# POLYPHENOLS OF THE LEAVES OF Hibiscus cannabinus

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We have studied the leaves of kenaf (Hibiscus cannabinus), family Malvaceae, collected in the period of technical ripeness in the environs of Tashkent.

The leaves were defatted by steeping them in chloroform and benzene. After the solvents had been driven off, the raw material was extracted successively five times each with ether, ethyl acetate, and 85% methanol. The extracts were concentrated in vacuum at 40°C.

When the ethereal extracts were chromatographed on Silufol plates in the chloroform–acetone–acetic acid–ethyl acetate–formic acid (10 : 7 : 1 : 25 : 0.5) system, three spots were revealed with diazotized p-nitroaniline having $R_f$ 0.38, 0.57, and 0.79. The preparative separation of this mixture on a paper chromatogram in the ethanol–ammonia–water (35 : 2 : 13) system yielded the substances with $R_f$ 0.57 and 0.79.

Substance (I), mp 201-203°C, $R_f$ 0.79, dissolved readily in ether, ethyl acetate, and methanol.

Substance (II) had mp 194°C, $R_f$ 0.57.

The cochromatography of the substances isolated with authentic samples of vanillic and protocatechuic acids showed their respective identities [1]. These acids are present in the free state in kenaf leaves.

The ethyl acetate extract contained substances of flavonoid nature which consisted mainly of compounds with $R_f$ 0.46 and 0.27 in the chloroform–methanol–acetone (3.5 : 1.5 : 0.5) system; they were revealed with a 1% ethanolic solution of $FeCl_3$ and a 1% butanolic solution of $AlCl_3$. When the extract was kept in the cold, it deposited yellow crystals of compound (III).

Substance (III) had mp 184°C (from ethanol), composition $C_{27}H_{30}O_{14}$, $[\alpha]_D^{20}$ – 220° (c 0.07; methanol). The acid hydrolysis of (III) gave an aglycone and a sugar. On the basis of its physiological properties, UV spectra with diagnostic reagents, and IR spectrum, the aglycone was characterized as 3,4',5,7-tetrahydroxyflavone (kaempferol) [2]. The sugar was identified as L-rhamnose.

The UV spectrum of the glycoside with ionizing and complex-forming reagents showed the presence of free hydroxy groups at $C_5$ and $C_4$, and the substitution of the hydroxyls at $C_3$ and $C_7$. The PMR spectrum of the acetyl derivative of the glycoside also confirmed the substitution of the hydroxyls in these positions [4].

In the course of the stepwise acid hydrolysis of (III) with 10% acetic acid, we isolated an intermediate product (IV) with mp 230-232°C, $[\alpha]_D^{20}$ – 176°C (c 0.08; methanol), which was identified as kaempferol 7-O-α-L-rhamnofuranoside [4].

The hydrolysis of (III) with 5% aqueous alkali [6] gave an intermediate product (V) with mp 172-175°C, $[\alpha]_D^{20}$ –164° (c 0.07; methanol). On the basis of UV spectra with diagnostic reagents, rate of hydrolysis, and a comparison of the specific molecular rotation with that of the phenylglycoside by Klyne's method [7], compound (V) was identified as kaempferol 3-O-α-L-rhamnofuranoside.

A quantitative gravimetric analysis of the products of the complete acid hydrolysis of (III) showed a ratio of kaempferol and rhamnose of 1 : 2.

The experimental results obtained enable substance (III) to be characterized as 4',5-dihydroxy-3,7-di-O-α-L-rhamnofuranosyloxyflavone [8].

This is the first time that vanillic acid (I), protocatechuic acid (II), and kaempferol 3,7-di-O-α-L-rhamnofuranoside (III) have been isolated from the leaves of H. cannabinus.

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# KAEMPFEROL GLYCOSIDES OF THE LEAVES OF *Laurocerasus officinalis*

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Continuing a chemical study of the flavonoid compounds of *Laurocerasus officinalis* Roem. (*Prunus laurocerasus*; common laurel cherry) [1, 2], we have isolated two kaempferol glycosides from the leaves of this plant.

To isolate the glycosides, an aqueous ethanolic extract was evaporated in vacuum and the aqueous residue was washed with chloroform and chromatographed on a column of polyamide. Elution was carried out with distilled water and with aqueous ethanol of various concentrations. The individual glycosides were obtained by the rechromatography of the fractions obtained on a polyamide column (elution with isopropanol).

Glycoside (I) formed pale yellow acicular crystals with mp 168-170°C, $[\alpha]_D^{20}$ −85° (c 0.2; $CH_3OH$). UV spectrum, $\lambda_{max}$, nm, ($C_2H_5OH$): 267, 351; + $CH_3COONa$: 273, 361; + NaOH: 276, 402; + $AlCl_3$: 276, 396.

Glycoside (II) formed yellow acicular crystals with mp 172-174°C, $[\alpha]_D^{20}$ −103.2° (c 0.2; $CH_3OH$). UV spectrum, $\lambda_{max}$, nm ($C_2H_5OH$): 268, 350; + $CH_3COONa$: 276, 360; + NaOH: 276, 404; + $AlCl_3$: 276, 396.

$R_f$ values of glycosides (I) and (II) (ascending method): 0.77, 0.76 (water-saturated phenol); 0.49, 0.52 (butan-1-ol–acetic acid–water (4:1:5)); 0.68, 0.64 (15% acetic acid); 0.80, 0.85 (ethyl acetate–formic acid–water (10:2:3)), respectively.

The spectral characteristics of glycosides (I) and (II) and of their aglycones in the UV region and qualitative reactions showed that the carbohydrate moieties were present in the C-3 positions [3, 4].

In a study of the products of acid hydrolysis of compound (I) (2 N HCl), kaempferol, D-galactose, and D-glucose were detected, and in the case of (II) kaempferol, D-galactose, and D-xylose. The percentage amounts of the aglycones (40 and 43.6%), and also the ratio of the intensities of absorption of the maxima of bands I in the UV region of the spectra of the glycosides and their aglycones (38 and 40%) showed that the glycosides under investigation were biosides [5].

When (I) and (II) were subjected to stepwise acid hydrolysis [6], monosides were formed which were identified in each case as kaempferol 3-$\beta$-D-galactoside (trifolin). The order of the bonds between the sugars was established by oxidative degradation with hydrogen peroxide and by enzymatic hydrolysis with rhamnodiastase [7]. The sizes of the oxide rings of the sugars and the forms of the bonds were determined by IR spectroscopy and polarimetric analysis [8].

According to the results obtained, glycoside (I) is kaempferol 3-O-(6-O-$\beta$-D-glucofuranosyl-$\beta$-D-galactopyranoside) and glycoside (II) is kaempferol 3-O-(6-O-$\alpha$-D-xylofuranosyl-$\beta$-D-galactopyranoside).

This is the first time that kaempferol glycosides with such combinations of sugars have been isolated.

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