Pyrolytic Formation of Polyaromatic Hydrocarbons From Steroid Hormones

Alfred A. Christy*, Monica I. Lian and George W. Francis

*Department of Science, Faculty of Engineering and Science, University of Agder, Serviceboks 422, N-4604 Kristiansand, Norway.

Department of Chemistry, University of Bergen, Allégaten 41, 5007 Bergen, Norway.

Abstract

Four steroid hormones namely androsterone, cholesterol, estrone and estradiol have been pyrolysed at 300, 400 and 500 ^{o}C and the pyrolysates from these have been analysed by GC-MS. The results indicate that these formed different products under the pyrolysis and most of them evolved into polycyclic aromatic hydrocarbons during their residence in the pyrolysis chamber at high temperatures. The products from the pyrolysates at all temperatures have been analysed for similarities and differences using multivariate data analysis. The products possessed some similarities on pyrolysis at 300 ^{o}C but were entirely different when pyrolysed at 500 ^{o}C. Androsterone and cholesterol formed a higher percentage of substituted PAH than estrone and estradiol. These compounds included carcinogens such as phenanthrene, methylphenanthrene, fluorene and its derivatives. The side chain of cholesterol had no effect on the products formed, while the presence of the aromatic ring in estrone and estradiol led to a higher percentage of phenol and its derivatives in the pyrolysates. Furthermore, estrone has been subjected to flash pyrolysis and the products formed have been compared with those which resulted from the long time pyrolysis: flash pyrolysis produced small amounts of PAH.

* Corresponding author.

Keywords

Pyrolysis, steroid hormones, polyaromatic hydrocarbons, GC-MS, chemometrics

Introduction

Steroids are products of the same biosynthetic pathway as the triterpenes and squalene acts as a precursor for both classes of compound. The steroids are an important class of secondary metabolites and include sterols, bile acids, cardiac aglycones, steroid hormones, adrenal steroids, the toad poisons, and the steroid sapogenins (Noller, 1966). The compounds share the steroid ring system (Hart, 1991) as shown in Fig. 1, with four conjoined rings where the first three have six, and the last five carbon atoms.

The steroid hormones can be grouped according to their functions and the largest group contains the male and female sex hormones (androgenes and estrogens respectively). All of these hormones are produced in specialized body tissues and after binding to specific carrier proteins are transported in the blood stream to the sites where they are to function (Cox, Nilson & Lehninger, 1993). The steroid hormones are soluble in fats, and migrate easily through cell membranes and into the cell cytosol. Estradiol (D) is one of the most important female sex hormones and it is produced in the ovaries. Estrone (C) is close to estradiol in molecular structure, the -OH group in estradiol at position 17 is replaced by a C=O group in estrone. Androsterone (A) is one of the male hormones responsible for considerable hormone activity and is produced in the testes. The first ring is not aromatic in male hormones like androsterone (A).

Cholesterol (B) is the most predominant sterol in animal body tissue. It is concentrated in the brain, the spinal cord, skin secretions and gallstones. It is mostly produced in the liver and is the precursor for the biosynthesis of steroid hormones in the sexual organs and adrenal cortex. It is notorious for its precipitation as gallstones and tendency to deposit along the walls of the arteries (Butler & Berlein, 1972) and often pointed to as a cause of coronary heart disease.

The first ring is not aromatic in cholesterol although it should be noted the molecule has a long side chain at position 17.

A preliminary study revealed that the thermal degradation of cholesterol provided a variety of different products although these were dominated by polycyclic aromatic hydrocarbons (PAH). Some of the products identified were carcinogenic and known to demonstrate mutagenic activity. During cooking, for example frying, smoking and grilling, steroid hormones might possibly decompose and to form polycyclic aromatic hydrocarbons (PAH). The carcinogenic effects of PAH are well established (Luch, 2005) and although, the concentrations of these PAH components might seem harmless, their cumulative effects could be dangerous. A survey of the literature revealed that a study of the pyrolysis of steroid hormones has not yet been undertaken.

The aim of this work was to examine the pyrolysis products of four selected steroids, namely androsterone, cholesterol, estrone and estradiol. The last two are female sex hormones and their molecules contain an aromatic ring. Androsterone and cholesterol lack the aromatic ring in their molecules, while cholesterol has a long aliphatic side chain at position 17. These were selected to study the effect of the presence of the aromatic ring and side chain on the products formed during pyrolysis.

.

Pyrolysis is the decomposition of materials by heat. Different types of pyrolytic methods are used for the thermal decomposition of substances. A detailed description of a variety of different pyrolytic techniques can be found in Voorhees (1984). The two reactions normally involved in the pyrolyis of organic compounds are often designated as pyrolysis and pyrosynthesis (Lee, Novotny & Bartle, 1981). During the first process, pyrolysis, the molecules are cleaved into

smaller unstable molecular particles, mostly comprising radicals. When the residence time is long these unstable entities may evolve into stable products of larger molecular weights in a process designated pyrosynthesis.

Experimental

Samples, Pyrolysis and GC-MS analysis

The compounds used in the pyrolysis experiments were cholesterol, androsterone, estrone and estradiol. Cholesterol (>97%) was purchased from Fluka Chemie AG. Androsterone (>97%), estrone (>99%) and estradiol (>97%) were purchased from Aldrich Chem.

Several ampoules, each of length 170 mm were made from quartz glass tubes with wall thickness of 1.7 mm and internal diameter of 13.6 mm. One end of the ampoule was drawn out in order to facilitate sealing. Aliquots of 25 mg of cholesterol, androsterone, estrone and estradiol were quantitatively weighed into four pre-weighed ampoules. The narrow ends of the ampoules were sealed and aluminum identity tags attached.

The ampoules were then placed in an electric oven set at and preheated to 300 ^{o}C. The pyrolysis of the samples was carried out for seven hours. The time of seven hours was selected to allow pyrolysis during the same working day. At the end of the pyrolysis, the ampoules were removed from the oven and placed in a drying oven set at 100 ^{o}C for 30 minutes in order to minimize stress on the walls of the ampoules. After the 30 minute cooling period, the ampoules were removed and placed on a test tube rack for further cooling. The products of the decomposed samples were then carefully extracted with l ml portions of hexane which had been shown to be an efficient solvent for the products formed in a preliminary study carried out on cholesterol. The

pyrolysis experiments were repeated with sets of fresh samples of the same four compounds at temperatures of 400 and 500 ^{o}C.

The GC-MS analysis was carried out on a Hewlett Packard (HP) 5890 series II gas chromatograph connected to a HP 5971A quadrupole mass spectrometer. The separation of the components in the sample was carried out on a 30 m x 0.25 mm I.D fused silica column coated with 0.25 μm 100% dimethylpolysiloxane stationary phase. The oven was temperature programmed from 50 ^{o}C, after a 3 minutes hold time, at 5 ^{o}C /min up to 250 ^{o}C. The temperature gradient was then changed to 20 ^{o}C /min up to 300 ^{o}C. Helium gas was used as the carrier gas.

The total ion chromatograms of the pyrolysates were acquired using the HP G1034C chemstation program. The peaks in the total ion chromatogram were then identified using a mass spectral library (NIST 98). The library identifications were further clarified and confirmed by examining and comparing the fragmentation patterns obtained with the molecular structure of the starting material.

The flash pyrolysis was carried out on a Pyrola 2000 instrument. A few μg of the estrone sample were placed in the flash pyrolysis instrument which was programmed to raise the temperature to 1080 ^{o}C in 8 milliseconds. The temperature was held at 1080 ^{o}C for 2 seconds. Separation of the components was carried out on a HP 5890 series II gas chromatograph equipped with a 30 m x 0.25 mm I.D. fused silica column containing 0.25 μm stationary phase *(*35% phenyl siloxane and 65% dimethyl polysiloxane). The detection of the components was carried out on a HP 5972 quadrupole mass spectrometer. The chromatograph oven was temperature programmed from 50

^{o}C, after a 2 minutes hold time, at 10 ^{o}C /min up to 150 ^{o}C**.** The temperature gradient was then changed to 25 ^{o}C/min up to 340 ^{o}C. The temperature was held at 340 ^{o}C for two minutes. Helium was used as the carrier gas. The peaks in the total ion chromatogram were identified using the procedure mentioned above.

Pyrolysis over an extended time (7 hours) was used here to study the products evolved during the pyrolysis period. Flash pyrolysis, as the name suggests, a technique that quickly raises the temperature of a sample to a higher level for decomposition, was used in an additional experiment to pyrolyse estrone. The technique gives only the products of decomposition. The products can be compared with the evolution products from the extended pyrolysis. We have selected GC-MS as the analytical technique for the identification and analysis of the pyrolysis products.

Data Analysis

When the products formed are large in number, comparison between the products formed from different samples becomes difficult. A chemometric technique, namely Principal Component Analysis (PCA), was used to identify similarities and relationships between the products formed. The theoretical basis and applications of the PCA technique can be found in Christy, Ozaki & Gregoriou (2001).

The peaks in the total ion chromatograms from the pyrolysates of all the samples including the flash pyrolysed sample were area integrated and recorded. A table containing the peak areas of the components identified as variables and the temperatures of pyrolysis as samples was

prepared. This table was used to acquire information regarding the nature of the components formed during the pyrolysis and as a data matrix for the multivariate data analysis.

The data analysis was performed using the SIRIUS multivariate data analysis program (Kvalheim & Karstang, 1997) on the data matrix of area percentages of the components. Since the numbers in the table indicate the percentage data of the components identified in the total ion chromatograms, pre-processing of the data was not necessary.

Results

Table 1 presents the concentrations as peak percentages of the major polycyclic aromatic hydrocarbons (PAH) found in the pyrolysates of all four steroids studied. The total ion chromatograms of the estrone samples pyrolysed at 300, 400 and 500 ^{o}C for seven hours are shown in Figures 2 a, b and c respectively, while those of the cholesterol samples pyrolysed at 300, 400 and 500 ^{o}C are shown in Figures 3 a, b and c respectively. An important aspect of this work was to find the extent of the formation of PAH during the pyrolysis and the percentages of the substituted and unsubstituted PAH in the total ion chromatograms are given in Table 2. Methylfluorene, phenanthrene and methylphenanthrene have proven mutagenic activity in human beings (Luch, 2005) and the extent of these hydrocarbons in the pyrolysates is presented for all the samples in Table 3.

Chemometric Results

Manual comparison of several variables for the purpose of similarity identification of samples is difficult if not impossible. Chemometric analysis makes this type of comparison simple.

The principal component analysis of the data matrix using temperatures as samples and peak areas as variables showed that 82% of the variance in the data could be explained by two principal components. A plot describing the scores of the samples on the plane spanned by the first two principal components is shown in Fig. 5. The letters A, B, C and D indicate the samples androsterone, cholesterol, estrone and estradiol respectively. The score plot shows that the products formed during the pyrolysis of these samples are similar when the temperature is low. When the temperature is high, the products formed differ. However, the products formed from the sample pair A and B are similar, as are those for the sample pair C and D. The samples on the score plot suggest that the samples A and B lie along PC1 (Principal Component 1) and the samples C and D lie along the direction of PC2. The samples A and B have almost zero score on PC2 and samples C and D have almost zero scores on PC1. Thus, the samples A and B have variables significantly different from the samples C and D. This difference arises where products are missing in one group of samples relative to the other or where the peak areas of the common components vary by several orders of magnitude from one group to the other. This observation was true for the products formed at pyrolysis temperatures of 500 ^{o}C**.** The same was true for the products formed at 400 ^{o}C**.**

A score plot from the principal component analysis between the samples and products formed at 300 ^{o}C is shown in Fig. 6. In this score plot, the samples A and D resemble each other in terms of the products formed compared to samples B and C. A similar score plot for samples and a loading plot for variables are shown in Fig. 7 for the case of pyrolysis at a temperature of 500 ^{o}C. Here the samples A and B lie close together and the samples C and D lie close together. This again shows the similarity in the products formed from the samples lying close to each other. The loading plot (variable plot) shows the variables that influence the samples to their specific

location in space, for example the variables 1, 5, 11, 33, 42, 58, 84 and 91 are dominant in the products from samples A and B. Likewise the variables 20, 28, 65 and 97 are dominant in the samples C and D.

The chemometric analysis also reveals that the side chain at position 17 on cholesterol has little influence on the products formed at 400 and 500 ^{o}C. However, at 300 ^{o}C, aliphatic compounds were detected in cholesterol compared to androsterone and the other steroids. At higher temperatures, cleavages and rearrangements resulted in formation of aliphatic molecules.

Discussion

Polycyclic aromatic hydrocarbons are known to be formed during incomplete combustion of organic compounds, especially at high temperatures (Pacakova & Leclercq, 1991)]. As previously noted, there are two processes, pyrolysis and pyrosynthesis involved in these transformations. Some simple PAH compounds can undergo many pyrosynthetic reactions (Lee, Novotny and Bartle, 1981). Long residence times at high temperatures allow an increase in the secondary reactions that can lead to larger PAH hydrocarbons (Cunliffe & Williams, 1998). However, the extent and types of PAH formed depend strongly on the chemical nature of the sample undergoing pyrolysis.

Thus the steroids examined in the present study are expected to have decomposed into smaller unstable molecular particles first and then undergone pyrosynthesis leading to the formation of stable PAH hydrocarbons during the long residence time in the pyrolysis chamber.
A comparison of the total ion chromatograms of the pyrolysed samples clearly shows the differences in the products formed. The analysis of the total ion chromatograms of all the

pyrolysed samples showed that there were hundreds of compounds formed during the pyrolysis at all three temperatures. A closer look at the Tables 1 and 2 clearly shows the formation of significant amounts of PAH after pyrolysis in the case of all four steroids. These PAH contained a higher proportion of substituted PAH than of unsubstituted PAH. Furthermore, androsterone and cholesterol formed a higher proportion of substituted PAH than of unsubstituted PAH at all three pyrolysis temperatures. Estrone and estradiol formed higher proportions of unsubstituted PAH compared to substituted PAH at 500 ^{o}C. At 400 ^{o}C, all of the steroids formed more substituted than unsubstituted PAH.

PAH hydrocarbons such as methylfluorene, phenanthrene and methylphenanthrene were present in all the pyrolysates. The data presented in the tables show without any doubt that cholesterol formed higher proportions of methylfluorene, phenathrene and methylphenathrene compared to the other three steroids, although all of the steroids examined produced these compounds at 400 ^{o}C. Cholesterol was decomposed and transformed into these compounds at 400 ^{o}C. Table 3 also shows that cholesterol led to formation of methylphenanthrene even at a temperature as low as 300 ^{o}C. This was an indication that the formation of the other two compounds, methylfluorene and phenanthrene from cholesterol, might start at a temperature lower than 400 ^{o}C.

The flash pyrolysis of estrone gave rise to a very small percentage of methylfluorene and phenanthrene, possibly reflecting the very limited residence time and the consequent limitations on pyrosynthesis, but the significance of this is as yet unknown.

Mechanism

The mechanism of formation of phenanthrene and methylphenathrene from cholesterol proceeds by way of the intermediate cholesta-3,5-diene (Badger, Donnelly & Spotswood, 1965). The cholesta-3,5-diene then undergoes cleavage, condensation and rearrangement leading to phenanthrene or methylphenathrene (Fig. 4). The methyl group in 1-methylphenanthrene can migrate to provide the more stable 2-methylphenanthrene during heating (Kvalheim et al., 1988). The formation of anthracene and its derivatives involves a rearrangement of the ring systems (Britt et al., 2001; Britt et al., 2003; Rushdi et al., 2003). Methylfluorene can also be formed from cholesta-3,5-diene (Fig. 4). The possible mechanism should involve the cleavage and condensation of the side chain in cholesterol. The formation of toluene in the pyrolysis of cholesterol at 400 and 500 ^{o}C supports this route. This is further reinforced by the fact that the products of pyrolysis of cholesterol at 300 ^{o}C do not include either fluorene or toluene.

Analysis of the products at all pyrolysis temperatures revealed that phenol is formed in significant amounts from estrone and estradiol as compared with formation from cholesterol and androsterone. This indicates that the aromatic ring A in estrone and estradiol was responsible for the formation of phenol in the products. The formation of phenol was not due to the presence of oxygen during the pyrolysis. This fact was supported in the case of estrone by the results of flash pyrolysis in a nitrogen atmosphere which gave phenol as one of the products.

The formation of PAH during the pyrolysis of steroids, especially cholesterol is interesting and of concern. Cholesterol is found in meat in considerable amounts and during frying and grilling of meat cholesterol decomposes and evolves into polycyclic aromatic hydrocarbons. Even although, there have been several reports on the formation of PAH in fried and grilled meat, the fact that

cholesterol might be one of the components in meat that contributed to the formation of these unwanted harmful substances was not brought to light.

Conclusion

The pyrolysis of androsterone, cholesterol, estrone and estradiol at 300, 400 and 500 ^{o}C has been studied. Polycyclic aromatic hydrocarbons make increasing contributions to the products formed during pyrolysis, and are of significant importance at 400 and 500 ^{o}C**.**

At 500 ^{o}C androsteron and cholesterol form a higher percentage of substituted PAH compared to estrone and estradiol, which form a higher proportion of unsubstituted PAH. All four steroids form phenanthrene, methylphenanthrene and methylfluorene at the pyrolysis temperature of 500 ^{o}C, while at 400 ^{o}C cholesterol produces significantly larger amounts of PAH, than do the other steroids.

The side chain on cholesterol seems to have little direct influence on the products formed. The chain is probably easily cleaved during pyrolysis and does not influence significantly the behaviour of the rest of the molecule. However, higher product concentrations of phenol and its alkyl derivatives found in estrone and estradiol than is the case for cholesterol and androsterone, may reflect the presence of an aromatic ring in the former compounds.

The present results show for the first time, that a significant contribution to the PAH found in fried or grilled food stuffs may be made by cholesterol. Grilling of fatty food may instead of stripping fat, actually contribute to the formation of harmful carcinogenic substances.

References

Badger, G. M., Donnelly, J. K., Spotswood, T. M. (1965) The formation of aromatic hydrocarbons at high temperatures. *Australian Journal of Chemistry*, 18, 1249-1266.

Britt, P. F., Buchanan, A. C., Kidder, M. M, Owens, C, Ammann, J. R., Skeen, J. T. and Luo, L. (2001) Mechanistic investigation into the formation of polycyclic aromatic hydrocarbons from the pyrolysis of plant steroids. *Fuel*, 80, 1727-1746.

Britt, P. F., Buchanan, A. C., Kidder, M. K., Owens, C. V. (2003) Influence of steroid structure on the pyrolytic formation of polycyclic aromatic hydrocarbons. *Journal of Analytical & Applied Pyrolysis,* 66, 71-95.

Butler, G. B., Berlein, K. D. (1972) Fundementals of Organic Chemistry, (p. 687 and p. 925.). New York: The Ronald Press Company.

Christy, A. A., Ozaki, Y., Gregoriou, V. G. (2001) Modem Fourier Transform Infrared Spectroscopy, (p. 285), Amsterdam: Elsevier Science.

Cox, M. M., Nilson, D. L., Lehninger, A. L. (1993) Principles of Biochemistry, (p. 254 and p. 744.). New York: Worth Publishers.

Cunliffe, A. M., Williams, P. T. (1998) Composition of oils derived from the batch pyrolysis of tyres. *Journal of Analytical & Applied Pyrolysis*, 44**,** 131-152.

Hart, H. (1991) Organic Chemistry, 8th Edn, (pp. 419-423). Boston: Hougton Mifflin Company.

Kvalheim, O. M., Christy, A. A., Telnæs, N., Bjørseth, A. (1988) Maturity determination of organic matter in coals using the methyl phenanthrene distribution. *Geochimica et Cosmochimica Acta*, 51, 1883-1888.

Kvalheim, O. M., Karstang, T. V. (1997). A general-purpose program for multivariate data analysis. *Chemometrics & Intelligent Laboratory Systems*, 2, 235–237.

Lee, M. L., Novotny, M. V., Bartle, K. D. (1981) Analytical Chemistry of Polycyclic Aromatic Compounds, (p. 441). Columbus, Ohio: Academic Press.

Luch, A. (2005) The Carcinogenic Effects of Polycyclic Aromatic Hydrocarbons, London, UK: Imperial College Press.

Noller, C. R. (1966) Chemistry of Organic Compounds, 3rd Edn. P. 972, Philadelphia and London: W.B. Sounders Company.

Pacakova, V., Leclercq, P. A. (1991). Gas chromatography-mass spectrometry and high performance liquid chromatographic analyses of thermal degradation products of common plastics. *Journal of Chromatography*, 55, 229-237.

Rushdi, A. I., Ritter, G., Grimalt, J. O., Simoneit, B. R. T. (2003) Hydrous pyrolysis of cholesterol under various conditions. *Organic Geochemistry*, 34, 799-812.

Voorhees, K. J. (1984) *Analytical Pyrolysis*, (p. 196). London: Butterworth & Co.

Figure captions

1. The steroid ring system and the steroid hormones androsterone (A), cholesterol (B), estrone (C) and estradiol (D).
2. The GC-MS total ion chromatogram of the pyrolysates from estrone at 300 (2a), 400 (2b) and 500 ^{o}C (2c). The numbers indicate some of the products presented in Table 1.
3. The GC-MS total ion chromatogram of the pyrolysates from cholesterol at 300 (3a), 400 (3b) and 500 ^{o}C (3c). The numbers indicate some of the products presented in Table 1.
4. The pathways of formation from cholesterol to phenanthrene, methylphenanthrene, fluorene and methylfluorene.
5. A score plot showing the scores of the samples pyrolysed at 300, 400 and 500 ^{o}C. The letters A, B, C and D indicate the samples androsterone, cholesterol, estrone and estradiol respectively.
6. A score plot (6a) and a loading plot (6b) of the samples pyrolysed at 300 ^{o}C. The letters A, B, C and D indicate the samples androsterone, cholesterol, estrone and estradiol respectively.

7. A score plot (7a) and a loading plot (7b) of the samples pyrolysed at 300 ^{o}C. The letters A, B, C and D indicate the samples androsterone, cholesterol, estrone and estradiol respectively.

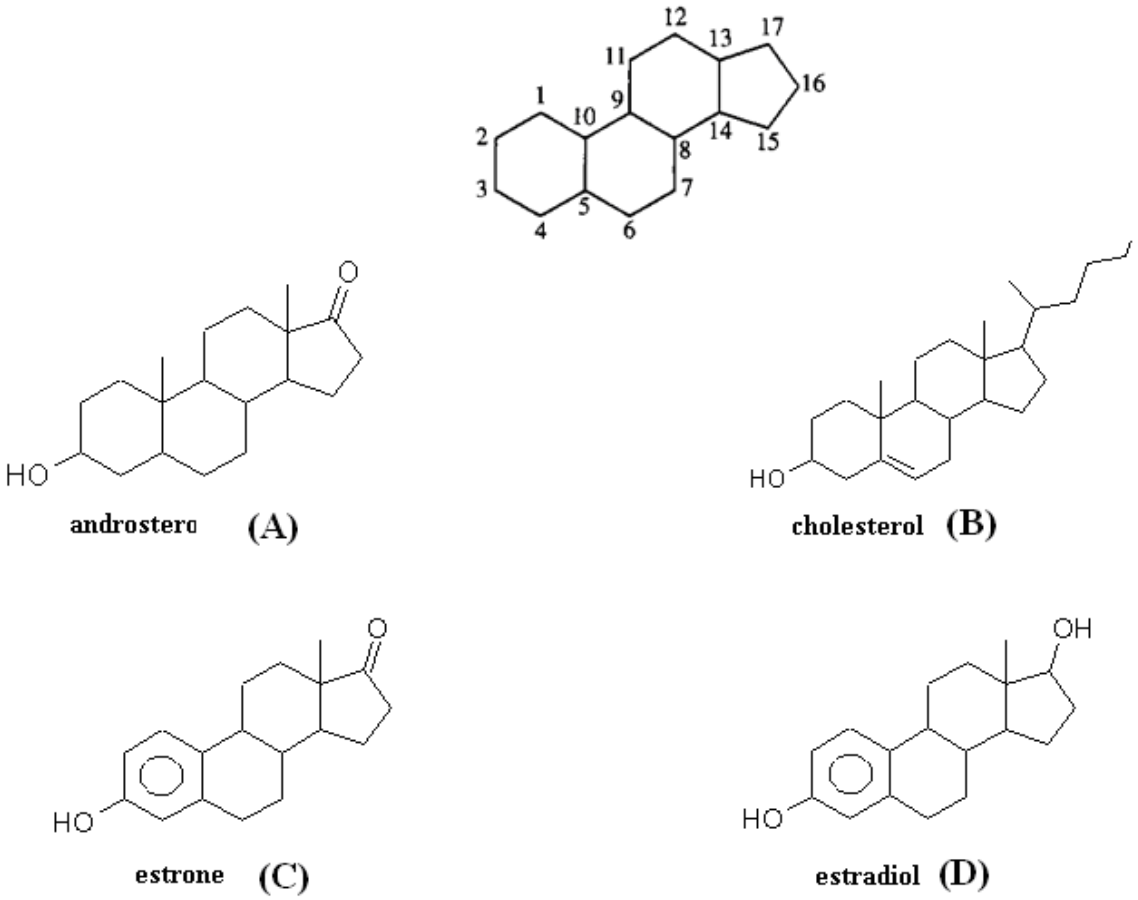


Fig. 1. The steroid ring system and the steroid hormones androsterone (A), cholesterol (B), estrone (C) and estradiol (D).

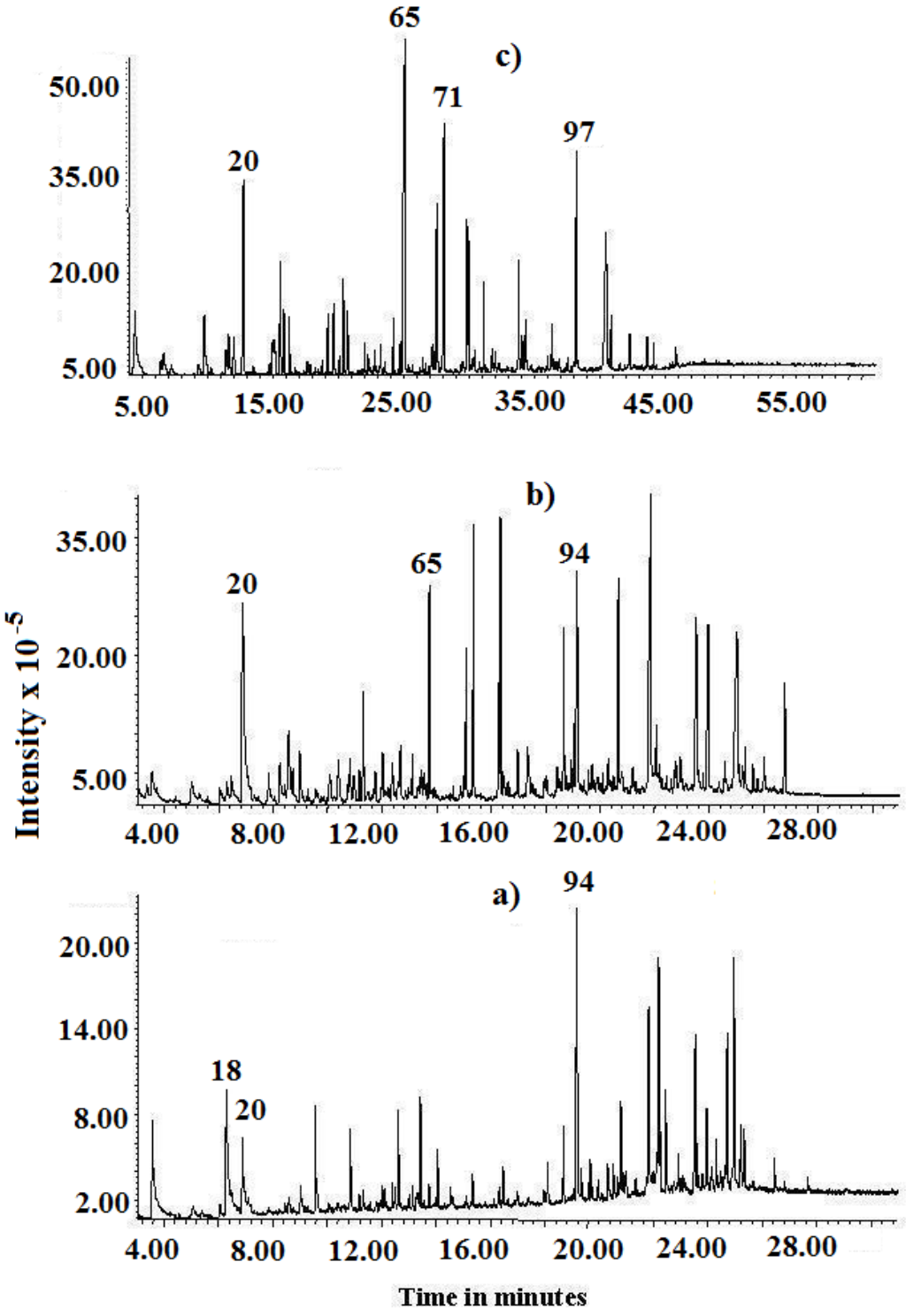


Fig. 2. The GC-MS total ion chromatogram of the pyrolysates from estrone at 300 (2a), 400 (2b) and 500^{o} C (2c). The numbers indicate some of the products presented in Table 1.

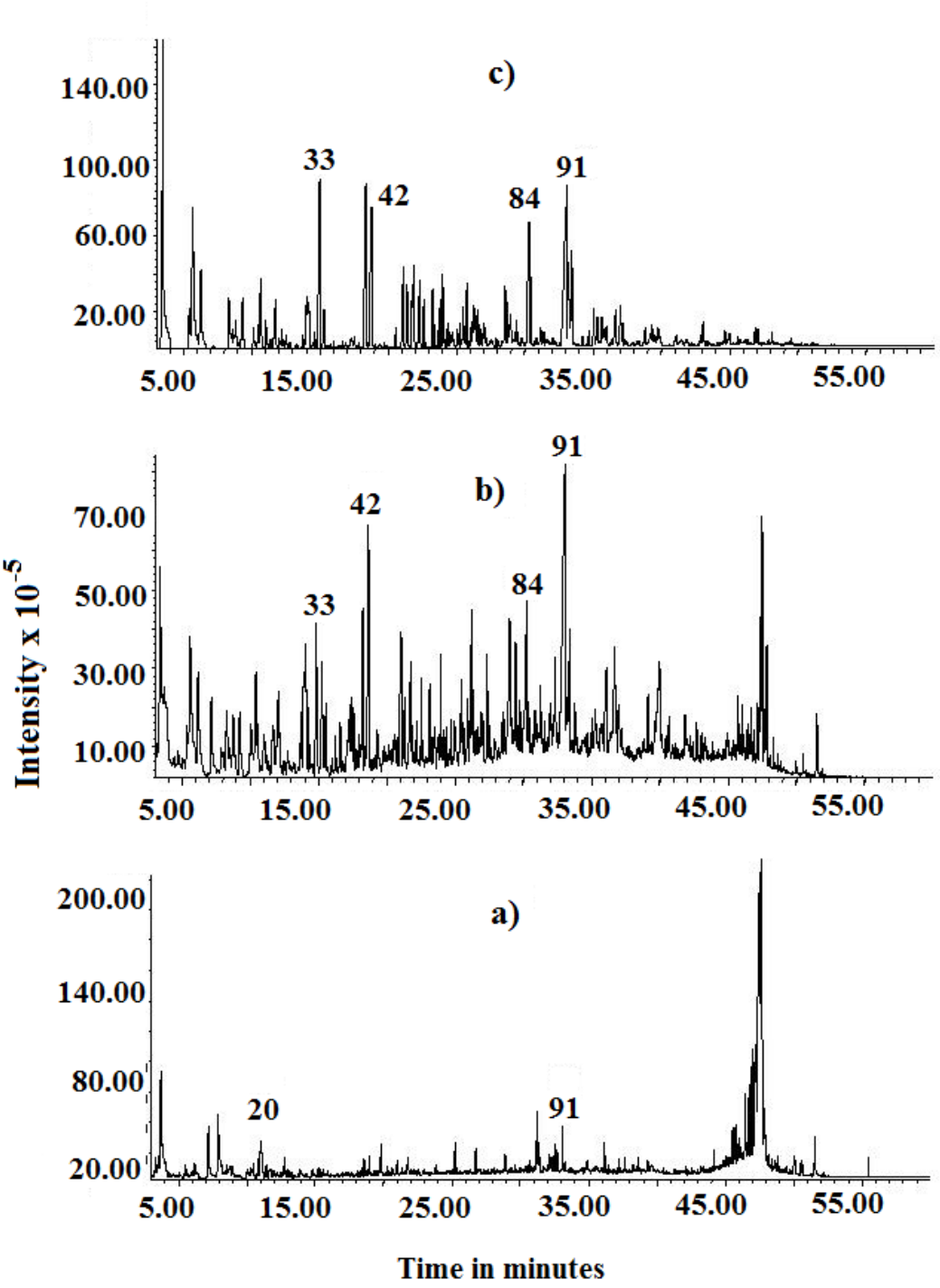


Fig. 3. The GC-MS total ion chromatogram of the pyrolysates from cholesterol at 300 (3a), 400 (3b) and 500^{o} C (3c).

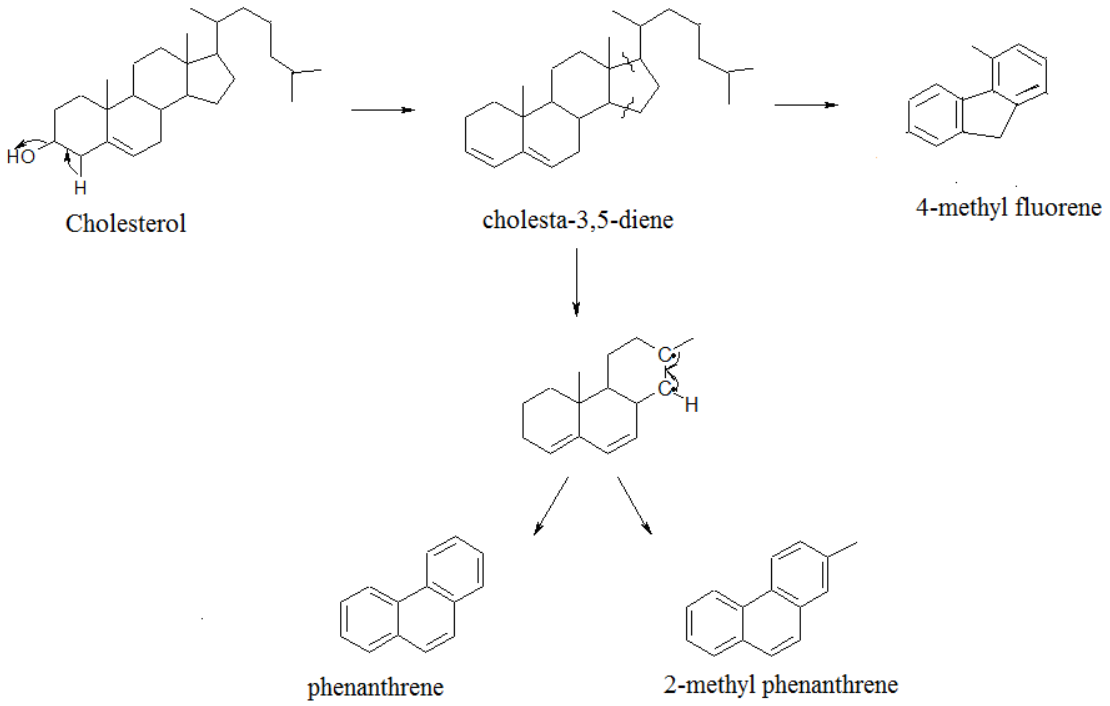


Fig. 4. The pathways of formation from cholesterol to phenanthrene, methylphenanthrene and methylfluorene.

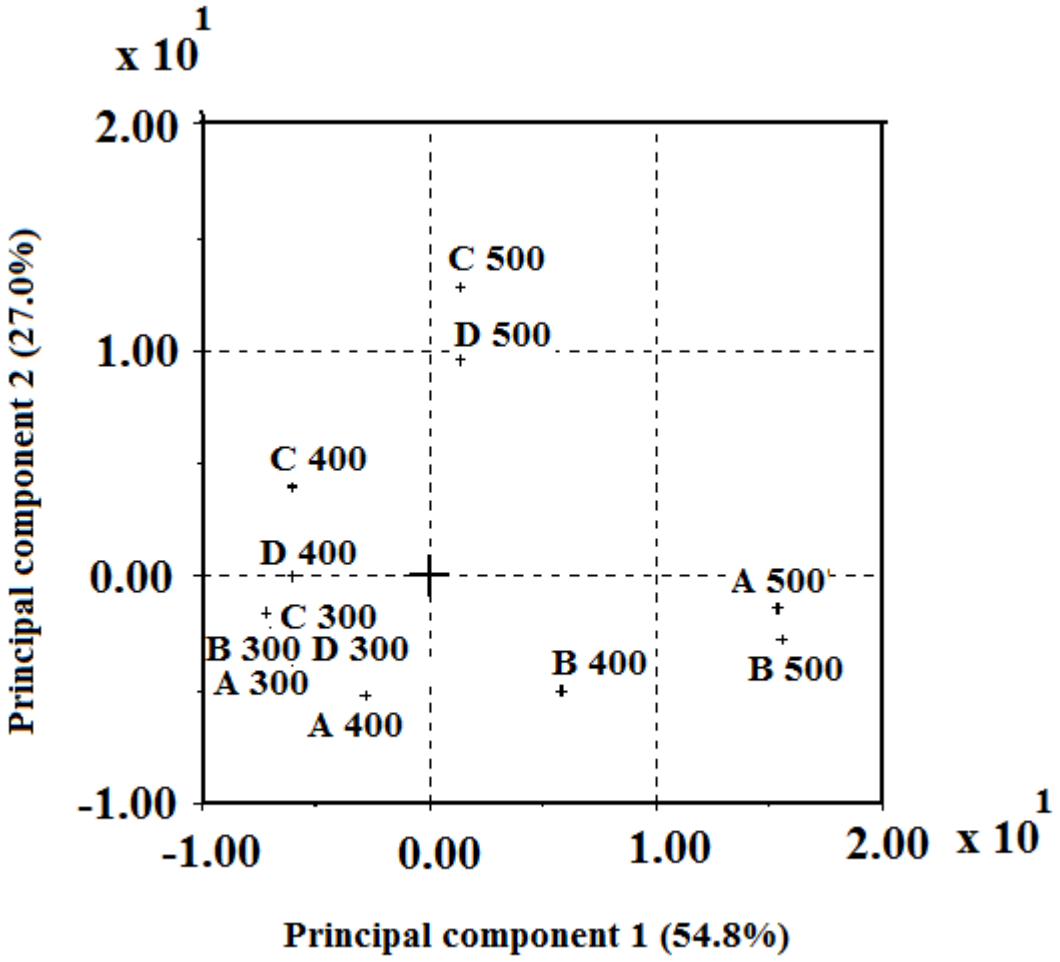
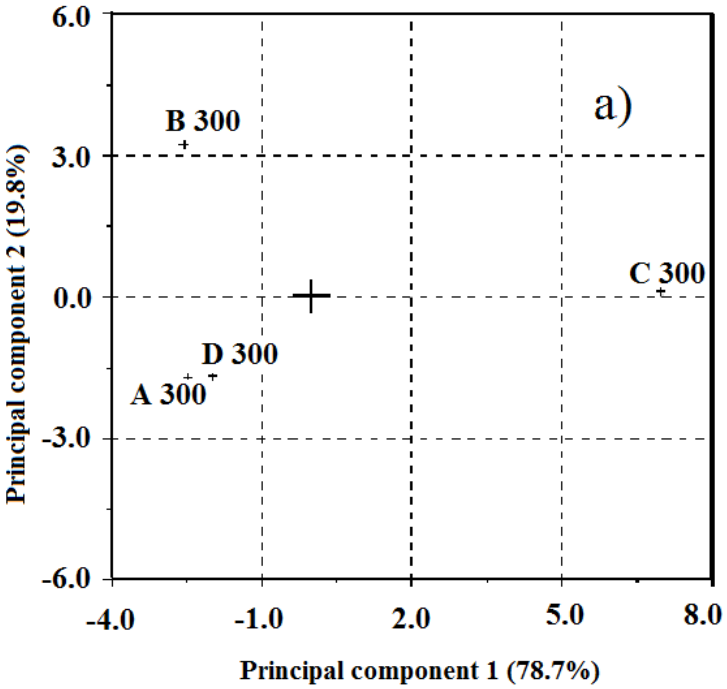


Fig. 5. A score plot showing the scores of the samples pyrolysed at 300, 400 and 500^{o}C. The letters A, B, C and D indicate the samples androsterone, cholesterol, estrone and estradiol respectively.


a)
B 300
C 300
D 300
A 300
6.0
3.0
0.0
-3.0
-6.0
-4.0
-1.0
2.0
5.0
8.0
Principal component 2 (19.8%)
Principal component 1 (78.7%)

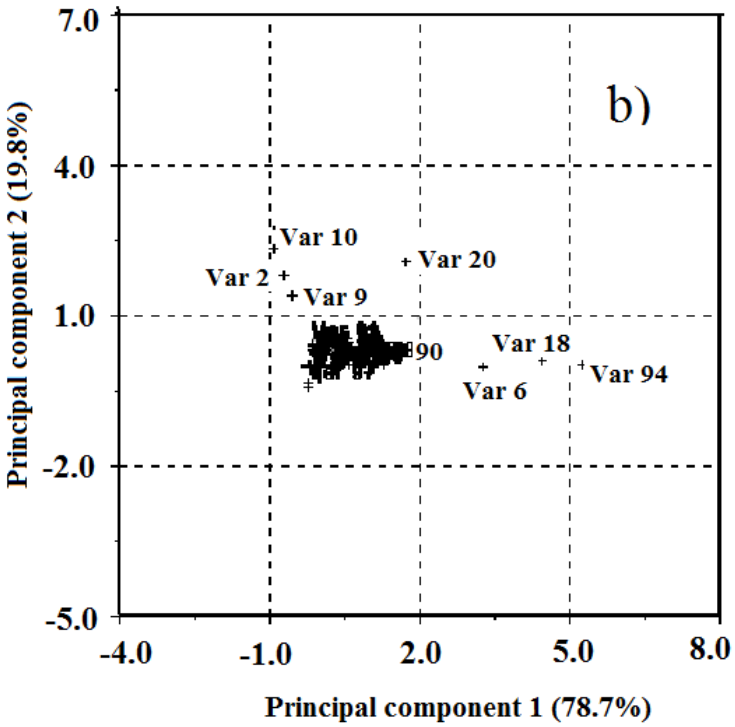
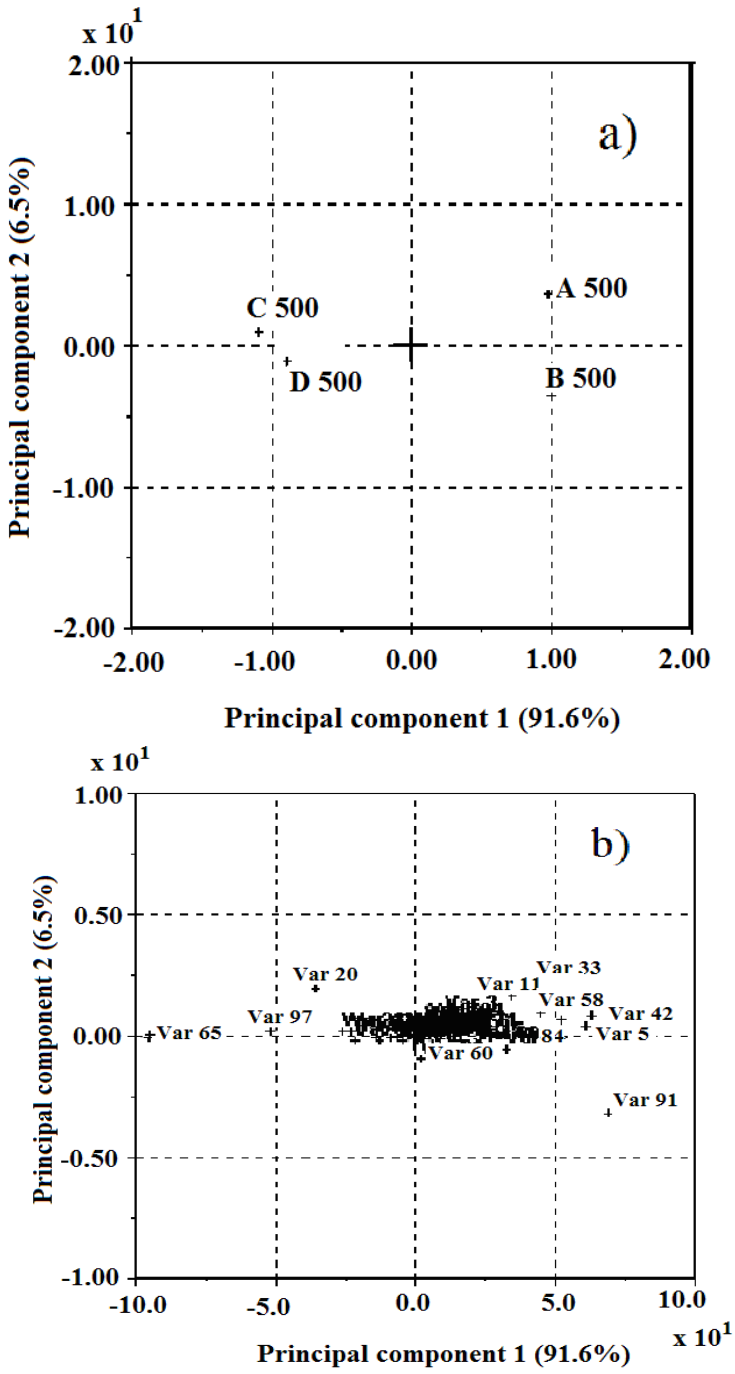


Fig. 6. A score plot (6a) and a loading plot (6b) of the samples pyrolysed at 300^{o}C. The letters A, B, C and D indicate the samples androsterone, cholesterol, estrone and estradiol respectively.



\Fig. 7. A score plot (7a) and a loading plot (7b) of the samples pyrolysed at 300^{o}C. The letters A, B, C and D indicate the samples androsterone, cholesterol, estrone and estradiol respectively.

Table 1. Some of the polycyclic aromatic hydrocarbons identified in the pyrolysates. The numbers in the colums
indicate the area percent of the peaks.

	300 degrees				400 degrees				500 degrees				108 degr
	A	B	C	D	A	B	C	D	A	B	C	D	C
(13) Benzofuran												0,08	
(17) Indene						0,4			2,08	1,6	1,24	1,22	
Indan, 1-methyl-					0,89	2,14	0,42	0,16					
(25) Benzofuran, 2-methyl- (25) Benzofuran, 7-methyl-									0,12	0,36			
(29) 1H-Indene, 2,3-dihydro-4-methyl- (29) 1H-Indene, 2,3-dihydro 5-methyl-						0,57		0,4	0,24	0,25	0,24	0,33	
(30) 1H-Indene, 1-methyl-						1,41			2,62	1,95	1,94	1,89	
(30) 2-Methylindene									0,23	0,94			
(33) Naphthalene		0,31				1,45			7,39	4,49	1,64	1,34	
(34) 1H-Indene, 1,1-dimethyl- (34) 1H-Indene, 1,3-dimethyl- (34) 1H-Indene, 2,3-dimethyl-				0,29		1,82			0,29	0,6	0,32	0,38	
(40) 2-Ethyl-1-H-indene									0,09	0,13			
(42) Naphthalene, 1-methyl- (42) Naphthalene, 2-methyl-		0,51			1,39	4,04			9,26	8,43	2,4	2,08	
(45) 1H-Indenol									0,08	0,09	1,46	1,6	
(53) 1H-Indene, 1,1,3-trimethyl-	0,82					0,4							
(56) Naphthalene, 1-ethyl- (56) Naphthalene, 2-ethyl-		0,24				1,42			1,83	1,69	0,58	0,66	
(57) 1,2,3-Trimethylindene	0,05												
Naphthalene, 1,2-dimethyl- Naphthalene, 1,3-dimethyl- Naphthalene, 1,4-dimethyl- Naphthalene, 1,5-dimethyl- Naphthalene, 1,6-dimethyl- Naphthalene, 1,7-dimethyl- (58) Naphthalene, 1,8-dimethyl- Naphthalene, 2,3-dimethyl- Naphthalene, 2,6-dimethyl- Naphthalene, 2,7-dimethyl-					3,37	3,03			6,22	4,96	1,01	1,26	
(62) Naphthalene, 2-(1-methylethyl)-					0,29	0,3				0,05			
(63) Acenaphthene						0,55			0,96	0,84	0,92	0,97	
(65)1-Naphthalenol (65) 2-Naphthalenol		0,14	0,51	0,24			3,01	2,31	2,11	2,1	12,37	10,98	
(66) Naphthalene, 1,4,6-trimethyl- (66) Naphthalene, 1,6,7-	0,25	0,11			4,76	3,61	0,16	0,22	2,05	2,69	0,24	0,24	

trimethyl- (66) Naphthalene, 2,3,6-trimethyl-													
(68) Dibenzofuran											0,13		
(69) 1-Naphthalenol, 2-methyl- (69) 1-Naphthalenol, 4-methyl-			0,66				0,35	0,36	0,13			0,17	
(70) Naphthalene, 1,2,3,4-tetramethyl-		0,11			0,93	0,54				0,11		0,06	
(71) Fluorene									1,7	1,48	0,18	0,15	
(72) 1-Isopropenylnaphthalene						0,64			0,91				
(77) Acenaphthenone									0,08				
(78) 2-Hydroxyfluorene							0,33				0,85	0,96	
9H-Fluorene, 1-methyl- 9H-Fluorene, 2-methyl- 9H-Fluorene, 3-methyl- 9H-Fluorene, 4-methyl- 9H-Fluorene, 9-methyl-		(79)				1,34			2,65	3,38	0,87	0,67	
(81) Phenanthrene, 1,2,3,4-tetrahydro- (81) Anthracene, 1,2,3,4-tetrahydro-					0,47	0,64							
(82) 1,7-Dihydroxynaphthalene								0,35					
(83) 2-Hydroxy-4 isopropylnaphthalene							0,49	0,21					
(84) Phenanthrene (84) Anthracene						2,91			4,53	5,39	1,62	1,86	
(87) 9H-Fluorene, 2,3-dimethyl-									0,78	0,99		0,08	
Anthracene, 1-methyl- Anthracene, 2-methyl- Anthracene, 9-methyl- Phenanthrene, 1-methyl- Phenanthrene, 2-methyl-	(91)	0,52			2,61	8,77			7,72	12,53	3	3,55	
(96) 1-Phenanthrenol											0,18	0,26	
(97) Benz[a]naphthalane, 2-hydroxy-				0,97		0,26	3,21	1,23	0,39	0,41	6,59	4,43	

Table 2. Percentages of unsubstituted and sustituted PAH in the pyrolysates.

Temperature	**300 deg. C**		
Steroid	**Unsub. PAH(%)**	**Subst. PAH (%)**	**Total PAH (%)**
Androsterone	0.0	1.1	1.1
Cholesterol	0.5	2.0	2.5
Estrogen	2.0	6.8	8.8
Estradiol	1.2	0.9	2.1
Temperature	**400 deg. C**		
Androsterone	0.5	16.9	17.4
Cholesterol	6.7	36.4	43.1
Estrogen	6.6	8.0	14.6
Estradiol	3.9	4.3	8.2
Temperature	**500 deg. C**		
Androsterone	14.2	37.7	51.9
Cholesterol	18.2	40.5	58.7
Estrogen	28.0	12.1	40.1
Estradiol	24.8	15.1	39.9
Temperature	**1080 deg. C**		
Estrogen	10.5	1.6	12.1

Table 3. The extent of the formation of methylfluorene, phenanthrene and methylphenanthrene.

Temperature		**300 deg. C**	
Steroid	**methyl fluorene**	**phenanthrene**	**methyl phenanth ./anthracene**
Androsterone	0.0	0.0	0.0
Cholesterol	0.0	0.0	0.5
Estrogen	0.0	0.0	0.0
Estradiol	0.0	0.0	0.0
Temperature		**400 deg. C**	
Androsterone	0.0	0.0	2.6
Cholesterol	1.3	2.9	8.8
Estrogen	0.0	0.0	0.0
Estradiol	0.0	0.0	0.0
Temperature		**500 deg. C**	
Androsterone	2.7	4.5	7.7
Cholesterol	3.4	5.4	12.5
Estrogen	0.9	1.6	3.0
Estradiol	0.7	1.9	3.6
Temperature		**1080 deg. C**	
Estrogen	0.02	0.18	0.0