PCCP

RSC Publishing

PAPER

View Article Online
View Journal | View Issue

Cite this: *Phys. Chem. Chem. Phys.*, 2013, **15**, 9407

Intramolecular hydrogen-bonding activation in cysteines: a new effective radical scavenger†

Luisa Haya,[a] Iñaki Osante,[b] Ana M. Mainar,[a] Carlos Cativiela[b] and Jose S. Urieta*[a]

Received 18th February 2013,
Accepted 17th April 2013

DOI: 10.1039/c3cp50743b

www.rsc.org/pccp

The challenge of developing organic molecules with improved antioxidant activities for a competitive marketplace requires, given the great amount of possibilities, much laboratory work. Nowadays, the ability of methodologies based on quantum chemistry to determine the influence of different modifications on a molecule core provides a powerful tool for selecting the most useful derivatives to be synthesized. Here, we report the results of the assessment of antioxidant activity for quaternary amino acids, specifically for cysteine derivatives. The effect of introducing different substituents on the cysteine core is evaluated by using DFT to obtain an adequate structure–antioxidant activity relationship. This theoretical study shows a small panel of targets among which (*R*)-*N*-acetyl-2-methylcysteine methyl ester **15** exhibits special features and relevant antioxidant activity. The conformational ^{1}H NMR study of this synthesized compound indicates the existence of an intramolecular C_7 member ring involving S–H···O=C substructure, which is reported for the first time in the literature for this amino acid unit. This unusual conformation seems to be the reason for the high antioxidant capacity experimentally found for this compound.

Introduction

Antioxidant is a term applied in medicine and nutrition to substances that, in low concentration levels, reduce reactive oxygen species (ROS) or reactive nitrogen species (RNS) before they can oxidize other molecules. They also bind heavy metals and react with other potentially harmful substances. Owing to these properties, antioxidant activity is very important for delaying the aging process in biological systems, preserving cells from damage and preventing the oxidation of food and pharmaceutical products. There is a wide range of compounds on the marketplace, either natural or synthetic, that have more or less pronounced antioxidant activity[1–3] comprising several sulphur derivatives,[4,5] among which the thiol-type antioxidants are probably the most significant for their good electron donor ability,[6] and for their interactions, as nucleophiles, with electrophilic groups of ROS or RNS species.[7]

The most common thiol-type antioxidants[8] are cysteine, *N*-acetylcysteine (NAC) and derivatives[9] such as γ-glutathione (GSH, γ-L-glutamyl-cysteinyl-glycine).[10] The importance of their chemical and biological properties[11,12] makes advisable a rationalised research focused on the cysteine-core. As occurs with many other substances,[13] it can be expected that the modification of the original molecule of cysteine would yield even more multipotent antioxidants. However, there is such a large number of possible combinations of substituents in different positions of cysteine that a method for screening a subset of target products with enhanced antioxidant activity would be very useful in order to reduce the amount of time that the synthesis and testing of all of the possibilities would involve.

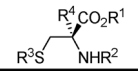
Therefore, in this paper a methodology based on quantum calculations is developed to assess "*a priori*" (*in silico*) the antioxidant activity of a set of modified cysteines. Four substitutions are considered, each corresponding to a different position in the core molecule of cysteine. The first focuses on the influence of a protecting group in carboxylic acid (R^1) dealing with either the free acid or the corresponding methyl ester. The second deals with the effect of introducing an acetyl protecting group into the amine (R^2). In the third one, the effect of replacing the hydrogen atom in the sulfhydryl group by a methyl group (R^3) is considered. Finally, an alkyl substituent which would presumably change the rigidity of the cysteine skeleton is introduced into the C^α-carbon (R^4). Table 1 shows

[a] *Group of Applied Thermodynamics and Surfaces (GATHERS), Aragon Institute for Engineering Research (I3A), Universidad de Zaragoza, C/Pedro Cerbuna 12, 50009, Zaragoza, Spain. E-mail: urieta@unizar.es; Fax: +34 976 761 202; Tel: +34 976 761 298*

[b] *Amino Acids and Peptides Group, Department of Organic Chemistry, Universidad de Zaragoza, C/Pedro Cerbuna 12, 50009, Zaragoza, Spain. E-mail: cativiela@unizar.es; Fax: +34 976 761 210; Tel: +34 976 761210*

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c3cp50743b

Table 1 Set of cysteines with different modifications considered in this work



No.	R^1	R^2	R^3	R^4
1	H	H	H	H
2	CH_3	H	H	H
3	H	$COCH_3$	H	H
4	H	H	CH_3	H
5	H	H	H	CH_3
6	CH_3	$COCH_3$	H	H
7	H	$COCH_3$	CH_3	H
8	H	H	CH_3	CH_3
9	CH_3	H	CH_3	H
10	H	$COCH_3$	H	CH_3
11	CH_3	H	H	CH_3
12	CH_3	$COCH_3$	CH_3	H
13	H	$COCH_3$	CH_3	CH_3
14	CH_3	H	CH_3	CH_3
15	CH_3	$COCH_3$	H	CH_3
16	CH_3	$COCH_3$	CH_3	CH_3

the complete array of modified cysteines studied in this work. The results pose the possibility of activating unusual intramolecular hydrogen bonding as a strategy to synthesize new effective radical scavengers from the cysteine core.

Computational methods

All the calculations reported in this work were performed using the Gaussian 09 program.[14] Gas-phase molecular geometries of all the cysteines in their neutral and radical cation forms were optimized using Density Functional Theory (DFT) with the hybrid B3LYP method and the 6-31+G(d,p) basis set. A frequency analysis was carried out to verify the nature of the minimum states of all molecules. Zero-point energies and thermal corrections together with entropies were used to convert internal energies to Gibbs energies at 298.15 K. Finally, in order to obtain consistent energy calculations, single point calculations were performed at the M06-2X/6-31+G(d,p) level of the B3LYP/6-31+G(d,p). The effect of different solvents was calculated using the polarizable continuum solvation model (PCM).

Redox potentials for modified cysteines were calculated using a thermodynamic cycle,[15–17] *via* eqn (1).

$$\Delta G_{(\mathrm{soln})}^{\circ} = \Delta G_{(\mathrm{g})}^{\circ} + \Delta\Delta G_{(\mathrm{solv})}^{\circ} \quad (1)$$

The Gibbs energy of the one electron oxidation reaction in the gas-phase, $\Delta G_{(\mathrm{g})}^{\circ}$, and the Gibbs energies of solvation, $\Delta G_{(\mathrm{solv})}^{\circ}$, for neutral and radical cation species were calculated following the procedure described above. The resulting value of the Gibbs energy in solution, $\Delta G_{(\mathrm{soln})}^{\circ}$, is related to the absolute formal redox potential, E_{abs}, according to eqn (2).

$$E_{\mathrm{abs}} = \Delta G_{(\mathrm{soln})}^{\circ}/nF \quad (2)$$

where n is the number of electrons transferred and F is the Faraday constant.

The ionization energies (IE) of the modified cysteines were computed according to the difference in the Gibbs energy state between cation radicals and neutral species, and from the HOMO energy applying Koopmans' theorem.[18]

Experimental section

The radical scavenging activities of cysteines were evaluated using DPPH (1,1-diphenyl-2-picrilhydrazyl) radical assays, following the method described by Brand-Williams *et al.*[19] with several modifications. Solutions with different concentrations of Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) in methanol (42 μl) were added to 2.5 ml of a 0.03 g l^{-1} DPPH solution in methanol. The decrease in absorbance of the DPPH radical at 514 nm was measured under agitation in a thermostated cell at 25 °C for two hours using a Thermo Scientific evolution 300 UV-Vis spectrophotometer. The exact initial and final DPPH concentrations in the reaction medium were calculated from a calibration curve. The results are reported as EC_{50}, that is, the amount of antioxidant necessary to reduce the DPPH concentration to 50%. The EC_{50} value obtained for Trolox was 0.23 ± 0.02 mol_{Trolox}/mol_{DPPH}.[20]

The EC_{50} for cysteines was determined from the Trolox EC_{50} value. A cysteine solution in water (42 μl) was added to 2.5 ml of a DPPH solution of 0.03 g l^{-1} in methanol. The decrease in absorbance was measured under the same working conditions as those used for Trolox. The difference between the initial and final DPPH concentrations was normalized to the cysteine concentration in the reaction mixture. The EC_{50} value of the cysteines was obtained by extrapolation of the normalized concentration to the Trolox EC_{50}. This method was tested with pure cysteine **1**, a compound whose antioxidant ability is commonly measured using the DPPH method.[21–23]

The procedures for the synthesis, isolation and spectroscopic characterization of compounds **2**, **5**, **6** and **15** are described in the ESI.†

Results and discussion

In silico prediction of antioxidant activity

In general, to reliably assess the antioxidant activity of a molecule it would be extremely advantageous to establish a relationship between that activity and one or several fundamental parameters of the molecule that could be calculated or experimentally determined. As the main factor governing the antioxidant activity of a molecule is its ability to transfer electrons, several properties can be chosen that, in principle, are intimately related to the ease with which an electron is released. Following previous studies on redox processes,[15] the HOMO energy of the molecule as well as the formal redox potential, and the first ionization energy for the formation of the most stable radical cation (on the sulphur atom) have been selected. Then, the existence of a correlation between the properties and a suitable experimental parameter accounting for the antioxidant activity needs to be checked to prove the usefulness of the property for gauging the activity. The experimental parameter chosen as an indicator of the reducing character is the EC_{50} determined by means of the DPPH radical assay.

Table 2 Experimental EC_{50} and calculated absolute redox potentials (E_{abs}), ionization potentials (IE) and HOMO energies for several cysteines

No	EC_{50}[a] (mol_{Cys}/mol_{DPPH})	E_{abs} (V)	IE (eV)	$-E_{HOMO}$ (eV)
1	0.42[b]	6.79	8.81	8.36
2	0.38	6.71	8.67	8.27
3	0.38[c]	6.69	8.45	8.26
5	0.39	6.69	8.65	8.33
6	0.36	6.67	8.33	8.18

[a] $n = 3$, $s = \pm 0.02$ where n and s are the number of replicates and the standard deviation, respectively. [b] Bibliographic EC_{50} (**1**) = 0.40 ± 0.02 mol_{NAC}/mol_{DPPH}. [c] EC_{50} (**3**) = 0.32 ± 0.02 mol_{CYS}/mol_{DPPH}.

Five cysteines that are commercially available or that can be easily synthesized (compounds **1**, **2**, **3**, **5** and **6**) were chosen to check the procedure. DFT calculations were used to obtain either directly HOMO energy or indirectly the absolute formal redox potential and ionization energy. The EC_{50} of the cysteines was also measured. The obtained results are gathered in Table 2.

Fig. 1 shows that there is a quite good linear correlation between the EC_{50} and each fundamental molecular property of the cysteines. Thus, any of the properties could be used to gauge the antioxidant activity. These results also indicate that the molecular properties are related to each other, as would be expected. In fact, a good correlation had already been obtained between the formal redox potential and the HOMO energy for a set of carbamates.[15] This is important because, although the formal redox potential is likely to be the more rigorous indicator from the theoretical point of view, the HOMO energy entails less computational cost allowing us, nevertheless, to estimate EC_{50} values with a very reasonable accuracy.

Once the correlation between HOMO energy and EC_{50} was established, HOMO energies (as well as the absolute redox potentials and ionization energies) were calculated and the EC_{50} estimated for all the remaining cysteine derivatives is included in Table 3. All of the derivatives exhibit better antioxidant activity

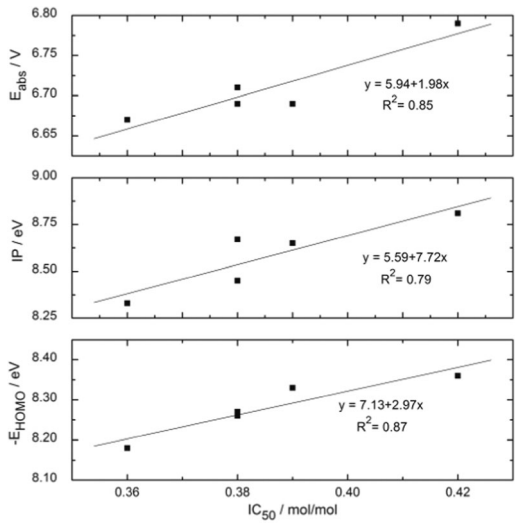


Fig. 1 EC_{50} *vs.* absolute redox potential, ionization potential and HOMO energy, respectively.

Table 3 Calculated absolute redox potentials in methanol (E_{abs}), ionization potentials (IE) and HOMO energies of several cysteines, together with their estimated EC_{50} from E_{HOMO}, E_{abs} and IE regressions

No	$-E_{HOMO}$ (eV)	EC_{50}[a] (mol_{Cys}/mol_{DPPH})	E_{abs} (V)	EC_{50}[b] (mol_{Cys}/mol_{DPPH})	IE (eV)	EC_{50}[c] (mol_{Cys}/mol_{DPPH})
1	8.36	0.41	6.79	0.43	8.81	0.42
2	8.27	0.38	6.71	0.39	8.67	0.40
3	8.26	0.38	6.69	0.38	8.45	0.37
4	7.88	0.25	6.27	0.17	8.19	0.34
5	8.33	0.40	6.69	0.38	8.65	0.40
6	8.18	0.35	6.67	0.37	8.33	0.35
7	7.91	0.26	6.27	0.17	7.91	0.30
8	7.86	0.25	6.37	0.22	8.25	0.34
9	7.81	0.23	6.27	0.17	8.07	0.32
10	7.87	0.25	6.50	0.28	8.26	0.35
11	8.23	0.37	6.66	0.36	8.52	0.38
12	7.88	0.25	6.57	0.32	8.43	0.37
13	8.08	0.32	6.21	0.14	7.86	0.29
14	7.78	0.22	6.39	0.23	8.13	0.33
15	7.78	0.22	6.45	0.26	8.16	0.33
16	8.01	0.30	6.18	0.12	7.78	0.28

[a] Estimated EC_{50} from $-E_{HOMO}$. [b] Estimated EC_{50} from E_{abs}. [c] Estimated EC_{50} from IE.

than pure cysteine but the improvement is remarkable in several compounds. These are *S*-methyl-L-cysteine **4**, *N*-acetyl-*S*-methyl-L-cysteine **7**, (*R*)-*S*-methyl-2-methylcysteine **8**, (*R*)-*S*-methyl-L-cysteine methyl ester **9**, (*R*)-*N*-acetyl-2-methylcysteine **10**, *N*-acetyl-*S*-methyl-L-cysteine methyl ester **12**, (*R*)-*N*-acetyl-*S*-methyl-2-methylcysteine **13**, (*R*)-*S*-methyl-2-methylcysteine methyl ester **14**, (*R*)-*N*-acetyl-2-methylcysteine methyl ester **15** (NAMCME) and (*R*)-*N*-acetyl-*S*-methyl-2-methylcysteine methyl ester **16**. Eight out of ten compounds are *S*-Me-type and the remaining two are thiol-type compounds.

It should be indicated that the more stabilized the radical cation the better are the EC_{50} values. This is to be expected. As is well known, antioxidant activity and the mechanism of the oxidation process are intimately related to the active centre. Therefore, in the scavenging process *S*-methyl and thiol-type compounds yield different types of products through different pathways. Mechanistically, the oxidation process of thiol compounds yields a thiyl radical that finally leads to the formation of a disulfide bond.[24] However, *in vitro* antioxidant activity of *S*-methylcysteines implies that the molecules can undergo, depending of the ROS, either one or two-electron oxidations to give, respectively, radical cations or sulfoxides.[25] However, despite these differences, the processes share a common key step, which is the base of our theoretical methodology: the loss of a first electron to yield a high unstable radical cation. In this way, an effective modification on the cysteine core must imply the radical cation stabilization. For this reason any electro-auxiliary group, *e.g.* a methyl group, attached to the sulphur atom will promote a logical enhancement of the antioxidant activity. Then, most of the cysteines with the best antioxidant activity have the sulphur atom protected, and their activity is due to the σ-electron density of the methyl group directly attached to the sulphur atom. But this line of argument makes noticeable the high antioxidant activity predicted for non

Table 4 Geometrical parameters for C_7 and C_5-membered ring hydrogen bonds (**10** and **15**) and non-intramolecular hydrogen bonds (**3** and **6**) at B3LYP/6-31+g(d,p)

No	Solvent	d(S–H···O=C)[a]	Φ	ψ	χ_1	d(N–H···O=C)[a]
10	Gas-phase	2.092	−178.0	177.8	−95.7	2.085
	Heptane	2.080	−177.7	177.7	−94.9	2.085
	Methanol	2.053	−176.4	177.0	−92.5	2.081
	DMSO	2.052	−176.3	176.9	−92.4	2.081
	Water	2.051	−176.2	176.9	−92.2	2.081
3	Gas-phase	3.696	−107.7	164.5	−66.1	2.520
	Heptane	3.794	−104.6	165.4	−66.1	2.618
	Methanol	3.907	−107.0	170.2	−65.4	2.783
	DMSO	3.913	−107.0	170.6	−65.3	2.790
	Water	3.917	−107.0	171.0	−65.2	2.796
15	Gas-phase	2.080	−177.3	178.2	−94.8	2.067
	Heptane	2.066	−176.5	178.1	−93.5	2.066
	Methanol	2.040	−175.1	177.4	−90.9	2.065
	DMSO	2.039	−175.0	177.4	−90.8	2.065
	Water	2.039	−175.0	177.3	−96.7	2.065
6	Gas-phase	3.697	−107.9	161.5	−66.1	2.501
	Heptane	3.752	−103.8	166.5	−65.6	2.564
	Methanol	3.990	−103.4	165.8	−65.8	2.795
	DMSO	3.992	−103.6	166.2	−65.7	2.799
	Water	3.994	−107.2	161.1	−66.1	2.802

[a] Distances (d) are in Å.

S-methylated compounds, thus implying a different strategy of the molecules to stabilize the radical cation species.

Then, regarding a theoretical answer for the stabilization in compounds **10** and **15** the geometrical parameters obtained for the minimum structures in the gas phase, listed in Table 4, suggest the formation of an intramolecular S–H···O=C (on the *N*-acetyl group) hydrogen bond. When comparing pairs of compounds (**10** *vs.* **3** and **15** *vs.* **6**) in which the quaternary methyl group (R^4) is, respectively, present or absent, shorter distances between functional groups are observed in the presence of the C^{α}-methyl group. In fact, in compounds **3** and **6**, the carbonyl O=C of the *N*-acetyl group is oriented away from hydrogen (S–H). Thus, the methyl group in C^{α} causes a restructuration of the torsional angles χ_1, ψ and ϕ promoting the approach of S–H and O=C of the *N*-acetyl group which could allow the formation of a S–H···O=C intramolecular hydrogen bond giving a seven-membered ring. At the same time the formation of the C_7 member ring is accompanied by the formation of a second intramolecular C_5 member ring if the distances between N–H and O=C on the carboxylic group, d(N–H···O=C), are taken into account. This C_5 member ring does not appear for molecules that are non-methylated in C^{α}. Besides, the values for the dihedral angles (ϕ,ψ) = (−177.3, 178.2) obtained by the DFT calculations in the gas phase are characteristic of a C_5 conformation due to the alkyl side chains attached to the C^{α}-carbon.[26] Fig. 2 shows, as an example, the differences between optimized structures of **6** and **15**. In conclusion, compounds **10** and **15** exhibit a structure consistent with the existence of two intramolecular hydrogen bonds, a structure never previously observed in a cysteine. What is more, it must also be noticed that these compounds are very

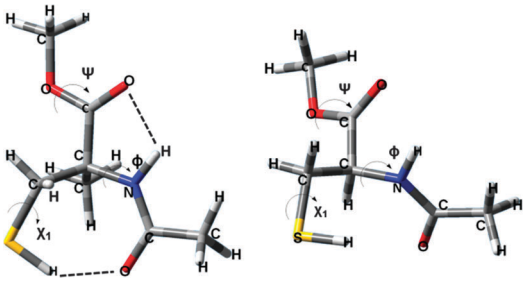
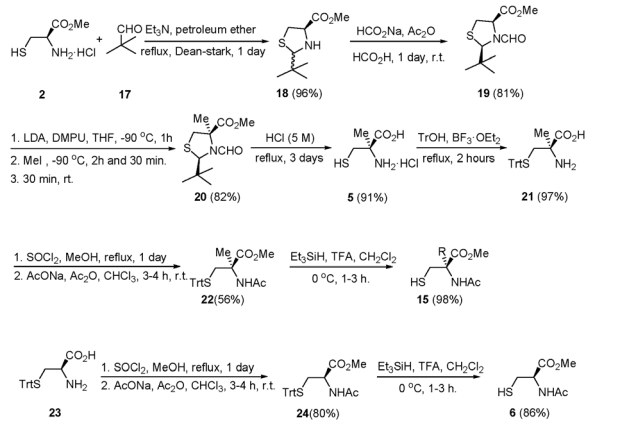


Fig. 2 Optimized structures obtained by DFT calculations for compounds **15** and **6**.

remarkable because of the intramolecular S–H···O=C hydrogen bond. Although the S–H···O=C hydrogen bond has been observed in other compounds,[27] it is very unusual. In fact, in the Cambridge Structural Database, out of 1811 Y-S-Z substructures only 26 show that hydrogen bond and only one of these is intramolecular.[28]

Taking into account these results, it can be proposed that the C^{α}-methyl group would activate intramolecular hydrogen bonds in **10** and **15** thus enhancing the stability of the neutral species and remarkably that of the radical cation, with the subsequent reduction of the ionization energy. It is likely that the cooperative effects existing between C_7 and C_5-membered rings would donate to the sulphur atom an amount of electron density similar to that provided by a methyl group leading to a similar antioxidant activity.

It has also been considered advisable to study the effect of different solvents on the structure of **10** and **15** to know whether and to what extent the intramolecular hydrogen bonds are maintained under the influence of different types of solute–solvent interactions. This is due to two reasons. On the one hand, as is well known, molecules in solution can adopt different conformations depending on the nature of the solvent. On the other hand, antioxidant activity is exerted and measured in specific media. Therefore, the PCM model, which provides good results in describing the behaviour in solution of compounds able to form intramolecular hydrogen bonds,[29,30] has been used to optimize the molecular geometry of compounds **3**, **6**, **10** and **15** in several solvents with increasing polarity, namely, heptane, methanol, DMSO, and water (Table 4). Compounds **3** and **6** form intermolecular hydrogen bonds with the solvents as indicated by the increase of distances d(S–H···O=C) and d(N–H···O=C) with increased solvent polarity. But for **10** and **15** the intramolecular distances d(S–H···O=C) and d(N–H···O=C) are not affected by the media. This could be indicative of the presence of a strong intramolecular hydrogen bond that is not affected by solvent nature. This particularity seems to be a result of the so called "structural effects" that do not involve the rupture of the hydrogen bonds. Either water or methanol molecules can form three or two-dimensional networks through intermolecular hydrogen bonds. These networks will have cavities of different sizes and shapes where 10 or 15 molecules could be inserted. This insertion would bring about an increase in the regularity



Scheme 1 Synthesis of (*R*)-*N*-acetyl-2-methylcysteine methyl ester **15** and *N*-acetyl-L-cysteine methyl ester **6**.

of the network by forcing intermolecular hydrogen bonds of the solvent to stretch in such a way that there are no changes in the conformation of the solute molecule in a state of infinite dilution.[31–33]

Experimental corroboration of the existence of intramolecular hydrogen bonds in C^{α}-quaternary cysteines and of their antioxidant activity

The surprising structures predicted for cysteines **10** and **15** as well as their good antioxidant activity were worth an experimental verification to test the validity of the method in the prediction of both EC_{50} and structures. Compound **10** was discarded because in non-polar media the carboxylic acids usually exist as dimers due to their tendency to ''self-associate'', and this would interfere with the verification of the existence of

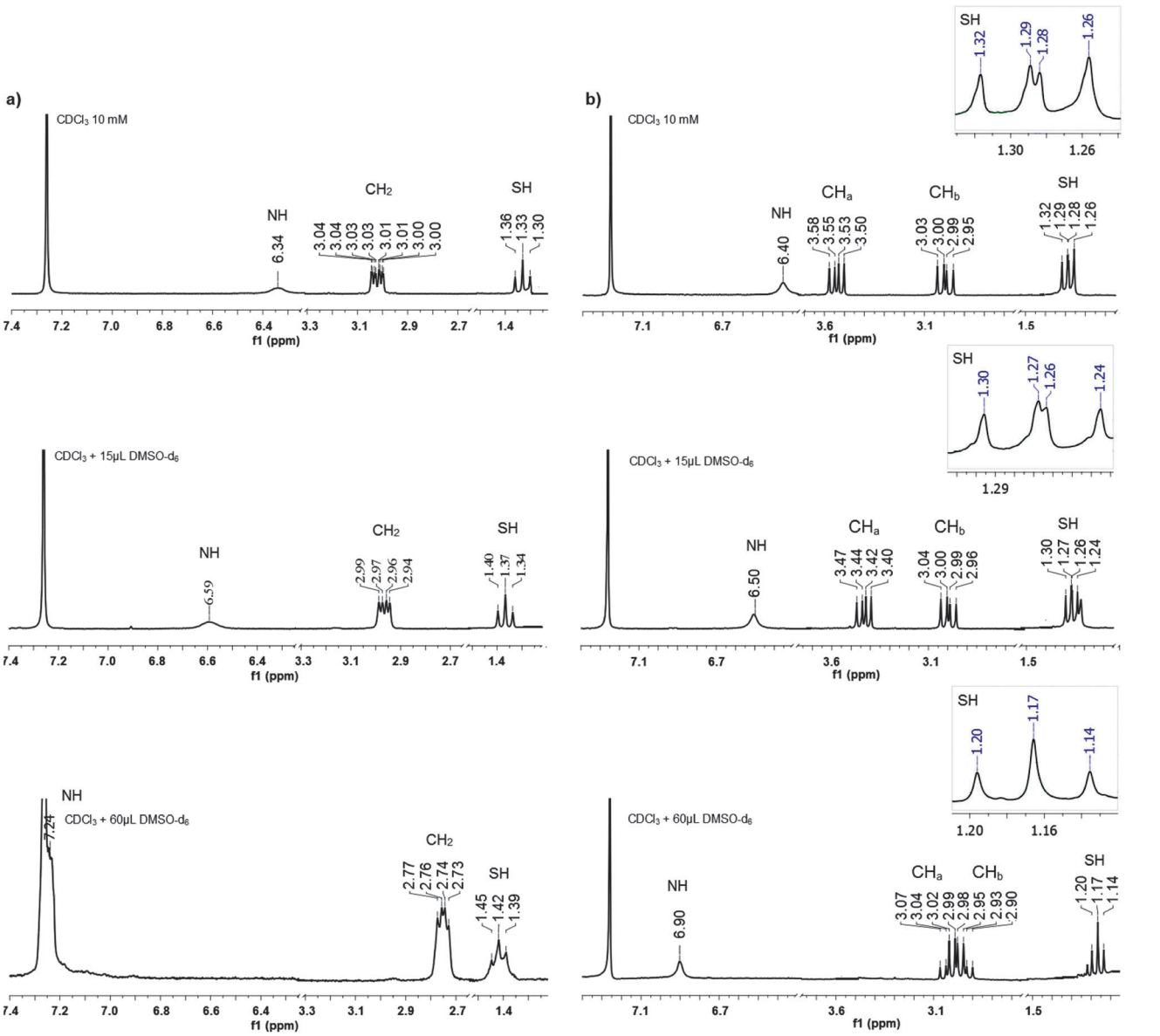


Fig. 3 Experimental ^{1}H NMR chemical shift in $CDCl_3$ when adding different portions of DMSO-d_6 (a) **6** and (b) **15**.

the intramolecular hydrogen bonds. Thus, (*R*)-*N*-acetyl-2-methylcysteine methyl ester **15** was synthesized. Scheme 1 shows the reaction path and the yields of compounds **15** and **6**. Then the conformational preferences of their molecules in solution were studied through ^{1}H NMR.

A ^{1}H NMR titration was performed on compounds **6** and **15** by increasing the polarity of the medium ($CDCl_3$) with the addition of increasing amounts of DMSO. In this sense, the ^{1}H NMR chemical shift of a proton involved in an intramolecular hydrogen bond would be scarcely disturbed if the polarity of the medium changes.

From the ^{1}H NMR spectrum, Fig. 3b, the S–H proton of compound **15** shows no change in chemical shift when increasing the polarity of the medium but at the same time it exhibits important structural changes. The S–H proton shows different coupling constants, J_{SH-CH_a} = 9.6 Hz and J_{SH-CH_b} = 8.5 Hz, with each proton in CH_2 (CH_aH_b) in pure $CDCl_3$ (doublet of doublets) but it shows the same coupling constant, $J_{SH-CH_aH_b}$ = 9.1 Hz, for both CH_aH_b protons when the concentration of DMSO increases to 60 μL (triplet). That is, CH_2 protons are diastereotopic (CH_aH_b) in pure $CDCl_3$ suggesting a substantial S–H···O=C intramolecular H-bonding. Different behavior can be observed for the non-hydrogen bound and non-methylated C^α compound **6** (Fig. 3a).

In the case of N–H its shift changed little with the addition of DMSO-d_6 to $CDCl_3$, the values of $\Delta\delta$ for the amide N–H protons for cysteines **15** and **6** are 0.50 and 0.90 ppm, respectively, as can be seen in Fig. 3. The amide N–H proton for the cysteine **15** was less shifted downfield and agrees with the values described in the bibliography for quaternary amino acids.[34,35] Then, the possibility of the amide N–H proton in **15** being involved in an intramolecular hydrogen bond N–H···O=C with the oxygen of the ester carbonyl group can be reasonably assumed. Therefore, the ^{1}H NMR study confirms that the thiol S–H and the amide N–H are involved in cooperative intramolecular hydrogen bonds with the oxygen of the acetyl group and the oxygen of the ester carbonyl group, respectively, as was predicted by the DFT calculations.

Finally, the experimental antioxidant activity (EC_{50}) found for (*R*)-*N*-acetyl-2-methylcysteine methyl ester **15** was 0.27 ± 0.02 mol_{Cys}/mol_{DPPH}. Considering the experimental antioxidant activity of compound **6**, 0.36 ± 0.02 mol_{Cys}/mol_{DPPH}, and the conformation found experimental and theoretically for both cysteines (in the gas-phase and at infinite dilution), the unusual structure of (*R*)-*N*-acetyl-2-methylcysteine methyl ester **15** is responsible for its effectiveness as a radical scavenger. Moreover, the EC_{50} value of **15** quite coincides with the extrapolated value (Table 3) in such a way that it constitutes a strong support for the validity of the prediction method proposed, especially if it is considered that the correlation was based on values of EC_{50} ranging from 0.36 to 0.42, with the extrapolated value being quite far away from that range.

In any case, compound **15** is a promising antioxidant with a reducing ability far greater than that of NAC (0.38 ± 0.02 mol_{Cys}/mol_{DPPH}) and similar to that of Trolox (0.23 ± 0.02 mol_{Trolox}/mol_{DPPH}) and ascorbic acid (0.25 $mol_{Ascorbic\ acid}/mol_{DPPH}$).[36] Besides, it could also be useful for synthetic oligomer design by profiting from its steric and dihedral angle constraints which could enable modulating their structural features for possible applications involving protein recognition.[37,38]

Conclusions

This paper describes a method devised to estimate antioxidant activity based on the correlation of HOMO energies, obtained by DFT calculations, of a subset of cysteines with their respective experimental EC_{50}. This procedure, combined with the PCM calculations, has been applied to an array of cysteines allowing us to discover intramolecular hydrogen bond activation in cysteines **10** and **15** by introducing a C^α-methyl group. The experimental structure of (*R*)-*N*-acetyl-2-methylcysteine methyl ester **15** shows two cooperative C_7 and C_5 member rings. This conformation had never been previously reported in a single cysteine and it is responsible for its remarkable antioxidant activity. Additionally, the experimental EC_{50} value of **15** coincides reasonably well with the value predicted using the method, thus confirming its validity.

Acknowledgements

The authors thank the Ministerio de Ciencia e Innovación-FEDER (grants CTQ2010-17436, CTQ2009-14629-CO2-02, CTQ2012-38219-C03-02 and IPT-2011-0860-060000) and the Gobierno de Aragón-FSE (research groups E40 and E52) for financial support. The authors thank Juan I. Pardo for helpful discussions. L.H. thanks UZ for a predoctoral contract and I.O. thanks CSIC for a postdoctoral contract.

Notes and references

1 N. F. L. Machado, C. Ruano, J. L. Castro, M. P. M. Marques and J. C. Otero, *Phys. Chem. Chem. Phys.*, 2011, **13**, 1012.
2 Z. Q. Liu, *Chem. Rev.*, 2012, **112**, 3329.
3 V. Vaidya, K. U. Ingold and D. A. Pratt, *Angew. Chem., Int. Ed.*, 2009, **48**, 157.
4 R. Amorati, P. T. Lynett, L. Valgimigli and D. A. Pratt, *Chem.–Eur. J.*, 2012, **18**, 6370.
5 M. Marí, A. Morales, A. Colell, C. García-Ruiz and J. C. Fernández-Checa, *Antioxid. Redox Signaling*, 2009, **11**, 2685.
6 P. Eaton, *Free Radicals Biol. Med.*, 2006, **40**, 1889.
7 L. Yuan, W. Lin, Y. Xie, B. Chen and S. Zhu, *J. Am. Chem. Soc.*, 2012, **134**, 1305.
8 Z. A. Wood, E. Schröder, J. R. Harris and L. B. Poole, *Trends Biochem. Sci.*, 2003, **28**, 32.
9 J. Oiry, P. Mialocq, J. Y. Puy, P. Fretier, N. Dereuddre-Bosquet, D. Dormont, J. L. Imbach and P. Clayette, *J. Med. Chem.*, 2004, **47**, 1789.
10 B. K. McMahon and T. Gunnlaugsson, *J. Am. Chem. Soc.*, 2012, **134**, 10725.
11 S. Chakravarthi, C. E. Jessop and N. J. Bulleid, *EMBO Rep.*, 2006, **7**, 271.

12 A. Rauk, D. Yu and D. A. Armstrong, *J. Am. Chem. Soc.*, 1998, **120**, 8848.
13 C. Viglianisi, M. G. Bartolozzi, G. F. Pedulli, R. Amorati and S. Menichetti, *Chem.–Eur. J.*, 2011, **17**, 12396–12404.
14 M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, Ö. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski and D. J. Fox, *Gaussian 09, Revision A.1*, Gaussian, Inc., Wallingford CT, 2009.
15 L. Haya, F. J. Sayago, A. M. Mainar, C. Cativiela and J. S. Urieta, *Phys. Chem. Chem. Phys.*, 2011, **13**, 17696.
16 A. Nafady, P. J. Costa, M. J. Calhorda and W. E. Geiger, *J. Am. Chem. Soc.*, 2006, **128**, 16587.
17 P. Winget, E. J. Weber, C. J. Cramer and D. G. Truhlar, *Phys. Chem. Chem. Phys.*, 2000, **2**, 1231.
18 T. Koopmans, *Physica*, 1933, **1**, 104.
19 W. Brand-Williams, M. E. Cuvelier and C. Berset, *Lebensm.-Wiss. Technol.*, 1995, **28**, 25.
20 M. H. Gordon, F. Paiva-Martins and M. Almeida, *J. Agric. Food Chem.*, 2001, **49**, 2480.
21 T. Yamaguchi, H. Takamura, R. Matoba and J. Terao, *Biosci., Biotechnol., Biochem.*, 1998, **62**, 1201.
22 Y. V. Yuan, D. E. Bone and M. F. Carrington, *Food Chem.*, 2005, **91**, 485.
23 M. S. Blois, *Nature*, 1958, **181**, 1199.
24 C. Jacob, G. I. Giles, N. M. Giles and H. Sies, *Angew. Chem., Int. Ed.*, 2003, **42**, 4742.
25 S. Luo and R. L. Levine, *FASEB J.*, 2009, **23**, 464.
26 C. Toniolo, G. M. Bonora, A. Bavoso, E. Benedetti, B. Di Blaso, V. Pavone and V. Pedone, *Macromolecules*, 1986, **19**, 472.
27 T. G. Heafield, G. Hopkins and L. Hunter, *Nature*, 1942, **149**, 218.
28 F. H. Allen, C. M. Bird, R. S. Rowland and P. R. Raithby, *Acta Crystallogr., Sect. B: Struct. Crystallogr. Cryst. Chem.*, 1997, **53**, 696–701.
29 P. A. Nielsen, P. Norrby, T. Liljefors, N. Rega and V. Barone, *J. Am. Chem. Soc.*, 2000, **122**, 3151.
30 P. I. Nagy and P. W. Erhardt, *J. Phys. Chem. B*, 2012, **116**, 5425.
31 A. Ben-Naim, *J. Phys. Chem.*, 1968, **72**, 2998.
32 A. M. Mainar, *PhD thesis*, University of Zaragoza, 2000.
33 A. M. Mainar, in 13th Annual Research Awards Presentation, Rev. Real Acad. Ciencias de Zaragoza, 2009, 64, 7.
34 D. Núñez-Villanueva, M. A. Bonache, L. Infantes, M. T. García-López, M. Martín-Martínez and R. González-Muñiz, *J. Org. Chem.*, 2011, **76**, 6592.
35 D. Yang, J. Qu, W. Li, D. P. Wang, Y. Ren and Y. D. Wu, *J. Am. Chem. Soc.*, 2003, **125**, 14452.
36 H. Hotta, S. Nagano, M. Ueda, Y. Tsujino, J. Koyama and T. Osakai, *Biochim. Biophys. Acta*, 2002, **1572**, 123.
37 Y. Imamura, N. Watanabe, N. Umezawa, T. Iwatsubo, N. Kato, T. Tomita and T. Higuchi, *J. Am. Chem. Soc.*, 2009, **131**, 7353.
38 W. S. Horne and S. H. Gellman, *Acc. Chem. Res.*, 2008, **41**, 1399.