SYNTHESES OF TRITIUM AND CARBON-14 LABELLED N-(3-DIMETHYL AMINOPROPYL)-N-(ETHYLAMINOCARBONYL)-6-(2-PROPENYL)ERGOLINE-8β-CARBOXAMIDE (CABERGOLINE), A POTENT LONG LASTING PROLACTIN LOWERING AGENT.

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SUMMARY

The syntheses of ^{3}H- and ^{14}C-labelled cabergoline (FCE 21336), namely N-(3-dimethylaminopropyl)-N-(ethylamino-carbonyl)-6-(2-propenyl)ergoline-8β-carboxamide 1 and its analogues are described.
Tritiated cabergoline ([^{3}H]cabergoline) 6, namely N-(3-di-methylaminopropyl)-N-(ethylaminocarbonyl)-6-(2-[2,3-^{3}H]-propenyl)ergoline-8β-carboxamide, was obtained, by catalytic reduction with tritium gas, according to two different synthetic procedures:

A - a three step route, starting from 6-(2-propargyl)-dihydro lysergic acid-methyl ester 3 gave [^{3}H]cabergoline, 97% radiochemically pure, with a specific radioactivity of 11.19 GBq/mmol and in an overall radiochemical yield of 3.5%.

B - a one step route, starting from 1-ethyl-3-(3-dimethyl-aminopropyl)-3-[6'-(2-propargyl)ergoline-8'β-carbonyl]-urea 5' yielded [^{3}H]cabergoline with a radiochemical purity >97%, specific radioactivity 1.06 TBq/mmol and radiochemical yield of 23%.

A modification of this last procedure also gave [^{3}H]dihydro cabergoline ([^{3}H]FCE 23411), namely N-(3-dimethylaminopropyl) -N-(ethylaminocarbonyl)-6-(2-[2,3-^{3}H]propyl)ergoline-8β-carboxamide 7, 97% radiochemically pure and with a specific radioactivity up to 4.03 TBq/mmol.

The synthesis of [^{14}C]cabergoline was carried out, in a three step route, by addition of potassium[^{14}C]cyanide to 6-(2-propenyl)-8β-chloroergoline 12 to give the expected N-(dimethylaminopropyl)-N-(ethylaminocarbonyl)-6-(2-propenyl)-ergoline-8β-[^{14}C]carboxamide 15', 97% radiochemically pure with a specific radioactivity of 2.09 GBq/mmol and an overall radiochemical yield of 16%.

Key words : ergoline, cabergoline, FCE 21336, antiprolactin agent, dihydrocabergoline, FCE 23411 [^{3}H]cabergoline, [^{3}H]dihydrocabergoline, [^{14}C]cabergoline.

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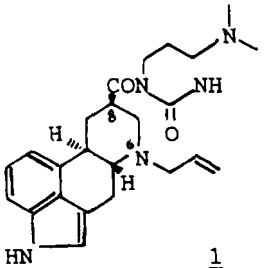
INTRODUCTION

Natural ergot alkaloids and synthetic ergoline derivatives display a remarkable variety of pharmacological activities depending mainly upon the nature and configuration of the substituent at position 8 [1,2].
Various ergot derivatives, e.g. bromocriptine, lisuride and pergolide, show potent dopamine agonistic properties and are useful as anti-Parkinson and prolactin lowering drugs [3].
Recently, compound 1 (cabergoline or FCE 21336) was found to be an extremely potent and long lasting prolactin lowering agent and was selected for further pharmaco-toxicological studies. The compound is now under extensive clinical evaluation [4,5].



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For pharmacokinetics studies and biotransformations of cabergoline in animals and man, a radiolabelled form was required.

[^{3}H]Cabergoline was obtained by partial catalytic reduction with tritium gas of the propargyl analogue of cabergoline 5' or from the compound 3 which has in position 6 the side chain 2-propargyl. Palladium/C poisoned with quinoline was employed as catalyst [6].

In vivo experiments with laboratory animals [7] showed that the biological stability of the label to be insufficient for metabolic studies. For this reason it was necessary to prepare [^{14}C]cabergoline. As stated by Marzoni G. et al. [8], the possible position of labelling site, without interfering with the integrity of the ring system, is the carbonyl group attached to the position 8. We expect that this radiolabel remains present in all the possible cabergoline metabolites containing the ergoline skeleton.

It was also necessary to develop a radioimmunological method (RIA) to determine the low levels of the drug in biological fluids of humans, given doses of cabergoline [9]. The necessity to prepare a radiolabelled hapten for RIA prompted us to obtain a compound with a specific activity as high as practicable. This goal was achieved by exhaustive tritiation of the cabergoline precursor 5' to give [^{3}H]dihydrocabergoline (FCE 23411), which was used, successfully in the cabergoline RIA assay [10].

RESULTS AND DISCUSSION

The easy availability of compound 3, starting from 6-nor-dihydrolysergic acid methylester 2 and the high yields of each step of synthesis, prompted us at first to prepare [^{3}H]cabergoline 6 according to the scheme 1 (route A). Unfortunately, the final compound was obtained in low yield (3.5%) and with the specific radioactivity not adequate for in vivo studies.

The second approach to prepare [^{3}H]cabergoline was chosen as shown in the scheme 1 (route B). The 6-propargyl derivative 5' was submitted to tritiation employing as catalyst, 10% Pd/C, containing 16% quinoline in dioxane, giving the expected product. The poisoned catalyst was used in order to avoid the overreduction to the fully saturated derivative. Nevertheless the 6-propyl derivative 7 was always present as a by-product in the crude reaction mixture. After purification by preparative-TLC, [^{3}H]cabergoline was obtained in 23% radiochemical yield and specific radioactivity of 1.06 TBq/mmol.

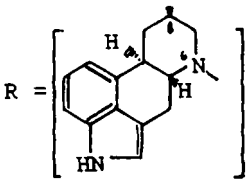
On the contrary, the complete reduction of 5' with tritium gas in presence of 5% Pd/C [5] yielded [^{3}H]dihydrocabergoline 7 with specific radioactivity up to 4.03 TBq/mmol.This compound was used as labelled hapten in cabergoline RIA assays. At this high specific radioactivity, self-decomposition becomes important and an appropriate preparative-TLC method was developed to purify the compound 7, when necessary.

[^{14}C]cabergoline was obtained according to the procedure depicted in the scheme 2. This synthetic route is similar to that described by Wheeler et al. [11], who prepared [17-^{14}C]pergolide mesylate.

6-(2-Propenyl)-dihydrolysergic acid methyl ester 8, readily available from dihydrolysergic acid [12], was converted to the hydrazide derivative 9 by means of hydrazine hydrate in refluxing methanol. Diazotation of this last compound to the corresponding acyl azide and further Curtius rearrangement in aqueous hydrochloric acid, afforded the amine 10 in good yield [13]. Reaction of the amine 10 with an excess of sodium nitrite in concentrated hydrochloric acid gave, after addition of stannous chloride, the chloro compound 12 in acceptable yield [14]. As a by-product of this reaction hydroxy compound 11 was also formed.

Concerning the stereochemistry at C-8, the reactions occur with complete retention of configuration at this chiral centre as clearly shown by the H-NMR data (JHH) of compounds 10, 12, 13 and 14, for protons H-9ax(β) and H-7ax(β): both have a diaxial relationship to the H-8ax (α).

SCHEME 1



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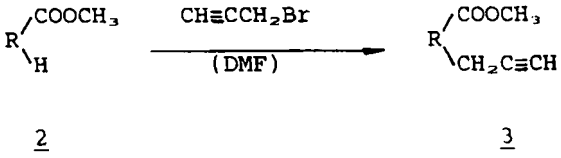
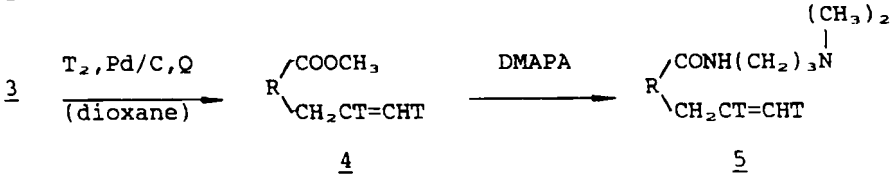
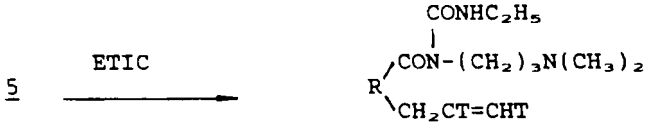


2 3

route A

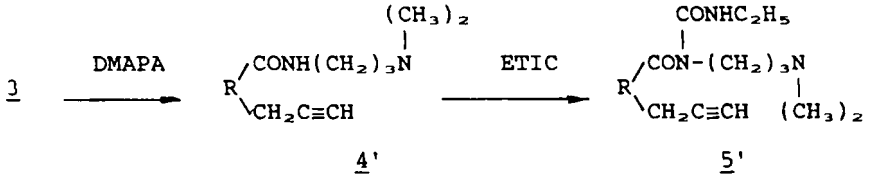


4 5

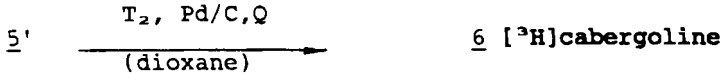


6 **[^{3}H]cabergoline**

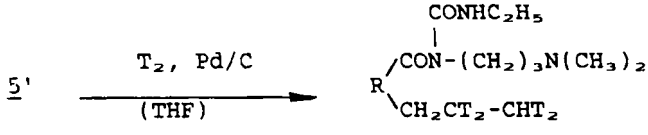
route B



4' 5'



6 **[^{3}H]cabergoline**



7 **[^{3}H]dihydrocabergoline**

T = [^{3}H] ; Q = quinoline
DMAPA = 3-dimethylamino-1-propylamine
ETIC = ethylisocyanate

SCHEME 2

$$\text{R-COOCH}_3 \xrightarrow[\text{(MeOH) reflux}]{N_2H_4.H_2O} \text{R-CONHNH}_2 \xrightarrow[H_2O]{NaNO_2,HCl} \text{R-NH}_2$$

8 9 10

$$10 \xrightarrow{NaNO_2,HCl,SnCl_2} \text{R-OH} + \text{R-Cl}$$

11 12

$$12 \xrightarrow[\text{(EtOH)}]{NaXN} \text{R-XN} \xrightarrow[(H_2O)\ \text{reflux}]{NaOH} \text{R-XOOH}$$

13 ; X=^{12}C 14 ; X=^{12}C

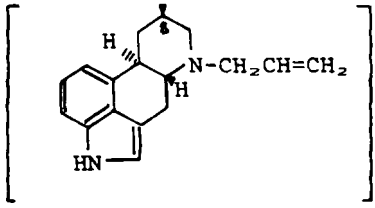
13'; X=^{14}C 14'; X=^{14}C

$$14,14' \xrightarrow[\text{(DMF,TEA)}]{EDPC} \text{R-XON(CH}_2)_3\text{N(CH}_3)_2 \text{ (CONHC}_2\text{H}_5) + \text{R-XONC}_2\text{H}_5 \text{ (CONH-(CH}_2)_3\text{N(CH}_3)_2)$$

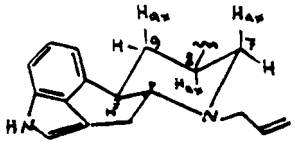
15 ; X=^{12}C 16 ; X=^{12}C

15'; X=^{14}C 16'; X=^{14}C

[^{14}C]cabergoline

R = 

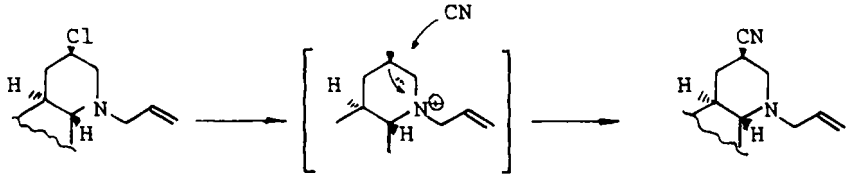
EDPC = N-ethyl-N'-(3-dimethylamino)propyl-carbodiimide



Coupling constants are in fact:

H-H Coupling	Compound			
	10	12	13	14
H-9ax — H-8ax	11.6	12.1	12.0	13.2
H-7ax — H-8ax	10.9	11.1	11.4	11.2

As is demonstrated by the above NMR data, in all these steps, the stereochemistry about carbon 8 remained unchanged.
The chloro compound 12 was considered a suitable precursor for labelling cabergoline with radiocarbon. In fact this compound, by reaction with NaCN in refluxing ethanol afforded the cyano compound 13. As shown above, the SN_2 displacement occurred with complete retention of the configuration by participation of the nitrogen ring.



Such participation has been observed in 2-(halomethyl)pyrrolidines and 3 chloro-1-ethylpiperidine [15-19].
Hydrolysis of the cyano compound 13 in ethanolic sodium hydroxyde, yielded the carboxylic acid compound 14, which was directly converted to cabergoline 15 and its regioisomer 16 by reaction with N-ethyl-N'-(3-dimethylamino)propyl carbodiimide (EDPC) in dimethylformamide, as previously described [4]. Cabergoline was recovered in pure form by column chromatography on silica gel. The complete stereochemical control and the good yields of the described reaction make this route not only attractive for radiocarbon labelling cabergoline but also for ergoline derivatives.
Therefore [^{14}C]cabergoline was obtained, following this route, from potassium[^{14}C]cyanide in an overall radiochemical yield of 16%.

EXPERIMENTAL

Thin layer chromatography (TLC)

TLC was carried out, where not specified, using Merck silica gel F 254 20x5 cm, 0.25 mm thick plates. The eluting solvent systems were as follows with proportions by volume:

A) chloroform:methanol (95:5)

B) cyclohexane:n-propanol:diethylamine(DEA) (7:2:1)

C) cyclohexane:ethyl acetate:DEA (2:7:1)

D) chloroform:methanol:ammonia 30% (70:30:0.25)

E) n-hexane:n-propanol:DEA (8:1:1)
(double migration)

F) n-hexane:acetone:DEA (5:4:1)

G) chloroform:methanol (4:1)

H) chloroform:methanol:ammonia 30% (80:20:2)

I) chloroform:methanol (9:1)

L) cyclohexane:ethyl acetate:methanol (4:2:1)

M) chloroform:methanol:ammonia 36 Be (80:20:3)

N) pyridine:n-butanol:ethyl acetate:dimethylformamide (DMF) (1:3:4:3)

O) benzene:ethyl acetate:DMF (1:5:4)

High performance liquid chromatography (HPLC)

Analyses were performed by using a Waters μBondapak C18, 10 μm (300 x 3.9 mm ID) column with a mobile phase of CH_3CN : KH_2PO_4 buffer (0.075 M) pH 3 (18 : 82 by volume); flow rate 1.0 ml/min ; UV detection 283 nm ; radiometric detection with heterogeneous cell (0.36 ml) Yttrium silicate.

Purification was performed with Waters μBondapak C18 (10 um) preparative column (30 cm x 7.8 mm ID) by using as mobile phase CH_3CN : Cl_3CCOOH 0.001 M (about pH 3) (20 : 80 by volume); flow rate 4 ml/min ; UV detection 283 nm.

Assay methods

Ultraviolet spectra were determined on a Beckman DU50 spectrophotometer. Measurements of radioactivity were carried out with a Packard 300C liquid scintillation counter using Rialuma (Lumac System A.G.) as liquid scintillation cocktail. Radiochemical analyses of TLC plates were performed with a Berthold 3832 automatic linear analyzer.
HPLC analyses and purification were carried out with a Perkin Elmer series 2x2 liquid chromatograph with LC 75 UV/VIS detector and Packard Trace 7130 on line with 512 kRAM 3270 IBM PC as radioactivity flow monitor.
Melting points were determined on a Buchi apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 125 spectrophotometer. ^{1}H-NMR were recorded on a Varian XL-200 spectrometer (chemical shifts are given in ppm (δ) downfield from tetramethylsilane). Mass spectra were recorded at 70 eV on a Varian MAT 311A mass spectrometer. All unlabelled compounds had IR, NMR and mass spectra that are fully consistent with their structure.

The precursors labelled with tritium were supplied by Amersham International plc.
Potassium [^{14}C]cyanide was purchased from Amersham International plc.

6-(2-Propargyl)-dihydrolysergic acid methyl ester (3)

To a stirred solution of 6-nor-dihydrolysergic acid methyl ester 2 (5 g, 18.5 mmoles) and ground potassium carbonate (3.32 g, 24 mmoles) in DMF (35 ml), a solution of 2-propargyl bromide (1.75 ml, 23.1 mmoles) in DMF (5 ml) was added dropwise at room temperature. The resulting mixture was stirred at room temperature for 2 hours and then poured into ice water. The precipitate was washed with water and dissolved in methylene chloride. After washing with brine, the organic phase was dried (Na_2SO_4) and evaporated to dryness. The residue was crystallized from ethanol affording 4.4 g (77% yield) of the pure compound 3, mp. 140-142°C.
TLC: system G, L (Rf=0.75, Rf=0.3 respectively).
IR (KBr) : 1710 cm^{-1} (CO); 3250 cm^{-1} (CH); 3400 cm^{-1} (NH).
NMR ($CDCl_3$, 200 MHz): 1.61 (ddd, J=12.0, 12.0, 12.0 Hz, 1H, H-9ax); 2.18 (t, J=2.4 Hz, 1H, C=CH); 2.67 (m, 2H, H-5ax, H-4ax); 2.84 (dd, J= 10.5, 10.5 Hz, 1H, H-7ax); 2.9-3.1 (m, 3H, H-8ax, H-9e, H-10ax); 3.17 (ddd, J=2.8, 2.8, 10.5 Hz, 1H, H-7e); 3.4 (m, 1H, H-4e); 3.48, 3.98 (two dd, J=2.4, 17.8 Hz, 2H, NCH_2); 3.75 (s, 3H, $COOCH_3$); 6.9-7.1 (m, 4H, H-12, H-13, H-14, H-2); 7.96 (bs, 1H, NH).
MS (EI): m/z 308 (100, $M^{+\cdot}$); 269 (65); 209 (65); 182 (28); 168 (50); 167 (60); 154 (95); 144 (34); 127 (53); 115 (25).

6-(2-[2,3-^{3}H]propenyl)dihydrolysergic acid methyl ester (4)

The partial hydrogenation of the compound 3 was carried out by Amersham Int. plc. according to the following general procedure. The compound (about 0.5 mmoles) is dissolved in 4 ml of dioxane and 10% Pd/C (about 30 mg) catalyst (prehydrogenated)is added. A 16% quinoline solution in dioxane (0.2 ml) is added. The hydrogenation is carried on at room temperature and normal pressure until an equivalent of hydrogen (about 12 ml) is absorbed. Catalyst is removed by filtration and the mixture evaporated to dryness. The solid is dissolved in benzene to give a solution of crude 4.

The crude compound (5.36 GBq; 63% radiochemically pure by radio-TLC system A) was TLC chromatographed (silica gel TLC plate Merck F254; 20x20 cm; 1 mm thick) using the system A as eluting solvent. The chromatographic band corresponding to the compound 4 was extracted from silica gel with a mixture methanol:chloroform 1:1 (by volume). The resulting product (82% radiochemically pure by radio-TLC system A) was diluted with "cold" 4 (58.2 mg, 0.188 mmoles) and the resulting mixture was repurified by flash-chromatography on silica gel (3 g) eluting with chloroform (15 ml) and then with a mixture chloroform:methanol 99:1 (50 ml). The fractions containing the expected compound were combined, yielding, after solvent evaporation, 2.32 GBq of the ester 4, 93% radiochemically pure (by radio-TLC; system A: Rf=0.58).

N-(3-Dimethylaminopropyl)-6-(2-[2,3-^{3}H]propenyl)ergoline-8β-carboxamide (5)

Ammonium chloride (189.4 mg, 3.54 mmoles) and 3-dimethylamino-1-propylamine (2 ml, 0.016 mmoles) were stirred at room temperature for 30 min and then at 120°C for about 30 min. The solution was evaporated to small volume (0.5 ml) and added to the compound 4 (1.10 GBq; 0.076 mmoles). The mixture was afterwards heated at 120°C for about 16 hours.

At the end of the reaction (checked by radio-TLC; system B) the excess of amine was distilled under vacuum. The crude product was purified by preparative TLC (silica gel plate Merck F254; 20x20 cm; 1 mm thick) using the system B as chromatographic eluent. The product was extracted from silica gel with a mixture of methanol:chloroform 1:1 (by volume). The recovered amide 5 (453 MBq) had a radiochemical purity of 98% (by radio-TLC; system B: Rf=0.22). This preparation was repeated in the same conditions with the same amounts, affording an additional quantity (551 MBq) of the final product 5.

N-(3-dimethylaminopropyl)-N-(ethylaminocarbonyl)-6-(2-[2,3-^{3}H] propenyl)ergoline-8β-carboxamide ([^{3}H]cabergoline)(6)

Compound 5 (827 MBq, 0.093 mmoles), toluene (1.7 ml) and ethylisocyanate (0.76 ml, 0.97 mmoles) were kept in a sealed glass ampoule at 100°C for about 72 hours. The ampoule was then frozen with liquid nitrogen, and, after opening, the reaction mixture was transferred into a 50 ml flask. The crude product was worked up by two subsequent preparative-TLC purifications (silica gel plates Merck F254 20x20 cm; 1 mm thick): the first one employed the system C, as chromatographic eluent, and the second one the system D. In both purifications the product was extracted from silicagel with acetone (10 ml each). The recovered [^{3}H]cabergoline 6 (166 MBq) had a specific radioactivity of 11.19 GBq/mmol.The UV spectrum (in methanol λ_{max} at 283 nm ; $E^{1\%}_{1cm}$ = 156) was concordant to that of the standard sample. The radiochemical purity was >97% (by radio-TLC; system C: Rf=0.34 and system D: Rf=0.50). The overall radiochemical yield from 3 was 3.5%.

N-(3-Dimethylaminopropyl)-6-(2-propargyl)ergoline-8β-carboxamide (4')

A solution of the compound 3 (8.8 g, 28 mmoles) and 3-dimethylamino-1-propylamine (18 ml) in glacial acetic acid (3 ml) was heated, under stirring, at 120°C for 18 hours. The resulting brown solution was evaporated. The solid residue was dissolved in ethyl acetate and washed with a saturated solution of sodium carbonate. The organic layer was washed with brine, stirred with charcoal, filtered and then dried (Na_2SO_4). The solution, evaporated to small volume, yielded 8.4 g (78% yield) of the pure amide 4', m.p. 212-214°C.
TLC: systems H, G (Rf=0.6, Rf=0.25 respectively).
IR (KBr): 1540 cm^{-1} (C=O; amide II); 1620 cm^{-1} (C=O; amide I); 3300 cm^{-1} (=CH); 3370 cm^{-1} (NH).
NMR ($CDCl_3$, 200 MHz) : 1.6 (m, 3H, H-9ax, N-CH_2-CH_2-CH_2); 2.15 (t, J=2.4 Hz, 1H, C=CH); 2.24 (s, 6H, N(CH_3)$_2$); 2.41 (m, 2H, CO-NH-CH_2-CH_2-CH_2-N); 2.6-3.0 (m, 6H, H-4ax, H-5ax, H-7ax, H-9e, H-10ax, H-8ax); 3.08 (m, 1H, H-7e); 3.3-3.5 (m, 3H, H-4e, CO-NH-CH_2-CH_2-CH_2); 3.46, 3.96 (two dd, J=2.4, 17.7 Hz, 2H, N-CH_2-C=); 6.9-7.1 (m, 4H, H-12, H-13, H-14, H-2); 7.80 (bm, 1H, NH-CH_2); 7.96 (bs, 1H, NH).
MS (EI): m/z 378 (31,$M^{+\cdot}$); 339 (29); 294 (26); 249 (47); 237 (45); 167 (69); 157 (60); 154 (100); 129 (66); 112 (69).

1-Ethyl-3-(3-dimethylaminopropyl)-3-[6'-(2-propinyl)ergoline -8'β-carbonyl]-urea (5')

Ethylisocyanate (50 ml) was added to a stirred suspension of the amide 4' (6.8 g, 17.9 mmoles) in toluene (350 ml). The resulting suspension was heated at reflux for 3 days. The

turbid solution was evaporated to dryness. The residue was dissolved in a small volume of acetone and percolated through a pad of silica gel. Acetone was used as eluting agent. The fractions containing the pure compound 5' were pooled and evaporated to dryness affording 3.9 g (48% yield) of the title compound, as a white foam.
TLC: systems H, G (RF=0.35, Rf=0.1 respectively).
IR (KBr): 1690 cm^{-1} (CO); 3290 cm^{-1} (CH); 3400 cm^{-1} (NH).
NMR ($CDCl_3$, 200 MHz) : 1.18 (t, J= 7.2 Hz, 3H, NH-CH_2-CH_3); 1.79 (ddd, J=12.2, 12.2, 12.2 Hz, 1H, H-9ax); 1.86 (m, 2H, CO-N-CH_2-CH_2-CH_2-N); 2.17 (t, J= 2.3 Hz, 1H, C=CH); 2.23 (s, 6H, $N(CH_3)_2$); 2.34 (t, J= 6.7 Hz, 2H, CO-N-CH_2-CH_2-CH_2-N); 2.6-3.1 (m, 6H, H-4ax, H-5ax, H-9e, H-10ax, CH_2-7); 3.30 (m, 2H, N-CH_2-CH_3); 3.40 (m, 2H, H-4e, H-8ax); 3.46, 3.96 (two dd, J= 2.3, 17.8 Hz, 2H, N-CH_2-C=); 3.84 (m, 2H, CO-N-CH_2-CH_2-CH_2-N); 6.9-7.1 (m, 4H, H-12, H-13, H-14, H-2); 7.93 (bs, 1H, NH); 9.45 (bm, 1H, NH-CH_2-CH_3).
MS (EI): m/z 449 (5, $M^{+\cdot}$); 378 (19); 339 (16); 249 (38); 207 (50); 173 (38); 167 (75); 154 (100); 129 (58); 84 (80).

N-(3-dimethylaminopropyl)-N-(ethylaminocarbonyl)-6-(2-[2,3-^{3}H] propenyl)ergoline-8β-carboxamide ([^{3}H]cabergoline(6).

The compound 5' was treated with tritium gas in presence of a catalyst by Amersham International plc. according to the procedure already described for the tritiation of the precursor 3 .

Tritiation of 14,9 mg of the compound 4' with 185 GBq of tritium gas gave 35.15 GBq of [^{3}H]cabergoline. Radiochemical purity (checked by radio-TLC; system E) was found to be 46.5%. A portion of this crude (175.8 MBq) was charged on a silica gel TLC plate (Merck F254; 20 x 20 cm ; 0.25 mm thick) using the system E as eluting solvent. The chromatographic band corresponding to cabergoline was removed and the product was taken up from silica gel by several extractions with acetone until disappearance of radioactivity. The combined extracts were filtered through a D_4 sintered-glass filter to give an acetonic solution (about 25 ml) containing 41.6 MBq of [^{3}H]cabergoline 6 with a radiochemical yield of 23.7% a specific radioactivity (calculated) of 1.06 TBq/mmol and a radiochemical purity >97% (by radio-TLC; system: D Rf=0.50 and system E: Rf=0.59).

N-(3-dimethylaminopropyl)-N-(ethylaminocarbonyl)-6-(2-[2,3-^{3}H] propanyl)ergoline-8β-carboxamide ([^{3}H]dihydrocabergoline (7)

The exhaustive tritiation of the compound 5' was performed by Amersham Int. plc. according to the standard procedure described below.
The propargyl compound 5' (9.9 mg) and 5% Pd/C (53 mg) in tetrahydrofuran (THF; 2 ml) were stirred in the dark with tritium gas (370 GBq) for 105 minutes.
Catalyst was removed by filtration and, after solvent evaporation, the crude 7 (24.05 GBq) was obtained 84% radiochemically pure (by radio-TLC; system E).
A purification performed by Amersham Int. plc. by preparative HPLC [Zorbax silica column (25cm x 10mm ID) eluting with a mixture THF:n-hexane:triethylamine (TEA) 650:350:10 (by volume), flow 2 ml/min], gave the compound 7 (3.7 GBq), 98% radiochemically pure(by radio-TLC; system E: Rf=0.64) with a specific radioactivity of 2.56 TBq/mmole.

A sample of impure 7 (44.4 MBq; 53% radiochemically pure

by radio-TLC; system F) was repurified by preparative-TLC (Merck F254 silica gel plate 20x20 cm; 0.25 mm thick) employing the system F as eluting solvent. The product was extracted from silica gel with acetone to give [^{3}H]dihydrocabergoline 7 (16.78 MBq), 97% radiochemically pure (by radio-TLC; system F: Rf=0.48).
Other preparations and purifications of 7 were performed with the same technique and the highest specific radioactivity of [^{3}H]dihydrocabergoline was found to be 4.03 TBq/mmol.

6-(2-Propenyl)-8β-hydrazinocarbonyl-ergoline (9)

Hydrazine hydrate (98%, 50 ml) was added to a stirred solution of 6-(2-propenyl)-8 -methoxycarbonyl-ergoline 8 (50 g, 0.16 moles) in methanol (300 ml). The resulting solution was heated at reflux for 3 hours. The solvent was removed in vacuo and the residue was taken up in ice-water (500 ml). The precipitate was filtered, washed with water, then crystallized from ethanol, affording compound 9 as shiny crystals (42 g, 84% yield); m.p. 205-207°C.
TLC : system G; Rf=0.68.
IR (KBr): 1540-1650 cm^{-1} (C=O); 3320 cm^{-1} (N-H)
NMR (DMSO-d_6) : 1.43 (1H,ddd, J=12.7, 12.7, 12.7 Hz, H-9ax); 2.28 (m, 1H, H-5ax); 2.38 (1H,J=11.0, 11.0 Hz, H-7ax); 2.4-2.6 (m, 3H, H-4ax, H-9e, H-8ax); 2.78 (m, 1H, H-10ax); 2.92 (m, 1H, H-7e); 3.1-3.5 (m, 3H, N-CH_2-CH, H-4e); 4.19 (bs, 2H, NH_2); 5.19 (m, 2H, =CH_2); 5.91 (m, 1H, =CH-); 6.7-7.2 (m, 4H, aromatic H's); 9.10 (bs, 1H, CO-NH-N); 10.61 (bs, 1H, NH-1).
MS (EI): m/z 310 (100, $M^{+\cdot}$); 269 (10); 251 (16); 249 (26); 209 (21); 182 (11); 167 (27); 154 (42); 144 (13); 127 (13).

6-(2-Propenyl)-8β-amino-ergoline (10)

To a stirred suspension of the compound 9 (49 g, 0.158 moles) in water (200 ml), a solution of 0.2N HCl (790 ml, 0.158 moles) was added at room temperature . The resulting solution was cooled at 0°C and then treated dropwise with a solution of 1M sodium nitrite (161 ml, 0.161 moles). A solution of 1M HCl (650 ml, 0.65 moles) was slowly added dropwise to this suspension. After 10 minutes of stirring, the crystalline suspension of the acylazide hydrochloride was heated, as fast as possible, at 80°C. When the effervescence had ceased, the resulting solution was made alkaline up to pH 11 with concentrated NH_4OH and kept at room temperature. The precipitate was filtered and washed with water. After drying, the crude amine 10 was crystallized from boiling ethyl acetate, giving pure 10 (32.5 g, 78% yield); m.p. 168-170°C.
TLC: system H; Rf=0.82.
IR (KBr): 1580, 1610 cm^{-1} (NH_2); 3410 cm^{-1} (NH)
NMR (DMSO-d_6) : 1.00 (ddd,1H, J=11.6, 11.6, 11.6 Hz, H-9ax); 1.50 (bm, 2H, NH_2); 1.92 (dd,1H, J=10.0, 10.0 Hz, H-7ax); 2.20 (m,1H, H-5ax); 2.4-3.0 (m,5H, H-4ax, H-7e, H-8ax, H-9e, H-10ax); 3.1-3.5 (m,3H, H-4e, N-CH_2); 5.19 (m,2H, =CH_2); 5.92 (m,1H, =CH-); 6.7-7.1 (m,4H, aromatic H's); 10.60 (bs,1H,NH-1).
MS (EI): m/z 267 (100, $M^{+\cdot}$); 249 (9); 225 (8); 223 (10); 209 (9); 183 (18); 181 (21); 167 (16); 154 (47); 127 (15).

6-(2-Propenyl)-8β-chloro-ergoline (12)

A solution of sodium nitrite (25 g, 0.362 moles) in water (30 ml) was added dropwise, at 0°C under a flow of nitrogen, to a stirred solution of the compound 10 (32.8 g, 0.123 moles)

in a mixture of THF (30 ml) and 5N HCl (24.5 ml, 0.123 moles). 5N HCl (90 ml, 0.45 moles) was slowly added to the resulting suspension and the stirring was continued at 0°C for 2 hours. The yellow suspension was treated with $SnCl_2 \cdot H_2O$ (30 g) and heated at 40°C for 1 hour. After concentration to small volume, the solution was basified up to pH 10 with concentrate NH_4OH and then extracted several times with ethyl acetate. The organic phase was washed with brine and dried (Na_2SO_4). Evaporation of the solvent afforded a crude residue, which was chromatographed on silica gel by elution with cyclohexane: ethyl acetate (2:1 by volume). After evaporation of the solvent and crystallization from ethanol, pure 12 was obtained (21.3 g, 60% yield); m.p.157-159°C.
TLC: system I; Rf=0.65.
IR (KBr): 790 cm^{-1} (C-Cl); 3200 cm^{-1} (NH).
NMR ($CDCl_3$) : 1.73 (ddd, 1H, J=12.1, 12.1, 12.1 Hz, H-9ax); 2.49 (dd, 1H, J=11.1, 11.1 Hz, H-7ax); 2.6-2.8 (m,2H, H-4ax, H-5ax); 2.9-3.2 (m,2H, H-9e, H-10ax); 3.3-3.6 (m,4H, H-4e, H-7e, N-CH_2); 4.24 (m,1H, H-8ax); 5.26 (m,2H, =CH_2); 5.95 (m,1H, =CH-); 6.9-7.2 (m,4H, aromatic H's); 7.91 (bs,1H, NH-1).
MS (EI): m/z 286 (100, $M^{+\cdot}$); 251 (31); 249 (21); 209 (27); 182 (24); 167 (35); 154 (76); 144 (28); 127 (36); 115 (15).

Continuing the elution with a mixture of cyclohexane: ethylacetate (1:1 by volume), 6-(2-propenyl)-8 -hydroxy-ergoline 11 (3.5 g) was obtained; m.p. 162-164 °C.
TLC: Silica gel Merck 60, F254, system chloroform:methanol 6:1 (by volume); Rf=0.75.
IR (KBr): 2970 cm^{-1} (OH); 3200 cm^{-1} (NH).
NMR (DMSO-d_6) : 1.12 (ddd, 1H, J=13.1, 13.1, 13.1 Hz, H-9ax); 2.01 (dd, 1H, J=10.5, 10.5 Hz, H-7ax); 2.21 (m,1H, H-5ax); 2.70 (m,2H, H-9e, H-10ax); 3.01 (dd,1H, J= 4.3, 10.5 Hz, H-7e); 3.1-3.5 (m,3H, H-4e, N-CH_2-CH); 3.72 (m,1H, H-8ax);4.80 (d.1H, 4.9 HZ, OH-8); 5.20 (m,2H, =CH_2); 5.95 (m,1H, =CH-); 6.7-7.2 (m,4H, aromatic H's); 10.6 (bs,1H, NH-1).
MS (EI): m/z 268 (100, $M^{+\cdot}$); 223 (12); 209 (8); 197 (7); 183 (17); 167 (24); 154 (79); 144 (12); 127 (30); 115 (11).

6-(2-Propenyl)-8β-cyano-ergoline (13)

A stirred solution of compound 12 (5.15 g, 19.2 mmoles) and NaCN (1.9 g, 38.7 mmoles) in a mixture of ethanol (50 ml) and water (20 ml) was heated at reflux for 3 hours. The solvent was removed "in vacuo" and the residue was taken up in ethyl acetate and washed with brine. After drying (Na_2SO_4) and removal of the solvent, the crude cyano derivative 13 was chromatographed on silica gel eluting with hexane:ethyl acetate (1:1 by volume). After crystallization from diethyl ether, pure 13 was obtained (4.8 g, 90% yield); m.p.152-154°C.
TLC : system L; Rf=0.43.
IR (KBr): 2120 cm^{-1} (CN); 3300 cm^{-1} (NH)
NMR ($CDCl_3$) : 1.72 (ddd,1H, J=12.0, 12.0, 12.0 Hz, H-9ax); 2.53 (dd,1H, J=11.4, 11.4 Hz, H-7ax); 2.5-2.8 (m,2H, H-4ax, H-5ax); 2.9-3.1 (m,3H, H-8ax, H-9e, H-10ax); 3.3-3.6 (m,4H, H-4e, H-7e, N-CH_2); 5.27 (m,2H, =CH_2); 6.9-7.3 (m,4H, aromatic H's); 7.93 (bs,1H, NH-1).
MS (EI): m/z 277 (100, $M^{+\cdot}$); 250 (7); 237 (34); 236 (31); 182 (16); 167 (27); 154 (99); 144 (9); 127 (39); 115 (12).

6-(2-Propenyl)-8β-carboxy-ergoline (14)

A stirred solution of the cyanide 13 (4.8 g, 17.3 mmoles) and 10M NaOH (40 ml, 0.4 ml) was heated at reflux overnight.

The solvent was removed "in vacuo" and the residue was dissolved in water (50 ml). After cooling, the stirred solution was slowly acidified with 12M HCl (33.5 ml, 400 mmoles). The resulting precipitate was filtered off, washed several times with cold water then with acetone to give the desired compound 14 (4.6 g, 89% yield); m.p. 203-205°C.
TLC : system M (Rf=0.55).
IR (KBr): 1600 cm^{-1} (COOH); 3000-3500 cm^{-1} (OH, NH)
NMR (DMSO-d_6) : 1.34 (ddd,1H, J=13.2, 13.2, 13.2 Hz, H-9ax); 2.28 (dd,1H, J=11.2, 11.2 Hz, H-7ax); 2.31 (m,1H, H-5ax); 2.5-2.9 (m,4H, H-4ax, H-8ax, H-9e, H-10ax); 3.1-3.6 (m,4H, H-4e, H-7e, N-CH_2-CH=); 5.20 (m,2H, =CH_2); 5.94 (m, 1H, =CH-); 6.7-7.2 (m,4H, aromatic H's); 10.62 (bs,1H,NH-1); 12.10 (bs,1H, COOH).
MS (EI): m/z 296 (100, $M^{+\cdot}$); 255 (15); 209 (35); 194 (10); 183 (26); 167 (46); 154 (95); 144 (67); 127 (52); 115 (22).

N-(3-Dimethylaminopropyl)-N-(ethylaminocarbonyl)-6-(2-propenyl)ergoline-8β-[^{14}C]carboxamide ([^{14}C]cabergoline)(15').

The synthesis was carried out in two parts using one half of the radioactive cyanide for each preparation. The adopted standard procedure is described below. Potassium [^{14}C]cyanide (445 umoles ; 925 MBq) was transferred with aqueous ethanol (water 10%) from the original ampoule into a 25 ml Erlenmeyer flask. In a 50 ml flask, one half of the above solution (12.5 ml) and the ergoline compound 12 (66.0 mg; 223 µmoles) in 12 ml of aqueous ethanol was added. The mixture was heated at reflux for 3 hours and, at the end of reaction (checked by radio-TLC; system L), the solvent was evaporated in a rotary evaporator to give a pale brown residue (crude 13'). The solid was dissolved in ethanol (6.7 ml) and 10M NaOH (4.5 ml) was added. The solution was warmed at reflux overnight. The complete conversion of compound 12 into 14' (84% radiochemically pure) was checked by radio-TLC (system G; Rf of 14'= 14= 0.22). The solution was adjusted to pH 7 with 12M HCl and then evaporated to dryness in a "vacuum manifold". The residue was dried under vacuum over phosphoric anhydride for about 24 hours. The solid was suspended in DMF (11 ml) and N-ethyl-N'-(3-dimethylamino)propyl-carbodiimide (EDPC; 85 mg; 444 umoles) and TEA (42 ul; 444 umoles) were added. The mixture was kept under stirring at room temperature for 6 hours. In order to complete the reaction, EDPC (85 mg) and TEA (42 ul) were successively added and the mixture was stirred overnight. At the end of reaction (checked by radio-TLC; system N : for 15'= 15 and 16'= 16 Rf = 0.41 and 0.28 respectively), the solvent was evaporated to dryness under vacuum by the aid of a "vacuum manifold" to give a solid, which was partially dissolved in ethylacetate (3x15 ml). The combined organic extracts were transferred into a separating funnel. The residue remaining in the flask was dissolved in 5% $NaHCO_3$ (40 ml) and added to the above extract.
The organic layer was successively washed with 5% $NaHCO_3$ (3x20 ml), filtered trough a Na_2SO_4 panel and evaporated to dryness to give 328 MBq of crude compound 15', 60% radiochemically pure (by radio-TLC; system N). In this mixture the regioisomer 16' of [^{14}C]cabergoline accounted for 30%.

The second preparation from the remaining radioactive cyanide yielded 288 MBq of crude 15', 58% radiochemically pure (rp), which was added to that of the previous preparation (Total = 616 MBq, about 59% rp).

A portion of this crude [^{14}C]cabergoline (535 MBq) was charged on a column (50x7 I.D. cm) containing 50 g of silica

gel Merck 60 (230-400 mesh ASTM) in CH_2Cl_2. The column was eluted with acetone recovering 182 fractions (20 ml each). The isolation of pure [^{14}C]cabergoline from these fractions was carried out with different procedures as described below. The combined fractions from 78 to 105, containing 114.7 MBq of compound 15' (72.9% rp by radio-HPLC), were concentrated to a small volume and purified by preparative HPLC affording [^{14}C]cabergoline (60.3 MBq; 98% rp by radio-HPLC). The fractions from 106 to 165 (181.7 MBq; 15'= 79% rp by radio-HPLC) were purified by preparative HPLC, then by preparative TLC (silica gel plate Merck F254 20x20 cm; 0.5 mm thick; eluent system O).The chromatographic band corresponding to 15' was removed and the product extracted from silica gel with acetone. The combined extracts yielded [^{14}C]cabergoline (74.7 MBq; 97% rp by radio-HPLC). The fractions from 167 to 173 (68.1 MBq; 15'= 62.3% rp radio-HPLC) were chromatographed by TLC as above yielding 15' (15.5 MBq), 97% rp. All the pure samples were combined yielding [^{14}C]cabergoline (150.5 MBq) with a radiochemical purity >97% (by radio-HPLC: t_R=15 min; by radio-TLC, system N: Rf=0.34) and a specific radioactivity of 2.09 GBq/mmol. The UV spectrum (in methanol λ_{max} at 283 nm $E^{1\%}_{1cm}$=156) was concordant to that of the standard sample. The overall radiochemical yield from potassium [^{14}C]cyanide was 16%.

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