BIOTRANSFORMATION OF RETICULINE INTO MORPHINANDIENONE, APORPHINE AND PROTOBERBERINE ALKALOIDS WITH RAT LIVER ENZYME

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(±)-Reticuline (1a) was biotransformed into morphinandienone alkaloid, pallidine (4a), and aporphine alkaloid, isoboldine (5a) in addition to protoberberine alkaloids, coreximine (3a) and scoulerine (2a). The transformation was stimulated by the presence of the following cofactors, NAD, NADP or NADPH. Deuterium labelled experiment revealed that the N-methyl group of (±)-reticuline was not intactly incorporated into protoberberines.

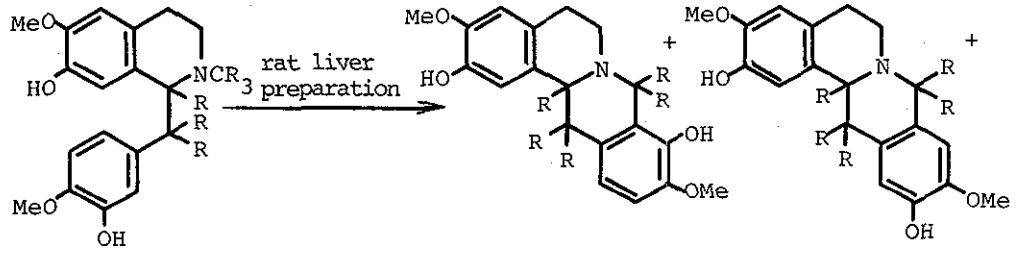
Recently we reported the biotransformation of radioactive 1-benzyl-1,2,3,4-tetrahydroisoquinolines into protoberberine alkaloids with rat liver enzyme.[1] The current interest about the drug-evoked aberrations of neuroamine metabolism, particularly concerning a possible biochemical basis for alcoholism,[2-8] has prompted us to investigate mammalian enzyme, which is responsible for the phenol oxidative coupling of 1-benzylisoquinoline

alkaloids. In the previous time,[1] the formation of aporphine and morphinandienone type alkaloids were not fully tested, because suitable carriers for the tracer experiments were not available.

Thus, (±)-reticuline (1a) (50 mg, 0.117 mmole), an important intermediate in the biogenesis of opium alkaloids, was incubated at 37°C for 2 h with a 9,000 g supernatant (20 ml) of 30 % rat liver homogenate in phosphate buffer at pH 7.4 in the presence of several cofactors as mentioned in Table 1. The products were isolated by preparative t.l.c. on silica gel followed by high pressure liquid chromatography (h.p.l.c.). The structure of the compounds obtained were determined on the basis of the above chromatographical behaviours and mass spectroscopy. Rf values of scoulerine (2a), coreximine (3a), pallidine (4a) and isoboldine (5a) on t.l.c. using silica gel (Merck HF_{254}) developing with chloroform-methanol (10 : 1 v/v) were 0.47, 0.39, 0.23 and 0.21, while retention times (tR) of these alkaloids on h.p.l.c. with μ-Bondapak-C_{18} (1 ft x 1/4 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. As mentioned in Table 1, yields of the above alkaloids were significantly increased by addition of NAD, NADP or NADPH as cofactor. No detectable amount of the ortho-ortho and para-ortho coupled products by phenolic oxidation was formed. Starting material was mainly recovered by the above reactions and norreticuline (6) could not be isolated as a pure form, because its chromatographical behaviours under the above conditions were similar to that of reticuline (1a). Treatment of

reticuline (1a) in phosphate buffer with magnesium chloride and NAD in the absence of the supernatant of rat liver gave only the starting material.

Furthermore, in order to study the intact transformation of reticuline to the alkaloids by the mammalian enzyme, 0.117 mmole of multiply deuterium labelled (±)-[$^2H_6$]reticuline (1b), prepared by the reduction of [N-C$^2H_3$]7-benzyloxy-1-(3-benzyloxy-4-methoxy-benzyl)-3,4-dihydro-6-methoxy-2-methylisoquinolinium iodide (7)



(1a) R=H

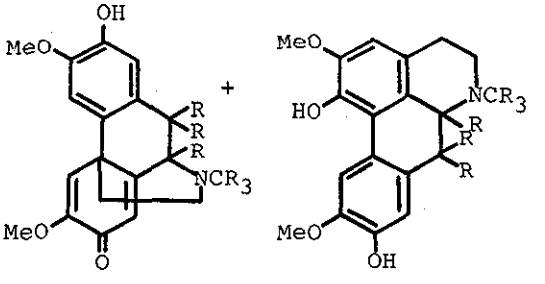
(1b) R=D

(2a) R=H

(2b) R=D

(3a) R=H

(3b) R=D



(4a) R=H

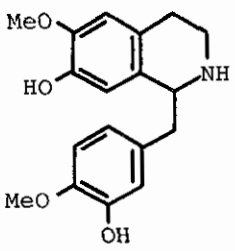
(4b) R=D

(5a) R=H

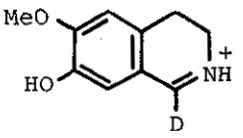
(5b) R=D

Table 1 Biotransformation of (±)-reticuline (1a) (50 mg, 0.117 mmole) with a rat liver 9000 g supernatant

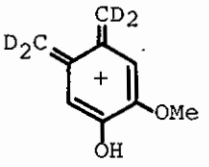
Cofactors added (mmole)	Yields of products mg (%)			
	Scoulerine (2a)	Coreximine (3a)	Pallidine (4a)	Isoboldine (5a)
$MgCl_2$ (0.246)	0.34 (0.89)	1.03 (2.69)	0.09 (0.24)	0.11 (0.29)
NAD (0.102), $MgCl_2$ (0.246)	1.79 (4.68)	5.37 (14.04)	0.51 (1.33)	1.02 (2.67)
NADP (0.101), $MgCl_2$ (0.246)	1.83 (4.8)	5.51 (14.40)	0.34 (0.89)	0.78 (2.04)
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)
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)



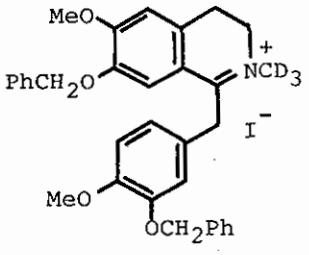
(6)



(8)



(9)



(7)

Table 2 Relative intensities in the mass spectra of unlabelled coreximine (3a) and [$^2H_5$]coreximine (3b) obtained from (±)-[$^2H_6$]-reticuline (1b)

unlabelled coreximine (3b)		[$^2H_5$]Coreximine (3b)	
m/e	%	m/e	%
324	3.67	328	9.05
325	2.97	329	20.81
326	38.14	330	73.08
327(M^+)	100.00	331	48.87
328	22.60	332(M^+)	100.00
329	4.24	333	34.84
		334	9.50

in deuterioacetic acid with zinc powder,[9] followed by usual debenzylation, was treated with the rat liver preparation in the presence of NADPH, magnesium chloride and nicotinamide. The mass spectra of isoboldine (5b) and pallidine (4b) showed respectively new parent ion at m/e 333, six mass unit higher than that corresponding to undeuteriated authentic samples and of intensity more than 95 % of hexadeuterio compounds. No significant amounts of the less deuterio compounds were detected.

On the other hand, the mass spectrum of coreximine (3b) exhibited two relatively strong peaks at m/e 332 and 330 as shown in Table 2. Furthermore the 3,4-dihydroisoquinolinium ion (8) appeared at m/e 179 as the base peak, while the ion (9) at m/e 154 formed by retro Diels-Alder fragmentation was accompanied with a rather strong peak at m/e 152. Scoulerine (2b) showed a mass spectrum similar to that of coreximine (3b). It was thus revealed that the N-methyl group of reticuline (1b) was not intactly incorporated into the protoberberines. A part of N-methyl group of reticuline would have been demethylated to give N-norreticuline (6), which would have been converted into tetrahydroprotoberberines incorporating one carbon unit.[4]

This demonstrated the ability of mammalian systems to evoke phenol oxidative coupling of 1-benzyltetrahydroisoquinoline and would strengthen the credibility of the hypothesis for alcohol addiction.[2-5]

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