CARDIAC GLYCOSIDES OF THE *Cheiranthus allioni*. XII*

I. F. Makarevich, A. I. Pavlii, and S. I. Makarevich

UDC 547.918+547.926+615.711.5

The seeds of *Cheiranthus allioni* hort. have yielded three new cardenolides the structures of which have been established and which have been named as 4-dehydrosarmentogenin (II), 4-dehydrosarmentogenin rhamnoside (I), and 4-dehydrosarmentogenin rhamnoglucoside (IV). (II) — $C_{23}H_{32}O_5$. m.p. 296-302°, $[\alpha]_D^{20}$ +26.2 ± 3° (in pyridine) is 3β,11α,14-trihydroxy-14β-card-4,20(22)-dienolide. (I) $C_{29}H_{42}O_9$, m.p. 268-275°, $[\alpha]_D^{20}$ −38.2 ± 3° (chloroform–ethanol) is 11α,14-dihydroxy-3β-α-L-rhamnopyranosyloxy-14β-card-4,20(22)-dienolide. (IV) $C_{35}H_{52}O_{14}$, $[\alpha]_D^{20}$ −44.1 ± 3° (methanol), is 3β-(4'-O-β-D-glucopyranosyl-α-L-rhamnopyranosyloxy)-14β-card-4,20(22)-dienolide. An independent synthesis of 4-dehydrosarmentogenin (II) has been carried out, starting from 3β,5,11α,14-tetrahydroxy-5β,14β-card-20(22)-enolide, which has confirmed its structure.

Cheiranthus allioni hort. (*Erysimum asperum*; plains erysimum) (family Cruciferae) is a unique plant with respect to the diversity of the set of cardiac glycosides and aglycons that it contains. In preceding communications published in this journal in 1969-1975, the isolation from the seeds of this plant and the structural determination of 29 glycosides composed of 9 aglycons and 6 monosaccharides have been described. These include cardenolides of both the A/B-cis and the A/B-trans series.

Continuing a study of plains erysimum, we have isolated another two new cardenolide glycosides and have provisionally called them Ch-(30) (I) and Ch-31 (IV).

Ch-30 (I) is a monoglycoside with the composition $C_{29}H_{32}O_9$, as shown by the results of elementary analysis and an investigation of hydrolysis products. After acid hydrolysis, performed by the Mannich–Siewert method [2], the new aglycon (II) and L-rhamnose were obtained. The elementary analysis and mass spectrum of the aglycon (II) showed that it had the composition $C_{23}H_{32}O_5$. The mass spectrum was characterized by a relatively intense peak of the molecular ion with m/z 388 and by fragments with m/z 370 $(M - H_2O)^+$, 352 $(M - 2H_2O)^+$, 334 $(M - 3H_2O)^+$, 319 $(M - 3H_2O - CH_2)^+$, 217, 201, 199, 179, 173, 145, 91, 67, 55.

The composition $C_{23}H_{32}O_6$ shows the "unsaturated" structure of the aglycon, i.e., that its molecule contains either a double C=C bond in the steroid nucleus or a carbonyl group. The presence of a carbonyl group was excluded by the characteristics of the optical rotatory dispersion spectrum and the IR spectrum. At the same time, they showed the presence of a double C=C bond. In addition to a band at 1620 cm^{-1} due to the C=C bond of a butenolide ring there was a well resolved band at 1630 cm^{-1} due to the stretching vibrations of an additional C=C bond present in the steroid part of the molecule. There was also a band at 810 cm^{-1} which is apparently due to the deformation vibrations of hydrogen atoms at a double bond.

The presence of alcoholic groups at C-3 and C-14, of a butenolide ring at C-17, and of their β-configuration in the aglycon is essential for cardenolides with a high biological activity. The β-configuration of the lactone ring was also confirmed by isomerization results: when the aglycon or the glycoside Ch-30 (I) was heated in absolute dimethylformamide in the presence of anhydrous sodium tosylate and sodium acetate, more highly polar cardenolides were formed, which was due to the transformation of the initial substances into 17α-cardenolides. As was to be expected, the reaction products were revealed on chromatograms by the Jensen reagent in the form of spots with a blue fluorescence in UV light, while the initial glycosides and the aglycon had a yellow fluorescence.

*For Communication XI, see [1].

All-Union Scientific-Research Drug Institute, Kharkov. Kharkov State Pharmaceutical Institute. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 119-125, January-February, 1987. Original article submitted September 2, 1986.

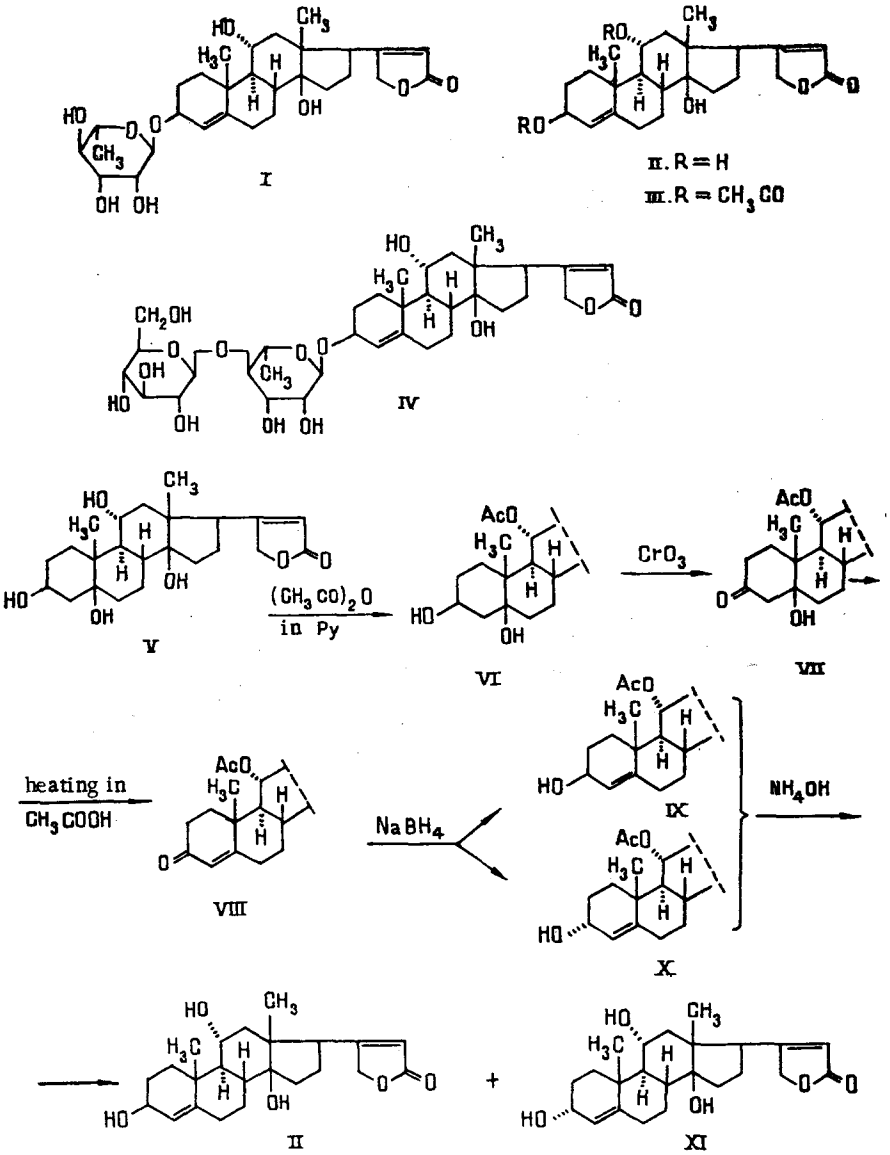
0009-3130/87/2301-0101$12.50

On oxidation with chromium trioxide, the aglycon formed a neutral reaction product, which excludes the presence of a primary OH group and of an aldehyde group in the initial compound.

When the aglycon was acetylated with acetic anhydride in pyridine and the course of the reaction was analyzed by a published method [3], it was found that it formed a diacetate, and the rates of acetylation of both OH groups were characteristic for secondary equatorial hydroxy groups, with a half-reaction time of less than 30 min (at 20°C).

The aglycon gave a positive reaction for a Δ^4 or a Δ^5 bond [4, 5]. On the basis of the following facts, the most probable position of the double bond in this case is 4:5. The optical rotatory dispersion spectrum of the aglycon consists of a smooth positive curve, which excludes a double bond in the 5:6 position and does not exclude but, on the contrary, confirms its presence in the 4:5 position [9]. Furthermore, the increased reactivity of the 3β-OH group observed on the acetylation of aglycon is obviously due to the influence of a closely adjacent double bond, i.e., it is characterized by the presence of a vinyl alcohol fragment.

The choice of the position of the second equatorial OH group amounted to deciding between the 11α, 12β, and 16β positions as those most frequently found. The 12β and 16β positions were excluded, since such cardenolides, after treatment with the Jensen reagent, fluoresce blue in UV light, while, as already mentioned the aglycons under investigation fluoresced yellow.



On the basis of the results obtained, we assumed that the new aglycon was 4-dehydrosarmentogenin (II). In the light of such a structure, it could be expected that in a reaction with concentrated sulfuric acid this aglycon and bipindogenin (V) should give similar colora-

tions changing with time, since bipindogenin can be converted under these conditions into (II) as an intermediate compound. In actual fact, the reactions with these aglycons proved to be practically identical.

For a definitive answer to the question of the structure of the aglycon, we performed the independent synthesis of 4-dehydrosarmentogenin and compared the cardenolides. They proved to be identical. The initial compound for the synthesis was bipindogenin (V). With the aid of the controlled acetylation of bipindogenin (V), the 11α-O-acetate (VI) was obtained, which was then oxidized, giving the 3-ketocardenolide (VII). The latter was converted with the aid of a dehydration reaction into the α,β-unsaturated ketone (VIII), the structure of which was confirmed by its UV spectrum; this had an intense absorption band with a maximum at 242 nm (log ε 4.24), which is typical for Δ^4-3-ketones, and also an absorption band with its maximum at 218 nm (log ε 4.17) due to the butenolide ring. The reduction of the Δ^4-3-ketone (VIII) with sodium tetrahydroborate yielded a mixture of epimers — the unsaturated alcohols (IX and X).

After the removal of the protective 11α-O-acetyl group and chromatographic separation, the cardenolides (II) and (XI) were obtained in the individual state. The 4-dehydrosarmentogenin synthesized in this way and the natural aglycon (II) were identical in their physicochemical properties, including their IR spectra. Thus, the new aglycon (II) is 3β,11α,14-trihydroxy-14β-card-4,20(22)-dienolide.

The increment of the molecular rotation of the L-rhamnose in the glycoside Ch-30 was minus 305 ± 27° which indicates an α-glycosidic bond. Consequently, glycoside Ch-30 (I) is 11α,14-dihydroxy-3β-α-L-rhamnopyranosyloxy-14β-card-4,20(22)-dienolide (I).

According to the results of elementary analysis and a molecular weight measurement, the second glycoside, Ch-31 (IV), had the composition $C_{35}H_{52}O_{14}$, i.e., it was a diglycoside. The molecular weight of the glycoside was determined by a spectrophotometric method [6]. On enzymatic hydrolysis it formed D-glucose and a monoglycoside identical with the above-described glycoside (I).

Ch-31 (IV) reacted with acetone in the presene of anhydrous copper sulfate to form an isopropylidene derivative. This indicated that in the L-rhamnose unit the cis-α-glycol grouping that is capable of such a reaction was free, i.e., there were free OH groups at C-2' and C-3'. In its turn, this unambiguously showed a 1 → 4 bond of the D-glucose residue with the L-rhamnose residue.

Analysis of the molecular rotations of the mono- and diglycosides showed that the D-glucose was attached by a β-glycosidic bond — the increment of the molecular rotation of the D-glucose moiety was minus 103 ± 37°.

Consequently, Ch-31 (IV) was 11α,14-dihydroxy-3β-(4'-O-β-D-glucopyranosyl-α-L-rhamnopyranosyloxy)-14β-card-4,20(22)-dienolide.

EXPERIMENTAL

IR spectra were obtained for the substances tableted in KBr on a IR-27G spectrometer. Mass spectra were taken on a Varian Ch-8 instrument, and optical rotatory dispersion spectra on a SPU-M automatic polarimeter. Elementary analyses were performed with the use of an automatic C-H-N analyzer. The results corresponded to those calculated for the suggested structures.

Glycosides Ch-30 and Ch-31 were isolated from the so-called medium-polar fraction the isolation of which was described in [7]. The substances were separated with the aid of chromatography on silica gel activated at 110°C (2 h); the ratio of the mixture to be separated and the adsorbent was 1:100. The eluents were mixtures of chloroform and ethanol with increasing polarity.

Ch-30 (I), $C_{29}H_{32}O_9$, mp 268-275°C (crystallization from methanol), $[\alpha]_D^{20}$ −38.2 ± 3°; chloroform–ethanol). Its biological activity, determined by S. I. Lutokhin using Hatcher's method, was 0.25 mg/kg weight of a cat.

Ch-31 (IV), $C_{35}H_{52}O_{14}$, $[\alpha]_D^{21}$ −44.1 ± 3° (c 0.65; methanol). The molecular weight determined by a spectrophotometric method [6] was 711; that calculated for the composition shown is 697.

Hydrolysis of the Glycoside Ch-30 (I). A solution of 0.2 g of the glycoside in 100 ml of acetone was treated with 1 ml of concentrated hydrochloric acid and the mixture was left at room temperature. Chromatographic analysis showed that the glycoside was hydrolyzed almost completely in the course of 5 days. The reaction mixture was diluted with 30 ml of water, and the acetone was evaporated off in vacuum. Crystals of the aglycon (II) deposited from the aqueous solution, and these were separated off and recrystallized from acetone. An additional amount of the aglycon was extracted from the aqueous solution with chloroform–ethanol (4:1). The aqueous solution after the usual elimination of HCl was used for chromatographic analysis and for the preparation of a phenylosazone. The monosaccharide showed the same R_f values as L-rhamnose in various solvent systems. The phenylosazone with mp 180-183°C was also identical with L-rhamnose phenylosazone according to the results of a mixed melting point and chromatogaphy.

4-Dehydrosarmentogenin (II). The aglycon (II) obtained from the glycoside Ch-30 (I), $C_{23}H_{32}O_5$, had mp 296-302°C (crystallized from acetone), $[\alpha]_D^{20}$ +26.2 ± 3° (c 0.4; pyridine). With concentrated H_2SO_4 it formed the following colorations changing with time: yellow-brown (0'); brown (19'); green (75'); blue-green (90'); and blue with a greenish tinge (120'). Acetylation of the aglycon with acetic anhydride in pyridine (21°C, 18 h) gave, after the usual working-up procedure, a diacetate (III) having mp 265-270°C with a molecular weight of 468 measured by a spectrophotometric method [6]; the calculated molecular weight for the composition $C_{27}H_{36}O_7$ is 472.

The reaction of the aglycon for a double bond in the 4:5 or 5:6 position [4, 5, 8] was positive.

Enzymatic Hydrolysis of the Glycoside Ch-31 (IV). A solution of 0.1 g of the glycoside in 2 ml of ethanol and 10 ml of an aqueous solution of an enzyme preparation obtained from the pancreatic juice of the grape snail were prepared separately. Then they were mixed and the mixture was left at 40°C for 24 h. A sample taken after this showed, on chromatographic analysis, that there was none of the initial glycoside in solution but there was a less polar cardenolide. The enzymes were precipitated with hot ethanol. The cardenolide was extracted from the solution with chloroform and chloroform–ethanol (2:1) and was crystallized from acetone. According to paper chromatography, the aqueous solution contained D-glucose.

The cardenolide obtained had mp 267-275°C, $[\alpha]_D^{20}$ −39.4 ± 3° (c 0.5; chloroform–ethanol). A mixture with the monoglycoside (I) gave no depression of the melting point: 267-275°C. The results of a chromatographic comparison also indicated the identity of these compounds.

Synthesis of 4-Dehydrosarmentogenin (II). A solution of 0.2 g of bipindogenin (V) in 5 ml of absolute pyridine was treated with 2.5 ml of acetic anhydride, and the mixture was left at 20°C for 80 min. Then the solution was diluted with ice-water and, after 30 min, it was extracted with chloroform (3 × 30 ml). The chloroform extract was washed free from pyridine with 5% hydrochloric acid (3 × 10 ml) and was then washed with water (4 × 5 ml) and was evaporated. The residue, which consisted of 11α-O-acetylbipindogenin (VI) containing the diacetate as an impurity was, without additional purification, dissolved in 5 ml of glacial acetic acid, and 2.7 ml of a 2% solution of CrO_3 in acetic acid was added over an hour. The reaction mixture was diluted with 100 ml of chloroform and was washed with 3% sulfuric acid (3 × 10 ml), with 5% sodium carbonate solution (5 × 5 ml), and with water (3 × 10 ml) and was evaporated. The ketone (VII) so obtained was dissolved in 100 ml of chloroform containing 3% of acetic acid and the solution was boiled for 2 h, the completeness of this dehydration reaction being monitored with the aid of TLC. The chloroform solution was washed with 3% sodium carbonate solution and with water to neutrality and was evaporated. The Δ^4-3-ketone (VIII) was purified by chromatography on a column of activity grade III alumina. Elution was performed with benzene, methylene chloride, and chloroform. This gave the ketone (VIII) with mp 157-160°C (from methanol–water). $\lambda_{max}^{C_2H_5OH}$: 242 nm (log ε 4.24), 218 nm (log ε 4.17).

Over 1 h, 100 mg of sodium tetrahydroborate was added in portions to a solution of 60 mg of the Δ^4-3-ketone (VIII) in 15 ml of 80% dioxane. Then, with cooling, the reaction mixture was diluted with water (50 ml) and was extracted with chloroform (100 ml) and with chloroform–ethanol (3:1) (3 × 40 ml). The combined extracts were washed with water to neutrality and were evaporated. The residue was dissolved in 10 ml of methanol and to this solution was added 3 ml of ammonia saturated methanol and the mixture was left at room temperature for 2 days.

Then it was evaporated and the resulting mixture of cardenolides was chromatographed on 10 g of activity grade III Al_2O_3 with elution by chloroform and chloroform–ethanol. Two products were isolated. One of them, having mp 293-301°C (from acetone), when chromatographed on

paper, proved to have the same R_f values as 4-dehydrosarmentogenin (II), and their IR spectra proved to be identical. A mixture melted without depression at 293-302°C. Cardenolide (XI) had mp 271-278°C.

SUMMARY

Two new cardiac glycosides have been isolated from the seeds of *Cheiranthus allioni* hort., and, from them, a new aglycon — 4-dehydrosarmentogenin, which is 3β,11α,14-trihydroxy-14β-card-4,20(22)-dienolide. An independent synthesis of this cardenolide starting from bipindogenin has been performed.

The glycosides 4-dehydrosarmentogenin rhamnoside and 4-dehydrosarmentogenin glucorhamnoside are, respectively, 11α,14-dihydroxy-3β-α-L-rhamnopyranosyloxy-14β-card-4,20(22)-dienolide and 11α,14-dihydroxy-3β-(4'-O-β-D-glucopyranosyl-α-L-rhamnopyranosyloxy)-14β-card-4,20(22)-dienolide.

LITERATURE CITED

1. I. F. Makarevich, D. D. Kolesnikov, I. P. Kovalev, V. T. Gordienko, and V. S. Kabanov, Khim Prir. Soedin., 754 (1975).
2. C. Mannich and G. Siewert, Chem. Ber., 75 737 and 750 (1942).
3. I. F. Makarevich, Khim. Prir. Soedin., 221 (1967).
4. K. F. Witter and Sh. Stove, Anal. Chem., 29, No. 1, 156 (1957).
5. R. Tschesche, G. Snatzke, J. Delgado, and A. G. Gonsales, Ann. Chem., 663, 157 (1963).
6. I. F. Makarevich and S. G. Kislichenko, Khim. Prir. Soedin., 125 (1972).
7. I. F. Makarevich, O. I. Klimenko, and D. G. Kolesnikov, Khim. Prir. Soedin., 188 (1969).
8. N. F. Komissarenko, Khim. Prir. Soedin. 38 (1971).
9. C. Djerassi et al., J. Am. Chem. Soc., 78, 3163 (1956).

SYNTHESIS OF URIDINE DIPHOSPHATE [1-^{3}H]GLUCOSE AND URIDINE DIPHOSPHATE [6-^{3}H]GLUCOSE

E. V. Snetkova, G. P. Akulov, L. S. Gordeeva, and Yu. L. Kaminskii

UDC 577.114:546 11.3

Information is given on the synthesis of tritium-labeled UDPG by the successive transformation of D-[1-^{3}H]glucose or D-[6-^{3}H]glucose by the action of the enzymes hexokinase (EC 2.7.1.1), phosphoglucomutase (EC 2.7.5.1), and UDPG-pyrophosphorylase (EC 2.7.7.9) under conditions ensuring the retention of the tritium label. Methods of obtaining tritium-labelled substrates and intermediate products of their enzymatic transformations in the synthesis of [^{3}H]UDPG are discussed.

The preparation of uridine diphosphate glucose (UDPG) labeled in the glycosidic moiety of the molecule is of interest in connection with the participation of this compound in numerous processes involving the transfer of a carbohydrate residue [1]. Both chemical [2] and enzymatic [3] methods have been used for the synthesis of nonradioactive UDPG. Chemical methods of obtaining labeled UDPG are unsuitable because of their multistage nature, the necessity for the insertion and removal of protective groups, and the relatively large scale of the synthesis. Enzymatic methods with the participation of the enzyme UDPG-pyrophosphorylase (EC 2.7.7.9) are frequently used for the synthesis of [^{14}C]UDPG [4], but their use in the synthesis of [^{3}H]UDPG sometimes leads to the almost complete "washing out" of the label [5]. It is apparently just this fact that is responsible for the absence of information on the synthesis of UDPG labeled with tritium in position 1 of the carbohydrate residue which, at least in free carbohydrates, is considered more labile than the other positions.

In the present paper we give information on this enzymatic synthesis of UDPG taking place with the retention of the label both in position 6 and in position 1 of the glycosidic residue.

V. G. Khlopin Radium Institute, Leningrad.

Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 125-128, January-February, 1987. Original article submitted June 5, 1986.