Stereochemical effects in the EI mass spectra of *cis*- and *trans*-fused octa/hexahydro-3,l-benzoxazines and -benzothiazines

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Dedicated to Professor Nikolai Zefirov on the occasion of his 70th birthday

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Abstract

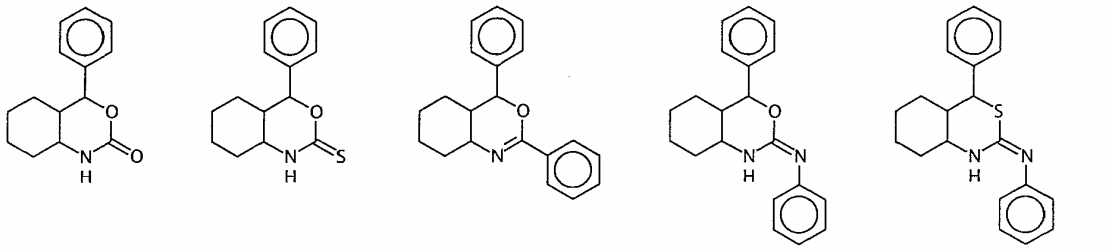
The electron ionization mass spectra of *cis*- and *trans*-fused 2-oxo- (**1**,**2**), and 2-thioxo-4-phenyloctahydro-2*H*-3,l-benzoxazines (**3,4**), 2,4-diphenylhexahydro-3,1-benzoxazines (**5,6**) and 2-phenylimino-4-phenylhexahydro-3,l-benzoxazines (**7**,**8**) and -benzothiazines (**9,10**) were recorded, and the fragmentation pathways established and compared to their 4-unsubstituted counterparts. Effects of stereochemistry, substitution, and (in the case of 2-phenylimino derivatives) of an intramolecular cyclization of the $[M-H]^+$ ions were observed in the mass spectra. In general, mass spectral behavior was similar for the isomeric compounds although they could usually be differentiated from each other based on the relative abundances of their characteristic fragment ions.

Keywords: Electron ionization, stereochemical effects, hexahydro-3,l-benzoxazines, intramolecular cyclization, substituent effects

Introduction

Saturated benzoxazines and -thiazines form a class of heterocyclic compounds which possess remarkable pharmaceutical activity.[1] They are also interesting from an organic synthesis and structural chemistry point of view.

As a continuation of our systematic mass spectrometric studies on 1,3- and 3,1-octahydrobenz-oxazines and related thiazines[2-8] the mass spectrometric behavior of *cis*- / *trans*-fused isomeric pairs shown in Scheme 1 is now studied.



Scheme 1. The compounds studied.

It has been shown earlier[5] that in the case of 2-thioxoperhydro-3,l-benzoxazines, *cis*- and *trans*-fused isomers gave similar spectra, although the *cis*-type compounds fragmented more easily. The isomers could, however, be distinguished from each other. The gas phase enolisa-tion or thioenolisation was not found which probably also reflects the lack of ring-chain tautomerism. This was also true for octahydro-3,1-benzoxazines[6] with a heteroaryl moiety at position 2. It was shown previously[7] that an intramolecular cyclization could occur between the ring nitrogen atom and the *ortho*-carbon of the phenylimino moiety at position 2 which was always accompanied[8] with a hydrogen loss from position 1.

The aim of this study was to find out whether the phenyl substitution at position 4 has any significant effect on the mass-spectral fragmentations and whether any cyclization occurs between an *ortho*- carbon of the phenylimino group and the ring nitrogen. It was also interesting to see how much the higher polarizability of sulfur as compared with that of oxygen atom alters the fragmentations. The possibility to differentiate *cis*- and *trans*-fused isomers based on their low-resolution mass spectra was also a question of interest.

Experimental Section

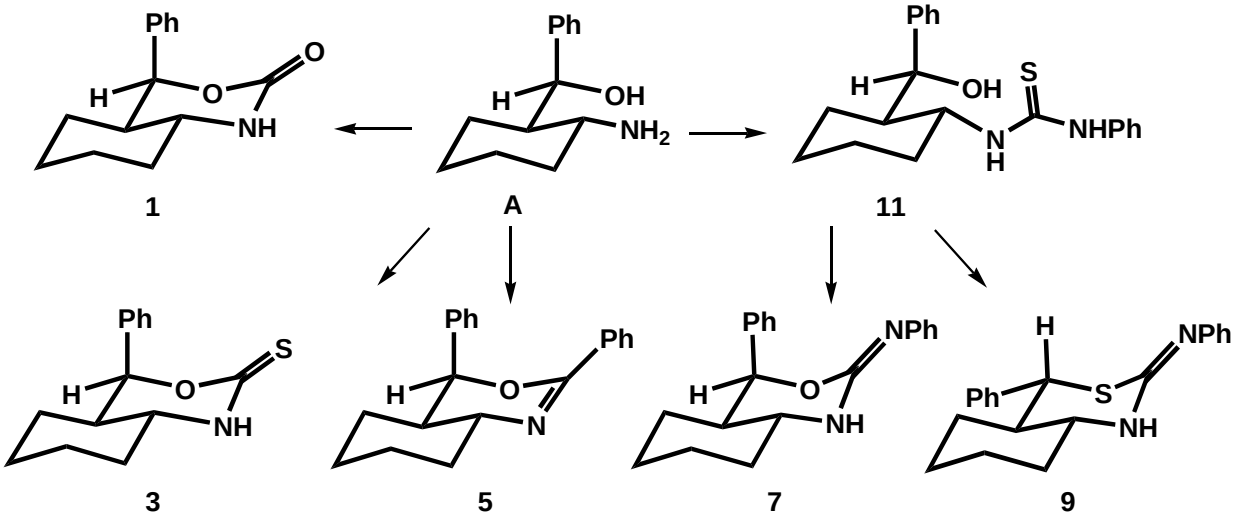
Synthesis. The synthetic pathways to the aliphatic cyclocondensed 1,3-oxazines and -thiazines studied are shown in Schemes 2 and 3. The syntheses of all compounds were described earlier[9] except for thiazines **9** and **10**. The reactions of **A** and **B** with ethyl chloroformate followed with sodium methoxide gave 2-oxo-octahydro-2*H*-3,1-benzoxazines **1** and **2** in good yields. The corresponding 2-thiones **3** and **4** were prepared by reacting **A** or **B** with carbon disulfide. The cyclizations of **A** and **B** with ethyl benzimidate resulted in 2,4-diphenyl-4a,5,6,7,8,8a-hexahydro-4*H*-3,1-benzoxazines **5** and **6** and the treatment of **A** and **B** with phenyl isothiocyanate provided the thiourea derivatives **11** and **12**, which were treated with methyl iodide and alkali to give 2-phenylimino-3,l-benzoxazines **7** and **8** with retention of configuration of the 4-phenyl substituent.[9]

When thioureas **11** and **12** were refluxed in ethanol containing dry HC1, thiazines **9** and **10** were obtained in good yields. In the ring closure it is possible to have either retention or inversion of configuration. According to NMR-measurements, both **9** and **10** attain inverted configurations.

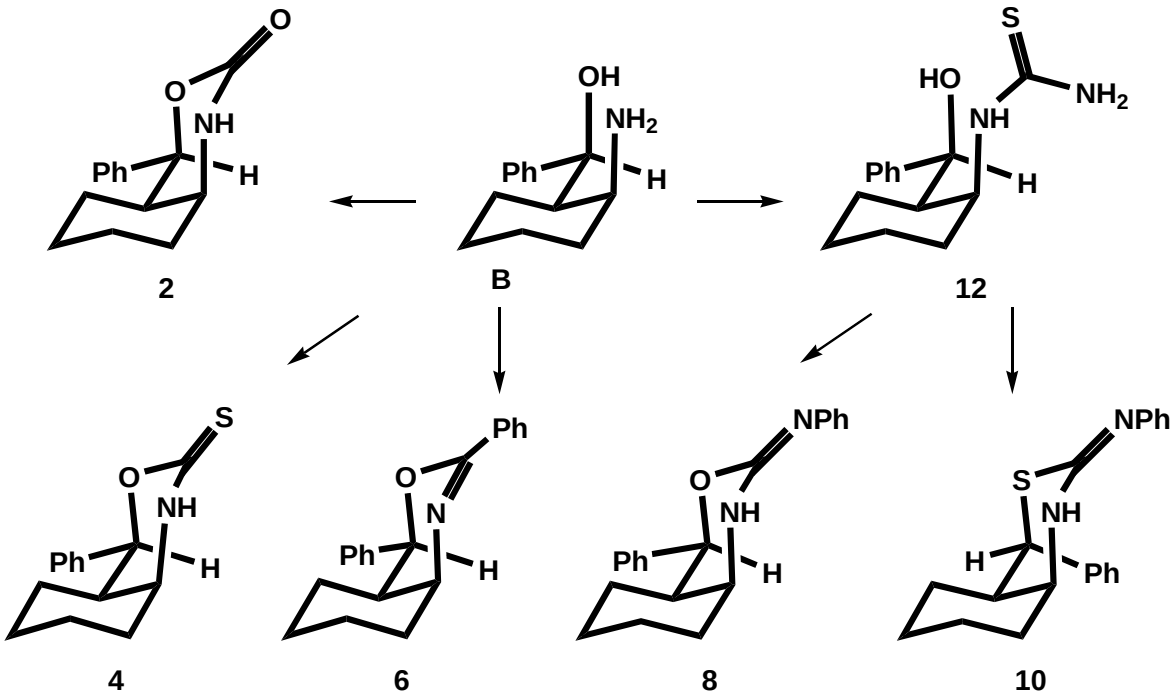
(4*R,4a*R**,8a*R**)-4-Phenyl-2(1*H*)-phenylimino-4a,5,6,7,8,8a-hexahydro-4*H*-3,1-benzothiazine (9).** Thiourea derivative **11** (0.50 g, 1.47 mmol) was dissolved in ethanol (10 mL) containing 22% of dry HCl. The reaction mixture was refluxed for 2 h. After evaporation the residue was dissolved in water (5 ml) and the solution neutralized with potassium carbonate and extracted with dichloromethane (3x5 ml). After drying (Na_2SO_4) and evaporation of the organic phase the residue was recrystallised from diisopropyl ether - ethyl acetate (ca. 9:1) providing **9** as white crystals in 72% yield, m.p. 225-228 °C. ^{1}H NMR ($CDCl_3$): 7.31-7.20 (m, 7H, H-arom.), 7.06-6.95 (m, 3H, H-arom.), 4.04 (d, 1H, H4, *J* = 10.7 Hz), 3.20 (td, 1H, H8a, *J* = 2×10 and 4 Hz), 1.98 (m, 1H, H8eq), 1.88 (br quartet, 1H, H4a, *J* = 3×10 Hz), 1.78 (br d, 1H, H7eq, *J* = 13 Hz), 1.67 (br d, 1H, H6eq, *J* = 13 Hz), 1.52-1.42 (m, 2H, H5eq and H8ax), 1.34 (m, 1H, H7ax), 1.20 (m, 1H, H6ax), 0.82 (m, 1H, H5ax). ^{13}C NMR ($CDCl_3$): 138.7 (very br), 128.7-128.8 (two overlapping peaks), 128.5, 128.1, 122.8, 122.2 (br), 57.7 (br, C8a), 51.9 (C4), 44.5 (br, C4a), 34.4 (br, C8), 28.8 (C5), 25.7 (C6), 24.5 (br, C7).

(4*R,4a*R**,8a*S**)-4-Phenyl-2(1*H*)-phenylimino-4a,5,6,7,8,8a-hexahydro-4*H*-3,l-benzothiazine (10).** This compound was prepared according to the above procedure from **12** in 83% yield, m.p. 245-247 °C. ^{1}H NMR ($CDCl_3$ + drop TFA): 11.3, 10.9 (exchangeable protons), 10.3 (br s, 1H, H1), 7.42-7.33 (m, 6H, H-arom.), 7.30-7.28 (m, 4H, H-arom.), 4.57 (d, 1H, H4, *J* = 9.2 Hz), 3.90 (m, 1H, H8a), 2.48 (m, 1H, H4a), 1.93 (m, 1H, H8x), 1.85-1.76 (m, 2H, H8y and H7x), 1.63-1.57 (m, 2H, H5x and H5y), 1.53-1.39 (m, 3H, H7y, H6x and H6y). ^{13}C NMR ($CDCl_3$ + drop TFA): 166.8 (1C, C2), 135.9 (br, 1C), 134.0 (1C), 129.7 (s, 2C), 129.5 (s, 2C), 129.4 (1C), 129.0 (1C), 128.2 (s, 2C), 126.7 (s, 2C), 51.9 (br, 1C, C8a), 46.6 (br, 1C, C4), 37.9 (1C, C4a), 29.8 (1C, C8), 26.5 (1C, C5), 22.9 (br, 1C, C7), 20.8 (br, 1C, C6).

Purity of the compounds was checked with ^{1}H NMR. The samples were fairly stable at room temperatures but some of them decomposed after a relatively small rise of temperature (e.g. in the ion source).



Scheme 2



Scheme 3

Mass spectrometry. The low resolution (R = 1000) EI^+ mass spectra were recorded on a VG7070E double-focussing mass spectrometer (VG Analytical, UK) equipped with an OPUS data system. Samples were introduced into spectrometer using water-cooled direct insertion probe at ambient temperatures. The ionization energy of 70 eV, trap current of 100 μA, source temperature of 180°C and acceleration voltage of 6 kV were used.

High resolution spectra (R = 8000) were obtained on a ZABSpec-oaTOF instrument (Fisons Instruments, UK). This instrument was also used for linked scan and MS/MS measurements. The source conditions were 70 eV / 200 μA / 160°C. The acceleration voltage was 8 kV. The collision energy in oaTOF chamber was 800 eV. The residual air was acting as the collision gas. In linked scan measurements, He was used as the CID target gas for the high energy (8 keV) collisions. The transmission of 30% was used. The accurate masses were obtained using peak matching with PFK as the reference compound. All the high-resolution measurements for the ions discussed in the text were within 5 ppm from the calculated values.

NMR spectroscopy. The NMR spectra of compounds **9** and **10** were recorded on a Bruker Avance 500 NMR spectrometer (500.13 MHz for ^1H and 125.76 MHz for ^{13}C) in $CDCl_3$ using TMS as an internal reference. The spectra were measured at 25 °C both with and without adding a drop of trifluoroacetic acid (TFA); for **10**, ^1H spectrum without TFA was recorded also at 55 °C. The experiments consisted of standard ^1H and $^{13}C\{^1H\}$ NMR, 1D gs-NOESY or NOE-difference, dqf-COSY, $^1H\{^{13}C\}$-HSQC and $^1H\{^{13}C\}$-HMBC, using vendor-supplied pulse programs.

Results and Discussion

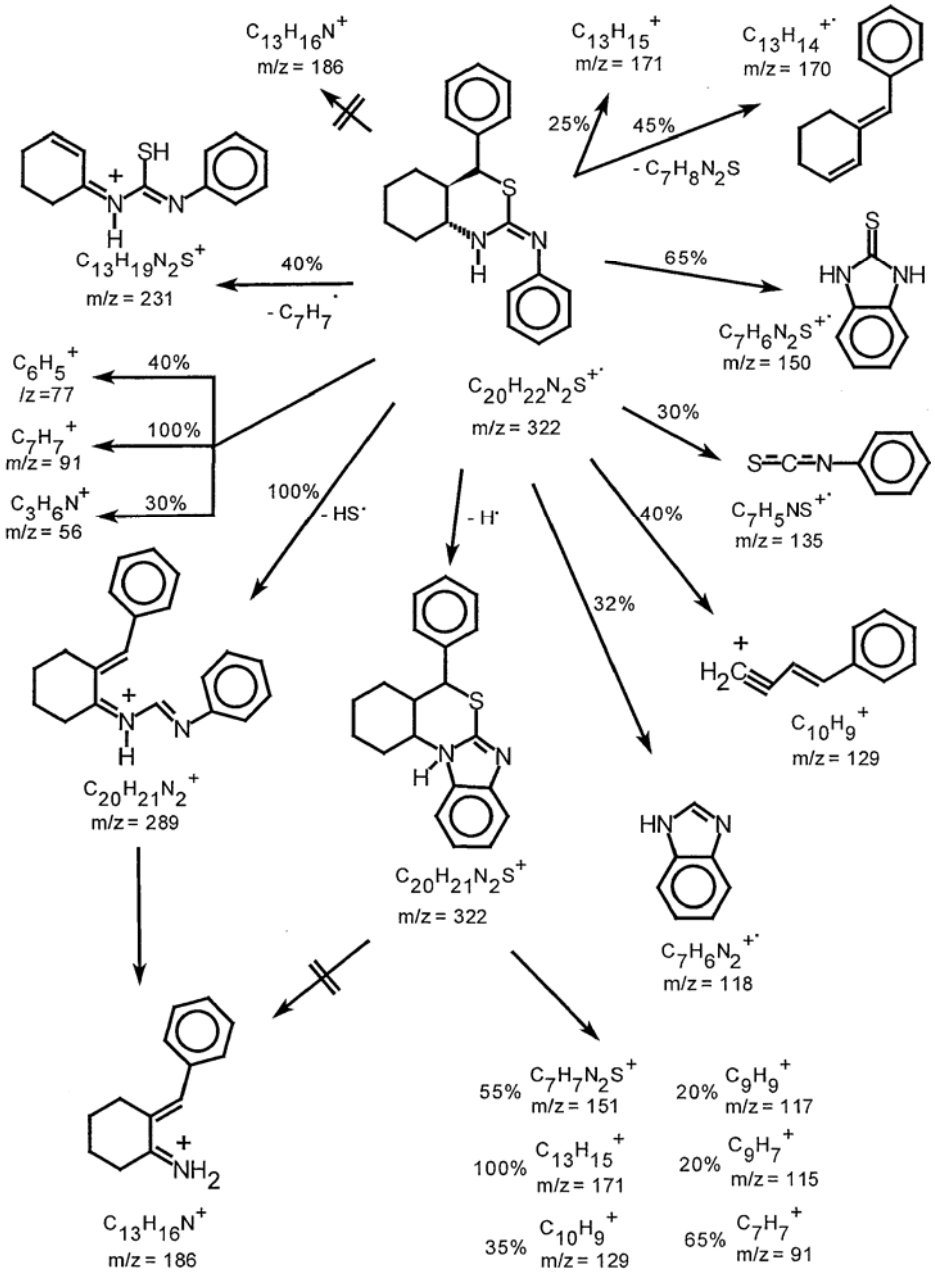
The structures of compounds **1**-**8** have been verified earlier.[9] Compound **9** displayed large vicinal diaxial couplings between protons H4 and H4a, and between H4a and H8a (10.7 and ca. 10 Hz, respectively), thus confirming both the *trans*-fusion and the axial position of H4 for this compound. Consistently with this, presaturation of the H4 resonance resulted in an NOE enhancement of protons H5ax and H8a. ^{1}H and ^{13}C signals were broadened at 25 °C to the point that some ^{13}C signals could not be observed, indicating the presence of dynamic processes (e.g. *endo*-*exo*-tautomerism of the double bond or *E*-*Z*-isomerism with respect to it). Addition of a drop of TFA resulted in sharper NMR lines with all carbon signals being visible. Compound **10** was only slightly soluble in $CDCl_3$ at 25 °C showing very broad ^{1}H resonances. At 55 °C, signals were sharper, though still broadened. Again, adding TFA increased the signal resolution, and dramatically improved as well the solubility, allowing measurements at 25 °C. In these conditions, **10** showed a large coupling (9.2 Hz) between H4 and H4a, indicating their mutual *trans* orientation, and thus also the predominance of *N*-*out* conformation (i.e. a conformation where N1 is equatorial w.r.t. the cyclohexane moiety). 1D NOESY confirmed that in the predominant ring-invertomer H4 is facing the cyclohexane ring (e.g. an NOE in H8ax due to H4 was observed). At 55 °C without the presence of TFA, the coupling between H4 and H4a was resolved in the H4 signal; *J*(H4,H4a) = 6.6 Hz. Assuming this coupling has a value of 10.7 Hz in the limiting *N*-*out* conformer (axial-axial relationship, cf. **9**) and 2.5 Hz in the *N*-*in* conformer (equatorial-equatorial relationship as shown in Scheme 3), we conclude that **10** exists in these conditions as a roughly 50:50 mixture of these conformers. A further indication for the fact that indeed the Ph is axial in compounds **3**, **5**, and **7** is their C-8a chemical shift which is around 50 ppm whereas for **10** it is ca. 58 ppm – so one can see the shielding effect of axial Ph in the former. To further prove this situation we remeasured the H4,H4a couplings for compounds **3** (5.0 Hz) and **5** (5.3 Hz) which are what one can expect from an eq,ax-type coupling between these protons.

The 70-eV EI mass spectra of compounds **1**-**10** are listed in Table 1 which shows that all compounds except 2-oxo-derivatives **1** and **2** exhibit intense molecular ion peaks under the EI conditions. The $M^{+\bullet}$ peaks are especially abundant for sulphur-containing derivatives **3**, **4, 9** and **10** and give rise to the base peaks in thiazines **9** and **10**. Note that $[M–H]^+$ ions were detected only in the spectra of 2-phenylimino-substituted compounds (**7**–**10**). An abundant loss of hydrogen occurred from their molecular ions ($[M–H]^+$ ions, 40-90% RA). This loss is most probably due to the intramolecular cyclization between the *ortho*-carbon of the iminophenyl group and the ring nitrogen (Scheme 4) – a phenomenon often observed in oxazines, thiazines and pyrimidinones with e.g. a benzyl or 2-phenylimino substituent in a position next to the ring nitrogen atom.[7] However, elimination of one of the bridgehead or benzylic hydrogen atoms is also likely. The possible hydrogen migration between 1-NH and 2-NPh moieties can further stabilize the structure thus formed.

Some similarities exist between the fragmentations of 2-phenylimino oxazines and corresponding thiazines. However, due to the greater ability of sulfur to maintain the charge some of the main fragmentation routes are different. The fragment ions appear to be less abundant and more numerous in the thiazine spectra. The differences between the LR spectra of

cis- and *trans*-fused isomers are very small in thiazines but in case of oxazines the isomers can be differentiated based on their LR spectra.

The main difference between the fragmentation routes of 2-phenylimino-oxazines (**7** and **8**) and -thiazines (**9** and **10)** is that the $M^{+\bullet}$ of oxazines do not release hydroxyl radicals although $SH^{\bullet}$ is released from the $M^{+\bullet}$ of thiazines. Also, the ion $C_{13}H_{16}N^{+}$ at *m/z* 186, which in oxazines is formed directly from $M^{+\bullet}$, can be obtained from the $M^{+\bullet}$ of thiazines only after the loss of $SH^{\bullet}$. Also, the ions $C_7H_5NS^{+\bullet}$ (*m/z* 135) are observed in the CID spectrum of $M^{+\bullet}$ of thiazines, although there is no counterpart $C_7H_5NO^{+\bullet}$ ions in the CID spectra of oxazines. This reflects the role of the sulfur atom which localizes the charge in the phenyliso-thiocyanate ions.



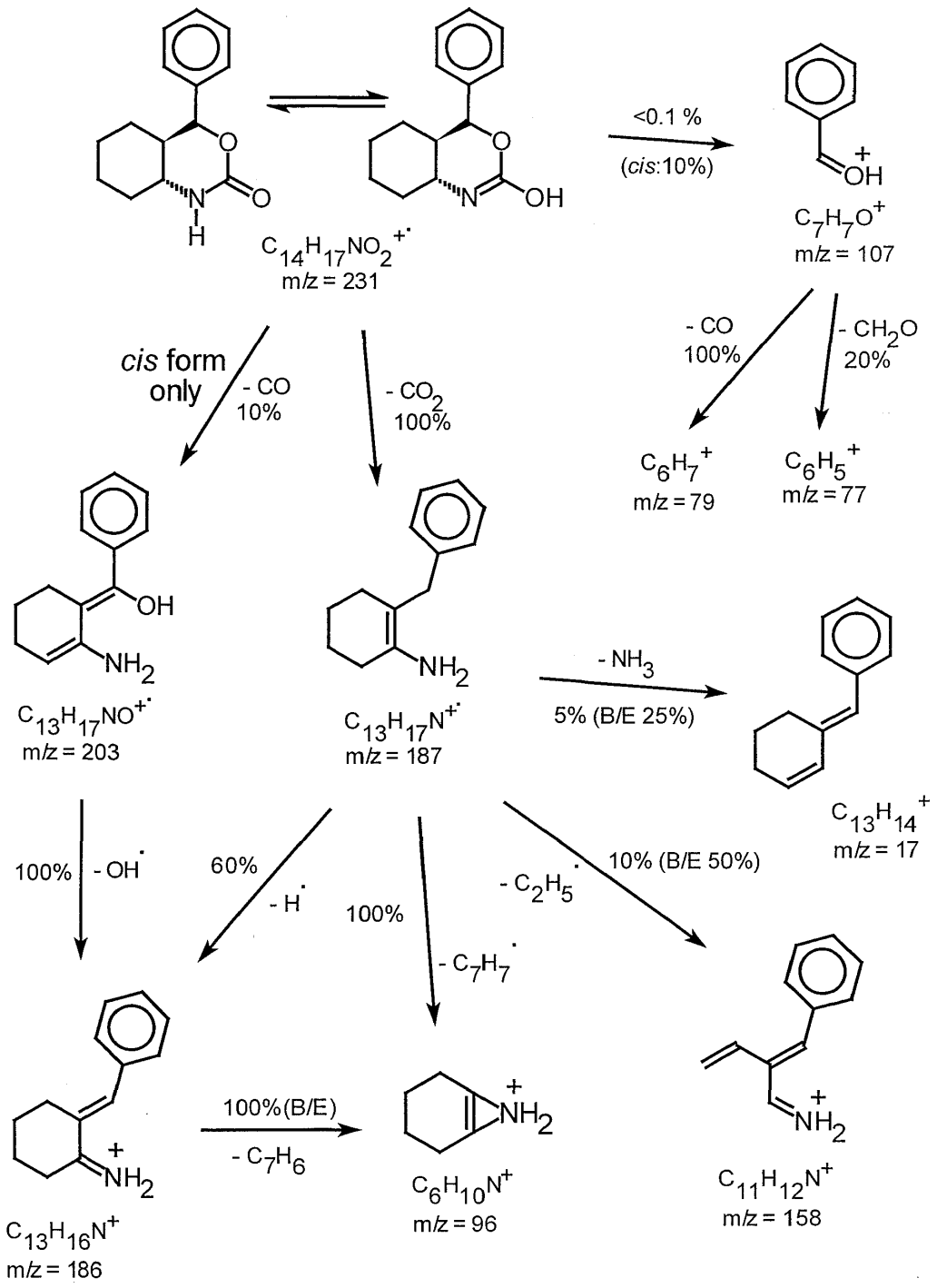
Scheme 4

Major differences can also be found between the CID-oaTOF spectra of $M^{+\bullet}$ ions of *cis*- and *trans*-fused isomers of these compounds. While the *trans*-fused isomers **7** (X = O) and **9** (X = S) give abundant cyclic $C_7H_6N_2X^{+\bullet}$ ions (100% and 65% RA, respectively), the base peak in the CID spectrum of *cis*-fused 2-phenylimino-oxazine **8** corresponds to the protonated phenylurea, $C_7H_9N_2O^+$ (*m/z* 137), which is formed through loss of $C_{13}H_{13}^{\bullet}$ from $M^{+\bullet}$. The *cis*-fused 2-phenylimino-thiazine **10** loses $SH^{\bullet}$ radical but very little of the (supposedly cyclic) ion $C_7H_6N_2S^{+\bullet}$. Furthermore, the loss of hydrogen from $M^{+\bullet}$ results in almost identical $[M–H]^+$ ions for the *cis*- and *trans*-fused isomers in both pairs of compounds. This agrees well with the $[M–H]^+$ cyclization hypothesis, but can also be due to a hydrogen rearrangement.

The behaviour of the 2-phenyl compounds **5** and **6** resembles that of the 2-oxo and 2-thioxo compounds. The ring-fusion isomers are easily differentiated on the basis of their EI spectra since the ions at *m/z* 200 and *m/z* 186 for the *trans* form **5** are much more abundant than for the *cis* form **6** (Scheme 6). Additional stereospecific fragmentations were revealed by their CID-oaTOF-spectra. Thus, the main fragmentation route of the *cis* form leads to the ions $C_{13}H_{14}^{\bullet}$ (*m/z* 170), but the *trans* form gives the ions $C_{13}H_{14}NO^+$ (*m/z* 200) instead. Although the oaTOF-CID spectra of $M^{+\bullet}$ for the *cis*- and *trans*-fused isomers are different, the loss of hydrogen makes the CID spectra of their $[M–H]^+$ ions quite similar. Stereochemical effects of ring annelation on the relative abundance of $[M–H]^+$ ions have been described in the literature,[10] but in this case the fragments and abundances of the high intensity ions are practically the same for both the *cis*- and *trans*-fused isomers, and only the relative abundances of some low intensity ions are different.

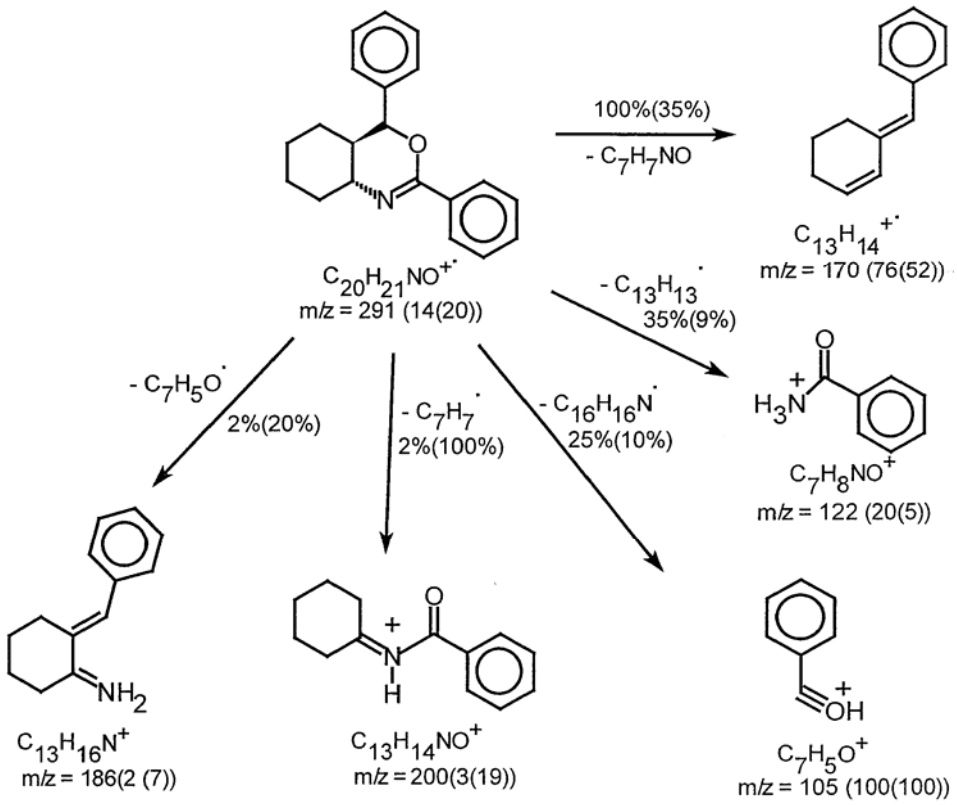
Thus, the CID spectra of compounds **7-10** show stereospecific fragmentation patterns unless the stereoisomers produce a common structure (in the case of $[M–H]^+$ ions).

The EI spectra of the 2-oxo isomers **1** and **2** show that they fragmented primarily *via* the loss of CO_2 from the $M^{+\bullet}$ ions (Scheme 5). They differed more clearly from each other than the corresponding thioxo-isomers **3** and **4**. For instance, the $M^{+\bullet}$ ions are less stable, and the $[M–CO_2]^{+\bullet}$ ions more abundant in the spectrum of the *trans*-fused **1** than in that of **2**.



Scheme 5

However, the EI spectra of *cis* and *trans*-fused isomers of 2-thioxo compounds **3** and **4** also provided a few peaks with sufficiently different abundancies which allowed their differentiation. Fragmentations of compounds **3** and **4** proceeded mainly by the losses of $HS^{\bullet}$, $COS^{\bullet}$, and $C_7H_7^{\bullet}$ from the $M^{+\bullet}$, similarly to earlier findings.[4,5]



Scheme 6

Conclusions

It was demonstrated for the 2-phenylimino substituted compounds that under EI conditions a hydrogen loss from their molecular ions is followed by cyclization to the ring nitrogen atom. This observation is in a good agreement with earlier results.

In general, the mass spectral behavior is similar for the isomeric compounds studied. The EI spectra of the isomeric 2-phenylimino thiazines are almost identical but the daughter-ion spectra of their molecular ions are different. The corresponding oxazines differ mainly by the higher stability of the molecular ion of the *trans* isomer as compared with that of the *cis* isomer, probably because the latter forms a protonated phenylurea ion more easily. The isomeric 2-phenyl oxazines produce similar EI mass spectra except that the loss of benzyl radical, $C_7H_7^{\bullet}$, from the molecular ion is the main pathway only for the *cis* isomer but not for the *trans* isomer. The spectra of the isomeric 2-thioxo compounds exhibit only fairly small differences but those of the 2-oxo-compounds are clearly different.

The stabilities of the thioxo-compounds under electron ionization do not practically differ from those of the 4-unsubstituted homologues, probably because the sulfur atom better stabilizes the ions due to charge localization. In contrast, the difference between the isomeric oxo-

compounds is much clearer. So, the 2-oxo-compounds studied are clearly less stable under electron ionization than their 4-unsubstituted homologues.

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