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Biotransformation of Reticuline into Coreximine, Scoulerine, Pallidine, and Isoboldine with Rat Liver Enzyme[1]

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(±)-Reticuline (**1**) was biotransformed into the protoberberine alkaloids, coreximine (**12**) and scoulerine (**10**), the morphinandienone alkaloid, pallidine (**14**), and the aporphine alkaloid, isoboldine (**16**). The transformation was stimulated by O_2 and the cofactor NAD, NADP, or NADPH, NADPH being more effective than the other cofactors. The *N*-methyl group of (±)-reticuline was not incorporated intact into protoberberines.

INTRODUCTION

In previous papers, we reported the biotransformation of 1-benzyl-1,2,3,4-tetrahydro-2-methylisoquinolines into protoberberines with rat liver enzyme, which required NADPH (*1*, *2*). However, the formation of aporphine- and morphinandienone-type alkaloids with the mammalian system was not fully tested, because suitable carriers for the tracer experiments were not available. The current interest in the drug-evoked aberrations of neuroamine metabolism, particularly as regards a possible biochemical basis for alcoholism (*3–10*), has prompted further investigation with the above enzymic system. In this paper we report the formation of morphinandienone- and aporphine-type alkaloids as well as protoberberines from (±)-reticuline (**1**) with rat liver enzyme and the fate of *N*-methyl group of reticuline in the above conversion.

PREPARATION OF SUBSTRATES

(±)-Reticuline (**1**) was prepared according to the known methods and characterized as the perchlorate (*1*, *2*, *11*, *12*). Furthermore, the synthesis of reticuline labeled with deuterium at multiple positions, including the *N*-methyl group, was required for studying the fate of the *N*-methyl group of reticuline during the biotransformation. We had already found that reduction of the 3,4-dihydroisoquinolines (**2**) with deuterioacetic acid and zinc powder readily gave the triduterio compounds (**3**) (*9*). This method had several merits, namely the yield was high and the reagents were inexpensive and readily available. Therefore, 7-benzyloxy-1-(3-benzyloxy-4-methoxybenzyl)-3,4-dihydro-6-methoxy-2-methylisoquinolinium iodide (**5**) was reduced with zinc powder in deuterioacetic acid, prepared from acetic anhydride and deuterium oxide. If zinc

[1] Dedicated to the memory of Professor S. Morris Kupchan.

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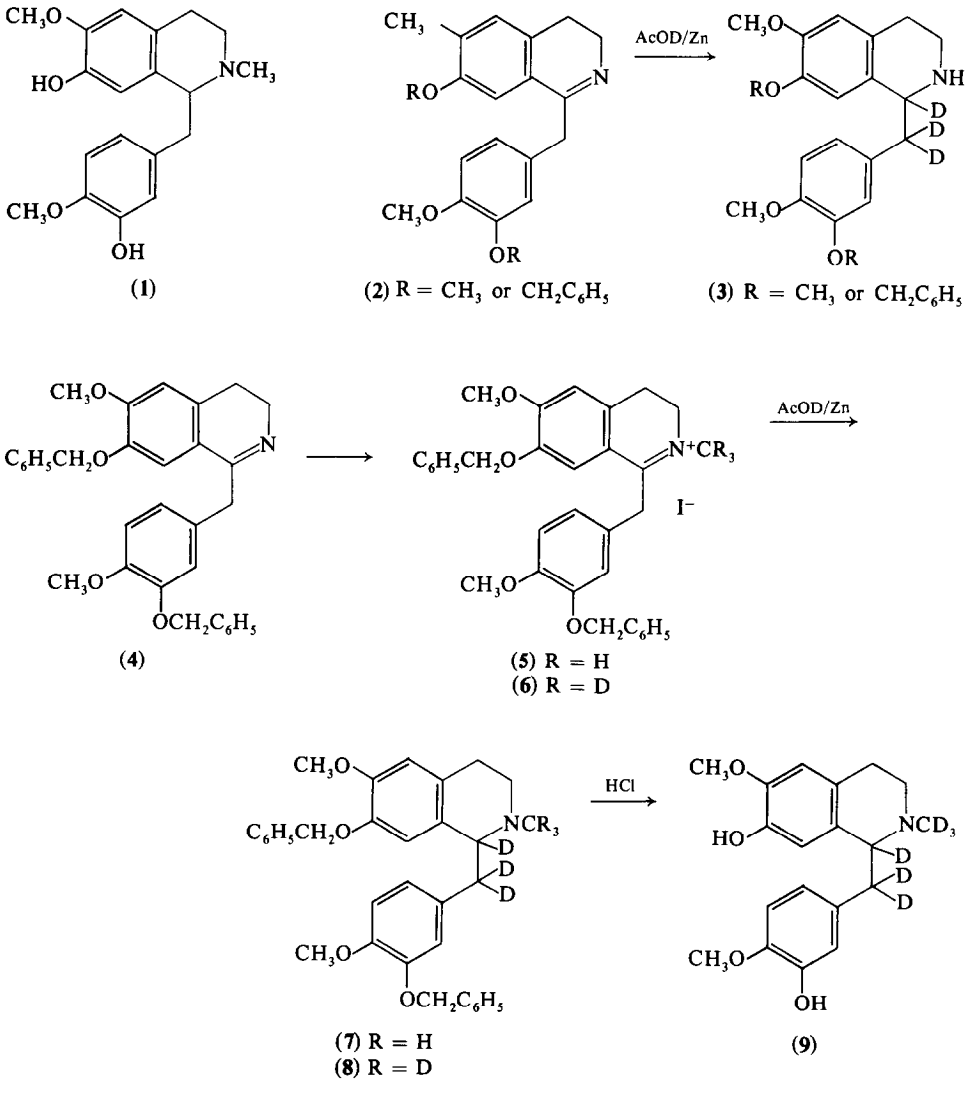
powder was immediately added to the hot solution of the methiodide (**5**) in deuterioacetic acid, the incorporation of deuterium at the benzylic α-position was incomplete. Addition of zinc powder to the hot solution of the methiodide (**5**) in deuterioacetic acid after heating for several hours under a nitrogen atmosphere gave the product (**7**), which



SCHEME 1

was fully labeled with three deuteriums, as indicated by mass spectroscopy of the product (**7**). It was thus considered that deuteriation at the benzylic α-position was due to its enamine character (*15*). (±)-Hexadeuterioreticuline (**9**) was prepared by the reduction of the [*N*-CD_3]isoquinolinium iodide (**6**) in deuterioacetic acid with zinc powder, followed by usual debenzylation of **8**.

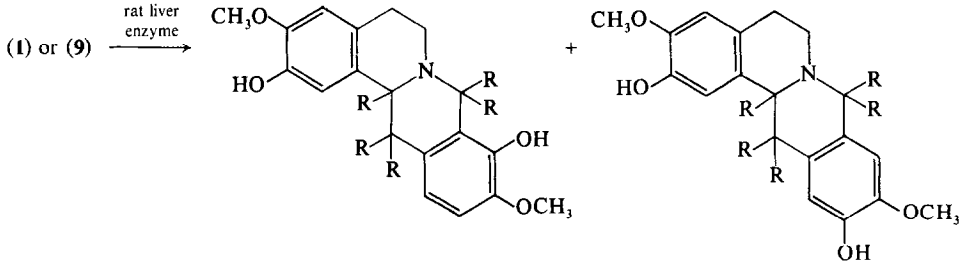
TABLE 1

BIOTRANSFORMATION OF (±)-RETICULINE (**1**) (50 mg, 0.117 mmol) WITH A RAT LIVER 9000*g* SUPERNATANT

Experimental number	Cofactors added (mmol)	Yields of products [mg, (%)]			
		Scoulerine (**10**)	Coreximine (**12**)	Pallidine (**14**)	Isoboldine (**16**)
1	$MgCl_2$ (0.246)	0.34 (0.89)	1.03 (2.69)	0.09 (0.24)	0.11 (0.29)
2	NAD (0.102), $MgCl_2$ (0.246)	1.79 (4.68)	5.37 (14.04)	0.51 (1.33)	1.02 (2.67)
3	NADP (0.101), $MgCl_2$ (0.246)	1.83 (4.8)	5.51 (14.40)	0.34 (0.89)	0.78 (2.04)
4	NADP (0.101), G-6-P) (0.203), nicotinamide (0.291), $MgCl_2$ (0.246)	1.54 (4.87)	5.62 (14.82)	0.44 (1.16)	1.16 (3.06)
5	NADPH (0.102), nicotinamide (0.292), $MgCl_2$ (0.246)	2.83 (7.41)	8.49 (22.24)	0.90 (2.35)	1.40 (3.66)

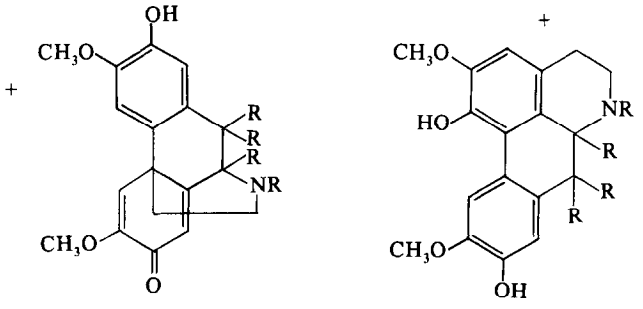
BIOTRANSFORMATION OF (±)-RETICULINE (1) AND (±)-HEXADEUTERIORETICULINE (9)

About 50 mg (0.117 mmol) of (±)-reticuline (**1**) perchlorate was incubated at 37°C for 2 hr with a 9000*g* supernatant (20 ml) of 30% rat liver homogenate in phosphate buffer at pH 7.4 in the presence of several cofactors, as in Table 1. The products were separated by preparative tlc on silica gel followed by high-pressure liquid chromatography (hplc). The structures of the compounds obtained were determined on the basis of mass spectroscopy and the following chromatographic behavior. R_f values of scoulerine (**10**), coreximine (**12**), pallidine (**14**), and isoboldine (**16**) on tlc using silica gel developing with chloroform–methanol (9 : 1 v/v) were 0.47, 0.39, 0.23, and 0.21, respectively. Retention times (*Rt*) of these alkaloids on hplc with μ-Bondapak-C_{18} (1 ft × $\frac{1}{4}$



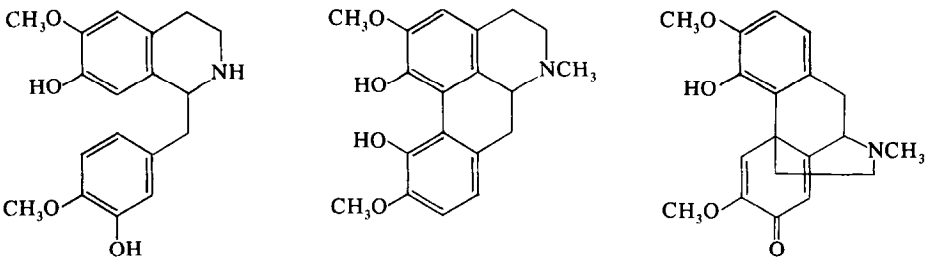
(**10**) R = H
(**11**) R = D

(**12**) R = H
(**13**) R = D



(**14**) R = H
(**15** R = D

(**16**) R = H
(**17**) R = D



(**18**) (**19**) (**20**)

SCHEME 2

in.) using methanol–water containing 0.5% (w/v) of ammonium carbonate (1 : 1 v/v) at 2.0 ml/min were 10.4, 10.6, 3.0, and 17.8 min, respectively. Therefore these alkaloids were cleanly separated by the above combined chromatographic procedures. Since starting material was mainly recovered in the above reactions, *N*-norreticuline (**18**) could not be obtained in a pure form, because its chromatographic behavior under the above conditions was similar to that of reticuline (**1**).

As mentioned in Table 1, yields of the above alkaloids were significantly increased by addition of NAD, NADP, or NADPH as a cofactor. It should be noted that NADPH was the most effective cofactor among these reagents. Treatment of (±)-reticuline (**1**) in a phosphate buffer at pH 7.4 with magnesium chloride and NAD in the absence of the supernatant of rat liver gave only starting material. Furthermore, after removal of air from the 9000*g* supernatant of rat liver homogenate under reduced pressure for 5 min, nitrogen was blown into the resulting mixture. With this enzymic mixture, reticuline (**1**) was treated under nitrogen in the presence of NAD and magnesium chloride, but only a small amount of the above alkaloids was obtained. On the other hand, a parallel experiment (after the degassing procedure, the incubation was carried out under an air atmosphere) yielded almost the same products as those in Experiment 2. There was a significant difference between the amount of the alkaloids formed in the above two experiments. This fact indicates that molecular oxygen is involved in the above oxidative reactions. It was thus assumed that reducing cofactor(s) such as NADPH, molecular oxygen, and probably transition metal(s) were responsible for the above enzymic oxidation.

No detectable amount of the *ortho–ortho* [(±)-corytuberine (**19**)]-coupled or *para–ortho* [(±)-salutaridine (**20**)]-coupled product was found on the above reactions.

(±)-Hexadeuterioreticuline (**9**) perchlorate was treated with the rat liver preparation in the presence of NADPH, magnesium chloride, and nicotinamide (Experiment 5). Mass spectra of products pallidine (**15**) and isoboldine (**17**) showed new parent ions at

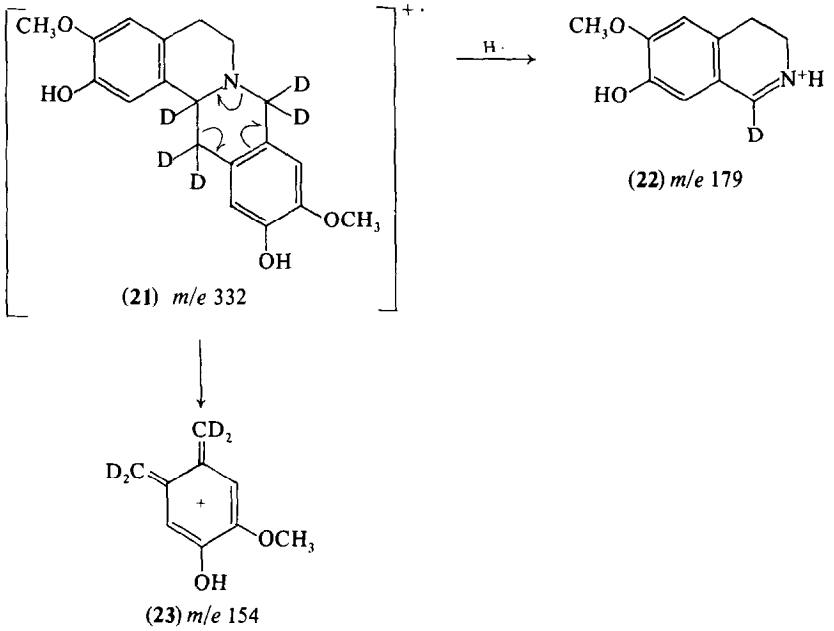
TABLE 2

RELATIVE INTENSITIES IN THE MASS SPECTRA OF UNLABELED COREXIMINE (**12**) AND [D_5]COREXIMINES (**13**) OBTAINED FROM (±)-[D_6]RETICULINE (**9**) AND A 1 : 1 MIXTURE OF UNLABELED (±)-RETICULINE (**1**) AND (±)-[D_6]RETICULINE (**9**)

Unlabeled coreximine (**12**)		[D_5]Coreximine (**13**)		[D_5]Coreximine (**13**) from a mixture of **1** and **9**	
m/e	%	*m/e*	%	*m/e*	%
324	3.67	328	9.05	324	5.05
325	2.97	329	20.81	325	7.21
326	38.14	330	73.08	326	37.30
327 (M^+)	100.00	331	48.87	327	100.00
328	22.60	332 (M^+)	100.00	328	33.06
329	4.24	333	34.84	329	19.19
		334	9.50	330	30.63
				331	16.22
				332 (M^+)	27.03
				333	11.35
				334	3.78

m/e 333, 6 mass units higher than that corresponding to undeuteriated authentic samples, and intensities indicated the presence of more than 95% hexadeuterio compounds. No significant amounts of less deuterated compounds were detected, indicating incorporation of the six deuterium atoms intact.

On the other hand, the mass spectrum of coreximine (**13**) with two relatively strong peaks at *m/e* 332 and 330 is shown in Table 2. Furthermore, the 3,4-dihydroisoquinolinium ion (**22**) appears at *m/e* 179 as the base peak, while the ion (**23**) at *m/e* 154 formed by retro Diels–Alder fragmentation (*16*) is accompanied by a rather strong peak at *m/e* 152. Scoulerine (**11**) showed a mass spectrum similar to that of coreximine (**13**). Thus, the *N*-methyl group of reticuline (**9**) was not incorporated intact into the protoberberines. On the other hand the deuteriums at C-1 and the benzylic α positions of reticuline remained throughout the transformation. A part of *N*-methyl group of



(**21**) *m/e* 332

(**22**) *m/e* 179

(**23**) *m/e* 154

SCHEME 3

reticuline (**1**) would have been demthylated to give *N*-norreticuline (**18**), which would have been converted into protoberberines incorporating 1 carbon unit (*6*).

Furthermore, 0.115 mmol of hexadeuterioreticuline (**9**) perchlorate was mixed with 0.117 mmol of nonlabeled **1** perchlorate, and the mixture was incubated with the 9000*g* supernatant (40 ml) in the presence of 0.111 mmol of NADPH, 0.580 mmol of nicotinamide, 0.193 mmol of glucose-6-phosphate (G-6-p), and 0.502 mmol of magnesium chloride under an air atmosphere. Intensities of the peaks formed at *m/e* 330 and 332 of coreximine (**13**) were nearly the same as those shown in Table 2. The observation of a relatively small peak at *m/e* 329 indicates little interchange of *N*-methyl groups of reticuline molecules. There is, therefore, little possibility of recombination between formaldehyde and *N*-nonreticuline (**18**) which formed by splitting from reticuline. This experiment again supported the intact incorporation of all six deuteriums of reticuline into pallidine and isoboldine.

EXPERIMENTAL

All melting points are uncorrected. Ultraviolet and infrared spectra were taken with Hitachi 124 and 215 spectrophotometers, respectively. Nuclear magnetic resonance spectra were measured with a JNM/PMX-60 spectrometer (tetramethylsilane as an internal reference), and mass spectra with an Hitachi RMU-7 spectrometer. High-pressure liquid chromatography was carried out with a Waters Associates ALC/GDC 200/R 401 instrument equipped with a column (1 ft × $\frac{1}{4}$ in.) packed with μ-Bondapak-C_{18}. Preparative tlc was carried out using Kieselgel HF_{254} (Merck).

[N-CD$_3$]-7-Benzyloxy-1-(3-benzyloxy-4-methoxybenzyl)-3,4-dihydro-6-methoxy-2-methylisoquinolinium iodide (**6**). To a solution of 7-benzyloxy-1-(3-benzyloxy-4-methoxybenzyl)-3,4-dihydro-6-methoxy-2-methylisoquinoline (**4**), obtained from 2.1 g of the corresponding hydrochloride (**14**) in a usual manner, in 22 ml of methanol was added 1 ml of methyl iodide-d_3, and the mixture was allowed to stand for 3 days at room temperature. After evaporation of the solvent and the reagent under reduced pressure, the residue was recrystallized from methanol–chloroform–ether to give 2.1 g of **6** as yellowish needles, mp 202–203°C, the nmr spectrum ($CDCl_3$) of which was superimposable on that of the corresponding undeuterated compound (**5**) (*11*, *12*) except for the disappearance of the *N*-methyl group.

(±)-*[N-CD$_3$,1,α,α-D$_3$]O,O-Dibenzylreticuline* (**8**). After heating a mixture of 4 ml of acetic anhydride and 4 ml of deuterium oxide in a steam-bath for 30 min, 400 mg of the above methiodide (**6**) was added to the resulting deuterioacetic acid. The mixture was further heated for 5 hr on a steam-bath, and 340 mg of zinc powder was then added to the above hot solution. After the resulting mixture had been heated for 1 hr on a steam-bath, 220 mg of zinc powder was further added and the mixture was heated at the same temperature for 5 hr. After filtration through Celite followed by washing with acetic acid, the filtrate was evaporated under reduced pressure, and the residue was partitioned between 10% ammonia and benzene. The aqueous layer was further extracted with benzene. The combined benzene layers were washed with a saturated aqueous sodium chloride solution and dried over anhydrous sodium sulfate. Evaporation of the solvent under reduced pressure gave 330 mg of a yellowish syrup, which was triturated with hexane to give **8** as colorless needles, mp 88.5–89°C.

nmr ($CDCl_3$): δ 2.54–3.29 (4H, m, C_3–H_2 and C_4–H_2); 3.80 (6H, s, 2 × OCH_3); 4.75 (2H, s, $OCH_2C_6H_5$); 4.98 (2H, s, $OCH_2C_6H_5$); 6.04 (1H, s, C_8–*H*); 6.04–6.75 (4H, m, 4 × ArH); 7.26 (10H, s, 2 × C_6H_5).

ms: *m/e* 515 (M^+).

(±)-*[N-CD$_3$,1,α,α-D$_3$]Reticuline* (**9**). A mixture of 280 mg of the above hexadeuterio compound (**8**), 14 ml of benzene, and 14 ml of concentrated hydrochloric acid was stirred for 24 hr at room temperature under a nitrogen atmosphere. The aqueous layer was evaporated under reduced pressure to give a residue, which was partitioned between 10% ammonia and chloroform. The aqueous layer was extracted with chloroform. The combined chloroform layers were washed with a saturated aqueous sodium chloride solution and dried over anhydrous sodium sulfate. Evaporation of the solvent yielded a yellow gum, which was converted to the perchlorate. Recrystallization from methanol–ether gave 170 mg of **9**·$HClO_4$ as crystals, mp 138.5–139.5°C (decomp.), which showed no depression on mixture melting point test with the undeuterio authentic sample (*11*, *12*).

nmr ($CDCl_3$): δ (free base) 2.61–3.30 (4H, m, C_3–H_2 and C_4–H_2); 3.74 (6H, s, 2 × OCH_3); 5.67 (2H, broad s, 2 × O*H*); 6.24 (1H, s, C_8–*H*); 6.44–6.72 (4H, m, 4 × Ar*H*).

Preparation of the 9000 g supernatant of rat liver and incubation with (±)-reticuline. Rats were killed by decapitation, and the livers were homogenized in three times of the volume of the phosphate buffer at pH 7.4 with a Potter–Elvehjem homogenizer, which was cooled with ice. The resulting homogenate was centrifuged for 30 min at 9000*g* and 0°C. The separated supernatant was immediately added to a solution of the cofactors mentioned in Table 1, the substrate in propylene glycole, and the phosphate buffer. The resulting mixture was shaken at 37°C under an air atmosphere for 2 hr and brought to pH 2 with concentrated hydrochloric acid. After basification with 10% ammonia, the resulting mixture was extracted five times with ethyl acetate. The combined ethyl acetate layers were washed with a saturated aqueous sodium chloride, dried over anhydrous sodium sulfate, and evaporated. The residue was purified by preparative tlc on silica gel in chloroform–methanol (9:1 v/v) followed by hplc of the separated fractions using μ-Bondapak-C_{18} (1 ft × $\frac{1}{4}$ in.) with methanol–water containing 0.5% ammonium carbonate (1:1 v/v) at 2 ml/min to give scoulerine (**10**) (*18*), coreximine (**12**), (*17*), pallidine (**14**) (*19*), and isoboldine (**16**) (*19*).

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