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1D and 2D NMR spectroscopy for identification of carbamide-containing biologically active compounds

Urea (carbamide) is the main end product of amino acids' metabolism in mammals. Extensive research in the field of urea chemistry has contributed to the creation of many biologically active and other compounds based on the carbamide fragment NH–CO–NH. The substituting groups of urea directly affect its properties and characteristics which are reflected in the NMR spectral data and this circumstance can be the basis for the identification of urea derivatives. In this work, chemical shifts in the NMR spectra of urea and its acyclic structure, barbituric series, imidazolidinone series and bicyclic structure derivatives were studied and identified. A system analysis was carried out to determine the effect of the type of substituents on the positions of signals of the NH-CO-NH fragment in the NMR spectra. The possibility of 2D NMR spectroscopy using to simplify the identification procedure for complex mixtures was shown in the paper. The combined use of 1D and 2D NMR spectroscopy is convenient and informative to establish the structure of biologically active compounds. These methods make it possible to determine the presence and type of impurities, as well as to establish the destruction processes leading to the corresponding impurities.

Keywords: urea, urea-containing compounds, barbiturates, glycoluril, imidazolidinone, NMR spectroscopy, hydrolysates, impurities.

Introduction

It is known that the urea (carbamide) **I** is a product of nitrogenous compounds' metabolism in mammals [1, 2]. In some sources there is an information about an independent biological role of the urea [3–5]. The urea **I** is presented in the form of resonance structures **Ia** and **Ib** (Fig. 1) to explain some of the physicochemical processes that occur with the participation of this compound. The studies carried out in the field of urea chemistry made it possible to create a variety of biologically active and other compounds of acyclic and heterocyclic structures containing the urea NH–CO–NH fragment [6–10].

Obviously, the substituting groups of urea directly affect its properties and characteristics which are reflected in the NMR spectral data, and this circumstance may be the basis for the identification of urea derivatives. In this study, the chemical shifts in the NMR spectra of various heterocyclic derivatives of ureas were studied and identified to assess the effect of the type of substituents on the NH–CO–NH fragment. There are heterocyclic urea derivatives of barbituric series (pyrimidine-2,4,6-trione **II**, 5,5-diethylpyrimidine-2,4,6-trione **III**, 5-ethyl-5-phenylpyrimidine-2,4,6-trione **IV**, 1-benzoyl-5-ethyl-5-phenylpyrimidine-2,4,6-trione **V**, 1-benzoyl-5-ethyl-5-isoamylpyrimidine-2,4,6-trione **VI**), of imidazolidinone series (imidazolidin-2,4-dione **VII**, 5,5-diphenylimidazolidine-2,4-dione **VIII**), bicyclic structure (2,4,6,8-tetraazabicyclo(3,3,0)-octanedione-3,7 (glycoluryl) **IX**, 4,6,8-tetramethyl-2,4,6,8-tetraazabicyclo (3.3.0) octanedione-3.7 **X**), and of acyclic urea (N-(benzyl)urea **XI**, 1-[(3-chlorophenyl)(phenyl) methyl] urea **XII**, N-(phenylacetyl) urea **XIII**). The structures of these compounds are shown in Figure 1.

Substances **II–XIII** were previously known and identified by various analytical methods [11, 12]. However, a systematic analysis of the effect of different chemical environments on the position of signals in the NMR spectra of biologically active compounds **II–XIII** has not yet been carried out. Previous NMR spectroscopy studies did not provide sufficient explanations for the chemical shifts of compounds **II–XIII** relative to the carbamide fragment of NH–CO–NH in accordance with the shielding and deshielding effects of the substituents.

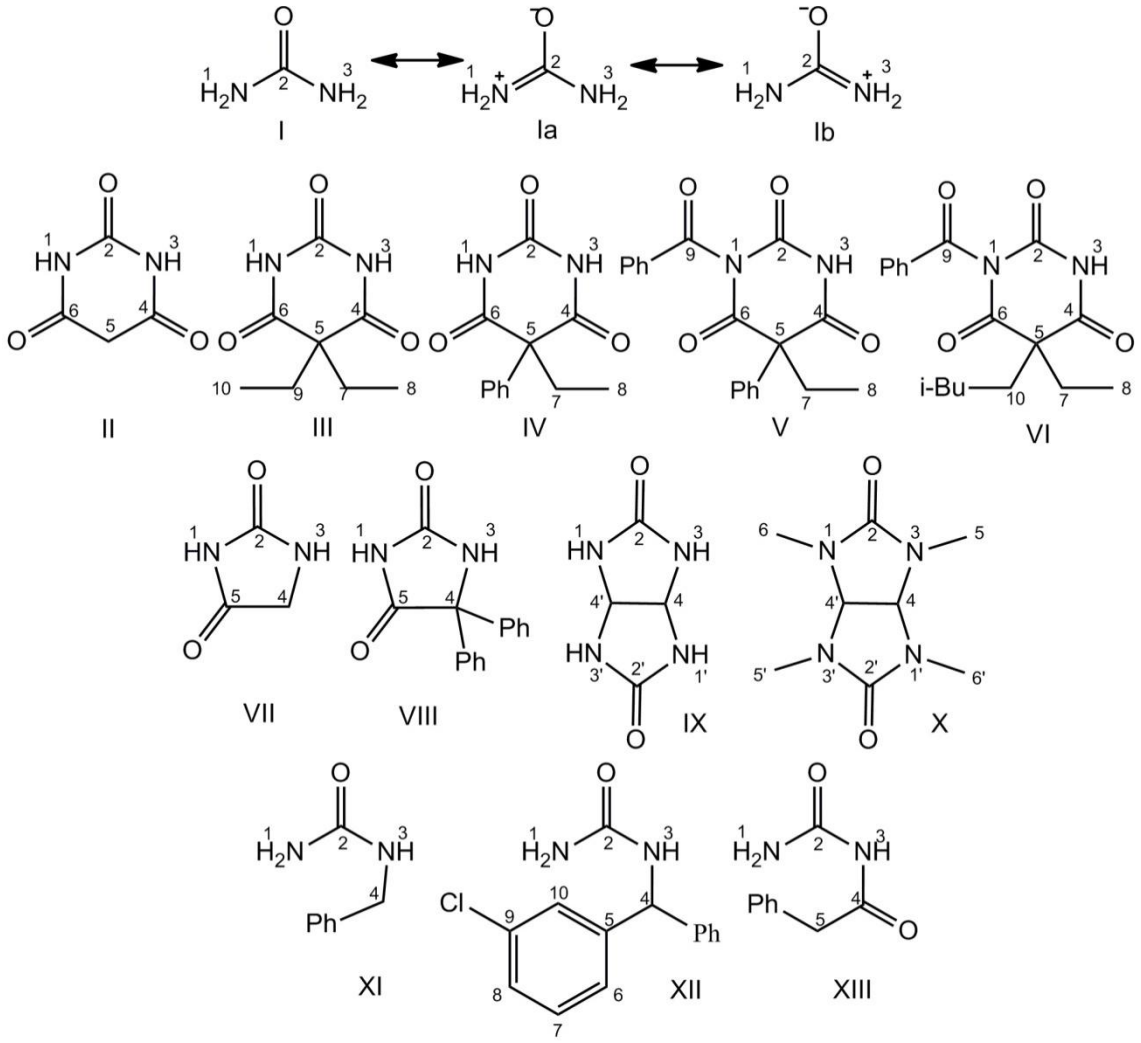


Figure 1. Structures of substances **I–XIII**

Therefore, the goal of our research was to identify and study the patterns of changes of chemical shifts in the 1D and 2D NMR spectra for a number of biologically active compounds **II–XIII**, urea **I** derivatives, and hydrolysates of these compounds **XIV–XXIV**.

Experimental

Samples of analyzed compounds **II–XIII** were provided by the Tomsk National Research Medical Center of the Russian Academy of Sciences (Scientific Research Institute of Mental Health). Samples of compounds **XIV–XVII** and **XVIII**, **XX** were synthesized according to the procedures [13, 14] and N-methylglycoluryls **XXI**–**XXIV** were obtained by the method described Kurgachev et al. [15].

NMR spectra were recorded on a Bruker AVANCE III HD spectrometer (Bruker Corporation, Germany) with an operating frequency of 400 and 100 MHz for ^{1}H and ^{13}C nuclei, respectively, in DMSO-d_6 solution (0.001 mol : 0.5 ml of solvent). The internal standard was tetramethylsilane (TMS).

Results and Discussion

First of all, the analyzed compounds **II–XIII** are structurally intramolecular bisacetylated urea **II–VI** and mono-acetylated urea **VII**, **VIII**, bicyclic bisurea **IX, X**, arylmethylurea **XI, XII** and linear acetylurea XIII. Table 1 presents the chemical shifts of the key protons and carbonyl carbon atoms of the urea **I** (as a parent) and the carbamide-containing compounds **II**–**XIII**.

Table 1

Chemical shifts in the NMR spectra for substances I–XVIII

Substance	^{1}H NMR spectrum,δ, ppm (*J*, Hz)					^{13}C NMR spectrum,δ, ppm (Hz)				
	1	3	4	5	Substituents	2	4	5	6	Substituents
I	5.93 (2H, s)	5.93 (2H, s)	–	–	–	161.5	–	–	–	–
II	11.12 (1H, s)	11.12 (1H, s)	–	3.46 (2H, s)	–	151.9	168.5	39.8	168.5	–
III	11.53 (1H, s)	11.53 (1H, s)	–	–	1.81 (4H, q, *J*=7.6, CH_2), 0.74 (6H, t, *J*=7.6, CH_3)	150.4	173.6	57.0	173.6	31,8 (C^7, C^9), 9.7 (C^8, C^{10})
IV	11.74 (1H, s)	11.74 (1H, s)	–	–	7.31–7.40 (5H, m, Ph), 2.31 (2H, q, *J*=7.2, CH_2), 0.87 (3H, t, *J*=7.6, CH_3)	150.3	172.0	60.4	172.0	126.7, 128.7, 129.6, 138.9 (Ph), 29.0 (C^7), 10.2 (C^8)
V	–	12.41 (1H, s)	–	–	7.42–7.50 (5H, m, Ph), 7.98 (2H, d, *J*=7.6, Ph), 7.78 (2H, dd, Ph), 7.59 (1H, dd, Ph), 2,5 (2H, q, *J*=7.2, CH_2), 0,99 (3H, t, *J*=7.2, CH_3)	149.0	170.8	61.4	171.0	169.1 (C^9), 129.2, 130.1, 130.87, 137.7 (Ph), 131.5, 126.8, 129.8, 136.5 (Ph), 29.0 (C^7), 10.3(C^8)
VI	–	11.76 (1H, s)	–	–	7.96 (2H, d, *J*=8.0, Ph), 7.81 (1H, dd, Ph,) 7.63 (2H, dd. Ph), 1.97–1.91 (2H, m, CH_2), 1.91–1.88 (2H, m, CH_2), 1.48–1.41 (1H, m, CH), 1.14–1.09 (2H, m, CH_2), 0.88 (3H, t, *J*=7.6, CH_3 (Et)), 0.81 (6H, t, *J*=6.4, $2CH_3$, (i-Bu))	149.4	172.8	57.5	173.1	169.5 (C^9), 130.5, 131.0, 131.7, 136.8 (Ph), 36.5 (C^{10}), 34.2 (C^7), 32.5(CH_2, i-Bu), 28.3 (CH, i-Bu), 22.9 (CH_3, i-Bu), 22.9 (CH_3, i-Bu), 10.0 (C^8)
VII	10.66 (1H, s)	7.72 (1H, s)	3.84 (2H, s)	–	–	158.8	47.7	174.4	–	–
VIII	11.34 (1H, s)	9.55 (1H, s)	–	–	8.00 (5H, m, Ph), 7.32 (5H, m, Ph)	156.6	70.5	175.3	–	140.2, 129.7, 128.3, 126.8 (Ph)
IX	7.16 (1H, s)	–	5.24 (2H, s)		–	160.3	64.6	–	–	–
X	–	–	5.06 (2H, s)	–	2.82 (12H, s)	159.1	71.9	–	–	30.4 ($C^{5,6}$)
XI	5.72 (2H, s)	6.56 (1H, t, *J* = 4.8)	4.23 (d. 2H, *J* = 6.3)	–	7.29 (5H, m, Ph),	159.5	43.4	–	–	128.7, 127.5, 127.0, 141.3 (Ph),
XII	7.04 (1H, d, *J* = 8.4)	5.60 (2H, s)	5.85 (1H, d, *J* = 8.8)	–	7.22–7.38 (9H, m, (Ph, Ar).	158.0	56.6	–	–	126.2, 126.4, 127.6, 144.1 (Ph), 135.8, 128.1, 126.9, 140.8 (Cl-Ar)
XIII	7.74 (2H, s)	10.40 (2H, s)	–	3.61 (2H, s)	7.23–7.33 (5H, m, Ph)	154.9	173.7	43.3	–	127.7, 129.2, 130.1, 135.5 (Ph)

The following patterns can be identified based on the data in Table 1:

1. Significant shielding of C=O group compared to the urea I (C^2-carbons, up to 12.5 ppm) occurs with intramolecular (compounds **II–VIII**) and linear acylation of the urea **I** (compound **IX**). While N-arylalkylation (**XI, XII**) and bicyclization (**IX, X**) causes a slight change in the chemical shifts of C=O groups (Δ = 2 ppm).

2. It is interesting to compare the chemical shifts of acyl carbonyl groups in the series of barbiturates (C^2-carbon). Any type of N- and C-substitution in the pyrimidinone cycle (**III–VI** compounds) causes a higher-field C^2-carbon shift compared to the chemical shifts of this carbon of the barbituric acid **II** itself (up to 2 ppm). This circumstance may be due to some changes in the geometry of the pyrimidinone cycle under the influence of substituents at C^2-carbon.

3. The chemical shifts of C^5-carbon in C,C-disubstituted derivatives of the barbituric acids **II–VI** are well distinguished in the ^{13}C NMR spectra: in the dialkyl-substituted **III, VI** (57.0–57.5 ppm), and in the phenylalkyl-substituted compounds **IV, V** (60.4–61.4 ppm).

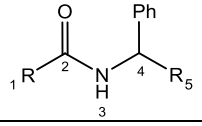
4. The data of the ^{1}H NMR spectra indicates that any type of N-substitution in the urea I leads to regular lower field shifts of NH-protons from 6.56 ppm (arylmethylation: acyclic compounds **XI–XIII**); up to 12.41 ppm (cyclic urea **VII, VIII**, barbituric derivative **V** and its analogues).

One of the main ways of transformation of the studied compounds **II–XIII** is the process of their hydrolysis or oxidative destruction in the biogenic medium. Initially, formation of the corresponding acylureas from compounds **II–VIII** or the corresponding amide from the urea **XII** and even from the urea itself can be

expected during the series of such type of reactions. In this regard, the ^{1}H and ^{13}C NMR spectra of a number of hydrolysates **XIV–XVII** of studied biologically active compounds **II–XIII** were taken and identified (Table 2).

Table 2

Chemical shifts in the NMR spectra of probable hydrolysates XI–XIV for compounds of urea derivatives II–X

					R_1 = $CH_3C(O)NH$, R_5=Ph (**XIV**); R_1 = i-$C_3H_7C(O)NH$, R_5=H **(XV)**; R_1 = H, R_5= 3-Cl-C_6H_4 (**XVI**); R_1 = (3-Cl-C_6H_4)(Ph)CHNH R_5=3-Cl-C_6H_4 (**XVII**)		
Substance	^{1}H NMR spectrum,δ, ppm (*J*, Hz)				^{13}C NMR spectrum,δ, ppm (Hz)		
	1	3	4	Substituents	2	4	Substituents
XIV	10.77 (1H, s)	9.49 (1H, d)	6.27 (1H, d)	7.41–7.53 (5H, m, Ph), 2.25 (3H, s, CH_3)	153.2	57.2	173.7 (COR_1), 142.5, 126.9, 128.9, 127.6 (Ph), 24.0 (CH_3)
XV	10.53 (1H, s)	9.02 (1H, d)	4.70 (2H, s)	7.45–7.50 (5H, m, Ph), 3.91 (1H, sep, CH), 1.17 (6H, d, CH_3, i-Pr)	153.6	42.6	174.6 (COR), 139.4, 128.5, 127.3, 127.0 (Ph), 38.0 (CH), 23.6 (CH_3)
XVI	9.52 (1H, s)	8.47 (1H, d)	6.61 (1H, d)	7.41–7.62 (9H, m, Ph, Cl-C_6H_4)	160.4	54.2	141.6, 128.6, 127.3, 127.2 (Ph), 144.8, 126.0, 130.4, 128.1, 133.3, 126.3, (Cl-C_6H_4)
XVII	7.01 (1H, d)	7.01 (1H, d)	6.01 (2H, d)	7.49–7.54 (9H, m, Ph, Cl-C_6H_4)	156.57	56.57	142.6, 126.9, 128.5, 127.0 (Ph, 3-Cl-C_6H_4)

According to the result of the initial hydrolytic transformations of barbituric acid derivatives **II–VI** and imidazolidinone compound **VIII**, formation of the corresponding N-acetylureas is quite predictable. Therefore, N-alkyl (aryl)-N-acylurea **XIV–XV** was used as a model substance in the NMR spectra. Its data were also compared with the spectra of compounds **II–VI**, **VIII**. It was found that the chemical shifts of carbonyl group (NH–CO–NH) of barbiturates **II–VI** is located in a higher-field (149–151 ppm) than in N-acylureas **XIV–XV** (153 ppm). However, the chemical shifts of NH-protons of the barbituric acid and its derivatives **II–VI** are more strongly deshielded (11.20–12.41 ppm) than those of N-alkyl(aryl)-N-acylureas **XIV, XV** (9.02–10.77 ppm). Detailed analysis of the chemical shifts values (Table 1) of the compared compounds **II–VI**, **VIII** makes it possible to distinguish intramolecular acylated urea **II–VI, VIII** from their acyclic intermediates **XIV, XV**.

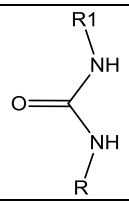
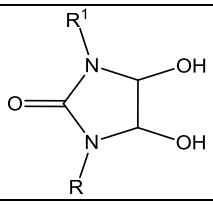
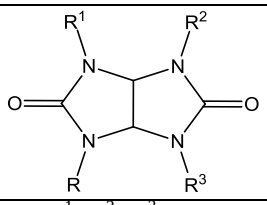
One of the most possible products of the hydrolysis of *m*-chlorobenzhydrylurea **XII** is *m*-chlorobenzhydrylformamide **XVI**. The chemical shifts of compounds **XVI** and m-chlorobenzhydrylurea **XII** were recorded and identified (Table 2). Comparison of the chemical shifts of these compounds makes it possible to reliably and distinguish them due to the perceptible difference in the chemical shifts of methine CH (Δ = 2.5 ppm), CO-carbons (Δ = 2.39 ppm), and NH-protons (Δ = 1.4 ppm).

In our opinion, the NMR identification of di(*m*-chlorobenzhydryl)urea **XVII** is extremely important, since the synthesis of compound **XII** always results in its di-derivative **XVII**, which accompanies the substance of the preparation as a minor impurity. When NMR spectra of compounds **XII** and **XVII** were compared, a significant difference of the chemical shift of the carbonyl groups was observed (approximately 2 ppm). The observed difference in the chemical shift of the C=O groups makes it possible to establish the presence of an impurity of compound **XVII** in substance **XII** by NMR. Using ^{13}C, ^{15}N NMR spectroscopy and quantum chemical calculations, it was shown that the CS in the ^{13}C spectra of C=O groups of urea does not correlate well with the electron density on the carbon atom [16]. It was found that the shielding of the carbonyl carbon atom in the di(*m*-chlorobenzhydryl) derivative of **XVII** in comparison with **XII** in the N-substitution of urea **I** is due to the presence of «bulky» radicals. The reason is an increase in steric stresses (compression) in compound **XVII** due to the «bulkiness» of symmetric diarylmethyl radicals, and an increase in the order of the amide bond, as a result.

The possible products of the initial hydrolytic transformations of hydantoin **VII** and a bicyclic urea **IX** were examined (Table 3). Thus, the formation of 4,5-dihydroxyimidazolidin-2-one **XVIII** is possible in the reaction mixture as an intermediate of synthesis of component compounds **VII, IX**, and the product of their long hydrolysis, therefore, its identification as an impurity is important.

Table 3

Chemical shifts in the NMR spectra of the probable products XVIII–XXIV of hydrolytic transformations of substances VII, IX and X

		
R=R[1]=H (**I**) R=R[1]=Me (**XIX**)	R=R[1]=H (**XVIII**) R=R[1]=Me (**XX**)	R=R[1]=R[2]=R[3]=H (**IX**); R=R[3]=Me, R[1], R[2]=H (**XXI**); R=R[2]=Me, R[1], R[3]=H (**XXII**); R=R[1]=Me, R[2]=R[3]=H (**XXIII**); R=Me, R[1]=R[2]=R[3]=H (**XXIV**);

Chemical shifts of ^{1}H NMR spectrum, δ, ppm (*J*, Hz)

Substance	CH_3	CH	CH	NH		OH
I	–	–		5.93 (2H, s)		–
XVIII		4.48 (2H, d)		6.98 (2H, s)		5.76 (2H, d)
IX		5.24 (2H, s)		7.16 (2H, s)		
XIX	2.51 (6H, s)	–		6.12 (2H, s)		
XX	2.65 (6H, s)	4.50 (2H, s)		–		6.24 (2H, s)
X	2.81 (12H, s)	5.06 (2H, s)		–		
XXI	2.78 (6H, s)	5.15 (1H, d)	5.18 (1H, d)	7.39 (2H, s)		
XXII	2.60 (6H, s)	5.10 (2H, s)		7.57 (2H, s)		
XXIII	2.64 (6H, s)	5.12 (2H, s)		7.54 (2H, s)		
XXIV	2.60 (3H, s)	5.14 (1H, d)	5.19 (1H, d)	7.20 (1H, s)	7.30 (2H, s)	

Chemical shifts of ^{13}C NMR spectrum, δ, ppm (Hz)

Substance	CH_3	CH	CH	C=O	
I	–	–		161.5	
XVIII	–	84.3		160.9	
IX	–	64.6		160.3	
XIX	26.8	–		160.8	
XX	27.2	86.1		158.3	
X	30.4	71.9		159.1	
XXI	29.7	60.6	75.6	159.5	160.2
XXII	27.4	67.4		159.7	
XXIII	28.2	76.7		158.2	
XXIV	27.6	62.5	69.9	159.8	161.8

A combined analysis of the chemical shifts of NH protons in the series of acyclic urea **I** (5.93 ppm), monocyclic urea **XVIII** (6.98 ppm) and bicyclic urea **IX** (7.16 ppm) allows them to be reliably distinguished in the NMR spectra. Probably, the cyclization (**XVIII**) of urea **I** affects the displacement of the chemical shift of the NH groups to 6.98 ppm, and the bicyclization deshielding this signal to 7.16 ppm. (**IX**). Methine protons in cycle **XVIII** are shifted by 0.76 ppm in a higher-field relative to CH–CH glycoluryl (**IX**), and, due to the structural features, resonate with a doublet. However, when comparing the chemical shifts of methine carbons in the ^{13}C NMR spectra, an antibatical shift of the peaks is observed: the CH–CH signals of urea **XVIII** are shifted to a weak field by 21.7 ppm relative to bicycle **IX**. The positions of the signals of the carbonyl C=O groups in the ^{13}C NMR spectra for substances I, **XVIII**, **IX** are almost the same, but the signal C=O group in acyclic urea **I** is the most deshielded. It can be noted that for the identification of compounds **I**, **XVIII**, **IX** proton NMR spectra are more informative.

In study Kurgachev et al [15], we isolated and characterized by chromatography and mass spectrometry analytical methods all possible N-methyl derivatives of glycoluril **XXI**–**XXIV**, which can be present as probable products of mebicar **X** transformation in hydrolysis conditions. In this study we compare the values of chemical shifts in the NMR spectra of N-methylglycoluryl (hereafter, MeGU) **X**, **XXI**–**XXIV** and N,N'-dimethylurea **XIX** (Table 3). However, it is also necessary to take into account the possible presence of

the monocyclic analogue of 1,3-dimethyl-4,5-dihydroxyimidazolidin-2-one **XX** as an intermediate in the synthesis of urea **X** and its half-life product, the NMR characteristics of which are described in Table 3.

^{1}H and ^{13}C NMR methods are highly informative for the identification of methyl derivatives of urea-containing compounds **XIX**–**XXIV**. The presence of CH_3-groups in MeGU X, **XXI**–**XXIV** leads to shielding of CH protons (5.24–5.06 ppm) and to deshielding of NH protons (7.16–7.55 ppm) of these compounds relative to **IX** in ^{1}H NMR spectra. A similar antibate effect was found in the ^{13}C NMR spectra for chemical shifts of compounds X, **XXI**–**XXIV**. There is a general deshielding of CH-carbons and a higher-field shift of C=O groups relatively to bisurea **IX** (Table 3).

In MeGU X, **XXI**–**XXIV** the most sensitive fragments to changes in the structure of the bicyclic skeleton are CH–CH fragments (Table 3), where the shielding range for ^{1}H signals is 0.18 ppm, and the range of changes for ^{13}C signals is observed from –4.0 ppm up to +12.1 ppm and –14.2 ppm for **XX** relative to MeGU X. Symmetric MeGUs X and **XXII**, **XXII** regularly give singlet peaks of CH-protons in the regions of 5.10, 5.12, and 5.06 ppm and annelated carbon atoms in the region of 67.4, 76.7, 71.9 ppm (Table 3). In asymmetric MeGUs **XXI** and **XXIV**, due to their nonequivalence, protons and carbons resonate in pairs in the regions 5.14–5.19 ppm, 5.15–5.18 ppm (^{1}H NMR) and 62.5–70.0 ppm, 60.6–75.6 ppm (^{13}C NMR). It is seen, that CH_3 groups have a symbiotic effect of de-shielding of CH signal in the ^{13}C NMR spectra of monocycles **XVIII** (84.3 ppm) and **XX** (86.1 ppm).

When comparing the chemical shifts of unsubstituted urea (**I**, **XVIII**, **IX**) and N,N'-methylureas (**X**, **XX**, **XIX**) (Table 3), it can be noted that N-methyl substituents cause weak shielding of the carbonyl atom carbon N–CO–N in ^{13}C NMR spectra. It can be determined by the effects of steric inhibition of conjugation in the amide fragment with a corresponding decrease in the order of the amide bond [16].

Thus, variations of the chemical shifts in the NMR spectra of substances **X**, **XX**–**XXIV** can be additionally caused by an increase in the effect of steric compression from methyl groups in the CH–N–Me fragment. This effect is progressive with an increase in the number of methyl groups in nitrogen atoms, as indicated by spectral data in Table 3.

In our opinion, the characteristics of the NMR spectra of compounds **XIV**–**XXIV** are suitable for determining the possible products of hydrolytic or oxidative transformations of urea-containing biologically active compounds **II**–**XIII**.

The ^{1}H (Fig. 2) and ^{13}C (Fig. 3) NMR spectra of the model mixture consisting of urea **I**, barbituric acid **II**, phenobarbital **IV**, N,N'-dimethylurea **XIX** and N-methylglycoluril **X** in DMSO-d_6 were for visual investigation of the environmental effect on chemical signals of C=O in a carbonyl fragment.

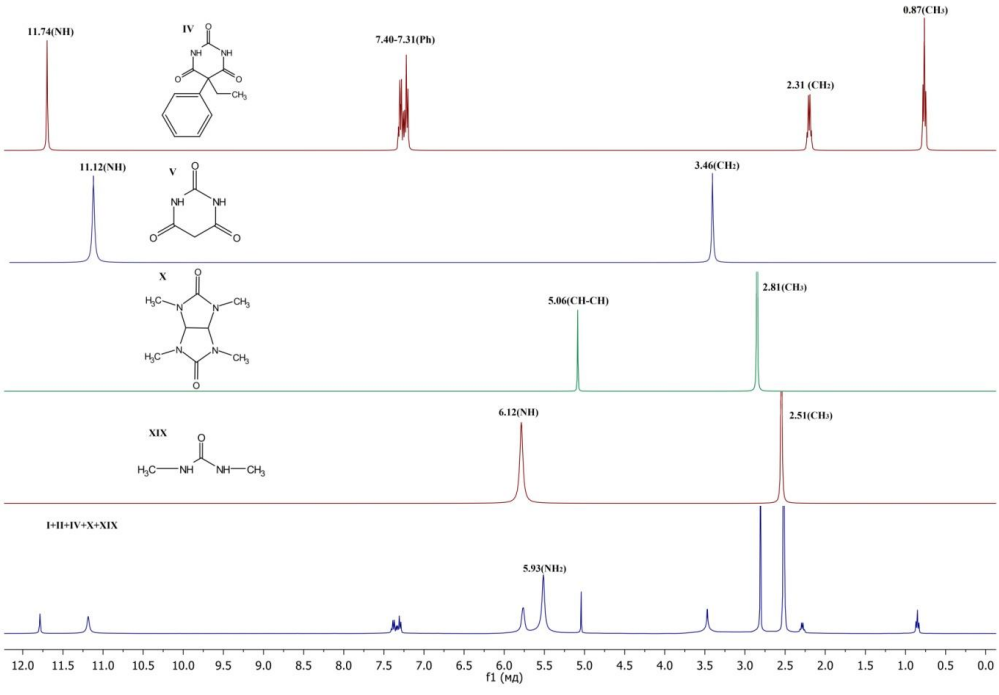


Figure 2. ^{1}H NMR spectrum of a model mixture of substances: urea **I**, barbituric acid **II**, phenobarbital **IV**, N,N'-dimethylurea **XIX** and N-methylglycoluryl **X** in DMSO-d_6 (δ 2.50 ppm)

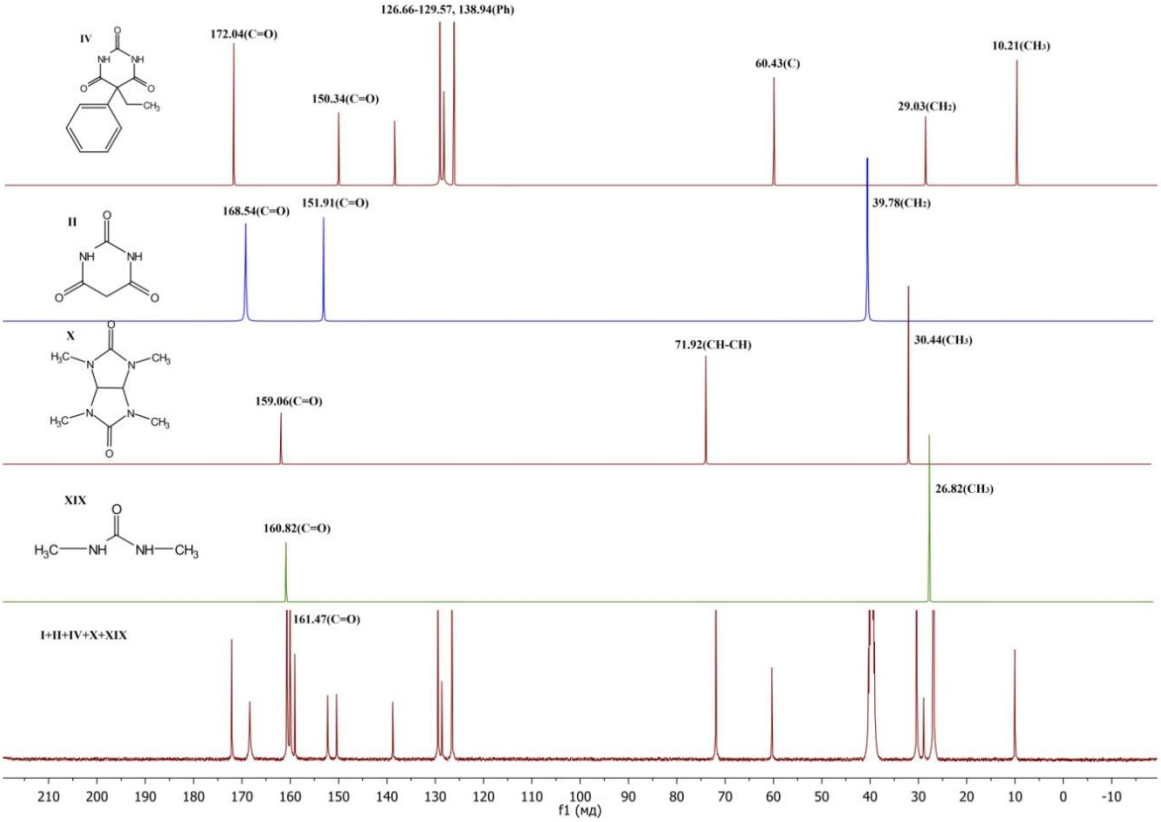


Figure 3. ^{13}C NMR spectrum of a model mixture of substances: urea **I**, barbituric acid **II**, phenobarbital **IV**, N,N'-dimethylurea **XIX** and N-methylglycoluryl **X** in DMSO-d_6 (δ 39.5 ppm)

Resonance signals can overlap with solvent peaks when registering 1D spectra (Fig. 2 and 3) of mixtures of compounds **I, II, IV, XIX**, and **X** in DMSO-d_6. But these substances are always distinguishable, since the same compound gives several signals in different regions of the NMR spectrum. In our example the difference in the chemical shifts of the urea NH–CO–NH fragment of compounds **I**, **II**, **IV**, **XIX**, and X is also noticeably. The position of the C=O signals depends on the substituent groups in NH–CO–NH.

Thus, the presented data of NMR spectra of the model mixture of substances **IV, X** and possible impurities **I, II, XIX** (Fig. 2 and 3) allows to reliably identify compounds and reliably distinguish them from each other. However, for this identification example (Fig. 2, 3), it is necessary to pre-record the spectra of the standards or to separate the components of the analyzed mixture.

In this paper, the possibility of using two-dimensional 2D NMR spectroscopy to simplify the identification procedure in the composition of complex mixtures was shown. Such widely recommended techniques [17–19] as ^{1}H–^{1}H COSY homonuclear correlation spectroscopy (Fig. 4) and heteronuclear correlation methods (^{1}H–^{13}C HSQC (Fig. 5) and ^{1}H–^{15}N HSQC (Fig. 6)) were used. Determination of correlations in the spectra of COSY (Fig. 4) and HSQC (Fig. 5, 6) between the functional groups of compounds **I, II, IV, XIX**, and **X** in the mixture was shown.

It can be seen (Fig. 4) that the protons NH–CO–NH of phenobarbital **IV** have common cross peaks with the protons of their own molecule. The signal at 11.99 ppm correlates with the protons of the phenyl substituent 7.13 ppm, with a quartet at 2.25 ppm and triplet at 0.84 ppm of ethyl radical, which also have a peak of interaction (0.84 ppm; 2.24 ppm). Barbituric acid protons **II** (NH–CO–NH) have a common cross-peak with the signal of the CH_2 group (11.24 ppm, 3.46 ppm). Signal of the NH-group of N,N'-dimethylurea **XIX** at 5.82 ppm correlate with the doublet of the methyl radical at 2.53 ppm, and the methyl protons CH_3 of N-methylglycoluril X have a cross-peak with the methine group CH–CH (2.81 ppm; 5.04 ppm). Urea **I** NH protons at 5.32 ppm correlate with themselves due to equivalence and there is no cross-peak in the ^{1}H–^{1}H COSY spectrum.

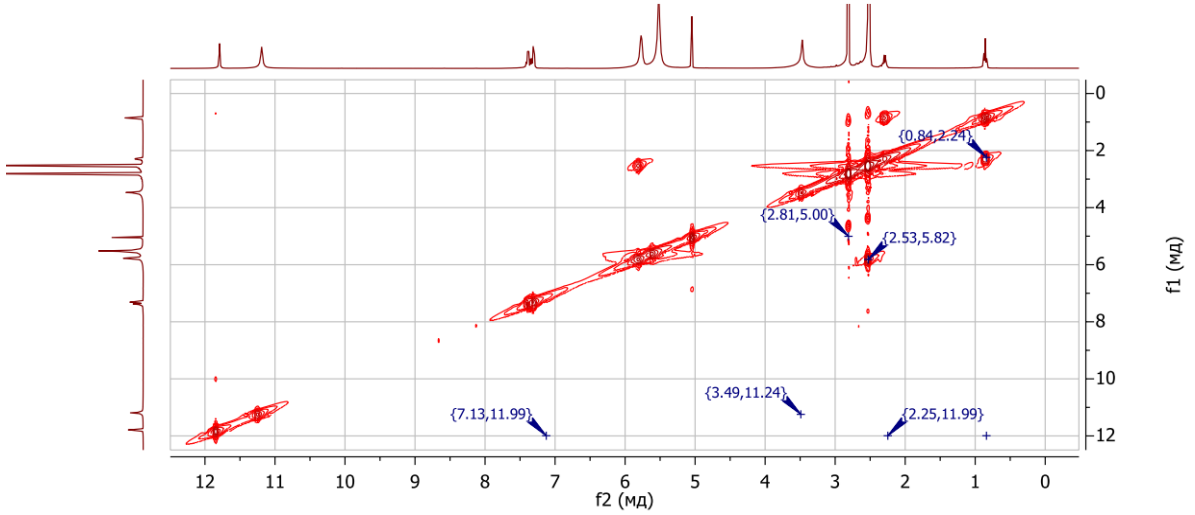


Figure 4. $^{1}H–^{1}H$ COSY spectrum of a model mixture of substances: urea **I**, barbituric acid **II**, phenobarbital **IV**, N,N'-dimethylurea **XIX** and N-methylglycoluril **X** in DMSO-d_6 (δ 2.50 ppm)

After the correlation of the proton groups of individual substances was established, the data of the heteronuclear $^{1}H–^{13}C$ HSQC spectrum were used (Fig. 5).

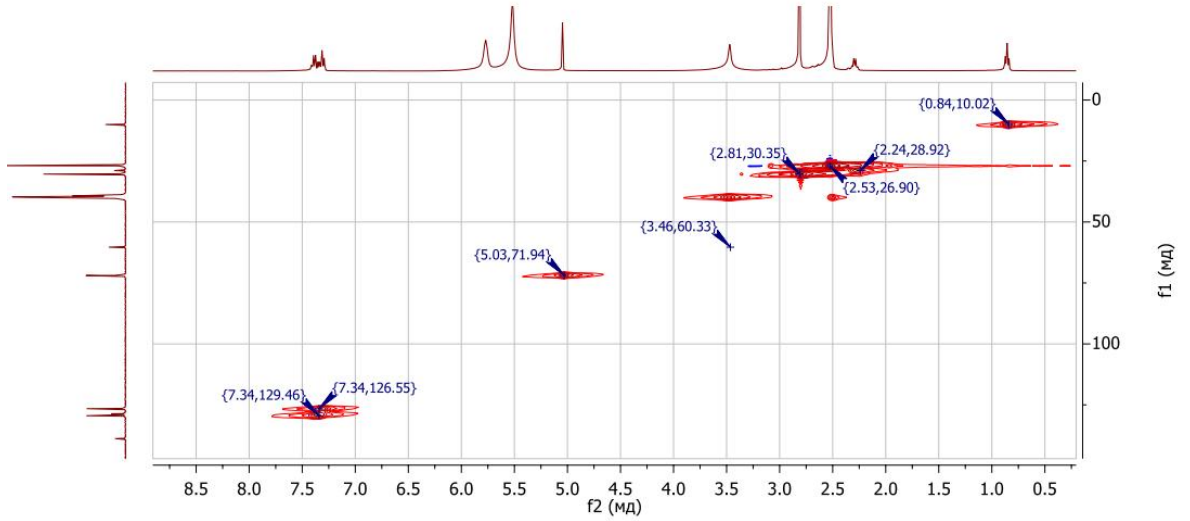
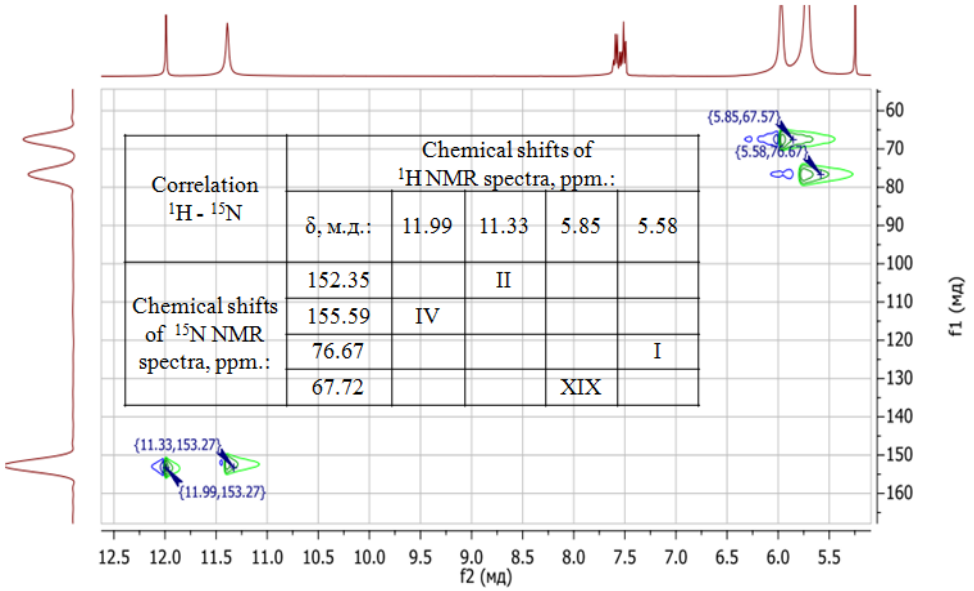


Figure 5. $^{1}H–^{13}C$ HSQC spectrum of a model mixture of substances: urea **I**, barbituric acid **II**, phenobarbital **IV**, N,N'-dimethylurea **XIX** and N-methylglycoluril **X** in DMSO-d_6 (δ: 2.50 ppm; 39.5 ppm)

It is shown (Fig. 5) that previously established multiplet signals of Ph-protons of compound **IV** at 7.34 ppm have cross peaks with carbon signals at 126.6 ppm and 129.5 ppm, probably the carbon signal of the *para* position at 128.7 ppm is hidden. The proton signals of ethyl radical IV (0.84 ppm and 2.24 ppm) correlate with the signals of carbons (10.0 ppm and 28.9 ppm), respectively. Protons of the CH_2 group of barbituric acid **II** at 3.46 ppm directly bonded to carbon at 60.3 ppm. The signal of the methine CH–CH group of the N-methylglycoluril **X** has a common peak in the regions: 5.04 ppm for ^{1}H and 71.9 ppm for ^{13}C, and methyl groups at 2.81 ppm correlate with a carbon signal of 30.4 ppm. Doublet of protons of the CH_3 group of N,N'-dimethylurea **XIX** at 2.53 ppm intersects with a carbon signal in the region of 26.9 ppm.

Chemical shifts of nitrogen atoms of ^{15}N amino groups of ureas **I**, **II**, **IV**, **XIX**, which have a direct N–H bond, can be determined in the $^{1}H–^{15}N$ HSQC spectrum (Fig. 6).



Correlation ^{1}H - ^{15}N	Chemical shifts of ^{1}H NMR spectra, ppm.: δ, м.д.:	11.99	11.33	5.85	5.58
Chemical shifts of ^{15}N NMR spectra, ppm.:	152.35		II		
	155.59	IV			
	76.67				I
	67.72			XIX	

Figure 6. ^{1}H–^{15}N HSQC spectrum of a model mixture of substances: urea **I**, barbituric acid **II**, phenobarbital **IV**, N,N'-dimethylurea **XIX** and N-methylglycoluril **X** in DMSO-d_6 (δ 2.50 ppm)

It can be seen (Fig. 6) that the chemical shift of the ^{15}N amino group of N,N'-dimethylurea **XIX** at 67.6 ppm is located in a stronger field relative to the signal ^{15}N of urea **I** (76.7 ppm) due to the electron-donating effect of methyl groups. A comparison of the ^{15}N chemical shifts of phenobarbital **IV** and barbituric acid **II** showed that the signals are in the same region (153.3 ppm), and this fact makes them indistinguishable. Almost twofold deshielding of the signals of the ^{15}N NH groups of compounds **II** and **IV** in comparison with the chemical shifts of urea **I** occurs due to intramolecular bisacetylation, causing an electron-withdrawing effect.

Conclusions

Thus, the data of chemical shifts of carbamide-containing biologically active compounds **I–XIII** (Table 1) make it possible to identify these substances, on the one hand, and, on the other, to reliably distinguish them in the ^{1}H and ^{13}C NMR spectra, including compounds of the same class of azaheterocycles (barbiturates **II–VI**, hydantoins **VII, VIII**, glycolurils **IX**, **X**, **XXI**–**XXIV**). It was shown on a model mixture of some urea-containing biologically active compounds **IV, X** and probable impurities **I**, **II**, **XIX** (Fig. 2, 3) that they can be easily identified by NMR spectra.

In addition, this study demonstrated the convenience of using two-dimensional 2D NMR spectroscopy to analyze a model mixture of **I, II, IV**, **XIX**, and **X** (Fig. 3–5), where the presence of standards and preliminary separation are not required to identify chemical shifts of compounds. The use of 2D methods of NMR spectroscopy can provide comprehensive information for the identification of all components of the analyzed sample.

In summary of the studies, it can be noted that the combined use of 1D and 2D NMR spectroscopy is convenient and informative to confirm the structure of biologically active compounds **I–XIII**. These methods make it possible to determine the presence and type of impurities **XIV**–**XXIV**, as well as to establish the destruction processes leading to the corresponding impurities **XIV**–**XXIV**.

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Мочевина құрамындағы биологиялық белсенді қосылыстарды анықтауға арналған 1D және 2D NMR спектроскопиясы

Мочевина (карбамид) — сүтқоректілердегі азотты қосылыстардың алмасуының өнімі екені белгілі. Мочевина химияның саласындағы біртұтас зерттеулері көптеген биологиялық белсенді және басқа қосылыстар, құрамында мочевина NH–CO–NH фрагменті бар құрылымдарын жасауға мүмкіндік берді. Несепнәрді алмастыратын топтар оның қасиеттері мен сипаттамаларына тікелей әсер етеді, олар ЯМР спектрлік мәліметтерде көрініс табады және бұл жағдай мочевина туындыларын анықтауға негіз бола алады. Жұмыста мочевина мен оның туындыларының ЯМР спектрлеріндегі химиялық ауысулар зерттелді және анықталды: ациклдік құрылым, барбитуралық сериялар, имидазолидинондар сериясы және бициклдік құрылым. Орынбасар типінің s-ге NH-CO-NH фрагментінің NMR спектрлеріндегі позицияларының әсерін анықтау үшін жүйелік талдау жүргізілді. Сондай-ақ, кешенді қоспалар құрамындағы сәйкестендіру процедурасын жеңілдету үшін 2D NMR спектроскопиясын қолдану мүмкіндігі көрсетілген. 1D және 2D NMR спектроскопиясын бірге қолдану қосылыстар құрылымын құру үшін ыңғайлы және ақпараттылыққа ие. Бұл әдістер қоспалардың болуы мен түрін анықтауға және тиісті қоспаларға әкелетін жою процестерін орнатуға мүмкіндік береді.

Кілт сөздер: құрамында карбамид бар қосылыстар, барбитураттар, NMR спектроскопиясы, гидролизаттар, қоспалар.

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1D и 2D ЯМР-спектроскопия для идентификации карбамидсодержащих биологически активных соединений

Известно, что мочевина (карбамид) является продуктом метаболизма азотистых соединений у млекопитающих. Целенаправленные исследования в области химии мочевины позволили создать множество биологически активных и других соединений ациклической и гетероциклической структур, содержащих NH–CO–NH карбамидный фрагмент. Заместители в молекуле мочевины напрямую влияют на ее свойства и характеристики, которые отражаются в данных спектров ЯМР, и это обстоятельство может быть основанием для идентификации мочевины и ее производных. В статье изучены и идентифицированы химические сдвиги в спектрах ЯМР мочевины и ее производных: ациклического строения, барбитурового ряда, имидазолидинонового ряда и бициклического строения. Был проведен системный анализ для определения влияния типа заместителей на положения сигналов фрагмента NH-CO-NH в спектрах ЯМР. Авторами показана возможность использования 2D ЯМР-спектроскопии для упрощения процедуры идентификации сложных смесей, где совместное использование 1D и 2D ЯМР-спектроскопии достаточно удобно и информативно для установления структур биологически активных соединений. Данные методы позволяют нам определить наличие и тип примесей, а также процессы деструкции, приводящие к соответствующим примесям.

Ключевые слова: мочевина, карбамидсодержащие соединения, барбитураты, гликолурил, имидазолидинон, ЯМР-спектроскопия, гидролизаты, примеси.

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