

citric was half this. The presence of such acids permits the more intensive assimilation of sugars by the microorganism, which, in the final account, leads to an increase in the weight of the fodder product obtained from aqueous extracts of the verdure.

The comminuted woody verdure of the pine *Pinus sylvestris* was extracted at 18–20°C for 15–20 min. The filtered extract was passed through KU-2 cation-exchange resin to eliminate impurities and to convert the acids into the free form. The total content of acids, calculated as malic, was determined by titration with 0.1 N NaOH. The concentrated eluate was extracted with ether, followed by repeated treatment with 5% sodium bicarbonate solution, decomposition of the salts with 2 N HCl, and extraction of the free acids with ether. The chromatographic investigation was carried out on a LKhM-72 chromatograph using a thermal conductivity detector. The acids were analyzed in the form of their methyl esters under the following conditions: rate of flow of carrier gas (He) 37 ml/min; column 200 × 0.4 cm; solid support Chromaton N-AWGMS (0.16–0.20 nm); stationary phase Silicone SE-30, 5% on the mass of the solid support. Temperature of the detector 300°C. The temperature was programmed at the rate of 3.6°C per minute in the interval of 60–290°C. Identification was performed by the method of adding the pure substances.

#### LITERATURE CITED

1. Z. A. Karepova, S. M. Repyakh, and V. L. Levdikova, *Khim. Prir. Soedin.*,
2. B. I. Osipov, *The Hydroaromatic Acids of Conifers* [in Russian], Novosibirsk (1979), p. 111.
3. G. A. Evdokimova et al., *Khim. Drev.*, **3**, 113 (1976).
4. Inventor's Certificate No. 765,359. *Byull. Izobret.*, No. 28 (1977).

#### HYDROXYCOUMARINS OF *Phaseolus vulgaris*

V. I. Dikhtyarev, V. N. Kovalev,  
and N. F. Komissarenko

UDC 547.99:635.652

We have previously [1] reported on the isolation of the hydroxycoumarin scopoletin from the herbage of *Phaseolus vulgaris* L. (kidney bean). Continuing a study of the coumarins of the epigeal part of this plant, we have isolated four substances, preliminarily designated compounds A–D.

Substance A fluoresced pale blue in UV light on a paper chromatogram, with color of the fluorescence changing to orange after treatment with an ethanolic solution of caustic soda [3]. It had the empirical formula  $C_{10}H_8O_4$  and was amorphous. In view of the identical elementary compositions and close  $R_f$  values in a number of solvent systems of the substance isolated and scopoletin, it was assumed that it was an isomer of scopoletin – 6-hydroxy-7-methoxycoumarin [3]. The isomer of scopoletin was obtained by methylating esculin with dimethyl sulfate in dry acetone in the presence of dry potassium carbonate followed by enzymatic hydrolysis with rhamnodiastase.

A comparison of the physicochemical properties of substance A with the 6-hydroxy-7-methoxy coumarin obtained showed their identity.

Substance B had the empirical formula  $C_9H_6O_3$ , mp 228–230°C and fluoresced bright blue in UV light. Its methylation gave a compound with the composition  $C_{10}H_8O_3$ , mp 117–118°C, identical with herniarin (7-methoxycoumarin). From its physicochemical properties, substance B was identified as umbelliferone (7-hydroxycoumarin) [2].

Substance C has the composition  $C_9H_6O_4$ , mp 268–272°C. On chromatograms it was revealed in UV light in the form of a blue spot which, after treatment with an ethanolic solution of caustic soda, fluoresced yellow. By its chromatographic behavior in various solvent systems and the absence of a depression of the melting point of a mixture with authentic material, substance C proved to be identical with esculetin (6,7-dihydroxycoumarin) [2].

Substance D had the composition  $C_{15}H_{16}O_9$ , mp 204–205°C. A preliminary study permitted its assignment to

---

Khar'kov Pharmaceutical Institute. Translated from *Khimiya Prirodnikh Soedinenii*, No. 3, p. 384, May–June, 1983. Original article submitted February 2, 1983.

the group of hydroxycoumarin glycosides. Esculetin and D-glucose were detected in the products of acid and enzymatic hydrolysis. On the basis of the  $R_f$  values in various solvent systems, spectral characteristics, and a mixed melting point with the authentic material, substance D proved to be identical with esculin (6- $\beta$ -D-glucopyranosyloxy-7-hydroxycoumarin) [2].

This is the first time that these coumarins have been isolated from the herbage of the kidney bean.

#### LITERATURE CITED

1. V. I. Dikhtyarev, V. N. Kovalev, and N. F. Komissarenko, *Khim. Prirodn. Soedin.*, 258 (1982).
2. G. A. Kuznetsova, *Natural Coumarins and Furocoumarins* [in Russian], Leningrad (1967).
3. É. Ya. Martynenko, N. V. Lobko, and N. F. Komissarenko, *Vinodelie Vinogradarstvo, SSSR*, 2, 51 (1982).

#### PHENOLIC COMPOUNDS OF *Artemisia gmelinii*

I. I. Chemesova, L. M. Belenovskaya,  
and L. P. Markova

UDC 547.972

We have studied the phenolic compounds of two samples of the epigeal part of *Artemisia gmelinii* Web. ex Stechm., collected in the early vegetation phase by the resources-prospecting section of a combined Soviet-Mongolian Complex Biological Expedition.

A preliminary comparative study by paper chromatography showed that the qualitative compositions of the two samples were similar. The plant material was extracted with 96% ethanol. The concentrated extract was treated with hot water, and then the aqueous fraction successively with chloroform and ethyl acetate.

The chromatographic separation of the chloroform extract on silica gel (L 100/160  $\mu$ ) with elution by chloroform yielded substance (I), and a mixture of chloroform and ethanol (19:1) gave substance (II). Reseparation of the individual fractions on silica gel (L 40/100  $\mu$ ) gave substances (III), (IV), (V), and (VI). By chromatography on polyamide, the ethyl acetate fraction yielded substance (VII).

Substance (I), with the composition  $C_{10}H_8O_4$ , mp 205°C, and substance (II) with the composition  $C_9H_8O_4$ , mp 104°C, were identified by IR and UV spectroscopy, and also by mixed melting points with authentic samples as scopoletin [1] and caffeic acid, respectively.

Substance (III) had the composition  $C_{17}H_{14}O_5$ , mp 168°C (96% ethanol).  $\lambda_{\max}^{\text{MeOH}}$  (nm): 272, 310 sh., 338; IR spectrum ( $\text{cm}^{-1}$ ): 1665, 1610, 1510, 1200, 1170, 1130, 1100, 840, 820. PMR spectrum ( $\text{CDCl}_3$ ,  $\delta$ , ppm): 7.81 (d, 2 H,  $J = 9$  Hz, H-2', H-6'); 6.97 (d, 2 H,  $J = 9$  Hz, H-5', H-3'); 6.54 (s, 1 H, H-3); 6.46 (d, 1 H,  $J = 2$  Hz, H-8); 6.34 (d, 1 H,  $J = 2$  Hz, H-6); 3.87 and 3.85 (s, s, 3 H each, 2  $\text{OCH}_3$ ). The demethylation product of (III) (pyridine hydrochloride, 170°C, 2 h), on comparison with an authentic sample, proved to be identical with apigenin. Analysis of the facts given and their comparison with the literature [2, 3] permitted the structure of 4',7-di-O-methyl apigenin to be established for (III).

Substance (IV) with the composition  $C_{16}H_{12}O_5$ , mp 262°C (96% ethanol)  $\lambda_{\max}^{\text{MeOH}}$  (nm) 272, 305, 340 and substance (V) with the composition  $C_{17}H_{14}O_7$ , mp 228°C (chloroform),  $\lambda_{\max}^{\text{MeOH}}$  (nm) 250 sh., 274, 348 were identified on the basis of the results of PMR spectroscopy and their IR spectra, and also of a comparison by the chromatographic method with authentic samples, as acacetin [4] and 4',5,7-trihydroxy-3',6-dimethoxyflavone, respectively [5, 6].

Substance (VI) had the composition  $C_{17}H_{14}O_6$ , mp 228°C (ethanol-chloroform);  $\lambda_{\max}^{\text{MeOH}}$  (nm) 245 sh., 255, 272, 349. The results of UV spectroscopy with complex-forming and ionizing additives permitted the assumption of the presence in (VI) of OH groups in the 4' and 5 positions. IR spectrum ( $\text{cm}^{-1}$ ): 3440, 1650, 1600, 1500, 1180, 1169, 1130. PMR spectrum (DMSO,  $\delta$ , ppm): 7.56 (m, 2 H, H-2', H-6'); 6.94 (d, 1 H,  $J = 8$  Hz, H-5'); 6.90 (s, 1 H, H-3); 6.78 (d, 1 H,  $J = 2$  Hz, H-8); 6.26 (d, 1 H,  $J = 2$  Hz, H-6); 3.90 and 3.84 (s, s, 3 H each, 2

---

V. L. Komarov Botanical Institute, Academy of Sciences of the USSR, Leningrad. Translated from *Khimiya Prirodnikh Soedinenii*, No. 3, pp. 384-385, May-June, 1983. Original article submitted December 17, 1982.