The Aberrant Biosynthesis of 2′-Nitro- and 2′-Bromo-papaverines†

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Incorporation of labelled racemic 2′-bromonor-reticuline (**5**) and 2′-nitronor-reticuline (**6**) into 2′-bromopapaverine (**2**) and 2′-nitropapaverine (**3**), respectively, has been studied in *Papaver somniferum* (Papaveraceae) plants, and specific incorporation of (**5**) and (**6**) into (**2**) and (**3**), respectively, has been demonstrated. Further, it has been shown that (**5**) and (**6**) are not metabolized by the plants to form papaverine (**1**).

Several reports are available on the potential of higher plants for carrying out transformations of organic molecules that they normally do not produce.[1–5] Further incorporation of an unnatural substrate into a normal product is also well documented.[6,7] We have studied the conversion of racemic 2′-bromonor-reticuline (**5**) and 2′-nitronor-reticuline (**6**) into 2′-bromopapaverine (**2**) and 2′-nitropapaverine (**3**), respectively, in *Papaver somniferum* (Papaveraceae). The normal biosynthesis of papaverine (**1**) from 1-benzyltetrahydroisoquinoline precursors in *P. somniferum* is well established.[8–12]

Initially L-[U-^{14}C]tyrosine (experiment 1) was injected into mature capsules of *P. somniferum* and papaverine (**1**) was isolated and found radioactive. The result indicated that the plants were actively biosynthesizing papaverine (**1**). In subsequent experiments, several labelled precursors were fed to the plants. The results of these feedings are recorded in the Table.

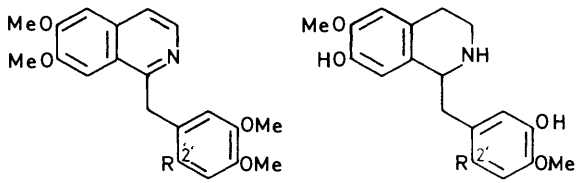
Feeding of (±)-2′-bromo[*aryl*-^{3}H]nor-reticuline (**5**) (experiment 2) to *P. somniferum* demonstrated that (**5**) was utilized by the plants to form 2′-bromopapaverine (**2**) but was poorly metabolized to papaverine (**1**). (±)-2′-Nitro[*aryl*-^{3}H]nor-reticuline (**6**) (experiment 3) was then fed and biosynthetic 2′-nitropapaverine (**3**) isolated. Thus the precursor (**6**) was again incorporated into (**3**), but its incorporation into papaverine (**1**) was negligible.

The regiospecificity of label in the biosynthetic 2′-bromopapaverine (**2**) derived from (±)-2′-bromo[3-^{14}C]nor-reticuline (**5**) was established as follows. Labelled (**2**) was treated with methyl iodide to give labelled 2′-bromopapaverine methiodide (**7**) having essentially the same molar radioactivity as the parent base. Reduction of (**7**) with sodium borohydride afforded labelled 2′-bromolaudanosine (**9**) with essentially no loss of radioactivity. Debromination of (**9**) with lithium aluminium hydride gave laudanosine (**10**) with essentially the same molar activity as the parent base. Labelled (**10**) was treated with methyl iodide to give the methiodide (**12**), which was converted into the corresponding methohydroxide (**13**). Hofmann degradation of (**13**) yielded a mixture of methine bases (**16**) and (**18**), which was hydrogenated to (**20**). Labelled (**20**) was converted into its methiodide (**21**) and then into the methohydroxide (**22**). A second Hofmann degradation of (**22**) afforded the methine (**23**) with practically no loss of radioactivity. Ozonolysis of (**23**) gave formaldehyde (91% of original activity).

The regiospecificity of label in the biosynthetic 2′-nitropapaverine (**3**) derived from the feeding of (±)-2′-nitro[3-^{14}C]nor-reticuline (**6**) (experiment 5) was established as follows. Labelled (**3**) was treated with methyl iodide to furnish 2′-nitropapaverine methiodide (**8**) with essentially the same molar radioactivity as the parent base. Reduction of (**8**) with sodium borohydride afforded 2′-nitrolaudanosine (**11**), which on treatment with methyl iodide gave the methiodide (**14**) with essentially no loss of radioactivity. Labelled (**14**) was passed through Amberlite IR-400 anion-exchange resin (OH^- form) to furnish the methohydroxide (**15**). Hofmann degradation of labelled (**15**) gave the methine base (**17**). Radioactive (**17**) was treated with methyl iodide to afford the methiodide (**24**), which was converted into the methohydroxide (**25**). A second Hofmann degradation of (**25**) furnished the methine (**26**) with essentially no loss of radioactivity. Ozonolysis of (**26**) gave formaldehyde (86% of original activity).

Table. Tracer experiments on *P. somniferum*

Expt.	Precursor fed	% Incorporation into (1)	(2)	(3)
1	L-[U-^{14}C]Tyrosine	0.03		
2	(±)-2′-Bromo[*aryl*-^{3}H]nor-reticuline (**5**)	0.0006	0.44	
3	(±)-2′-Nitro[*aryl*-^{3}H]nor-reticuline (**6**)	0.002		0.29
4	(±)-2′-Bromo[3-^{14}C]nor-reticuline (**5**)		0.61	
5	(±)-2′-Nitro[3-^{14}C]nor-reticuline (**6**)			0.41



(**1**) R = H
(**2**) R = Br
(**3**) R = NO_2

(**4**) R = H
(**5**) R = Br
(**6**) R = NO_2

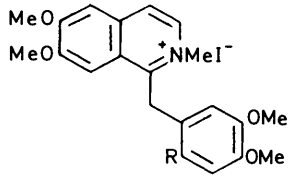
The preceding experiments thus demonstrated that the enzyme system in *P. somniferum* plant can metabolize 2′-bromonor-reticuline (**5**) and 2′-nitronor-reticuline (**6**) to 2′-bromopapaverine (**2**) and 2′-nitropapaverine (**3**), respectively. However, the abnormal precursors (**5**) and (**6**) are not metabolized by the plants to form papaverine (**1**).

Experimental

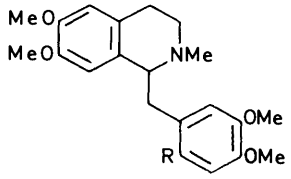
For general directions (spectroscopy details and counting method) see ref. 13.

Synthesis of Precursors.—The racemates of 2′-bromonor-reticuline[14] (**5**) and 2′-nitronor-reticuline[15,16] (**6**) were prepared by standard procedures.

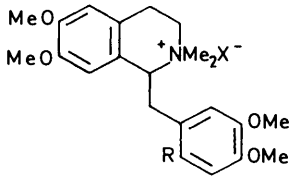
† Papaverine is 6,7-dimethoxy-1-veratrylisoquinoline; nor-reticuline is 1,2,3,4-tetrahydro-1-(3-hydroxy-4-methoxybenzyl)-6-methoxyisoquinolin-7-ol.



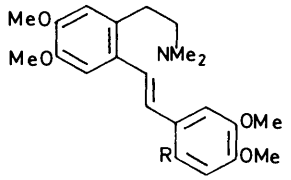
(7) R = Br
(8) R = NO_2



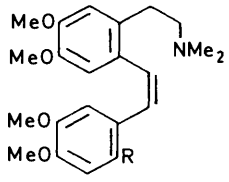
(9) R = Br
(10) R = H
(11) R = NO_2



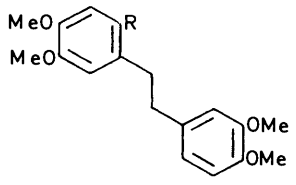
(12) R = H, X = I
(13) R = H, X = OH
(14) R = NO_2, X = I
(15) R = NO_2, X = OH



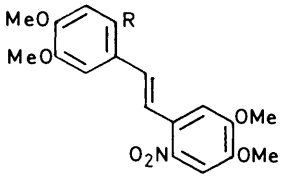
(16) R = H
(17) R = NO_2



(18) R = H
(19) R = NO_2



(20) R = $[CH_2]_2NMe_2$
(21) R = $[CH_2]_2\overset{+}{N}Me_3\ I^-$
(22) R = $[CH_2]_2\overset{+}{N}Me_3\ OH^-$
(23) R = $CH{=}CH_2$



(24) R = $[CH_2]_2\overset{+}{N}Me_3I^-$
(25) R = $[CH_2]_2\overset{+}{N}Me_3\ OH^-$
(26) R = $CH{=}CH_2$

Labelling of Precursors.[17]—To a mixture of $SOCl_2$ (0.02 ml) and tritiated water (0.2 ml; 150 mCi) was added (±)-2′-bromonor-reticuline (**5**) (100 mg). The mixture was heated under N_2 (sealed tube) for 110 h at 100 °C. Work-up in the usual way afforded (±)-2′-bromo[*aryl*-^{3}H]nor-reticuline (**5**). (±)-2′-Nitro[*aryl*-^{3}H]nor-reticuline (**6**) was similarly prepared.

(±)-2′-Bromo[3-^{14}C]nor-reticuline (**5**) and (±)-2′-nitro[3-^{14}C]nor-reticuline (**6**) were prepared by total synthesis by standard procedures for introduction of ^{14}C label.[18]

Synthesis of Inactive 2′-Bromopapaverine (**2**) *and 2′-Nitropapaverine* (**3**).—Both (**2**) and (**3**) were prepared according to reported procedures from papaverine (**1**).[19,20]

Feeding Experiment.—The hydrochlorides of 2′-bromonor-reticuline (**5**) and 2′-nitronor-reticuline (**6**) were dissolved in water (1 ml) containing dimethyl sulphoxide (0.2 ml). The solution of the precursors was injected into the mature capsules of *Papaver somniferum* (Papaveraceae) plants. The plants were kept alive for 6—8 days to metabolize the precursor and then worked up for the bases of interest.

Isolation of 2′-Bromopapaverine (**2**).—The young plants (typically 400 g, wet wt.) of *P. somniferum* were macerated in EtOH (500 ml) with inactive (**2**) hydrochloride (150 mg) and NH_4OH (0.2 ml) and left for 12 h. The EtOH was decanted and the plant material was percolated with fresh EtOH (5 × 500 ml). The combined ethanolic percolate was concentrated under reduced pressure to furnish a green viscous mass, which was extracted with 10% hydrochloric acid (4 × 30 ml). The acidic extract was basified with ammonia (pH 8) and liberated bases were extracted with $CHCl_3$ (5 × 25 ml). The extract was washed with water, dried (Na_2SO_4), and evaporated under reduced pressure. The crude base so obtained was subjected to preparative t.l.c. on silica gel GF_{254} (solvent $CHCl_3$–MeOH, 98:2) to furnish radioactive 2′-bromopapaverine (**2**) (110 mg).

Isolation of 2′-Nitropapaverine (**3**).—The precursor-fed plants of *P. somniferum* (450 g, wet wt.) were macerated in EtOH (500 ml) with radioinactive 2′-nitropapaverine (**3**) (150 mg) and NH_4OH (0.2 ml). The plant material was extracted with EtOH and the extract worked up as earlier to give radioactive (**3**) (90 mg).

Degradation of the Biosynthetic 2′-Bromopapaverine (**2**) *Derived from* (±)-2′-*Bromo*[3-^{14}Cl]*nor-reticuline* (**5**).—A solution of labelled 2′-bromopapaverine (**2**) (450 mg) (molar activity 2.98 × 10^4 disint. min^{-1} $mmol^{-1}$) in MeOH (30 ml) was refluxed with MeI (7 ml) for 4 h to yield *2′-bromopapaverine methiodide* (**7**) (395 mg) (molar activity 2.90 × 10^4 disint. min^{-1} $mmol^{-1}$), m.p. 176—178 °C (from MeOH–Et_2O) (Found: C, 44.9; H, 4.1; N, 2.3. $C_{21}H_{23}BrINO_4$ requires C, 45.0; H, 4.1; N, 2.5%). Labelled (**7**) (380 mg) in MeOH (25 ml) was treated with $NaBH_4$ (400 mg) at *ca.* 5—10 °C with stirring for 1 h. Work-up in the usual way afforded 2′-bromolaudanosine (**9**) (355 mg) (molar activity 2.86 × 10^4 disint. min^{-1} $mmol^{-1}$), m.p. 179—180 °C (from MeOH), m.p. 69—70 °C (from aq. EtOH) (lit.,[19] 71—73 °C from aq. EtOH) (Found: C, 57.8; H, 5.83; N, 3.3. Calc. for $C_{21}H_{26}BrNO_4$: C, 57.8; H, 6.0; N, 3.2%), δ_H (90 MHz; $CDCl_3$) 2.43 (3 H, s, NMe), 3.59 and 3.48 (3 H each, each s, 2 × OMe), 3.71 (6 H, s, 2 × OMe), and 5.98, 6.43, 6.46, and 6.9 (1 H each, each s, 4 × ArH).

Labelled (**9**) (350 mg) in dry tetrahydrofuran (THF) (15 ml) was added dropwise to a stirred suspension of lithium aluminium hydride (250 mg) in dry THF (35 ml). The mixture was stirred at 55 °C for 3 h and then worked up to give labelled laudanosine (**10**) (290 mg) (molar activity 2.85 × 10^4 disint. min^{-1} $mmol^{-1}$), m.p. 113—114 °C (from MeOH–Et_2O) (lit.,[10] 114—115 °C). Laudanosine (**10**) (288 mg) in MeOH (8 ml) was refluxed with MeI (4 ml) for 2 h to give radioactive laudanosine methiodide (**12**) (300 mg) (molar activity 2.81 × 10^4 disint. min^{-1} $mmol^{-1}$), m.p. 210—212 °C (lit.,[21] 212—213 °C) having essentially the same molar radioactivity as the parent base. A solution of the radioactive (**12**) (298 mg) in MeOH (100 ml) was passed through a column of freshly generated Amberlite IR-400 anion-exchange resin (OH^- form) to furnish the corresponding methohydroxide (**13**). The methohydroxide (**13**) in MeOH (10 ml) and KOH [3.5 g in water (3 ml)] was refluxed for 2 h. The resulting mixture was worked up to give a mixture of methines (**16**) and (**18**) (200 mg) (molar activity 2.77 × 10^4 disint. min^{-1} $mmol^{-1}$). The mixture of (**16**) and (**18**) (195 mg) in EtOH (20 ml) was hydrogenated in the presence of PtO_2 (60 mg) to give the

radioactive methine (**20**) (190 mg) (molar activity 2.75×10^4 disint. min^{-1} $mmol^{-1}$); picrate, m.p. 152—154 °C (from EtOH) (lit.,[10] 153—156 °C).

The radioactive (**20**) (180 mg) in MeOH (5 ml) was refluxed with MeI (3 ml) for 3 h to give the radioactive methine methiodide (**21**) (200 mg) (molar activity 2.75×10^4 disint. min^{-1} $mmol^{-1}$), m.p. 196—197 °C (lit.,[10] 197 °C) with practically no loss of radioactivity. The methohydroxide (**22**) was prepared from (**21**) as already described. Compound (**22**) was then refluxed with KOH [3 g in H_2O (2 mol)] for 2 h and the resulting mixture was worked up in the usual manner to afford 1-(4,5-dimethoxy-2-vinylphenyl)-2-(3,4-dimethoxyphenyl)-ethane (**23**) (140 mg) (molar activity 2.71×10^4 disint. min^{-1} $mmol^{-1}$), m.p. 73—74 °C (lit.,[10] 73—74 °C) with no loss of radioactivity.

Ozonised oxygen was passed through a solution of radioactive (**23**) (130 mg) in EtOAc (200 ml) at −78 °C for 30 min. The solvent was removed. To the residue, water (35 ml), Zn dust (440 mg), and $AgNO_3$ (24 mg) were added and the mixture was refluxed for 20 min, then distilled. The distillate was collected in a solution of dimedone (420 mg) in aqueous EtOH (3:1; 150 ml); the solution was left for 1 h at ambient temperature, concentrated to 10 ml, and left overnight. The product was filtered off, washed with water, dried, and chromatographed on a column of silica gel. Elution with C_6H_6 and C_6H_6–$CHCl_3$ (t.l.c. control) gave formaldehyde dimedone adduct (20 mg), m.p. 188—189 °C (from MeOH–Et_2O) (lit.,[10] 188 °C) (molar activity 2.70×10^4 disint. min^{-1} $mmol^{-1}$; 91% of original activity).

Degradation of the Biosynthetic 2′-Nitropapaverine (**3**) *Derived from* (±)-*2′-Nitro*[3-^{14}C]*nor-reticuline* (**6**).—A solution of labelled (**3**) (250 mg) (molar activity 4.12×10^3 disint. min^{-1} $mmol^{-1}$) in MeOH (50 ml) was refluxed for 4 h with MeI (10 ml) to afford the 2′-nitropapaverine methiodide (**8**) (205 mg), m.p. 232 °C (decomp.), [lit.,[20] 230 °C (decomp.)] (molar activity 4.08×10^3 disint. min^{-1} $mmol^{-1}$). A solution of this labelled (**8**) (200 mg) in MeOH (60 ml) was treated with $NaBH_4$ (800 mg) at 5—10 °C. The mixture was stirred for 1.5 h and then worked up as usual to afford 2′-nitrolaudanosine (**11**) (160 mg) (molar activity 4.07×10^3 disint. min^{-1} $mmol^{-1}$); *hydrochloride*, m.p. 120—121 °C (Found: C, 62.7; H, 6.5; N, 6.9. $C_{21}H_{26}N_2O_6$ requires C, 62.7; H, 6.5; N, 7.0%); δ_H (90 MHz; $CDCl_3$) 2.32 (3 H, s, NCH_3), 3.59, 3.63, 3.7 and 3.8 (3 H each, each s, 4 × OCH_3), and 6.28, 6.3, 6.42, and 7.41 (1 H each, each s, 4 × ArH); *m/z* 206 (base peak). Radioactive (**11**) (150 mg) was diluted with inactive (**11**) (150 mg) and crystallized; hydrochloride, m.p. 120—121 °C (molar activity 2.02×10^3 disint. min^{-1} $mmol^{-1}$). This sample of (**11**) (300 mg) in MeOH (10 ml) was refluxed with MeI (5 ml) to furnish *2′-nitrolaudanosine methiodide* (**14**) (285 mg), m.p. 222—223 °C (from MeOH) with essentially no loss of radioactivity (molar activity 1.97×10^3 disint. min^{-1} $mmol^{-1}$) (Found: C, 48.0; H, 5.3; N, 5.2. $C_{22}H_{29}IN_2O_6$ requires C, 48.5; H, 5.3; N, 5.15%). The labelled (**14**) (275 mg) in MeOH (100 ml) was passed through a column of freshly generated Amberlite IR-400 anion-exchange resin (OH^- form) to afford the methohydroxide (**15**). The radioactive (**15**) (240 mg) in MeOH (5 ml) was heated with KOH [4 g in H_2O (4 ml)] for 4 h; the product was worked up as usual to afford the *methine base* (**19**) (205 mg), m.p. 145—146 °C (from Et_2O–hexane) with essentially same molar radioactivity as the parent base (molar activity 1.92×10^3 disint. min^{-1} $mmol^{-1}$) (Found: C, 63.4; H, 6.8; N, 6.7. $C_{22}H_{28}N_2O_6$ requires C, 63.5; H, 6.7; N, 6.7%); $\lambda_{max.}$(MeOH) 215, 290, 325, and 380 nm; δ_H (90 MHz; $CDCl_3$) 2.2 (6 H, s, 2 × NCH_3), 3.78, 3.8, 3.82, and 3.9 (3 H each, each s, 4 × OCH_3), 7.03 and 7.48 (1 H each, each d, *J* 15 Hz, 2 × olefinic H), and 6.6, 6.98, 7.02 and 7.5 (1 H each, each s, 4 × ArH); *m/z* 416 (M^+).

The labelled (**19**) (200 mg) in MeOH (10 ml) was refluxed with MeI (5 ml) for 4 h to afford the radioactive *methiodide* (**24**) (190 mg), m.p. 199—200 °C (from MeOH) with no loss of radioactivity (molar activity 1.91×10^3 disint. min^{-1} $mmol^{-1}$) (Found: C, 49.5; H, 5.5; N, 5.2. $C_{23}H_{31}IN_2O_6$ requires C, 49.5; H, 5.55; N, 5.0%). This product (**24**) was converted into the corresponding methohydroxide (**25**) (150 mg) as described earlier. Hofmann degradation of (**25**) afforded the methine (**26**) (85 mg), m.p. 168—169 °C (from MeOH–Et_2O) with essentially same molar radioactivity as the parent base (molar activity 1.85×10^3 disint. min^{-1} $mmol^{-1}$) (Found: C, 64.5; H, 5.6; N, 3.8. $C_{20}H_{21}NO_6$ requires C, 64.7; H, 5.7; N, 3.8%); $\lambda_{max.}$(MeOH) 224, 260, 300, and 390 nm; δ_H (90 MHz; $CDCl_3$), 3.79 (9 H, s, 3 × OCH_3), 3.86 (3 H, s, OCH_3), 5.0 to 5.7 (3 H, m, $ArCH{=}CH_2$), 6.8, 6.89, 6.9 and 7.41 (1 H each, each s, 4 × ArH), and 7.02 and 7.39 (1 H each, each d, *J* 15 Hz, ArCH=CHAr); *m/z* 371 (M^+). Ozonolysis of (**26**) was carried out as described earlier to give radioactive formaldehyde dimedone adduct (10 mg), m.p. 187 °C (from MeOH–Et_2O) (lit.,[10] 188 °C) (molar activity 1.77×10^3 disint. min^{-1} $mmol^{-1}$; 86% of original activity).

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