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Hydrous pyrolysis of cholesterol under various conditions

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Abstract

The alteration products of cholesterol subjected to hydrothermal conditions have been analyzed by gas chromatography–mass spectrometry. Four sets of experiments were conducted at temperatures ranging between 150 and 300 °C for 24 h. The reaction mixtures of the first set included only cholesterol and water, whereas those of the second, third and fourth sets also contained oxalic acid, natural sediment and montmorillonite, respectively. The alteration rate and the number of observed alteration products increased with higher temperature and acidity of the reaction mixtures. At lower temperatures cholestenes and cholestenones were major compounds. Cholestene concentrations increased at higher temperatures in all sets and were highest at 200 °C in acidic medium, at 250 °C in the presence of sediment and at 300 °C with montmorillonite. Cholestane concentrations also increased at elevated temperatures, being greatest in the absence of both sediment and montmorillonite. Diacholestenes were detected in an acidic medium at all temperatures and with montmorillonite at >200 °C. Monoaromatic steroid hydrocarbons were found above 200 °C. Thus, backbone rearrangements were the major alteration processes and bond cleavage (cracking) was predominant in an acidic medium, whereas aromatization was enhanced in the presence of both sediment and montmorillonite. These products confirm that reductive biomarker alteration in hydrothermal systems occurs rapidly and at high temperatures (>250 °C).

1. Introduction

Steroids are ubiquitous in eucaryotes and affect an amazing array of cellular functions. The steroid skeleton has been widely used as an indicator of diagenesis and biological origin (biomarker) of organic matter (e.g., Boon et al., 1979; de Leeuw and Baas, 1986; Gagosian, 1977; Grimalt et al., 1990; Huang and Meinschein, 1976; Simoneit, 1985; Venkatesan and Kaplan, 1987; Volkman, 1986; Wakeham et al., 1980). Steroids are also source tracers for biogenic material in complex mixtures of dissolved and particulate matter in the geosphere, especially in the marine realm (e.g., Brault and Simoneit, 1988; Gaskell and Eglinton, 1975; Morris and Brassell, 1988; Taylor et al., 1981). In some cases, terrestrial riverine and eolian inputs are also an important steroid source (e.g., Gagosian, 1977; Hatcher et al., 1977; Simoneit et al., 1983; Takada et al., 1994).

Hydrous pyrolysis occurring in nature (i.e., rapid thermal alteration) is currently of great interest in research concerning such processes as hydrothermal petroleum generation (e.g., Simoneit, 1990, 1992a,b, 1993), biomass burning (e.g., Simoneit et al., 1993, 2000), and organic thermochemistry (e.g., Katritzky et al., 1990). Steroids are one group of natural product precursors which can be followed as they are converted to various derivatives by these processes. Alteration of sterols by thermal stress yields stenones, sterenes, steradienes, stanones and steranes (Mackenzie et al., 1982; Seifert and Moldowan, 1978, 1979; Brault and Simoneit, 1988; Simoneit, 1994). Sterols can be hydrogenated in

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hydrothermal systems and produce stanols (Brault and Simoneit, 1988). Currently, laboratory hydrous pyrolysis is without doubt one of the most widely applied organic geochemical techniques for artificial maturation studies of sediments (e.g., Comet et al., 1986; Eglinton et al., 1986; Huizinga et al., 1987a,b; Lewan, 1985), crude oils (e.g., Curiale et al., 1992), and individual compounds (e.g., Smith et al., 1989; Leif et al., 1992).

The first investigations on the thermal alteration of steroids were carried out with one of their major representatives, cholesterol, by Diels and Linn (1908). Research on dry pyrolysis of steroids and especially cholesterol was active until the 1950s (e.g., Heilbron and Sexton, 1928; Falk et al., 1949; de Fazio and Banchetti, 1952). Further investigations on the thermal decomposition of cholesterol under hydrothermal conditions were conducted by Tyler and Cane (1982). They treated cholesterol with supercritical steam for 30 min and the main products were cholestane and coprostane. Abbott et al. (1995) studied the thermal degradation of 5α(H)-cholestane by "dry" and "wet" (hydrous) pyrolysis. In this latter case the main products were dimethylperhydrophenanthrenes, 5α(H)-androstane, pregnanes and series of C_{22}–C_{27} steranes. A C_{27} diasterane and a C_{27} spirosterene were also recognized. The unsaturated products made up 90% of the aliphatic hydrocarbons. However, in the "dry" pyrolysis the saturated hydrocarbons dominated (>62%). Spirosterenes were absent but otherwise the same degradation products were observed with "dry" thermal alteration. These experiments helped to understand the mechanisms involved in the hydrothermal alteration of cholesterol to hydrocarbon products. However, some of the steroids observed in hydrothermally-altered sediment samples (e.g., Simoneit, 1994), which were postulated to originate from sterols, were not detectable under supercritical reaction conditions used by Tyler and Cane (1982).

The first mechanisms for clay catalyzed rearrangements of sterols were postulated by Rubinstein and Albrecht (1975). Clays and other minerals appear to have a major influence in the thermal alteration of biomarker precursors to products (Rubinstein et al., 1975; Eglinton et al., 1986; Huizinga et al., 1987a,b). However, a general survey of cholesterol and its alteration products under hydrothermal conditions (e.g., reductive hydrous pyrolysis with a clay matrix) and in the absence of bulk sediment or kerogen has not been carried out. Such data are needed to aid the interpretation of the alteration pathways of natural products to biomarkers in hydrothermal systems. Hence, in this paper we report all alteration products from cholesterol under hydrothermal conditions to provide insight on the possible transformation mechanisms at high temperatures.

2. Experimental procedures

2.1. Experiments

We used 316 stainless steel vessels constructed with Sno-Trik high-pressure couplings (Leif, 1993; Leif and Simoneit, 1995) to study the alteration products of cholesterol under hydrothermal conditions. They were capable of handling system pressure to 60,000 psi (413,682 kPa, Sno-Trik Company). The internal capacities of the vessels were 286±0.018 μL. Before each experiment, the reaction vessels, sediments and montmorillonite were placed in a glove bag and flushed with N_2 for about 5 min.

Each experimental set consisted of four series with particular reaction conditions. Series 1 was the basic mixture of doubly distilled water (Burdick and Jackson) and cholesterol (Aldrich, 98%, contains 2% cholest-4-en-3-one per GC-MS). The addition of pre-extracted solid oxalic acid (99.5%, EM Science) to series 1 gave series 2. Addition of pre-extracted natural sediment (from ODP Leg 169, Hole 1037B) and montmorillonite (K10, surface: 200±20 m^2/g, Fluka) to series 1 gave series 3 and series 4 conditions, respectively.

Aqueous oxalic acid ($C_2H_2O_4$) degrades at 160–230 °C to formic acid (CH_2O_2) and CO_2 following first order kinetics (Crossey, 1991):

$$C_2H_2O_4 \rightarrow CH_2O_2 + CO_2 \quad (1)$$

The decomposition of formic acid in the presence of excess water and at high temperatures proceeds according to reactions (2) and (3) (Palmer et al., 1993):

$$CH_2O_2 \rightarrow H_2 + CO_2 \quad (2)$$

$$CH_2O_2 \rightarrow CO + H_2O \quad (3)$$

$$2C_2H_2O_4 \rightarrow 3CO_2 + CO + H_2O \quad (4)$$

Thus, the net reaction (4) provides hydrogen for reduction. The estimated pH of the aqueous oxalic acid at 25 °C is about 0.54.

Blank experiments (i.e., without cholesterol) were carried out with all reactants to insure that the alteration products were not impurities originating from oxalic acid, doubly distilled water, sediment (clay and silt to fine grained sand) or montmorillonite.

A volume of 286±0.018 μl of standard cholesterol (248 ng/μl dichloromethane) was added to the reaction vessel, followed by solvent removal by flushing with N_2, and then the same amount of doubly distilled water was added. Therefore, the basic mixture (series 1) consisted of 248 ng cholesterol per μl of doubly distilled water (total 71 μg of cholesterol). The series 2 reaction composition consisted of 1.43±0.04 mol/l (409 μM total) oxalic acid solution. The series 3 condition was obtained

by adding 12.6±0.7 mg of natural sediment to the series 1 reaction mixture. The series 4 condition was obtained by adding 10.9±1.0 mg of montmorillonite (K10) to the series 1 reaction mixture. The vessels were filled to capacity, sealed and placed immediately into an oven for one day. The temperature settings were 150 °C, 200 °C, 250 °C and 300 °C + <1 °C for all series.

Upon removal from the oven, the vessels were cooled to room temperature. They were opened gradually and carefully in order to release the pressure (due to CO_2 and other gases generated during the experiment). Despite this precaution, a minor portion of fluid sample was sometimes expelled during opening in experiments with oxalic acid mixtures. Each sample was immediately transferred by Pasteur pipette to a glass vial. Each vessel was rinsed three times with methylene chloride/methanol (3:1 v/v) which was added to the vial giving a total volume of approximately 1500 µl. Each sample was then concentrated under nitrogen flush and room temperature to approximately 50 µl before the gas chromatography–mass spectrometry (GC–MS) analysis.

2.2. Analysis

The analysis of the alteration products and sterol standards was carried out by GC–MS on a Hewlett-Packard 6890 GC coupled to a 5973 Mass Selective Detector using a DB-5 (J and W Scientific, Agilent) fused silica capillary column (30 m×0.25 mm i.d., 0.25 µm film thickness) and helium as carrier gas. The GC was temperature programmed from 65 °C (2 min initial time) to 300 °C at 6 °C min^{-1} (isothermal for 20 min final time). The MS was operated in the electron impact mode at 70 eV ion source energy. Data were acquired and processed with a Hewlett-Packard Chemstation and compounds were identified by GC retention index and by comparison of mass spectra with those of authentic standards, literature and library data, and characterized mixtures. Unknown compounds were characterized by interpretation of the fragmentation pattern of their mass spectra. Relative concentrations of reaction products were estimated from ratios of peak areas, which assumes that the detection response is linear over the range of concentrations observed and that the response factors are similar for the different steroidal compounds.

3. Results and discussion

The experimental survey was conducted over a 150° temperature range from 150 °C to 300 °C. The conversion rate of cholesterol increased with increasing temperature and acidity of the reaction mixture (Fig. 1, Table 1). At low temperature (e.g., 150 °C) the acidity of the reaction mixture enhanced the conversion rate from 7.9% (series 1) to 70.6% (series 2), whereas the sediment and clay increased the conversion rate only slightly (to 11.3 and 14.4%, respectively). The incremental percent conversion of cholesterol increases in the order of oxalic acid montmorillonite > water > sediment. 100% conversion

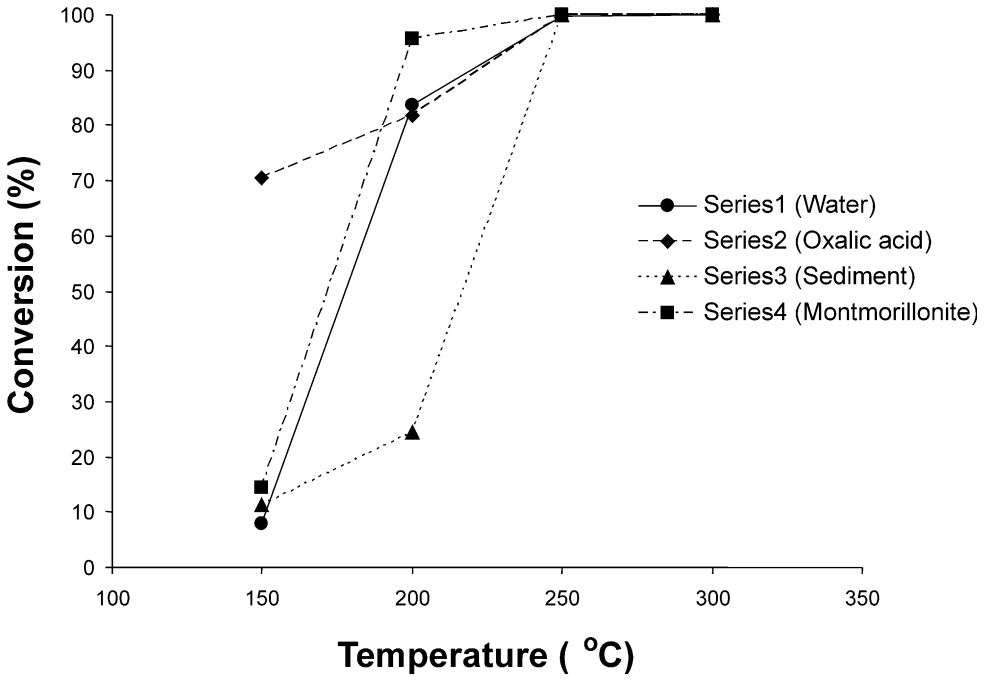

Fig. 1. The percent conversion of cholesterol in the four series of reaction mixtures versus the temperature of the experiments.

Table 1
Relative concentrations (in % of the total starting cholesterol) of biomarker groups found in experiment series 1–4 as a function of temperature

Compound groups and parameters[a]	Series (relative concentration)															
	1 (DDW)				2 (oxalic acid sol.)				3 (DSDP sediment)				4 (Montmorillonite)			
	150 °C	200 °C	250 °C	300 °C	150 °C	200 °C	250 °C	300 °C	150 °C	200 °C	250 °C	300 °C	150 °C	200 °C	250 °C	300 °C
Cholestanes		T[b]	58	56	2	1	49	34			1	7	T	T	4	30
Cholestenes		3	16	10	T	73	15	21	T	2	59	11	T	11	8	37
Cholestadienes	1	4	T		25	2	2		1	6			2	4		
Diacholestenes			23	T	22	3	32	45		T	3	21		36	38	5
Diacholestadienes	5	T			T	3	T					T		41	27	
Sterols (without cholesterol)	4	T		31	T				7	3	T	T	7			
Cholestatrienes		T	T	T	14	T	T			T	3	3		6	11	
Anthracholestenes											5	T				
Cholestenones		6				T			2	9	13		2			
Cholestadienones	1	41			7	T			2	4			2			
Cholestanones											8					
Others[c]		T	T	T						T	2	8		6	11	11
%Conversion	**8**	**84**	**100**	**100**	**71**	**83**	**100**	**100**	**11**	**25**	**100**	**100**	**14**	**96**	**100**	**100**
Reduction	**0**	**3**	**56**	**56**	**0**	**0**	**46**	**34**	**0**	**0**	**1**	**11**	**0**	**0**	**4**	**35**
Oxidation	**6**	**47**	**0**	**0**	**37**	**5**	**0**	**0**	**9**	**23**	**21**	**10**	**13**	**13**	**10**	**T**
Backbone rearrangement	**5**	**51**	**37**	**10**	**93**	**78**	**76**	**59**	**11**	**23**	**80**	**62**	**13**	**57**	**61**	**32**
U:R	**0.08**	**0.24**	**0.20**	**0.29**	**0.14**	**0.13**	**0.19**	**0.11**	**0.12**	**0.09**	**0.18**	**0.64**	**0.14**	**0.68**	**0.52**	**0.51**
(Δ^4/Δ^5)-Cholestene	**1.35**	**2.14**	**2.49**	**5.88**	**T**	**1.81**	**V.H.[b]**	**V.H.**	**T**	**3.86**	**1.48**	**V.H.**	**1.14**	**1.68**	**V.H.**	**V.H.**
(5α/5β)-Cholestane	**1.27**	**0.79**	**1.60**	**3.31**	**T**	**0**	**1.31**	**1.92**	**0**	**T**	**T**	**2.5**	**T**	**T**	**2.14**	**2.00**

[a] Parameters are given in bold type: reduction (hydrogenation) all cholestanes, cholestenes and diacholestenes; oxidation (dehydrogenation) all cholestadienes, diacholestadienes, cholestatrienes, anthracholestenes, cholestenones, cholestadienones and others. U:R is the ratio of unresolved (UCM) to resolved compounds.

[b] T = trace; V.H. = very high.

[c] *seco*-Cholestenes, androstenones, pregnenes, cholanthrene.

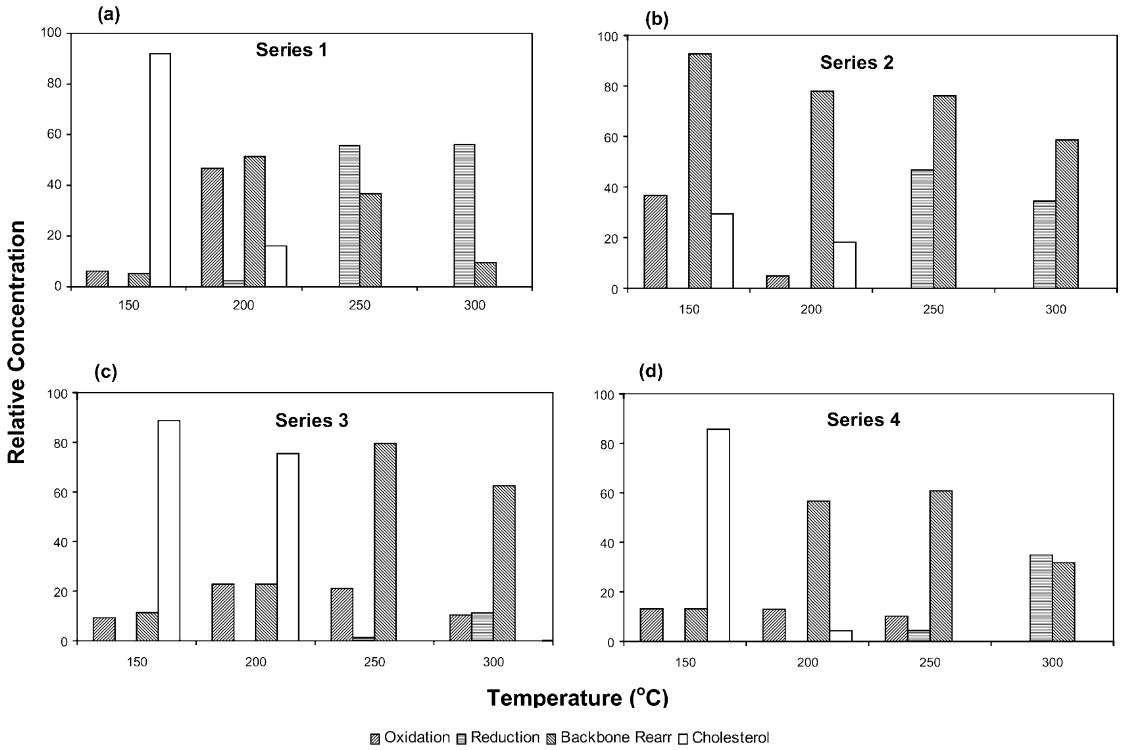

Fig. 2. The relative concentrations of the alteration product group and cholesterol shown as their chemical class for each temperature of the series 1–4 (a–d) experiments.

occurred at temperatures above 250 °C in all series (cf., Fig. 1 and Table 1).

More products were generated with increasing temperature and acidity of the reactants. The series 1 experiments at 150 °C yielded the smallest number of alteration products and this increased with temperature, acidity and catalyst substrate, with highest numbers found under series 4 conditions at 300 °C. This considerable increase in the alteration products also resulted in an UCM (unresolved complex mixture of branched and cyclic compounds) in the gas chromatograms with an increase in unresolved:resolved (U:R) ratios at 300 °C from 0.29 in series 1 to 0.65 and 0.51 in series 3 and 4, respectively (Table 1).

The alteration products can be divided into three major compound classes: steranes, sterenes and diasterenes, and aromatic steroid hydrocarbons. There are some general trends recognizable. First, the overall oxidation products (mainly dehydrogenation) (e.g., stenones, aromatics) in all series decrease with increasing temperature above 200 °C (Fig. 2). On the other hand, the reduction products increase with temperature, whereas the backbone rearrangement compounds increase to a maximum at 200–250 °C and then decrease (Table 1 and Fig. 2). Generally, the backbone rearrangement processes maximize and stabilize at around 250 °C in the presence of sediment or montmorillonite and at <200 °C in their absence.

The sterenes, steranes and diasterenes are minor components at lower temperature (<200 °C) and the aromatic steroids are significant only at high temperatures (>200 °C) and in the presence of clay (Table 1). Sterenes and diasterenes (also oxygenated derivatives) the predominant product classes >200 °C for sediment and clay (Table 1). These three major compound classes are further subdivided into 14 compound groups (Table 1).

3.1. Steroidal compounds

Alteration products with a sterane backbone are the major derivatives and their ratios change with increasing temperature and decreasing pH (Table 1). The steranes (only 5α- and 5β-cholestane present) in experiment series 1 and 2 are detectable only in traces ($<0.2\%$) at low temperature and increase to a range between 34 and 58% at 250 °C and above. Only a minor increase is detectable in the cholestane concentration with the sediment matrix for all experiments and reaches the maximum (6.85% of the total, Table 1) at 300 °C. In the presence of clay the production of cholestane is higher than that with sediment, and reaches a maximum (30%) at 300 °C. Thus, reduction to steranes is a dominant

process >200 °C in these experiments, consistent with what has been observed in the natural hydrothermal systems (e.g., Simoneit, 1985, 1994). 5β-Cholestane (coprostane) and 5α-cholestanone are found only after hydrous pyrolysis >250 °C. Key ion plots of *m/z* 217 for steranes or *m/z* 218 for the [5α,14α,17α(H)] and [5α,14β,17β(H)] configurations, respectively, indicate that the stereochemistry does not change at positions C-14 and C-17. Thus, full maturation to the 5α,14β,17β(H)-configuration probably requires longer time, or temperatures >300 °C. 5α,14α,17α(H)-Cholestane is the major sterane produced. However, epimerization occurred above 200 °C at position 5 to yield coprostane. It seems that epimerization occurs only slowly and is kinetically hindered to reach thermodynamic equilibrium (Mackenzie et al., 1982; Seifert and Moldowan, 1986). Hence, a reaction time much longer than one day would be necessary to increase isomerization. One proposed pathway postulates that ster-4-enes and ster-5-enes are the precursors of the 5α,14α,17α(H)-steranes and they in turn are epimerized to the 5α,14β,17β(H)-steranes (Rubinstein et al., 1975; Seifert and Moldowan 1979, 1981). However, other experiments suggest that the latter and former compounds can be formed from ster-7-ene precursors via stera-2,8(14)-dienes and stera-2,14-dienes, ster-8(14)-enes, and ster-14-enes (ten Haven et al., 1986; Peakman and Maxwell, 1988; Peakman et al., 1989). The second postulate may best explain why we find no 5α,14β,17β(H)-steranes, because the precursor molecules are only present in trace amounts (Δ^7-, Δ^8-,

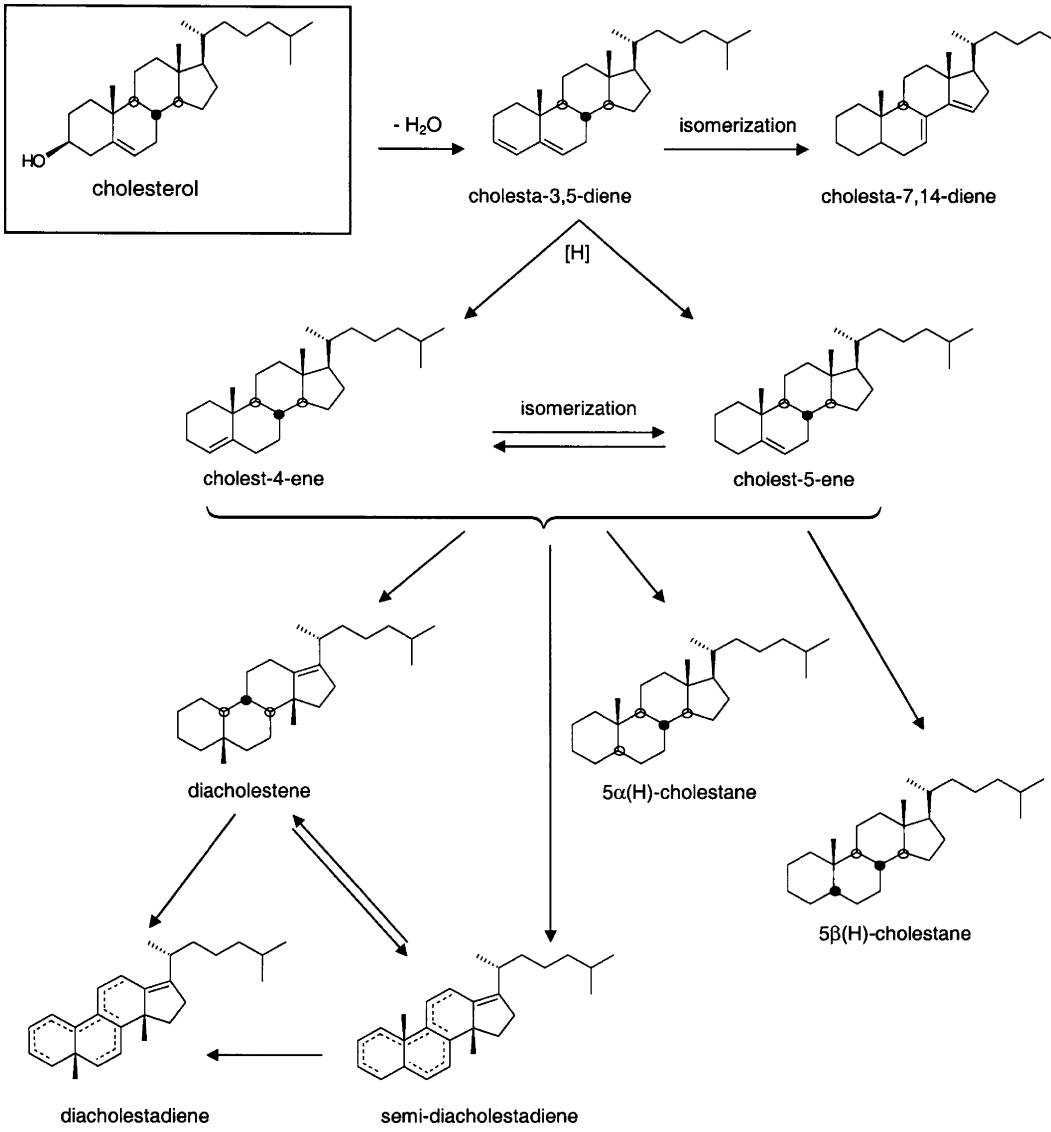

Fig. 3. Alteration scheme for cholesterol to its hydrous pyrolysis products—cholestadienes, cholestenes, cholestanes and diacholestenes.

and Δ^{14}-cholestene) or completely absent (*AD*- and *AC*-ring cholestadienes) in these experiments (Fig. 3).

Sterenes (mainly cholestenes) are predominant alteration products with increasing temperatures (Table 1). As reported by Giger and Schaffner (1981) and Rullkötter et al. (1981), we also observe cholest-4-ene and cholest-5-ene as the major sterenes for all experiments over the temperature range. The ratio of Δ^4/Δ^5-cholestenes indicates that in general cholest-4-ene is more stable than cholest-5-ene. Thus, in all experiment series the ratio increases dramatically, and only cholest-4-ene is dominant at temperatures above 250 °C, except in the series 1 experiments where cholest-5-ene survives to higher temperature and the ratio increases steadily from 1.35 at 150 °C to 5.88 at 300 °C (Table 1). The generally accepted pathway suggests that cholesta-3,5-diene is the precursor

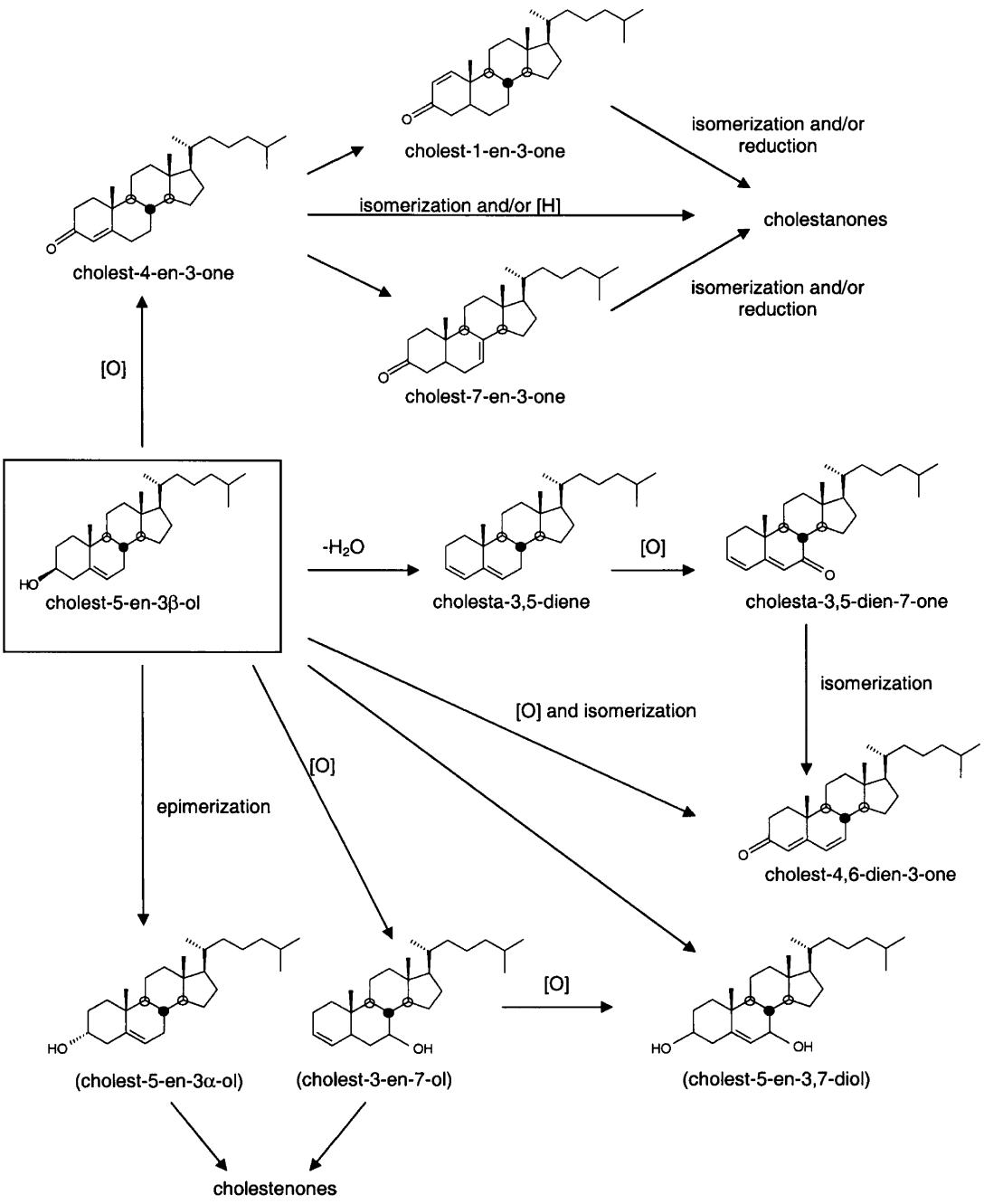

Fig. 4. Alteration scheme for cholesterol to its hydrous pyrolysis products—cholestadienones, cholestenones and cholestenols.

of cholest-4-ene and cholest-5-ene (e.g., Mackenzie et al. 1982) (Fig. 3).

At lower temperatures (<200 °C) traces of Δ^3-, Δ^7-, $\Delta^{8(9)}$-, $\Delta^{8(14)}$-, and Δ^{14}-cholestenes are also found. These cholestenes are less stable and hence, are "short-lived" intermediates, which probably isomerize to the more stable cholest-4-ene and cholest-5-ene or other compounds.

The cholestadienes have maximum concentrations in all experimental series between 150 and 200 °C and decrease with increasing temperature. A significant amount of cholestadienes was detected in series 2 at 150 °C. Cholestadienes (especially $\Delta^{3,5}$) are known to be formed by dehydration during simulated cholesterol alteration (Rhead et al., 1971). They represent one of the major alteration products in most of our experiments below 250 °C (Table 1). The direct dehydration of cholesterol leads to the formation of cholesta-3,5-diene with the double bonds in the *A–B* ring system. Probable isomerization of the predominant cholesta-3,5-diene leads to the formation of cholesta-7,14-diene, which is detectable in trace amounts (Fig. 3). The concentrations of other cholestadienes were, if produced, below 0.05% of the total product concentration.

As shown in Fig. 2, cholesten-3-ones have been detected at temperatures ⩽200 °C in all experiment series and totally disappear above 250 °C. However, cholestan-3-one, cholestan-4-one and coprostan-3-one are detectable at 250 °C and represent the major stanones occuring in minor amounts (Table 1). The precursor of the ketones is probably cholesterol which is oxidized to cholestenones and other analogues (Fig. 4).

Sterols (C_{27}) other than cholesterol (e.g., cholest-5-en-3α-ol, cholest-3-en-7-ol, Fig. 4) are in general minor by-products of the alterations (Table 1). The formation of cholest-5-en-3,7-diols occurs only <200 °C (Fig. 4). At higher temperatures the sterols are not stable and alter

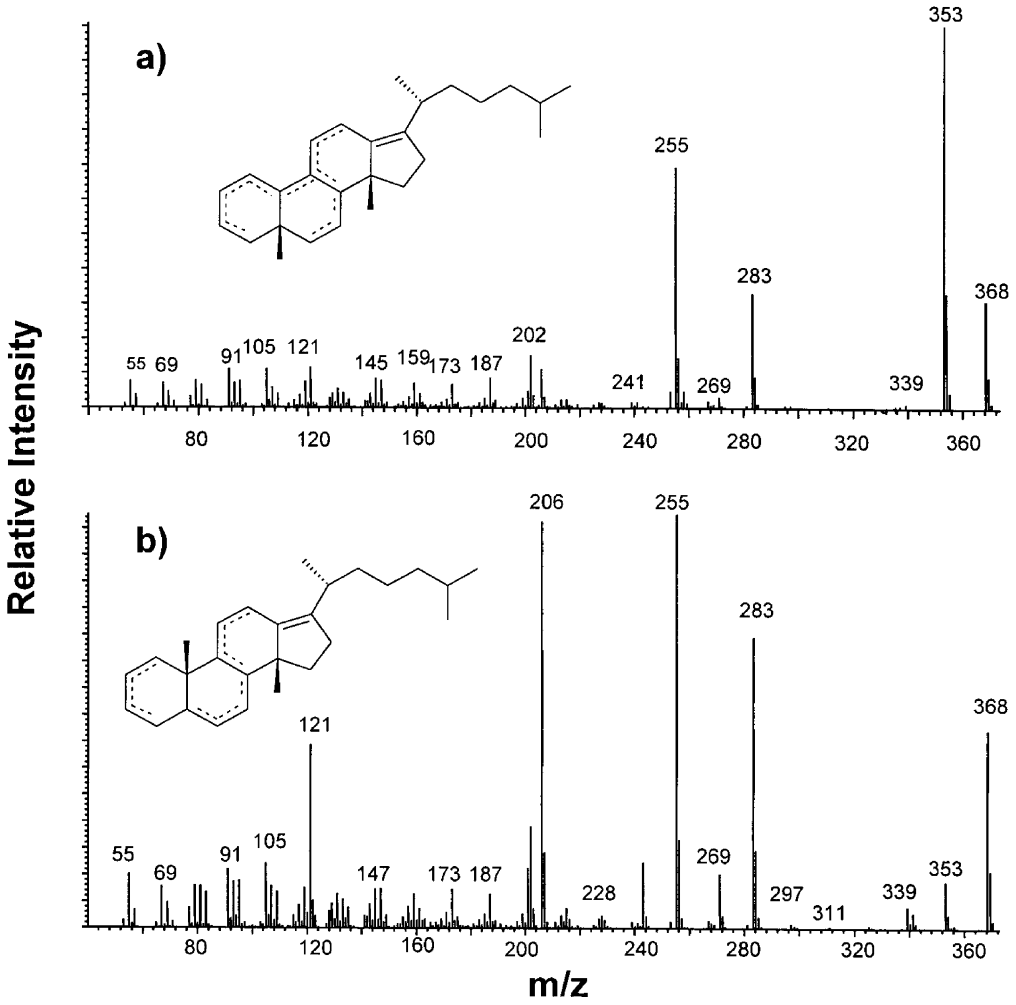

Fig. 5. Characteristic mass spectra of: (a) a diacholestadiene, (b) a *semi*-diacholestadiene, (c) a 9,10-*seco*-cholesta-1,3,5(10)-triene, and (d) a 19-nor-*anthra*-cholestene.

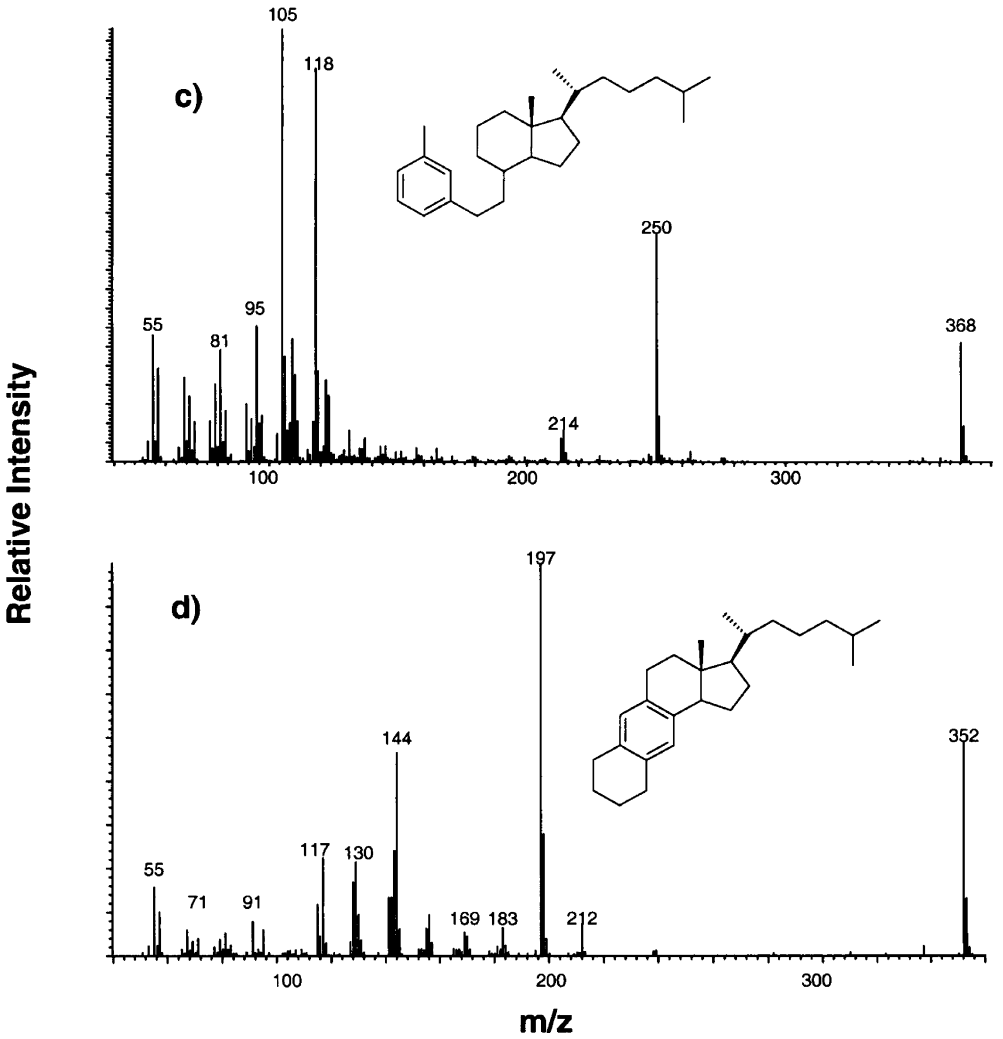

Fig. 5 (*continued*)

further by oxidation to their ketone analogues or by dehydration to sterenes.

The 20R- and 20S-diasterenes were first reported by Rubinstein et al. (1975). Diacholestenes formed in our experiments and their concentrations increased with increasing temperatures and reached a maximum between 250 and 300 °C, mainly in acidic medium and in the presence of montmorillonite (Table 1). Their concentrations are low below 200 °C except in acidic medium and represent more than 37% of the total product concentration at temperatures >250 °C. The rearrangements are catalyzed by the superacid sites of the montmorillonite (Sieskind et al., 1979) and/or the low pH of the reaction mixture (Peakman et al., 1992). The rearrangement products were predominantly found at temperatures between 200 and 300 °C. Under acidic conditions (e.g., series 2) the maximum rearrangement products were found at 150 °C and decreased slightly with increasing temperature. The formation of diacholestadienes reached a maximum at temperatures between 150 and 250 °C in all series with a significant amount in the presence of clay. They are trace components in the other experiment series. The diacholestadienes have characteristic mass spectra with major ions at *m*/*z* 368 ($M^{\cdot+}$), 353 (base peak), 283, 255, and 202 (Fig. 5a). The position of the second double bond is unclear. Semi-diacholestadienes, as reported by Hoffmann (1984), are also found where one of the methyl groups is still at position C-10 and not at C-4 (Fig. 3). Their characteristic mass spectra show fragment ions at *m*/*z* 368 ($M^{\cdot+}$), 283, 255, 206 (base peak) and 121 (Fig. 5b). There is evidence for the postulate, that cholest-4-ene and cholest-5-ene are the precursors of the diacholestenes (Mackenzie et al., 1982; Rubinstein et al., 1975). Hence, with rising temperatures the concentrations of the cholestenes decrease and the concentrations of the

diacholestenes increase. *seco*-Cholestene was detected in series 3 at temperatures >200 °C, with a maximum concentration at 250 °C. The 9,10-*seco*-cholesta-1,3,5(10)-trienes have characteristic mass spectra with major fragment ions at m/z 368 ($M^{\cdot+}$), 250, 118 and 105 (base peak) (Fig. 5c), however, only one isomer was detected in these experiments. Also the cracking products from loss of the A,B rings (Fig. 6) as reported by Jiang et al. (1988) were not detected.

Androstene and pregnene derivatives are found in traces above 250 °C (Fig. 6). The androstenes are mostly represented as the ketone analogs and two pregnene

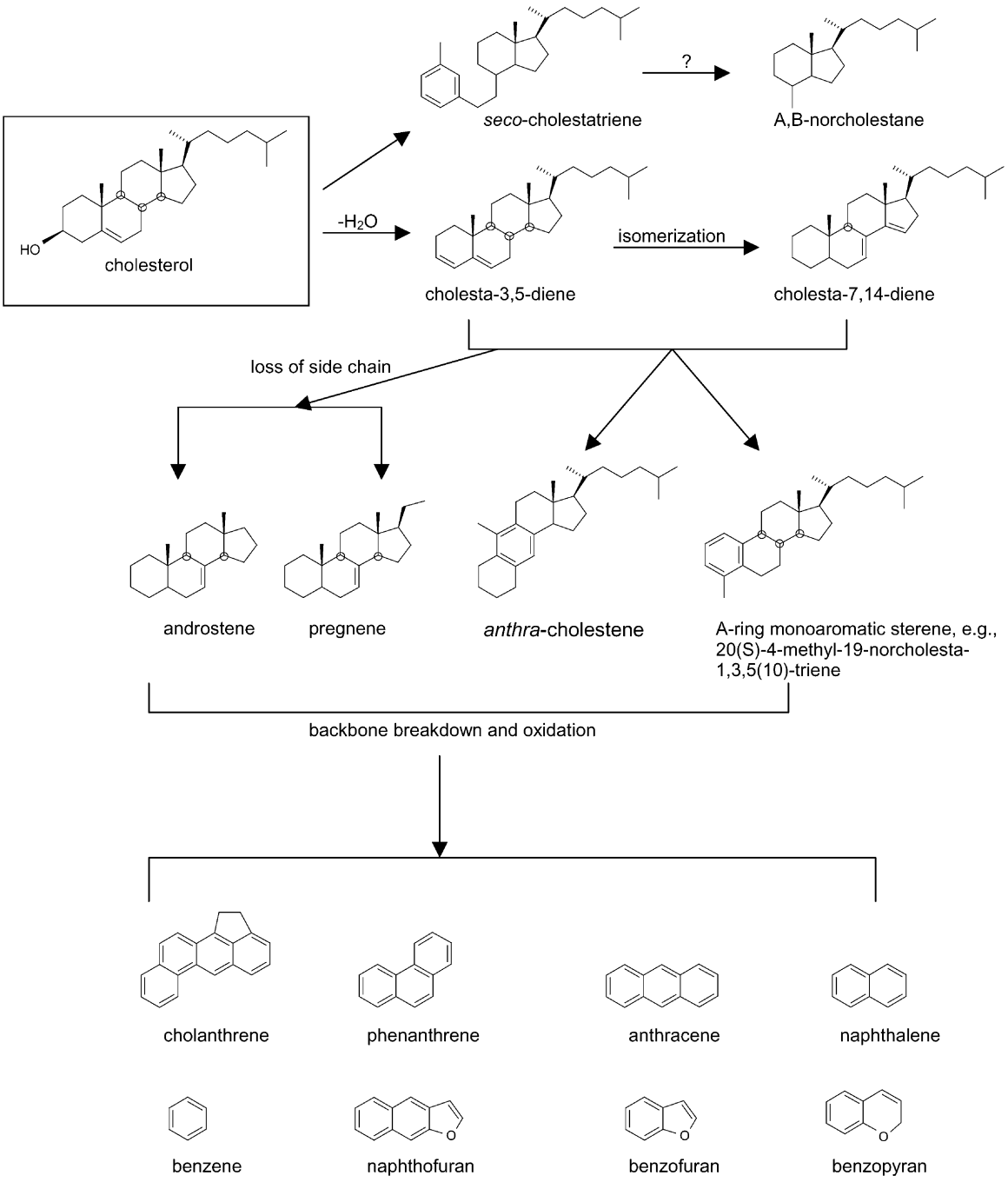

Fig. 6. Alteration scheme for cholesterol to its hydrous pyrolysis products—loss of side chain, backbone rearrangement, ring opening, and aromatic compounds. For mechanistic details see Hoffmann (1984).

compounds, i.e., 7-pregnene and 14-pregnene are found. The catagenetic pathways include cleavage (cracking) of the C-17–C-20 bond for the androstenes or the C-20– C-22 bond for the pregnenes, besides oxidation processes due to the thermal stress.

3.2. Aromatic steroids

The genesis and concentrations of aromatic compounds generally increase parallel to the temperature increase. This is the result of higher thermal stress which favors aromatization of the sterene *A–C* ring system and some bond cleavage. Also, the acidity of the reaction mixture and lower pH due to the superacid sites in the clay matrix catalyze the latter processes as well as the backbone rearrangements (Peakman et al., 1992; Sieskind et al., 1979).

Formation of *anthra*-cholestenes (Hoffmann, 1984) is observed at intermediate temperatures. However, the *anthra*-cholestenes have a maximum concentration at 250 °C (series 3 only) (Table 1). The 20R conformation (as in cholesterol) is predominant for all *anthra*-cholestenes, and the 20S isomers are detectable only in traces below 200 °C. The transformation of 20R to 20S involves reactive intermediates which are also precursors for several other pathways. A significant amount of a 19-nor-*anthra*-cholestene is also present and its typical mass spectrum has major ions at m/z 352 (M^+), 221, 197 (base peak), 144, 130 and 117 (Fig. 5d). This compound indicates rapid rearrangement and dehydrogenation with concomitant loss of a CH_3 group, as was described for the triaromatic steroid hydrocarbons in hydrothermal petroleum from Guaymas Basin (Simoneit et al., 1992).

At lower temperatures *anthra*-cholestenes and monoaromatic steroid hydrocarbons (e.g., 4-methyl-19-norcholesta-1,3,5(10)-triene) are the only aromatic compounds observed. Their decrease above 250 °C and the possibility of subsequent formation of a variety of other aromatic compounds indicates that aromatization probably commences in ring-A. However, it has also been postulated that aromatization for geological samples starts in ring-C and proceeds to form ring-ABC triaromatic compounds (Brassell et al., 1983; Mackenzie et al., 1982). Cholanthrene (1,2-dihydrobenz[j]aceanthrylene, $C_{20}H_{14}$) is a trace product (0.6% at 300 °C, series 4).

The breakdown of the triaromatic steroid ring system leads to trace levels of smaller moieties such as naphthalene, phenanthrene, anthracene, indene, benzene, etc. (Fig. 6). The formation of furan and pyran analogs of naphthalene and benzene are also of interest. Diels' hydrocarbon, as was described for hydrothermal petroleum from Guaymas Basin (Simoneit et al., 1992), is not found, i.e. it is below 0.05% of the total product concentration.

In summary the experiments suggest that sterols via stenones and steradienones are reduced to stanols as proposed by Edmunds et al. (1980). Sterols also form steradienes, whereas stanols produce sterenes, i.e. ster-2-enes (Dastillung and Albrecht, 1977; Gagosian and Farrington, 1978), which isomerize to ster-4-ene (major) and ster-5-ene (minor) (Rubinstien et al., 1975; Table 1). The sterenes are reduced to steranes under these hydrothermal conditions. Under acidic and higher temperature conditions, the sterenes also undergo rearrangement and form diasterenes. Diasteranes (Mackenzie et al., 1982) and spirosterenes (Peakman et al., 1984), which were not detected in the temperature and time ranges of these experiments, may need higher temperature and/or longer time to form. The major steranes under such conditions would be non-rearranged 5α- and 5β-steranes (Table 1). Longer time and higher temperature are required to form the other sterane isomers. Monoaromatic steroids are anticipated to form, whereas triaromatic steroids will possibly form under acidic medium if heated for longer time or at higher temperature.

4. Conclusions

A survey of the alteration products from cholesterol after hydrous pyrolysis under hydrothermal conditions over a temperature range from 150 to 300 °C revealed the presence of at least 14 steroid classes. The major alteration pathways at lower temperature (<200 °C) are oxidation processes yielding mainly cholestadienes, diacholestenes, *anthra*-cholestenes, cholestenones and C_{27}-sterols. In the intermediate temperature range (200–250 °C) the alteration pathways are dominated less by oxidation processes and the concentrations of cholestenes, diacholestenes, cholestenones and C_{27}-sterols reach their maximum. Above 200 °C backbone rearrangement processes yield predominantly diacholestenes, diacholestadienes and *seco*-cholestatriene.

It should be noted that the fully hydrogenated derivatives (e.g., cholestanes) are significant products only at higher temperature (e.g., >250 °C), and decreased slightly in the presence of substrates such as sediment and/or clay in these hydrothermal simulation experiments. At temperatures above 250 °C, reduced compounds showed a relative increase. Backbone rearrangements are favored at high temperatures in the presence of sediment, montmorillonite, and acidic medium, but extensive hydrogenation (i.e., diacholestanes) is not observed.

Diacholestenes, aromatic compounds (e.g., phenanthrene, anthracene, naphthalene, cholanthrene) and the UCM are the significant products above 275 °C. They are derived from acid catalyzed rearrangement and cracking reactions. Androstene and pregnene derivatives are detectable only above 300 °C.

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