I. F. Makarevich, M. Ya. Tropp, D. G. Kolesnikov

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Abstract

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I. F. Makarevich, M. Ya. Tropp, D. G. Kolesnikov

Erycordin and Desglucoerycordin–New Cardiac Glycosides

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From the herb of the treacle-mustard *Erysimum cheiranthoides* L., we have obtained, along with other cardenolides, a cardiac glycoside previously designated as glycoside L. Now, after studying the chemical nature of this substance and establishing its difference from the cardiac glycosides described previously, we propose to call it erycordin. Proof of the chemical structure of erycordin is the subject of the present communication. The yield of crystalline glycoside, based on the weight of dry raw material, is 0.015–0.02%. Its biological activity is 0.13–0.15 mg/kg of cat body weight. After recrystallization from acetone–water it melts at 189–192°/201–203°; $[\alpha]_D^{20} - 25.3 \pm 3^\circ$ (C 1.101, ethanol). It gives positive reactions for cardenolides: Legal, Raymond, and Kedde; it reacts negatively with the Keller–Kiliani and Webb–Levy reagents ([3]), which indicates the absence of 2-deoxy sugars. In conc. H_2SO_4 it dissolves with a yellow coloration, which after 1.5 hours changes to a long-persisting red-violet.

The glycoside, as well as all the substances described below, was analyzed after drying in vacuum at 0.1 mm Hg at 80° over P_2O_5 for 3 hours.

Found, %: C 57.29; H 7.82

$C_{35}H_{54}O_{14} \cdot 2H_2O$. Calculated, %: C 57.21; H 7.95

Molecular weight found 736.1 (lactone titration), calculated 734.85. The molecular weight and empirical formula correspond to a steroid diglycoside. The UV spectrum shows only one absorption maximum at 220 mμ ($\log \varepsilon = 4.15$), characteristic of a butenolide ring. The IR spectrum* also shows the presence of a butenolide ring (bands with frequencies 1800 cm^{-1}, 1735^{-1}, and 1860 cm^{-1}) and has no absorption bands characteristic of an aldehyde group (see scheme on p. 850).

The glycoside is cleaved both by the pancreatic juice of the grape snail and by an enzyme preparation from the fungus *Aspergillus oryzae*. After fermentation and appropriate treatment ([4,5]), a monoglycoside (yield 87.7% of theory) and a monosaccharide were obtained in crystalline form. The monosaccharide melts at 145–146°; $[\alpha]_D^{19} + 53.0 \pm 3^\circ$ (C 0.787, aqueous solution after 2 hours).

By paper chromatography, using the osazone obtained and mixed samples, the monosaccharide was identified as *d*-glucose (IV).

The monoglycoside (desglucoerycordin) (II) has m.p. 162–164°; $[\alpha]_D^{18} -21.12 \pm 2^\circ$ (C 1.657, methanol). In conc. H_2SO_4 it dissolves with a brown coloration, which after 2 min changes to yellow, and after 45 min to red-violet.

Found, %: C 62.34; H 8.33
$C_{29}H_{44}O_9 \cdot H_2O$. Calculated, %: C 62.79; H 8.36

Molecular weight found 558.2 (lactone titration), calculated 554.68. On acetylation with acetic anhydride in pyridine, the monoglycoside forms a tetraacetate, m.p. 133–137°; $[\alpha]_D^{22} - 5.03 \pm 3^\circ$ (C 0.715, methanol).

Found, %: C 62.79; H 7.55
$C_{37}H_{52}O_{13}$. Calculated, %: C 63.05; H 7.44

* The infrared spectra were obtained by I. P. Kovalev on an IKS-14 spectrometer with LiF and NaCl prisms in Vaseline oil.

Mol. wt. found 709.2 (Rast method ([6])), calculated 704.78.

The number of acetyl groups, determined by the Kuhn and Roth method ([7]), also corresponds to the tetraacetate of a monoglycoside (III). After hydrolysis of desglucoerycordin by Mannich–Siewert ([8]), a monosaccharide was isolated in crystalline form: it melts at 125–129°, forms an osazone, melting at 181–182°; $[\alpha]_D^{18} + 7.0 \pm 5^\circ$ (C 0.328, methanol); it gives the reactions of Votoček–Oshima–Tollens ([9]) and Rosenthaler ([10]) typical for methylpentoses. The data obtained, as well as paper-chromatographic comparisons, make it possible to identify this sugar as *d*-gulomethylose (VI).

From the aglycone portion of the hydrolysate, consisting of three cardenolide substances, after chromatography on aluminum oxide, erycordigenin (aglycone) and two anhydro compounds of the aglycone were obtained in crystalline form. Erycordigenin (V) melts at 236–239°; $[\alpha]_D^{18} + 29.2 \pm 3^\circ$ (C 0.889, methanol). It gives positive Legal, Raymond, and Kedde reactions and a negative reaction with tetranitromethane. In conc. H_2SO_4 it dissolves with a yellow coloration, which after 2 h 35 min changes to greenish-yellow.

Found, %: C 70.78; H 8.73
$C_{23}H_{34}O_5$. Calculated, %: C 70.74; H 8.77

Mol. wt. found 395.1 (Rast method), calculated 390.52.

The UV spectrum shows one absorption maximum at 220 mμ ($\log \varepsilon = 4.11$), which characterizes the presence of a butenolide ring. In a 2% methanolic solution of sodium hydroxide, the aglycone is completely isomerized within 2 hr. A portion of the isoericordigenin ($\sim$ 30%), in which cleavage of the lactone ring

had occurred, was separated by treating the chloroform solution with a $2N$ aqueous solution of Na_2CO_3. Isoericordigenin (X) melts at 234-239°, gives negative Raymond and Kedde reactions. The IR spectrum, unlike that of the original aglycone, does not reveal a double C = C bond.

$C_{23}H_{34}O_5$. Found % : C 70.67; H 8.89
Calculated % : C 70.74; H 8.77

Molecular weight found 393.02 (Rast method), calculated 390.52.

The formation of an isoaglycone under the influence of alkali indicates the presence of a tertiary OH group at C_{14} and its *cis* arrangement with respect to the butenolide ring. The presence in ericordigenin of secondary OH groups at C_{12}, C_{15}, or C_{16}, likewise capable of participating in the formation of isocompounds, is excluded, as is evident from the following discussion. Acetylation of the aglycone with acetic anhydride in pyridine leads to formation of the diacetate, m.p. 183-187°, $[\alpha]_D^{20} + 25.1 \pm 3^\circ$ (C 0.933, methanol).

$C_{27}H_{38}O_7$. Found % : C 68.19; H 7.92
Calculated % : C 68.32; H 8.07

Molecular weight found 477.0 (Rast method), calculated 474.57.

Further information on the structure of ericordigenin was obtained after its oxidation with CrO_3 ([13]) in acetic acid solution. An acid (VIII) was isolated in amorphous form (yield $\sim$ 40%). The methyl ester (IX), obtained by the action of an ethereal solution of diazomethane on a methanolic solution of acid (VIII), likewise has not yet been crystallized. Both acid (VIII) and its methyl ester (IX) give positive Raymond and Kedde reactions, indicating preservation of the butenolide ring. On treatment with acetic anhydride in pyridine, ester (IX) is not changed.

Since an angular aldehyde group at C_{10} in ericordigenin is excluded on the basis of spectroscopic data, the formation of an acid on oxidation indicates the presence in it of a primary alcohol group. The position of the primary alcohol group in ericordigenin is most probably at C_{19}. Consequently, of the five oxygen atoms of the aglycone, two are included in the lactone ring, the third and fourth in OH groups at C_3 and C_{19}. The fifth oxygen atom is part of the tertiary (non-acetylating) alcohol group at C_{14}. In this aglycone, the 17β-position of the butenolide and the 3β-position of the OH group follow (II b) from the high biological activity of the glycosides of which it is a constituent—ericordin (see above) and desglucoericordin (66000 LED).

Among natural aglycones of known structure with five oxygen atoms, only coroglaucigenin (XI) has an OH group at C_{19} ([12,13]). Ericordigenin has the same oxygen-containing substituents as coroglaucigenin. The substituents at C_3, C_{14}, and C_{17}, as in coroglaucigenin, have the β-configuration, but these

aglycones are quite clearly distinguishable by their physicochemical properties: by melting point, optical activity, and IR absorption spectra; in paper chromatography the spots of these substances are located at different levels.

Taking into account that in cardenolides the fusion of rings C and D is *cis*, that of rings B and C is *trans*, while rings A and B may be fused either *cis* or *trans* (IIa), we believe that ericordigenin, in contrast to coroglaucigenin, has a *cis* fusion of rings A and B and, correspondingly, the β-configuration of the hydrogen atom at C_5. Confirmation of this is provided by the magnitude of the difference in molecular rotations of ericordigenin and coroglaucigenin, which approaches (see Table 1) the difference in molecular rotations of digitoxigenin (XII) and uzarigenin (XIII).

Thus, the structure of ericordigenin can be expressed by the most probable formula (V). T. Reichstein and co-workers ([17,24]) by chemical means

(by reduction of cannogenin) cannogenol, which has the same structure as that which we propose for ericordigenin; the physicochemical properties of these substances are close. Evidently, the natural aglycone obtained by us—ericordigenin—and the product of partial synthesis—cannogenol—are identical with one another.

Table 1

Comparison of differences in molecular rotations of aglycones

Substance	$[M]_D$
Digitoxigenin (A/B-*cis*), mol. wt. 374.5, $[\alpha]_D + 18.1^\circ$	$+67.78^\circ$ [14]
Uzarigenin (A/B-*trans*), mol. wt. 374.5, $[\alpha]_D + 14.0^\circ$	$+52.43^\circ$ [15,16]
$[M]_D$ digitoxigenin $-[M]_D$ uzarigenin	$+15.35^\circ$
Ericordigenin (A/B-*cis*), mol. wt. 390.5, $[\alpha]_D + 29.2^\circ$	$+114.03^\circ$
Coroglaucigenin (A/B-*trans*), mol. wt. 390.5, $[\alpha]_D + 25.7^\circ$	$+100.36^\circ$ [12,13]
$[M]_D$ ericordigenin $-[M]_D$ coroglaucigenin	$+13.67^\circ$

Comparison of the molecular rotations of ericordin (I), desglucoericordin (II), and ericordigenin according to Klyne' s rule [18] shows that both glucose and gulomethylose are linked by β-glycosidic bonds (see Table 2).

Table 2

Comparison of molecular rotations according to Klyne

Substance	$[M]_D$
Ericordin $C_{35}H_{54}O_{14} \cdot 2H_2O$, mol. wt. 734.85, $[\alpha]_D - 25.3^\circ$	-183.52°
Desglucoericordin $C_{29}H_{44}O_9$, mol. wt. 536.66 $[\alpha]_D - 21.1^\circ$	-113.23°
Ericordigenin $C_{23}H_{34}O_5$, mol. wt. 390.52 $[\alpha]_D + 29.2^\circ$	$+114.03^\circ$
Rotation of the *d*-glucose moiety in ericordin	-70.29°
Rotation of the *d*-gulomethylose moiety in desglucoericordin	-227.26°
α-methyl-*d*-glucopyranoside, mol. wt. 194.18 $[\alpha]_D + 158.9^\circ$	$+308.6^\circ$ (22,23)
β-methyl-*d*-glucopyranoside, mol. wt. 194.18, $[\alpha]_D - 34.2^\circ$	-66.4° (22,23)
Rotation of the β-*d*-gulomethylose moiety in desglucoheirotoxin	-222.0° (23)

Consequently, the structure of ericordin may be represented most probably by formula (I), and that of desglucoericordin by formula (II). Such cardenolides have not hitherto been described in the literature.

The glycoside Zh obtained by us directly from the plant is identical in physicochemical properties with desglucoericordin; therefore their study was carried out simultaneously.

Kharkov Scientific Research
Chemical-Pharmaceutical Institute

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Note: Figure translations are in progress. See original paper for figures.