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LIQUID CHROMATOGRAPHY OF SEMISYNTHETIC ERGOT PREPARATIONS

I. NICERGOLINE

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SUMMARY

An impurity in medical nicergoline preparations was identified as its 5'-chloro analogue by ^{1}H, ^{13}C NMR and mass spectrometry. Liquid chromatography can readily be applied to a study of the reaction mixtures arising during the synthesis of nicergoline.

INTRODUCTION

A considerable number of lumi-I derivatives of ergot compounds have been prepared by addition of one molecule of water in a dilute acidic medium upon illumination[1]. Their structures and stereochemistries have been reported by Bernardi *et al.*[2]. 10α-Methoxydihydrolysergol (II) and its 1-methyl derivative (V) were used for the preparation of a large series of esters[3] among which nicergoline (Fig. 1), trade-name Sermion, the 5-bromonicotinic acid ester (VIII), proved to be an α-adrenergic blocking agent[4].

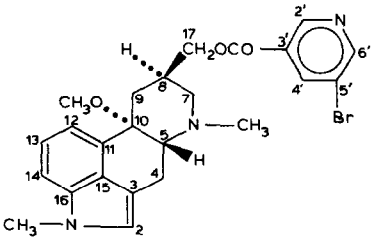

Fig. 1. The structure of nicergoline.

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The preparation of the 5'-chloro analogue of nicergoline (VII) was described by Arcari *et al.*[4]. Reduction of 10α-methoxydihydrolysergic acid methyl ester (I) afforded 10α-methoxydihydrolysergol (II). The esters VII and VIII were prepared by condensation of 1-methyl-10α-methoxydihydrolysergol (V) with an excess of the corresponding acyl chloride in pyridine.

Compounds VII and VIII have now been studied by a liquid chromatographic method based on results published earlier[5,6].

EXPERIMENTAL

Reagents

Drug standards were obtained from Galena (Opava, Czechoslovakia). Acetonitrile (Fluka, Buchs, Switzerland) was of analytical reagent grade; other solvents (Lachema, Brno, Czechoslovakia) were of analytical reagent grade and were doubly distilled before use. The stationary phases were Separon Si C 18 (Laboratory Equipments, Prague, Czechoslovakia) in ready packed columns: A, 25 × 0.4 cm, particle size 10 μm; B, 25 × 0.6 cm, particle size 7 μm. Amino bonded phases in ready packed columns were also employed: C, Perkin-Elmer LC column NH_2, 25 × 0.46 cm, particle size 10 μm; D, MicroPak NH_2 (Varian), 50 × 0.8 cm, particle size 10 μm.

Instruments

Analytical liquid chromatography was carried out on a Perkin-Elmer liquid chromatograph Series 3b with LC 85 spectrophotometric detector and Sigma 15 chromatography data station. Chromatographic conditions: column A, mobile phase, water–acetonitrile–triethylamine (1084:895:21), flow-rate 1.8 ml min^{-1}, UV detection (288 nm); column C, mobile phase, hexane–ethanol (7:3), flow-rate 0.6 ml min^{-1}, UV detection (288 nm).

Semi-preparative liquid chromatography was carried out on an apparatus consisting of a VCM 300 high-pressure micro pump and a variable-wavelength UV detector (both from Development Workshop, Czechoslovak Academy of Sciences, Prague, Czechoslovakia). Chromatographic conditions: column B, see column A; column D, mobile phase, diethyl ether–ethanol (4:1), flow-rate 2.5 ml min^{-1}, UV detection (288 nm).

Melting points were determined on a Kofler block and are not corrected. Specific rotations were determined on a Perkin-Elmer 141 polarimeter.

Mass spectra were measured on a Varian MAT 311 instrument under the following conditions: energy of ionizing electrons, 70 eV; ionizing current, 1 mA; ion source temperature, 200°C; direct inlet system operated at 100–180°C. The elemental composition of the ions was determined by a peak-matching technique (±5 ppm; perfluorokerosene standard).

^1H and ^{13}C NMR spectra were measured on a Jeol FX-60 NMR spectrometer (59.797 and 15.035 MHz, Fourier transform mode, deuteriochloroform, 25°C, tetramethylsilane as internal standard). Chemical shifts (expressed on the δ scale) were calculated from the digitally obtained address differences (computer data calculation) (±0.004 and ±0.06 ppm, respectively).

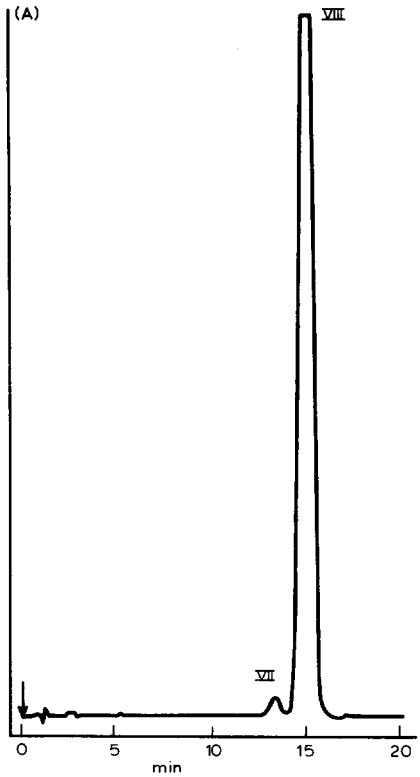


Fig. 2. Chromatogram of nicergoline preparation on Separon Si C 18 (particle size 10 μm), ready-packed column (25 × 0.4 cm). Mobile phase: water–acetonitrile–triethylamine (1084:895:21). Flow-rate: 1.8 ml min^{-1}. UV detection: 288 nm.

RESULTS

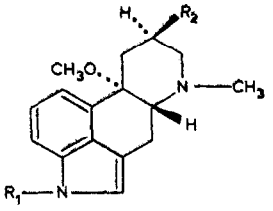
Liquid chromatography (LC)

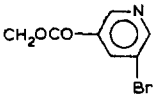
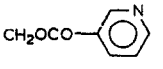
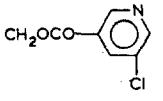
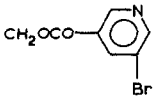
LC analysis of the semisynthetic ergot preparation nicergoline revealed two peaks on the chromatogram, one of which corresponded to nicergoline. The other peak had a retention time that did not correspond to any standard at our disposal and had to be isolated by semi-preparative LC in order to determine its structure. The concentration of the unknown component was in all cases lower than 1.5% (Fig. 2). Semi-preparative LC was carried out on column B and the chromatographic cycle was repeated twenty times to obtain a sufficient amount of a pure standard. To prevent possible structural changes through isomerization or degradation, the fraction containing the unknown component was immediately evaporated to dryness under reduced pressure at 5–10°C. The resulting standard was used for identification. It contained residual amounts of acetonitrile and triethylamine even after prolonged evaporation under high vacuum. The standard was again dissolved in a small amount of chloroform and purified by semi-preparative LC on MicroPak NH_2 (column D). The pure standard was used for identification.

For determination of the capacity factors of all compounds under study, the

TABLE I

STRUCTURES AND CAPACITY FACTORS OF THE INVESTIGATED ALKALOIDS



Compound	R_1	R_2	*Capacity factors, k'*	
			NH_2	*RP-18*
I	H	$COOCH_3$	3.65	3.38
II	H	CH_2OH	6.65	1.25
III	H		3.12	9.40
IV	CH_3	$COOCH_3$	1.94	6.00
V	CH_3	CH_2OH	3.53	2.50
VI	CH_3		2.88	6.25
VII	CH_3		1.78	16.00
VIII	CH_3		1.68	18.25

Perkin-Elmer NH_2 column (C) and Separon Si C 18 column (A) were used (Table I).

Identification

The empirical formula of the unknown compound, $C_{24}H_{26}N_3O_3Cl$ (as determined by peak-matching) differs from that of nicergoline, $C_{24}H_{26}N_3O_3Br$, only in the type of halogen. The corresponding molecular ion is a doublet at *m/z* 439 and 441 (relative intensities 29 and 11%). Other doublets were observed for M − 15 at *m/z* 424 and 426 (relative intensities 14 and 6%), for M − 32 at *m/z* 407 and 409 (relative intensities 60 and 52%), elemental composition $C_{23}H_{22}N_3O_2Cl$, and for the substituent at C(17), *m/z* 157 and 159 (relative intensities 10 and 4%). Analogously, the spectrum of nicergoline contained $^{79}Br/^{81}Br$ doublets. Molecular ions were observed at *m/z* 483 and 485 (relative intensities 18 and 18%); M − 15 at *m/z* 468 and 470 (relative intensities both 10%); M − 32 at *m/z* 451 and 453 (relative intensities

TABLE II

COMPARISON OF ^{13}C NMR CHEMICAL SHIFTS OF COMPOUNDS I, VII AND VIII

Abbreviations: S = singlet; D = doublet; T = triplet; Q = quartet. Data for compound I from ref. 7.

Carbon	*Multiplicity*	*I*	*VII*	*VIII*
Ergoline part				
2	D	118.6	121.2	121.4
3	S	111.1	110.2	110.3
4	T	22.2	22.2	22.4
5	D	69.4	69.9	70.0
7	T	58.5	60.3	60.7
8	D	37.4	31.3	31.6
9	T	30.0	29.9	30.2
10	S	73.5	73.4	73.6
11	S	129.1	129.6	129.7
12	D	115.6	114.7	115.0
13	D	121.7	123.7	123.3
14	D	110.8	108.8	109.0
15	S	126.0	126.2	126.3
16	S	134.2	135.0	135.2
17	T	174.6	68.5	68.6
N(1)-CH_3	Q	–	32.6	32.8
N(6)-CH_3	Q	43.6	43.4	43.4
O-CH_3	Q	49.5	49.4	49.5

Carbon	*Multiplicity*	*VIII*	*VII*	*VIII – VII*
Acid part				
2′	D	148.8	148.3	0.5
3′	S	127.5	127.0	0.5
4′	D	139.6	136.5	3.1
5′	S	120.7	132.2	−11.5
6′	D	154.6	152.3	2.3
COO^-	S	164.0	163.9	0.1

84 and 100%) and substituent on C(17) at *m/z* 201 and 203 (relative intensites both 24%). Ions at *m/z* 283, 267, 251, 250, 249, 248, 237, 235, 233, 221, 207, 198, 181, 172 and 168 are common to both compounds. Therefore, we suggest that the unknown compound is the 5′-chloro analogue of nicergoline (VII).

The ^1H NMR spectra of nicergoline (VIII) and its 5′-chloro analogue (VII) are consistent with their structures. VII: 2.07 t (J = 11.2 Hz, 1H), 2.47 s (3H), 3.00 s (3H), 3.78 s (3H), 4.29–4.56 mt (2H), 6.80 s (1H), 7.00–7.39 mt (3H), 8.27 dd (J = 2.4 and 1.5 Hz, 1H), 8.75 d (J = 2.4 Hz, 1H), 9.00 d (J = 1.5 Hz, 1H). VIII: 2.06 t (J = 10.4 Hz, 1H), 2.48 s (3H), 3.00 s (3H), 3.78 s (3H), 4.29–4.60 mt (2H), 6.80 s (1H), 6.95–7.33 mt (3H), 8.43 dd (J = 2.4 and 1.8 Hz, 1H), 8.85 d (J = 2.4 Hz, 1H), 9.14 d (J = 1.8 Hz, 1H). They differ slightly only in the chemical shifts of the pyridine protons.

The ^{13}C NMR spectra of VII and VIII exhibit the expected number of signals with appropriate off-resonance multiplicities. A comparison with model 10α-methoxydihydrolysergic acid methyl esters[7] allows confirmation of the 5β, 8β and 10α

stereochemistry (Table II). However, there are some differences at carbons 7, 8 and 17 (Fig. 1) due to the replacement of CO_2Me by a CH_2OCO group and at carbons 2, 3 and 16 caused by the methylation in the position 1. The ^{13}C chemical shifts of the ergoline carbons of VII and VIII are identical within the experimental error. The differences in the pyridine carbon resonances are attributable to known halogen substituent effects[8].

Preparation of D-1,6-dimethyl-10α-methoxy-8β-(5-chloronicotinoyl)oxymethylergoline (VII)

A suspension of V[4] (228 mg, 0.762 mmol) in absolute dichloromethane (2.5 ml) was supplemented with triethylamine (93 mg, 0.91 mmol) and a solution of 5-chloronicotinic acid chloride[9] (134 mg, 0.762 mmol) in 5 ml dichloromethane was added dropwise with stirring and cooling to 18–20°C. The solution was stirred for 4 h at room temperature, washed with 0.1 *M* $NaHCO_3$ and water, dried (Na_2SO_4) and the solvent removed by distillation. The residue (300 mg) was chromatographed on a silica gel column (Merck, Art. 7754) with chloroform as eluent. Fractions containing individual compounds were pooled, the solvent was removed by evaporation and the product recrystallized from diethyl ether. M.p. 130–132°C (127–128°C)[4]. Optical rotation, $[\alpha]_D = -21.27°$ ($c = 0.1$, pyridine).

DISCUSSION

A study of the relationship between the structures of the compounds under investigation and their retention volumes showed that, on the amino-bonded stationary phases, N(1)-methylated derivatives exhibit a marked reduction in retention volume as compared with unsubstituted compounds (*cf.*, I and IV; II and V; III and VIII; Table I). This is probably due to loss of the interaction of the N(1)-hydrogen with a free electron pair from the stationary phase nitrogen. The N(1)-methylation results in a lowering of the basicity of the molecule which is reflected in increased retention on the reversed phase.

Substitution by a halogen in position 5′ of the pyridine moiety results in a decrease in electron density on the nitrogen of the pyridine ring and an increase in the polarizability of the molecule. This effect results in increased retention of the substituted alkaloids (compare VII and VIII, Table I). Examination of the purity of nicergoline preparations is thus more readily achieved by chromatography on a reversed phase which makes it possible to detect trace amounts of the impurity VII. The amount of VII in nicergoline was in all cases below 1.5%. The pharmaceutical preparation Sermion (Ch. B. 1044) contained a compound with an identical retention volume and at a concentration of 1.65%. The probable source of this compound (VII) is 5-chloronicotinic acid formed during bromination of nicotinic acid in thionylchloride.

The above LC method is suitable for testing the efficiency or kinetics of formation of individual intermediates in nicergoline synthesis and checking their purity. Thus, the study of the formation of compound VIII during condensation of V and 5-bromonicotinic acid, especially its kinetic evaluation, is best done by using an amino-bonded stationary phase which permits the detection of small amounts of the product. Consequently, both quantitative and qualitative control of individual substances is readily achieved using an appropriate combination of phases.

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