Erysimum GLYCOSIDES

IX. SINAPOYL ESTER OF ERYSIMOSIDE IN Erysimum diffusum

A. M. Navruzova, V. A. Maslennikova, and N. K. Abubakirov

UDC 547.918:547.926+615.711.5

Cardiac glycosides acylated by acetic acid in the sugar moiety are not infrequently found in nature. They are found particularly often among the glycosides of Digitalis (lanatosides A, B, and C; α-, β-, and γ-acetyldigitoxins, etc.). The hydroxy group present in the aglycones at C_{16} is sometimes esterified by formic, acetic, or (in one case) isovaleric acids. No aromatic carboxylic acids have been found in compounds with cardenolides.

Maksyutina [1] first drew attention to the fact that in plants of the genera Cheiranthus, Erysimum, and Syrenia there are cardenolide glycosides acylated with sinapic (4-hydroxy-3,5-dimethoxycinnamic) acid. Externally, these glycosides are distinguished by the fact that on paper or thin-layer chromatography their spots fluoresce bright blue in UV light and on treatment with Raymond's reagent they are colored not deep blue, like ordinary cardenolides, but green. This paper [1], published in the form of a brief communication, has unfortunately not attracted its due attention, possibly because of the absence of experimental development.

Erysimum diffusum Ehrh. (synonym E. canescens Roth.) has previously been shown to contain strophanthidin, erysimin [2-5] (synonym: helveticoside [6]), desglucocheirotoxin [4, 5], canescein [4, 5], erysimoside [3-5], cheirotoxin [4], glucocanescein [4], erycanoside [7], eryscenoside [7], and a glycoside of undetermined structure – erydiffuside [5]. The main component of the mixed glycosides of the plant is erysimoside [3], the amount of which in the seeds exceeds 2%.

In the present paper we give the results of a study of a new glycoside from the seeds of this plant which we have called, after determining its structure, sinapoylerysimoside [1]. Besides this compound, the mixture contains another two or three compounds among the most polar glycosides which fluoresce blue in UV light.

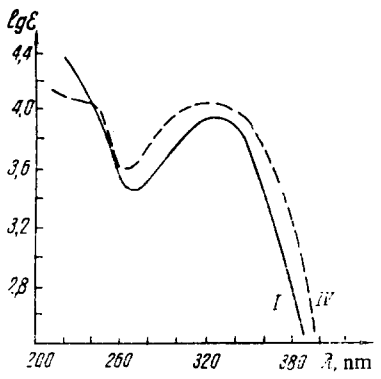


Fig. 1. UV spectra of sinapoylerysimoside (I) and of sinapic acid (IV).

From the nature of its absorption in UV light, compound (I) differs sharply from all known cardenolide glycosides (Fig. 1). The intensity of its absorption at 218 nm is very high (log ε 4.37). Another intense maximum (log ε 3.97) is found at 330 nm.

In the IR spectrum, on the absorption of an α,β-unsaturated γ-lactone (the butenolide ring of a cardenolide) is superposed the absorption of an α,β-unsaturated ester of sinapic acid (IV). Consequently, the regions of absorption of the carbonyl group (1710-1740 cm^{-1}) and of a conjugated double bond (1605-1635 cm^{-1}) are considerably broadened.

We were unable to record the mass spectrum of sinapoylerysimoside in an ionization field. Under electron impact, however, compound (I) gives a series of fragmentary ions that are characteristic for strophanthidin [8] – 386 ($M - H_2O$), 368 ($M - 2H_2O$), 358 ($M - H_2O - CO$), 350 ($M - 3H_2O$), 340 ($M - 2H_2O - CO$)

Institute of the Chemistry of Plant Substances, Academy of Sciences of the Uzbek SSR. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 750-755, November-December, 1973. Original article submitted September 5, 1972.

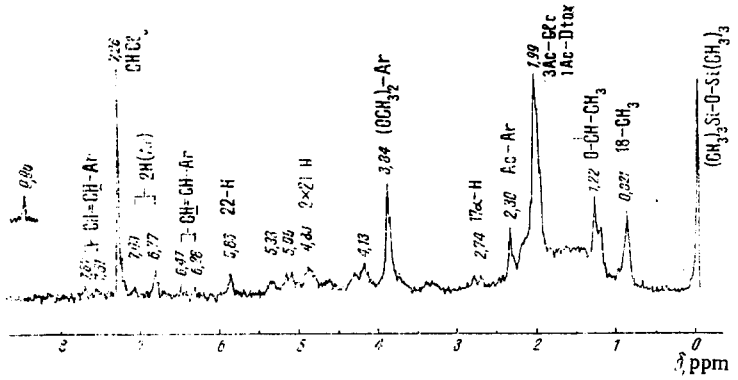


Fig. 2. NMR spectrum of sinapoylerysimoside pentaacetate (II) (30 mg in 0.4 ml).

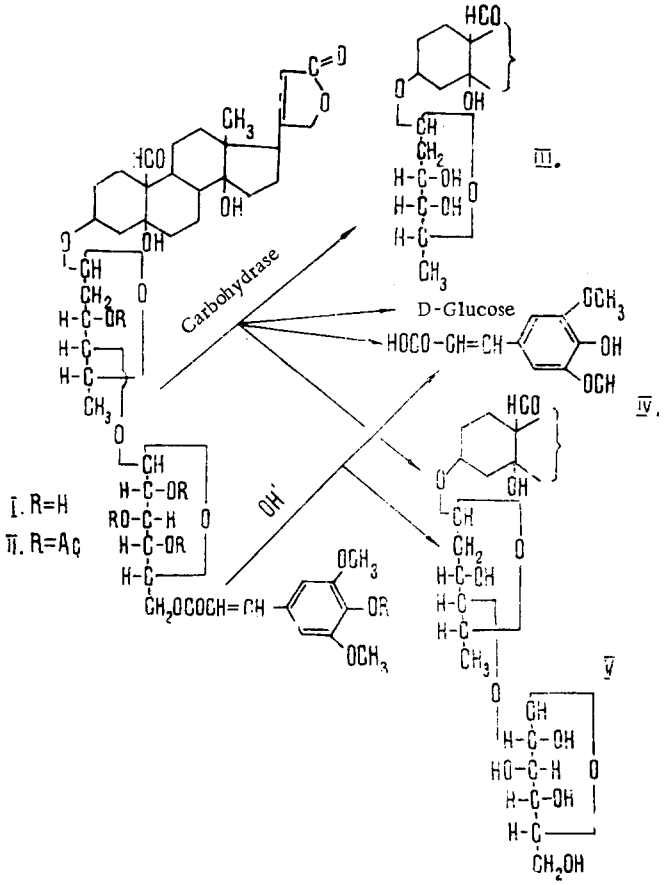
and 322 (M − $3H_2O$ − CO), 307 (M − 97) − and for sinapic acid (IV) − 224 (M^+), 223 (M − 1), 180 (M − 1 − CH_3 − CO). It is characteristic that in the mass spectrum of sinapoylerysimoside pentaacetate (II) the ion with m/e 207, corresponding to the radical $[HO(OCH_3)_2C_6H_2CH=CHCO]^+$, and the ion with m/e 206, corresponding to the preceding ion, are the maximum ions.

Particularly striking is the NMR spectrum of sinapoylerysimoside pentaacetate (II) (Fig. 2). It reflects all the elements of the structure of sinapic acid. The two free protons of the benzene ring resonate at 6.77 and 7.03 ppm. The ethylenic protons give a quartet of the AB type with signals at 6.28, 6.47, 7.51, and 7.67 ppm. The magnitude of the spin−spin coupling constant (J_{AB} = 16 Hz) probably shows the trans configuration of the acid. In the spectrum of erysimoside acetate (spectrum not given), in the high-frequency region there are no other signals whatever beyond the proton at C_{22} of the aglycone and only in the very weak field in the form of a sharp singlet does the proton of the aldehyde group appear. The same proton in the spectrum of compound (II) resonates at 9.96 ppm. The protons of the two methoxy groups appear clearly in the form of a six-proton singlet at 3.84 ppm. As was to be expected, the signal of the aromatic acetyl group appears in a weaker field (2.30 ppm) than the signals of the acetyl groups of the sugar residues. The fact that the phenolic hydroxyl proved to be acetylated shows that this group takes no part in the formation of the bond of the sinapic acid with the erysimoside (V). Consequently, we have isolated a cardenolide glycoside including sinapic acid.

The results of the experimental investigations confirmed those obtained by instrumental methods. The hydrolysis of the glycoside (I) in a methanolic solution of ammonia gave erysimoside (V) and sinapic acid (IV). Enzymatic hydrolysis using snail pancreatic juice took place fairly rapidly and after only 15 min erysimoside (V) and an intense spot of erysimin (III) were found on a chromatogram in addition to that of the initial compound. After about an hour, hydrolysis was absolutely complete, the spot of the sinapoylerysimoside (I) had disappeared, and from the hydrolyzate, after the usual working up, it was possible to isolate in the individual state erysimin (III), erysimoside (V), D-glucose, and sinapic acid (IV). In contrast to Maksyutina [1], who experimented with the sinapoyl ester of glucoerysimoside, we found no compound of D-glucose with sinapic acid in the hydrolyzate. Under the conditions that we used, the carbohydrase of the snail juice simultaneously acted on the glucosidic bond (between the D-glucose and D-digitoxose) and ester bond.

Thus, the sinapoylerysimoside is represented by structural formula (I). The site of attachment of the sinapic acid (IV) to the hydroxy group at C_6 of the D-glucose we showed roughly by a consideration of the fact that esters of a primary hydroxy group saponify very readily. From biogenetic considerations it appears unlikely that the bond was effected through C_4, since the plant contains glucosyl-(1″→4″)-erysimoside simultaneously with erysimoside and its sinapoyl ester [9].

It is possible that the original group of natural compounds of the type of sinapoylerysimoside is an exceptional advantage of plants of the family Cruciferae, where this group may be connected genetically with a complex glucoside of mustard oils − sinalbin. It is worth noting that sinapoyl esters of anthocyanidin glycosides (rubrobrassicin and matthiolanin) have been found only in plants of the family Cruciferae − in the leaves of red cabbage and in the flowers of Matthiola incana R. Br. [10].



EXPERIMENTAL

For paper chromatography (PC) we used paper of the "medium" type, for thin-layer chromatography (TLC) silica gel of type KSK with 5% of gypsum and the following solvent systems: 1) benzene–chloroform–methanol (5 : 5 : 2); 2) chloroform saturated with formamide; 3) toluene–butan-1-ol–water (1 : 1 : 1); 4) chloroform–isoamyl alcohol (4-methylbutan-1-ol)–water (1 : 1 : 1); 5) acetic acid–hydrochloric acid–water (30 : 3 : 10); 6) butan-1-ol–acetic acid–water (4 : 1 : 5); 7) water-saturated butan-1-ol; and 8) hexane–acetone (1 : 1). The cardenolides were revealed with Raymond's reagent and the sinapic acid derivatives by their fluorescence in UV light.

The UV spectra were taken on an SF-4 spectrophotometer, the IR spectra on a UR-20 instrument (KBr), the mass spectra on an MKh-1303 instrument at an ionizing voltage of 40 eV, and the NMR spectra on a JNM-4H-100/100 MHz instrument (with hexamethyldisiloxane, HMDS, as internal standard).

Isolation of Sinapoylerysimoside. The benzene-defatted seeds (1 kg) of E. diffusum were extracted repeatedly with ethanol. The ethanolic extract was concentrated to a volume of 0.3 liter, and 1 liter of acetone was added to precipitate the sugars. The settled viscous mass was separated off and was washed twice with acetone, and the combined ethanolic–acetone solution was evaporated in vacuum to 130 ml. The concentrated extract, resembling a syrup in external appearance, was mixed with 300 ml of ether. After the ethereal extraction of the residue of fats and hydrophobic substances, the residual viscous mass was dissolved in 100 ml of methanol and the solution was diluted with 1 liter of water. The aqueous methanolic solution was extracted with chloroform (3-4 × 300 ml) and then with a mixture of chloroform and isopropanol. The chloroform–isopropanol fraction, which contained mainly erysimoside and other polar glycosides, was not studied in detail. The chloroform fraction (weighing, after evaporation, 10 g) contained mainly monoglycosides and, among them, a substance with a blue fluorescence in UV light. In systems 1 (TLC) and 3 and 4 (PC) it was more polar than erysimin, slightly more polar than desglucocheirotoxin, and less polar than erysimoside.

To isolate the sinapoylerysimoside we used preparative chromatography on a glass plate (35 × 35 cm) with a fixed layer of silica gel. On the starting line, 50- to 80-mg portions of the total glycosides

from the chloroform extract were deposited and chromatography was performed in system 1. The zone with the blue fluorescence was scraped off and eluted with methanol. After evaporation, the eluate was rechromatographed on plates under the same conditions until a chromatographically individual compound was obtained. In this way, from 10 g of the combined substances we isolated 204 mg of sinapoylerysimoside.

Almost at the starting line, where the glucoerysimoside usually appears, another band fluorescing in UV light was found. It contained several glycosides which have not yet been studied.

Sinapoylerysimoside (I), $C_{46}H_{62}O_{18} \cdot H_2O$, a chromatographically homogeneous substance, was purified by reprecipitation with ether from methanolic solution. The product was an amorphous yellow powder soluble in chloroform and methanol, more sparingly in ethanol, and only slightly in water, and insoluble in ether and benzene, $[\alpha]_D^{20}$ +33.6 ± 3° (c 2.2; methanol), $\lambda_{max}^{C_2H_5OH}$ (see Fig. 1) 218, 330 nm (log ε 4.37, 3.97); ν_{max}^{KBr} 3410 (OH), 1736, 1725, 1712 (C=O), 1632, 1625 (conjugated C=C), 1605, 1518, and 1462 cm^{-1} (benzene ring).

Mass spectrum, 110°C, strophanthidin fragments: 386 (M − 18), 368 (M − 36), 358 (M − 46), 350 (M − 54), 340 (M − 64), 322 (M − 82, maximum), 307 (M − 97), 212 (M − 192), 197 (M − 207), 160 (M − 244), 111 (M − 293), 91 (M − 313); fragments of sinapic acid: 224 (M^+), 223 (M − 1), 180 (M − 44), 165 (M − 59), 97 (M − 127).

Penta-O-acetylsinapoylerysimoside (II), $C_{56}H_{72}O_{23}$. Over 25 min, in small portions, 50 mg of sinapoylerysimoside was added to a mixture of 1.0 ml of acetic anhydride, 1.0 ml of pyridine, and 4 mg of $Mg(ClO_4)_2$ heated to 55°C. The reaction mixture was stirred at the same temperature for another 15 min. The solvents were distilled off in vacuum to dryness, the residue was dissolved in 0.2 ml of methanol, and the solution was poured into 12 ml of ice water. The precipitate that deposited was dissolved in 0.5 ml of ethanol and reprecipitated with ether. Yield 36 mg. The individuality and purity of the acetate were checked by TLC in system 8. Amorphous light-yellow powder, $[\alpha]_D^{20}$ + 40.1 ± 2° (c 1.1; methanol). NMR spectrum: see Fig. 2.

Mass spectrum, 130°C, strophanthidin fragments: 368 (M − 36), 358 (M − 46), 350 (M − 54), 340 (M − 64, maximum), 322 (M − 82), 111 (M − 293); fragments of the acetate of sinapic acid: 249 (M − 17, medium intensity); fragments of sinapic acid: 224 (M^+), 207 (M − 17, maximum), 206 (M − 18), 181 (M − 43), 180 (M − 44), 165 (M − 59), 97 (M − 127).

Alkaline Hydrolysis of Sinapoylerysimoside. The glycoside (I) (40 mg) was dissolved in 2 ml of methanol saturated with ammonia. After two days, 4 ml of water was added, the solution was neutralized with 0.1 N H_2SO_4 to pH 6, and it was extracted successively with ether, chloroform, and chloroform–ethanol (2 : 1).

The residue after the evaporation of the ether (10 mg) was recrystallized from ethanol. Crystals deposited in the form of yellow needles with mp 189°C. Literature data for sinapic acid: 192°C [11]. On PC in system 5, the acid (IV) had R_f 0.89 and was revealed by diazotized sulfanilic acid in the form of a pink spot [12]. In UV light, the substance showed a bright blue fluorescence $\lambda_{max}^{C_2H_5OH}$ (see Fig. 1) 218, 240, 320 nm (log ε 4.09; 4.07; 4.06); ν_{max}^{KBr} 3410 (OH), 2855 (CH_3 in Ar–OCH_3) 1720, 1630, 1615 (C=C–COOH), 1602, 1512, 1460 cm^{-1} (benzene ring).

The chloroform–ethanolic fraction was evaporated to dryness, and the residue (22 mg) was chromatographed on a column of silica gel. The organic phase was eluted with the solvents of system 3. This gave crystals of erysimoside (V) with mp 228-230°C, $[\alpha]_D^{20}$ + 24.4 ± 2° (c 2.15; methanol). In systems 1 and 4 (TLC) and 3 (PC) the substance migrated at the same level as an authentic sample of erysimoside.

Enzymatic Hydrolysis of Sinapoylerysimoside. The glycoside (I) (86 mg) was dissolved in 2 ml of ethanol, the solution was diluted with 20 ml of water, 0.5 ml of snail pancreatic juice was added, and the solution was placed in a thermostat with a constant temperature of 36°C. The course of hydrolysis was monitored every 15 min by TLC in system 1. Even the first sample showed the presence of erysimoside (V) and erysimin (III). As fermentation proceeded, the spot of the initial compound on the chromatograms became weaker, and after 1 h it had disappeared completely. After 2 h the reaction was stopped and the hydrolyzate was first extracted with ether and then with chloroform and with chloroform–ethanol (2 : 1). Evaporation of the ethereal extract and recrystallization of the residue (18 mg) from ethanol gave sinapic acid with mp 190°C. The chloroform extract (38 mg) was chromatographed on a column of silica gel.

Elution was performed with the organic phase of system 3. The substance isolated in this way had mp 170-172°C, $[\alpha]_D^{20}$ + 30.2 ± 2° (c 1.8; methanol) and in systems 1 and 2 (TLC) it behaved identically with erysimin (III).

The chloroform–ethanol fraction (21 mg) was separated by preparative chromatography on silica gel in system 1. This gave 11 mg of a crystalline substance with mp 228-230°C the behavior of which was identical with that of erysimoside in systems 1 (TLC) and 3 and 4 (PC).

The aqueous hydrolyzate was evaporated, and D-glucose was found in the residue in systems 6 and 7 (PC), the spot being revealed with aniline phthalate.

SUMMARY

A new cardenolide glycoside – sinapoylerysimoside, having the structure of 3-O-[4'-O-(6"-sinapoyl-β-D-glucopyranosyl)-β-D-digitoxopyranosyl]strophanthidin – has been isolated from the seeds of Erysimum diffusum Ehrh.

LITERATURE CITED

1. N. P. Maksyutina, Khim. Prirodn. Soedin., 293 (1965).
2. V. V. Feofilaktov and P. M. Loshkarev, Dokl. Akad. Nauk SSSR, 94, 709 (1954).
3. V. A. Maslennikova, F. S. Khristulas, and N. K. Abubakirov, Dokl. Akad. Nauk SSSR, 124, 822 (1959); Zh. Obshch. Khim., 31, 2069 (1961).
4. Liu Yung-lung and P. M. Loshkarev, Med. Prom. SSSR, No. 4, 11 (1962); No. 7, 18 (1963).
5. V. A. Maslennikova, G. L. Genkina, R. U. Umarova, A. M. Navruzova, and N. K. Abubakirov, Khim. Prirodn. Soedin., 173 (1967).
6. W. Nagata, C. Tamm, and T. Reichstein, Helv. Chim. Acta, 40, 41 (1957).
7. Š. Bauer, L. Masler, D. Šikl, and O. Bauerova, Tetrahedron Lett., 1103 (1966).
8. P. Brown, F. Brüschweiler, G. R. Pettit, and T. Reichstein, Org. Mass Spectrom., 5, 573 (1971).
9. I. F. Makarevich, Khim. Prirodn. Soedin., 566 (1970).
10. H. H. Stroh, Z. Naturforsch., 146, 699 (1959); W. Segffert, Z. Pflanzenzücht, 44, 4 (1960) cited by J. B. Harborne, Fortsch. Chemie Organ. Naturstoffe, 20, 165 (1962).
11. I. Heilbron, Dictionary of Organic Compounds, 4th ed., Eyre and Spottiswoode, London (1965), Vol. 3, p. 1672.
12. E. C. Bate-Smith, Chem. and Ind., 1457 (1954).