Journal of Chromatography, 105 (1975) 393–395

CHROM. 8057

Note

Ergot alkaloids*

II. Determination of agroclavine by gas–liquid chromatography

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(Received September 13th, 1974)

The ergot alkaloid agroclavine has recently been shown to be a potent inhibitor of the early stage of pregnancy in the rat and mouse[2] and also causes the failure of established lactation in the rat and mouse[3]. To aid the study of these important pharmacological properties we were interested in the use of gas–liquid chromatography (GLC) as a rapid, accurate method for the determination of agroclavine.

Apart from attempts to identify and detect lysergic acid diethylamide[4,5], GLC has only been used once to separate and identify ergot alkaloids[6]. That study used the non-polar liquid phases SE-30, JxR and OV-17 and the more polar XE-60 to chromatograph and partially separate a wide variety of underivatized clavine alkaloids and some lysergic acid metabolites. We were interested in the use of suitable derivatives which would both increase the temperature stability of the alkaloids and change retention times. Trifluoroacetyl and trimethylsilyl derivatives have been successfully used for GLC of indoles such as tryptophan[7], tryptamines[8] and related compounds[9].

EXPERIMENTAL

All determinations were carried out on a Pye Model 104 chromatograph equipped with flame ionization detectors using glass columns (5 ft. × ¼ in.). The stationary phase was acid-washed silanized Chromosorb W (80–100 mesh). The liquid phases used were SE-30, SE-52 and OV-17 and these were conditioned on the stationary phase under helium at the recommended temperatures. The injection ports and detectors were maintained at 20° above the maximum temperature attained in a run. The carrier gas was nitrogen and the flow-rate 50 ml/min. The operating temperatures and details of temperature programming experiments are given in Tables I and II.

Preparation of derivatives

All reactions were carried out in Pierce reaction vials (0.3-ml capacity) fitted with a PTFE-lined cap.

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* For Part I, *cf.* ref. 1.

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The trifluoroacetyl (TFA) derivatives were prepared as follows: The preparation 1-agroclavine (0.1–1 mg) was reacted with trifluoroacetic anhydride (0.3 ml) at room temperature for 1 h, or, the preparation 2-agroclavine (0.1–1 mg) was dissolved in a suitable dry solvent (dichloromethane or pyridine) and reacted with trifluoroacetic anhydride (0.1 ml) at room temperature for 1 h. Samples were either injected directly or evaporated to dryness with nitrogen and dissolved in dry chloroform for injection.

The trimethylsilyl (TMS) derivatives were prepared as follows: (i) Agroclavine (0.5 mg) in dry pyridine (0.1 ml) was reacted with N,O-bis(trimethylsilyl)acetamide (0.2 ml) at 80° overnight and injected directly. (ii) Agroclavine (0.1–1 mg) was reacted with the following mixture (0.3 ml): N,O-bis(trimethylsilyl)trifluoroacetamide–N-trimethylsilyldiethylamine–trimethylchlorosilane–pyridine (90:30:1:10) for 10 min at 100°, left to stand overnight at room temperature, and injected directly.

RESULTS AND DISCUSSION

Agroclavine reacted quantitatively with the silylating agents under the conditions described to yield a single derivative. The retention times of TMS-agroclavine under a variety of conditions on different columns are shown in Table I. A linear response of the detector to the amount of TMS-agroclavine injected was observed. As expected, the large increase in molecular weight on derivatization led to increased retention times for TMS-agroclavine compared to agroclavine. On these non-polar silicone phases, retention times are primarily affected by molecular weight changes. The use of temperature programming, however, easily overcomes this difficulty. The peaks observed were symmetrical and with the columns described (10% SE-30, 10% OV-17 and 3% SE-52) showed no tendency to tail, despite the presence of the tertiary amine in the ergoline ring system. The use of 1–3% SE-30 led to extensive tailing of TMS-agroclavine, presumably by partial adsorption to active sites in the solid support. For our work, involving detection of agroclavine in the microgram range, the use of the relatively high percentage of liquid phase was most satisfactory. The OV phases showed very long retention times and offered no advantages over the SE phases.

The chromatographic conditions and retention times for peaks observed after

TABLE I

CHROMATOGRAPHIC CONDITIONS AND RETENTION TIMES FOR THE GLC OF TMS-AGROCLAVINE

Column	*Operating conditions (°C)*	*Retention time (min)*
10% SE-30	210°, isothermal	38.2*
10% SE-30	210° for 10 min, then 2°/min	31.5
10% SE-30	215° for 5 min, then 6°/min	16.0
3% SE-52	198° for 4 min, then 8°/min	10.8
10% OV-17	220°, isothermal	77.5

* Agroclavine (underivatized) under these conditions (210°, isothermal) had a retention time of 24.8 min.

TABLE II

CHROMATOGRAPHIC CONDITIONS AND RETENTION TIMES FOR THE GLC OF TRIFLUOROACETYLATED AGROCLAVINE

Column	*Operating conditions*	*Preparation*	*Retention times (min)*
10% SE-30	202° isothermal	1	23.2 (1)*, 45.2 (5)
10% SE-30	210° isothermal	2	20.0 (2), 24.4** (1), 28.6 (4)

* Numbers in parentheses refer to relative areas of peaks.
** Underivatized agroclavine.

trifluoroacetylation of agroclavine are shown in Table II. We were unable to produce a single product or reproducibly obtain a consistent mixture of products. Reactions under as nearly as possible identical conditions gave variations in the areas of the peaks obtained and in the amount of decomposition. The time of reaction, temperature of reaction, solvent and ratio of reagent to reactant were varied but no further useful information was obtained. Similar difficulties of multiple derivatization have been noted for tryptamines[8]. Trifluoroacetylation usually offers the advantage of giving more volatile compounds and consequently allowing the use of lower column temperatures, but in the case of the highly reactive ergot alkaloids the unsatisfactory results on derivatization preclude its use in analytical determinations. The trifluoroacetylation of agroclavine is complex. Presumably the derivative with a shorter retention time than agroclavine is the expected derivative with the indole N–H trifluoroacetylated. This probably undergoes a facile hydrolytic cleavage in the presence of traces of moisture and acids and bases. The decomposition was more marked in the presence of solvents, particularly pyridine. The peaks of longer retention time must be further reaction products and one possibility is trifluoroacetylation of the indole ring, most likely in the two position. This could result from rearrangement of the N-trifluoroacetyl derivative under the acidic reaction conditions.

The TMS-agroclavine derivative is easily and cleanly formed and appears to be a suitable derivative for the analytical determination of agroclavine. Preliminary investigations have indicated that other clavine hydrocarbons, festuclavine and pyroclavine, can also be chromatographed satisfactorily as their TMS-derivatives, but this method is unsatisfactory for hydroxylated clavine alkaloids.

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