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Note

A preliminary investigation of the high-speed liquid chromatography of some ergot alkaloids*

There has been a considerable interest in recent years in sensitive, specific analytical methods for the ergot alkaloids. Many of these compounds have legitimate medicinal uses, however certain members of this group, notably D-lysergic acid diethylamide (LSD), have become well known by virtue of their non-medical use, based on their hallucinogenic properties. Much has been written in the past decade on analytical procedures for "drugs of abuse" (such as LSD) (*cf.* ref. 1).

Many of the methods proposed for the ergot alkaloids have been based on the native fluorescence of these bases[2,3]. The combination of thin-layer chromatography and *in situ* spectrophotometric determination on thin-layer plates[4–7] or fluorescence measurement in solution following elution of the sample from the chromatogram[8] has been the basis of some investigations. The technique of high-speed liquid chromatography (HSLC)[9–11] has gained increasing popularity with analysts in the past few years, and some studies using several commercial instruments for the separation and determination of some ergot alkaloids have recently been reported[12]. In the current investigation, the fluorescence of these compounds has been used for the first time for their detection in HSLC.

Experimental

Chemicals. The majority of the ergot alkaloids used in these investigations were obtained as gifts from either Sandoz Ltd. (Basle, Switzerland) or from Dr. L. C. VINING (Dalhousie University). D-Lysergic acid and agroclavine were purchased from the Koch-Light Laboratories Ltd. D-Lysergic acid diethylamide was supplied by the Department of National Health and Welfare (Canada).

Thin-layer chromatography. Thin-layer chromatography was carried out on commercially available Merck Silica Gel F_{254} thin-layer plates (layer thickness, 0.25 mm), using a variety of chloroform–methanol mixtures as solvent systems. The spots were visualised on the developed chromatoplates by viewing in ultraviolet light (UVS-11 lamp, Ultra-violet Products Inc.).

Fluorescence measurements. The fluorescence spectra of the ergot alkaloids were measured in solution (20–100 p.p.m.) using an Aminco-Bowman SPF Spectrophotofluorimeter to determine the optimum choice of primary and secondary filters for the fluorescence detector. The excitation wavelength was, in all cases, set at 350 nm.

High-speed liquid chromatography. Experiments were carried out on two instruments which had been built in the laboratories[13,14] of the Dalhousie University Chemistry Department and also on one commercially available instrument, a Chroma-

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tronix Model 3100 equipped with UV-detector. One of the experimental instruments was equipped with a Haskel air-driven piston pump capable of operating to 500 atm and a Pharmacia UV monitor operated at 254 nm[13], the other instrument consisted of a three-head diaphragm pump which could be operated to 350 atm (Type 54, Orlita K.G., Giessen, G.F.R.) and was equipped with a fluorescence detector built from a modified Turner 111 fluorimeter[14].

All fittings and tubing used in the instruments built in the laboratory were of stainless steel. Columns were 1000 × 2.4 mm seamless stainless steel. A stop-flow valve was used to permit direct on-column injection of the sample with these instruments. Column packings ("Zipax" and "Corasil") were pre-conditioned according to manufacturer's specifications and the columns were then packed as previously described[13,14]. The same columns were also used with the commercial instrument which was equipped with the standard sample injection loop system.

Results and discussion

The fluorescence maxima of a number of ergot alkaloids and related lysergic acid derivatives were measured in acetone solution with the excitation wavelength set at 350 nm. The observed fluorescence maxima were, in all cases, between 390 and 410 nm. Therefore the primary filter chosen was a Turner 110-81 (or Corning 7-60), whilst the secondary filter used was a Turner 110-816 (or Kodak-Wratten 2A). The intensities of the fluorescence observed were somewhat variable depending on the chemical structure of the compound used (see Table I).

Further experiments were conducted to determine the effect of various solvents on the fluorescence intensity of the compounds studied using ergotamine as a test substance. Measurements were made in either pure ethanol or in 50:50 mixtures of

TABLE I

EMISSION MAXIMA AND RELATIVE FLUORESCENCE INTENSITIES FOR SOME ERGOT ALKALOIDS IN ACETONE SOLUTION

λ_{ex} = 350 nm, concentration = 100 p.p.m.

Compound	λ_{max} *emission (nm)*	*Rel. intensity (%)*
Ergotamine	400	40.2
Ergotaminine	397	100.0
Ergocristine	400	21.0
Ergocryptine	403	22.1
Ergocryptinine	397	62.2
Ergocornine	402	18.3
Ergocorninine	398	83.8
Ergosine	398	56.1
Ergine[a]	393	1.1
Erginine	393	61.5
D-Lysergic acid	392	32.8
Ergotrate	403	28.9
Isosetoclavine	393	82.2
Elymoclavine[a]	394	0.3
Agroclavine	392	0.5
D-Lysergic acid diethylamide	397	62.2

[a] Concentration = 20 p.p.m., due to low solubility of compound in acetone.

TABLE II

INFLUENCE OF SOLVENT ON FLUORESCENCE INTENSITY OF ERGOTAMINE (4.5×10^{-3} *M*)
λ_{ex} = 350 nm.

Solvent	*Relative intensity (%)*
100% Ethanol	100
50% Diisopropyl ether[a]	130
50% Cyclohexane[a]	113
50% Acetone[a]	96
50% Hexane[a]	91
50% Benzene[a]	109
50% Chloroform[a]	13

[a] 50% Mixtures with absolute ethanol.

ethanol with acetone, hexane, cyclohexane, diisopropyl ether and chloroform. Little variation in fluorescence intensity was noted except in the case of the chloroform–ethanol mixture where the observed intensity was only *ca.* 10–15% of that found in other solvents used due to quenching. The results are summarised in Table II. The sensitivity of the fluorescence detector is thus limited when chloroform is used in the solvent system.

It is well known that thin-layer chromatographic systems can often be usefully applied to HSLC separations[15]. The use of chloroform–methanol systems for the separation of ergot alkaloids and lysergic acid derivatives on silica gel thin layers is well established[5,6,16]. Therefore the use of various methanol–chloroform mixtures containing from 1% to 15% methanol as possible solvent systems in HSLC using "Corasil" columns was investigated using both UV and fluorescence detector systems. Some experiments were carried out with "Zipax" columns with a fluorescence detector. A large number of solvent systems containing chloroform–methanol mixtures alone or, in some cases, with additional solvents were investigated. Whilst many proved to be unsatisfactory, the separation of ergotamine and ergocristine on a "Corasil" column using fluorescence detection is shown in Fig. 1, using chloroform–methanol–ethyl acetate–acetic acid (60:20:50:3) as solvent.

It may be seen from Fig. 1 that no problems of tailing are encountered with these compounds under such conditions and the resolution could undoubtedly be improved by increasing the number of theoretical plates in the column. It can also be seen from Fig. 1 that the lowering of the operating pressure improves the separation of these compounds. The detection sensitivity was definitely reduced by the presence of chloroform in the solvent system, however the presence of more polar solvents in the mixture counteracted the negative effect to some extent.

Experiments comparing UV-detection with fluorescence detection for simple chloroform–methanol mixtures strikingly illustrated the effect of the chloroform in quenching the fluorescence. Compounds which were eluted at the solvent front were readily detected by their fluorescence, but their detection became increasingly difficult as their retention time on the column increased. Compounds which were retained for several minutes could not be detected at the 1-μg level. This can be explained by assuming that the fluorescence properties of the compounds travelling in the solvent front are influenced mainly by the original solvent in which they were

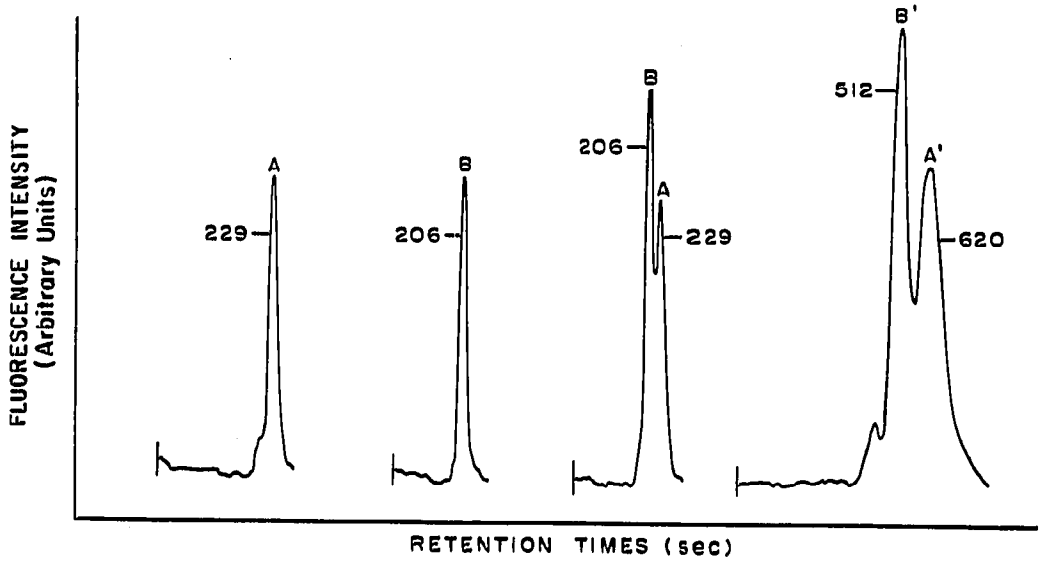

Fig. 1. High-speed liquid chromatography of ergotamine (A), ergocristine (B), alone, and in a mixture on a "Corasil" column with fluorescence detection at 300 p.s.i. (A, B) and 150 p.s.i. (A', B'). Solvent system, chloroform–methanol–ethyl acetate–acetic acid (60:20:50:3); injection volume, 2 μl; flow-rate, 110 sec/ml. Retention times as indicated.

injected (*i.e.* ethanol). However, compounds retained on the column are in a predominantly chloroform environment, and thus their fluorescence is substantially quenched. It may be expected that other solvents containing several chlorine atoms would cause similar problems.

Work carried out with the apparatus built in the Dalhousie University laboratories using UV detection showed that separations of many of these compounds could be achieved by using a methanol–chloroform solvent and varying polarity (*i.e.* ratio of solvent components) of the solvent. Variation of operating pressure also could be used to advantage in separations of this type. Other polar solvents tried (*e.g.* acetonitrile) did not appear very promising, due to severe tailing. Other less polar phases (hexane, diethyl ether) as substitutes for chloroform were also ineffective.

Some preliminary separations are shown in Table III. It can be clearly seen that the stereoisomers ergotamine and ergotaminine can be readily separated using the methanol–chloroform systems. Furthermore they could be separated from structurally different compounds such as agroclavin.

Further studies with these systems were then carried out using a commercially available instrument (Chromatronix 3100). In an attempt to improve the poor column efficiencies, due to tailing, the "Corasil" was partially deactivated by the addition of a larger amount of water to the column material. (This represented a change from 0.9% to 2.0% water deactivation.)

Using the 2% water-deactivated column and 4% methanol in chloroform solvent at 1000 p.s.i. the compounds examined may readily be separated into four groups as shown in Table IV.

Using a 0.9% water-deactivated column and the same operating conditions as those mentioned above, the capacity factors for the ergot alkaloids examined are as

TABLE III

R^1 VALUES[a] FOR SOME ERGOT ALKALOIDS ON "CORASIL" COLUMN

Column length = 1 m. Solvent systems: 1 = methanol/chloroform (5:100), 2 = Acetonitrile/chloroform (8:100), 3 = methanol/hexane (8:100), 4 = methanol/ether (3:100), 5 = methanol/chloroform (2:100), 6 = methanol/chloroform (3:1000), 7 = methanol/chloroform (15:100).

Compound	*Solvent system*						
	1	*2*	*3*	*4*	*5*	*6*	*7*
Ergotaminine	1.09	T[b]	7.87 (T)	T	1.29	1.25	1.08
Ergotamine	1.58	T	11.74 (T)	—	—	2.66	—
Ergocristine	1.12	T	8.13	T	1.42	1.25	—
Ergocryptine	1.09	T	4.65	T	1.39	1.23	—
Agroclavine	3.18 (T)	T	—	—	—	4.92 (T)	1.55
Ergotrate	—	T	—	—	—	—	—
Elymoclavine	—	T	—	—	—	—	2.43
Column pressure (p.s.i.)	1000	1000	1000	500	1000	500	500

[a] $R^1 = \frac{R_t - R_0}{R_0}$, where R_t = retention time of compound, R_0 = retention time of solvent.

[b] T = tailing.

TABLE IV

SEPARATIONS ACHIEVED ON A 2%-WATER-DEACTIVATED "CORASIL" COLUMN (1000 × 2.5 mm) USING AS SOLVENT METHANOL–CHLOROFORM (4:100) AT 1000 p.s.i. ON A CHROMATRONIX 3100 HIGH-SPEED LIQUID CHROMATOGRAPH

Band	*R_t (sec)*	*R^1*	*Compounds in band*
I	45 (= R_0)		ergocornine ergocryptinine
II	70	1.56	ergotaminine ergocristine ergocornine ergocryptine ergosinine
III	160	3.56	ergotamine ergosine
IV	not eluted at these column conditions		all other compounds tested

follows: ergotamine, 2.42; ergotaminine, 1.53; ergosine, 2.62; ergosinine, 1.69; ergocornine, 2.09; ergocorninine, 1.69; ergometrine, 7.78; D-lysergine, 4.00; D-isolysergine 2.60; penniclavine, 8.93; agroclavine, 4.22; isosetoclavine, 3.47; ergotrate (maleate), 10.89; D-lysergic acid diethylamide bitartrate, 3.07; ergocristine, 1.51; ergocryptine, 1.76; ergocryptinine, 1.53; (flow-rate 45 sec/ml).

Conclusions

These preliminary studies have shown that HSLC has excellent possibilities for the analysis of ergot alkaloids and related compounds such as the lysergic acid derivatives. Separations may be achieved using a variety of columns and operating conditions, and even stereoisomers may be resolved. While the results achieved with fluorescence detection in this investigation are preliminary, this method of detection should offer advantages of both selectivity and sensitivity once more suitable solvent

systems are found. Further experiments are now being carried out with other solvent systems and column packings to develop a qualitative and quantitative method of analysis for these compounds based on high-speed liquid chromatographic separation with fluorescence detection.

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