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KINETICS OF THE ACETYLATION OF e- AND a-HYDROXYLS IN CARDENOLIDES

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It is known [1, 2] that equatorial hydroxy groups are more readily acylated than axial ones. This correct conclusion was apparently obtained empirically, since we have found no kinetic studies. In the present paper, we show

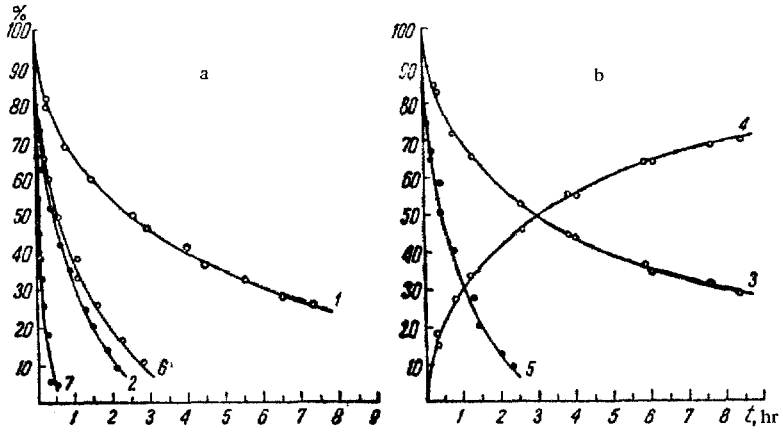
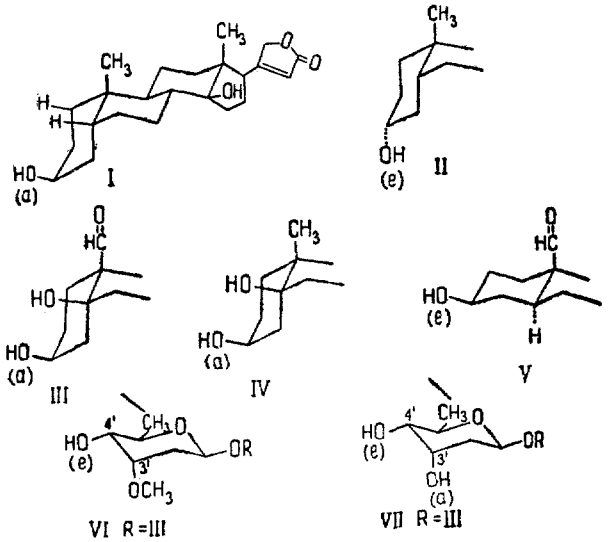


Fig. 1. Kinetic curves of the change in the content of digitoxigenin (1), 3-epidigitoxigenin (2), cymarin (6), erysimin (7), strophanthidin (3), and corotoxigenin (5) and for the formation of strophanthidin acetate (4) in the acetylation reaction.

how large are the differences in dependence on their conformations of the rates of acetylation of the OH groups of digitoxigenin (I), 3-epidigitoxigenin (II), strophanthidin (III), periplogenin (IV), corotoxigenin (V), cymarin (VI), and erysimin (VII).

The substances were acetylated under predetermined conditions with acetic anhydride in pyridine. The course of the reaction was analyzed with quantitative paper chromatography by a known method [3]. The kinetic curves were plotted mainly on the basis of the change in the contents of the initial compounds as a function of the reaction time, and in individual cases with respect to the acetates formed. For comparative estimates of the rates of the reactions, we took the time during which 50% of a given substance was converted into the acetyl derivative.

The results obtained (Fig. 1, table) show that the rates of acetylation differ markedly according to the conformation of the OH groups. The equatorial hydroxyls of the aglycones are acetylated four and more times faster than the axial hydroxyls. The "half-period" of acetylation of the former is about 35 min, while for the axial hydroxyl groups it is more than 150 min. The most indicative pair of cardenolides with different conformations of the OH groups on the same carbon atom is formed by digitoxigenin (I) and 3-epidigitoxigenin (II). The latter acetylates 4.8 times faster than the former.



The lower reactivity of the axial hydroxyls is explained by the fact that they are more strongly subjected to steric screening from the direction of the two, frequently axial, substituents on the neighboring carbon atoms. Thus,

Rate of 50% Acetylation of Cardenolides

Substance	Conformation of the OH group acetylated	Time, min	Relative rate	
			with respect to digitoxigenin	with respect to 4'-O-acetyl erysimin
Digitoxigenin (I)	*a*	154	1.0	0.8
Strophanthidin (III)	*a*	180	0.9	0.7
Periplogenin (IV)	*a*	175	0.9	0.7
3-Epidigitoxigenin (II)	*e*	32	4.8	3.8
Corotoxigenin (V)	*e*	36	4.3	3.3
4'-O-Acetylerysimin	*a* at $C_{3'}$	120	1.3	1.0
Cymarin (VI)	*e* at $C_{4'}$	38	4.1	3.2
Erysimin (VII)	*e* at $C_{4'}$	5	30.8	24.0

in digitoxigenin (I) the axially-located hydrogen atoms at C_1 and C_5 exert a screening influence on the hydroxyl at C_3. In 3-epidigitoxigenin (II), the equatorial hydroxyl is freer and is more remote from the substituents mentioned. Strophanthidin (III) and periplogenin (IV) acetylate more slowly than digitoxigenin. This is connected with the presence of an intermolecular hydrogen bond between the hydroxyls at C_3 and C_5 [4] and also with the more voluminous substituent at C_5. The axial hydroxyls in the cardiac aglycones at C_1 and C_{11} acetylate particularly slowly, since the approach of the reagent to them is hindered to a still greater extent by the adjacent voluminous substituents. At the same time, equatorial hydroxyls on these carbon atoms are comparatively free and readily acetylated.

Judging from the rate of acetylation of erysimin, it can be assumed that OH groups in sugars are more reactive than those in a steroid nucleus. This is probably due to the lower conformational stability of substituents in carbohydrates and to their capacity for assuming positions somewhat deviating from the "pure" axial or equatorial positions under the influence of external factors. In the steroids, unlike the sugars, the polycyclic system is bound fairly rigidly, and therefore substituents in it have a high conformational stability.

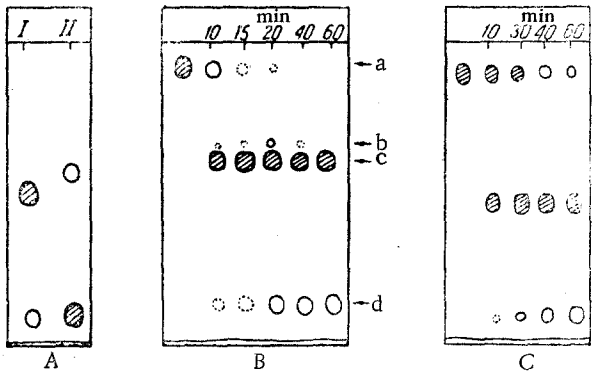


Fig. 2. Chromatograms of the acetylation products [solvent system: methyl ethyl ketone–m-xylene (1 : 1)/formamide [6], chromatography for 3 hr]. A) Digitoxigenin (I) and 3-epidigitoxigenin (II) (70 min); B) a) initial erysimin; b) 3'-O-acetylerysimin; c) 4'-O-acetylerysimin; d) 3',4'-di-O-acetylerysimin; C) digoxigenin.

The great difference in the rates of acetylation of e- and *a*-hydroxyls makes it possible to use them to determine the conformations of OH groups in natural steroid compounds. Our experiments show that for these purposes in the majority of cases it is unnecessary to plot kinetic curves. In order to obtain a rapid answer to the question of the conformation of a secondary hydroxyl, the following simplified method can be used. Five to six samples must be taken over 1.5 hr from the beginning of the acetylation of the substance and be subjected to paper chromatography. On the developed chromatograms it can clearly be seen with the naked eye that after 1–1.5 hr a compound with an e-hydroxyl undergoing acetylation is mainly converted into the less polar acetyl derivative, while in the case of a compound with an *a*-hydroxyl the bulk of it still remains unchanged. Figure 2 shows a chromatogram with samples 1 hr 10 min after the beginning of the acetylation of digitoxigenin and 3-epidigitoxigenin. When the compound contains two secondary hydroxyls one of which is axial and the other equatorial, the chromatogram looks approximately as is shown in Fig. 2 for erysimin and digitoxigenin. The initial compound disappears fairly rapidly, being converted mainly into the intermediate monoacetate (acetylation predominantly at the e-hydroxyl). The intermediate compound is then slowly converted into the diacetate at the rate characteristic for the acetylability of *a*-hydroxyls.

By making use of the difference in the rates of acetylation of e- and *a*-hydroxyls, we have synthesized [5] k-strophanthin-β from erysimoside, and cymarin (VI) from erysimin (VII) [7]. In both cases, the acetylation reaction was used to introduce a protective group at the e-hydroxyl. By breaking off the reaction after a predetermined time, the axial hydroxyl could be kept free and in a subsequent reaction it was subjected to methylation. The protective acetyl groups were then eliminated by ammoniacal hydrolysis.

Experimental

All the cardenolides mentioned above were dried before analysis. The formamide used for the quantitative paper chromatography was purified by passage through activated carbon and alumina.

A separate calibration curve was constructed for each cardenolide studied. For this purpose, 12 mg of the substance was dissolved in 3 ml of ethanol–formamide (3 : 1), and equal volumes of the solutions were transferred by means of a micropipet to chromatograms cut to a definite pattern. After chromatography and drying of the chromatograms in air for 30 min, the corresponding sections were cut out at the level of reference samples, chopped into small pieces, placed in flasks with firmly ground-in stoppers, covered with 5 ml of ethanol in each case, and left for 2 hr with stirring from time to time. Then 5 ml of a reagent consisting of 95 parts (by volume) of a 1% aqueous solution of picric acid and five parts of a 10% aqueous solution of caustic soda was added. Each solution was shaken for 4 min and was filtered into a quartz cell through a no. 2 glass filter, and the extinction was measured in an FEK-56 photoelectric colorimeter-nephelometer (filter with maximum light transmission at a wavelength of 490 mμ). The highest value of the extinction was reached 12–15 min after the addition of the reagent. The calibration graphs were constructed in the form of the extinction as a function of the number of grams of substance.

For acetylation, 12 mg of a cardenolide was dissolved in 2 ml of absolute pyridine, 1 ml of acetic anhydride was added, and the mixture was left at 19–20° C. The change in the amount of initial compound or the accumulation of acetyl derivative as the reaction proceeded was determined by taking samples for chromatographic-colorimetric analysis. The solutions were deposited on sheets of paper and the chromatograms were treated as described above. After the determination of the extinction of the sample taken, the number of grams of substance in the volume of the reaction mixture taken for analysis was found from the calibration graph. Then the percentage content of the substance was calculated and was referred to the moment of taking the sample for analysis. When a sufficient number of data had been obtained, the kinetic curve was constructed in the form of the content of the compound as a function of the time of the reaction.

The time from the beginning of deposition of the sample on the sheet of chromatographic paper to the beginning of chromatography was 2–3 min. Assuming that an error was possible due to the occurrence of the acetylation reaction after the deposition of the solution on the paper, we carried out several experiments in which the reaction was stopped after predetermined intervals of time by the addition of ice to the reaction mixture, and the cardenolides were separated from the solution with subsequent quantitative analysis of their composition. The experiments showed that the analyses using the first and second methods of taking samples did not differ appreciably. Consequently, we subsequently used only the first method, which demands the minimum consumption of time.

Conclusions

1. Kinetic investigations of the rate of acetylation of e- and α-hydroxy groups in cardenolides have been carried out. It has been shown that equatorial hydroxyls in aglycones acetylate more than four times as fast as axial hydroxyls.

2. On the basis of the acetylation of reaction, a simple method has been proposed for determining the conformations of secondary OH groups in steroid compounds.

3. The possibility has been shown of using the acetylation reaction for introducing protective groups on e-hydroxyls.

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