SYNTHESIS OF TRITIUM LABELLED METERGOLINE, NICERGOLINE, 1-DEMETHYLNICERGOLINE AND OF ^{3}H, ^{14}C DOUBLE LABELLED NICERGOLINE

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SUMMARY

[9,10-^{3}H]Metergoline (9) has been prepared starting from 1-methyllysergamide (6) which was reduced with tritium gas to 1-methyl-[9,10-^{3}H]dihydrolysergamide. The latter was converted to 9 in two steps with a 42% overall radiochemical yield. [G-^{3}H] and [17-^{3}H]nicergoline were prepared starting from generally labelled 11 and from 14 respectively. The latter was obtained by reducing the ester (13) with sodium boro[^{3}H]-hydride. Similarly, 1-demethyl-[17-^{3}H]nicergoline (15a) was also obtained. [Carboxyl-^{14}C] 5-bromonicotinic acid (18) was prepared from K^{14}CN via 3-[^{14}C]cyanopyridine (16) which was converted into 17 and finally brominated. Double labelled nicergoline (15b) was obtained by condensation of 14 and 18. Radiochemical yield of 15, 15a, and 15b from 14 and 14a was approximately 40% after the chromatographic purification step.

Key words: ergolines, [9,10-^{3}H]metergoline, [17-^{3}H]nicergoline, [^{3}H, ^{14}C]nicergoline, [carboxyl-^{14}C]-5-bromonicotinic acid.

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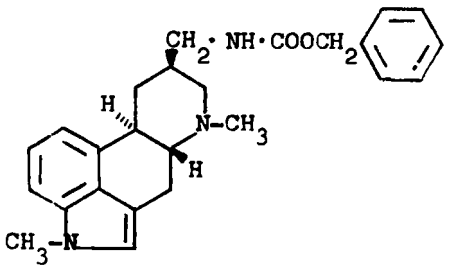
Metergoline, 8β-[(carboxyamino)methyl]-1,6-dimethyl-ergoline benzyl ester (1), and nicergoline, 10-methoxy-1,6-dimethyl-ergoline-8β-methanol 5-bromonicotinate (2), are valuable pharmacodynamic agents of clinical interest. Metergoline shows specific antiserotonine activity[1,2] and prolactin inhibition properties[3]. Nicergoline exhibits vasodilating and receptor blocking activity[4] and is currently used for the treatment of disorders due to inadequacy of cerebral blood flow in the aged[5-8]. The labelled drugs were required for metabolic studies in laboratory animals and in man, in which milligram doses of the compound are usually administered. In contrast with the importance of ergot alkaloids in pharmacology and in medicine, only a scarce number of reports concerning the radiolabelling of compounds of this class are found in literature[9-13]. The synthetic radiolabelling methods reported in this paper make use of direct labelling of lysergic acid derivatives and avoid previously used procedures involving biosynthesis of labelled lysergic acid starting from tritium or radiocarbon labelled tryptophane[10,13,14].



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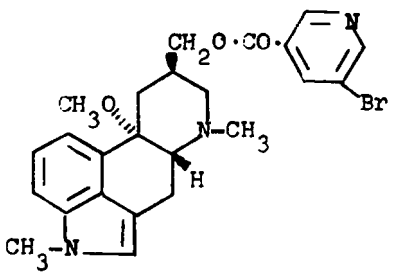
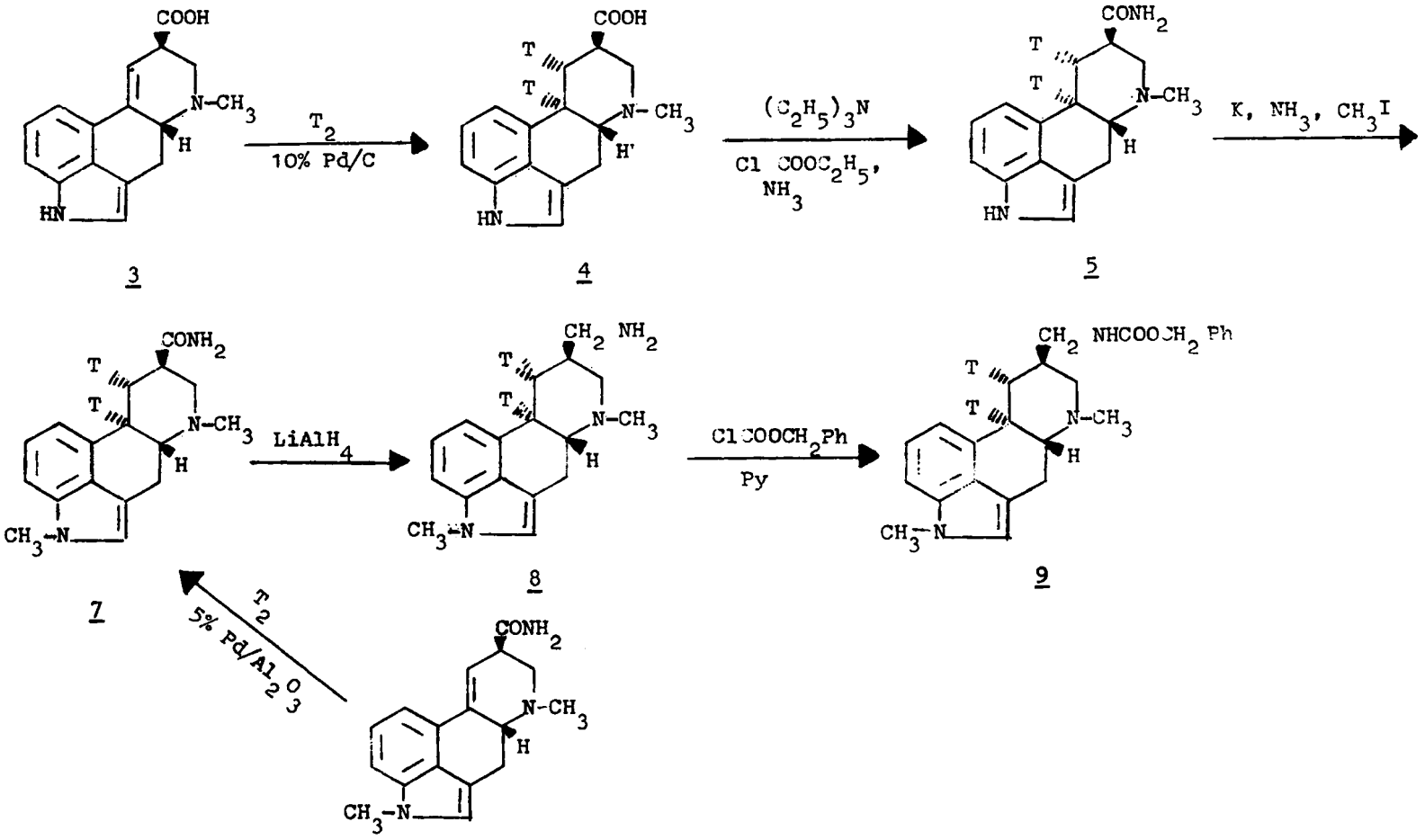


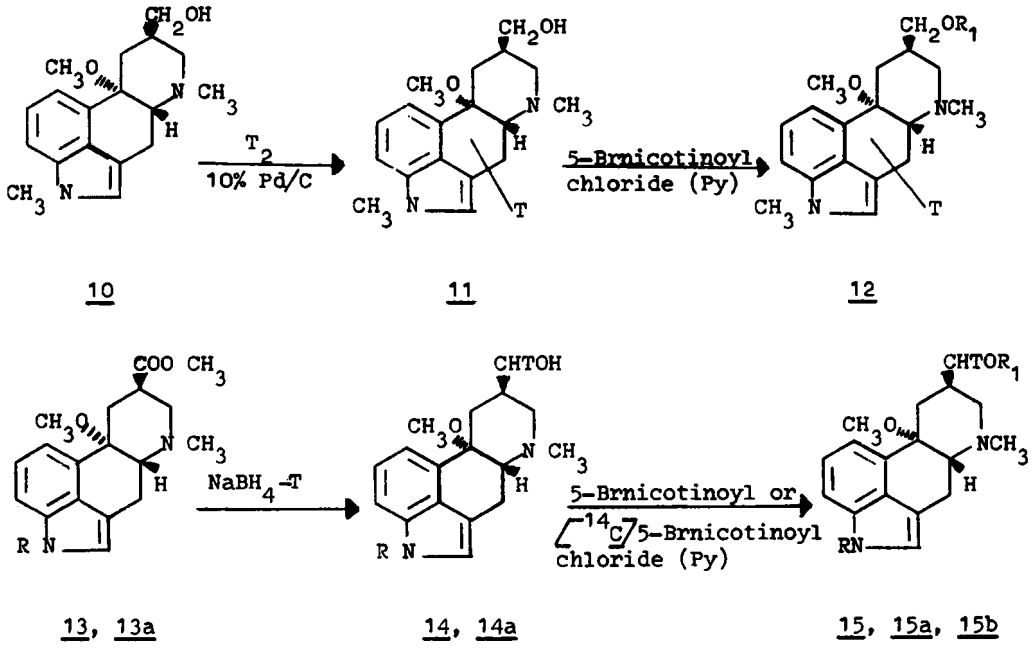
2

The succesfull synthesis of [9,10-^{3}H]metergoline (9) starting from lysergic acid (3, scheme 1)[12] prompted us to improve the radiochemical yield using 1-methyl-lysergamide (6)



3 4 5 7 8 9 6

Scheme 1. Synthesis of [9,10-^{3}H]metergoline.



10 11 12

13, 13a 14, 14a 15, 15a, 15b

	R	R_1
13	CH_3	-
13a	H	-
14	CH_3	-
14a	H	-
15	CH_3	CO-(3-Br-pyridyl)
15a	H	CO-(3-Br-pyridyl)
15b	H	*CO-(3-Br-pyridyl)

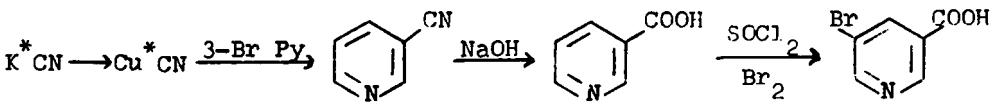
Scheme 2. Synthesis of single or double labelled nicergoline and of 1-demethylderivative.

as the substrate for the catalytic tritium reduction of the 9,10 double bond. This reaction, performed using 2.4 Ci of tritium, allowed the recovery of 17 with specific radioactivity of 276 mCi/mM, which was subsequently treated with $LiAlH_4$ to give 8 in 84% radiochemical yield. Reaction of 8 with carbobenzoxy chloride afforded the desired 9 in 50% radiochemical yield. Specific radioactivity of the final product was 150.8 mCi/mM due to the addition of unlabelled substance for optimal recovery during the working up of 8. It appears clearly that much higher values for the specific radioactivity of 9 can be obtained increasing the amount of tritium used in the catalytic reduction step.

Tritium labelling of nicergoline was first attempted by the catalytic exchange method exposing 10-methoxy-1,6-dimethyl-ergoline-8β-methanol (10) to 2 Ci of tritium gas in the presence of 10% Pd/C catalyst. Following this procedure and after esterification of the generally labelled alcohol 11 with 5-bromonicotinic acid [G-^{3}H]nicergoline (12), with specific radioactivity of 1.04 mCi/mM, was obtained (scheme 2). In order to make available a product endowed with higher specific radioactivity and specifically labelled at a stable C-H bond, the alternative procedure outlined in scheme 2 was investigated. The ester 13, obtained by N-methylation[15] of methyl 10-methoxy-6-methyl-ergoline-8β-carboxylate (13a)[16] was treated with 750 mCi of sodium boro [^{3}H] hydride to give 14 with specific radioactivity 87.9 mCi/mM in 62.5% chemical yield. Acylation of 14 with 5-bromonicotinic acid chloride afforded [17-^{3}H] nicergoline (15) with specific radioactivity 88 mCi/mM. Similarly, starting from 13a and using 300 mCi of sodium boro [^{3}H] hydride, 14a was obtained with specific radioactivity 37.2 mCi/mM. Subsequent acylation gave 1-demethyl-[17-^{3}H] nicergoline with specific radioactivity 36.7 mCi/mM in 41% radiochemical yield. Both 15 and 15a were recovered

after silica gel column chromatography and showed radiochemical purity higher than 98%.

Further investigations were performed on the synthesis of double labelled nicergoline possessing the different labels one in the alcohol moiety and the other in the acid residue. Synthesis of ^{14}C labelled 5-bromonicotinic acid was achieved as outlined in scheme 3[17-19]. This procedure allows the pre-



16 17 18

Scheme 3. Synthesis of [carboxyl-^{14}C]-5-bromonicotinic acid.

paration of [carboxyl-^{14}C]nicotinic acid (17) in acceptable overall radiochemical yield (40% from KCN), via the nitrile 16, using normal laboratory equipment. We consider this an advantage over the synthetic method of Murray et. al.[20] which, although being reported to give better yields in crude 17 from $Ba^{14}CO_3$, requires the rather complex apparatus for the preparation and carbonation of organolithium compounds. Conversion of 17 to 5-bromonicotinic acid (18) was achieved following an established procedure[19] in 63% yield. Overall radiochemical yield of 18 from K^*CN was therefore 25%. Compound 18 was used for the acylation of 14 to give double labelled nicergoline with specific radioactivity ^3H, 26.3 mCi/mM and ^{14}C, 23.9 mCi/mM.

EXPERIMENTAL

Melting points were taken on a Kofler microscopic hot stage and are uncorrected. Specific radioactivity was measured by liquid scintillation technique using a Packard Tricarb 3375 spectrometer. Chemical and radiochemical homogeneity

were tested by tlc on silica gel plates Merck 60 F 254 with the following solvent systems:

A. Methanol: chloroform (1:1 v/v)

B. Chloroform: methanol: acetic acid: water (80:20:14:6 v/v)

C. Methanol: chloroform (9:1 v/v)

D. Ethyl acetate: dimethylformamide: n-butanol: pyridine (4:3:3:1 v/v)

E. Cyclohexane: ethyl acetate: diethylamine (7:2:1 v/v)

F. Ethanol: chloroform: 25% ammonia: water (7:4:4:2 v/v)

The plates were scanned in a Packard chromatogram scanner model 7201. Chemical titre of products was determined by UV measurements in a PYE-Unicam spectrophotometer.

/9,10-^{3}H/1,6-Dimethyl-ergoline-8β-carboxamide (7)

The reduction was carried out in a small flask equipped with a magnetic stirring bar and connected with a reaction device made up of a gas measuring tube joined with a Toepler pump and/or a gas leveling bulb. Product (6) (155 mg 0.55mM), in an aqueous mixture of tritiated methyl and ethyl alcohol (3 ml, radioactivity not determined)* was mixed with 310 mg of 5% Pd/Al_2O_3 catalyst. Tritium gas (about 2.4 Ci), after dilution with hydrogen and transferred with the aid of the Toepler pump to the gas measuring tube, was introduced into the reaction flask and the system was vigorously stirred until 15 ml (at S.T.P.) of gas were absorbed (120 minutes). The residual hydrogen was removed and the solvents were evaporated at reduced pressure. The labile tritium was removed by treating twice the crude compound with 2 ml of ethanol and then evaporating "in vacuo". The remaining solid mixture was suspended in 5 ml of ethanol, filtered over a layer of carbon DARCO G 60 (50 mg) in a sintered glass funnel and the solids were washed with hot ethanol. The combined filtrate and washings (20 ml) were concentrated. Traces of insoluble solid

* Freshly distilled dioxane may also be used and compound (6) is completely hydrogenated in spite of its low solubility.

were removed by filtration and the filtrate was evaporated to dryness. The crude product was crystallized from a small volume of acetone yielding 105 mg (67%) of (7), spec. act. 975 μCi/mg or 276 mCi/mM (0.37 mM, 102 mCi, m.p. 246-248°). Tlc on silica gel with System A gave a single radioactive spot, in agreement with an authentic sample.

/9,10-^{3}H/-8β (Aminoethyl)-1,6-dimethyl-ergoline (8)

Lithium aluminum hydride (120 mg) was slowly added to a solution of 7 (105 mg 0.37 mM, 102 mCi) in 15 ml dry tetrahydrofuran. After stirring at room temperature for 30 minutes, the mixture was heated at reflux for two hours, then cooled to 0°C. The excess of reagent was decomposed by the slow addition of 5 ml of 10% water in THF (v/v) while stirring and cooling in ice. The mixture was stirred for one hour at room temperature then filtered and the solids washed with hot THF (15 ml) containing 52 mg of unlabelled 8. The combined mother liquors and washings were concentrated "in vacuo" to a small volume (3-5 ml), then diluted with 25 ml of $CHCl_3$. The yellow solution was washed twice with 5 ml of water. Dessication over sodium sulfate and solvent evaporation left 8 as a yellow oil which crystallized spontaneously (155mg, 85.8 mCi), radiochemical yield was 84%. Tlc on silica gel with System B showed a chemical and radiochemical purity of about 95%. The product was used in the following reaction without further purification.

/9,10-^{3}H/Metergoline (9)

Carbobenzoxychloride (0.15 ml), diluted with 0.5 ml of $CHCl_3$, was added to a cooled (-10°C) and stirred solution of 8 (153 mg 85.8 mCi) in dry pyridine. Stirring was maintained for 10 minutes at 0°C and then for 20 minutes at room temperature. The red solution was transferred in a separatory funnel with the aid of 25 ml of $CHCl_3$, and washed successively with NaOH 1N (5 ml), $NaHCO_3$ 5% (5 ml) and 2x5 ml of water. The

organic layer, after drying over sodium sulfate, was filtered and concentrated under reduced pressure. The traces of pyridine were removed by two evaporations under reduced pressure with 5 ml of toluene. The residual yellow brown oil, dissolved in benzene (5 ml), was then chromatographed on a 5x120 mm column of 1 g of Al_2O_3 Woelm N, Akt I, and eluted with benzene. The eluate was collected in 15 ml fractions for an overall volume of 300 ml. The pooled residues from fractions 3 through 25 were rechromatographed in the same manner described above. The combined fractions, concentrated and crystallized from a small volume of diethyl ether, gave 120 mg of 9 (42.7 mCi, m.p. 145°-147°C). The purity of the product, checked radiochemically scanning the thin-layer-chromatogram (System C) and spectrophotometrically (291 nm in ethanol), showed to be 97% and 95% respectively; spec. act. 374 μCi/mg or 150.8 mCi/mM (based on spectrophotometric titre). Radiochemical yield was 50%.

/G-^{3}H/-10-Methoxy-1,6-dimethyl-ergoline-8β-methanol (11)

A mixture of 10-methoxy-1,6-dimethyl-ergoline-8β-methanol (10) (624 mg) and 620 mg of 10% Pd/C catalyst was treated for two weeks at room temperature with 2 Ci of tritium gas in a "break-seal" ampoule. At the end of the exchange period, the excess of tritium gas was evacuated from the ampoule. The solid mixture was then dissolved in 100 ml of $CHCl_3$ and filtered. After removal of labile tritium by shaking vigorously with water (3 x 50 ml), the solution was evaporated to dryness. The crude product gave, after several crystallizations from aqueous acetone, 444 mg of 11 (71%), spec. activity 3.53 μCi/mg or 1.06 mCi/mM. The radiochemical purity, tested by tlc (Systems B and D) was sufficient for the next step.

/G-^{3}H/Nicergoline (12)

The solution of 11 (440 mg, 1.53 mCi) in dry pyridine (10 ml) was slowly added to a stirred and cooled (0°C) solu-

tion of 5-bromonicotinoyl chloride hydrochloride prepared in the usual manner[19-20] from 420 mg of 5-bromonicotinic acid with freshly distilled thionyl chloride in dry pyridine (10 ml). Stirring was maintained for 15 minutes at 0°C and then for 90 minutes at room temperature. At the end of the reaction 2 ml of water were added and the solution was kept at room temperature overnight. The mother liquor was evaporated "in vacuo" to dryness and the residue was dissolved in 8 ml of water. The aqueous solution was adjusted to pH 9 (NaOH 2N) and extracted with several portions of $CHCl_3$ (4 x30ml). The combined extracts were washed with 5% sodium bicarbonate and water saturated with NaCl until the aqueous phase remained neutral. The organic phase was dried (Na_2SO_4) and evaporated to dryness. The foamy crude product was crystallized from a small volume of dry diethyl ether (6 + 8 ml) yielding 490 mg (67%) of 12, spec. act. 2.15 μCi/mg or 1.04 mCi/mM (1.05 mCi, m.p. 137-138°C). The radiochemical purity checked by tlc (Systems C, D and E) was higher than 96%.

[17-^{3}H]-10-Methoxy-1,6-dimethyl-ergoline-8β-methanol (14)

Sodium (9 mg) in dry methanol (6.5 ml) was added, under nitrogen, in a 25 ml flask equipped with magnetic stirrer and reflux condenser to 498 mg of 13 (1.52 mM). The sample was stirred and, after complete solution, sodium boro[^{3}H]hydride (750 mCi, 12.5 Ci/mM, the Radiochemical Centre-Amersham) plus 62 mg of "cold" $NaBH_4$ were added. The mixture was stirred for 30 minutes at room temperature and then heated at reflux for 90 minutes. After cooling at room temperature an additional amount of $NaBH_4$ (127 mg) was added and the reaction mixture was again heated at reflux temperature until reduction was complete (90 minutes, checked by tlc, System B). Unreacted $NaBH_4$ was decomposed by addition of 8 ml of water and stirring was continued with gentle reflux for an hour. The crude

tritiated product was dissolved in chloroform by extraction of the aqueous mother liquors in a separatory funnel (5 x10 ml). The combined extracts, washed with water and dried over sodium sulfate were concentrated under vacuum to a small volume. By the addition of acetone a precipitate was obtained. Crystallization from the same solvent gave 285 mg (0.95 mM; 83.5 mCi) of 14 (62.5%), spec. act. 293 μCi/mg or 87.9 mCi/mM. Its purity was shown to be 98% by tlc,(single spot Rf=0.36 in System B).

[17-^{3}H]Nicergoline (15)

[17-^{3}H]Nicergoline was obtained in the same manner as described above [see: [G-^{3}H]nicergoline (12)] starting from 14 (285mg; 0.95 mM, 83.5 mCi) and 270 mg (1.33 mM) of 5-bromo-nicotinic acid. The crude product (520 mg), whose radiochemical purity was unsatifactory (88-90%), was purified as follows: the brown oil was placed on a column of silica gel (5 g) Merck 60 (70-230mesh ASTM). The column was eluted with benzene containing increasing amounts of ethyl acetate, up to 50% (v/v). The combined fractions containing the pure product were evaporated to dryness. The residue was crystallized from benzene and diethyl ether to give 111mg of 15, spec. act. 181.8 μCi/mg or 88 mCi/mM, radiochemically pure (over 99% by tlc, Systems C, D and E). The mother liquors, by addition of 100 mg of "cold" nicergoline, afforded a second crop of 15 (154 mg) with spec. act. 82.9 μCi/mg or 40.1 mCi/mM and 99% radiochemical purity. Overall radiochemical yield 39.5%. Chemical purity, determined spectrophotometrically at 288 nm in EtOH, showed to be higher than 98% for both lots.

[17-^{3}H]-10-Methoxy-6-methyl-ergoline-8β-methanol (14a)

In the same manner, 135 mg of crude 14a (90%), spec. act. 130 μCi/mg or 37.2 mCi/mM were obtained from 164 mg (0.52 mM) of 13a and 300mCi of sodium[^{3}H]hydride plus 100 mg of unlabel-

led 13a. The product was directly used in the following reaction without further purification.

[17-^{3}H]-1-Demethyl-nicergoline (15a)

Crude 14a (135 mg, 0.47 mM, 130 μCi/mg or 37.2 mCi/mM) and 5-bromonicotinic acid (124 mg, 0.61 mM) were condensed as described above for 15 giving an oily residue (137 mg). This residue was chromatographed on a column (1x10 cm) of 5 g of silica gel and eluted using a gradient elution from benzene-ethyl acetate (3:2) to ethyl acetate. The collected and combined fractions containing the pure product yielded 92 mg of 15b, spec. act. 78 μCi/mg or 36.7 mCi/mM, 41% radiochemical yield. The product was 98% radiochemically pure by tlc (Systems C, D and E) and identical in every respect to an authentic unlabelled sample.

[Carboxyl-^{14}C]-5-bromonicotinic acid (18)

To 130 mg of [^{14}C]copper(I)cyanide (1.45 mM, about 43mCi), prepared from potassium[^{14}C]cyanide (50 mCi, Radiochemical Centre, Amersham, England) according to the procedure of Reid and Weaver[17], 3-bromopyridine (3.3 g) was added. The mixture was stirred at 160°-170° for 30 minutes and then distilled under vacuum (16 mm). The distillate was dissolved in ethanol (10 ml) and sodium hydroxyde (0.5 g) was added. The mixture was refluxed with stirring for three hours, then the solvent was evaporated. The remaining oil was poured into water (5 ml) and continuously extracted with ether for 12-14 hours. The aqueous solution was adjusted to pH 3 and the product was recovered by continuous ether extraction for two days. Dessication over sodium sulfate of the extract and solvent evaporation gave [carboxyl-^{14}C]nicotinic acid (83 mg, 0.67 mM; 20 mCi, m.p. 234°-235°) radiochemically pure by tlc (silica gel and solvent System F). The product was dissolved in ether, tranferred in a glass ampoule and after addition of 20 mg (0.16 mM) of unlabelled nicotinic acid, the solution was evaporated to dryness.

After addition of 4 ml of freshly distilled thionyl chloride, the solution was refluxed for two hours. The excess of thionyl chloride was removed, first by evaporation at reduced pressure, and then by twofold evaporation with 3 ml of anhydrous benzene.

Bromine, previously distilled over phosphorus pentoxide (about 150 mg) was added to the resulting acid chloride hydrochloride in an ampoule which was promptly sealed. The mixture was heated in an oil bath for 14 hours at 160°C. The ampoule was opened, the solid was dissolved in 3 N NaOH (10 ml) and the solution was continuously extracted with ether for six hours. The aqueous solution was then adjusted to pH 3 with diluted sulphuric acid and extracted with ether for 14 hours. The extracts were dried (Na_2SO_4) and the solvent evaporated to give a residue which was crystallized from a small volume of ethyl alcohol yielding 107 mg (36.5% based on Cu(I)CN) of [carboxyl-^{14}C]-5-bromonicotinic acid (18), spec. act. 118.9 μCi/mg or 24 mCi/mM (0.53 mM, 12.72 mCi, m.p. 182°-184°), with radiochemical purity 98% by tlc (System F).

[17-^{3}H]-10-Methoxy-1,6-dimethyl-ergoline-8β-methanol 5-bromo-carboxyl-[^{14}C]nicotinate ([^{3}H, ^{14}C]nicergoline, 15b)

The synthesis of the double labelled nicergoline was carried out in the same manner as described above for 12 and 15 by acylating 72.5 mg of 14 (0.24 mM, 91.5 μCi/mg or 27.5 mCi/mM, 6.63 mCi) with 51 mg of 18 (0.25 mM, 118 μCi/mg or 24.1 mCi/mM, 6.06 mCi) as the chloride hydrochloride. The dry crude product (72 mg) was purified by preparative tlc (silica gel plate 20x20 cm Merck 60 F 254, 2mm thickness) and developing with System E. The double labelled nicergoline was eluted with ethyl acetate. The ethyl acetate solution was evaporated to dryness and the residue dissolved in 50 ml of 95% ethanol. The title by UV (288 nm, $E^{1\%}_{1cm}$=1.87) and the radioactivity for ^{3}H and ^{14}C, determined on this solution, indicated the presence of 48 mg of 15b spec. act. ^{3}H 54.4 μCi/mg or 26.3 mCi/mM (yield 39.4%),

spec. act. ^{14}C 49.4 μCi/mg or 23.9 mCi/mM (yield 39.1%). The radiochemical purity tested by tlc (Systems C,D and E) was higher than 97%. This product, after evaporation of ethanol, was dissolved in dry benzene (50 ml) and stored in the dark at 10° to avoid decomposition.

ACKNOWLEDGMENT

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