceous earth	F & M Scientific Corp., Avondale, Pa., U.S.A.
DMMCS	dimethylmonochlorosilane	Applied Science Laboratories, State College, Pa., U.S.A.
Epon Resin 1001	bisphenol–epichlorohydrin polymer	Alltech Associates, Arlington Heights, Ill., U.S.A.
F-50, etc.	see Versilube	
Factice 31-B	polymer from soybean oil	Carter-Bell Manuf. Co., Springfield, N.J., U.S.A.
Florisil	magnesium silicate	Applied Science Laboratories, State College, Pa., U.S.A.
Gas-Chrom	diatomaceous earth	Applied Science Laboratories, State College, Pa., U.S.A.
Gas Quat L	trioctadecylmethylammonium bromide	Applied Science Laboratories, State College, Pa., U.S.A.
GE-F-50	see Versilube	Applied Science Laboratories, State College, Pa., U.S.A.
HCP	ethylene–propylene copolymer	DuPont de Nemours, Wilmington, Del., U.S.A.
HFBA	heptafluorobutyric anhydride	PCR Inc., Gainesville, Fla., U.S.A.
HI-EFF-8BP	cyclohexanedimethanol succinate	Applied Science Laboratories, State College, Pa., U.S.A.
Hyamine hydroxide	diisobutylcresoxyethoxyethyl dimethyl benzylammonium hydroxide	J.T. Baker, Phillipsburg, N.J., U.S.A.
ITLC	(Instant Thin-Layer Chromatography) adsorbent-impregnated glass-fiber paper	Gelman Instruments, Ann Arbor, Mich., U.S.A.
JXR	dimethylpolysiloxane	Supelco, Bellefonte, Pa., U.S.A.
KGn, Kalignost	sodium tetraphenylborate	Eastman, Rochester, N.Y., U.S.A.
L-45	methyl silicone	General Electric, Schenectady, N.Y.
LA-1	*n*-dodecenal(trialkylmethyl)amine	Rohm & Haas, Philadelphia, Pa., U.S.A.
Lipidex	hydroxyalkoxypropyl Sephadex	Pharmacia, Uppsala, Sweden
Michrome No. 64	primuline	E. Gurr Ltd., London SW 14, Great Britain
Micropak Si 60	silica gel	Varian Associates, Walnut Creek, Calif., U.S.A.
MSTFA	N-methyl-N-trimethylsilyltrifluoroacetamide	Supelco, Bellefonte, Pa., U.S.A.
NGS	neopentyl glycol succinate	Applied Science Laboratories, State College, Pa., U.S.A.
NPGA	neopentyl glycol adipate	Applied Science Laboratories, State College, Pa., U.S.A.
OV-1, OV-101	methyl silicones	Applied Science Laboratories, State College, Pa., U.S.A.
OV-17, OV-25	phenyl methyl silicones	Applied Science Laboratories, State College, Pa., U.S.A.
OV-225	cyanopropyl phenyl methyl silicone	Applied Science Laboratories, State College, Pa., U.S.A.

Continued on p. XII

Commercial products *(continued)*

Trade designation	Chemical nature	Source
Permaphase ETH	ether-bonded controlled-porous surface beads	DuPont de Nemours, Wilmington, Del., U.S.A.
Permaphase ODS	octadecylsilane bonded to Zipax	DuPont de Nemours, Wilmington, Del., U.S.A.
PhSi	see XE-61	
Plaskon CTFE-2300	trifluoroethylene polymer	Allied Chemical Corp., Morristown, N.J., U.S.A.
PMPE	polymetaphenoxylene	Varian Associates, Walnut Creek, Calif., U.S.A.
Polygram Sil G	silica gel on polyester sheets	Macherey, Nagel & Co., Düren, G.F.R.
Polyimide		Pennzoil United, Shreveport, La., U.S.A.
Poragel PN	polystyrene gel	Waters Associates, Milford, Mass., U.S.A.
Porasil A	porous silica	Waters Associates, Milford, Mass., U.S.A.
PZ-176	polyphenyl ether sulfone	Pennzoil United, Shreveport, La., U.S.A.
QF-1	fluoroalkyl polysiloxane	Applied Science Laboratories, State College, Pa., U.S.A.
Regisil	BSTFA+TMCS (99:1)	Regis Chemical Co., Morton Grove, Ill., U.S.A.
SCX	strong cation exchanger	DuPont de Nemours, Wilmington, Del., U.S.A.
SE-30	methylpolysiloxane	Analabs, North Haven, Conn., U.S.A.
SE-30 "ultraphase"	"improved" methyl silicone	Phase Separation, Queensferry, Great Britain
SE-52	methyl phenyl silicone	General Electric, Schenectady, N.Y., U.S.A.
Sephadex	cross-linked dextran	Pharmacia, Uppsala, Sweden
Sephadex LH-20	hydroxypropyl ether of dextran	Pharmacia, Uppsala, Sweden
SI-100	silica	Merck, Darmstadt, G.F.R.
Silanox 101	silica	Cabot, Boston, Mass., U.S.A.
SILAR-5CP	cyanoalkyl phenyl silicone	Applied Science Laboratories, State College, Pa., U.S.A.
Silica Gel 1B-F	flexible precoated TLC sheet	Baker, Phillipsburg, N.J., U.S.A.
Silica Gel F	silica plus fluorophor	Merck, Darmstadt, G.F.R.
Silica Gel G	silica plus gypsum	Merck, Darmstadt, G.F.R.
Silica Gel HS	silanized silica	Merck, Darmstadt, G.F.R.
SP-400	chlorophenyl silicone	Supelco, Bellefonte, Pa., U.S.A.
SP-525	aromatic hydrocarbon	Supelco, Bellefonte, Pa., U.S.A.
SP-1000	modified Carbowax 20M	Supelco, Bellefonte, Pa., U.S.A.

SP-2401	trifluoropropyl methyl silicone	Supelco, Bellefonte, Pa., U.S.A.
Spherosil XOA-400	silica	Pechiney, Saint Gobain, France
Supelcoport	GC support	Supelco, Bellefonte, Pa., U.S.A.
Sylon-CT	silanizing solution	Supelco, Bellefonte, Pa., U.S.A.
TCTFA	1,1,3-trichlorotrifluoroacetone	Allied Chemical Corp., Morristown, N.J., U.S.A.
TMCBA	tetramethylcyclobutanediol adipate	Applied Science Laboratories, State College, Pa., U.S.A.
TMDS	tetramethyldisilazane	Applied Science Laboratories, State College, Pa., U.S.A.
TMSDEA	trimethylsilyldiethylamine	Supelco, Bellefonte, Pa., U.S.A.
Versilube	methyl chlorophenyl silicone	Applied Science Laboratories, State College, Pa., U.S.A.
Vydac	porous silica layer on solid core	Applied Science Laboratories, State College, Pa., U.S.A.
XE-60	cyanoethyl silicone	Applied Science Laboratories, State College, Pa., U.S.A.
XE-61	phenyl methyl silicone	General Electric, Schenectady, N.Y., U.S.A.
Z	ethylene glycol, succinic acid, and methyl siloxane copolymer	General Electric, Schenectady, N.Y., U.S.A.
Zipax	porous-layer support	DuPont de Nemours, Wilmington, Del., U.S.A.
Zorbax SIL	porous silica microspheres	DuPont de Nemours, Wilmington, Del., U.S.A.

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Chapter 1

Introduction

The steroids constitute a very large group of natural and synthetic compounds with a broad range of biological activities. Because they are of considerable importance in medicine, there has been a great deal of interest in various aspects of steroid chemistry, including their analysis. In biological extracts, steroids usually occur in low concentration and invariably in association with numerous structurally related compounds. The latter may be present in considerably higher concentrations, but may not have comparable biological activities. Thus, it is desirable that analytical methods for steroids be both specific and sensitive [112].

As in the analysis of other groups of products, there is a strong trend toward instrumentation and automation in steroid analysis. Because most physical methods of analysis are not sufficiently selective, the analytical samples must be purified and, preferably, fractionated. The most efficient fractionation method available to the steroid chemist is chromatography. This is one reason for the strong association between steroid chemistry and chromatography. The other reason is that scientists who develop instrumental methods of analysis, including chromatographic methods, look toward the steroid analyst as a relatively well-endowed user with many challenging problems.

One of these problems is that the associated steroids in an analytical sample are often analogous or isomeric compounds with very similar physical and chemical properties. Another problem is that steroids may belong to several solubility classes, ranging from rather hydrophilic to very lipophilic compounds. Some of these compounds are highly reactive and even unstable, whereas others are extremely sluggish and may even be devoid of analytically useful functional groups.

So far, no single chromatographic method has been able to overcome all of these problems, and the analyst must have the ability to select the technique appropriate to the goal [770]. Thus, in addition to the obvious constraints of his knowledge and skill, available time and facilities, the analyst is limited by the chemical nature and physical condition of the steroids in samples of biological or synthetic origin. A few generalizations about the selection of techniques are in order, although many exceptions will be found in the examples of chromatographic analyses presented in the following pages.

As a rule, such methods as ion exchange and electrophoresis are only suitable for ionic or ionogenic substances and are therefore largely inapplicable to neutral steroids. Generally speaking, partition chromatography will be more successful in separating the homologs of the more hydrophilic steroids, whereas adsorption chromatography is more apt to resolve mixtures of analogous or isomeric steroids having a more lipophilic character. For crude or bulky samples, old-fashioned column chromatography is still the method of choice, although it is not as efficient with respect to resolution, labor, and time as other chromatographic techniques. Qualitative analysis is most efficiently performed by thin-layer chromatography (TLC), because a number of samples and reference compounds can be tested simultaneously. For quantitative analysis and for the best resolution, gas chromatography (GC) is preferred, but high-pressure liquid chromatography (HPLC) has several potential

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advantages over GC. It provides larger capacity and greater choice of parameters and it usually requires no derivatization, while offering advantages of speed, convenience, and sensitivity that rival GC.

Chapter 2

Liquid column chromatography

2.1. SORBENTS

As the specific examples of liquid column chromatography (LC) in this volume show, silica is by far the most useful sorbent for steroids. Some of the important work on the relation between the structure of silica gel and the chromatographic behavior of steroids goes back to 1961, but it must be mentioned here because it was not covered in Neher's book. Klein [566] has systematically studied the effect of surface area, pore volume, and average pore diameter of different types of silica on the resolution of sterol acetates by LC. Only when the pore diameter is large relative to the size of the sterol molecule can the molecule be attracted by a flat surface. Thus, 24-dehydrosterols, which have a smaller cross-section than sterols with a nuclear double bond, are more strongly adsorbed than the latter on silica gels with small pore diameters.

Activation of silica gel by heat increases the range of surface energies and results in broadening or trailing of chromatographic zones [567]. Deactivation, which is best accomplished by exposure to an atmosphere of controlled humidity, will improve the performance of a silica gel only if it is adjusted to the surface structure of that particular silica.

The water content of silica depends on the water content of the solvent [69]. By gradually increasing it, one can change the chromatographic system continuously from an adsorption to a partition system. It is even possible to achieve some sort of gradient elution effect by using a wet nonpolar solvent as eluent for a dry silica column.

An example of the use of silica columns is the preliminary isolation of steroids from a crude lipid extract [429]. Up to 1 g of mixture, dissolved in pentane–diethyl ether (4:1) can be fractionated on a column, 1 cm in internal diameter (I.D.), which has been packed with 7 g silicic acid (activity grade IIB), slurried in pentane–diethyl ether. Neutral lipids, sterols, and steryl esters are eluted by 150 ml pentane–diethyl ether (4:1), other steroids by 150 ml acetone–chloroform (2:1), and more polar lipids by 150 ml methanol.

Another example is the use of Silica Gel G with a water content of 10% in a 5-cm × 1.8-cm column [210]. About 10–15 mg of lipid mixture can be fractionated on such a column by elution with a series of solvents. Thus, 60 ml petroleum ether (b.r. 30–75°) elutes the hydrocarbons, 50 ml 6% diethyl ether in petroleum ether the cholesteryl esters, and 160 ml of 10% ethyl acetate in petroleum ether the triglycerides, followed, in the last 60 ml, by cholesterol.

The activity and thus the chromatographic properties of alumina can be reversibly modified *in situ* by passing organic solvents with different water content through a column of alumina [283]. Useful separations can be achieved on alumina thus deactivated. Alumina, although quite selective, is now discredited by the various alterations the more active grades have been reported to produce, and it is rarely used nowadays.

However, an interesting method of labeling steroids by alumina chromatography should be mentioned [569]. By taking advantage of the enolization of ketosteroids on basic

TABLE 2.1

ELUTION VOLUMES OF STEROIDS ON DIFFERENT GELS IN DIFFERENT SOLVENTS EXPRESSED AS PER CENT OF TOTAL BED VOLUME [278]

Compound	LH-20				G-25-36			G-15-38	G-25-40
	Dichloro-methane	Isopropanol	Propanol	Methanol	Dichloro-methane	Propanol	Methanol	Dichloro-methane	Dichloro-methane
5α-Cholestane	45.5	69.0	70.0	–	54.5	75.5	–	47.0	57.0
5α-Cholestan-3-one	45.0	72.5	73.5	76.0	53.0	79.0	98.0	45.0	55.0
5α-Cholestan-3β-ol	61.5	69.0	70.0	76.0	67.0	71.0	91.0	54.0	64.0
5β-Cholestan-3-one	–	74.5	75.0	–	–	–	–	–	55.0
5β-Cholestan-3β-ol	59.0	70.5	72.0	–	64.5	74.0	93.0	–	–
5β-Cholestane-3,12-dione	–	77.5	–	–	–	82.0	–	–	–
5β-Cholestane-3α,12α-diol	73.5	72.5	72.0	–	80.0	72.5	84.5	59.0	68.5
5β-Cholestane-3,7,12-trione	44.0	90.0	85.0	73.5	52.0	100.5	94.0	44.0	53.0
5β-Cholestane-3α,7α,12α-triol	151.5	77.5	75.0	73.5	132.5	74.0	82.5	74.5	87.0
3β-Methoxy-5-cholestene	42.0	68.0	69.0	76.0	53.0	75.5	100.5	45.0	55.0
3β-Acetoxy-5-cholestene	43.5	70.5	71.5	76.0	–	82.5	102.5	43.0	54.0
5α-Pregnane	48.5	74.5	77.0	–	59.5	–	–	52.0	62.5
Progesterone	48.0	88.0	86.5	77.5	56.5	92.5	93.0	–	57.0
5α-Androstane	51.0	78.0	78.5	–	62.5	83.5	–	54.5	–
5α-Androstan-17-one	50.0	84.5	83.0	83.5	59.5	90.5	100.5	52.5	–
5α-Androstan-17β-ol	71.0	81.5	81.5	83.5	77.0	80.5	95.0	63.5	–
Estrone	239.5	115.5	114.0	99.0	202.5	133.0	92.0	138.0	–
Estradiol	–	113.5	109.5	98.0	–	113.0	91.0	–	–

alumina, HTO on the column can be made to exchange with H in such ketosteroids as 5α-cholest-7-en-3-one, and sizeable quantities of tritiated ketosteroids of high purity (5–10 mCi/mmole) can be prepared by simple chromatographic development. This procedure can also be utilized for analytical purposes [568]. A mixture of ketosteroids passing through a column of tritiated alumina becomes self-labeled, and the individual compounds are readily detected and measured in submicrogram quantities by monitoring the column effluent.

Ion-exchange resins are useful sorbents for partition chromatography of nonionic compounds. Seki and Matsumoto [954] have pioneered in the application of partially esterified cation exchangers to steroids (*cf.* p.99). Columns of neutral resins, such as polystyrene with 2% divinylbenzene cross-linkages, can be developed with benzene to fractionate mixtures of lipids, including sterols and steryl esters [1056].

Nyström and Sjövall [800] began to use lipophilic dextran preparations as supports for the stationary phase in reversed-phase partition chromatography of lipids in 1964. Methylated Sephadex G-25 was mixed with lipid solvents in which the sorbent does not float, *e.g.*, chloroform–methanol (1:1), and the slurry was poured into a chromatographic tube. The same solvent mixture eluted cholesterol, some of its esters, and various bile acids from this column, in generally decreasing order of polarity, but other solvent mixtures and other lipids gave other elution orders [801,802]. The effects noted were clearly not due to reversed-phase partition alone, but gel permeation, ordinary partition, and perhaps also adsorption may have contributed to them, depending on the degree of cross-linking and methylation of the dextran preparation and the chloroform/methanol ratio of the eluent.

The theoretical basis of steroid chromatography on lipophilic Sephadex gels was studied in more detail by Eneroth and Nyström [278]. Table 2.1 shows the per cent total bed volume (PTV), *i.e.* the milliliters of each solvent which would have been required to elute the compounds shown from a Sephadex column, if its total volume had been 100 ml. Sephadex LH-20 is a cross-linked dextran with hydroxypropyl ether groups, and Sephadex G-15 and G-25 are dextran gels which exclude polysaccharides above a molecular weight of 1500 and 5000, respectively. The second number in the Sephadex G series denotes the per cent methoxyl content of the dextran. Generally, the PTV value of steroids without hydroxyl groups increases with the polarity of the solvent and with the methoxyl content of the sorbent, whereas the reverse is true of steroids carrying hydroxyl groups. The elution order with nonpolar solvents, such as dichloromethane, follows the general order of increasing polarity of the samples, whereas polar solvents usually elute steroids in decreasing order of polarity. For any given sample and solvent combination, the more porous gel gives the higher PTV value. Thus, combinations of a relatively nonpolar gel with a relatively polar solvent exhibit reversed-phase partition behavior, whereas ordinary partition chromatography may be at work in combinations of relatively polar gels with relatively nonpolar solvents.

Ellingboe *et al.* [268] have prepared a number of long-chain alkyl ethers of Sephadex and tested them as column packing materials for partition and reversed-phase partition chromatography of sterols, bile acids, and steroid hormones. The reversed-phase systems, such as Sephadex G-25 with a hydroxyalkyl (C_{15}–C_{18}) group content of 71% by weight, eluted with methanol–heptane (19:1), separated C_{27}, C_{28}, and C_{29} sterols; Sephadex with

TABLE 2.2

PER CENT TOTAL BED VOLUME OF STEROIDS ON A HYDROXYCYCLOHEXYL SEPHADEX COLUMN ELUTED WITH BENZENE [23]

Steroid	OH*	PTV**
5α-Cholestan-3-one		55.8
4-Cholesten-3-one		56.6
5-Cholesten-3-one		55.3
5α-Cholestan-3β-ol (cholestanol)	*e*	88.4
5α-Cholestan-3α-ol (epicholestanol)	*a*	82.9
5β-Cholestan-3α-ol (epicoprostanol)	*e*	87.0
5β-Cholestan-3β-ol (coprostanol)	*a*	87.0
5-Cholesten-3β-ol (cholesterol)	*e*	94.4
5-Cholesten-3α-ol (epicholesterol)	*a*	70.1
3β-Hydroxy-5α-androstan-17-one (epiandrosterone)	*e*	114
3α-Hydroxy-5α-androstan-17-one (androsterone)	*a*	104
3α-Hydroxy-5β-androstan-17-one (etiocholanolone)	*e*	107
3β-Hydroxy-5β-androstan-17-one	*a*	104
3α-Hydroxy-5β-pregnan-20-one	*e*	99.5
3β-Hydroxy-5β-pregnan-20-one	*a*	99.5
5-Cholestene-3β,7β-diol	*e*	170
5-Cholestene-3β,7α-diol	*a*	162
5β-Cholan-7β-ol	*e*	67.0
5β-Cholan-7α-ol	*a*	67.0
11α-Hydroxypregn-4-ene-3,20-dione	*e*	115
11β-Hydroxypregn-4-ene-3,20-dione	*a*	108
5α-Androstan-17β-ol	ψ-*e*	103
5α-Androstan-17α-ol	ψ-*a*	95.2
5-Pregnene-3β,20β-diol	more hindered	170
5-Pregnene-3β,20α-diol	less hindered	183
4-Pregnene-3,20-dione (progesterone)		62.9
3β-Hydroxy-5-androsten-17-one (dehydroepiandrosterone)		108
3β-Hydroxy-5β-pregnan-20-one		97.2
3β-Hydroxy-5-pregnen-20-one (pregnenolone)		105
5α-Pregnane-3β,20β-diol		193
5-Pregnene-3β,20β-diol		211

*Conformation: *a* = axial; *e* = equatorial.
**Per cent total bed volume.

55% hydroxyalkyl (C_{11}–C_{14}) group content, eluted with methanol–water–1,2-dichloroethane (7:3:1), separated bile acids; and a partition column of Sephadex with 58% hydroxyalkyl (C_{15}–C_{18}) group content, eluted with heptane–chloroform (4:1), separated various pregnane derivatives. Using the lipophilic dextran with 50% hydroxyalkyl groups in columns eluted with benzene or benzene–isopropanol (3:1), Brooks and Keates [118] further investigated the chromatographic behavior of many steroids.

After experimenting with various other lipophilic dextran gels [20], Anderson *et al.* [23] observed a considerable enhancement in selectivity when dextran gels were substituted with hydroxycyclohexyl residues. Elution with benzene gave the PTV values shown in Table 2.2. Pairs of steroids with epimeric hydroxyl groups were generally

resolved, unless they had Rings A and B *cis*-fused (5β-steroids). Moreover, this chromatographic system separated 5-unsaturated steroids from their saturated 5α-analogs. In both 5α- and Δ^5-steroids, the equatorial alcohols were more retarded than their axial epimers. This is illustrated in Fig. 2.1. The mechanism underlying this separation appears to be adsorption of the steroids to the gel, more specifically, hydrogen bonding to its ether linkages.

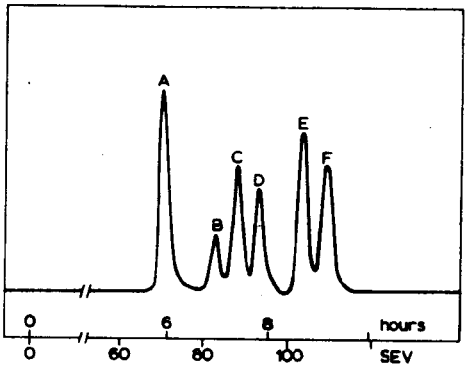

Fig. 2.1. Separation of epimeric 3-hydroxysteroids on a hydroxycyclohexyl Sephadex column (1 m × 3 mm I.D.), eluted with benzene. A = epicholesterol; B = epicholestanol; C = cholestanol; D = cholesterol; E = androsterone; F = epiandrosterone. SEV = standard elution volume. (Reproduced from *J. Chromatogr.*, 82 (1973) 340, with permission; [23].)

The use of LH-20 columns is recommended for clinical analyses, where groups of steroids must be isolated from blood or urine samples (*cf.* Chapter 12, Sections 2 and 3), particularly for purposes of radioimmunoassay or competitive protein binding assay [100, 142,754,821,955]. They have also been found useful in biosynthetic studies of plants for the convenient separation of carotenoids from sterols [1029]. Various other aspects of chromatography on lipophilic Sephadex have been reviewed [978]. For instance, it is used in recycling and capillary column chromatography with automatic detection systems [804]. Marker dyes facilitate the location of steroid fractions in routine applications [248].

Lipophilic Sephadex also exhibits cation-exchange properties when electrolytes are present in the solvents or samples [981]. This effect can be exploited for the isolation of conjugated steroids from biological sources. For instance, from a column of methylated Sephadex, eluted with a 1:1 mixture of chloroform and a 0.02 *M* methanolic solution of some salt, free steroids emerge before steroid monosulfates, which are followed by disulfates. The mixed steroid sulfates in *ca.* 5 ml plasma can be separated on a 4-g column of Sephadex LH-20 by elution of the monosulfates with 30–60 ml chloroform–0.01 *M* NaCl in methanol (1:1) and then of the disulfates with 65–115 ml methanol [514]. The separation of individual steroid sulfates can be accomplished by liquid–liquid partition chromatography on Celite columns [138]. The use of polyamide columns for the isolation of steroid conjugates has also been reported [807].

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