Phytochemistry, 1978, Vol 17, pp 891–893 © Pergamon Press Ltd Printed in England

0031-9422/78/0501-0891 $02.00/0

BIOTRANSFORMATION OF (*RS*)-RETICULINE AND MORPHINAN ALKALOIDS BY CELL CULTURES OF *PAPAVER SOMNIFERUM**

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(*Received* 5 *December* 1977)

Key Word Index—*Papaver somniferum*; Papaveraceae; cell culture; biotransformation; racemic resolution; reduction; morphinan alkaloids; reticuline; scoulerine; cheilanthifoline; codeinone; codeine

Abstract—(*RS*)-Reticuline was stereospecifically converted to (−)-(*S*)-scoulerine and (−)-(*S*)-cheilanthifoline by cell cultures of *Papaver somniferum* and (−)-(*R*)-reticuline was recovered as an optical pure compound by racemic resolution. (−)-Codeinone was converted in high yield to (−)-codeine in both cell culture and enzyme preparation, but the other morphinans, thebaine, codeine and morphine, were not metabolized.

INTRODUCTION

Although the opium poppy, *Papaver somniferum* L. contains as the main components morphinan type alkaloids, poppy callus tissues, lack such alkaloids, but contain instead benzophenanthridine, protopine and aporphine type alkaloids [1, 2]. These data suggest that the callus tissues cannot biosynthesize the alkaloids derived from (−)-(*R*)-reticuline, an important intermediate of the opium alkaloids such as morphine [3, 4], but can biosynthesize only those from (+)-(*S*)-reticuline. In the present paper, we describe the ability of the *Papaver somniferum* callus tissues to metabolize (*RS*)-reticuline and morphinan alkaloids.

RESULTS

Biotransformation of (RS)-*reticuline*

(*RS*)-reticuline (total 300 mg) was administered to *Papaver somniferum* suspension cell cultures. After shaking for 3 days, the cultures were harvested. The callus and medium were separately extracted with $CHCl_3$ at pH 13 (crude alkaloids; 118.7 and 106 mg, respectively) and 3 metabolites were detected by TLC (R_f 0.60, 0.47 and 0.22; $CHCl_3$–MeOH, 9:1). These metabolites (**1–3**) were isolated by silica gel column chromatography and PLC on silica gel.

Compound **1** (R_f 0.60, 1.5 mg) was isolated as crystals, recrystallized from MeOH, mp 170° (decomp.), and its formula $C_{19}H_{19}O_4N$ determined by high resolution MS. The main MS fragmentation peaks were observed at *m/e* 325 (M^+), 177 and 148. The peaks at *m/e* 177 and 148 suggested a protoberberine base [5, 6], containing a hydroxy and a methoxy group in A ring and a methylenedioxy group in D ring. The NMR spectrum ($CDCl_3$, δ, ppm) also showed the presence of a methoxy group (3H, OMe) at 3.85 and a methylenedioxy group (2H, $-CH_2-$) at 5.90. Moreover, the NMR analysis revealed the proton of C-8 methylene as AB-quartet at 3.48 and 4.21 with the coupling constant of 16 Hz, indicating the presence of methylenedioxy group in 9 and 10 position of D ring [7]. Therefore, **1** is (−)-(*S*)-cheilanthifoline ($[\alpha]_D^{20}$ −315°), an identification confirmed by the comparison of mp, IR, TLC and GC–MS with an authentic sample.

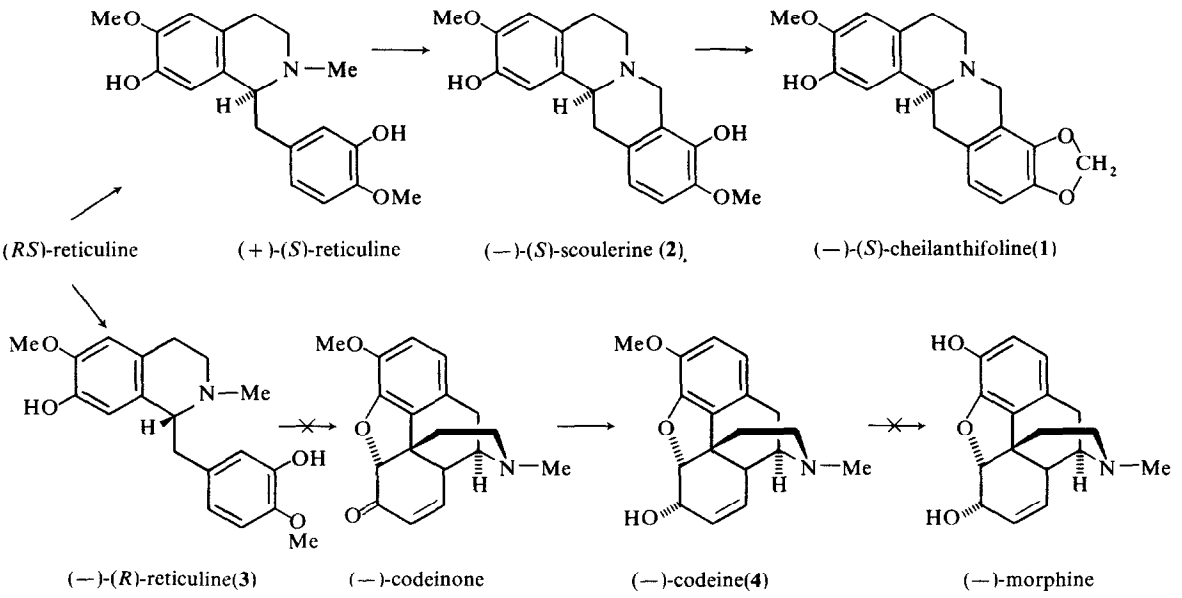
Compound **2** (R_f 0.47, 44.1 mg) was isolated as oil and its formula $C_{19}H_{21}O_4N$ determined by high resolution MS. Its UV and IR spectra were similar to those of **1**, indicating a protoberberine base having a hydroxy group (3560 cm^{-1}). The main MS fragmentation peaks were observed at *m/e* 329 (M^+), 177, 150 and 135. The peaks at *m/e* 177 and 150 showed the fragment with a hydroxy and methoxy group in A, B and C, D ring, respectively. The NMR spectrum showed a broad singlet at 5.26 (2H, 2-OH), a singlet at 3.85 (6H, 2-OMe) and AB-quartet at 3.48 and 4.21 with a coupling constant of 16 Hz. Therefore, **2** is (−)-(*S*)-scoulerine with $[\alpha]_D^{20}$ −246°, and identified by TLC and the spectral comparison with an authentic sample.

Compound **3** (R_f 0.22, 28.7 mg) was isolated as oil. Its formula $C_{19}H_{23}O_4N$ and R_f value on TLC were the same as those of the administered substrate. The MS, NMR and IR data were also very similar to those of (*RS*)-reticuline, except its $[\alpha]_D^{20}$ −59°. Moreover, its dimethyl ether (by CH_2N_2) showed (−)-(*R*)-laudanosine by the spectral identification with the authentic sample. From these data, **3** must be optically pure (−)-(*R*)-reticuline. As regards the other metabolites, the mixture of sanguinarine and its derivatives was confirmed on TLC (R_f 0.70), and protopine by GLC and GC–MS.

Biotransformation of (−)-*codeinone*

(−)-Codeinone (15 mg) was administered to the poppy suspension culture as described above. After shaking for 3 days, the callus and medium were respectively extracted with $CHCl_3$–*iso*-PrOH (4:1) at pH 8.5 (total alkaloids, 8.7 mg). The (−)-codeinone administered to the suspension culture was almost metabolized on day 3, and a new product (**4**) was detected by TLC and GLC.

* Part 30 in the series 'Studies on Plant Tissue Cultures'. For Part 29, see Furuya, T., Ayabe, S. and Kobayashi, M. (1976) *Tetrahedron Letters* 2539. Part of this work was presented in 5th Symposium for Plant Tissue Culture at Sendai, Japan (9 July 1976).



Scheme 1. Biotransformation of (*RS*)-reticuline and codeinone by cell culture of *Papaver somniferum*.

4 (3.6 mg) was isolated by silica gel column chromatography. It was a powder, recrystallized from petrol, mp 153–155°, and its formula $C_{19}H_{21}O_4N$ by high resolution MS, increasing by two hydrogen molecules by comparison with that of codeinone. The IR spectrum newly revealed the absorption at 3550 cm^{-1} (—OH) instead of the C=O in codeinone. The NMR analysis showed the C_5 proton doublet at 4.88 with a coupling constant of 6.5 Hz, indicating that the conformation of C_6 (—OH) was *R* (equatorial) [8]. Therefore, **4** is (−)-codeine and was identified by IR, NMR, mmp and $[\alpha]_D^{20}$ −121.3° comparison with authentic (−)-codeine. The biotransformation ratio was 60.8% at 3 days and 66.7% at 8 days. The reduction by the crude enzyme preparation was also observed in almost the same yield as that in cell culture. The other morphinan alkaloids, thebaine, codeine and morphine, were not metabolized by the poppy cell cultures.

DISCUSSION

In our earlier reports [1, 2], we suggested that *Papaver somniferum* callus was unable to biosynthesize morphine and related alkaloids, which were derived from (−)-(*R*)-reticuline and produced in high content by the original plant. In biotransformation experiments, it was first shown that only (+)-(*S*)-reticuline obtained by racemic resolution, was stereospecifically cyclized to convert to (−)-(*S*)-scoulerine (biotransformation ratio 14.7%) and (−)-(*S*)-cheilanthifoline (0.5%) and (−)-(*R*)-reticuline (9.6%) remained unchanged in both callus and medium after one month. Thus *Papaver somniferum* callus lacks the phenol oxidation enzyme [4, 9] catalyzing the reaction from (−)-(*R*)-reticuline to salutaridine, but shows the presence of the berberine bridge enzyme, converting (+)-(*S*)-reticuline to scoulerine, which was reported in *Macleaya cordata* callus (Papaveraceae) by Rink and Böhm [10, 11]. However, *Macleaya* plant itself contained in high yield the alkaloids biosynthesized from (+)-(*S*)-reticuline, but not those from (−)-(*R*)-reticuline. It is also interesting that (*RS*)-reticuline administered to rat was converted to scoulerine and coreximine by a similar metabolic pathway to that proposed in plants, but they were both racemic compounds [12].

We have now investigated the ability of the poppy callus to metabolize thebaine, codeinone, codeine and morphine and found the only reaction to occur was the stereospecific reduction of (−)-codeinone to (−)-codeine. This reduction is well known to occur with steroids in cell cultures of *Digitalis*, *Nicotania* and *Dioscorea* [13–18], but the reduction of alkaloids by poppy cell cultures has not been demonstrated before.

EXPERIMENTAL

Mps are uncorr. IR spectra were taken in KBr; NMR spectra were determined in $CDCl_3$ using tetramethylsilane as int. ref.; MS were run, using a direct insertion probe.

Tissue culture and administration of (RS)-*reticuline*, (−)-*codeinone and other morphinan alkaloids* The tissue culture used was derived from the capsule of opium poppy (*Papaver somniferum* L. var. Ikkanshu) in 1967 [1, 2] and subcultured for about 10 yr on the modified Murashige and Skoog's tobacco medium containing 1 ppm 2,4-D, 0.1 ppm kinetin, 7% coconut milk and 3% sucrose. The suspension cultures were grown on the same medium for 3 weeks. The medium (250 ml) was dispensed in 1 l. flasks. After 3 weeks culture at 26° in a shaker, 25 mg of (*RS*)-reticuline hydrochloride was administered on each flask under sterile condition. The other alkaloids, thebaine, codeinone, codeine and morphine phosphate, were administered (5 mg/flask) as described above.

Extraction procedure. The cell cultures, harvested with Nylon cloth after 3 days incubation, were homogenized in cold MeOH, filtered and the residue was refluxed with MeOH. The combined soln was concd under red. pres. and acidified with N HCl. The acidic soln was extracted with *n*-hexane to remove neutral and acidic fractions. The aq. soln obtained was made basic to pH 13 with NH_4OH and extracted repeatedly with $CHCl_3$. In the

biotransformation of morphinan alkaloids, the aq. soln was made basic to pH 8.5 and extracted with $CHCl_3$–*iso*-PrOH (4:1). The medium separated from calluses in the both experiments was concd and extracted as above.

Biotransformation of (−)-codeinone by a crude enzyme preparation. By the following procedures at 0–4° the crude enzyme prepn was obtained. 340 g (fr. wt) of the callus was homogenized in an equal vol. of 0.1 M borate buffer, pH 7.6 with Teflon homogenizer. The ppt. from centrifugation at 15000 *g* for 20 min, was suspended in 20 ml of the same buffer and used as crude enzyme for the experiment. The reaction mixture in a total vol. of 10 ml containing 10^{-2} mM NADH, 1 mg codeinone, 5 ml crude enzyme and borate buffer was incubated at 30° for 16 hr. The reaction was stopped by the addition of 12% NH_4OH to pH 8.5 and extracted with the same procedure as above. The alkaloidal fraction obtained from $CHCl_3$–*iso*-PrOH (4:1) was estimated by GLC.

Estimation of the metabolites of (−)-codeinone. The biotransformation ratio from (−)-codeinone to (−)-codeine was determined by GLC under the following operating condition. The R_t values of (−)-codeinone and (−)-codeine were 10.6 and 8.8, respectively. GLC operating condition; 2 m × 3 mm column of Diasolid ZS; oven 220°, detector block 240°, N_2 carrier gas 40 ml/min. JMS-OIS mass spectrometer connected to JGC-20K gas chromatograph was used. 1 m × 4 mm column of 1% OV-1 on chromosorb W at 250°, ionizing energy 70 eV.

Acknowledgements—We are grateful to Prof. C. Tani, Kobe Women's College of Pharmacy and Prof. I. Nishioka, Faculty of Pharmaceutical Sciences, Kyushu University, for their kind supplies of the authentic samples of (−)-(*S*)-scoulerine and (−)-(*S*)-cheilanthifoline, respectively. Thanks are also due to the members of the Analytical Centre, this university for NMR, MS and microelemental analysis. This work was supported in part by the Grant-in-Aid for Scientific Research from the Ministry of Education of Japan.

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