UDC 547

# FLAVONOID DIGLYCOSIDES OF PRUNUS SPINOSA

V. A. Makarov, A. L. Shinkarenko, V. I. Litvinenko, and I. P. Kovalev

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In an investigation of the flavonoid composition of the leaves of Prunus spinosa L. (blackthorn) growing in the southern Caucasus, we have found a complex mixture of compounds among which substance 1 has a chromatographic behavior similar to that of the diglycosides from this plant described previously [1,2].

We isolated substance 1 by chromatographic separation of the mixture of flavonoids on Kapron. This flavonoid consists of a light yellow crystalline powder, mp 173-174° C (from dilute ethanol), giving a single spot on two-dimensional chromatography. Quantitative acid hydrolysis yielded L-rhamnose, L-arabinose, and kaempferol with a yield of 50%. A spectral study in the UV region with diagnostic reagents showed the presence of free 5'- and 4'-hydroxy groups in the glycoside. Consequently, in it the kaempferol is substituted in positions 3 and 7.

The stepwise acid hydrolysis of the diglycoside formed a monoglycoside which was isolated and characterized as the 7-rhamnoside. It remained to be assumed that the L-arabinose is present in position 3, but to prove this it was necessary to obtain the 3-glycoside. Acid and enzymatic hydrolysis did not give satisfactory results. In view of this, we have developed a new method for the hydrolysis of glycosides in an alkaline medium by means of which it was possible to split off the sugar in position 7, alone. This gave two monoglycosides (3A and 3B), which were isolated in the individual state and identified as kaempferol 3-rhamnoside and 3-arabinoside, respectively.

On the basis of the results obtained, it may be assumed that substance 1 is a mixture of two diglycosides: kaempferol 3,7-dirhamnoside and kaempferol 3-arabinosido-6-rhamnoside. A whole series of methods was attempted for their separation, of which repeated crystallization from dilute ethanol yielded the individual compounds (1A and 1B).

On stepwise acid hydrolysis, the dirhamnoside IA formed the 7-rhamnoside 2A, the physicochemical properties, polarometric analysis (table), and UV and IR spectra of which showed to be kaempferol 7-α-L-rhamnofuranoside.

Alkaline cleavage led to the isolation of the 3-glycoside 3A, which was characterized as kaempferol 3-α-L-rhamnofuranoside. Thus, on the basis of the investigation carried out we came to the conclusion that the diglycoside 1A is the 3,7-di-α-L-rhamnofuranoside.

From the mother liquors after the separation of the dirhamnoside 1A we obtained the diglycoside 1B, which, on acid hydrolysis, yielded the 7-rhamnoside 2B and on alkaline hydrolysis kaempferol 3-arabinoside (3B). The monosides isolated were studied in detail and found to be kaempferol 3-α-L-arabofuranoside and kaempferol 7-α-L-rhamnofuranoside. The nature of the oxide rings was confirmed by IR spectroscopy and the rates of acid hydrolysis. Consequently, the diglycoside 1B is kaempferol 3-α-L-arabofuranosido-7-α-L-rhamnofuranoside. We have called this glycoside, a new compound, ternoside.

## Experimental

Isolation of the diglycosides. The comminuted air-dry leaves of the blackthorn (Prunus spinosa L., family Rosaceae) (1.2 kg), prepared immediately after flowering (April-May) were extracted with 70% ethanol in an apparatus of the

Polarimetric Analysis of the Flavonoid Glycosides

| Substance | $M$ | $[\alpha]_D$ deg | $[M]_D$ | $K_{Ph}$ | $[M]_D \cdot K_{Ph}$ | Form of the bond | Form of the sugar |
|---|---|---|---|---|---|---|---|
| Kaempferol 7-rhamnoside (2A) | 432 | —176 | —760 | 0.55 | —4'8 | α | Furanose |
| Kaempferol 7-rhamnoside (2B) | 432 | —176 | —760 | 0.55 | —418 | α | |
| Kaempferol 3-rhamnoside (3A) | 432 | —164 | —708 | 0.55 | —389 | α | |
| Kaempferol 3-arabinoside (3B) | 418 | —154 | —644 | 0.54 | —348 | α | |
| Phenyl α-L-rhamnofuranoside | 240 | —170 | —410 | 1.0 | —410 | α | |
| Phenyl α-L-arabofuranoside | 225 | —159 | —358 | 1.0 | —358 | α | |

Soxhlet type. The extract was evaporated in vacuum to an aqueous residue and was freed with chloroform from lipophilic substances. The aqueous solution was chromatographed on a column of Kapron (10 × 80 cm) and substance 1 was eluted with 25-35% ethanol. The eluates were evaporated to small bulk (1/5) and left at 3-5° C for crystallization. This gave light yellow acicular crystals of substance 1 with mp 173-174° C, $R_f$ 0.69 (15% acetic acid), 0.80 [butan-1-ol—acetic acid—water (4 : 1 : 5)], and 0.80 [formic acid—ethyl acetate—water (10 : 2 : 3)]. Yield 0.4%

The repeated recrystallization of substance 1 from dilute ethanol gave two substances: **1A** and **1B**, differing from one another in the composition of the sugars.

Substance **1A** has mp 186–187° C (from dilute ethanol), $[\alpha]_D^{20}$ −255° (c 0.1; methanol); $\lambda_{max}$ in methanol 266 and 347 mμ; $\lambda_{max}$ with sodium acetate 265 and 351 mμ; $\lambda_{max}$ with sodium ethoxide 270, 398 mμ; $\lambda_{max}$ with zirconyl nitrate 274, 345, and 399 mμ; $\lambda_{max}$ with zirconyl nitrate and citric acid 265 and 345 mμ.

Substance **1B** has mp 177–180° C (from dilute ethanol), $[\alpha]_D^{20}$ −240° (c 0.1; methanol), $\lambda_{max}$ in methanol 265 and 350 mμ; $\lambda_{max}$ with sodium acetate 262 and 360 mμ; $\lambda_{max}$ with sodium ethoxide 270 and 400 mμ; $\lambda_{max}$ with zirconyl nitrate 275, 350, and 400 mμ; $\lambda_{max}$ with zirconyl nitrate and citric acid 265 and 350 mμ.

Acid hydrolysis of the diglycosides. A. Complete hydrolysis. The glycosides **1A** and **1B** (0.253) were hydrolyzed with 2% $H_2SO_4$ in 50% ethanol in the boiling water bath for 2 hr.

The aglycones were separated on a layer of Kapron, washed with water, and eluted with 80% ethanol. The glycoside **1A** yielded 0.1227 g and **1B** 0.1237 g of the aglycone, amounting to 49.1 and 49.5%, respectively.

The aqueous fractions of the hydrolysates were neutralized with barium carbonate, and the sugars were analyzed by paper chromatography. Only L-rhamnose was found in **1A**, while L-rhamnose and L-arabinose were found in equivalent amounts in **1B**. To identify the sugars, their osazones were prepared. The mixture of rhamnose and arabinose osazones from **1B** was separated on the basis of their different solubilities in hot water [1].

B. Stepwise hydrolysis. The diglycosides **1A** and **1B** (0.5 g each) were hydrolyzed with 15% acetic acid (100 ml) with heating in the boiling water bath for 2 hr. The hydrolysis products were separated on a polyamide column, giving kaempferol and the monosides **2A** and **2B**, respectively. Both monosides had mp 232–233° C (from ethanol), $[\alpha]_D^{20}$ −176° (c 0.08; methanol); $\lambda_{max}$ in methanol 266 and 367 mμ; $\lambda_{max}$ with sodium acetate 266 and 367 mμ; $\lambda_{max}$ with sodium ethoxide 270 and 439 mμ; $\lambda_{max}$ with zirconyl nitrate 265 and 455 mμ; $\lambda_{max}$ with zirconyl nitrate and citric acid 265 and 425 mμ; $R_f$ 0.18 (15% acetic acid), 0.85 (BAW, 4 : 1 : 5), 0.71 [formic acid–ethyl acetate–water (10 : 2 : 3)].

Alkaline hydrolysis of the diglycosides. The glycoside **1A** or **1B** (0.5 g) was dissolved in 100 ml of 0.5% aqueous KOH solution and hydrolyzed in the boiling water bath for 2 hr. The solutions were neutralized and the products purified on a layer of Kapron. **1A** and **1B** yielded the monosides **3A** and **3B**, respectively.

The monoside **3A** has mp 173–175° C (from ethanol), $[\alpha]_D^{20}$ −164° (c 0.1; methanol); $\lambda_{max}$ in methanol 265 and 345 mμ; $\lambda_{max}$ with sodium acetate 273 and 360 mμ; $\lambda_{max}$ with sodium ethoxide 273 and 385 mμ; $\lambda_{max}$ with zirconyl nitrate 265 and 395 mμ; $\lambda_{max}$ with zirconyl nitrate and citric acid 265 and 345 mμ; $R_f$ 0.53 (15% acetic acid), 0.87 (BAW, 4 : 1 : 5), 0.83 [formic acid–ethyl acetate–water (10 : 2 : 3)].

After acid hydrolysis, **3A** yielded kaempferol and L-rhamnose.

The monoside **3B** has mp 228–230° C, $[\alpha]_D^{20}$ −154° (c 0.1; methanol); $\lambda_{max}$ in methanol 265 and 350 mμ; $\lambda_{max}$ with sodium acetate 274 and 367 mμ; $\lambda_{max}$ with sodium ethoxide 273 and 387 mμ; $\lambda_{max}$ with zirconyl nitrate 275 and 395 mμ; $\lambda_{max}$ with zirconyl nitrate and citric acid 265 and 350 mμ; $R_f$ 0.41 (15% acetic acid), 0.86 (BAW, 4 : 1 : 5), 0.80 [formic acid–ethyl acetate–water (10 : 2 : 3)].

On acid hydrolysis, **3B** formed kaempferol and L-arabinose in equimolar amounts.

## Conclusions

1. Two glycosides (**1A** and **1B**) have been isolated from blackthorn leaves gathered in the spring.
2. A chemical investigation of the glycosides has been carried out, and it has been established that:

a) glycoside **1A** is kaempferol 3,7-di-α-L-rhamnofuranoside;

b) glycoside **1B** is kaempferol 3-α-L-arabofuranoside-7-α-L-rhamnofuranoside. This is a new compound which we have called ternoside.

## REFERENCES

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