

# FT-IR i NMR spektroskopska istraživanja derivata salicilne kiseline. II. Usporedba 2-hidroksi- i 2,4- i 2,5-dihidroksi derivata

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## FT-IR and NMR spectroscopic studies of salicylic acid derivatives. II. Comparison of 2-hydroxy- and 2,4- and 2,5-dihydroxy derivatives

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The 2,4- and 2,5-dihydroxybenzamides (8, 9) were synthesized from their corresponding methyl esters. The structures and the spectral properties of investigated salicylic acid (1), 2,4- and 2,5-dihydroxy benzoic acids (2, 3), their methyl esters (4–6) and amides (7–9) were analyzed by means of FT-IR and one- and two-dimensional homo- and heteronuclear <sup>1</sup>H and <sup>13</sup>C NMR spectroscopies. Comparison of FT-IR and NMR spectral data of investigated compounds showed that the spectral characteristics of 2,4-dihydroxy benzoic acid derivatives are more similar to those of 2-hydroxy benzoic acid (salicylic acid) derivatives than to those of 2,5-dihydroxy benzoic acid derivatives. The results suggest that the spatial orientation of amide protons in 2,4-dihydroxy benzamide resembles more that in salicylamide than that in 2,5-dihydroxy benzamide.

**Keywords:** salicylic acid, 2,4- and 2,5-dihydroxy benzoic acids, methyl esters, amide derivatives, FT-IR, 1D and 2D homo- and heteronuclear <sup>1</sup>H and <sup>13</sup>C NMR

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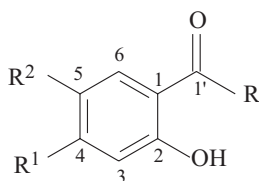
In addition to analgesic, antipyretic, and antiinflammatory properties, salicylates possess also some other actions that have been proven to be therapeutically beneficial (1, 2). More attention has been recently paid to the ability of salicylates to inhibit platelet aggregation. Unfortunately, a number of side-effects are associated with the use of salicylates, most notable being gastrointestinal disturbances such as dyspepsia, gastroduodenal bleeding, gastric ulcerations and gastritis (3, 4).

Salicylic acid is 2-hydroxy benzoic acid, the simplest aromatic carboxylic acid, which together with its hydroxyl derivatives are not only of importance as NSAIDs, but also as semi-products of biosynthesis of aromatic amino-acids in plants (phenolic acids), metabolites of numerous exogenous toxic substances including drugs, and endogenous catecholamines (5). It is well known that salicylic acid and its 2,4- and 2,5-dihydroxy derivatives are present in many medicinal plants, e.g., *Matricaria recutita* L.

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The pharmacological effect, as well as the side-effects of salicylic acid, are due to its structural features, so the active moiety for the cyclooxygenase (COX) inhibition appears to be the salicylate anion, while side-effects appear to be associated with the carboxylic acid functional group. Substitution on either the carboxyl or phenolic hydroxyl groups may affect potency and toxicity, while placing the phenolic hydroxyl group *meta*- or *para*- to the carboxyl group abolishes NSAID activity (1). Since metabolism affects pharmacological as well as toxicological effects of xenobiotics and drugs, the differentiation of structurally related metabolites is of biomedical interest (6).

Pharmacological effects and biotransformation pathways of salicylic acid and its derivatives are well known (1, 7–10), and the effect of the structural features on the physico-chemical properties and on the bioactivity of these compounds were investigated in numerous theoretical and experimental studies (11–22). For the physico-chemical properties of phenolic carboxylic acids, intra- and intermolecular hydrogen bonding is very important. This type of interaction is structure-determining, hence it is of great importance to rationalise the geometry of organic molecules and biomolecules (23–25). Although the hydrogen bond is fairly weak compared to other types of molecular interactions (e.g., electrostatic and van der Waals forces, as well as effects related to electron-transfer and/or hydrophobic interactions), it considerably determines the properties of the biological systems (26). Hydrogen bond strongly depends on the type of atoms that build up the hydrogen bridge. Information gained from FT-IR and NMR spectroscopies certainly enable clarifying the influence of structural parameters on the physico-chemical properties of H-bonded compounds.



- 1: R = OH, R<sup>1</sup> = R<sup>2</sup> = H, 2-hydroxy benzoic acid (salicylic acid)
- 2: R = R<sup>1</sup> = OH, R<sup>2</sup> = H, 2,4-dihydroxy benzoic acid ( $\beta$ -resorcylic acid)
- 3: R = R<sup>2</sup> = OH, R<sup>1</sup> = H, 2,5-dihydroxy benzoic acid (gentisic acid)
  
- 4: R = OCH<sub>3</sub>, R<sup>1</sup> = R<sup>2</sup> = H, methyl 2-hydroxybenzoate (methyl salicylate)
- 5: R = OCH<sub>3</sub>, R<sup>1</sup> = OH, R<sup>2</sup> = H, methyl 2,4-dihydroxybenzoate
- 6: R = OCH<sub>3</sub>, R<sup>1</sup> = H, R<sup>2</sup> = OH, methyl 2,5-dihydroxybenzoate (methyl gentisate)
  
- 7: R = NH<sub>2</sub>, R<sup>1</sup> = R<sup>2</sup> = H, 2-hydroxybenzamide (salicylamide)
- 8: R = NH<sub>2</sub>, R<sup>1</sup> = OH, R<sup>2</sup> = H, 2,4-dihydroxybenzamide
- 9: R = NH<sub>2</sub>, R<sup>1</sup> = H, R<sup>2</sup> = OH, 2,5-dihydroxybenzamide (gentisamide)

Scheme 1

The finding that the amide protons orientation is opposite in gentisamide to that in salicylamide, observed by NMR measurements in DMSO-*d*<sub>6</sub>, initiated the study of the substituent effect in 2-hydroxy- and 2,4- and 2,5-dihydroxy benzoic acids and their methyl esters and amides. Salicylic acid and its derivatives could serve as a good model system for investigating H-bonding and the effect of additional hydroxyl substitution in aromatic moieties.

The aim of this work was to study the substituent effects on the spectral properties of salicylic acid derivatives (**1** to **9**, Scheme 1).

## EXPERIMENTAL

Melting points were determined on a Boëtius Microheating Stage (Franz Küstner Nachf. KG, Germany) and remained uncorrected. Elemental analyses were performed by means of a CHN-LECO-932 elemental analyzer (LECO Corporation, USA).

Salicylic acid, 2,4-dihydroxy and 2,5-dihydroxy benzoic acid, methyl salicylate, methyl 2,4-dihydroxy- and 2,5-dihydroxybenzoate and salicylamide were purchased from Sigma-Aldrich (USA) and were used without further purification. 2,4-Dihydroxy- (**8**) and 2,5-dihydroxybenzamides (**9**) were prepared by a modified procedure according to Bray *et al.* (7). Synthesized amides were purified by column chromatography (silica gel 70–230 mesh ASTM, 0.063–0.200 mm, Kemika, Croatia) using ethyl acetate as a mobile phase. Thin-layer chromatography (TLC) was performed on a 2-mm thick silica gel sheets Kieselgel 60 F<sub>254</sub> (Merck, Germany) using the following solvent mixtures: benzene/ether/acetic acid/methanol (60:30:9:1), benzene/ether/acetic acid/water (50:40:9:1) and acetone/ethyl acetate/water (5.4:1). Spots were detected under UV light (254 nm) using iron(III)-chloride as a reagent.

All other chemicals were of analytical grade and were commercially purchased.

### *Spectral analyses*

Infrared absorption spectra were obtained in the range from 4000 to 450 cm<sup>-1</sup> by using a Perkin-Elmer Paragon 500 FT-IR spectrometer. Solid samples dispersed in KBr pellets were used.

The <sup>1</sup>H and <sup>13</sup>C one- and two-dimensional NMR spectra were recorded with a Varian Gemini 300 spectrometer (Varian, USA) operating at 300 MHz and 75.5 MHz for the <sup>1</sup>H and <sup>13</sup>C nucleus, respectively, and with a Bruker Avance DRX500 spectrometer (Bruker, Germany), operating at 500 MHz and 125 MHz for the <sup>1</sup>H and <sup>13</sup>C nucleus, respectively. Experiments were performed in DMSO-*d*<sub>6</sub> at 20 °C in 5-mm NMR tubes. Chemical shifts  $\delta$  in ppm are referred to TMS as the internal standard.

The following spectra were recorded on a Gemini 300 spectrometer: <sup>1</sup>H, <sup>13</sup>C broadband proton decoupling, gated proton decoupling, APT, COSY-45, LRCOSY-45, NOESY and HETCOR. Digital resolutions in one-dimensional <sup>1</sup>H NMR spectra were 0.20 Hz, and in <sup>13</sup>C NMR spectra 0.60 Hz per point. In all experiments, proton decoupling was performed by Waltz-16 modulation. In two-dimensional experiments, standard pulse sequences were used. COSY-45 and LRCOSY-45 spectra were measured in the magnitude mode, while NOESY

spectra in the phase-sensitive mode. In COSY-45 and NOESY spectra, 1024 points in F2 dimension and 256 increments in F1 dimension, subsequently zero-filled to 1024 points, were used. Each increment was obtained with 16 scans, 3000 Hz spectral width and a relaxation delay of 1 s. Thus, the digital resolution was 5.9 Hz per point and 11.7 Hz per point in F2 and F1 dimensions, respectively. The time delay (D3) in LRCOSY-45 was set to 0.3 s. NOESY spectra were measured with several mixing times (0.45–1.2 s). HETCOR spectra were recorded with 2048 points in F2 dimension and 256 increments in F1 dimension, zero-filled to 512 points. Increments were recorded with 180 scans, relaxation delay of 1 s and spectral width of 20000 Hz in F2 and 4500 Hz in F1 dimensions. The corresponding digital resolutions were 19.53 and 17.6 Hz per point in F2 and F1 dimensions, respectively.

The following spectra were recorded on a Bruker Avance DRX500 spectrometer:  $^1\text{H}$ , APT, HMBC. Absolute value HMBC spectra were measured with pulsed field gradients. The relaxation delay was 1.5 s, while spectra were recorded with 8–16 scans per increment. The spectral width was 6600 Hz in the acquisition domain F2 and 31000 Hz in the time domain F1. Data were collected into the 2048 x 256 acquisition matrix and processed using a 2K x 1K transformed matrix with zero filling in F1 domain. The delay for the long-range couplings was set to 60 ms in HMBC spectra.

### Syntheses

*General procedure for preparation of 2,4-dihydroxy- (8) and 2,5-dihydroxybenzamide (9).* – Amides **8** and **9** were prepared by addition of 1.69 g (0.10 mol) methyl 2,4-dihydroxy- or 2,5-dihydroxybenzoate in 10 mL of ammonia (pro analysi, 25% in water). The reaction mixture was stirred for 24 hours at room temperature. The excess of ammonia and methanol formed during the reaction were removed under reduced pressure. Upon addition of 10 mL of water, the product was extracted by ether. Ether extracts were collected, washed with water, and after separation the ether layer was dried over anhydrous sodium sulfate and evaporated under reduced pressure to give amide **8** or **9**. Crude products were purified by column chromatography using ethyl acetate as a mobile phase.

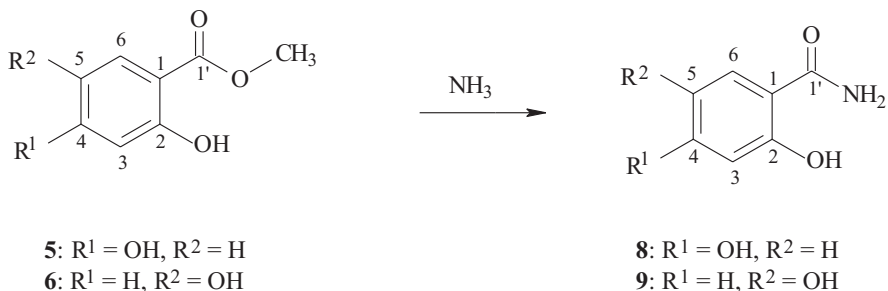
Physical-chemical data for compounds **8** and **9** are as follows:

**8**: yield 0.71 g (42.0%), m.p. 244–245 °C,  $M_r$  153.13, analysis for  $\text{C}_7\text{H}_7\text{NO}_3$  (%): calcd. (found) C 54.90 (55.21), H 4.61 (4.33), N 9.14 (9.41);  $R_{f1}$  0.53 (benzene/ether/acetic acid/methanol, 60:30:9:1),  $R_{f2}$  0.43 (benzene/ether/acetic acid/water, 50:40:9:1),  $R_{f3}$  0.86 (acetone/ethyl acetate/water, 5:4:1); IR (KBr)  $\nu$ : 3437vs, 3373s, 3320s, 3216vs, 1671vs, 1632vs, 1515s, 1428s, 1392s, 1332s, 1304s, 1249s, 1165s, 1115s, 975m, 840m, 762m, 708w, 612m and 521m  $\text{cm}^{-1}$ .

**9**: yield 0.54 g (32.1%), m.p. 217–218 °C (215–216 °C in ref. 7),  $M_r$  153.13, analysis for  $\text{C}_7\text{H}_7\text{NO}_3$  (%): calcd. (found) C 54.90 (54.63), H 4.61 (4.58), N 9.14 (9.25);  $R_{f1}$  0.36 (benzene/ether/acetic acid/methanol, 60:30:9:1),  $R_{f2}$  0.31 (benzene/ether/acetic acid/water, 50:40:9:1),  $R_{f3}$  0.83 (acetone/ethyl acetate/water, 5:4:1); IR (KBr)  $\nu$ : 3444s, 3393s, 3350s, 3274s, 2971m, 2873m, 1665s, 1577s, 1495s, 1424s, 1353s, 1269s, 1234s, 1138m, 106m, 934w, 825m, 791s, 731w, 667m, 638w and 550w  $\text{cm}^{-1}$ .

## RESULTS AND DISCUSSION

For the purpose of spectroscopic investigation, amides 2,4- and 2,5-dihydroxybenzamide were prepared by ammonolysis in the reaction of the corresponding methyl esters, methyl 2,4- and 2,5-dihydroxybenzoate with ammonia. This reaction proceeds by the mechanism where the nucleophile is ammonia and the leaving group is methyl alcohol (Scheme 2):



Scheme 2

Salicylic acid (1), 2,4- and 2,5-dihydroxy benzoic acids (2, 3), and their corresponding methyl esters, *i.e.*, methyl salicylate (4), methyl 2,4- and 2,5-dihydroxybenzoates (5, 6) as well as amides, *i.e.*, salicylamide (7), 2,4- and 2,5-dihydroxybenzamides (8, 9) were

Table I. Characteristic stretching absorption bands ( $\nu$ ,  $\text{cm}^{-1}$ ) in FT-IR spectra (KBr) of compounds 1 to 9

| Compd. | Wavenumber, $\nu$ ( $\text{cm}^{-1}$ ) |                                   |                     |
|--------|--|-----------------------------------|---------------------|
|        | $\nu_{\text{OH}}$                      | $\nu_{\text{C=O}}$                | $\nu_{\text{NH}_2}$ |
| 1      | 3238                                   | 1662                              | –                   |
| 2      | 3374<br>3300–2500                      | 1650                              | –                   |
| 3      | 3310<br>3300–2500                      | 1670                              | –                   |
| 4      | 3186                                   | 1686                              | –                   |
| 5      | 3338<br>3186                           | 1642                              | –                   |
| 6      | 3339<br>3227                           | 1678                              | –                   |
| 7      | 3398                                   | 1676 (amide I)<br>1590 (amide II) | 3364<br>3190        |
| 8      | 3450<br>3216                           | 1672 (amide I)<br>1612 (amide II) | 3372<br>3326        |
| 9      | 3446<br>3274                           | 1666 (amide I)<br>1576 (amide II) | 3396<br>3348        |

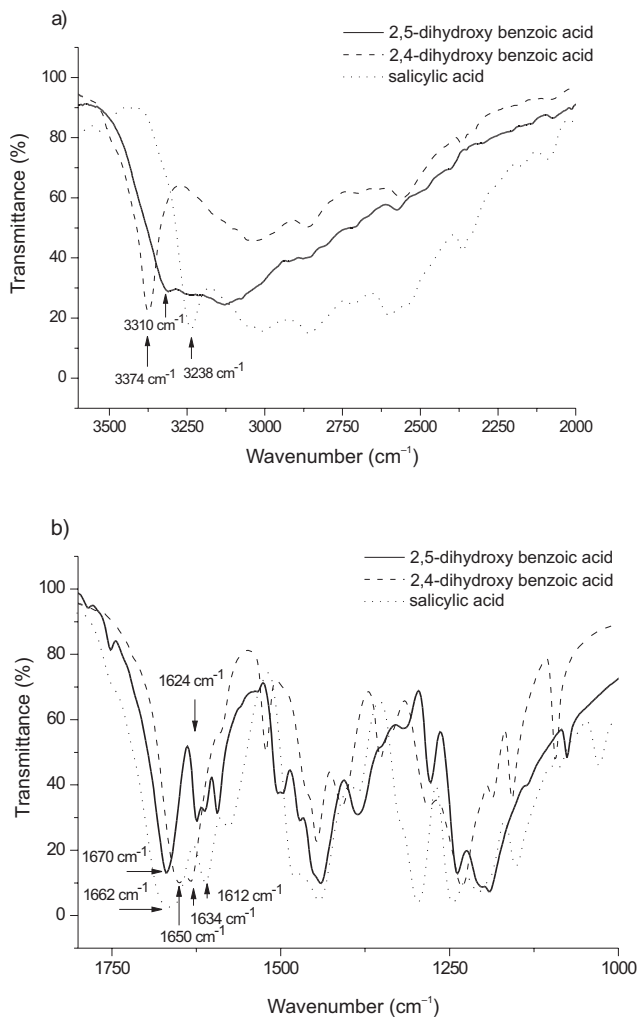


Fig. 1. FT-IR spectra of salicylic acid (1), 2,4- (2) and 2,5-dihydroxy benzoic acid (3) in the region of: a) 4400–2000 cm<sup>-1</sup>, and b) 1800–1000 cm<sup>-1</sup>.

analyzed by FT-IR and one- and two-dimensional homo- and heteronuclear <sup>1</sup>H and <sup>13</sup>C NMR methods.

Derivatives of carboxylic acids are characterized by several intense absorptions in the infrared spectrum (27). The most prominent absorption bands are in the carbonyl stretching region (1660–1800 cm<sup>-1</sup>). Their exact position depends on the type of acid derivative. Thus, carboxylic acids usually absorb in the region 1700–1725 cm<sup>-1</sup>, while esters are at a somewhat higher frequency (1735–1750 cm<sup>-1</sup>) and amides at a slightly lower frequency (1670–1690 cm<sup>-1</sup>). However, the presence of other substituents or H-bonding in the molecule gives rise to additional shifting of these bands. In addition to the carbonyl stretching absorption, the acids themselves exhibit a strong, broad absorption of O-H

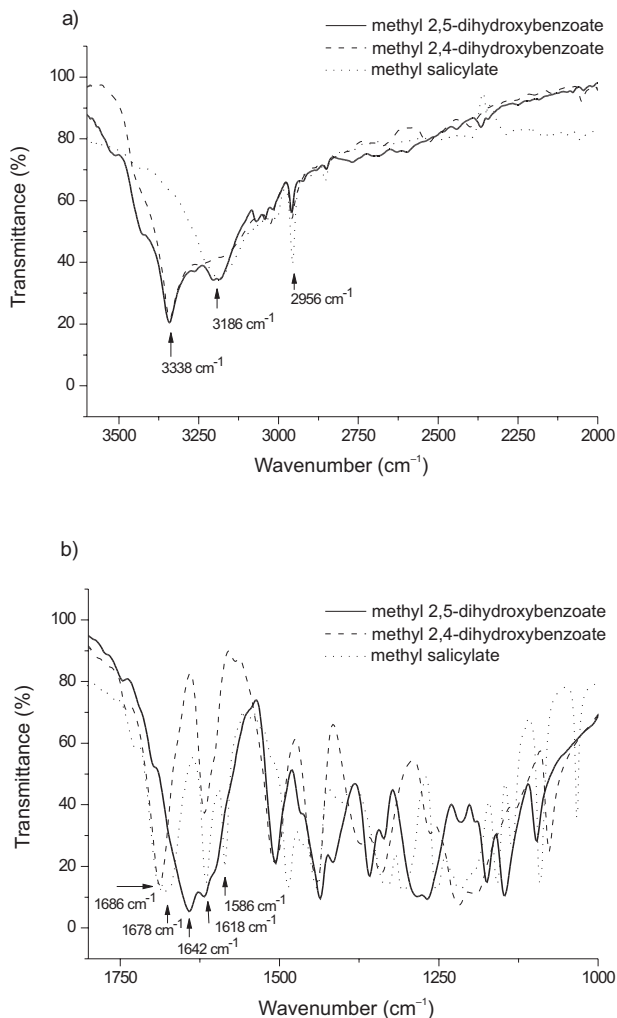


Fig. 2. FT-IR spectra of methyl salicylate (4), methyl 2,4- (5) and methyl 2,5-dihydroxy benzoate (6) in the region of: a) 4400–2000  $\text{cm}^{-1}$ , and b) 1800–1000  $\text{cm}^{-1}$ .

stretching. Due to the strongly hydrogen-bonded hydroxyl group, this band is spread over the range of 3500–2500  $\text{cm}^{-1}$ . This absorption is one of the broadest absorptions in an infrared spectrum, and it is frequently more than 600  $\text{cm}^{-1}$  wide, hence quite easy to recognize (23, 27).

The characteristic stretching absorption bands of the investigated compounds 1–9 are collected in Table I. Comparison of the IR spectrum of 1 (one hydroxyl group at 2-position, Scheme 1) with the spectra of 2 and 3 (two hydroxyl groups at 2,4- and 2,5-positions) showed significant differences in the OH-absorption region, both in band width and frequency (Fig. 1a). Thus, in compound 1, the broad absorption band spreads from 3523  $\text{cm}^{-1}$  to 2086  $\text{cm}^{-1}$ , having several peaks. The peak with the highest absorption in-



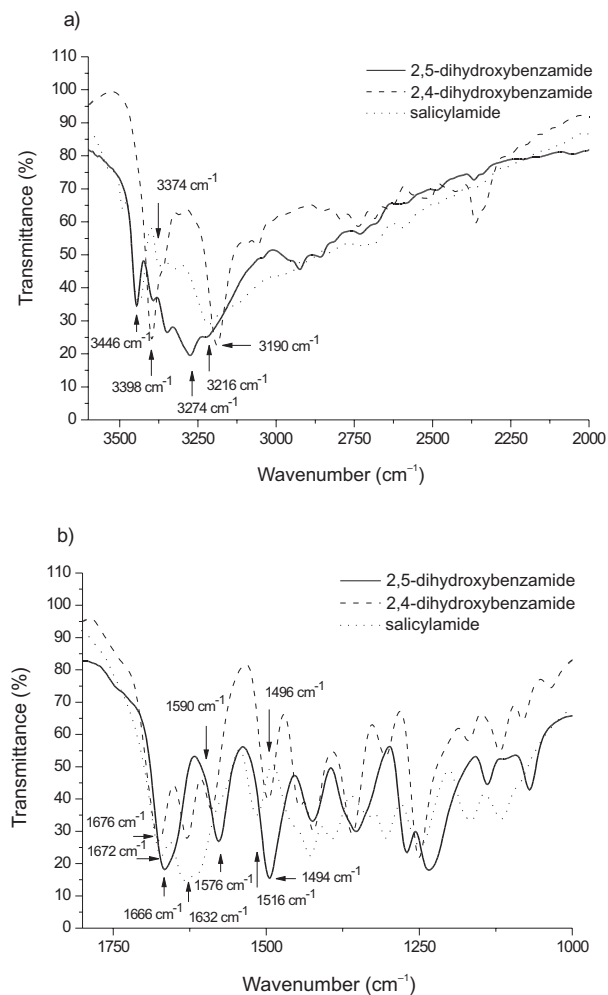


Fig. 3. FT-IR spectra of salicylamide (7), 2,4- (8) and 2,5-dihydroxybenzamide (9) in the region of: a) 4400–2000  $\text{cm}^{-1}$ , and b) 1800–1000  $\text{cm}^{-1}$ .

tensity is at 3238  $\text{cm}^{-1}$ . In compound 2, the OH absorption band spreads from 3514  $\text{cm}^{-1}$  to 2138  $\text{cm}^{-1}$ , while in compound 3 from 3532  $\text{cm}^{-1}$  to 2138  $\text{cm}^{-1}$ . The highest absorption peak in 2 is at 3374  $\text{cm}^{-1}$ . In compound 3, the broad absorption band spreads from 3310  $\text{cm}^{-1}$  to 3132  $\text{cm}^{-1}$ . These broadened bands correspond to different strengths of the intra- and intermolecular H-bonding, which arise from the different number and position of OH groups. The same is reflected on the carbonyl absorption bands in compounds 1 to 3, 1662  $\text{cm}^{-1}$ , 1650  $\text{cm}^{-1}$  and 1670  $\text{cm}^{-1}$ , respectively (Fig. 1b).

In esters (4 to 6), the OH absorption region is less spread than in the corresponding acids, which is expected due to the substitution of the methyl group for the OH group in COOH moiety. The 2-OH band in methylsalicylate (4) is at 2956  $\text{cm}^{-1}$ . In both 5 and 6, the additional OH band is at 3338  $\text{cm}^{-1}$  (Fig. 2a). The carbonyl region of esters revealed

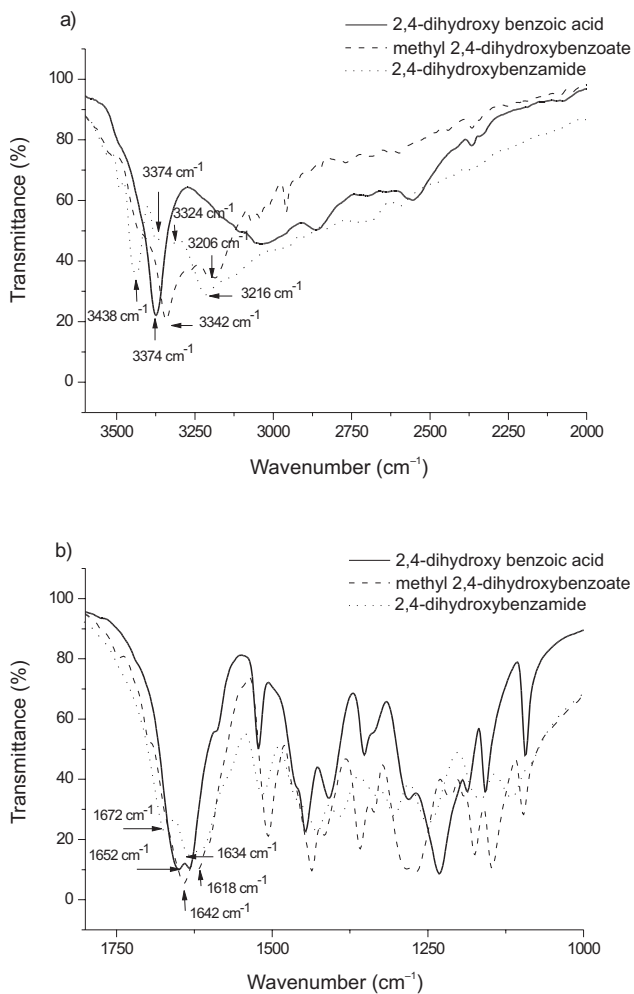


Fig. 4. FT-IR spectra of 2,4-dihydroxybenzamide (8) comparing to its corresponding acid (2) and ester (5) in the region of: a) 4400–2000 cm<sup>-1</sup>, and b) 1800–1000 cm<sup>-1</sup>.

expected differences in the corresponding acids. The differences between carbonyl absorption in esters arise due to the different number and positions of hydroxy groups. Namely, in methylsalicylate (4) and methyl 2,5-dihydroxybenzoate (6) the carbonyl shift is in accordance with the expectation for esters, *i.e.*, towards slightly higher wavenumbers, while in methyl 2,4-dihydroxybenzoate (5) carbonyl absorption is shifted towards lower wavenumbers. The latter is due to the fact that the 4-OH group increases the ability of 2-OH intramolecular H-bonding (Fig. 2b).

Primary amides give rise to medium-intensity N-H stretching absorptions in the same region as for the O-H absorptions. The typical range for N-H stretching is 3250–3400 cm<sup>-1</sup>. Primary amides give two bands in this region. According to the findings that 2-OH group in 7 forms the N...HO type of hydrogen bond, two distinguished N-H ab-

Table II.  $^1\text{H}$  NMR data for compounds 1 to 9 in  $\text{DMSO-}d_6$

| H-atom          | $^1\text{H}$ NMR, $\delta$ (ppm), $^nJ_{\text{H,H}}$ (Hz) |                                       |  |  |  |  |                        |  |  |
|-----------------|---|---------------------------------------|--|--|--|--|------------------------|--|--|
|                 | 1   | 2                                     | 3                                      | 4                                      | 5                                      | 6                                      | 7                      | 8                                      | 9                                      |
| COOH            | 13.44, bs   | 10.78                                 | 13.25                                  | –                                      | –                                      | –                                      | –                      | –                                      | –                                      |
| NH <sub>2</sub> | –   | –                                     | –                                      | –                                      | –                                      | –                                      | 8.43, s<br>7.92, s     | 8.09, s<br>7.58, s                     | 8.24, s<br>7.74, s                     |
| C2-OH           | 11.52, bs   | 10.78                                 | 10.25                                  | 10.62                                  | 10.74, s                               | 9.92, s                                | 13.07                  | 13.28, s                               | 12.13, s                               |
| C4-OH           | –   | 10.78                                 | –                                      | –                                      | 10.46, s                               | –                                      | –                      | 10.07, s                               | –                                      |
| C5-OH           | –   | –                                     | 11.40                                  | –                                      | –                                      | 9.19, s                                | –                      | –                                      | 8.98, s                                |
| CH <sub>3</sub> | –   | –                                     | –                                      | 3.92                                   | 3.84, s                                | 3.04, s                                | –                      | –                                      | –                                      |
| H-3             | 6.96, d<br>$^3J = 7.5$                                    | 7.20, s                               | 7.60, d<br>$^3J = 8.7$                 | 7.02, dd<br>$^3J = 8.4$<br>$^4J = 0.8$ | 6.32, d<br>$^3J = 2.2$                 | 6.79, d<br>$^3J = 8.9$                 | 7.85, d<br>$^3J = 7.9$ | 6.18, d<br>$^4J = 2.3$                 | 6.86, dd<br>$^3J = 8.6$<br>$^4J = 2.0$ |
| H-4             | 7.52, t<br>$^3J = 7.7$                                    | –                                     | 6.32, dd<br>$^3J = 8.6$<br>$^4J = 2.3$ | 7.54, dt<br>$^3J = 7.2$<br>$^4J = 1.7$ | –                                      | 6.69, dd<br>$^3J = 8.9$<br>$^4J = 3.0$ | 7.38, t<br>$^3J = 7.3$ | –                                      | 6.69, d<br>$^3J = 8.6$<br>$^4J = 2.0$  |
| H-5             | 6.92, t<br>$^3J = 7.4$                                    | 6.89, d<br>$^3J = 9.0$<br>$^4J = 1.3$ | –                                      | 6.95, dt<br>$^3J = 6.1$<br>$^4J = 1.1$ | 6.38, dd<br>$^3J = 8.7$<br>$^4J = 2.3$ | –                                      | 6.85, t<br>$^3J = 7.6$ | 6.23, dd<br>$^3J = 8.7$<br>$^4J = 2.3$ | –                                      |
| H-6             | 7.82, d<br>$^3J = 7.7$                                    | 6.69, d<br>$^3J = 9.0$                | 6.26, d<br>$^4J = 2.3$                 | 7.79, dd<br>$^3J = 7.9$<br>$^4J = 1.6$ | 7.64, d<br>$^3J = 8.7$                 | 7.13, d<br>$^4J = 3.0$                 | 6.89, d<br>$^3J = 7.9$ | 7.64, d<br>$^3J = 8.6$                 | 7.20, d<br>$^4J = 2.0$                 |

bs – broadened singlet, s – singlet, d – doublet, t – triplet, dd – doublet of doublets, dt – doublet of triplets

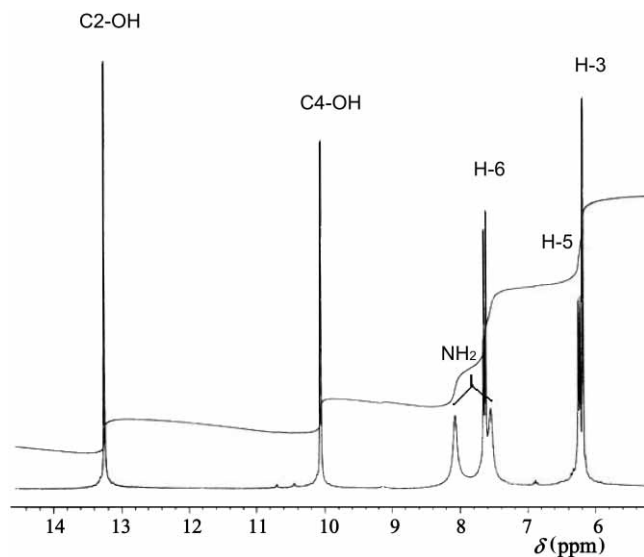


Fig. 5. The  $^1\text{H}$  NMR spectrum of 2,4-dihydroxybenzamide (8) displaying two separated signals for amide protons.

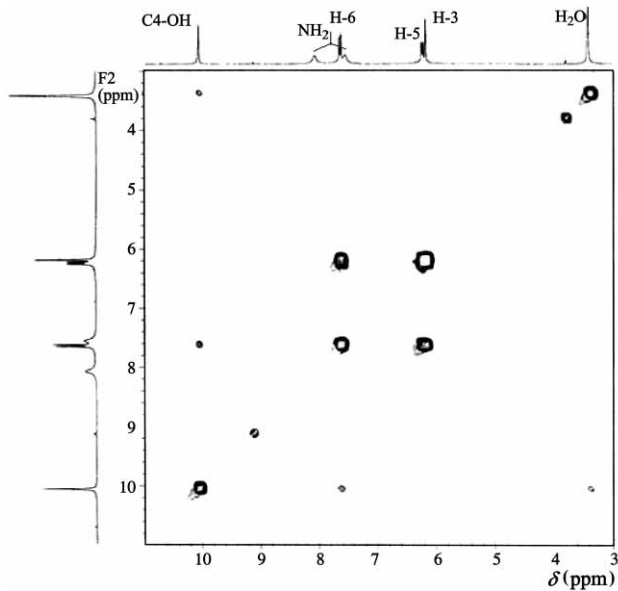


Fig. 6. The LRCOSY-45 spectrum of 2,4-dihydroxybenzamide (8) displaying the five-bond H-H coupling between H-6 and C4-OH protons.

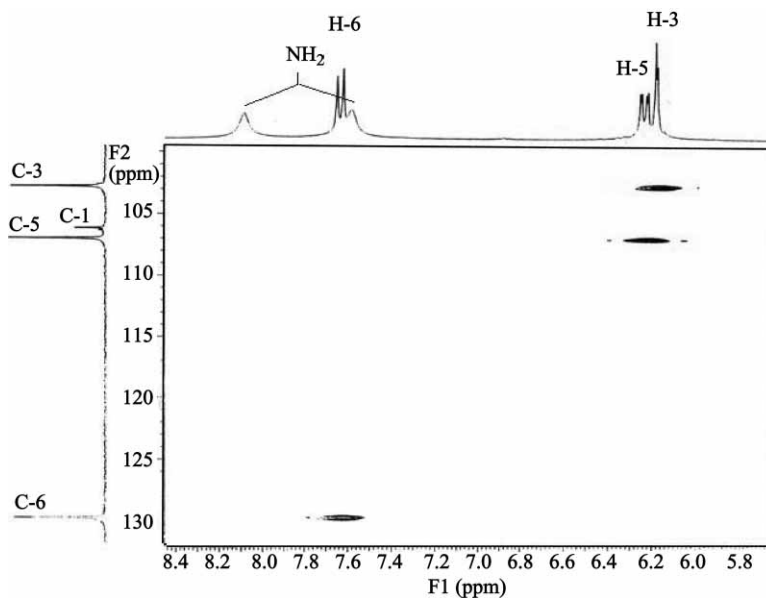


Fig. 7. The HETCOR spectrum of 2,4-dihydroxybenzamide (8) showing C-H connectivities in the aromatic moiety.

sorptions at 3364 cm<sup>-1</sup> and at 3190 cm<sup>-1</sup> have been assigned to NH stretching absorption bands of the primary amide moiety (28).

The NH region in the IR spectrum of **8** is more similar to that of **7** than to that of **9** (Fig. 3a). This is in accordance with findings for corresponding acids and esters. The same similarity was found in the carbonyl region of amides **7** and **8** (Fig. 3b). In Fig. 4a and b, FT-IR spectra of 2,4-dihydroxybenzamide are compared to the corresponding acid and methyl ester.

The <sup>1</sup>H NMR data are collected in Table II. In acids **1** to **3**, the <sup>1</sup>H NMR chemical shifts of carboxylic protons are shifted more downfield than those of hydroxyl protons. Aromatic protons have chemical shifts, H-H coupling constants and splitting patterns in accordance with the structure of acids as well as esters **4** to **6**. In all investigated amides (**7** to **9**), two separated signals for NH<sub>2</sub> were observed due to restricted rotation around the C-N bond. Thus, the NH<sub>2</sub> protons chemical shifts in **7** are at 8.43 ppm and 7.92 ppm, in **8** at 8.09 ppm and 7.58 ppm (Fig. 5), while in **9** at 8.24 ppm and 7.74 ppm. The hydroxyl proton chemical shift at C-2 was observed at 13.07 ppm in **7**, 13.28 ppm in **8**, and 12.13 ppm in **9**. One can see that the chemical shift values of 2-OH resemble more each other in **7** and **8** than in **8** and **9**, which is in agreement with different substituent effects of C4-OH in **8** and C5-OH in **9**. Different influences of C4-OH and C5-OH were observed in FT-IR spectra as well. On the basis of these results, one can assume that the spatial orientation in 2,4-dihydroxybenzamide (**8**) is more similar to that in salicylamide (**7**) than to that in 2,5-dihydroxybenzamide (**9**). The <sup>1</sup>H NMR spectrum of **8** is shown in Fig. 5, the LRCOSY45 in Fig. 6 and its HETCOR spectrum in Fig. 7.

Table III. <sup>13</sup>C NMR data for compounds **1** to **9** in DMSO-d<sub>6</sub>

| Compd.   | <sup>13</sup> C NMR, δ (ppm) |        |        |        |        |        |        |                         |
|----------|------------------------------|--------|--------|--------|--------|--------|--------|-------------------------|
|          | C-1                          | C-2    | C-3    | C-4    | C-5    | C-6    | C-1'   | C-2' (CH <sub>3</sub> ) |
| <b>1</b> | 113.02                       | 161.29 | 117.21 | 135.76 | 119.28 | 130.40 | 172.08 | –                       |
| <b>2</b> | 118.81                       | 150.29 | 113.61 | 155.53 | 115.88 | 124.91 | 172.96 | –                       |
| <b>3</b> | 104.65                       | 163.75 | 102.61 | 132.23 | 164.35 | 108.27 | 172.27 | –                       |
| <b>4</b> | 112.65                       | 160.24 | 117.25 | 135.52 | 119.19 | 129.79 | 169.37 | 52.21                   |
| <b>5</b> | 106.48                       | 162.87 | 102.62 | 164.37 | 108.48 | 131.70 | 169.74 | 52.08                   |
| <b>6</b> | 109.79                       | 147.01 | 115.56 | 111.53 | 150.65 | 121.30 | 166.73 | 49.78                   |
| <b>7</b> | 114.57                       | 161.31 | 117.60 | 134.23 | 118.52 | 128.29 | 172.34 | –                       |
| <b>8</b> | 106.16                       | 163.55 | 102.76 | 162.65 | 106.99 | 129.66 | 172.49 | –                       |
| <b>9</b> | 114.96                       | 153.64 | 117.92 | 121.98 | 149.15 | 113.83 | 171.79 | –                       |

The <sup>13</sup>C NMR data are collected in Table III. The number of carbon signals and their chemical shifts correspond to the structure of investigated compounds. Due to the presence of several substituents, the <sup>13</sup>C chemical shifts do not comply with the additivity rule. However, the assignments were confirmed by HMBC spectra.

## CONCLUSIONS

The spectral properties of salicylic acid derivatives and 2,4- and 2,5-dihydroxy benzoic acid derivatives (methyl esters and amides), **1** to **9**, were analyzed by means of FT-IR, one- and twodimensional homo- and heteronuclear  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectroscopy. The results showed that the FT-IR and NMR spectral characteristics of 2,4-dihydroxy benzoic acid derivatives (**2**, **5**, **8**) are more similar to those of salicylic acid derivatives (**1**, **4**, **7**) than to those of 2,5-dihydroxy benzoic acid derivatives (**3**, **6**, **9**). The results also suggest that the spatial orientation of amide protons in 2,4-dihydroxybenzamide (**8**) resembles more on the orientation of amide protons in salicylamide (**7**) than those in 2,5-dihydroxybenzamide (**9**).

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*Abbreviations.* COSY – Homonuclear Correlated Spectroscopy, FT-IR – Fourier Transform Infrared Spectroscopy, HETCOR – Heteronuclear Chemical Shift Correlation, HMBC – Homonuclear Multiple Bond Coherence, NMR – Nuclear Magnetic Resonance, NOESY – Nuclear Overhauser and Exchange Spectroscopy, NSAID – Nonsteroidal Antiinflammatory Drugs.

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S A Ž E T A K

**FT-IR i NMR spektroskopska istraživanja derivata salicilne kiseline.  
II. Usporedba 2-hidroksi- i 2,4- i 2,5-dihidroksi derivata**

MILENA JADRIJEVIĆ-MLADAR TAKAČ i DRAŽEN VIKIĆ-TOPIĆ

Spektroskopska svojstva derivata salicilne kiseline i 2,4- i 2,5-dihidroksi derivata benzojeve kiseline (metilnih estera i amida), **1** do **9**, analizirana su pomoću FT-IR te jedno- i dvodimenzijске homo- i heteronuklearne  $^1\text{H}$  i  $^{13}\text{C}$  NMR spektroskopije. Dobiveni rezultati pokazuju da su FT-IR i NMR spektralne karakteristike derivata 2,4-dihidroksi-benzojeve kiseline (**2**, **5**, **8**) sličnije karakteristikama derivata salicilne kiseline (**1**, **4**, **7**) nego derivata 2,5-dihidroksi-benzojeve kiseline (**3**, **6**, **9**). Rezultati istraživanja ukazuju da je prostorna orijentacija amidnih protona 2,4-dihidroksibenzamida (**8**) sličnija orijentaciji amidnih protona salicilamida (**7**) nego 2,5-dihidroksibenzamida (**9**).

*Ključne riječi:* salicilna kiselina 2,4- i 2,5-dihidroksi-benzojeva kiselina, metilni esteri, amidi, FT-IR, 1D i 2D homo- i heteronuklearna  $^1\text{H}$  i  $^{13}\text{C}$  NMR

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