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# Dihydroergotoxine: Separation and Determination of Four Components by High-Performance Liquid Chromatography

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Received December 16, 1976, from *Analytical Research and Development, Pharmaceutical Department, Sandoz Ltd., CH 4002 Basle, Switzerland.* Accepted for publication April 18, 1977.

**Abstract** □ The evaluation of a new high-performance liquid chromatographic method is described. It permits the separation and determination of the four components of dihydroergotoxine (dihydroergocristine, dihydroergocornine, dihydro-α-ergocryptine, and dihydro-β-ergocryptine) in a single step. On reversed-phase microparticles, complete baseline separation is possible with different mobile phases containing about $10^{-2}$ *M* base. The analysis of dihydroergotoxine mesylate drug substance or its dosage forms can be carried out in about 15 min. No reference substance is required for the determination of the proportions of the components. This method is simple and exhibits high accuracy, reproducibility, and selectivity. It permits the analytical control of dosage forms containing dihydroergotoxine mesylate to ensure that they comply with the specifications for the drug substance used in clinical and pharmacological studies.

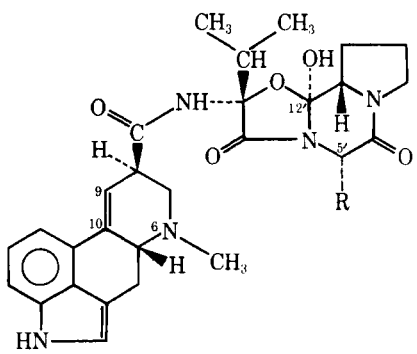
**Keyphrases** □ Dihydroergotoxine—high-performance liquid chromatographic analyses of four components in bulk drug and pharmaceutical formulations □ High-performance liquid chromatography—analyses, four components of dihydroergotoxine in bulk drug and pharmaceutical formulations □ Antiadrenergic agents—dihydroergotoxine, high-performance liquid chromatographic analyses of four components in bulk drug and pharmaceutical formulations

Ergotoxine is the name given to a particular group of ergot alkaloids derived from lysergic acid; they contain peptide moieties with similar chemical structures and have almost the same chemical and physical properties. These alkaloids are produced together by many strains of *Claviceps purpurea.*

Ergotoxine was believed to consist of three components: ergocristine (I), ergocornine (II), and ergocryptine (1, 2). In 1967, however, ergocryptine was shown to be composed of two isomers (3, 4), designated α-ergocryptine (III) and β-ergocryptine (IV) (5), differing only in one isomeric amino acid.

## BACKGROUND

Ergotoxine, the actual product from *Claviceps,* is not used as such but in the form of the 9,10-dihydro derivative, generally called dihydroergotoxine (V–VIII). In the mesylate form, it is now widely used for the treatment of symptoms of impairment of mental function in the elderly.



I: R = $CH_2C_6H_5$
II: R = $CH(CH_3)_2$
III: R = $CH_2CH(CH_3)_2$
IV: R = $CH(CH_3)CH_2CH_3$
V: R = $CH_2C_6H_5$; 9,10-dihydro
VI: R = $CH(CH_3)_2$; 9,10-dihydro
VII: R = $CH_2CH(CH_3)_2$; 9,10-dihydro
VIII: R = $CH(CH_3)CH_2CH_3$; 9,10-dihydro

Proof of safety and efficacy was obtained with material[1] containing the four constituents in specified proportions, representing the amounts found in natural ergotoxine isolated from selected, cultivated strains of *C. purpurea.* This defined dihydroergotoxine mesylate[2] consists of equal amounts of the mesylates of dihydroergocristine (V), dihydroergocornine (VI) and dihydroergocryptine (both isomers taken together), the ratio of dihydro-α-ergocryptine (VII) to dihydro-β-ergocryptine (VIII) being 2:1.

An analytical method is required to check the compliance of active ingredient and dosage forms with these specifications[3]. No simultaneous

[1] Hydergine, Sandoz Ltd.
[2] Dergocrine, generic name proposed to WHO.
[3] The tolerance ranges are 30.3–36.3% for V and VI as well as for the sum of VII and VIII and 1.5:1–2.5:1 for the ratio of VII to VIII.

chromatographic separation of the four components has been reported previously.

Incomplete separation without distinguishing between the isomers VII and VIII by paper chromatography (6–11), TLC (12, 13), high-performance liquid chromatography (HPLC) (14, 15), and countercurrent distribution (16) was described. A TLC separation of VII and VIII, derived from a system previously proposed (17), was reported[4]. However, the simultaneous separation into all four components failed because VIII and VI showed the same $R_f$ value.

Amino acid analysis, according to Moore and Stein (18), has been the only technique available for the identification and determination of the four components in the drug substance. The peptide moieties of the alkaloids were hydrolyzed, and the resulting amino acids were determined by ion-exchange chromatography and subsequent reaction with ninhydrin. This indirect determination, however, is usually inadequate for the analysis of pharmaceutical dosage forms.

This paper describes a method, based on HPLC separation, suitable for the analysis of the drug substance and the various liquid and solid dosage forms. Because of its accuracy, reproducibility, and simplicity, this method is suggested for quality control, *e.g.*, to verify compliance with the established specifications.

## EXPERIMENTAL

**Apparatus**—Two solvent delivery systems were used[5]. Sample injections were made with three systems[6] using 20–50 μl. The detectors[7] were set at 280 nm. Peak integrations were performed with a laboratory data system[8]. A pH meter[9] and an NMR spectrometer[10] were used.

**Reagents and Solvents**—Water was double distilled in a quartz apparatus. The solvents and reagents were analytical grade and were used without further purification; they were triethylamine[11], acetonitrile for pesticide residue analysis[12], methanol[12], absolute ethanol[12], tartaric acid[12], ammonium carbonate[12], and ammonium acetate[12].

The pH 2–13 range was studied using commercial buffer solutions[13]. Phosphate buffers, $5 \times 10^{-2}$ *M* or $1 \times 10^{-1}$ *M*, were used for the pH 9–13 range after adjustment to the appropriate pH with sodium hydroxide.

**Packings and Columns**—Several types (A[14], B[15], and C[16]) of chemically bonded reversed-phase materials were used to pack columns (25 cm long, 3–4 mm i.d., for 10-μm materials or 10–15 cm long, 3–4 mm i.d., for 5-μm materials) according to the procedures described for microparticles, *e.g.*, the balanced density technique (19–21), the dynamic slurry packing technique (22), and the viscosity technique (23–25). Some columns were glass coated[17]. In addition, prepacked columns were used with Phases B[18], C[19], and D[20].

**Samples**—Sample solutions, 1 mg/ml, were prepared by dissolving the drug substance[21] in methanol or the mobile phase.

Twenty tablets[1] of 1 or 1.5 mg were stirred without grinding in 20 ml of tartaric acid–water–ethanol (0.03:2:1 w/v/v) for about 15 min. The suspensions were centrifuged, and the solutions were injected directly into the column. Liquid dosage forms[1] required no preparation.

**Assay**—The sample injection size was 50 μl, corresponding to the following amounts of dihydroergotoxine mesylate: 50 μg for 1-mg tablets, 75 μg for 1.5-mg tablets, 15 μg for the injection, and 50 μg for the oral solution.

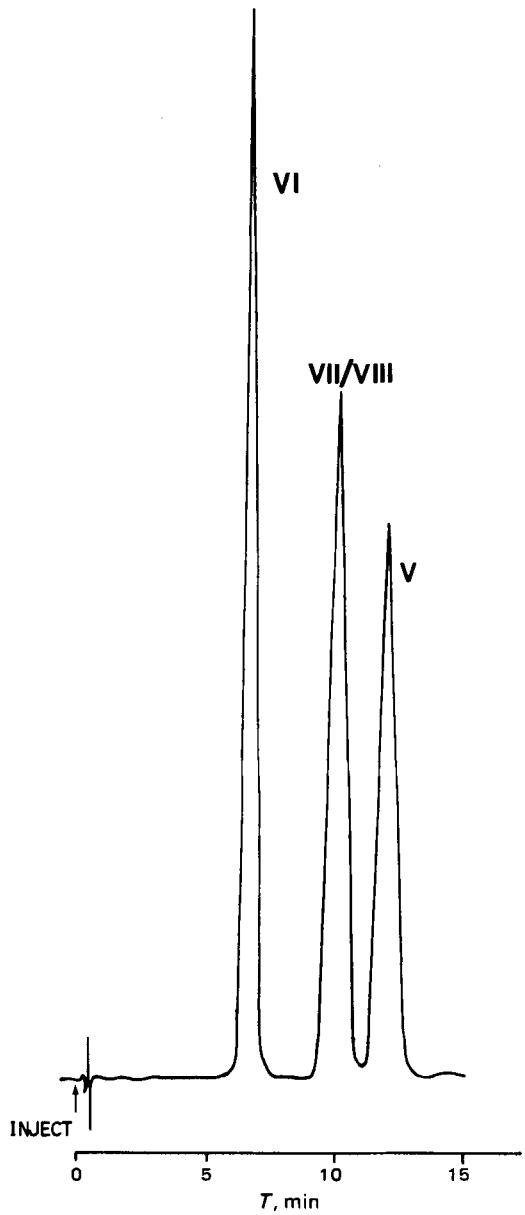



**Figure 1**—*Dihydroergotoxine mesylate in an oral solution; separation of components V, VI, and VII/VIII. The conditions were: column, 30 cm × 4.6 mm i.d.; stationary phase, C, 10 μm; mobile phase, acetonitrile–$10^{-2}$ M ammonium carbonate in water (0.82:1 v/v); flow, 7 ml/min; pressure, 5000 psi; detection, 280 nm and 0.4 absorbance unit; and injection, 50 μl of oral solution = 50 μg of dihydroergotoxine mesylate.*

Chromatographic conditions for different stationary phases were: Phase C, 5 μm in a 15-cm column, 3 mm i.d., water–acetonitrile–triethylamine (32:8:1 v/v/v) with a 1.0-ml/min flow, corresponding to a linear velocity of 0.3 cm/sec and a pressure of 4000 psi; Phase B, water–acetonitrile–triethylamine (75:40:1 v/v/v) with a 0.8-ml/min flow, corresponding to a linear velocity of 0.3 cm/sec and a pressure of 2000 psi; and Phase D, water–acetonitrile–triethylamine (22.8:11.4:1 v/v/v) with a 2.0-ml/min flow, corresponding to a linear velocity of 0.4 cm/sec and a pressure of 5000 psi.

The general procedure for evaluating the proportions of the components is demonstrated with the following example of 1-mg tablets[1]. Peak areas found were 16.960 (V), 17.079 (VI), 10.602 (VII), and 6.011 (VIII). Molecular weights of the mesylates are 707.8 (V), 659.8 (VI), and 673.8 (VII and VIII). The corrected peak areas are obtained by multiplication of the found areas with the corresponding molecular weights: 11,969 (V), 11,269 (VI), 7144 (VII), and 4050 (VIII). The sum of these corrected peak areas, 34,432, corresponds to 100%. Consequently, the relative amounts of the components are: 34.8% (V), 32.7% (VI), and 32.5% (VII/VIII), and the ratio of VII to VIII is 1.8:1.

[4] R. Brunner, Chemical Production Department, Sandoz Ltd., personal communication.
[5] Model 6000 A, Waters Associates, Milford, Mass.; and model FLM 50/2, Lewa, Leonberg, Germany.
[6] Model 70-10 loop injector, Rheodyne, Berkeley, Calif.; model U6K, Waters Associates, Milford, Mass.; and autosampler system, Varian.
[7] Model 1030 B, Hewlett-Packard; and model LC 55, Perkin-Elmer.
[8] Model 3352 B, Hewlett-Packard.
[9] Model E 500, Metrohm, Herisau, Switzerland.
[10] Model HX-90E, 22.63 MHz, Bruker Physik, Karlsruhe, Germany.
[11] Puriss., Fluka, Buchs, Switzerland.
[12] Merck, Darmstadt, Germany.
[13] Titrisol, Merck, Darmstadt, Germany.
[14] Nucleosil $C_{18}$, 10 μm, Macherey and Nagel, Düren, Germany.
[15] LiChrosorb RP 8, 5 and 10 μm, Merck, Darmstadt, Germany.
[16] LiChrosorb RP 18, 5 and 10 μm, Merck, Darmstadt, Germany.
[17] Scientific Glass Engineering, Melbourne, Australia.
[18] LiChrosorb RP 8, 7 μm, 25 cm long, 3 mm i.d., Merck, Darmstadt, Germany.
[19] LiChrosorb RP 18, 5 μm, 25 cm long, 3 mm i.d., Merck, Darmstadt, Germany.
[20] μ-Bondapak $C_{18}$, 10 μm, 30 cm long, 4 mm i.d., Waters Associates, Milford, Mass.
[21] Sandoz Ltd., Basle, Switzerland.

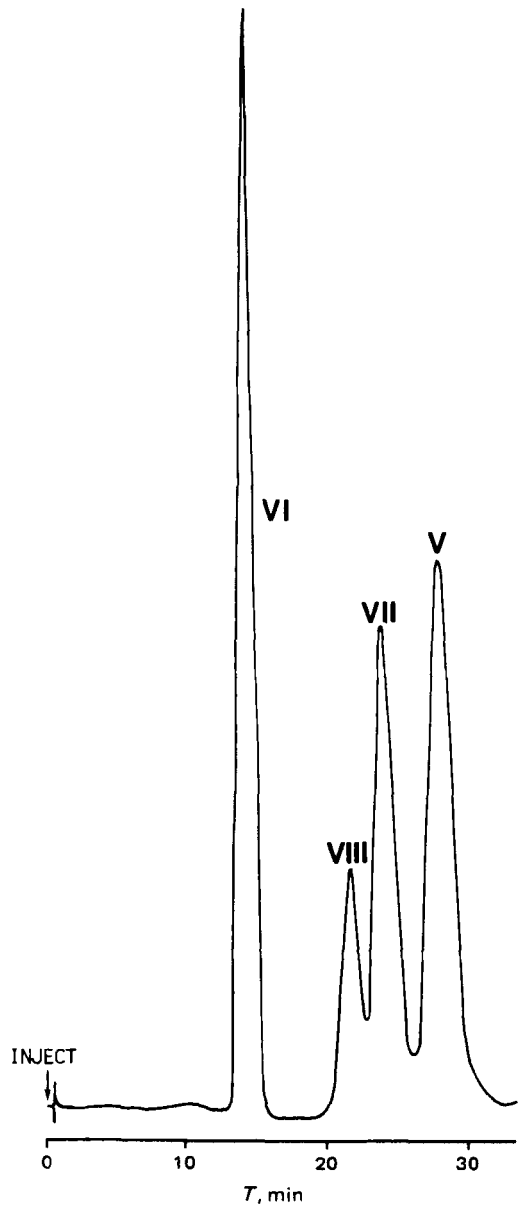



**Figure 2**—*Sparation of dihydroergotoxine mesylate drug substance into its constituents using a neutral mobile phase. The conditions were: column, 30 cm × 4 mm i.d.; stationary phase, D; mobile phase, methanol–$10^{-1}$ M ammonium acetate in water (1.1:1 v/v); flow, 3.3 ml/min; pressure, 5500 psi; detection, 280 nm and 0.02 absorbance unit; and injection, 30 µl of methanolic solution = 30 µg of drug substance.*

## RESULTS AND DISCUSSION

The problem of separating all four components (V–VIII) of dihydroergotoxine mesylate has been tackled in various ways by HPLC. With normal phase partition chromatography, a complete separation into three components, V, VI, and VII/VIII, was achieved, but isomers VII and VIII were only partly separated[22]. The selectivity factor $\alpha$ for this critical pair of compounds was reported to be 1.06.

A separation of components V, VI, and VII/VIII on reversed-phase porous layer beads[23] was described (14) and improved (15) by using the corresponding microparticles[20]. At the same time, determination of the components of dihydroergotoxine mesylate in various dosage forms with reversed-phase microparticles was begun in this laboratory. A representative chromatogram for an oral solution[1] is given in Fig. 1. The chromatographic system is useful for quality control and stability testing.

[22] R. Stampfli and R. Krattiger, Chemical Production Department, Sandoz Ltd., personal communication.

[23] Corasil $C_{18}$, Waters Associates, Milford, Mass.

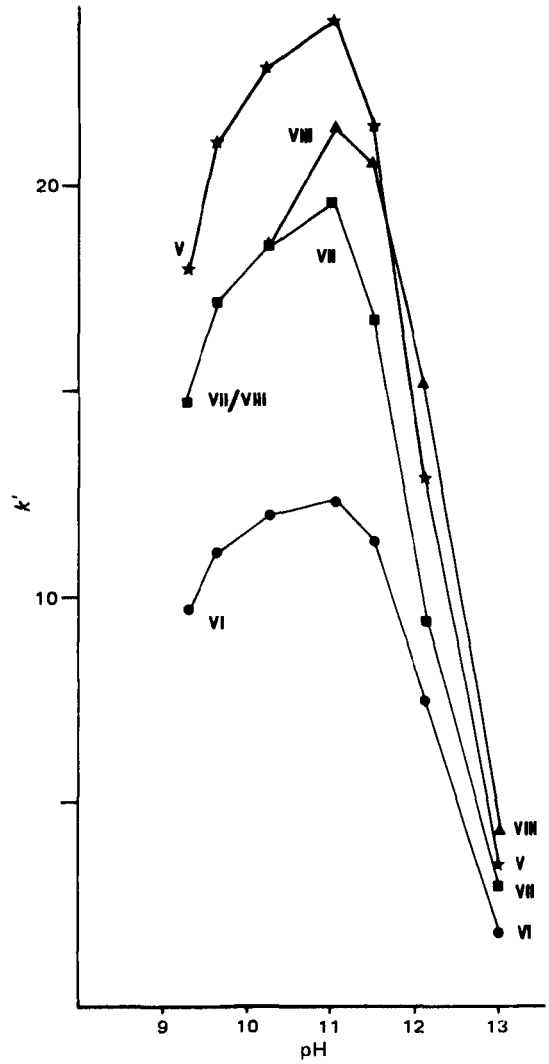



**Figure 3**—*Capacity factors k′ of V–VIII as a function of the pH of the mobile phase (aqueous part of phosphate buffers and sodium hydroxide mixed with acetonitrile, 1.67:1). Stationary Phase A was used.*

Proper selectivity of the mobile phase is achieved by using a salt concentration of at least $10^{-3}$ *M* and a pH value adjusted to $7 \leq \text{pH} \leq 9$, *e.g.*, with a mobile phase of acetonitrile–$10^{-2}$ *M* ammonium carbonate in water (0.54:1 v/v).

Insufficient stability was observed for some columns of any reversed-phase material because of the alkaline conditions of a mobile phase with acetonitrile and ammonium carbonate. New experiments showed that the ammonium carbonate solution can be replaced by systems buffered to pH ~ 7.5, prepared either with 0.33 *M* phosphate buffer or with 0.1% (v/v) triethylamine in water and adjusted to the required pH with 1 *N* acetic acid. The ratio of acetonitrile to the aqueous solution is the same as when ammonium carbonate solution is used.

A partial separation of VII and VIII was observed only on columns with extremely high performance ($n > 3000$, determined at the peak of VI). It was, therefore, necessary to improve the selectivity of the phases. Replacement of acetonitrile by methanol led to a slight change in the capacity factors ($k'$) of VII and VIII. To improve the selectivity of this methanolic phase, the effect of certain salts and acids added to the mobile phase was investigated.

A mobile phase of methanol–$10^{-1}$ *M* ammonium acetate in water (1:1–1.2:1) gave reproducible separations of all four components (V–VIII) on Phase D in a column with at least 1000 theoretical plates (determined in the system at the peak for VI). The capacity factors ($k'$) were: 21 (VI), 33 (VIII), 36 (VII), and 42 (V), thus leading to a selectivity factor $\alpha$ for the critical pair of isomers, VII and VIII, of about 1.1 and to a resolution $R_S$ of about 0.9 (Fig. 2).

The advantage of this chromatographic system is that the pH of the mobile phase (pH ~7) causes no difficulties with the stability of the

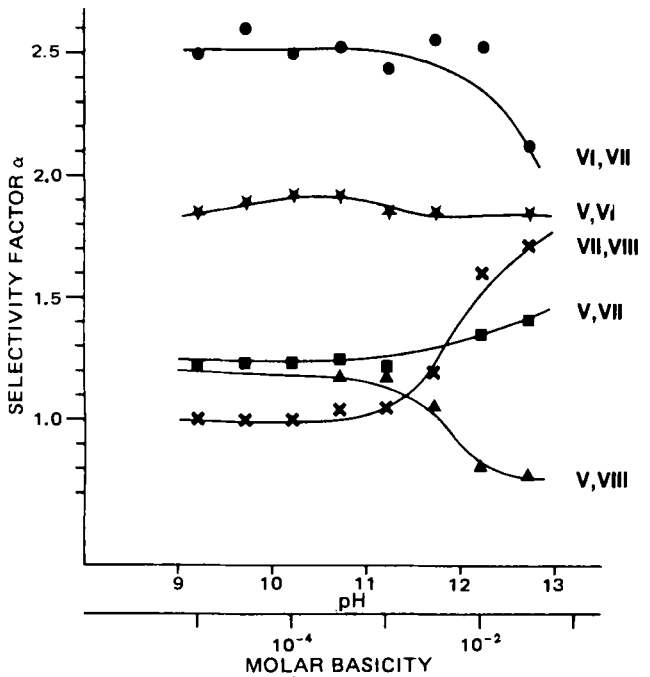



**Figure 4**—*Selectivity, α, of the different pairs of compounds as a function of the basicity of the mobile phase, corresponding to the pH of the aqueous part (mixed with acetonitrile, 1.67:1). Stationary Phase C was used.*

stationary phase used. Disadvantages result from the high capacity factors ($k'$), particularly for electronic peak integration.

An improvement of selectivity in drug analysis by pH variation of the mobile phase was reported. Twitchett and Moffat (26) described the correlation between the pKa values of various drugs and their retention time in reversed-phase HPLC. As a result, the optimum pH can be predicted from the pKa value. Hartmann and Rödiger (27) reported that improved selectivity in separation of penicillins and cephalosporins could be obtained by modifying the pH.

For dihydroergotoxine mesylate, a pH variation of the aqueous part of the mobile phase between 2 and 13 influenced the selectivity of the chromatographic system.

Mobile phases with pH < 9 (buffer–acetonitrile) did not improve selectivity. On the other hand, Fig. 3 demonstrates specific influences on the selectivity at pH ≥ 10 by showing the $k'$ values as a function of pH. With increasing basicity, the retention time of VIII becomes longer in comparison with that of the other components, thereby improving the separation between VII and VIII. But at the same time, the separation between V and VIII becomes worse in an intermediate region at pH ~ 11. If the pH is increased still further, the $k'$ value of VIII becomes higher than that of V, and a further improvement of resolution is achieved.

The results for the critical components VI–VIII appear to indicate that the separation of VII and VIII requires a pH above 10.5 using a phosphate buffer system. In the pH 10.5–11.5 range, the peak of VIII emerges between the peaks of VII and V; finally, at pH > 12, VIII is eluted as the last peak.

Figure 4 shows that the selectivities of the pairs VI and VII, V and VI, and V and VII are more or less independent of pH changes. The dramatic variation of the pairs VII and VIII and V and VIII is solely due to the effect of pH on the retardation of VIII. The reasons for this particular effect cannot be found in the ionization constants of the nitrogen in position 6 of the molecules[24].

All explanations of this successful separation remain purely hypothetical. Factors that may influence the retention behavior of VIII are: (*a*) changes in solvation or conformation, (*b*) specific ionization of the amide groups, and (*c*) specific ionization of the 12′-hydroxyl group. Preliminary $^{13}$C-NMR investigations were carried out to study the influence of basicity on the carbon skeleton of the molecules.

**Table I—Results Deduced from the Calibration for the Proportions of the Four Components, Including the Relative Standard Deviation Found by Repeating the Analyses in the Most Relevant Range under Constant Conditions (100% = 1 mg of Dihydroergotoxine Mesylate/ml)**

| Component | Proportion | | *RSD*, % |
|---|---|---|---|
| | Calculated, % | Found, % | |
| V | 4.8 | 4.5 | |
| | 9.6 | 9.3 | |
| | 19.2 | 19.1 | |
| | 24.0 | 24.1 | |
| | 28.8 | 28.9 | |
| | 30.2 | 30.7 | 1.0 ($n$ = 3) |
| | 33.2 | 33.4 | 0.7 ($n$ = 9) |
| | 37.2 | 37.5 | 0.3 ($n$ = 3) |
| | 38.4 | 38.7 | |
| VI | 4.7 | 4.7 | |
| | 9.5 | 9.5 | |
| | 18.9 | 18.7 | |
| | 23.6 | 23.6 | |
| | 28.4 | 28.7 | |
| | 30.4 | 30.0 | 0.6 ($n$ = 4) |
| | 33.4 | 32.9 | 0.2 ($n$ = 6) |
| | 37.5 | 36.8 | 1.0 ($n$ = 3) |
| | 37.8 | 37.8 | |
| VII | 4.8 | 4.7 | |
| | 9.5 | 9.8 | |
| | 19.1 | 19.0 | |
| | 20.8 | 20.5 | 0.3 ($n$ = 3) |
| | 21.8 | 21.3 | 0.5 ($n$ = 3) |
| | 22.8 | 22.5 | 0.4 ($n$ = 4) |
| | 23.8 | 23.7 | 0.4 ($n$ = 2) |
| | 23.9 | 24.2 | |
| | 28.6 | 29.0 | |
| | 38.2 | 38.3 | |
| VIII | 4.8 | 4.6 | |
| | 9.0 | 9.2 | 0.9 ($n$ = 8) |
| | 9.7 | 9.6 | |
| | 11.0 | 11.2 | 1.5 ($n$ = 3) |
| | 14.0 | 14.1 | 0.9 ($n$ = 4) |
| | 19.2 | 18.5 | |
| | 24.0 | 23.9 | |
| | 28.7 | 28.5 | |
| | 38.3 | 38.0 | |

Solutions of VII and VIII in dimethyl sulfoxide[25] yielded signals that did not vary as a function of basicity. Nevertheless, there is some evidence for variations in the chemical shifts of isomers VII and VIII in the critical range of pH > 10 when the solvent is as similar as possible to the mobile phase used in HPLC[26].

The conclusion to be drawn from the results of variation in pH is that sufficient selectivity can be achieved only when the concentration of the base is at least $10^{-2}$ $M$. This value is far outside the range recommended for chromatographic support materials based on silica gel (pH < 8–9) (24). In fact, the columns cannot resist such an alkaline mobile phase and usually fail after about 2–4 days.

Methylating the remaining silanol groups of the stationary phase produced some improvement in the stability, but it was not sufficient. Therefore, the replacement of the inorganic bases and buffer systems by organic amines was studied[27]. The influence of methanol and acetonitrile was investigated at the same time.

Successful separation of the four components (V–VIII) of dihydroergotoxine was obtained with triethylamine in the specified concentration ($10^{-2}$ $M$) together with either methanol or acetonitrile to produce the optimum selectivity. Typical conditions for Phase C are water–methanol–triethylamine (25:3.6:1 v/v/v) or water–acetonitrile–triethylamine (32:8:1 v/v/v). The acetonitrile system has the following advantages over the methanol system: (*a*) better selectivity, particularly for the pair V and VII; (*b*) lower pressure and, consequently, fewer technical problems with pumps, injectors, *etc.*; and (*c*) a longer lasting stationary phase since silica gel is not dissolved.

In accordance with the results from pH variation, the important modifier of the selectivity is the amount of the amine. At least 0.5–1% triethylamine (equivalent to 3.5–7 × $10^{-2}$ $M$ in the mobile phase) is

[24] Maulding and Zoglio (28) reported the pKa values in aqueous solution of V, VI, and VII/VIII to be practically equal (pKa ~ 6.74). A. Wehrli, Chemical Research Department, Sandoz Ltd., determined the following $pK_{MCS}$ values according to the method described by Simon (29): 5.83 (V), 5.78 (VI), 5.81 (VII), and 5.84 (VIII).

[25] F. Erni and H. Loosli, Sandoz Ltd., showed that $^{13}$C-NMR spectra can be used to determine the proportions of the four components in dihydroergotoxine mesylate drug substance dissolved in dimethyl sulfoxide.

[26] H. Bethke and H. Loosli, to be published.

[27] H. P. Keller, R. Stampfli, and A. Wehrli, to be published.

**Table II—Determination of the Proportions of the Four Components in Dihydroergotoxine Mesylate and Its Preparations, Including the Relative Standard Deviation Found by Repeating the Analyses under Constant Conditions**

| Sample | Proportion of V Mesylate | Proportion of VI Mesylate | Proportion of Sum of VII Mesylate and VIII Mesylate | Ratio of VII Mesylate to VIII Mesylate |
|---|---|---|---|---|
| Dihydroergotoxine mesylate (10 determinations) | 33.9 ± 0.9% | 35.0 ± 0.8% | 31.2 ± 0.5% | 1.9 ± 1.7% |
| Oral solution[a] (eight determinations) | 33.6 ± 0.7% | 32.2 ± 0.8% | 34.2 ± 0.4% | 2.2 ± 1.8% |
| Injection solution[a] (10 determinations) | 33.7 ± 0.7% | 34.2 ± 0.9% | 32.1 ± 0.3% | 2.1 ± 1.7% |
| Tablets[a], 1 mg (seven determinations) | 34.9 ± 0.6% | 32.7 ± 0.9% | 32.4 ± 0.5% | 1.8 ± 1.2% |
| Tablets[a], 1.5 mg (eight determinations) | 34.5 ± 0.4% | 33.8 ± 0.4% | 31.7 ± 0.4% | 2.0 ± 1.4% |

[a] Hydergine, Sandoz Ltd.

necessary for sufficient separation. With larger amounts, the retention times of all components are reduced and the separation between VI and VII is practically unchanged whereas the selectivity for the pair V and VIII is improved. A similar decrease in selectivity is observed for the pair V and VII. Therefore, about 2–3% triethylamine should be optimum, producing sufficient resolution while permitting the analysis to be completed within a reasonable time.

On the other hand, in the range of 20–30%, the amount of acetonitrile has little influence on system selectivity. It only affects the retention times, which decrease with increasing acetonitrile concentration. These results and further experience led to typical chromatographic conditions (Fig. 5) for different reversed stationary phases (see *Experimental*). Under these conditions, baseline separations are achieved for all adjoining peaks. The capacity factors vary between $k' \sim 6$ for VI and $k' \sim 35$ for VIII. By slight modifications of the mobile phase, the system can be adjusted to yield optimum separation and minimum chromatographic time, generally about 15 min.

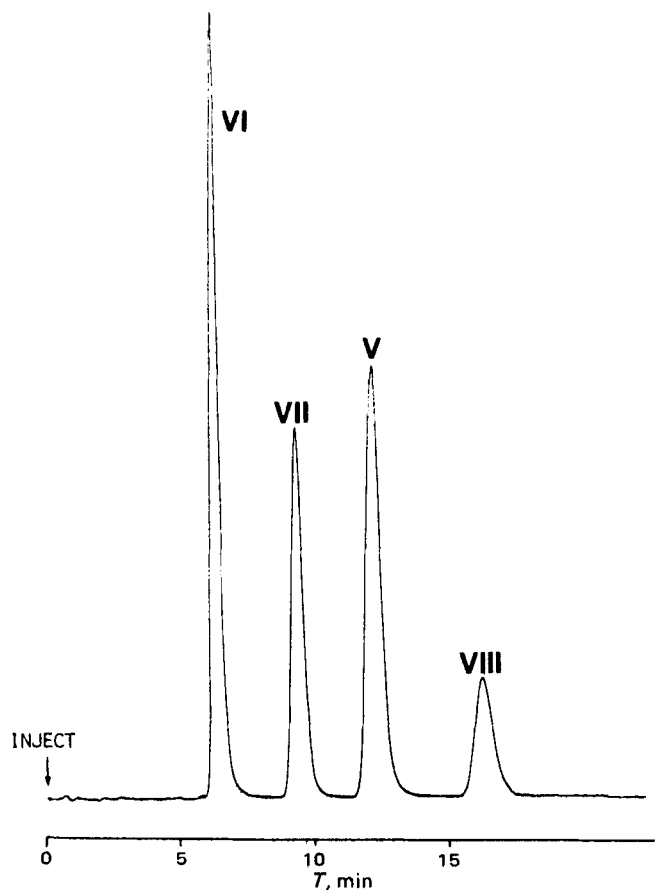



**Figure 5**—*Separation of dihydroergotoxine mesylate oral solution into its constituents using the optimum mobile phase of pH ~ 12. The conditions were: column, 15 cm × 3 mm i.d. glass-coated steel; stationary phase, C, 5 μm; mobile phase, water–acetonitrile–triethylamine (32:8:1 v/v/v); flow, 1.0 ml/min; pressure, 5500 psi; detection, 280 nm and 0.2 absorbance unit; and injection, 20 μg of dihydroergotoxine mesylate in 20 μl of water–acetonitrile (4:1 v/v).*

Several columns have been in use, some for more than 2 months for hundreds of routine analyses of dihydroergotoxine mesylate drug substance and its dosage forms. The columns are at least as stable as under conditions of low alkalinity (pH 8 with inorganic salts), although the measured pH is about 12.

The top of the column must be inspected routinely and a small amount of the stationary phase must be added, if necessary. Washing the column with water, acetonitrile, or alcohols should be avoided because it adversely affects stability.

Since all peaks show baseline separation, electronic integrators can be used for the quantitative determination. Since the indole group of the lysergic acid constitutes the chromophore of all four components, the molar ratios can be calculated directly from the peak areas obtained at 280 nm. This fact has been verified by calibration experiments, in which the proportions of the single components ranged from 5 to 50% calculated on the total amount of dihydroergotoxine mesylate. At the 95% confidence level, the measured values did not differ significantly from the expected values (Table I).

The precise setting of the detection wavelength is not critical, as was demonstrated by varying it from 275 to 285 nm with 2-nm bandwidth. Calibration with an external standard is not necessary for the determination of the proportion of each component. Consequently, a high level of accuracy is required for integration of the peaks, especially the smallest peak (VIII). Proper column performance, an injected amount of about 20–50 μg of dihydroergotoxine mesylate, and an optimum setting of the peak detection system of the integrator are essential.

For the drug substance dihydroergotoxine mesylate, the proportions are labeled as weight ratios rather than as molar ratios. Therefore, the area of each peak in the chromatogram must be multiplied by the molecular weight of the relevant mesylate. These corrected values give the amount of each component by weight; the proportions can be calculated by using the 100% method (example given in the *Experimental* section).

The proportions of the components were determined for dihydroergotoxine mesylate and for different dosage forms. The results and the standard deviations of the analyses are summarized in Table II. There is a good agreement with the specifications given in the introduction as well as good reproducibility, which demonstrates the suitability of the method for routine analysis.

The preparation of the samples is simple. The drug substance must be dissolved in the mixture of water and acetonitrile used in the mobile phase; liquid dosage forms require no preparation; and an extract of tablets is prepared in a single step (*cf., Experimental*).

Further investigations showed that the HPLC system is not only suitable for the determination of the relative amounts of each component but also for the determination of dihydroergotoxine mesylate as the sum of all components[28].

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This report is the 84th paper on ergot alkaloids. For the previous report in this series, see E. Schreier, *Helv. Chim. Acta*, **59**, 585 (1976).

# Biosynthesis of Morphine Alkaloids in *Papaver bracteatum* Lindl.

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**Abstract** □ Administration of 1-$^{3}$H-*N*-methyl-$^{14}$C-(±)-reticuline to *Papaver bracteatum* gave good incorporation of carbon-14 into thebaine and a decrease in the tritium to carbon-14 ratio indicative of racemization. The incorporation of carbon-14 and the extent of tritium loss were the same whether reticuline was administered to the intact plant or to isolated leaves. Carrier dilution with cold codeine, codeinone, and morphine showed only insignificant incorporation of radioactivity into codeine and none at all into codeinone and morphine. When codeinone was administered to the living plant, it was converted to codeine rapidly and efficiently, but no *O*-demethylation to morphine could be detected. The experimental data indicate that the biosynthesis of thebaine in *P. bracteatum* proceeds by the same pathway as in the opium poppy. The limiting step in the sequence is the demethylation of the enol ether group of thebaine to neopinone.

**Keyphrases** □ Morphine alkaloids—biosynthesis in *Papaver bracteatum*, radiochemical study □ Alkaloids, morphine—biosynthesis in *Papaver bracteatum*, radiochemical study □ Biosynthesis—morphine alkaloids in *Papaver bracteatum*, radiochemical study □ *Papaver bracteatum*—biosynthesis of morphine alkaloids, radiochemical study □ Radiochemistry—study of biosynthesis of morphine alkaloids in *Papaver bracteatum*

In 1963, Neubauer and Mothes (1) reported on a strain of *Papaver bracteatum* Lindl. that produced thebaine in high yield but apparently contained neither codeine nor morphine. *P. bracteatum* is closely related to *P. orientale* and *P. pseudo-orientale*, which do not synthesize significant amounts of thebaine. They can be differentiated from *P. bracteatum* by cytological examination (2) and chemotaxonomic tests (3). In contrast to the opium poppy (*P. somniferum*), which produces a large number of alkaloids, several in appreciable concentrations, *P. bracteatum* contains mainly thebaine, which may account for 98% of the alkaloid content[1] (1, 4). Isolation and purification of thebaine from *P. bracteatum* are, therefore, relatively simple.

In recent years, considerable interest has developed in this plant as a source of thebaine; in the laboratory, thebaine can be converted to codeine and other narcotic analgesics and antitussives (6–8). Thebaine is also the raw material for naloxone and related narcotic antagonists and for the interesting *endo*-ethenotetrahydrothebaines (9). In 1972, a collaborative research project on *P. bracteatum* was initiated by the United Nations Narcotics Laboratory, which has been coordinating investigations carried out in many countries (10).

The purposes of the present investigation were to study the biosynthetic pathways of hydrophenanthrene alkaloids in *P. bracteatum* and to explore possible sites of biosynthesis.

## BACKGROUND

The biosynthesis of morphine alkaloids in the opium poppy has been

[1] Several varieties, or chemical races, of *P. bracteatum* have been found, differing somewhat in alkaloid composition. The Arya II variety from western Iran has a particularly high content of thebaine (5).