SYNTHESIS AND MASS SPECTRAL STUDY OF TRANS- AND CIS-(±)-3-PHENYL-3,4-DIHYDROISOCOUMARIN-4-CARBOXAMIDES

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Abstract. A series of trans- and cis- (±)-3-phenyl-3,4-dihydroisocoumarin-4-carboxamides (4a-c) were synthesized and the electron impact (EI) ionization mass spectral fragmentation was described. For all studied compounds, both diastereomers follow the same fragmentation pattern, but differ on the abundance of the base peaks, which shows the opportunity to distinguish 3,4-dihydroisocoumarin diastereomers using mass spectrometry.

Key words: Isocoumarin, Dihydroisocoumarin, Diastereomers, Homophthalic anhydride, Mass Specrometry.

INTRODUCTION

3,4-dihydroisocoumarins possess an aryl substituent in position 3 are the core of various natural [1] and synthetic [2] compounds which exhibit a wide variety of pharmacological activities [1–5]. Because C-3 and C-4 carbon atoms are stereogenic centers both diastereomers (cis- and trans-) are possible for this type of compounds (see Fig. 1). The relative configuration can be attributed on the basis of the value of the vicinal coupling constant ($J_{3,4}$) between H-3 and H-4 atoms in the $^1$H NMR spectra [6, 7], but in some cases [8] the intermediate value (2–6 Hz) of the observed coupling constant (being characteristic both for the trans- and cis- diastereomers in synclinal conformation) can lead to wrong interpretation of the experimental data and thus to erroneously proposed relative configuration. This problem can be avoided if another spectral method distinguishes both diastereomers. Thus, the aim of this work is to study the fragmentation pattern of trans- and cis-(±)-
3-phenyl-3,4-dihydroisocoumarin-4-carboxamides (4a-c) by electron impact mass spectrometry (EIMS) in order to distinguish trans- and cis- diastereomers.

RESULTS AND DISCUSSION

Synthesis of compounds

Scheme 1 shows the reaction outcome and the synthetic path to compounds 4a-c. In the first synthetic step, following a previously reported by us protocols, [9,10] homophthalic anhydride (1) reacts with benzaldehyde to give the corresponding trans- and cis-(±)-3-phenyl-3,4-dihydroisocoumarin-4-carboxylic acids (2). It is noteworthy that the ratio among the isomers depends on the catalyst used in the reaction, so both basic (DMAP) and acidic catalysts (BF$_3$•Et$_2$O) were used. The cis-/trans-isomeric products were separate successfully by fractional recrystallization and the separated isomers were converted into corresponding acyl chlorides 3a-c in boiling benzene in the presence of thionyl chloride. Treatment of the latter with cyclic secondary amines (NuH) gave the target products 4a-c, which were isolated as crystalline products and purified by recrystallization from appropriate solvents.

![Chemical diagram](image)

The structure of the newly synthesized products was characterized by spectral methods (IR and $^1$H NMR) and elemental analysis. The interpretation of the $^1$H NMR spectra is in agreement with the literature data for compounds of this class [9,10] and showed the following characteristic signals: the signals for H-4 and H-3 protons appear as doublets within 3.99–4.61 and 5.67–5.94 ppm respectively; the
two groups of protons close to the amide nitrogen are unequivalent and thus independent signals in the region 2.43–3.62 ppm are observed for each proton; the signals for proton H-8 appear at lower field (7.77–8.04) compared to the other aromatic protons. This phenomenon is an indication for proximity of H-8 to the lactone carbonyl group. The relative configuration of trans- and cis-4a-c can be defined on the basis of the vicinal coupling constant value ($J_{3,4}$) between H-3 and H-4 atoms in the $^1$H NMR spectra (see Fig. 1). In general, values of 10–13 Hz suggest trans-configuration [6,7] and preferred antiperiplanar (ax,ax) conformation of the H-3 and H-4 atoms, but values of $J_{3,4} = 3–6$ Hz suggest, synclinal (sc or gauche) conformation and did not allow a distinction between cis (eq,ax) and trans (eq,eq) configuration of H-3 and H-4 atoms. On the other hand, 3,4-dihydroisocoumarins are conformationally flexible compounds, [8] and thus one can define incorrect relative configuration if the existing conformational equilibrium between conformers I and II is neglected. However, to overcome this problem another spectral method could be applied. It is known that the mass spectrometry is a proven technique for the differentiation of stereoisomers, but to the best of our knowledge no literature data is available for diastereomeric differentiation by MS of 3,4-disubstituted 3,4-dihydroisocoumarins. In this direction, we carried out preliminary mass spectral study on three of the synthesized diastereomeric couples.

**Figure 1.** Possible conformational equilibrium for trans- and cis-4a-c.
Mass spectral characterization

The fragmentation pattern for the studied compounds is shown in Scheme 2 and is summarized in Table 1. The molecular ion peaks (M⁺) for 4a, 4b and 4c appear at m/z 335, 337 and 351, respectively. The relative abundances of M⁺ is in the range 1–12%, so that, only a small fraction of the total ion current is carried by those peaks. The fragmentation proceeds further in two directions – path a, due to ionization of the amide group, and path b – due to ionization of the pyranone ring. The most important fragmentation in 4a-c arises from α-cleavage of the amide function, resulting in the formation of isocoumarin fragment [i-cum] of mass 222, 223 and 224 for 4b, 4a and 4c, respectively. Following the fragmentation path a, further elimination of phenyl radical yield a cation at m/z 145. This is followed by consequent elimination of two CO molecules furnishing cations at m/z 117 and 89. The loss of CO₂ and CO from [i-cum] furnished radical cations at m/z 178 and 194. The fragmentation path b gives characteristic fragments regarding the amide group. Loss of a molecule benzaldehyde from M⁺ yield radical cations of type i in all cases, which afford radical cations ii and iii after consequent loss of two molecules CO. It is noteworthy that the fragmentation pattern shown in Scheme 2 is followed by all the compounds studied, and thus, not characteristic fragment can be attributed towards one of the isomers. However, an interesting result appears from the analysis of Table 1. Although the isomeric compounds show the same characteristic fragment ions, clear differences are observed in the relative abundance for the base peaks. The [i-cum] fragment appears as the base peak in the spectra of the cis-isomers only. In contrast, the formed [i-cum] fragments from the trans-isomers are always of less abundance. That difference in the formation of [i-cum] could be explained by the different stability of the two diastereomers. According to the observed values of 3J₃,₄ of about 11 Hz we assign conformation in solution with both equatorial bulky substituents at C3 and C4 atoms (trans-I) for compound trans-4a-d (see Fig. 1). In the case of compounds cis-4a-d the preferred conformation in solution can not be determined only from 3J₃,₄, because of the same value (3–6 Hz) in conformations cis-I and cis-II, but since one of the substituents is always in axial position (unpreferable conformation) it could be concluded that the cis-isomers are of higher energy (related to the trans-isomers). Thus, the formation of the [i-cum] fragment from cis-4a-c is expected to be faster than those formed from trans-4a-c, since the formation of [i-cum] from the cis-isomers needs less activation energy.
### Table 1. EI mass spectra of trans- and cis-4a-c.

<table>
<thead>
<tr>
<th>Compound</th>
<th>m/z (relative abundance, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M⁺</td>
</tr>
<tr>
<td>cis-4a</td>
<td>335 (12) 223 (100)</td>
</tr>
<tr>
<td>trans-4a</td>
<td>335 (2) 223 (2)</td>
</tr>
<tr>
<td>cis-4b</td>
<td>337 (12) 222 (100)</td>
</tr>
<tr>
<td>trans-4b</td>
<td>337 (2) 223 (2)</td>
</tr>
<tr>
<td>cis-4c</td>
<td>351 (1) 224 (100)</td>
</tr>
<tr>
<td>trans-4c</td>
<td>351 (9) 224 (61)</td>
</tr>
</tbody>
</table>

**Scheme 2.** EI fragmentation pattern of trans- and cis-4a-c.

**CONCLUSION**

The present mass spectral study on isomeric 3,4-disubstituted 3,4-dihydroisocoumarins shows that trans- and cis-isomers of this type could be differentiated by mass spectral analysis, since under EI conditions, the relative abundance of the...
[i-cum] fragment is greater in the cis-isomers. In order to understand the observed phenomenon in details, the synthesis of more complete series of diastereomeric couples of dihydroisocoumarine type is now in progress.

EXPERIMENTAL

Melting points were determined on a Kofler microscope Boetius PHMK 0.5 and are uncorrected. The IR spectra were acquired in chloroform on a Specord 75 and are reported in reciprocal centimeters. The 1H-NMR spectra were obtained on a DRX Bruker Avance NMR spectrometer at 250 MHz in corresponding solvents given in parentheses. The chemical shift is given in ppm (δ) relative to tetramethylsilane as internal standard. Elemental analyses were obtained in the relevant laboratories at the Faculty of Chemistry, University of Sofia. The mass spectra were obtained after preliminary GC separation on Trace GC (Thermo Electron) instrument equipped with a quadruple MS detector DSQ (Thermo Electron). The separation was carried out on DB-5 MS column. The mass spectrometer operates in scan-mode (70 eV ionization potential).

**cis/trans-1-Oxo-3-phenyl-isochroman-4-carboxylic acid (cis-/trans-2).**

**DMAP catalyzed reaction:** To a mixture of homophthalic anhydride (1) (1 g, 0.006 mol) and 1.1 equiv. of benzaldehyde (0.72 g, 0.007 mol) in 7 ml dry chloroform 1 equiv. DMAP was added. The reaction mixture was stirred for 2 h at room temperature. At the end of the reaction (TLC) the obtained carboxylic acids were extracted with 10 % sodium hydrogen carbonate. The aqueous layer was acidified (pH = 3) with 15 % hydrochloric acid and extracted with ethyl acetate. The organic layer was dried with sodium sulfate, filtered and then the solvent was evaporated under reduced pressure. The mixture of cis-2 and trans-2 isomers was separated by fractional crystallization from dichloromethane (yield: cis-2 0.124 g, 12%; trans-2 0.698 g, 70 %).

**BF3.Et2O catalyzed reaction:** To a mixture of homophthalic anhydride (1) (1.81 g, 0.011 mol) and benzaldehyde (1.19 g, 0.011 mol) in 15 ml dry chloroform BF3.Et2O (15.78 g, 0.111 mol) was added. The reaction mixture was stirred for 8 h at room temperature. At the end of the reaction (TLC) the reaction mixture was diluted with ethyl acetate and was washed with 15 % hydrochloric acid and then with water (pH = 7). The organic layer was dried (sodium sulfate), filtered and then evaporated under reduced pressure. The mixture of cis-2 and trans-2 isomers was separated by fractional crystallization from dichloromethane (yield: cis-2 1.053 g, 35 %; trans-2 1.608 g, 54 %).
General procedure for synthesis of compounds (cis-/trans-4a-c). To a suspension of cis-/trans-2 acid in dry benzene, thionyl chloride (4 equiv.) was added. The reaction mixture was stirred at 80 °C for 2 h. Then, the solvents were evaporated under reduce pressure and the residue was dissolved in dry chloroform. To the cooled solution (0 °C, ice bath) 3 equiv. of the corresponding amine were added and the reaction mixture was stirred for 30 minutes. At the end of the reaction (TLC), the reaction mixture was diluted with ethyl acetate and washed with water (pH = 7). The organic layer was dried (sodium sulfate), filtered and the solvent was then evaporated under reduced pressure. The products cis-/trans-4a-c were isolated after recrystallization.

trans-4-(Piperidine-4-carbonyl)-3-phenyl-isochroman-1-on (trans-4a). This compound was obtained as colorless needles from ethyl acetate (yield: 0.216 g, 72 %), mp 181-182 °C; IR: 1620 cm⁻¹ (CONH), 1720 cm⁻¹ (COOR); ¹H NMR (CDCl₃): δ = 0.64-0.90 (1H, m, CH₂), 1.06-1.31 (1H, m, CH₂), 1.31-1.60 (4H, m, CH₂), 3.13-3.39 (3H, m, CH₂), 3.80-3.93 (1H, m, CH₂), 4.61 (1H, d, H-C, J = 10.8), 5.84 (1H, d, H-C, J = 10.8), 7.10 (1H, d, H-Ar, J = 7.7), 7.34-7.44 (3H, m, H-Ar), 7.44-7.54 (3H, m, H-Ar), 7.59 (1H, dt, H-Ar, J = 1.5, 7.6), 8.19 (1H, dd, H-Ar, J = 1.4, 7.7). Anal. Calcd. For C₂₁H₂₁NO₃ (335.40): C, 75.20; H, 6.31; N, 4.18. Found: C, 75.50; H, 5.98; N, 4.18.

cis-4-(Piperidine-4-carbonyl)-3-phenyl-isochroman-1-on (cis-4a). This compound was obtained as colorless needles from ethyl acetate (yield: 0.184 g, 61 %), mp 281-282 °C; IR: 1630 cm⁻¹ (CONH), 1730 cm⁻¹ (COOR); ¹H NMR (CDCl₃): δ = 1.17-1.46 (6H, m, CH₂), 2.70-2.80 (1H, m, CH₂), 3.08-3.29 (2H, m, CH₂), 3.37-3.46 (1H, m, CH₂), 4.50 (1H, d, H-C, J = 3.5), 5.74 (1H, d, H-C, J = 3.5), 7.20 (1H, dd, H-Ar, J = 1.3, 7.4), 7.38-7.52 (6H, m, H-Ar), 7.57 (1H, dt, H-Ar, J = 1.6, 7.4), 8.18 (1H, dd, H-Ar, J = 1.6, 7.5). Anal. Calcd. For C₂₁H₂₁NO₃ (335.40): C, 75.20; H, 6.31; N, 4.18. Found: C, 75.40; H, 5.90; N, 4.16.

trans-4-(Morpholine-4-carbonyl)-3-phenyl-isochroman-1-on (trans-4b). This compound was obtained as white crystals from dichloromethane/ethyl acetate (yield: 0.255 g, 85 %), mp 221-223 °C; IR: 1630 cm⁻¹ (CONH), 1720 cm⁻¹ (COOR); ¹H NMR (CDCl₃): δ = 2.60-2.73 (1H, m, CH₂), 3.04-3.49 (5H, m, CH₂), 3.58 (1H, ddd, CH₂, J = 2.9, 5.1, 11.4 Hz), 3.69-3.77 (1H, m, CH₂), 4.56 (1H, d, H-C, J = 10.9), 5.74 (1H, d, H-C, J = 10.9), 7.05 (1H, d, H-Ar, J = 7.7), 7.33-7.39 (3H, m, H-Ar), 7.41-7.46 (3H, m, H-Ar), 7.56 (1H, dt, H-Ar, J = 1.5, 7.6), 8.30 (1H, dd, H-Ar, J = 1.3, 7.8, H-8). Anal. Calcd. For C₂₀H₁₉NO₄ (337.37): C, 71.20; H, 5.68; N, 4.15. Found: C, 71.67; H, 5.48; N, 4.14.
**cis-4-(Morpholine-4-carbonyl)-3-phenyl-isochroman-1-on (cis-4b).** This compound was obtained as colorless prisms from dichloromethane/methanol (yield: 0.167 g, 56 %), mp 302-303 °C; IR: 1635 cm⁻¹ (CONH), 1720 cm⁻¹ (COOR); ¹H NMR (CDCl₃): δ = 2.44-2.61 (1H, m, CH₂), 2.63-2.77 (1H, m, CH₂), 3.14-3.41 (4H, m, CH₂), 3.43-3.63 (2H, m, CH₂), 4.42 (1H, d, H-C, J = 3.4), 5.76 (1H, d, H-C, J = 3.5), 7.19 (1H, dd, H-Ar, J = 1.4, 7.3), 7.37-7.54 (6H, m, H-Ar), 7.58 (1H, dt, H-Ar, J = 1.7, 7.4), 8.22 (1H, dd, H-Ar, J = 1.7, 7.5). Anal. Calcd. For C₂₀H₁₉NO₄ (337.37): C, 71.20; H, 5.68; N, 4.15. Found: C, 71.20; H, 5.35; N, 4.33.

**trans-4-(Hexyl-4-carbonyl)-3-phenyl-isochroman-1-on (trans-4c).** This compound was obtained as colorless needles from ethyl acetate (yield: 0.183 g, 61 %), mp 171-172 °C; IR: 1540 cm⁻¹ (NH), 1650 cm⁻¹ (CONH), 1720 cm⁻¹ (COOR); ¹H NMR (CDCl₃): δ = 0.89 (3H, t, CH₃, J = 6,8), 1.03-1.39 (8H, m, CH₂), 3.06-3.33 (2H, m, CH₂-N), 3.99 (1H, d, H-C, J = 8.3), 5.59 (1H, brs, H-N), 5.94 (1H, d, H-C, J = 8.3), 7.28 (1H, d, H-Ar, J = 7.5), 7.32-7.44 (5H, m, H-Ar), 7.45-7.54 (1H, m, H-Ar), 7.63 (1H, dt, H-Ar, J = 1.5, 7.6), 8.19 (1H, dd, H-Ar, J = 1.4, 7.7). Anal. Calcd. For C₂₂H₂₅NO₃ (351.44): C, 75.19; H, 7.17; N, 3.99. Found: C, 75.48; H, 6.65; N, 4.12.

**cis-4-(Hexyl-4-carbonyl)-3-phenyl-isochroman-1-on (cis-4e).** This compound was obtained as white crystals from ethyl acetate (yield: 0.202 g, 67 %), mp 200-201 °C; IR: 1540 cm⁻¹ (NH), 1650 cm⁻¹ (CONH), 1730 cm⁻¹ (COOR); ¹H NMR (CDCl₃): δ = 0.85 (3H, t, CH₃, J = 7,0), 0.91-1.04 (2H, m, CH₂), 1.94-1.33 (6H, m, CH₂), 2.84-3.06 (2H, m, CH₂-N), 4.00 (1H, d, H-C, J = 3.4), 5.43 (1H, brs, H-N), 5.83 (1H, d, H-C, J = 3.4), 7.34-7.49 (4H, m, H-Ar), 7.49-7.59 (3H, m, H-Ar), 7.64 (1H, dt, H-Ar, J = 1.5, 7.5), 8.24 (1H, dd, H-Ar, J = 1.5, 7.7). Anal. Calcd. For C₂₂H₂₅NO₃ (351.44): C, 75.19; H, 7.17; N, 3.99. Found: C, 75.40; H, 6.47; N, 3.99.

**REFERENCES**


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