BIOTRANSFORMATION OF ISOQUINOLINE ALKALOIDS WITH RAT LIVER MICROSOMES

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Abstract —— Optically active (+)-reticuline (1) was biotransformed into (-)-coreximine (2), (-)-scoulerine (3), (+)-isoboldine (4) and (-)-pallidine (5) with retention of the chirality by the incubation with rat liver microsomes. On the other hand, the racemate of reticuline formed the racemates of the above alkaloids on the some treatment. The S-adenosylmethionine was partially incorporated into the berberine bridge during the biotransformation of reticuline into the protoberberines.

In the previous papers,[1,2] we reported the biotransformation of reticuline into several alkaloids by the whole rat experiments and by the incubations with the 9,000 g supernatant of rat liver homogenate. We further studied the following three subjects in the biotranformation, (a) stereospecificity, (b) involvement of the N-oxide intermediate, and (c) origin of the berberine bridge.

NON STEREOSPECIFICITY

It was previously supported by the reverse dilution method that the coreximine (2) formed from (±)-[N-$^{14}CH_3$]-reticuline by incubation with homogenate of rat liver was the racemate.[1] However the chiralities of the phenol oxidative coupling products, isoboldine (4) and pallidine (5) were not known. Therefore the biotransformations of both optically active (+)-reticuline and the racemate were investigated.

On the incubation with rat liver microsomes[3] in the presence of NADPH, magnesium chloride and nicotinamide in phosphate buffer at pH 7.4 and 37°C, (+)-reticuline (1) was converted into (-)-coreximine (2), (-)-scoulerine (3), (+)-isoboldine (4)

and (-)-pallidine (5), while (±)-reticuline produced the racemates of these alkaloids. The yields and optical rotations of products were summarized in Table 1. Although the conditions of the biotransformation were not optimized, the rates of the conversion into the alkaloids did not depend on the chirality of the substrate. The stereochemistry was retained through the biotransformation of (+)-reticuline. It was made clear that the above biotransformation using rat liver microsomes is not stereospecific.

Scheme 1

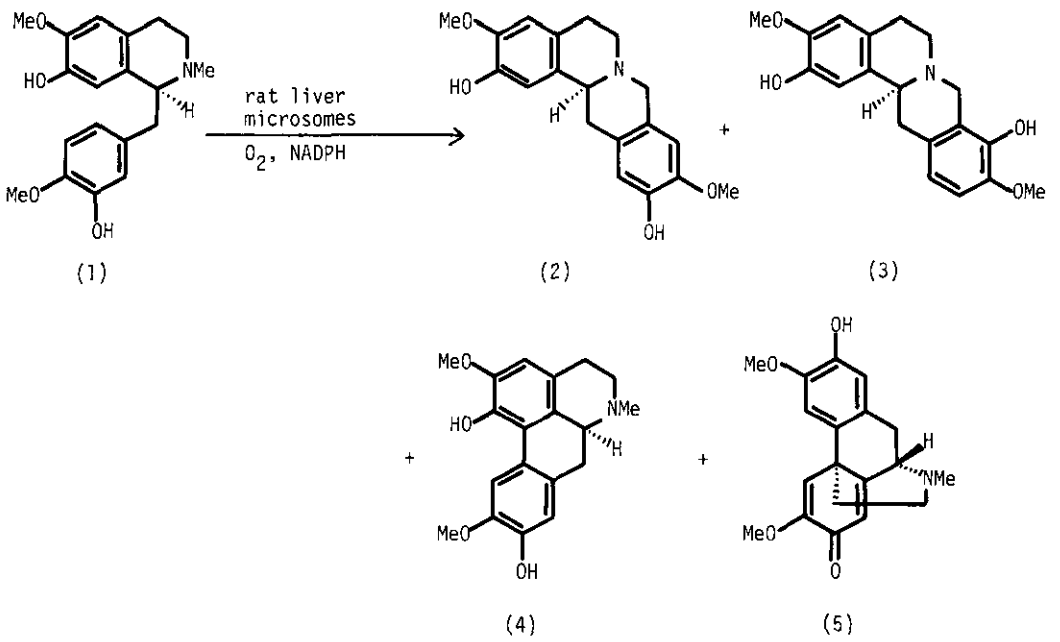


Table 1. Yields and Optical Rotations of Products ($[\alpha]_D^{18}$ (methanol))

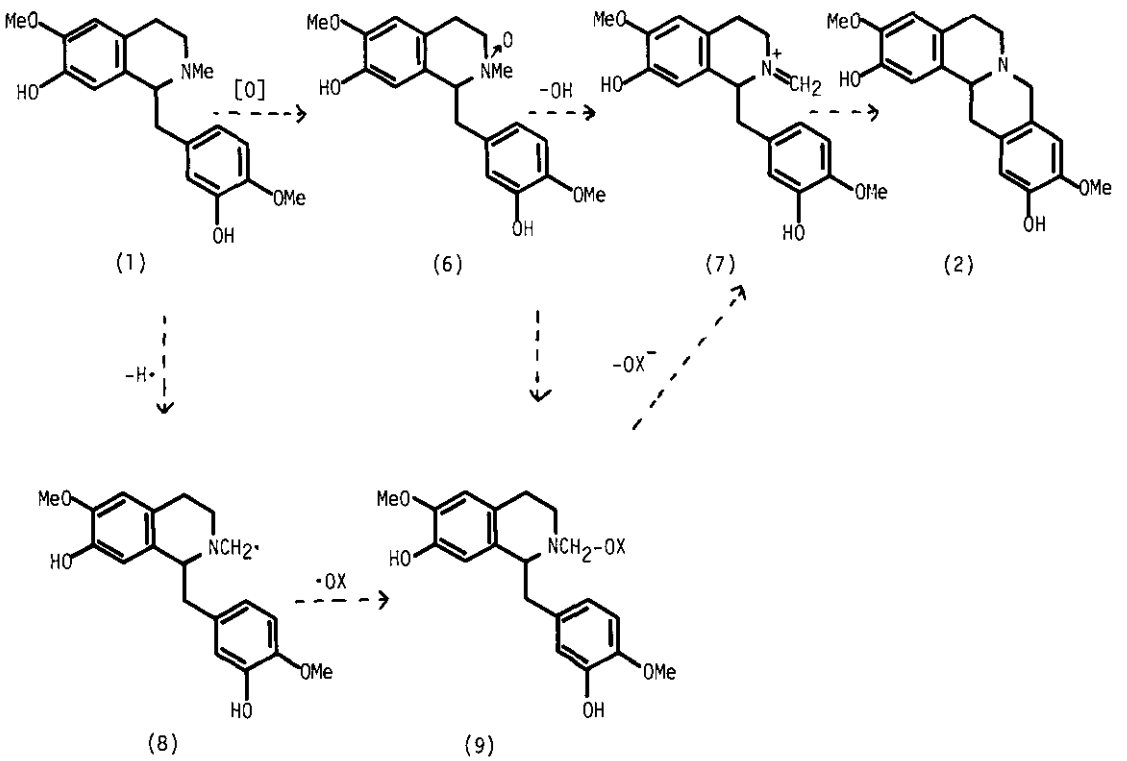
Substrate	Coreximine (2)	Scoulerine (3)	Isoboldine (4)	Pallidine (5)
(+)-Reticuline	21.1 % (-330^o)[a]	7.9 % (-240^o)[b]	7.5 % ($+45^o$)[c]	5.2 % (-28^o)[d]
(±)-Reticuline	19.0 % ($\pm 0^o$)	8.1 % ($\pm 0^o$)	5.5 % ($\pm 0^o$)	4.7 % ($\pm 0^o$)

Reported optical rotations: a) $[\alpha]_D^{20}$ -350^o (methanol)[4]; b) $[\alpha]_D^{9}$ -285.2^o (methanol)[5]; c) $[\alpha]_D^{20}$ $+62^o$ (chloroform)[6]; d) $[\alpha]_D^{18}$ -32^o (ethanol)[6].

N-OXIDE INTERMEDIATE

Protoberberines would be produced via the imine (7), which formed by the oxidation of the N-methyl group. Possible mechanisms[1] for the formation of the immonium cation (7) are outlined in Scheme 2, in which the N-oxide (6) or the radical intermediate (8) would be concerned. In order to test the involvement of the N-oxide intermediate, (±)-reticuline N-oxide (6)[7] was treated with the rat liver microsomes under the same conditions as above. No formation of isoboldine and pallidine was observed. Formations of a very small amount (>0.1 %) of coreximine and scoulerine was detected by tlc analysis, but these must be neglected because the conversion of reticuline N-oxide into the protoberberines easily occurred on the treatment with inorganic salts.[7] It is thus probable that the N-oxide intermediate is not concerned in the biotransformation.[8] However the participation of the N-oxide in the biosynthesis of the alkaloids should be tested in the future using plant enzymes.

Scheme 2



ORIGIN OF BERBERINE BRIDGE

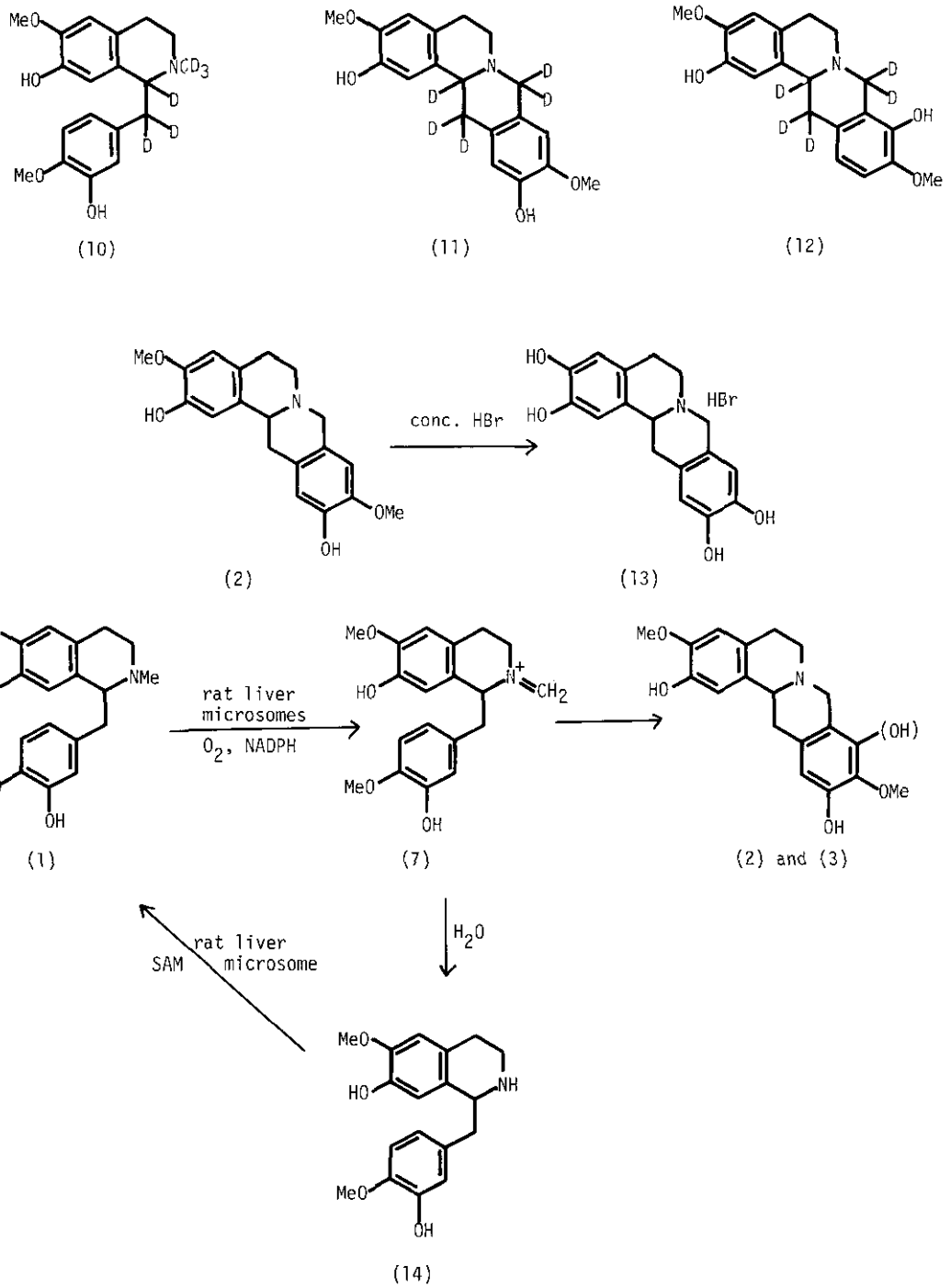
In the biogenesis of berberine alkaloids, it was established that the N-methyl group of the phenolic 1-benzylisoquinoline is incorporated into the berberine bridge.[9,10] However, by the incubation of the hexadeuterioreticuline (10) with the 9,000 g supernatant of rat liver homogenate, a partial loss of the N-methyl group during the biotransformation into the coreximine (11) and the scoulerine (12) was observed.[2] This fact indicated that a part of N-methyl group of reticuline was demethylated to N-norreticuline (13), which was further converted into protoberberines incorporating one carbon unit. Therefore the origin of the carbon unit was studied using radioactive methionine and S-adenosylmethionine (SAM).

On incubation of (±)-reticuline perchlorate and ^{14}C-[S-Me]-methionine in the presence of NADPH, NADP, glucose-6-phosphate, magnesium chloride and nicotinamide with 9,000 g supernatant of rat liver homogenate, the radioactivity of the methionine was not incorporated into coreximine (this result was verified by the dilution method).

On the other hand, the radioactivity of ^{14}C-[S-Me]-S-adenosylmethionine was incorporated into coreximine on the similar treatment using the radioactive S-adenosylmethionine. Demethylation of the resulting radioactive coreximine (2) (25,179 dpm/mmole) by heating with concentrated hydrobromic acid yielded the radioactive tetrahydroxyprotoberberine (13) (21,660 dpm/mmole).

The small decrease of the activity during the demethylation would be attributed to the interchange between the O and S-methyl groups. It was thus, from the above result, determined that N-methyl groups of reticuline (1) was oxidized to the imine (7), which was cyclized to protoberberines or hydrolized to the N-nor-compound (14). The resulting 14 was methylated with S-adenosylmethionine in the microsomes and then underwent further oxidative cyclization to protoberberines.[11]

Scheme 3


MeO
HO
NCD3
D
OMe
OH
(10)
(11)
(12)
(2)
conc. HBr
HBr
(13)
NMe
rat liver
microsomes
O2, NADPH
CH2
(OH)
(1)
(7)
(2) and (3)
H2O
SAM
rat liver
microsome
NH
(14)

EXPERIMENTAL

Ir spectra were taken with Hitachi 215 spectrometer and nmr spectra were measured with a JNM/PMX-60 spectrometer (tetramethylsilane as an internal reference). Optical rotations were determined with a JASCO-PIP-SL automatic polarimeter using a 0.5 dm cell. The radioactivities were measured by liquid scintillation counting with a Packard (Model 3380) Tri-Carb liquid scintillation counter equipped with an absolute activity analyzer (Model 544). High pressure liquid chromatography was carried out with a Hitachi 635 instrument equipped with a column (1 ft x 0.25 in.) packed with μ-Bondapak-C_{18}. Preparative tlc was carried out using Kieselgel HF_{254} (Merck).

Preparation of Rat Liver Microsomes and Incubation with (+)-and (±)-Reticuline and (±)-Reticuline N-Oxide

Three rats (about 200 g weight) were fasted overnight prior to decaptation. After serial washings with 1.15 % potassium chloride, the livers were homogenized in three times of the volume of 1.15 % potassium chloride solution with a Potter-Elvehjem homogenizer, which was cooled with ice. The resulting homogenate was centrifuged for 30 min at 9,000 g and 0°C. The separated supernatant was further centrifuged for 1 h at 105,000 g at the same temperature to give the microsomal pellets, which were homogenized in the same volume of the phosphate buffer at pH 7.4. (±)-Reticuline (76 mg), (+)-reticuline (70 mg) and (±)-reticuline N-oxide (80 mg) was respectively incubated for 2 h at 37°C with one third of the above microsomal solution in the presence of NADPH (100 mg), nicotinamide (74 mg) and magnesium chloride hexahydrate (100 mg). The resulting mixture was brought to pH 2 with concentrated hydrochloric acid, then basified with 10 % ammonia and extracted with ethyl acetate. The extract was dried (Na_2SO_4) and evaporated. The residue was purified by preparative tlc followed by high pressure liquid chromatography according to the previously reported procedure.[2] The results from (+)- and (±)-reticuline were summarized in Table 1.

Incubation of (±)-Reticuline and ^{14}C-[S-Me]-S-Adenosylmethionine with the 9,000 g Supernatant of Rat Liver Homogenate

The liver obtained from a rat (210 g) was homogenized in three times of the volume of the phosphate buffer at pH 7.4 with a Potter-Elvehjem homogenizer cooled with ice. The resulting homogenate was centrifuged for 30 min at 9,000 g and 0°C. The separated supernatant was immediately added to a solution of (±)-reticuline perchlorate (3.1 mg) in propylene glycol. After addition of NADPH (4.9 mg), NADP (6.7 mg), nicotinamide (3.5 mg), glucose-6-phosphate (7.0 mg), magnesium chloride (4 mg) and ^{14}C-[S-Me]-S-

adenosylmethionine (10 μCi), the mixture was shaken at 37°C under an air atmosphere for 2 h and brought to pH 2 with concentrated hydrochloric acid. After basification with 10 % ammonia followed by addition of (±)-coreximine (60 mg), the resulting mixture was extracted with ethyl acetate. The extract was dried (Na_2SO_4) and evaporated to give a residue, which was partitioned between 2 % hydrochloric acid and ether. The aqueous layer was basified with 10 % ammonia and extracted with chloroform. The extract was washed with brine, dried (Na_2SO_4) and evaporated to give a residue, which was recrystallized from methanol to constant activity (25,179 dpm/mmole).

(±)-2,3,10,11-Tetrahydroxyprotoberberine Hydrobromide (13)

A mixture of (±)-coreximine (20 mg) and 48 % hydrobromic acid (20 ml) was refluxed for 16 h under nitrogen. Evaporation of the reagent gave a powder which was recrystallized from methanol to afford the hydrobromide (13) (19 mg) as fine needles, mp > 260°C. Anal. Calcd. for $C_{17}H_{17}NO_4 \cdot HBr$: C, 53.70; H, 4.77; N, 3.68. Found: C, 53.70; H, 4.74; N, 3.48.

The ^{14}C-labelled coreximine (25,179 dpm/mmole, 17 mg) obtained by the above incubation was reacted with 48 % hydrobromic acid (2 ml) as above and the tetrahydroxyprotoberberine formed was purified by recrystallization from methanol to constant activity (21,660 dpm/mmole).

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