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CHROMATOGRAPHIC SEPARATION OF OVARIAN DERMOID CYST LIPIDS AND PROSTAGLANDINS

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SUMMARY

Using Florisil column chromatography the ovarian dermoid cyst lipids were fractionated. Temperature-programmed gas chromatography showed the presence of C_{14} to C_{40} saturated hydrocarbons. The cholesteryl ester fraction had a high content of myristic acid, 17.5 %. There were no marked differences in the fatty acid composition of glycerides between mono-, di- and triglycerides except for some differences in the content of saturated fatty acids.

A glass fiber paper chromatographic method was developed for the separation and identification of prostaglandins. The following components were identified: PGA, PGB, PGE_1, PGE_2, $PGF_{1\alpha}$ and $PGF_{2\alpha}$.

INTRODUCTION

The composition of prostaglandins and lipids in ovarian dermoid cysts has not been reported to date. Ovarian dermoid lipids were isolated and separated into biologically active and non-biologically active lipid classes. Quantitative analysis of lipids was made by Florisil column chromatography. The fatty acid composition of sterol esters, glycerides, free fatty acids and phospholipids was analyzed by gas chromatography employing polar and non-polar packings. Prostaglandins, pharmacologically active derivatives of prostanoic acid ($C_{20}H_{38}O_2$) have been determined by thin-layer chromatogaphy(TLC) [1-3], paper chromatography[4], enzymatic assay[5], fluorescence[6], absorption spectroscopy[7] and gas chromatography (GC)[8]. The present report is also concerned with the development of a glass fiber paper chromatographic method for the separation and identification of primary prostaglandins, as well as dehydrated prostaglandins.

EXPERIMENTAL PROCEDURES

All glassware and related equipment employed in this study were thoroughly de-fatted with chloroform–methanol and ethyl ether, and the AnalaR (AR) grade solvents were re-distilled before use. A nitrogen atmosphere was maintained to minimize oxidation during various procedures. The reference fatty acid methyl esters and hydrocarbons came from Applied Science Laboratories, Inc. The prostaglandins were obtained from the Upjohn Company. Ovarian dermoid cysts were obtained

immediately after surgical excision and the lipids were extracted with chloroform–methanol (C–M) (2:1) (w/v) following the method of FOLCH *et al.*[9]. Subsequent processing yielded a light yellow semi-solid mass (50 %).

Fractionation of lipids by Florisil column chromatography

A sample of the total lipids in hexane was chromatographed over Florisil by the method of CARROLL[10]. Elution of the column with hexane; hexane and 5 %, 15 %, 25 % and 50 % ethyl ether; 98 % ethyl ether + 2 % methanol; and 95 % ethyl ether + 4 % acetic acid separated the hydrocarbons, sterol esters, triglycerides, sterol, diglycerides, monoglycerides and free fatty acids. The phospholipids were in a final fraction eluted with absolute methanol. The purity of the samples was checked by TLC[11]. The results are presented in Table I.

The mono-, di- and triglycerides, were transesterified by the use of sodium methoxide and methanol according to the procedure of LUDDY *et al.*[12]. The phospholipid fraction was hydrolyzed by heating with 5 % hydrochloric acid in methanol for 2 h[13], a procedure also applied to the sterol esters and free fatty acids. The results of GLC separation of the methyl esters are summarized in Table II.

TABLE I

COMPOSITION OF OVARIAN DERMOID CYST LIPIDS

Component	*Weight % composition*
Hydrocarbons	19.5
Cholesterol esters	16.9
Triglycerides	25.7
Cholesterol	6.8
Diglycerides	5.1
Monoglycerides	3.4
Free fatty acids	1.7
Phospholipids	20.9

Gas chromatography

A Barber Colman Model 5000 gas chromatograph equipped with dual hydrogen flame detector was used. The two U-shaped borosilicate columns measured 8 ft. × 1/4 in. O.D. and contained 3 % SE-30 on 80–100 mesh Gas-Chrom P. The column, injector and detector temperatures were 250°, 250° and 270° respectively; in case of hydrocarbons the column temperature programmed from 100–350°. Helium was the carrier gas at 65 ml/min. With the system containing 15 % DEGS on 80–100 mesh Gas-Chrom P, the column, injector and detector temperatures were 200°, 200° and 250° in the order stated. Helium was introduced at 65 ml/min. The samples were dissolved in ether and volumes of 2 µl injected. Hydrogenation of the sample was described elsewhere[11]. Tentative assignments for each peak were obtained from a semilogarithmic plot of relative retention time *vs.* chain length and degree of unsaturation of standard mixtures of fatty acid methyl esters.[14]

Saponification

The sample in amount of 2.0 g was refluxed with 8 ml of 20 % sodium hydroxide in 95 % ethanol overnight. Processing of the mixture yielded 0.5 g or 25 % unsaponi-

fiables (UNS). The UNS in petroleum ether was fractionated[15] on alumina (Alcoa F-20) which had been determined by prior tests to be essentially free of absorbed lipid, eluting with successive portions of petroleum ether, petroleum ether plus 5% and 10% chloroform, 100% chloroform and absolute methanol. The results are as shown in Table III. The fraction of hydrocarbons which was obtained from

TABLE II

FATTY ACID COMPOSITION OF OVARIAN DERMOID CYST LIPIDS

All GLC values in the tables are relative (area) percentages, values are of pool sample.

Acid[a]	*Free acid*	*Glycerides*			*Sterol esters*	*Total phospholipid*
		Mono-	*Di-*	*Tri-*		
11:0	T[b]	0.2	0.2	0.3		T
12:0	0.8	0.7	1.0	0.6	2.5	0.9
13:0	0.2	0.2	0.3	0.3	0.7	0.3
14:0	7.2	7.9	10.7	7.3	17.5	7.0
15:0	6.0	7.6	9.5	6.6	13.1	6.0
16:0	22.4	27.0	31.2	25.1	30.4	22.5
16:1	19.1	23.2	25.4	20.2	27.7	20.0
17:1	1.0	0.5	1.7	1.2	0.5	0.9
18:0	2.8	2.7	1.2	4.2	0.4	2.8
18:1	12.1	9.7	9.0	10.3	6.6	11.7
18:2	4.4	4.5	1.2	5.4		7.9
20:0	0.5	0.7	0.5	1.0	0.2	T
20:1	0.7	0.4	0.5	1.2		T
22:Br[c]	3.4	1.3	2.0	2.4		
20:4						5.4
22:0	3.3	3.8	0.6	3.9		5.1
23:0	1.1	1.3	0.2	0.6		
24:Br	0.3	0.2	2.4	0.6		
24:0	14.7	8.1	2.4	8.8	0.4	9.5
Saturated	60.7	61.7	62.2	61.7	65.2	54.1
Unsaturated	39.3	38.3	37.8	38.3	34.8	45.9
Mono Unsaturated	34.9	33.8	36.6	32.9	34.8	32.6
Branched	3.7	1.5	4.4	3.0		
Odd	10.3	9.8	11.9	9.0	14.3	7.2
Even	89.7	90.2	88.1	91.0	85.7	92.8

[a] Number of C atoms: number of double bonds.
[b] T = Trace.
[c] Br = Branched.

TABLE III

ALUMINA CHROMATOGRAPHY OF UNSAPONIFIABLE FRACTION OF OVARIAN DERMOID CYST

Fraction	*Eluent*	*Weight %*
I	Petroleum ether	32.4
II	5% Chloroform in petroleum ether	9.2
III	10% Chloroform in petroleum ether	0.1
IV	Chloroform	0.1
V[a]	Methanol	58.1

[a] Fraction contains alcohols and sterol.

the Florisil column was chromatographed in petroleum ether over silica gel (100–200 mesh) and two petroleum ether-eluted fractions were collected. The third fraction was eluted with benzene. The compositions of the fractions are presented in Table IV.

TABLE IV

HYDROCARBONS OF OVARIAN DERMOID CYST SILICA GEL CHROMATOGRAPHY

Temperature programmed gas chromatographic separation over SE-30.

Relative carbon number	*Relative percentage area*		
	Petroleum ether		*Benzene Fraction III*
	Fraction I[a]	*Fraction II*	
C_{14}	4.1		
C_{15}	1.4		
C_{16}	1.4		
C_{17}	2.8		
C_{18}	4.1		
C_{19}	2.8		
C_{20}	8.3		6.6
C_{21}	5.5		5.8
C_{22}	6.9		0.7
$C_{22.5}$			0.7
C_{23}	8.3		0.8
C_{24}	8.3		4.5
$C_{24.5}$			1.6
C_{25}	8.3		2.3
C_{26}	6.9	0.1	9.0
$C_{26.5}$		T[b]	
C_{27}	6.9	T	9.4
C_{28} + squalene	5.5	42.5	24.9
$C_{28.5}$		5.3	
C_{29}	5.5	3.1	16.2
$C_{29.5}$		1.3	5.5
C_{30}	4.1	0.4	
$C_{30.3}$		0.8	
$C_{30.5}$		4.7	1.6
C_{31}	4.1	2.1	
$C_{31.5}$		1.0	5.8
C_{32}	2.8	5.9	
$C_{32.3}$		1.3	
$C_{32.5}$		0.8	1.0
C_{33}	1.4	2.5	
$C_{33.5}$		3.4	1.3
C_{34}	0.4	0.8	
$C_{34.5}$			0.3
C_{35}	0.2	0.8	
$C_{35.5}$			1.0
C_{36}		7.6	
C_{37}		0.8	
$C_{37.5}$			0.3
C_{38}		8.4	
$C_{38.5}$		2.1	
C_{39}		2.1	
$C_{39.5}$		1.3	
C_{40}		0.8	0.6

[a] Fraction I contains the saturated hydrocarbons and does not contain squalene.
[b] T = Trace.

Extraction of prostaglandins

Thirty ml of ethanol was added to 1.5 g ovarian dermoid cyst lipids and stirred well. The solution was acidified with 1 *N* hydrochloric acid to pH 3, extracted with 60 ml of petroleum ether which was discarded and then extracted with 60 ml of ether. The ether was washed once with water and dried with anhydrous sodium sulfate and evaporated to dryness at low temperature with a stream of nitrogen. The weight of prostaglandins recovered from the ether layer was 1.1 mg.

Separation of prostaglandins in classes by column chromatography[1]

Up to 50–60 μg of total prostaglandins were loaded onto a micro column of 1.0 g of SilicAR CC-4, slurried in *n*-hexane–ethyl acetate (2:1). A and B prostaglandins were eluted with 30 ml of *n*-hexane–ethyl acetate (2:1) giving Fraction I. Type E prostaglandins were eluted with 30 ml of *n*-hexane–ethyl acetate (2:3) (Fraction II), and F prostaglandins were eluted with 30 ml of ethyl acetate–acetone (95:5) (Fraction III). The composition of Fraction I, II and III are 32.0 %, 28.0 % and 40.0 %, respectively. Glass fiber paper chromatography showed that the isolated fractions were not pure.

Glass fiber paper chromatography[16]

The separation of prostaglandins was performed on silica gel impregnated glass fiber paper chromatograms which were prepared as follows:

Glass fiber paper (No. 934 AH, obtained from H. Reeve Angel Co., Clifton, N.J., U.S.A.) was impregnated with a supersaturated silicic acid solution which subsequently gels. The supersaturated silicic acid solution was prepared by mixing 30 ml of ammonium chloride solution (5 % w/v in water) with 100 ml of potassium silicate solution (approximately 2 % aqueous, prepared by making a 15 to 1 dilution of "Potassium Silicate-Electronics 200", Electrochemicals Dept., E. I. DuPont, Wilmington, Del., U.S.A.). Glass filter paper was cut to 20 × 20 cm size and dipped singly into the coating mixture for about 15 sec. A clean glass rod was passed across both surfaces to drain excess fluid. Small binder clips were used to suspend the paper vertically over a hot plate for 60 min to dry and then hung in a furnace at 400° for 60 min. The papers were stored in a clean covered container until used.

The solvent system consisted of ethyl acetate–water–methanol (110:10:1) equilibrated overnight before use. Samples of the prostaglandins in ether, unknown and standard weighed, were applied 2.0 cm from the edge of the chromatogram, the latter dried at 25°, and then developed. Ascending development was conducted at 25° for 15 min, after which time the straight line solvent front was marked and the chromatogram dried. The sample and reference compounds were detected by spraying with concentrated sulfuric acid and charring at 180°. The relative percentages were calculated using a densitometer. The results are shown in Table V and a typical separation in Fig 1. The sequence of separation from the origin is $PGF_{2\alpha} - PGF_{1\alpha} - PGE_2 - PGE_1 - PGA + PGB$.

Determination of total A and B compounds[17]

The determination of the relative percentage of A and B compounds of the (20 μg) prostaglandin was performed by measuring the chromophore at 278 nm

using a Beckman spectrophotometer. The percentages of prostaglandin A and B compounds are found to be 35 % and 65 %, respectively.

TABLE V

RELATIVE PERCENTAGE DISTRIBUTION OF PROSTAGLANDINS IN OVARIAN DERMOID CYST LIPIDS

PG = Prostaglandin.

Prostaglandins	*Batch I*	*Batch II*	*Batch III*
PGA + PGB	22.2	30.0	23.6
PGE_1	8.8	12.3	13.1
PGE_2	13.2	15.0	15.6
$PGF_{1\alpha}$	24.4	17.4	21.1
$PGF_{2\alpha}$	31.4	25.3	26.6

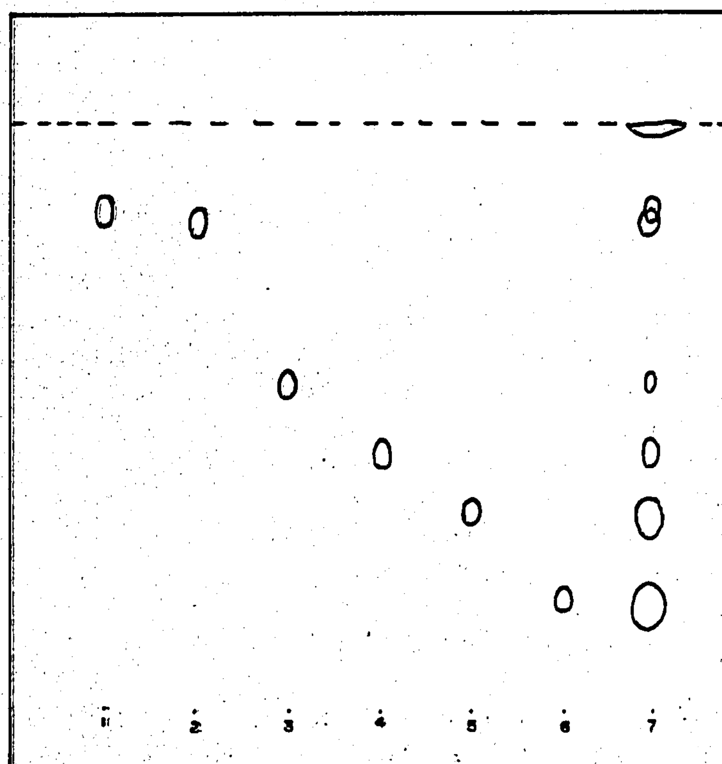

Fig. 1. Silica gel impregnated glass fiber paper chromatogram of the prostaglandins from ovarian dermoid cyst lipids. The developing solvent was ethyl acetate–water–methanol (110:10:1). The dotted line indicates the solvent front. 1 = Prostaglandin B (R_F value 0.87); 2 = prostaglandin A (R_F value 0.85); 3 = prostaglandin E_1 (R_F value 0.58); 4 = prostaglandin E_2 (R_F value 0.46); 5 = prostaglandin $F_{1\alpha}$ (R_F value 0.36); 6 = prostaglandin $F_{2\alpha}$ (R_F value 0.21); 7 = sample of ovarian dermoid cyst prostaglandins; material at solvent front is contamination of lipids.

RESULTS AND DISCUSSION

The resolution of hydrocarbons was achieved by column chromatography of Fraction I (obtained by Florisil column) in petroleum ether over silica gel. Saturated hydrocarbons occurred in the initial petroleum ether cut and olefinic types were removed by benzene and cut II of petroleum ether (Table IV). Fractions II and III

contained 42.5 % and 24.9 % squalene, respectively. Temperature-programmed gas chromatography showed the presence of C_{14} to C_{40} hydrocarbons in the derived cuts. The paraffinic hydrocarbons are bona fide constituents of ovarian dermoid cyst lipids. The composition of free, glyceride, sterol ester and phospholipid fatty acids derived from the Florisil-chromatographed fractions is presented in Table II. Essentially, the same peaks occurred among glycerides and free fatty acids, and the saturated acid levels exceeded those of the unsaturated members. The fatty acid findings on trans-esterification of the mono-, di- and triglycerides are worthy of comment. The percentage composition of the acids was nearly the same except in the decrease of the level of 18:2 and 24:0 in diglycerides. Free fatty acids contained the highest amount of 24:0.

Polyunsaturated acids were prominent in phospholipids with the 18:2 and 20:4 constituent at 7.9 % and 5.4 %, respectively. The acids from the sterol esters were high in hexadecanoic acid, but very low in octadecanoic acid in contrast to the other mixture. No branched acids were found in sterol esters and phospholipid fatty acids. The ratio of olefinic to saturated acids ranged from 0.63–0.69, but amounted to 0.85 for the phospholipids and lower, 0.53, for the sterol ester components.

Glass fiber paper chromatographic method was used for the separation and identification of prostaglandins. The results are summarized in Table V. The dominating fraction is $PGF_{2\alpha}$ among primary prostaglandins. Glass fiber paper chromatography is more sensitive than thin-layer chromatography, while column chromatography gives poor resolution. A combination of several chromatographic techniques, shown to be of value in the elucidation of ovarian dermoid cysts lipids composition, might also be applied to others.

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