# STRUCTURE OF ACETYLPECTOLINARIN, A NEW ACYLATED FLAVONOID FROM PLANTS OF THE GENUS Linaria

L. P. Smirnova, G. G. Zapesochnaya, V. I. Sheichenko, and A. I. Ban'kovskii

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We have previously [1, 2] reported the isolation from some species of toadflax of a new flavone glycoside – acetylpectolinarin (I). On acid hydrolysis it gave the aglycone pectolinarigenin (5,7-dihydroxy-4',6-dimethoxyflavone) and the sugars glucose and rhamnose. The UV-spectroscopic examination of the glycoside and the aglycone with diagnostic reagents showed that in the glycoside the 5-hydroxy group is free and, consequently, both the sugars are attached at position 7. Stepwise hydrolysis with the production of the 7-glucoside of pectolinarigenin showed that the terminal sugar is the rhamnose.

In the IR spectrum of (I) (Fig. 1) there is an absorption band at 1730 $cm^{-1}$ that is characteristic for an ester grouping. Alkaline saponification under mild conditions (and also brief heating with diluted acids) led to the formation of pectolinarin (II) and acetic acid. An analysis showed that (I) corresponds to the formula $C_{31}H_{36}O_{16} \cdot H_2O$ and it contains one acetic acid residue, which is present in the carbohydrate part of the molecule. The acetylation of (I) gave a heptaacetate (III) analogous to the acetate (II).

For natural flavonoid O-glycosides, three disaccharides containing glucose and rhamnose are known: rutinose, neohesperidose, and rungiose (6-, 2-, and 3-O-$\alpha$-L-rhamnopyranosyl-$\beta$-D-glucopyranose, respectively). To determine the structure of the carbohydrate part of the molecule we performed the quantitative oxidation of (I) and (II) with $NaIO_2$ by the Fleury–Lange method [3]. Substance (II) absorbed 1 mole of $NaIO_4$ in 2 h, 2 moles in 24 h, and 3.5 moles in 4 h, which is evidence in favor of a 1,6-linkage of the sugars (calculated: 4 moles of $NaIO_4$). The oxidation of (I) took place at first far more slowly; at the moment when two moles of $NaIO_4$ had been absorbed, the curves became practically identical and did not differ subsequently. At the beginning of the reaction, apparently, the acetyl group is split off, which may explain the initial lag and the subsequent equalization of the rates of the reactions. It is known that the rate of periodate oxidation depends on the stereochemistry of the neighboring hydroxy groups and falls in the sequence ae > ee ≫ aa [4]. In the molecules of (I) and (II) there is one axial-equatorial grouping – in positions 2 and 3 of the rhamnose. The results of the periodate oxidation permit the assumption that the acetyl group in (I) substitutes one of these hydroxyls or is adjacent to them and interferes with oxidation.

The final choice between the position of attachment of the acetyl group was made on the basis of an analysis of the nuclear magnetic resonance spectra of compounds (I), (II), and (III).

In the NMR spectrum of the trimethylsilyl ether of (II), the signals of the anomeric protons of the glucose (5.05 ppm, J = 6.0 Hz) and of the rhamnose (4.28 ppm, J = 2.0 Hz) are characteristic for the pyranose

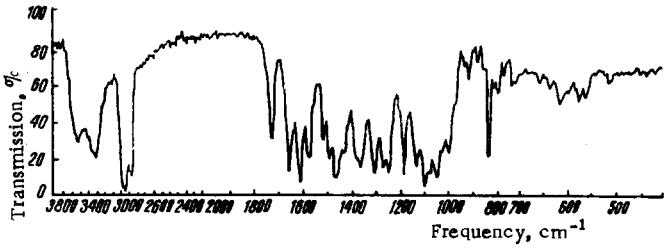


Fig. 1. IR spectrum of acetylpectolinarin.

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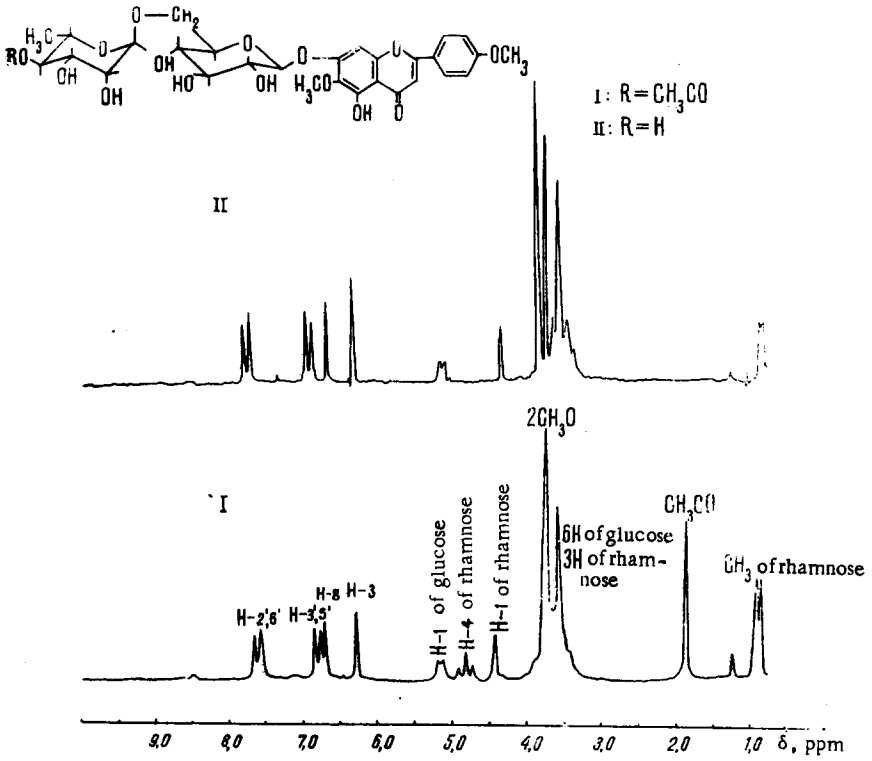



Fig. 2. NMR spectrum in $CCl_4$ of acetylpectolinarin (I) and pectolinarin (II).

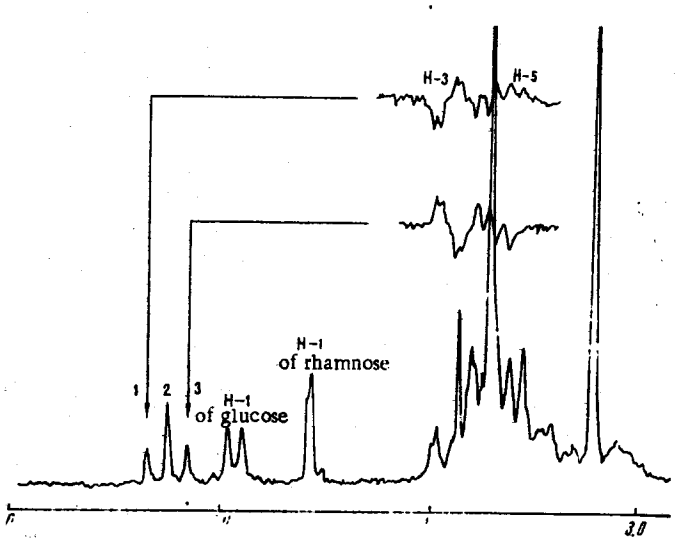



Fig. 3. NMR spectrum in $C_6H_6$ of acetylpectolinarin and the INDOR spectrum.

form of these sugars in conformations C1 and 1C, respectively [5-9]. The methyl group of rhamnose appears in the form of a broad peak at 0.82 ppm. These signals (4.28 and 0.82 ppm) are characteristic of rhamnose in rutinosides [10]. The integration of the 4.5-5.6 and 3.4-4.4-ppm regions in the NMR spectrum of (III) (ratio 8:4) permitted its unambiguous assignment to the rutinosides, since it is known [10] that in the NMR spectra of full acetates, all the methine protons adjacent to acetyl or to two ester bonds are located in a weaker field than methylene and methine protons adjacent to a single ester grouping. For the neohesperidosides and rungiosides [11], this ratio is 7:5. Furthermore, in rutinosides, the signal of the proton at $C_1$ of the rhamnose is easily distinguished in comparison with the others in the weak-field region [10] (in our case, 4.64 ppm).

The spectrum of (I) differs from the spectrum of (II) (Fig. 2) by the presence of a sharp doublet (0.78 ppm, J = 6.0 Hz) of a methyl group, the signal of an acetyl group (1.83 ppm), and a triplet with an intensity

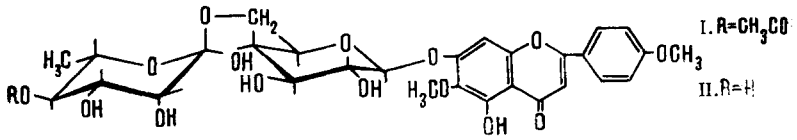
of 1 proton unit at 4.79 ppm ($J_1 = J_2 = 9.5$ Hz), which must be assigned to the hemiacyl proton. The two coupling constants of 9.5 Hz do not enable the signal at 4.79 ppm to be assigned unambiguously to the protons of the rhamnose or the glucose: in both molecules there are chains of three carbon atoms, the protons of which have the trans arrangement with respect to one another in pairs.

To prove the position of attachment of the acetyl group it was necessary to investigate the structure of the signals of the protons vicinal to the hemiacyl proton, for which we made use of the INDOR method [12].

The INDOR spectrum of the trimethylsilyl ether of (I) in $C_6D_6$ was obtained on the first and second lines of the triplet. It can be seen from the spectrum (Fig. 3) that one of the signals is a quartet, the distance between the first and second lines of which is 3.2 Hz and the distance between the 2nd and 3rd lines is 9.5 Hz. In glucose, all the protons are present in the trans position with respect to one another; larger (> 8 Hz) values of the coupling constant correspond to them [6]. Thus, the small constant (3.2 Hz) shows that the acetyl group is located in the rhamnose residue. Two large constants for vicinal protons in rhamnose are realized only for the $C_4$ position, i.e., only this position is possible for the acetyl group.

Although a broad peak of a $CH_3$ group in the 0.8-1.0 ppm region is characteristic for rutinosides [10], in compound (I) this signal appears in the form of a clear doublet, which can be explained by the influence of the neighbouring acetyl group.

Thus, the new acylated flavonoid which we have called acetylpectolinarin has structure (I).




## EXPERIMENTAL

The IR spectra were taken on a UR-10 spectrophotometer (mulls in paraffin oil), the UV spectra on a Hitachi EPS-3T instrument, the NMR spectra on a Varian AA-100D instrument (standard: HMDS; $\delta$ scale), and the mass spectra on a Varian CH-8 instrument. Gas-liquid chromatography was performed on a Varian 2700 instrument and paper chromatography on Leningrad paper of type "M" ["slow"] in the following solvent systems: 1) 15% $CH_3COOH$; 2) water-saturated phenol; 3) benzene–butan-1-ol–pyridine–water (5 : 1 : 3 : 3); 4) butanol–acetic acid–water (4 : 1 : 5); and 5) water-saturated butanol. The flavonoids were revealed with a 1% ethanolic solution of $AlCl_3$, the sugars with aniline phthalate, and the diethylamine salts of the fatty acids with a 0.04% solution of Bromocresol Green in butanol.

The analyses of all the compounds corresponded to the calculated figures.

Isolation of Acetylpectolinarin (I). The comminuted raw material of the Linaria vulgaris, L. vulgariformis, L. kurdica, L. popovii, L. sessilis and L. kokanica was extracted with 96% ethanol, the extract was evaporated, and the residue was treated with chloroform and chromatographed on polyamide. The fractions containing the substance with $R_f$ 0.72 (system 1) were combined and evaporated, and the residue was recrystallized from methanol. This gave yellow crystals with the composition $C_{31}H_{36}O_{16} \cdot H_2O$, mp 242.5-244°C, $[\alpha]_D^{20}$ −95.9° (c 1, pyridine) −95.0° (c 0.4; formamide).

UV spectrum: $\lambda_{max}$ (in ethanol) 277, 328-329 nm; $\lambda_{max}$ (ethanol + $C_2H_5ONa$) 298 nm; $\lambda_{max}$ (ethanol + $CH_3COONa$) 277, 328 nm; $\lambda_{max}$ (ethanol + $AlCl_3$) 300 and 347 nm.

The NMR spectrum is given in Fig. 2. For INDOR the spectrum was taken in $C_6D_6$ (see Fig. 3). The shift of the signal of one of the $CH_3O$-groups (3.2 ppm) permits it to be assigned to position 4' [13].

Acetylation of Acetylpectolinarin. Preparation of (III). A mixture of 1 g of (I) [or (II)] and 50 ml of $(CH_3CO)_2O$ was heated at 100°C for 4.5 h in the presence of a few drops of pyridine and anhydrous sodium acetate. After cooling, the reaction mixture was poured into 0.5 liter of ice water. The precipitate that deposited was recrystallized from a mixture of diethyl ether and petroleum ether. This yielded small colorless crystals with the composition $C_{29}H_{27}O_{15}(CH_3CO)_7$ with mp 123-125°C, $[\alpha]_D^{18}$ −39.6° (c 1.2, chloroform).

NMR spectrum: doublet at 7.65 ppm, J = 9, 2H (H-2; 6'); doublet at 6.9 ppm, J = 9, 2H (H-3; 5'); singlet at 6.95 ppm, 1H (H-8); singlet at 6.4 ppm, 1H (H-3); doublet at 4.64 ppm, J = 2, 1H (H-1 of rhamnose); singlets at 3.75 and 3.70 ppm, 6H (2 $CH_3O$ groups); singlets at 2.36, 3H, 1.90-1.98, 15H, and 1.77 ppm, 3H ($CH_3CO$ groups); doublet at 1.05 ppm, J = 6, 3H ($CH_3$ of rhamnose); and multiplets at 4.8-5.34 ppm, 7H and at 3.5-4.0 ppm, 4H (protons of the sugars).

Acid Hyrolysis of (I). A. Production of Pectolinarigenin. A mixture of 0.74 g of (I), 100 ml of 20% sulfuric acid, and 15 ml of methanol were heated at 100°C for 2 h. After elimination of the methanol, the crystals of pectolinarigenin were filtered off and recrystallized from ethanol. Yield 0.35 g (47.3%), $C_{17}H_{14}O_{16}$ mp 218-219°C, mol. wt. 314 (mass spectrometry). UV spectrum: $\lambda_{max}$ (absolute ethanol) 277, 322 nm.

The diacetate of the aglycone [$(CH_3CO)_2O$, $CH_3COONa$, 100°C, 4 h)] had mp 154.5-155°C, and the IR spectrum lacked the absorption bands of OH groups.

B. Isolation of Glucose and Rhamnose. The acid filtrate was neutralized on Dowex-1 anion-exchange resin ($HCO_3$ form), concentrated in vacuum, and chromatographed on paper in systems 2, 3, and 4 in the presence of markers; glucose and rhamnose were found.

From another portion of the neutralized filtrate a mixture of the osazones of the sugars was obtained, which was treated with a small amount of cooled acetone. The rhamnose osazone passed into solution and, after recrystallization it had mp 181°C, while the glucose osazone had mp 204°C. Neither osazone gave a depression of the melting point in admixture with a corresponding authentic sample.

Alkaline Saponification. A. Production of Pectolinarin. A solution of 0.35 g of (I) in 5.5 ml of 1% aqueous KOH was left at 20°C for 20 min. The mixture was acidified with 5% HCl to pH 5.5. The gelatinous precipitate that deposited was filtered off and washed with water, and recrystallized from methanol. Light yellow crystals, $C_{29}H_{34}O_{15}$, with mp 277°C deposited. A mixture with authentic pectolinarin showed no depression of the melting point, and their IR spectra coincided.

B. Detection of Acetic Acid. The acid aqueous filtrate was extracted with ether, the extract was concentrated, and diethylamine was added to the residue to a pH of 10. The diethylamine acetate so formed was identified by paper chromatography in system 5. The presence of an acetic acid residue in (I) was also confirmed by GLC.

Stepwise Hydrolysis. A mixture of 2.0 g of (I), 20 ml of 10% $H_2SO_4$, and 40 ml of methanol was heated at 100°C for 2 h. After evaporation of the methanol and cooling, the precipitate that had deposited was filtered off. Rhamnose and traces of glucose were found in the acid hydrolysis solution. The residue, which contained the aglycone, a monoglycoside and (II) [(I) was absent] was washed with chloroform (5 · 50 ml) and chromatographed on polyamide. The column was eluted with chloroform; when 2% of methanol was added, pectolinarigenin glucoside was eluted: $C_{23}H_{24}O_{11}$, mp 253-256°C, $[\alpha]_D^{14} -68.1°$ (c 0.94; pyridine), $R_f$ 0.35 (system 1).

UV spectrum: $\lambda_{max}$ (methanol) 277, 330 nm; $\lambda_{max}$ (methanol + $CH_3ONa$) 290 nm; $\lambda_{max}$ (methanol + $CH_3COONa$) 277, 330 nm. NMR spectrum: doublet at 7.70 ppm, J = 9, 2H (H-2', 6'); doublet at 6.88 ppm J = 9, 2H (H-3', 5'); singlet at 6.46 ppm, 1H (H-8); singlet at 6.42 ppm, 1H (H-3); doublet at 4.96 ppm, J = 6.5, 1H (H-1 of glucose); singlet at 3.80 ppm, 6H (2 $CH_3O$ groups); multiplet at 3.4-3.9 ppm, 6H (protons of the sugars).

Periodate Oxidation. With heating in a 100-ml measuring flask, 0.0891 g of (I) was dissolved in 45 ml of ethanol. The solution was cooled, 50 ml of a $15.78 \cdot 10^{-3}$ M solution of $NaIO_4$ was added with a pipette, and the contents of the flask were made up to the mark with ethanol and were carefully mixed. A solution of 0.0751 g of (II) was prepared similarly. In a blank experiment, 50 ml of $NaIO_4$ solution was added to a 100-ml flask and was made up to the mark with ethanol.

The process of periodiate oxidation was monitored in the following way: a sample of the reaction mixture (5 ml) was treated with 10 ml of a saturated solution of $NaHCO_3$, 20 ml of a $5.08 \cdot 10^{-3}$ M solution of $NaAsO_2$, and 2 ml of a 20% solution of KI. The mixture was kept in the dark for 15 min, and then the excess of $NaAsO_2$ was titrated with a 0.01 N solution of $I_2$ with the addition as indicator of 2 drops of a 1% solution of soluble starch. In the calculation of the consumption of $NaIO_4$, the amount consumed in the oxidation of the ethanol (from the results of the blank experiment) was deducted.

## CONCLUSIONS

1. It has been established that acetylpectolinarin, a new flavonoid from some species of Linaria, is 7-[6-O-(4-acetyl-α-L-rhamnopyranosyl) β-D-glucopyranosyl]-5,7-dihydroxy-4',6-dimethoxyflavone.

2. It has been found that the presence of the acetyl group in position 4 of the rhamnose residue sharply reduces the rate of the periodate oxidation of the cis-2,3-diol grouping of the rhamnose in the rutinoside.

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