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RETENTION INDICES OF SOME ERGOPEPTINES IN REVERSED-PHASE HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY*

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SUMMARY

The chromatographic behaviour of some naturally occurring ergot alkaloids of the peptide type ("ergopeptines") and their isomers was examined by reversed-phase high-performance liquid chromatography. A satisfactory separation of eight natural ergot alkaloids and their isomers on LiChrosorb RP-18 is achieved with a neutral mobile phase of acetonitrile–water at pH 7 without addition of alkaline modifiers such as triethylamine or carbonate. For a better correlation of retention data with chemical structure, retention indices have been calculated using the homologous series of 2-ketoalkanes as reference compounds. On the octadecylsilica columns, the differences in retention indices (ΔI values) were nearly constant for particular compounds or molecular fragments, even when the absolute I values differed by 100 units or more.

INTRODUCTION

The natural ergot alkaloids can be obtained by extraction of strains of the fungus "claviceps purpurea". One of the four main structural groups of ergot alkaloids are the peptide alkaloids. They are also called "ergopeptines" and consist of a D-lysergic acid, linked by a peptide bond to a tricyclic peptide moiety of the cyclol type. The biological and pharmaceutical activities of the ergopeptines depend on their configuration; only derivatives of the natural D-lysergic acid show a physiological activity, whereas the derivatives of the D-isolysergic acid are much less active[1]. Conventionally these isomers are distinguished by the endings "-ine" (--- lysergic acid) and "inine" (--- isolysergic acid). Isomerization occurs very rapidly in alkaline solution, more slowly during storage or extraction of sclerotia[1].

In recent years, the qualitative and quantitative analysis of ergot alkaloids has often been performed by high-performance liquid chromatography (HPLC) using silica gel[2,3], alkylamine-modified silica gel[4] and especially octyl and octadecyl phase[3,5] as stationary phases. In order to get good separations on octadecylsilica columns the use of alkaline mobile phases is often employed[3,5]. This is achieved by

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adding carbonate[3] or triethylamine[5] to the mobile phase. The disadvantage of this method is the inherent instability of the support material in reversed-phase (RP) columns towards alkaline solutions and the danger of isomerization during analysis. This can be partially overcome by the use of mobile phases buffered to pH 7.

In 1956 the first paper concerning a relationship between the chromatographic behaviour on cellulose paper and the chemical structure of some ergot alkaloids was published by Macek[6]. Since the ergopeptines represent a closely related family of compounds, differing only by two hydrocarbon substituents at positions R_1 and R_2 of the peptide moiety, these compounds could also be used as model substances for correlating the chromatographic properties on reversed-phase columns with chemical structure. In gas chromatography (GC) the Kováts retention index scale is already widely used in this manner. Recently, some efforts have been made toward establishing a retention index scale in LC, which should allow a better reproducibility and a better documentation of LC data and results[7–11], and some investigations concerning the linearity of retention plots for homologous series in RPLC have been published[7,8,12,13]. Good results have been obtained with the 2-ketoalkanes[7] and the aryl alkyl ketones[8] as a base for the calculation of retention indices.

In this work, retention indices for ergopeptines have been calculated using the 2-ketoalkanes as standard compounds. It is shown that the use of such indices allows more precise statements about the chromatographic behaviour and the correlation of structure and retention data for these alkaloids.

EXPERIMENTAL

Instrumentation

HPLC was performed using a Waters liquid chromatography pump M-6000 A with a Waters U6K injector. The detector was a variable wavelength UV–VIS spectrophotometric detector (190–800 nm), Perkin-Elmer Model LC-55 with an 8-μl flow-cell. It was connected to a Varian Integrator Model CDS-101.

Columns

Columns (250 × 4 mm I.D.), packed with LiChrosorb RP-18, 5 μm were used as received (Merck, Darmstadt, F.R.G.). Other columns (250 × 4.6 mm I.D. and 120 × 4.6 mm I.D.) were slurry-packed with LiChrosorb RP-18, 5 μm (Merck) and Nucleosil-5 C_{18} (Macherey & Nagel, Düren, F.R.G.) in our laboratory.

Reagents and chemicals

All solvents and chemicals were of analytical reagent grade.

Acetone and butanone were obtained from Merck, pentanone to decanone from Fluka (Buchs, Switzerland) and the other ketones from Analabs (Antechnika, F.R.G.). All ergot alkaloids were obtained from Dr. Rentschler, Arzneimittel (Laupheim, F.R.G.).

Chromatography

Chromatography was performed at room temperature. All of the reversed-phase columns were used with a buffered mobile phase (Na_2HPO_4–KH_2PO_4, adjusted to pH 7.0). The actual solvent composition for calculation of the retention

indices was acetonitrile–phosphate buffer pH 7 (50:50). Flow-rate: 1.0 ml/min. Pressure: ≈200 bar. Detection: UV, 280 nm (ketones) and 310 nm (alkaloids). Ketone reference solution: ≈ 5 μg/μl of each compound dissolved in methanol. Injection volume: 20 μl. Alkaloid solution: 10–60 ng/μl of each compound dissolved in acetonitrile. Injection volume: 20 μl.

The maximum of the solvent peak was used for measuring the dead time of the chromatographic system.

Calculation of retention indices

The retention indices were calculated as for the Kováts indices in GC:

$$I\,(\mathrm{x}) = 100 \cdot \frac{\log k'\,(\mathrm{x}) - \log k'\,(n)}{\log k'\,(n+1) - \log k'\,(n)} + 100\,n \qquad (1)$$

The constancy of the system was checked by injection of standard homologous series before and after each measurement of alkaloid retention.

In analogy to the work of Baker and Ma[7], acetone was assigned a value of 300. The calculation of retention indices is based on the assumption of a linear relationship between the logarithm of the capacity factor (log k') and the number of carbon atoms, n, in a standard homologous series[12]:

$$\log k' = pn + q \qquad (2)$$

Fig. 1 shows that plots of log k' *versus* carbon number, n, were linear over the examined concentration ranges in acetonitrile–water (50:50 to 71.5:28.5) and methanol–water (60:40 to 75:25).

The retention indices in Table II are average values from three measurements; the difference between these measurements did not exceed 3 index units.

RESULTS AND DISCUSSION

As mentioned before, the ergopeptines differ only by the two substituents R_1 and R_2 in their peptide moiety. Table I shows the different structures of the allkaloids examined.

A good separation of the alkaloids under reproducible conditions is necessary. It is possible to separate these substances and their isomers ("-inines") under neutral conditions by buffering the mobile phase to a pH of 7.0 by means of a phosphate buffer. A chromatogram of this separation under neutral conditions is shown in Fig. 2. Separation of the alkaloids at neutral pH could have some advantages, because under alkaline conditions the danger of partial isomerization during analysis is always present. When the content of isomerization products in a drug or a crude plant extract is to be analysed it is therefore often better to work under non-alkaline chromatographic conditions.

With the 2-ketoalkanes as reference compounds, retention indices (I values) can be calculated. Table II shows I values for some natural ergopeptines on columns of different dimensions (1 and 2) and different stationary phases (3). It is obvious that, with the same mobile phases, differences in column packing are reflected in

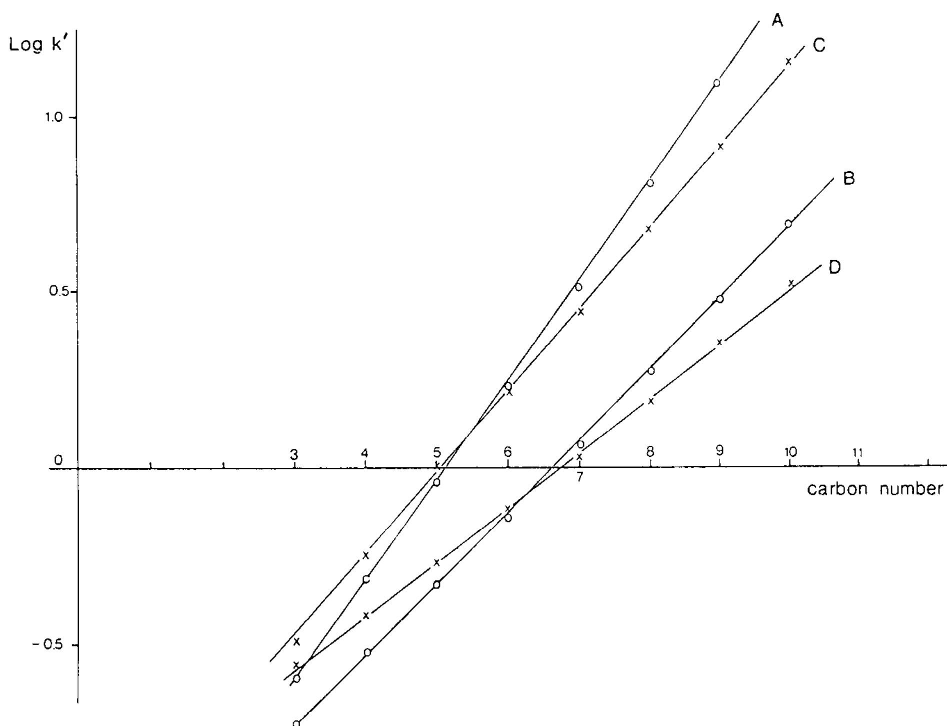
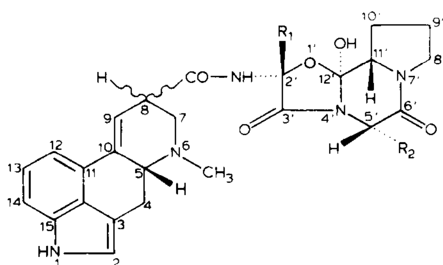


Fig. 1. Plot of log *k'* *vs.* carbon number of 2-ketoalkanes. Mobile phases: A, methanol–water (60:40); B, methanol–water (75:25); C, acetonitrile–water (50:50); D, acetonitrile–water (71.5:28.5).

TABLE I

STRUCTURES OF THE ERGOPEPTINES EXAMINED



Alkaloid	R_1	R_2
Ergosine	Methyl	Isobutyl
Ergotamine	Methyl	Benzyl
Ergostine	Ethyl	Benzyl
Ergocornine	Isopropyl	Isopropyl
α-Ergokryptine	Isopropyl	Isobutyl
β-Ergokryptine	Isopropyl	*sec.*-Butyl
Ergocristine	Isopropyl	Benzyl

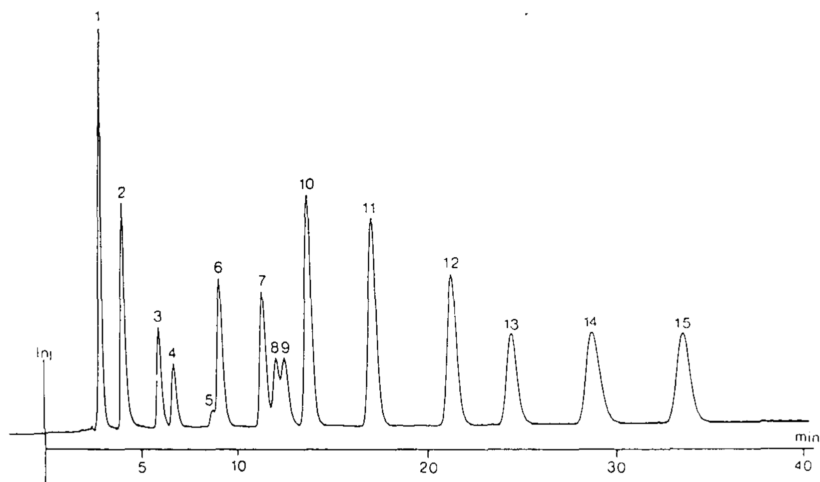


Fig. 2. Separation of eight natural ergot alkaloids and isomers under neutral conditions. Stationary phase: LiChrosorb RP-18, 5 μm. Mobile phase: acetonitrile–0.02 *M* phosphate buffer (pH 7.0) (50:50). Peaks: 1 = ergobasine; 2 = ergobasinine; 3 = ergosine; 4 = ergotamine; 5 = ergostine; 6 = ergocornine; 7 = α-ergokryptine; 8 = β-ergokryptine; 9 = ergocristine; 10 = ergosinine; 11 = ergotaminine; 12 = ergocorninine; 13 = ergostinine; 14 = α-ergokryptinine; 15 = ergocristinine.

changes in retention index, as expected. In some cases these changes can exceed 100 index units. It is therefore useful to calculate differences in retention indices (ΔI values), which reflect a change in substituents R_1 and/or R_2 through addition or removal of C_xH_y fragments or substitution of one fragment for another, *i.e.*, phenyl for isopropyl. From Table III, it is evident that the ΔI values of ergopeptines are nearly constant on the three columns examined, even when the I values differed by 100 units or more.

One of the important recent approaches to a correlation of chemical structure

TABLE II

RETENTION INDICES OF SOME ERGOPEPTINES AND ISOMERS

Mobile phase: acetonitrile–water (50:50) buffered to pH 7.0. Columns: 1, Hibar RT 250-4 (Merck); LiChrosorb RP-18, 5 μm; 2, LiChrosorb RP-18, 5 μm (250 × 4.6 mm); 3, Nucleosil C_{18}, 5 μm (120 × 4.6 mm) (Mackerey & Nagel). I values referred to 2-ketoalkanes as standards.

Alkaloid	*I (1)*	*I (2)*	*I (3)*
Ergosine	567	676	–
Ergotamine	606	718	661
Ergocornine	688	782	730
α-Ergokryptine	739	838	783
β-Ergokryptine	756	848	–
Ergocristine	761	867	808
Ergosinine	800		
Ergotaminine	846		
Ergocorninine	892		
α-Ergokryptinine	954		
Ergocristinine	983		

TABLE III

ΔI VALUES DERIVED FROM FRAGMENTAL CHANGES AT THE SUBSTITUENTS R_1 AND R_2 IN THE PEPTIDE MOIETY OF ERGOPEPTINES

Increment	ΔI *(1)*	ΔI *(2)*	ΔI *(3)*	ΔI
Ergocornine → α-ergokryptine (+ CH_2 at R_2)	53	56	56	54
α-Ergokryptine → ergocristine (isopropyl for phenyl at R_2)	25	22	29	25
Ergocornine → ergocristine (isopropyl for phenyl; + CH_2 at R_2)	78	78	85	80
α-Ergokryptine → β-ergokryptine (2-methyl for 1-methyl at R_2)	–	18	10	14
Ergosine → ergotamine (isopropyl for phenyl at R_2)	–	39	42	40
Ergotamine → ergostine (+ CH_2 at R_1)	69	79	64	71
Ergotamine → ergocristine (+ CH + CH_3 at R_1)	147	157	149	151
Ergosine → α-ergokryptine (+ CH + CH_3 at R_1)	–	174	162	168

with hydrophobic properties is the calculation of hydrophobic fragmental constants[14,15]. Applying the extended method of Hansch and Leo[14], a larger contribution to hydrophobicity is expected from the isopropyl group than from the phenyl group. The data in Table III allow the statement that the substitution of phenyl for isopropyl at position R_2 of the molecules results in an increased retention (+ 25 *I* units). This example shows that the retention index scale can be a useful tool for a limited prediction of retention behaviour on reversed-phase columns. Furthermore, it is obvious that caution is required when hydrophobic parameters and molecular constants or fragments are translated into chromatographic data in reversed-phase liquid chromatography.

Regarding the ΔI values in Table III, it is seen that an additional CH_2 fragment at position R_1 results in an increase of +71 units, whereas at position R_2 this gives an enhancement only of +54 units. Thus substituent R_1 contributes more the the chromatographic retention of this molecular family of compounds than does substituent R_2. These results might be useful for a prediction of the separation of other natural or synthetic ergopeptines or their derivatives. The use of retention indices can result in a faster optimization of separation or in a better identification of ergopeptine compounds in a drug or a plant extract of unknown alkaloid composition.

CONCLUSIONS

A good separation of ergot alkaloids can be achieved on LiChrosorb RP-18 with acetonitrile–water as mobile phse, buffered to pH 7 by means of a 0.02 *M* phosphate buffer. The homologous series of 2-ketoalkanes are good standards for the calculation of retention data. Retention indices of ergopeptines are very helpful for prediction of chromatographic behaviour in RP-HPLC. The differences in retention

indices (ΔI values) for particular ergopeptines proved to be nearly constant on octadecylsilica columns of different dimensions (internal diameter, length) and different stationary phases (LiChrosorb and Nucleosil). These ΔI values reflect such alterations in the peptide part of the molecules as addition or removal of hydrocarbon fragments. The use of retention indices in HPLC therefore allows a good estimation of chromatographic behaviour and gives improved possibilities for identification by means of retention data.

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