GLYCOSYLATION OF CARDENOLIDES.

VIII. STRUCTURES OF THE BY-PRODUCTS OF THE SYNTHESIS BY THE ORTHOESTER METHOD OF STROPHANTHIDOL RHAMNOSIDES

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The by-products formed in the preparation of strophanthiodol 19-rhamnoside and 3,19-bisrhamnoside by the orthoester method are 3β,5-dihydroxy-8,19-epoxy-5β,14β,17α-card-20(22)-enolide and its 3-α-L-rhamnoside. 3β,5-Dihydroxy-8,19-epoxy-5β,14α,17β-card-20(22)-enolide has been obtained by the action of a solution of HBr in nitromethane on strophanthidol.

We have previously reported the synthesis of strophanthidol 3,19-bis-α-L-rhamnoside (IV) by the condensation of strophanthidol with the rhamnose orthoester (II) in the presence of $HgBr_2$ [1]. The process also led to compound (V), which, according to its PMR spectrum, is a monorhamnoside. In its physicochemical constants, this substance differs from strophanthidol 3-L-rhamnoside (convallaxol (III)). Neither is it strophanthidol 19-L-rhamnoside (X). The chemical shifts of the proton at C-1 of the rhamnose residues in compounds (V) and (X) do not coincide with one another. In the PMR spectrum of rhamnoside (X), the signals of the methylene protons are unresolvable, since they are superposed on the signals of protons geminal to hydroxy group. In product (V), however, the signal of one of the methylene protons at C-19 is shifted upfield and appears distinctly at 3.27 ppm. (See scheme on following page.)

In a comparison of the indices of the PMR spectra of strophanthidol (I) and product (V), it can be seen that the signals of the C-19 methylene protons of strophanthidol are located in a weaker field and have a larger spin–spin coupling constant than in the case of substance (V) (Table 1).

Acetylation of the glycoside (V) yielded the triacetate (VI), in the mass spectrum of which there is a peak of an ion with m/z 273. This peak must be assigned to the pyronium ion of rhamnose triacetate. In the acetate (VI) the chemical shifts of the protons at C-19 have changed little in comparison with compound (V). Only additional signals are those of three acetyl groups. Consequently, acetylation has not affected the aglucone moiety.

The facts given indicate that in the by-product of the condensation of strophanthidol (I) with the rhamnose orthoester (II) the nature of the oxygen function at C-19 has changed. In glycoside (V) there is no hydroxy group at C-19. It appears to us to be most likely that the by-product (V) includes an 8,19-oxygen bridge.

It is known that transformations of this type take place in an acid medium [2]. Consequently, an attempt was made to obtain the genin of the glycoside (V) directly from strophanthidol (I) by treating it with an anhydrous solution of HBr in nitromethane. However, the product (VII) synthesized in this way differed considerably from compound (V). While the signals of the protons at C-19 in products (V) and (VII) are completely comparable (see Table 1), the methylene protons at C-21 resonate differently. In the PMR spectrum of compound (VII) there are two doublets with J = 18 Hz (with deuteropyridine as solvent) or a triplet with J = 18 Hz (with deuterochloroform as solvent), which best corresponds to the 17β configuration of the butenolide ring. In the case of glycoside (V), this is a broadened singlet which corresponds to the 17α configuration [3].

We give the chemical shifts of protons of cardenolides with different configurations at C-14 and C-17 (according to the literature [3], δ, ppm, 0 — TMS):

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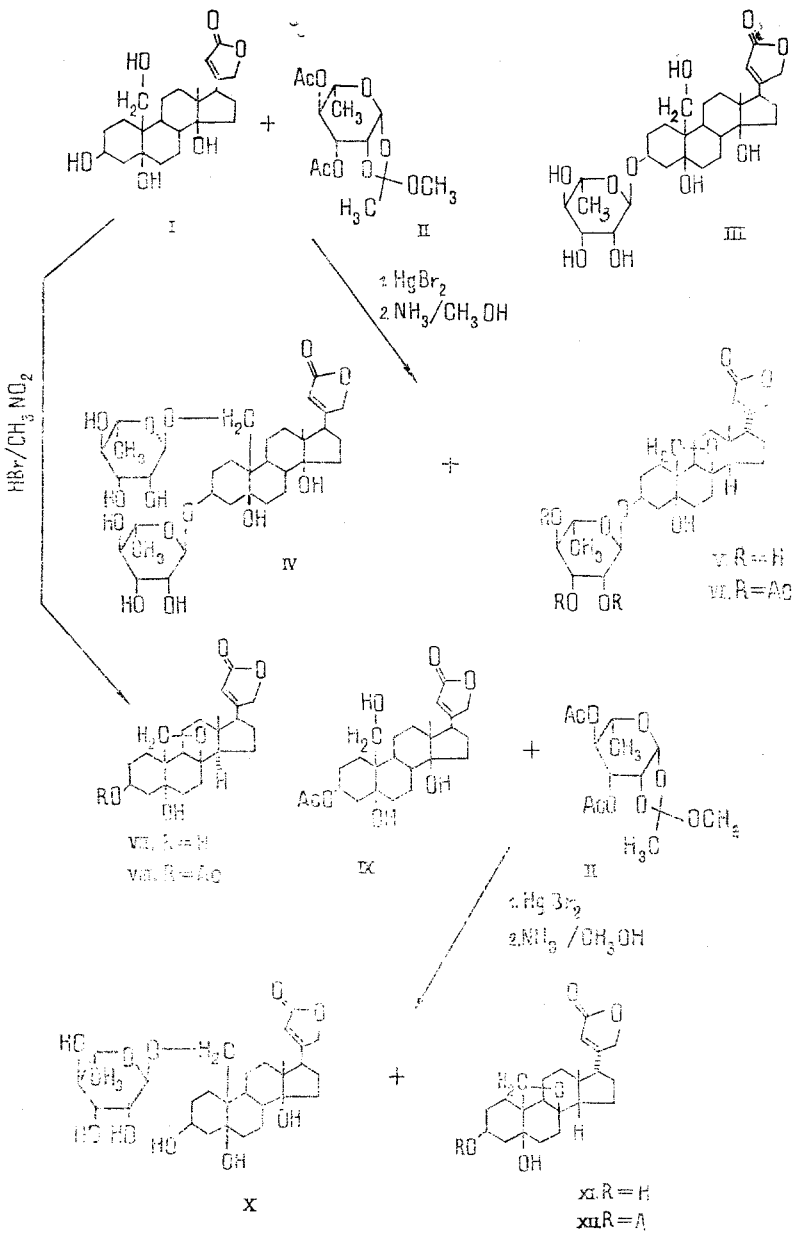

Configuration	17-H	21-H	Solvent
14α; 17β	Signal unresolvable	Quartet	$CDCl_3$
14β; 17β	"	"	"
14β; 17α	2.57-2.60	Singlet	"
14β-OH; 17α	3.22-3.25	"	"
"	3.40-3.43	"	C_5D_5N
14β-OH; 17β	2.80-2.84	Quartet	$CDCl_3$
"	2.83-2.86	"	C_5D_5N

As an analysis of the features of the optical rotatory dispersion of the cardenolides differing by the configurations at C-14 and C-17 shows, only the 14α,17β-isomers have a negative Cotton effect, and in all other cases this effect is positive [4]. The negative Cotton effect observed for substance (VII) has permitted us to assign it to compounds with a 14α, 17β-configuration.

In the PMR spectrum of the acetate (VIII) there is the signal of only one acetyl group, and the signal of the proton at C-3 is shifted downfield by 0.85 ppm as compared with the signal of the proton at the same carbon atom of the initial compound (VII). The chemical

TABLE 1. Chemical Shifts of the Protons at C-17, C-18, C-19, and C-21 of Strophanthidol and its Derivatives (δ, ppm; 0 — HMDS)

Compound	18-H	19-H	17-H	21-H	Solvent
I	0,92	3,72; 4,36* J=11,0 Hz	2,71	4,88; 5,18*; J=18,0 Hz	C_5D_5N
VI	0,88	†	2,65	4,87; 5,17* J=18,0 Hz	"
V	0,93	3,27; 4,23* J=8,5 Hz	2,35	4,73 u.s.	"
IV	0,91	3,35; 4,30* J=8,5 Hz	2,38	4,72 u.s.	"
VII	0,74	3,36; 4,35* J=8,0 Hz	‡	4,59; 4,85* J=18,0 Hz	"
"	0,76	3,37; 4,09* J=8,5 Hz	‡	4,66, τ J=18,0 Hz	$CDCl_3$
VIII	0,70	3,31; 4,32* J=8,0 Hz	‡	4,58; 4,80* J=18,0 Hz	C_5D_5N
X	0,87	†	2,61	4,79; 5,11* J=18,0 Hz	"
XI	0,93	3,31; 4,32* J=8,0 Hz	2,40	4,70 u.s.	"
"	0,94	3,31; 4,00* J=8,0 Hz	2,50 (2,55)	4,65 u.s. (4,70)	$CDCl_3$

*The values of the centers of the doublets are given.
†The signals of the protons at C-19 are unresolvable because they are superposed on the signals of protons geminal to hydroxy groups.
‡The signal of the 17-H proton is unresolvable since it is present in the "methylene hump."
**In parenthesis are the values of the chemical shift recalculated to TMS as standard.

shifts of the protons at C-19 of substances (VII) and (VIII) are completely comparable (see Table 1). Consequently, compound (VII) contains one acetylatable hydroxy group which is present on the third carbon atom. In the mass spectra of compounds (VII) and (VIII) there are the peaks of the molecular ions with m/z 388 and 430, respectively, which, together with the features of their PMR spectra, show the detachment of water from the hydroxy groups of strophanthidol at C-19 and C-14 with the formation of an oxygen bridge. By analogy with the transformation of strophanthidine into pseudostrophanthidine [2], we consider that compound (VII) is 3β,5-dihydroxy-8,19-epoxy-5β,14α,17b-card-20(22)-enolide. It may also be called 14α-pseudostrophanthidol.

By the reaction of strophanthidol 3-acetate (IX) with the rhamnose orthoester (II) under the same conditions as in the preparation of strophanthidol 3,19-bisrhamnoside (IV) [1], followed by saponification of the reaction products, in addition to strophanthidol 19-rhamnoside (X) we obtained a product (XI). A comparison of PMR spectra shows that the chemical shifts of the protons of the steroid part of glycoside (V) and of compound (XI) have similar values (see Table 1). Substance (XI) may be considered as the aglycone of rhamnoside (V).

In the PMR spectrum of compound (XI), the protons at C-21 give a broadened singlet at 4.70 ppm, which confirms the 17α-configuration of the butenolide ring. It is also known that the signal of the proton at C-17 in 17α,14β-hydroxycardenolides appears at 3.22-3.25 ppm, and in the 17α,14β-deoxycardenolides at 2.57-2.60 ppm [3]. In the PMR spectrum of compound (XI), the proton at C-17 resonates at 2.55 ppm, which corresponds to our hypothesis of the absence of a hydroxy group at C-14 and is in harmony with literature information [2] on the formation of 8,19-epoxy compounds.

Compound (XI) is 3β,5-dihydroxy-8,19-epoxy-5β,14β,17α-card-20(22)-enolide and glycoside (V) is the 3-O-α-L-rhamnoside of the aglycone (XI). The configuration of the glycosidic bond in rhamnoside (V) was determined by the method of molecular rotation differences [5].

The comparable values of the chemical shifts and spin—spin coupling constants of the protons at C-19 in compounds (V), (VII), and (XI), the molecular peaks of the ions at m/z

388 in the mass spectra of substances (VII) and (XI) and at m/z 430 in the mass spectra of compounds (VIII) and (XII) — all these facts indicate that the compounds mentioned have similar structures with 8,19-epoxy groupings.

It is possible that compounds of this type are found fairly frequently as by-products on glycosylation in the presence of mercury salts of triterpene aglycones [6] and cardenolide aglycones capable of forming internal ethers or esters.

It is interesting to note that under the action of a methanolic solution of HCl on cardenolides the configurations at C-14 and C-17 do not change [7] while under the action of HBr the configuration at C-14 changes, and under the conditions of the orthoester method epimerization takes place at C-17.

EXPERIMENTAL

For thin-layer chromatography (TLC) we used type KSK silica gel with 5% of gypsum. Nitromethane was purified by a method described previously [8]. The 3,4-di-O-acetyl-β-L-rhamnose 1,2-(methyl orthoacetate) was obtained under the conditions given by Mazurek and Perlin [9].

Optical rotatory dispersion curves were measured on a Jasco spectropolarimeter. Molecular weights were determined with the aid of MKh-1310 mass spectrometer. IR spectra were obtained on a UR-20 instrument (KBr) and PMR spectrum on a JNM-4H-100 instrument (100 MHz, δ, ppm, 0 — HMDS). Arbitrary symbols: u.s. — unresolved singlet; d — doublet; t — triplet; q — quartet; m — multiplet; a single prime denotes the signals of the protons of the rhamnose residue attached to C-3 and two primes that attached to C-19.

The yields were calculated on the initial cardenolide compound.

Strophanthidol 3,19-Bisrhamnoside and 3β,5-Dihydroxy-8,19-epoxy-5β,14β,17α-card-20(22)-enolide 3-O-α-L-rhamnopyranoside (V) from (I). A solution of 406 mg (1 mmole) of strophanthidol (I) and 608 mg (2 mmole) of the rhamnose orthoester (II) in 15 ml of nitromethane was heated to the boil. After 5 ml of solvent had been distilled off, 10.8 mg (0.03 mmole) of mercuric bromide was added. After half an hour, another 304 mg (1 mmole) of the rhamnose orthoester was added and the reaction mixture was boiled for 45 min. Then a few drops of pyridine was added, the contents of the vessel were evaporated to dryness, the residue was dissolved in 10 ml of absolute methanol, and 4 ml of methanol saturated with ammonia was added to the solution. After 20 h, the solution was evaporated to half its original volume, and then 60 ml of water was added and the methanol was evaporated off completely. The residual aqueous solution was extracted with ether (4 × 200 ml), with chloroform (4 × 200 ml), and with chloroform—ethanol (4:1) (6 × 200 ml). The dry residue from the chloroform—ethanol extract was chromatographed on silica gel and was eluted with chloroform containing gradientwise increasing amounts of methanol.

This led to the isolation of 250 mg (35.8%) of compound (IV), $C_{35}H_{54}O_{14}$ mp 200-201.5°C (from ethanol), $[\alpha]_D^{24}$ −29.5 ± 3° (c 0.87; ethanol). $\lambda_{max}^{C_2H_5OH}$: 218 nm (log ε 4.20). ν_{max}^{KBr} (cm^{-1}): 3400-3500 (OH), 1780, 1740, 1630 (butenolide ring). PMR spectrum (C_5D_5N), ppm: 0.88 (3 H at C-18, s); 1.54 (6 H at C-6' and C-6", m); 2.65 (H at C-17, m); 4.87 and 5.17 (2 H at C-21, q, centers of doublets, J = 18 Hz); 5.12 (H at C-1", u.s), 5.33 (h at C-1', u.s); 6.00 (H at C-22, s).

The dry residue from the chloroform extract was chromatographed on silica gel with elution by chloroform containing gradientwise increasing amounts of acetone. This gave 103 mg (25.4%) of compound (V), $C_{20}H_{42}O_9$, mp 240-242°C (from acetone), $[\alpha]_D^{24}$ +21.0 ± 2° (c 0.93, methanol), $\lambda_{max}^{C_2H_5OH}$: 217 nm (log ε 4.21). ν_{max}^{KBr} (cm^{-1}): 3400-3550 (OH), 1780, 1760, 1740, 1628 (butenolide ring). PMR spectrum (C_5D_5N), ppm: 0.93 (3 H at C-18, s); 1.54 (3 H at C-6', d, J = 6 Hz); 2.35 (H at C-17, m); 3.27 and 4.23 (2 H at C-19, q, centers of doublets J = 8.5 Hz); 4.73 (2 H at C-21, u.s); 5.35 (H at C-1', u.s); 5.88 (H at C-22, u.s).

3β-5-Dihydroxy-8,19-epoxy-5β,14β,17α-card-20(22)-enolide 3-O-(2',3',4'-Tri-O-acetyl-α-L-rhamnopyranoside) (VI) from (V). A solution of 90 mg of the glycoside (V) in 3.6 ml of pyridine cooled to 0°C was treated with 2.5 ml of acetic anhydride, and the mixture was left at room temperature for 20 h. The contents of the reaction vessel were evaporated to dryness, the residue was dissolved in chloroform—ether (1:3), and the solution was washed suc-

cessively with dilute solutions of HCl and of Na_2CO_3 and with water. After the solvents had been dissolved, the dry residue was crystallized from ethanol–ether. This gave 94 mg (95%) of the acetate (VI), $C_{35}H_{48}O_{12}$, with mp 212-215°C, $[\alpha]_D^{24}$ +14.7 ± 3° (c 0.92; chloroform), ν_{max}^{KBr} (cm^{-1}): 3530 (OH), 1780, 1750 (C=O), 1625 (double bond of a butenolide ring), 1250-1220 (C–O–C). Mass spectrum, m/z: 273 $[Rha(OAc)_3 - H_2O]^+$. PMR spectrum (C_5D_5N), ppm: 0.91 (3 H at C-18, s); 1.23 (3 H at C-6', d, J = 6 Hz); 1.86, 1.89, and 1.97 (9 H of 3 Ac at C-2', C-3', and C-4', s); 2.38 (H at C-17, m); 3.35 and 4.30 (2 H at C-19, q, centers of doublets, J = 8.5 Hz); 4.05-4.15 (H at C-5' and H at C-3, m); 4.72 (2 H at C-21, u.s); 5.22 (H at C-1', u.s); 5.40-5.70 (3 H at C-2', C-3', and C-4', m); 5.88 (H at C-22, u.s).

Strophanthidol 19-O-α-L-Rhamnopyranoside (X) and 3β,5-Dihydroxy-8,19-epoxy-5β,14β,17α-card-20(22)-enolide (XI) from (IX). A solution of 900 mg (2 mmole) of strophanthidol 3-acetate (IX) and 880 mg (2.9 mmole) of the rhamnose orthoester (II) in 15 ml of nitromethane was boiled in a two-necked flask. The solvents were distilled off and fresh nitromethane was added at such a rate that the volume of the reaction mixture remained constant. After the first few milliliters of nitromethane had been distilled off, 10.8 mg (0.03 mmole) of $HgBr_2$ was added to the reaction mixture and heating was continued under the same condition for another 75 min. Then a few drops of pyridine was added and the solution was evaporated to dryness. The residue was dissolved in 20 ml of methanol and 4 ml of methanol saturated with ammonia was added. After 18 h, the mixture was evaporated to dryness, and the residue was chromatographed on silica gel with elution by chloroform containing gradientwise increasing amounts of ethanol. This gave 100 mg (12.8%) of compound (XI) (crystals from acetone), 100 mg (12.3%) of strophanthidol (I), and 500 mg (45.0%) of amorphous strophanthidol 19-rhamnoside (X).

Compound (XI), $C_{23}H_{32}O_5$ had mp 210-213°C (decomp.) $[\alpha]_D^{22}$ +78.0 ± 2° (c 0.82; chloform). ν_{max}^{KBr} (cm^{-1}): 3300-3370 (OH), 1780, 1760, 1631 (butenolide ring). PMR spectrum (C_5D_5N), ppm: 0.93 (3 H at C-18, s), 2.40 (H at C-17, m); 3.31 and 4.31 (2 H at C-19, q, centers of doublets, J = 8.5 Hz); 4.35 (H at C-3, m); 4.70 (2 H at C-21, s); 5.84 (H at C-22, u.s). PMR spectrum ($CDCl_3$), ppm: 0.94 (3 H at C-18, s); 2.50 (H at C-17, m); 3.33 and 4.00 (2 H at C-19, q, centers of doublets, J = 9 Hz); 4.15 (H at C-3, m); 4.65 (2 H at C-21, s); 5.69 (H at C-22, u.s). ORD (dioxane, c 2.10): $[M]_{236}$ + 8220; $[M]_{250}$ + 3510; $[M]_{270}$ + 1850; $[M]_{300}$ + 1380. M^+388.

Strophanthidol 19-rhamnoside (X), $C_{29}H_{44}O_{10}$, had $[\alpha]_D^{24}$ +1.6 ± 2° (c 1.03; methanol). ν_{max}^{KBr} (cm^{-1}): 3300-3500 (OH); 1785, 1745, 1710, 1630 (butenolide ring). PMR spectrum (C_5D_5N), ppm: 0.87 (3 H at C-18, s); 1.48 (3 H at C-6", d, J = 6 Hz); 2.61 (H at C-17, m); 3.55-4.40 (7 H at C-3, C-19, C-2", C-3", C-4", and C-5", m); 4.79 and 5.11 (2 H at C-21, q, centers of doublets, J = 18 Hz); 5.04 (H at C-1", u.s); 5.90 (H at C-22, u.s). According to the literature: mp 169-172°C (aqueous ethanol), $[\alpha]_D^{19}$ 0 ± 2° (methanol) [10].

3β-5-Dihydroxy-8,19-epoxy-5β,14β,17α-card-20(22)-enolide 3-O-Acetate (XII) from (XI). Substance (XI) was acetylated and the product was worked up as described above for the preparation of the acetate (VI). Compound (XII), $C_{25}H_{34}O_6$, had mp 242-248°C (decomp.; from ethanol); $[\alpha]_D^{25}$ +58.0 ± 2° (c 0.36; chloroform). ν_{max}^{KBr}, cm^{-1}: 3550 (OH), 1780, 1750, 1730 (C=O), 1630 (double fond of a butenolide ring). M+430.

3β,5-Dihydroxy-8,19-epoxy-5β,14α,17β-card-20(22)-enolide (VII) from (I). A solution of 76 mg of strophanthidol (I) in 10 ml of absolute nitromethane was treated with 2 ml of an 8.8% solution of anhydrous HBr in absolute nitromethane, and the mixture was left at −5°C for 15 min. Then it was neutralized with pyridine and evaporated to dryness, the residue was dissolved in chloroform, and the solution was washed with water. The chloroform was distilled off, and the reaction products were chromatographed on silica gel with elution by the chloroform–benzene–methanol (5:5:2) solvent system. This gave 28 mg (39.0%) of substance (VII) (crystals from acetone), $C_{23}H_{32}O_5$, mp 230-235°C (decomp.), $[\alpha]_D^{24}$ + 41.5 ± 2° (c 1.16; chloroform). ν_{max}^{KBr} (cm^{-1}): 3300-3450 (OH), 1790, 1750, 1630 (butenolide ring). ORD (dioxane, c 2.34): $[M]_{265}$ − 5400; $[M]_{227}$ + 22.800; a = 282.

PMR spectrum (C_5D_5N), ppm: 0.74 (3 H at C-18, s); 3.36 and 4.35 (2 H at C-19, q, centers of doublets. J = 8 Hz); 4.27 (H at C-3), 4.59 and 4.85 (2 H at C-21, q, centers of

doublets, J = 18 Hz); 5.91 (H at C-22, u.s). PMR spectrum ($CDCl_3$), ppm: 0.76 (3 H at C-18, s); 3.37 and 4.09 (2 H at C-19, q, centers of doublets, J = 8.5 Hz); 4.17 (H at C-3), 4.66 (2 H at C-21, t, J = 18 Hz); 5.75 (H at C-22, u.s). M^+ 388.

3β,5-Dihydroxy-8,19-epoxy-5β,14α,17β-card-20(22)-enolide 3-O-Acetate (VIII) from (V II). Substance (VII) was acetylated and the products were worked up in just the same way as described above in the preparation of the the acetate (VI). The product (VIII), $C_{25}H_{34}O_6$, had mp 210-215 °C (decomp. from ethanol) $[\alpha]_D^{22}$ +60.0 ± 2° (c 1.30; chloroform). ν_{max}^{KBr}, cm^{-1}: 3480 (OH); 1795, 1765, 1710 (C=O); 1635 (double bond of a butenolide ring). PMR spectrum (C_5D_5N), ppm: 0.70 (3 H at C-18, s); 1.86 (3 H in Ac, s); 3.31 and 4.32 (2 H at C-19, q, centers of doublets, J = 8 Hz); 4.58 and 4.80 (2 H at C-21, q, centers of doublets, J = 18 Hz); 5.12 (H at C-3, m); 5.86 (H at C-22, u.s). M^+ 430.

SUMMARY

In the condensation of strophanthidol with an orthoester of rhamnose, in addition to the expected strophanthidol 3,19-bisrhamnoside a monorhamnoside with an epoxy grouping in the aglycone moiety is formed. The aglycone of this glycoside can be obtained directly by the condensation of strophanthidol 3-acetate with the rhamnose orthoester. The configuration of the butenolide ring changes in the process of orthoester condensation. It has been shown that the product with the epoxy grouping has the structure of 3β,5-dihydroxy-8,19-epoxy-5β,14β,17α-card-20(22)-enolide.

An attempt has been made to obtain this product by treating strophanthidol with hydrobromic acid in nitromethane. This gave a different cardenolide compound likewise containing an 8,19-epoxy grouping, but in which the butenolide ring had not undergone epimerization.

LITERATURE CITED

1. N. Sh. Pal'yants and N. K. Abubakirov, Khim. Prir. Soedin., 125 (1977).
2. T. Kubota and M. Ehrenstein, J. Org. Chem., 29, 342 (1964).
3. K. Tori and K. Aono, Annu. Rep. Shionogi Res. Lab., 15, 130 (1965).
4. F. Burkhardt, W. Meier, A. Fürst, and T. Reichstein, Helv. Chim. Acta, 50, 607 (1967).
5. W. Klyne, Biochem. J., 47, No. 4, xli (1950).
6. A. A. Akimaliev, N. Sh. Pal'yants, N. K. Alimbaeva, and N. K. Abubakirov, Khim. Prir. Soedin., 668 (1979).
7. W. Merkel and M. Ehrenstein, Helv. Chim. Acta, 52, 2156 (1969).
8. A. Ya. Khorlin, A. F. Bochkov, and N. K. Kochetkov, Khim. Prir. Soedin., 6 (1966).
9. M. Mazurek and A. S. Perlin, Can. J. Chem., 43, 1918 (1965).
10. V. T. Chernobai, Zh. Obshch. Khim., 34, 3852 (1964).