Experimenteller Teil

1. Allgemeiner Teil und Geräte [6]

2. 2-Isopropyl-5-methyl-1,4-benzochinon-arylimine 8, 11 und 12

Die Lösung von 5 mmol Sulfonamid **6** oder **10** und 5 mmol Thymol (**4**) in 70 ml 3 M wässriger Natronlauge wird bei Raumtemperatur und unter Rühren tropfenweise mit 2,5 ml 13% iger Natriumhypochloritlösung versetzt. Nach einer Stunde Rühren bei Raumtemperatur wird das jeweilige Farbprodukt erschöpfend mit Essigsäureethylester extrahiert, die organische Phase mit Wasser neutral gewaschen, mit Natriumsulfat getrocknet und nach Einengen im Vakuum sc aufgearbeitet. Sc-/Dc-Fließmittel: Essigsäureethylester/Cyclohexan (50 + 1).

2.1. 2-Isopropyl-5-methyl-1,4-benzochinon-4-[(2-aminophenyl)imin] (**8**)

Ausbeute: 195 mg (15%) blauschwarze Kristalle vom Schmpt. 78 °C. Dc: $R_f = 0.68$. MS (E.I., 70 eV) m/z (rel. Int.): 254 (M^+, 11), 239 (7), 221 (9), 211 (44), 196 (19), 106 (68), 53 (61), 43 (100). IR (KBr, cm^{-1}): 3320, 1642, 1619, 1604, 1510. ^{1}H NMR ($CDCl_3$, δ, ppm): 0,95 (d, $^3J = 6,2$ Hz, 6 H, $CH(CH_3)_2$); 2,21 (s, 3 H, CH_3); 2,85 (sept., $^3J = 6,2$ Hz, 1 H, $CH(CH)_2$); 3,95 (s, breit, 2 H, NH_2); 6,62 (s, 1 H, 6-H); 6,72 (d, $^3J = 8,4$ Hz, 1 H, 5′ oder 6′-H); 6,81 (d, $^3J = 8,4$ Hz, 1 H, 5′ oder 6′-H); 6,85–6,95 (m, 2 H, 3′, 4′-H); 7,04 (s, 1 H, 3-H); UV/Vis (CH_2Cl_2, nm): λ_{max} (log ε) = 275 (4,22), 320 (sh), 507 (3,77).
$C_{16}H_{18}N_2O$ (254,3)

2.2. 2-Isopropyl-5-methyl-1,4-benzochinon-4-[(4-amino-6-chlor-3-sulfamoylphenyl)imin] (**11**)

Ausbeute: 120 mg (7%) magenta Kristalle vom Schmpt. 224 °C. Dc: $R_f = 0,20$. MS (E.I., 70 eV) m/z (rel. Int.): 369 (M^+, ^{37}Cl, 17), 367 (M^+, ^{35}Cl, 28), 332 (62), 304 (24), 317 (100), 290 (26), 120 (41), 92 (44), 42 (27). IR (KBr, cm^{-1}): 3300, 2990, 2940, 1725, 1660, 1640, 1590. ^{1}H MNR (DMSO-d_6, δ, ppm): 1,14 (d, $^3J = 6,8$ Hz, 6 H, $CH(CH_3)_2$); 2,35 (s, 3 H, CH_3); 3,13 (sept., $^3J = 6,2$ Hz, 1 H, $CH(CH_3)_2$); 3,95 (s, breit, 2 H, NH_2); 6,71 (s, 1 H, 6-H); 6,98 (s, 1 H, 3-H); 7,12 (s, breit, 1 H, 5′-H); 7,23 (s, 1 H, 2′-H); UV/Vis (CH_2Cl_2, nm): λ_{max} (log ε) = 277 (4,09), 309 (sh), 514 (3,88).
$C_{16}H_{18}N_3O_3CIS$ (369,9, 367,9)

2.3. 2-Isopropyl-5-methyl-1,4-benzochinon-4-[(4-hydroxy-5-isopropyl-2-methyl-phenyl)imin] (**12**)

Ausbeute: 234 mg (15%) blauviolette Kristalle vom Schmpt. 134 °C nach sc Isolierung mit Diethylether/Essigsäureethylester (5 + 2). Dc (Diethylether/Essigsäureethylester (5 + 2)): $R_f = 0,80$. MS (E.I., 70 eV) m/z (rel. Int.): 311 (M^+, 65), 293 (19), 253 (23), 164 (17), 135 (20), 90 (39), 41 (52), 39 (100). IR (KBr, cm^{-1}): 3340, 2970, 1635, 1610, 1605, 1520. ^{1}H NMR ($CDCl_3$, δ, ppm): 1,04 (d, $^3J = 7,1$ Hz, 6 H, $CH(CH_3)_2$), chinoid); 1,22 (d, $^3J = 6,6$ Hz, 6 H, $CH(CH_3)_2$, aromat.); 2,19 (s, 3 H, CH_3, chinoid); 2,30 (s, 3 H, CH_3, aromat.); 3,08 (sept., $^3J = 7,1$ Hz, 1 H, $CH(CH_3)_2$, chinoid); 3,25 (sept., $^3J = 6,6$ Hz, 1 H, $CH(CH_3)_2$, aromat.); 5,67 (s, breit, 1 H, OH), 6,46 und 6,91 (je 1 s, 1 H, 3, 6-H), 6,56 und 6,74 (je 1 s, 1 H, 3′, 6′-H). UV/Vis (CH_2Cl_2, nm): λ_{max} (log ε) = 274 (4,07), 340 (sh), 555 (3,93).
$C_{20}H_{25}NO_2$ (311,4)

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Capillary electrophoretic analysis of hydroxycinnamic acids from *Ononis arvensis* L.

J. Spilková [1], P. Bednář [2] and R. Štroblíková[1]

Ononis arvensis L. (*Fabaceae*) [1] has been widely cultivated for the production of the drug *Radix ononidis* used in the treatment of urinary tract infections [2]. Constituents of roots of *O. arvensis* L. are very similar to those of the roots of *O. spinosa* L. [3], both species can yield the drug *Radix ononidis* [4].

The main constituents of the roots are the triterpene alcohol α-onocerin [5], the isoflavonoids ononin [6], formononetin, onogenin, trifolirhizin [7] and the flavonols kaempferol and trifolin [7].

The aerial part has been used in folk medicine to treat of urinary tract infections and skin diseases. The main constituents are ononin [6] quercetin and its glycosides and α-onocerin [3].

In plants, flavonoids are often accompanied by phenolcarboxylic acids such as the analogues of cinnamic acid. They are widely distributed in medicinal plants, fruits and vegetables and have been studied as potential antioxidants of plant origin.

Capillary electrophoresis has been used for the analysis of these compounds in recent time [8]. We used this analytical method for the identification and simple control of the content of caffeic, chlorogenic and ferulic acids in *O. arvensis*.

Chlorogenic acid, caffeic acid and ferulic acid were identified in the methanolic extracts of *Ononidis radix* and *Ononidis herba*. Both, the identification in the electrograms and the control of the content were carried out using the standard addition of the studied acid. Two analyses were performed. The extract was analysed at first run and both the extract and the standard solution were injected in the second one.

A linear relationship between the injected volume and the time of injection (Hagen – Poiseuille equation [10]) can be considered under the same experimental conditions (constant viscosity of the analyte, fast and reproducible velocity of the vacuum establishment) so it was possible to use the following equation to determine the content:

$$c = \frac{A \cdot c_{st} \cdot t_{st}}{A' \cdot (t + t_{st}) - A \cdot t}$$

where A or A′, is the peak area of the studied component in the analysed mixture or the peak area of the studied compound plus standard addition, respectively, t or t_{st} is the injection time of analyte or the standard addition, respectively and c or c_{st} is the concentration of the component in the analysed mixture or the concentration of the standard solution, respectively.

Electropherogram of methanolic extract of *Ononidis radix is* shown in the Fig. The contents of hydroxycinnamic acids are given in the Table. The contents of hydroxycinnamic acids in *Ononidis radix* and *Ononidis herba* is in the range of 10^{-3}–10^{-2}%. The aerial part of *O. arvensis* contains approximately twice the amounts of hydroxycinnamic acids than the roots.

The present study demonstrates the successful use of capillary electrophoresis for the determination of caffeic

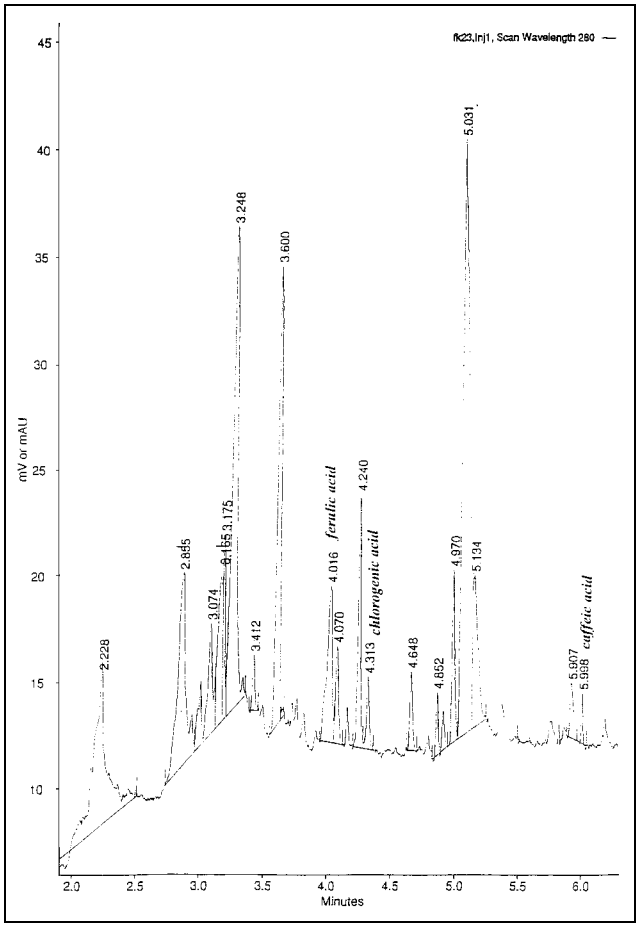

Fig.: Electropherogram of methanolic extract of *Ononidis radix*

Table: Contents of studied phenolic acids in Radix et Herba ononidis

Acid	Radix (%)	Herba (%)
Caffeic	2.7×10^{-3}	3.2×10^{-3}
Chlorogenic[a]	4.2×10^{-3}	5.2×10^{-3}
Ferulic	1.6×10^{-2}	3.5×10^{-2}

Determination at 280 nm,of chlorogenic acid at 320 nm
Electropherogram of methanolic extract of *Ononidis radix*

acid, chlorogenic acid and ferulic acid in the extracts from *O. arvensis* roots and aerial parts. The described method may serve as a valuable tool in assessing the quality of phytopharmaceutical products from *Ononis arvensis*.

Experimental

1. Chemicals

Solutions were prepared from chromatographic or analytic reagent-grade chemicals (Lachema, Brno, Czech Rep.; Merck, Darmstadt, Germany). As standards were used caffeic acid, ferulic acid and chlorogenic acid (all p.a., Fluka, Buchs, Switzerland) in methanol at a concentration of 0.01 mg · ml^{-1}; 0.1 mol · l^{-1} borate buffer (pH = 9.5, adjusted with 0.2 mol · l^{-1} sodium hydroxide) was used as background electrolyte for all the experiments.

2. Extraction of hydroxycinnamic acids from the plants

Hydroxycinnamic acids were extracted from 20 g of dried, pulverised roots and aerial parts of *O. arvensis* as described [9]. The dried extracts thus obtained were dissolved in 1 ml of methanol.

3. Apparatus and capillary conditioning

All the analyses were performed on a Spectraphoresis 100 with UV/VIS detection (Thermoseparation products, USA) equipped with a 75 cm fussed-silica capillary of I.D. 75 μm; (effective length 45 cm). The detection was carried out at 280, resp. 320 nm. The inlet electrode was the anode. Anions of phenolcarboxylic acids were taken along by the electroosmotic flow to the detector. The capillary was flushed with 1 mol · l^{-1} NaOH, deionized water and running buffer before analysis, 5 min each of them. Samples were loaded by applying a vacuum (injection time 0.2–0.6 s). The applied voltage was adjusted at 30 kV. The current was approximately 83 μA.

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