# CARDENOLIDE ALKYLIDENEGLYCOSIDES

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*Alkylidene derivatives of cardiac glycosides were synthesized for increased lipophilicity. The synthesis was based on reaction of aldehydes and ketones with the natural glycosides erysimin, digoxin, and digoxigenin-bis-digitoxoside. A total of 19 biologically active compounds was prepared.*

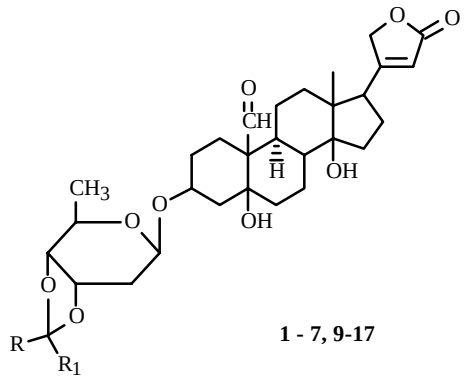
**Key words:** cardenolides, cardenolide-glycosides, alkylidene derivatives, erysimin, isopropylidene-erysimin, bioavailability, absorption from gastrointestinal (GI) tract, biological activity.

Many highly active cardenolide glycosides are not used orally (tablets, capsules, granules) because of very low absorption from the gastrointestinal (GI) tract. These include convallatoxin, erysimin, erysimoside, K-strophanthoside, K-strophanthin-$\beta$, cheirotoxin, erychroside, glucoerysimin, convalloside, helveticosol, convallatoxol, and others. This is despite the fact that these glycosides are available for industrial production.

The bioavailibility of polar glycosides can be increased substantially by increasing their lipophilicity. This is achieved by acetylation, partial methoxylation, or production of alkylidene derivatives.

Of these two methods, the less complicated is the synthesis of alkylidene derivatives, which gives it an advantage.

Alkylidene derivatives can be prepared for those glycosides in which the structure contains a *cis*-$\alpha$-glycol group [1]. Of the glycosides enumerated above, erysimin, convallatoxin, convalloside, and cheirotoxin contain such a group.



**1 - 7, 9-17**

R = $CH_3$– **1, 3**; $C_2H_5$– **2, 17**; HO– **4**; $H_3C$, $H_3C$N– **7**; $H_3CO$– **9**; $H_2N$– **11**

$BrCH_2$–C(=O)–$CH_2$–O– **12**; **13**; –CH=CH– **14**; HO, $H_3CO$ **15**; $C_3H_7$– **16**

**5:** $RR_1$ = -$CH_2(CH_2)_3CH_2$-; **6:** $RR_1$ = -$CH_2(CH_2)_2CH_2$-; **10:** $RR_1$ = -$CH_2$-$CH(CH_3)CH_2CH_2$-

**1, 2:** $R_1$ = $CH_3$; **3, 4, 7, 9, 11 - 17:** $R_1$ = H

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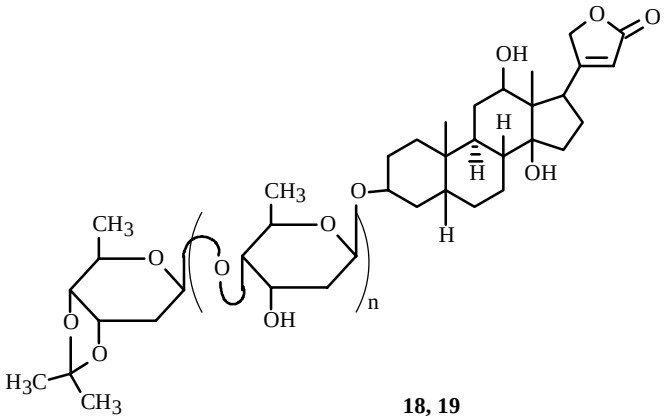
TABLE 1. Alkylidene Derivatives of Erysimin

| Compound | Molecular formula | mp, °C | $[\alpha]_D^{20}$ |
|---|---|---|---|
| **1** | $C_{32}H_{46}O_9$ | 220-223 | +37.2±2 ° ($CHCl_3$) |
| **2** | $C_{33}H_{48}O_9$ | 188-190 | +32.7±2 ° (MeOH) |
| **3** | $C_{31}H_{44}O_9$ | 182-185 | +30.7±2 ° (MeOH) |
| **4** | $C_{36}H_{46}O_{10}$ | 110-112 | +37.3±3 ° ($CHCl_3$) |
| **5** | $C_{35}H_{50}O_9$ | 131-133 | +33.0±2 ° (MeOH) |
| **6** | $C_{34}H_{48}O_9$ | 133-136 | +35.4±2 ° ($CHCl_3$) |
| **7** | $C_{38}H_{51}O_9N$ | 77-79 | +18.7±7 ° ($CHCl_3$) |
| **9** | $C_{36}H_{48}O_{10}$ | 143-147 | +25.2±2 ° (MeOH) |
| **10** | $C_{36}H_{52}O_8$ | 118-121 | +46.0±2 ° ($CHCl_3$) |
| **11** | $C_{38}H_{48}O_8N$ | 105-107 | +45.9±3 ° ($CHCl_3$) |
| **12** | $C_{45}H_{54}O_{11}Br$ | 187-190 | +49.4±3 ° ($CHCl_3$) |
| **13** | $C_{36}H_{47}O_9$ | 118-120/138-140 | +24.2±2 ° ($CHCl_3$) |
| **14** | $C_{38}H_{49}O_9$ | 136-140 | +47.2±3 ° ($CHCl_3$) |
| **15** | $C_{37}H_{48}O_{11}$ | 83-85 | +44.5±3 ° ($CHCl_3$) |
| **16** | $C_{33}H_{49}O_9$ | 117-119 | +42.5±3 ° ($CHCl_3$) |
| **17** | $C_{32}H_{47}O_9$ | 114-116 | +25.0±2 ° ($CHCl_3$) |

These glycosides react with aldehydes and ketones in the presence of dehydrating agents. Most often anhydrous $CuSO_4$ or $ZnCl_2$ is used for this. If the glycoside is soluble in the aldehyde or ketone used, for example, in acetaldehyde, acetone, methylethylketone, cyclopentanone, and cyclohexanone, the problem is technically simplified. Such a solution can be treated with $CuSO_4$ or $ZnCl_2$ and stirred, monitoring the course of the reaction using TLC. If necessary, the reaction can be carried out with heating. In other instances, the glycoside can be dissolved in a suitable organic solvent, treated with the aldehyde (or ketone) and a Lewis salt, and refluxed. An instance was noted where the reaction had to be carried out with cooling. Such a situation arose using propionaldehyde $CH_3CH_2CHO$, which especially quickly reacted with erysimin and heated the reaction mixture. Considering the high volatility of propionaldehyde (bp 48.8°C), the reaction was carried out with cooling.

The product yields, as a rule, are rather high, 70-85% of theoretical. The structures were confirmed by elemental analysis and PMR spectra. Thus, the PMR spectrum of **2** had the following signals for the cardenolide glycoside: 0.72 ppm, C-13 $-CH_3$; 0.9 ppm, D-digitoxose $-CH_3$; 4.90 ppm, butenolide ring $CH_2$; 5.90 ppm, CH of butenolide ring double bond; 10, CHO; and for the isobutylidene: 1.15 ppm, 6H triplet for two methyls of this fragment. The PMR spectrum of **15** had the following signals for the cardenolide glycoside: 0.70 ppm, C-13 $-CH_3$; 3.74 ppm, $-OCH_3$; 4.01 ppm, butenolide ring $CH_2$; 5.90, 1H signal in butenolide ring; 10.0, CHO; 6.90, aromatic protons. The presence of signals for aromatic protons (6.90 ppm, 3H) and methoxyls (3.74 ppm, 3H) indicated that the structure included vanillin. The PMR spectrum of **16** contained signals due to cardenolide glycoside protons that were analogous to those for **2** and, moreover, had a 6H multiplet for two methyls at 0.85 ppm, one of which was from D-digitoxose; the second, butylidene.



**18, 19**

**18:** n = 2; **19:** n = 1

The method for preparing the isopropylidene derivatives was successfully used to prove the chemical structure of several cardiac diglycosides such as convalloside [2], cheirotoxin [3], convallatoxoloside [2], erycordin [2], evobioside [4], and hellebrin [2]. These glycosides contain L-rhamnose, D-gulomethylose, and D-glucose (terminal monosaccharide). L-Rhamnose and D-gulomethylose were bonded directly to the aglycon. It was established that the *cis*-$\alpha$-glycol groups (on C2′ and C3′) in them were free and formed the isopropylidene derivatives, on the basis of which the unambiguous conclusion was made that the D-glucose was bonded to C4′.

The alkylidene glycosides in which R and $R_1$ were different were difficultly separated mixtures of geometric isomers.

Of the synthesized compounds (**1-19**), isopropylidene erysimin (**1**) deserves special attention. This compound retains the high biological activity of the natural glycoside of erysimin and is 70% absorbed from the GI tract. Its synthesis is simple and provides a high yield and purity. Considering the availability of erysimin (produced from seeds of *Erysimum canescens* Roth in ~3% yield), all conditions for fabricating a new medicinal preparation for oral use in cardiology are fulfilled.

## EXPERIMENTAL

The course of reactions, their completion, and the purity of products were monitored using TLC on Sorbfil plates with elution by $CHCl_3$:$CH_3OH$ (95:5-85:15). Elemental analyses were performed on a Model 1106 C—H—N—S analyzer and agreed with those calculated for all compounds.

**Erysimin Methylethylketonide (2).** Dried erysimin (2 g) was dissolved in methylethylketone (22 mL), treated with anhydrous finely ground $CuSO_4$ (6 g), and stirred at room temperature. The salt was separated by filtering the mixture through a dense layer of fine cellulose (under vacuum). The filtering layer was 4 mm thick. The filter was washed with $CHCl_3$ (10 mL). The filtrate was treated with methanol (3 drops) and saturated ammonia and evaporated in vacuo. The resulting amorphous white powder was recrystallized from ethanol (2.5 mL) to afford white crystals of **2** (1.328 g, 60.4%), $C_{33}H_{48}O_9$, mp 188-190°C, $[\alpha]_D$ +32.7 ± 2° (*c* 1.2, MeOH).

The mother liquor was evaporated with heating to ~1 mL and left for 3 h at room temperature to afford an additional batch of **2** (0.4 g, 1.728 g total, 78.5%).

**(*p*-Hydroxy-, *m*-methoxy)-phenylmethylidene-3′,4′-erysimin (15).** Erysimin (1.2 g) and vanillin (1.5 g) were dissolved in $CHCl_3$ (40 mL). The solution was evaporated with heating to about 25 mL (moisture removed using azeotropic distillation), treated with anhydrous $CuSO_4$ (4 g), and refluxed for 15 min. The product that was worked up as described above contained about 15% of the aglycon strophanthidin as an impurity and was purified by chromatography over a column of $Al_2O_3$ (Brockman activity III) with elution by $CCl_4$:$CHCl_3$, then $CHCl_3$, and $CHCl_3$:$CH_3OH$ to afford **15** (0.62 g, 41.3%), mp 83-85°C, $[\alpha]_D$ +44.5 ± 2° (*c* 0.9, $CHCl_3$), $C_{37}H_{48}O_{11}$.

***n*-Butylidene Erysimin (16).** Erysimin (1.2 g) was dissolved in $CHCl_3$ (30 mL) and treated with butyraldehyde (0.55 g) and anhydrous $CuSO_4$ (3.2 g). The mixture was refluxed and stirred for 20 min with TLC monitoring. The desired product was worked up as above to afford *n*-butylidene erysimin (EtOH) (0.87 g, 68%), mp 117-119°C, $[\alpha]_D$ +42.5 ± 2° ($CHCl_3$), $C_{33}H_{47}O_9$.

The remaining compounds, with the exception of **18** and **19**, were synthesized analogously to these three examples.

**Isopropylidene-3‴,4‴-*O*,*O*-digoxin (18).** The synthesis of **18** proceeded slowly, about 24 h at room temperature. The product was purified by chromatography over a column of $Al_2O_3$ with elution by $CHCl_3$:$CH_3OH$ (88:12) to afford **18**, only 22% of calculated, mp 140-142°C, $[\alpha]_D$ +38.4 ± 2° (*c* 1.2, MeOH), $C_{44}H_{48}O_{14}$.

Compound **19** was synthesized in the same way in 22 h.

**Biological Tests.** 3′,4′-*O*,*O*-Isopropylidene erysimin (**1**) exhibited Hatcher biological activity at 0.1 mg/kg in cats. The working solution was injected i.v. at 1:100,000 (Candidate of Medical Sciences N. A. Kisten′).

The degree of absorption from the GI tract (70%) was determined (Candidate of Biological Sciences Zh. A. Lyubetskaya) as usual. Pigeon stomach was administered a certain dose of **1**. After 20-30 min the animal was "titrated" until the heart stopped in systole by administering i.v. a working solution at 1:100,000. Establishing how much compound was used to titrate the animal, the usual calculation determined how much compound was absorbed from the GI tract from the i.p. dose.

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