FLAVONOIDS FROM *Ephedra sinica* STAPF

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From the above-ground part of *Ephedra sinica* STAPF seven flavonoids were isolated — apigenin, tricin, kaempferol, apigenin-5-rhamnoside, herbacetin, and 3-methoxyherbacetin; one flavonoid glycoside was characterized as kaempferol rhamnoside.

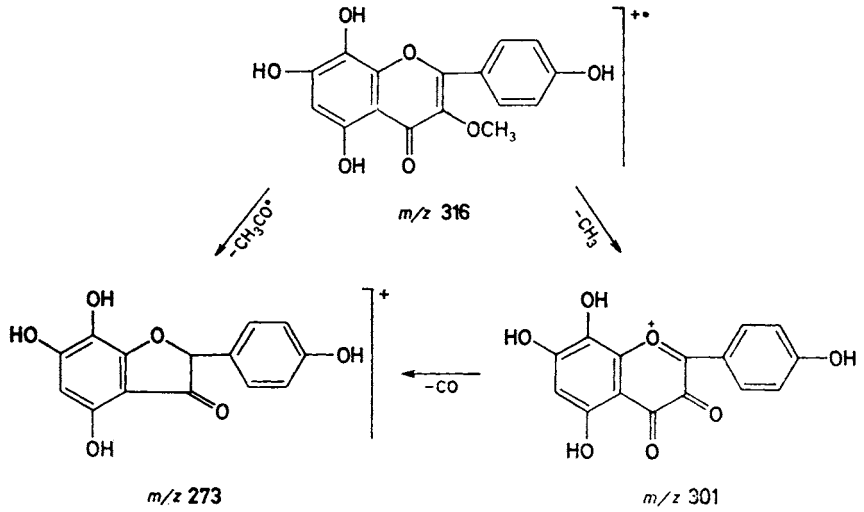
The presence of alkaloids related to ephedrine is characteristic of the species of *Ephedra*. The best known species is *Ephedra sinica* STAPF, growing in Mongolia and adjacent Chinese provinces. It has been used in China for several millenia in the form of a preparation called "ma huang" as a hypotensive, antiasthmatic, sympathomimetic and adstringent drug[1].

Similarly as in other *Ephedra* species other components (except alkaloids) were not much investigated (cf. ref.[2]). Therefore we were interested in flavonoid compounds from the above-ground part of the plant, collected in Mongolia. A methanolic extract, displaying the presence of nine flavones in two-dimensional paper chromatography, was extracted with chloroform, ethyl acetate and butanol. The ethyl acetate extract was chromatographed on silica gel and individual fractions were further separated by preparative thin-layer chromatography. For characterization of individual components conventional spectral methods (UV, IR) and mass spectra[3] were used.

In this way, tricin, kaempferol, apigenin, herbacetin and further apigenin-5-O--rhamnoside were isolated. The flavonoids present in dry material amounted to 0·3%, of which apigenin and tricin represented half the sum of the flavonoids, being present in an approximately 1 : 1 ratio.

In addition to these substances we also succeeded in proving the presence of an aglycone which according to IR spectrum contained a methoxy group. The presence of this group also followed from the ^{1}H NMR spectrum (3·89 s). The UV data indicated the presence of OH groups on carbon atoms C-5 nad C-7. The difference between the main absorption maxima of the methanolic solution was 52 nm, which indicated the presence of a substituent on C-3 (ref.[3]). The mass spectrum gave the

composition $C_{16}H_{12}O_7$ (molecular ion m/z 316), which corresponds to an aglycone with four hydroxy and one methoxy group. The fragmentation represented in Scheme 1 shows that the position of the methoxy group is at C-3. These data indicated that the compound is 3-methoxyherbacetin, described quite recently for the first time by Reitman and James[4].



SCHEME 1

Finally the presence of a flavonoid glycoside was detected, which gave kaempferol and rhamnose on acid hydrolysis. The very small amount of the glycoside prevented us from determining the position of glycosylation in kaempferol.

EXPERIMENTAL

The melting points were determined on a Kofler block. The UV spectra were measured on a Specord UV-VIS (Zeiss, Jena) in methanol. The IR spectra were taken on a UR-20 (Zeiss, Jena) instrument, in chloroform, and the mass spectra on an AEI/MS 902 instrument, at 70 eV. For paper chromatography Whatman No 1 paper was used and for TLC silica gel Merck CF_{254}. For comparison authentic samples of apigenin, tricin, kaempferol and herbacetin were used.

Isolation of Flavonoids

The plant was collected in the Central Gobi ajmak (province), about 10 km east of Mandalgovi (Mongolia). The herbarium specimen is deposited in the Chemical Institute of the Mongolian Academy of Sciences, Ulan Batar. The dried and ground aerial part of the plant (2·5 kg) was thoroughly extracted with methanol. After evaporation of the solvent in a vacuum the residue was extracted consecutively with chloroform, ethyl acetate and butanol. The fraction obtained with ethyl acetate (26·2 g) was chromatographed on silica gel (1·0 kg) with chloroform–ethyl acetate and ethyl acetate–methanol (increasing polarity). Further separation of the mixture of

flavonoids was carried out by preparative paper chromatography (using the upper phase of the mixture 1-butanol–acetic acid–water 4 : 1 : 5) and TLC on silica gel (benzene–methanol 8 : 2 or chloroform–methanol 8 : 2).

Characterization of Flavonoids

Tricin. Yellow needles, m.p. 283–285°C (ref.[5] 291–292°C). UV spectrum λ_{max}, nm (MeOH): 242, 267, 354; (MeOH–NaOMe): 263, 275, 322 sh, 418 sh; (MeOH + CH_3COONa): 272, 320 sh, 368; (MeOH + CH_3COONa–H_3BO_3): 287, 354; (MeOH + $AlCl_3$): 253, 276, 303, 368, 392; (MeOH + $AlCl_3$–HCl): 253, 276, 303, 362, 382. Mass spectrum: 330 (M^+-$C_{17}H_{14}O_7$). IR spectrum (cm^{-1}): 3 200–3 460 (OH); 2 855, 2 960 (OCH_3); 1 670 (CO); 1 520, 1 470, 1 625 (arom.).

Kaempferol. M.p. 278–280°C (ref.[5] 275–277°C). UV spectrum, λ_{max}, nm (MeOH): 266, 297 sh, 322, 368; (MeOH + NaOMe): 277, 416; (MeOH + $AlCl_3$): 267, 300 sh, 350, 422; (MeOH + $AlCl_3$–HCl): 268, 300 sh, 346, 423; (MeOH + CH_3COONa): 272, 303 sh, 389; (MeOH + CH_3COONa + H_3BO_3): 267, 370. Mass spectrum: 286 ($M^+ - C_{15}H_{10}O_6$). In paper chromatography, using two systems[3] and comparison with a standard, R_F 0·75 and 0·03.

Apigenin. M.p. 346–347°C (ref.[5] 347°C). UV spectrum, λ_{max}, nm (MeOH): 268, 300 sh, 336; (MeOH + NaOMe): 275, 324, 394; (MeOH + CH_3COONa): 274, 300, 378; (MeOH + + CH_3COONa + H_3BO_3): 268, 300 sh, 338; (MeOH + $AlCl_3$): 276, 300, 348, 386; (MeOH + + $AlCl_3$ + HCl): 276, 298, 342, 384.

Herbacetin. M.p. 259–273°C (ref.[5] 280–283°C). UV spectrum, λ_{max}, nm (MeOH): 275, 327, 386; (MeOH + NaOMe): decomp.; (MeOH + $AlCl_3$): 295, 335, 370, 490; (MeOH + $AlCl_3$ + + HCl): 270, 280, 365, 447; (MeOH + CH_3COONa): decomp.; (MeOH + CH_3COONa + + H_3BO_3): decomp.

Apigenin-5-O-rhamnoside. UV spectrum, λ_{max}, nm (MeOH): 372, 304 sh, 332; (MeOH + + NaOMe): 281, 326, 394; (MeOH + $AlCl_3$): 276, 304, 350, 384 sh; (MeOH + $AlCl_3$ + HCl): 277, 305, 350, 376 sh; (MeOH + CH_3COONa): 278, 300 sh, 354; (MeOH + CH_3COONa + + H_3BO_3): 275, 345. The hydrolysis of the glycoside was carried out in 5% H_2SO_4 in a standard manner[3], while the identification of the sugar component was carried out by TLC in ethyl acetate–pyridine–water (12 : 5 : 1), and comparison with a standard.

3-Methoxyherbacetin. UV spectrum, λ_{max}, nm (MeOH): 278, 320, 330; (MeOH + CH_3ONa): 275, 324, 375; (MeOH + $AlCl_3$): 264, 276, 303, 362, 368; (MeOH + $AlCl_3$ + HCl): 264, 274, 303, 358, 368; (MeOH + CH_3COONa): 284, 303, 378; (MeOH + CH_3COONa + H_3BO_3): 274, 300 sh, 338. Mass spectrum: 316 (M^+), 301 (M–15), 273 (M–43). IR spectrum (cm^{-1}): 3 200–3 400 (OH); 2 870–2 890 (OCH_3); 1 670 (CO); 1 625, 1 575, 1 500 (arom.). ^1H NMR spectrum ($C^2H_3SOC^2H_3$): 3·89 s, 3 H (OMe); 6·56 s, 1 H (H-6); 6·90 d, 2 H (H-3′, H-5′); 8·24 d, 2 H (H-2′, H-6′).

Kaempferol rhamnoside. UV spectrum, λ_{max}, nm (MeOH): 268, 328, 368; (MeOH + CH_3ONa): 279, 323, 408; (MeOH + $AlCl_3$): 270, 357, 413; (MeOH + $AlCl_3$ + HCl): 267, 357 sh, 382; (MeOH + CH_3COONa): 277, 389; (MeOH + CH_3COONa + H_3BO_3): 275, 369. IR spectrum (cm^{-1}): 3 300–3 450 (OH); 2 860–2 950 (OCH_3); 1 660 (CO); 900, 1 040, 1 070 (glycosidic component). Mass spectrum (aglycone): 286 (M^+), 121, 153. The hydrolysis of the glycoside was carried out in a similar manner as in the above-mentioned apigenin-5-O-rhamnoside.

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