NMR study of diastereoisomerism of 2-(1-aminoethyl)bicyclo[2.2.1]heptane and its hydrochloride (deitiforin)

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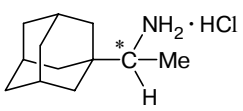
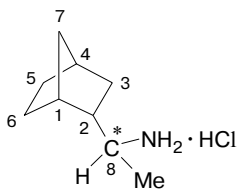
The assignment of the signals for the H and C atoms of four diastereomers (without their separation) of 2-(1-aminoethyl)bicyclo[2.2.1]heptane (**1**) and its hydrochloride (**2**) (the antiviral drug deitiforin) was performed for the first time by two-dimensional ^{1}H and ^{13}C NMR spectroscopy. The effects of the substituent at position 2 of norbornane on the chemical shifts of the α-, β-, and γ-carbon atoms of the bicycle were examined using the increments for alkanes. The changes in the chemical shifts of the C(6) and C(7) atoms are substantially larger than those for the other C atoms, which made it possible to identify the *exo* and *endo* forms. Each of these forms exists as a mixture of two diastereomers. The effect of the positive charge of the N atom on the γ-protons, which are closely spaced, but separated by a number of covalent bonds, was considered on going from amine **1** to hydrochloride **2**. Based on significant changes in shielding of these H atoms, the configurations of the asymmetric center in the $CHMe(NH_2)$ substituent of the diastereomers were established.

Key words: 2-(1-aminoethyl)bicyclo[2.2.1]heptane, deitiforin, ^{1}H and ^{13}C NMR spectroscopy, diastereoisomerism, anti-influenza drug.

Chemotherapy is the most commonly used, simple, and inexpensive means for the prophylaxis and treatment of influenza. An empirical search for virus inhibitors exhibiting anti-influenza activity among different classes of organic compounds demonstrated that amines containing alicyclic skeleton fragments selectively suppress influenza virus reproduction without disturbance of the vital functions in the organism as a whole.[1—3] These compounds are efficient at rather low concentrations, weakly toxic, and highly selective. Drugs of this class, *viz.*, amantadine (1-aminoadamantane hydrochloride)[4—6] (USA, 1966) and rimantadine (USSR, 1974; later on, in USA),[5—8] are widely used in public-health practice. 2-(1-Aminoethyl)bicyclo[2.2.1]heptane (**1**) whose hydrochloride **2** is known as the drug deitiforin[7—10] is a promising anti-influenza drug of this class. Rimantadine and deitiforin contain the identical side fragments with the asymmetric center (*R*/*S*)-*R** = $CHMeNH_2$, but differ in the structure of the hydrocarbon skeleton.



Rimantadine



Deitiforin (**2**)

The *exo* form of amine **1**, which was prepared from the *exo* isomer of 2-acetylbicyclo[2.2.1]heptane by oximation of ketone followed by reduction with a Raney nickel alloy, was proposed as an antiviral drug in 1969.[11] More recently, it was demonstrated that catalytic hydrogenation of 2-acetylbicyclo[2.2.1]hept-5-ene oxime afforded a mixture of the *exo* and *endo* isomers of amine **1**, which is (in the form of hydrochloride) also an active virus inhibitor.[9,10]

The antiviral activity of 2-substituted bicyclo[2.2.1]heptanes, including the 1-aminoethyl derivative, has long been known[11,12] and has been well studied for the drug deitiforin.[13—26] As an anti-influenza drug, deitiforin is equal to rimantadine from the standpoint of the protective effect in the treatment of the influenza infection,[14] and it can not only efficiently suppress virus-specific growth, but can also selectively act on virus-infected cells.[15]

Deitiforin possesses lower toxicity[16] and exhibits noticeable activity not only against influenza A viruses[17,18] and parainfluenza,[19] but also (unlike rimantadine) against influenza B viruses.[18] Deitiforin does not affect extension of resistant strains among epidemic influenza A viruses, *i.e.*, it does not give rise to drug resistance.[19,20] In addition, the efficiency of deitiforin was examined depending on the concentration (for the influenza FPV virus) and it was demonstrated that the use of this drug in the treatment of influenza does not

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cause complications associated with acuteness of bacterial infections.[21]

However, the mechanism of antiviral activity of deitiforin remains unknown.[17,22—24] Thus, the question of which biochemical reactions are affected by deitiforin upon interactions of influenza virus with cells is still open. The antiviral effect may result from the fact that deitiforin inhibits activation of protein kinase C in the corresponding lymphoidal cells.[22] This effect can be realized through hydrophobic bonds present in the nonpolar portion of the molecule in the membrane.[25] The protective action of deitiforin against toxicosis is probably associated with its ability to prevent penetration of the M protein into the membrane.[23] Most likely, deitiforin has the virus-specific action because it does not cause structural-morphological and functional changes in cells.[26]

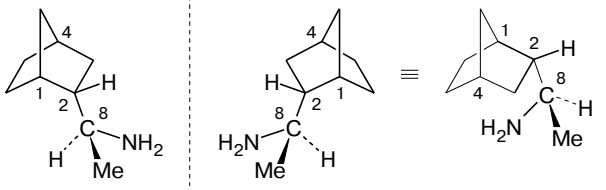
Anti-influenza chemodrugs possessing a rigid skeleton belong to a group of molecular hindrances, which efficiently block penetration of virus proteins through cell membranes.[27] Using rimantadine and adapromine* as examples, it has been demonstrated that this activity is closely related to their molecular structures.[28] The establishment of the relationship between the pharmacological action and the chemical structure in the case of deitiforin** can enable one not only to examine the effect of the molecule as a whole, but also to reveal the effect of the different mutual arrangement of the molecular fragments resulting from stereoisomerism.

Identification of the biologically active stereoisomer and its directed synthesis are topical problems in the pharmaceutical industry because the use of smaller amounts of the active compound in a drug reduces the cost of the production of the substance and leads to a decrease in the drug dose, which, in turn, results in an increase in the average therapeutic drug dose.

The only attempt to obtain a quantitative estimate of the ratio between the *exo* or *endo* isomers in deitiforin has been reported.[10] According to the data from ^{1}H NMR spectroscopy of derivatives of amine **1** (sulfonamides and ureas), only two isomers were experimentally detected.[10] These isomers were formally assigned to the *exo* or *endo* forms (the ratios were 55—60 and 40—45%, respectively). It was assumed that this ratio is also typical of the initial amine **1**. It should be noted that, strictly speaking, the observed isomers could be two diastereomers of only one of the forms (*exo* or *endo*).

It is worthy of note that 2-R-norbornanes (R = Me, CH_2OH, *etc.*) studied previously[31] do not contain asymmetric centers in the substituents and, consequently, occur only as two diastereomers (*exo* and *endo* forms). The *exo* and *endo* forms of the amine derivatives (sulfonamides, ureas, and thioureas) of the bicyclo[2.2.1]hept-5-ene series (norbornene) were also identified by ^{13}C NMR spectroscopy.[32—34] However, in the latter case, these diastereomers were preliminarily isolated as individual compounds by fine fractional distillation.

If the molecule contains four asymmetric centers, the number of theoretically possible stereoisomers is equal to $2^n = 16$. However, only one-half of these stereoisomers can exist in the case of amine **1** or deitiforin **2**, which have the framework structure with the asymmetric C(1) and C(4) bridgehead atoms, because the rigid bicyclic structure allows for only two groups of isomers in which the C(1) and C(4) atoms should adopt consistent configurations, *i.e.*, (1*R*,4*S*) or (1*S*,4*R*). Therefore, eight stereoisomers of racemic amine **1** and deitiforin **2** represent four enantiomeric pairs (diastereomers). One of these pairs is shown below.



(1*R*,2*S*,4*S*,8*S*)-*endo*-**1** (1*S*,2*R*,4*R*,8*R*)-*endo*-**1**

These enantiomeric pairs can be divided into two groups depending on the orientation of the $CHMeNH_2$ substituent at the C(2) atom in the bicycle. Two pairs occur as two racemic *endo* diastereomers (1*R*,2*S*,4*S*,8*R*/*S*) corresponding to the *endo* orientation of the substituent. The remaining two pairs (1*R*,2*R*,4*S*,8*R*/*S*) form two other diastereomers with the *exo* orientation of the substituent. For simplicity, only the (1*R*,4*S*) enantiomers are shown below.

(1*R*,2*R*,4*S*,8*R*/*S*)-*exo*-**1** (1*R*,2*S*,4*S*,8*R*/*S*)-*endo*-**1**

R* = CH(Me)NH_2

Experimental

The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker DRX-500 spectrometer (500.13 MHz for ^{1}H and 125.76 MHz for ^{13}C). The chemical shifts, which are given in the δ scale relative to Me_4Si for the H atoms and relative to $CDCl_3$ (δ = 77.0) for the C atoms, are listed in Tables 1 and 2, respectively. We used standard two-dimensional NMR techniques (HSQC, HMQC, HMBC, TOCSY, and ROESY).

* These C(1)-substituted adamantane derivatives, C*H(Me)NH_2 · HCl (rimantadine) and C*H(Et)NH_2 · HCl (adapromine) containing one asymmetric C atom, exist as racemic mixtures of enantiomers.

** It has been reported that the mechanism of action of deitiforin on the influenza virus reproduction differs from that of rimantadine.[29] If virus mutants resistant to rimantadine arise in the course of the influenza epidemy,[30] the design of a new drug becomes an urgent problem.

Table 1. The ^{13}C chemical shifts for 2-(1-aminoethyl)bicyclo[2.2.1]heptane (**1**) and its hydrochloride (**2**)

R*	Diastereomer		δ								
			C(1)	C(2)	C(3)	C(4)	C(5)	C(6)	C(7)	C(8)	Me
$CH(Me)NH_2$	*exo* (1*R*,2*R*,4*S*)	(8*R*)	39.04	52.00	35.54	36.70	28.52	30.51	35.23	51.09	22.97
		(8*S*)	38.03	51.79	35.52	36.68	28.48	30.34	35.89	50.85	21.16
	endo (1*R*,2*S*,4*S*)	(8*R*)	38.13	49.55	34.63	36.82	30.19	22.39	40.00	49.24	22.63
		(8*S*)	39.04	50.22	35.26	37.01	29.92	22.55	39.81	50.22	22.48
$CH(Me)NH_2 \cdot HCl$	*exo* (1*R*,2*R*,4*S*)	(8*R*)	38.30	46.86	35.54	36.48	27.91	29.55	35.66	52.86	18.10
		(8*S*)	37.84	46.83	34.96	36.25	27.91	29.81	35.54	51.61	16.46
	endo (1*R*,2*S*,4*S*)	(8*R*)	37.77	44.73	34.17	36.56	29.89	22.07	39.37	50.06	18.26
		(8*S*)	38.42	45.14	34.97	36.82	29.55	22.66	39.44	52.04	18.26

Table 2. The ^{1}H chemical shifts for 2-(1-aminoethyl)bicyclo[2.2.1]heptane (**1**) and its hydrochloride (**2**)

R*	Diastereomer		δ												
			H(1)	H(2)	H(3)		H(4)	H(5)		H(6)		H(7)		H(8)	Me
					exo	*endo*		*exo*	*endo*	*exo*	*endo*	*syn*	*anti*		
$CH(Me)NH_2$	*exo* (1*R*,2*R*,4*S*)	(8*R*)	2.07	1.08	1.18	1.36	2.22	1.43	1.01	1.43	1.01	1.23	1.03	2.45	1.10
		(8*S*)	2.20	1.11	0.98	1.31	2.18	1.41	1.07	1.45	1.05	1.22	1.03	2.42	0.95
	endo (1*R*,2*S*,4*S*)	(8*R*)	2.25	1.50	1.66	0.59	2.15	1.49	1.05	1.49	1.38	1.30★	1.24★	2.66	1.03
		(8*S*)	2.16	1.48	1.77	0.81	2.19	1.48	1.09	1.43	1.31	1.32★	1.27★	2.64	1.04
$CH(Me)NH_2 \cdot HCl$	*exo* (1*R*,2*R*,4*S*)	(8*R*)	2.15	1.62	1.39	1.51	2.40	1.45	1.10	1.45	1.18	1.20	1.09	2.88	1.42
		(8*S*)	2.55	1.65	1.03	1.40	2.25	1.45	1.10	1.49	1.20	1.20	1.12	2.85	1.27
	endo (1*R*,2*S*,4*S*)	(8*R*)	2.45	1.95	1.65	0.58	2.12	1.44	0.99	1.42	1.22	1.36★	1.31★	3.02	1.26
		(8*S*)	2.11	1.95	1.75	0.98	2.17	1.43	1.22	1.43	1.25	1.36★	1.27★	3.02	1.24

★ We failed to perform the assignment of the *syn*/*anti* signals for the H(7) protons.

2-Acetylbicyclo[2.2.1]hept-5-ene oxime (a mixture of isomers) was prepared by condensation of cyclopentadiene with methyl vinyl ketone followed by oximation. Hydrogenation of the resulting compound to form amine **1** (specimen *1*) was carried out in the presence of Raney nickel according to a known procedure[10] in a laboratory reactor equipped with a magnetic stirrer at 80 °C under a hydrogen pressure of 20 atm. We used Pr^iOH as the solvent. Deitiforin (**2**) was obtained by acidification of the amine with HCl followed by recrystallization from Pr^iOH, m.p. 282—285 °C. According to the results of titration with perchloric acid (99.2%), the content of the major compound ($C_9H_{17}N \cdot HCl$) corresponded to the requirements.[35] Specimen *2* was isolated from specimen *1* by chromatography.

Results and Discussion

The ^{13}C NMR spectra of the specimens containing four diastereomers* of amine **1** or deitiforin **2** (specimens *1*) have more than 30 signals out of 36 theoretically possible signals. For these specimens, attempts to assign the signals to particular diastereomers failed even with the use of 2D NMR spectroscopy.

The ^{13}C NMR spectra of specimens *2* of amine **1** and the corresponding deitiforin **2** have only 18 intense signals, whereas the intensities of the remaining 18 signals were no higher than 10%. The simpler NMR spectral patterns of specimens *2* made it possible to perform the assignment of all signals in the ^{1}H and ^{13}C NMR spectra for both diastereomers. As shown below, specimen *2* represents a mixture of two (1*R*,2*R*,4*S*,8*R*/*S*) diastereomers of the *exo* form.

Based on the ^{1}H and ^{13}C NMR spectra of specimen *1* (a mixture of four diastereomers), the assignment of the remaining signals to two (1*R*,2*S*,4*S*,8*R*/*S*) diastereomers of the *endo* form was carried out taking into account the signals of the *exo* form identified previously (see Tables 1 and 2).

To a first approximation, the effect of particular substituents on the chemical shifts of the α-, β-, γ-, and δ-carbon atoms in alkanes is constant and additive, which makes it possible to introduce increments of the substituents $\Delta\delta^{13}C = \delta^{13}C(R) - \delta^{13}C(H)$. Since the usual effect of the substituent through covalent bonds depends insignificantly on the orientation of these bonds, empirical correlations between the chemical shifts and the molecular structure are of most use in analysis of ^{13}C NMR spectra of alkanes.[36] The possibility of the employment of increments of the substituents for the molecules of amine **1** and its hydrochloride **2** is supported by the fact that the chemical shifts of the C atoms in the (8*R*) and (8*S*) diastereomers are similar both for the *exo* and *endo* forms (the differences are at

* Hereinafter, this term is taken to mean racemic diastereomers, *i.e.*, 1 : 1 mixtures of enantiomers.

Table 3. Increments of the substituents for 2-R-bicyclo[2.2.1]heptanes $\Delta\delta^{13}C = \delta^{13}C(R) - \delta^{13}C(H)$

R	$\Delta\delta^{13}C$							
	C(1)	C(2)	C(3)	C(4)	C(5)	C(6)	C(7)	C(8)
				exo				
CH_3 [31]	6.7	6.7	10.1	0.5	0.2	−1.1	−3.7	22.3
CH_2OH [31]	1.8	15.1	4.4	−0.2	0.2	−0.7	−3.3	66.4
$CH(Me)NH_2$*	1.77	21.79	5.43	−0.11	−1.57	0.32	−3.14	50.97
$CH(Me)NH_2 \cdot HCl$*	1.27	16.74	5.15	−0.44	−2.19	−0.42	−3.10	52.23
				endo				
CH_3 [31]	5.4	4.5	10.6	1.4	0.5	−7.7	0.2	17.4
CH_2OH [31]	1.7	12.8	4.0	0.4	0.2	−7.2	1.4	64.3
$CH(Me)NH_2$*	1.76	19.57	4.84	0.13	0.12	−7.50	1.16	49.16
$CH(Me)NH_2 \cdot HCl$*	1.29	14.83	4.47	−0.11	−0.38	−7.74	0.70	51.05

* Calculated from the average values for the (8*R*/*S*) diastereomers.

most 1.0 ppm, see Table 1). The effects of the substituents on the chemical shifts of the C atoms in 2-methylbicyclo[2.2.1]heptanes are presented in Table 3.

The α- and β-effects

In the case of 2-methylbicyclo[2.2.1]heptane, the presence of the CH_3 group causes deshielding of the α-C atom by 6.7 ppm and deshielding of the β-C atom by 6.7 (for C(1)) and 10.1 ppm (C(3)) compared to nonsubstituted norbornane[31] (see Table 3). It is known that the β-effect of the CH_3 group in alkanes (the increment is 9.4 ppm) is larger than the α-effect (the increment is 9.1 ppm).[37,38] The β-effect of the CH_3 group decreases as the H atoms of the methyl group are successively replaced. Hence, in the remaining three compounds (see Table 3), the corresponding β-effects at the C(1) (1.2—2.2 ppm) and C(3) (4.4—5.4 ppm) atoms are substantially smaller than the α-effect at the C(2) atom (6.6—15.1 ppm). The β-effect of the substituent at the C(2) atom on the signal for the C(3) atom is always more substantial than that on the signal for the C(1) atom, as has also been observed previously for other norbornanes.[31] Therefore, the side groups at position 2 of the norbornane bicycle in amine **1** ($R = CH(Me)NH_2$) and its hydrochloride **2** ($R = CH(Me)NH_2 \cdot HCl$) exhibit the usual α- and β-effects.

However, if 2-methylnorbornane (for which δC(2) is 36.8 and 34.6 for the *exo* and *endo* isomers, respectively[31]) is considered for comparison and the increments of the effects of the substituents in alkanes on β-C (9.4 for CH_3,[36] 11.3 for NH_2,[36] and 7.5 for NH_3^+ [37]) are taken into account, the values for amine **1** (δC(2) 57.6 and 55.4) and deitiforin (**2**) (δC(2) 53.7 and 51.5) are regularly larger than the corresponding experimental values by more than 5.6 and 6.6 ppm, respectively (see Table 1). Analogously, the calculated chemical shifts of the C(8) atom are also larger than the experimental values, on the average, by 7.6 (**1**) and 3.3 ppm (**2**). The upfield shifts of the experimental values for **1** and **2** compared to the values, which were calculated for acyclic hydrocarbons using the additive scheme, result, most likely, from bond strain in norbornane (the energy is ~12 kJ mol^{-1})[38] and substantial steric interactions of the substituent R in the amine and deitiforin molecules.

The γ- and δ-effects

In the case of aliphatic hydrocarbons, the classical γ- and δ-effects are substantially weaker than the α- and β-effects.[36] In the case of alicyclic compounds in which some H atoms are involved in van der Walls interactions, the signals for the corresponding C atoms are shifted upfield due to polarization of the C—H bonds (steric compression). In this case, the γ-effect is of most importance for sterically strained structures.[36] The increments of the ^{13}C chemical shifts were analyzed for a wide range of norbornane derivatives.[39—41]

For all isomers of four compounds, including amine **1** and deitiforin (**2**) (see Table 3), the substituent R has insignificant effects on the C(4) (γ-effect) and C(5) (δ-effect) atoms, which is also typical of other 2-substituted norbornanes.[31] However, the γ-effect of this substituent on the C(6) and C(7) atoms is abnormally high.

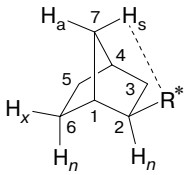
For specimens *2* of the amine and its hydrochloride, the changes in the chemical shifts of the C(7) atoms with respect to the norbornane core ($\Delta\delta^{13}C$ are −3.14 and −3.10, respectively) are substantially larger than the increments for the C(6) atoms ($\Delta\delta^{13}C$ are 0.32 and −0.42, respectively; see Table 3). Previously, it has been demonstrated that this is the distinguishing characteristic feature of the *exo* isomers of all 2-substituted norbornanes studied previously.[31,39] Based on this fact, we assigned the *exo* configuration to two diastereomers prevailing in specimen *2*. However, in the case of the *endo* isomers of amine **1** and hydrochloride **2**, the increments for the C(6) atom are, on the contrary, more significant ($\Delta\delta^{13}C$ are −7.50 are −7.74, respectively), the changes for C(7) being small ($\Delta\delta^{13}C$ are 1.16 and 0.70, respectively; see Table 3).

Apparently, the signals for the C(6) or C(7) atoms are shifted upfield due to the steric γ-*gauche* interaction

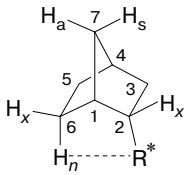
Table 4. Effect of the orientation of the substituent R on $\delta^{13}C$ for 2-R-bicyclo[2.2.1]heptane $\Delta\delta^{13}C = \delta^{13}C(endo) - \delta^{13}C(exo)$

R	Δδ							
	C(1)	C(2)	C(3)	C(4)	C(5)	C(6)	C(7)	C(8)
CH_3 [27]	−1.3	−2.2	0.5	0.9	0.3	−6.6	3.9	−4.9
CH_2OH [27]	−0.1	−2.3	−0.4	0.6	0	−6.5	4.7	−2.1
$CH(Me)NH_2$	−0.01	−2.33	−0.59	0.03	1.69	−7.82	4.30	−1.80
$CH(Me)NH_2 \cdot HCl$	0.02	−1.91	−0.68	0.33	1.81	−7.32	3.80	−1.18

of the substituent R with one of the H atoms at C(6) or C(7).



exo-R(2)—*syn*-H(7)



endo-R(2)—*endo*-H(6)

The shift of the signal for the C(7) atom in the *exo* isomer is smaller than that for the C(6) atom in the *endo* isomer (see Table 3), which suggests that the *exo*(2)—*syn*(7) interaction is weaker than the *endo*(2)—*endo*(6) interaction, *i.e.*, in the case of steric interactions, the substituent R is located more closely to the C(6) atom than to the C(7) atom.

For the C(4) atom, the γ-effect is, evidenlty, insignificant because its H atom is directed away from the substituent R*.

Exo and *endo* orientations of the substituent R

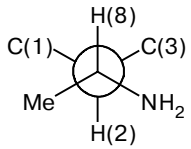
For the *exo* and *endo* isomers of amine **1** and deitiforin **2**, the differences in the chemical shifts $\Delta\delta^{13}C = \delta^{13}C(endo) - \delta^{13}C(exo)$ for the C(6) (−7.82 and −6.71) and C(7) atoms (4.30 and 3.80, see Table 4) are noticeably larger in magnitude than those for all other single-type C atoms. For four structurally similar compounds presented in Table 4, the observed differences for the C(2) atoms (for which the α-effect is most significant) in the *exo* and *endo* isomers are actually no larger than 2.3 ppm. For the C(1), C(3) (β-effect), and C(4) (γ-effect) atoms, the effect of the orientation of the substituent at position 2 is at most 1.3 ppm. Therefore, the differences in shielding of the C(6) (upfield) and C(7) (downfield) atoms due to nonbonded steric interactions, which are observed on going from the *exo* to *endo* diastereomers, are noticeably larger than the changes for the remaining C atoms and these differences are typical not only of 2-substituted norbornanes,[31] but also of compounds **1** and **2.**

For all compounds under consideration, the signals for the C atom of the substituent R adjacent to the bicycle (C(8)) are observed at somewhat higher field in the spectra of the *endo*-isomers compared to those of the *exo* isomers. For the CH_3 group containing three H atoms, this regularity is more pronounced and the difference between the chemical shifts $\Delta\delta^{13}C$ is −4.9 for the CH_3 group (see Table 4). The replacement of one H atom by the OH group leads to a decrease in this difference to −2.1 ppm, this difference being decreased to −1.37 and −1.18 when two H atoms are replaced.

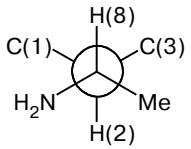
This fact is in agreement with the above-mentioned γ-effect of the substituent R exerted on the C(6) and C(7) atoms, which is manifested in the stronger nonbonded *endo*(2)—*endo*(6) contact in the *endo*-diastereomers of amine **1** and hydrochloride **2**. For the same reason, the resonance signal for the C(8) atom of the substituent R, which is involved in the interaction as the second member, is also more substantially shifted upfield in the spectra of the *endo*-diastereomers (see Table 2).

Proton resonance

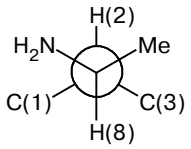
In amine **1**, three hindered conformers, which differ by the angle of rotation of the aminomethyl fragment about the C(8)—C(2) bond, can occur for each diastereomer.[10] According to the results of molecular-mechanics calculations (the MMX method), the total steric energy of one of these conformers is noticeably smaller than those of the remaining two conformers.* Below are shown the most stable conformers of each diastereomer.



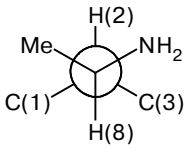
exo-(8*R*)



exo-(8*S*)



endo-(8*R*)



endo-(8*S*)

* The authors concluded[10] that the *S* diastereomeric forms of the amine are thermodynamically more stable both for the *exo* and *endo* isomers. Apparently, the authors meant the 8*S* configuration of the asymmetric $CHMeNH_2$ substituent for the (1*R*,2*R*,4*S*,8*S*) and (1*R*,2*S*,4*S*,8*S*) enantiomers of the *exo* and *endo* isomers, respectively. However, an analogous conclusion is also true for the (1*S*,2*S*,4*R*,8*R*) and (1*S*,2*R*,4*R*,8*R*) enantiomers for which the *R* confuguartion rather than the *S* configuration of the substituent at the C(2) atom is thermodynamically more stable.

In all cases, the H(2) and H(8) protons are in *trans* orientations. The most stable conformers of the (8*R*) and (8*S*) diastereomers differ only in that the NH_2 and Me groups actually change places. Apparently, this reasoning is also true for the symmetrically similar diastereomers of hydrochloride **2**.

Evidently, there is free rotation between three possible conformers in solution. According to the results of calculations,[10] these conformers should differ in occupancy, which introduces an additional uncertainty of the effect of the substituent R on shielding of the protons. Despite the uncertainty of the assignment of the signals for the *syn* and *anti* H(7) atoms in the diastereomers of the amine and deitiforin with the *endo* orientation of the substituent R, analysis of the chemical shifts made it possible to follow some regularities of their changes. Let us consider the particular ^1H chemical shifts.

H(1). The resonance of the bridgehead H(1) proton in two of four diastereomers of hydrochloride **2** is noticeably shifted downfield (δ 2.55 and 2.45) relative to the H(1) protons in other diastereomers for which the chemical shifts (δ 2.25—2.07, see Table 2) are close to that for nonsubstituted norbornane (δ 2.20).[42,43] This anomalous decrease in shielding observed in the spectra of two diastereomers of hydrochloride **2** is, most likely, due to the proximity of the positively charged ammonium N atom in the most favorable conformers.

Along with the electronic and magnetic effects, the positively charged NH_3^+ group, which substantially surpasses the NH_2 and CH_3 groups in polarity (σ_I = 0.60 as compared to 0.10 and 0 for the NH_2 and CH_3 groups, respectively) makes a purely electrostatic contribution to shielding of the H(1) atom.[43] Due to electrostatic forces, the NH_3^+ group distorts the charge cloud on the adjacent C—H bonds resulting in a decrease in the spherical symmetry of the electron distribution at the proton. In this case, the diamagnetic contribution to shielding decreases and the resonance is shifted downfield, which is observed for virtually all H atoms in molecules **2** (see below).

Based on this fact, we performed the assignment for the (8*R*/*S*) diastereomers of deitiforin **2**. Thus, the signal at δ 2.55 was assigned to the *exo* diastereomer in which the amino group is located in proximity to H(1), *i.e.*, the diastereomer has the (8*S*) configuration, whereas the signal at δ 2.45 was assigned to the *endo*-diastereomer with the (8*R*) configuration.

The assignment of the diastereomers of amine **1** was carried out using the same criterion. In the above-considered diastereomers of the amine, the observed decrease in shielding of the H(1) protons (δ 2.20 and 2.25) is less pronounced due to the absence of the positive charge on the N atom (see Table 2).

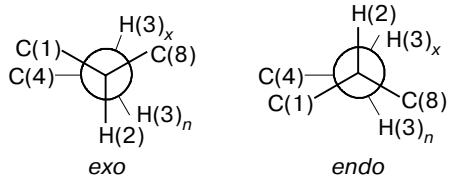
It should be noted that these arguments are consistent with the ^{13}C NMR spectra. In the above-considered two diastereomers, *viz.*, in *exo*-(8*S*) and *endo*-(8*R*), the signals for the C(1) atoms are observed at higher field (by δ ~1) compared to those in the spectra of the other two diastereomers both of the salt and the amine due to the γ-effect (see Table 1).

H(2) and H(3). The H(2) atom in the *exo* diastereomers of the amine has the *endo* orientation and its signal (δH 1.08 and 1.11) is observed at substantially higher field than the signal for the *exo*-oriented H(2) atom in the *endo* isomers (δH 1.50 and 1.48, see Table 2). These values are close to the signals for the H atoms in the *endo* and *exo* positions of nonsubstituted norbornane (δ 1.18 and 1.49, respectively).[43] In both cases, larger shielding of the *endo*-proton occurs due to anisotropy of the C(1)—C(6) bond. For an analogous reason, the shielding of the proton in the axial position in cyclohexane (in the case of slow inversion) is 0.5 ppm larger than that of the equatorial proton.[36] An analogous regularity was observed in the case of deitiforin; however, the higher electronegativity of the ammonium N atom leads to an approximately equal increase in the chemical shifts of the H(2) protons in the *exo*/*endo* positions (by 0.4—0.5 ppm; see Table 2).

The electronic effects of the substituent R on the chemical shifts of the more remote geminal H(3) protons through the covalent bonds are insignificant, and shielding of the latter is determined primarily by the anisotropic effect of the C(2)—C(8) bond depending on the *cis*,*trans* orientation in the eclipsed configuration of the C(2)—C(3) atoms. In accordance with the cone of magnetic anisotropy,[36] the presence of the C(2)—C(8) bond leads to an upfield shift of the signal for the *cis*-proton and to a downfield shift of the signal for the *trans*-proton.

For the *endo* diastereomers, the C(2)—C(8) and C(1)—C(6) bonds are located on the same side of the C(1)—C(2)—C(3)—C(4) plane. In this case, the anisotropic effects of these bonds exerted on shielding of the *exo*-,*endo*-H(3) protons are summed. The effect of the $CH(Me)NH_2$ substituent in the *endo* position is such that the high-field signal for the *endo*-proton is shifted upfield by −0.59 (8*R*) and −0.37 ppm (8*S*), whereas the low-field signal for the *exo*-proton is shifted downfield by 0.17 (8*R*) and 0.18 ppm (8*S*) (see Table 2). In this case, the differences in their shielding are increased to 1.07 (8*R*) and 0.96 ppm (8*S*) relative to nonsubstituted norbornane (1.49 − 1.18 = 0.31 ppm).[36]

In the case of the *exo* diastereomers, the C(2)—C(8) and C(1)—C(6) bonds are located on opposite sides of the C(1)—C(2)—C(3)—C(4) plane, and their effects on the geminal H(3) protons compensate each other. Since the atoms at the C(2)—C(3) bond in the *exo* and *endo* forms of the bicycle adopt an eclipsed conformation,



exo *endo*

the arrangement of the C(2)—C(8) bond relative to the geminal H(3) protons remains virtually unchanged and,

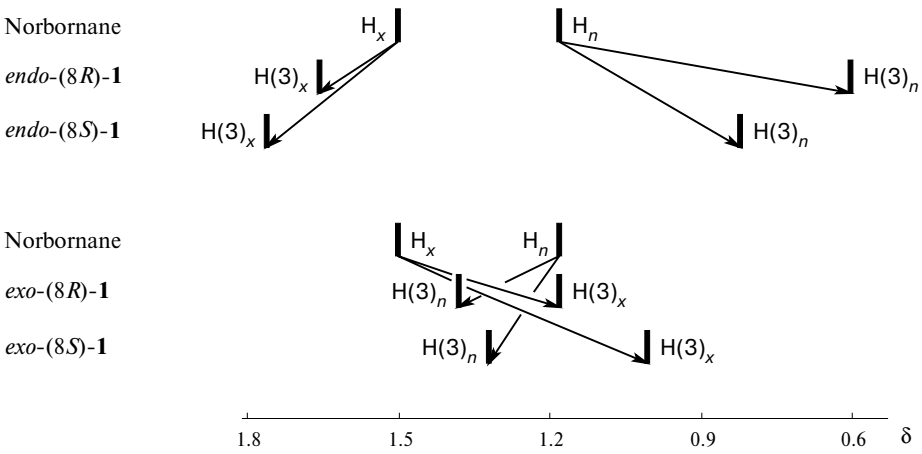


Fig. 1. Shielding of the H(3) protons.

consequently, the anisotropic effect of this bond on the H(3) protons in the *cis* and *trans* positions with respect to the substituent R is similar to that in the *endo* isomer.

Therefore, shielding of the *cis*-$H(3)_x$ and *trans*-$H(3)_n$ protons in the *exo* diastereomer of the amine is increased and decreased, respectively, *i.e.*, the low-field signal for the *exo*-$H(3)_x$ proton is shifted upfield by 0.18 (8*R*) and 0.13 ppm (8*S*), whereas the high-field signal for the *endo*-$H(3)_n$ proton is shifted downfield by −0.31 (8*R*) and −0.51 ppm (8*S*) (see Table 2). In this case, not only does the difference in their shielding decrease, but it also changes sign (−0.18 (8*R*) and 0.33 ppm (8*S*); Fig. 1).

Analogous regularities are also observed for the *exo* and *endo* diastereomers of deitiforin (**2**) (see Table 2).

H(7). Shielding of the H(7) proton in all *endo* diastereomers of **1** and **2** is analogous to that in nonsubstituted norbornane (δ 1.20)[42] and their differences are at most 0.09 ppm (see Table 2). However, in the spectra of the *exo* diastereomers in which the substituent R is located more closely to the bridge, the signals for the protons (*syn*-H(7)) are shifted downfield and the differences are increased to 0.20 ppm (see Table 2). Shielding of the $H(7)_s$ protons is decreased compared to that of the $H(7)_a$ protons due to the proximity of the substituent R.

Effect of the positive charge of N^+

An additional information on the structures of the diastereomers can be obtained from the analysis of the effect exerted by the ammonium N atom on the chemical shifts of the protons. On going from amine **1** to deitiforin (**2**), the signals for virtually all protons (including H(5) and H(6)) are shifted downfield to a greater or lesser extent. It should be noted that the ΔδH values for the H(2) atom at the β position (0.54 and 0.54 for the *exo* diastereomers; and 0.47 and 0.45 for the *endo* diastereomers) are larger than the corresponding values for the H(8) protons at the α position (0.43 and 0.43 for the *exo* diastereomers; and 0.38 and 0.36 for the *endo* diastereomers). This anomaly, *i.e.*, the larger changes for the more remote proton, may be due to the higher stability of the most favorable conformers (see the chart in the preceeding paragraph) in which the amino group is always located in the *cis* position with respect to the H(2) atom.

Since the effect of the positive charge in saturated systems is rapidly damped out as the distance to the charged center increases (see Ref. 36, p. 84), the change in shielding $\Delta\delta^1H$ for the remote γ-protons observed on going from amine **1** to its hydrochloride **2** occurs through space due to the field effect and is determined by the proximity of these protons to the N atom. For example, for two pairs of the diastereomers with the asymmetric (8*R*) center, the $\Delta\delta^1H$ value (0.35) for the H(1) proton located in proximity to the N atom in the *exo* form and $\Delta\delta^1H$ for the H(1) and $H(6)_n$ protons (0.20 and −0.16, respectively) in the *endo* form are substantially larger than the changes for the other H atoms, which have close contacts with the CH_3 group (δ 0.05 and −0.01 for $H(3)_x$ and $H(3)_n$, respectively) (Table 5). At the same time, the reverse situation is observed in the case of the (8*S*) diastereomers in which the amine and methyl groups formally change places: the changes, which are maximum in the above-considered case, become minimum and vice versa (see Table 5). It should be noted

Table 5. Effect of the positive charge of the N atom in hydrochloride **2** on the chemical shifts of selected protons

Diastereomer		$\Delta\delta^1H = \delta^1H(\mathbf{2}) - \delta^1H(\mathbf{1})$		
		H(1)	$H(3)_x$	
exo-(1*R*,2*R*,4*S*)	(8*R*) (N...$H(3)_x$)	0.08	0.21	
	(8*S*) (N...H(1))	0.35	0.05	
		H(1)	$H(6)_n$	$H(3)_n$
endo-(1*R*,2*S*,4*S*)	(8*R*) (N...H(1), $H(6)_n$)	0.20	−0.16	−0.01
	(8*S*) (N...$H(3)_n$)	−0.05	−0.06	0.18

that shielding of the remote $H(3)_x$ proton in the *endo* isomers, which are characterized by the maximum nonequivalence of the H(3) protons, remains unchanged in both diastereomers (δ 1.66 and 1.65 in (8*R*); and δ 1.77 and 1.75 in (8*S*)), *i.e.*, it is independent of the arrangement of the amine group.

For two *endo* diastereomers of amine **1**, the signals for the $H(3)_x$ (δ 1.66 and 1.77) and $H(3)_n$ (δ 0.59 and 0.81) protons are located separately from all other signals, which made it possible to determine the vicinal spin-spin coupling constants $^3J_{H(2)-H(3)n}$ = 5.3 Hz and $^3J_{H(2)-H(3)_x}$ = 11.6 Hz. The large values of the latter constant and the cross-peaks observed for the closely spaced H(2) and $H(3)_x$ protons in the ROESY experiment confirmed the *exo* position of the H(2) proton in the *endo*-(8*R*/*S*) diastereomers (see Scheme 2).

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