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

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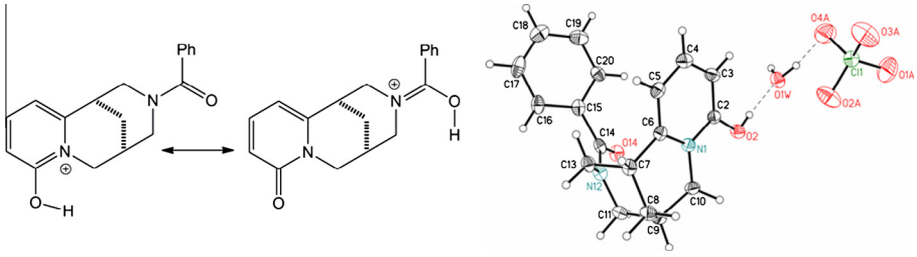
HIGHLIGHTS

- 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



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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.

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

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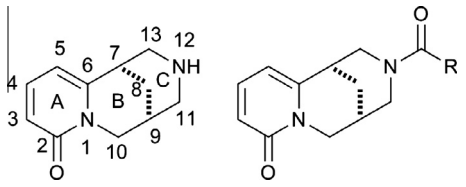

Fig. 1. Cytisine (**1**) and its derivatives: *N*-benzoylcytisine (**2**), where $R = COC_6H_5$; *N*-acetylcytisine (**3**), where $R = COCH_3$, and *N*-propionylcytisine (**4**), where $R = COC_2H_5$.

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 ^{13}C NMR spectra of free bases (–)-*N*-acetylcytisine (**3**), (–)-*N*-propionylcytisine (**4**) and (–)-*N*-benzoylcytisine (**2**) in DMSO-d_6. 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 ^{13}C 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-d_6 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 ^{13}C NMR spectrum of the protonated *N*-benzoylcytisine (**2**·**HClO$_4$**) measured in CD_3OD in r.t. reveals only one set of signals of carbon atoms (Table 1). Although, in the same conditions (r.t., CD_3OD) the ^1H NMR spectrum of **2**·**HClO$_4$** 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 ^{13}C 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 – CD_3OD (Table 1), while in aprotic solvent – DMSO-d_6 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 ($\Delta\delta$) values of C10, C11

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

At C	**1** CD_3OD	**1**·**HClO$_4$** CD_3OD	**2** [13] CD_3OD *trans/cis*	**2**·**HClO$_4$** CD_3OD	**3**·**HClO$_4$** CD_3OD *trans/cis*	**4**·**HClO$_4$** CD_3OD *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.