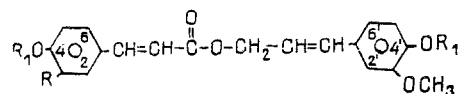


In a study of the composition of propolis of the "aspen" type and its source - secretions of the axillary buds of the *Populus tremula* - three new conjugates have been isolated and identified: the ferulate, p-coumarate, and p-hydroxybenzoate of trans-coniferyl alcohol. The structures of the compounds were shown by the methods of NMR and low- and high-resolution mass spectrometry.

As a result of a chemical study of propolis and its sources - in particular, the secretions of the axillary buds of the aspen *Populus tremula* - a series of compounds consisting of conjugates of hydroxycinnamic acids [1] and trans-coniferyl alcohol [2] has been isolated and identified.

In a further study of the composition of the buds of *Populus tremula* and propolis of the "aspen" type, we have isolated and identified three new conjugates: the ferulate (I), p-coumarate (III), and p-hydroxybenzoate (V) of trans-coniferyl alcohol.

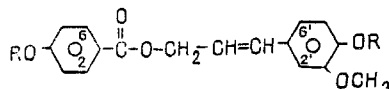


I. $R = OCH_3$, $R_1 = H$

II. $R = OCH_3$, $R_1 = COCH_3$

III. $R = R_1 = H$

IV. $R = H$, $R_1 = COCH_3$



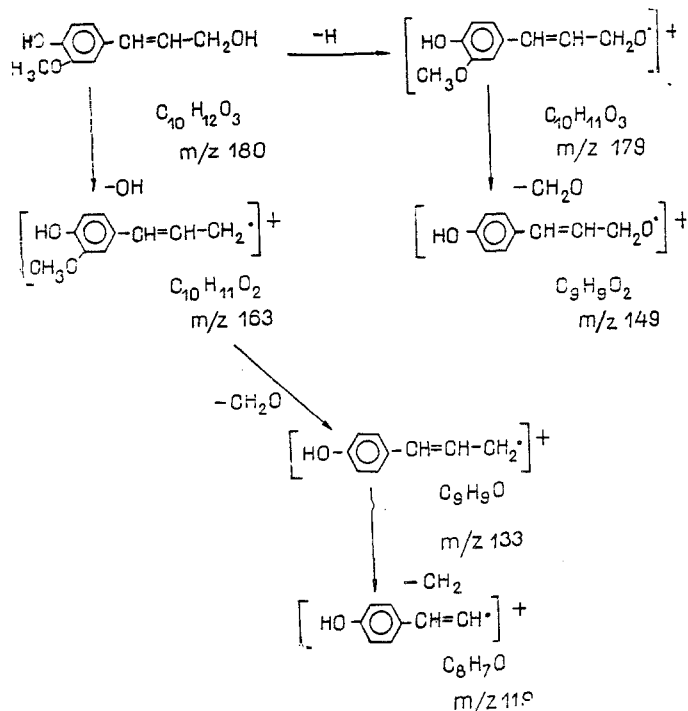
V. $R = H$

VI. $R = COCH_3$

The spots of compounds (I), (III), and (V) were readily detected on Silufol chromatographic plates from their intense cherry-red coloration. The conjugates of trans-coniferyl alcohol are labile compounds and they were therefore isolated in accordance with [2], using column chromatography on alumina and silica gel. Each of the fractions of the conjugates (I), (III), and (V) obtained as the result of isolation was separated into two parts. One of them was treated with acetic anhydride in pyridine and the acetates obtained as a result - (II), (IV), and (VI) - were purified by PTLC on silica gel. The other part was subjected to additional purification by column chromatography and comparative thin-layer chromatography.

In the mass-spectra of the conjugates (I), (III), and (V), in addition to the peaks of the M^+ ions with m/z 356, 326, and 300, respectively, there was a group of peaks with the same m/z values - 119, 133, 149, 163, 179, and 180 - which showed the presence of fragments of coniferyl alcohol in structures (I), (III), and (V). Analysis of the mass spectrum of trans-coniferyl alcohol, obtained by the enzymatic hydrolysis of coniferin (trans-coniferyl alcohol β -D-glucoside) [2], showed the presence in it of a similar group of peaks of ions with the same m/z values. Measurement of the accurate mass values performed for

this group of peaks in compounds (I)-(VI) and also of trans-coniferyl alcohol acetate showed their practical complete coincidence. The NMR spectra of compounds (II), (IV), and (VI) and of trans-coniferyl alcohol [2] were in good agreement with results of the mass-spectrometric analysis. The combination of results obtained confirmed the presence in conjugates (I), (III), and (V) of fragments of trans-coniferyl alcohol and also permitted the composition and structure of the main characteristic ions of trans-coniferyl alcohol formed as the result of the breakdown of the molecule under the action of electron impact (scheme) to be determined.



Main directions of the fragmentation of the trans-coniferyl alcohol molecule under the action of electron impact.

So far as concerns the structure of the acid moieties of conjugates (I), (III), and (V), simple calculation performed on the basis of differences in molecular masses between the conjugates investigated and the coniferyl alcohol fragment showed that the most probable acids present in them may be ferulic, p-coumaric, and p-hydroxybenzoic [2].

Analysis of the mass spectra of (I-VI) showed that in actual fact they contained, in addition to the peaks of the ions of the trans-coniferyl alcohol fragments, fairly intense peaks of ions with m/z values differing from them. Thus, for example, in the mass spectrum of (II) the peaks of ions with m/z 119, 147, 164, 177, and 194 were observed, the intensity of the m/z 177 peak being the highest. In the mass spectrum (IV), the peaks of ions with m/z 119, 147, and 164 were observed with the m/z 147 peak having the greatest intensity, and the mass spectrum of (VI) was characterized by the presence of peaks of ions with m/z 121 and 138, the m/z 121 group being the greater.

To prove the hypothesis of the presence of aromatic and phenylpropanoid acids in the conjugate studied we investigated the mass spectra of ferulic acid, ethyl acetoferulate [3], p-coumaric acid and its acetate, and also p-hydroxybenzoic acid.

As a result of the mass-spectrometric analysis of the acids it was found that the mass spectra of ferulic and its derivatives contained intense peaks of ions with m/z 119, 147, 164, 177, and 194, the peak of the ions at m/z 177 being the maximum. The mass spectra of p-coumaric acid and its acetate were characterized by the presence of intense peaks of ions with m/z 119, 147, 164, the intensity of the peak at m/z 147 being the maximum. The mass spectrum of p-hydroxybenzoic acid was characterized by the presence of intense peaks of ions with m/z 121 and 138 with the maximum intensity for the m/z 121 peak.

A comparison of the results of high-resolution mass spectrometry for the acid moieties of the conjugates and the acids investigated showed the practically complete coincidence of the values of the accurate masses for the corresponding ions, which, in its turn, confirmed the hypothesis of the presence of fragments of ferulic, p-coumaric, and p-hydroxybenzoic acids in the conjugates studied.

The structures of the conjugates (I), (III), and (V) that had been isolated were determined from a combination of all the mass-spectrometric and NMR results obtained.

EXPERIMENTAL

Compounds (I), (III), and (V) were isolated from 28 g of propolis of the "aspen" type and 22 g of aspen buds as in [2]. Samples of ferulic and p-coumaric acids were obtained from Sigma (USA). Ethyl acetoferulate was obtained in accordance with [3]. Coniferin - trans-coniferyl alcohol β -glucoside - was obtained from the V. I. Vernadskii Institute of Geochemistry of the USSR Academy of Sciences.

Low-resolution mass spectra were taken on a MKh-1309 instrument with an ionization potential of 70 eV at 80-100°C. High-resolution mass spectra were taken on a Varian MAT-44 instrument. The values found for the ions in the mass spectra corresponded to the calculated figures. NMR spectra were taken on a Varian XL-100 instrument with TMS as internal standard. IR spectra were taken on a UR-20 instrument.

Compound (I). R_f 0.24, cherry-red coloration.* The amount obtained from aspen buds was 12.5 mg and from the propolis of the "aspen" type 24.5 mg.

Mass spectrum, m/z (I, %): M^+ 356 (15) $C_{20}H_{20}O_6$ 356.1260, 194 (58) $[(OH)(OCH_3)-C_6H_3-CH=CH-COOH]^+$ 194.0579, 180 (10) 180.0788, 179 (42) 179.0708, 177 (100) $[(OH)(OCH_3)-C_6H_3-CH=CH-CO]^+$ 177.0552, 164 (15) $[(OH)C_6H_4-CH=CH-COOH]^+$ 164.0472, 163 (60) 163.0759, 149 (21) 149.0603, 147 (80) $[(OH)C_6H_4-CH=CH-CO]^+$ 147.0446, 133 (25) 133.0653, 119 (30) 119.0497.

Compound (II). R_f 0.50, deep blue coloration. The amount obtained from aspen buds was 20.0 mg and from propolis of the "aspen" type 60.0 mg. Mass spectrum, m/z (I, %): M^+ 440 (10) $C_{24}H_{24}O_8$ 440.1471, 398 (38) $[M-CH_2CO]^+$ $C_{22}H_{22}O_7$ 398.1364, 356 (60) $[M-2CH_2CO]^+$ $C_{20}H_{20}O_6$ 356.1259, 194 (63) $[(OH)(OCH_3)C_6H_3-CH=CH-COOH]^+$ 194.0579, 180 (30) 180.0787, 179 (49) 179.0708, 177 (100) $[(OH)(OCH_3)C_6H_3-CH=CH-CO]^+$ 177.0552, 164 (15) $[(OH)C_6H_4-CH=CH-COOH]^+$ 164.0472, 163 (70) 163.0759, 149 (22) 149.0603, 147 (37) $[(OH)C_6H_4-CH=CH-CO]^+$ 147.0446, 133 (23) 133.0653, 119 (20) 119.0497. NMR spectrum (δ , ppm, CCl_4): 2.20 (6H, s, 2 $OCOCH_3$), 3.74 (6H, s, 2 OCH_3), 4.76 (2H, d, $J = 6$ Hz, $-OCH_2-$), 6.22 (1H, d, $J = 16$ Hz, $-CH=CH-CH_2-O-$), 6.32 (1H, d, $J = 16$ Hz, $-CH=CH-CO-$), 6.60 (1H, m, $J = 16$ Hz, $-CH=CH-CH_2-O-$), 6.90 (3H, s, H-2', H-5', H-6'), 7.00 (3H, m, H-2, H-5, H-6), 7.58 (1H, d, $J = 16$ Hz, $-CH=CH-CO-$).

IR spectrum, (paraffin oil, ν , cm^{-1}): 1780, 1720, 1640, 1600, 1510.

Compound (III). R_f 0.19, cherry-red coloration. The amount obtained from the aspen buds was 10.4 mg and from propolis of the "aspen" type 15.5 mg. Mass spectrum, m/z (I, %): M^+ 326 (8) $C_{19}H_{18}O_5$ 326.1153, 180 (10), 180.0788, 179 (42) 179.0708, 164 (13) $[(OH)-C_6H_4-CH=CH-COOH]^+$ 164.0472, 163 (68) 163.0759, 149 (25) 149.0603, 147 (100) $[(OH)C_6H_4-CH=CH-CO]^+$ 147.0446, 133 (90) 133.0653, 119 (72) 119.0497.

Compound (IV). R_f 0.5, deep blue coloration. The amount obtained from aspen buds was 25.0 mg and from propolis of the "aspen" type 35.5 mg.

Mass spectrum, m/z (I, %): M^+ 410 $C_{23}H_{22}O_7$ (25) 410.1366, 368 (95) $[M-CH_2CO]^+$ $C_{21}H_{20}O_6$ 368.1260, 326 (33) $[M-2CH_2CO]^+$ $C_{19}H_{18}O_5$ 326.1153, 180 (10) 180.0788, 179 (53) 179.0708, 164 (38) $[(OH)C_6H_4-CH=CH-COOH]^+$ 164.0472, 163 (77) 163.0759, 149 (30) 149.0603, 147 (100) $[(OH)C_6H_4-CH=CH-CO]^+$ 147.0446, 133 (95) 133.0653, 119 (76) 119.0497.

NMR spectrum (δ , ppm, CCl_4): 2.18 (6H, s, 2 $OCOCH_3$), 3.76 (3H, s, $-OCH_3$), 4.76 (2H, d, $J = 6$ Hz, $-OCH_2-$), 6.24 (1H, d, $J = 16$ Hz, $-CH=CH-CH_2-O-$), 6.34 (1H, d, $J = 16$ Hz, $-CH=CH-CO-$), 6.50 (1H, m, $J = 16$ Hz, $-CH=CH-CH_2-O-$), 6.90 (3H, s, H-2', H-5', H-6'), 6.82 (2H, d, $J = 9$ Hz, H-3, H-5), 7.48 (2H, d, $J = 9$ Hz, H-2, H-6), 7.62 (1H, m, $J = 16$ Hz, $-CH=CH-CO-$).

*The values of R_f are given for the benzene-acetone (9:1) system. Coloration of the spots after spraying the plate with concentrated H_2SO_4 .

IR spectrum (paraffin oil, ν , cm^{-1}): 1750, 1720, 1630, 1600, 1500.

Compound (V). R_f 0.14, cherry-red coloration. The amount obtained from aspen buds was 5.0 mg and from the propolis of the "aspen" type 11.6 mg.

Mass spectrum, m/z (I, %): M^+ 300 (5) $\text{C}_{17}\text{H}_{16}\text{O}_5$ 300.0998, 180 (10) 180.0787, 179 (12) 179.0708, 163 (32) 163.0759, 149 (11) 149.0603, 138 (20) $[(\text{OH})\text{C}_6\text{H}_4\text{COOH}]^+$ 138.0317, 133 (21) 133.0653, 121 (100) $[(\text{OH})\text{C}_6\text{H}_4\text{CO}]^+$ 121.0290, 119 (12) 119.0497.

Compound (VI). R_f 0.50, deep-blue coloration. The amount obtained from aspen buds was 10.8 mg and from the propolis of the "aspen" type 20.3 mg.

Mass spectrum, m/z (I, %): M^+ 384 (8) $\text{C}_{21}\text{H}_{20}\text{O}_7$ 384.1209, 342 (96) $[\text{M} - \text{CH}_2\text{CO}]^+$ $\text{C}_{19}\text{H}_{18}\text{O}_6$ 342.1102, 300 (5) $[\text{M} - 2\text{CH}_2\text{CO}]^+$ $\text{C}_{17}\text{H}_{16}\text{O}_5$ 300.0998, 180 (10) 180.0789, 179 (17), 179.0708, 163 (40), 163.0759, 149 (18) 149.0603, 138 (17) $[(\text{OH})\text{C}_6\text{H}_4\text{COOH}]^+$ 138.0317, 133 (25) 133.0653, 121 (100) $[(\text{OH})\text{C}_6\text{H}_4\text{CO}]^+$ 121.0290, 119 (12) 119.0497.

PMR spectrum, (δ , ppm, CCl_4): 2.22 (6H, s, 2 OCOCH_3), 3.82 (3H, s, $-\text{OCH}_3$), 4.92 (2H, d, $J = 6$ Hz, $-\text{OCH}_2-$), 6.30 (1H, d, $J = 16$ Hz, $-\text{CH}=\text{CH}-\text{CH}_2-\text{O}-$), 6.70 (1H, m, $J = 16$ Hz, $-\text{CH}=\text{CH}-\text{CH}_2-\text{O}-$), 6.92 (2H, d, $J = 9$ Hz, H-3, H-5), 7.10 (3H, m, H-2', H-5', H-6'), 8.08 (2H, d, $J = 9$ Hz, H-2, H-6).

IR spectrum (film of the substance, ν , cm^{-1}): 1780, 1720, 1640, 1610, 1510.

The diacetate of trans-coniferyl alcohol was obtained in accordance with [2].

Mass spectrum, m/z (I, %): 264 M^+ (8) $\text{C}_{14}\text{H}_{16}\text{O}_5$ 264.0998, 222 (75) $[\text{M} - \text{CH}_2\text{CO}]^+$ $\text{C}_{12}\text{H}_{14}\text{O}_4$ 222.0892, 180 (30) $[\text{M} - 2\text{CH}_2\text{CO}]^+$ 180.0798, 179 (48), 179.0708, 163 (28) 163.0759, 133 (20) 133.0653, 119 (58) 119.0497, 103 (62), 91 (100).

Ferulic Acid. M^+ 194 (92) $\text{C}_{10}\text{H}_{10}\text{O}_4$ 194.0579, 177 (100) $\text{C}_{10}\text{H}_9\text{O}_3$, 164 (32) $\text{C}_9\text{H}_8\text{O}_3$ 164.0474, 147 (33) $\text{C}_9\text{H}_7\text{O}_2$, 119 (36) $\text{C}_8\text{H}_7\text{O}$ 119.0497.

Ethyl Acetoferulate. M^+ 264 (7) $\text{C}_{14}\text{H}_{16}\text{O}_5$, 222 (20) $\text{C}_{12}\text{H}_{14}\text{O}_4$, 194 (14) $\text{C}_{10}\text{H}_{10}\text{O}_4$ 194.0579, 177 (100) $\text{C}_{10}\text{H}_9\text{O}_3$ 177.0552, 164 (71) $\text{C}_9\text{H}_8\text{O}_3$ 164.0474, 147 (15) $\text{C}_9\text{H}_7\text{O}_2$ 147.0446, 119 (8) $\text{C}_8\text{H}_7\text{O}$ 119.0497.

p-Coumaric Acid. M^+ 164 (90) $\text{C}_9\text{H}_8\text{O}_3$ 164.0474, 147 (100) $\text{C}_9\text{H}_7\text{O}_2$ 147.0446, 119 (27) $\text{C}_8\text{H}_7\text{O}$ 119.0497.

Acetate of p-Coumaric Acid. M^+ 206 (47) $\text{C}_{11}\text{H}_{10}\text{O}_4$, 164 (90) $\text{C}_9\text{H}_8\text{O}_3$ 164.0474, 147 (100) $\text{C}_9\text{H}_7\text{O}_2$ 147.0446, 119 (32) $\text{C}_8\text{H}_7\text{O}$ 119.0497.

p-Hydroxybenzoic Acid. M^+ 138 (53) $\text{C}_7\text{H}_6\text{O}_3$ 138.0317, 121 (100) $\text{C}_7\text{H}_5\text{O}_2$ 121.0290.

SUMMARY

Three new natural compounds have been isolated from aspen buds and propolis of the "aspen" type: the ferulate, p-coumarate, and p-hydroxybenzoate of trans-coniferyl alcohol.

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