CARDIAC GLYCOSIDES OF ERYSIMUM CONTRACTUM

I. F. Makarevich, K. V. Zhernoklov,
T. V. Slyusarskaya, A. O. Magomedova,
T. N. Terekhova, and G. T. Sirenko

UDC 547.92/93+615.224:547.918

The cardenolide composition of Erysimum contractum Somm. et Lew. family Brassicaceae (Cruciferae) has been investigated. The seeds of this plant have yielded strophanthidin, erysimin, erysimoside, erycordin, and new cardiac glycoside which has been called nigrescigenin digitoxoside. It has mp 141-145°C, $[\alpha]_D^{20}$ +16.0 ± 2° (c 0.75; methanol), $C_{29}H_{42}O_{10}$. On the basis of chemical transformations and spectral investigations it has been established that the new glycoside is 3-β-D-digitoxopyranosyloxy-5,11α,14-trihydroxy-19-oxo-5β,14β-card-20(22)-enolide. The total content of cardiac glycosides in the seeds of this plant amounted to 3.2%, including 1.26% of erysimoside.

Erysimum contractum Somm. et Lev., family Brassicaceae (Cruciferae) is a new source of cardiac glycosides which has not previously been studied in phytochemical respect. The object of our investigations was the seeds of this plant, which were grown and collected on an experimental plot of the Institute.

The comminuted seeds were defatted with petroleum ether, which gave a fatty oil in an amount of 25% of the weight of the initial material. When the glycosides were extracted and purified as described below, the total glycosides were obtained in the form of a light brown powder with a yield of 6.0% on the weight of the initial raw material.

Analysis with the aid of paper chromatography of the total glycosides obtained showed that they included not less than 13 compounds of cardenolide nature. The preparative separation of the weakly polar and medium-polar glycosides was carried out with the aid of adsorption chromatography on columns of silica gel, while the polar glycosides were separated by partition column chromatography on cellulose.

Six cardenolides were isolated in the individual state, and five of them were identified as strophanthidin, erysimin, erysimoside, and erycordin; two of them have been previously designated E.sp. 4 and E.sp. 5. Glycoside E.sp. 4 proved to be a new monoglycoside (I). Glycoside (I) contained a 2-deoxysugar residue, as was shown by a positive Keller–Kiliani reaction. Spectral investigations showed the presence in the molecule of glycoside (I) of an aldehyde group: the IR region of the spectrum contained absorption bands at 2760 and 1720 cm^{-1}; the PMR spectrum showed a one-proton signal in the 9.92 ppm region.

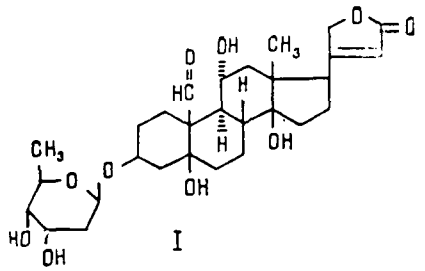
On the acid hydrolysis of (I), performed under mild conditions, the aglycon and a monosaccharide were obtained, and these were identified from their properties and also by a direct comparison with authentic samples as nigrescigenin and D-digitoxose, respectively. The PMR spectrum showed that the D-digitoxose residue in glycoside (I) was attached by a β-glycosidic bond and was present in the pyranose form. The proton at the anomeric carbon atom gave a signal in the region of 4.55 ppm with the SSCC J = 7.0 Hz. Analysis of molecular rotations, performed in accordance with Klyne's rule [2], confirmed the β-configuration of the glycosidic bond: ΔC for D-digitoxins = −12 ± 19°; compare: $[M]_D$ for methyl β-D-digitoxopyranoside = 60°, and $[M]_D$ for methyl α-D-digitoxopyranoside = +311°C [6].

Of the two theoretically possible sites of attachment of the carbohydrate component, C-3 and C-11, C-3 is the most probable for the following reasons. In the first place, all known natural cardiac glycosides contain a sugar residue at C-3. In the second place, the presence of a carbohydrate component at C-11 is unlikely because of steric factors.

All-Union Scientific-Research Institute of the Chemistry and Technology of Drugs, Kharkov. G. S. Skovroda Kharkov State Pedagogic Institute. Translated from Khimiya Prirodnykh Soedinenii, No. 1, pp. 58-62, January-February, 1991. Original article submitted March 11, 1990; revision submitted August 13, 1990.

 0009-3130/91/2701-0050$12.50 © 1991 Plenum Publishing Corporation

Nevertheless, to confirm the structure of (I), we performed the following investigations. In nigrescigenin, the OH group at C-3 is axial, and that at C-11 is equatorial, which permits them to be distinguished by various methods, including the rates of the acetylation reactions. For this purpose, we carried out the "exhaustive" acetylation of glycoside (I), and then hydrolyzed the reaction product, splitting out the carbohydrate component. The monoacetate of the aglycon so obtained was subjected to controlled further acetylation at the OH group liberated after the splitting out of the D-digitoxose residue. It was established at the time of half-reaction here exceeded 2 h. This showed (see [8]) that this OH group was equatorial and difficult to acetylate. Consequently, it is possible to draw the following unambiguous conclusion: the D-digitoxose residue of glycoside (I) is attached at C-3, i.e., at the axial secondary OH group.



I

Thus, the new cardiac glycoside (I) is 3-O-β-D-digitoxopyranosyloxy-5,11α,14-trihydroxy-19-oxo-5β,14β-card-20(22)-enolide (I). We propose the following semitrivial name: nigrescigenin digitoxoside.

The main glycosides of E. compactum are erysimoside, glucoerysimoside, and the unknown highly polar glycoside E.sp. 5. We have made a semiquantitative analysis of the amounts of some glycosides in the erysimum, and also a quantitative analysis of the total amount of cardenolide glycosides both in the seeds and in the purified total material that had been isolated. This showed that the E. compactum seeds contained 3.2% of glycosides, while the purified total material contained 50% of them:

Glycoside	Amount in the purified total cardenolides, %	Amount in the seeds, %
Erysimoside	24.1	1.26
Erysimin	4.5	0.22
Glycoside E.sp. 5	9.2	0.45

The facts given above show that the plant investigated is a favorable source of erysimoside, erysimin, and the aglycon strophanthidin, which is a component of these glycosides. Erysimin can be obtained both directly from the plant and also, mainly, from the erysimoside and glucoerysimoside after their enzymatic hydrolysis. With respect to its erysimoside content, the plant under investigation is comparable with hoary erysimum (E. canescens) [7], although it is somewhat inferior to the latter. Its content of the glycoside E.sp. 5 is fairly high.

EXPERIMENTAL

Elementary analysis was carried out with the aid of an automatic C–H–N analyzer; the results of the analyses corresponded to the calculated figures. PMR spectra were taken on a Tesla BS-497 instrument (100 MHz). Melting points were determined on a Kofler block. The purity of the substances and the analysis of the fractions were monitored by paper chromatography with the use of the following systems: chloroform–tetrahydrofuran (1:1)/formamide; toluene–butan-1-ol (1:2)/water; and butan-1-ol–acetic acid–water (4:1:2).

Isolation of the Purified Total Glycosides and their Analysis. Erysimum compactum seeds (16 kg) were ground in a ball mill and were defatted with petroleum ether. The defatted raw material was dried to eliminate the last traces of petroleum ether and was exhaustively extracted with 95% and 80% ethanols. The extracts were concentrated to an alcohol concentration of 35-40%. The aqueous alcoholic solution was purified with toluene by a method described previously [5]. The glycosides were extracted from the purified solution successively with chloroform, chloroform–ethanol (2:1), and butanol. Evaporation of the

chloroform and the ethanol-chloroform extracts yielded 970 g of a light brown powder, and that of the butanol extracts 160 g of a viscous brown mass. The latter contained the most polar cardenolide glycosides.

With the aim of a quantitative determination of the total amount of cardiac glycosides, a weighed sample of the total material obtained (dry) was dissolved in 40% ethanol, and the solution was filtered through a threefold amount of alumina. Then the amount of cardenolide glycosides was determined in the usual way using the photocolorimetric picrate method [4] (with erysimoside as standard).

Preparative Chromatographic Separation. Part of the total glycoside material obtained was separated on a 100-fold amount of silica gel activated at 120°C. Chloroform and mixtures of chloroform and ethanol of increasing polarity were used as eluents. This led to the isolation of five cardenolides in the individual state. The glycoside E.sp. 5, obtained with other cardenolides as impurities, was additionally chromatographed on a column of cellulose. Elution with the toluene–butan-1-ol (1:2)/water system gave an individual compound. The ratio of dry cellulose and the mixture to be separated amounted to 200:1.

Strophanthidin, mp 142-146°C (from 40% ethanol), $[\alpha]_D^{20}$ +43 ± 2° (c 1.0; methanol) [1].

Erysimin, mp 178-183°C (from 30% ethanol), $[\alpha]_D^{20}$ +27.1 ± 2° (c 1.0; methanol) [1].

Erysimoside, mp 170-173°C (from isopropanol), $[\alpha]_D^{21}$ +18.8 ± 2° (c 1.2; methanol) [1].

Erycordin, mp 201-203°C (from ethanol), $[\alpha]_D^{20}$ −25.2 ± 2° (c 1.0; methanol) [1].

Nigrescigenin digitoxoside (I), $C_{29}H_{42}O_{10}$, mp 141-145°C (from methanol–benzene), $[\alpha]_D^{21}$ +16.0 ± 2° (c 0.75; methanol).

E.sp. 5, mp 169-174°C (from methanol–ethyl ether), $[\alpha]_D^{20}$ −2.6 ± 2° (c 0.9; methanol).

The semiquantitative analysis of the main glycosides was performed by a known method [3].

Hydrolysis of Nigrescigenin Digitoxoside (I). A solution of 0.2 g of the glycoside in 10 ml of ethanol was treated with 10 ml of 0.1 N sulfuric acid solution. The reaction mixture was heated in a flask fitted with a reflux condenser at 75-80°C for 40 min. The acid was neutralized with barium carbonate. The solution was filtered. The aglycon was extracted from the solution with chloroform (2 × 10 ml) and with chloroform–ethanol (4:1) (3 × 10 ml). The combined ethanolic-hydrochloric acid solutions were washed with water (5 ml) and evaporated. The aglycon so obtained was crystallized from methanol–ethanol: mp 222-230°C; $[\alpha]_D^{20}$ +23.8 ± 2° (c 0.65; methanol). In its properties and comparative chromatographic characteristics it was identical with a sample of nigrescigenin.

The aqueous phase containing the carbohydrate component was evaporated to dryness. The residue was crystallized from acetone–diethyl ether and recrystallized from acetone. The crystals, obtained in the form of large prisms, had mp 108-111°C. A direct comparison (mixed melting point, paper chromatography) showed the identity of the monosaccharide obtained with D-digitose.

LITERATURE CITED

1. I. F. Makarevich, É. P. Kemertelidze, S. G. Kislichenko, V. V. Zatula, A. A. Reznichenko, D. G. Kolesnikov, and I. P. Kovalev, Cardenolides and Bufadienolides [in Russian], Metsniereva, Tbilisi (1976).
2. K. Klyne, Biochem. J., No. 4, 41 (1959).
3. I. F. Makarevich, Yu. A. Chernyaev, N. E. Vorob'ev, L. V. Evseeva, and A. I. Alistrenko, Farm. Zh., No. 4, 71 (1987).
4. N. E. Vorob'ev and N. P. Dzyuba, Farmatsiya, No. 3, 22 (1971).
5. I. F. Makarevich and Yu. A. Chernyaev, USSR Inventor's Certificate No. 1172560; Byull. Isobret., No. 30, 31 (1985).
6. W. E. Zorbach and T. A. Payne, J. Am. Chem. Soc., 80, 5564 (1958).
7. V. A. Maslennikova, G. L. Genkina, R. U. Umarova, A. M. Navruzova, and N. K. Abubakirov, Khim. Prir. Soedin., No. 3, 173 (1967).
8. I. F. Makarevich, Khim. Prir. Soedin., No. 4, 221 (1968).