INFLUENCE OF SPATIAL EFFECTS ON THE OXIDATION OF CARDENOLIDES AND BUFADIENOLIDES CONTAINING ALDEHYDE GROUPS

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The reactivity of a number of aglycones and glycosides with a cardiac action containing a carbonyl group at C_{10} is considered. The study was based on the use of the oxidation reaction in the presence of diethylamine, the excess of which after the binding of the hydrochloric acid liberated in the reaction was back-titrated with percholoric acid in methanol. The rate constants of 15 compounds have been determined and it has been established that their reactivity depends on their structure: the nature of the linkage of the A/B rings of the steroid skeleton, the position of a double bond in it, the introduction of a sugar or acetyl group in position 3 of the aglycon, and the nature of the sugar.

In papers published previously [1, 2] the difficulty of performing the oximation reaction of some cardenolides belonging to the trans-A/B series has been shown.

We have carried out a kinetic study of the oximation of cardenolides and bufadienolides containing a carbonyl group at C_{10} of the steroid nucleus with the aim of determining the influence of the type of A/B ring linkage, the presence of a double bond in the 4:6 or 5:6 position, and the nature of the sugar components in glycosides.

An oximation reaction based on the titration with perchloric acid in methanol of the excess of diethylamine binding the hydrochloric acid liberated as the result of the reaction of hydroxylamine with the carbonyl group at C_{10} of the steroid skeleton was used. On the basis of the results obtained, graphs have been plotted of the time dependence of the logarithm of the change in the concentration of the substances under investigation for first- and second-order reactions. In both cases, a linear relationship was observed, but for the second-order reaction the line did not pass through the origin. This fact, along with the considerably greater concentration of hydroxylamine (0.2518 M) as compared with the initial concentration of the substances under investigation (0.0018 M; more than 100-fold) shows that in spite of the bimolecular nature of the oximation reaction, in this case it can be assigned to the pseudo-first-order reactions.

Graphs of the dependence of the instantaneous concentrations of the substances under investigation on the reaction time are shown in Fig. 1. The rate constants of the reactions of the substances under investigation with hydroxylamine hydrochloride are given in Table 1. As follows from the table, these magnitudes are not identical, which can be explained by the linkage of rings A and B with one another in the steroid skeleton by the presence of double bonds in the 4:5 and 5:5 positions in them, and by the structure of the sugar component, which affect the conformations of cardenolide and bufadienolide molecules.

In the first place, glucosides and aglycons containing a carbonyl group at C_{10} and belonging to the cis-A/B series (I) react with hydroxylamine almost an order of magnitude faster than glycosides and aglycons belonging to the trans-A/B series (III).

Strophanthidin (I) has a rate constant an order of magnitude higher than corotoxigenin (III). This can be explained by the fact that the carbonyl group of strophanthidin does not participate in the formation of an intramolecular hydrogen bond with the hydroxy groups at C_3 and C_5; the hydrogen bond in strophanthidin [3] fixes the position of ring A in the chair form (I). Then the formation of the semiacetal form of the OH group at C_3 with the aldehyde

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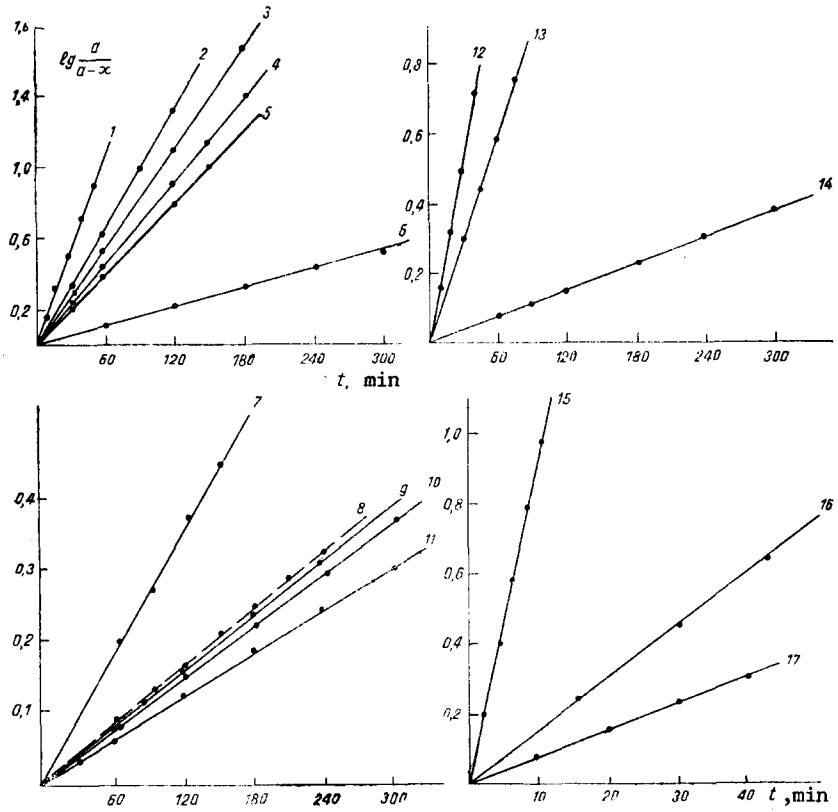
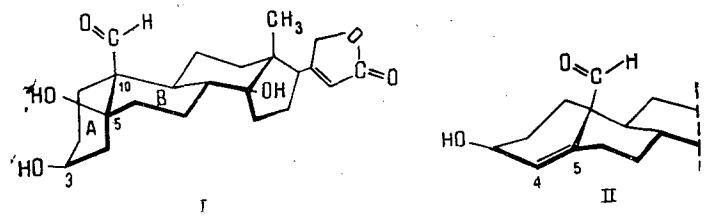


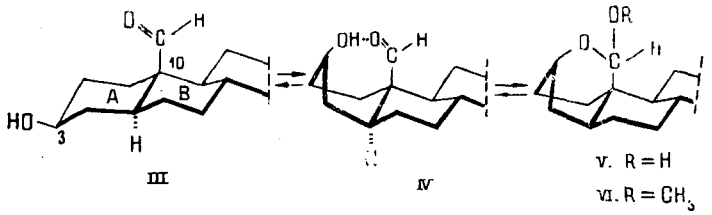
Fig. 1. Graphs of the time dependence of the concentrations of: 1) strophanthidin; 2) cymarin; 3) strophanthidin acetate; 4) convallatoxin; 5) glucostrophanthidin; 6) scorpioside; 7) glucocorotoxigenin; 8) corotoxigenin acetate; 9) bovoside; 10) corotoxigenin; 11) coronillobioside; 12) strophanthidin; 13) pachygenin; 14) corotoxigenin; 15) hyrcanogenin; 16) erysimin; 17) convalloside.

group is impossible. Corotoxigenin (III) contains two polar functional groups (hydroxyl at C_3 and carbonyl at C_{10}) thanks to which in an acid medium the chair-boat equilibrium of ring A is shifted in the direction of the predominant boat state through the formation of an intramolecular hydrogen bond between the C_3 hydroxy group and the carbonyl group at C_{10} (IV). The formation of the semiacetal form (V) in an acid medium was shown by the production of the crystalline methylal of corotoxigenin (VI) which was not reduced with sodium tetrahydroborate and did not undergo acylation which shows the conversion of the aldehyde into an acetal group (VI) [4], and this was also confirmed by the optical rotation dispersion spectra and IR spectra [5].



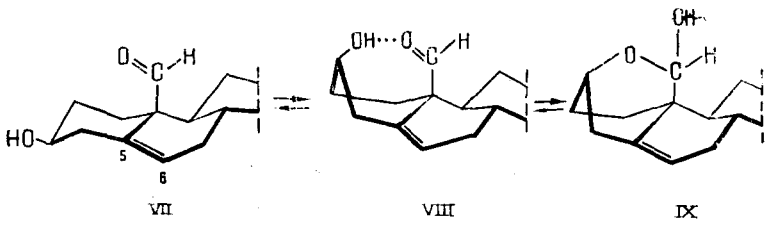
I II

Since the oximation process is performed in the presence of such reagents as hydroxylamine hydrochloride, diethylamine, and methanol, corotoxigenin is possibly present in the aldehyde and semiacetal forms (forms (III-V)), which lowers the rate of oximation as compared with strophanthidin (I), which is present only in the aldehyde form. In addition to this, in the case of glycosides of the trans-A/B series (II), greater steric hindrance is created for the penetration of the reagent to the carbonyl group than in the case of glycosides of the cis-A/B series [6]



III IV V. R = H VI. R = CH_3

Double bonds in various positions in the steroid skeleton also affect the reactivity of the aglycon.



VII VIII IX

In a comparison of the reactivities of strophanthidin (I), hyrcanogenin (Δ^4-strophanthidin, II) and pachygenin (Δ^5-strophanthidin, VII) it can be seen that the rate constant for hyrcanogenin (II) is an order of magnitude higher. As has been shown in [4], a double bond in the Δ^4 position (hyrcanogenin) and the Δ^5 position (pachygenin) is responsible for a number of characteristic features in the reaction properties of aglycons containing a carbonyl group at C_{10}: a Δ^4 bond, unlike a Δ^5 bond, imparts a high positive rotation to a steroid; the optical rotatory dispersion spectrum of hyrcanogenin has a positive Cotton effect, while that of pachygenin has a negative effect. Pachygenin (VII) forms a methylal and a semacetal (IX) while hyrcanogenin does not give derivatives under these conditions. The latter fact is explained by the spatial remoteness of the OH group at C_3 of hyrcanogenin from the aldehyde group at C_{10} because of the 4:5-double bond, which does not permit ring A to assume the boat form, as is observed in pachygenin (VII) or corotoxigenin (III or V). Pachygenin (VII) is capable of assuming the semiacetal form (IX) but the rate of its oximation is higher than that of corotoxigenin (III) because of the 5:6-double bond which changes the valence angles of the C_5 atom, decreasing the mobilities of the C_4–C_5 and C_5–C_6 bonds and imparting to ring A a conformation close to the half-boat conformation. This interferes with the ring-closure of the hydroxyl at C_3 with the aldehyde group to form a semiacetal (IX) and increases the accessibility of the carbonyl group for the reagent. Thus, genins with a double bond at C_4 possess a considerably greater reactivity than genins with a double bond at C_5.

When an acetyl group is introduced into the C_3 position of strophanthidin, the rate constant of the reaction falls to one half, while the introduction of the same group into corotoxigenin has no appreciable effect on the rate constant of the reaction. The decrease in the rate of the reaction for strophanthidin acetate as compared with strophanthidin depends on the steric hindrance to the approach of the reagent to the carbonyl group at C_{10} due to the voluminous axial substituent, an acetoxy group, at C_3.

In a study of the influence of the carbohydrate component in cardiac glycosides on the rate of oxidation of the aldehyde group at C_{10} we took as a basis the following considerations: all sugars in the pyranose form of the β-D series have the Cl conformation of the α-L series the 1C conformation [8], and those of the β-D series in the furanose form the E conformation [9]. The investigation was carried out on glycosides with identical algycons: for the cis-A/B series, strophanthidin (Table 1, substances 3-8), and for the trans-A/B series, corotoxigenin (substances 11 and 12).

It was established that the introduction of a sugar component has an influence on the rate constant of the oximation reaction of glycosides both of the cis-A/B series and of the trans-A/B series (see Table 1).

In the cis-A/B series, on passing from the aglycon strophanthidin to monosides – convallatoxin (α-L-rhamnose) and glucostrophanthidin (β-D-glucose) – the rate of the reaction falls by a factor of 2.3, and the rate constant of the oximation of the bioside convalloside

TABLE 1. Rate Constants of Oxiмation according to the Structure of Cardenolides and Bufadienolides containing Aldehyde Groups

Substance	Sugar residue in a glycoside	conformation of sugar ring	Substituent in the ring of the sugar residue				Oximation rate constant, m^{-1}
			2	3	4	5	
cis-A/B series							
1. Strophanthidin	—	—	—	—	—	—	$4.01 \cdot 10^{-2}$
2. Strophanthidin acetate	—	—	—	—	—	—	$2.15 \cdot 10^{-2}$
3. Convallatoxin	α-L-rhamnopyranose	1C	aOH	eOH	eOH	CH_3	$1.77 \cdot 10^{-2}$
4. Convalloside	α-L-rhamnopyrnose-(4←1)-O-β-D-giucopyranose	1C C1	aOH eOH	eOH eOH	eOH	CH_3 CH_2OH	$1.82 \cdot 10^{-2}$
5. Erysimin	β-D-digitoxopyranose	C1	—	aOH	eOH	CH_3	$3.35 \cdot 10^{-2}$
6. Cymarin	β-D-cymaropyranose	C1	—	$aOCH_3$	eOH	CH_3	$2.59 \cdot 10^{-2}$
7. Glucostrophanthidin	β-D-glucopyranose	C1	eOH	eOH	eOH	CH_2OH	$1.63 \cdot 10^{-2}$
8. Scorpioside	β-D-glucopyranose	E	qeOH	qe OH	CHOH	CH_2OH	$4.04 \cdot 10^{-3}$
trans-A/B series							
9. Cortoxigenin	—	—	—	—	—	—	$2.92 \cdot 10^{-3}$
10. Cortoxigenin acetate	—	—	—	—	—	—	$3.04 \cdot 10^{-3}$
11. Glucocorotoxigenin	β-D-glucopyranose	C1	eOH	eOH	eOH	CH_2OH	$7.05 \cdot 10^{-3}$
12. Coronillobioside	β-D-glucopyranose-(4←1)-β-D-glucopyranose	C1	eOH	eOH	eOH	CH_2OH	$2.33 \cdot 10^{-3}$
13. Bovoside (bufadienolide)	α-L-thevetopyranose	C1 1C	eOH eOH	eOH $eOCH_3$	eOH eOH	CH_2OH CH_3	$3.08 \cdot 10^{-3}$
Δ^4 series							
14. Hyrcanogenin (Δ^4-strophanthidin)	—	—	—	—	—	—	$2.21 \cdot 10^{-1}$
Δ^5 series							
15. Pachygenin (Δ^5-strophanthidin)	—	—	—	—	—	—	$2.29 \cdot 10^{-2}$

a — axial substituent; e — equatorial substituent; qe — quasi-equatorial substituent.

(α-L-rhamnose-(4 ← 1)-β-D-glucose) falls by the same factor. Consequently, the introduction of a sugar component into the aglycon strophanthidin interferes with the occurrence of the oximation reaction obviously through the influence of a spatial effect, and the introduction of a second sugar component does not lead to a decrease in the rate constant as compared with the monosides.

A marked decrease in the rate of the reaction is observed on the introduction of the sugar D-glycopyranose into the strophanthidin molecule. Thus, the rate constant for the oximation of scorpioside is an order of magnitude lower than that of strophanthidin. It is obvious that flucofuranose has a greater steric effect than D-glucopyranose or L-rhamnopyranose which lower the reactivity of the aldehyde group by a factor of 2.5 relative to strophanthidin (see Table 1).

A hydroxy group, in position 2 of the carbohydrate residue has a substantial influence on the reactivity of the aldehyde group in the cardiac glycosides of the cis-A/B series. Its absence from D-digitoxose (erysimin) and D-cymarose (cymarin) lowers the rate constant of the reaction by factors of 1.2 and 1.5 relative to that for strophanthidin (see Table 1).

In the trans-A/B series the sugar components likewise affect the rate constant of the oximation of glucosides. The introduction of D-glucopyranose into corotoxigenin increases the rate constant by a factor of 2.3, which can be explained by the hindrance to the passage of ring A of the aglycon into the boat form caused by the carbohydrate residue. An increase in the size of the carbohydrate chain in coronillobioside decreases the rate of the reaction only slightly. This is possibly due to the screening of the aldehyde group by the terminal D-glucose residue, as has been observed by Tschesche et al. [10] in glucobovoside A.

For the bufadienolide glycoside bovoside the same characteristics are observed as for the cardenolides of the trans-A/B series.

EXPERIMENTAL

The substances for analysis were dried in high vacuum over phosphorus pentoxide at 78.4°C for 3 h. The purity of the aglycons and glycosides was determined by thin-layer chromatography using plates of the Silufol type and the following solvent systems: 1) benzene–butanol (1:1) saturated with water; 2) chloroform–ethanol (3:1); 3) benzene–ethanol (3:1); 4) chloroform–acetic acid–methanol (85:2:13); 5) methylene chloride–methanol–formamide (80:19:1); and 6) ethanol [11].

Method of Determination. A weighed sample of substance (0.0018 mole) was placed in a 50-ml measuring flask and was dissolved in methanol freed from carbonyl-containing compounds, and the volume of the liquid in the flask was made up to the mark with methanol. From the resulting solution, 5 ml was transferred into a flask with a ground-in stopper, 5 ml of the reagent (prepared by dissolving 3.5 g of hydroxylamine hydrochloride and 0.12 g of diethylamine in 100 ml of methanol) was added, and the mixture was kept for a definite time at 20°C. Then the excess of reagent was titrated with a 0.02 N solution of perchloric acid in methanol in the presence of a 0.3% solution of thymol blue in methanol as indicator until the appearance of a pink coloration. The concentrations of the substances under investigation (M) were calculated from the difference in milliliters consumed in the titration of a control experiment and in an experiment with a weighed sample of substance. Graphs of the time dependence of the logarithms of the instantaneous concentrations of the substances under investigation for a first-order reaction were plotted from the results obtained.

The rate constants of the reaction were calculated from the first-order equation [12]

$$K = \frac{1}{t} \ln \frac{a}{a - x}$$

SUMMARY

1. The rate constants of the oximation reaction of 15 compounds consisting of cardenolide and bufadienolide aglycons and glycosides have been determined.

2. On the basis of the results of a study of the kinetics of the oximation reactions of cardenolides and bufadienolides it has been established that the reactivity of a carbonyl group at C_{10} depends on the nature of the linkage of the A/B rings of the steroid skeleton, the position of a double bond in it, and the nature of a sugar component in position 3 of the aglycon.

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TRITERPENE GLYCOSIDES OF ALFALFA.

III. MEDICOSIDE I

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On the basis of chemical transformations and with the aid of physicochemical results, the structure of glycoside I isolated from the roots of the plant Medicago sativa has been established as hederagin 3-O-[O-α-L-arabinopyranosyl-(1 → 2)-β-D-glucopyranosyl-(1 → 2)-α-L-arabinopyranoside] 28-O-β-D-glucopyranoside. Compound (I), $C_{52}H_{84}O_{22}$, mp 210-212°C, $[\alpha]_D^{21}$ +38.4° (c 1.48; methanol). Acid hydrolysis of (I) led to hederogenin (II) – $C_{30}H_{48}O_4$, mp 326-330°C, $[\alpha]_D^{23}$ + 84.2° (c 0.19; pyridine. The Hakomori methylation of glycoside (I) yielded the permethylate (IV) – $C_{65}H_{110}O_{22}$ $[\alpha]_D^{23}$ +41.6° (c 1.79; methanol). The GLC analysis of the products of the methanolysis of compound (IV) showed the presence of 3,4,6-tri-O-methyl-D-glucopyranose, 2,3,4,6-tetra-O-methyl-D-glucopyranose, 3,4-di-O-methyl-L-O-arabinopyranose, and 2,3,4-tri-o-methyl-L-arabinopyranose. The alkaline hydrolysis of glycoside I gave compound (III) with mp 230-233°C, $[\alpha]_D^{21}$ +35.2° (c 0.21; methanol), which was identified as medicoside C. Details of the PMR spectrum are given for compound (IV) and of the IR spectrum for compound (I).

We have continued a study of the triterpene glycosides of Medicago sativa L. (family Fabaceae). The column chromatography on silica gel of the combined triterpene glycosides from a new batch of the roots yielded medicagenic acid 3-O-β-D-glucopyranoside (substance A), medicoside C (substance C) and medicoside G (substance G) [1, 2], and also three more polar compounds which we have called medicosides I, J, and L. In the present paper we consider the determination of the structure of medicoside I (I).

The acid hydrolysis of medicoside I led to hederagenin (II), which was identified from its physicochemical constants and IR spectrum. It was established with the aid of gas-liquid chromatography (GLC) that compound (I) contained D-glucose and L-arabinose residues in equimolar amounts.

The IR spectrum of glycoside (I) contained absorption bands due to an ester group, which showed the presence of an acyloside chain. The alkaline hydrolysis of the medicoside gave compound (III), the GLC analysis of which showed the presence of D-glucose and L-arabinose residues in a ratio of 1:2. The physicochemical constants, spectral characteristics, and, and the R_f value on a thin-layer chromatogram (TLC) showed the identity of glycosides (III) and native medicoside C, isolated previously [2].

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