Constituents of propolis of Czechoslovak origin. V*

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The ethanolic extract of crude propolis was divided into five fractions by treatment with mutually immiscible solvents, namely into the interphase, and methanolic, ethyl acetate, light petroleum, and aqueous portions. Separation of the interphase afforded a new flavonoid — 3,7-dihydroxy-5--methoxyflavone (galangin 5-methyl ether), whilst the methanolic portion furnished (2*R*,3*R*)-3,7-dihydroxy-5-methoxyflavanone (pinobanksin 5--methyl ether), and (2*E*,4*E*)-5-phenyl-2,4-pentadienoic acid (cinnamylideneacetic acid). Structure of these products was determined by spectral means.

Из этанольного экстракта прополиса экстракцией во взаимно не смешивающихся растворителях было получено пять фракций: граничная, метанольная, этилацетатная, петролейно-эфирная и водная. Хроматографическим делением из граничной фракции был получен новый флавоноид — 3,7-дигидрокси-5-метоксифлавон (5-метиловый эфир галангина), а из метанольной фракции (2*R*,3*R*)-3,7-дигидрокси-5-метоксифлаванон (5-метиловый эфир пинобанксина) и (2*E*,4*E*)-5-финил--2,4-пентадиеновая кислота (циннамилиденуксусная кислота). Строение выделенных соединений было установлено на основании спектроскопических данных.

Propolis is a polycomponent product of bee activity; it has been utilized especially in folk medicine and abroad, also in pharmaceutical industry and cosmetology. The principal components of propolis were reported to be aromatic acids, alcohols, aldehydes, and flavonoid aglycones [1].

The ethanolic extract of propolis evaporated to dryness under reduced pressure was dissolved in chloroform and taken into water. The insoluble interface

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was removed as an interphase. The aqueous portion was repeatedly extracted with ethyl acetate. The chloroform solution was evaporated and the solid residue was shaken with a light petroleum and 90 % methanol mixture [2]. The interphase and the methanolic portion were separately chromatographed on a silica gel-packed column. Rechromatography of the selected fractions on polyamide and Sephadex LH-20 afforded compounds of molecular formulas $C_{16}H_{12}O_5$, and $C_{16}H_{14}O_5$ and $C_{11}H_{10}O_2$ from the interphase and methanolic portion, respectively.

The first compound was amorphous, light brown in colour, its melting point was 301—304 °C. Its UV spectrum in methanol showed bands at $\lambda = 260$ nm and 350 nm (band II and I), characteristic of 3-hydroxyflavones. A bathochromic shift of band I by 60 nm evoked by addition of $AlCl_3$ solution remained unchanged after addition of hydrochloric acid. This shift is typical of the presence of a free hydroxyl group at C-3 in the vicinity of a C-4 carbonyl. Addition of sodium methoxide associated with a bathochromic shift of band I by 45 nm and formation of a little intense band at $\lambda = 320$ nm were indicative of the presence of a free hydroxyl group at C-7. This fact was also evidenced by a bathochromic shift of band II caused by addition of sodium acetate.

The IR spectrum of this compound revealed strong vibration bands of a hydroxyl group, a double C=C bond, and out-of-plane vibrations of hydrogens of an aromatic ring. On the other hand, the vibration band of a carbonyl is very low, and is, at the same time shifted towards higher wavenumbers, as usually found with flavonoids.

The molecular formula of this compound was determined by high-resolution mass spectral measurement. The presence of intense peaks at m/z M − H_2O, M − CO, and M − CHO evidenced the methoxyl group at C-5, *i.e.* in the neighbourhood of the C-4 carbonyl, with which it interacted under cleavage of carbon monoxide from C-3. The intense peak at m/z M − CO − H_2O is in favour of the previous statement.

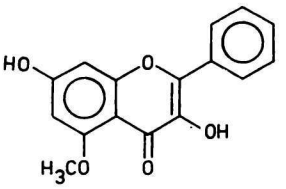
The methoxyl group was located at C-5 also from the arguments of ^{1}H NMR spectral data. The position of H-6 signal in the decoupled spectrum remained unchanged after irradiation, and simultaneously, the signal of the hydroxyl hydrogen at $\delta \approx 12$ ppm, typical of a hydroxyl at C-3, missed in the plain spectrum. According to spectral data this compound was assigned the structure of 3,7-dihydroxy-5-methoxyflavone (galangin 5-methyl ether, *I*). Compound *I* was isolated from the natural material for the first time.

The UV spectrum of the second compound, obtained from the methanolic portion revealed an absorption band at $\lambda = 288$ nm and shoulders at $\lambda = 240$ nm and 380 nm, typical of dihydroflavonols. Addition of $AlCl_3$ solution did not cause any shift of the band I, and consequently, no free hydroxyl group at C-5 could be present; on the other hand, addition of either sodium methoxide

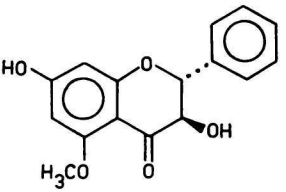
or acetate resulted in a bathochromic shift of band II, which indicated the presence of a free hydroxyl group at C-7. The IR spectrum showed significant vibration bands attributable to the respective hydroxyl and carbonyl groups, and out-of-plane vibrations of hydrogens of an aromatic ring.

Intense peaks in its mass spectrum at m/z M − CHO, M − C_6H_6 − CHO, and M − C_8H_7O evidenced the presence of hydroxyl and methoxyl groups in ring A and an unsubstituted ring B of the flavonoid under investigation.

The CD curve disclosed two positive maxima at $\lambda = 221$ nm and 329 nm and a negative one at $\lambda = 284$ nm. The course of the CD curve was analogous with that of pinobanksin ((2*R*, 3*R*)-3,5,7-trihydroxyflavanone), and therefore, the second compound (*II*) is its 5-methyl ether. So far, this substance was reported to be a constituent of propolis of Bulgarian origin [3].



I



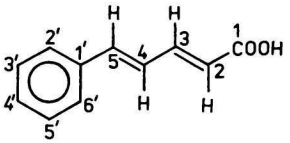
II

Rechromatography of another fraction of the methanolic portion over silica gel afforded colourless flaky crystals (from benzene—acetone). The IR spectrum revealed absorption bands ascribable to vibrations of out-of-plane hydrogens of an aromatic ring, and further bands of a CH=CH (*trans*) arrangement and hydroxyl groups, and a diffuse one of a carbonyl group.

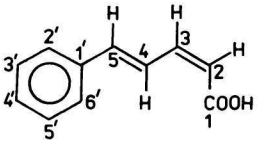
The high-resolution mass spectral measurement determined the molecular formula as being $C_{11}H_{10}O_2$; moreover, one active hydrogen was found after labellation.

The chemical shift and coupling constant data of the alkene hydrogens in the ^{1}H NMR spectrum could not be directly interpreted and therefore, they were obtained by analysis of the four-spin system by an ABCD simulation-interactive SPINI method available with the Varian XL-200 spectrometer. The ^{13}C NMR shifts were gained from the proton—decoupled ^{13}C NMR spectra; the respective resonances were attributed both from their signal intensities and by comparison with chemical shift values of structurally close compounds. Geometrical arrangement at the C=C double bond was determined from coupling constants of the native and the synthesized compounds. The $J_{2,3} = 15.2$ Hz and $J_{4,5} = 15.6$ Hz values evidently indicated the *trans*, *trans* arrangement. The synthetic *cis*, *trans* isomer had $J_{2,3} = 11.4$ Hz and $J_{4,5} = 15.6$ Hz. Signals of

protons of the monosubstituted benzene ring of the natural compound were found at $\delta = 7.32$—7.51 ppm, whereas those of the synthetic *cis*, *trans* isomer at $\delta = 7.28$—7.58 ppm.



III



IV

According to arguments presented, the structure of this compound was shown to be (2*E*, 4*E*)-5-phenyl-2,4-pentadienoic acid (*III*). This aromatic acid has not been reported in propolis as yet.

Experimental

Melting points were determined on a Boetius micro hot-stage, the UV spectra of methanolic solutions and the IR spectra of KBr pellets were measured with UV VIS Specord (Zeiss, Jena) and Perkin—Elmer, model 477 spectrophotometers, respectively. The mass spectra were recorded with an AEI-MS 902 apparatus. The ^{13}C NMR spectra of deuterodimethyl sulfoxide solutions and the ^{1}H NMR spectra of deuteromethanolic solutions were taken with the respective Bruker 300 MHz (FT-mode) and Varian XL-200 (200 MHz, FT-mode) instruments, tetramethylsilane being the internal reference. The CD curve of methanolic solutions was traced with Jobin—Yvon Mark V spectrometer.

The representation of components in fractions on Silufol UV-254 sheets was visualized with a UV $\lambda = 365$ nm light; solvent systems: benzene—ethyl acetate—formic acid ($\varphi_r = 7:3:1$) for compounds *I* and *II*, and benzene—butanone ($\varphi_r = 98:2$) for compound *III*. Carriers used for column chromatography: *a*) silica gel Silpearl floated according to [4], solvent systems: benzene—ethyl acetate ($\varphi_r = 9:1$ to $7:3$) for compounds *I* and *II*, and benzene—butanone ($\varphi_r = 95:5$) for *III*; *b*) polyamide Woelm, solvent system benzene—butanone—methanol ($\varphi_r = 100:1:1$ to $100:8:8$); *c*) Sephadex LH-20, solvent system methanol—water ($\varphi_r = 8:2$). Chemicals of anal. grade (Lachema, Brno) were employed for the synthesis of samples for comparison.

3,7-Dihydroxy-5-methoxyflavone (I)

The interphase prepared according to [2] was chromatographed on silica gel No. 3 with benzene—ethyl acetate, the individual fraction volume being 50 cm^3. Rechromatography of fraction *2* on a polyamide-packed column and purification over Sephadex afforded a light-brown amorphous compound (22 mg), m.p. = 301—304 °C, $R_f = 0.30$.

UV spectrum (CH_3OH), λ/nm (log (ε/(m^2 mol^{-1}))): 238 sh, 260 (4.23), 280 sh, 350 (4.20), 410 sh; λ_{max}($AlCl_3$): 227 sh, 249, 304 sh, 326, 410; λ_{max}($AlCl_3$ + HCl): 227 sh, 249, 304 sh, 326, 410; λ_{max}(NaOMe): 245 sh, 275, 330 sh, 395; λ_{max}(NaOAc): 275, 373; λ_{max}(NaOAc + + H_3BO_3): 264, 282 sh, 304 sh, 345, 413. IR spectrum (KBr), $\tilde{\nu}$/cm^{-1}: 3240, 2940, 2840, 1740, 1600, 1520, 1270, 825, 770. Mass spectrum, *m/z*: 284 (M^+), 265, 256, 255, 238, 105, 77.^{1}H NMR spectrum, δ/ppm: 10.7 (bs, 1H, C-3—OH), 8.8 (bs, 1H, C-7—OH), 8.13 (2H, H-2′, H-6′), 7.43—7.56 (m, 3H, H-3′, H-4′, H-5′), 6.52 (d, 1H, $J_{6,8}$ = 2 Hz, H-8), 6.37 (d, 1H, H-6), 3.85 (s, 3H, C-5—OCH_3).

3,7-Dihydroxy-5-methoxyflavanone (II)

The methanolic portion obtained according to [2] was chromatographed on silica gel No. 4; the fraction containing the title product was purified on polyamide. Recovery: 25 mg of flaky crystals, m.p. = 248 °C (subl. at 225 °C), R_f = 0.25. UV spectrum (CH_3OH), λ/nm (log (ε/(m^2 mol^{-1}))): 230 sh, 240 sh, 288 (3.88), 320 sh; λ_{max}($AlCl_3$): 240 sh, 315; λ_{max}($AlCl_3$ + HCl): 230 sh, 240 sh, 288, 320 sh; λ_{max}(NaOMe): 250, 327; λ_{max}(NaOAc): 255 sh, 325; λ_{max}(NaOAc + H_3BO_3): 230 sh, 240 sh, 288, 320 sh. IR spectrum (KBr), $\tilde{\nu}$/cm^{-1}: 3440, 3200, 3050, 2940, 1650, 1590, 1250, 820. Mass spectrum, *m/z*: 286 (M^+), 257, 179, 167, 138, 120, 91. CD spectrum (CH_3OH), Θ/°: 841 (329), −2112 (284), 6732 (221).

Natural (2E,4E)-5-phenyl-2,4-pentadienoic acid (III)

The methanolic portion as above was chromatographed on silica gel No. 3 and the proper fraction was rechromatographed on silica gel No. 4 to afford compound *III* (106 mg) as colourless crystals, m.p. = 128—130 °C, R_f = 0.32. UV spectrum (CH_3OH), λ/nm (log (ε/(m^2 mol^{-1}))): 225 (0.96), 231 (0.99), 238 (0.75), 301 (1.18). IR spectrum (KBr), $\tilde{\nu}$/cm^{-1}: 3400, 3055, 3020, 2400—2900 (diff.), 1680, 1620, 1002, 960, 756, 718. Mass spectrum, *m/z*: 174 (M^+), 157, 129, 128, 85, 83. ^{1}H NMR spectrum, δ/ppm: 7.32—7.51 (m, 5H, H-2′, H-3′, H-4′, H-5′, H-6′), 6.95 (1H, $J_{4,5}$ = 15.6 Hz, $J_{3,5}$ = −0.9 Hz, $J_{2,5}$ = 0.7 Hz, H-5), 6.91 (1H, $J_{3,4}$ = 11.2 Hz, $J_{2,4}$ = −0.7 Hz, H-4), 6.55 (1H, $J_{2,3}$ = 15.2 Hz, H-3), 6.00 (1H, H-2). ^{13}C NMR spectrum, δ/ppm: 135.79 (C-1′), 128.83 (C-2′, C-6′), 127.34 (C-3′, C-5′), 125.93 (C-4′), 146.96 (C-5), 120.23 (C-4), 141.63 (C-3), 129.30 (C-2), 172.30 (C-1).

Synthetic (2E,4E)-5-phenyl-2,4-pentadienoic acid (III)

This compound was synthesized according to [5] from cinnamic aldehyde (9.0 g) and malonic acid (9.0 g) in quinoline (20 cm^3) at 130 °C, till evolution of CO_2 ceased. The mixture was then poured onto crushed ice, acidified with dilute H_2SO_4 (300 cm^3) and after 1 h the product was worked up and crystallized from ethanol—water (φ_r = 7 : 3) to give 4.0 g of *III*. The spectral data were identical with those of the isolated compound *III*.

Synthetic (2E,4Z)-5-phenyl-2,4-pentadienoic acid (IV)

This acid was obtained according to [5] from cinnamic aldehyde (9.0 g) and malonic acid (9.0 g) by heating in pyridine (20 cm^3) at 100 °C for 6 h. The mixture was worked up as above to give 6.8 g of compound *IV*, m.p. = 165 °C. ^{1}H NMR spectrum, δ/ppm: 7.28—7.58 (m, 5H, H-2′, H-3′, H-4′, H-5′, H-6′), 6.88 (1H, $J_{4,5} = 15.6$ Hz, $J_{3,5} = -0.7$ Hz, $J_{2,5} = 0.9$ Hz, H-5), 8.11 (1H, $J_{3,4} = 11.5$ Hz, $J_{2,4} = -1.1$ Hz, H-4), 6.86 (1H, $J_{2,3} = 11.4$ Hz, H-3), 5.76 (1H, H-2). ^{13}C NMR spectrum, δ/ppm: 136.10 (C-1′), 128.76 (C-2′, C-6′), 127.65 (C-3′, C-5′), 124.80 (C-4′), 147.11 (C-5), 116.49 (C-4), 142.45 (C-3), 129.25 (C-2), 171.98 (C-1).

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