CHROM. 5240

Capillary column chromatography of steroids: Evaluation of stationary phases

Natural mixtures, such as steroid metabolites of biologic origin, are quite complex in composition. Gas phase chromatographic techniques are presently the most important method for their determination. High-resolution glass capillary columns[1-3] were recently applied successfully to the separation of mixtures of steroids[4,5]. While large numbers of theoretical plates may be necessary to resolve some components, it is quite evident that there are limitations in the column length for analyses of high-boiling compounds. Many substances with very similar structures can often be resolved on the basis of column selectivity; however this is of limited use in the analysis of complex mixtures. The analysis of tobacco smoke[6-8] is a good example of this kind of problem. A need for complementary use of "non-selective" and "selective" columns in biochemical analyses also reflects this situation.

Different analytical problems encountered in the analysis of steroids require various degrees of separation efficiency and selectivity. Therefore, the selected pairs of model steroidal compounds and glass capillary columns coated with a number of stationary phases were tested in this work to establish a more general knowledge of the problems involved. The large number of available liquid phases is substantially reduced in the case of steroids due to the requirements of high temperature stability. In addition to the traditional phases used in steroid work, some new potential substrates were studied.

The relative volatility $\alpha_{2,1} = \frac{t_{R2} - t_{air}}{t_{R1} - t_{air}}$ of a given pair of solutes may be used as a simple measure of selectivity. It can further be related to the number of theoretical plates necessary for a given resolution[9]. The fact that retention data obtained with capillary columns are not compatible with those obtained on packings with identical

TABLE I

STATIONARY PHASES USED FOR PREPARATION OF CAPILLARY COLUMNS

Stationary phase	*Supplier*	*Solvent*	*% of phase in the coating solution*	*conditioned overnight at °C*
SE-30 Silicone gum	General Electric	Toluene	2	320
OV-101 Silicone oil	Supelco, Inc.	Toluene	5	290
OV-17 Silicone oil	Supelco, Inc.	Toluene	5	270
OV-210 Silicone oil	Supelco, Inc.	Acetone	5	240
SP-400 Silicone fluid	Supelco, Inc.	Toluene	5	270
Dexsil 300-GC	Analabs	Toluene	7	340
Polyimide	[a]	Toluene + Isopropanol (90:5)	2	280
STAP	Varian Aerograph	Acetone	5	230
Cyclohexane-dimethanol succinate	Applied Science Laboratories, Inc.	Methylene chloride	10	230

[a] Obtained from R. G. MATHEWS AND R. D. SCHWARTZ, Pennzoil United, Inc., Shreveport, La.

phases[10,4] further justifies this study. The data obtained here may be used as an approximate guide for selecting a capillary column for particular problems encountered in steroid analysis.

Experimental

Glass capillary columns 20 m in length were prepared according to procedures previously described[1,2,11,12]. The stationary phases used are listed in Table I. The following pairs of steroids were selected for measurements:

(I) Androsterone (3α-hydroxy-5α-androstan-17-one); androstanediol (5α-androstane-3α, 17β-diol).

(II) Epiandrosterone (3β-hydroxy-5α-androstan-17-one); androsterone (3α-hydroxy-5α-androstan-17-one).

(III) Etiocholanolone (3α-hydroxy-5β-androstan-17-one); androsterone (3α-hydroxy-5α-androstan-17-one).

(IV) Cholestanol (5α-cholestan-3β-ol); cholesterol (5-cholesten-3β-ol).

(V) Stigmasterol (5,22-cholestadien-24-ethyl-3β-ol); cholesterol (5-cholesten-3β-ol).

Their silylation was carried out with N,O-bis(trimethylsilyl)acetamide (Supelco, Inc.). Instrumental conditions for the gas chromatography of the steroid derivatives were essentially those described in our earlier publication[4]. The relative volatilities $\alpha_{2,1}$ for the pairs I–III were measured at 195° and IV–V at 215°. The temperature stabilities of the columns were compared by observing a base line drift within a selected temperature range under the same conditions of temperature programming and recorder sensitivity.

Duplicate capillary columns were used for each stationary phase. Efficiencies of 2000–5000 theoretical plates/m were found with all stationary phases studied.

Results and discussion

The molecular weight, shape of molecules, and polarity are three fundamental physical properties which influence the chromatographic behavior of steroids. Although steric factors may sometimes be quite complex in nature and cause irreg-

TABLE II

VALUES OF RELATIVE VOLATILITY MEASURED FOR MODEL STEROIDS ON DIFFERENT STATIONAIRY PHASES

Stationary phase	*195°*			*215°*	
	$\alpha_{2,1}(I)$	$\alpha_{2,1}(II)$	$\alpha_{2,1}(III)$	$\alpha_{2,1}(IV)$	$\alpha_{2,1}(V)$
SE-30	0.88[a]	1.25	1.03	1.02	1.33
OV-101	1.09	1.38	1.08	1.01	1.45
OV-17	1.31	1.32	1.10	0.99[a]	1.48
OV-210	1.91	1.26	1.08	1.05	1.27
SP-400	0.87[a]	1.39	1.07	0.99[a]	1.40
Dexsil 300 GC	1.08	1.18	1.11	1.05	1.37
Polyimide	1.32	1.47	1.14	1.04	1.38
STAP	2.14	1.45	1.21	0.95[a]	1.38
Cyclohexane-dimethanol succinate	2.41	1.64	1.36	0.97[a]	1.55

[a] Reversed order of elution.

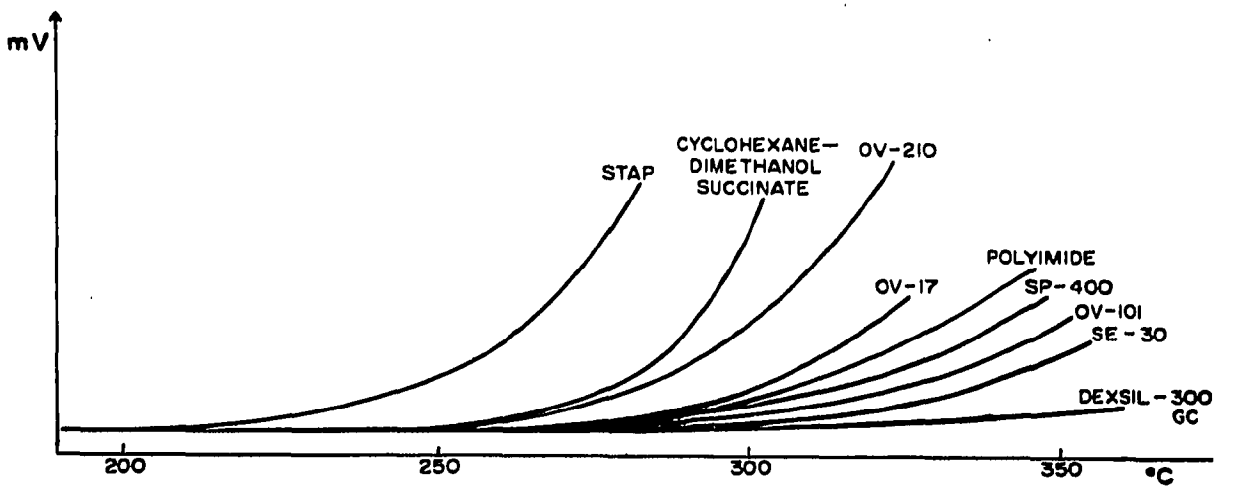

Fig. 1. Relative bleeding rate of 20 m long columns coated with various stationary phases (programming rate: 4°/min.).

ularities[13,14], the interaction of steroids with the stationary phase can be utilized for identification purposes. The stationary phases used in gas chromatography of steroids were classified by HORNING *et al.*[15] and the basic retention rules established[16,17]. However, NOVOTNY AND ZLATKIS[4] observed that "methylene units" measured on glass capillary columns were not identical with packed column data. This is due to the participation of the solid material in the separation process as shown by TESARIK AND NOVOTNY[10]; however, a simple explanation of its mechanism cannot readily be suggested.

It can be seen from Table II that the basic rules for retention of steroid trimethylsilyl derivatives also apply in capillary column work. The axial steroids are eluted

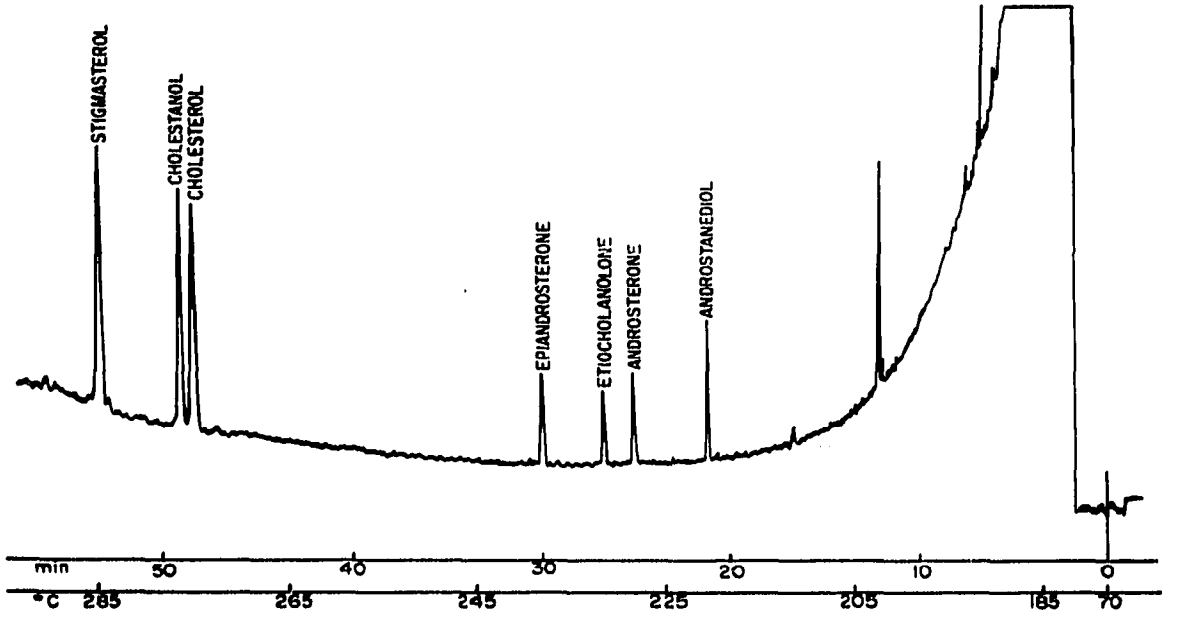

Fig. 2. Chromatogram of the steroid standard mixture on the polyimide phase. Conditions: 20 m × 0.3 mm; injector, 250°; detector, 280°.

before equatorial ones and 5β-structures are retained in the columns longer than their 5α-analogs. With the exception of SE-30 and SP-400 (chlorophenyl silicone fluid), androsterone is eluted after androstanediol, the latter being of higher molecular weight, but with fully protected polar groups. A remarkable selectivity of STAP (polyester, a modified Carbowax) and cyclohexanedimethanol succinate for all measured pairs is unfortunately offset by their low temperature stability (Fig. 1). A short capillary column with a moderate number of theoretical plates yielded complete separations of androsterone and etiocholanolone with all of the phases studied.

Although OV-17, SP-400, STAP and cyclohexanedimethanol succinate appear to possess a selectivity for the double bond of cholesterol, the fluoroalkyl silicone OV-210, Dexsil 300-GC and the polyimide phase can resolve cholesterol and cholestanol quite easily (Fig. 2). The order of elution here remains unchanged. At least 100,000 theoretical plates may be necessary with the rest of silicone stationary phases for such a resolution. It is also evident that these particular sterols cannot be resolved on any of the stationary phases listed in Table I with packed columns. Although many of these substrates can be used in the capillary column work, the superiority of Dexsil and the polyimide phases is quite evident because of their selectivity and unusual temperature stability. The preparation and chromatographic properties of the polyimide phases and the high-temperature applications of Dexsil will be reported elsewhere[18,19].

This work was supported in part by the Robert E. Welch Foundation (Grant No. E-363).

Department of Chemistry,
University of Houston,
Houston, Texas 77004 (U.S.A.)

M. NOVOTNY
A. ZLATKIS

1 M. NOVOTNY AND K. TESARIK, *Chromatographia*, 1 (1968) 332.
2 K. TESARIK AND M. NOVOTNY, in H. G. STRUPPE (Editor), *Gas-Chromatographie 1968*, Akademic-Verlag GmBH, Berlin, 1968.
3 K. GROB, *Helv. Chim. Acta*, 48 (1965) 1362.
4 M. NOVOTNY AND A. ZLATKIS, *J. Chromatogr., Sci.*, 8 (1970) 346.
5 J. A. VOELLMIN, *Chromatographia*, 3 (1970) 223.
6 K. GROB, *Beitr. Tabakforsch.*, 3 (1966) 403.
7 K. B. BARTLE, L. BERGSTEDT, M. NOVOTNY AND G. WIDMARK, *J. Chromatogr.*, 45 (1969) 256.
8 K. GROB AND J. A. VOELLMIN, *J. Chromatogr., Sci.*, 8 (1970) 218.
9 J. H. PURNELL, *J. Chem. Soc. (London)*, (1960) 1268.
10 K. TESARIK AND M. NOVOTNY, *Chromatographia*, 2 (1969) 284.
11 M. NOVOTNY AND K. D. BARTLE, *Chromatographia*, 3 (1970) 272.
12 M. NOVOTNY, L. BLOMBERG AND K. D. BARTLE, *J. Chromatogr., Sci.*, 8 (1970) 390.
13 C. J. W. BROOKS, *Monograph No. 2*, The Society for Analytical Chemistry, London, 1964, p. 18.
14 B. A. KNIGHTS AND G. H. THOMAS, *Anal. Chem.*, 34 (1962) 1046.
15 E. C. HORNING, W. J. A. VANDENHEUVEL AND B. G. CREECH, in D. GLICK (Editor), *Methods of Biochemical Analysis*, Vol. XI, Interscience Publishers, New York, 1963.
16 W. J. A. VANDENHEUVEL AND E. C. HORNING, *Biochim. Biophys. Acta*, 64 (1962) 416.
17 W. J. A. VANDENHEUVEL, W. L. GARDINER AND E. C. HORNING, *J. Chromatogr.*, 26 (1967) 387.
18 R. G. MATHEWS, R. D. SCHWARTZ, M. NOVOTNY AND A. ZLATKIS, to be published.
19 M. NOVOTNY AND A. ZLATKIS, to be published.

Received December 15th, 1970