CARDENOLIDES OF SECURIGERA SECURIDACA. II.

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In the preceding communication [1], it was reported that a freshly-obtained alcoholic aqueous extract of the seeds of Securigera securidaca (L.) Dergen u. Dërfler (hatchetvetch) contains a single steroid compound (previously called substance III). A chemical study of this substance has shown that it is a new cardiac bioside, and for it we propose the name securidaside.

In developing a method for obtaining securidaside, two other cardenolide compounds were found. On further investigation, one of them proved to be the aglycone securigenin, and the other its monoglycoside, which we have called securiside.

The present paper describes methods of isolating individual cardenolides of Securigera and gives data obtained in a preliminary chemical investigation. By using the principle of the self-fermentation of the bioside by the enzymes of the seeds [2, 3], we have obtained, by full and stepwise enzymatic hydrolysis of the basic diglycoside of the seeds, considerable amounts of securiside and securigenin. Figure 1 shows schematically the chromatographic behavior of the products of enzymatic hydrolysis of the cardenolides of securigera.

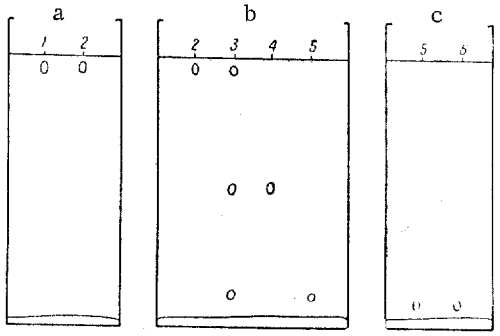


Fig. 1. Schematic drawings of the chromatograms of the enzymatic hydrolysis of the cardenolides of hatchetvetch. Benzene-n-butyl alcohol (1: 1)-water system: a) unfermented seed; b) after 35 hr fermentation: c) after 96 hr fermentation; 1) extracts from the unfermented seeds; 2) securidaside; 3) extract from the partially fermented seeds; 4) securiside; 5) extract obtained from the seeds after complete fermentation; 6) securigenin.

Securigenin consists of a cardiac aglycone $C_{23}H_{32}O_6$, mp 234-235°, $[\alpha]_D^{20}$ + 82° ± 2° (c 1.0; methanol). It gives a monoacetyl derivative which shows the presence in the aglycone of one secondary hydroxyl group capable of being acetylated.

The UV spectrum of securigenin exhibits two absorption maxima: at 217 mμ (log ε 4.25) (lactone ring) and 308 mμ (log ε 2.19) (aldehyde group).

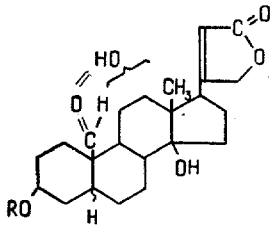
The presence of a five-membered lactone ring and an aldehyde group is also confirmed by the IR spectrum with absorption bands in the 1800 and 1745 cm^{-1} regions (carbonyl group of a lactone ring) and in the 1718 cm^{-1} region (aldehyde group).

Securiside, $C_{28}H_{42}O_{10}$, mp 192-194°, $[\alpha]_D^{20}$ + 27° ± 2° (c 0.51; methanol), is a monoglycoside of securigenin. The hydrolysis of securiside gives securigenin and a monosaccharide with mp 162-165°, the latter behaving in paper chromatography similarly to D-xylose. The presence of D-xylose is confirmed by positive furfural and phloroglucinol tests, which are characteristic for pentoses, and by the formation of an osazone giving no depression of the melting point with D-xylose osazone.

Up to the present time, the only cardiac glycosides that have been found to contain D-xylose are erychroside and gypsobioside [5].

Securidaside, $C_{34}H_{52}O_{15}$, mp 177-179°, $[\alpha]_D^{20}$ + 4° ± 2° (c 1.01; methanol), is a diglycoside. The stepwise enzymatic hydrolysis of securidaside first gave D-glucose and the monoside securiside and then securigenin and D-xylose.

On the basis of the results obtained, the following structures may be proposed for the compounds isolated.



I. (R = H) securigenin

II. (R = D-xylose) securiside

III. (R = D-xylose-D-glucose) securidaside.

Experimental

Isolation of securidaside. Two kilograms of the comminuted seeds were defatted with petroleum ether and were then extracted with 70% alcohol until a negative Legal reaction was obtained. The qualitative composition of the extract was followed by paper chromatography (cf., Fig. 1, a). After concentration of the alcoholic solution in vacuum, 230 g of extract was obtained. The extract was dissolved in three liters of water and the solution was extracted with a mixture of chloroform and alcohol (2: 1). The chloroformic-alcoholic extract was concentrated under vacuum, the residue (85 g) was dissolved in three liters of water, and with constant stirring 1 kg of alumina was added in small portions. After a short period of stirring, the alumina was filtered off and washed with water until a negative reaction for cardenolides was obtained.

The purified aqueous solution was concentrated under vacuum to two liters, and the cardenolides were extracted with a mixture of chloroform and alcohol (2: 1). After elimination of the solvent, the residue (33.5 g) was chromatographed on a column of alumina (1.2 kg). The column was washed with pure and with dilute alcohol. The process of elution of the cardenolides is shown in Fig. 2.

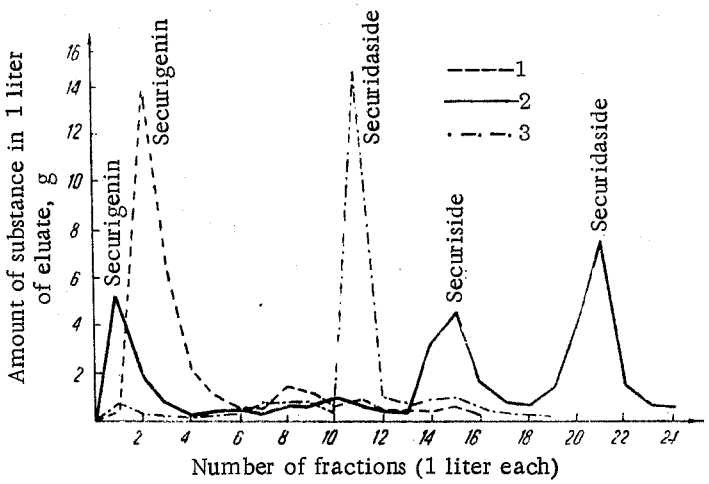


Fig. 2. Scheme of the elution of the cardenolides of Securigera from an alumina column: 1) elution of securigenin; 2) elution of securigenin, securiside, and securidaside; 3) elution of securidaside.

Fractions 10-16, containing the securidaside, were combined, the solvent was evaporated off, and the residue was crystallized from isopropyl alcohol. This gave 13.7 g of crystalline securidaside.

Isolation of securidaside, securiside, and securigenin. Two kilograms of comminuted and defatted seeds were covered with 8 liters of water and left for fermentation in the thermostat at 40-42°. The hydrolysis of the glycosides was followed by paper chromatography (cf., Fig. 1, b). After 36 hr, the fermentation mixture was extracted with ethyl alcohol. The completeness of the extraction of the cardenolides was checked by Legal's reaction.

Evaporation of the alcoholic solution in vacuum gave 306 g of extract. This was dissolved in 4 liters of water and the glycosides were extracted with a mixture of chloroform and alcohol (2: 1). The chloroformic extracts were evaporated in vacuum, giving 93 g of residue.

The residue was dissolved in 500 ml of 50% alcohol, after which 250 g of activated alumina was added with constant stirring, and this was then filtered off and was washed with 50% alcohol until the reaction for cardenolides was negative.

The aqueous alcoholic solution was concentrated in vacuum to 4 liters and the cardenolides were extracted with a mixture of chloroform and alcohol (2: 1). Evaporation of the chloroformic-alcoholic extract in vacuum gave 66 g of residue, which was dissolved in a mixture of chloroform and alcohol (9: 1) and transferred to a column of alumina (2.3 kg). The column was washed successively with a mixture of chloroform and alcohol, pure alcohol, and dilute alcohol. The process of chromatographing the cardenolides is illustrated in Fig. 2.

The evaporation and crystallization of fractions 1-5 (containing the securigenin) gave 4.5 g of crystals (from methanol), fractions 14-18 gave 4.1 g of securiside (from ethyl alcohol), and fractions 22-23 gave 6.2 g of securidaside (from isopropyl alcohol).

Isolation of securigenin. Two kilograms of the comminuted seeds defatted with petroleum ether were covered with 10 ml of water and left to ferment in the thermostat at 40-42°. The fermentation process was checked by paper chromatography (cf., Fig. 1, c). After four days enzymatic hydrolysis, the mixture was extracted with ethyl alcohol until the

Legal reaction was negative. After evaporation in vacuum, the alcoholic extract gave 285 g of residue, which was dissolved in chloroform and subjected to chromatography on a column of alumina (1.9 kg). The process of chromatographing the securigenin is shown in Fig. 2.

After evaporation and crystallization from methanol of fractions 1-10 containing the securigenin, 10.6 g of securigenin was obtained.

Summary

The seeds of Securigera securidaca have been found to contain a cardiac glycoside securidaside, which is securigenin xyloglucoside. By stepwise enzymatic hydrolysis, securidaside can be decomposed into the monoxyloside securiside or into the aglycone and the sugar component.

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