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

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The importance of GC-data for the structural elucidation of novel isomers by GC–MS is discussed. A gas chromatographic–mass spectrometric study of mixtures of the stereoisomers of perhydroxanthene (PHX) has been carried out. Separation has been accomplished on a column packed with graphitized thermal carbon black (GTCB) and on a capillary column of high efficiency. In accordance with the mass spectral evidence together with the known *cis-syn-cis*, *trans-anti-cis* and *trans-syn-trans* isomers four novel stereoisomers have been found. Three of them, *cis-anti-cis*, *trans-syn-cis* and *trans-anti-trans* are theoretically expected, whereas the fourth novel isomer is surprising and has been preliminarily assigned as conformer B of *cis-syn-cis* PHX. The stereospecificity of the fragmentation of PHX isomers under electron impact appears almost suppressed by the predominant formation of a stable oxonium ion $[M - C_3H_7]^+$. The similarity of the mass spectra complicates the structural elucidation of novel isomers based on the qualitative relationships between their retention on the column packed with GTCB and hypothetical molecular structures.

Introduction

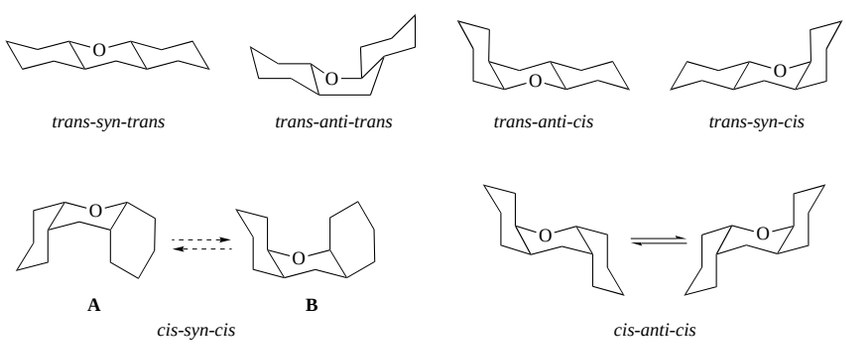
Perhydroxanthene (PHX), the oxygen-containing analogue of perhydroanthracene (PHA), theoretically exists as six diastereomers, as with perhydrothioxanthene (PHTX) studied previously.[1] By analogy with PHA and PHTX the inversion of the middle ring of the conformationally mobile *cis-cis*-isomers is possible. Inversion of the *cis-anti-cis*-PHX forms two equivalent conformations, whereas the conformers **A** and **B** of *cis-syn-cis*-PHX are different.

PHX was synthesised at first by Braun by means of a catalytic hydrogenation of xanthone with a nickel catalyst.[2] However, the stereochemical composition of the reaction products was not studied. The results of the structural elucidation by ^{13}C NMR spectroscopy of the *cis-syn-cis*- and *trans-anti-cis*-PHX obtained by catalytic reduction of bicyclic 1,5-diketones were reported much later.[3,4] On the basis of this data Yudovich and co-workers inferred an absence of the inversion of *cis-syn-cis*-PHX and the preference for conformer **A** which is more stable. This important fact was interpreted in terms of the shorter C–O bond, as compared with the C–C bond (1.42 and 1.54 Å, respectively), and the resulting increase of 1,3-diaxial intramolecular interactions in the molecule of conformer **B**.[4,5] The possibility of the stereochemically directed synthesis of these isomers has also been demonstrated.[3,4] There is evidence for the preferred formation of the *trans-syn-trans*-PHX from the ion hydrogenation of *sym*-octahydroxanthene.[6,7] The existence of the other three stereoisomers is not confirmed in the literature. Moreover, to the best of our knowledge, we are unaware of GC–MS studies ever having been performed on PHX.

Similarly to PHTX,[1] it might be expected that *trans-anti-trans*, *trans-syn-cis* and *cis-anti-cis* isomers of PHX occur in mixtures in minor amounts. Thus, we are dealing with a common problem in the chemistry of isomeric organic compounds—the search for and the structure elucidation of novel isomers that are of low abundance in mixtures and that are difficult to separate. The possibilities of using NMR spectroscopy, commonly used for structure assignments, in this case are limited by its relatively low sensitivity. Therefore, the idea of extending the potential of GC–MS as a more sensitive method to solve this problem is very enticing. However, a conventional mass spectrum in most cases is just sufficient to establish the relationship of a novel isomer to its family, but cannot be used for the structure elucidation without additional data. In this respect, as we have emphasised before,[1] the consideration of the GC-data seems to be of great interest.

In order to contribute to the structure elucidation of novel isomers the gas chromatography must satisfy two important conditions: (*i*) it should provide the complete separation of all stable isomers expected in a sample; and (*ii*) it should be possible to predict the separation order of isomers on the basis of their hypothetical structure.

The accomplishment of the first condition is now rather simple. Modern capillary gas–liquid chromatography provides the highest separation efficiency and good sensitivity when coupled to MS because of the sharp profiles of the GC peaks. The second condition is the most important and much



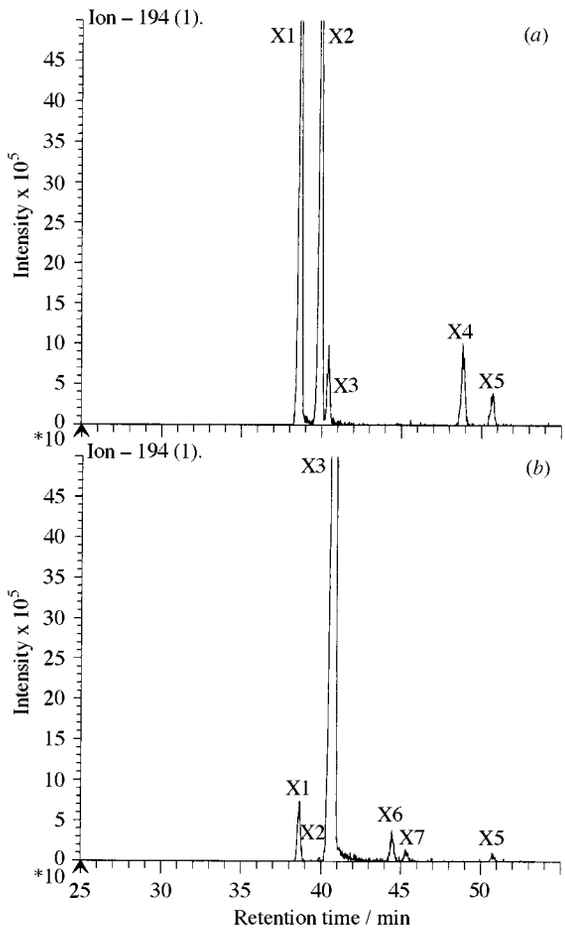


Fig. 1 Mass chromatograms for ions with *m/z* 194 of mixture 1 (*a*) and mixture 2 (*b*) of the stereoisomers of perhydroxanthene on DB-1, 60 m × 0.25 mm, 170 °C

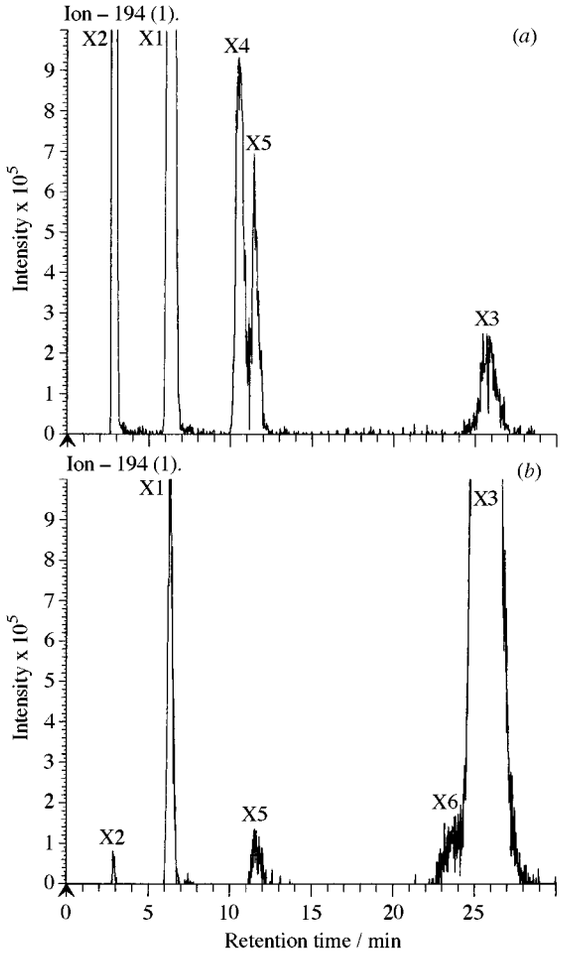


Fig. 2 Mass chromatograms for ions with *m/z* 194 of mixture 1 (*a*) and mixture 2 (*b*) of the stereoisomers of perhydroxanthene on GTCB, 2 m × 1 mm, 230 °C

more difficult. It implies the possibility of establishing reliable relationships between retention parameters and hypothetical molecular structures. It was emphasised previously that only gas-solid chromatography with graphitized thermal carbon black (GTCB) as an adsorbent could fulfil this requirement.[1,8,9]

In spite of its simplicity, a qualitative concept of location of stereoisomer molecules on the flat surface of GTCB has contributed to the structure elucidation of perhydroanthracenes (PHA), perhydrophenanthrenes (PHP) and perhydrothioxanthenes (PHTX) provided that their mass spectra appeared distinct.[1,9] This paper presents the results of a GC–MS study of the two mixtures of PHX stereoisomers having been synthesised according to the above-mentioned methods and kindly donated by Yudovich and co-workers and Blinokhvatov.[3,6]

Results and discussion

The first step in this study was the separation and the search of all PHX stereoisomers expected in the mixtures. This was carried out using a highly efficient capillary column with a non-polar stationary liquid phase. The mass chromatogram for the molecular ions (*m/z* 194) shown in Fig. 1(*a*) corresponds to the separation of mixture 1, obtained by reduction of bicyclic 1,5-diketones,[3,4] and exhibits five peaks (labelled X1–5). Each peak corresponds, in accordance with the mass-spectral evidence, to a PHX stereoisomer. The retention time of the stereoisomer X1 coincides with the retention time of an individual *trans-anti-cis* isomer also donated by the authors.[3,4] On the basis of the ^{13}C NMR data[4,5] the second predominant component X2 of this mixture corresponds to the *cis-syn-cis* isomer (conformer **A**).

The separation of the mixture 2, obtained by the ion hydrogenation of *sym*-octahydroxanthene,[6,7] was carried out under the same conditions. The mass chromatogram for the molecular ions exhibits six peaks, all of those, in accordance with the mass-spectral evidence, corresponds to PHX stereoisomers [Fig. 1(*b*)]. As was expected from the synthesis conditions and ^{13}C NMR data,[7] there is one prominent peak X3 on this chromatogram, which most likely corresponds to *trans-syn-trans*-PHX. The labelling of GC-peaks of mixture 2 has been carried out by comparison of the coincidence of their retention times with the retention times of stereoisomers of mixture 1. The result of the comparison was unexpected. Only four stereoisomers (X1, X2, X3 and X5) were found in both mixtures (their retention times coincide), whereas X4, X6 and X7 have different retention times. That is, instead of six theoretically expected diastereomers in both mixtures seven compounds were found with similar mass spectra. According to the synthesis conditions, the actual and possible by-products have structures and, therefore, mass spectra quite distinct from PHX. One could surmise regardless of the synthesis conditions the existence of structural isomers of PHX, but in that case their mass spectra most probably would also differ. Therefore, there are formal grounds to presume the existence of seven stable stereoisomeric forms of PHX. If this is the case, in view of the conformational immobility of the *cis-syn-cis*-PHX emphasised earlier,[4,5] the only stereoisomeric form, which could be considered as a seventh, is conformer **B** of this isomer.

The elution order of PHX stereoisomers from the capillary column coated with the non-polar stationary liquid phase differs compared to the stereoisomers of PHA and PHTX.[1,9] In particular, *cis-syn-cis*-PHX is the second species to appear on

the chromatogram (X2, Fig. 1), whereas *cis-syn-cis* isomers of PHA and PHTX are the fifth isomers to elute. This fact may be related to the conformational immobility of *cis-syn-cis*-PHX. *Trans-syn-trans* isomers of PHA and PHTX have the lowest retention time, whereas *trans-syn-trans*-PHX is the third species to elute (X3, Fig. 1). Thus, taking into account the complicated nature of intermolecular interactions of stereoisomers with the liquid phase, no assumptions about the four unknown isomers (X4–7) could be made.

The mass chromatograms for the ions with *m*/*z* 194 from the separation of the mixtures 1 and 2 on the column packed with GTCB are shown in Fig. 2. The GC peaks in Figs. 1 and 2 were related to each other in accordance with the abundance of isomers in the mixtures. The most abundant components of the mixtures corresponding to the known stereoisomers elute from this column in the expected order according to their molecular geometry and in a similar way to their congeners of PHA and PHTX: *cis-syn-cis* (X2) the first, *trans-anti-cis* (X1) the second and *trans-syn-trans* (X3) the last. The small unresolved peak (X6) near *trans-syn-trans*-PHX (X3) may most probably be assigned to *trans-anti-trans*-PHX, which is nearly flat [Fig. 2(*b*)]. The isomer corresponding to the GC-peak X7 on the chromatogram of Fig. 1(*b*) is not separated on the column packed with GTCB and an appropriate peak on the chromatogram of Fig. 2(*b*) is absent.

The mass spectra (70 eV) of the stereoisomers of PHX, in contrast to PHTX studied before,[1] appeared to be similar (Fig. 3). As with most of the cyclic compounds, in particular PHTX, PHX is stable to electron impact, which is demonstrated by the high peak intensities of the molecular ions (*m*/*z* 194). The major fragmentation process of PHX, in the same way as PHTX,[1] leads to formation, *via* β-cleavage, of a stable onium ion

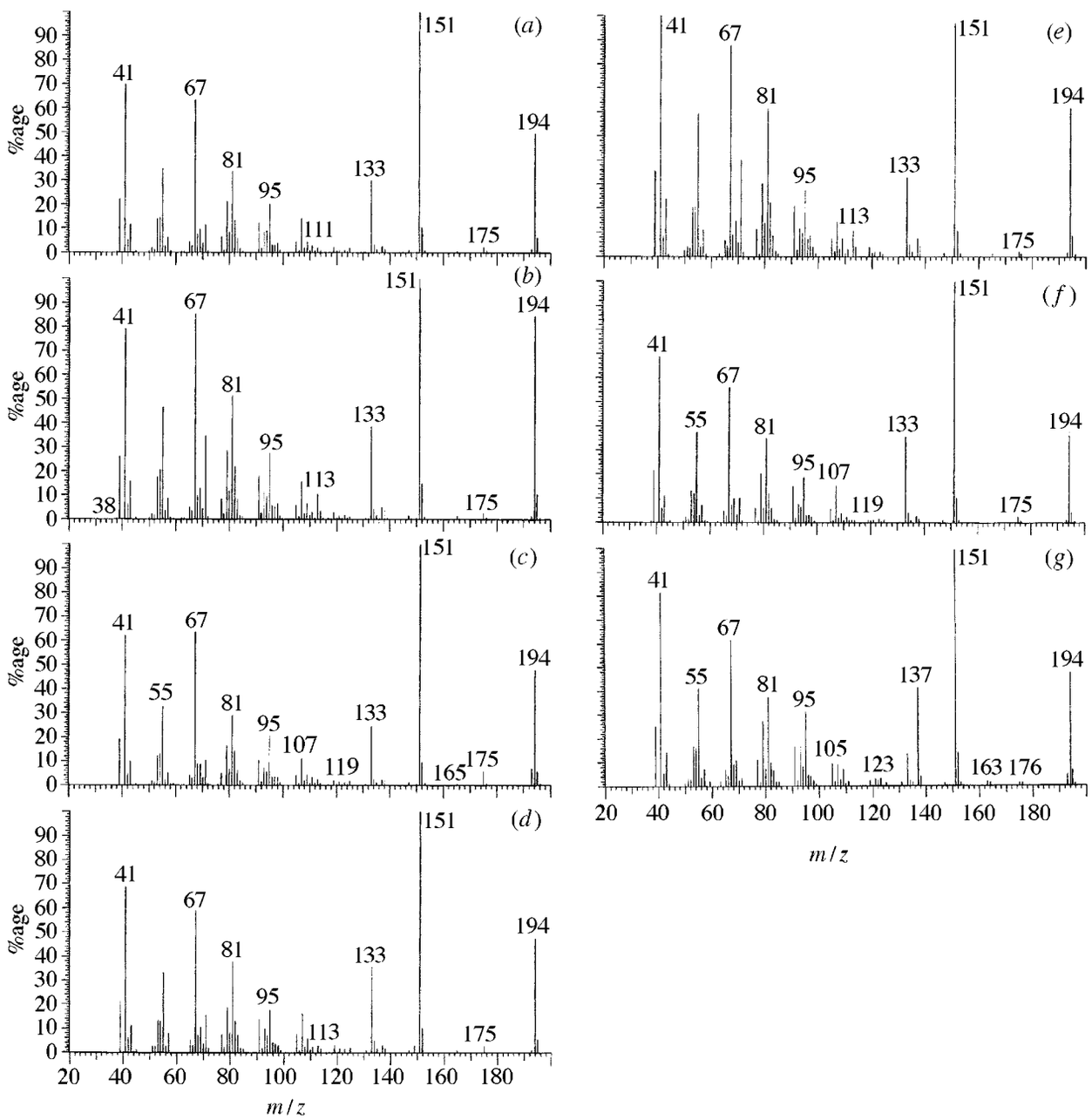
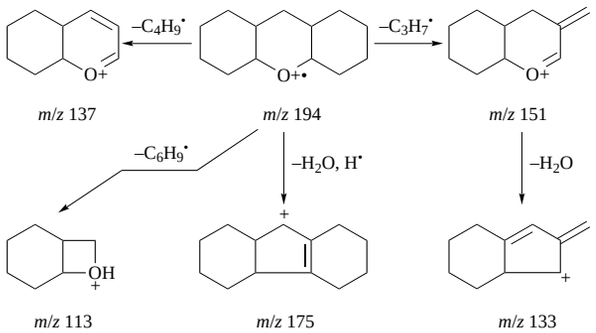


Fig. 3 Mass spectra of the stereoisomers of perhydroxanthene (70 eV): (*a*) *trans-anti-cis*, (*b*) *cis-syn-cis* (**A**), (*c*) *trans-syn-trans*, (*d*) *trans-syn-cis*, (*e*) *cis-anti-cis*, (*f*) *trans-anti-trans*, (*g*) *cis-syn-cis* (**B**)



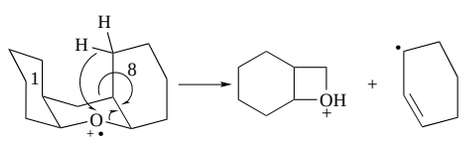
Scheme 1

$[M - C_3H_7]^+$ with *m*/*z* 151, in which the charge is delocalized over two conjugate double bonds (Scheme 1).

The elimination of $C_2H_5^{\bullet}$ and $C_4H_9^{\bullet}$ radicals also proceeds *via* a β-cleavage, but contribution of ions with *m*/*z* 165 and 137 to the total ion current (TIC) is small (with the exception of *m*/*z* 137 in the mass spectra of stereoisomer X7, Fig. 3). In a similar way to PHTX, the fragmentation of PHX is characterised by processes involving the elimination of the heteroatom from the molecular ions (*m*/*z* 175 and 176).

However, in contrast to PHTX, alternative fragmentation processes *via* an α-cleavage are negligible. This fact is in keeping with the results reported earlier by Keyes and Harrison who investigated the energetic characteristics of competing fragmentation pathways occurring *via* α- and β-cleavages in a number of sulfur-containing compounds and compared them with similar processes of oxygen-containing analogues.[10] As has been ascertained, the activation energies of these processes in sulfides are quite similar, whereas in the case of oxygen-containing analogues the activation energy of processes with β-cleavage is *ca*. 125 kJ mol^{-1} lower. A similar difference in competitive processes proceeding *via* α- and β-cleavage is presumed for PHTX and PHX. If this is the case the similarity of the mass spectra of PHX stereoisomers and therefore the lack of stereospecificity of their fragmentation, as compared with PHTX stereoisomers,[1] seems to be related to the predominant formation of an oxonium ion $[M - C_3H_7]^+$ (*m*/*z* 151) and the suppression of competitive processes. At the same time, due to this process most of the intramolecular interactions, which are responsible for the stereospecificity of fragmentation, disappear with the loss of the side ring.

However, with detailed consideration some differences in mass spectra of stereoisomers X2, X5 and X7 have been detected. The intensities of the peak of *m*/*z* 113 in the mass spectra of the isomer *cis-syn-cis* (X2) and isomer X5 comprise 1.09 and 1.04% of the TIC respectively, that is about four times as much as compared with other isomers, in particular, *trans-anti-cis* (0.27%, Fig. 3). The formation of $[C_7H_{13}O]^+$ ions (*m*/*z* 113) probably proceeds through a rearrangement process involving α-cleavage (Scheme 2). An important factor promoting the occurrence of this rearrangement seems to be the proximity of the oxygen atom and the axial CH_2 groups (C1, C8) of the side rings, which is inherent to only the *cis-cis*-isomers (Scheme 2). This stereospecific feature enables elucidation of the X5 isomer as *cis-anti-cis*.



Scheme 2

In the mass spectra of stereoisomer X7 the abundance of the ions $[M - C_4H_9]^+$ (*m*/*z* 137) is about one order greater than for the other isomers (Fig. 3). This distinction may be related to the *cis-syn-cis* (**B**) isomer which is less stable due to strong non-valence intramolecular interactions compared with the *trans-syn-cis*-PHX.

If this is the case, together with the known stereoisomers of PHX [*trans-anti-cis* (X1), conformer **A** of *cis-syn-cis* (X2) and *trans-syn-trans* (X3)], according to the data obtained four novel isomers may be assigned as follows: X4, *trans-syn-cis*, X5, *cis-anti-cis*; X6, *trans-anti-trans*; and X7, *cis-syn-cis* (**B**). However, allowance must be made for the fact that only the elucidation of the *cis-anti-cis* and *trans-anti-trans* isomers is supported by the arguments based on the stereospecificity of fragmentation and GC-retention and given previously whereas the assignment of *trans-syn-cis* and *cis-syn-cis* (**B**) is just hypothesis.

From the results obtained it is concluded that the qualitative relationship between retention parameters and molecular geometry which is acceptable in the case of isomers having distinct mass spectra (for example PHTX[1]), do not help much in the structural elucidation of novel isomers having similar mass spectra. Now it is possible to solve this problem by the quantitative calculation of the retention order of novel adsorbates using the approach published recently.[11] The results of the application of this new quantitative concept to the further structural elucidation of the stereoisomers of PHX will be presented in the next paper of this series.

Experimental

GC separation was carried out in a glass column 2 m in length with 1 mm internal diameter, packed with HT GTCB Sterling MT (7.6 m^2 g^{-1}) of particle diameter 0.15–0.20 mm and on a DB-1 capillary column (J & W Scientific) 60 m in length and with 0.25 mm internal diameter using a Varian 3740 gas chromatograph. Mass spectra were obtained using a model MAT 112S, at ionizing energy 70 eV and ionizing chamber temperature 220 °C.

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References

1 Part 1: V. G. Adeeva, M. S. Bobyleva, N. S. Kulikov, V. G. Kharchenko and L. M. Yudovich, *J. Chem. Soc.*, *Perkin Trans. 2*, 1992, 965.
2 J. Braun and O. Bayer, *Chem. Ber.*, 1926, **59**, 2317.
3 V. G. Kharchenko, L. M. Yudovich and N. S. Smirnova, *Zh. Org. Khim.*, 1987, **23**, 576.
4 V. G. Kharchenko, N. S. Smirnova and L. M. Yudovich, *Khim. Geterotsikl. Soedin.*, 1990, 1011.
5 L. M. Yudovich, Ph.D. Thesis, Saratov, 1988.
6 A. F. Blinokhvatov, Ph.D. Thesis, Saratov, 1970.
7 V. G. Kharchenko and A. F. Blinokhvatov, *Khim. Geterotsikl. Soedin.*, 1978, 1615.
8 A. V. Kiselev, *Intermolecular Interactions in Adsorption and Chromatography*, Vyshaya Shkola, Moscow (in Russian), 1986.
9 A. V. Kiselev, N. S. Kulikov and G. Curthoys, *Chromatographia*, 1984, **18**, 297.
10 B. G. Keyes and A. G. Harrison, *J. Am. Chem. Soc.*, 1968, **90**, 5671.
11 N. S. Kulikov, *Adsorpt. Sci. Technol.*, 1997, **15**, 115.

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