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Thermochemical decomposition of cholic acid and its derivatives

N. Shopova *, Ts. Milkova

Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria

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Abstract

The thermal decomposition reactions of cholic acid (**1**), methyl cholate (**2**), 3α-acetoxy-7α,12α-dihydroxy-5β-methylcholanate (**3**), 3α,7α-diacetoxy-12α-hydroxy-5β-methylcholanate (**4**), 3α,7α-diacetoxy-12α-oxo-5β-methylcholanate (**5**) and 3α,7α,12α-triacetoxy-5β-methylcholanate (**6**) were studied by DTA and TGA.

The thermal decomposition of cholic acid proceeds as a one stage process in the range 200–420°C. The esterification of the carboxylic group of cholic acid results in considerable increase of the thermal stability of compound (**2**). The endothermic peaks at about 100°C occurring only in compounds (**1**) and (**2**) can be attributed to desolvation and release of hygroscopically bound water.

The thermal decomposition reactions of the dried compound (**2**) and compounds (**3**), (**4**) and (**5**) are found to proceed in two stages. During the first stage, the thermal degradation of (**5**) leads to the formation of a completely deacetylated compound and two monodeacetylated compounds with the predominant product resulting from the elimination of the acetate group at C_3. Degradation of the carbon skeleton takes place during the second stage.

Keywords: Cholic acid; Decomposition; DTA; Methyl cholate; Steroid; TGA

1. Introduction

The recent increasing interest in the thermochemical behaviour of steroids is obviously connected with the pharmacological problems. Thermoanalytical data are

* Corresponding author.

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required for every stage of pharmaceutical preparation [1–5]. The purity of chenodeoxycholic acids, which are used in a pharmaceutical preparation for the solubilization of gallstones, was investigated by thermoanalytical analysis [6]. In view of the fact that cholesteryl esters have important physiological functions, Barrel et al. [7] studied their mesophase transition temperature. This is the first reported DTA study of the polymorphism of steroids. A thermochemical study of estradiol monovalerate and estriol by De Maury and Masse [8] provided an explanation of thermal stability, decomposition kinetics and the degree of purity of the investigated compounds. Alexander et al. [9] demonstrated that the specific decomposition reactions of the steroid derivatives offer opportunities for their identification in different compositions.

The absence of literature data on the thermochemical properties of cholic acid and its derivatives, which are required for synthesis of chenodeoxycholic and ursodeoxycholic acids (very important pharmaceuticals used as gallstone dissolving agents) [10,11] evoked the present investigations.

The purpose of this investigation is to study the thermal behaviour of cholic acid (**1**), methyl cholate (**2**), 3α-acetoxy-7α,12α-dihydroxy-5β-methylcholanate (**3**), 3α,7α-diacetoxy-12α-hydroxy-5β-methylcholanate (**4**), 3α,7α-diacetoxy-12α-oxo-5β-methylcholanate (**5**) and 3α,7α,12α-triacetoxy-5β-methylcholanate (**6**) under non-isothermal conditions in the 230–500°C range. The mechanism of the thermal decomposition and the effect of the structure of the investigated steroids on the thermoanalytical parameters were also discussed.

2. Experimental

Cholic acid (99%) with m.p. 197–199°C was purchased from Fluka and used "as is". Methyl cholate was prepared from cholic acid and crystallized from methanol. 3α-Acetoxy-7α,12α-dihydroxy-5β-methylcholanate, 3α,7α-diacetoxy-12α-hydroxy-5β-methylcholanate, 3α,7α,12α-triacetoxy-5β-methylcholanate, and 3α,7α-diacetoxy-12α-oxo-5β-methylcholanate were synthesized using the procedure described in [10].

Differential thermal analysis was conducted on a derivatograph Q-1500 (MOM-Budapest) under the following conditions: a heating rate of 2.5°C per minute, platinum crucibles and Al_2O_3 as reference substance. The experiments were performed in a self-generating atmosphere.

The ^1H NMR spectra were measured on a Bruker WM-250 spectrometer in $CDCl_3$.

The MS measurements were conducted on a JEOL JMS D-300 spectrometer (EI, 70 eV).

3. Results and discussion

The thermal decomposition of cholic acid proceeds as a one-stage process in the range 200–420°C, as indicated by the TG curves (Fig. 1). The endothermic peak at

115°C, accompanied by mass losses, is due to desolvation and release of hygroscopically bound water, and is dependent upon the conditions of crystallization [6].

An endothermic peak at 180°C is observed on the DTA curve of cholic acid (Fig. 1), which is indicative of its phase transition (solid–liquid state). The melting of the sample is followed by a slow mass loss in the 200–300°C range, probably due to the occurrence of a non-intensive evaporation process.

The enthalpy changes of cholic acid registered above 300°C can be related to the processes of evaporation, dehydration (endo effect at 340°C) and decarboxylation (exo effect at 360°C). The endothermic peak at 386°C is probably due to degradation of the carbon skeleton of cholic acid.

The esterification of the carboxylic group of cholic acid increases the thermal stability of the derivatized compound (**2a**). Although the phase changes of methyl cholate are registered at a much lower temperature (145°C, Fig. 2a) than for cholic acid (180°C, Fig. 1), the starting decomposition temperature of (**2a**) is shifted to higher temperatures (Fig. 2a, Table 1).

The data on the thermal decomposition of methyl cholate demonstrate one stage process in the 270–450°C range. As for cholic acid, the endothermic peak at about 100°C appears to be due to the release of hydroscopically bound water and desolvation. This is confirmed by thin-layer chromatography of the initial compound and of the dried product (**2b**). The last product was obtained after thermal treatment of the initial methyl cholate at 105°C and continuously separation of the gas phase. Additionally, the melting temperature and T_i of the dried product are identical to those of the initial methyl cholate (compare Figs. 2a and 2b).

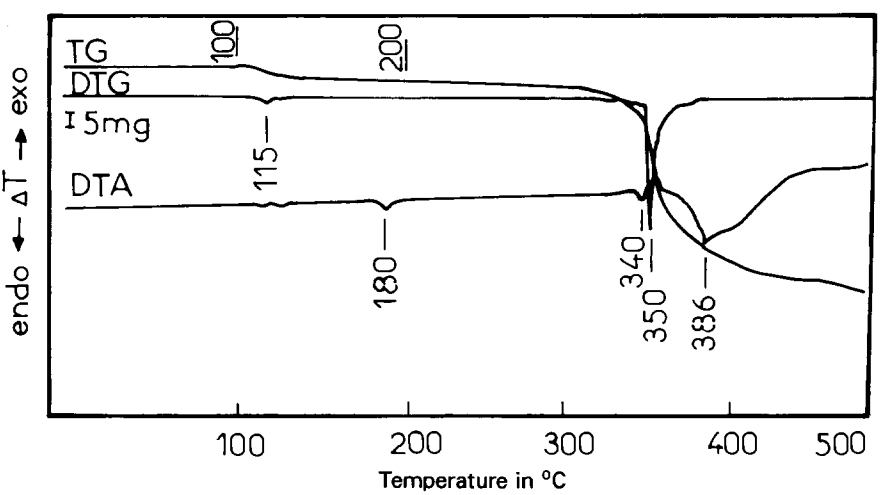
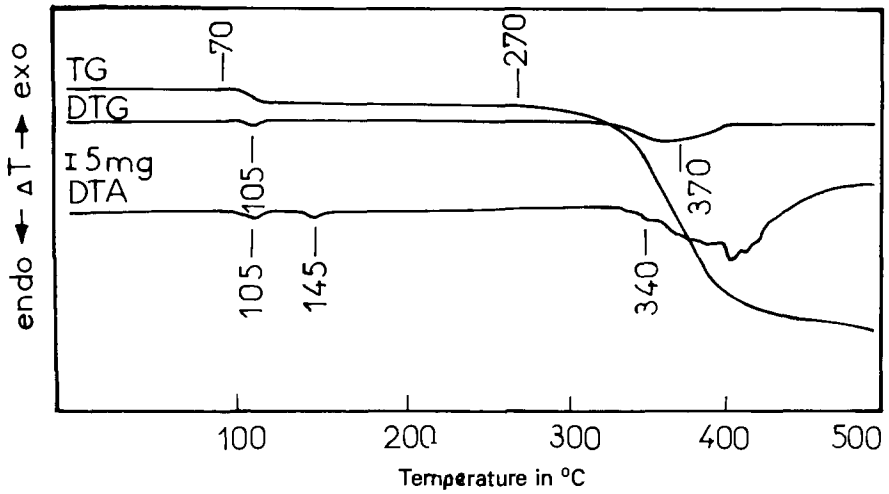
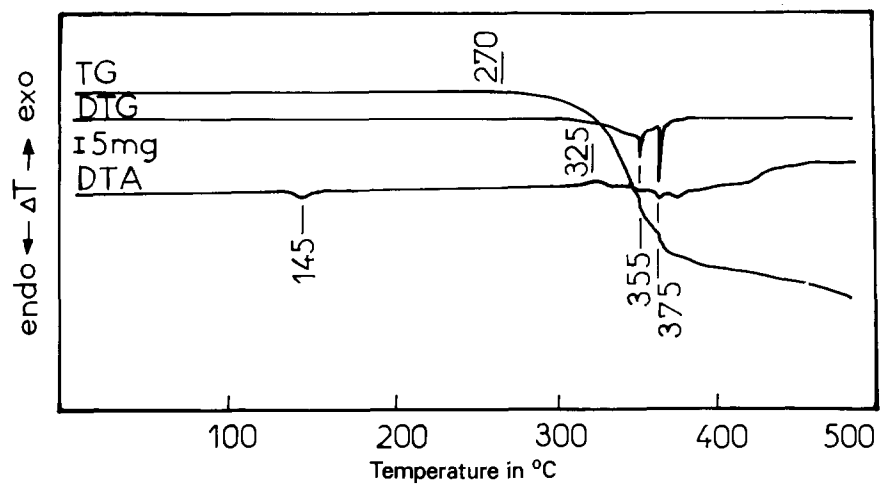


Fig. 1. Thermal decomposition curves of cholic acid.



(a)



(b)

Fig. 2. Thermal decomposition curves of (a) methyl cholate, and (b) dried methyl cholate.

Unlike the initial compound, the dried methyl cholate appears to undergo a two-stage thermal decomposition with W_{max} at 355 and 375°C (Fig. 2b). The insignificant exotherm peak at 325°C could not be ascribed to decarboxylation and most probably may be related to thermooxidative changes in the structure. The presence of three –OH groups in the molecule of methyl cholate allows for various types of oxidation reactions. It is known that the oxidation of –OH groups of methyl cholate takes place selectively, depending on steric factors [12]. In order to follow the influence of –OH groups and the changes in the carbon skeleton during the thermal decomposition of steroids, we undertook some investigations on the following derivatives of cholic acid: 3α-acetoxy-7α,12α-dihydroxy-5β-methylcholanate (**3**), 3α,7α-diacetoxy-12α-hydroxy-5β-methylcholanate (**4**), 3α,7α-diace-

Table 1
Thermal decomposition of cholic acid and its derivatives

No.	Steroid	Stage	Temperature interval of decomposition/°C	$T_{w\,max}$ [a]/°C	Thermal effect/°C	
					Endo	Exo
1	Cholic acid	1	200–420	350	115, 180 [b]	360
2a	Methyl cholate	1	270–440	370	105, 145 [b]	
2b	Methyl cholate	1	270–355	355	145 [b]	325
	dried	2	355–380	375		
3	3α-Acetoxy-7α,2α-					
	dihydroxy-5β-	1	195–350	340	140 [b]	330
	methylcholanate	2	350–385	370	375	
4	3α,7α-Diacetoxy-					
	12α-hydroxy-5β-	1	240–340	335	175 [b]	305
	methylcholanate	2	340–390	365	370, 380	
5	3α,7α-Diacetoxy-					
	12α-oxo-5β-methyl	1	265–355	350	165 [b]	
	cholanate	2	355–400	365	375	
6	3α,7α,12α-Triace-					
	toxy-5β-methyl-					
	cholanate	1	220–400	340	90 [b], 375	

[a] DTA peaks. [b] Melting point.

toxy-12α-oxo-5β-methylcholanate (**5**), and 3α,7α,12α-triacetoxy-5β-methylcholanate (**6**). The analysis of the TGA/DTA data of (**3**), (**4**) and (**5**) (Figs. 3–5) reveals that the thermal decomposition of these compounds occurs in two stages (Table 1).

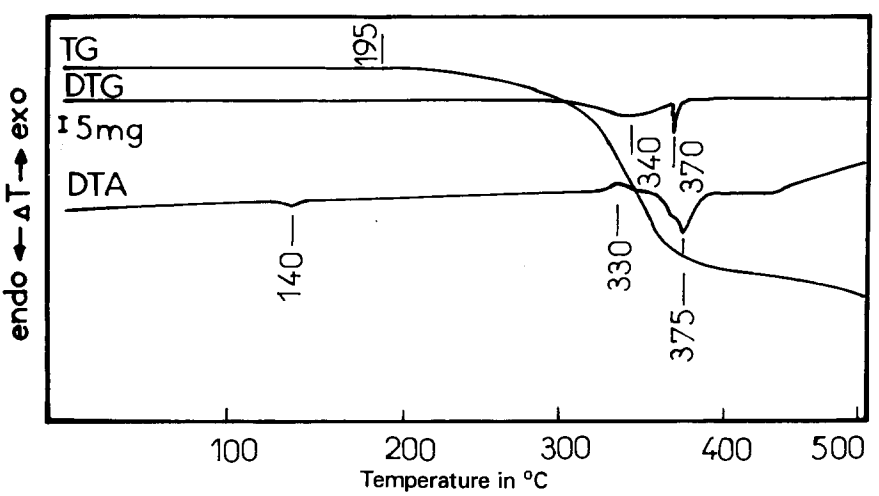


Fig. 3. Thermal decomposition curves of 3α-acetoxy-7α,12α-dihydroxy-5β-methylcholanate.

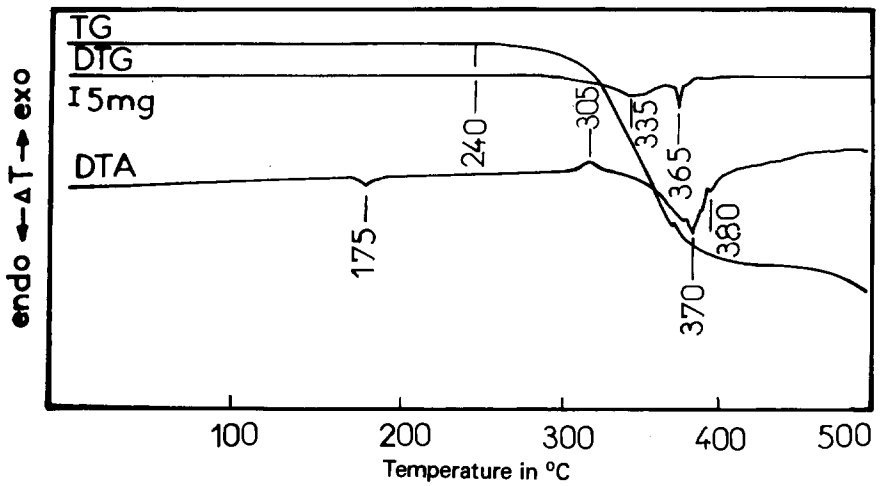


Fig. 4. Thermal decomposition curves of 3α,7α-diacetoxy-12α-dihydroxy-5β-methylcholanate.

The DTA curve of 3α-acetoxy-7α,12α-dihydroxy-5β-methylcholanate has a low intensive exotherm peak at 330°C, similar to that observed for methyl cholate (Fig. 2b). Therefore, it appears that the thermal changes of methyl cholate and of 3α-acetoxy-7α,12α-dihydroxy-5β-methylcholanate (at 325 and 330°C, respectively) proceed according to a similar mechanism.

According to the literature data [12], in the presence of two axial –OH groups at C_7 and C_{12} in the steroid molecule the hydroxyl group at C_7 is oxidized preferentially. Under our experimental conditions we could reasonably expect that the thermochemical changes of (**3**) and (**4**) can affect both axial –OH groups at C_7 and

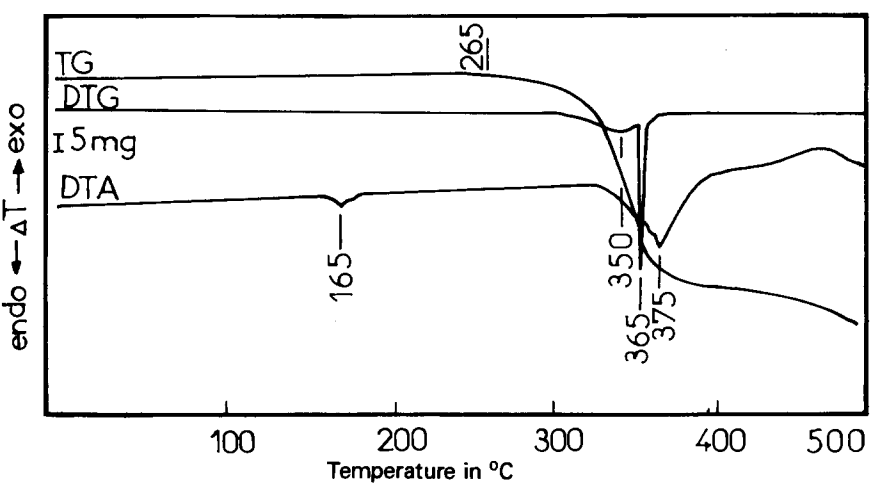
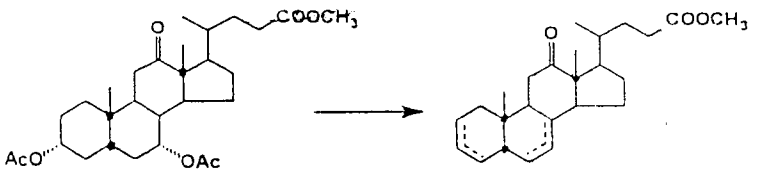


Fig. 5. Thermal decomposition curves of 3α,7α-diacetoxy-12-oxo-5β-methylcholanate.



Scheme 1

at C_{12}. The low intensive exotherm peak at 305°C, registered on the DTA curve of 3α,7α-diacetoxy-12α-hydroxy-5β-methylcholanate (Fig. 4) can be assigned to the oxidation of the only $C_{12}OH$ group. The absence of a similar exotherm peak on the DTA curve of (**5**) (Fig. 5) confirms this assumption.

The study of the thermochemical mechanism of the 3α,7α-diacetoxy-12α-oxo-5β-methylcholanate is of special interest because of the lack of hydroxyl groups. For this reason, we isolated the product from thermochemical treatment (**5**) at 340°C and conducted a column-chromatographic separation on silica gel using an increasing concentration of Et_2O in hexane. Two fractions were isolated

Fraction 1
^{1}H NMR (δ, ppm) −3.659 (s, 3H, $-OCH_3$); 5.399–5.612 (m, 3H).
MS (EI, m/z, (%)) 384 (100) M^+, 269 (15).

Fraction 2
^{1}H NMR (δ, ppm) 1.952 (s, $C_{(3)}OCOCH_3$); 2.002 (s, $C_{(7)}OCOCH_3$); 3.658 (s); 3.665 (s); 4.652 (m, $>C_{(3)}(H)OCOCH_3$); 4.890 (m, $>C_{(7)}(H)OCOCH_3$); 5.4361–5.561 (m).
MS (EI, m/z, (%)) 444 (60) M^+, 384 (100); 369 (5); 329 (10); 269 (22).

The singlet in the ^{1}H NMR spectrum of the non-polar fraction 1 at δ 3.659 is assigned to $-OCH_3$ and the multiplet in the range δ 5.396–5.612 to olefinic protons. This observation proves that the side chain of the initial ketone has not changed at 340°C. The absence of signals characteristic of acetate groups from one side, and the presence of olefinic protons from the other, indicate that Fraction 1 is a completely deacetylated product (Scheme 1).

The presence of the most intensive molecular peak (m/z 384) in the MS of Fraction 1 also confirms the structure of this compound.

The data of the MS and ^{1}H NMR of the polar fraction 2 reveal a mixture of two monodeacetylated products (Scheme 2).

The occurrence of M^+, (m/z 444) and an intensive $M-60^+$, (m/z 384) in the MS of Fraction 2 indicate the formation of a monodeacetylated product. However, by this method it is impossible to determine which of the two acetate groups (at C_3 or at C_7) has been eliminated during the thermal reaction.

In the ^{1}H NMR spectrum of Fraction 2 two multiplets at 4.652 and 4.890 ppm are observed, characteristic of $>C_3(H)OCOCH_3$ and $>C_{(7)}(H)OCOCH_3$ respectively [10]. The occurrence of olefinic protons, two singlets for $-OCH_3$ groups and two singlets for the acetate groups with integral ratio 3 : 1 indicate that Fraction 2

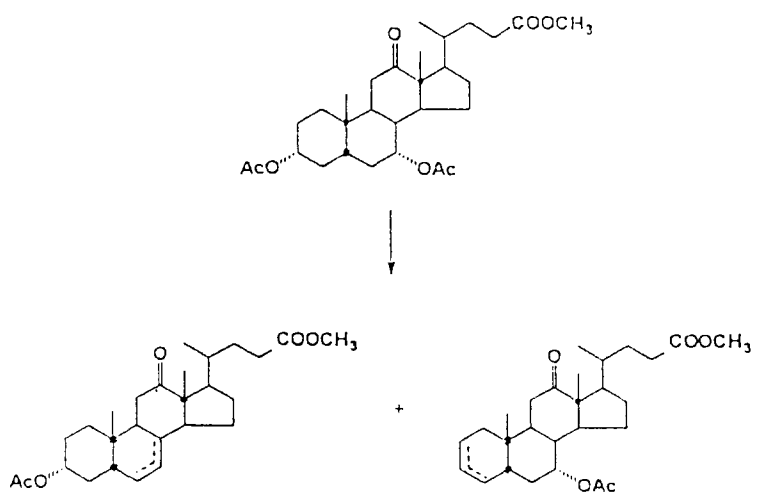
is a mixture of two monodeacetylated products in the ratio given above (Scheme 2). These results support the fact that the elimination of the acetate group at C_3 dominates.

Regardless of an intensive loss of mass (12%) at 365°C during the second stage of the thermal decomposition of 3α,7α-diacetoxy-12α-oxo-5β-methycholanate (Fig. 5), we did not observe individual compounds by thin-layer chromatographic investigation.

The thermal destruction of methyl cholate, 3α-acetoxy-7α,12α-dihydroxy-5β-methylcholanate, and 3α,7α-diacetoxy-12α-hydroxy-5β-methylcholanate is similar in the second stage of the reaction, but the most intensive reaction in this stage is observed for 3α,7α-diacetoxy-12α-oxo-5β-methylcholanate (Figs. 3–5).

The thermal decomposition of 3α,7α,12α-triacetoxy-5β-methylcholanate proceeds as a one-stage process in the temperature range 220–400°C. The absence of exothermy can be regarded as additional evidence that the positive enthalpy changes for (**2**), (**3**) and (**4**) are the result of oxidation. The DTA curves of all investigated compounds have endotherms in the range 370–390°C, indicating that cleavage of the carbon skeleton takes place during the second stage of the reaction.

It has been established that while 3α,7α-diacetoxy-12α-oxo-5β-methylcholanate undergoes an intensive thermal destruction (Fig. 5) in the second stage, similar thermochemical changes for the triacetate have not been observed (Fig. 6). This observation gives us grounds to state that the C_{12} keto group is the reason for such a destruction. Most probably the –OH group at C_{12} is oxidized to a keto group and this leads to thermal destruction of methyl cholate, 3α-acetoxy-7α,12α-dihydroxy-5β-methylcholanate and 3α,7α-diacetoxy-12α-hydroxy-5β-methylcholanate. The presence of a side chain does not exert a steric influence on the oxidation of –OH at C_{12} [12].



Scheme 2

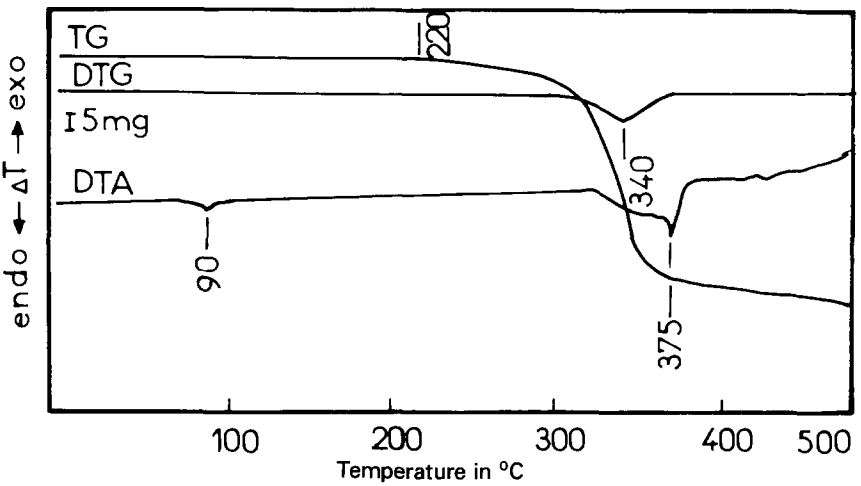


Fig. 6. Thermal decomposition curves of 3α,7α,12α-triacetoxy-5β-methylcholanate.

4. Conclusions

(1) The thermal decomposition of cholic acid proceeds as a one-stage process in the 200–420°C range. The esterification of cholic acid increases the thermal stability of the derivatized compound.

(2) The thermal decomposition reactions of the dried methyl cholate (**2b**), 3α-acetoxy-7α,12α-dihydroxy-5β-methylcholanate (**3**), 3α,7α-diacetoxy-12α-hydroxy-5β-methylcholanate (**4**), 3α,7α-diacetoxy-12α-oxo-5β-methylcholanate (**5**) are found to proceed in two stages.

(3) The formation of a completely deacetylated compound and two monodeacetylated compounds was established during the thermal degradation of (**5**) during the first stage (340°C). The presence of a C_{12} keto group in the molecule is responsible for this destruction.

(4) Degradation of the carbon skeleton takes place during the second stage of the thermal decomposition of the dried methyl cholate (**2b**), 3α-acetoxy-7α, 12α-dihydroxy-5β-methylcholanate (**3**), 3α,7α-diacetoxy-12α-hydroxy-5β-methylcholanate (**4**), 3α,7α-diacetoxy-12α-oxo-5β-methylcholanate (**5**).

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