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ISOLATION AND ANALYSIS OF SQUALENE ALCOHOLS FROM RAT PREPUTIAL GLANDS

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Indexing Words

rat preputial glands; squalene alcohols; thin layer chromatography; column chromatography; infrared spectrometry; gas chromatography; gas chromatography-mass spectrometry

Sumiko MURAKAMI. *Isolation and Analysis of Squalene Alcohols from Rat Preputial Glands.* Kobe J. Med. Sci. 19, 91-110, June 1973—— The total lipids of rat preputial glands which were extracted with chloroform: methanol (1:1 v/v) were separated into neutral lipids and phospholipids by a silicic acid column chromatography. The neutral lipids were further separated into 6 fractions (a, b, c, d, e and f) by a florisil column chromatography. One major component of unsaponifiable materials of fractions (b and c) was purified by a preparative thin layer chromatography and a silicic acid column rechromatography. The lipid thus isolated was a single spot on TLC in three different solvent systems. For the structural studies of the purified lipid, infrared spectrometric, gas chromatographic and gas chromatograph-mass spectrometric analyses were carried out.

The infrared spectrum of the lipid was characteristic for polyprenols. The GLC analysis of hydrogenated lipid showed that the original material was composed of 2 isomeric alcohols. The mass spectrum of the lipid was characteristic for a polyprenyl hydrocarbon; squalene. The mass spectra of hydrogenated one and trimethylsilyl ether derivatives of the lipid suggested that the alcohols have the formula $C_{30}H_{49}OH$ which is the same with presqualene alcohol. However, these alcohols which contain six double bonds instead of five (double bonds) and one cyclopropane ring of presqualene alcohol, are named squalene alcohols. The squalene alcohols are more similar to betulaprenol-6 which was isolated from birch wood. Betulaprenol-6 has 6 isoprenoid units and a hydroxyl group in position of terminal C_1. The squalene alcohols reported here have a hydroxyl group in position of C_8.

INTRODUCTION

Few reports were published about the study of the lipids of the rat preputial

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glands. Patterson (1960) and Burgess and Wilson (1963) studied the effects of steroid hormones on squalene and on cholesterol synthesis in the rat preputial glands. The author found out a new lipid fraction, while analyzing lipids of the preputial gland. The new lipid was isolated and purified to study its structural analysis. The new lipid was composed of 2 isomeric alcohols, which were named squalene alcohols.

The structure of squalene alcohols are similar to that of presqualene alcohol. Presqualene alcohol was yielded after chemical reduction of presqualene pyrophosphate by $LiAlH_4$ (Epstein and Rilling, 1970) and after hydrolysis by yeast microsomes (Edmond, Popják, Wong and Williams, 1971). Presqualene alcohol biosynthesized from farnesyl pyrophosphate was found by high resolution mass spectrometry to be $C_{30}H_{49}OH$ and five double bonds and one cyclopropane ring. The squalene alcohols had the same formula $C_{30}H_{49}OH$, six double bonds and no cyclopropane ring by mass spectrometric analysis. Lindgren (1965) reported that the alcohols in birch wood were compounds with the general formula: $H-[CH_2-C(CH_3)=CH-CH_2]_n-OH$ (n being 6, 7, 8 and 9), and proposed the name betulaprenols for the alcohols. The difference between squalene alcohols and betulaprenol-6 which has 6 isoprene units is in the position of a hydroxyl group; squalene alcohols at C_3 and betulaprenols at terminal C_1.

MATERIALS AND METHODS

Materials : Solvents were distilled or otherwise reagent grade. Reference compounds; squalene, cholesterol, cholesterol palmitate, tripalmitoyl glycerol, octadecyl alcohol and bathyl alcohol were purchased from Nakarai Chemicals LTD, Kyoto, Japan and phosphatidyl choline, phosphatidyl ethanolamine and sphingomyelin from Sigma Chemical Co., Missouri, U.S.A. An alcohol acetate standard was synthesized from octadecyl alcohol (Inouye, Onodera, Kitaoka and Hirano, 1956).

Animals: Preputial glands were obtained from normal adult rats of Wister and Sprague-Dawley by excision immediately after the animals were killed by decapitation.

Column chromatography: Column chromatographic separations were carried out on Mallinckrodt silicic acid (100 mesh): celite 454 (Nakarai Chemicals LTD), (2 : 1 v/v) columns (30×3 cm and 40×1.5 cm) and florisil (100 - 200 mesh, Nakarai Chemicals LTD) columns (30×3 cm).

Thin layer chromatography (TLC): The glass plates were coated with Silica Gel G (Merk). Four solvent systems were used: (1) chloroform: methanol: water (95:35:4 v/v), (2) petroleum ether: ethyl ether: acetic acid (90:20:1 or 95:5:1 v/v), (3) benzene: ethyl acetate (7:1 v/v) and (4) hexane: ehtyl ether (95:5 v/v). Spots were made visible by charring with 50% sulfulic acid. The preparative TLC was performed using 5 mm thick Silica Gel G.

Infrared spectrometry (IR): The infrared spectrum was measured with Hitachi Model 215 infrared spectrometer.

Gas liquid chromatography (GLC): GLC separations were carried out with: (1) a Shimadzu Model GC-3AF instrument equipped with Shimadzu Model R-101 recoder, with 6 ft×3.5 mm coiled glass columns; the packings were 3% SE-30 and 3% OV-17 (Nishio Industry Co., LTD, Osaka, Japan) on 80-100 mesh acid-washed and silanized Chromosorb W, and (2) a Barber-Colman Model 5000 instruments equipped with Keithley Model 417 picoanmeters and Texas Instruments recorders, with 12 ft×3.5 mm W-shaped glass columns; the packings were 1% SE-30 and 1% OV-17 on 100-200 mesh acid-washed and silanized Gas Chrom P (Horning, Van den Heuvel and Creech, 1963). The flow rates (nitrogen) were 40-50 ml/min measured with the column at 200°C.

Mass spectrometry (MS): Mass spectrometric studies were carried out with the following. (1) a LKB Model 9000 gas chromatograph-mass spectrometer was used; the column packing was 1% SE-30. The accelerating voltage was either 70 eV or 22.5 eV and the current was 60 μa. The ion source temperature was either 250°C or 350°C. (2) a JEOL JMS 01SC gas chromatograph-mass spectrometer was used; the column packing of 3% OV-17. The accelerating voltage was 40 eV and the current was 150 μa. The ion source temperature was 250°C.

Catalytic hydrogenation of sample: 1-2 mg of the sample dissolved in 1 ml of ethanol was hydrogenated for 3 hours at room temperature in the presence of about 10 mg of platinum oxide. The catalyst was centrifuged off and the supernatant was dried on a rotary evaporater. The residue was dissolved in hexane and analyzed by GLC.

Derivative formation: One milligram of sample was heated at 60°C for one hour in 0.1 ml of bis trimethyl-trifluoroacetamide (BSTFA) and 0.05 ml of trimethyl chlorosilane (TMCS). An aliquot 3 μl of this solution was analyzed by GLC directly.

RESULTS

Isolation and purification of squalene alcohol: The preputial glands which were dissected free of fat were homogenized with aceton. The total lipids were extracted with chloroform: methanol mixture (C: M, 1: 1, v/v) 4 times (Table 1).

Table 1 The wet weight and total lipids of the rat prepuitial glands.

Sex	Rat	Number	Rat preputial glands Wet weight(ww)	Total lipids(tl)	% of tl/ww
Male	Wister	28	2.52g	0.375 g	15
	Sprague-Dawley	120	10.02	1.684	17
	SUM	148	12.54	2.059	
Female	Wister	65	4.87	0.656	14
	Sprague-Dawley	86	5.75	0.898	16
	SUM	151	10.62	1.554	

Phospholipids were separated from neutral lipids by a silicic acid column chromatography. Columns were prepared by pouring a silicic acid-celite 454 mixture slushy in C: M (98: 2 v/v) into a column (30×3 cm). The total lipids in C: M (98: 2) were separated into 3 fractions (A, B and C) with C: M (98: 2), C: M (1: 1) and methanol, respectively by stepwise elution (Fig. 1-I and 1-II). Fraction A containing almost neutral lipids was 77%, fraction B containing phosphatidyl ethanolamine, 8－9% and fraction C containing phosphatidyl choline, 10－11% of total lipids in weight. Each fraction was assayed phosphorus (King, 1932); fraction A was not detected, B 3.0－3.1 % and C 2.2－2.4 % of lipids weight of each fraction.

Fraction A was fractionated further on a florisil column (30×3 cm) with mixtures of hexane (H) and ethyl ether (E); H, H: E (9: 1 v/v), H: E (7: 3) and methanol by stepwise elution. The materials in each tube were detected with TLC and were recombined to make 6 fractions (a, b, c, d, e and f, in Fig. 1-III). Each fraction was weighted; (a) was a few percent of fraction A, (b) about 25% (22-28%), (c) 20% (17－24%), (d) 27%, (e) 10% and (f) 15%.

Fractions b and c were saponified with 2N KOH in 80 % methanol for 3 hours. Non saponifiable lipids were extracted with ethyl ether 5 times and washed with water 5 times (Fig. 2-I). Non saponifiable lipids were subjected to rechromatography on 40×1.5 cm silicic acid column using stepwise elution with H, H: E (9: 1 v/v), H: E (8: 2), H: E (7: 3) and methanol. The major component of this H: E (9: 1) fraction was purified with preparative TLC. Rechromatography and preparative TLC were repeated once more to yield 12.7 mg of purified squalene alcohol from male

rats and 12.7 mg from female rats (Fig. 2-II). After saponification, fractions from Wister and Sprague-Dawley were combined for the following purification between the same sex, because of no difference on TLC and GLC. The squalene alcohol thus isolated was a single spot on TLC in three different solvent systems (systems 2, 3 and 4).

Infrared spectrometry: The infrared spectrum of the squalene alcohol shown in Fig. 3, was characteristic for the polyprenols which had reported by Wellburn, Stevenson, Hemming and Morton (1967). The absorptions expected were for C-H stretching at 2860 cm^{-1} ($-CH_2-$), 2920 cm^{-1} ($-CH_2-$, $-CH_3$) and 2970 cm^{-1} ($-CH_3$), for C-H bending at 1440 cm^{-1} ($C-CH_3$) and 1380 cm^{-1} ($C-CH_3$, $C-(CH_3)_2$), for C=C stretching at 1660 cm^{-1}, and for O—H stretching at 3400 cm^{-1}.

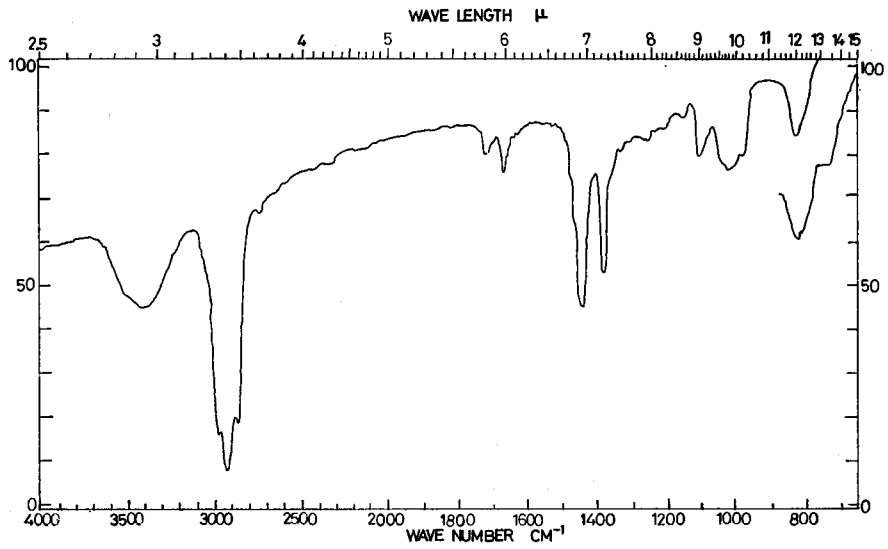


Fig. 3 Infrared spectrum of squalene alcohol. Spectrum was recorded by the use of KBr-disk.

Gas liquid chromatography: Squalene alcohol isolated from female rats preputial glands was dissolved in hexane and subjected GLC on 3% SE-30 column. As is shown in Fig. 4-I, four peaks (1, 2, 3, and 4) appeared. The major peak 2 had a similar retention time to squalene and peak 1 to squalane. Squalene alcohol isolated from male rats showed the same pattern as that of female. The same pattern was obtained on 3% OV-17 column. Fig. 4-II and 4-III show a GLC separation of hydrogenated and its trimethylsilyl (TMS) derivatives of squalene alcohol of male rats, respectively. Both of the peaks 1 had the same retention time as the squalane.

Mass spectrometry: Mass spectra of untreated squalene alcohol corresponding to peak 2 in Fig. 4-I and standard squalene are shown in Fig. 5. Peak 2 gave a hydrocarbon with a molecular weight of 408 ($C_{30}H_{48}$), which is probably dehydrosqualene or its isomer. The mass spectrum obtained from untreated one was characteristic for a polyprenyl hydrocarbon: (i) the base peak was at m/e 69, and (ii) the parent ion underwent typical fragmentations by losing successively 69 and 68 mass units (ions at m/e 339, 271, 203 and 135). The cracking pattern of this peak was very similar to that of squalene (mol wt 410) except that all of the major fragments, from the parent ion down to the ions at m/e 93 and 95, were found at 2 mass units lower than in the spectrum of squalene, and much more similar to that of presqualene alcohol (Popják and others, 1969). The most probable structure of the hydrocarbon on the basis of the mass spectrum is either I or II, but it was not possible to deduce the positions of the two double bonds marked with arrows in these structures, not an uncommon difficulty in the analysis of mass spectra of olefins. Peak 2 in Fig. 4-I had very similar retention time to that of the squalene, but the squalene alcohol had more polarity than the squalene on TLC (Fig. 2-II). This means that the squalene alcohol is dehydrated during GLC procedure.

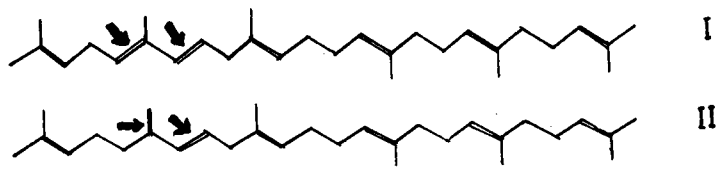


Fig. 6 shows the mass spectra of hydrogenated squalene alcohol. The mass spectrum of squalane in Fig. 6 was obtained with peak 1 in Fig. 4-II which is the same as that of reference squalane. The mass spectra of the hydrogenated (2) and (3) in Fig. 6 were corresponding to peak 2 and 3 in Fig. 4-II, respectively. Both hydrogenated (2) and (3) had peaks at m/e 437 (M-1) and m/e 420 (M-18), which are common in mass spectra of alcohols. The molecular weights of both hydrogenated squalene alcohols were 438 which were 16 mass units higher than squalane (mol wt 422). Thereby, hydrogenated squalene alcohol (2) and (3) may be isomers. The mass spectrum of untreated squalene alcohol was very similar to squalene and hydrogenated one was similar to the squalane which is saturated squalene, with some exceptions for the following. (i) The base peak was in squalene at m/e 71, hydrogenated (2) at m/e 83 and (3) at m/e 57. (ii) The peaks at m/e 157 and m/e 311 were considered

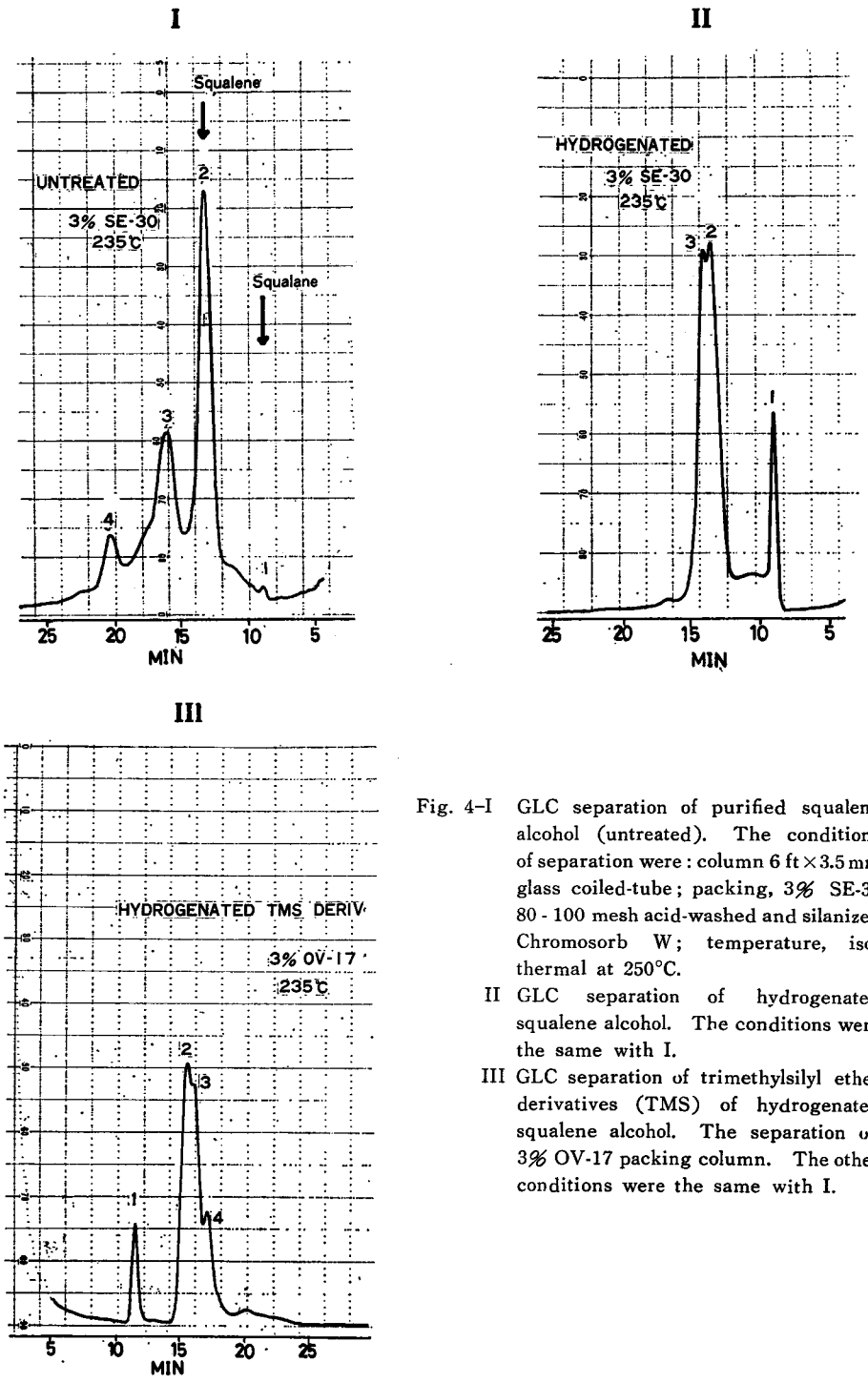


Fig. 4-I GLC separation of purified squalene alcohol (untreated). The conditions of separation were : column 6 ft × 3.5 mm glass coiled-tube ; packing, 3% SE-30 80 - 100 mesh acid-washed and silanized Chromosorb W ; temperature, isothermal at 250°C.

II GLC separation of hydrogenated squalene alcohol. The conditions were the same with I.

III GLC separation of trimethylsilyl ether derivatives (TMS) of hydrogenated squalene alcohol. The separation on 3% OV-17 packing column. The other conditions were the same with I.

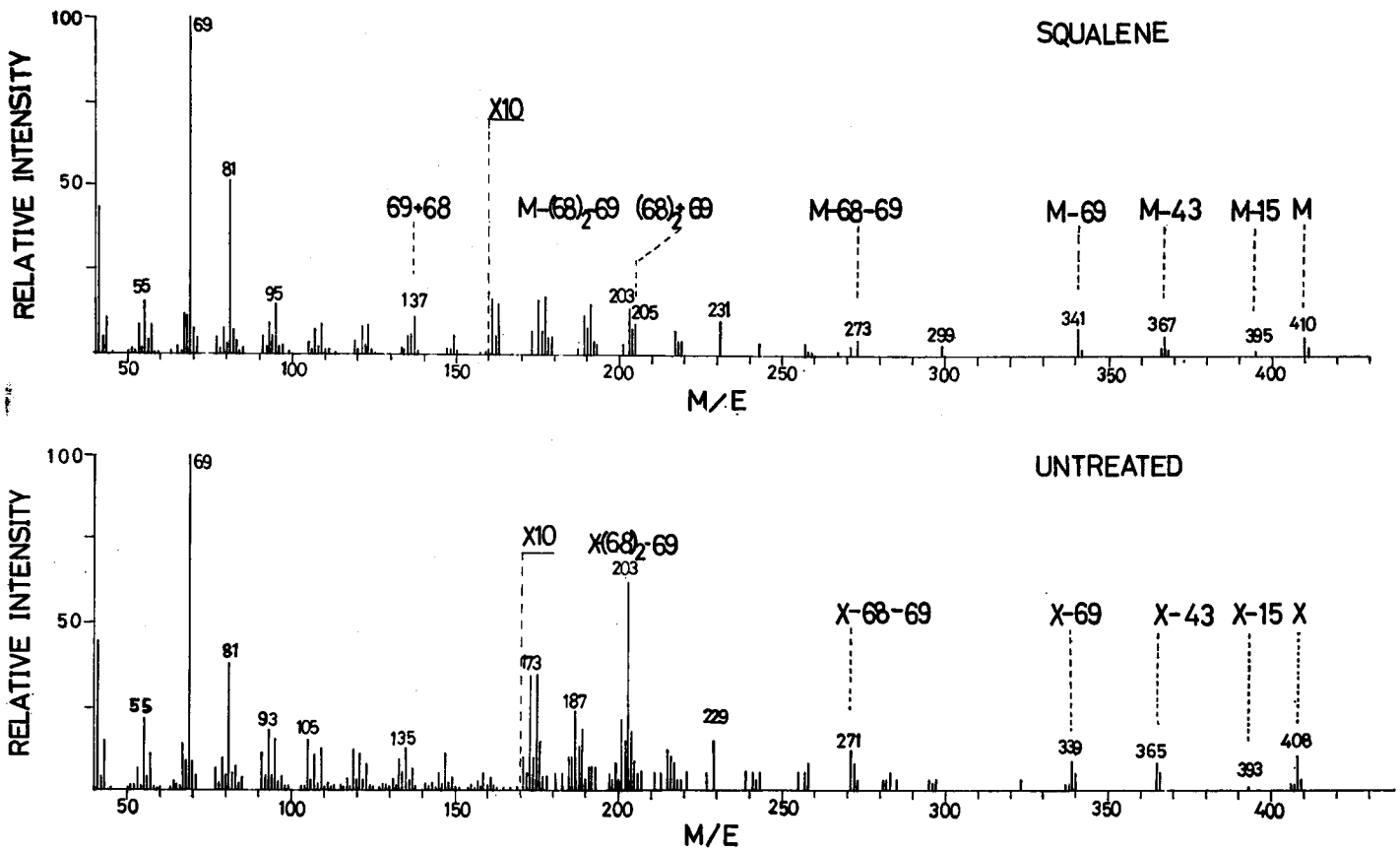
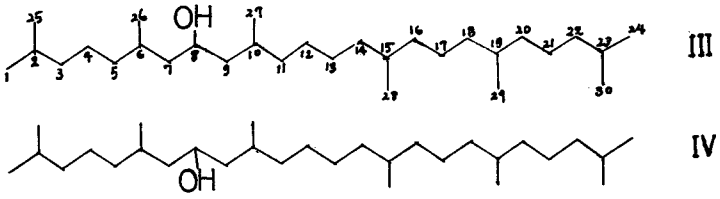


Fig. 5 Mass spectra for peak 2 in Fig. 4-I (untreated) and reference squalene. JEOL JMS 01SC gas chromatograph-mass spectrometer was used.

due to be α-cleavage to furnish stable oxonium ions (Budzikiewicz, Djerassi and Williams). These two ions indicate that the hydrogenated squalene alcohol have a hydroxyl group in position of C_8 (structure III or IV).



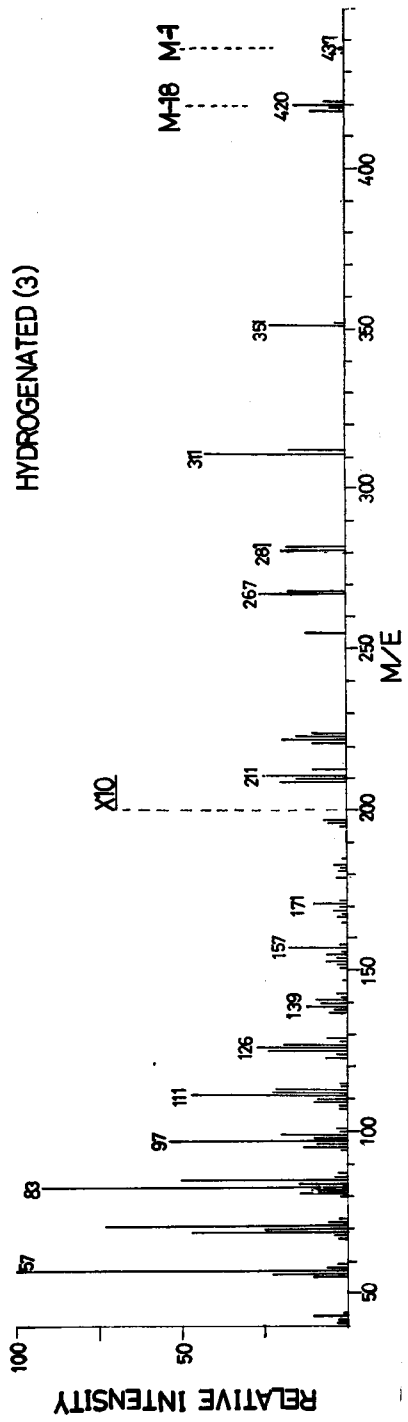
(iii) Series ions at m/e 157 $\xrightarrow{-H_2O}$ m/e 139 $\xrightarrow{-C_2H_4}$ m/e 111 $\xrightarrow{-CH_2}$ m/e 97 $\xrightarrow{-CH_2}$ m/e 83 $\xrightarrow{-CH_2}$ m/e 69 were present. And (iv) the ions at m/e 129 (157$-C_2H_4$) and m/e 353 (M-85) in hydrogenated (2). The occurrence of the peaks at m/e 129 and m/e 353 may suggest that positional isomers of a hydroxyl group were present.

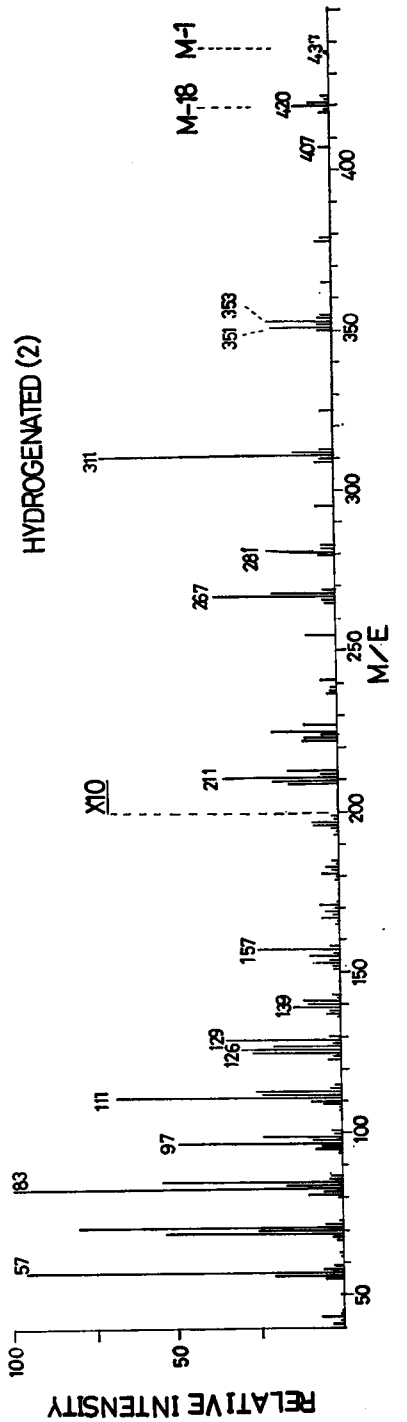
Hydrogenated squalene alcohol was trimethylsilylated by silylating reagents. Both mass spectra of hydrogenated TMS (2) and (3) which are corresponding to peak 2 and 3 in Fig. 4-III, had the parent ions at m/e 510 and the base peaks at m/e 229 (Fig. 7). The peaks at m/e 510 (M), m/e 495 (M-15) and m/e 420 (M-90) indicate the empirical formula. These molecular weights (510) were 72 mass units (TMS-H) higher than hydrogenated one (438). The peaks at m/e 229 and m/e 383 were corresponding to m/e 157 and m/e 331 in Fig. 6, respectively. In the hydrogenated TMS ether derivative (3), the peaks at m/e 201 (229-C_2H_4) and m/e 425 (M-85) were corresponding to m/e 129 and m/e 353 in hydrogenated squalene alcohol, respectively.

These mass spectra (Fig. 6 and 7) indicated that the hydrogenated squalene alcohols had formular $C_{30}H_{61}OH$. They also suggested that the hydrogenated squalene alcohols had the carbon skeleton of squalane in which position C_8 is substituted with a hydroxyl group.

Squalene alcohol from Wister rats preputial glands was prepared by the same procedure in this text, converted to TMS ether and analyzed by GLC on 1 % SE-30 and 1% OV-17 packed 12 ft W-shaped columns, programed temperature 1°C/min from 200°C (Fig. 8). Squalene alcohol had one peak on both columns.

The spectrum of this TMS ether derivative (Fig. 9) had the parent ion at m/e 498 and the base peak at m/e 69. The parent ion underwent typical fragmentations by losing successively 69 and 68 mass units (ions at m/e 429, 361, 293, 225 and 157) and by losing 69, 68 plus 90 (OTMS ion) mass units (ions at m/e 339, 271, 203 and 135). The ion at m/e 73 is due to TMS ion. The peaks at m/e 498 (M), 483


HYDROGENATED (3)
M-18
M-1
X10
RELATIVE INTENSITY
M/E


HYDROGENATED (2)
M-18
M-1
X10
RELATIVE INTENSITY
M/E

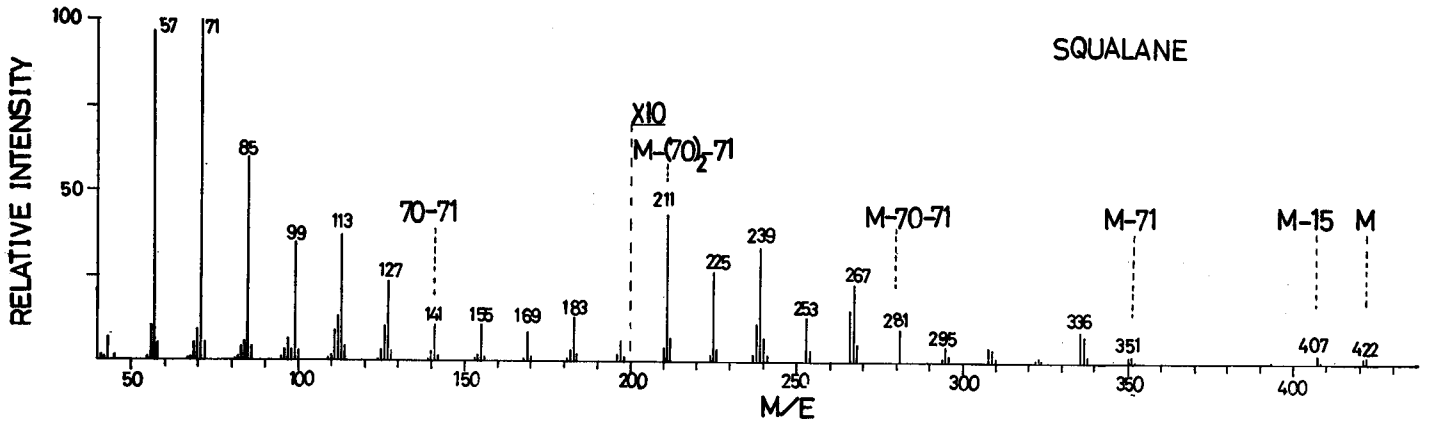


Fig. 6 Mass spectra for peak 2 and 3 in Fig. 4-II, respectively (hydrogenated (2) and (3)), and for peak 1 in Fig. 4-II (squalane). LKB Model 9000 gas chromatograph-mass spectrometer was used; accelerating voltage, 22.5 eV; ion source temperature 350°C.

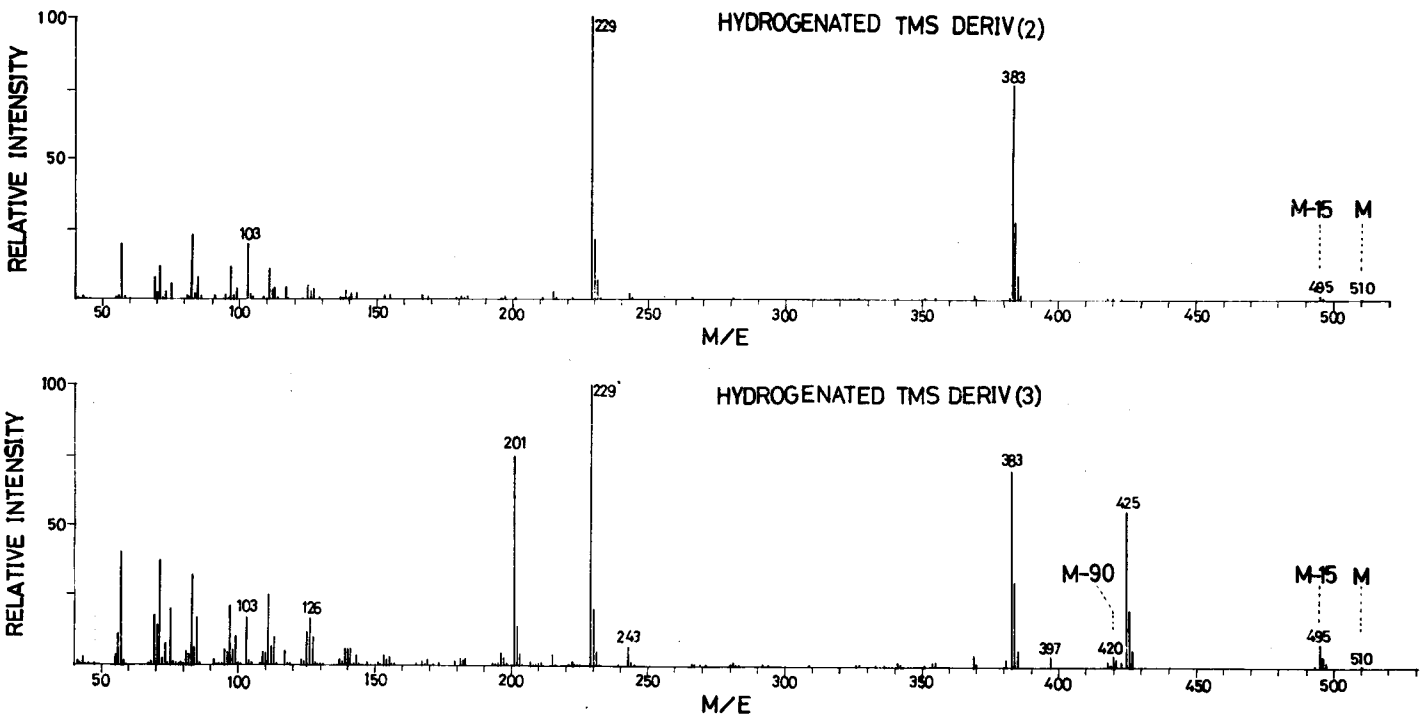


Fig. 7 Mass spectra for peak 2 and 3 in Fig. 4-III, respectively (hydrogenated TMS (2) and (3)). The conditions were the same with Fig. 6.

(M-15) and 408 (M-90) indicate the empirical formula. The ion at m/e 415 (M-83) was also present. Further possible fragmentations were shown as in the following:

$$\underset{(135\ m/e)}{C_{10}H_5} \xleftarrow[HOTMS]{-90} \underset{(225\ m/e)}{C_{10}H_{16}OTMS} \xrightarrow[C_5H_8]{-60} \underset{(157\ m/e)}{C_5H_8OTMS} \xrightarrow[C_2H_2]{-26} \underset{(131\ m/e)}{C_3H_6OTMS}$$

The molecular weight was 498.4245 (calc. 498.4335) by high resolution mass spectrum.

The hydroxyl group of the squalene alcohol was converted to deutriated trimethylsilyl ether by silylating reagent (N, O-bis (trimethy-D9 silyl) acetamide) and catalyzer (TMCS). Both mass spectra of TMS (D-9) derivatives (Fig. 10) had the parent ion at m/e 507 which was 9 mass units higher than m/e 498 (in Fig. 9). This evidence suggested that squalene alcohol has one hydroxyl group. The base peak was 69 m/e in TMS (D-9) derivative (1) and 234 m/e in (2). The peaks of losing successively 69 and 68 mass units from parent ion (ions at m/e 438, 370, 302, 234 and 166 in Fig. 10) were 9 mass units higher than the peaks corresponding to those in TMS derivatives (ions at m/e 429, 361, 293, 225 and 157 in Fig. 9). That indicates these fragments containing a hydroxyl group. The peaks of losing successively 69, 68 plus 99 (TMS D-9) units from the parent ion (ions at m/e 339, 271, 203 and 135) were containing no hydroxyl group. The peaks at m/e 507 (M), 489 (M-18) and 408 (M-99) indicate the empirical formula. The peak at m/e 82 is due to TMS (D-9) ion. These TMS (D-9) derivatives of squalene alcohols had the same molecular weight (507) and qualitatively the same fragmentation. The evidences indicate that (1) and (2) are isomers. They also supported that the parent ion at m/e 408 in untreated squalene alcohol was dehydrated. Then the original molecular weight of the squalene alcohol may be 426. According to that, the molecular weight of hydrogenated squalene alcohol is 12 mass units higher than untreated one, that is, the squalene alcohols have 6 double bonds. The squalene alcohols had the same carbon skeleton as squalene and their formula was $C_{30}H_{49}OH$.

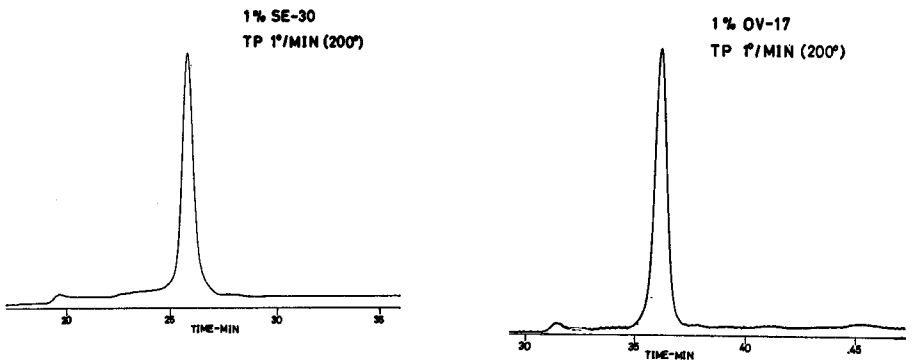


Fig. 8 GLC separation of trimethylsilyl (TMS) derivatives of squalene alcohol. The conditions of separation were; column, 12 ft×3.5 mm glass W-tube; packing, 1% SE-30 and 1% OV-17 on 100–200 mesh acid-washed and silanized Gas Chrom P, temperature programed from 200°C at 1°C/min.

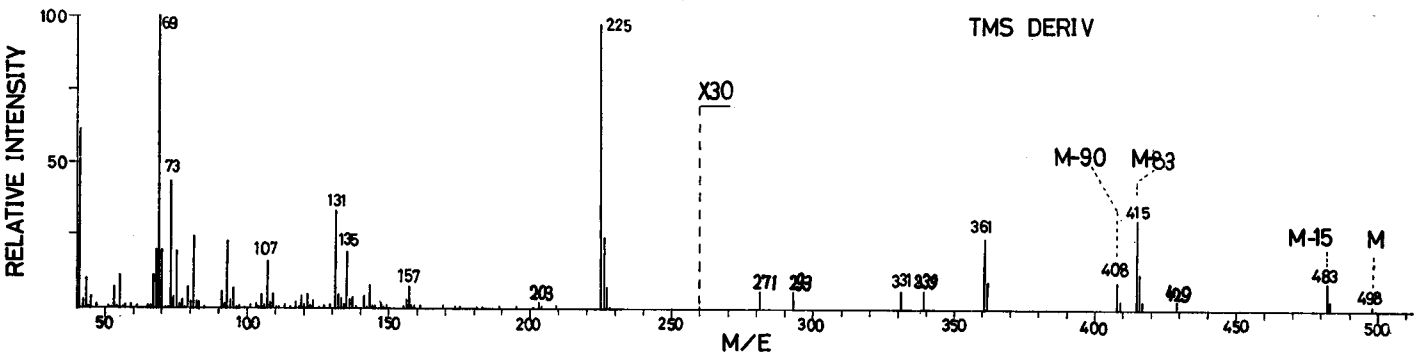


Fig. 9 Mass spectrum TMS ether derivative of squalene alcohol. LKB Model 9000 gas chromatograph-mass spectrometer was used; accelerating voltage, 70 eV; ion source temperature 250°C, 1% SE-30 column.

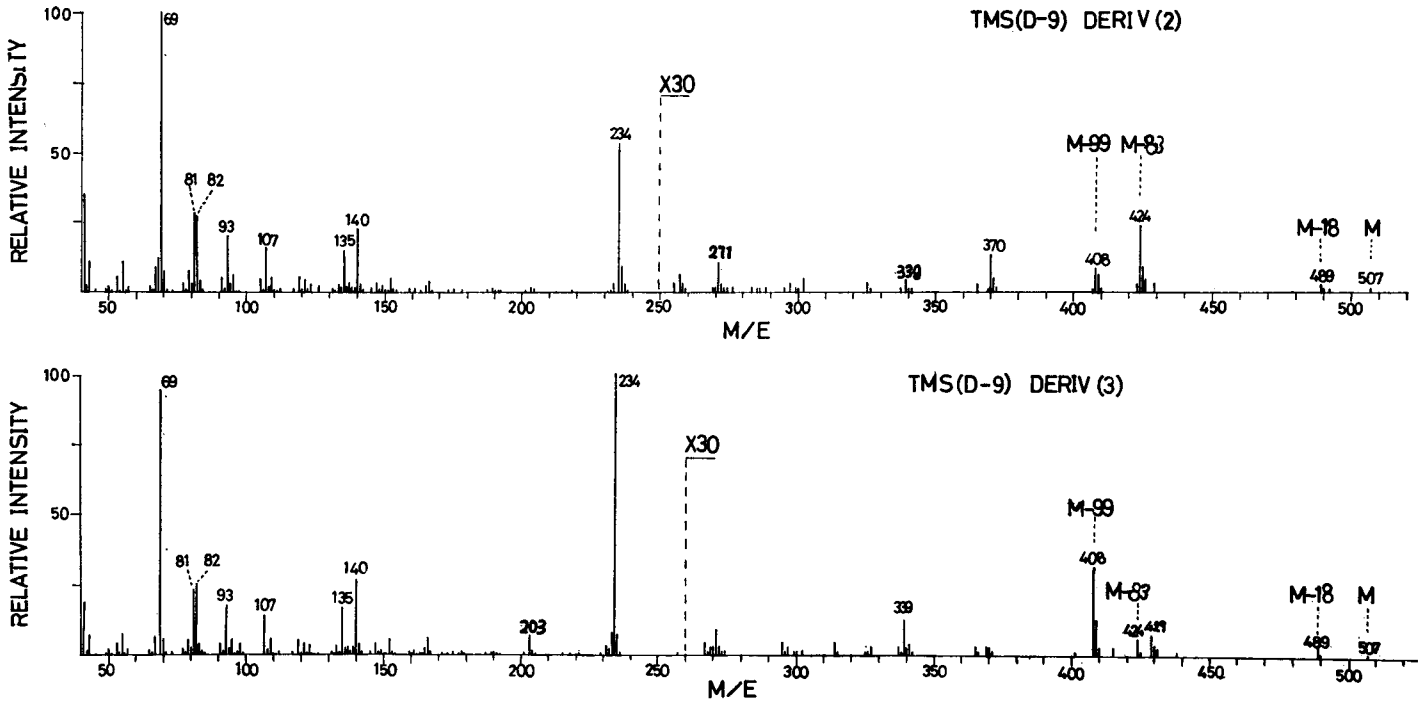


Fig. 10 Mass spectra of deuteriated trimethylsilyl (TMS D-9) ether derivatives. Gas chromatograph-mass spectrometer used was the same with Fig. 9.

DISCUSSION

Squalene alcohols were isolated from the rat preputial glands. The squalene alcohols seemed to occur as free alcohols in neutral lipids. The squalene alcohols are included in fraction c (Fig. 2-I) but they were not clear and difficult to separate from other fractions. After saponification, fraction c (NS) had clear spots of squalene alcohols. The squalene alcohols were also present as esterified form. When the spot belonging to cholesterol ester in fraction b was isolated and saponified, non saponifiable lipids were composed of cholesterol and squalene alcohol on TLC. The squalene alcohols were homogeneous but still had a little the less polar substances which may be squalene and squalane for behaviour on TLC and GLC. The squalene alcohols isolated were gradually degraded to the less polar substances on TLC. No differences of the squalene alcohols were observed between strains and sex by TLC, GLC and MS.

The mass spectrometric studies suggested that squalene alcohols were triterpenols; in formula $C_{30}H_{49}OH$ (mol wt 426) which had the carbon skeleton of squalene and a hydroxyl group in position of C_8. The molecular ion was absent in the spectrum of untreated squalene alcohol, and the ion at m/e 408 which was 2 mass units lower than squalene (mol wt 410) should be losing water (18 mass units) from molecular ion. The mass spectra of hydrogenated one had no molecular ion but M-1 (437 m/e); M-1 is observed usual in an alcohol. The peaks at m/e 311 and 157 suggested that position C_8 in carbon number had a hydroxyl group. The hydroxyl group in position C_8 was supported by the ions at m/e 383 and m/e 229 in the mass spectra of hydrogenated TMS derivatives of squalene alcohols (Fig. 7), by the ion at m/e 225 in Fig. 9 and by the ion at m/e 234 in Fig. 10.

The studies on the mechanism of squalene biosynthesis were estsblished in the occurrence of an intermediate between farnesyl pyrophosphate and squalene by Rilling (1966) and Popják and others (1969) and this intermediate was identified as 2-(2, 6, 10-trimethyl-1, 6, 9-undecatriene)-3-methyl-3-(4, 8-dimethyl-3, 7-nonadien)-cyclopropylcalbinyl-pyrophosphate, by Epstein and Rilling (1970). They were converted an alcohol after chemical reduction and enzymatic hydrolysis of presqualene pyrophosphate. The alcohol had the formula $C_{30}H_{49}OH$, and had 5 double bonds and a cyclopropane ring. The squalene alcohols had the same formula, but had 6 double bonds and no ring by structural analysis.

The betulaprenols (C_{30-45}) in birch wood had been analyzed by Lindgren (1955). He proposed the general formula for those alcohols; $H-[CH_2-C(CH_3)=CH-CH_2]_n-OH$. The difference is in the position of a hydroxyl group attached between the betulaprenol-6 (n=6) and squalene alcohols in their structure. The betulaprenols have a hydroxyl group in terminal C_1, while squalene alcohols in position C_8.

In the preputial glands of the rats, biosynthesis of squalene as a model of the sebaceous glands was studied by Wilson (1963). The preputial glands had a large amount of squalene and cholesterol (free and esterified) in Fig. 1-II and 1-III. The best studied and most widespread mechanism for the conversion of lanosterol to cholesterol is Bloch pathway in most tissues (Bloch, 1959). On the other hand, Kandutsch and Russell (1959, 1960a-b) on the basis of their studies in a tumor of mouse preputial gland have concluded that cholesterol can be synthesized by a second series of

reactions. And this Kandutsch and Russell pathway does operate in rat preputial glands (Wilson, 1963). In rat preputial glands, whether the squalene alcohols work like presqualene alcohol, are intermediate between squalene and lanosterol or terpenes, or are merely unsaturated alcohols and waxes, meight be an interesting poroject for future study.

SUMMARY

1) The lipids (3.6 g) were extracted with chloroform : methanol (1 : 1) from the preputial glands (23 g) of the rats. Phospholipids (23%) were separated from neutral lipids (77%) by a silicic acid column chromatography. The neutral lipids were further separated into 6 fractions (a, b, c, d, e and f). After saponification of fraction b and c (about 45% of neutral lipids in weight), one major component of unsaponifiable lipids was purified by a silicic acid rechromatography and preparative TLC to yield 25.4 mg. The purified lipid was a single spot on TLC in three different solvent systems and was studied its structural analysis.

2) The infrared spectrum of the lipid was characteristic for the polyprenols. The mass spectrum of the lipid was hydrocarbon with a molecular weight of 408 ($C_{30}H_{48}$) and its cracking pattern was similar to that of squalene. The GLC and GC-MS analysis of hydrogenated lipid showed that the original lipid was composed of 2 isomeric alcohols and that the molecular weight was 438. The mass spectra of the TMS and hydrogenated TMS derivatives of the lipid had a molecular weight of 498 and 510, respectively. These mass spectra indicate that the lipids have a hydroxyl group in position C_8 and 6 double bonds. These alcohols, the formula $C_{30}H_{49}OH$, were named squalene alcohols.

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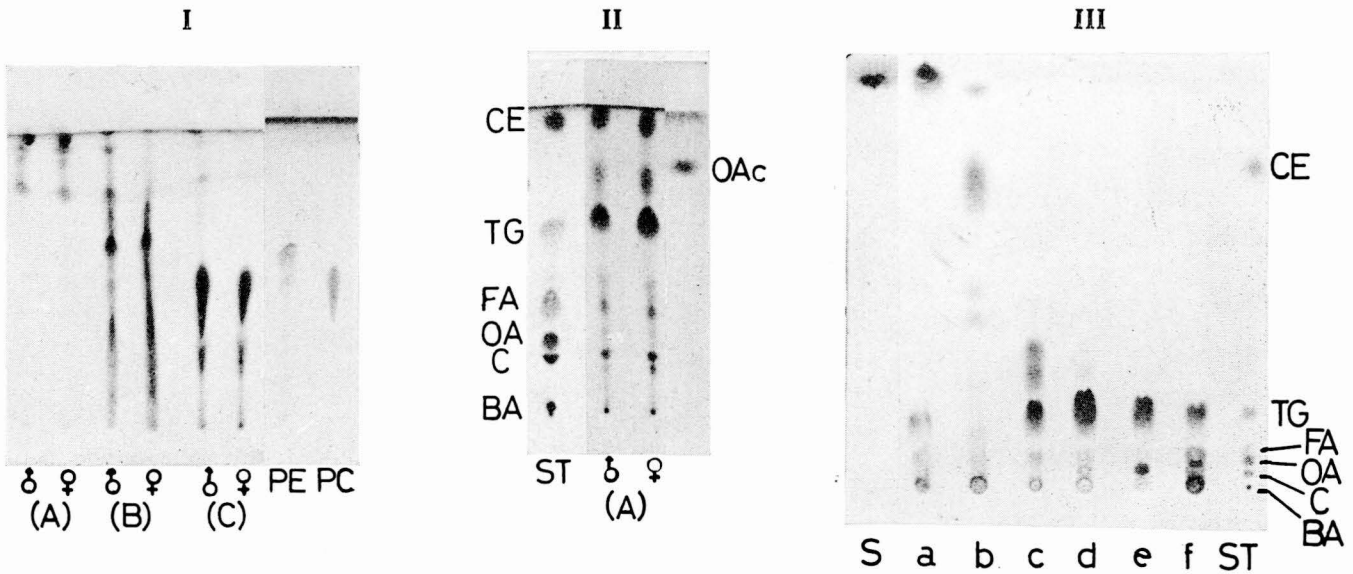

Fig. 1-I Thin layer chromatograms (TLC) of fraction A, B and C (in this text) from the preputial glands of rats. Fraction A contained the most neutral lipids. B and C; phospatidyl ethanolamine (PE) and phospatidyl choline (PC) were identified,respectively. Solvent system used was chloroform : methanol : water (95 : 35 : 4).

II TLC of fraction A and standards (ST). The symbols at the left side of the chromatogram are standards : cholesterol palmitate (CE), tripalmitoyl glycerol (TG), palnitic acid (FA), octadecyl alcohol (OA), cholesterol (C) and bathyl alcohol (BA). (OAc) is octadecyl acetate. Solvent system used was petroleum ether : ethyl ether : acetic acid (90 : 20 : 1).

III TLC of fractions a, b, c, d, e and f (in this text). The major lipids of each fraction : (a) hydrocarbon ; squalene, (b) cholesterol ester (c) unknoen and triglyceride, (d) triglyceride, (e) triglyceride and cholesterol and (f) more polar lipids. Their symbols are the same as II, and (s) is squalene. Solvent system used was petroleum ether : ethyl ether : acetic acid (95 : 5 : 1).

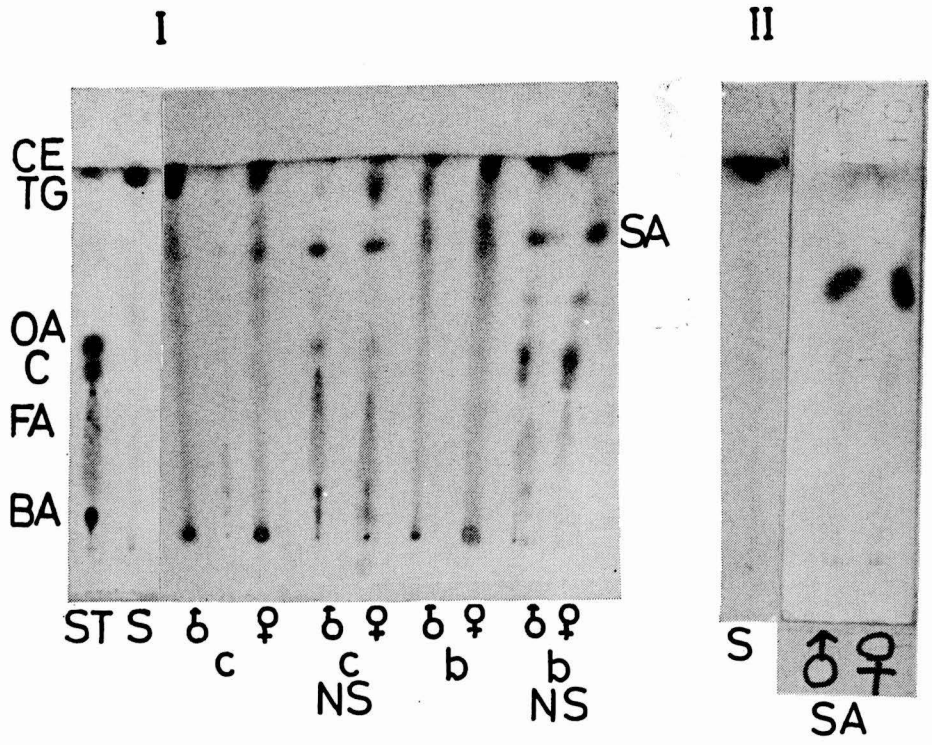

Fig. 2-I TLC of fractions b and c, and these nonsaponifiable fractinos (NS). The symbols are the same with Fig. 1-II. (SA) is squalene alcohol. Solvent system used was benzene : ethyl acetate (7 : 1).

II TLC of purified squalene alcohol. Solvent system used was the same with I.