

Rate Constants for Methanolysis and Alkaline Hydrolysis of a Group of C¹⁴ Labelled (Radioactive) Steroidal Esters Using a Radiochemical Procedure

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Summary: Rate constants for methanolysis of a group of C¹⁴ labelled methyl esters of steroidal carboxylic acids were determined by a radiochemical procedure in order to examine the effect of interposition of a methylene group between the steroidal ring system and the carbomethoxy group on the differential reactivity of epimeric esters. The results are of interest regarding the factors controlling the differential rate of alkaline hydrolysis of acyl derivatives of epimeric axial and equatorial alicyclic alcohols.

For specific comparison with the methyl ester rate constants, those for the hydrolysis of the epimeric 5-acetoxy-5- α -cholestane were also measured, again by a radiochemical procedure.

We carried out a kinetic study of methanolysis and alkaline hydrolysis of a group of C¹⁴ labelled radioactive steroidal esters (synthesised by the authors), in order to investigate the differential reactivity between axial and equatorial epimers when these reactions were sensitive to steric compression and to examine the effect of interposition of a methylene group between the steroidal ring system and the carbomethoxyl group on the differential reactivity of epimeric esters.

The steroidal esters used are conveniently classified as those which contain three different types of reaction centres, these being -CH₂COOC¹⁴H₃, -COOC¹⁴H₃ and -O-CO-C¹⁴H₃. These reactions involve transition states, whose steric requirements are greater than those of ground states. According to the equation:

$$\Delta G_{AX}^{\ddagger} = G_{Eq}^{\ddagger} + (G_{AX}^{\ddagger} - G_{Eq}^{\ddagger}) \cdot \Delta G^{\circ}$$

$$\text{and } (G_{AX}^{\ddagger} - G_{Eq}^{\ddagger}) > \Delta G^{\circ}$$

it follows that $\Delta G_{AX}^{\ddagger} > \Delta G_{Eq}^{\ddagger}$ and therefore the axial isomers react more slowly.

ΔG° is the free energy difference between axial and equatorial isomer in the ground state, $G_{AX}^{\ddagger} - G_{Eq}^{\ddagger}$ is the

larger difference in energy between the transition states.

Methanolysis or hydrolysis of C¹⁴ radioactive esters were carried out at low temperatures and an accurate estimate of the amount of ester hydrolysed was determined by the fall in C¹⁴ contents of the total ester. The fall in C¹⁴ content of steroidal esters containing reaction centres -CH₂COOC¹⁴H₃ and -COOC¹⁴H₃ was carried out by a process of methanolysis which involves an exchange reaction using CH₃ONa to obtain the corresponding unlabelled steroidal esters. A similar fall in C¹⁴ content of steroidal esters containing -O-CO-C¹⁴H₃ group was carried out by alkaline hydrolysis using sodium hydroxide to obtain the corresponding unlabelled steroidal alcohols and sodium acetate. A comparison of the reaction rate constant for the exchange reaction or for hydrolysis, at the same temperature for a particular ester of axial or equatorial orientation determines the ratio of the corresponding reaction rates. The reaction follows a pseudounimolecular rate law as the amount of alkali used was large as compared to the steroidal esters, shown in Table 1.

The rate determining step is presumed to be the formation of the intermediate complex in all the three types of esters as shown in equations (1 to 9)

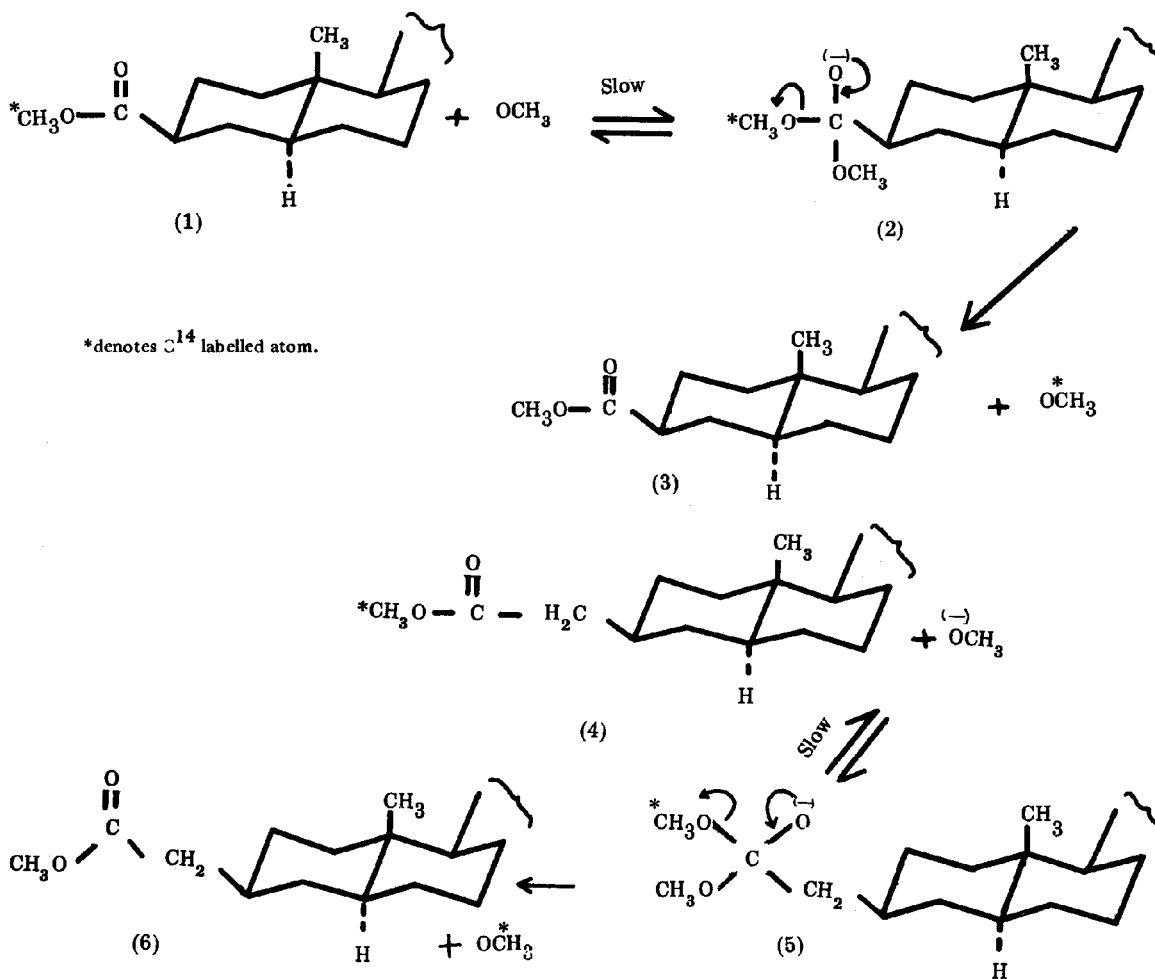
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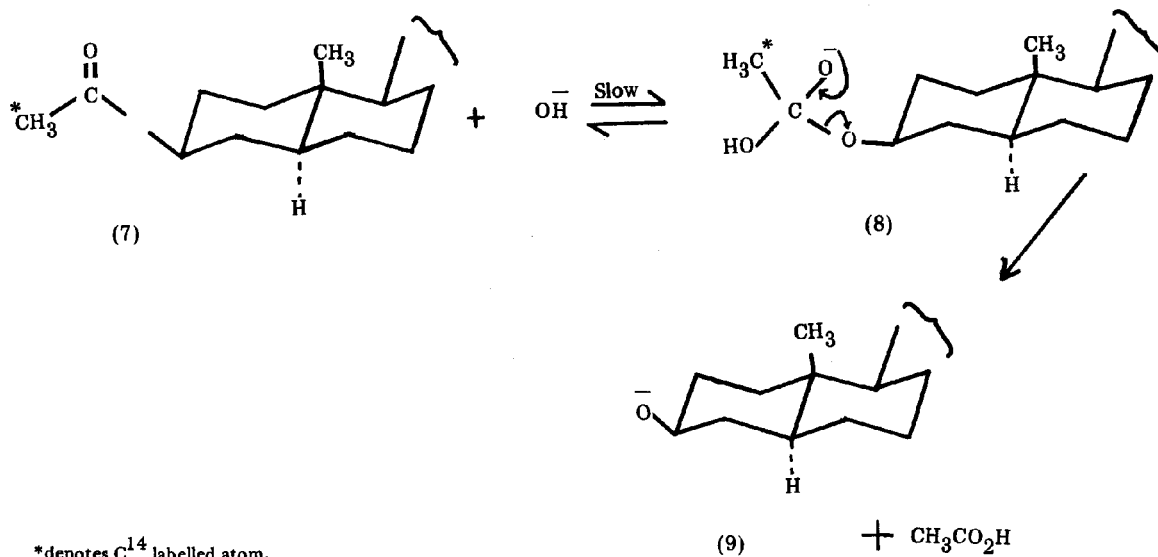
Table 1. Kinetic investigation of C^{14} labelled radioactive steroidal esters.

Compound	Temperature (Centigrade $^{\circ}$)	Half Life $t_{1/2} \times 10^{-3}$ sec.	Rate $10^4 K \text{ Sec.}^{-1}$	Ratio
				$\frac{K_{\text{Eq.}}}{K_{\text{Ax.}}}$
Ch-COOC $^{14}\text{H}_3$ (Eq.)	3.5	10.2	0.6837	3.8
Ch-COOC $^{14}\text{H}_3$ (Ax.)		39.88	0.179	
Ch-CH $_2$ COOC $^{14}\text{H}_3$ (Eq.)	3.5	9.972	0.7059	1.32
Ch-CH $_2$ COOC $^{14}\text{H}_3$ (Ax.)		13.176	0.531	
Ch-O-COC $^{14}\text{H}_3$ (Eq.)	6	17.88	0.396	2.23
Ch-O-COC $^{14}\text{H}_3$ (Ax.)		40.080	0.174	

(i) Ch-stands for 5α - cholestanyl - radical).

(ii) Radioactive samples were counted on Packard Tricarb Series 3003 Liquid Scintillation Spectrometer.



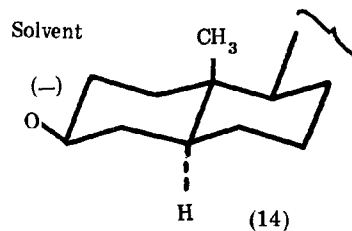
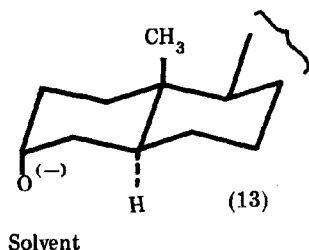
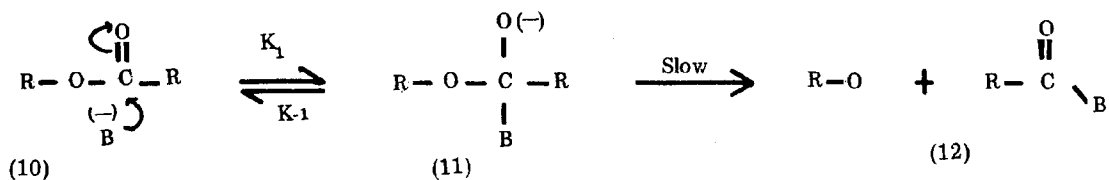


From the above results it is observed that the reaction rates of equatorial and axial epimers in the alkaline methoxy — exchange reaction of methyl 5 α -cholestane-3-carboxylate was found to be 4:1. The differential reactivity of the epimers is attributed to the reactivity of the ester group, which is directly attached to the steroid residue and is subjected to 3:5 related (syn-axial) steric compression in the axial epimer. This will cause the axial esters to have a higher value of activation energies, which in turn will lower the rate of methanolysis for the axial epimer.

A similar high ratio¹ of 17:1 for the reaction rates of equatorial to axial epimers in the alkaline hydrolysis of 4-*t*-butylcyclohexane carboxylate was observed. In the study of the Menchutkin reaction involving quaternisation of the epimeric 3-dimethylamino-5 α -cholestane² using methyl iodide, the ratio was even more marked, being approximately 100:1 for the equatorial and axial epimers. The ratio of reaction rates for methanolysis was found to have fallen to 1:1 (approx.) in case of epimers of methyl-5 α -cholestan-3-ylacetate. This was because of the increase in bulk in the transition state at a reaction centre two bond-lengths away from the steroid residue. The reaction centre will therefore be free of steric compression in both the axial and the equatorial epimer and consequently no significant difference in the energy of activation between the epimers is expected. This in turn will cause the axial and equatorial epimers to react at an approximate similar rate. Thus the quaternisation of axial and equatorial 3-dimethylaminomethyl-5 α -cholestane³, in which the reaction centre was two bond lengths away from the steroid residue, also reacted at a similar rate-ratio (1:1).

The ratio of reaction rates for the hydrolysis of axial and equatorial epimers of 3-acetoxy-5 α -cholestane was found to be 1:2. The slow rate of alkaline hydrolysis of axial ester requires a special explanation as the bulk of the electric charge is built up at a reaction centre which is two bond-lengths away from the steroid residue, and so the steric compression exerted at the reaction centre is very similar whether it be axial or equatorial. Further, C-C-N, C-C-C and C-O-C bondings in various compounds are approximately of the same size and shape and the interposed methylene group and the oxygen atom in particular are occupying a similar spatial volume. Therefore, the reason for the faster reaction rate of the equatorial than axial steroidal epimers is probably due to the relatively lower hindrance to solvation of the alkoxy oxygen atom in the equatorial as compared to the axial isomer. It should be pointed out that the alkaline hydrolysis of an ester normally involves an unstable intermediate (11).

Both the formation of this intermediate and its decomposition would result in solvation around the alkoxy - oxygen atom in each case and this seems to be the critical feature of the observed differential reactivity⁴. The alkoxy - oxygen atom may become partly negatively charged during the course of the reaction and this will require solvation. The solvent molecule will "freeze" around the anion to increase effectively the bulk of the group. The degree of solvation of the transition state complex will be less in the axial ester (because of 3:5 related (syn-axial) interactions) than in the equatorial epimer. Thus the solvent sphere will make the anion more hindered in the axial position (13) than for a similar transition state in the equatorial epimer (14).



Therefore the differential ease of solvation of the epimers is the obvious explanation for the lower rate of hydrolysis obtained in case of axial isomer. This has also been observed in the case of axial primary amines^{3,5}, which are weaker bases than their equatorial epimers because solvation of the ammonium ion (which lowers the energy of the ground state) is more hindered for the axial compound.

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