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Impacts of Steric Compression, Protonation, and Intramolecular Hydrogen Bonding on the ^{15}N NMR Spectroscopy of Norditerpenoid Alkaloids and Their Piperidine-Ring Analogues

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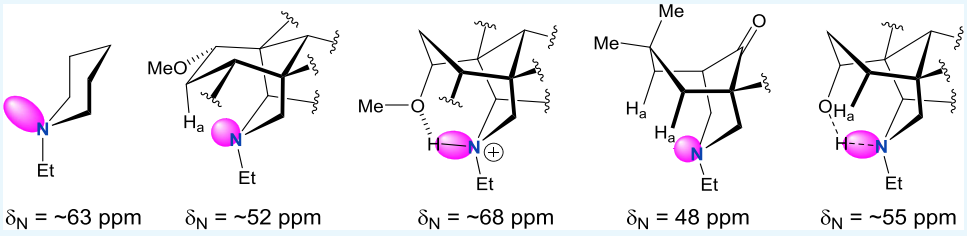

ABSTRACT: ^{1}H−^{15}N HMBC spectra of norditerpenoid alkaloids and their synthetic azabicyclic analogues were obtained to investigate the impacts of the through-space effect of steric compression, protonation, and formation of intramolecular hydrogen bonding on the ^{15}N NMR spectroscopy of these natural products and their piperidine-containing analogues. A rare ^{15}N NMR effect of steric compression is demonstrated in half-cage A/E-rings of norditerpenoid alkaloid free bases and their synthetic azabicyclic analogues, in which the distribution of the lone pair of electrons of the tertiary amine *N*-atom is sterically restricted by bridged cycloalkanes, e.g., cyclopentane, cyclohexane, and cycloheptane rings. This results in significant changes in the ^{15}N chemical shift, typically by at least ∼10 ppm. The lone pair of electrons of the *N*-atom in the piperidine ring are sterically compressed whether the bridged cyclohexane ring adopts a chair or boat conformation. The ^{15}N chemical shifts of 1α-OMe norditerpenoid alkaloid free bases significantly increase ($\Delta\delta_N \geq 15.6$ ppm) on alkaloid protonation and thence the formation of an intramolecular hydrogen bond between N^+-H and 1α-OMe. The intramolecular hydrogen bonds between the *N*-atom and 1α-OH of 1α-OH norditerpenoid alkaloid free bases, karacoline, condelphine, and neoline stabilize their A-rings, adopting an unusual twisted-boat conformation, and they also significantly increase δ_N of the tertiary amine *N*-atom.

INTRODUCTION

For nonisotopically enriched samples, the sensitivity of ^{15}N NMR is limited by the low natural abundance of ^{15}N nuclei (0.36%, $I = 1/2$) and its intrinsic low receptivity. Typically, compared with the ^{1}H signals in a sample, the signal-to-noise ratio will be of the order of 10^4 smaller. This essentially precludes direct measurement without recourse to extended acquisition times or extremely concentrated samples. These limitations can be overcome through judicious use of inverse-detected ^{15}N NMR experiments, e.g., ^{1}H−^{15}N heteronuclear multiple-bond correlation (^{1}H−^{15}N HMBC) spectroscopy.[1,2] Chemical shifts of ^{15}N nuclei (δ_N) in simple aliphatic amines, e.g., *N*-Et-piperidine (**1**), *N*-Me-piperidine (**2**), and N-H-piperidine (**3**), have been reported,[3−6] and steric and electronic effects of *N*α- and *N*β-substituents on δ_N have also been investigated.[6,7] A rare, indeed possibly the only case that has demonstrated the through-space ^{1}H NMR effect of steric compression caused by a secondary amine deshielding a proton that is close to the *N*-atom in space has been reported for imino[14]annulene (**4**, Figure 1).[8] We are investigating the effects of such a steric compression in the synthetic azabicycles (**5**−**12**) and their related norditerpenoid alkaloids (**13**−**21**)[9,10] and how such steric interactions impact the tertiary amine using ^{1}H−^{15}N HMBC spectroscopy to report on the environment of the *N*-atom.

RESULTS AND DISCUSSION

^{1}H−^{15}N HMBC spectroscopic experiments were carried out on three sample piperidines (**1**−**3**, Table 1), synthetic azabicycles (**5**−**12**, Table 2), and their related norditerpenoid alkaloids (Table 3), aconitine (**13**), mesaconitine (**14**), crassicauline A (**15**), lappaconitine (**16**), lycoctonine (**17**), methyllycaconitine (**18**, MLA), karacoline (**19**), condelphine (**20**), and neoline (**21**). In addition, several protonated forms of these compounds (**1′**−**3′**, **13′**, and **15′**−**17′**) have been studied. All of the ^{1}H−^{15}N HMBC spectra were externally calibrated with a $MeNO_2$

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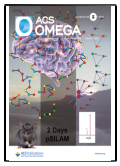

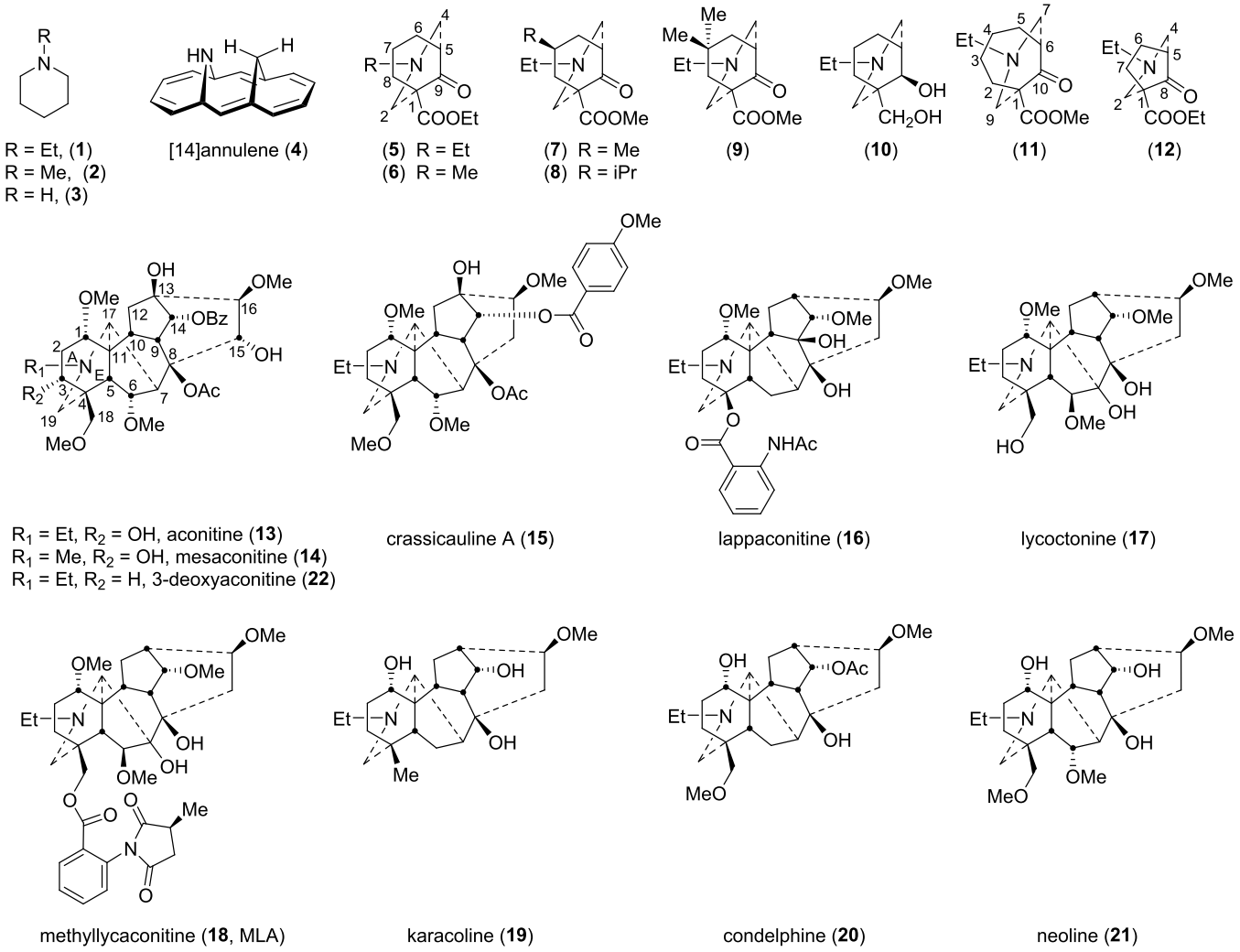

Figure 1. [14]Annulene (**4**), synthetic azabicycles (**5**−**12**), and norditerpenoid alkaloids (**13**−**22**).

Table 1. δ_N of Piperidines (δ in ppm)

Compound	Solvent	δ_N	Compound	Solvent	δ_N
N-Et piperidine (**1**)	$CDCl_3$	64.1	*N*-Me piperidine (**2**)	$CDCl_3$	51.3
	CD_3OD	63.0		D_2O[a], D_2O[a,b]	51.2
	d_6-DMSO	62.3	*N*-Me piperidine HCl salt (**2'**)	$CDCl_3$	57.8
	D_2O[a]	62.7		D_2O	54.7
	D_2O[a,b]	62.6	*N*-H piperidine (**3**)	$CDCl_3$	49.8
N-Et piperidine HCl salt (**1'**)	$CDCl_3$	65.5		D_2O	49.0
	CD_3OD	64.5		D_2O[b]	48.5
	d_6-DMSO	64.4	*N*-H piperidine HCl salt (**3'**)	$CDCl_3$[a]	53.9
	D_2O	64.3		D_2O	48.6

[a]With additional two drops of DMSO-d_6. [b]With additional drops of NaOD solution (30% in D_2O, w/w), pD ∼ 13.

solution (50% in $CDCl_3$, v/v) following the IUPAC guidelines.[11,12]

Steric Compression. In $CDCl_3$, synthetic A/E-[3.3.1]-azabicyclic free bases (**5**−**8** and **10**) and their relative 1α-OMe norditerpenoid alkaloid free bases (**13**−**18**) show the rare NMR effect of steric compression on 7-H_a (2-H_a) as the chemical shifts of 7-H_a (2-H_a) are larger than those of 7-H_e (2-H_e). The chemical shift of an axial proton (∼1.1 ppm) attached to cyclohexane is normally at a higher field than that of its geminal equatorial proton (1.6 ppm) by ∼0.5 ppm due to the magnetic

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Table 2. Key NMR Data of Synthetic [3.3.1]Azabicycles (δ in ppm)

Compound	Solvent	δ_N	$\Delta\delta_{7\text{-H}}$	δ (7-H)[a]	
				a (*endo*)	e (*exo*)
A/E-[3.3.1]azabicycle (**5**, *N*-Et)	$CDCl_3$	51.5	1.33	2.86	1.53
A/E-[3.3.1]azabicycle (**6**, *N*-Me)	$CDCl_3$	39.7	1.29	2.84	1.55
7-Me A/E-[3.3.1]azabicycle (**7**)	$CDCl_3$	52.0	-	3.42	-
7-iPr A/E-[3.3.1]azabicycle (**8**)	$CDCl_3$	52.0	-	3.02	-
7,7-diMe A/E-[3.3.1]azabicycle (**9**)	$CDCl_3$	47.7	-	-	-
A/E-[3.3.1]azabicyclic diol (**10**)	$CDCl_3$	53.6	1.11	2.59	1.48
	CD_3OD	53.2	1.16	2.58	1.42
	d_6-DMSO	53.7	1.18	2.47	1.29
	D_2O[b]	55.3	0.45	2.04	1.59
[4.3.1]azabicycle (**11**)	$CDCl_3$	50.9	$\Delta\delta_{3\text{-H}} = 0.68$	δ (3-$H_{a'}$) = 2.04	δ (3-$H_{e'}$) = 1.36
			$\Delta\delta_{4\text{-H}} = 0.45$	δ (4-$H_{a'}$) = 1.96	δ (4-$H_{e'}$) = 1.51
[3.2.1]azabicycle (**12**)	$CDCl_3$	50.4	$\Delta\delta_{6\text{-H}} = 0.00$	δ (6-$H_{a'}$) = δ (6-$H_{e'}$) = 1.95	
			$\Delta\delta_{7\text{-H}} = 0.13$	δ (7-$H_{e'}$) = 2.38	δ (7-$H_{a'}$) = 2.25

[a]Orientation label: a = axial, e = equatorial, a′ = pseudo-axial, and e′ = pseudo-equatorial. Chemical shifts of overlapping signals were extracted from HSQC. [b]With additional two drops of DMSO-d_6.

anisotropic effect,[13,14] and the reversed order of chemical shifts that are observed in [3.3.1]azabicyclic compounds (**5**−**8**, **10**, and **13**−**18**) is indicative of the electron cloud of 7-H_a (2-H_a) being repulsed by the lone pair of electrons of the tertiary amine *N*-atoms in such half-cage [3.3.1]azabicycles. Therefore, these 7-H_a (2-H_a) are deshielded and their chemical shifts increase.[15] Similar effects are displayed in [4.3.1]- and [3.2.1]azabicycles (**11** and **12**). The values of $\Delta\delta_{7\text{-H}}$ of synthetic [3.3.1]azabicyclic free bases (**5**−**8** and **10**, ≥1.11 ppm) are significantly larger than those of $\Delta\delta_{2\text{-H}}$ of natural alkaloid free bases (**13**−**18**, ≤0.37 ppm), as the synthetic [3.3.1]azabicycles (**5**−**8** and **10**) adopt true-chair/true-chair conformations, and the A/E-[3.3.1]-azabicycles of the natural norditerpenoid alkaloid free bases (**13**−**18**) are in twisted-chair/twisted-chair conformations due to through-space repulsion between the 12-$H_{e'}$/O-atom of 1α-OMe acting on the A-rings and 19-H_a/6α-$H_{e'}$ (6α-OMe) acting on the E-rings.[10,16]

It is notable that the chemical shifts δ_N for *N*-Et, *N*-Me, and N-H piperidine (**1**−**3**) occur at 64.1, 51.3, and 49.8 ppm (in $CDCl_3$, Table 1), respectively. Likewise, similar differences for δ_N between *N*-Et (**5**, **7**, **8**, and **10**) and *N*-Me (**6**) bicyclic piperidine analogues were obtained. Shifts of synthetic *N*-Et [3.3.1]azabicyclic free bases (**5**, **7**, **8**, and **10**) (in $CDCl_3$, Table 2) and *N*-Et 1α-OMe norditerpenoid alkaloid free bases (**13** and **15**−**18**) are found at ∼50 ppm (in $CDCl_3$, Table 3), and those of *N*-Me [3.3.1]azabicyclic free bases (**6**) and *N*-Me mesaconitine (**14**) resonate at ∼39 ppm (in $CDCl_3$). Thus, the *N*-alkyl substituents (Et, Me, H) sensitively influence δ_N in simple piperidine. In the half-cage molecules, the typical ^{15}N shift is reduced by ∼12 ppm when compared with such piperidines. This could be a result of the deformation in size and hybridization of the orbitals for the lone pair of electrons of the tertiary amine *N*-atoms.[3]

To investigate solvent effects on chemical shift, the ^{15}N shifts for *N*-Et piperidine (**1**) were recorded in $CDCl_3$, CD_3OD, DMSO-d_6, D_2O, and NaOD solutions (in D_2O, pD ∼13) resulting in a value of ∼63 ppm, thus showing negligible differences. Similarly, the ^{15}N shifts of diol (**10**) were obtained from solutions in $CDCl_3$, CD_3OD, DMSO-d_6, and D_2O and were typically ∼54 ppm. Therefore, the difference of δ_N between simple piperidines (**1**−**3**) and [3.3.1]azabicycles (**5**−**8**, **10**, and **13**−**18**) is not caused by solvent effects.

The distribution of the *N*-atom lone pair of electrons in piperidines (**1**−**3**) is not restricted by the steric hindrance caused by bridged cyclohexane rings (Figure 2a), but those in the half-cage [3.3.1]azabicycles adopting chair/chair conformations are restricted by 7-H_a (2-H_a) (Figures 2b,f); therefore, the distribution of the *N*-atom lone pair of electrons are concentrated around the *N*-atom increasing the electron density and decreasing the δ_N by ∼10 ppm.

Similar results of δ_N were observed in synthetic [4.3.1]- and [3.2.1]azabicycles (**11** and **12**), which resonate at 50.9 and 50.4 ppm (Table 2), respectively; these results show that the amine *N*-atoms are shielded by *endo* protons attached to C3 and C4 of [4.3.1]azabicycles (**11**, Figure 2c) and *endo* protons attached to C6 and C7 of [3.2.1]azabicycles (**12**, Figure 2d).

N-Et 7,7-diMe [3.3.1]azabicycle (**9**) adopts a boat/chair conformation, and its δ_N is smaller than those of *N*-Et [3.3.1]azabicycles (**5**, **7**, **8**, and **10**) which adopt chair/chair conformations. The lone pair of electrons of 7,7-diMe [3.3.1]azabicycle (**9**) are restricted by 6-H_a and 8-H_a (Figure 2e),[17] thus the electron density of the *N*-atom of 7,7-diMe [3.3.1]azabicycle (**9**) is more concentrated in comparison with those of [3.3.1]azabicycles (**5**, **7**, **8**, and **10**). The fact that such ^{15}N shift effects can be larger has been previously reported by Roberts and co-workers.[3] From our studies, we conclude that

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Table 3. Key NMR Data of the Selected Norditerpenoid Alkaloids (δ in ppm)

	Compound	Solvent	δ_N (amine)	$\Delta\delta_{2\text{-H}}$	δ (2-H)[a]	
					a (b)	e (f)
1α-OMe	aconitine (**13**)	$CDCl_3$	52.9	0.37	2.38	2.01
	aconitine HCl salt (**13'**)	D_2O[b]	70.3	0.63	2.32	1.69
	mesaconitine (**14**)	$CDCl_3$	38.8	0.17	2.31	2.14
	crassicauline A (**15**)	$CDCl_3$	50.7	0.34	2.29	1.95
	crassicauline A HCl salt (**15'**)	D_2O	67.0	0.33	1.98	1.65
	lappaconitine (**16**)	$CDCl_3$	52.3	0.12	2.23	2.11
	lappaconitine HBr salt (**16'**)	D_2O[b]	67.9	0.27	2.17	1.90
	lycoctonine (**17**)	$CDCl_3$	47.3	0.07	2.15[d]	2.08
	lycoctonine HCl salt (**17'**)	D_2O[b,c]	67.2	0.61	2.02	1.41
	MLA (**18**)	$CDCl_3$	46.9	0.08	2.10	2.02
1α-OH	karacoline (**19**)	$CDCl_3$	56.1	0.04	1.62, 1.58[e]	
	condelphine (**20**)	$CDCl_3$	54.2	0.04	1.60	1.56
	neoline (**21**)	$CDCl_3$	54.5	0.07	1.56	1.49
		$CDCl_3$[f]	54.5	0.07	1.56	1.49
		$CDCl_3$[g]	54.3	0.06	1.55	1.49

[a]Orientation label: a = axial, e = equatorial in a chair conformation, b = bowsprit, and f = flagpole in a boat conformation. Chemical shifts of overlapping signals were extracted from HSQC. [b]With additional two drops of DMSO-d_6. [c]With additional drops of DCl (35% in D_2O), pD ~2. [d]Chemical shift of this overlapping signal was extracted from COSY. [e]No evidence was obtained to identify the orientation of these protons unambiguously. [f]With additional two drops of pyridine-d_5. [g]With additional two drops of pyridine-d_5 and two drops of NaOD solution (30% in D_2O, w/w).

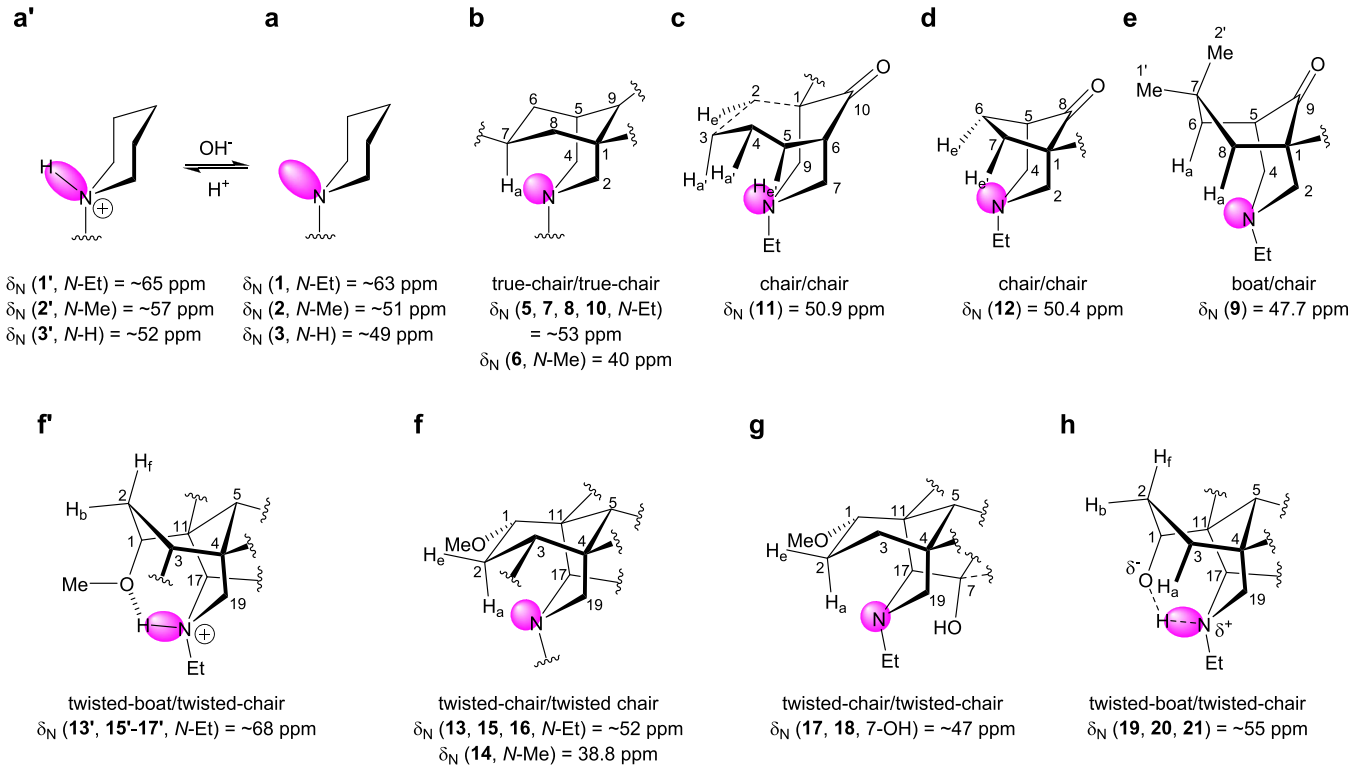

Figure 2. A/E-[3.3.1]azabicyclic frames of norditerpenoid alkaloids and their analogues. Pink: lone pair of electrons of the *N*-atom.

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the ^{15}N shift effects make them useful reporter nuclei of the substitution pattern. Indeed, a possible reason is a difference in size and hybridization of the orbitals for the unshared electrons in such tertiary amines,[4] and whether the substituents adopt axial or equatorial positions.[3]

Due to the proximity in space between 7-OH and the *N*-atom (Figure 2g), the amine δ_N of norditerpenoid alkaloid free bases with *N*-Et and 7-OH (**17** and **18**) resonate at ∼47 ppm, slightly lower than those of alkaloid free bases with *N*-Et and 7-H (**13**, **15**, and **16**), which resonate at ∼52 ppm.

Protonation. δ_N of *N*-Et piperidine (**1**) resonates at ∼63 ppm (in $CDCl_3$, CD_3OD, DMSO-d_6, and D_2O), showing only a small difference[4] from its HCl salt (**1′**) resonating at ∼65 ppm (in $CDCl_3$, CD_3OD, DMSO-d_6, and D_2O). δ_N of *N*-Et (**1**), *N*-Me (**2**), and N-H free bases (**3**) were also measured in NaOD solutions (in D_2O, pD ∼13), and these δ_N have no significant difference from those of their corresponding free bases (**1**−**3**) obtained in D_2O.

The A/E-[3.3.1]azabicyclic rings of protonated 1α-OMe norditerpenoid alkaloid salts (**13′** and **15′**−**17′**) adopt twisted-boat/twisted-chair conformations, which are stabilized by hydrogen bonds between N^+-H and 1α-OMe (Figure 2f′).[10,16] The δ_N of norditerpenoid alkaloid free bases (**13** and **15**−**17**; resonating at 52.9, 50.7, 52.3, and 47.3 ppm, respectively; in $CDCl_3$) are at a higher field than those of their protonated forms (**13′** and **15′**−**17′**; 70.3, 67.0, 67.9, and 67.2 ppm, respectively; in D_2O), and the δ_N of these natural products (**13** and **15**−**17**) increase by 17.4, 16.3, 15.6, and 19.9 ppm, respectively, on protonation. The $\Delta\delta_N$ (≥15.6 ppm) between norditerpenoid alkaloids (**13** and **15**−**17**) and their salts (**13′** and **15′**−**17′**) are further supported by the recently reported $\Delta\delta_N$ = 18.0 ppm between 3-deoxyaconitine (**22**) (δ_N = 40.7 ppm, in acetone-d_6) and its trifluoroacetate salt (δ_N = 58.7 ppm, in acetone-d_6).[18]

The space that the lone pair of electrons of the *N*-atoms in piperidines (**1**−**3**) occupy (Figure 2a) is similar to that of their HCl salts (**1′**−**3′**) (fixed in the N^+−H-bonds; Figure 2a′); hence, the δ_N of simple piperidines (**1**−**3**) show only a small change on protonation. The distribution of the lone pair of electrons of the *N*-atoms in norditerpenoid alkaloid free bases (**13** and **15**−**17**) are restricted by 2-H_a through space (Figure 2f), which is near the *N*-atoms, and these electrons are fixed by N^+−H-bonds and are away from the *N*-atom (Figure 2f′), leading to a decrease in the electron density when the alkaloids (**13** and **15**−**17**) are protonated.

Intramolecular Hydrogen Bonding. The A-ring of 1α-OH norditerpenoid alkaloid free bases adopts twisted-boat conformations[10] stabilized by H-bonds between tertiary amine *N*-atom and 1α-OH (Figure 2h).[19,20] Compared with δ_N (47.7 ppm, in $CDCl_3$) of 7,7-diMe [3.3.1]azabicycle (**9**), which adopts a boat/chair conformation, δ_N of 1α-OH norditerpenoid alkaloid free bases (**19**−**21**) are higher field, resonating at ∼55 ppm for all three. The lone pair of electrons of the *N*-atom in 7,7-diMe [3.3.1]azabicycle (**9**) are significantly compressed by 6-H_a and 8-H_a through space (Figure 2e). The lone pair of electrons of the *N*-atom in 1α-OH natural alkaloids (**19**−**21**) are shared and fixed by intramolecular H-bond to 1α-OH. Thus, the electrons are distributed away from the *N*-atom, leading to an increase of δ_N (Figure 2h), typically of 6−8 ppm compared with δ_N of boat/chair analogue (**9**). Neoline (**21**) in $CDCl_3$ was treated with additional pyridine-d_5 and NaOD solution (30% in D_2O, w/w) successively for cleaving the intramolecular H-bond. In comparison with those of neoline (**21**) in $CDCl_3$, no notable change was observed in the ^{1}H NMR and ^{1}H−^{15}N HMBC spectra of neoline (**21**) in basified $CDCl_3$, demonstrating that this intramolecular H-bond is stable, and it holds the A-ring in a twisted-boat conformation.

CONCLUSIONS

A rare ^{15}N NMR spectroscopic effect of steric compression has been demonstrated in the A/E-rings of several norditerpenoid alkaloid free bases and their synthetic azabicyclic analogues using ^{1}H−^{15}N HMBC spectroscopy. The distribution of the tertiary amine *N*-atom lone pair of electrons is restricted in the half-cage azabicycles; therefore, the electron density of the *N*-atom increases and its δ_N decreases. δ_N of norditerpenoid alkaloids bearing 1α-OMe significantly increase on protonation. The intramolecular hydrogen bonds between the *N*-atom and 1α-OH of 1α-OH norditerpenoid alkaloid free bases not only stabilize the A-rings, adopting twisted-boat conformation, but they also increase the δ_N of the tertiary amine *N*-atom. Thus, ^{1}H−^{15}N HMBC spectroscopy has been demonstrated to be an excellent reporter for the analysis of the electron density of substituted piperidine alkaloids. It is particularly useful for certain norditerpenoids with complex substitution patterns and half-cage skeleta.

EXPERIMENTAL SECTION

General Methods. *Chemicals and Materials.* Condelphine (98%) was donated by Carbosynth Ltd. (U.K.). Mesaconitine was extracted and then purified by sulfuric acid acid-base cycling from the ground roots of *Aconitum napellus*. After column chromatography to homogeneity, it was indistinguishable from a commercial sample (Sigma-Aldrich, U.K.). Aconitine (95%) and crassicauline A (98%) were purchased from Sigma-Aldrich (U.K.); lappaconitine hydrobromide (98%) and neoline (98%) were purchased from Carbosynth (U.K.); and lycoctonine (99%), methyllycaconitine perchlorate (99%), and karacoline (98%) were purchased from Latoxan (France). All other chemicals were purchased from Sigma-Aldrich (U.K.) and used as received.

Deuterated solvents, chloroform-*d* ($CDCl_3$), methanol-d_4 (CD_3OD), dimethyl sulfoxide-d_6 (DMSO-d_6), deuterium oxide (D_2O), pyridine-d_5, and NaOD solution (30% in D_2O, w/w) were used for NMR experiments (99.8% D atom, Cambridge Isotope Laboratories, USA). All other solvents were of HPLC grade, ≥99.9% purity (FisherScientific, U.K. and VWR, U.K.). Petroleum ether (FisherScientific, U.K.) specifically refers to the 40−60 °C distillate.

Instrumentation. ^{1}H NMR spectra were recorded on a Bruker Avance III (^{1}H Larmor precession frequency 500 MHz) spectrometer at 25 °C. Chemical shifts were expressed in parts per million (ppm) downfield shift from tetramethylsilane (TMS) or 3-(trimethylsilyl)propionic-2,2,3,3-d_4 acid sodium salt (TMSP) as internal or external standards, and residual (protio) solvent peaks were also used as internal standards if required. Chemical shifts (δ_H) were reported as position (accurate δ_H of overlapping signals were extracted from two-dimensional (2D) NMR spectra, e.g., HSQC, COSY, and NOESY), relative integral, multiplicity, and assignment. Multiplicity was abbreviated: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet; br = broad. Coupling constants (*J*) are line separations (absolute values expressed in hertz, Hz), rounded and rationalized to 0.1 Hz.

^{13}C NMR spectra were recorded with complete proton decoupling on a Bruker Avance III (^{13}C Larmor precession

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frequency 125 MHz) spectrometer at 25 °C as well as 2D NMR experiments including HSQC, HMBC, and H2BC. Chemical shifts are expressed in ppm downfield shift from TMS or TMSP as internal or external standards, and solvent peaks were also used as internal standards if required, and they were reported as position (δ_C), number of attached protons (CH_3, CH_2, CH, quat = quaternary), and assignment.

$^1H-^{15}N$ HMBC spectra were recorded on a Bruker Avance III (^{15}N Larmor precession frequency 51 MHz) spectrometer at 25 °C. The spectra were externally calibrated with a $MeNO_2$ solution (50% in $CDCl_3$, v/v), and chemical shifts (δ_N) are expressed in ppm downfield shift from TMS or TMSP as internal or external standards.

Positive-ion $[M + H]^+$ mode mass spectrometry was performed, on samples dissolved in methanol, on an Agilent ESI-Q-TOF mass spectrometer. High-resolution mass spectrometry (HR-MS) was within 5 ppm error unless otherwise stated.

A PerkinElmer 65 spectrum FT-IR spectrometer was used to obtain the IR spectra.

Removal of solvents by evaporation in the procedures specifically refers to the use of a Buchi R-114 rotary evaporator with warming samples to 40 °C on a Buchi B-480 water bath and in vacuo (50−500 millibar). Residual solvents were removed under high vacuum for ∼14 h and the NMR data of the products were recorded.

Chromatography. Flash chromatography was performed using silica gel 60A 35−70 μm (Fluorochem Ltd, U.K. and Sigma-Aldrich, U.K.) with the indicated solvents. Thin-layer chromatography (TLC) was performed using 0.2 mm thick precoated silica gel plates (Merck KGaA 60 F254). Compounds were visualized by ultraviolet radiation (UV, λ = 254 nm) and by staining with different reagent(s) including iodine vapor, potassium permanganate aq. solution (0.05 M), *p*-anisaldehyde solution (*p*-anisaldehyde: conc. aq. H_2SO_4/H_2O/acetic acid = 3:2:50:40, v/v), ninhydrin solution (0.2% w/v ninhydrin in EtOH), or Dragendorff's reagent: bismuth subnitrate (1.7 g), acetic acid (20 mL), H_2O (80 mL), and 50% w/v solution of potassium iodide in H_2O (100 mL) were mixed and stored as a stock solution. Stock solution (10 mL) and acetic acid (20 mL) were mixed and made up to 100 mL with H_2O to give Dragendorff's reagent.

Synthesis and Structural Evaluation. Details of preparation, ESI-MS and NMR spectroscopy (^1H, ^{13}C, COSY, HSQC, HMBC, NOESY), structural analysis, and crystallographic data were reported in our previous works.[9,10]

*Synthesis of Ethyl 3-Methyl-9-oxo-3-azabicyclo[3.3.1]-nonane-1-carboxylate (**6**).*[21] A solution of ethyl cyclohexanone-2-carboxylate (4.44 mmol, 0.748 mL, 95%), 2.2 equiv formaldehyde (9.768 mmol, 0.713 mL, 38% aq v/v), and 1.1 equiv of methylamine (4.88 mmol, 0.608 mL, 33% in EtOH) in EtOH (25 mL) was stirred at 40 °C for 2 days under an atmosphere of anhydrous nitrogen. After the solvents were removed by evaporation, the crude product was purified by chromatography over silica gel (12.5% EtOAc in petroleum ether) to yield the title compound (280 mg, 28%) as a yellow oil. R_f = 0.36 (12.5% EtOAc in petroleum ether). HR-ESI-MS: *m*/*z* calcd for $C_{12}H_{20}NO_3$: 226.1443, found: 226.1443 $[M + H]^+$ and *m*/*z* calcd for $C_{12}H_{19}NO_3Na$: 248.1263, found: 248.1262 $[M + Na]^+$. IR: ν_{max} (NaCl)/cm^{-1} 1733 (ester, C═O), 1717 (ketone, C═O). δ_H (500 MHz, $CDCl_3$): 1.26 (t, *J* = 7.1 Hz, 3H, OCH_2CH_3), 1.49−1.57 (m, 1H, 7-H_e), 2.00−2.09 (m, 1H, 6-H_e), 2.10−2.17 (m, 1H, 6-H_a), 2.15−2.29 (m, 1H, 8-H_e), 2.25 (s, 3H, NCH_3), 2.40−2.45 (m, 1H, 5-H_e), 2.50 (dddd, *J* = 14.2, 12.3, 6.3, 2.0 Hz, 1H, 8-H_a), 2.59 (dd, *J* = 11.2, 3.8 Hz, 1H, 4-H_a), 2.76−2.89 (m, 1H, 7-H_a), 2.96 (dd, *J* = 11.3, 1.9 Hz, 1H, 2-H_a), 3.04 (dt, *J* = 11.2, 2.3 Hz, 1H, 4-H_e), 3.11 (dd, *J* = 11.3, 2.2 Hz, 1H, 2-H_e), 4.19 (q, *J* = 7.1 Hz, 2H, OCH_2CH_3). δ_H (125 MHz, $CDCl_3$): 13.81 (OCH_2CH_3), 20.17 (C7), 34.01 (C6), 36.76 (C8), 44.76 (NCH_3), 47.06 (C5), 58.54 (C1), 60.90 (OCH_2CH_3), 62.27 (C4), 63.98 (C2), 170.88 (ester), 212.33 (C9).

*Preparation of Piperidinium HCl Salts (**1′**−**3′**) Crassicauline A HCl Salt (**15′**), and Lycoctonine HCl Salt (**17′**).* The free base (**15** or **17**, ∼20 mg) in MeOH (3 mL) was acidified with conc. aq. HCl (∼37%) to pH = 2; then, all of the solvents and HCl residue were removed by evaporation to afford the HCl salts as white solids.

Characterization data and related spectra (Figures S1−S121) are all presented in the Supporting Information.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c01648.

Characterization data and related spectra (Figures S1−S121) (PDF)

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Notes

The authors declare no competing financial interest.

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