CROATICA CHEMICA ACTA
CCACAA **77** (3) 491–500 (2004)
ISSN-0011-1643
CCA-2952
Original Scientific Paper

Synthesis of Selected Naturally Occurring Glucosides of Volatile Compounds. Their Chromatographic and Spectroscopic Properties*

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RECEIVED JANUARY 15, 2004; REVISED APRIL 16, 2004; ACCEPTED APRIL 20, 2004

Key words
glucosides of volatiles
synthesis of glucosides
1D and 2D NMR spectra
glucoside tetraacetates
GC-MS spectra

Naturally occurring glucosides of benzyl alcohol, (±)-menthol, (+)-borneol, thymol, carvacrol and eugenol were synthesized by the Koenigs-Knorr-Zemplén method (yields 19.5–52.2 %). Their β-D-glucopyranosidic structures were determined by one- and two-dimensional homo- and heteronuclear ^{1}H and ^{13}C NMR spectroscopy. The β-configuration was additionally confirmed by the hydrolysis with β-glucosidase. Tetraacetyl-β-D-glucopyranosides, as intermediates, were GC-MS analyzed. Diastereomeric β-glucoside tetraacetates of (±)-menthol were well separated on the HP-101 column. The mass spectra of glucopyranoside tetraacetates were mutually compared, as well as with the spectra of their aglycones.

INTRODUCTION

Volatile compounds are present in plants (aroma compounds) as well as their non-volatile glycosides. The glycosides of volatiles were detected in almost 170 plants and belong to 50 families.[1–3] Volatile compounds can be liberated from the non-volatile glycosides by an enzymatic/chemical hydrolysis and by pyrolysis.[3–5] These glycosides are involved in the flower fragrance formation, in the release of aroma compounds of fruits or spicy materials and in the aroma formation of tea and vine. Moreover, they have been identified, along with other glycosides, in the plants whose extracts are widely used in folk medicine. From the physiological point of view, such glycosides are considered as storage forms of aroma compounds, and glycosylation could act as a protective mechanism against toxicity of hydrophobic compounds. The sugar moieties of these glycosides are mostly monosaccharidic and disaccharidic.[2] Glucose is the most common monosaccharide found in naturally glycosides. Almost all natural glycosides are β-anomers. Aliphatic and terpene alcohols, phenylpropanes with related compounds and C_{13}-norisoprenoids were identified among volatile aglycons.[1–4] Eugenol, thymol, geraniol, menthol, terpinen-4-ol, 1-octen-3-ol, 3-hexen-1-ol, benzyl alcohol, 2-phenylethanol, carvacrol, borneol and other compounds are ubiquitous aglycones in aromatic plants. Some of these

* Partially presented at the 18th Croatian Congress of Chemists and Chemical Engineers, Zagreb, February 2003.

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glycosides can be of pharmacological interest as well as useable for food additives and cosmetics.

O-glycosides are usually prepared by synthesis from protected and activated glycosyl donors with different leaving groups and alcohols or phenols as glycosyl acceptors.[6] Some terpenyl glycosides were synthesized by the Koenigs-Knorr method[6–8] with silver compounds as promoters (Ag_2O, Ag_2CO_3, Ag_2SiO_3). Better yields of the glycosides of hindered alcohols can be obtained with silver trifluoromethanesulfonate, which requires the presence of a very hindered base 2,6-di(*tert*-butyl)-4-methylpiridine as trifluormethanesulphuric acid scavenger.[7] The trichloroacetimidate method of Schmidt and co-workers[9] is often superior to the Koenigs-Knorr method, particularly for hindered glycosyl acceptors. Namely, *O*-glycosyl trichloroacetimidates act as strong glycosyl donors under relatively mild acid catalysis. Anomeric pure products can be prepared by the enzymatic synthesis (reversed hydrolysis or transglycosylation method) in organic cosolvent,[10] but with limited yields.

The aim of this study was to synthesize the most common naturally occurring glucosides of volatile compounds. Therefore, β-glucopyranosides of (±)-menthol, (+)-borneol, thymol, carvacrol, eugenol and benzyl alcohol were synthesized by the Koenigs-Knorr-Zemplén method. They were identified by one- and two-dimensional homo- and heteronuclear ^1H and ^{13}C NMR spectroscopy, and β-configuration was additionally confirmed by the hydrolysis with β-glucosidase. Furthermore, chromatographic and mass spectrometric properties of the intermediary glucoside tetraacetates were determined.

EXPERIMENTAL

Reagents

Thymol (99 %), carvacrol (97 %), eugenol (99 %) and benzyl alcohol (99 %) were purchased from Fluka Chemie, Buchs, Switzerland, while (+)-borneol (98 %) and (±)-menthol (99 %) were purchased from Sigma-Aldrich, Germany. Silica gel for column chromatography (Kieselgel 60, 0.040–0.063 mm), precoated silicagel plates (Kieselgel 60, thickness 0.2 mm) for thin layer chromatography, solvents and other applied reagents were obtained from Merck, Darmstadt, Germany. All solvents were dried according to standard procedures. β-Glucosidase was obtained from Fluka Chemie, Buchs, Switzerland.

*General Synthesis of 2,3,4,6-Tetra-*O*-Acetyl-β-D-Glucopyranosides*

α-Acetobromglucose (2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide) was prepared from dry glucose, acetic anhydride, red phosphorus and bromine.[8] Perchloric acid was used as catalyst. The obtained α-acetobromglucose was purified by recrystallization from ether–petrolether (volume ratio, ψ = 1:1), with a sharp melting point (m.p. 89 °C).

Tetraacetyl-β-glucopyranosides **1a**–**1c** were prepared by the reaction of α-acetobromglucose (12.1 mmol) with relevant alcohols (20–40 mmol). Tetraacetyl-β-glucopyranosides **1d**–**1f** were synthesized by the reaction of α-acetobromglucose and the appropriate phenols (16.7 mmol) or phenolates (16.7–20 mmol). Freshly prepared, dried and finely powdered silver oxide (for alcohols and phenols) or silver carbonate (for phenolates) were used as promoters. Powdered anhydrous calcium sulphate (5.0 g) was used for water binding. Reagents, promoter (3.0 g) and calcium sulphate were added into ether (20 mL), or THF (20 mL) for sodium eugenolate, and the reaction mixture was mixed on a shaker for 24 hours at room temperature, in a dark place. After filtration of the reaction mixture, the solvent was removed by distillation and the remaining glucosyl acceptor by steam distillation. The oily residue was separated from water, solved in a small volume of warm ethanol and crystallized to give tetraacetyl-β-glucopyranosides. After recrystallization, the obtained tetraacetates were analyzed by GC-MS.

General Procedure for Deacetylation of Tetraacetyl-β-D-Glucopyranosides

The obtained tetraacetates (**1a**–**1f**) were solved in methanol (20 mL) and deacetylated with sodium methoxide (5 mmol) for 30 minutes (transesterification by Zemplén). The solution was neutralized with an ion-exchange resin (Amberlite-IR-120H^+). β-Glucopyranosides were obtained as oily syrups. After crystallization and recrystallization, their structures were determined by NMR-spectroscopy. To additionally confirm β-configuration, the glucosides were hydrolyzed with β-glucosidase, and the liberated aglycones were identified by GC-MS.

General Procedure for Enzymatic Hydrolysis of β-Glucopyranosides

The obtained glucosides (**2a**–**2f**) were separately dissolved in 5 mL of 0.1 mol dm^{-3} citrate buffer, pH = 5.5. β-Glucosidase from almonds (20 mg, 5–8 IU/mg) was added to the glucosidic solutions along with 3 mL pentane to trap the liberated aglycones. The hydrolysis was carried out for 70 h at 30 °C in a shaking water bath. After hydrolysis, the pentane layer was separated and the remaining aglycones were extracted from the aqueous layer with pentane (3 × 2 mL). The combined pentane extract was dried over Na_2SO_4 and concentrated to a final volume of 0.5 mL, and 1 μL was used for GC-MS analysis.

Thin Layer Chromatography

Prepared glucosides **(2a**–**2f)** and aglycones liberated after enzymatic hydrolysis were chromatographed on silicagel plates. The mixture of EtOAc, EtOH and conc. ammonia, ψ = 6:3:1, was applied as solvent for the glucosides, and the mixture of hexane and EtOAc, ψ = 85:15, was used as solvent for the aglycones. The plates were sprayed with vanillin–sulphuric acid and heated to 120 °C for 10 minutes. Individual compounds were detected as dark spots (glucosides) or different coloured spots (aglycones) on a white background.

NMR Measurements

One- and twodimensional homo- and heteronuclear ^{1}H and ^{13}C NMR spectra were recorded with a Bruker AV-600 spectrometer, operating at 600.133 MHz for the ^{1}H nucleus and 150.917 MHz for the ^{13}C nucleus. Samples of glucosides were measured from DMSO-d_6 solutions at 27 °C (300 K) in 5 mm NMR tubes. Chemical shifts, in ppm, were referred to TMS as internal standard. FID resolution in ^{1}H NMR and ^{13}C NMR spectra was 0.29 Hz and 0.54 Hz per point, respectively. The following measurement techniques were used: standard ^{1}H, ^{13}C gated proton decoupling, APT, COSY, NOESY, HMQC and HMBC. Proton decoupling was performed by Waltz-16 modulation. The COSY with standard $\pi/2$ pulse sequence was measured using 2048 points in F2 dimension and 512 increments in F1 dimension. The latter was subsequently zero-filled to 1024 points. Increments were obtained by 2 scans each, 8012.82 Hz spectral width and a relaxation delay of 1.486 s. The FID resolution was 3.91 Hz/point and 15.65 Hz/point in F2 and F1 dimensions, respectively. The NOESY spectra were measured in the phase-sensitive mode, with a mixing time of 0.89 s and 16 scans per each increment. The spectral width was 6127.45 Hz, 2048 points in F2 dimension and 512 increments in F1 dimension, subsequently zero-filled to 1024 points. The resulting FID resolution was 2.99 Hz/point and 11.96 Hz/point in F2 and F1 dimensions, respectively. The HMQC spectra ($^1J_{C,H}$ was set to 145 Hz) were recorded with 2048 points in F2 dimension and 256 increments in F1 dimension, subsequently zero-filled to 1024 points. For each increment, 48 scans were collected using a relaxation delay of 1.5 s. The spectral widths were 6067.96 Hz (F2) and 25000 Hz (F1), with the corresponding resolution of 2.96 and 97.65 Hz/point in F2 and F1 dimensions, respectively. The HMBC spectra were measured with 2048 points and 8012.82 Hz spectral width in F2 dimension and a relaxation delay of 1.500 s. The additional delay of 0.065 s was used to detect the long-range C, H couplings. The spectral width in F1 dimension was 33560 Hz, while 256 increments were recorded, each by 48 scans. The FID resolution was 3.912 and 131.08 Hz per point in F2 and F1 dimensions, respectively. The 2D NMR spectra, except for NOESY, were measured in the pulsed field gradient mode (z-gradient).

Gas Chromatography-Mass Spectrometry Analysis

Analysis of liberated aglycones and glucoside tetraacetates was performed on a GC-MS Hewlett-Packard (model 5890 with a mass selective detector model 5971A, Hewlett Packard, Vienna, Austria). GC operating conditions for the aglycones: column HP-20M (Carbowax 20M, Hewlett Packard, Vienna, Austria), 50 m × 0.2 μm i.d., film thickness 0.2 mm; column temperature programmed from 70 °C (4 minutes isothermal) to 180 °C at a rate of 4 °C min^{-1}, as in our previous papers[4,5] and for glucoside tetraacetates: column HP-101 (dimethylpolysiloxane, Hewlett Packard, Vienna, Austria), 25 m × 0.2 mm i.d., film thickness 0.2 μm; column temperature programmed from 150 °C (4 minutes isothermal) to 220 °C at a rate of 10 °C min^{-1}; carrier gas: helium; flow rate: 1 mL min^{-1}; injector temperature: 250 °C; volume injected: 1 μL; split ratio: 1:50. MS conditions: ionization voltage: 70 eV; ion source temperature: 280 °C; mass range: for the aglycones 30–300 mass units and for tetraacetate glucosides 30–600 mass units. Individual spectra of glucoside tetraacetates were obtained by scanning pure compounds after the synthesis. Spectra of aglycones were also scanned from authentic compounds and compared with spectra from the database (Wiley library) as well as spectra published by Adams.[11]

*Benzyl-β-glucopyranoside (**2a**)*

Compound **2a** was obtained by the general Koenigs-Knorr-Zemplén procedure using benzyl alcohol (20 mmol) and it was recrystallized from ethanol. Yield: 50.5 %; m.p. 106 °C; R_F = 0.32. ^{1}H NMR (DMSO-d_6) δ/ppm: 4.77 (d, 1H, J = 12.23 Hz), 4.52 (d, 1H, J = 12.23 Hz), 7.33 (d, 2H, J = 7.36 Hz), 7.28 (d, 2H, J = 7.53 Hz), 7.21 (d, 1H, J = 7.27 Hz). ^{13}C NMR (DMSO-d_6) δ/ppm: 138.08, 128.15, 127.63, 127.36, 69.49.

*Menthyl-β-glucopyranoside (**2b, 2b'**)*

Compounds **2b, 2b'** were obtained by the general Koenigs-Knorr-Zemplén procedure with racemic menthol (40 mmol) and were recrystallized from acetone. Yield: 28.0 %; m.p. 74–78 °C; R_F = 0.42. ^{1}H NMR (DMSO-d_6) δ/ppm: 3.19 (m, 1H), 2.40 (m, 1H), 2.15 (m, 1H), 2.12 (m, 1H), 2.01 (m, 1H), 1.95 (m, 1H), 1.55 (m, 2H), 1.45 (m, 2H), 1.26 (m, 1H), 1.07 (m, 1H), 1.05 (m, 1H), 0.82 (m, 6H), 0.80 (m, 4H), 0.75 (m, 7H), 0.70 (m, 2H), 0.67 (m, 6H). ^{13}C NMR (DMSO-d_6) δ/ppm: 79.92, 48.17, 47.49, 43.38, 40.39, 34.12, 34.05, 31.14, 30.98, 24.60, 23.98, 22.71, 22.57, 22.29, 21.17, 20.97, 16.03, 15.64.

*Bornyl-β-glucopyranoside (**2c**)*

Compound **2c** was obtained by the general Koenigs-Knorr-Zemplén procedure with (+)-borneol (20 mmol) and was recrystallized from cyclohexane-hexane. Yield: 52.2 %; m.p. 119–120 °C; R_F = 0.39. ^{1}H NMR (DMSO-d_6) δ/ppm: 3.88 (d, 1H, J = 9.50 Hz), 2.02 (m, 1H), 1.97 (m, 1H), 1.57 (m, 1H), 1.52 (t, 1H, J = 4.50 Hz), 1.11 (m, 1H), 1.03 (m, 1H), 1.00 (m, 1H), 0.75 (s, 3H), 0.74 (s, 6H). ^{13}C NMR (DMSO-d_6) δ/ppm: 82.39, 48.59, 47.59, 44.23, 35.52, 27.82, 26.32, 19.69, 18.74, 13.52.

*Thymyl-β-glucopyranoside (**2d**)*

Sodium thymolate was prepared in the reaction of thymol (16.7 mmol) and sodium in diethyl ether. Tetraacetate of thymyl glucoside was prepared and deacetylated according to the standard procedure. Glucoside **2d** was recrystallized from water–ethanol. Yields: 19.5 % (sodium thymolate as glucosyl acceptor) and 5 % (thymol as glucosyl acceptor); m.p. 99–101 °C; R_F = 0.43. ^{1}H NMR (DMSO-d_6) δ/ppm: 6.98 (d, 1H, J = 7.73 Hz), 6.82 (s, 1H), 6.69 (d, 1H, J = 7.57 Hz), 3.27 (heptet, 1H, J = 6.90). ^{13}C NMR (DMSO-d_6) δ/ppm: 154.62, 135.64, 134.12, 125.39, 122.40, 115.84, 25.62, 22.96, 22.69, 20.90.

*Carvacryl-β-glucopyranoside (**2e**)*

Sodium carvacrolate (16.7 mmol) was prepared by the same procedure as thymolate, and tetraacetatae of carvacryl glucoside was prepered and deacetylated according to the standard procedure. The glucoside **2e** was recrystallized from water–ethanol. Yields: 34.5 % (sodium carvacrolate as glucosyl acceptor), 8 % (carvacrol as glucosyl acceptor); m.p. 100–103 °C; R_F = 0.42. ^{1}H NMR (DMSO-d_6) δ/ppm: 6.95 (d, 1H, J = 7.70 Hz), 6.87 (s, 1H), 6.69 (d, 1H, J = 7.60 Hz), 2.73 (heptet, 1H, J = 6.86 Hz), 2.08 (s, 3H), 1.10 (d, 6H, J = 6.80 Hz). ^{13}C NMR (DMSO-d_6) δ/ppm: 155.55, 147.19, 129.94, 123.93, 119.31, 112.98, 33.22, 23.89, 23.78, 15.61.

*Eugenyl-β-glucopyranoside (**2f**)*

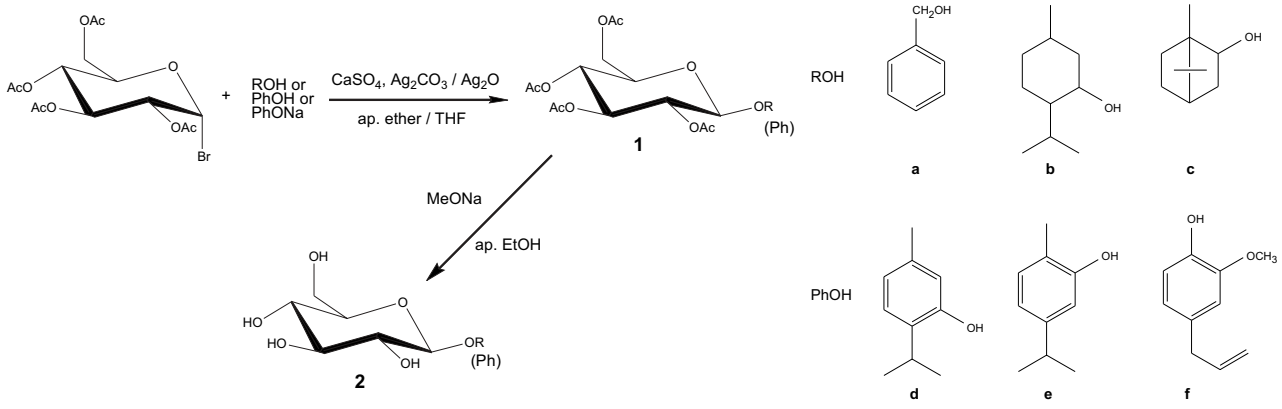
Sodium eugenolate (20 mmol) was obtained from eugenol and sodium ethoxide in an acid-basic reaction. Liberated ethanol was removed by distillation under reduced pressure. Residual sodium eugenolate was dissolved in THF and eugenyl glucoside was prepared and deacetylated according to the standard procedure. Glucoside **2f** was recrystallized from ethanol. Yields: 41.0 % (eugenolate as glucosyl acceptor) and 3 % (eugenol as glucosyl acceptor); m.p. 124–126 °C; R_F = 0.34**.** ^{1}H NMR (DMSO-d_6) δ/ppm: 6.93 (d, 1H, J = 8.30 Hz), 6.73 (s, 1H), 6.60 (d, 1H, J = 8.30 Hz), 5.87 (m, 1H), 4.98 (m, 2H), 3.67 (s, 3H), 3.23 (d, 2H, J = 6.70 Hz). ^{13}C NMR (DMSO-d_6) δ/ppm: 148.92, 144.90, 137.90, 133.48, 120.32, 115.62, 115.52, 55.68, 39.05.

RESULTS AND DISCUSSION

Tetraacetyl-β-glucopyranosides of thymol, carvacrol, eugenol, benzyl alcohol, (±)-menthol and (+)-borneol were prepared by the Koenigs-Knorr reaction. This glucosylation is the reaction of nucleophilic substitution at saturated carbon where α-acetobromglucose is the substrate, and alcohols, phenols or phenolates are the nucleophiles. It is mainly bimolecular S_N2 nucleophilic substitution with total inversion of the product configuration. Tetraacetyl-β-glucopyranosides synthesis is presented in Scheme 1.

Ordinary nucleophiles, but also substrates, are sterically hindered and require a promoter to bind the leaving groups. Water is undesirable, since it is a competitive nucleophile and calcium sulphate added in the reaction mixture binds water. The yields of glucoside tetraacetates depend on the substrate species but also on reagent nucleophilicity, promoter, solvent and binding of water. After deacetylation of tetraacetates and recrystallization, glucosides **2a**–**2f** were obtained as pure crystal products with sharp melting points (except diastereomers **2b** and **2b'**). The glucoside yields ranged from 5 to 52.2 %. The most reactive were unhindered nucleophiles such as benzyl alcohol and borneol. Phenolates, as better nucleophiles, gave higher β-glucoside yields (19.5–41.0 %) compared to phenols (3–8 %). Sodium thymolate was less reactive in comparison with carvacrolate due to the steric hindrance of thymol (Scheme 1). Sodium eugenolate was the most reactive. Eugenyl-β-glucoside tetraacetate was prepared from sodium eugenolate and α-acetobromglucose. It was not possible to prepare sodium eugenolate from eugenol and sodium in ether by the method applied for the other two phenolates. Namely, eugenol has a double bond in a side-chain and hydrogen in *status nascendi* easily hydrogenates eugenol. Therefore, eugenolate was obtained from eugenol and sodium ethoxide in an acid-basic reaction. This reaction is favourable owing to the large difference in relative acidity of eugenol and ethanol (equilibrium constant = K_a (eugenol) / K_a (ethanol) = $10^{-10}/10^{-16} = 10^6$). Furthemore, the generated ethanol is the most volatile of all substances in the reaction mixture and can be removed by distillation under reduced pressure.

The glycosides prepared by the synthesis or isolated from the plant material can be analyzed by gas chromatography after conversion into their volatile derivatives (methyl and trimethylsilyl ethers or trifluoracetate and acetate esters). Tetraacetyl-β-glucopyranosides can be prepared from glucosides with acetanhydride in pyridine. Many of these acetates are volatile and suitable compounds for gas chromatography. Gas chromatographic



Scheme 1.

and mass spectrometric properties of the prepared tetraacetyl-β-D-glucopyranosides **1a–1f** and the related aglycones **a–f** were determined. The results are given in Tables I and II.

TABLE I. Retention times and mass spectra of tetraacetyl-β-glucopyranosides **1a–1f**

Glucoside tetraacetate	$t_{ret.}$ / min	Mass spectra *m/z* (%)
Benzyl alcohol (**1a**)	27.42	91(100), 43(97), 139(30), 97(22), 152(14), 44(12), 92(9), 65(7), 77(2), 331(2), 245(4), 81(2), 271(2), 439(M^+)
Menthol[(a)] (**1b**)	27.82	43(100), 83(66), 69(26), 98(25), 81(23), 55(24), 138(21), 115(20), 57(19), 157(18), 95(17), 109(14), 169(9), 242(9), 41(8), 200(8), 145(5), 71(4), 123(2), 229(1)
Menthol[(a)] (**1b'**)	30.44	43(100), 83(66), 69(26), 98(25), 81(23), 55(24), 138(21), 115(20), 57(19), 157(18), 95(17), 109(14), 169(9), 242(9), 41(8), 200(8), 145(5), 71(4), 123(2), 229(1)
(+)-Borneol (**1c**)	31.32	43(100), 109(65), 81(63), 169(56), 137(53), 95(19), 69(15), 67(9), 110(9), 139(8), 145(4), 331(4), 121(2), 242(1)
Thymol (**1d**)	34.44	43(100), 109(54), 169(51), 127(15), 135(12) 44(11), 115(7), 81(5), 91(4), 145(4), 150(4), 331(4), 69(2), 211(2), 271(1)
Carvacrol (**1e**)	40.05	43(100), 109(71), 169(61), 127(17), 135(12), 115(8), 150(7), 44(5), 81(5), 91(4), 145(4), 331(4), 69(2), 211(2), 187(1), 229(1), 271(1)
Eugenol (**1f**)	59.73	43(110), 109(55), 169(49), 44(20), 127(16), 164(12), 81(8), 103(8), 77(4), 145(4), 331(4), 91(2), 131(2), 211(2), 229(1)

[(a)] (+) or (–)-menthyl-β-glucopyranoside tetraacetate. These are two resolved diastereoisomeric derivates of menthol (**1b**, **1b'**), but correct isomers were not determined.

TABLE II. Retention times and mass spectra of aglycones **a–f**

Aglycones	$t_{ret.}$ / min	Mass spectrum *m/z* (%)
Benzyl alcohol (**a**)	27.42	79(100), 108(84), 77(74), 107(65), 51(35), 91(14), **M^+(108)**
Menthol[(a)] (**b**)	27.57	95(100), 81(94), 71(86), 41(65), 123(50), 55(47), 43 (35), 57 (33), 138(30), 109(17)
(+)-Borneol (**c**)	30.17	95(100), 110(23), 139(8), 67(8), 121(6), 41(6)
Thymol (**d**)	32.48	135(100), 150(26), 91(14), 107(6), 117(9), 136(9), 77(6), 121(4), 79(4), 78(3), 151(3), **M^+(150)**
Carvacrol (**e**)	36.11	135(100), 150(31), 91(20), 77(12), 107(11), 136(10), 79(7), 117(4), 121(4), 78(3), 151(3), **M^+(150)**
Eugenol (**f**)	58.12	164(100), 77(49), 103(42), 91(38), 149(39), 131(44), 104(28), 55(24), 121(12), 165 (10), **M^+(164)**

[(a)] (±)-Menthol is a racemic mixture, unresolved on achiral stationary phases.

Prepared tetraacetyl-β-D-glucopyranosides **1a–1f** had different retention times (Figure 1 and Table I), even diastereomeric tetraacetyl-β-glucosides of (±)-menthol were satisfactorily separated (Figure 2).

Menthyl- (**1b**) and benzyl- (**1a**) glucoside tetraacetates were insufficiently separated in the mixture (Figure 1). The spectra of glucoside tetraacetates mainly contained more or less abundant characteristic ions of the aglycones and other ions such as acetyl ion (intensive) and the ions generated by the combination of different parts of glucoside tetraacetate. However, some unspecific fragment ions resulting from sugar cleavage were also present in the mass range below the parent ions of the aglycones, and could make the identification of some aglycones difficult. The ion $C_5H_5O^+$ (*m/z* = 81) was found in all spectra. Fragment ions with high masses were 331, 271, and 211. The peak *m/z* = 331 represents the glucopyranoside-tetraacetate ion $C_6H_7(OOCCH_3)_4O^+$. In further discussion the obtained mass spectra of glucopyranoside tetraacetates were compared with the spectra of their aglycones (Tables I and II).

The spectrum of benzyl alcohol (**a**) contains the $C_6H_7^+$ peak (*m/z* = 79) as the most intensive ion. The next most abundant peak was the parent ion (M^+, *m/z* = 108). Other abundant ions were phenyl cation $C_6H_5^+$ (*m/z* = 77) and $C_6H_5CH{=}OH^+$ (*m/z* = 107). The less intensive ones were the tropylium (cycloheptatrienyl) ion $C_7H_7^+$ (*m/z* = 91) and $C_4H_3^+$ (*m/z* = 51). These ions are characteristic of aromatic compounds.[12,13] The mass spectrum of tetraacetyl-β-glucopyranoside of benzyl alcohol (**1a**) contained tropylium ion (*m/z* = 91) as the most intensive peak. Acetyl ion, CH_3CO^+ (*m/z* = 43), was also intensive, which is characteristic of acetate esters. It is possible to distinguish other peaks with *m/z* = 139, 97, 152, 92, 245, 331, 65, 81, while the small signal with 439 mass units was of the parent ion.

Menthol (**b**) is a monocyclic secondary alcohol. Reactions through menthol elimination and rearrangement give ion $C_7H_{11}^+$ (*m/z* = 95) as the most intensive peak in the spectrum (Table II, **b**). This ion is accompanied by abundant ions $C_6H_9^+$ (*m/z* = 81), C_3H_7–$C_6H_8^+$ (*m/z* = 123) and C_2H_5–$C_6H_8^+$ (*m/z* = 109). They are characteristic ions of cycloalkenes. The $C_3H_7CO^+$ peak (*m/z* = 71) is characteristic of cycloalkanols, similarly as peaks *m/z* = 57 and 43. These ions are non-specific and could arise from $C_3H_5O^+$ or $C_4H_9^+$ (propanoyl or buthyl ions) or could arise from $C_2H_3O^+$ or $C_3H_7^+$ (acetyl or propyl ions). The peak *m/z* = 55 is also non-specific ($C_4H_7^+$ or $C_3H_3O^+$) and belongs to cycloalkanes or buthyl esters. The ion $C_{10}H_{18}^+$ (*m/z* = 138) originates from the parent ion decreased by 18 mass units (M^+–H_2O), which is especially useful in recognizing alcohol. (±)-Menthol racemic mixture in the Koenigs-Knorr synthesis produced two diastereomeric tetraacetyl-β-glucopyranosides (**1b**, **1b'**). These diastereomers (generally separable) were not

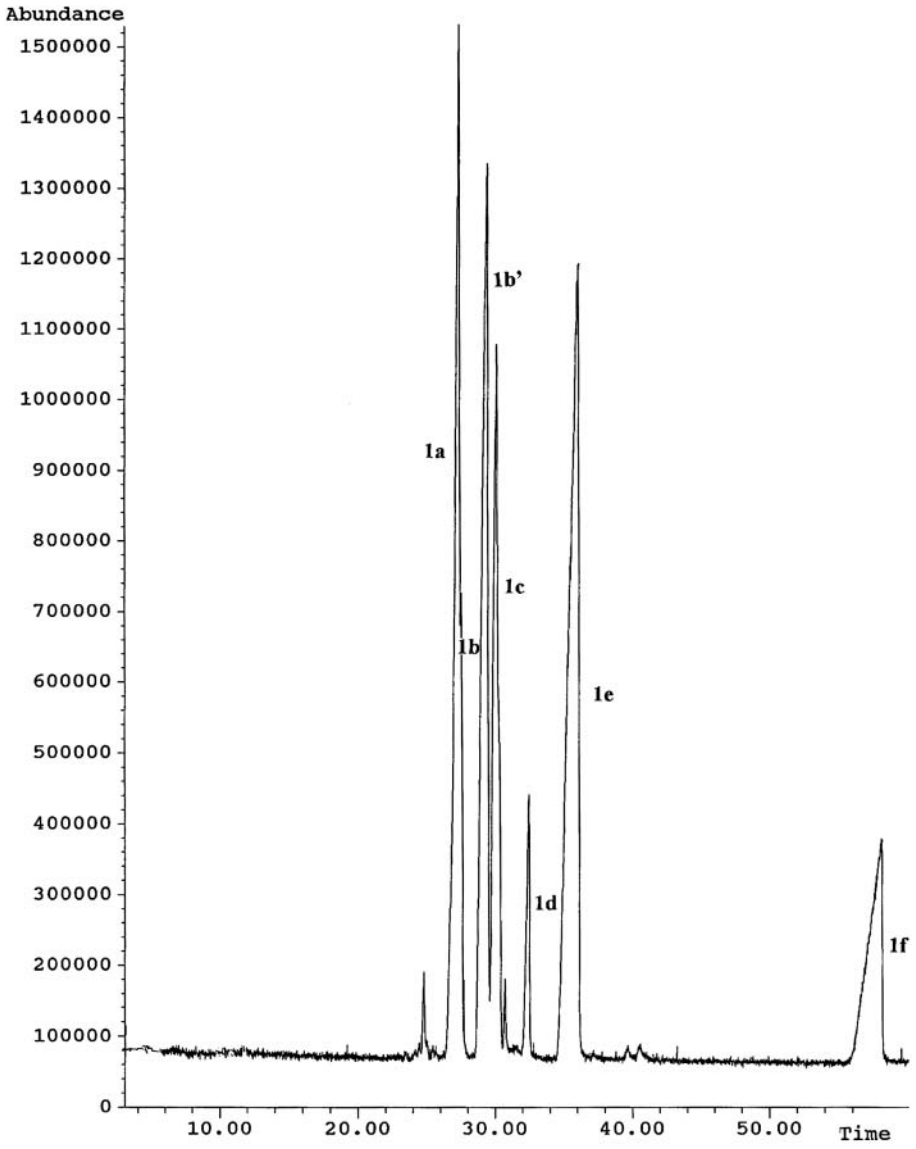


Figure 1. Total ion chromatogram of glucoside tetraacetates on the HP-101 column. The symbols in the figure are presented in Scheme 1.

separated from each other by recrystallization. Gas chromatography separated them with a mass ratio **1b** : **1b'** = 1 : 1 and with different retention times (27.82 and 30.44 min), but identical mass spectra (Figure 2, Table I). Two diastereomeric menthyl-β-glucopyranosides were not separated by TLC. Glucoside tetraacetates (**1b**, **1b'**), as separable derivatives of menthol, are suitable for resolution of the (±)-menthol racemic mixture. Therefore, it is interesting to note that glucoside tetraacetates could be used for resolution of other terpenic alcohols according to the following sequence: (±)-alcohol → glucoside tetraacetate → separation, deacetylation, hydrolysis. The mass spectrum of tetraacetyl-β-glucopyranosides of (±)-menthol, except for the acetyl ion as the most intensive, contained the same peaks as the menthol spectrum, but with smaller total abundance. New peaks in the spectrum were *m*/*z* = 242, 200, 157, 115, 169, 98 and 229.

Borneol is a secondary bicyclic alcohol (**c**). The peak *m*/*z* = 95 ($C_7H_{11}^+$) is comprised in the spectrum of borneol as the most intensive ion (Table II, **c**). This ion, generated by reactions of borneol elimination and rearrangement, is the characteristic ion of cycloalkenes as well as ions $C_8H_{14}^+$ (*m*/*z* = 110) and $C_5H_7^+$ (*m*/*z* = 67). The less abundant peaks were *m*/*z* = 139 ($C_9H_{17}O^+$, characteristic of cycloalkyl carbonyl compounds) and *m*/*z* = 121 (characteristic of terpenes and derivatives).[12,13] The spectrum contains very low abundance (below 1 %) of the parent ion (*m*/*z* = 154). Bornyl-β-glucopyranoside tetraacetate spectrum included the acetyl ion as the most intensive and the same ions as the spectrum of borneol, but with lower abundance. The other important peaks in the spectrum were 81, 109, 137 and 169 (highly intensive), 331 and 145 (Table I**, 1c**).

Spectra of carvacrol and thymol (*o*- and *m*-isomers; **d**,**e**) are similar, but their retention times are different. The spectra of both phenols illustrate several fragmentation characteristics of the hydroxyl group on an aromatic ring. The intensive parent ion (*m*/*z* = 150) and slightly

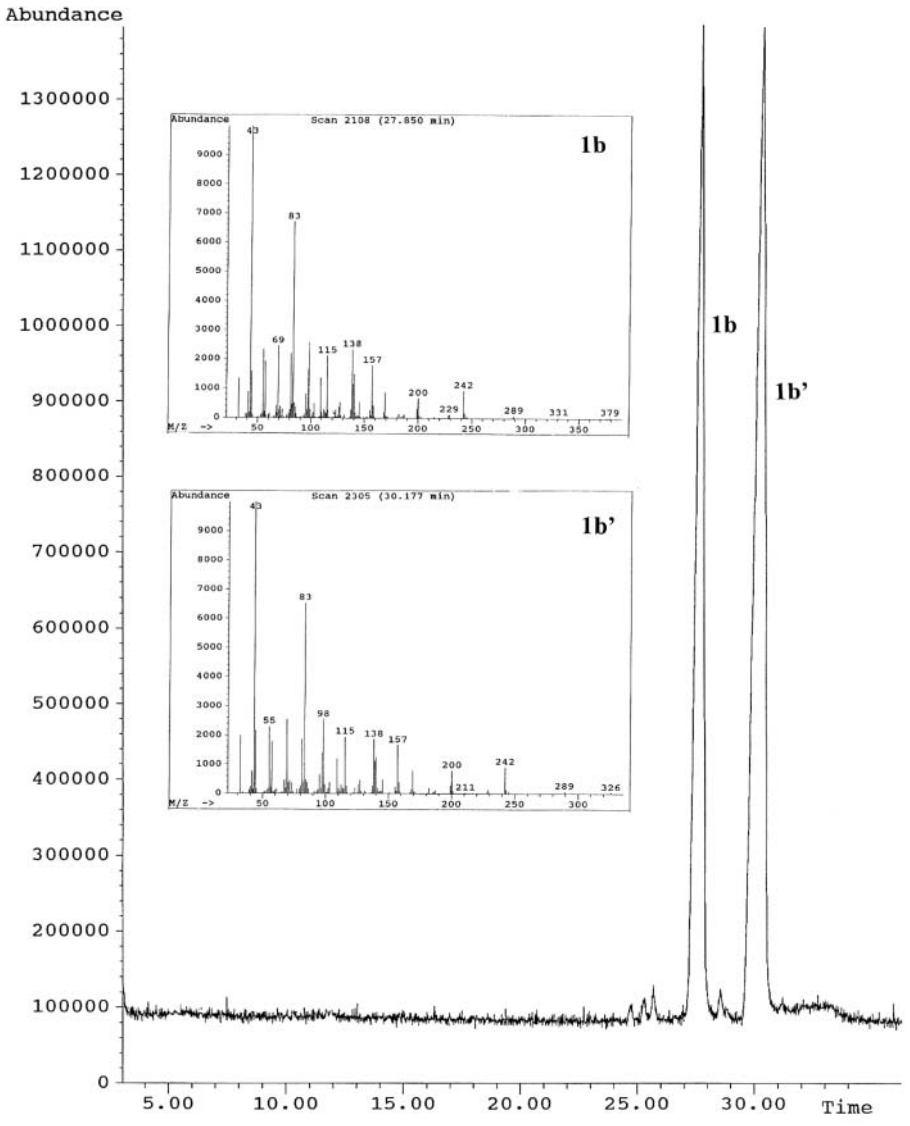


Figure 2. Gas chromatogram and mass spectra of diastereomeric menthyl-β-glucoside tetraacetates obtained from (±)-menthol: **1b**, (+) or (–)-menthyl-β-glucoside tetraacetate; **1b'**, (–) or (+)-menthyl-β-glucoside tetraacetate.

intensive ion m/z = 151 (Table II, **d**,**e**) were found in both spectra. The large abundance of the parent ion is characteristic of phenols.[13] The most intensive ion in both spectra is m/z = 135 ($C_9H_{11}O^+$). Ions such as tropylium m/z = 91, phenyl cation $C_6H_5^+$ (m/z = 77), $C_6H_6^+$ (m/z = 78) and $C_6H_7^+$ (m/z = 79) belong to aromatic compounds. The $(M\text{-}CO)^+$ peak (m/z = 122) was accompanied by $(M–CHO)^+$ ion $C_8H_9O^+$ with m/z = 121, which was useful for recognizing phenols. Both spectra contain slightly intensive ions with m/z = 136, 117 (135 is decreased by 18 mass units) and 107. Non-specific ions with masses 45 and 73 are O-indicators. The spectrum of carvacryl-β-glucopyranoside tetraacetate (**1e**) was also very similar to the spectrum of thymyl-β-glucopyranoside tetraacetate (**1d**). Their retention times were different. Both spectra contained acetyl ion as the most intensive one and almost the same ions as the thymol and carvacrol spectra, but with smaller total abundance. The parent ion of thymol and carvacrol (m/z = 150) and the peak m/z = 91 of tropylium ion can be seen in both spectra. Both spectra contained highly intensive ions with m/z = 109, 169, 127 and 135, while the ions with m/z = 115, 81, 145, 331, 187 and 211 were less abundant. The ion with m/z = 81 is characteristic of pyrans.

Eugenol spectrum (**f**) contains the parent ion (m/z = 164) as the most intensive ion (Table II, **f**). The peak with m/z = 165 is also intensive. Other abundant ions were $C_9H_9O_2^+$ (m/z = 149) and $C_9H_7O^+$ (m/z = 131). Ions with m/z = 77, 78, 79 and 91 are characteristic of aromatic compounds, and the peak m/z = 121 is characteristic of hydoxybenzenes.[12,13] Furthermore, the spectrum contains ions with m/z = 103, 104 and 55. The spectrum of eugenyl-β-glucopyranoside tetraacetate also contained acetyl ion as the most abundant one (Table I, **1f**). The peaks with m/z = 109, 169, 127 are also intensive. They were not identified in the spectrum of eugenol. The peak m/z = 81 is characteristic of pyrans. Peaks m/z = 103 and 131 were identified in the eugenol spectrum as distinct from peaks

TABLE III. ^{1}H NMR chemical shifts (δ/ppm)[a] and H-H coupling constants (^nJ/Hz)[b] of β-D-glucopyranosides **2a**–**2f**

	Benzyl- (**2a**)	Menthyl- (**2b, 2b'**)[c]	Bornyl- (**2c**)	Thymyl- (**2d**)	Carvacryl- (**2e**)	Eugenyl- (**2f**)
H-1	4.17 (1H) ^3J = 7.78 (d)	4.11 (1H) (**2b'**) ^3J = 7.79 (d) 4.07 (1H) (**2b**) ^3J = 7.74 (d)	4.00 (1H) ^3J = 7.81 (d)	4.68 (1H) ^3J = 7.34 (d)	4.70 (1H) ^3J = 7.48 (d)	4.78 (1H) ^3J = 7.32 (d)
H-2	2.98 (1H) (m)	2.86–2.80 (2H) (m) (**2b** and **2b'**)	2.85 (1H) ^3J = 7.72 (t)	3.17 (1H) (m)	3.19 (1H) (m)	2.98 (1H) ^3J = 7.62 (t)
H-3	3.09 (1H) ^3J = 8.60 (t)	3.07–3.01 (2H) (m) (**2b** and **2b'**)	3.04 (1H) ^3J = 8.19 (q)	3.19 (1H) (m)	3.20 (1H) (m)	3.22 (1H) (m)
H-4	3.03 (1H) ^3J = 8.80 (t)	3.00–2.94 (2H) (m) (**2b** and **2b'**)	3.00 (1H) (m)	3.09 (1H) (m)	3.09 (1H) (m)	3.19 (1H) (m)
H-5	3.04 (1H) (m)	3.33 (2H) (m) (**2b** and **2b'**)	2.95(1H) (m)	3.21 (1H) (m)	3.24 (1H) ^3J = 9.73, 6.03, 2.00 (ddd)	3.10 (1H) (m)
H-6A	3.64 (1H) ^2J = 11.59 (d) ^3J = 3.95 (d)	3.58 (2H) (m) (**2b** and **2b'**)	3.57 (1H) ^2J = 9.27 (d)	3.63 (1H) ^2J = 11.59 (d) ^3J = 1.37 (d)	3.63 (1H) ^2J = 11.78 (d) ^3J = 5.19 (d)	3.59 (1H) ^2J = 11.69 (d) ^3J = 2.77 (d)
H-6B	3.41 (1H) ^2J = 11.71(d) ^3J = 5.47 (t)	3.36 (2H) (m) (**2b** and **2b'**)	3.37 (1H) (m)	3.39 (1H) ^2J = 11.80 (d) ^3J = 5.64 (t)	3.39 (1H) ^2J = 11.71(d) ^3J = 6.15 (t)	3.37 (1H) ^2J = 11.81(d) ^3J = 5.79 (t)
OH-2	5.08 (1H) ^3J = 4.00 (d)	4.82 (2H) ^3J = 4.74 (d) (**2b** and **2b'**)	4.89 (1H) (s)	5.15 (1H) (s) br.[d]	5.18 (1H) ^3J = 4.97 (d)	5.13 (1H) ^3J = 4.47 (d)
OH-3	4.94 (1H) (s) br.[d]	4.80 (2H) ^3J = 5.54 (d) (**2b** and **2b'**)	4.86 (1H) (s)	5.03 (2H) (s) br.[d]	5.00 (1H) ^3J = 4.33 (d)	5.02 (1H) ^3J = 5.03 (d)
OH-4	4.89 (1H) (s) br.[d]	4.79 (2H) ^3J = 5.47 (d) (**2b** and **2b'**)	4.78 (1H) (s)	4.51 (1H) (s) br.[d]	4.94 (1H) ^3J = 5.34 (d)	4.94 (1H) ^3J = 4.89 (d)
OH-6	4.49 (1H) ^3J = 4.23 (t)	4.30 (1H) (**2b**) ^3J = 5.67 (d) 4.14 (1H) (**2b'**) ^3J = 5.36 (d)	4.32 (1H) ^3J = 4.00 (t)	4.32 (1H) (s) br.[d]	4.50 (1H) ^3J = 5.76 (t)	4.45 (1H) ^3J = 5.53 (t)

[a] Recorded in DMSO-d_6 solutions. Referred to TMS. Number of protons in brackets.

[b] Resolution ±0.29 Hz; *n* denotes the number of bonds between nuclei in spin-spin coupling; (s) singlet, (d) doublet, (t) triplet, (q) quartet, (m) complex multiplet splitting.

[c] Two diastereoisomeric menthyl-β-glucopyranosides exist (**2b** and **2b'**), since (±)-menthol was used in the synthesis. For the majority of protons the signals of two forms are overlapped, giving complex multiplets. In a few cases, separate signals were observed and hence two values are given.

[d] Signal is broadened.

with *m*/*z* = 145, 211 and 331, which were contained in the spectrum of eugenyl glucoside tetraacetate.

The β-pyranoside structure of investigated glucosides was confirmed by one- and twodimensional homo- and heteronuclear ^{1}H and ^{13}C NMR measurements in dimethylsulfoxide-d_6 solutions. This was determined on the basis of specific chemical shifts, H–H and C–H spin-spin couplings. The assignment was substantiated by connectivities in homo- (COSY, NOESY) and heteronuclear (HMQC, HMBC) correlation spectra as well. The number of signals and their integrals showed the existence of only one anomeric form. The glycone moiety ^{1}H and ^{13}C data of investigated glucosides are displayed in Tables III and IV, respectively (corresponding data for the aglycone moiety are given in the experimental section). One can see that the anomeric H-1 chemical shifts of glucosides are in the range 4.00–4.78 ppm, while anomeric C-1 chemical shifts are in the range 100.0–104.4 ppm. The observed values are in agreement with the β-configuration. In model α-anomers, the H-1 chemical shifts are downfield (4.50–5.50 ppm) and the C-1 chemical shifts are upfield (95–97 ppm) in comparison with those of β-anomers.[14–16] The one-bond C–H spin-spin couplings ($^1J_{CH}$) at C-1, amounting to 156–160 Hz, also corre-

TABLE IV. ^{13}C NMR chemical shifts (δ/ppm)[a] of β-D-glucopyranosides **2a**–**2f**

	Benzyl- (**2a**)	Menthyl-[b] (**2b, 2b'**)	Bornyl- (**2c**)	Thymyl- (**2d**)	Carvacryl- (**2e**)	Eugenyl- (**2f**)
C-1	102.10	104.36 (**2b**) 100.06 (**2b'**)	101.91	101.30	101.06	100.32
C-2	73.54	73.87 (**2b**) 73.36 (**2b'**)	73.51	73.49	73.35	73.26
C-3	76.77	76.89 (**2b**) 76.58 (**2b'**)	76.85	77.04	77.04	76.99
C-4	70.16	70.42 (**2b**) 70.15 (**2b'**)	70.15	69.89	69.92	69.73
C-5	76.98	76.60 (**2b**) 75.96 (**2b'**)	76.72	76.91	76.80	76.87
C-6	61.17	61.51 (**2b**) 61.32 (**2b'**)	61.17	60.83	60.78	60.72

[a] Recorded in DMSO-d_6 solutions. Referred to TMS.

[b] Two values of chemical shifts for each C-atom due to the presence of two diastereoisomeric menthyl-β-glucopyranosides **2b** and **2b'**.

spond to β-configuration, since the values of $^1J_{CH}$ in model α-compounds are *ca*. 10 Hz higher than those presented here.[15] A very reliable test of anomeric form is the value of the three-bond H-H spin-spin coupling ($^3J_{HH}$) between H-1 and H-2, which in α-glucopyranosides (H_{eq}, H_{ax}) amounts to 3.5–4.0 Hz, while in β-forms (H_{ax}, H_{ax}) it amounts to 7.5–8.0 Hz,[18–20] as was found here. Besides the mentioned ^{1}H and ^{13}C NMR spectral data, which unambiguously prove the β-pyranoside form of synthesized glucosides, some other features of their spectra are interesting as well.

Thus, in compounds **2a**–**f** the chemical shifts of H-2, H-3, H-4 and H-5 are in a narrow range from 2.85 to 3.33 ppm; hence their signals are partially overlapped. From COSY and NOESY analyses we determined that δ(H-3), δ(H-4) and δ(H-5) are very similar and somewhat greater than δ(H-2). All these protons show small variations of chemical shifts, obviously due to different substitution at C-1. The greatest changes were observed for the H-2 ($\Delta\delta$ = 0.34 ppm, *i.e,. ca.* 200 Hz) and H-5 ($\Delta\delta$ = 0.38 ppm, *i.e., ca.* 230 Hz), since the former is close to the substitution site, while the latter is in a spatial 1,3-diaxial interaction with H-1. Since measurements were performed in dimethylsulfoxide-d_6 solutions, the proton signals of all four OH groups are present, participating in spin-spin and dipole-dipole interactions with other protons. Thus, the signal of *gem* H-6_B (**2a**, **2d, 2e**, **2f**) appears as a quasi-quintet instead of the expected doublet of doublets (*dd*). The quintet arises from the overlapped doublet of triplets (*dt*), since additional spin-spin interaction with OH-6 exists. In **2a,** the signal of *gem* H-6_A remains *dd*, with smaller 3.95 Hz splitting arising from the interaction with OH-6, but not H-5, which was proven by the COSY spectrum. The signals of OH groups in **2a–f** showed a chemical shift range of 4.14–5.18 ppm. As in case of the glycone protons, the hydroxyl protons also show small variations in chemical shifts depending on substitution at C-1. The greatest change, $\Delta\delta$ (0.36 ppm, *i.e., ca.* 220 Hz), was found for OH-2. Other OH groups displayed rather smaller differences. The sequence of OH chemical shifts remains constant in all investigated glucosides and is: δ(OH-2) > δ(OH-3) > δ(OH-4) > δ(OH-6).

From Tables III and IV one can see that two diastereomeric menthyl-glucopyranosides were detected. They are both β-anomers, arising from the use of the (±)-menthol in the synthesis. For all protons in the glycone moiety, except for H-1 and OH-6, the signals of two forms are partially and/or completely overlapped, giving rather complex multiplet patterns. In Figure 3, the anomeric part of the ^{1}H spectrum of β-(±)-menthyl-glucopyranoside is displayed. Two sets of signals for H-1 (doublets) and OH-6 (triplets) exist**,** since two diastereoisomeric species are present.

In ^{13}C spectra of β-(±)menthyl-glucopyranosides, the doublets of signals for all carbons were observed in correspondence with two diastereoisomeric forms. In a glycone moiety, the greatest difference of chemical shifts of signals for the corresponding carbons in two diastereomeric forms was observed for the anomeric C-1 (100.06 and 104.36 ppm). C-1 is spatially the closest of all glycone carbons to the differently orientated O-substituted (+)- and (–)-menthyls. In all investigated glucosides, HMQC and HMBC spectra confirm that the C-3 and C-5 signals have very similar or sometimes even identical chemical shifts and that these signals are always more deshielded than the C-2 and C-4 signals, for which δ(C-2) >

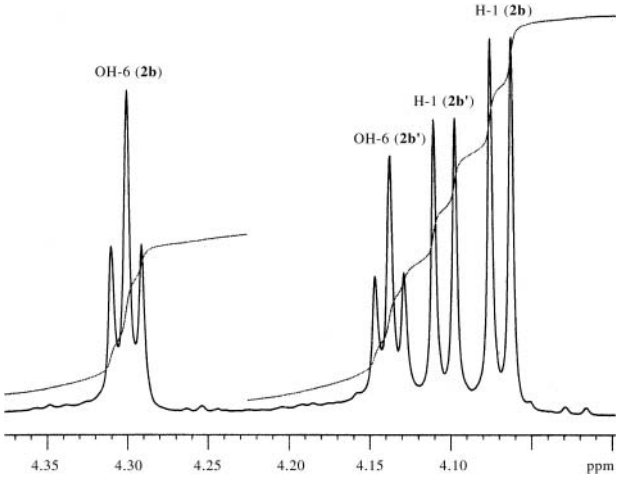


Figure 3. The anomeric part of the 600 MHz ^{1}H spectrum of β-(±)-menthyl-glucopyranosides. Two sets of the H-1 (doublets) and OH-6 (triplets) signals are visible due to the presence of two diastereomeric forms (**2b** and **2b'**).

δ(C-4) is valid. Therefore, one can establish that the general sequence for glycone ^{13}C chemical shifts is: δ(C-3) ~ δ(C-5) ~ δ(C-2) ~ δ(C-4).

The β-configuration of prepared glucosides was additionally confirmed by hydrolysis with β-glucosidase. All the prepared glucosides liberated aglycones, which were identified by TLC and GC-MS.

CONCLUSIONS

The glucosides of ubiquitous volatiles were prepared by the Koenigs-Knorr-Zemplén reaction. Their β-glucopyranosidic structures were determined on the basis of specific chemical shifts, H–H and C–H spin-spin coupling constants obtained from 1D and 2D homo- and heteronuclear ^{1}H and ^{13}C NMR spectra. Additionally, β-anomers were determined by hydrolysis with β-glucosidase. Glucoside tetraacetates are suitable for GC-MS analysis and for the resolution of racemic menthol. The spectra of glucoside tetraacetates mainly contained the more or less abundant characteristic ions of the aglycones and other ions generated by the combination of different parts of glucoside tetraacetate.

Acknowledgement. – The financial support from the Croatian Ministry of Science and Technology (Projects 0011010 and 0098059) is gratefully acknowledged.

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SAŽETAK

Sinteza odabranih prirodnih glukozida hlapljivih spojeva. Njihova kromatografska i spektroskopska svojstva

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Prirodni glukozidi benzil-alkohola, (±)-mentola, (+)-borneola, timola, karvakrola i eugenola sintetizirani su po Koenigs-Knorr-Zemplén metodi (prinosi 19,5–52,2 %). Njihova β-glukopiranozna struktura određena je jednodimenzijskom i dvodimenzijskom homo- i heteronuklearnom ^{1}H i ^{13}C NMR spektroskopijom. β-Konfiguracija je dodatno potvrđena hidrolizom s β-glukozidazom. Tetraacetil-β-glukopiranozidi, kao intermedijeri, analizirani su GC-MS sustavom. Dijastereomerni tetraacetati β-glukozida (±)-mentola bili su dobro odijeljeni na HP-101 koloni. Maseni spektri tetraacetata glukozida uspoređeni su međusobno i sa spektrima njihovih aglikona.