SINAPOYL ESTER OF GLUCOERYSIMOSIDE IN *Erysimum marschallianum*

N. P. Maksyutina

UDC 547.918/926

Cardiac glycosides acylated with sinapic acid have been detected relatively recently [1-4].

In the present papaer we give information on a new steroid glycoside acylated with sinapic acid (I) obtained from the seeds of *Erysimum marschallianum*, which we have called sinapoylglucoerysimoside.

When sinapoylglucoerysimoside was treated with a 1-2 N solution of alkali, glucoerysimoside (II) and a salt of sinapic acid (III) were formed. Acid hydrolysis of sinapoylglucoerysimoside gave the aglycone strophanthidin (IV), glucose, digitoxose, and sinapic acid.

Enzyme preparations of β-glucosidases (snail pancreatic juice, the fungus *Aspergillus oryzae*) cleave sinapoylglucoerysimoside with the formation of products changing with the time. In the first few minutes of hydrolysis the main products are erysimoside (VI) and sinapoylglucose (VII). On subsequent fermentation, erysimin (V), sinapic acid, and glucose appear in the hydrolysis products. The full hydrolysis of sinapoylglucoerysimoside was complete after 100 min, and erysimoside, erysimin, and sinapoylglucose were isolated from the hydrolyzate.

The formation of erysimoside and sinapoylglucose on enzymatic hydrolysis, and also the formation of glucoerysimoside and of potassium sinapate on alkaline saponification of the glycoside (I) shows that the sinapic acid is present in the terminal glucose of the glycoside. The position of acylation of the terminal glucose of the glycoside (I) with sinapic acid has not been established but is shown provisionally at C_6 (see scheme on next page).

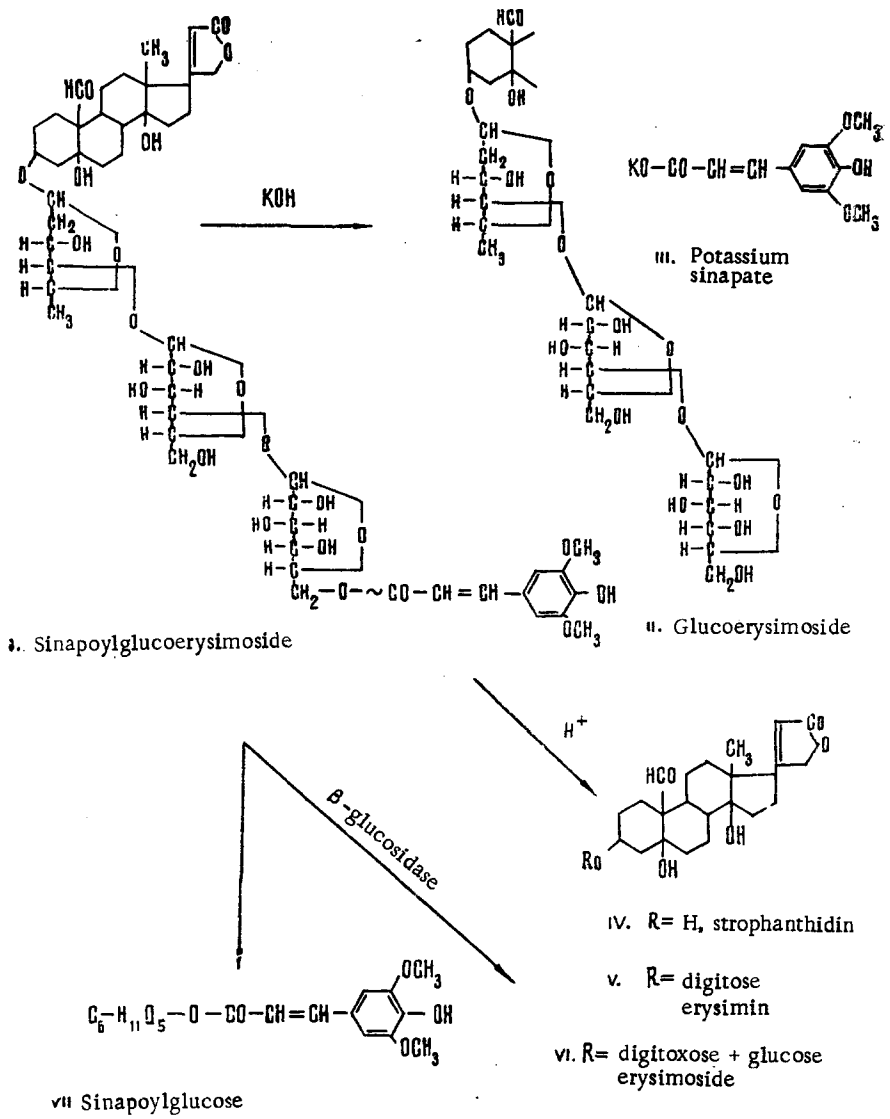
EXPERIMENTAL

To purify the aqueous extracts we used polyamide powder prepared by the acetic acid method. For paper chromatography we used Filtrak FN, Ogsznak, chromatographic board, and the following solvent systems: 1) chloroform–isopropanol–formamide (19:1:1); 2) toluene–acetic acid–water (4:1:5); 3) butan-1-ol–pyridine–water (10:3:3); 4) butan-1-ol–acetic acid–water (4:1:2); 5) methyl ethyl ketone–butan-1-ol (1:1)–buffer solution (50%); 6) benzene–ethyl acetate–acetic acid–formamide (23.5 : 74.5 : 2:1).

The cardenolides were revealed by Raymond's reagent and the sinapic acid derivatives by fluorescence in UV light. The UV spectra were taken on an SF-4 A spectrophotometer, and the IR spectra on a UR-20 instrument (KBr).

Isolation of Sinapoylglucoerysimoside. The seeds of *Erysimum marshallianum* (2 kg) were extracted with ethanol, the extract was evaporated, and the residue was dissolved in 400 ml of water and purified on a column containing 0.4 kg of Kapron. The first eluates, containing no cardiac glycosides, were discarded, the fraction with a positive reaction for steroid glycosides and containing substances with a bright blue fluorescence in UV light were collected separately and were separated by preparative chromatography on 150 sheets of chromatographic board in system 6 for 18 h. A zone with a bright blue fluorescence and a positive Raymond's reaction was eluted from the chromatographic strips with water and was repurified in system 6 for two days (with flow), and then in system 4 for 18 h. The substance was eluted from the chromatographic strip with water and freeze-dried. This gave 920 mg of an amorphous substance – sinapoylglucoerysimoside, $C_{52}H_{72}O_{23}$, chromatographically homogeneous, in the form

Kiev Institute for the Further Training of Doctors. Translated from Khimiya Prirodnykh Soedinenii No. 5, pp. 603-606, September-October, 1975. Original article submitted June 28, 1974.



of a yellow amorphous powder soluble in water, formamide, ethanol, and dimethylformamide, and insoluble in ether.

The chromatographic mobility of sinapoylglucoerysimoside in system 1 in relation to erysimoside after chromatography for 20 hours was 6.6; $\lambda_{max}^{C_2H_5OH}$ (nm) 218, 236 (shoulder), 303, and 334; λ_{max}^{KBr} 3280 cm^{-1} (OH), 1738, 1640, 1627, 1560, 1520, 1465, 1410.

Alkaline Hydrolysis of Sinapoylglucoerysimoside. A solution of 240 mg of the glycoside (I) in 2 ml of 50% formamide was treated with 2 ml of a 2 N solution of caustic potash, and the mixture was heated at 80°C for 2 h. Then it was neutralized with 1 N hydrochloric acid to pH 6.8-7.0 and was shaken several times successively with ether, chloroform, and chloroform–ethanol (2:1).

The residue after the evaporation of the ether (≈50 mg) was recrystallized from ethanol. Yellow crystals deposited, mp 192°C, giving no depression of the melting point with an authentic sample of sinapic acid.

In UV light, the acid obtained showed a bright blue fluorescence; $\lambda_{max}^{C_2H_5OH}$ 218, 240, 320 nm.

The ethanolic-chloroformic extracts were evaporated to dryness and the residue was dissolved in 150 ml of chloroform with heating and was chromatographed on 10 g of alumina. It was eluted first with chloroform and then with chloroform–ethanol. After evaporation the eluates were analyzed for glycosides by paper chromatography in system 1.

A fraction eluted by chloroform–ethanol (2:1) yielded 84 mg of glucoside (II), $C_{41}H_{62}O_{19}$,

which was identified as glucoerysimoside [5] by its chemical properties, IR absorption spectrum, and the products of enzymatic hydrolysis.

Acid Hydrolysis of Sinapoylglucoerysimoside. Sinapoylglucoerysimoside (100 mg) was hydrolyzed with a 0.1% solution of hydrochloric acid (3 ml) at 45°C for 30 min. The hydrolyzate was treated several times with ether and then with chloroform. The ethereal extracts after evaporation was chromatographed preparatively on paper in system 2.

The bands gave blue fluorescence, were separated and treated with ethanol. The ethanolic extracts were evaporated, giving 21 mg of a substance identified as sinapic acid by its chemical reactions in the absence of a depression of the melting point with an authentic sample.

The chloroform extract was purified on alimina. After purification, 16 mg of a substance $C_{23}H_{32}O_6$, $[\alpha]_D^{20}$ + 44.8° (methanol), identified by its IR spectrum as strophanthidin (IV), was obtained.

The aqueous hydrolyzate, after treatment with the ether and chloroform and neutralization with AB-16 anion-exchange resin was shown by paper chromatography to contain digitoxose and glucose.

Enzymatic Hydrolysis of Sinapoylglucoerysimoside. A. A solution of 25 mg of sinapoylglucoerysimoside in 1.5 ml of water was treated with 20 mg of an enzyme preparation of snail pancreatic juice, and the solution was placed in a thermostat (40°C). The course of hydrolysis was followed by chromatography on paper every 20 min in system 1. In the first sample, apart from the initial glucoside traces of erysimoside and erysimin were detected. In the subsequent samples, the accumulation of erysimoside and of erysimosin continued with a simultaneous decrease in the amount of initial glycoside. The initial glycoside had disappeared completely after 1 h 40 min. The largest amount of erysimoside was also found after hydrolysis for 1 h 40 min.

In the subsequent samples, the amount of erysimoside fell, and that of erysimin increased. The erysimoside was completely hydrolyzed after 4 h.

B. The products of the hydrolysis of glycoside (I) were isolated after the chromatographic separation of the hydrolyzates on paper in system 1.

To isolate erysimoside and sinapoylglucose, the enzymatic hydrolysis of 175 mg of glycosides (I) was performed for 2 h, and to isolate the erysimin and sinapic acids, a similar amount of glycoside was hydrolyzed for 5 h. This gave 44 mg of erysimoside, 36 mg of sinapic acid, 74 mg of erysimin, and 38 mg of sinapoylglucose, which were identified by comparison with authentic samples through their absorption spectra and the products of partial hydrolysis.

SUMMARY

A new cardenolide glycoside — sinapoylglucoerysimoside — having the structure of strophanthidin 3-O-[O-sinapoyl-β-D-glucopyranosyl-(1→4) O-β-digitoxopyranoside —has been found in the seeds of *Erysimum marshallianum*.

LITERATURE CITED

1. N. P. Maksyutina, Khim. Prirodn. Soedin., 293 (1965).
2. N. P. Maksyutina, Author's Abstract of Doctoral Dissertation, Kiev (1970).
3. Yu. G. Barisyuk, N. P. Maksyutina, T. V. Zinchenko, M. I. Borisov, and K. E. Koreshchuk, Farmats. Zh., No. 5, 6 (1967).
4. A. M. Navruzova, V. A. Maslennikova, and N. K. Abubakirov, Khim. Prirodn. Soedin., 750 (1973).
5. I. F. Makarevich, Khim. Prirodn. Soedin., 566 (1970).