# FLAVONOIDS OF *Agastache rugosa*

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Acacetin, tilianin, linarin, and the new acylated flavone glycoside agastachoside have been isolated from the plant *Agastache rugosa* for the first time. On the basis of IR, UV, NMR, and mass spectra the structure of 6"-O-acetyl-7-β-D-glucopyranosyloxy-5-hydroxy-4'-methoxyflavone has been established for agastachoside.

From the herb *Agastache rugosa* (Fisch. et Mey) Kuntze (wrinkled giant hyssop), family Lamiaceae, collected in the flowering phase in the collection nursery of the North Caucasus Zonal Experimental Station of VILR [All-Union Scientific-Research Institute of Medicinal Plants] we have isolated four flavonoid compounds with mp 261-263°C (I), 234-236°C (II), 206-209°C (III), and 267-269°C (IV).

Judging from their mobilities in polar and nonpolar systems in chromatography on Silufol plates and on paper, and also from the colors of the spots on examination in UV light before treatment with $AlCl_3$ (dark spots) and afterwards (fluorescing green), compound (I) was a flavone, and compounds (II), (III), and (IV) were flavone glycosides.

The IR spectrum of compound (I) had absorption bands characteristic of carbonyl (1660 $cm^{-1}$), phenol (3170 $cm^{-1}$), and methoxy (2940 $cm^{-1}$) groups [1]. There were free hydroxy groups in positions 5 and 7 (UV spectroscopy) and the methoxy group was present in position 4, as can be seen from the NMR spectrum (singlet at δ 3.70 ppm) and the mass spectrum (m/e 132). Compound (I) was 5,7-dihydroxy-4'-methoxyflavone (acacetin) [2, 3].

Spectral investigations in the UV region with additives showed that the carbohydrate moiety in compounds (II), (III),and (IV) was present at $C_7$ [4]. The mass spectra of these compounds each had a fragment with m/e 284. This shows that the aglycone was acacetin. The IR and NMR spectra of the aglycones obtained by the hydrolysis of compounds (II), (III),and (IV) were identical with those of acacetin. Mixtures of acacetin with the aglycones of these compounds showed no depression of the melting point.

The acid hydrolysis of compound (II) gave acacetin and glucose. In the NMR spectrum of (II) in [D]pyridine a double at δ 5.70 ppm (1 H) with an SSCC of 7 Hz was due to the proton of the glycosidic center of β-glucose.

Compound (II) was 7-β-D-glucopyranosyloxy-5-hydroxy-4'-methoxyflavone and was identical with tilianin [2].

Substance (III) differed from (II) by a higher mobility in systems of low polarity. The IR spectrum of (III) contained an absorption band at 1710 $cm^{-1}$, which is characteristic for the carbonyl group of an ester at an alcoholic hydroxyl [1].

The alkaline saponification of (III) yielded tilianin and acetic acid. The presence of the latter was shown by the preparation of its diethylammonium salt and a comparison of it with markers by paper chromatography. Compound (III) belonged to the comparatively small group of acylated glycosides [5-18]. The position of attachment of the acetyl group in it was established with the aid of the NMR spectrum of its trimethylsilyl derivative (Fig. 1).

The signal of the anomeric proton (doublet at δ 4.88 ppm with J = 7 Hz) showed a β linkage of D-glucopyranose with the aglycone, while a signal at 4.35 ppm (2 H) is related to geminal protons and showed the attachment of the acetic acid in position 6" of the glucose, a singlet at 1.97 ppm (3 H) representing the protons of the acyl residue. Compound (III) had the structure of 6"-O-acetyl-7-β-D-glucopyranosyloxy-5-hydroxy-4'-methoxyflavone. It is a new acylated glycoside and we have called it agastachoside.

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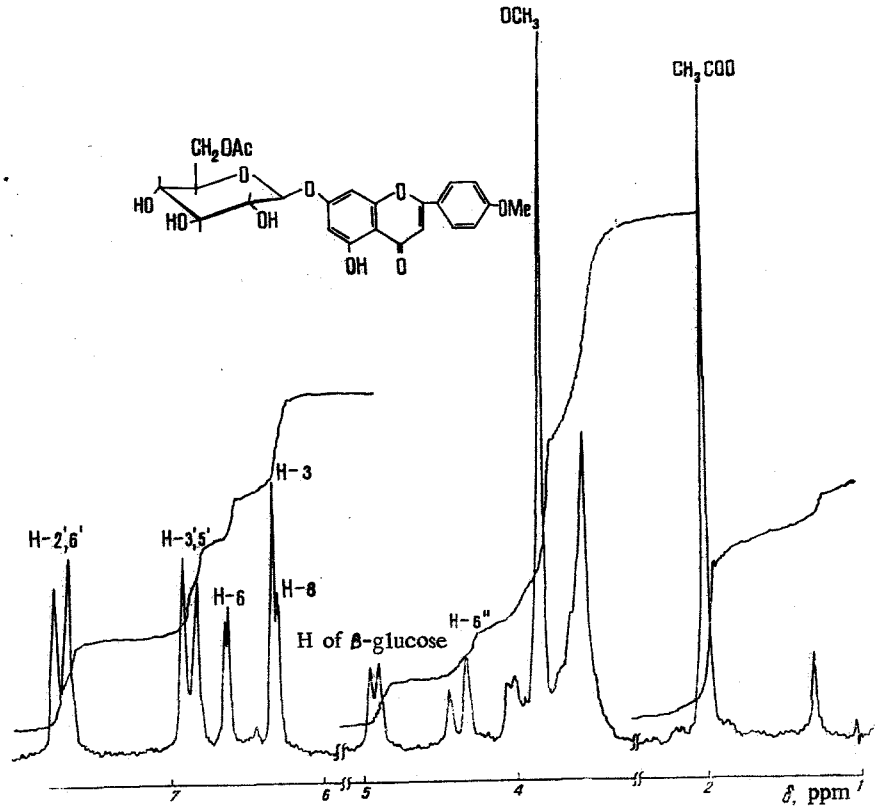


Fig. 1. NMR spectrum of the trimethylsilyl ether of agastachoside.

Compound (IV) was a bioside the acid hydrolysis of which gave acacetin, D-glucose, and L-rhamnose. Stepwise acid hydrolysis yielded tilianin and rhamnose and, therefore, the rhamnose is terminal in the biose. This can also be seen from the NMR spectrum of the trimethylsilyl ether of compound (IV). A doublet at δ 5.00 ppm (1 H), J = 7 Hz, relates to the proton of the glycosidic center of β-glucose attached in position 7 of the aglycone; a broadened singlet at 4.36 ppm is the signal of the proton of the glycosidic center of β-rhamnose attached in position 6", a singlet at 3.80 ppm is the signal of a methoxy group, and a multiplet at 1 ppm is that of the methyl group of rhamnose.

Compound (IV) has the structure of 5-hydroxy-4'-methoxy-7-[O-α-L-rhamnopyranosyl-(1 → 6)-β-D-glucopyranosyloxy]flavone and is identical with linarin [19-21].

## EXPERIMENTAL

IR spectra were obtained on a UR-20 instrument (paraffin oil), UV spectra on a Specord UV-VIS, NMR spectra on a Varian HA-100D, and mass spectra on a Varian CH-8. Melting points were determined on a Kofler block, and elementary analyses were performed on a Hewlett-Packard 185 B automatic CHN analyzer. Chromatographic monitoring was carried out by TLC (Silufol) in system 1) chloroform—methanol (8:2) and by PC in systems 2) 15% acetic acid, 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 aluminum trichloride, the sugars with aniline phthalate, and the diethylammonium salts of the fatty acids with a 0.04% solution of Bromocresol Blue in butanol.

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

Isolation of the Flavonoids. The air-dry comminuted herb wrinkled giant hyssop (1.3 kg) was exhaustively extracted with 96% ethanol at room temperature. The extract was concentrated in vacuum and the residue was treated with chloroform and was chromatographed on a column of polyamide (4 × 30 cm).

The column was first washed with water to eliminate aromatic acids and sugars and then with 96% ethanol. The ethanol was distilled off in vacuum and 8.3 g of the resulting combined flavonoids was chromatographed on a column of silica gel L 100/250 μ (3.5 × 60 cm). Elution was performed with chloroform and then with mixtures of chloroform and methanol with gradually increasing concentrations of methanol, 150-200-ml fractions being collected. The separation was monitored by chromatography on Silufol plates in system 1.

Fractions 10-12 on elution with chloroform–methanol (86:4) contained substance (I), fractions 23-26 (chloroform–methanol (90:10)) contained (II), fractions 29-32 (chloroform–methanol (90:10)) contained (III), and fractions 41-45 (chloroform–methanol (70:30)) contained (IV).

The fractions were evaporated in vacuum and all the substances were recrystallized from dioxane–water (1:1).

Substance (I) formed yellow acicular crystals soluble in chloroform, pyridine, and DMSO; $C_{16}H_{12}O_5$, $M^+$ 284. $\lambda_{max}$ (nm): MeOH 271, 328; NaOMe 278, 368; NaOAc 280, 300, 342; NaOAc + $H_3BO_3$ 272, 340; $AlCl_3$ 278, 350; $AlCl_3$ + HCl 278, 345. NMR spectrum in [D]pyridine (ppm): d 7.92 (J = 9 Hz, H-2', 6'); d 7.02 (J = 9 Hz, H-3', 5'); s 6.84 (H-3); d 6.72 (J = 2.5 Hz, H-6); d 6.64 (J = 2.5 Hz, H-8); s 3.70 ($CH_3O$).

Substance (II) formed light yellow acicular crystals soluble in formamide, pyridine, and DMSO; $C_{22}H_{22}O_{10}\cdot H_2O$. $\lambda_{max}$ (nm): MeOH 270, 325; NaOMe 290, 370; NaOAc 278, 342; NaOAc + $H_3BO_3$ 270, 325; $AlCl_3$ and $AlCl_3$ + HCl 278, 348, 390, NMR spectrum in [D]pyridine (ppm): d 7.86 (J = 9 Hz, H-2', 6'); d 7.04 (J = 9 Hz, H-3', 3'); d 7.00 (J = 2.5 Hz, H-6); s 6.85 (H-3); d 6.76 (J = 2.5 Hz, H-8), 3.00-6.00 (6 H of glucose); s 3.75 ($CH_3O$).

Acid Hydrolysis of (II). A mixture of 15 mg of compound (II) and 3 ml of 30% sulfuric acid was heated on the boiling water bath for 2 h. The precipitate formed was shown by TLC and mass spectrometry ($M^+$ 284) to contain acacetin, while glucose was found in the neutralized and evaporated aqueous residue by PC in systems 3 and 4.

Substance (III) formed light yellow acicular crystals insoluble in water, chloroform, and ether, sparingly soluble in ethanol and methanol, and soluble in formamide, pyridine, and DMSO; $C_{24}H_{24}O_{11}$. $\lambda_{max}$ (nm): MeOH 270, 325; NaOMe 295; NaOAc 302, 340; NaOAc + $H_3BO_3$ 270, 325; $AlCl_3$ and $AlCl_3$ + HCl 272, 347. NMR spectrum of the TMS ether (ppm): d 7.66 (J = 9 Hz, H-2", 6"); d 6.82 (J = 9 Hz, H-3', 5'); d 6.58 (J = 2.5 Hz, H-6); s 6.28 (H-3); d 6.24 (J = 2.5 Hz, H-8); d 4.88 (J = 7 Hz, H of β-glucose); 4.35 (J = 12 Hz, H-6"); s 3.80 ($CH_3O$); 3.44-4.04 (6 H of glucose); s 1.97 ($OCOCH_3$).

The trimethylsilyl ethers of compounds (III) and (IV) were obtained as described previously [21].

Alkaline Saponification of (III). Substance (III) (0.3 g) was dissolved in 5 ml of 1% aqueous KOH and the solution was left at 20°C for 20 min. Then it was acidified with 5% HCl to pH 5.5. The jelly-like precipitate that deposited was filtered off, washed with water, and recrystallized from a mixture of dioxane and water (1:1). Light yellow crystals, $C_{22}H_{22}O_{10}$, mp 234-236°C, deposited. A mixture with tilianin showed no depression of the melting point, and their IR spectra were identical.

The acidic aqueous filtrate was extracted with ether, the extract was concentrated, the residue was treated with diethylamine to pH 10. The diethylammonium acetate formed was identified by paper chromatography in system 5.

Substance (IV) formed white acicular crystals insoluble in water, chloroform, and ether, sparingly soluble in ethanol and methanol, and soluble in formamide, pyridine, and DMSO; $C_{28}H_{32}O_{14}$. $\lambda_{max}$ (nm): MeOH 270, 330; NaOMe 282, 372; NaOAc 278, 342; NaOAc + $H_3BO_3$ 270, 330; $AlCl_3$ and $AlCl_3$ + HCl 315, 345. NMR spectrum of the TMS ether (ppm); d 7.74 (J = 9 Hz, H-2', 6'); d 6.74 (J = 9 Hz, H-3', 5'); s 6.45 (H-3); d 6.56 (J = 2.5 Hz, H-6); d 6.24 (J = 2.5 Hz, H-8); d 5.00 (J = 7 Hz, H of β-glucose); s 4.36 (H of β-rhamnose), s 3.80 ($CH_3O$); 3.40-3.90 (10 H of glucose and rhamnose).

Acid Hydrolysis of (IV). A mixture of 0.03 g of substance (IV), 5 ml of 20% sulfuric acid, and 1 ml of methanol was heated at 100°C for 2 h. After the methanol had been driven off, the crystals were filtered off and were recrystallized from a mixture of methanol and chloroform. The aglycone was identified as acacetin by TLC, mass spectrometry ($M^+$ 284), and UV and IR spectroscopy. The acid filtrate was neutralized and evaporated, and glucose and

mannose were detected in the aqueous residue by paper chromatography in systems 3 and 4 in the presence of markers.

The stepwise hydrolysis of (IV) was carried out as described by Smirnova et al. [11]. When the hydrolysis products were subjected to TLC, compounds (IV) and (III) (tilianin) were detected, while rhamnose and traces of glucose were found in the acid hydrolysis solution by the PC method.

## SUMMARY

From the epigeal part of *Agastache rugosa* we have isolated acacetin, tilianin, linarin, and a new acylated flavone glycoside which we have called agastachoside, for which the structure of 6"-O-acetyl-7-β-D-glucopyranosyloxy-5-hydroxy-4'-methoxyflavone has been established. This is the first time that acacetin, tilianin, and linarin have been isolated from this plant.

## LITERATURE CITED

1. L. Bellamy, Infrared Spectra of Complex Molecules, 2nd ed. Wiley, New York (1958).
2. Z. P. Pakudina and A. S. Sadykov, The Distribution in Plants and the Physicochemical Properties of Flavones, Flavonols, and Their Glycosides [in Russian], Tashkent (1970).
3. T. A. Geissmann, The Chemistry of Flavonoid Compounds, Pergamon, Oxford (1962).
4. V. I. Litvinenko and N. P. Maksyutina, Khim. Prir. Soedin., 420 (1965).
5. S. Z. Ivanova, G. G. Zapesochnaya, V. I. Sheichenko, S. A. Medvedeva, and N. A. Tyukavkina, Khim. Prir. Soedin., 196 (1978).
6. S. Z. Ivanova, G. G. Zapesochnaya, S. A. Medvedeva, and N. A. Tyukavkina, Khim. Prir. Soedin., 200 (1978); 332 (1978).
7. G. G. Zapesochnaya, S. Z. Ivanova, V. I. Sheichenko, N. A. Tyukavkina, and S. A. Medvedeva, Khim. Prir. Soedin., 570 (1978).
8. G. G. Zapesochnaya and G. P. Shnyakina, Khim. Prir. Soedin., 806 (1978).
9. G. G. Zapesochnaya and G. P. Shnyakina, Khim. Prir. Soedin., 720 (1975).
10. L. P. Smirnova, K. I. Boryaev, and A. I. Ban'kovskii, Khim. Prir. Soedin., 96 (1974).
11. L. P. Smirnova, G. G. Zapesochnaya, V. I. Sheichenko, and A. I. Ban'kovskii, Khim. Prir. Soedin., 313 (1974).
12. T. T. Pangarova, G. G. Zapesochnaya, and E. L. Nukhimovskii, Khim. Prir. Soedin., 667 (1974).
13. T. T. Pangarova and G. G. Zapesochnaya, Khim. Prir. Soedin., 712 (1975).
14. L. S. Teslov and G. G. Zapesochnaya, Khim. Prir. Soedin., 256 (1976).
15. T. D. Rendyuk, A. I. Shreter, V. L. Shelyuto, and V. I. Glyzin, Khim. Prir. Soedin., 282 (1977).
16. G. G. Zapesochnaya, S. Z. Ivanova, S. A. Medvedeva, and N. A. Tyukavkina, Khim. Prir. Soedin., 193 (1978).
17. J. B. Harborne, T. J. Mabry, and H. Mabry, The Flavonoids, Chapman and Hall, London (1975), p. 400.
18. H. Fujiwara, G. Nonaka, A. Jagi, and J. Nishiona, Chem. Pharm. Bull., 24, No. 3, 407 (1976).
19. K. W. Merz and Y. H. Wu, Arch. Pharm., 274, 126 (1936).
20. H. Wagner, L. Hörhammer, and W. Kirchnez, Arch. Pharm., 293, 1053 (1960).
21. V. L. Shelyuto, V. I. Glyzin, G. N. Yurchenko, and L. P. Smirnova, Khim. Prir. Soedin., 400 (1978).
22. T. J. Mabry, K. R. Markham, and M. B. Thomas, The Systematic Identification of Flavonoids, Springer, New York (1970), p. 4.