Gas chromatography–mass spectrometry of the stereoisomers of heterocyclic compounds. Part 2b.[1] Perhydroxanthenes

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The quantitative relationships between the retention of the stereoisomers of perhydroxanthene (PHX) on a column packed with graphitized thermal carbon black (GTCB) and their hypothetical molecular structures optimized by molecular mechanics have been established. From the GC–MS data obtained the structure of four novel stereoisomers have been elucidated. Along with the theoretically expected *trans-anti-trans-*, *trans-syn-cis-* and *cis-anti-cis-*isomers, the unusual stable stereoisomeric form of PHX has been found and elucidated as conformer B of the *cis-syn-cis-*isomer. The evidence for this assignment based on the obtained GC- and MS-data is discussed.

Introduction

In our previous work the existence of seven stereoisomeric forms of perhydroxanthene (PHX), three of which are known [*cis-syn-cis* (**A**), *trans-anti-cis*, *trans-syn-trans*], was established.[1] The structure elucidation of the four novel PHX stereoisomers appeared more difficult, as compared with PHTX studied earlier,[2] due to the similarity of the electron impact mass spectra and the existence of the seventh theoretically unexpected stable stereoisomeric form.[1] Nevertheless, *cis-anti-cis* and *trans-anti-trans* isomers were the preliminary assignments. From the data obtained it might be concluded that a qualitative relationship between the retention order and molecular geometry of isomers cannot be considered to be reliable if their mass spectra are indistinguishable or very similar. In this case, the order of the separation of isomers must be established quantitatively.

The retention parameters and, consequently, separation order on columns packed with graphitized thermal carbon black (GTCB) of adsorbates of known structure can be calculated accurately using Kiselev's molecular-statistical method (chromatoscopy).[3,4] This method, although suitable for our purpose, cannot be used without the proper selection of atomic coordinates of an adsorbate molecule. According to the traditional approach, molecular parameters were experimentally obtained by independent methods (electron diffraction, X-ray diffraction). In some cases (mainly for aromatic compounds), the molecular geometry has been represented as a simple combination of previously known structural parameters.[4] Obviously, this approach is not valid in the case of novel compounds. Therefore, recently a new approach based on computerized modeling of hypothetical molecular structures has been developed in which allowance is made for the effect of intramolecular interactions.[5] Eleven stereoisomers of perhydroanthracene (PHA) and perhydrophenanthrene (PHP), the *cis-cis-*isomers of which are characterized by strong 1,3- and 1,4-diaxial non-valence intramolecular interactions, were selected as a test mixture. The order of their separation on the column packed with GTCB was calculated using both approaches and compared with the experimental results, which were obtained previously.[6] The order of separation calculated by the traditional approach appeared erroneous, whereas the results based on molecular modeling with regard to intermolecular interactions agreed with the experimental results.[5] Therefore, in this study the order of the separation of the PHX stereoisomers was calculated on the basis of the computerized molecular modeling.

Calculations

Molecular mechanics was used to optimize the hypothetical molecular structures of PHX stereoisomers. For this purpose the PCMODEL program (modified version of Still's MODEL program, Columbia University) based on the MM2 force field, developed by Allinger, was used. The final atomic coordinates obtained after energy minimization have been used for further molecular-statistical calculations.

The order of retention of the PHX stereoisomers on the column packed with GTCB was determined by molecular-statistical calculations of their adsorption equilibrium constants (Henry constants, K_1). Henry constants have been calculated using eqn. (1) for the adsorption of quasi-rigid molecules

$$K_1 = V_{A,1} = \tfrac{1}{4}\pi\iint(2\pi kT/\Phi''_z)^{\frac{1}{2}}\exp(-\Phi_0/kT)\sin\Theta\, d\Theta\, d\psi \qquad (1)$$

on a model of the mathematically uniform surface of GTCB,[7] where $V_{A,1}$ is the retention volume per unit surface area of the adsorbent, Φ_0 is the potential energy corresponding to molecule–adsorbent interactions, Φ''_z is the second derivative of this potential energy with respect to the distance z between the GTCB surface and the center of mass of the molecule at fixed values of the Euler angles Θ and ψ at the corresponding equilibrium distance z_0. The potential energy Φ_0 was calculated in the atom–atom approximation using three semi-empirical Buckingham–Cornor atom–atom potential functions [eqns. (2)–(4)],[7,8] where r is the distance between the interacting atoms

$$\varphi_{C(sp3)\cdots C(GTCB)} = -1.386 \times 10^{-3}r^{-6} - 2.148 \times 10^{-5}r^{-8} + 1.890 \times 10^{5}\exp(-35.7r) \qquad (2)$$

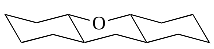
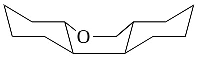
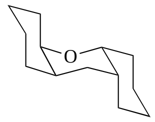
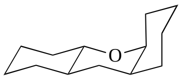
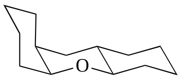
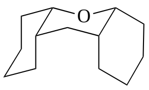
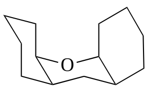
$$\varphi_{H\cdots C(GTCB)} = -0.498 \times 10^{-3}r^{-6} - 0.950 \times 10^{-5}r^{-8} + 3.60 \times 10^{4}\exp(-35.7r) \qquad (3)$$

$$\varphi_{O\cdots C(GTCB)} = -1.005 \times 10^{-3}r^{-6} - 1.628 \times 10^{-5}r^{-8} + 7.234 \times 10^{4}\exp(-35.7r) \qquad (4)$$

of the adsorbed molecule and the adsorbate (expressed in nm) and φ is the atom–atom potential function (expressed in kJ mol^{-1}). The calculations were performed according to the procedure described previously.[9]

For each isomer a series of parallel calculations with different initial arrangements of its molecule on a flat GTCB

Table 1 Structures percentage in mixtures, thermodynamic adsorption characteristics and intensities of the characteristic ions in the mass spectra of the stereoisomers of PHX

Isomer	Structure	Percentage in mixtures		$K_1^{230\,°C}$/ $cm^3\,m^{-2}$	$-\Delta U_1$/ kJ mol^{-1}	Intensities of characteristic ions in mass spectra (70 eV), %TIC									
		1	2			*m/z* 194	176	175	165	151	137	133	113	107	95
trans-syn-trans		4.6	93.8	44	69	7.3	0.11	0.89	0.09	15.4	0.31	3.8	0.39	1.7	3.1
trans-anti-trans		—	2.0	41	68	5.4	0.08	0.35	—	15.0	0.38	5.3	0.18	2.3	2.8
cis-anti-cis		3.5	0.5	17	62	6.1	0.13	0.20	0.12	9.7	0.74	3.2	1.0	1.4	2.7
trans-syn-cis		7.4	—	14	61	6.7	—	0.35	0.15	14.1	0.41	5.0	0.40	2.3	2.5
trans-anti-cis		48.3	2.9	9.5 9.6[a]	60 63[a]	7.1	0.10	0.36	0.09	14.4	0.35	4.3	0.27	2.0	2.9
cis-syn-cis (**A**)		36.2	0.1	3.9 4.3[a]	52 56[a]	9.0	0.08	0.28	0.15	10.6	0.54	4.1	1.1	1.6	2.9
cis-syn-cis (**B**)		—	0.7	10	58	6.0	0.16	0.15	—	12.6	5.2	1.7	—	1.1	3.9

[a] Experimentally obtained values.

surface was performed. The result with the highest value for the potential energy of the intermolecular molecule–adsorbent interaction was selected.

The results are shown as a dependence of ln K_1 on the reciprocal column temperature, $1/T$, which can be approximated by eqn. (5), where $V_{A,1}$ is the retention volume at zero

$$\ln K_1 = \ln V_{A,1} = A + B/T \qquad (5)$$

sample size per unit surface area. The coefficients A and B are related to the entropy ($\overline{\Delta S}_1$) and internal energy ($\overline{\Delta U}_1$) (the differential heat of adsorption q_1) by the relationships given in eqns. (6)[3] and (7). Subscript 1 indicates small (zero) surface coverage.

$$A = \overline{\Delta S}_1/R + 1 \qquad (6)$$

$$B = -\overline{\Delta U}_1/R \qquad (7)$$

Results and discussion

The plots of the dependence of the calculated values of ln K_1 on the reciprocal column temperature $1/T$ for the optimized hypothetical molecular models of PHX stereoisomers are shown in Fig. 1 (straight lines). The Henry constants for the known isomers *cis-syn-cis* (**A**) and *trans-anti-cis* (the most abundant in mixture 1, Table 1) were measured experimentally according to the procedure described earlier.[7] The experimentally measured values of ln K_1 (dots) for PHX appeared close to the calculated values (Fig. 1, Table 1). From this good match it can be inferred that the determination of the retention characteristics of other PHX stereoisomers is also accurate. Thus, the theoretically expected stereoisomers of PHX elute from the column packed with GTCB in the following order: *cis-syn-cis* (**A**), *trans-anti-cis*, *trans-syn-cis*, *cis-anti-cis*, *trans-anti-trans* and *trans-syn-trans* (Fig. 1).

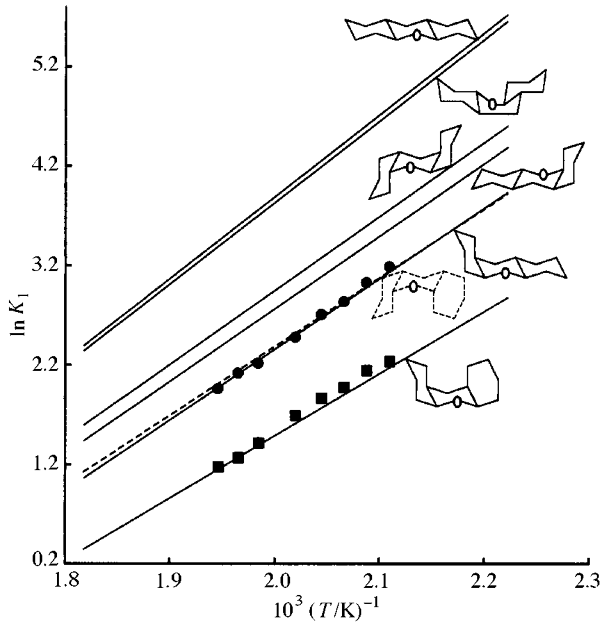


Fig. 1 Calculated (straight lines) and experimental (dashed line) dependence of ln K_1 on $1/T$ for the adsorption of the stereoisomers of perhydroxanthene on GTCB

The theoretically unexpected stereoisomer X7 of mixture 2, assumed to be conformer **B** of *cis-syn-cis*-PHX, coeluted from the column packed with GTCB with one of the other stereo-

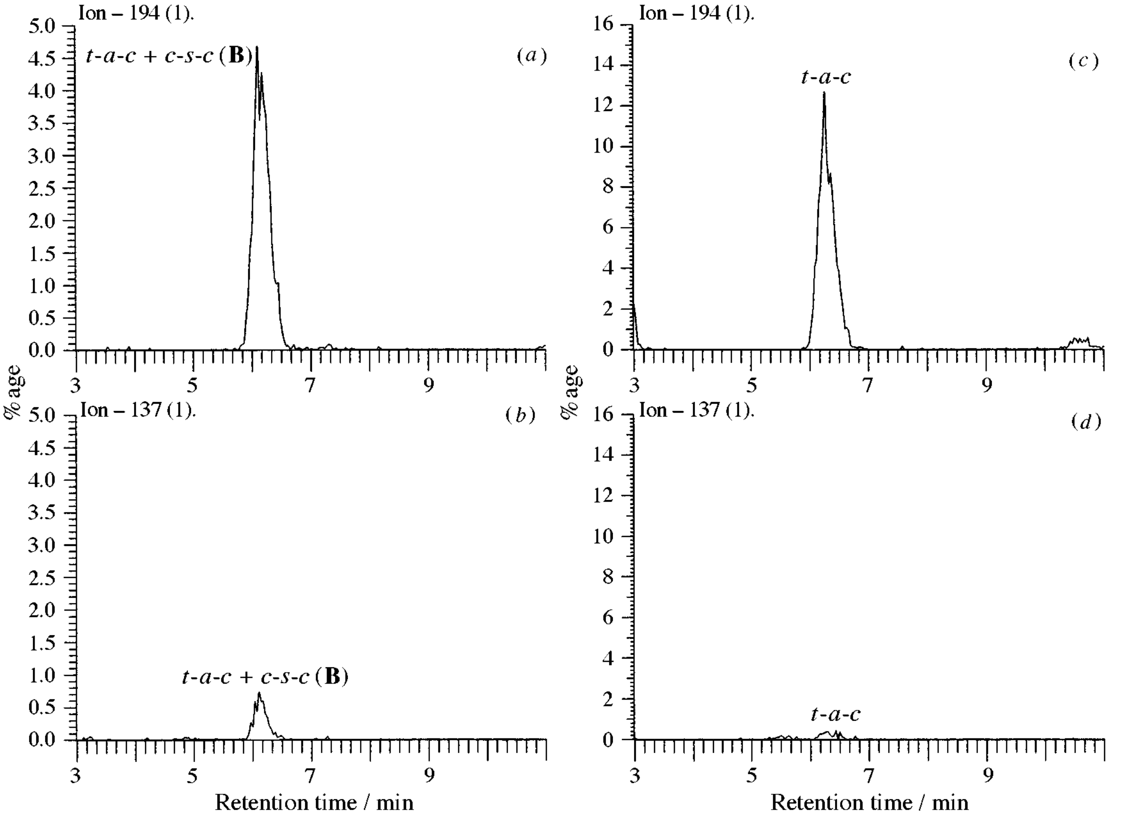


Fig. 2 Part of mass chromatograms for ions with *m*/*z* 194 and 137 of mixture 1 [(*c*) and (*d*)] and mixture 2 [(*a*) and (*b*)] of the stereoisomers of perhydroxanthene on GTCB, 230 °C

isomers.[1] The hypothetical molecular structure of this conformer was considered on the assumption that all rings are in the chair conformation. A possible intermediate conformation with the middle ring as a boat was not considered because the structure is not quasi-rigid and the calculations according to eqn. (1) would not give unambiguous solution. The plot of the dependence of the calculated values of ln K_1 on the reciprocal column temperature $1/T$ for the optimized hypothetical molecular model of this conformer is shown in Fig. 1 (dashed line). The result appeared very close to the retention parameters of *trans-anti-cis*-PHX, that is the corresponding GC-peaks on the chromatogram would overlap (Fig. 1, Table 1).

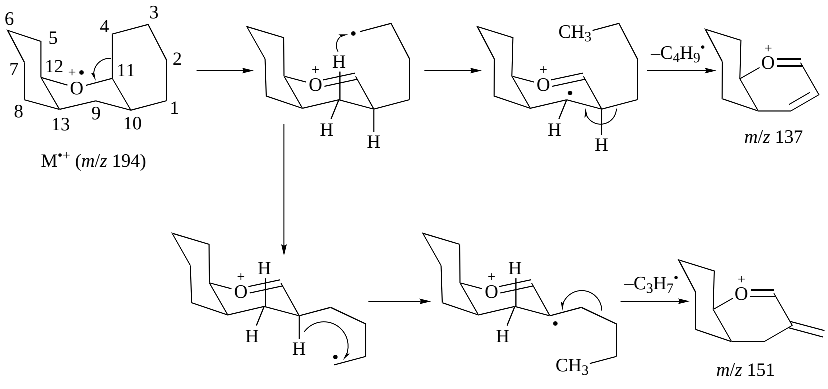
The formation of the ions $[M - C_4H_9]^+$ (*m*/*z* 137) as the stereospecific fragmentation pathway of the stereoisomer X7 was considered to be confirmation of this fact. The intensity of the corresponding peak in the mass spectra is 15 times greater than those in the mass spectra of *trans-anti-cis*-PHX (Table 1). Therefore, in the case of the coelution of the isomers X7 and *trans-anti-cis*, an increase in the signal of the ions $[M - C_4H_9]^+$ (*m*/*z* 137) should be observed. Fig. 2 shows the sections of the mass chromatograms for ions with *m*/*z* 137 and 194 of mixtures 1 and 2, corresponding to the retention time of *trans-anti-cis* PHX. The increased GC-peak in the mass chromatogram for ions with *m*/*z* 137 of mixture 2 clearly demonstrated the presence of the stereoisomer X7 [Fig. 2 (*a*) and (*b*)], as compared to mixture 1 in which this isomer is absent [Fig. 2 (*c*) and (*d*)]. The obtained ratio of the areas of the GC-peaks corresponding to the ions with *m*/*z* 137 and 194 (S_{137} and S_{194}) is 0.17, which is close to the expected value $S_{137}/S_{194} = 0.19$, calculated with regard to the percentages of these isomers in mixture 2 and their mass spectra (Table 1).

Under the assumption that stereoisomer X7 is conformer **B** of *cis-syn-cis*-PHX it is important to compare its fragmentation under electron impact with the fragmentation of conformer **A**, to consider in particular the mechanism of the stereospecific elimination of the radical $C_4H_9^{\bullet}$ from the molecular ions *via* β-cleavage. A similar process was studied before as the stereospecific feature of the fragmentation of PHA isomers.[10] $[M - C_4H_9]^+$ ions were predominantly formed from *cis-syn-cis*-PHA, which is involved in strong *syn*-axial intramolecular interactions. However, the formal comparison in this case is incorrect because for isomer *cis-syn-cis* (**A**) this process is not inherent. In contrast to PHA, the removal of intramolecular tension in molecular ions of *cis-anti-cis*-PHX occurs through elimination of the radical $C_3H_7^{\bullet}$ followed by the formation of the stable oxonium ion (*m*/*z* 151).[1] The intensity of the peak with *m*/*z* 137 in the mass spectra of conformer **A** is 10 times smaller than those in the mass spectra of conformer **B** (Table 1). To explain this phenomenon we considered the competitive rearrangement processes of the formation of the ions $[M - C_4H_9]^+$ (*m*/*z* 137) and $[M - C_3H_7]^+$ (*m*/*z* 151) under the assumption of their dependence on the spatial positioning of the migratory hydrogen atoms.

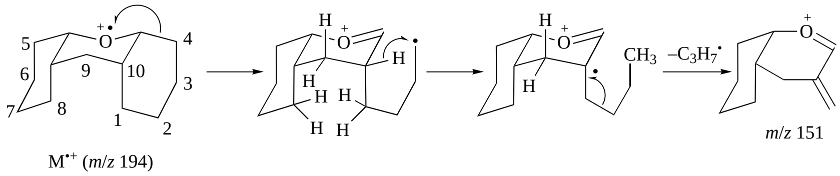
The methylene groups presenting a side ring of the molecular ions become mobile *via* β-cleavage and their rotation promotes the migration of a hydrogen atom to the radical center (Schemes 1 and 2). In the transition state of the molecular ion of conformer **B** rotation about the C(1)–C(1) bond brings together the radical center and a hydrogen atom at either C(9) or C(10). In the first case the result of this rearrangement is the ion $[M - C_4H_9]^+$, whereas in the second the ion $[M - C_3H_7]^+$ is formed (Scheme 1).

The free rotation about the C(1)–C(10) bond in the transition state of the molecular ion of conformer **A** is not possible due to strong non-valence interactions of hydrogen atoms at C(1) and C(8). Rotation about the C(1)–C(2) bond is more likely, resulting in the migration of the hydrogen atoms at C(10) and the formation of an ion with *m*/*z* 151. The hydrogen atoms at C(9) in this transition state appear more distant from the radical center and the competitive elimination of the radical $C_4H_9^{\bullet}$ is negligible (Scheme 2).

Thus, these arguments are in keeping with the assignment of X7 to the conformer **B** of *cis-syn-cis*-PHX. However, the existence of both conformers of the *cis-syn-cis*-isomer in the absence



Scheme 1



Scheme 2

of their interconversion under the experimental conditions implies the high energetic barrier of this process. The interconversion does not occur under synthesis conditions either, explaining the fact that conformer **B** is absent from mixture 1 (Table 1). The nature of this phenomenon might be related to the lone pairs of the oxygen atom helping to stabilize both conformers.

Thus, it may be concluded that the first attempt to calculate the retention order of novel stereoisomers in terms of their hypothetical molecular structures with the aim of their structural elucidation by GC–MS has been successful. Due to the applied quantitative concept, the theoretically expected but previously unknown stereoisomers *trans-anti-trans*, *trans-syn-cis* and *cis-anti-cis*, were found and elucidated according to their molecular structure. The seventh unexpected isomer in terms of GC- and MS-data obtained was elucidated as conformer **B** of *cis-syn-cis*-PHX.

Experimental

The measurements of Henry's constants were carried out according to the procedure previously described in detail[7] using a Varian 3740 gas chromatograph equipped with a flame ionization detector. A glass column 40 cm in length and with 2 mm internal diameter, packed with HT GTCB Sterling MT (7.6 m^2 g^{-1}) of particle diameter 0.22–0.25 mm was used.

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