Gas Chromatography—Mass Spectrometry of the Stereoisomers of Heterocyclic Compounds. Part 1. Perhydrothioxanthenes

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A gas chromatographic–mass spectrometric study of a mixture of the stereoisomers of perhydrothioxanthene has been carried out. Separation was accomplished on columns filled (HT GTCB) with hydrogen treatment-graphitized thermal carbon black and on WCOT capillary columns. The stereospecific features of the retention and the fragmentation of the six stereoisomers under electron impact have allowed the identification of all of them; five of these were previously unknown. The fragmentation of *trans*-fused stereoisomers is characterized by predominant cleavage of middle-ring bonds, while the fragmentation of *cis*-fused stereoisomers proceeds through the cleavage of side-ring bonds.

The present paper introduces a series of experimental works concerned with the separation and identification of hetero-analogues of perhydroanthracene (PHA) and their structural congeners. All five stereoisomers that might be envisioned for PHA are actually known.[1] Among the heterocyclic analogues of PHA, perhydroacridine (PHAc) is the most well-studied. Theoretically, it possesses six stereoisomers,[2,3] of which four have been identified structurally. Evidence has been reported on the occurrence of two perhydroxanthene stereoisomers and one perhydrothioxanthene (PTHX) isomer.[4,5]

The isolation of an individual stereoisomer from a mixture is an arduous task, the successful accomplishment of which by conventional methods cannot be assumed. This causes a serious limitation on the structural elucidation of stereoisomers, especially those available in minor amounts. The most promising route to a solution of this problem is the use of gas chromatography–mass spectrometry (GC–MS) because of its high sensitivity, structure selectivity and separation power.

Identification by this method can be carried out using retention indices and/or mass spectra provided that both are sufficiently different. However, this implies the availability of *a priori* information about the retention parameters and mass spectra for stereoisomers of known spatial structure; otherwise, the assignment of any novel stereoisomer becomes practically impossible.

One route to a solution of this problem is to study the relationship between the spatial structures of isomers, the peculiarities of their GC retention and molecular-ion fragmentation. The establishment of such relationships allows significant augmentation of the diagnostic value of characteristics obtained by GC–MS experiments and the facilitation of stereoisomer assignment.

A. V. Kiselev and co-workers[6–9] have shown the possibility of wide and promising applications for graphitized thermal carbon black (GTCB) in resolving geometrical isomers by GC. As distinct from many sorbents, the retention on GTCB is mainly due to dispersive intermolecular interaction and is dependent to a large degree on the geometrical structure of molecules. For this reason, adsorption on GTCB can be used, along with the separation technique, in studying correlations between the retention indices and geometrical molecular parameters.

Among tricyclic saturated systems, the stereoisomers of PHA and perhydrophenanthrene (PHP) have been studied in greater detail.[10–13] Their mixtures were completely resolved on high-efficiency GTCB-packed columns, which allowed us to record mass spectra and to identify completely the constituent isomers.[14] The results obtained have provided a demonstrative example of the advantageous combination of GTCB gas-adsorption chromatography and mass spectrometry for the separation, identification and structural study of isomer molecules.

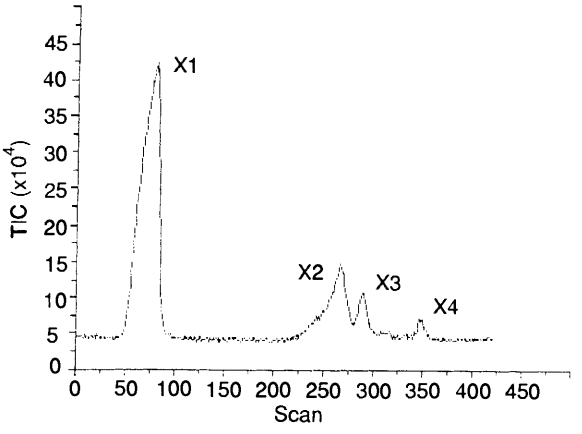


Fig. 1 Chromatogram of perhydrothioxanthenes on HT GTCB, 40 cm × 2 mm, temperature programming 150–220 °C, 10 °C min^{-1}, He flow rate 20 cm^3/min^{-1}

This paper presents the results of a study on the PHTX stereoisomers by GC–MS and ^{13}C NMR spectroscopy. To the best of our knowledge, we are unaware of similar studies ever having been performed on PHTX, although the mass spectra of PHTX and some of its homologues have been reported in the literature, but in those cases the authors studied a mixture of isomers without any separation.[15,16]

Results and Discussion

Until now, only one of the six PHTX stereoisomers, the *cis-syn-cis* isomer, has been studied in any detail.[5] An analysis of the known synthetic methods has provided evidence that the synthesis of PHTX as reported[17] yields an individual *trans-anti-cis* isomer. Simultaneously, PHTX as produced by catalytic hydrogenation of *sym*-octahydrothioxanthene on a palladium catalyst is a mixture of stereoisomers which we have chosen as the object of our investigation.

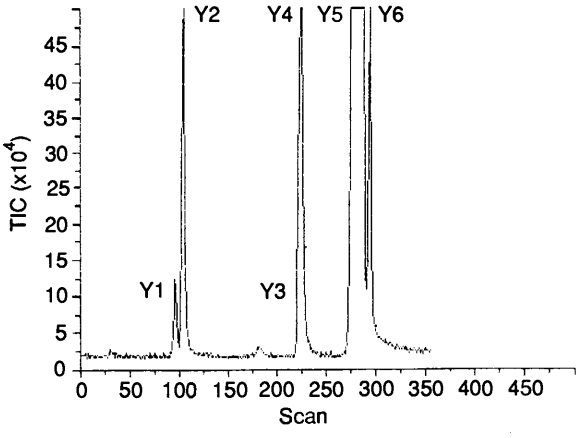


Fig. 2 Chromatogram of perhydrothioxanthenes on SP-1000, 60 m × 0.25 mm, temperature 150 °C, He flow rate 1 cm^3 min^{-1}

The PHTX stereoisomers were resolved using both a microbore HT GTCB-packed column (Fig. 1) and a high-efficiency capillary column with SP-1000 as a liquid phase (Fig. 2). Under the same conditions, the retention times of pure *cis-syn-cis* and *trans-anti-cis* PHTX isomers as standards were measured; the structures of these species were identified by ^{13}C NMR spectroscopy.[5]

The chromatogram shown in Fig. 1 exhibits four peaks (labelled X1–X4), each corresponding, in accordance with the mass-spectral evidence, to a PHTX stereoisomer. The retention times, measured under the same conditions for individual *cis-syn-cis* and *trans-anti-cis* PHTX isomers used as standards did not differ from those for the first two components of the mixture implying, by analogy with PHA, that these isomers were eluted in succession from the column as their molecular structure became increasingly more planar. Pursuing the PHA analogy, one may presume that *trans-syn-cis*-PHTX, differing from the *trans-anti-cis* isomer only in the position of the sulfur atom, shares a common peak with the latter isomer as they leave the HT GTCB-packed column (capable of resolving stereoisomers with spatially different structure). By contrast, *trans-syn-trans*-PHTX, whose more planar structure provides for a larger number of contacts with the flat surface of HT GTCB, is the last to leave the column (Fig. 1). The peak X3 in the chromatogram may presumably be assigned to *cis-anti-cis*-PHTX; the *trans-anti-trans* isomer as the thermodynamically least-stable species is expected to be present in the mixture in trace (if any) amounts.

This line of reasoning is in good accord with the ^{13}C NMR spectroscopic results which provide evidence for the occurrence of four stereoisomers in the mixture studied. Along with *cis-syn-cis* and *trans-anti-cis* isomers, the isomers *trans-syn-cis*- and *trans-syn-trans*-PHTX are also present. The ^{13}C NMR chemical shifts of these species are summarized in Table 1. It is noteworthy that no signals that might have been asssigned in the ^{13}C NMR spectrum to the *trans-anti-trans* isomer were observed, which lends support to the earlier suggestion as to the non-occurrence of this species in the mixture. Also, the ^{13}C NMR evidence provides no evidence for the presence of the *cis-anti-cis* isomer, which should be attributable to the conformational flexibility of this species thus rendering its identification uncertain at ambient temperature.

The mass spectra of the mixture components that have been resolved on the SP-1000 liquid-phase capillary column show a close correlation between the recorded six-peak chromatogram (peaks labelled Y1–Y6) and the number of theoretically expected PHTX isomers (Fig. 2). A comparison of these mass-spectra (Fig. 3) reveals that they differ among themselves to a much greater extent than the mass spectra of the previously-studied PHA[14] and PHAc[20] stereoisomers. This fact is indicative of a stereoselective fragmentation of PHTX isomers and may be thus helpful in their identification.

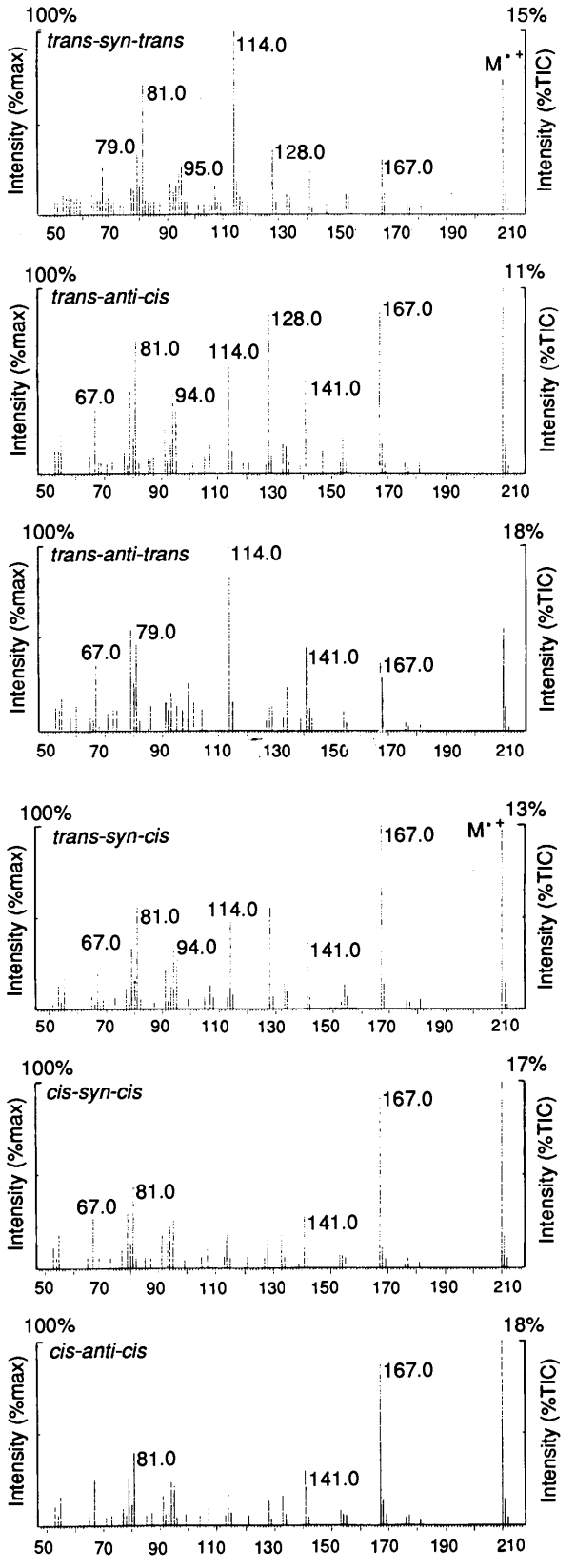
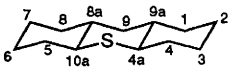
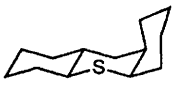
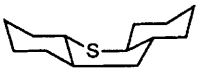
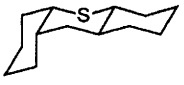
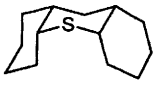
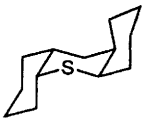


Fig. 3 Mass spectra of the stereoisomers of perhydrothioxanthene (70 eV)

A comparison of the mass spectra of the X1–X4 components (whose elution order from the GTCB-packed column has been discussed above) with those of the Y1–Y6 components gives the

Table 1 Structural formulae, percentage in mixture and spectral characteristics [δ_C(ppm)] for the stereoisomers of PHTX[9]

Isomer[b]	Structural formula	Percentage in mixture	$C^1(C^8)$	$C^2(C^7)$	$C^3(C^6)$	$C^4(C^5)$	C^9	$C^{4a}(C^{10a})$	$C^{8a}(C^{9a})$
trans-syn-trans		0,5	34.25	—	—	—	38.35	47.76	44.73
trans-anti-cis		4,7	25.56 (34.36)	27.07 (26.40)	21.03 (26.70)	31.74 (32.42)	41.42	44.32 (48.05)	37.62 (37.62)
trans-anti-trans		0,06	—	—	—	—	—	—	—
trans-syn-cis		7,2	34.57 (33.96)	20.05 (26.51)	27.76 (26.93)	29.75 (32.14)	34.14	42.49 (41.95)	45.41 (37.90)
cis-syn-cis		85,2	33.25	23.61	25.11	32.34	32.34	43.33	37.93
cis-anti-cis		2,3	—	—	—	—	—	—	—

[a] The assignment was performed according to Vierhapper increments[18] and the data in ref. 19. [b] We succeeded in distinguishing only four signals for *trans-syn-trans*-PHTX because other signals were overlapped by more intensive resonance signals due to the other isomers.

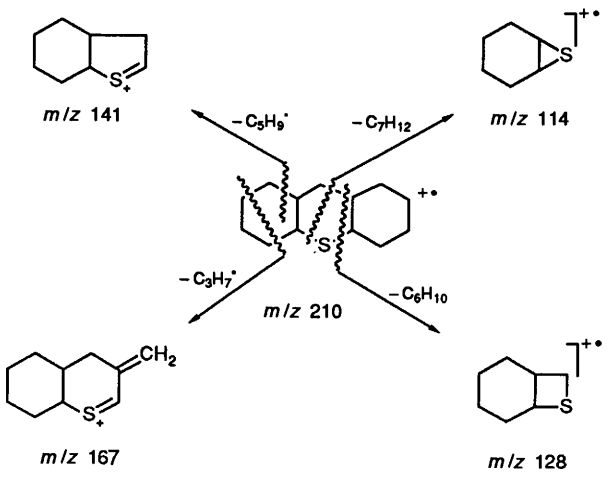
following sequence for the exit of PHTX stereoisomers from the SP-100 column: Y1 = X4, *trans-syn-trans*; Y2 = X2, *trans-anti-cis*; Y3, *trans-anti-trans*; Y4, *trans-syn-cis*; Y5 = X1, *cis-syn-cis*; and Y6 = X3, *cis-anti-cis* Figs. 1 and 2).

In assigning the Y3 and Y4 stereoisomers, the previously suggested co-elution of the structurally congeneric *trans-anti-cis* and *trans-syn-cis* isomers from the GTCB-packed column has been taken into account, which is further borne out by the similarity of the mass spectra of the Y2 and Y4 components. The *trans-anti-trans* isomer (Y3), like its hydrocarbon PHA analogue,[11] is expected to elute from the GTCB-packed column between the *cis-anti-cis* and *trans-syn-trans* isomers; however, taking into account the low efficiency of the column and the small concentration of Y3 component in the mixture (0.06%), its peak could not be detected against the zero-line drift background in the chromatogram. Thus, an alternative assignment for the Y3 and Y4 isomers is hardly possible, since the Y4 concentration (7.2%) is two orders of magnitude higher than that of Y3, and the Y4 isomer, were it present in the mixture, would have certainly been recorded as a fifth peak in the chromatogram (Fig. 1).

Thus, a comparison of the chromatograms (Figs. 1 and 2) shows that, on replacement of GTCB by a polar liquid phase, the elution order for PHTX stereoisomers is drastically changed and, in the latter case, is chiefly determined by dipole–dipole interactions between the adsorbate and the liquid phase. As distinct from GTCB, the complicated nature of these interactions precludes a reliable structural correlation for the isomers and, consequently, any attempt to predict their retention by liquid phase is merely speculative. It may be presumed, though, that the elution order is affected by the degree of shielding and, consequently, of the exposure to interaction of the large sulfur atom which is easily polarized by the hydrogen atoms of the side rings. The conformational flexibility of the stereoisomeric PHTX molecules might also be a contributing factor.

The complete GC separation of PHTX stereoisomers has enabled the recording of the mass spectra shown in Fig. 3. As with most cyclic compounds, PHTX is stable enough to electron impact (70 eV) which is manifested by the high peak intensities of the molecular ions. The PHTX stereoisomer fragmentation, similar to that of PHA, is chiefly determined by two groups of competing processes. The first group includes the processes leading to a cleavage of the side-ring bonds (β-cleavage). A major process in this group is the formation of a stable cation $[M - C_3H_7]^+$ with *m/z* 167, in which the charge is delocalized over two conjugate double bonds—a feature typical of many heterocyclic compounds. $[M - C_2H_5]^+$ ions (*m/z* 181) are also indicative of this group; however, their contribution to the total ion current (TIC) is rather small. The second group embraces the processes attended by a cleavage of the middle-ring bonds to produce ions with *m/z* 141, 128 and 114 (so-called α-cleavage). In either group, the fragment ions contain a heteroatom. The processes involving the elimination of HS and H_2S particles (*m/z* 177 and 176) from the molecular ions are of minor importance. Hypothetical structural formulae for the fragment ions produced by competing degradations of the PHTX molecular ions are shown in Scheme 1; their mass-spectral peak intensities, normalized to TIC, are summarized in Table 2.

The results in Table 2 show that the fragmentation specificity for the molecular ions of PHTX isomers is a major factor in determining the contributions due to β-cleavage ($[M - C_3H_7]^+$ ions, *m/z* 167) and α-cleavage ($[C_6H_{10}S]^{\bullet+}$ ions, *m/z* 114 and $[C_7H_{12}S]^{\bullet+}$ ions, *m/z* 128), these peaks exhibiting the



Scheme 1

Table 2 Mass spectra of the stereoisomers of PHTX (70 eV)[a]

	% TIC					
m/z	*t-s-t*	*t-a-c*	*t-a-t*	*t-s-c*	*c-s-c*	*c-a-c*
211	1.8	1.7	2.4	1.8	2.8	2.6
210	12.0	11.0	10.0	12.0	17.0	18.0
168	0.7	1.8	0.8	1.8	2.3	2.3
167	4.7	10.0	6.4	13.0	16.0	16.0
155	1.0	1.0	1.0	1.0	1.0	1.0
154	1.7	2.0	1.7	1.7	1.2	1.1
147	1.1	1.2	—	0.8	—	—
141	3.7	5.6	7.9	4.7	4.7	5.2
134	0.7	1.5	4.0	1.2	0.8	0.7
133	0.7	1.8	—	1.7	3.1	2.7
129	0.7	1.0	2.0	0.8	—	—
128	5.8	9.7	1.5	7.4	2.5	2.5
115	3.0	1.3	—	1.0	0.6	1.2
114	15.0	6.5	18.0	5.8	3.0	3.8
107	2.6	1.9	—	1.7	1.7	1.6
105	0.5	1.0	—	0.8	1.0	1.0
95	4.1	0.4	2.4	3.6	4.2	3.9
94	3.4	0.5	—	4.0	3.7	4.2
93	2.5	2.0	3.6	1.7	2.1	2.0
91	2.7	2.7	2.6	2.7	3.0	2.9
87	0.5	1.0	2.7	0.9	1.0	1.1
85	1.1	0.9	—	0.6	0.8	0.9
81	11.0	7.9	8.2	7.3	7.5	7.3
80	2.4	2.2	4.6	2.0	2.2	2.1
79	5.1	4.9	9.6	4.2	5.0	4.7
77	2.2	1.4	—	1.4	1.7	1.6
67	4.0	4.0	6.3	3.7	4.6	4.4
55	2.1	2.3	3.1	2.4	2.9	2.9
53	1.8	1.3	2.1	1.6	1.8	1.8

[a] *t, trans*; *c, cis*; *s, syn*; *a, anti.*

greatest difference in intensity. In the first place, this is true for the stereoisomers having differently-fused rings. The mass spectra of stereoisomers exhibiting a similar ring fusion: *trans-syn-trans* (Y1) and *trans-anti-trans* (Y3), *trans-anti-cis* (Y2) and *trans-syn-cis* (Y4), *cis-syn-cis* (Y5) and *cis-anti-cis* (Y6) are not very different.

Peaks with *m/z* 167 are the most prominent in the mass spectra of *cis*-fused stereoisomers (Y5 and Y6), where as the intensity of the peaks corresponding to the competing processes yielding the ions with *m/z* 114 and 128 is rather low (Fig. 3, Table 2).

A reversed trend is observed in the mass spectra of *trans*-fused isomers: the peak due to the $[C_6H_{10}S]^{\cdot+}$ ion (*m/z* 114) is predominant whereas the peak intensity of the $[C_7H_{12}S]$ ion (*m/z* 128) is greater than that of the $[M - C_3H_7]^+$ ion (*m/z* 167) (Fig. 3).

Table 3 Contribution ratio for the competing fragmentation processes followed by α- and β-cleavage

t-s-t	*t-a-t*	*t-a-c*	*t-s-c*	*c-s-c*	*c-a-c*
0.22	0.33	0.62	0.98	2.9	2.5

In the PHTX stereoisomer series, the fragmentation of *trans-cis* isomers (Y2 and Y4) is the least selective, whereas the mass-spectral peaks of $[C_6H_{10}S]^{\cdot+}$ (*m/z* 114), $[C_7H_{12}S]^{\cdot+}$ (*m/z* 128), and $[M - C_3H_7]^+$ (*m/z* 167) ions, corresponding to the aforementioned competing processes of monomolecular degradation, exhibit an intermediate intensity (Fig. 3, Table 2). In other words, its fragmentation is characterized by diagnostic features of both *cis*- and *trans*-fused isomers in roughly equal measure. However, the peculiarity of fragmentation of *trans-cis*-fused isomers is the primary formation of $[C_7H_{12}S]^{\cdot+}$ ions (*m/z* 128) due to α-cleavage (Fig. 3, Table 2).

The stereospecific features considered above, concerning the PHTX isomers fragmentation (involving the competing processes of α- and β-cleavage) can conveniently be estimated by means of the factor *K*, eqn. (1), where I_{167}, I_{128} and I_{114} are

$$K = I_{167}/(I_{128} + I_{114}) \quad (1)$$

the peak intensities for ions $[M - C_3H_7]^+$, $[C_7H_{12}S]^{\cdot+}$ and $[C_6H_{10}S]^{\cdot+}$, respectively, expressed as a percentage relative to the TIC (Table 3). As follows from Table 3, for *trans*-fused side rings this factor is much smaller than for *cis*-fused rings, whereas for *trans-cis* stereoisomers it takes an intermediate value.

Thus, using data obtained by GTCB gas–solid and gas–liquid chromatography, mass spectrometric and ^{13}C NMR spectroscopic methods we have found for the first time the five stereoisomers of PHTX: *trans-syn-trans*, *trans-anti-cis*, *trans-anti-trans*, *trans-syn-cis* and *cis-anti-cis*. The fragmentation of PHTX isomers under electron impact differ considerably, which enables their easy identification using the methods described earlier.

Experimental

The mixture of PHTX isomers was produced by catalytic hydrogenation of *sym*-octahydrothioxanthene on a palladium catalyst.[17] GC separation was carried out in a glass column 40 cm in length and 2.0 internal diameter, packed with HT GTCB Sterling MT (7.6 $m^2\ g^{-1}$) of particle diameter 0.22–0.25 mm and capillary column 60 m in length and 0.25 mm internal diameter with a SP-1000 liquid phase (Supelco, Inc.) using a Varian 3740 gas chromatograph. Mass spectra were obtained using a model MAT 212, at ionizing energy 70 eV and ionizing chamber temperature 180 °C.

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Paper 1/06429K
Received 23*rd December* 1991
Accepted 30*th January* 1992