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DIGITOGENIN–GITOGENIN ISOMORPHOUS SUBSTITUTION IN THE MIXED CRYSTAL FROM *DIGITALIS LANATA* EHRH.

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A crystalline compound is isolated from *Digitalis lanata* Ehrh. and its molecular and crystal structures are studied by single crystal X-ray diffraction. The X-ray diffraction results show that the crystal is mixed and consists of a crystalline hydrate of isomorphically substituted digitogenin and gitogenin molecules in the 38:62 ratio. The data on the mole ratio are confirmed by high-performance liquid chromatography.

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Research papers on glycosides (saponins) and their hydrolysis products – sapogenins (aglycons) hold an important place among publications on steroid chemistry and biochemistry. In plants, they are usually found as complex mixtures. It became possible to isolate them as individual substances and determine their structure only owing to the widespread use of chromatography and physical research methods.

Previously, we have studied the crystal and molecular structure of tigogenin isolated from the aerial part of woolly foxglove [1]. In furtherance of this research, here we report the isolation of new sapogenins typical of the aerial part of *Digitalis lanata* Ehrh. and the determination of their structures.

EXPERIMENTAL

Sapogenins were obtained from the aerial part of woolly foxglove according to the following procedure: plant roughage collected in the flowering phase (primary seed nursery of the JSC International Research and Production Holding "Phytochemistry", Karaganda) was extracted with 70% ethanol. Lipophilic components were removed from the extract by treating with petroleum ether. The product was purified from water-soluble constituents by extraction with isobutanol. Then the extract was subjected to acid hydrolysis (5% H_2SO_4 in a mixture of 1,3-dioxane : water = 1:1, with solvent boiling for 4 h). Column chromatography on alumina was used to isolate a substance from the hydrolyzate, and then by subsequent recrystallization from a cyclohexane–ethyl acetate mixture in a 7:3 ratio we obtained crystals of **1** with T_{melt} of 283-285 °C and R_f = 0.38.

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The chromatographic analysis was carried out on a High Pressure Laboratory Chromotograph by reverse-phase high-performance liquid chromatography. An analytical column filled with the Zorbax SB-C_{18} sorbent was the stationary phase; 1:1 (vol./vol.) tetrahydrofuran–water was the mobile phase.

Single crystal X-ray diffraction study was performed on a Bruker P4 diffractometer (MoK_α radiation, graphite monochromator, ω-scanning, $2\theta \le 50°$) at a temperature of 153 K. 5001 reflections were measured, including 4987 independent ($R_{int} = 0.0637$). The crystals are orthorhombic: $a = 7.493(5)$ Å, $b = 19.844(12)$ Å, $c = 33.859(15)$ Å, $V = 5034(5)$ Å^3, $Z = 8$, $(0.38(C_{27}H_{44}O_5)\ 0.62(C_{27}H_{44}O_4)\cdot H_2O)$, space group $P2_12_12_1$, $d_{calc} = 1.181$ g/cm^3, $\mu = 0.079$ mm^{-1}. The initial set of measured intensities was processed and the absorption correction was applied using the SAINT and SADABS programs from the APEX2 software [2].

The structure was solved by a direct method. The positions and thermal parameters of non-hydrogen atoms were refined in the anisotropic approximation by full-matrix LSM. A solvate water molecule was found in the crystal structure. Note an incomplete occupancy of the O3 and O3A (0.38(1)) atomic sites in two crystallographically independent molecules, which is indicative of cocrystallization of two compounds: **2** with the OH group at the C15 atom (38%) and **3** without the OH group (62%). Hydrogen atoms were placed in geometrically calculated positions and included in the refinement with a riding model. We failed to locate hydrogen atoms of the OH groups and the second H atom of hydration water from the difference maps.

2469 independent reflections with $I \ge 2\sigma(I)$ and 591 refined parameters were used in the calculations. The final divergence factors $R1 = 0.0636$, $wR_2 = 0.1350$ (for reflections with $I \ge 2\sigma(I)$), $R1 = 0.1479$, $wR_2 = 0.1702$ (for all reflections), GOOF = 0.987. The residual density peaks: $\Delta\rho = 0.226$ e/Å^3 and –0.210 e/Å^3. The structure was solved and refined using the SHELXS-97 [3] and SHELXL-97 [4] software. The CIF file containing full information on the studied structure has been deposited with the Cambridge Crystallography Data Center (CCDC 680350).

RESULTS AND DISCUSSION

The structure of crystalline compound **1** was determined by X-ray diffraction. The single crystal X-ray diffraction results showed that the crystal is a crystalline hydrate of a mixture of two compounds (solid solution): the molecules of digitogenin (**2**) and gitogenin (**3**). The detailed analysis of the atomic site occupancy evidenced that in a mixed crystal the content of compound **2** is 38% and that of compound **3** is 62%. There is an isomorphous substitution in the crystal of **1**: the hydroxyl O(3)–H group at the C(15) atom in the molecule of **2** is substituted with the hydrogen atom in the molecule of **3**. In general, the composition of the crystal of **1** is expressed by the formula $0.38(C_{27}H_{44}O_5)\cdot 0.62(C_{27}H_{44}O_4)\cdot H_2O$. The general view of **2** and **3** molecules is shown in Fig. 1. The configuration of chiral centers correlates with the absolute configuration in a (25R)-1α,2α-epoxy-3α-hydroxy-5α-spirostan-6-one molecule [5].

The existence of two compounds (**2** and **3**) in the crystal of **1** was confirmed by high-performance liquid chromatography. From the chromatography results it was established that the sample under study included two compounds: digitogenin **2** with a content of 36.95% and gitogenin **3** with a content of 61.43%; the retention times were 6.15 min and 4.73 min respectively. There are also impurities of an unknown composition (1.62%). Without regard for the impurity content, the fractions of digitogenin **2** and gitogenin **3** are 36.95% and 61.43% respectively, which agrees with the X-ray diffraction data.

In addition to **2** and **3** molecules with isomorphous substitution, the crystal of **1** contains two crystallographically independent molecules (**1a** and **1b**). In the literature, such cases of cocrystallization of symmetrically independent molecules are considered as a special case of mixed crystals [6].

The bond lengths in **1a** and **1b** molecules are similar, whereas the bond angles are distorted as compared to normal values [7]. All six-membered cycles in two crystallographically independent molecules take the conformation of a slightly *distorted chair* (the asymmetry parameters for the **1b** molecule are given in parentheses). For example, for the

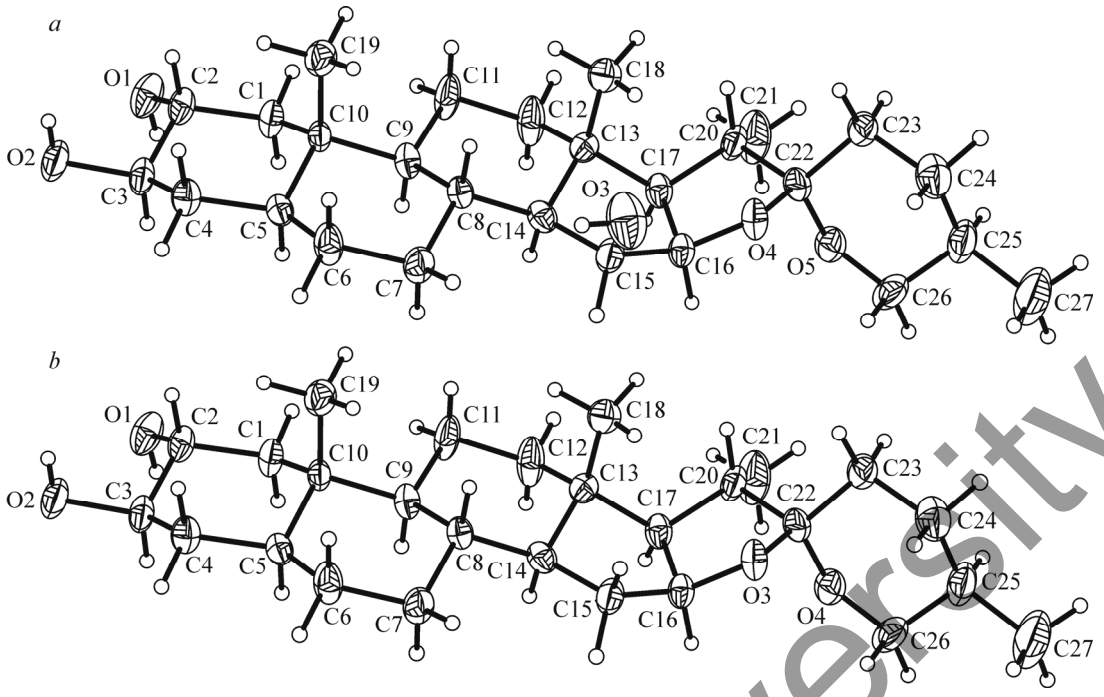

Fig. 1. Structural components of the solid solution: molecule of **2** (digitogenin) (*a*); molecule of **3** (gitogenin) (*b*).

C(1)C(2)C(3)C(4)C(5)C(10) cycle $\Delta C_S^2 = 1.6°$ and $\Delta C_2^{4,5} = 1.8°$ ($\Delta C_S^1 = 2.0°$ and $\Delta C_2^{1,2} = 2.7°$), for C(5)C(60C(7)C(8)C(9)C(10) $\Delta C_S^7 = 1.8°$ and $\Delta C_2^{7,8} = 1.7°$ ($\Delta C_S^5 = 0.9°$ and $\Delta C_2^{5,6} = 3.3°$), for C(8)C(9)C(11)C(12)C(13)C(14) $\Delta C_S^{14} = 1.6°$ and $\Delta C_2^{13,14} = 2.4°$ ($\Delta C_S^9 = 3.4°$ and $\Delta C_2^{9,11} = 1.2°$), and for O(5)C(22)C(23)C(24)C(25)C(26) $\Delta C_S^{23} = 1.0°$ and $\Delta C_2^{25,26} = 4.2°$ ($\Delta C_S^{24} = 0.8°$ and $\Delta C_2^{24,25} = 2.1°$).

The O(4)C(16)C(17)C(20)C(22) heterocycle in the first crystallographically independent **1a** molecule (data for the **1b** molecule are given in parentheses) has an intermediate conformation between a *distorted envelope* with the oxygen atom deviating to the β side by 0.52 Å (0.51 Å) from the plane of the other atoms and a distorted O(4),C(22) *half-chair* ($\Delta C_S^{O4} = 7°$ and $\Delta C_2^{4,22} = 11.3°$; $\Delta C_S^{O4} = 04.2°$ and $\Delta C_2^{4,22} = 12.3°$). The five-membered C(13)C(14)C(15)C(16)C(17) carbocycle also has an intermediate conformation between a *distorted envelope* with the C(14) atom deviating to the α side by 0.64 Å (0.63 Å) and a *distorted* 13,14 *half-chair* ($\Delta C_S^{14} = 13.1°$ and $\Delta C_2^{13,14} = 5.1°$; $\Delta C_S^{14} = 10.4°$ and $\Delta C_2^{13,14} = 8.5°$).

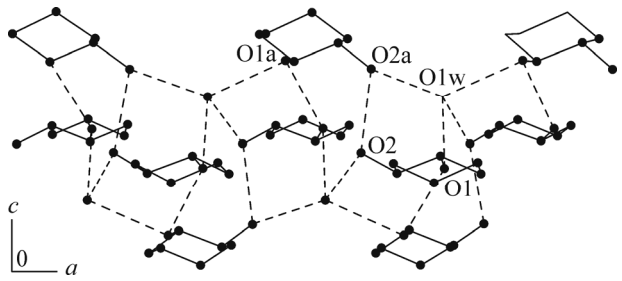

Fig. 2. Packing of fragments in **2**, **3** molecules and hydration water in the crystal cell; the projection is perpendicular to the *b* axis. Hydrogen atoms are omitted. The O…O contacts corresponding to hydrogen bonds are indicated by dashed lines.

Methyl groups at the C(10), C(13) atoms and the hydroxyl group at the C(15) atom have the β-axial orientation and the methyl groups at the C(20), C(25) atoms and the hydroxyl groups at the C(2) and C(3) atoms have the equatorial orientation. The conformation of the cycles of the titled compounds corresponds to those in previously considered aglycons, such as methanol solvate 25R,5α-spirostan-1β,3β-diol [8], 3-hydroxyspirostan-2-yl acetate [9], and 20-epitigogenin acetate [10].

The O(1), O(2), O(1a), O(2a), and O(1w) atoms of **2** and **3** molecules in the crystal are involved in intramolecular hydrogen bonds: O(1)–H…O(1w) (–0.5+*x*, 0.5–*y*, 1–*z*) (O…O of 2.76 Å), O(2)–H…O(2a) (–0.5+*x*, 0.5–*y*, 1–*z*) (2.81 Å), O(1a)–H.O(1)(1+*x*, *y*, *z*) (2.85 Å), O(2a)…O(1w) (*x*, *y*, *z*) (2.78 Å), O(1w)–H.O(2) (*x*, *y*, *z*) (2.78 Å), and O(1w)…O(1a) (–1+*x*, *y*, *z*) (2.91 Å). They form infinite ribbons in the (*x* 0 0) plane along the [*a* 0 0] direction. The arrangement of the molecular fragments in the crystal in the projection on the *ac* plane is shown in Fig. 2.

CONFLICT OF INTERESTS

The authors declare that they have no conflict of interests.

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