CORONILLOBIOSIDOL – A CARDENOLIDE GLYCOSIDE FROM SEEDS OF Coronilla scorpioides

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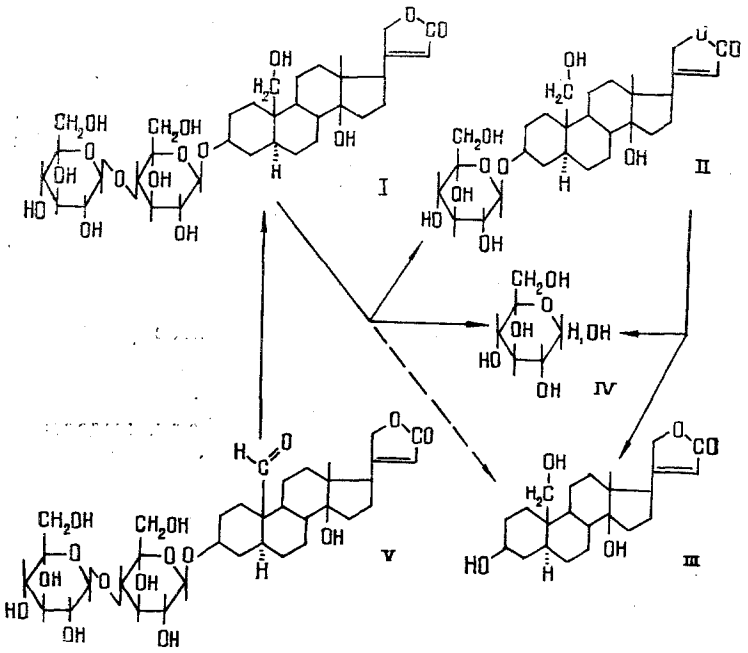
The seeds of Coronilla scorpioides have yielded a new cardenolidic bioside, which has been called coronillobiosidol and has the structure of 3β-O-[O-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl]-14β, 19-dihydroxy-5α-card-20(22)-enolide.

Continuing a study of the cardenolides of Coronilla scorpioides Koch. (scorpion coronilla) [1], in addition to coronillobioside [2] and scorpioside [3], we have isolated a new glycoside which we have called coronillobiosidol (I). In the UV spectrum of substance (I) there is a single maximum in the 219 nm region (log ε 4.12) which is characteristic for the butenolide ring of a cardenolide. Hydrolysis of glycoside (I) with the enzymes of the grape snail [1] led to the formation of D-glucose (IV) and of an aglycon (III), which was identified as 3β, 14β, 19-trihydroxy-5α-card-20(22)-enolide (coroglaucigenin). From its molecular mass, the glycoside was a bioside. To confirm this, we carried out stepwise hydrolysis with rhamnodiastase. In the cleavage products the main component detected was glucocoroglaucigenin (II) [1] with very small amounts of coroglaucigenin (III) and D-glucose (IV).



Scheme of the transformation of coroglaucigenin

The ease of splitting out of the terminal D-glucose residue indicated the possibility of its attachment by a 1 → 4-glucosidic bond in the disaccharide moiety of substance (I), since a 1 → 2-glycosidic bond is not hydrolyzed by enzymes because of steric hindrance. If a 1 → 6-bond were present, the splitting out of a biose could have been expected [5]. Exhaustive methylation of the initial substance followed by hydrolysis [3] gave 2, 3, 6-trimethoxy- and 2, 3, 4, 6-tetramethoxy-D-glucoses.

The configurations of the glycosidic bonds were determined by Klyne's method [3, 6]. It was established that both glucose residues in the glycoside had β-bonds. To confirm the results obtained, coronillobioside (V) was subjected to reduction with sodium tetrahydroborate,

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as a result of which a glycoside identical with the bioside (I) under investigation was obtained. Thus, the structure of coronillobiosidol can be represented as 3β-O-[O-β-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl]-14β,19-dehydroxy-5α-card-20(22)-enolide.

EXPERIMENT

General remarks are similar to those in [1].

For the isolation of the cardenolides, see [1]. The evaporated extract from 0.75 kg of defatted seeds was treated with mixtures of chloroform and ethanol in ratios of 3:1 and 2:1. After evaporation, 6.0 g and 48.5 g of residues, respectively, were obtained. By partition chromatography on a 4 × 80 cm of silica gel (with the mobile phase butan-1-ol–toluene (1:3-3:1, and the stationary phase water), the sum of the cardenolides of the chloroform-ethanol fraction was separated.

When cardenolides appeared in the eluates, the collection of 50-ml fractions was begun. Their compositions were monitored by paper chromatography in the toluene–butanol (3:1 and 1:3) system. Fractions having the same cardenolide composition were combined, evaporated, and crystallized. First, 135 mg of scorpioside was eluted, with 272-276°C (from ethanol), $[\alpha]_D^{20}$ −8.0° (c 0.5; methanol), and then 123 mg of coronillobioside mp 232-239°C (from propanol), $[\alpha]_D^{20}$ −9° (c 0.5; methanol); they were identified by comparison with glycosides isolated from the seeds of the plant under investigation previously [2, 3]. On further elution, 153 mg of glycoside (I) was obtained.

Substance (I) (coronillobiosidol), $C_{35}H_{54}O_{15}$, formed colorless crystal clusters with mp 195-201°C (from propanol and diethyl ether); $[\alpha]_D^{20}$ −4.0° (c 0.5; methanol).

Enzymatic hydrolysis. A. A solution of 18 mg of substance (I) in 3.0 ml of water was treated with 15 mg of grape snail enzyme. The experiment was then continued as described in [1]. As a result, the aglycon coroglaucigenin was obtained, with mp 247-250°C (from ethanol); with respect to melting point, R_f values on paper chromatography in the solvent system benzene–chloroform (9:1)–formamide, and in a thin layer on Silufol plates in the chloroform–methanol (9:1) system was identical with an authentic sample of coroglaucigenin (I).

Chromatography in the butan-1-ol–acetic acid–water (4:1:2) and phenol–water (95:5) systems revealed D-glucose.

B. The stepwise hydrolysis of the glycoside (I) under investigation was carried out with the enzyme rhamnodiastase obtained from buckthorn seeds [5].

The glycoside (32 mg) was dissolved in 4 ml of water, 30 mg of rhamnodiastase was added and the mixture was left at 35-37°C. The hydrolysis of the substance was monitored by paper chromatography every 30 min. After 62 h, a very small amount of coroglaucigenin (III), traces of the initial substance (I), and the glycoside (II) were detected in the fermented mixture.

After the precipitation of the enzyme, it was filtered off and the solution was evaporated to a dry residue, which was dissolved in 5 ml of water; this solution was treated with chloroform (3 × 3 ml) to eliminate coroglaucigenin, and then glycoside (II) was extracted with chloroform–ethanol (4:1).

When the residue after the evaporation of the chloroform-ethanol mixture was crystallized from methanol, 9 mg of compound (II) was obtained, with mp 177-183°C, which was identified as glucocoroglaucigenin (II) by its physicochemical properties and its R_f value in parallel chromatography using the toluene–butan-1-ol (3:1)–water (35%) system [1].

Methylation of Coronillobiosidol (I). Glucoside (II) (100 mg) was methylated as described in [3]. In a hydrolysate of the methylated glycoside, 2, 3, 6-trimethoxy- and 2, 3, 4, 6-tetramethosy-D-glucose were detected by paper chromatography [7].

Reduction of Coronillobioside (V) to Coronillobiosidol (I). A solution of 80 mg of coronillobioside in 10 ml of ethanol, was cooled to (5-7)°C, and 50 mg of sodium tetrahydroborate was added in three portions. The completeness of reaction was monitored by paper chromatography in the toluene–butanol-(1:3)–water (35%) system. After 2 h, the reaction mixture was evaporated, the residue was dissolved in 10 ml of distilled water and the reduced substance was extracted with chloroform–ethanol (2:1). The residue after the evaporation of the solvents was crystallized from isopropanol. This gave 65 mg of a substance

$C_{35}H_{54}O_{15}$, mp 196-200°C $[\alpha]_D^{20}$ − 3.9° (c 0.5; methanol). From its R_f values in various solvent systems and a mixed melting point, the reduced substance was identical with the glycoside (I) that had been isolated.

SUMMARY

A new cardenolidic bioside has been isolated from the seeds of Coronilla scorpioides; it has been called coronillobiosidol and is 3β-O-[O-β-D-glucopyranosyl)-(1 → 4)-β-D-glucopyranosyl]-14β, 19-dihydroxy-5α-card-20(22)-enolide.

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IMMOBILIZATION OF Oospora lactis LIPASE

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A number of new sorbents for the immobilization of lipases have been synthesized from Silokhrom and Porokhrom, and also from microcrystalline cellulose. The conditions for the immobilization of lipases have been selected and some of their properties have been studied.

At the present time, immobilized lipases are being widely used in scientific investigations [1, 2] and various biotechnological processes [3, 4]. At the same time, the search for new approaches to immobilization and the selection of a suitable support continues to remain an urgent one.

The aim of our investigations was to obtain an immobilized lipase with the aid of traditional approaches to the immobilization of enzymes and to evaluate its stability and some catalytic properties.

As the support, we used Silokhrom and Porokhrom, and the biospecific sorbent Liposorb-4 which we had synthesized previously [5].

To obtain a sorbent with a greater sorption capacity, partial optimization of the conditions of immobilization was carried out by the mathematical planning of experiment work. The factors varied were the concentrations of 3-aminopropyl-3-ethoxysilane and of 2,4-toluylene diisocyanate, and the temperature and time of the reaction; as a parameter for evaluating the sorbent we used the amount of adsorbed protein and the lipase activity determined before and after contact with the sorbent. Under the conditions of a batch reactor, the following optimum conditions for the modification of the sorbents were established: for the first sorbent – temperature 90 to 95°C; reaction time 5 h; ratio of 3-aminopropyl-3-ethoxysilane to Silokhrom 1:4; and for the second sorbent, respectively, 78-80°C, 6 h, and ratio of 2,4-toluylene diisocyanate to Porokhrom 1:5.

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