Comparison of Mass Spectral Fragmentation Patterns of Isomeric N-Substitued-2-methoxycarbonyl-9-azabicyclo[4.2.1]- and [3.3.1]nonane Skeletons

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Anatoxin-a, known as a potent toxin, is a naturally occurring alkaloid. It possesses unusual 9-azabicyclo [4.2.1] ring system.[1,2] However, as we reported previously,[3] in the course of its total synthesis, palladium-catalyzed aminocarbonylation of N-substituted cyclooct-4-enamines yielded a mixture of N-substituted 2-methoxycarbonyl-9-azabicyclo[4.2.1]- and [3.3.1]nonane isomeric intermediates at different ratios. The ratios were dependent on the nature of the N-substituent of unsaturated amine cyclization precursors. The accurate structural discrimination between the two isomers was thus prerequisite for the isolation of the desired [4.2.1] isomers in pure form.

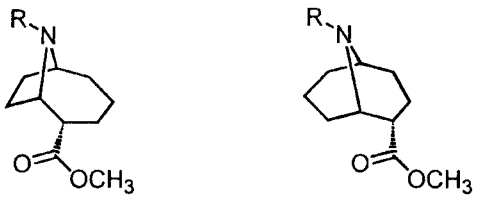
In the literature, nuclear resonance spectroscopy (NMR)[3-7] and mass spectrometry (MS)[8-10] have been employed for the structural identification of bicyclic compounds. Conformational analyses by NMR require very complicated homonuclear correlation experiment (COSY) or successive homonuclear decoupling experiments resorting to high field NMR instruments.[3-7] Therefore, a simpler identification method is desired for the rapid diagnosis in the routine works.

In contrast to the NMR methods, the electron impact (EI) MS technique was known to distinguish more readily between isomeric N-methyl-chloro-9-azabicyclo[4.2.1]- and [3.3.1]nonane skeletons by providing the accurate molecular weights and characteristic fragmentation patterns.[8-10] It was based on the presence of a prominent peak at m/z 82 corresponding to the five-membered N-methylpyrrolidine ring cation for the [4.2.1] isomer in its mass spectrum, while the mass spectrum of [3.3.1] isomer showed a characteristic peak at m/z 96 corresponding to six-membered N-methylpiperidine ring cation.

This work discusses our recent investigations on the mass spectrometric fragmentation patterns as a more definite distinction between the N-substituted 2-methoxycarbonyl-9-azabicyclo[4.2.1]- and [3.3.1]nonane isomers. Effects of four different N-substituents such as methoxycarbonyl, *p*-toluenesulfonyl, methanesulfonyl and benzyl groups on the patterns were examined.

Experimental

Synthesis and Chromatographic Separation. Eight N-substituted 2-methoxycarbonyl-9-azabicyclo[4.2.1]- and [3.3.1]nonane skeletons (Figure 1) were investigated in this study. They were obtained by intramolecular palladium-catalyzed aminocarbonylation reaction of four cyclooct-4-enamines substituted with methoxycarbonyl, benzyl, methanesulfonyl or *p*-toluenesulfonyl functions as the N-substituent, in the course of the total synthesis of anatoxin-a as reported earlier.[3] Each reaction mixture was subjected to isomeric separation by preparative high performance liquid chromatography (HPLC) in normal phase adsorption mode on a liquid chromatograph equipped with a Jasco Pu-986 pump (Kyoto, Japan) and a Waters 410 differential refractometer (Milford, MA, USA). A YMC-Pack Sil (Kyoto, Japan) was used as the silica column (25 cm × 20 mm I.D., 5 μm particle size) and hexane containing ethyl acetate (25% v/v) as the mobile phase at 8 mL min^{-1} in isocratic mode.



[4.2.1] [3.3.1]

R=CO_2CH_3 (I), $CH_2C_6H_5$ (II), SO_2CH_3 (III), $SO_2C_6H_4CH_3$ (IV)

Figure 1. Structures of 2-methoxycarbonyl-9-azabicyclononanes substituted with N-methoxycarbonly(I), N-benzyl(II), N-methanesulfonyl(III) and N-*p*-toluenesulfonyl(VI) functions.

NMR Spectroscopy. ^1H NMR and ^{13}C NMR spectra were acquired in $CDCl_3$ at 25 °C on a Varian Unity Inova 500 MHz spectrometer (Palo Alto, CA, USA).

Gas Chromatography-Mass Spectrometry. EI mass spectra were acquired on a Hewlett-Packard HP model 5890A series II gas chromatograph, interfaced to an HP 5970B mass spectrometer (70 eV, electron impact mode), which was on-line to an HP 59940A MS ChemStation. An Ultra-2 (SE-54 bonded phase) capillary column (25 m × 0.20 mm I.D., 0.11 μm film thickness) was used in the split injection mode (10 : 1). The inlet pressure of helium as the carrier gas was set to 82.7 kPa. The oven temperature was initially 150 °C for 2 min and then raised to 280 °C at 4 °C min^{-1} for N-methoxycarbonyl bicyclononanes while the initial temperatures were 220 °C for N-*p*-toluenesulfonyl bicyclononanes. For the N-methanesulfonyl and N-benzyl bicyclononanes, the initial temperatures were 200 °C. The injector and interface temperatures were 260 and 280 °C, respectively. The mass range scanned was 50-650 u at a rate of 0.99 scan s^{-1}.

Results and Discussion

Upon isomeric separation under the present HPLC conditions, each bicyclic ring isomer studied was obtained in pure form. And their structures were confirmed by high field NMR techniques as reported elsewhere.[3]

Each bicycle obtained in pure form was subjected to GC-

Table 1. Mass spectral data of N-substituted-2-methoxycarbonyl-9-azabicyclo[4.2.1]- and [3.3.1]nonanes

N-Substituent	Isomer	Mass (% relative abundance)								
		$[M]^+$	$[M-43]^+$	$[M-101]^+$	$[M-115]^+$	$[M-R]^+$	$[M-R-32]^+$	$[M-R-60]^+$	$[M-115-R+H]^+$	Other ion
CO_2CH_3	[4.2.1]	241(17)	198(1)	140(24)	126(100)	182(16)	150(5)	122(5)	68(18)	
	[3.3.1]	241(20)	198(17)	140(100)	126(5)	182(28)	150(3)	122(6)	68(10)	
$CH_2C_6H_5$	[4.2.1]	273(45)	230(2)	172(32)	158(45)	182(3)	150(5)	122(3)	68(9)	91(100)
	[3.3.1]	273(27)	230(7)	172(49)	158(4)	182(4)	150(3)	122(3)	68(4)	91(100)
SO_2CH_3	[4.2.1]	261(4)	218(ND*)	160(2)	146(61)	182(95)	150(100)	122(28)	68(97)	
	[3.3.1]	261(8)	218(6)	160(35)	146(ND*)	182(100)	150(14)	122(19)	68(15)	
$SO_2C_6H_4CH_3$	[4.2.1]	337(17)	294(1)	236(3)	222(37)	182(100)	150(45)	122(20)	68(56)	91(100)
	[3.3.1]	337(17)	294(10)	236(28)	222(ND*)	182(100)	150(8)	122(14)	68(8)	91(100)

*ND: not detected

MS analysis. The EIMS data are summarized in Table 1. As in rigid saturated bicyclic ring compounds, the molecular ion peaks were intense in all spectra of bicycles studied except for the two N-methanesulfonyl substituted isomers, which yielded weak but discernible molecular ion peaks.

Fragmentation of molecular ions involving cleavage of side chains only yielded fragment ion peaks that were not diagnostic in discriminating between [4.2.1] and [3.3.1] isomers except for the peak at m/z 150 in sulfonamides. $[M-31]^+$ and $[M-59]^+$ ions generated by the loss of methoxy and methoxycarbonyl functions from the side chain, respectively, were present in all spectra at low abundance. The commonly observed peaks at m/z 182 corresponding to $[M-R]^+$ ions are formed by the loss of the N-substituents that are bound to each nitrogen atom backbone. It constituted the base peaks for the sulfonamides. However, they were less abundant for the carbamates, indicating that S-N bond is much weaker compared to the C-N bond. This $[M-R]^+$ ions were much less intense (3-4%) for N-benzyl bicyclononanes because of the preferential benzylic cleavage, forming tropylium ions at m/z 91 as the base peaks in the spectra of benzylamine isomers. Tropylium ions were also very intense in the spectra of N-*p*-toluenesulfonyl bicyclononanes due to their benzenoid structure. $[M-R-32]^+$ ion at m/z 150 is most likely formed by the further loss of methoxy side function plus an additional hydrogen atom from $[M-R]^+$ fragment ion. It was barely observable for carbamates and benzyl amines as expected. However, it constituted the base peak for methanesulfonylated [4.2.1] isomer and very intense (45%) for the *p*-toluenesulfonylated [4.2.1] isomer. Very interestingly its abundance was much lower in the spectra of their corresponding [3.3.1] isomers of sulfonamides, thus permitting us to distinguish them from [4.2.1] isomers. Another common ion observed at m/z 122 is possibly formed by the consecutive loss of methoxycarbonyl function plus an additional hydrogen atom from $[M-R]^+$ ion. They were barely observable for the carbamates and benzyl amines like the m/z 150 ion, but intense for the sulfonamides due to the more favorable cleavage of the S-N bond

As is characteristic of azabicyclic compounds,[8] our [4.2.1] and [3.3.1] isomers showed very different ring cleavage patterns in the relative intensities of $[M-115]^+$, $[M-101]^+$

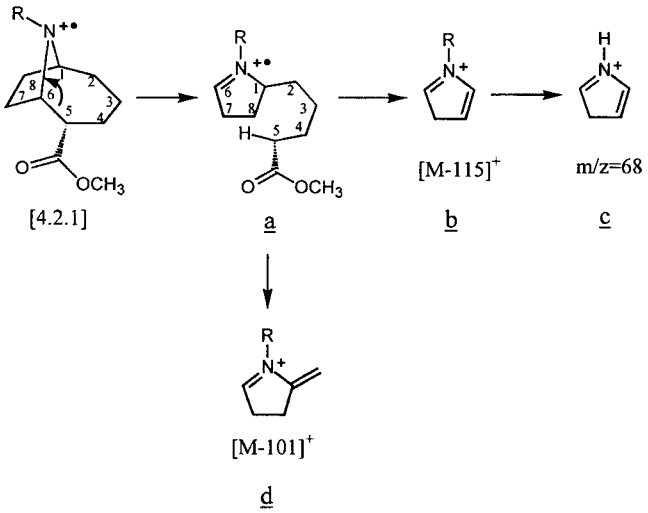


Figure 2. Fragmentation of N-substituted 2-methoxycarbonyl-9-azabicyclo[4.2.1]nonanes.

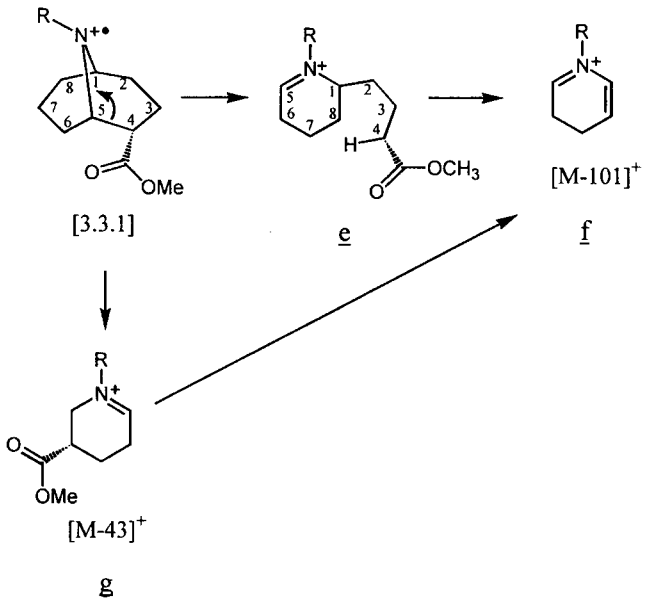


Figure 3. Fragmentation of N-substituted 2-methoxycarbonyl-9-azabicyclo[3.3.1]nonanes.

fragment ions. $[M-115]^+$ ions which are generally accepted as N-substituted five-membered pyrrolidine ring cations[10]

were very intense (37 to 100%) in all spectra of [4.2.1] isomers but trace or undetectable in spectra of their corresponding [3.3.1] isomers, thus readily distinguishing [4.2.1] isomers from [3.3.1] isomers. On the contrary all of the [3.3.1] isomers yielded characteristic $[M-101]^+$ ions of higher intensity, which correspond to the six-membered N-substituted piperidine ring cations.[10] However, they were present at comparatively lower abundance in the spectra of [4.2.1] isomers which were thus easily differentiated from [3.3.1] isomers.

Besides these characteristic fragment ions, m/z 68 ions were found to be more intense in the spectra of the [4.2.1] isomers than the [3.3.1] isomers, particularly in the spectra of sulfonamides. Another characteristic $[M-43]^+$ ions were observed in all spectra of [3.3.1] isomers, but were barely or not detectable in the spectra of the corresponding [4.2.1] isomers. $[M-43]^+$ ions and m/z 68 ions appear to be useful in further confirmation of the desired [4.2.1] isomers predistinguished from [3.3.1] isomers by comparing the abundances of $[M-115]^+$ and $[M-101]^+$ fragment ion peaks.

The formation of $[M-115]^+$ ions present only in [4.2.1] isomers may be explained by the favored cleavage of the C 5-C6 bond next to C-N bond, leading to five-membered intermediates **a** (Figure 2). Subsequently they decompose by cleavage of the C1-C2 linkage with migration of a hydrogen (C8) to the carboxyl oxygen, yielding the five-membered $[M-115]^+$ ions **b**. Further loss of R function from **b** accompanied by migration of a hydrogen atom to the ring may yield diagnostic ion at m/z 68 **c** which loses a hydrogen atom to form m/z 67 ion. Less abundant $[M-101]^+$ ions **d** may be formed by the less favored cleavage of C2-C 3 bond instead of C1-C2 bond.

Similar ring cleavage process (C4-C5 cleavage) for [3.3.1] isomers may yield six-membered intermediates **e** which decompose to form $[M-101]^+$ ions **f** (Figure 3). The characteristic $[M-43]^+$ fragment ions **g** occurring only in [3.3.1] isomers are assumed to be formed by loss of $CH_2CH_2CH_3$ from molecular ions through the cleavage of C 1-C8 and C5-C6 bonds with migration of a hydrogen atom from C4.

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Synthesis and Anion Binding Properties of Urea Derivatized *p-tert*-Butylcalix[6]arenes

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In comparison with the large variety of ligands which have been described for cation receptors,[1-3] the development of selective host molecules for anions is still in its infancy. The ligands for complexation of anions need to have comparatively large cavities, which have so far proved difficult to be synthesized. In addition, as the charge density of anions is low, the electrostatic forces with them are weaker than those with cations. Selective complexation of anions is more demanding than that of cations in the view of the higher free energies of solvation of anions and the frequently occurring pH dependency of anion complexation.[4,5] Anions have a wide variety of geometries[6] which have to be taken into account in the development of selective anion receptors.

Reinhoudt and co-workers have reported that a selective complexation of Cl^- over Br^- and I^- can be achieved by the neutral urea receptors derived from the lower rim of calix[4]arene[7] and that three urea groups at the lower rim of calix[6]arene are well suited for complexation of tricarboxylate.[8] Both systems complex anions exclusively through hydrogen bonding. The use of hydrogen bonding as sole interaction for the binding of anions implies that recognition is most pronounced in non-competitive solvents. The advantage of using hydrogen bond is that a hydrogen bond is highly directional in character. Correct orientation of the hydrogen bond donors and/or acceptors can provide selective anion recognition. The urea moiety is a powerful hydrogen bond donor as was recently shown by Hamilton *et al.*[9] in the complexation of dicarboxylate anion.

In order to develop the selective anion receptors, here we