Genistein complexes with amines: structure and properties

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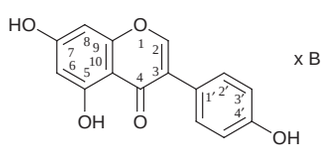
New amine complexes of genistein in the crystal and the solution state have been studied by X-ray crystallography and by ^{1}H and ^{13}C NMR spectroscopy. The gas-phase structures have been modelled with *ab initio* quantum chemical calculations. The morpholine–genistein hydrogen bonded complex has been investigated by all the above methods whereas the triethylamine, morpholine and piperazine complexes have been investigated with ^{1}H NOE and ^{13}C NMR spectroscopy. The X-ray results show the genistein–morpholine complex to be formed as a result of proton transfer from the genistein OH group at position C7 to the morpholine nitrogen atom. This complex also has the lowest total energy when compared to other possible complexes. The NMR measurements in solution indicate that the protonated amine is in fast exchange between various interaction sites, the most stable complex being formed at position C7 as in the crystal. The *ab initio* quantum mechanical calculations show that this position is also the best for interactions. The ^{13}C NMR chemical shifts calculated theoretically are in agreement with experimental values.

Introduction

Genistein, 5,7-dihydroxy-3-(4′-hydroxyphenyl)-4*H*-chromen-4-one, $C_{15}H_{10}O_5$ (**1**), is a natural isoflavone. Recently genistein has



1 B = none
2 B = HN O
3 B = HN NH
4 B = Et_3N

aroused interest in medical research owing to its diversified interaction with topoisomerase II DNA cleaving agent,[1] as an inhibitor of tyrosine-specific protein kinase[2] or as an immunosuppressant investigated *in vivo*.[3] The tyrosine kinase inhibitors have also been employed as antiproliferative agents *in vitro*. Genistein selectively discriminates normal and malignant mononuclear cells in large animals and humans through inhibition of DNA topoisomerase synthesis.[4]

We started to investigate the mechanisms of immunosuppressive action of these compounds at the cellular and molecular levels. Our investigations into the biological activity of various genistein derivatives have shown the amine complexes to exhibit immunosuppressant activity,[3a] and induced us to explore the structure of these complexes to facilitate the evaluation of the pharmacophore in the genistein moiety.

This paper describes part of the research program devoted to the investigation of the molecules that mediate in immunosuppression by affecting the mitogen-induced transition of lymphocytes and which are very interesting candidates for use in immunosuppression therapy.[3b] Moreover, as a prelude to possible clinical use, we have investigated[3] the efficacy and safety of genistein derivatives in rodent recipients of heart allografts.

Despite the wide interest in the biological activity of genistein and its derivatives, their electronic and geometrical parameters have been very little investigated. Recently, some data concerning the X-ray structure of genistein,[5] ^{1}H[6,7] and ^{13}C[6,8] NMR spectra and also quantum mechanical calculations[9] have been reported.

The aim of the present study is to examine the structure of the genistein (**1**) complexes with morpholine (**2**), piperazine (**3**) and triethylamine (**4**), by X-ray crystallography, NMR spectroscopy and by quantum mechanical calculations. Comparison of the structure and properties of the parent compound with its amine complexes may yield valuable information about the domains contributing to its pharmacophore.

Experimental

Synthesis

Genistein was synthesized by a reported method.[10] The complexes with amines were obtained according to standard methods by dissolving genistein in methanol containing a

Table 1 Crystal data and structure refinement parameters for the genistein–morpholine complex **2**

Empirical formula	$C_{19}H_{19}NO_6$
Formula weight	357.35
Temperature	293(2) K
Wavelength	1.541 78 Å
Crystal system	Orthorhombic
Space group	*Pbca*
Unit cell dimensions	
a	12.7127(9) Å
b	14.854(1) Å
c	17.770(2) Å
Volume	3355.7(5) Å^3
Z	8
Density (calculated)	Mg m^{-3}
Absorption coefficient	0.886 mm^{-1}
F(000)	1504
Crystal size	0.4 × 0.35 × 0.3 mm
Theta range for data collection	4.98–75.17°
Index ranges	$0 \leqslant h \leqslant 9$, $0 \leqslant k \leqslant 18$, $-22 \leqslant l \leqslant 22$
Reflections collected	3891
Independent reflections	2461 [R(int) = 0.0186]
Absorption correction	Not applied
Refinement method	Full-matrix least-squares on F^2
Data/restraints/parameters	2461/0/251
Goodness-of-fit on F^2	1.013
Final R indices [$I > 2\sigma(I)$]	$R = 0.0356$, $wR(F^2) = 0.0958$
R indices (all data)	$R = 0.0490$, $wR(F^2) = 0.1014$
Largest diff. peak and hole	0.183 and −0.186 e Å^{-3}

relevant amine in excess and crystallizing the resulting solid from the reaction mixture.

X-Ray crystal structure

X-Ray-grade crystals were obtained by crystallization from chloroform–methanol. A slightly irregular columnar yellow crystal, 0.3 × 0.35 × 0.4 mm in size, was used to produce the diffraction diagrams. The crystal was placed in a KUMA KM-4 single-crystal κ-axis diffractometer. Graphite monochromated Cu-Kα radiation was used to produce diffraction patterns. The unit cell parameters were obtained by the least-squares treatment of 25 reflections observed at angles $40 \leqslant 2\theta \leqslant 50°$. Reflections, 3891 in total, were measured up to $\theta = 76°$, including equivalent reflections. The systematic absences (0*kl*, *k* odd; *h*0*l*, *l* odd; *hk*0, *h* odd) led to the choice of the *Pbca* space group. The structure was solved by using the direct methods of the SHELXS86[11] program and refined by applying the SHELXL93[12] program. The E-map revealed almost all non-hydrogen atoms. The rest of them were found from the subsequent $\Delta\rho$ synthesis. After isotropic and anisotropic refinements of heavy atoms, the $\Delta\rho$ map revealed all hydrogens. In the last cycles of full matrix refinement the non-hydrogen atom positions were refined together with their anisotropic displacement parameters. The hydroxy and amino hydrogen atom positions together with their isotropic displacement coefficients were also refined. The remaining hydrogens were restrained to retain standard geometry and their isotropic thermal coefficients to be 1.2 times larger than the respective parameters of the carbon atoms. All structural parameters are collected in Table 1.

Full crystallographic details, excluding structure factor tables, have been deposited at the Cambridge Crystallographic Data Centre (CCDC). For details of the deposition scheme, see 'Instructions for Authors', *J. Chem. Soc.*, *Perkin Trans. 2*, available *via* the RSC Web pages (http://www.rsc.org/authors). Any request to the CCDC for this material should quote the full literature citation and the reference number 188/128.

NMR measurements

NMR measurements were performed using a Gemini 200 MHz Varian instrument to record broadband decoupled ^{13}C NMR spectra and a Varian INOVA 500 MHz NMR spectrometer to run nuclear Overhauser effect (NOE) spectra and to make other measurements. The solute, 50 mg, was usually dissolved in 0.7 ml of [^{2}H$_6$]DMSO for ^{13}C measurements and 2 mg was dissolved for NOE experiments. The ^{13}C NMR spectra were typically run using a spectral range of 10 kHz, a tip angle of 45° and collecting 64 k data points with TMS as internal reference. The GHMBC (gradient heteronuclear multiple bond correlation) spectra were used for specific assignment of long range H, C spin–spin coupling constants in ambiguous cases, as for the C6 carbon atom. A standard Varian program on the INOVA 500 MHz spectrometer was used with the parameters optimized for 8 or 3.3 Hz. The GHSQC (gradient heteronuclear single quantum correlation) spectra were used for specific assignment of CH resonances.

The T_1 experiments of proton resonances were measured on degassed samples used for NOE measurements. The 90° pulse-width, 5 s acquisition on a 9 kHz spectral width and 131 k data points were used. The standard inversion–recovery program was applied using 10 time increments and 16 s delay.

The NOE measurements were run in degassed samples sealed under argon. The irradiation power was kept low enough to achieve selectivity and the spectra were recorded using the standard Varian program with a 15 s irradiation time, no relaxation delay, 5 s acquisition and 90° pulse. The spectra were acquired in the absorption mode and NOE spectra corrected for saturation factor were calculated by using an auxiliary program described earlier.[13]

Quantum-chemical calculations

The *ab initio* quantum-mechanical calculations of genistein and its complexes were performed to estimate the geometry and energetics for the most stable complexes as well as to calculate the ^{1}H, ^{13}C, ^{15}N and ^{17}O chemical shifts. At the theoretical level we investigated the complex of genistein with the morpholine molecule, one out of several complexes studied experimentally.

The *ab initio* Hartree–Fock (HF) and the Møller–Plesset (MP2) molecular orbital calculations were carried out with the GAUSSIAN94 program,[14] using the split-valence 6-31G** basis set.[15] Geometry optimizations of genistein, morpholine and eight genistein–morpholine complexes were performed based on analytical calculations of the first derivatives of energy. The basis set superposition error (BSSE) was estimated by the counterpoise method.[16–19] The chemical shifts were calculated, based on the CHF-GIAO approach[20] to the optimized geometrical configurations of eight complexes using the double zeta (DZP) basis set of Hansen and Bouman.[21] This basis set is composed of (31/7) AO (atomic orbitals) contracted to [2s1p] for hydrogen atoms and (721/221/1) AO contracted to [3s3p1d] for C, N and O atoms. The GIAO approach internally extends the basis set with higher angular momentum orbitals, which are necessary for the correct description of the perturbed system. That is why, in particular, calculations with the DZP basis set provide quite good results for organic molecules by the GIAO method. This basis set has been previously found to be efficient in the chemical shift calculations for C, N and O atoms.[22–24]

Results and discussion

X-Ray structure

Crystal data and structure parameters for the genistein–morpholine complex (**2**) are presented in Table 1. Selected bond lengths and angles are shown in Table 2. The reaction of genistein with morpholine is the standard acid–base reaction, where the most acidic proton is transferred from the acid to the base (in this case the proton from the O7 hydroxy group, Fig. 1). All the hydroxy and amino hydrogens are involved in inter- and intra-molecular hydrogen bonding formation (see Fig. 2 and Table 3). The chromenone skeleton is planar and makes a dihedral angle with the phenyl substituent of 64.97(5)°. The structure found differs significantly from the structure of pure

Table 2 Selected bond lengths (Å) and angles (°) in the crystal of complex **2**

O1–C2	1.347(2)	C2–O1–C9	119.4(1)
O1–C9	1.373(2)	C3–C2–O1	125.5(2)
C2–C3	1.338(2)	C2–C3–C4	118.0(2)
C3–C4	1.459(2)	C2–C3–C1′	122.0(2)
C3–C1′	1.482(3)	C4–C3–C1′	120.0(1)
C4–O4	1.263(2)	O4–C4–C10	122.1(2)
C4–C10	1.422(3)	O4–C4–C3	121.3(2)
C5–O5	1.359(2)	C10–C4–C3	116.6(1)
C5–C6	1.366(3)	O5–C5–C6	119.7(2)
C5–C10	1.421(2)	O5–C5–C10	119.0(2)
C6–C7	1.413(2)	C6–C5–C10	121.3(1)
C7–O7	1.312(2)	C5–C6–C7	121.1(2)
C7–C8	1.407(2)	O7–C7–C8	120.9(1)
C8–C9	1.370(2)	O7–C7–C6	120.6(2)
C9–C10	1.406(2)	C8–C7–C6	118.5(2)
C1′–C2′	1.386(2)	C9–C8–C7	119.2(1)
C1′–C6′	1.393(3)	C8–C9–O1	117.0(1)
C2′–C3′	1.385(3)	C8–C9–C10	123.5(2)
C3′–C4′	1.378(3)	O1–C9–C10	119.5(2)
C4′–O4′	1.360(2)	C9–C10–C5	116.2(2)
C4′–C5′	1.389(2)	C9–C10–C4	120.9(2)
C5′–C6′	1.376(3)	C5–C10–C4	122.9(1)
N4″–C5″	1.475(3)	C2′–C1′–C6′	117.4(2)
N4″–C3″	1.478(3)	C2′–C1′–C3	122.4(2)
C5″–C6″	1.497(3)	C6′–C1′–C3	120.2(2)
C6″–O1″	1.411(3)	C3′–C2′–C1′	121.5(2)
O1″–C2″	1.417(3)	C4′–C3′–C2′	120.4(2)
C2″–C3″	1.499(3)	O4′–C4′–C3′	123.1(2)
		O4′–C4′–C5′	118.1(2)
		C3′–C4′–C5′	118.8(2)
		C6′–C5′–C4′	120.5(2)
		C5′–C6′–C1′	121.3(2)
		C5″–N4″–C3″	110.3(2)
		N4″–C5″–C6″	109.1(2)
		O1″–C6″–C5″	111.1(2)
		C6″–O1″–C2″	109.6(2)
		O1″–C2″–C3″	111.1(2)
		N4″–C3″–C2″	109.5(2)

Table 3 Hydrogen bond geometry in crystal structure of complex **2**

	Distance/Å			Angle
D–H···O[a]	D–H	D···O	H···O	DH···O
O5–H5···O4	1.00(3)	2.589(2)	1.65(3)	154(2)
N4″–H4″a···O7	1.06(2)	2.646(2)	1.59(3)	175(2)
N4″–H4″b···O4[b]	0.91(2)	2.765(2)	2.01(2)	140(2)
O4′–H4′···O7[c]	0.90(3)	2.626(2)	1.74(3)	169(2)

[a] D stands for donor atom. [b,c] Symmetry transformations used to generate equivalent atoms: [b] $0.5 + x$, $1.5 - y$, $-z$; [c] $-0.5 - x$, $1 - y$, $-0.5 + z$.

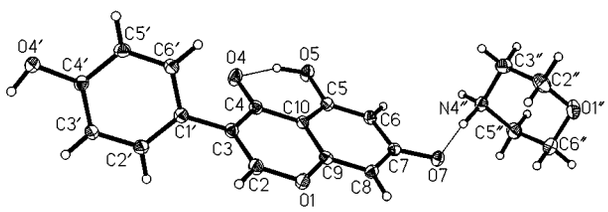


Fig. 1 The molecular structure of the genistein–morpholine complex **2** in the crystal. The non-hydrogen atoms are shown as the 20% probability ellipsoids.

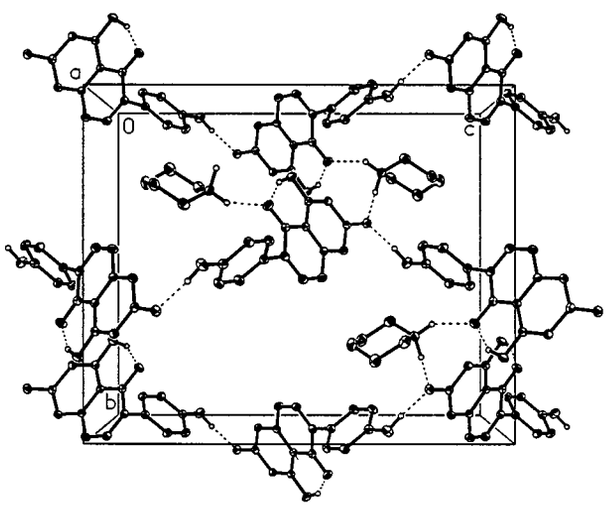


Fig. 2 View of crystal packing. All types of hydrogen bonds are shown as dashed lines. Somes hydrogen atoms, not involved in hydrogen bonding, are omitted for the clarity. The non-hydrogen atoms are shown as the 20% probability ellipsoids.

genistein[5] in both the molecular geometry of genistein and the hydrogen bond pattern. The transfer of a proton from the O7 hydroxy oxygen atom causes a shortening of the C–O distance (1.356 and 1.312 Å in genistein and in the present structure, respectively). The negative charge on the oxygen and the shortening of the C–O bond lead consecutively to the lengthening of the two adjacent C–C bonds of the ring. The other fragments of the molecule remain almost unaffected, including the dihedral angle between the two ring fragments. In the crystal packing the main difference is caused by the morpholinium cation which donates two hydrogens to hydrogen bond formation. In both structures, the O7 oxygen atom acts as the acceptor in the hydrogen bond with the O4′–H4′ hydroxy group. The oxygen atom O5, apart from being a donor in an intramolecular hydrogen bond, also acts as the acceptor for the O7–H7 hydroxy group in genistein and for the N4″–H4″ amino group in morpholine, respectively. In that structure, there is no evidence for a CH···O hydrogen bond interaction, which is an additional stabilizing factor in the crystal of pure genistein.

NMR measurements in solution

The proton coupled ^{13}C NMR spectra used for assignments of carbon resonances of compounds **1** and **2** are given in Table 4. In addition, the assignments of CH resonances were confirmed by the GHSQC experiment and quaternary carbon atom resonances were assigned using the information obtained from the GHMBC experiment. These experiments were also used to assign the H6 and H8 proton resonances.

The ^{13}C NMR spectra were used to monitor changes in the chemical shifts due to complexation in the genistein skeleton. The ^{13}C chemical shifts of genistein are compared with shifts of carbon atoms in relevant amine complexes (Fig. 3). The graph clearly indicates the O7–H7 hydroxy group being primarily engaged in complexation with the amine and the O4′–H4′ group to be complexed to a smaller extent. This is judged from the amount of downfield shift of the respective carbon resonance assigned to a particular phenolic group and carbon atoms in the *ortho* positions. These results indicate that a complex equilibrium exists in the solution, where the amine is complexed on either side of the molecule. It can also be judged from Fig. 3 that piperazine is more strongly complexed than morpholine or triethylamine.

The nuclear Overhauser effect was measured in [$^2H_6$]DMSO as solvent in order to confirm the ^{13}C results and to evaluate the degree of twist from planarity of the flexible aromatic ring.

In addition to intramolecular dipolar NOEs some effects were observed in genistein, upon irradiation of water, which are due to saturation transfer from water to exchanging OH protons. These effects show that, while the H5 proton shows a negligible rate of exchange with water, k_{OH}, the O7–H7 hydroxy group has the largest rate of exchange and hence NOEs observed on irradiation of either O7–H7 or H_2O are nearly the same for H3′, H6 and H8. The origin of small negative NOEs

Table 4 Proton coupled ^{13}C NMR spectra of compounds **1** and **2**[a]

	C2	C3	C4	C5	C6	C7	C8	C9	C10	C1′	C2′	C3′	C4′
Compound **1**													
δ	153.1	121.3	180.3	162.2	99.0	164.3	93.7	157.7	104.7	122.7	130.0	115.1	157.5
^{1}J(H)	195.6				162.0		164.5				159.5	158.5	
^{2}J(H)		2.7(2)		3.4(6) 5.2(OH5)		3.3(6,8)		3.9(8)		3.4(2′)			2.6(3′)
^{3}J(H)		7.2(2′)	6.7(2)		4.6(8) 7.2(OH5)		4.6(6)	8.1(2)	5.5 (6,8,OH5)	6.8(2)	7.3(2′)[b]	5.2(3′)[b]	9.0(2′)
^{4}J(H)						1.7(OH5)							
Compound **2**													
δ	152.8	121.4	180.2	162.2	99.3	165.3	93.8	157.5	104.4	122.7	130.0	115.2	157.8
^{1}J(H)	196.7				161.6		165.2				158.8	158.8	
^{2}J(H)		2.9(2)		3.3(6)	4.6(8)	3.2(6,8)	4.6(6)	4.1(8)		3.2(2′)			2.8(3′)
^{3}J(H)		7.8(2′)	6.9(2)					8.2(2)	5.0(6,8)	6.9(2)	7.3(2′)[b]	5.0(3′)[b]	9.2(2′)

[a] Chemical shifts (δ) in ppm from TMS and spin–spin couplings of a given carbon to a proton in parentheses, ^{n}J(H), in Hz. [b] Assignment not confirmed.

Table 5 Major NOEs in genistein[a]

	NOEs observed for proton								
	OH5	OH7	OH4′	H2	H2′	H3′	H8	H6	H_2O
δ	12.9	10.9	9.6	8.3	7.3	6.8	6.3	6.2	3.3
T_1/s	2.65	1.65	1.67	3.30	2.10	1.86	3.10	3.08	1.62
k_{OH}/s^{-1}	0.04	6.30	1.20						
Irradiated proton									
OH5[b]									
OH7	−5.0		−51.8			4.4	11.7	19.3	−20.0
OH4′		−10.9				9.2	1.2	1.9	
H2					6.0				
H2′				21.9		−2.3			
H3′					−1.9				
H8[b]									
H6[b]									
H_2O	−9.9	−91.4	−67.2			5.8	10.2	16.8	

[a] Chemical shifts δ are given in ppm from internal TMS. Exchange rates of OH protons, k_{OH}, are given in s^{-1}. [b] No NOE observed.

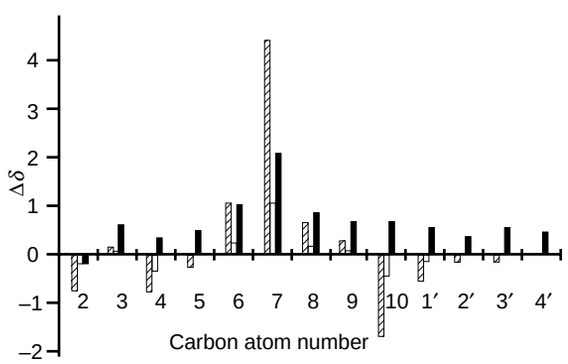


Fig. 3 Chemical shift differences $\Delta\delta$ in ^{13}C NMR spectra of complexes **2** □, **3** (▨) and **4** (■) relative to genistein

on mutual irradiation of H2′ and H3′ is not clear from the present experiments. The exchange of the three hydroxy groups with water and protons of ammonium-like ion N^+–H is fast in amine complexes, therefore saturation transfer is not observable for any of them. The observed NOE enhancements are shown in Tables 5 and 6. Comparison of the effects in complexes with those observed in pure genistein reveals additional intermolecular effects observable in the genistein skeleton upon irradiation of the N–CH_2 groups in amines. They are observed for all protons in the skeleton, thus confirming the exchange of protonated base between positions 7 and 4′.

Quantum chemical calculations

As a starting point, the optimized genistein moiety structure, after a full scan of the C2–C3–C1′–C6′ dihedral angle at the 6-31G level (Fig. 4), was used. Apparently, the angle of minimum energy structure is very close to the value found in the crystal phase.[5] The *ab initio* 6-31G** energies for genistein, morpholine and genistein–morpholine complexes are presented in Table 7, which contains the results of energy calculations for monomers and the eight complexes, *i.e.* the total energy $E_{6\text{-}31G^{**}}$, the interaction energy ΔE and interaction energy ΔE_{BSSE} corrected for BSSE. The most stable complex **2a** (structures **2a**–**h** are given in Fig. 5) with an interaction energy equal to −6.68 kcal mol^{-1}, contains a hydrogen bond between the O7–H7 group of genistein and a lone pair of electrons from the morpholine nitrogen atom N4″. The C7–O7 and O7–H7 distances in the complex have changed slightly (by −0.034 and 0.007 Å, respectively) while the N4″–H4″ bond is changed by ±0.003 Å in comparison to free morpholine. This is in line with the changes observed by the X-ray method. The interaction energy of the second structure **2b** is of the same order, *i.e.* −6.30 kcal mol^{-1}. This structure is characterized by the hydrogen bond between the O4′–H4′ group of genistein and the electron lone pair from the morpholine nitrogen atom N4″. Structure **2c** is very close energetically to this structure, with a stabilization energy of −6.24 kcal mol^{-1}, where the hydrogen bond is formed between the genistein O4′–H4′ group and the morpholine O1″

Table 6 Intermolecular NOEs (%) for genistein protons observed in complexes with amines. NOEs are observed upon irradiation of N–CH_2 signals in amine complexes

	NOEs				
	H2	H2′	H3′	H6	H8
Morpholine	0.5	0.4	0.5	0.8	0.3
Piperazine	1.4	0.9	1.1	1.8	1.9
Triethylamine	2.1	1.1	1.2	2.0	1.6

Table 7 Total energies E and interaction energies ΔE and ΔE_{BSSE} counterpoint corrected

System	$E_{6\text{-}31G^{**}}$/ hartrees	ΔE/ kcal mol^{-1}	ΔE_{BSSE}/ kcal mol^{-1}
Morpholine	−286.013 420		
Genistein **1**	−948.194 142		
2a	−1234.220 254	−7.96	−6.68
2b	−1234.220 545	−8.15	−6.30
2c	−1234.220 070	−7.85	−6.24
2d	−1234.219 123	−7.25	−5.70
2e	−1234.212 898	−3.35	−2.67
2f	−1234.212 885	−3.34	−1.91
2g	−1234.212 476	−3.08	−1.31
2h	−1234.211 412	−2.42	−0.55

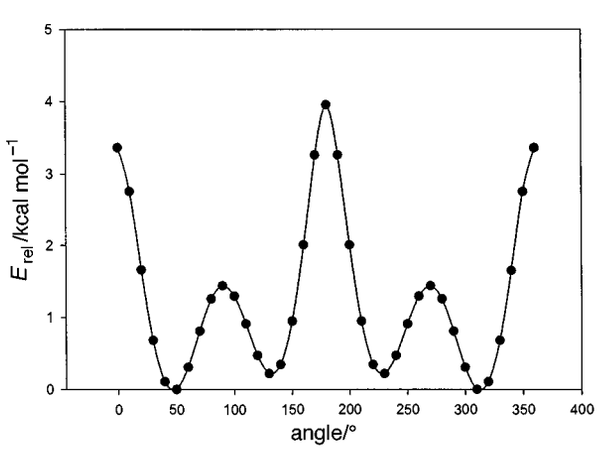


Fig. 4 The relative energy (E_{rel}) of different rotamers of genistein as a function of the C2–C3–C1′–C6′ torsion angle

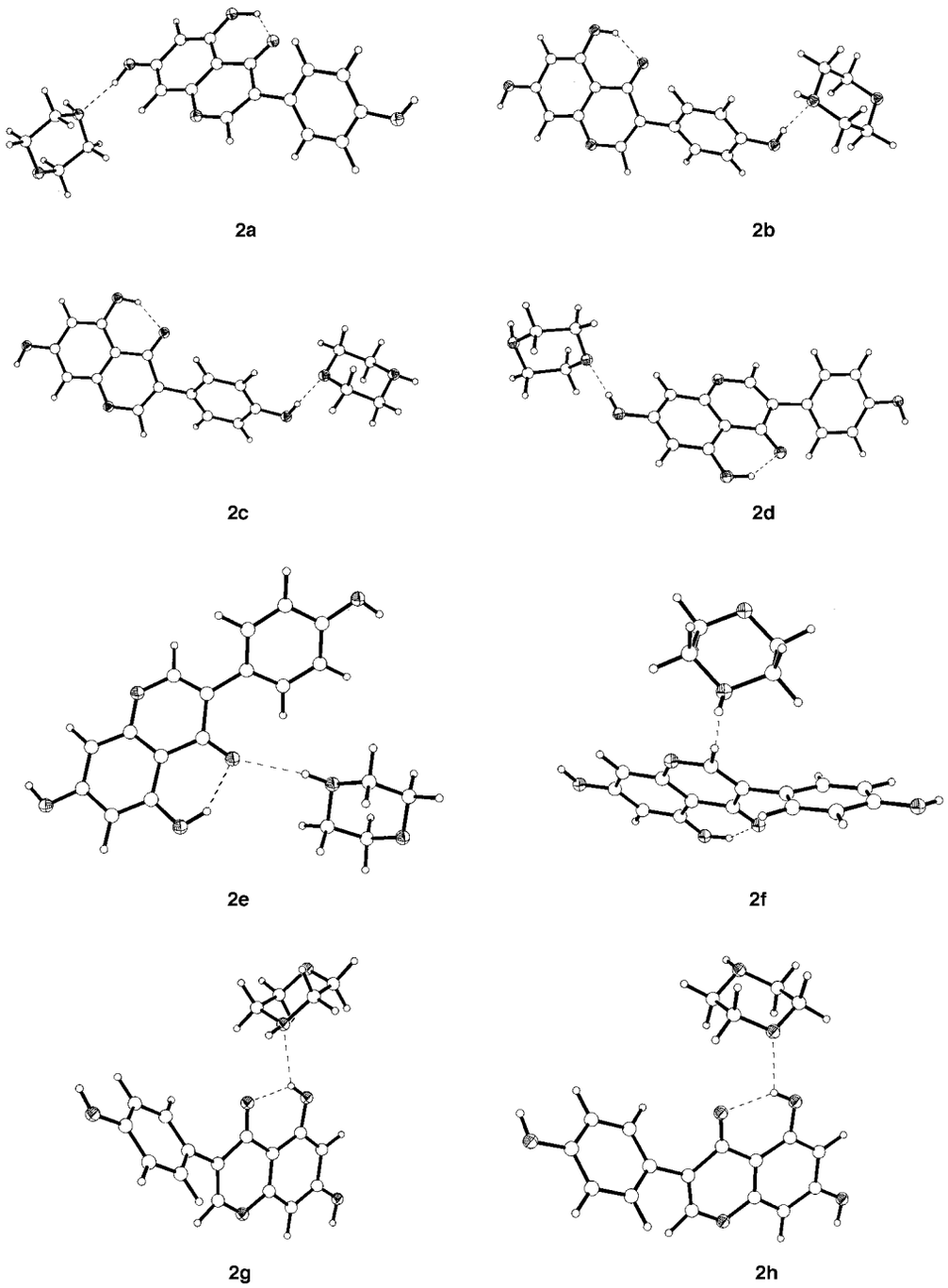


Fig. 5 Structures of genistein–morpholine complexes **2** optimized at the HF/6-31G** level

oxygen atom. The structure **2d** is stabilized by $\Delta E_{BSSE} = -5.70$ kcal mol^{-1} due to the hydrogen bond formed by the genistein O7–H7 group and the O1″ atom of the morpholine molecule.

Four other structures (**2e**–**2h**, Fig. 5) have a much lower energy of interaction ΔE_{BSSE} due to hydrogen bond formation (C4=O4 · · · H4″–N4″, **2e**; C2–H2 · · · N4″, **2f**; O5–H5 · · · N4″ and N4″–H4″ · · · O4=C4, **2g**; O5–H5 · · · O1″, **2h**).

The most stable structures **2a** and **2b** were also confirmed to occur in the solution by observing the intermolecular NOEs (Table 6). The theoretically calculated shielding constants for all nuclei of the complexes are presented in Table 8. The most stable complex **2a** contains the O7–H7 · · · N4″ hydrogen bond, therefore, the biggest changes occur in the shielding constants for O7 (*ca.* 10 ppm) and C7 (2.57 ppm). The chemical shift of the O4 oxygen atom is quite sensitive to the interaction (changed by *ca.* 4 ppm).

The correlation between theoretical chemical shifts ($\delta_{^{13}C,TMS} - \delta_{^{13}C,i}$, where i = free or complexed genistein) and experimental values is depicted in Fig. 6. Both linear correlations are equivalent at the confidence level 0.95, and are fairly good (correlation coefficients are equal to 0.9959 and 0.9955, respectively). The experimental data refer to measurements in the solution and, as is well known, the solvent effect on the shielding constant σ(X) can be large. Thus, this may in part be the source of differences between the computed and measured $\sigma(^{13}C)$. There are two main approximations in our calculations, which might offset the results: limitations of the basis set and the lack of inclusion of correlation effects. Further improvement of the basis set would have minimal effect on the discussed results.[22–24]

Conclusion

The results described were intended to characterize the domains of the genistein pharmacophore and to disclose changes induced by the complexation with amines. The tech-

Table 8 Calculated shielding constants (ppm) for genistein (**1**), morpholine (**m**) and genistein–morpholine complexes (**2a**–**h**)

Atom	1 + m	2a	2b	2c	2d	2e	2f	2g	2h
O1	179.19	179.63	179.41	179.60	179.19	178.73	177.38	180.14	180.93
C2	38.75	38.91	39.06	39.12	38.82	38.54	35.17	38.97	39.19
C3	71.28	71.64	70.83	70.55	71.79	71.33	72.37	71.24	71.11
C4	9.34	9.33	9.10	9.13	9.26	9.10	9.01	10.52	10.84
C5	22.54	22.86	22.56	22.53	23.03	22.19	22.51	22.77	22.64
C6	98.79	98.43	98.98	99.05	98.43	98.62	98.99	97.99	98.46
C7	23.71	21.13	23.83	23.91	21.36	23.52	23.83	24.02	24.15
C8	107.82	107.92	107.90	107.95	107.55	108.03	107.92	107.82	107.98
C9	29.42	29.36	29.33	29.31	29.46	29.36	29.17	29.13	29.22
C10	93.96	95.37	93.87	93.81	95.16	94.02	93.84	93.56	93.48
O4	−86.07	−80.33	−86.00	−87.02	−80.44	−83.05	−81.29	−92.96	−98.21
O5	236.36	238.25	236.50	236.48	238.43	232.27	236.34	228.09	230.91
O7	238.36	228.99	238.71	238.92	236.84	238.14	238.86	239.33	239.66
H2	24.75	24.76	24.77	24.79	24.80	24.75	23.18	24.81	24.80
H5	21.56	21.70	21.54	21.52	21.69	21.60	21.50	21.79	21.72
H6	25.47	25.61	25.49	25.50	25.55	25.41	25.48	25.42	25.51
H7	28.01	23.10	28.04	28.05	24.25	28.01	28.05	28.06	28.08
H8	26.16	26.09	26.18	26.19	25.88	26.17	26.17	26.17	26.19
C1′	70.14	69.51	72.36	72.23	69.45	69.80	69.20	69.53	69.41
C2′	57.16	57.26	57.02	57.59	57.29	56.94	52.43	56.87	56.86
C3′	78.26	78.44	77.90	78.38	78.47	78.17	81.59	78.17	78.20
C4′	34.15	34.41	31.81	31.97	34.45	34.22	34.56	34.32	34.39
C5′	81.36	81.45	81.87	80.84	81.46	81.54	78.87	81.56	81.69
C6′	52.82	52.75	53.06	52.96	52.75	52.91	56.23	53.07	53.06
O4′	255.15	255.63	249.05	255.38	255.70	255.20	255.86	255.34	255.49
H2′	24.69	24.69	24.73	24.78	24.71	24.67	23.97	24.71	24.69
H3′	24.93	24.99	24.98	25.04	24.96	24.92	25.39	24.94	24.94
H4′	28.45	28.49	23.41	25.02	28.49	28.46	28.49	28.47	28.50
H5′	25.37	25.39	25.36	25.06	25.39	25.39	25.03	25.41	25.45
H6′	23.99	23.96	24.07	24.09	23.97	24.00	24.68	24.13	24.09
O1″	331.58	331.48	333.48	327.11	327.55	332.08	332.19	332.46	331.44
C2″	134.98	134.31	136.36	134.38	133.38	135.24	135.77	135.06	135.34
C3″	153.11	154.17	153.69	153.43	154.18	153.44	154.01	153.35	153.18
N4″	236.98	236.48	234.26	237.80	241.87	237.74	236.04	235.31	237.33
C5″	153.11	154.19	153.50	153.56	154.18	153.38	153.13	153.76	153.22
C6″	134.98	134.31	136.55	134.69	133.39	135.13	135.52	135.16	135.31
H2″a	28.94	28.74	28.71	28.70	29.01	28.85	29.34	28.92	28.35
H2″b	28.95	29.14	28.84	28.67	28.72	28.91	29.62	28.73	28.97
H3″a	29.67	29.30	29.73	29.50	29.88	29.20	29.92	29.65	29.35
H3″b	30.02	29.88	29.47	29.86	29.30	30.02	30.60	29.98	29.96
H4″	31.97	31.85	31.57	31.77	32.13	31.94	31.97	30.98	31.98
H5″a	29.67	29.30	29.82	29.48	29.88	30.14	29.67	29.60	29.46
H5″b	30.02	29.88	29.49	29.87	29.30	29.51	29.94	29.73	29.97
H6″a	28.94	28.74	28.65	28.66	29.01	28.96	28.95	28.90	28.65
H6″b	28.95	29.14	28.82	28.67	28.72	28.88	29.06	28.70	28.89

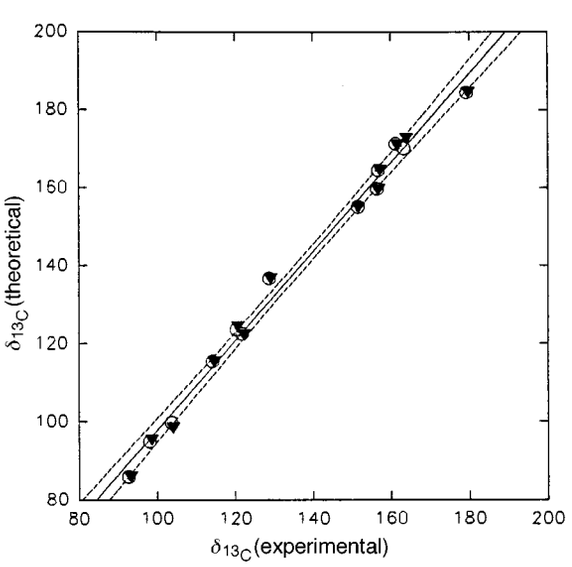


Fig. 6 Correlation between calculated and experimental values of ^{13}C chemical shifts in genistein **1** and its complex **2a**. The 0.95 confidence range is also shown. ○ Data for genistein **1**, ▼ data for genistein–morpholine complex **2a**.

niques applied have enabled us to establish the structure of the crystalline complex with morpholine, bond lengths and angles along with the distribution of the electronic charge and the nature of the intra- and inter-molecular processes regarding the hydroxy proton exchange and protonated amine in the complex.

The present data indicate three major motifs which may contribute to the genistein pharmacophore in its various biological configurations: (1) a planar motif consisting of condensed aromatic and unsaturated pyrone rings and a six-membered ring created by the strong intramolecular hydrogen bond between the carbonyl group in position 4 and the O5–H5 proton. (2) Flexible *p*-hydroxyphenyl in position 3, twisted from the plane of chromenone by *ca*. 64°. (3) Dynamic protons on oxygen O7 and O4′ capable of facilitating exchange of bound protons or amine cation in aqueous media.

The complexation with amines does not alter the condensed aromatic rings appreciably except that the C7–O7 bond is shortened and the negative charge is increased on C6 and C8 carbon atoms as disclosed by quantum mechanical calculations and crystallographic measurements, even though these data refer to the gas phase and the solid state, respectively. The degree of twist of the flexible aromatic ring is not changed as can be judged from the fact that values of NOEs measured

between H2 and H2′ protons in pure genistein and in its amine complex were the same. The most important change upon complexation with amines is the increase in the exchange rate of the hydroxy groups with H_2O leading to their coalescence and to the broadening of a water signal.

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