cis-CONIFERYL ALCOHOL AND ITS GLUCOSIDE FROM THE BARK OF BEECH (*Fagus silvatica* L.)*

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cis-Coniferyl alcohol (*III*) and its 1-O-β-D-glucopyranoside *I* called faguside were isolated as the main components of a methanolic and aqueous extract of beech bark. Spectral data and chemical degradations were used for the structure determination of these two new natural compounds.

Analyses of non-polar light petroleum and benzene extracts of beech bark were published some years ago[1-3]. In our case[2,3] the extracts were obtained after working up a saponified material and its subsequent separation by adsorption column chromatography on silica gel to individual fractions which were further analyzed or separated by gas chromatography. The individual components were identified directly in the spectra or they were isolated by preparative gas chromatography and further investigated. Attention was paid later on mainly to the methanolic and aqueous polar extracts[4].

From the ethyl acetate fraction of the aqueous extract after elimination of oligomeric components a noncrystalline substance, *I*, was isolated by a combination of column chromatographies on polyamide and silica gel, which contained a hydroxyl (3 430 cm^{-1}), a methoxyl (2 900 cm^{-1}) and an aromatic ring (1 600 and 1 520 cm^{-1} in its IR spectrum. After acetylation it gave a crystalline derivative, *II*, of the composition $C_{26}H_{32}O_{13}$ and molecular mass M^+ 552 (high resolution mass spectrometry). Another ion at *m/e* 331 ($C_{14}H_{19}O_9$) is the residue of the terminal peracetyl hexose. The strong ion *m/e* 510 ($M^+ - 42$), formed on elimination of $CH_2=C=O$ indicates the presence of an aromatic acetoxyl group. ^{1}H-NMR spectrum of acetate *II* confirms the presence of one aromatic acetoxyl at δ 2·30 (3 H), and 4 aliphatic acetoxyl groups at δ 2·00 (3 H), 2·01 (6 H) and 2·05 (3 H). In the IR spectrum of acetate *II* the bands of the aromatic ring (1 600 and 1 520 cm^{-1}) are present together with a conjugated double bond (1 640 and 1 580 cm^{-1}) and a methoxyl (2 840 cm^{-1}). The bands of the hydroxyl group are absent, while a new band of a carboxyl group (1 755 cm^{-1}) appeared. From this it follows that compound *I* is a monoglycoside

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containing hexose and an aglycone of the phenylpropenol type with one aromatic methoxyl, δ 3·82 (3 H). The glycosidic bond is not composed, as usual, of a phenolic hydroxyl (formation of an aromatic acetoxyl in acetate *II*), but of the aliphatic hydroxyl of the propenol moiety of monolignol. After the acetylation of the product of acid hydrolysis of acetate *II* penta-acetyl-β-D-glucose (66%) and penta-acetyl-α-D-glucose (34%) were detected in the product by gas chromatography. In the product of enzymatic hydrolysis of acetate *II* a substance was detected by gas chromatography, identical with the main component of the phenolic fraction of the methanolic extract of beech bark. However this compound *III* was different from all presently known monolignols.

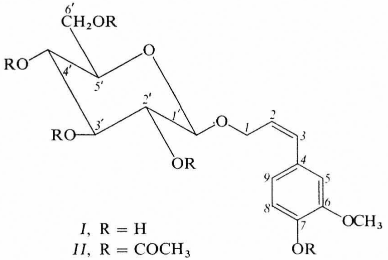
Compound *III* was isolated from the methanolic extract after the elimination of oligomeric components and subsequent alkaline hydrolysis, and after the separation of the hydrolysate to a neutral, an acidic and a phenolic fraction. During the working up of the unsaponified methanolic extract this substance was the main component of the phenolic fraction too (up to 0·7%, calculated per extract). Substance *III* of the composition $C_{10}H_{12}O_3$ and molecular mass 180 (mass spectrometry) contains in its molecule – according to IR spectrum – an aromatic ring (1605 and 1527 cm^{-1}) a conjugated double bond (1620 cm^{-1}) a phenolic hydroxyl (3170 cm^{-1}), an aliphatic hydroxyl (3450 cm^{-1}) and a methoxyl (2940 and 2955 cm^{-1}). The UV spectrum shows a maximum at 261 nm ($\log \varepsilon = 3·88$) and an inflexion at 293 nm ($\log \varepsilon = 3·42$). From the ^{1}H-NMR specturm it follows that the aromatic ring is trisubstituted (presence of three aromatic protons, multiplet at δ 6·76) and the double bond conjugated with the aromatic ring is disubstituted (2 protons on the double bond, doublet at δ 6·50 and multiplet at δ 5·76). On the double bond a —CH_2OH group is also bound (allylic splitting of the doublet of two protons at δ 4·41). The singlet of the three protons at δ 3·86 corresponds to the methoxyl on the aromatic ring, which was also determined by analysis. The correctness of the assignment of the above mentioned chemical shifts of individual protons of the proposed structure was confirmed by the analysis of the spectra of diacetate *IV*, methyl ether *V*, and dihydro derivative *VI*, as well as by the correlation with the spectra of authentic coniferyl alcohol (*VII*) and its dihydro derivative (identical with *VI*) and also by literature data[5] for isoeugenol and acetylated *trans*- and *cis*-isoeugenols. The values of the chemical shifts and the coupling constants obtained by first order analysis of all substances (derivatives and models) are listed in Table I. From the values of the coupling constants $J_{2,3} = 12·3$ Hz of compounds *III* and *IV* and their comparison with the $J_{2,3} = 16·0$ Hz of authentic coniferyl alcohol (*trans*), as well as of the constants of *cis*- and *trans*-isoeugenols it follows that the double bond of the analyzed substance *III* is *cis* substituted. In the infrared spectrum there is no band in the 960 cm^{-1} region, characteristic of the *trans* grouped hydrogens on the double bond (in the model *trans*-coniferyl alcohol the band is at 968 cm^{-1}), which also confirms the above conclusion indirectly. The determination of the position of the substituents on the ring was carried out by ozonization of the investigated compound *III* and parallelly also of coniferyl alcohol (*VII*). In the reaction mixture after the decomposition of the ozonide vanillin and vanillic acid were identified by gas chromatograohy in both instances. On the basis of this analysis the structure of *cis*-coniferyl alcohol was assigned to the isolated compound *III*. In order to confirm this a direct proof was carried out by comparison of the ^{1}H-NMR spectra of dihydro derivatives of the investigated compound *III* and authentic coniferyl alcohol (*VII*). The spectra of both dihydro derivatives were identical. During hydrogenation a small amount of hydrogenolytic product, dihydroeugenol, was also formed in both cases in addition to dihydroconiferyl alcohol (*VI*). The presence of dihydroeugenol was determined by mass spectral measurement.

TABLE I

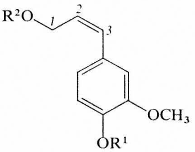
^{1}H-NMR Spectra of *cis*-Coniferyl Alkohol and Its Derivatives, Compared with Model Substances

Substance[a]	3 H_{ar}	OCH_3	OR^1	OR^2	2 $H_{(1)}$	$H_{(2)}$	$H_{(3)}$	$J_{1,2}$	$J_{2,3}$
II[b]	6·80 m	3·82	2·30	—	4·50 m	4·79 m	6·59 d	6·0	12·0
III	6·76 m	3·86	5·60	1·55	4·41 dd[c]	5·76 m	6·50 d	6·0	12·3
IV	6·80 m	3·75	2·22	2·00	4·78 dd[c]	5·74 m	6·56 d	6·3	12·3
V	6·77 m	3·86	3·86	1·76	4·41 dd[c]	5·76 m	6·49 d	6·0	12·0
VI	6·66 m	3·78	5·85	2·60	3·62 t	1·87 m	2·60 t	6·3	8·2
VII	6·85 m	3·86	5·81	1·84	4·27 d	6·15 m	6·55 d	5·0	16·0
VIII	6·90 m	3·88	3·88	1·67	4·30 d	6·19 m	6·59 d	5·0	15·6
Isoeugenol(*trans*)[d]	6·80 m	3·83	5·69	—	1·83 d	5·88 m	6·27 d	4·9	15·8
trans-Isoeugenol[d] acetate	6·90 m	3·81	2·26	—	1·86 d	6·07 m	6·37 d	5·6	15·5
cis-Isoeugenol[d] acetate	7·88 m	3·77	2·23	—	1·83 q	5·72 m	6·37 d	7·1	11·8

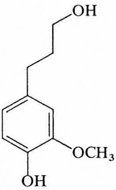
[a] Measured on a Tesla BS-477 instrument, 60 MHz; solvent deuteriochloroform, internal standard tetramethylsilane, chemical shifts in δ-scale, splittings in Hz, all data from first-order analysis; [b] protons of glucose acetate residue at δ 4·10—4·70 (m, 2 $H_{(6')}$, $H_{(5')}$, $H_{(1')}$), δ 4·90—5·30 (m, $H_{(2')}$, $H_{(3')}$, $H_{(4')}$) and protons of acetate groups at δ 2·00 (3 H), 2·01 (6 H), 2·05 (3 H); [c] additional splitting from allylic long-range coupling $J_{1,3} \approx 1$ Hz; [d] parameters from the literature[5].



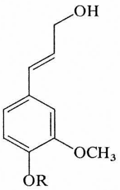
I, R = H
II, R = $COCH_3$



III, $R^1 = R^2 = H$
IV, $R^1 = R^2 = COCH_3$
V, $R^1 = CH_3$, $R^2 = H$



VI



VII, R = H
VIII, R = CH_3

The analysis and the spectral data of *cis*-coniferyl alcohol (*III*) described above served for a definite determination of the structure of glucoside *I*. A comparison of ^{1}H-NMR spectra of acetyl derivative *II* and of *cis*-coniferyl alcohol acetate *IV* (Table 1) confirmed the presence of a *cis*-substituted conjugated double bond (δ 6·59, d $H_{(3)}$ and 5·79, m $H_{(2)}$, $J_{2,3}$ = 12·0 Hz) in the aglycone moiety of the glycoside. In the ^{1}H-NMR spectrum of acetate *II* the doublet at δ 4·56 was assigned on the basis of double resonance technique to the anomeric proton of the sugar component (partly overlapped by the signal of the —CH_2—O— protons of the aglycone). The coupling constant value of the anomeric proton, $J_{1',2'}$ = 7·5 Hz, indicates a *trans*-diaxial arrangement of the protons at the $C_{(1')}$ and $C_{(2')}$ carbons and thus a β character of the glycoside bond. These spectral data, together with the above-mentioned analysis, show that the isolated glycoside must have the structure *I*, *i.e.* 1-O-(β-D-glucopyranoso-)-3-(3-methoxy-4-hydroxyphenyl)-2-propene.

cis-Coniferyl alcohol (*III*) and its 1-O-β-D-glucoside *I* are new natural compounds with a double bond unusual in monolignols, and with a rather unusual character of the binding of the phenylpropane unit with the sugar component. This peculiarity may be of importance in considerations on the structure and the biogenesis of lignin from beech bark.

EXPERIMENTAL

The melting points were determined on a Koffler block and they are not corrected. The infrared spectra were recorded on a double-beam spectrophotometer UR 10, Zeiss, Jena. Preliminary spectra were measured on a Unicam 200 spectrophotometer. The ultraviolet spectra were recorded on a Specord, Zeiss, Jena instrument. The ^{1}H-NMR spectra were measured on a Tesla BS-477 (60 MHz) and a Varian HA 100 instrument (double resonance technique). The molecular weights were determined by means of mass spectroscopy on a high-resolution AEI MS-902 spectrometer. Gas chromatography was carried out on a Carlo Erba, model GI, apparatus. The plant material was obtained by collection of beech bark in the region of Rača near Bratislava, in 1970, and it was dried at 55°C. Silica gel according to Pitra[6] was used for column adsorption chromatography (Service Laboratories, Lysolaje, Prague), as well as silica gel PHH (Spolana, Neratovice) dried at 120°C for 24 h and deactivated with 10 or 15% of water. Further polyamide powder prepared from Silamide (Povážské Chemical Works, Žilina) was also used. For thin-layer chromatography Kieselgel GF_{254} according to Stahl (Merck) was used. Detection of organic compounds on TLC was carried out with phosphomolybdic acid, concentrated sulfuric acid, also diluted with ethanol (1 : 1) and containing vanillin. For phenolic substances diazotized benzidine was also used.

Isolation of the Compounds

Dried, ground bark was boiled shortly with water and the hot aqueous extract was withdrawn from the boiling-flask by suction. Water was then added into the flask and the procedure was repeated 3 times. The combined aqueous extracts were partly evaporated and then extracted gradually with ether, chloroform and ethyl acetate. The ethyl acetate fraction was separated on a polyamide column with water–methanol mixture (with gradual increase of methanol concentration). The fractions obtained were further separated on a silica gel column with dichloromethane–methanol (6 : 1) mixture.

In the second case the dried and ground bark was extracted gradually with light petroleum and methanol in an extractor of the Soxhlet type. Oligomeric components were precipitated from the methanolic extract with water (about 80%). After their elimination a part of the extract was submitted to alkaline hydrolysis (with a 5% NaOH solution in water–methanol 1 : 1) and the neutral fraction (8·4%) was extracted with ether from the hydrolysate after evaporation of methanol and a part of water. The alkaline aqueous fraction was acidified with 1M-H_2SO_4 (to pH 4) and extracted with ether. From the ethereal extract the acid fraction (3·2%) was obtained by repeated extraction with a saturated $NaHCO_3$ solution in water, and the phenolic fraction (6·8%) by evaporation of ether. The second (non-hydrolysed) part of the methanolic extract was concentrated and the residue extracted with ether. The extract was extracted with a saturated $NaHCO_3$ solution (acid fraction), 5% aqueous NaOH solution (phenolic fraction), and the residue was evaporated to dryness (neutral fraction). The phenolic fraction was separated chromatographically on a silica gel column with a mixture of chloroform–methanol, using 100 : 0 → 100 : 1 → 100 : 3 gradient. The obtained fractions were analyzed by TLC and combined to united fractions according to the constituents. These combined fractions were further separated by chromatography.

1-O-β-D-Glucopyranoside of *cis*-Coniferyl Alcohol (*I*)

From the ethyl acetate fraction of the aqueous extract an individual, non-crystalline substance was isolated by repeated chromatography on polyamide and silica gel. IR spectrum (KBr): 3 430, 2 900, 1 600, 1 520 cm^{-1}. The substance was further purified after acetylation.

Peracetyl derivative II: A mixture of compound *I*, acetic anhydride and a few frops of pyridine was heated to boiling point and slowly cooled. The excess of acetic anhydride was decomposed with water, the mixture was neutralized with a saturated $NaHCO_3$ solution, and extracted with ether. After evaporation of ether the crude acetyl derivative was crystallized from methanol, m.p. 112—113°C, $[\alpha]_D^{24}$ −45·8° ($CHCl_3$), IR spectrum (KBr): 2 936, 2 840, 1 755, 1 640, 1 580, 1 600, 1 520, 840 cm^{-1}. ^{1}H-NMR spectrum: recorded in Table I. Mass spectrum: M^+ 552, *m/e* 510, 450, 390, 347, 331, 179, 178, 169, 164, 163, 131, 109, 103. For $C_{26}H_{32}O_{13}$ (552·5) calculated: 56·52% C, 5·84% H; found: 56·43% C, 5·92% H.

cis-Coniferyl Alcohol (*III*)

Compound *III* was isolated as a main constituent (about 15%) from the combined chromatographic fractions of the phenolic part of the methanolic extract by repeated chromatography on a silica gel column, using dichloromethane for elution. It was also isolated in the same manner from the extract after saponification, but the yield was lower. After crystallization from dichloromethane it had m.p. 104—106°C; IR spectrum (KBr): 3 450, 3 170, 2 955, 2 940, 1 620, 1 605 and 1 527 cm^{-1}, UV spectrum (ethanol): 261 nm (log ε = 3·88), inflexion at 293 nm (log ε = = 3·42). Mass spectrum: M^+ 180, *m/e* 152, 149, 147, 137, 124, 122, 109 and 91. For $C_{10}H_{12}O_3$ (180·2) calculated: 66·65% C, 6·71% H; found: 66·70% C, 6·74% H.

Acetyl Derivative *IV*

This was prepared similarly as *II*. The crude product was purified by column chromatography on silica gel using dichloromethane for elution. The chromatographically pure substance is a viscous liquid. ^{1}H-NMR spectrum is recorded in Table I.

Methyl Derivatives *V* and *VIII*

A diazomethane solution (10 ml) in ether was added to a solution of the substances *III* and *V* (100 mg) in methanol (1 ml). The mixture was allowed to stand at room temperature until the conversion was complete (followed by TLC). After elimination of excess diazomethane and evaporation of the solvents the product was purified by chromatography on silica gel with dichloromethane. The ^{1}H-NMR spectra of methyl derivatives of *V* and *VIII* are recorded in Table I.

Dihydroconiferyl Alcohol (*VI*)

A solution of coniferyl alcohol (*VII*) (100 mg) in ethanol (10 ml) was hydrogenated at atmospheric pressure and room temperature in the presence of a 30% Pd/C catalyst (50 mg). After 20 min hydrogenation the consumption of hydrogen corresponded to the saturation of one double bond. The catalyst was filtered off, the solvent evaporated and the residue chromatographed on a silica gel column with dichloromethane–ether (5 : 1). The product obtained represented a chromatographically pure, liquid dihydro derivative. Mass spectrum: M^+ 182 m/e 164, 149, 137, 122. ^{1}H-NMR spectrum is recorded in Table I. An identical dihydro derivative *VI* was prepared from *cis*-coniferyl alcohol (*III*) in the same manner.

The mass spectra were recorded and interpreted by Dr L. Dolejš and some ^{1}H-NMR *spectra* (100 MHz) *by Dr M. Buděšínský (both of the Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Prague). The infrared and the ultraviolet spectra were measured by Dr E. Paulinyová, the gas chromatographies were carried out by Dr E. Váradiová, and technical assistance provided by Dr M. Plšková and Mr V. Gulár (all of the State Forest Products Research Institute, Bratislava). We express our gratitude to all of them.*

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Note added in proof: A compound which is a dihydro derivative of our glucoside *I* was isolated from the leaves of *Pinus contorta* by Higuchi R,, Aritomi M., Donnelly D. M. X.: Phytochemistry *16*, 1007 (1977).