# The amide protonation of (−)-N-benzoylcytisine in its perchlorate salts

Anna K. Przybył *, Maciej Kubicki, Marcin Hoffmann

*Faculty of Chemistry, A. Mickiewicz University in Poznań, Umultowska 89b, 61-614 Poznań, Poland*

## HIGHLIGHTS

- The new salts of (−)-N-benzoylcytisine were characterized by spectroscopic methods.
- The energy calculations for tautomeric forms of the salts is presented.
- The structures were established by crystallographic analysis.
- In crystal, the protonation is on oxygen atom at cyclic amide, not at benzoic moiety.
- N-benzoylcytisinium salts protonation is at the oxygen atom instead nitrogen atom.

## GRAPHICAL ABSTRACT
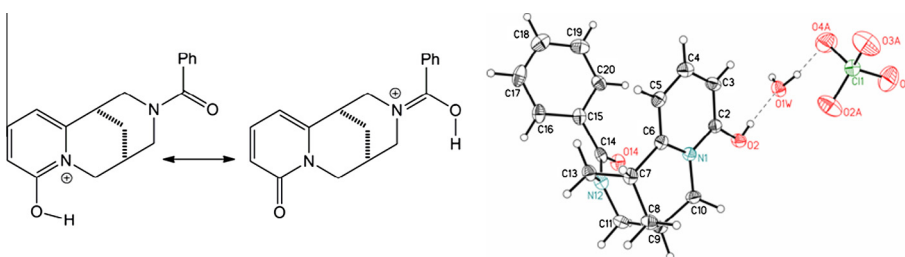
## ABSTRACT

The $^{13}$C NMR spectrum of (−)-N-benzoylcytisine perchlorate does not show a double set of signals typical of amide compounds, although this effect has been observed for the other diamine derivatives of cytisine. This observation means that in solution there must be the state of equilibrium between two forms of the cation with the protonated amide groups. DFT calculations have indeed indicated two preferred tautomeric forms with protonated oxygen atoms of amide groups. In the solid state however, according to X-ray analysis of perchlorate and perchlorate hydrate of N-benzoylcytisine the oxygen atom of the amide group in the six-membered ring A is preferred protonation site as compared with the oxygen in benzoic moiety. (−)-N-benzoylcytisine salt is the first compound from among the known derivatives of quinolizidine alkaloids that are not N-oxides, in which in solid state only the oxygen atom at cyclic amide is protonated instead of nitrogen atom or oxygen in benzoic moiety.

© 2014 Elsevier B.V. All rights reserved.

## Introduction

Amide bonds continue attracting the attention of the chemists, biologists etc. because of their profound importance in living systems; for instance, they determine the interactions of biologically active structures like peptides or proteins. Here we attempt to address the interesting aspect of protonation possibilities of a tertiary amide, taking as an example the certain cytisine derivative containing two such groups, one cyclic – embedded in a six-membered ring, and the other acyclic, connecting the aromatic substituent with the cytisine molecule.

(−)-Cytisine (**1**, Fig 1) is a naturally occurring quinolizidine alkaloid extracted from seeds of *Laburnum anagyroides* and other *Leguminosae* plants. (−)-Cytisine has been used as a smoking cessation aid (Tabex®), and is also a very promising compound for development of new drugs for potential treatment of the central nervous system disorders, particularly Alzheimer's and Parkinson's diseases. On the basis of the hitherto studies of certain cytisine derivatives it has been found that introduction of substituents modifying the molecular structure also changes the pharmacological properties of these compounds, that is the affinity and inner

* Corresponding author. Tel.: +48 618291004; fax: +48 618291555.
  E-mail address: annaprz@amu.edu.pl (A.K. Przybył).
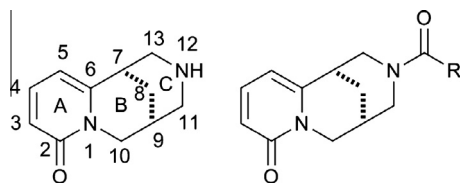
**Fig. 1.** Cytisine (**1**) and its derivatives: *N*-benzoylcytisine (**2**), where R = COC₆H₅; *N*-acetylcytisine (**3**), where R = COCH₃, and *N*-propionylcytisine (**4**), where R = COC₂H₅.

activity towards certain subtypes of nACh receptors and the affinity to ganglionic and centric receptors [1–3]. Moreover, among *N*-substituted cytisines some compounds with analgesic activities have been found [2–4]. It is generally accepted that biologically active compounds are usually polyfunctional derivatives whose protonation strongly depends on acid–base properties of individual functional groups, intra- and intermolecular interactions. The calculations in the gas phase and in water ($pK_a$ = 6,11 for cytisine) have shown – not surprising – that the oxygen is clearly a more basic site, which is consistent with the results of this study [5,6].

Upon protonation of quinolizidine and bisquinolizidine compounds their monosalts can be easily formed. The aim of this study is to explain the influence of the structure of *N*-cytisine derivatives (with additional proton-accepting groups) on the preferred protonation site. The explanation of the hierarchy of protonation sites in cytisine derivatives can help in understanding the mechanisms of binding these molecules to the nAChRs. Up to now, some of the *N*-substituted cytisines have been docked into a rat and human nAChR models based on the extramolecular domain of a molluscan acetylcholine binding protein and the results agreed with the binding data [7,8].

In this paper, we are presenting the results of the NMR spectroscopy, DFT calculations, and X-ray studies of the perchlorate salt of *N*-benzoylcytisine.

## Results and discussion

It is obvious that protonation of amides can take place at the oxygen or nitrogen atoms [6]. The calculation of molecular orbitals of strained amides and their *N*- and *O*-protonated forms reported by Greenberg indicated that the *N*-protonated form was favoured over the *O*-protonated one [10].

However, from the thermodynamical point of view the oxygen atom is much preferred position in this process [9,11]. Free,

unsubstituted cytisine contains three heteroatoms, but only two of them can be considered as potential basic sites: the oxygen (C2=O) in the pyridone ring (ring A, Fig. 1) and the nitrogen atom (N12) in the piperidine ring (ring C).

In (−)-*N*-benzoylcytisine (**2**, Fig. 1), free electron pairs of both nitrogen atoms N1 and N12 are strongly involved in the conjugated bond systems of lactams and amide groups, respectively [12,13]. Owing to this fact, the protonation at either of these atoms is highly improbable, and for this reason it was expected that the protonation will take place at either of oxygen atoms O2 or O14.

In our previous paper [13] we reported the chemical shifts of carbon atoms in the ¹³C NMR spectra of free bases (−)-*N*-acetylcytisine (**3**), (−)-*N*-propionylcytisine (**4**) and (−)-*N*-benzoylcytisine (**2**) in DMSO-d₆. These derivatives of cytisine occur in solution as mixtures of two conformers *cis* and *trans*, giving double sets of signals (both in ¹H NMR and ¹³C NMR).[13] The same difficulties to assign the chemical shifts have been encountered in the spectrum of the perchlorate salt of **2** taken in DMSO-d₆ at r.t. It was finally deemed necessary to measure the spectrum at the coalescence temperature (100 °C) [13]. In contrast to the spectra of free bases **2**, **3** and **4** and also to the spectra of the perchlorate salts of **3** and **4**, the ¹³C NMR spectrum of the protonated *N*-benzoylcytisine (**2·HClO₄**) measured in CD₃OD in r.t. reveals only one set of signals of carbon atoms (Table 1). Although, in the same conditions (r.t., CD₃OD) the ¹H NMR spectrum of **2·HClO₄** still shows not completely overlapped signals of the mixture of isomers, proving that the perchlorate salt of **2** in solution still occurs in a specific conformational equilibrium.

At the beginning, we supposed that the intermolecular hydrogen bond O—H⋯O with the protonated oxygen atom (O2 or O14) as a hydrogen-bond donor and solvent oxygen atom as an acceptor or vice versa, had blocked the rotation of the substituent at N12, so the ¹³C NMR spectrum of this salt showed only one set of signals. However, such situation has not been observed in other protonated cytisine derivatives **3** and **4** in which the small substituents (small steric hindrance) are not bulky enough to stiffen the structures of the molecules (Table 1). It should be mentioned that such situation was observed only in protic solvent – CD₃OD (Table 1), while in aprotic solvent – DMSO-d₆ a double set of signals was observed which suggests that the possibility of the solvent acting as hydrogen bond donor is more probable. Unfortunately, the NMR data did not bring a decisive answer as to the protonation site in *N*-benzoylcytisine (**2**). The signals of the lactam carbon atoms appeared as expected at low fields in the range 162–173 ppm, but insignificant changes in chemical shift (Δδ) values of C10, C11

**Table 1**
¹³C NMR chemical shifts [ppm] of *N*-benzoylcytisine (**2**) and the salts of **2** and *N*-acetylcytisine (**3**), *N*-propionylcytisine (**4**).

| At C | **1** CD₃OD | **1·HClO₄** CD₃OD | **2** [13] CD₃OD *trans/cis* | **2·HClO₄** CD₃OD | **3·HClO₄** CD₃OD *trans/cis* | **4·HClO₄** CD₃OD *trans/cis* |
|---|---|---|---|---|---|---|
| 2 | 165.9 | 165.9 | 165.4/165.3 | 165.1 | 163.5/163.1 | 162.9/162.5 |
| 3 | 116.8 | 118.5 | 117.4/117.2 | 116.8 | 114.8/115.6 | 116.5/117.1 |
| 4 | 141.3 | 142.2 | 141.5/141.8 | 142.2 | 144.6/144.9 | 145.5/145.7 |
| 5 | 108.2 | 110.1 | 108.6/109.1 | 109.8 | 114.5/113.9 | 113.9/113.3 |
| 6 | 153.1 | 148.3 | 150.8/151.0 | 151.3 | 153.0/153.5 | 153.7/154.2 |
| 7 | 36.5 | 32.9 | 36.4/ 36.0 | 36.4 | 36.5/35.9 | 36.4/35.9 |
| 8 | 26.7 | 24.1 | 26.4/26.5 | 26.5 | 20.7/21.2 | 25.8/25.7 |
| 9 | 28.9 | 26.4 | 29.1/29.3 | 29.2 | 28.8/28.9 | 28.8/28.7 |
| 10 | 51.1 | 50.8 | 49.6/50.2 | 50.9 | 52.6/52.5 | 53.5/52.95 |
| 11 | 53.1 | 49.8 | 49.1/50.3 | 49.9 | 49.4/48.5 | 49.6 [a] |
| 13 | 54.2 | 49.6 | 55.9/54.7 | 55.9 | 54.4/53.0 | 53.0/52.2 |
| 14 | | | 172.8/173.3 | 173.3 | 172.5/172.4 | 175.8/175.7 |
| 15 | | | – | – | 25.9/25.7 | 26.7/27.0 |
| 16 | | | – | – | – | 9.7/9.8 |
| 1′ | | | 136.1/136.5 | 136.2 | | |
| 2′/6′ | | | 129.4/130.0 | 131.1 | | |
| 3′/5′ | | | 127.4/127.6 | 127.5 | | |
| 4′ | | | 131.1/130.0 | 129.5 | | |

[a] Two overlapped signals.

and C13 were observed. The protonation of the nitrogen atom N1 or N12 should result in a shift of the corresponding signals (C6, C10, C11 and C13) towards lower field, while the protonation of the oxygen atom O2 or O14 should shift the signals assigned to C2 and C14 towards higher field.

As the NMR analysis did not bring an expected answer, we decided to use molecular modelling (DFT) and these data provided a very interesting result. DFT level of protonated cytisine confirms that the conjugation effect (O=C—N< and $^-$O—C=N<$^+$) strongly reduces the basicity of the pyridone nitrogen (ring A) and increases the basicity of the oxygen atom [6].

Therefore, the oxygen atom should be the favoured site of protonation in molecules with an amide group. It seems that this calculation can be applied to (−)-N-benzoylcytisine salt in which oxygen atoms (O2 and O14) are available to a proton.

For protonated N-benzoylcytisine the relative energies of the possible isomeric forms A–B (Fig. 2) of the studied molecular cation calculated at different basis sets and exchange correlation functionals [14] are presented in Table 2. The results for other structures are presented in Supplementary Information. The lowest energy structure is A in which proton is attached to the carbonyl oxygen atom (C2—O2), where it favours phenol-like tautomeric form. The next tautomer in the energetic sequence, higher in energy by ca. 10 kcal mol$^{-1}$, is the B structure in which proton is bound to the oxygen atom (O14) from the benzoic moiety. The tautomeric forms with protonated N atoms that show much higher energy than O-protonated tautomeric forms (20–40 kcal mol$^{-1}$, Supplementary Table 1).

In the crystal structures of both (−)-N-benzoylcytisine perchlorate ($\mathbf{2 \cdot HClO_4}$) its monohydrate ($\mathbf{2 \cdot HClO_4 \cdot H_2O}$) protonation undoubtedly took place on O2 atom (Figs. 3 and 4). The positions of the appropriate hydrogen atoms were determined, in either case, by (i) localization of the hydrogen atom in the difference Fourier map, (ii) successful refinement of this atom without any constraints and (iii), by the geometry of the neighbourhood, for instance C2—O2 bond lengths are 1.3156(18) Å in $\mathbf{2 \cdot HClO_4}$ and 1.299(2) Å in $\mathbf{2 \cdot HClO_4 \cdot H_2O}$, as compared with appropriate values for C14—O14 bonds, of 1.2467(17) Å and 1.248(2) Å.

Overall geometries of both cations 2 in the crystal structures are similar (Table 3), and close to the typical ones. The cytisine skeleton is stiff. The rings A are planar, rings B are close to the sofa conformation; and finally the rings C are almost ideal chairs close to the $D_{3d}$ symmetry.

In the crystals of $\mathbf{2 \cdot HClO_4}$ the cations are connected by O2—H···O14 intermolecular hydrogen bonds into infinite chains, and the cations and anions are connected by Coulombic interactions and weak C—H···O hydrogen bonds (Fig. 3), while in $\mathbf{2 \cdot HClO_4 \cdot H_2O}$ the water molecule acts as kind of a 'spacer': it accepts the O—H(cation)···O(water) hydrogen bonds and acts as a donor in O—H···O(anion) (Fig. 4) and O—H···O14 ones.
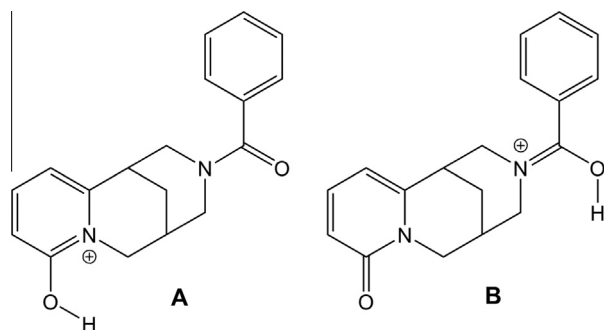


**Fig. 2.** Molecular structures of (−)-N-benzoylcytisine (**2**) with different protonation patterns. **A** – with protonated oxygen at amide group in ring A. **B** – with protonated oxygen at amide group at ring C.

**Table 2**
Relative energies of two tautomeric forms **A** and **B** of the molecular cation studied.

| Structure | Relative energy (kcal mol$^{-1}$) | | |
|---|---|---|---|
| | B3LYP/6-31G(d) | B3LYP/6-311++G(d,p) | PBE/6-311++G(d,p) |
| A | 0.0 | 0.0 | 0.0 |
| B | 9.6 | 11.4 | 11.4 |

The results of crystallographic analyses supplied the information about the preferred protonation sites in the solid state, that could not be transferred to the results of analyses in solution (NMR). However, similar result of amide protonation has been obtained in dutasteride hydrochloride salt with two secondary amide moieties. It was found that the favoured site of protonation depends on the resonance contribution and acceptor strength of amide oxygen [9] Although, for quinolizidine and bisquinolizidine alkaloids such a protonation in solid state has been observed for the first time. It has been only reported the N-oxide oxosparteine derivatives have an extremely strong proton-acceptor centre on oxygen atom, but – to the best of our knowledge – there are no published X-ray data of the salts of dioxo-bisquinolizidine alkaloids like 2,15-dioxosparteine or 2,17-dioxosparteine, that contain two amide moieties. Upon protonation of 15-oxosparteine-N1-oxide·HCl (**5**) and 17-oxosparteine-N1-oxide·HClO$_4$ (**6**) (Fig. 4) the N1$^+$—O—H group is formed and in the crystal structure the short intermolecular hydrogen bonds between the lactams and N1-oxygen atoms (N1$^+$—O1—H···O15 and N1$^+$—O1—H···O17, respectively) are formed. Such bonds (N16$^+$—O16—H···O2) have been also observed in the structures of 2-oxosparteine-N16-oxide HClO$_4$ (**7**) and 17-β-methyl-α-isolupanine-N16-oxide HClO$_4$ 0.5H$_2$O (**8**) (Fig. 5) [16–20].

## Experimental details

### Procedure

N-benzoylcytisine (**2**) was obtained according to literature [13]: **2** was dissolved in methanol and 60% HClO$_4$ solution in methanol were added to pH = 6.0. A yellow powder of perchlorate salt (**2**) was precipitated. Recrystalization from ethanol (yield 72%), m.p. 239 °C.; $^1$H NMR (300 MHz, MeOD-d$_6$, ppm); $\delta$ 8.07 (1H, dd, C4—H, J = 8.8; 7.4 Hz), phenyl ring: 7,54–7.42 (5H, m, C2′—H, C3′—H′, C4′—H, C—5′—H, C6′—H), 7.18 (1H dd, C3—H, J = 7.4; 0.8 Hz); 7.11 (1H, dd, C5—H, J = 8.9, 1.3 Hz); 8.81–3.53 (6H, m, C10—Hα, C10—Hβ, C11—Hα, C11—Hβ, C13—Hα, C13—Hβ); 3.33–3.30 (1H + CD$_3$OD, m, C7—H); 3.01 (1H, b.s., C9—H); 2.30–2.17 (2H, m, C8—Hα, C8—Hβ).

N-acetylcytisine (**3**) was obtained according to literature [13,21]: **3** was dissolved in methanol and 60% HClO$_4$ solution in methanol were added to pH = 6.0. A yellow powder of salt of **3** was precipitated. Recrys. from ethanol (yield 71%), m.p. 187–188 °C.

N-propionylcytisine (**4**) was obtained according to lit. [13,21]: **4** was dissolved in methanol and 60% HClO$_4$ solution in methanol were added to pH = 6.0. A yellow powder of the salt of **4** was precipitated. Recrys. from ethanol (yield 75%), m.p. 167–169 °C.

### NMR spectra

1D correlation spectra were recorded on a Bruker AVANCE 600 (600.31 MHz for 1H and 150.052 MHz for 13C) spectrometer, with a 5 mm triple – resonance inverse probe head (1H/31P/BB) with actively shielded z gradient coil (90_ 1H pulse width 90 ls, 13C pulse width 13.3 ls). 2D spectra were acquired and processed using standard Bruker software. Spectral width of 6313.13 and 25,000 Hz were used for 1H and 13C, respectively. Relaxation delays of 2.0 s
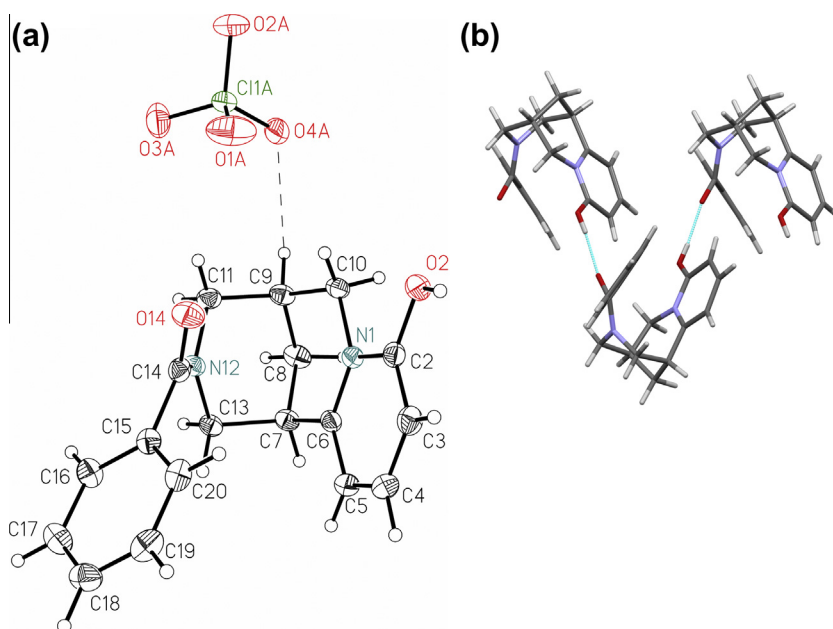
**Fig. 3.** (a) Perspective view of the (−)-N-benzoylcytisine perchlorate (2·HClO₄) as seen in the crystal structure [15]. The ellipsoids are drawn at 50% probability level, hydrogen atoms are shown as spheres of arbitrary radii; dashed line denotes the weak hydrogen bond. (b) A part of infinite chain of hydrogen-bonded cations.
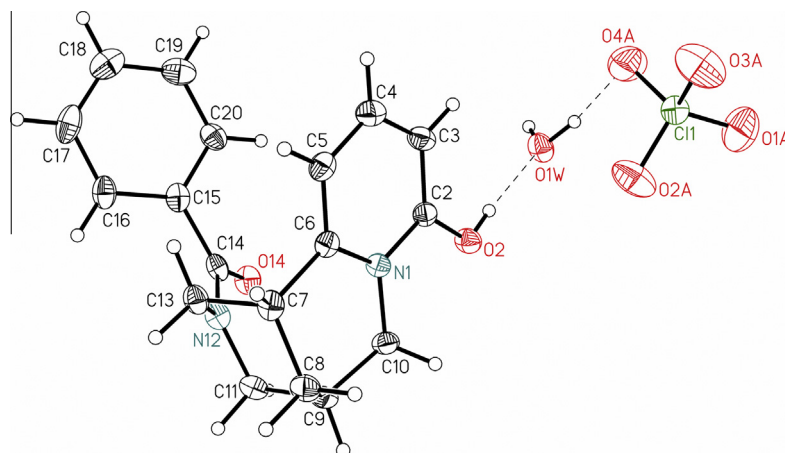


**Fig. 4.** Perspective view of the (−)-N-benzoylcytisine perchlorate hydrate (2·HClO₄·H₂O) as seen in the crystal structure [15]. The ellipsoids are drawn at 50% probability level, hydrogen atoms are shown as spheres of arbitrary radii; dashed lines denote the hydrogen.

**Table 3**
Selected geometrical parameters (Å, °) with esd's in parentheses.

|  | 2·HClO₄ | 2·HClO₄·H₂O |
| --- | --- | --- |
| N1—C2 | 1.3591(19) | 1.368(2) |
| N1—C6 | 1.3807(17) | 1.378(2) |
| N1—C10 | 1.4953(18) | 1.495(2) |
| C2—O2 | 1.3156(18) | 1.299(2) |
| C11—N12 | 1.4721(18) | 1.468(2) |
| N12—C13 | 1.4641(18) | 1.470(2) |
| N12—C14 | 1.3421(19) | 1.345(2) |
| C14—O14 | 1.2467(18) | 1.248(2) |
| C7—C13—N12—C14 | −108.37(15) | −111.89(19) |
| C9—C11—N12—C14 | 113.39(15) | 113.85(18) |
| C11—N12—C14—C15 | 167.98(12) | 176.79(16) |
| C13—N12—C14—C15 | −26.9(2) | −16.1(2) |
| C11—N12—C14—O14 | −9.3(2) | −2.5(3) |
| C13—N12—C14—O14 | 155.81(13) | 164.69(17) |
| N12—C14—C15—C16 | −50.0(2) | −50.2(2) |
| N12—C14—C15—C20 | 133.29(15) | 134.36(17) |
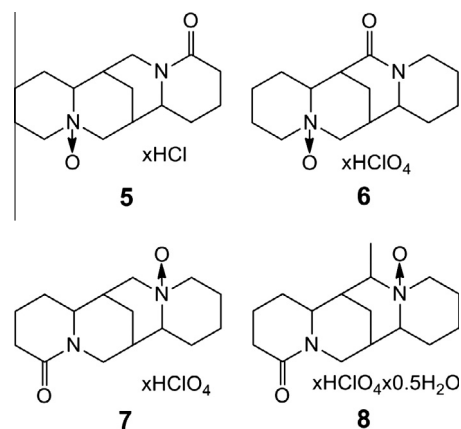


**Fig. 5.** The structure of 15-oxosparteine-N1-oxide·HCl (**5**), 17-oxosparteine-N1-oxide·HClO₄ (**6**), 2-oxosparteine-N16-oxide·HClO₄ (**7**) and 17-β-methyl-α-isolupanine-N16-oxide·HClO₄·0.5H₂O (**8**).

were used for all 2D experiments and the mixing time 0.8 s for 1H—1H NOESY spectrum was applied. All 2D spectra were collected with 2 K points in F2 and 256 increments (F1) with 4 (g-COSY) and 64 (g-HSQC) transients each and zero filling in F2 to 2048 _ 1024 data matrix.

### X-ray diffraction

Data were collected at 130($\mathbf{1}$) K by the $\omega$-scan technique on an Agilent Technologies Xcalibur four-circle diffractometer with Eos CCD detector and graphite-monochromated Mo K$\alpha$ radiation ($\lambda$ = 0.71069 Å). The data were corrected for Lorentz-polarization as well as for absorption effects. Precise unit-cell parameters were determined by a least-squares fit of 19,545 ($\mathbf{2 \cdot HClO_4}$) and 3249 ($\mathbf{2 \cdot HClO_4 \cdot H_2O}$) reflections of the highest intensity, chosen from the whole experiment. The calculations were mainly performed within the WinGX program system. The structures were solved with direct methods (SIR92) and refined with the full-matrix least-squares procedure on F$^2$ (SHELXL97). The scattering factors incorporated in SHELXL97 were used.

The function $\sum w(|F_o|^2 - |F_c|^2)^2$ was minimized, with $w^{-1} = [\sigma^2(F_o)^2 + (A.P)^2 + B.P]$ ($P = [Max\ (F_o^2,\ 0) + 2F_c^2]/3$). All non-hydrogen atoms were refined anisotropically, all hydrogen atoms in 1 were found in the difference Fourier map and isotropically refined; in 2 only OH hydrogens were treated in this way, all other were placed in idealized positions and refined as 'riding model' with isotropic displacement parameters.

Crystal data: $\mathbf{2 \cdot HClO_4}$: $C_{18}H_{19}N_2O_2^+ \cdot ClO_4^-$, $M_r$ = 394.80, orthorhombic, P2$_1$2$_1$2$_1$, $a$ = 7.8331(3) Å, $b$ = 14.6905(6) Å, $c$ = 15.6218(6) Å, $V$ = 1797.63(12) Å$^3$, $Z$ = 4, $d_x$ = 1.46 g cm$^{-3}$, $F(000)$ = 824, $\mu$ = 0.25 mm$^{-1}$, 36,002 reflections collected, 3904 unique ($R_{int}$ = 0.072), Final $R$ = 0.027, $wR_2$ = 0.071, $S$ = 0.95, $\Delta\rho$ in the final $\Delta F$ map 0.20/$-$0.21 e Å$^{-3}$. $\mathbf{2 \cdot HClO_4 \cdot H_2O}$: $C_{18}H_{19}N_2O_2^+ \cdot ClO_4^- \cdot H_2O$, $M_r$ = 412.82, monoclinic, P2$_1$, $a$ = 7.3699(7) Å, $b$ = 10.7915(8) Å, $c$ = 12.2632(11) Å, $\beta$ = 105.853(8), $V$ = 938.22(14) Å$^3$, $Z$ = 2, $d_x$ = 1.46 g cm$^{-3}$, F(000) = 432, $\mu$ = 0.25 mm$^{-1}$, 5645 reflections collected, 3568 unique ($R_{int}$ = 0.011), Final $R$ = 0.030, $wR_2$ = 0.083, $S$ = 1.08, $\Delta\rho$ in the final $\Delta F$ map 0.46/$-$0.36 e Å$^{-3}$.

### Computational methods

Initial tautomers of molecular cations with different protonation patterns were generated with the help of Marvin [14] and were subjected to full geometry optimization using the popular Becke [22] three parameter hybrid exchange and Lee–Yang–Parr correlation density functional (B3LYP) [23]. Full geometry optimization was performer at 6-31G(d) basis set followed by single point energy calculations at 6-311++G(d,p) basis set because it contains both polarized and diffusion functions focused on all atoms. Particularly, diffuse functions have a serious effect on the systems where electrons are relatively far from the nucleus including molecules with lone pairs [24]. The optimized structures were characterized by harmonic frequency calculations at B3LYP/6-31G(d) level of theory. The analytical harmonic vibrational wave numbers were positive values, confirming that the local minima on the potential energy surface had been found. Finally, to verify performance of B3LYP method calculations with PBE exchange correlation functional [25] were carried out. Complete optimized structures in the Cartesian coordinate format are available in Supplementary data. Electronic Supplementary Information (ESI) available: CCDC 862379 ($\mathbf{2}$) and 862378 ($\mathbf{2 \cdot H_2O}$) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif or e-mail: deposit@ccdc.cam.ac.uk See DOI:10.1039/b000000x/.

### Conclusions

In conclusion, we present many aspects of protonation possibilities in the perchlorate salt of N-benzoylcytisine, the cation with two tertiary amide moieties, one cyclic, in the six-membered ring A and the other acyclic with a benzoic group. The energy calculations for the isolated cations of (−)-N-benzoylcytisine (DFT level of theory) showed that the lowest energy tautomeric form of the cation had protonated oxygen atom in the six-membered cyclic ring (A), and this form is favoured over the tautomeric form with protonated oxygen atom in benzoic moiety (F) and both these O-protonated forms have lower energy the structures with protonated nitrogen atoms in amide moieties B–E. It turned out that we obtained two crystal forms containing this cation: (−)-N-benzoylcytisinium perchlorate ($\mathbf{2 \cdot HClO_4}$) and (−)-N-benzoylcytisinium perchlorate monohydrate ($\mathbf{2 \cdot HClO_4 \cdot H_2O}$). The X-ray data showed that also in the periodic conditions, in the crystal lattice, in both structures the protonation takes place at the O2 atom. This may indicate that the protonation pattern observed in the crystal structure results from energetic preferences of the isolated molecular cation rather than from the influence of the crystal lattice and intermolecular hydrogen bonds.

In solution the salt occurs in equilibrium between two forms and protonation can take place on oxygen atom, at either lactam or amide moieties. 1D and 2D NMR spectra (300 MHz and 600 MHz) of N-benzoylcytisine were analyzed and the chemical shifts have been assigned to the corresponding atoms of the cytisine derivatives salts ($\mathbf{2}$–$\mathbf{4}$). Surprisingly, to the best of our knowledge, N-benzoylcytisinium perchlorate ($\mathbf{2}$) is the first compound among the salts of quinolizidine alkaloids derivatives other than N-oxides, in which the protonation in the solid state has been observed at the oxygen atom but not at nitrogen atom. These observations of the protonation of amide moieties can help e.g. in the explanation of intermolecular interactions in binding affinity of cytisine derivatives to nACh receptors.

### Acknowledgements

### Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.saa.2014.02.192.

### References

[1] C.C. Boido, T. Vito, F. Sparatore, Farmaco 58 (2003) 265–277.
[2] Y.E. Slater, L.M. Houlihan, P.D. Maskell, R. Exley, I. Bermudez, R.J. Lukas, A.C. Valdivia, B.K. Cassels, Neuropharmacology 44 (2003) 503–515.
[3] T.S. Rao, L.D. Correa, R.T. Reid, G.K. Lloyd, Neuropharmacology 35 (1996) 393–405.
[4] T.W. Seale, S. Singh, G. Basmadjan, Neuroreport 9 (1998) 201–205.
[5] R.B. Barlow, L.J. McLeod, Br. J. Pharmacol. 35 (1969) 161–174.
[6] E.D. Raczyńska, M. Makowski, E. Górnicka, M. Darowska, Int. J. Mol. Sci. 6 (2005) 143–156.
[7] B. Tasso, C.C. Boido, E. Terranova, C. Gotti, L. Riganti, F. Clementi, R. Artali, G. Bombieri, F. Meneghetti, F. Sparatore, J. Med. Chem. 52 (2009) 4345–4357.
[8] J.A. Abin-Carriquiry, M.P. Zunini, B.K. Cassels, S. Wonnacott, F. Dajas, Bioorg. Med. Chem. Lett. 20 (2010) 3683–3687.
[9] J.B. Nanubolu, B. Srihar, K. Ravikumar, CrystEngComm 14 (2012) 2571–2578.
[10] a) A. Greenberg, D.T. Moore, T.D. DuBois, J. Am. Chem. Soc. 118 (1996) 8658–8668;

b) A. Greenberg, C.A. Venanzi, J. Am. Chem. Soc. 115 (1993) 6951–6957;
c) K. Tani, B.M. Stolz, Nature 441 (2006) 731–734;
d) Ch. Cox, H. Wack, T. Lectka, Angew. Chem. Int. Ed. 38 (1999) 798–800.

[11] a) A.J. Wiliams, J. Am. Chem. Soc. 98 (1976) 5645–5651;
b) A. Parkins, S.M. Harte, A.E. Goeta, C.C. Wilson, New J. Chem. 28 (2004) 718–721;
c) V.W. Day, A. Hossain, S.O. Kang, D. Powell, G. Lushington, K. Bowman-James, J. Am. Chem Soc. 129 (2007) 8692–8693.

[12] A.K. Przybył, Z. Nowakowska, Rapid Commun. Mass Spectrom. 25 (2011) 1193–1197.

[13] A.K. Przybył, M. Kubicki, J. Mol. Struct. 985 (2011) 157–166.

[14] <http://www.chemaxon.com/products/marvin/> accessed 10.05.12.

[15] G.M. Sheldrick, Acta Cryst. A64 (2008) 112–122.

[16] Wł. Boczoń, B. Kozioł, Pol. J. Chem. 74 (2000) 1249–1258.

[17] T. Borowiak, I. Wolska, J. Mol. Struct. 374 (1996) 97–109.

[18] A. Katrusiak, Pol. J. Chem. 68 (1994) 65–72.

[19] A. Katrusiak, Z. Kałuski, J. Crystallogr. Spectrosc. Res. 19 (1989) 193–201.

[20] A. Katrusiak, M. Ratajczak-Sitarz, Z. Kałuski, Acta Cryst. 43C (1987) 2386–2389.

[21] J. Rouden, A. Ragot, S. Gouault, D. Cahard, J.-C. Plaquevent, M.-C. Lasne, Tetrahedron: Asymmetry 13 (2002) 1299–1305.

[22] A.D. Becke, J. Chem. Phys. 98 (1993) 5648–5652.

[23] C. Lee, W. Yang, R.G. Parr, Phys. Rev. B 37 (1988) 785–789.

[24] M. Hoffmann, J. Rychlewski, J. Am. Chem. Soc. 123 (2001) 2308–2316.

[25] J.P. Perdew, K. Burke, M. Ernzerhof, Phys. Rev. Lett. 77 (1996) 3865–3868.