FT-IR i NMR spektroskopska istraživanja derivata salicilne kiselina. I. Gentizinamid- metabolit salicilamida

Jadrijević-Mladar Takač, Milena; Vikić Topić, Dražen; Govorčinović, Tihana

Source / Izvornik: **Acta Pharmaceutica, 2004, 54, 163 - 176**

Journal article, Published version
Rad u časopisu, Objavljena verzija rada (izdavačev PDF)

Permanent link / Trajna poveznica: https://urn.nsk.hr/urn:nbn:hr:163:224656

Acta Pharm. 54 (2004) 163–176 Original research paper

FT-IR and NMR spectroscopic studies of salicylic acid derivatives. I. Gentisamide – a metabolite of salicylamide

MILENA JADRIJEVIĆ-MLADAR TAKAČ[1]*
DRAŽEN VIKIĆ TOPIĆ[2]
TIHANA GOVORČINOVIĆ[1]

[1] *Department of Medicinal Chemistry Faculty of Pharmacy and Biochemistry University of Zagreb, 10000 Zagreb Croatia*

[2] *NMR Center, Ruđer Bošković Institute POB 180, 10002 Zagreb, Croatia*

Received April 14, 2004
Accepted August 23, 2004

Gentisamide (GAM, 2,5-dihydroxybenzamide), a minor first-pass metabolite of salicylamide (SAM, 2-hydroxybenzamide), was studied using FT-IR, 1D and 2D homo- and heteronuclear ^{1}H and ^{13}C NMR spectroscopy.

GAM was isolated from human urine eight hours after oral administration of SAM. FT-IR, ^{1}H and ^{13}C NMR spectra unequivocally confirmed the chemical structure of GAM through chemical and substituent shifts, coupling constants and connectivities in COSY, NOESY, HETCOR and HBMC spectra.

From NOESY spectra of GAM in DMSO-d_6, it was concluded that the amide protons are oriented toward the *ortho*-proton at C-6. Obtained results indicate that the presence of the additional phenol group at C-5 in GAM favours the formation of intramolecular hydrogen bonding of the O…HO type between C2-OH proton and oxygen atom of the amide group.

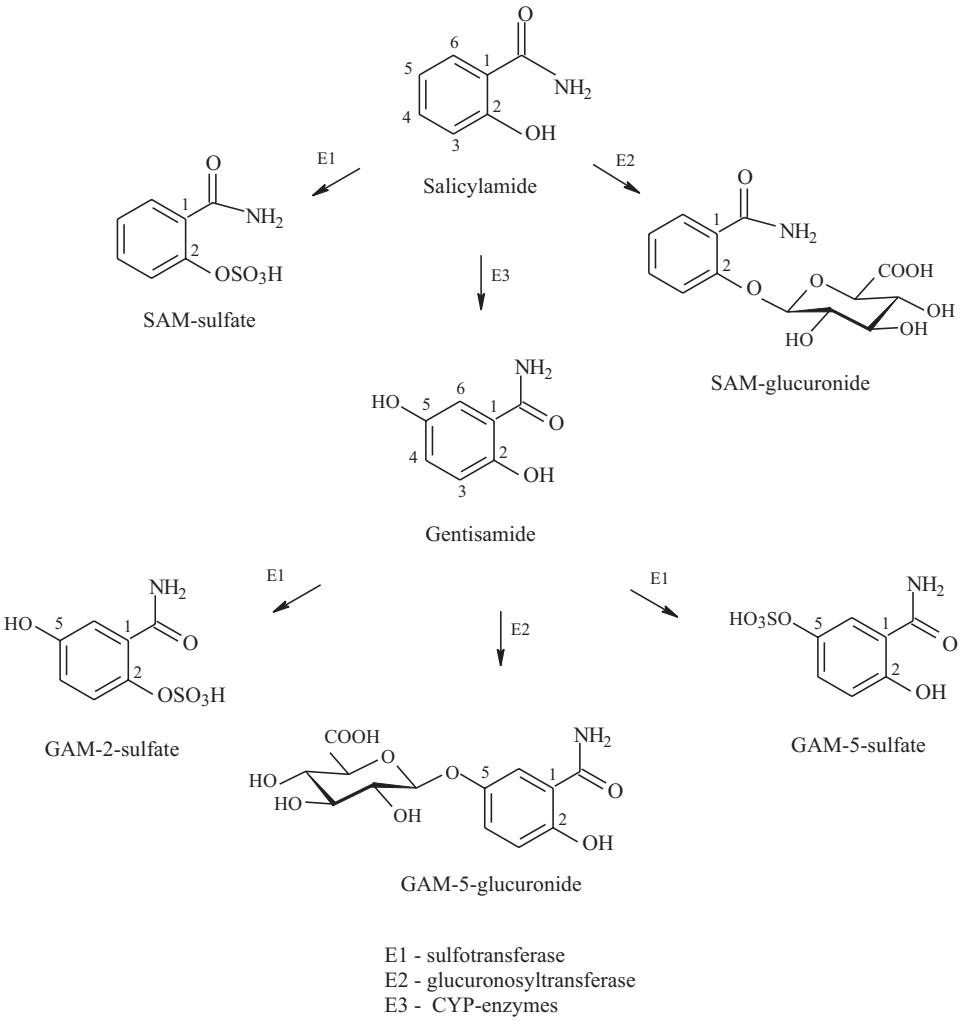
Keywords: gentisamide, NSAID, salicylamide, biotransformation, FT-IR, 1D and 2D homo- and heteronuclear ^{1}H and ^{13}C NMR

A number of events preceding the interaction of a drug with the biological target and/or pharmacological effect have to be considered, and these involve absorption, distribution, metabolism and elimination. In order to assess the importance of each of these factors on drug action, both structural and physico-chemical properties of the drug should be taken into account (1). In biological systems, properties such as electrostatic bonds, hydrogen bonds, van der Waals bonds, as well as effects related to electron-transfer and the hydrophobic effects are of major importance. Although the hydrogen bond is fairly weak compared to other interactions, it is of paramount importance in biological systems. Investigations of drug metabolism, its biotransformation pathways and structure of formed metabolites are of toxicological, pharmacological and biomedical interest (2).

* Correspondence, e-mail: mladar@pharma.hr

However, differentiation of structurally related metabolites in biological material could sometimes be very complex (3).

On the other hand, the separation, identification and quantitative analysis of aromatic carboxylic acids and their derivatives are necessary because of their importance as nonsteroidal antiinflamatory drugs (NSAID), semi-products of biosynthesis of aromatic amino acids in plants (phenolic acids), metabolites of numerous toxic substances, including metabolites of drugs, as well as endogenous catecholamines (adrenaline, noradrenaline and dopamine) (4).



Scheme 1. Biotransformation of salicylamide (SAM) and gentisamide (GAM) (8).

The NSAID salicylamide (SAM) is an effective analgesic for relieving minor muscle aches, headaches, pain associated with sinus congestion as well as for reducing fever (5). Although SAM is not as effective as acetylsalicylic acid (ASA) or paracetamol, it is still used in combined medicinal products for symptoms associated with cold and influenza. The mechanism of its action is based on inhibition of arachidonic acid cyclooxygenase (COX) and the metabolic pathway of SAM is well known (6–11). After oral administration, it is rapidly metabolized to its first-pass metabolites, SAM-glucuronide, SAM-sulfate and gentisamide (GAM) as a minor metabolite (Scheme 1) (8). The sulfation of SAM is the main pathway of biotransformation while glucuronidation and hydroxylation are of lesser importance. GAM, the 5-hydroxylated metabolite of SAM, formed in the body by catalytic action of cytochrome P450 (CYP) enzymes, undergoes further biotransformation to its monoconjugated metabolites GAM-sulfate and GAM-glucuronide, thus providing secondary metabolites of SAM (7, 10).

Numerous chromatographic and spectroscopic methods were used in monitoring and detection of SAM and its metabolites (12–20). Although nuclear magnetic resonance is now a unique and powerful method in detection and quantitative determination of drugs and their metabolites, as well as toxic substances in the urine and other body fluids (3, 21–24), data about the use of NMR in the structural analysis of SAM metabolites are scarce (25, 26).

The GAM isolated from biological material was studied using FTIR, 1D and 2D homo- and heteronuclear ^{1}H and ^{13}C NMR spectroscopies (27, 28), and its chemical structure was unequivocally determined by using chemical and substituent shifts, coupling constants and connectivities in COSY, NOESY, HETCOR and HBMC spectra.

EXPERIMENTAL

All chemicals used were of analytical grade. Melting point was determined on the Boëtius Microheating Stage (Franz Küstner Nachf. KG, Germany) and remained uncorrected.

Spectral analyses

The IR spectra (KBr pellets) were recorded on a FT-IR Perkin Elmer Paragon 500 spectrophotometer (Perkin Elmer, UK).

The ^{1}H and ^{13}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 ^{1}H and ^{13}C nucleus, respectively, and a Bruker Avance DRX500 spectrometer (Bruker, Germany), operating at 500 MHz and 125 MHz. All experiments were performed in DMSO-d_6 at 20 °C in 5-mm NMR tubes. Chemical shifts δ in ppm are referred to TMS as the internal standard.

The following spectra were recorded on a Gemini 300 spectrometer: broadband proton decoupling, gated proton decoupling, APT, COSY-45, long-range COSY-45, NOESY, and HETCOR. Digital resolution in one-dimensional ^{1}H NMR spectra was: 0.20 Hz and in ^{13}C NMR spectra 0.60 Hz per point. In all experiments, proton decoupling was performed by

Waltz-16 modulation. Standard pulse sequences were used in two-dimensional experiments. The COSY-45 and delayed COSY-45 spectra were measured in the magnitude mode, while NOESY spectra in the phase-sensitive mode. In COSY-45, delayed 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 delayed COSY-45 spectra were measured with delay time, D3 of 0.25 s. The NOESY spectra were measured with several mixing times (0.45–1.2 s). The 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 resolution was 19.53 and 17.6 Hz per point in F2 and F1 dimensions, respectively.

The HMBC pulsed field gradient spectra were recorded with a Bruker Avance DRX500 spectrometer. Absolute value HMBC spectra were recorded with a relaxation delay of 1.5 s and 8–16 scans per increment. The spectral width was 6600 Hz in the acquisition domain F2 and 31000 Hz in time domain F1. Data were collected into the 2048 × 256 acquisition matrix and processed using a 2K × 1K transformed matrix with zero filling in the F1 domain. In HMBC spectra, the delay for the long-range couplings was set at 60 ms.

GAM isolation

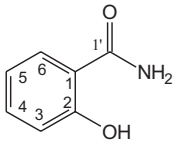
GAM was isolated from urine samples of ten healthy volunteers following overnight fasting, namely eight hours after oral self-administration of tablets containing 1000 mg of salicylamide. The official form and written consent were obtained from the subjects prior to the experiment. Voluntary subjects were not under any other drug therapy.

Collected urine samples (2500 mL) were divided into five portions and each 500 mL of urine was refluxed for 5 hours following addition of 250 mL of sulfuric acid (c = 3 mol L^{-1}). The metabolites freed from their conjugated forms were extracted by ether up to almost complete depletion of the acidic urine layer. Ether extracts were collected, dried by addition of anhydrous sodium sulfate, filtered off and evaporated. Salicylamide, salicylic acid, gentisamide and gentisic acid were separated by column chromatography and identified in the crude oily residue (silica gel 70–230 mesh ASTM, 0.063–0.200 mm, Kemika, Croatia, using chloroform/methanol 19:1). Separated components were additionally purified by column chromatography using acetone/ethyl acetate (17:3) as solvent mixture. For the qualitative analysis, thin-layer chromatography was performed (2-mm thick silica gel sheets Kieselgel 60 F_{254}, Merck, Germany) using the following solvent mixtures: benzene/ether/acetic acid/methanol (60:30:9:1), chloroform/methanol (19:1) and ethyl acetate/acetone (7:3). Spots were detected under UV light (254 nm) using iron(III)-chloride as a reagent: salicylamide and salicylic acid gave violet while gentisamide and gentisic acid gave blue spots. GAM (m.p. 217–218 °C, 215–216 °C in ref. 9) was isolated in an amount corresponding to about 5% of administered salicylamide. This is in agreement with the literature values of 3–15% (9).

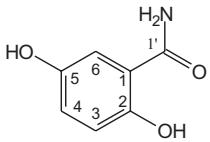
RESULTS AND DISCUSSION

The first-pass minor 5-hydroxy metabolite of salicylamide, the gentisamide, was isolated from urine after hydrolysis in a small quantity, amounting to 5% of the administered dose of SAM. Salicylic acid (SA), gentisic acid (GA) and SAM were extracted together with GAM in significant quantities, yielding almost 75% of the total extracted mass. The losses can be attributed to early urine collection and incomplete extraction, as well as to the presence of other components, detectable only by thin-layer chromatography.

In this work, we have studied the structural features of GAM, influence of 5-OH group on the FT-IR and NMR spectroscopic characteristics of the GAM molecule, which also contributed to other physico-chemical properties of GAM and can be of importance for its biological behaviour. The study of biotransformation of GAM alone in liver perfusate of rats (7) revealed that GAM-5-glucuronide was the major metabolite of GAM while GAM-2-sulfate was a minor metabolite. This should not be understood only as a consequence of the physico-chemical properties and reactivity of 5-OH group compared to C2-OH but the steric hindrance should be also taken into account.



Salicylamide (SAM)



Gentisamide (GAM)

FT-IR spectrum of GAM (Fig. 1, Table I) revealed several absorption bands in the region of stretching vibrations of O-H and N-H bonds. Thus, the absorption bands at ν 3393 and 3350 cm^{-1} were assigned to NH_2 group, while the absorption band at ν 3446

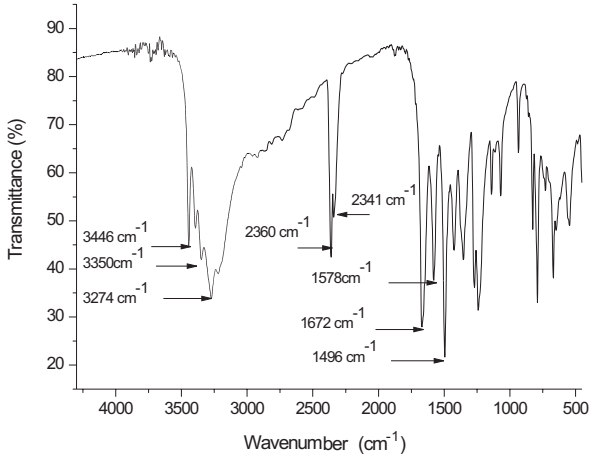


Fig. 1. FT-IR spectrum (KBr) of gentisamide.

cm^{-1} was assigned to O-H bond at C-5. The absorption band of the other hydroxyl group at C2-OH is most probably overlapped with the broadened band spreading from 3400 to 2700 cm^{-1}. These findings indicate the possible association through H-bonding in the structure of GAM. In the FT-IR spectrum of SAM (Fig. 2, Table I), the stretching absorption bands observed at ν 3398 and 3190 cm^{-1} were assigned to O-H and N-H bonds. The absorption bands at higher wavenumbers were attributed to O-H stretching, and those at lesser wavenumbers to N-H stretching. Although primary amides generally show two moderate absorption bands for NH_2 in this region (one for asymmetric and the other for symmetric vibrations), this probability was rejected here because of a significant distance between these two absorption bands ($\Delta\nu$ = 3398 – 3190 = 208 cm^{-1}) and their absorption intensities (T_{3398} = 24.6% and T_{3190} = 22.6%). The broad absorption band in the FT-IR spectrum of SAM from 3500 to 2500 cm^{-1} also points to the presence of H-bonding.

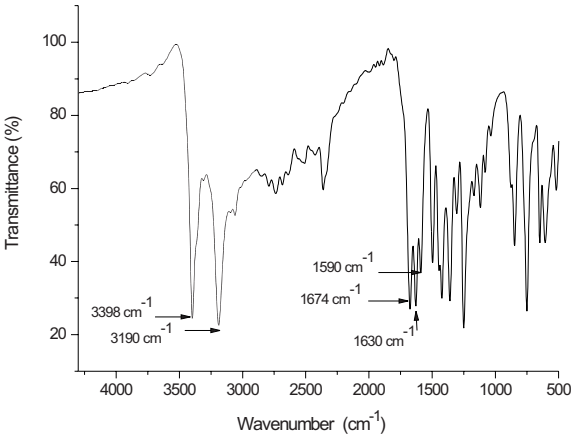


Fig. 2. FT-IR spectrum (KBr) of salicylamide.

Table I. Stretching absorption bands in FT-IR spectra (KBr) of salicylamide and gentisamide

Compound	Wavenumber, ν (cm^{-1})		
	ν_{OH}	$\nu_{C=O}$	ν_{NH2}
Salicylamide	3397	1674 (amide I)	3397
		1589 (amide II)	3189
Gentisamide	3444	1665 (amide I)	3393
	3274	1577 (amide II)	3350

In the region of stretching absorption of C=O group in primary amides, two characteristic bands were observed at 1674 and 1589 cm^{-1} for SAM and at 1665 and 1577 cm^{-1} for GAM. The absorption bands at higher wavenumbers were assigned to the amide I

band and the bands at lower wavenumbers to the amide II band, which is typical of primary amides. FT-IR spectra of GAM and SAM are displayed in Figs. 3a and b.

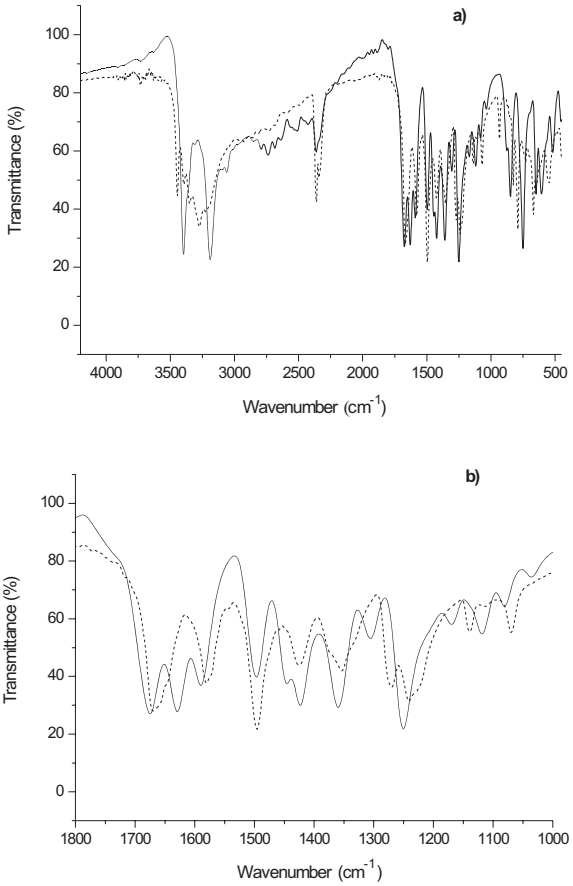


Fig. 3. FT-IR spectra (KBr) of salicylamide (——) and gentisamide (·····) in the region of: a) 4500 to 500 cm^{-1} (asorption bands at 2360 and 2341 cm^{-1} in both spectra belong to stretching asymmetric and symmetric vibrations of CO_2), and b) 1800–1000 cm^{-1}.

NMR investigations of SAM and its derivatives involved so far the acidic-base proton exchange and showed that *N*-methyl-SAM undergoes exceptionally rapid base-catalyzed proton exchange and specific base-intermolecular general acid catalysis (25). It was also concluded from the same study that *o*-hydroxyl group in SAM acts as an intramolecular catalyst in acidifying the NH proton of amide. Additionally, Brown *et al.* (26) concluded from deuterium nuclear quadrupole resonance spectra that the intramolecular H-bond in SAM at 77 K is non-linear and that the angle of the O-H…O is smaller than 150°. On the basis of electron-absorption spectra and quantum chemical calculations, El Shahawy (13) also found that the $CONH_2$ group is oriented toward OH group and that the proton of *ortho*-hydroxyl group is bound to *N*-atom of $CONH_2$ group by H-bond. These findings were confirmed by the crystal structure and solid-state infrared

patterns of SAM derivatives, *i.e.*, intramolecular hydrogen bonding between the amide proton and phenol oxygen, and intermolecular hydrogen bonds between the amide carbonyl oxygen and the phenol proton were found (14).

In this work, on the basis of the NMR study of GAM in DMSO-d_6, the orientation of the amide protons toward H-6 in GAM was found, while orientation of SAM amide protons was in agreement with the theoretical and experimental results of other authors. Thus, ^{1}H NMR spectra of GAM and SAM in DMSO-d_6 revealed two signals for amide protons ($CONH_2$) at δ 8.24 and 7.74 ppm in the ^{1}H NMR spectrum of GAM and at δ 8.43 and 7.92 ppm in the ^{1}H NMR spectrum of SAM (Table II, Figs. 4a and b). The observation of two distinct signals for amide protons was explained by hindered rotation of the C-N bond in the amide moiety, which is typical of amides. Additionally, the signal of the *ortho*-hydroxyl proton (C2-OH) was observed at 13.07 ppm in the proton spectrum of SAM, while in the ^{1}H NMR spectrum of GAM two signals at 12.13 and at 8.98 ppm were assigned to C2-OH and C5-OH, respectively. In each ^{13}C NMR spectrum of SAM and GAM, seven signals for seven chemically and magnetically non-equivalent carbon atoms were observed (Table II).

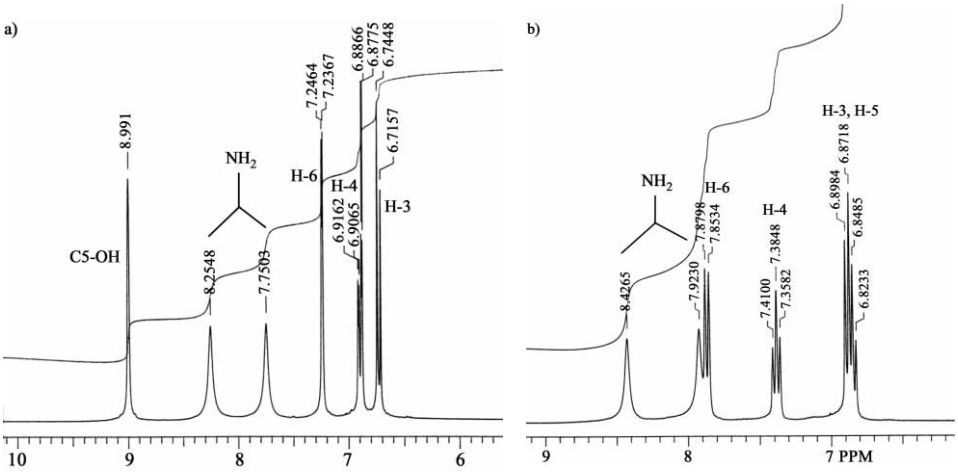


Fig. 4. Part of ^{1}H NMR spectrum of: a) gentisamide displaying amide and aromatic protons as well as the proton in C5-OH, b) salicylamide displaying amide and aromatic protons.

The results obtained from HETCOR (Fig. 5) and HMBC spectra (Fig. 6) enabled reliable assignment of carbon and proton signals in GAM. From the HETCOR spectrum, the correlations between each carbon atom and its attached proton were observed and these correlations were helpful and important in distinguishing between substituted aromatic C atoms and those which are not substituted. Additionally, the correlations in HMBC spectrum allow observation of couplings between C and H atoms through more than one bond and enable unambiguous distinguishing of protons in hydroxyl groups at C-2 and C-5. These characteristic findings from the HMBC spectrum of GAM were as fol-

Table II. ^1H and ^{13}C NMR spectral data, chemical shifts and coupling constants for salicylamide and gentisamide

Atom	Salicylamide	Gentisamide
	^{1}H-NMR δ ppm (DMSO-d_6)	
C2-OH	13.07	12.13, s
C5-OH	–	8.98, s
$CONH_2$	8.43, s 7.92, s	8.24, s 7.74, s
H-3	7.85, d ^3J = 7.9 Hz	6.86, dd ^3J = 8.6 Hz, ^4J = 2.0 Hz
H4	7.38, t ^3J = 7.3 Hz	6.69, d ^3J = 8.6 Hz, ^4J = 2.0 Hz
H-5	6.85, t ^3J = 7.6 Hz	–
H-6	6.89, d ^3J = 7.9 Hz	7.20, d ^4J = 2.0 Hz
	^{13}C NMR δ ppm (DMSO-d_6)	
C-1′	172.34	171.79
C-1	114.57	114.96
C-2	161.31	153.64
C-3	117.60	117.92
C-4	134.23	121.98
C-5	118.52	149.15
C-6	128.29	113.83

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

lows: C2-OH proton (12.13 ppm) is in correlation with C-1 (114.96 ppm) and C-3 (117.92 ppm) through three bonds, with C-2 (153.64 ppm) through two bonds and with carbon atom in the C=O group (171.79 ppm) through four bonds, while the proton in C5-OH (8.98 ppm) is in correlation with C-6 (113.83 ppm) and C-4 (121.98 ppm) through three bonds, and with C-5 (149.15 ppm) through two bonds (Fig. 6).

It was concluded on the basis of the NOESY spectrum of GAM (Fig. 7a) that the amide protons are oriented toward the *ortho*-proton at C-6. In turn, the hydroxyl group at the C-2 is H-bonded to the oxygen atom in the carbonyl group of the amide moiety. Con-

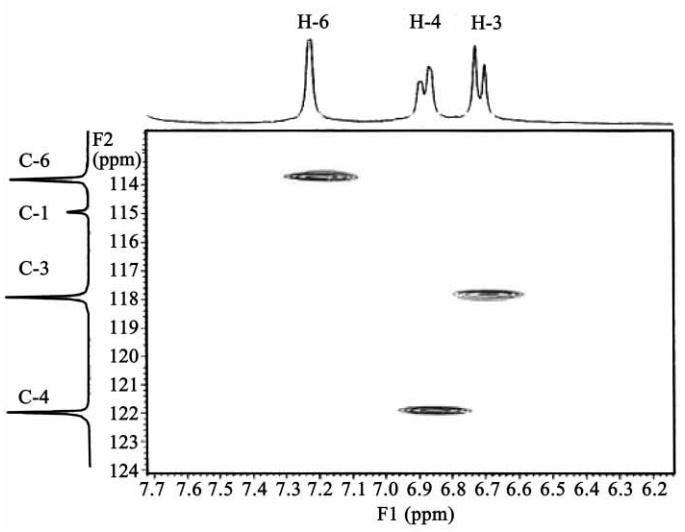


Fig. 5. A part of the HETCOR spectrum of gentisamide.

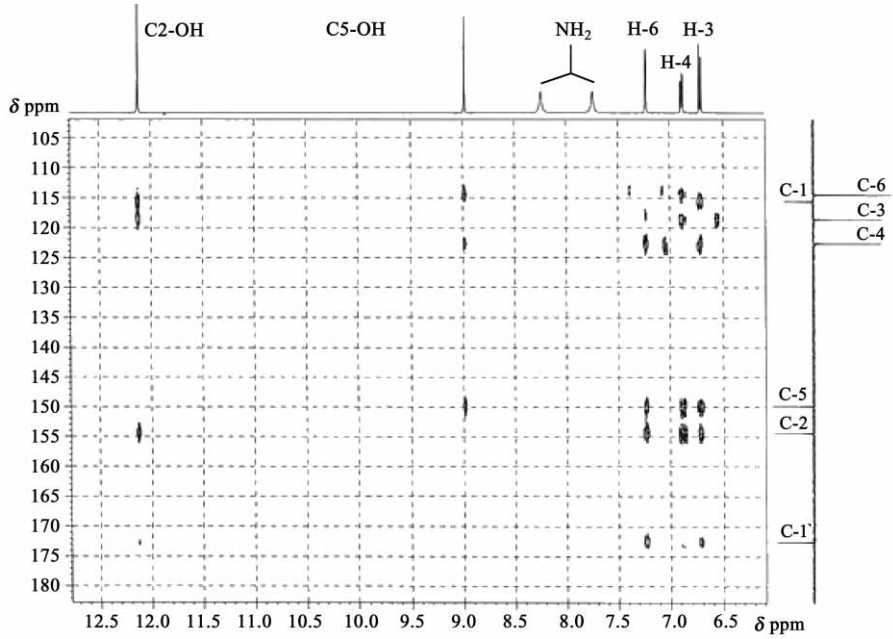


Fig. 6. The HMBC spectrum of gentisamide.

trary to that, theoretical and gas phase data for SAM suggested amino group orientation toward the *ortho*-hydroxyl group at C-2, due to strong intramolecular hydrogen bonding of the N...HO type (11, 12). Here, we have revealed the same situation for SAM in DMSO-d_6 by NMR spectroscopy (Fig. 7b).

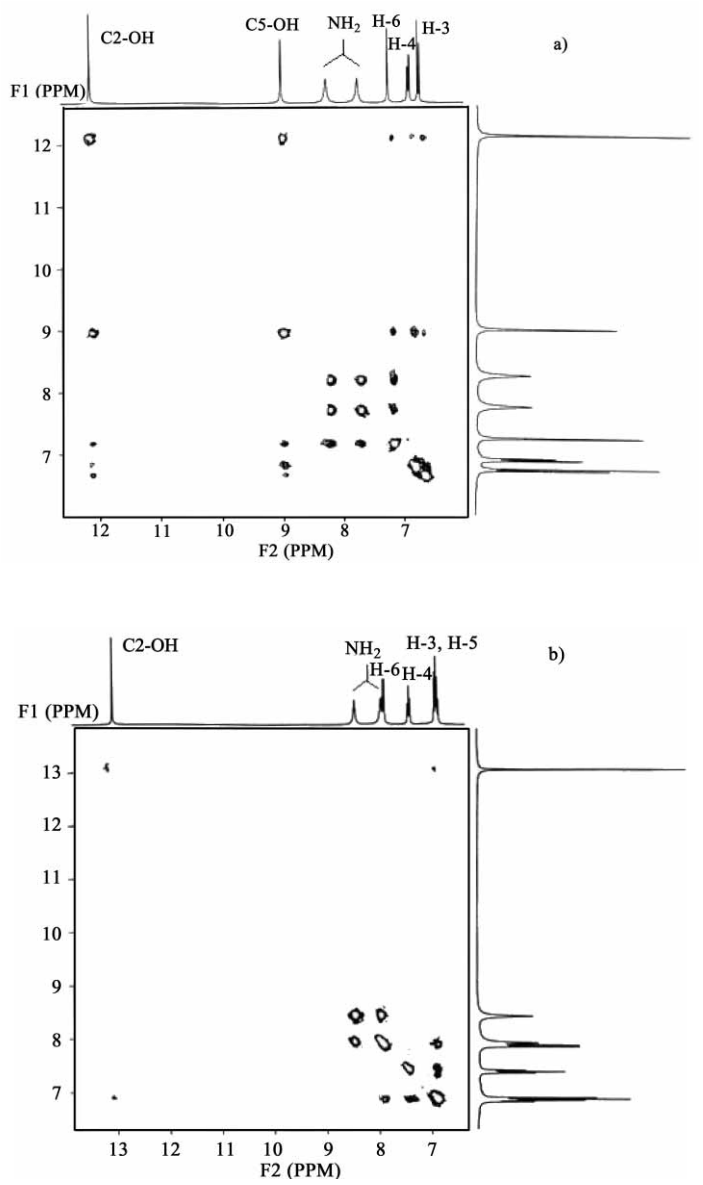
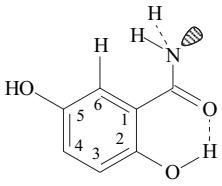


Fig. 7. The NOESY spectrum of: a) gentisamide, b) salicylamide.

It could be concluded from these findings that the presence of an additional phenol group at C-5 in GAM favours the formation of the intramolecular hydrogen bonding of the O…HO type as shown below. This was confirmed by high-temperature (100 °C) NMR measurements.



CONCLUSIONS

The structure of gentisamide isolated from human urine C was determined by means of FT-IR, one- and twodimensional homo- and heteronuclear ^{1}H and ^{13}C NMR spectroscopy. The H-bonding was observed by FT-IR spectra. On the basis of NMR spectra it was concluded that the NH_2 group is oriented toward H-6. It means that intramolecular H-bonding of O…HO type exists between C2-OH and C=O groups of GAM, which is contrary to the findings for SAM, where H-bonding of N…OH type exists between the C2-OH proton and the amide nitrogen atom.

Acknowledgements. – We are grateful for the financial support of the Ministry of Science, Education and Sports of the Republic of Croatia, grants No. 0006543 and No. 0098059.

Abbreviations. – COX – cyclooxygenase, COSY – Homonuclear Correlated Spectroscopy, CYP – cytochrome P450 enzyme, FT-IR – Fourier Transform Infrared spectroscopy, GA – gentisic acid, GAM – gentisamide, HETCOR – Heteronuclear Chemical Shift Correlation, HMBC – Homonuclear Multiple Bond Coherence, NMR – Nuclear Magnetic Resonance, NOESY – Nuclear Overhauser and Exchange Spectrosopy, NSAID – Nonsteroidal Antiinflamatory Drugs, SA – salicylic acid, SAM – salicylamide.

REFERENCES

1. U. Hacksell, Structural and physicochemical factors in drug action, in *A Textbook of Drug Design and Development* (P. Krogsgaard-Larsen, T. Liljefors and U. Madsen, Eds.), 2nd ed., Harwood Academic Publishers, Amsterdam 1996, p.p. 35–59.
2. N. P. E. Vermuelen, Role of metabolism in chemical toxicity, in *Cytochromes P450: Metabolic and Toxicological Aspects* (C. Ioanides, Ed.), CRC Press, Boca Raton 1996, p.p. 29–53.
3. U. Holzgrabe, I. Wawer and B. W. K. Diehl, *NMR Spectroscopy in Drug Development and Analysis*, Wiley-VCH Verlag GmbH, Weinheim 1999.
4. M. Waksmundzka-Hajnos, Chromatographic separations of aromatic carboxylic acids, *J. Chromatogr. B* **717** (1998) 93–118.
5. J. G. Hardman and L. E. Limbird (Eds.), *Goodman and Gilman's, The Pharmacological Basis of Therapeutics*, 10th ed., McGraw Hill, New York 2001.

6. X. Xu, H. Hirayama and K. S. Pang, First-pass metabolism of salicylamide. Studies in the once-through vascularly perfused rat intestine-liver preparation, *Drug Metab. Dispos.* **17** (1989) 556–563.
7. H. Hirayama and K. S. Pang, First-pass metabolism of gentisamide: influence of intestinal metabolism formation of conjugates. Studies in the once-through vascularly perfused intestine-liver preparation, *Drug Metab. Dispos.* **18** (1990) 580–587.
8. X. Xu, K. Bing, K. Tang and K. S. Pang, Sequential metabolism of salicylamide exclusively to gentisamide 5-glucuronide and not gentisamide sulfate conjugate in single-pass in situ perfused rat liver, *J. Pharmacol. Exp. Ther.* **253** (1990) 965–973.
9. H. G. Bray, B. E. Rayman and W. V. Thorpe, The fate of certain organic acids and amides in the rabbit, *Biochemistry* **43** (1948) 561–567.
10. M. Koike, K. Sugeno and M. Hirata, Sulfoconjugation and glucuronidation of salicylamide in isolated rat hepatocytes, *J. Pharm. Sci.* **70** (1981) 308–311.
11. R. G. Tirona and K. S. Pang, Sequestered endoplasmatic reticulum space for sequential metabolism of salicylamide. Coupling of hydroxylation and glucuronidation, *Drug Metab. Dispos.* **24** (1996) 821–833.
12. M. E. Morris, V. Yuen and K. S. Pang, Competing pathways in drug metabolism. I. An identical, anterior enzymatic distribution for 2- and 5-sulfoconjugation and a posterior localization for glucuronidation of gentisamide in the rat liver, *J. Pharmacokinet. Biopharm.* **16** (1988) 633–656.
13. S. A. El-Shahawy, Spectroscopic structural studies of salicylic acid, salicylamide and aspirin, *Spectrochim. Acta* **44A** (1988) 903–907.
14. M. C. Etter, Z. Urbanczyk-Lipkovska, T. M. Ameli and T. W. Panunto, Intra-versus intramolecular hydrogen bonds in salicylamide derivatives, *J. Crystallogr. Spectrosc. Res.* **18** (1988) 491–508.
15. J. Catalan, F. Toriblo and A. U. Acuna, Intramolecular hydrogen bonding and fluorescence of salicylaldehyde, salicylamide, and *o*-hydroxyacetophenone in gas and condensed phases, *J. Phys. Chem.* **86** (1982) 303–306.
16. J. Nakamura, M. Katayama, K. Nishida and H. Sasaki, An assessment of indomethacin-induced mucosal damage in vivo by measuring the metabolism of salicylamide in rabbit intestine, *Chem. Pharm. Bull.* **40** (1992) 1261–1265.
17. J. Nakamura, M. Katayama, K. Nishida and H. Sasaki, An assessment of salicylic acid-induced mucosal damage in vivo by measuring the metabolism of salicylamide in rabbit intestine, *Chem. Pharm. Bull.* **40** (1992) 815–818.
18. S. Zaugg, X. Zhang, J. Sweedler and W. Thormann, Determination of salicylate, gentisic acid and salicyluric acid in human urine by capillary electrophoresis with laser-induced fluorescence detection, *J. Chromatogr. B: Biomed. Sci. Appl.* **752** (2001) 17–31.
19. S. R. Gautam, V. Chungi, A. Hussain, S. Babhair and D. Papadimitrou, A direct and sensitive method for the determination of salicylamide in microplasma samples by high-perfomance liquid chromatography using fluorescence detection, *Anal. Lett.* **14** (1981) 577–582.
20. A. G. De Boer, J. M. Gubbens-Stibbe, F. H. De Koning, A. Bosma and D. D. Breimer, Assay of underivatized salicylamide in plasma, saliva and urine, *J. Chromatogr.* **162** (1979) 457–460.
21. J. Vion-Dury, F. Nicoli, G. Torri. M. Kriat, M. Sciaky, A. Davin, P. Viout, S. Confort-Gouny and P. J. Cozzone, High resolution NMR spectroscopy of physiological fluids: from metabolism to physiology, *Biochimie* **74** (1992) 801–807.
22. M. Kriat, S. Confort-Gouny, J. Vion-Dury, P. Viout and P. J. Cozzone, Two-dimensional ^{1}H NMR spectroscopy of normal and pathological human plasma: complete water suppression and further assignment of resonances, *Biochimie* **74** (1992) 913–918.
23. M. Kriat, S. Confort-Gouny, J. Vion-Dury, M. Sciaky, P. Viout and P. J. Cozzone, Quantitation of metabolites in human blood serum by proton magnetic resonance spectroscopy. A comparative study of the use of formate and TSP as concentration standards, *NMR Biomed.* **5** (1992) 179–184.

24. M. Kriat, J. Vion-Dury, S. Confort-Gouny, R. Fevre, P. Viout, M. Sciaky, H. Sari and P. J. Cozzone, Analysis of plasma lipids by NMR spectroscopy: application to modifications induced by malignant tumours, *J. Lipid Res.* **34** (1993) 1009–1019.
25. C. L. Perrin, E. R. Johnston, C. P. Lollo and P. A. Kobrin, NMR studies of base-catalysed proton exchange in amides, *J. Amer. Chem. Soc.* **103** (1981) 4691–4696.
26. T. L. Brown, L. G. Butler, D. Y. Curtin, Y. Hiyama, I. C. Paul and R. B. Wilson, Deuterium nuclear quadrupole resonance spectra of non-linear hydrogen bonds, *J. Amer. Chem. Soc.* **104** (1982) 1172–1177.
27. R. M. Silverstein, G. C. Bassler and T. C. Morril, *Spectrometric identification of organic compounds*, 5th ed., John Wiley, New York 1991.
28. H. O. Kalinowski, S. Berger and S. Braun, *^{13}C-NMR-Spektroskopie*, John Wiley, Chichester 1991.

S A Ž E T A K

FT-IR i NMR spektroskopska istraživanja derivata salicilne kiseline. I. Gentizinamid – metabolit salicilamida

MILENA JADRIJEVIĆ-MLADAR TAKAČ, DRAŽEN VIKIĆ-TOPIĆ i TIHANA GOVORČINOVIĆ

Gentizinamid (GAM), metabolit prvog prolaska salicilamida (SAM), analiziran je uporabom FT-IR, te jednodimenzijskom i dvodimenzijskom homo- i heteronuklearnom ^{1}H i ^{13}C NMR spektroskopijom.

GAM je izoliran iz ljudskog urina osam sati nakon oralne primjene SAM-a, a strukturna analiza provedena je pomoću FT-IR, ^{1}H i ^{13}C NMR uporabom kemijskih i supstituentnih pomaka, konstanti spin-spin sprege, te povezanosti u COSY, NOESY, HETCOR i HBMC spektrima.

Iz NOESY spektara u DMSO-d_6 zaključeno je da su protoni amidne skupine gentizinamida usmjereni prema *orto*-protonu na C-6 položaju. Rezultati ukazuju na to da prisustvo dodatne fenolne skupine C5-OH u GAM favorizira stvaranje intramolekulske vodikove veze O...HO tipa između C2-OH protona i kisika amidne skupine.

Ključne riječi: gentizinamid, salicilamid, NSAID, biotransformacija, FT-IR, 1D i 2D homo- i heteronuklearna ^{1}H i ^{13}C NMR

Farmaceutsko-biokemijski fakultet Sveučilišta u Zagrebu, Zagreb

Institut Ruđer Bošković, Zagreb