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NMR and Computational Study on the Anomeric Effect in *cis*/*trans*-3,4-Dihydro-2-alkoxy-4-substituted-2*H*,5*H*-pyrano[3,2-*c*][1]benzopyran-5-one Derivatives

Rita Annunziata,[1]* Laura Raimondi,[1] Gian Mario Nano,[2] Giovanni Palmisano[2] and Silvia Tagliapietra[2]

[1] Dipartimento di Chimica Organica e Industriale, Università degli Studi di Milano, Via Golgi 19, 20133 Milan, Italy
[2] Dipartimento di Scienza e Tecnologia del Farmaco, Università di Torino, Via Giuria 9, 10125 Turin, Italy

The configuration and conformation of *cis*/*trans*-3,4-dihydro-2-alkoxy-4-(alkyl- or aryl-substituted)-2*H*,5*H*-pyrano[3,2-*c*][1]benzopyran-5-one derivatives were studied by combined NMR and computational analyses. The *cis*/*trans* configurational assignments of all diastereoisomers were achieved via 2D NOESY experiments. The conformational analysis of *cis* compounds, performed via NMR and computational studies, allowed the establishment of a preference for the conformer with both substituents in the pseudo-equatorial orientations. For *trans* derivatives only the molecular mechanics analysis produced meaningful results, suggesting a general prevalence for the conformer bearing the 2-alkoxy group in a pseudo-axial and the 4-substituent in a pseudo-equatorial orientation.

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INTRODUCTION

The pyranocoumarins in which the pyran ring is fused to the heterocyclic ring of 2*H*-1-benzopyran-2-one (coumarin) are well known compounds. Indeed, many such products derived from prenylation of the heterocyclic ring *ortho* to a hydroxyl followed by cyclization have been found in secondary metabolites of plants.[1] Our previous studies aimed at elucidating the chemistry of coumarin derivatives led to the development of new procedures for the synthesis of various natural heterocyclic adducts.[2] As part of this study, we synthesized the 3,4-dihydro-2-alkoxy-4-substituted-pyranocoumarins **1**–**13** (Scheme 1) that are unusual among natural products and that have been less studied from a chemical point of view.

This paper reports a configurational and conformational investigation on this new series of pyrano derivatives by combined NMR spectroscopy and molecular mechanics calculations. Our efforts were directed towards a detailed understanding of the conformational preference of these compounds. Moreover, the particular feature of 2-alkoxy-4-substituted-pyranocoumarins **1c,t**–**13c,t** provides the possibility of investigating the anomeric effect in these systems. [The anomeric effect is the preference for the axial position exhibited by six-membered heterocyclic substituted at C-2 with an electronegative group; this can be used to measure the stability of an axial over an equatorial substitution over the expected value in cycloexane (where the equatorial substituent is favoured)[3]].

* Correspondence to: R. Annuziata. E-mail: research@iumchz.chimorg.unimi.it

RESULTS AND DISCUSSION

The reaction of 4-hydroxycoumarin with aldehydes gives 3-arylidenepyranochromandiones which were found to combine *in situ* with a variety of enol ethers to afford the corresponding 3,4-dihydro-2-alkoxy-4-substituted-2*H*,5*H*-pyrano[3,2-*c*][1]benzopyran-5-ones **1**–**13** (Scheme 1). These inverse electron demand [4 + 2] hetero-Diels–Alder reactions are dominated by HOMO (dienophile)–LUMO (diene) interactions and are facilitated by the presence of electron-donating groups in the dienophile and of electron-withdrawing groups in the 4π partner.

Under the described conditions, the 4-chlorophenyl (**4**) and the 3-indole (**11**) derivatives were obtained as single diastereoisomers, and compounds **1**–**3**, **5**–**10**, **12** and **13** as pairs of 2,4-*cis*/*trans* separable diastereoisomers. In principle each *cis* and *trans* diastereoisomer can exist in at least two conformers *A*,*B* and *A*′,*B*′ (see Fig. 1), featuring the OR and the X residues in a pseudo-axial or pseudo-equatorial position (the conformations of many unsaturated six-membered heterocyclic rings are usually considered in terms of equilibria of interconverting half-chair forms[4]).

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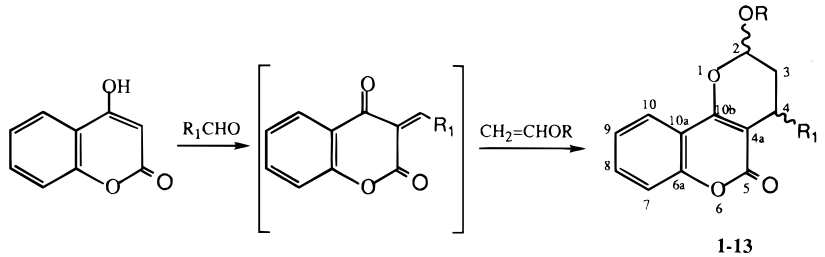

1-13

Compound	R	R_1	Cis / Trans Ratio
1	OCH_2CH_3	C_6H_5	60 / 40
2	"	o-$NO_2C_6H_4$	80 / 20
3	"	p-$NO_2C_6H_4$	75 / 25
4	"	p-ClC_6H_4	100 / 0
5	"	o-$CH(O)C_6H_4$	70 / 30
6	"	9-Anthryl	78 / 22
7	"	p-$NMe_2C_6H_4$	85 / 15
8	"	3-Pyridinyl	56 / 44
9	"	2-Furyl	67 / 33
10	"	CMe_3	40 / 60
11	"	1H-Indol-3-yl	100 / 0
12	$OCMe_3$	C_6H_5	68 / 32
13	"	o-$NO_2C_6H_4$	63 / 37

Scheme 1.

Except for **10**, the *cis* isomer was always predominant (see Scheme 1), probably being favored by the absence of a steric interaction between the two diequatorial substituents in the *B* conformer.

Configurational analysis

In addition to standard ^1H and ^{13}C NMR spectra, proton–proton decoupling, carbon–proton selective decoupling, 2D COSY and NOESY[5] experiments were performed to obtain the complete assignment of proton chemical shifts and elucidate unambiguously the structures of **1–13**. Generally, the knowledge of vicinal ^1H–^1H heterocyclic coupling constants ($^3J_{HH}$) and/or ^1H,^{13}C resonances has played a key role in the determination of the configuration and conformation of pyranosyl rings. These parameters can be obtained from the 1D and 2D ^1H NMR spectra; the $^3J_{HH}$ constants frequently have been correlated with the molecular structure and conformation via appropriate relationships.[6] The relevant ^1H and ^{13}C chemical shift assignments are reported in Table 1 and are in agreement with the spectroscopic data relative to the coumarin adducts previously described.[2]

In order to establish unequivocally the *cis*/*trans* configuration of diastereoisomers **1–13** a nuclear Overhauser effect (NOE) study was performed. In the *cis* isomers

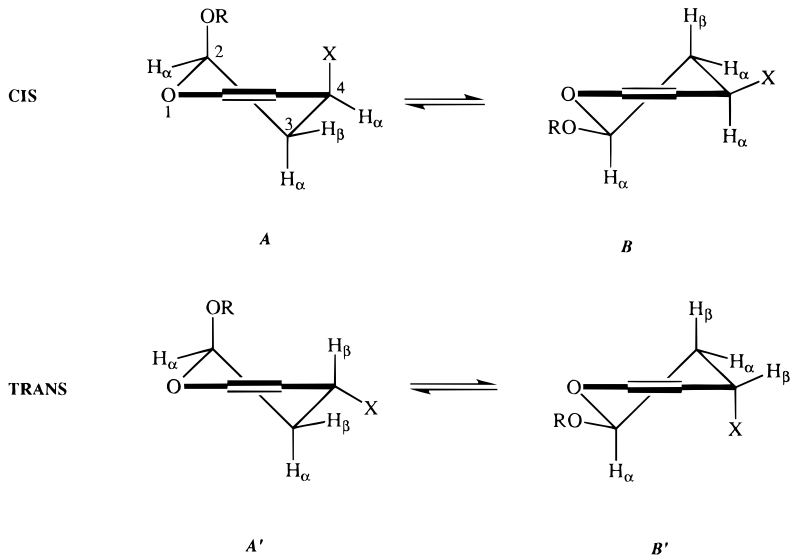

Figure 1. *A*, *B* and *A'*, *B'* are the proposed conformers for *cis* and *trans* diastereoisomers, respectively.

Table 1. Significant ^1H and ^{13}C chemical shifts (δ, ppm) and ^1H–^1H coupling constants (*J*, Hz) of compounds 1–13 in $CDCl_3$ solvent

	δ (ppm)			J_{cis} (Hz)		J_{trans} (Hz)		δ (ppm)		
	H-2	H-3[a]	H-4	H_2,H_3	H_4,H_3	H_2,H_3	H_4,H_3	C-2	C-3	C-4
1 *cis*	5.43	2.47; 2.32	4.15	2.5	6.0	4.6	6.0	100.1	35.8	35.1
trans	5.27	2.28	4.26	3.0[b]	6.0	7.7[b]	6.0	99.3	35.8	35.4
2 *cis*	5.50	2.58; 2.28	4.77	2.6	7.8	4.0	4.0	99.65	30.5	33.7
trans	5.37	2.62; 2.41	4.83	2.5	6.4	6.5	6.4	99.0	31.0	34.7
3 *cis*	5.49	2.47; 2.30	4.08	2.9	7.5	2.9	2.9	99.15	34.3	33.9
trans	5.33	2.38; 2.13	4.29	2.6	6.0	6.0	6.0	98.5	34.9	35.7
4 *cis*	5.46	2.43; 2.30	4.09	2.8	7.0	4.0	4.5	99.5	35.0	33.9
5 *cis*	5.33	2.57; 2.22	5.27	2.6	6.5	2.6	4.5	99.7	30.1	34.2
trans	5.22	2.40; 2.17	5.22	4.5[c]	7.0[c]	7.0[c]	2.0[c]	99.25	31.3	35.0
6 *cis*	5.57	2.85; 2.62	5.68	2.0	7.2	10.0	12.3	103.0	32.5	34.2
trans	5.74	2.80; 2.47	5.74	1.8[c]	7.2[c]	1.5[c]	12.3[c]	98.8	33.8	27.8
7 *cis*	5.41	2.43; 2.27	4.06	2.5	6.7	5.5	6.7	100.4	34.7	36.4
trans	5.23	2.24	4.18	5.0	6.5	5.0	6.5	99.6	34.6	35.9
8 *cis*	5.48	2.43; 2.33	4.13	3.0	7.0	3.0	3.5	99.2	34.4	31.8
trans	5.27	2.33; 2.17	4.22	2.6	6.0	7.0	6.0	98.75	35.7	32.9
9 *cis*	5.46	2.57; 2.20	4.17	3.5	7.0	3.5	3.5	98.9	31.2	27.7
trans	5.37	2.45; 2.13	4.28	2.3	5.7	9.0	3.0	100.15	32.2	29.8
10 *cis*	5.62	2.43; 1.78	2.87	5.0	1.7	10.0	6.5	100.6	32.9	38.1
trans	5.01	2.28; 2.08	2.97	5.2	3.8	5.2	8.7	102.4	31.1	37.85
11 *cis*	5.20	2.56; 2.22	4.57	2.5	2.5	9.0	5.0	100.6	33.3	27.8
12 *cis*	5.67	2.40; 2.27	4.16	2.5	7.0	5.5	7.0	95.1	37.3	35.0
trans	5.50	2.33; 2.12	4.25	2.5	5.8	8.0	4.7	94.0	37.0	35.5
13 *cis*	5.73	2.55; 2.29	4.79	3.5	5.0	3.5	3.5	94.4	34.8	30.6
trans	5.63	2.55; 2.12	4.82	2.0	6.5	6.0	6.5	93.7	36.0	31.2

[a] AB system.
[b] The J_{cis} and J_{trans} assignment can be reversed since the methylenic protons H-3 are less than 10 Hz apart.
[c] The coupling constant assignments are tentative since H-2 and H-4 isochronous.

the H-2 and H-4 protons must be spatially close in the *B* conformer (giving rise to an appreciable NOE), whereas in the *trans* isomers a weak or non-existent NOE effect [depending on the two dihedral angles C(2)—C(3)—C(4)—H and H—C(2)—C(3)—C(4)] can be expected. The 2D NOESY phase-sensitive pulse sequence[7] was employed with a mixing time, τ_m, closer to the relaxation time T_1 of the heterocyclic pyranosyl protons. The NOESY experiments were performed on compounds **2–4**, **6–9**, **11** and **13** as single diastereoisomers and on **1**, **5**, **10** and **12** as *cis*/*trans* mixtures of different composition. All 2D NOE spectra of *cis* derivatives reveal a magnetization transfer between the H-2 and H-4 hydrogens. This configurational assignment was confirmed by the fact that both H-2 and H-4 displayed a strong correlation with the same diastereotopic methylene proton H-3, shown as H-3_α in Fig. 1. In contrast, in the *trans* isomers, H-2 and H-4 were strongly correlated with different H-3 methylene protons: H-2 with H-3_α and H-4 with H-3_β (see Fig. 1). Indeed, the H-3 methylene protons give an AB system (here and subsequently, A and B are the low- and high-field hydrogens, respectively) generally separated enough (**2–6** and **8–13**) to distinguish the corresponding cross peaks in the NOESY maps. It was impossible to distinguish these correlations for the *trans* compounds **1** and **7**, the chemical shifts of H-3_A and H-3_B being less than 10.0 Hz apart, and for **5** and **6**, H-2 and H-4 being isochronous.

As a typical example, the 2D NOESY map of **12** (in a 50:50 *cis–trans* mixture) is displayed in Fig. 2.

From the NOESY spectra we could see that, with the exception of **6** and **9**, in the *cis* compounds H-3_α (H-3 proton on the same side of H-2 and H-4) is always H-3_A of the AB system. In agreement with the general behaviour of six-membered rings[8] (an equatorial proton is at lower field than an axial proton), the above trend should suggest that the *cis* derivatives prefer H-3_α and, consequently, for substituents at C-2 and C-4, the pseudo-equatorial position (see Fig. 1, conformer *B*). This hypothesis has to be accepted cautiously, however. First, because in the heterocyclic ring there are additional steric relationships to the heteroatom which may have long-range effects on chemical shifts. Second, the presence of the coumarin moiety and of the double bond can exert influences of the same order of magnitude as the difference between axial and equatorial shifts.

When the chemical shifts of H-3 protons were more than 10.0 Hz apart, a suitable trend was also observed with *trans* compounds: H-3_α (*cis* to H-2) and H-3_β (*cis* to H-4) were the H-3_B and H-3_A protons of the AB system, respectively. The derivative **9** was an exception to this trend.

Several aspects of the data of Table 1 must be discussed. The H-2 proton exhibits a lower field resonance in the *cis* diastereoisomers, with the exception of **6**. Indeed, in the *trans*-anthryl derivative **6**, the anisotropic effect of the very large aromatic ring and a large preference (see below) for the *A′* conformation (Fig. 1), bearing H-2 in a pseudo-equatorial position, prevail. The trend is reversed for H-4, which is more upfield in

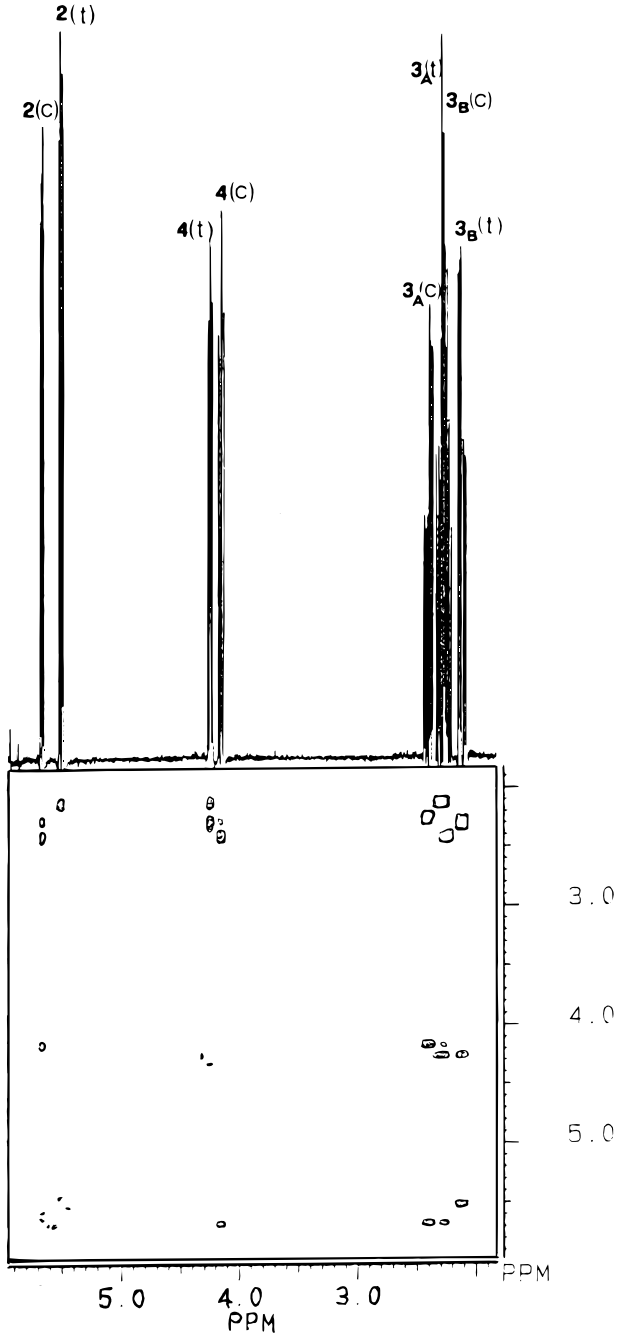

Figure 2. (^{1}H,^{1}H) NOESY contour plot for **12** in an equimolecular mixture of *cis* (c) and *trans* (t) isomers, recorded at 28 °C. The aromatic region is not displayed. Only the positive contours are plotted. The experiment was performed in $CDCl_3$ with a mixing time of 1.5 s and a relaxation delay of 6.0 s (see Experimental).

the *trans* than in the *cis* derivatives, **5** being an exception. Apart from **5** and **6**, these results seem to suggest a general rule: the chemical shifts of H-2 and H-4 can be considered diagnostic for the *cis*/*trans* configurational assignment of 3,4-dihydro-2-alkoxy-4-substituted-2*H*, 5*H*-pyrano[3,2-*c*][1]benzopyran-5-one derivatives.

Conformational studies

The *cis*/*trans* configuration of **1**–**13** having been assigned, our investigation was focused on the conformational analysis of these compounds by NMR and computational studies. Indeed, the variations in magnitude of the NOE effects and/or coupling constants between the protons of the pyranosyl ring indicated that not all compounds have the same conformational preference. The position of the conformational equilibrium is influenced by the usual conformational factors, such as the preference of a bulky, non-polar group for the equatorial position, the 1,3-axial–pseudo-axial repulsion, etc. Additionally, other effects must be taken into account: the anomeric effect of the alkoxy group and the allylic effect (preference for the pseudo-axial orientation) due to the double bond conjugated with a carbonyl group. Conformational studies on other unsaturated heterocyclic systems showed that many six-membered cyclic compounds, including benzodioxans[9] and 2,3-dihy-

dropyrans,[10] prefer to exist in the rapid interconverting half-chair form. The half-chair conformation is commonly assumed for the dihydropyran rings. However, a sofa conformation,[11] in which all ring atoms but C-2 are coplanar, has also been proposed. Clark-Lewis[12] suggested that the energy difference between the half-chair and the sofa form, which can arise when the double bond conjugates with a carbonyl group present in the near ring, is very small.

Only a few conclusions can be derived from the analysis of the vicinal heterocyclic coupling constants. Even the *cis*/*trans* configuration assignment could not be established from the coupling constants of H-2 and/or H-4 with H-3 protons.[13] Indeed, if it is assumed that the 4-substituent is in the equatorial position on a half-chair dihydropyran ring and that all the vicinal *J*'s have the same sign,[14] the Karplus relationship predicts that $J_{3\alpha,4} + J_{3\beta,4}$ will be significantly smaller for 2,4-*trans* than for 2,4-*cis* isomers. In contrast, in our case, for the average value the sum of the ${}^3J_{cis} + {}^3J_{trans}$, concerning H-2/H-3 or H-4/H-3, was not always smaller in the *trans* than the *cis* isomers (see Table 1). Generally, for each *cis*–*trans* pair, the J_{cis} of H-2 and H-4 with H-3 showed very similar values in both *cis* and *trans* derivatives. For the heteroatom effect, $J_{\text{H-2,H-3}} < J_{\text{H-4,H-3}}$ was found. Higher but not meaningful differences showed the J_{trans} values of H-2 and H-4 with H-3 in *cis*/*trans* isomers. This coupling constant behavior seems to indicate a fast equilibrium between the two half-chair forms in both series of diastereoisomers.

Several previous papers emphasized the limitations of NMR methods that use the Karplus equation to derive quantitative data for dihydropyranyl ring compounds. Nevertheless, Günther and Jikeli[15] reported for 5,6-dihydro-2-alkoxy-4-substituted-2*H*-pyran systems that in the *trans* compounds the 4-phenyl and the 2-alkoxy groups prefer the pseudo-equatorial and the pseudo-axial position, respectively. In *cis* compounds a slight dominance of the conformer with both groups in a pseudo-axial position was indicated but the 2,3-annulation alters this preference due to *peri* interactions with the 4-substituent. These conclusions were subsequently supported by other workers.[16] In our system the annulation effect was further increased by the presence of a vicinal carbonyl group which conjugates with the double bond of the dihydropyran ring.

We tried to calculate the percentages of the *A*, *B*, *A*′ and *B*′ conformers using the well known time-average equation $J_{(av)} = p_A J_A + p_B J_B$, assuming as limiting coupling constants the values found for the 9-anthryl derivative **6**: $J_{4\alpha,\text{H-}3\beta} = 12.3$ Hz (*cis* conformer *B*) and $J_{4\beta,\text{H-}3\alpha} = 1.5$ Hz (*trans* conformer *B*′) (see Table 1). Unfortunately, the Karplus equation does not allow a quantitative analysis of the conformational composition at the equilibrium, because the conformational preferences determined with this method show a random behavior.

Therefore, a different approach was attempted via NMR experiments. The 2D NOESY spectra of **1**–**13** provided a clear picture of the conformational preferences. Indeed, the essential feature of a quantitative 2D NOESY experiment is the relationship between the intensity of the cross peaks and the inter-proton distances which are closely connected with the conformer populations. The maps revealed interesting variations in the volume of the cross peaks due to the NOE between the pyran ring protons, indicating that not all compounds have the same conformational preferences. The analysis of these inter-proton distances can be taken as an indicator of the consistency of the NOE approach to determine the conformational equilibrium in solution.

In our cases the proton–proton distances (r_{ij}) between H-2 and H-4 and between H-2, H-4 and H-3 methylenic protons could be obtained from the s_{kl} values between H-3_A and H-3_B, separated by a known distance of 1.79 Å [this value, obtained from the molecular mechanics calculations (see below) was employed instead of usual 1.75 Å], using the equation[17]

$$r_{ij}/r_{kl} = (s_{kl}/s_{ij})^{1/6} \quad (1)$$

Indeed, for isotropic overall motion under extreme narrowing conditions, the s_{kl}/s_{ij} values (cross-relaxation due to dipole–dipole relaxation between the spins *k*, *l* and *i*, *j*, respectively) can be measured experimentally from the 2D maps by integrated volumes of the relative cross peaks. Subsequently, the correlation between the H-2 and H-4 distances and the conformer populations in the case of the *cis* diastereoisomers of **1**–**13** was performed using the time-average equation

$$r_{2,\,4(\text{av})} = p_{\text{ax, ax}}\, r_{2,\,4(\text{ax, ax})} + p_{\text{eq, eq}}\, r_{2,\,4(\text{eq, eq})} \quad (2)$$

in the assumption that the limiting H-2/H-4 distances relative to the pure conformers *A* and *B* are $r_{2,\,4(\text{ax, ax})} =$ 2.6 Å and $r_{2,\,4(\text{eq, eq})} = 4.3$ Å, respectively. These values were obtained for the limiting *cis* conformers *A* and *B* performing molecular mechanics studies.

Compounds **1**–**13** were subjected to molecular mechanics calculations to confirm the NMR-derived conformational analysis. We used the MM2* force field (an implementation of Allinger's MM2 force field[18] included in version 4.5 of the MacroModel/Batchmin package).[19] A conformational search was performed using the systematic pseudo-Monte Carlo/energy minimization (MC/EM) procedure of Goodman and Still.[20] The search proceeds by altering, in a systematic way, the torsional angles of a starting structure (a minimum energy conformation). Thus, a new geometry is generated, and is subjected to an energy minimization. This process produces a new minimum energy conformation, which is tested for duplication with previously found conformations (for details, see Experimental). The procedure alternates random changes of coordinates (which allow wide sampling of the potential energy surface) and energy minimizations. The MC/EM procedure has been proved to be among the most effective methods at finding all (or nearly all) low-energy conformations of flexible molecules (for a review on the problem of locating all minima on the conformational potential energy surface, see Ref. 21). All calculations were performed *in vacuo*. Both referees observed that a solvent model could be used instead of an *in vacuo* calculation. In MacroModel/Batchmin, only GB/SA solvent models[22] are implemented, and those models still present some inadequacies. Owing to the low polarity of the chloroform ($\varepsilon = 4.8$), *in vacuo* calculations compare successfully with NMR data; thus this protocol was adopted in the present study (see, for instance, Refs 2b and 23). A complete evaluation of the electrostatic

Table 2. Equilibrium ratio of conformers *A* and *B* for *cis* and *A′* and *B′* for *trans* diastereoisomers relative to the dihydropyran ring of compounds 1–13 (data obtained from 2D NOESY experiments and MM calculations)

	cis				*trans*	
	NOESY data		MM data		MM data	
Compound	*A*	*B*	*A*	*B*	*A′*	*B′*
1	42	58	45	55	62	38
2	13	87	19	81	88	12
3	41	59	48	52	76	24
4[a]	48	52	48	52	[a]	
5	26	74	34	66	76	24
6	3	97	0	100	100	0
7	50	50	52	48	62	38
8	60	40	58	42	71	29
9	70	30	75	25	14	63
10	10	90	[b]		[b]	
11[a]	35	65	42	58	[a]	
12	12	88	64	36	54	46
13	30	70	36	64	84	16

[a] The *trans* diastereoisomer was not obtained.
[b] Only one conformation was observed: see text.

effects for these compounds and their reproduction with molecular mechanics methods was beyond the scope of the present study (for instance, also the problem of a correct evaluation of the atomic charges within the force field should be addressed on a rigorous basis!). For a review of electrostatic treatments in molecular mechanics force fields, see Ref. 24. For the *cis* isomers, the agreement between the MC/EM and the NMR data, reported in Table 2, is excellent. The pseudo-diequatorial conformation *B* is the most stable for **1**–**6**, **11** and **13**, mainly because of the steric requirements of the R_1 substituent. The steric effects clearly overwhelm the anomeric effects, which would favor the pseudo-diaxial conformation *A*. For instance, on passing from **2** to **3**, i.e. moving the nitro group from the *ortho* to the *para* position on the phenyl ring, the pseudo-diequatorial conformation *B* drops from >80% to 52–58% of the equilibrium conformational mixture, a value similar to those found for **1**, **4** and **7** (see Table 2). A carbonyl group is less sterically demanding than a nitro group, and this is reflected in the difference of the *A*/*B* ratios for **2** *vs.* **5**. The limiting case is the *cis*-9-antracenyl derivative **6**, where only the diequatorial conformation *B* is significantly populated at 301 K.

This behavior shows that the anomeric effect and the conjugation of the coumarin carbonyl group with the double bond of the pyran ring destabilize the 4-aryl group in the pseudo-equatorial position only when its steric hindrance is low. To conform this, we can compare compounds **1** and **12** with the same substitution at C-4 (phenyl group) and different substitution at C-2 (*O*-ethyl group for **1** and *O*-*tert*-butyl group for **12**). In this case, changing the steric requirement of the alkoxy group, the percentage of the diequatorial conformer *B* goes from 58% for derivative **1** to 88% for derivative **12** (see NMR data in Table 2). The disagreement between NMR and MC/EM data for **12** stands as the only exception, and can be ascribed to an overestimation (for MC/EM calculations) of the steric repulsion between the phenyl ring and the *tert*-butyl group in the *A* conformation.

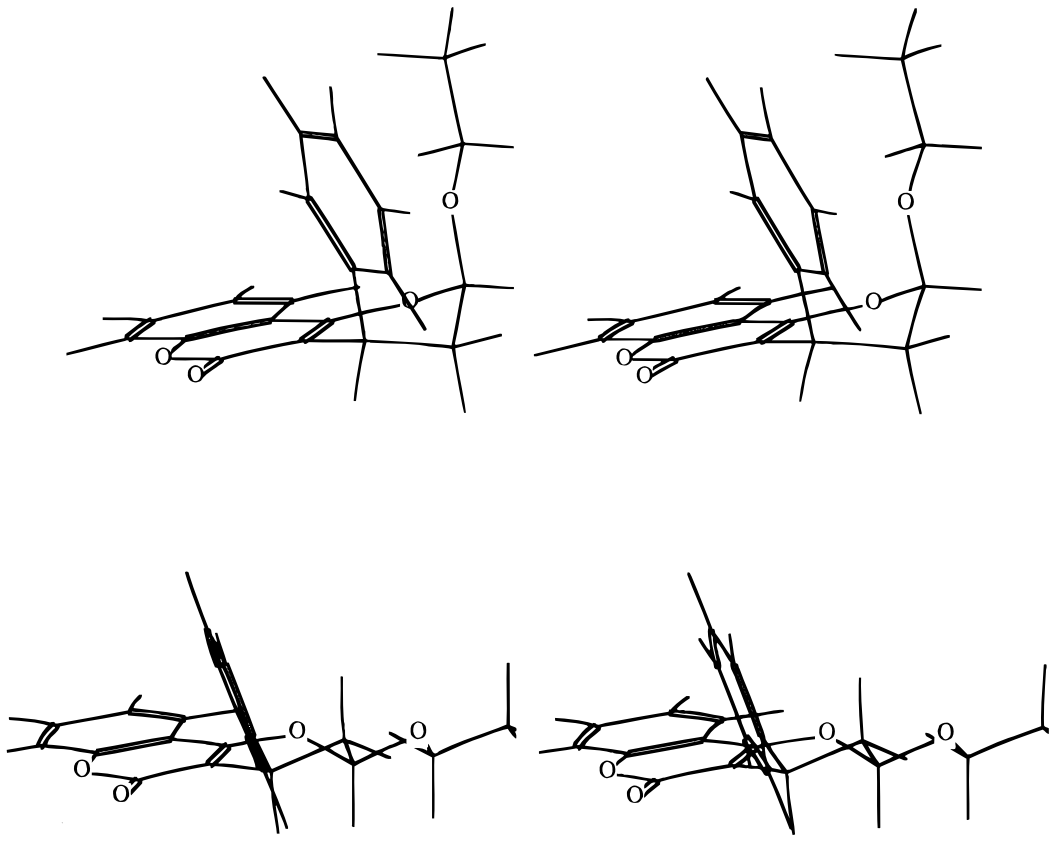

Figure 3. Minimum energy conformations for *cis*-**1**. Top, *A* conformation; bottom, *B* conformation.

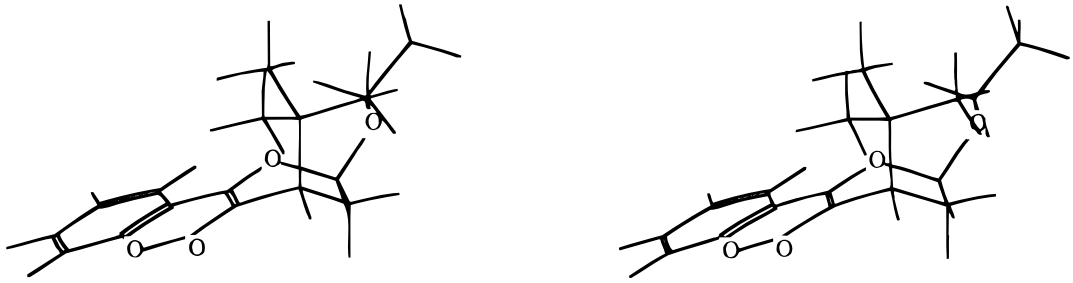

Figure 4. Minimum energy conformation for *cis*-**10**.

In Fig. 3 are shown the two lowest energy *A* and *B* conformations located for *cis*-**1**. The dihydropyran ring features three sp^2 atoms, hence a straightforward assignment of its conformation as half-chair or sofa is not always immediate. Generally, the *A* conformations are of the sofa-type, while the *B* family (in which the two substituents are in a pseudo-equatorial arrangement) prefers the half-chair conformation. A limiting case is that of **10**, as clearly appears from the stereoview shown in Fig. 4: the dihydropyran ring is in a distorted boat conformation, where the *tert*-butyl residue is almost pseudo-axial (to avoid the steric repulsion with the C=O group of the coumarin ring) and the *O*-ethyl group is neither pseuso-axial nor pseudo-equatorial! The steric effect due to the *tert*-butyl group is not present in all the other compounds, featuring always a flat aromatic ring; in these cases, the preference for the pseudo-equatorial conformation is reinforced by a favorable electrostatic interaction between the two almost stacked π systems: the aryl ring and the carbonyl group.

The pseudo-axial *A* conformation becomes predominant when the R_1 substituent is a heteroaromatic ring (**8** and **9**); for the 3-indole derivative **11** the steric effect

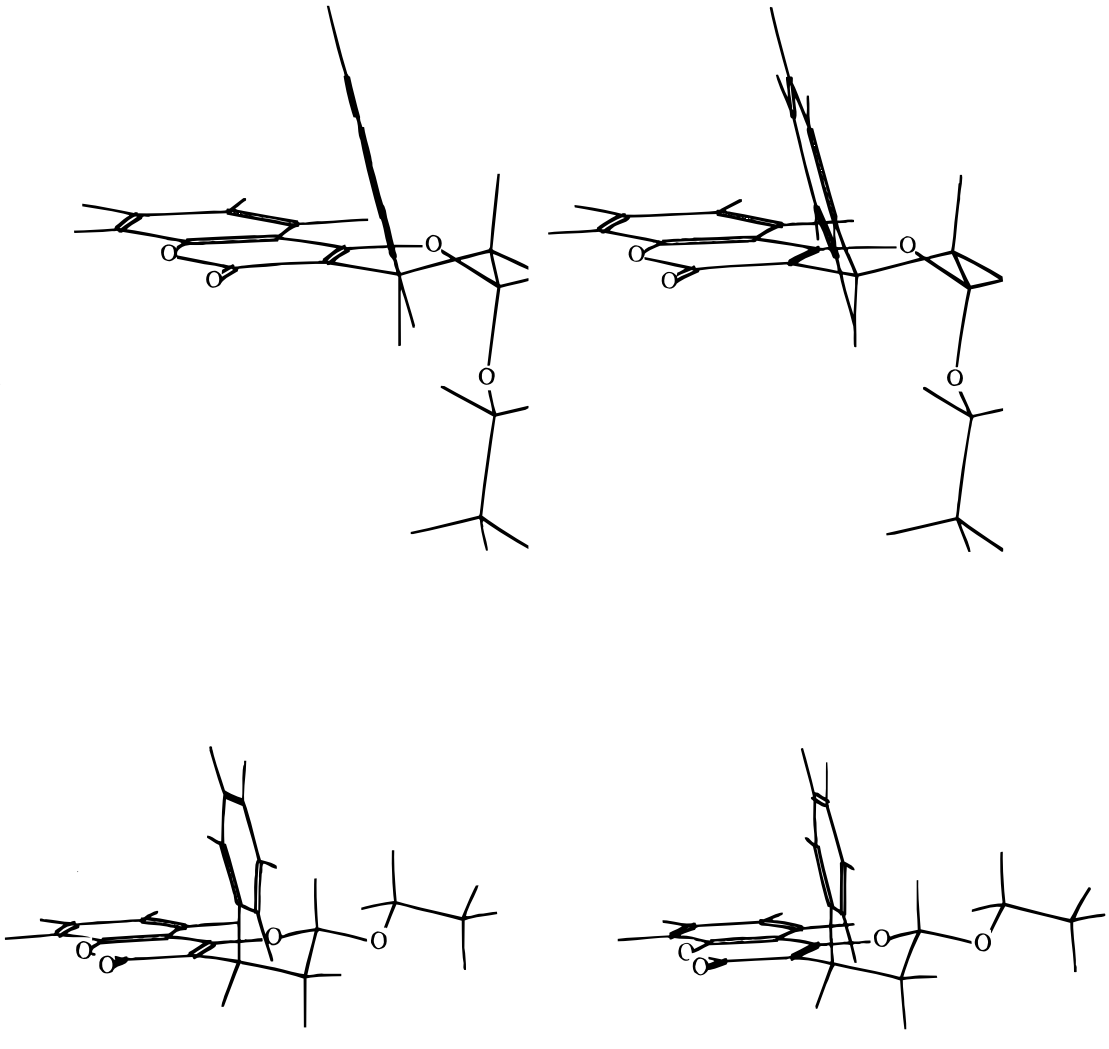

Figure 5. Minimum energy conformations for *trans*-**1**. Top, *A*′ conformation; bottom, *B*′ conformation.

due to the bulkiness of R_1 prevails. This is due to the electrostatic interactions between the carbonyl oxygen and the oxygen (**9**) or the nitrogen atom (**8**) of the heteroaromatic ring. These interactions destabilize the pseudo-diequatorial *B* conformer. The increasing preference for the axial conformer observed on replacing the nitrogen (*A*:*B* ratio = 60:40) with a more electronegative oxygen atom (*A*:*B* ratio = 68:32) seems to support this explanation.

It has been possible to compare the data obtained *via* both NMR and MC/EM experiments only in the case of *cis* isomers. Indeed, an accurate analysis of all NMR and MC/EM data allowed us to recognize the impossibility of performing an analogous study for the *trans* derivatives. The *trans* H-2 and H-4 protons are too far away to allow appreciable Overhauser effects. Moreover, the distances between H-2, H-4 and both H-3 protons in the *A*′/*B*′ conformers are alike, as confirmed by MC/EM calculations.

For the *cis* derivatives, excluding **12**, NMR- and MC/EM-derived *A*:*B* ratios show a quantitative correspondence. We can thus assume that the MC/EM-calculated ratios for *trans* isomers are at least qualitatively reliable, an assumption derived from the impossibility of obtaining significant NOE data in these cases, as stated before.

The calculated *A*′:*B*′ ratios are also given in Table 2. In all cases except *trans*-**10**, the OR group adopts a pseudo-axial conformation. This effect is reinforced by the bulkiness of the R_1 groups, which obviously prefer to occupy the pseudo-equatorial position for steric reasons (compare **1**, **3** and **7** with **5**, **2** and **9**). On passing from an *O*-ethyl to an *O*-*tert*-butyl substituent, the anomeric effect is obviously counteracted by the steric hindrance of the OR group (compare **1** with **12**). Figure 5 shows the *A*′ and *B*′ lowest energy conformations for **1**. In all cases, the dihydropyran ring features a half-chair conformation. A significant exception is **9**, where the 2-furyl residue prefers the axial position, probably to avoid the electrostatic interactions[22–24] between the furan oxygen and coumarin carbonyl oxygen. Again, in the case of **10** only one conformation is observed in the MC/EM simulation.

CONCLUSION

The *cis*/*trans* 3,4-dihydro-2-alkoxy-4-substituted-2*H*, 5*H*-pyrano[3,2-*c*][1]benzopyran-5-one compounds **1**–**13** were investigated by 1D and 2D NMR analysis and MC/EM techniques to determine their *cis*/*trans* configuration and their conformational preferences. The 2D NOESY spectra gave us the possibility of assigning with certainty the *cis*/*trans* configuration. Moreover, the NMR analysis revealed a chemical shift behavior for the H-2 and H-4 protons correlated with the relative configuration; indeed, H-2 exhibited a lower field resonance in the *cis* than in the *trans* isomers whereas for H-4 the trend was reversed. A conformational analysis via both NMR and MC/EM data was performed only for *cis*-**1**–**13**; for the *trans* isomers only the computational approach was possible. In both cases, the anomeric effect shows up unless it is counteracted by strong steric effects.

EXPERIMENTAL

Synthetic procedure

The pyranocoumarins **1**–**13** were prepared from the corresponding aldehydes R_1CHO and the ethyl and/or *tert*-butyl enol ether with the following general procedure. A mixture of 1.0 mmol of 4-hydroxycoumarin, 1.5 mmol of aldehyde, ethylendiammonium diacetate (10.0 mg), ethyl vinyl ether (6.8 mmol) and powdered over-dried 4 Å molecular sieves (1.0 g) in dry dioxane (15 ml) was refluxed under nitrogen until thin-layer chromatography (TLC) indicated the disappearance of 4-hydroxycoumarin. Then the mixture was cooled, filtered through Celite and concentrated under reduced pressure. The residue was purified by column chromatography on Merk silica gel (70–230 mesh) using mixtures with 5–15% ethyl acetate in hexane as eluent. Except for **4** and **11**, the cycloadducts were obtained as mixtures of *cis*/*trans* diastereoisomers with the ratios reported in Scheme 1 and with yields between 50% and 70%. Compounds *trans*-**1** (m.p. 141–142 °C), *cis*-**2** (m.p. 177–180 °C), *trans*-**2** (m.p. 110–113 °C), *cis*-**4** (m.p. 136–138 °C), *cis*-**8** (m.p. 118–120 °C) and *trans*-**8** (m.p. 136–137 °C) were recovered as crystalline solids after on crystallization from diethyl ether.

NMR measurements

^1H and ^{13}C NMR spectra were acquired on a Bruker AM 300 spectrometer (^1H at 300.133 MHz and ^{13}C at 75.47 MHz) in 5 mm sample tubes; all experiments were performed at 28 °C in $CDCl_3$ as solvent. The $^{13}C\{^1H\}$ spectra were obtained using Waltz decoupling and were exponentially multiplied to give 0.8 Hz line broadening before Fourier transformation.

For all 2D NOESY NMR experiments, the samples were prepared by dissolving 5–6 mg of the required pyranocoumarin derivative in 0.75 ml of $CDCl_3$. The solution was degassed to remove any dissolved oxygen. Pure absorption 2D spectra were recorded using the NOESY pulse sequence $90°$–t_1–$90°$–τ_m–$90°$–t_2 and the method of phase cycling described by Marion and Wüthrich[5] with time-proportional phase incrementation (TPPI). The following parameters and procedures are commonly employed: spectral width 2800 Hz, 1024 × 1024 data matrix, 256 time increments of 80 transients each; Fourier transformations were carried out with zero-filling only in f_1, and using the shifted sine-bell apodization function in both dimensions. A mixing time of 1.5 s and a relaxation delay of 6.0 s were used.

The 2D COSY spectra were recorded with a 1024 × 1024 data matrix and 512 time increments of 16 scans each, in magnitude mode, and processed with zero-filling in f_1 and unshifted sine-bell apodization function.

The temperature employed in all 2D experiment was 28 °C.

Computational details

All calculations were performed on a SGI-Iris workstation and visualized using version 4.5 of the MacroModel/Batchmin package.[19] In each case, 1000 structures were generated from a minimum energy conformation with a systematic pseudo-Monte Carlo procedure[20] and minimized with the MM2* force field.[18,19] This procedure invokes a systematic search in place of a purely random search: the search begins at low torsional resolution (120°), searches all angles without duplicating coverage, then doubles the resolution, and so on. Torsional memory was activated during the search to prevent retracting of points in conformational space when starting from different geometries. At each step, the least-used structures were used as starting geometries for the generation of the new conformation (usage-directed MC/EM). All unique conformations were located for each compound in the first 200–300 MC/EM steps, while only duplicate conformers were located in the subsequent 700–800 steps; thus, all searches were stopped after location and minimization of 1000 structures (1000 MC/EM steps).[21] The searches were performed by varying the significant dihedral angles up to $\pm 180°$: four angles were varied in the dihydropyran ring (namely the torsions around bonds 1–10b, 1–2, 3–4 and 4–4a; see Scheme 1 for numbering) together with the torsions of its two substituents for a total of 6–8 angles (depending on the structure). The 1–2–3–4 torsional angle was selected for closure of the dihydropyran ring. A chirality check was imposed on the stereogenic carbons to avoid epimerization during the MC/EM procedure. The minimization procedure was performed using the truncated Newton conjugated gradient minimizer[25] until the energy gradient root mean square fell below 0.01 kJ $Å^{-1}$ mol^{-1}. All calculations were performed in *in vacuo* (see Conformational studies).[22–24] Only conformers within 12 kcal mol^{-1} (1 kcal = 4.184 kJ) from the global minimum of the search were saved. For elimination of duplicate conformers, only heavy atoms were compared: two structures were considered to be the same unless the least-squares superimposition of the compared atoms found one or more pair of equivalent atoms separated by more than 0.25 Å (default). All other parameters were the default parameters in MacroModel/Batchmin 4.5. A Boltzmann distribution was used to evaluate the ratios and the average distances mentioned in the text; only a 2.5 kcal mol^{-1} energy window was used for this purpose at 301 K.

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