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COMPLETE ASSIGNMENT OF ^1H - AND ^{13}C -NMR SPECTRA OF ANTHRANILIC ACID AND ITS HYDROXY DERIVATIVES AND SALICYLIC ACID AND ITS AMINO DERIVATIVES[†]

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Abstract. We report on the detailed NMR spectral analyses of amino- or/and hydroxy-substituted benzoic acids: anthranilic (AA), 3-hydroxyanthranilic (3-HAA), 5-hydroxyanthranilic (5-HAA), salicylic (SA), 4-aminosalicylic (4-ASA) and 5-aminosalicylic (5-ASA) acids. According to a literature survey, there are limited, unassigned or even incorrectly assigned spectral data to these benzoic acid derivatives. In order to amend the situation, a complete assignment of ^1H - and ^{13}C -NMR spectra of these compounds, recorded in perdeuteriodimethyl sulfoxide (DMSO-d_6), based on a combination of 1D- and 2D-NMR experiments, including ^1H - ^1H COSY, NOESY, HSQC and HMBC, was performed.

Key words: ^1H - and ^{13}C -NMR, 1D- and 2D-NMR, anthranilic acid, 3- and 5-hydroxyanthranilic acids, salicylic acid, 4- and 5-aminosalicylic acids

1. INTRODUCTION

Salicylic and anthranilic acids (Fig. 1) are simple benzoic acids with a hydroxyl- or amino-group in the *ortho*-position, respectively. Salicylic (2-hydroxybenzoic) acid (SA) is a plant metabolite well-known for its ability to ease aches and pains and reduce fevers, and it is used as an anti-inflammatory drug (Madan and Levitt, 2014). It is an active metabolite of salicin, a glycoside isolated from *Salix alba*, a plant species used since ancient times to provide relief from pain and inflammation. Furthermore, it is an important active metabolite of aspirin (acetylsalicylic acid), a widely used non-steroidal anti-inflammatory drug (Delaney, 2010). Salicylic acid is a keratolytic agent (WHO,

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Dedicated to Professor Radosav Palić on the happy occasion of his 70th birthday.

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1997) and, like other hydroxy acids, it is a key ingredient in many skin-care products for the treatment of seborrheic dermatitis, acne, psoriasis, calluses, corns, keratosis pilaris, acanthosis nigricans, ichthyosis, and warts (Madan and Levitt, 2014). It is widely used in organic synthesis and functions as a plant hormone (Raskin, 1992).

Two amino derivatives of salicylic acid, 4-aminosalicylic acid (*para*-aminosalicylic acid, 4-ASA or PAS) and 5-aminosalicylic acid (5-ASA) (Fig. 1) are used to treat inflammatory bowel diseases, such as ulcerative colitis and mild-to-moderate Crohn's disease (Kruis et al., 2001; Sandborn et al., 2007; Daniel et al., 2004). As derivatives of salicylic acid, they are thought to possess significant antioxidant potential (Simmonds et al., 1999). 4-ASA is an antibiotic drug used to treat tuberculosis (Fox et al., 1999), while 5-ASA is an anti-inflammatory drug, sold under the name mesalazine (INN, BAN) or mesalamine (USAN) ("Mesalazine", PharmGKB).

Anthranilic (2-aminobenzoic) acid (AA) is an intermediary metabolite in the anabolism and catabolism of tryptophan (Wiklund and Bergman, 2006). Industrially, it is an intermediate in the production of azo dyes, pigments and saccharin (Wiklund and Bergman, 2006; Wouters and Quéré, 2011) and alongside its esters, it is used in preparing perfumes (to imitate jasmine and orange), pharmaceuticals (loop diuretics e.g. furosemide) and as a UV-absorber (Wouters and Quéré, 2011).

3-Hydroxyanthranilic (3-HAA) and 5-hydroxyanthranilic acids (5-HAA) (Fig. 1) are also metabolites of tryptophan and they could form either by monooxygenation of AA or from 3- or 5-hydroxy-L-kynurenine (Fujigaki et al., 1998). They are neurotoxics (Smith et al., 2009; Krause et al., 2011) and hypoglycemic agents (Armarego and Chai, 2003). 3-HAA induces macrophage/monocyte apoptosis under certain conditions, which may be relevant to pathophysiology of inflammatory conditions (Morita et al., 1999). It has been shown to inhibit nitric oxide synthase expression and activity in macrophages (Sekai et al., 1997). Moreover, 3-HAA may be a free radical scavenger and a carcinogen (Boylard and Watson, 1956).

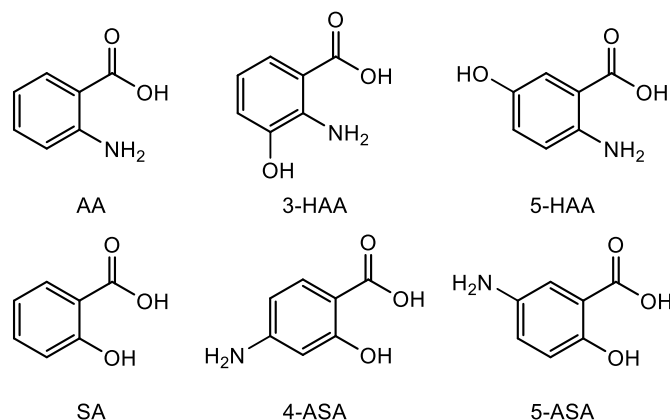


Fig. 1 Structures of the investigated compounds: anthranilic (AA), 3-hydroxyanthranilic (3-HAA), 5-hydroxyanthranilic (5-HAA), salicylic (SA), 4-aminosalicylic (4-ASA), 5-aminosalicylic (5-ASA) acids

Recently, we have identified a new natural anthranilic acid derivative – isopropyl *N*-methylantranilate (ternantranin), and a related compound, methyl *N*-methylantranilate from the essential oil of *Choisya ternata* Kunth, a plant species used in Mexican folk medicine (Radulović et al., 2011). These two *N*-methylantranilic acid esters were demonstrated to possess a number of important pharmacological properties: peripheral and central antinociceptive activity (Radulović et al., 2011; Gomes Pinheiro et al., 2014), anti-inflammatory activity (Gomes Pinheiro et al., 2015), anxiolytic and antidepressant potential, the effect on the onset and duration of diazepam-induced sleep (Radulović et al., 2013a), as well as nephro- (Radulović et al., 2015), hepato- (Radulović et al., 2013b) and gastro- (Radulović et al., 2013c) protective activities. In the continuation of our investigations on anthranilic (*ortho*-aminobenzoic) acid derivatives and considering the importance of the before mentioned amino- or/and hydroxy-substituted benzoic acids, we decided to analyze in detail ^1H - and ^{13}C -NMR spectra of AA, 3- and 5-HAA, SA, 4- and 5-ASA, recorded in perdeuteriodimethyl sulfoxide ($\text{DMSO-}d_6$). Although NMR spectral data for these compounds are available in the literature (Regulska et al., 2009; Esaki et al., 1996; Rajkumar et al., 2012; Jadrijević-Mladar Takac and Vikić Topić, 2004; da Silva et al., 2008; Peng et al., 2004) or from commercial sources (BIORAD; WSS; ACD), the reported studies provided limited and often unassigned data, or where assignment was attempted, it was based solely on “chemical” logic and not on the use of 2D NMR spectra. Moreover, the ^{13}C - ^1H coupling constants are often absent from the existing reports, probably due to the duration of the recording of such coupled ^{13}C NMR spectra. To address the paucity of fully assigned NMR spectral data for these compounds, ^1H - and ^{13}C -NMR spectral data were fully assigned based on a combination of 1D- and 2D- NMR experiments (^1H - ^1H COSY, NOESY, HSQC and HMBC). Also the substituent effects on the spectral properties of these benzoic acid derivatives were discussed.

2. EXPERIMENTAL

All the compounds were commercially available and were used as received. The purity was higher than 99.9% according to GC-MS (on a Hewlett-Packard 6890N gas chromatograph equipped with a fused silica capillary column DB-5MS) and/or NMR analyses.

All NMR spectra were recorded at 25 °C using perdeuteriodimethyl sulfoxide ($\text{DMSO-}d_6$) as the solvent with tetramethylsilane (TMS, Me_4Si) as an internal standard. Chemical shifts are expressed in δ (ppm) and referenced to TMS ($\delta_{\text{H}} = 0$ ppm) in ^1H NMR spectra or to $\text{DMSO-}d_6$ ($\delta_{\text{H}} = 2.50$ ppm, $\delta_{\text{C}} = 39.52$ ppm) in ^{13}C NMR and heteronuclear 2D spectra. Scalar couplings are reported in Hertz. Typically, 20 mg of sample was dissolved in 1 ml of $\text{DMSO-}d_6$, and 0.7 ml of the solution transferred into a 5 mm Wilmad, 528-TR-7 NMR tube.

The ^1H - and ^{13}C -NMR spectra of AA, 3- and 5-HAA, SA, 4- and 5-ASA were recorded on a Bruker Avance III 400 MHz NMR spectrometer (^1H at 400 MHz, ^{13}C at 100 MHz), equipped with a 5-mm dual $^{13}\text{C}/^1\text{H}$ probe head. The ^1H spectra were recorded with 16 scans, 1 s relaxation delay, 4 s acquisition time, 0.125 Hz digital FID resolution, 51 280 FID size, with 6410 Hz spectral width, and an overall data point resolution of 0.0003 ppm. The ^{13}C spectra were recorded with Waltz 161H broadband decoupling, 1024 scans, 0.5 s relaxation delay, 1 s acquisition time, 0.5 Hz digital FID resolution, 65

536 FID size, 31 850 Hz spectral width, and an overall data point resolution of 0.005 ppm. The DEPT-135 and DEPT-90 spectra were recorded by the standard Bruker Pulse Program Dept135 and Dept90 with 256 scans, respectively. The relaxation delay for each above measurement was 2 s.

Standard pulse sequences were used for 2D spectra. ^1H - ^1H COSY and NOESY spectra were recorded at spectral widths of 5 kHz in both $F2$ and $F1$ domains; $1\text{ K} \times 512$ data points were acquired with 32 scans per increment and the relaxation delays of 2.0 s. The mixing time in NOESY experiments was 1 s. Data processing was performed on a $1\text{ K} \times 1\text{ K}$ data matrix. Inverse-detected 2D heteronuclear correlated spectra were measured over 512 complex points in $F2$ and 256 increments in $F1$, collecting 128 (HSQC) or 256 (HMBC) scans per increment with a relaxation delay of 1.0 s. The spectral widths were 5 and 27 kHz in $F2$ and $F1$ dimensions, respectively. The HSQC experiments were optimized for C–H couplings of 160 Hz; the HMBC experiments were optimized for long-range C–H couplings of 10 Hz. Fourier transforms were performed on a 512×512 data matrix. $\pi/2$. Shifted sine-squared window functions were used along $F1$ and $F2$ axes for all 2D spectra.

3. RESULTS AND DISCUSSION

The NMR spectra of anthranilic acid and its 3- and 5-hydroxy derivatives, as well as salicylic acid and its 4- and 5-aminoderivatives were recorded in $\text{DMSO}-d_6$ (Fig. 1). The ^1H - and ^{13}C -NMR data are summarized in Tables 1 and 2. Chemical shifts, multiplicities and the observed coupling constants were in accordance with the structure of the investigated acids. The number of carbon signals corresponded to the expected number of carbon atoms of the investigated compounds, while the DEPT90/135 spectra showed only the existence of CH groups among protonated carbons. The ^{13}C - ^1H coupling constants, previously unavailable in the literature, were derived from the proton-coupled ^{13}C NMR spectra. A number of resolved long-range coupling constants (two- and three-bond couplings) alongside one-bond coupling of directly attached carbon and hydrogen atoms were observable in these spectra (Table 2).

Table 1 ^1H NMR (400 MHz) spectral data ($\text{DMSO}-d_6$) for AA, 3- and 5-HAA, SA, 4- and 5-ASA

H atom	^1H NMR, δ (ppm), multiplicity (J in Hz)					
	AA	3-HAA	5-HAA	SA	4-ASA	5-ASA
H-3	6.74, dd (8.4, 1.0)	-	6.61, d (8.8)	6.97, dd (8.6, 0.9)	5.98, d (2.1)	6.70, d (8.7)
H-4	7.22, ddd (8.4, 7.0, 1.6)	6.81, dd (7.6, 1.5)	6.78, dd (8.8, 2.9)	7.52, ddd (8.6, 7.1, 1.7)	-	6.90, dd (8.7, 2.9)
H-5	6.50, ddd (8.1, 7.0, 1.0)	6.38, dd (8.2, 7.6)	-	6.93, ddd (7.9, 7.1, 0.9)	6.10, dd (8.7, 2.1)	-
H-6	7.69, dd (8.1, 1.6)	7.22, dd (8.2, 1.5)	7.11, d (2.9)	7.82, dd (7.9, 1.7)	7.43, d (8.7)	7.20, d (2.9)
-COOH	8.58, br s,	8.18, br s, 3H	8.16, br s, 3H	11.64, br s,	6.03, br s, 2H	6.41, br s, 4 H
-OH	3H*	9.57, br s, 1H	8.60, br s, 1H	2H**	11.42, br s, 1H	
-NH ₂					12.36, br s, 1H	

*No –OH group present in the molecule; ** No –NH₂ group present in the molecule.

Table 2 ^{13}C NMR (100 MHz) spectral data (DMSO- d_6) for AA, 3- and 5-HAA, SA, 4- and 5-ASA

C atom	^{13}C NMR, δ (ppm), multiplicity (J in Hz)					
	AA	3-HAA	5-HAA	SA	4-ASA	5-ASA
C-1	109.6 C, ddd ($^3J=8.0, 5.4,$ $^2J=1.8$)	110.0 C, d ($^3J=8.8$)	110.0 C, d ($^3J=6.4$)	113.1 C, dd ($^3J=7.4, 5.6$)	100.1 C, dd ($^3J=7.7, 5.0$)	114.3 C, d ($^3J=4.7$)
C-2	151.5 C, pseudo t ($^3J=7.7$)	141.1 C, pseudo t ($^3J=6.7$)	144.8 C, dd ($^3J=8.5, 7.0$)	161.2 C, m	163.5 C, dd ($^3J=8.4,$ $^2J=3.0$)	154.7 C, m
C-3	116.4 CH, dd ($^1J=162.7,$ $^3J=8.1$)	144.5 C, dd ($^3J=9.7, ^2J=1.4$)	117.7 CH, d ($^1J=159.7$)	117.1 CH, dd ($^1J=161.6, ^3J=7.6$)	98.6 CH, dd ($^1J=157.9,$ $^3J=4.8$)	117.1 CH, m*
C-4	133.8 CH, ddd ($^1J=157.6,$ $^3J=9.1, ^2J=1.4$)	116.7 CH, ddd ($^1J=156.4,$ $^3J=9.0, ^2J=1.5$)	123.1 CH, dd ($^1J=158.9,$ $^3J=5.9$)	135.6 CH, ddd ($^1J=159.9,$ $^3J=9.1, ^2J=1.5$)	155.8 C, br d ($^3J=10.2$)	123.7 CH, m*
C-5	114.6 CH, dd ($^1J=162.7,$ $^3J=8.1$)	114.0 CH, d ($^1J=161.2$)	146.6 C, pseudo dt ($^3J=9.9,$ $^2J=3.1$)	119.2 CH, dd ($^1J=163.6, ^3J=8.0$)	106.3 CH, dd ($^1J=161.0,$ $^3J=5.5$)	136.1 C, m
C-6	131.2 CH, ddd ($^1J=159.9,$ $^3J=7.8, ^2J=1.4$)	121.4 CH, ddd ($^1J=162.0,$ $^3J=8.0, ^2J=1.4$)	115.3 CH, dd ($^1J=158.8,$ $^3J=4.7$)	130.3 CH, ddd ($^1J=162.3,$ $^3J=8.4, ^2J=1.4$)	131.5 CH, br d ($^1J=159.6$)	116.1 CH, d ($^1J=159.8$)
COOH	169.6 C, br d ($^3J=3.8$)	169.8 C, br d ($^3J=3.6$)	169.4 C, dd ($^3J=4.4,$ $^5J=1.5$)	172.0 C, br d ($^3J=3.9$)	172.1 C, br d ($^3J=3.5$)	171.8 C, dd ($^3J=4.3,$ $^5J=1.3$)

m* – second-order multiplets, where it was only possible to determine one-bond ^{13}C -H coupling (Fig. 4).

The assignment of all proton and carbon-13 NMR signals was made possible only by the use of 2D NMR (^1H - ^1H COSY, NOESY, HSQC and HMBC spectra) and correlations observed in these spectra gave rise to the assignments given in Tables 1 and 2. For example, the chemical shifts of non-protonated carbons C-2 and C-5 of 5-HAA were inferred from the HMBC spectrum due to the existence of cross-peaks between H-3 at 6.61 and H-4 at 6.78 ppm and carbons at 144.8 and 146.6 ppm (Fig. 2). A coupling between a carbon and a proton of an aromatic core through three bonds is stronger than a coupling through two bonds. As the cross-peak between H-4 and the carbon at 144.8 ppm was more intense than the one between H-4 and C-atom at 146.6 ppm, and the intensity of the cross-peak between H-3 and carbon at 146.6 was higher than the one between H-3 and the carbon signal at 144.8 ppm, the signal at 144.8 ppm was assigned to C-2, while the one at 146.6 was assigned to C-5. This assignment was additionally corroborated by the analysis of ^1H -coupled ^{13}C NMR spectra. The carbon signal at 144.8 ppm appeared as a doublet of doublets (dd) with coupling constants of 8.5 and 7.0 Hz, which was indicative of a carbon atom that has two *meta*-protons, while the carbon signal at 146.6 ppm appeared as a pseudo doublet of triplets (pseudo dt) with the ^{13}C -H coupling constants 9.9 and 3.1 Hz, 3J and 2J , respectively.

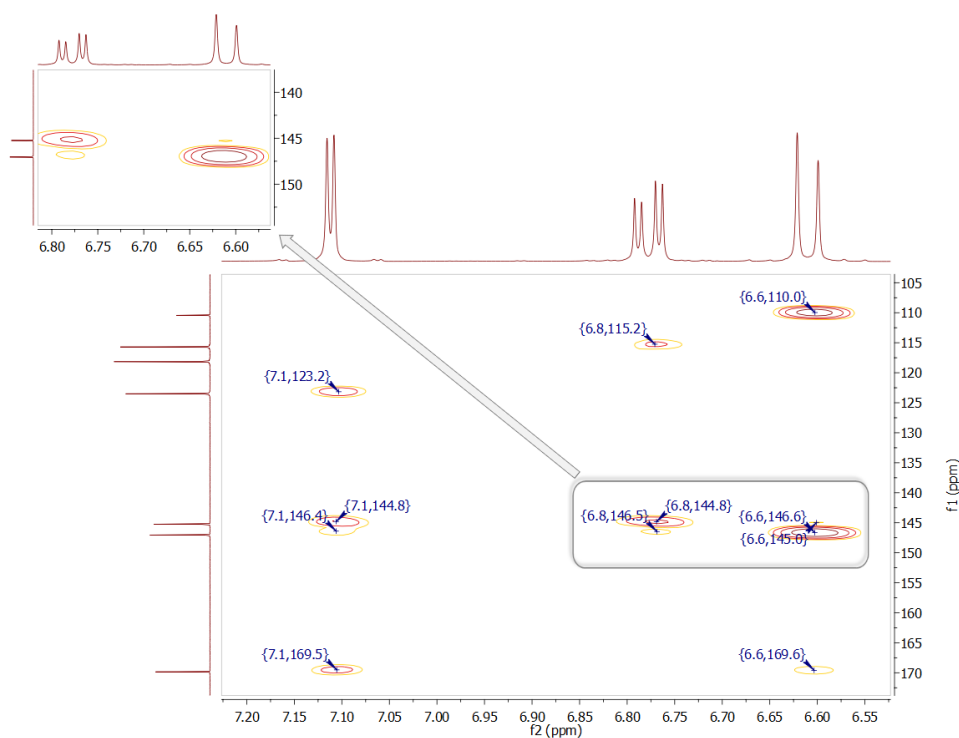


Fig. 2 Expansions (^1H : 6.5-7.2; ^{13}C : 105-175 ppm) of the HMBC spectrum of 5-HAA

The location of exchangeable protons ($-\text{COOH}$, $-\text{NH}_2$ and/or $-\text{OH}$) was revealed from a careful inspection of the NOESY spectra of the investigated acids. For example, the NOESY spectrum of 4-ASA is shown in Fig. 3. The cross-peaks originating from the dipolar coupling of H-5 and H-6, protons that are in close proximity one to each other, are distinguished from (chemical) exchange cross-peaks by their opposite phases. The opposite phases are represented in different colors in the spectrum. Dipolar coupling is represented in blue, while the cross-peaks originating from the coupling of exchangeable protons and diagonal (auto) peaks are depicted in orange color. A peak originating from residual water (atmospheric moisture) at about 3.4 ppm could be observed in the ^1H NMR spectra of all investigated compounds (except SA and 5-ASA) and the NOESY spectra of these compounds displayed cross-peaks between the exchangeable protons and the water signal.

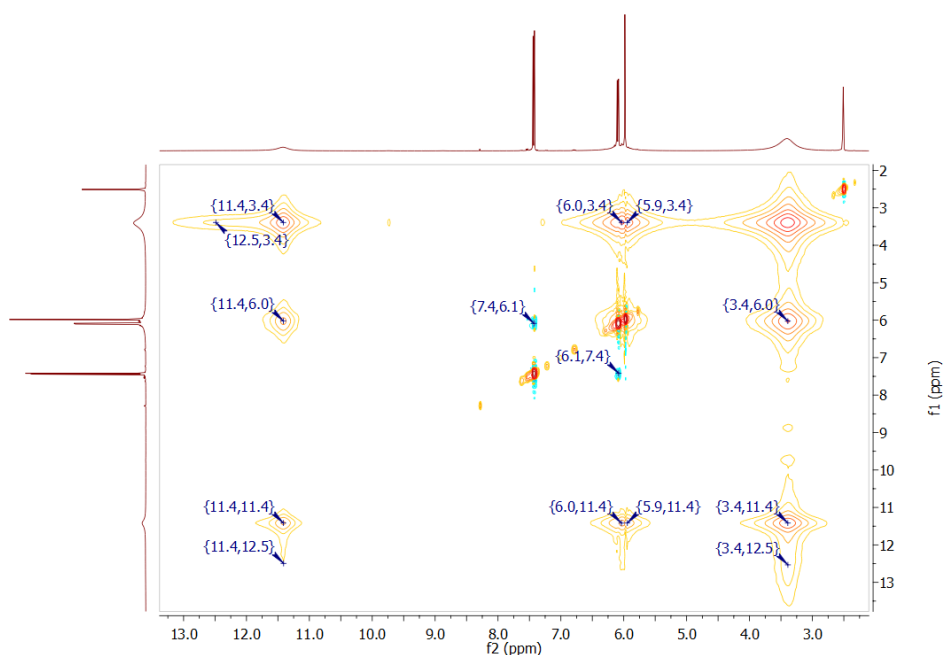


Fig. 3 NOESY spectrum of 4-ASA

Chemical shifts of C-1 of hydroxy derivatives of anthranilic acid (3-HAA – 110.0 ppm, 5-HAA – 110.0 ppm) were close in value to that of anthranilic acid (109.6 ppm). On the other hand, the chemical shifts of C-1 of 4-ASA (100.1 ppm) were shifted towards lower values in comparison with the one of SA (113.1 ppm) and 5-ASA (114.3 ppm). As the electron donating hydroxyl group in 3-HAA and 5-HAA is *meta*- to C-1, it is not expected to significantly influence the electron density at C-1 and consequently its δ value. In general, as expected, both $-\text{OH}$ and $-\text{NH}_2$ groups altered (increased) the electron density of carbon atoms in the *ortho*- and *para*-positions relative to either of them. This resulted in a shielding effect and moving of the corresponding carbon resonances to higher field in comparison with AA and SA. The *meta*-positioned carbons relative to $-\text{OH}$ and $-\text{NH}_2$ groups showed no significant influence from the presence of the two electron-donating groups and displayed similar chemical shifts to those of AA and SA. The interchange between amino- and hydroxyl-groups, as in the pair of AA and SA, as well as the pair of 5-HAA and 5-ASA, does not influence significantly the value of the chemical shift of core carbons except for the carbon directly attached to the group. This suggests a similar electron donating capacity of the two groups, and the greatest deviations can be observed for the *para*-positioned carbon in respect to the $-\text{NH}_2$ or $-\text{OH}$ groups (up to 5 ppm).

The carboxylic carbon atom resonated at around 170 ppm in the ^{13}C NMR of anthranilic acid and its 3- and 5-hydroxy derivatives, while the spectra of salicylic acid and its 4- and 5-amino derivatives displayed the resonance of this carbon at somewhat lower field (around 172 ppm). This carbon appeared as a broad doublet, with a coupling constant of around 4 Hz, except in the case of 5-HAA and 5-ASA, where this broad signal was resolved into a doublet of doublets with coupling constants of *ca.* 4 (3J coupling to H-6) and 1.5 Hz (4J , i.e. “W”, coupling to H-3). The mentioned resolution originates from the lack of additional long-range coupling between H-5 and –COOH in 5-substituted derivatives. One could expect a similar resolution of –COOH signal in 3-HAA. Although the width at half-height of this signal did decrease (compared to the analogous signal from AA), for some 1.5 Hz, due to the lack of 3J with H-3, it did not result in any significant signal resolution. These values of ^{13}C -H coupling constants were in accordance with the literature values (Ihrig et al., 1972).

Furthermore, ^{13}C -H coupling constants of the investigated benzoic acids showed the following trends: (1) $^3J_{\text{CH}}$ couplings are larger (6–12 Hz) than the $^2J_{\text{CH}}$ couplings (0–4 Hz) and easier to discern (in the case of 3-HAA, C-4 displayed a three-bond coupling of 9 Hz to H-6, and a coupling constant of 1.5 Hz to H-5 through two bonds); (2) The magnitude of the $^3J_{\text{CH}}$ couplings are directly proportional to the increased electronegativity of the substituent (in the case of AA, $J(\text{C-6-H-4}) = 7.8$ Hz, while in SA, $J(\text{C-6-H-4})$ increased to 8.4 Hz); (3) If a substituent is on the coupling pathway, the 3J values decrease with increased electronegativity (in the case of 3-HAA, $J(\text{C-4-H-6}) = 9.0$ Hz, while in 5-HAA, $J(\text{C-4-H-6})$ decreased to 5.9 Hz). This is in agreement with other di- or tri-substituted aromatic systems (DiMichele et al., 2006, and references cited therein).

In the proton-coupled ^{13}C NMR spectrum of 5-ASA, the signals at 117.1 ppm and 123.7 ppm, assigned to C-3 and C-4, respectively, appeared as asymmetric multiplets which indicated that they represented second-order multiplets, although initially first-order ones were expected (Fig. 4). The high-field parts of the multiplets arising from one bond and longer-range couplings differed from the low-field halves. Interestingly, the protons directly attached to the two mentioned carbons displayed first-order signals. Douglas and Shapiro (1980) reported that “unexpected” second-order effects could be encountered even when the relative proton shifts are large compared to proton-proton coupling constants. One could regard C-3-H-3-H-4 and C-4-H-4-H-3 as two ABX systems due to the near equality of the relative proton shift, in frequency units, to the difference between one-bond and long-range ^{13}C -H couplings. The appearance of strong second-order features when $1/2|\nu_{\text{A}} - \nu_{\text{B}}|$ is nearly equal to $1/4|J(\text{AX}) - J(\text{BX})|$ is to be expected whenever $J(\text{AB})$ is as large as a few Hz (Douglas and Shapiro, 1980). Since in our case $1/2|\nu_{\text{H-3}} - \nu_{\text{H-4}}| = 39.6$ Hz, according to $1/4|J(\text{H-3-C-3}) - J(\text{H-4-C-3})| = 39.6$ Hz, and knowing that $J(\text{H-3-C-3}) = 160.4$ Hz, we could estimate the value of $J(\text{H-4-C-3})$ to be around 2 Hz, what is in agreement with the values of the corresponding two-bond couplings found in the other herein investigated derivatives.

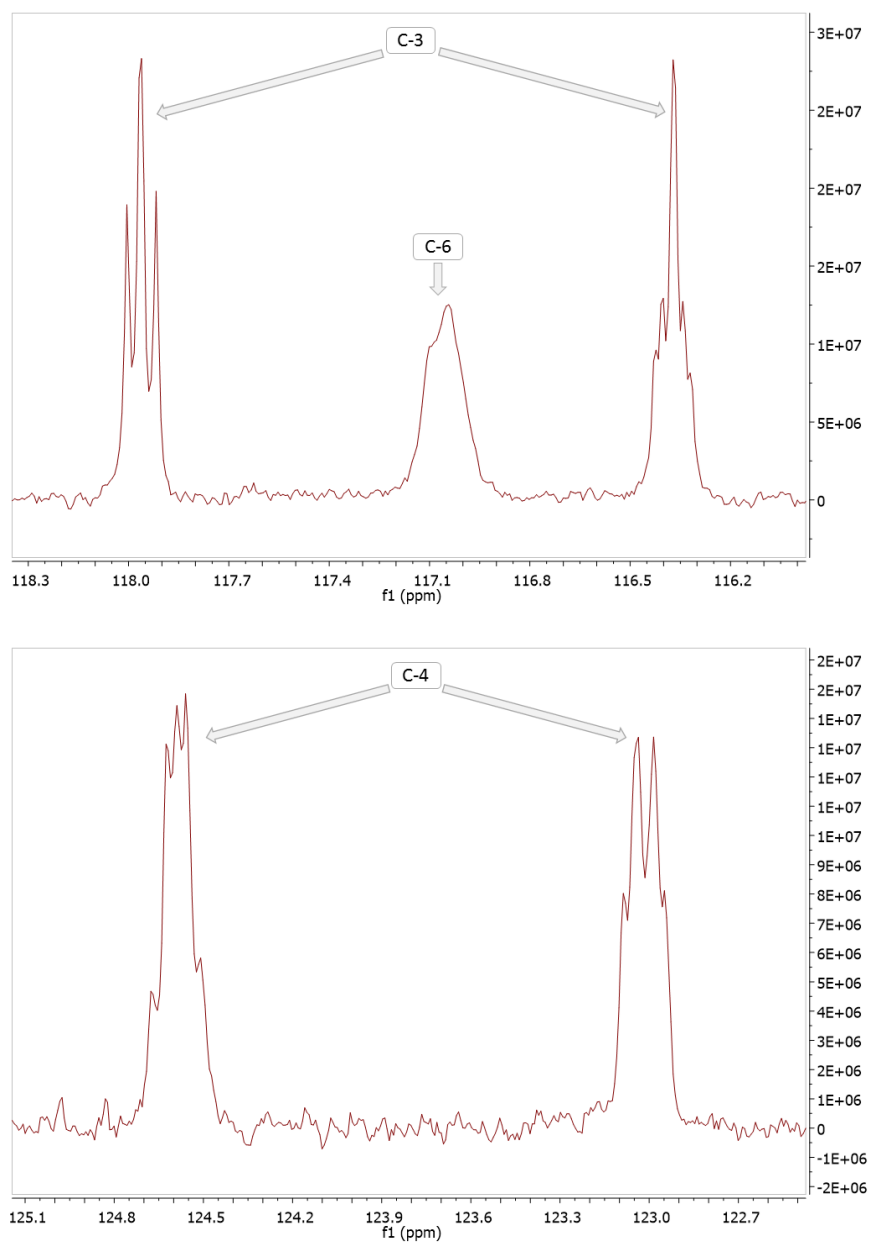


Fig. 4 Second-order multiplets (signals corresponding to C-3, up, and C-4, down, at 117.1 and 123.7 ppm, respectively) observed in the proton-coupled ^{13}C NMR of 5-ASAA literature survey (SciFinder search of the CAS database) on the NMR data of these amino- or/and hydroxy-substituted benzoic acids revealed the following:

- 1) Interestingly, the NMR spectra of AA recorded in DMSO-*d*₆ were very similar to ¹H- and ¹³C-spectral data for AA in D₂O (Regulska et al., 2009); however, the signals of C-3 and C-5 appear to be interchanged.
- 2) ¹H- and ¹³C-spectral data for 3-HAA were in accordance with Esaki et al. (1996) and Rajkumar et al. (2012); although Esakai with his coworkers did not report the chemical shifts of exchangeable protons, while in the ¹H NMR spectrum recorded by Rajkumar et al. all exchangeable protons resonated at the same δ .
- 3) NMR data for 5-HAA are available only from commercial sources (BIORAD, WSS, ACD), but since not being the subject of a scientific investigation, they were not assigned.
- 4) ¹H- and ¹³C-spectral data for SA were in accordance with Jadrijević-Mladar Takac and Vikić Topić (2004).
- 5) Da Silva et al. (2008) reported the following NMR data for 4-ASA: ¹H NMR: δ = 7.93 (s, *J* = 8.5 Hz, 2H), 7.80 (d, *J* = 8.5 Hz, 1H), 7.00 (dd, *J* = 8.5, 1.8 Hz, 1H), 7.08 (d, *J* = 8.5 Hz, 1H), 6.09–6.0 (s, 2H); ¹³C NMR: δ = 171.87 (C-7), 159.99 (C-2), 153.00 (C-4), 132.51 (C-6), 107.59 (C-1), 107.53 (C-5), 102.53 (C-3), which were neither in agreement with our data, nor in accordance with the investigated structure.
- 6) Although according to SciFinder, Peng et al. (2004) should have published the NMR data of 5-ASA, the paper in question does not contain these data.

The literature survey on NMR data of anthranilic, 3- and 5-hydroxyanthranilic, salicylic, 4- and 5-aminosalicylic acids revealed limited, unassigned or even incorrectly assigned spectral data. Our study provided a complete assignment of NMR spectral data based on a combination of ¹H- and ¹³C-NMR 1D- and 2D-experiments and in this way bridges the gap existing in the literature with regard to NMR spectral data for these simple amino- or/and hydroxy-substituted benzoic acids.

REFERENCES

- ACD, Advanced Chemistry Development, Inc., Toronto, ON, Canada.
- Armarego, W.L.F., Li Lin Chai, C., 2003. Purification of Laboratory Chemicals, fifth ed. Butterworth-Heinemann, Burlington, MA, USA.
- BIORAD, Bio-Rad Laboratories, Inc., Hemel Hempstead, Hertfordshire, United Kingdom.
- Boyland, E., Watson, G., 1956. *Nature*, 177, 837–838. doi:10.1038/177837a0
- Da Silva, M., Menezes, C.M., Ferreira, E.I., Leite, C.Q., Sato, D.N., Correia, C.C., Pimenta, C.P., Botelho, K.C., 2008. *Chem. Biol. Drug. Des.*, 71, 167–172. doi:10.1111/j.1747-0285.2007.00621.x
- Daniel, F., Seksik, P., Cacheux, W., Jian, R., Marteau, P., 2004. *Inflamm. Bowel Dis.*, 10, 258–260. doi:10.1097/00054725-200405000-00013.
- Delaney, T.P., 2010. Salicylic Acid, in: Davies, P.J. (Ed.), *Plant Hormones (Biosynthesis, Signal Transduction, Action!)*. Springer Netherlands 2010, pp. 681–699. doi:10.1007/978-1-4020-2686-7_29
- DiMichele, L., Menzel, K., Mills, P., Frantz, D., Nelson, T., 2006. *Magn. Reson. Chem.*, 44, 1041–1043. doi:10.1002/mrc.1891
- Douglas, A.W., Shapiro, M., 1980. *Org. Magn. Resonance*, 14, 38–39. doi:10.1002/mrc.1270140108
- Esaki, H., Onozaki, H., Kawakishi, S., Osawa, T., 1996. *J. Agric. Food Chem.*, 44, 696–700. doi:10.1021/jf950454t
- Fox, W., Ellard, G.A., Mitchison, D.A., 1999. *Int. J. Tuberc. Lung. Dis.*, 3(10 Suppl 2), S231–279.
- Fujigaki, S., Saito, K., Takemura, M., Fujii, H., Wada, H., Noma, A., Seishima, M., 1998. *Arch. Biochem. Biophys.*, 358, 329–335. doi:10.1006/abbi.1998.0861

- Gomes Pinheiro, M.M., Miltojević, A.B., Radulović, N.S., Abdul-Wahab, I.R., Boylan, F., Dias Fernandes, P., 2015. PLoS One, 10, e0121063. doi: 10.1371/journal.pone.0121063
- Gomes Pinheiro, M.M., Radulović, N.S., Miltojević, A.B., Boylan, F., Dias Fernandes, P., 2014. Eur. J. Pharmacol., 727, 106-114. doi: 10.1016/j.ejphar.2013.12.042
- Ihrig, A.M., Marshall, J.L., 1972, J. Am. Chem. Soc., 94, 3268–3269. doi:10.1021/ja00764a080
- Jadrijević-Mladar Takac, M., Vikić Topić, D., 2004. Acta Pharm., 54, 177–191.
- Krause, D., Suh, H.S., Tarassishin, L., Cui, Q.L., Durafourt, B.A., Choi, N., Bauman, A., Cosenza-Nashat, M., Antel, J.P., Zhao, M.L., Lee, S.C., 2011. Am. J. Pathol., 179, 1360–1372. doi:10.1016/j.ajpath.2011.05.048
- Kruis, W., Schreiber, I., Theuer, D., Brandes, J. W., Schütz, E., Howaldt, S., Krakamp, B., Hämling, J., Mönnikes, H., Koop, I., Stolte, M., Pallant, D., Ewald, U., 2001. Gut, 49, 783–789. doi:10.1136/gut.49.6.783
- Madan, R.K., Levitt, J., 2014. J. Am. Acad. Dermatol., 70, 788–792. doi:10.1016/j.jaad.2013.12.005
- "Mesalazine". PharmGKB. <https://www.pharmgkb.org/drug/PA450384#tabview=tab2&subtab=31>
- Morita, T., Saito, K., Takemura, M., Maekawa, N., Fujigaki, S., Fujii, H., Wada, H., Takeuchi, S., Noma, A., Seishima, M., 1999. Adv. Exp. Med. Biol., 467, 559–563. doi:10.1007/978-1-4615-4709-9_69
- Peng, Y., Song, G., Ding, F., 2004. Indian Journal of Chemistry, Section B: Organic Chemistry Including Medicinal Chemistry, 43B, 2021-2023
- Radulović, N.S., Jovanović, I., Ilić, I.R., Randjelović, P.J., Stojanović, N.M., Miltojević, A.B., 2013c. Life Sci., 93, 840-846. doi: 10.1016/j.lfs.2013.10.002
- Radulović, N.S., Miltojević, A.B., McDermott, M., Waldren, S., Parnell, J.A., Pinheiro, M.M., Dias Fernandes, P., de Sousa Menezes, F., 2011. J. Ethnopharmacol., 135, 610-619. doi:10.1016/j.jep.2011.03.035
- Radulović, N.S., Miltojević, A.B., Randjelović, P.J., Stojanović, N.M., Boylan, F., 2013a. Phytoter. Res., 27, 1334-1338. doi: 10.1002/ptr.4877.
- Radulović, N.S., Randjelović, P.J., Stojanović, N.M., Ilić, I.R., Miltojević, A.B., 2013b. FU Phys. Chem. Technol., 1, 67-73. doi:10.2298/FUPCT1301067R
- Radulović, N.S., Randjelović, P.J., Stojanović, N.M., Ilić, I.R., Miltojević, A.B., Stojković, M.B., Ilić, M., 2015. Life Sci. 135, 110-117. doi: 10.1016/j.lfs.2015.05.022
- Rajkumar, M., Alfred Cecil Raj, S., Renuga, V., 2012. Mater. Manuf. Process., 27, 1217–1220. doi:10.1080/10426914.2012.663132
- Raskin, I., 1992. Plant Physiol., 99, 799–803. doi:10.1104/pp.99.3.799
- Regulska, E., Samsonowicz, M., Świsłocka, R., Lewandowski, W., 2009. J. Mol. Struct., 936, 162–170. doi:10.1016/j.molstruc.2009.07.033
- Sandborn, W.J., Feagan, B.G., Lichtenstein, G.R., 2007. 26, 987–1003. doi:10.1111/j.1365-2036.2007.03455.x
SciFinder, Version 2007.1; Chemical Abstracts Service: Columbus, OH, 2007; RN 58-08-2 (accessed Aug 15, 2015).
- Sekkai, D., Guittet, O., Lemaire, G., Tenu, J.-P., Lepoivre, M. 1997. Arch. Biochem. Biophys. 340, 117–123. doi:10.1006/abbi.1997.9913
- Simmonds, N.J., Millar, A.D., Blake, D.R., Rampton, D.S., 1999. Aliment. Pharmacol. Ther., 13, 363–372. doi:10.1046/j.1365-2036.1999.00484.x
- Smith, A. J., Smith, R.A., Stone, T.W., 2009. Neurotox. Res., 15, 303–310. doi:10.1007/s12640-009-9034-0
- Wiklund, P., Bergman, J., 2006. Curr. Org. Synth., 3, 379–402. doi:10.2174/157017906777934926
- WHO, World Health Organization, 1997. WHO Model Prescribing Information: Drugs Used in Skin Diseases.
- Wouters, J., Rome, S., Quéré, L., 2011. Monographs of most Frequent Co-Crystal Formers, in: Wouters, J., Quéré, L. (Eds.), Pharmaceutical Salts and Co-crystals (RSC Drug Discovery). Royal Society of Chemistry, London, UK, pp. 343. doi:10.1039/9781849733502-00338
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**POTPUNA ASIGNACIJA ^1H - I ^{13}C -NMR SPEKTARA
ANTRANILNE KISELINE I NJENIH HIDROKSI-DERIVATA
I SALICILNE KISELINE I NJENIH AMINO-DERIVATA**

U ovom radu dati su NMR podaci za antranilnu (AA), salicilnu (SA) i regioizomerne (amino)hidroksibenzojeve kiseline, 3-hidroksiantranilnu (3-HAA), 5-hidroksiantranilnu (5-HAA), 4-aminosalicilnu (4-ASA) i 5-aminosalicilnu (5-ASA) kiselinu. Kako je pretragom literature utvrđeno da su do sada objavljeni NMR podaci za pomenute derivate benzojeve kiseline nepotpuni, neasignirani ili čak i pogrešno asignirani, izvršena je kompletna asignacija signala u ^1H - i ^{13}C -NMR spektrima snimljenim u deuterisanom dimetil-sulfoksidu (DMSO-d_6) kombinovanjem ^1H - i ^{13}C -NMR sa ^1H - ^1H COSY, NOESY, HSQC i HMBC eksperimentima.

Ključne reči: ^1H - i ^{13}C -NMR, 1D- i 2D- NMR, antranilna kiselina, 3- i 5-hidroksiantranilna kiselina, salicilna kiselina, 4- i 5-aminosalicilna kiselina