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GOMPHACIL — A CARDENOLIDE GLYCOSIDE OF *Gomphocarpus fruticosus*

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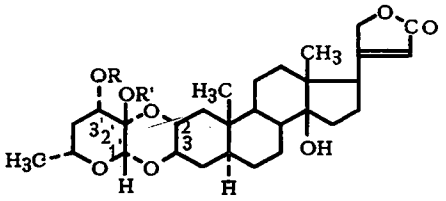
The leaves of Gomphocarpus fruticosus *(L.) R.Br. have yielded the new acetylated glycoside gomphacil, which is (3β-O-1'α,2α-O-2'α)-(3'α-acetoxy-2'β-hydroxy-4',6'-dideoxyhexulosido)-14β-hydroxy-5α-card-20(22)-enolide.*

We have previously [1, 2] reported the isolation from *Gomphocarpus fruticosus* (L.) R.Br., fam. Asclepiadaceae Lindl., of uzarigenin, deglucouzarigenin, gomphoside, gomphotin, and gomphotoxin. Continuing the study of the cardenolides of the leaves of this plant, we have isolated from the chloroform fraction a cardiac glycoside that has been called gomphacil (**1**). In this paper we consider the determination of its structure.

Gomphacil has the molecular formula $C_{31}H_{44}O_9$. On the basis of qualitative reactions [3, 4] and IR (absorption bands at 1633 and 1750 cm^{-1}) and UV (maximum in the 220 nm region (log ε 4.36)) spectra, the substance was assigned to the cardenolides. The positive course of the curve in the optical rotatory dispersion spectrum showed the *trans-* linkage of rings *A/B* in the steroid nucleus. The high-vacuum thermal decomposition of (**1**) led to the formation of methylreductic acid [1, 5], which showed the presence in it of a 6-deoxyhexosone as the sugar residue. When (**1**) was treated with a methanolic ammonia solution, the compound was hydrolyzed to acetic acid and glycoside (**2**), in which a glycol group resistant to the formation of an acetonide was detected, which is possible when the OH groups are in the *trans-* position. On the basis of its physicochemical characteristics and chemical properties, the deacetyl derivative of gomphacil was identified as gomphoside (**2**) [1].



1. R=Ac; R_1 =H
2. R=R_1 =H
3. R= R_1 =Ac

In the substance under investigation (**1**) we found one acetyl group [6]. Gomphacil acetate (**3**) was identical with diacetylgomphoside. This permitted the conclusion that the glycoside isolated (**1**) was a monoacetylgomphoside. To establish the position of attachment of the acetyl group we attempted the oxidation of gomphacil with CrO_3 in glacial acetic acid. As a result we recovered the initial substance, which is possible if the acetyl residue substitutes the OH group in position 3' of the 4',6'-hexuloside part of glycoside (**1**).

Thus, the structure of gomphacil may be represented as (3β-O-1'α,2α-O-2'α)-(3'α-acetoxy-2'β-hydroxy-4',6'-dideoxyhexulosido)-14β-hydroxy-5α-card-20(22)-enolide.

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EXPERIMENTAL

For the general experimental part, see [1, 2].

Isolation of Gomphacil (1). Substance (1) was isolated from the mother solutions of the chloroform fraction after the separation of gomphotin, gomphoside, and gomphotoxin [1] by chromatography on a column of alumina (activity grade III) in a ratio of sorbent to mixture to be separated of 70:1. The column was eluted with benzene and then with mixtures of benzene and chloroform having concentrations of the latter increasing from 30 to 100%. The efficiency of the separation of the cardenolides was monitored by paper chromatography in the solvent system benzene–chloroform (7:3)/formamide. Substance (1) was the first to emerge from the column. On crystallization from MeOH, colorless elongated plates deposited with mp 268-271°C, $[\alpha]_D^{20}$ +26 ± 3° (*c* 0.1; MeOH). An alcoholic solution of (1), gave positive reactions with the Legal and Raymond reagents [3, 4].

Optical rotatory dispersion of gomphacil (MeOH; *c* 0.1): 623 nm +26°; 589 nm +26°; 455 nm +65°; 420 nm +90°; 380 nm +152°; 360 nm +199°; 340 nm +323°; 320 nm +637°; 300 nm +804°.

Calculated %: C 68.87; H 8.05. $C_{31}H_{44}O_9$ (550.3). Found %: C 69.37; H 7.99.

High-Vacuum Thermal Decomposition of (1). A small retort was charged with 100 mg of the substance under investigation and it was then connected to a vacuum system at a pressure of 0.01-0.02 mm Hg and was heated to 240-245°C. After this, the experiment was continued as described in [1]. The crystals obtained melted at 83-84°C and had the molecular formula $C_6H_8O_3$. They were identified as methylreductic acid.

The benzidine-periodate test [1] for substance (1) was negative, showing the absence of a free glycol grouping.

Deacylation of (1). A solution of 20 mg of (1) in 3 ml of methanol was treated with 1 ml of ammonia-saturated methanol, and the mixture was left for 2 h. The residue after evaporation of the solvent was crystallized from methanol and water. The crystals that deposited (16 mg) melted at 236-244°C, $[\alpha]_D^{20}$ +15 ± 2° (*c* 0.6; MeOH), $C_{29}H_{42}O_8$. They were identified as gomphoside [1]. The mother solution was evaporated, the residue was dissolved in 1.5 ml of distilled water, and the solution was acidified with HCl to pH 2-2.5 and treated with 3 ml of diethyl ether. After concentration at room temperature to 0.5 ml, the ethereal extract was chromatographed on FN-3 paper in the solvent system MeOH-25% NH_4OH (8:2). When the chromatogram was treated with a 0.02% solution of Bromomethyl Blue in 70% alcohol with the addition of ammonia or caustic soda, a yellow spot appeared at the level of acetic acid. One acetyl group was found in gomphacil (1) [6].

Acetylation of Gomphacil (1). After 80 mg of (1) had been dissolved in 2 ml of anhydrous pyridine and 2 ml of acetic anhydride, the reaction and the isolation of the acetyl derivative were carried out as described in [1]. The acetate obtained, (3), with mp 252-254°C, $[\alpha]_D^{20}$ +32 ± 2° (*c* 0.5; $CHCl_3$), molecular formula $C_{33}H_{48}O_{10}$ was identified as gomphoside diacetate (3) [1].

Oxidation of (1). With stirring, a solution of 60 mg of (1) in 1 ml of glacial acetic acid was treated with 2 ml of a 2% solution of CrO_3 in glacial acetic acid. After 4 h, 1 ml of MeOH was added to the reaction mixture, and after 2 h it was evaporated to a resinous residue, which was dissolved in chloroform-alcohol (4:1). Then the solution was washed successively with 2 ml of 2 N H_2SO_4, water, 2 ml of 2 N $NaHCO_3$ solution, and again with 2 ml of water. The residue after evaporation was crystallized from diethyl ether and hexane. The crystals obtained (49 mg) were identified as the initial substance (1).

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