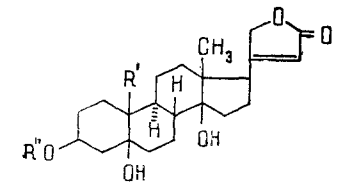
19-NORCARDENOLIDES

I. F. Makarevich and L. N. Dikan'

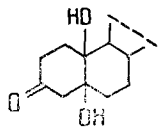
UDC 547.918+547.926+615.711.5

The products of the autooxidation of the cardenolides strophanthidin and erysimin in solutions have been studied. It has been established that the autooxidation of 19-oxocardenolides forms, together with 19-carboxylic acids, a complex mixture of 19-norcardenolides. The bulk of them consists of 10β-OH compounds and a smaller proportion of 10β-H compounds. Autooxidation affects both the functional group at C-10 and its transformation and also other groupings and bonds in ring A and, to some extent, in ring B, including the oxidation of the 3β-OH group, degradation of the 5β-OH group, and cleavage of the C_5—C_{10} bond with the formation of 5,10-seco-19-norcardenolides. A possible mechanism of the formation of 19- norcardenolides is discussed.

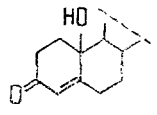
The formation of 19-norcardenolides as the products of the autooxidation of 19-oxocardenolides has been observed by Wartburg et al. [1]. On the autooxidation of strophanthidin and cymarin they obtained — in addition to the corresponding 19-carboxylic acids — 10β-hydroxy-19-norperiplogenin and 10β-hydroxy-19-norcymarin. However, the bulk of the products of this reaction have remained uninvestigated.



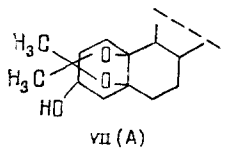
I. R' = R'' = H (B)
II. R' = OH, R'' = H (D)
III. R' = COOH, R'' = H
IV. R' = OH, R'' = β-D-digitoxose



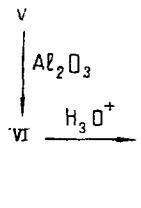
V

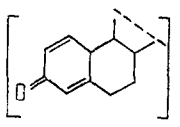


VI (C)

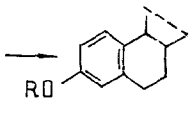


VII (A)

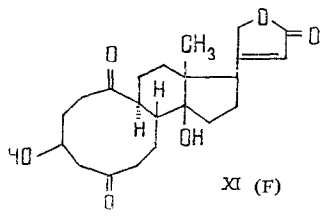




VIII



IX. R = H
X. R = CH.



XI (F)

All-Union Scientific-Research Institute of Drug Chemistry and Technology, Khar'kov. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 71-76, January-February, 1985. Original article submitted March 20, 1984.

0009-3130/85/2101-0067$09.50

TABLE 1. Properties of the 19-Norcadenolides Obtained

Name of substance	Molecular formula	mp, deg C	$[\alpha]_D$, deg
10β-H-19-Norperiplogenin (I, B)	$C_{22}H_{32}O_5$	128—130	+32,3±2 (MeOH)
10β-Hydroxy-19-norperiplogenin (II, D)	$C_{22}H_{32}O_6$	125—129	+37,2±2 (MeOH)
10β-Hydroxy-19-norerysimin (IV)	$C_{28}H_{42}O_9$	136—139	+23,6±2 (MeOH)
5,10β,14-Trihydroxy-3-oxo-19-nor-5β,14β-card-20(22)-enolide (V, E)	$C_{22}H_{30}O_6$	209—212/ 236—240	+47,6±3 (MeOH)
10β,14-Dihydroxy-3-oxo-19-nor-14β-card-4,20(22)-dienolide (VI, C)	$C_{22}H_{28}O_5$	265—270	+37,0±3 (MeOH)
3β,14-Dihydroxy-5β,10β-O-isopropylidene-19-nor-14-card-20(22)-enolide (VII, A)	$C_{25}H_{36}O_6$	186—190/ 221—225	+62,8±3(MeOH-chlf)
3,14-Dihydroxy-19-nor-14β-card-1,3,5(10), 20(22)-tetraenolide (IX)	$C_{22}H_{26}O_4$	250—255	+98,0±4 (MeOH)
14-Hydroxy-3-O-methoxy-19-nor-14β-card-1,3,5(10),20(22)-tetraenolide (X)	$C_{23}H_{28}O_4$	140—142/ 159—163	+84,3±2 (chlf)
3,4-Dihydroxy-5,10-dioxo-5,10-seco-19-nor-14β-card-20(22)-enolide (XI)	$C_{22}H_{30}O_6$	227—230	−10,8±2 (MeOH)

In the study of cardiac glycosides we have repeatedly observed that on storage in solutions 19-oxocardenolides give a fairly complex mixture of derivatives. Thus, when strophanthidin was kept in acetone with the access of air from five to ten and more cardenolides are formed, depending on the time of autooxidation. We have isolated seven of these products in the individual state.

The initial mixture was separated into acidic and neutral fractions with the aid of ordinary sodium carbonate extraction. From the acid fraction, which consisted of two cardenolides, we obtained one, predominating quantitatively, in the pure form by direct crystallization and we identified it as strophanthidin-19-carboxylic acid by direct comparison with a sample of this compound. The second, more polar cardenolide, of the acid fraction, as we assume, consisted of strophanthidin-19-percarboxylic acid.

The cardenolides present in the neutral fraction were separated with the aid of partition column chromatography on cellulose. This led to the isolation of six compounds in the pure form these being denoted in order of increasing polarity by the letters A, B, C, D, E, and F. The autooxidation of erysimin gave a 19-nor glycoside, in addition. The properties of the compounds isolated are given in Table 1.

Cardenolide D (II), $C_{22}H_{32}O_6$. Its mass spectra contained the peak of the molecular ion with m/z 392, and was also characterized by the following fragments: 374 ($M-H_2O$), 356 ($M-2H_2O$), 338 ($M-3H_2O$), 320 (M—72), 281, 267, 253, 221, 207, 203, 185. The IR spectrum had a strong absorption band with a maximum at 3425 cm^{-1} due to the stretching vibrations of OH groups; four bands in the regions of 2955 cm^{-1} (CH, CH_2, CH_3), 1780 and 1736 cm^{-1} (C=O of a butenolide ring), and 1623 cm^{-1} (C=C of a butenolide ring) are typical for cardenolides. There were no absorption bands characteristic for aldehyde or carboxy groups.

From its composition and properties, cardenolide D was identical with the 10β-hydroxy-19-norperiplogenin (II) described by Wartburg et al. [1].

Cardenolide E (V), $C_{22}H_{30}O_6$. The mass spectrum contained the peak of the molecular ion with m/z 390 (medium intensity) and also fragments with *m/z* 372 ($M-H_2O$), 354 ($M-2H_2O$), 336 ($M-3H_2O$), 320, 281, 262, 249, 229, 219, 207, 201, 195. The IR spectrum indicated the presence of two carbonyl broups, one of which was present as a component of a butenolide ring (bands in the 1785 and 1750 cm^{-1} region), while a band in the 1705 cm^{-1} region characterized the presence of a keto group.

In the light of the results obtained, we assumed that cardenolide E consisted of the product of the oxidation of 10β-hydroxy-19-norcardenolide (II) at C-3. In fact, the directed oxidation of cardenolide (II) with chromium trioxide enabled us to obtain the 3-ketocardenolide (V), which proved to be identical with substance E. Thus, compound E was 5,10β, 14-trihydroxy-3-oxo-19-nor-5β, 14β-card-20(22)-enolide (V).

Cardenolide C (VI), $C_{22}H_{28}O_5$. Its mass spectrum was characterized by the presence of a strong peak of the molecular ion with m/z 372 and by fragments with *m/z* 354 ($M-H_2O$), 336 ($M-2H_2O$), 326, 268, 244, 226, 219, 201, 187 . The IR spectrum showed, in addition to the usual cardenolide bands, absorption in the 1656 cm^{-1} region characteristic for the stretching vibrations of an α,β-unsaturated ketone and also two bands in the 1620 and 1630 cm^{-1} regions belonging to the stretching vibrations of two C=C bonds conjugated with carbonyl groups. On the basis of these facts, the structure of cardenolide C was represented by formula (VI). Its formation in the autooxidation of strophanthidin must be regarded as the result of the dehydration of the 5β-OH group in cardenolide E (V) under the influence of carboxylic acids. A confirmation of the correctness of the proposed structure of (VI) is the observation of the fact that in acetic acid cardenolide (V) was converted into (VI). Consequently, cardenolide C was 10β,14-dihydroxy-3-oxo-19-nor-14β-card-4,20(22)-dienolide (VI).

Cardenolide B (I), $C_{22}H_{30}O_5$. Its mass spectrum was characterized by the presence of a weak peak of the molecular ion with m/z 376, and also by the following fragments: 358 ($M-H_2O$), 354, 340 ($M-2H_2O$), 322 ($M-3H_2O$). The cardenolide formed a monoacetate under the action of acetic anhydride in pyridine and, judging from the time of half-reaction, which was 180 min, the OH group undergoing acetylation was typically axial (see [2]) and was included in an intramolecular hydrogen bond similar to that in periplogenin. In the region of the stretching vibrations of OH groups, the IR spectrum had a broad unsymmetrical band with a maximum at 3375 cm^{-1}, which confirmed the presence of an intramolecular hydrogen bond between the hydroxyls.

On the basis of the results obtained, and also in view of the molecular rotation of cardenolide B ($[M]_D$ +121 ± 7°), we consider that it was 3β, 5,14-trihydroxy-19-nor-5β,14β-card-20(22)-enolide (I). Also in harmony with this structure is the capacity for the cardenolide for giving a 3β,5β-O-cyclosulfoxide derivative with thionyl chloride.

Cardenolide A (VII), $C_{25}H_{36}O_6$. Its mass spectrum was characterized by the following fragments: *m/z* 417 ($M-CH_3$), 357 ($M-CH_3-CH_3COO$), 339 ($M-C_3H_6O_2-H_2O$), 321 ($M-C_3H_6O_2-2H_2O$). The PMR spectrum showed the presence in the cardenolide of three methyl groups: signals in the 0.90 ppm region — a three-proton signal of an 18-CH_3 group — and in the 1.35 ppm region — a six-proton signal belonging to a gem-dimethyl grouping.

On the basis of the results obtained, the hypothesis was formulated that cardenolide A consisted of the isopropylidene derivative of compound (II). An independent synthesis of 5β,10β-O-isopropylidene-19-norperiplogenin (VII) from (II) and a direct comparison of the compounds obtained confirmed their identity. Thus, cardenolide A was 3β,14-dihydroxy-5β, 10β-O-isopropylidene-19-nor-14β-card-20(22)-enolide (VII).

The formation of the isopropylidenecardenolide (VII) obviously took place on the prolonged residence of 10β-hydroxy-19-norperiplogenin (II) in acetone under the catalytic action of cardenolide-19-carboxylic acids.

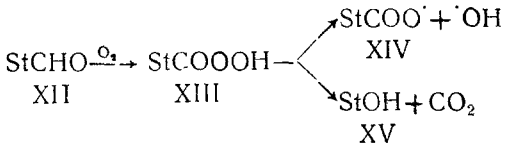
Cardenolide F (XI), $C_{22}H_{30}H_6$. Of all the autooxidation products, this compound proved to be the most unexpected. In its investigation it was established that it was a 5,10-seco-19-norcardenolide, representing one of the conformers of the 5,10-secocardenolides [3].

Cardenolides with an Aromatic Ring A (IX and X). The 3-ketocardenolide (V) is a convenient starting compound for obtaining the corresponding derivatives with an aromatic ring A. The transformation of (V) into the aromatic steroid (IX) took place by the acid dehydration of the 5β and 10β-OH groups and the simultaneous conversion of the unsaturated ketone (VIII) acid. The performance of the reaction in a methanolic solution of hydrochloric acid led to the production of the methyl ether of the aromatic cardenolide (X), and the use of alumina as dehydrating reagent led to the splitting out of only the 5β-OH group with the quantitative formation of the 4-anhydro ketone (VI).

10β-Hydroxy-19-norerysimin (IV), $C_{28}H_{42}O_9$. When erysimin was subjected to autooxidation in acetone, the main reaction products obtained were erysimin-19-carboxylic acid and 10β-hydroxy-19-norerysimin. Acid hydrolysis of the latter led to the formation of 10β-hydroxy-19-norperiplogenin (II) and D-digitose.

Thus, the autooxidation of 19-oxocardenolides takes place both at the aldehyde group and at the secondary OH group at C-3. The autooxidation products undergo further fairly far-reaching transformations, all, or almost all, the compounds formed in these processes being 19-norcadenolides.

The autooxidation reaction apparently takes placed through the formation of the peracids (XIII), which are decarboxylated with conversion into the 10β-hydroxy-19-norcardenolide (XV) or decompose into two radicals:



St — steroid moiety of the molecule

The latter catalyze a chain of autooxidation with the formation both of ordinary 19-carboxylic acids and also of peracids. The StCOO· radical is also capable of undergoing decarboxylation by adding ·OH or ·H (chain termination), with the formation of compounds of types (XV) and (I), respectively.

The high-quality chromatographic monitoring of the autooxidation process has shown that 19-carboxylic acids do actually predominate initially as the reaction product. Then their amount decreases with the accumulation of 19-nor compounds. The fact that the 19-carboxylic acids are the precursors of the 19-nor compounds is shown by an observation that we made using pure strophanthidin-19-carboxylic acid. In acetone, the formation of 10β-hydroxy-19-norperiplogenin (II) from it was observed after only 17 h.

Oxidation at C-3 probably takes place through hydroperoxides, which eliminate a molecule of water, being converted into 3-oxo compounds. When a β-OH group is present, the latter are readily dehydrated, forming 3-oxo-5-anhydrosteroids.

EXPERIMENTAL

General Information. The elementary analysis of the substances was performed with the aid of a Hewlett–Packard O-H-N analyzer. The results of the analyses corresponded to those calculated for the structures given. Mass spectra were taken on Varian CH-8 and MKh-1303 instruments; PMR spectra, on a Tesla BS-497 instrument (100 MHz) in C_5D_5N (0 — TMS); and IR spectra, in a JR-27G instrument (the substances being tableted with KBr).

Preparation of the 19-Norcardenolides. With heating, 10 g of strophanthidin was dissolved in 200 ml of acetone and the solution was left in a loosely closed flask in a place protected from the light for 30 days. Then the solution was evaporated in vacuum. The dry residue was dissolved in chloroform–ethanol (2:1) (350 ml), the solution was extracted with 1 N sodium carbonate solution (50 ml × 2), and the extract was washed with water (25 ml × 3) and evaporated. This gave 7.6 g of neutral fraction.

The sodium carbonate solutions, after acidification with sulfuric acid (110 ml of 1 N H_2SO_4) and ordinary extraction with chloroform–ethanol yielded 2.2 g of cardenolide carboxylic acids. By crystallization from aqueous ethanol, followed by two recrystallizations from the same solvent, strophanthidin-19-carboxylic acid was obtained in the pure form with mp 154-156°C, $[\alpha]_D^{20} + 58.7 \pm 2°$ (c 1.0; methanol).

The neutral fraction, by direct crystallization from aqueous ethanol followed by two crystallizations from the same solvent, yielded 2.3 g of pure 10β-hydroxy-19-norperiplogenin (D, II). The remaining 5.3 g of neutral cardenolides was chromtographed on a column containing 1500 g of cellulose using the toluene–1-butanol (4:1 to 3:1)/water solvent system. This gave cardenolides A, B, C, D, E, and F in the pure form.

Erysimin (3 g) was subjected to autooxidation in acetone solution for 12 days. The products were separated into neutral and acid fractions in a manner similar to that described above. This yielded 1 g of neutral fraction, which was chromatographed on alumina to give 10β-hydroxy-19-norerysimin (IV) in the pure form. In the process of structure determination, a sample of the glycoside was hydrolyzed with 0.05 N sulfuric acid at 80°C for 40 min. The hydrolysate was neutralized with sodium carbonate and was analyzed by paper chromatography. This showed the presence of D-digitose and an aglycone identical with cardenolide (II, D).

Aromatization of Ring A. 5,10β-14-Trihydroxy-3-oxo-19-nor-5β,14β-card-20(22)-enolide (V) (0.1 h) was heated in a 1% aqueous ethanolic solution of hydrochloric acid at 70°C for 2 h. The course of the reaction was monitored with the aid of thin-layer chromatography. After its completion, the reaction product was extracted with chloroform. The chloroform solution, after the usual washing out of acid, was evaporated and the residue was crystallized from aqueous acetone, which gave 3,14-dihydroxy-19-nor-14β-card-1,3,5(10),20(22)-tetraenolide (IX).

The methyl ether (X) of the aromatic cardenolide (IX) was obtained by keeping compound (V) in 1% methanolic hydrochloric acid at room temperature for 17 h and crystallizing the reaction product from methanol.

Selective Splitting Out of the 5β-OH Group in Compound (V). With heating, 0.1 g of the ketocardenolide (V) was dissolved in 7 ml of ethanol—ethyl acetate, 10 g of alumina that had been activated at 170°C for 1 h was added, and the mixture was left in a tightly sealed flask at room temperature for 60 h, the reaction being monitored with the aid of TLC. During the time mentioned, the initial cardenolide (V) was transformed completely into the less polar unsaturated ketone (VI). The latter was desorbed with chloroform—ethanol (3:1) and crystallized from methanol. The compound obtained, with mp 264-270°C, was identical with cardenolide B (VI), which was isolated as a product of the autooxidation of strophanthidin.

SUMMARY

It has been established that the autooxidation of 19-oxocardenolides forms — in addition to 19-carboxylic acids — a complete mixture of 19-norcardenolides. The greater part of them consists of 10β-OH compounds, and a smaller part of 10β-H compounds. Autooxidation affects both the functional group at C-10 and its transformation and also other groupings and bonds in ring A and, to some extent in ring B, including the oxidation of the 3β-OH group, the dehydration of the 5β-OH group, and the cleavage of the C_5—C_{10} bond with the formation of 5, 10-seco-19-norcardenolides.

A possible mechanism of the formation of 19-norcardenolides is discussed.

LITERATURE CITED

1. A. V. Wartburg, J. Binkert, E. Angliker, and J. Renz, US Patent No. 3,211,719; Helv. Chim. Acta, 45, 2122 (1962).
2. I. F. Makarevich, Khim. Prir. Soedin., 221 (1968).
3. I. F. Makarevich, Khim. Prir. Soedin., 76 (1985) [following paper in this issue].