PHYTOCHEMICAL STUDY OF Centaurea cyanus

V. I. Litvinenko and V. N. Bubenchikova

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Flavonoid aglycons (quercetin, kaempferol, isorhamnetin, apigenin, luteolin, hispidulin) and their glycosides, and caffeic, chlorogenic, neochlorogenic, and isochlorogenic acids have been isolated from the epigeal part of the Centaurea cyanus L. and have been identified, and ten amino acids have also been identified.

The flowers of the cornflower are used in scientific medicine as diuretic and cholagogic agents [1]. However, the epigeal part of the plant has scarcely been studied and is not used.

Having investigated the epigeal part of the cornflower collected in the flowering phase on the territory of the Tsentral'no-Chernozemnyi Reserve (Kursk province), we have established the presence of 14 flavonoids, four hydroxycinnamic acids, and ten amino acids. As the result of chromatographing the total material from aqueous acetone extracts on columns of polyamide sorbents, six flavonoid aglycons, eight flavonoid glycosides, and four hydroxycinnamic acids were isolated. On the basis of qualitative reactions, IR and UV spectroscopy, the products of acid hydrolysis, and the results of elementary analysis [2], six substances (1-6) were assigned to the class of flavonoids, and eight (7-14) to the class of flavones.

Substances (1, 2, 3, 7, 8, and 9) gave positive Bryant reactions, which showed their aglycon nature, the other flavonoids being glycosides. Substances (4, 5, 6, 10, 11, and 12), according to the results of quantitative acid hydrolysis, comparisons of the UV spectra of the glycosides and their aglycons, and their molecular masses were assigned to the monoglycosides, and substances (13 and 14) to the biosides. D-Glucose was detected in the products of the acid hydrolysis of the monosides, and D-apiose and D-glucose from the biosides. The stepwise acid hydrolysis of the biosides yielded luteolin and apigenin 7-glucoside. Consequently, these glycosides were biosides in which a glucose residue was attached directly to the aglycon and an apiose residue to the glucose. On alkaline hydrolysis of these biosides, no splitting out of a carbohydrate substituent, as is the case with biosides having a 1 → 2-bond between the sugar residues, was observed.

Substance (1) – $C_{15}H_{10}O_7$, mp 310-312°C, UV spectrum: 375, 265 sh., 265 nm – was quercetin [3].

Substance (2) – $C_{15}H_{10}O_6$, mp 275-277°C, UV spectrum: 370, 265 nm – kaempferol [3].

Substance (3) – $C_{16}H_{12}O_6$, mp 311-314°C, UV spectrum: 370, 265 sh., 255 nm; the demethylation of product of substance (3) being identical with an authentic sample of quercetin – was isorhamnetin [3].

Substance (4) – $C_{21}H_{20}O_{12}$, mp 247-249°C, UV spectrum: 375, 255 nm – was quercetin 7-O-β-D-glucoside (quercimeritrin) [4].

Substance (5) – $C_{22}H_{22}O_{12}$, mp 254-258°C, UV spectrum: 370, 255 nm – was isorhamnetin 7-O-β-D-glucoside.

Substance (6) – $C_{21}H_{20}O_{11}$, mp 251-253°C, UV spectrum: 370, 265 nm – was kaempferol 7-O-β-D-glucoside.

Substance (7) – $C_{15}H_{10}O_5$, mp 340-345°C, UV spectrum: 335, 270 nm – was apigenin [5].

Substance (8) – $C_{15}H_{10}O_6$, mp 328-330°C, UV spectrum: 350, 265 sh., 225 nm – was luteolin [6].

Substance (9) – $C_{16}H_{12}O_6$, mp 290-292°C, UV spectrum: 335, 285 nm; the product of the

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demethylation of substance (9) being identical with an authentic sample of scutellarein – was hispidulin [7].

Substance (10) – $C_{21}H_{20}O_{10}$, mp 245-250°C, UV spectrum: 325, 270 nm – was apigenin 4'-O-β-D-glucoside.

Substance (11) – $C_{21}H_{20}O_{10}$, mp 225-227°C, UV spectrum: 335, 270 nm – was apigenin 7-O-β-D-glucoside (cosmossiin) [5].

Substance (12) – $C_{21}H_{12}O_{11}$, mp 266-268°C, UV spectrum: 350, 255 nm – was luteolin 7-O-β-D-glucoside (cynaroside) [5].

Substance (13) – $C_{26}H_{28}O_{14}$, mp 235-237°C, UV spectrum: 335, 270 nm – was apigenin 7-apioglucoside (apiin) [3].

Substance (14) – $C_{26}H_{28}O_{15}$, mp 259-261°C, UV spectrum: 350, 255 nm – was luteolin 7-apioglucoside (graveobioside) [3].

We have previously reported the isolation from cornflowers of hydroxycinnamic acids: caffeic, chlorogenic, and neochlorogenic [8]. On investigating the composition of the hydroxycinnamic acids of the epigeal part, we established the presence of four substances which were identified as caffeic acid (substance (15)), chlorogenic acid (substance (16)), and neochlorogenic acid (substance (17)) [8]. Substance (18) – $C_{16}H_{18}O_9$ – could not be obtained in the crystalline state. R_f 0.45-0.55 (15% CH_3COOH). Fusion with KOH gave protocatechuic acid, and alkaline hydrolysis led to the formation of caffeic and D-quinic acids [9]. A chromatographic comparison of substance (18) with an authentic sample of isochlorogenic acid showed their identity.

The qualitative composition of the amino acids was studied by the procedure of [10]. Arginine, serine, methionine, proline, glutamic acid, tryptophan, alanine, phenylalanine, arginine [sic], and threonine were detected.

EXPERIMENTAL

Melting points were determined on a Kofler stage. Spectral characteristics were obtained on a SF-16 instrument in methanol (UV), and on a UR-20 instrument, using tablets with KBr (IR), and specific rotations were measured on a Zeiss polarimeter.

Analysis of the individual substances was carried out by PC in the following systems: 15%, 30%, 60%, and 2% acetic acids, benzene–ethyl acetate–acetic acid (50:50:1), and butan-1-ol–acetic acid–water (4:1:2).

Isolation of the Flavonoids and Hydroxycinnamic Acids. The comminuted air-dry raw material (3 kg) was exhaustively extracted with 80% acetone (18 liter) in the boiling water bath. The combined extracts were evaporated to an aqueous residue (2 liter) and were freed from lipophilic impurities with carbon tetrachloride (5 × 2 liter). In view of the diversity of the polarities of the complex mixtures of flavonoids, the purified aqueous extract was fractionated by selective extraction with diethyl ether (5 × 2 liter – fraction 1) and with a mixture of butanol and butyl acetate in a ratio of 1:1 (5 × 2 liter – fraction 2). On standing, fraction 2 deposited a precipitate (fraction 3). All the fractions obtained were analyzed by paper chromatography in the given solvent system. Fraction 1 contained flavonoid aglycons; 2, flavonoid glycosides and hydroxycinnamic acids; and 3, flavonoid glycosides. The mixture of flavonoids and hydroxycinnamic acids was separated by successive chromatography on polyamide sorbent. As a result of the chromotography of fraction 1 (with mixtures of chloroform and ethanol having increasing concentrations of the latter), aglycons (1), (2), (3), (7), (8), and (9) of the classes of flavones and flavonols were isolated in the individual state. From fraction 2, aqueous alcohol isolated substances (10) and (12-18), and fraction 3 (with isopropanol as the eluent) yielded substances (4), (5), (6), and (11).

Acid Hydrolysis. A glycoside (substance (4), (6), or one of (10-14)) in an amount of 0.01 g was dissolved in 10 ml of 10% hydrochloric acid in 50% acetic acid, and the solution was heated in the water bath in a flask with a reflux condenser for 2-4 h. After the end of hydrolysis, the reaction mixture was treated in a separatory funnel with diethyl ether. Each ethereal extract was dried with anhydrous sodium sulfate and was evaporated to 2-3 ml, and the aglycons were extracted (with ethyl ether) from the concentrate, while sugars remained in the aqueous solution. As a result of hydrolysis, substance (4) yielded quercetin and D-glucose; substance (5), isorhametin and D-glucose; substance (6), kaempferol and

D-glucose; substance (12), luteolin and D-glucose; substance (13), apigenin, D-glucose, and D-apiose; and substance (14), luteolin, D-glucose, and D-apiose.

To perform stepwise hydrolysis, 0.01 g of one of the glycosides (substance (13) or (14)) was mixed with 10 ml of 0.05% hydrochloric acid and the mixture was heated in a flask with a reflux condenser for 1.5-2 h with the taking of samples after 5, 10, 20, 30, 45, 60, 90, and 120 min followed by chromatography of the products of acid hydrolysis [2] (apigenin 7-glucoside was obtained from substance (13) and luteolin 7-glucoside from substance (14)).

Alkaline Hydrolysis. A solution of 0.02 g of one of the glucosides under investigation (substance (13) or (14)) in 10 ml of 2% aqueous potassium hydroxide was heated in a flask with a reflux condenser in the water bath for 3 h. Samples for analysis were taken after 5, 10, 20, 30, 60, and 120 min, and these were neutralized with hydrochloric acid to pH 3-5 and were chromatographed in 15- and 30% acetic acids [11]. No changes were observed in the initial glycosides.

Demethylation. A 0.01 g sample of substance (3) or (9) was dissolved in a melt of pyridinium hydrochloride (2-3 g) and the solution was heated at 180-220°C in a flask of a reflux condenser for 1 h. Then the reaction mixture was rapidly cooled to 20-25°C and was diluted with a 10-fold volume of distilled water. The precipitates were separated off and were crystallized from ethanol [12]. As a result, substance (3) yielded quercetin, and substance (9) scutellarein.

Identification of the Amino Acids. The amino acids were detected in an aqueous extract from the epigeal part of the plant by paper chromatography in the butan-1-ol–acetic acid–water (4:1:2) system in comparison with authentic samples. The chromatograms were revealed with a 0.2% ethanolic solution of ninhydrin with heating in a drying chest at 100-105°C for several minutes. Red-violet spots indicating the presence of amino acids [10] were observed.

CONCLUSIONS

From the epigeal part of the cornflower Centaurea cyanus L. six flavonoid aglycons (quercetin, kaempferol, isorhamnetin, apigenin, luteolin, and hispidulin), eight flavonoid glucoside (quercetin, kaempferol, isorhamnetin, apigenin, luteolin, and hispidulin), eight flavonoid glucoside (quercimeritrin, isorhamnetin 7-O-β-D-glucoside, kaempferol 7-O-β-D-glucoside, apigenin 4'-O-β-D-glucoside, cosmossiin, cynaroside, apiin, and graveobioside), and four hydroxycinnamic acids (caffeic, chlorogenic, neochlorogenic, and isochlorogenic acids) have been isolated in the individual state and have been identified, and ten amino acids have also been identified. This is the first time that kaempferol, luteolin, hispidulin, isorhamnetin, apigenin 4'-O-β-D-glucoside, isorhamnetin 7-O-β-D-glucoside, kaempferol 7-O-β-D-glucoside, and isochlorogenic acid have been isolated from the cornflower.

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