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Studies on Morphine Alkaloids. VII.[1] Microbial Transformation of 14β-Bromocodeinone[2]

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In the microbial transformation by *Trametes sanguinea*, 14β-bromocodeinone (IV) was first converted enzymatically into 14β-bromocodeine (VI), which was gradually affected chemically to form VII, II, and VIII as the final products.

Microbial transformation of morphine alkaloid has been studied systematically by Tsuda, *et al.*[4] and the transformation of codeinone derivatives with a substituent in C_{14} position by *Trametes sanguinea* (Japanese name: Hiirotake) was reported[4c] to show the following changes.

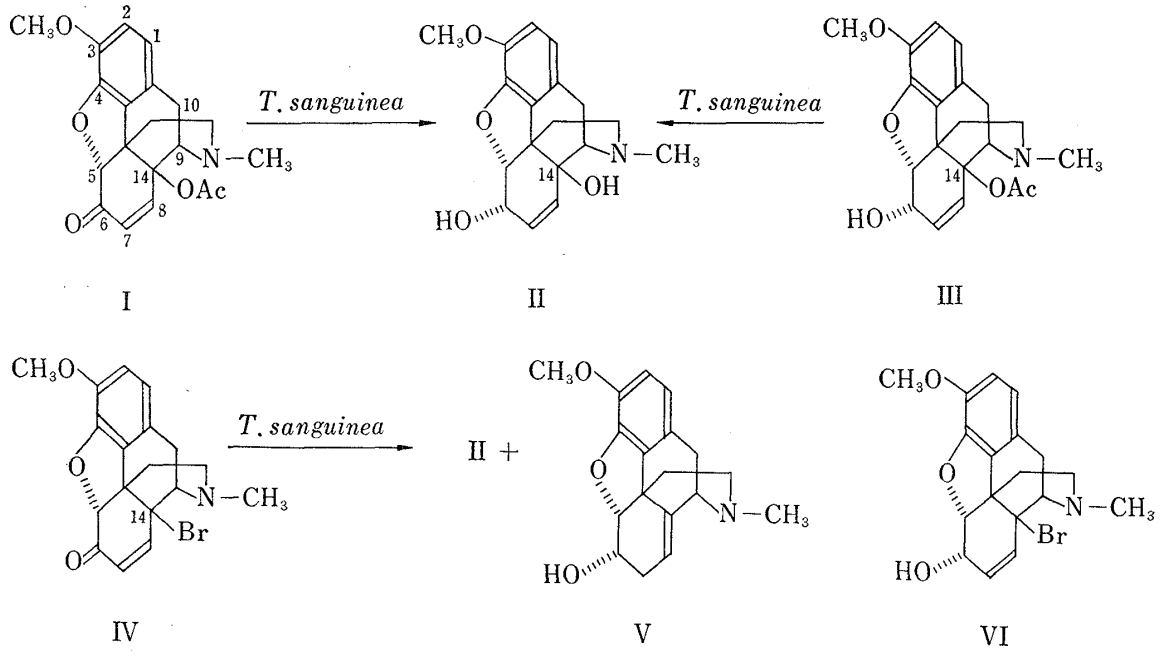

Chart 1

As shown in Chart 1, 14β-acetoxycodeinone (I) and 14β-bromocodeinone (IV) are transformed by *Trametes sanguinea* into 14β-hydroxycodeine (II) with the reduction of carbonyl

1) Part VI: K. Abe, Y. Nakamura, M. Onda, and S. Okuda, *Chem. Pharm. Bull.* (Tokyo), **17**, 1917 (1969).
2) This paper also constitutes part XXV of the series entitled "Studies on Microbial Transformation," Part XXIV: S. Okuda, H. Isaka, M. Iida, Y. Minemura, H. Iizuka, and K. Tsuda, *Yakugaku Zasshi*, **87**, 1003 (1967).
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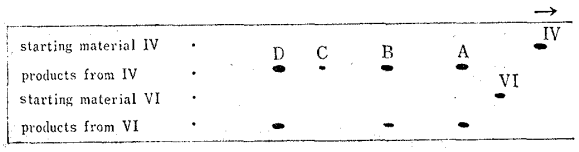
4) *a*) K. Iizuka, M. Yamada, J. Suzuki, I. Seki, K. Aida, S. Okuda, T. Asai, and K. Tsuda, *Chem. Pharm. Bull.* (Tokyo), **10**, 67 (1962); *b*) M. Yamada, K. Iizuka, S. Okuda, T. Asai, and K. Tsuda, *ibid.*, **10**, 981 (1962); *c*) *Idem, ibid.*, **11**, 206 (1963); *d*) M. Yamada, *ibid.*, **11**, 356 (1963); *e*) K. Aida, K. Uchida, K. Iizuka, S. Okuda, and T. Uemura, *Biochem. Biophys. Res. Commun.*, **22**, 13 (1966).

group at C_6 and further substitution of C_{14} position with a hydroxyl group. Transformation of 14β-acetoxycodeine (III) also gives the same result as in the case of I, and the use of IV sometimes gives a small amount of neopine (V), besides II.

In the present series of work, detailed examinations on this transformation were made using compounds having a bromine substituent in C_{14} position as a substrate, and examinations were also carried out on the chemical changes produced in each of these compounds.

Incubation was carried out with 14β-bromocodeinone (IV) and 14β-bromocodeine (VI) by the method previously reported.[4] After incubated in an M-2 medium[5] at 30° for 22 days, the cultural solution was extracted with chloroform and the product thereby obtained were examined by thin-layer (TLC) and gas-liquid chromatography (GLC).



TLC. (*Rf* value)[a]	0.25	0.35	0.50	0.70	0.85	0.90
GLC. (R.R.T.)[b]	0.90	1.14	1.18	2.93	—	—

Fig. 1. Results of Microbial Transformation (TLC and GLC)

a) silica gel plate, solvent system: $CHCl_3$: MeOH=9:1
b) relative retention time using codeine (RT=9.2 min) as standard

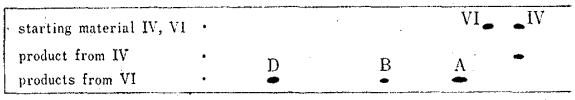


Fig. 2. Results of Blank Test (TLC)

As shown in Fig. 1, three major products, A, B, and D were obtained from either IV or VI, and also a small amount of C, was obtained when IV was used as a substrate. Of these products, B and C correspond respectively to II and V described above.

Then as a blank test, IV and VI were incubated under the same conditions of the medium, temperature, and time, as in microbial transformation but without using any microorganism. The results were shown in Fig. 2. These data indicate that IV is recovered entirely, without any change, under these cultural conditions. On the other hand, VI undergoes change under these conditions, even in the absence of any microorganism, and the result was the same as in microbial transformation.

Considering the fact that both IV and VI give the same product, except compound C, in microbial transformation and that this is the same as the result of blank test on VI, it may be assumed that microbial transformation of IV goes through the route shown in Chart 2.

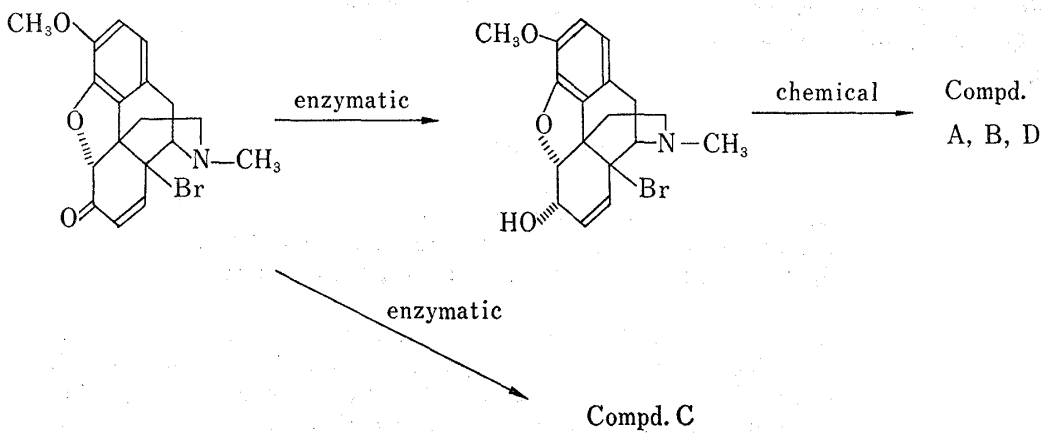
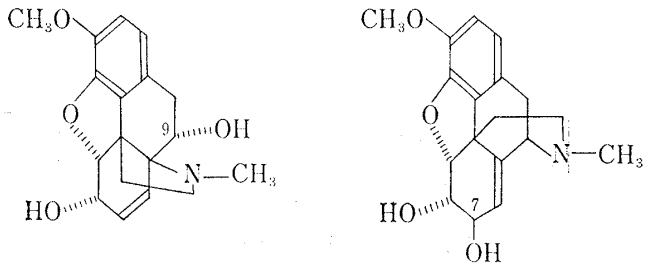


Chart 2

5) Consisting of glucose 1.0%, peptone 0.2%, meat-extract 0.1%, yeast-extract 0.1%, and corn-steep liquor 0.3%.

In this scheme, IV would be converted into VI enzymatically by *Trametes sanguinea* and then VI is changed chemically under the cultural conditions employed into the three compounds A, B, and D, irrespective of the presence or absence of a microorganism. From the quantitative aspect of the products, majority of the reaction seems to have gone through this route but it became clear that there is also a route in which IV is directly transformed into V by the microorganism without passing through VI.

Each of the products were then examined. The above mentioned reaction extract from the cultural solution of IV was separated by column chromatography over alumina (activity grade III) into following four products. The first fractions eluted with benzene were recrystallized from *n*-hexane to give crystal A, mp 194—195°. The product from fractions eluted with benzene–ethyl acetate (1:1) gave crystal B, mp 159—160°, that from ethyl acetate fractions gave crystal C, mp 127—127.5°, and that from ethyl acetate–methanol (3:1) gave crystal D, mp 167—168°. These products were purified by recrystallization and then identified as VII, II, V, and VIII respectively by mixed melting point test and from infrared (IR) and nuclear magnetic resonance (NMR) spectral data (Chart 3).



VII VIII

Chart 3

Some changes of 14β-bromocodeine (VI) in various solvnts have already been reported[6] and its transformation in water is known to involve solvolytic reactions to form VII, II, and VIII. Consequently, the transformation of VI in the present reaction is thought to be due to water in the medium and this point was examined further. Changes in VI when incubated in a medium of different pH's by the use of a phosphate buffer were examined and it was found that the starting material disappeared after incubation at 30° for four days at pH 5.4 (same as the incubation medium used), 7.0, 8.0, or 9.0, and the products formed were the same as those described above. Since VI is sparingly soluble in these buffer solutions, the time required for the reaction was long but if VI were dissolved in aqueous tetrahydrofuran and pH of the solution adjusted with the phosphate buffer, the compound underwent transformation in 5—10 hours of incubation, giving the same result.

From the result of the present experiments, it has become clear that, in the microbial transformation of IV, enzymatic reaction by *Trametes sanguinea* first converts IV into VI which is gradually affected by water in the medium to form VII, II, and VIII as the final products.

Experimental

Melting points were determined on a micro hot-stage and were uncorrected. Gas–liquid chromatography were performed on Barber Colmann Model 10, with argon ionization detector. Column; 1% XE-60 (Nitril silicon) on chromosorb W, Temp; Column: 190°, cell: 230°, flash heater: 215°, carrier gas pressure: 2 kg/cm^2.

Microbial Transformation of 14β-Bromocodeinone (IV) with *Trametes sanguinea*——To a culture solution of *Trametes sanguinea*, (1000 ml), incubated at 30° for 4 days with M-2 medium consisting of 1.0% glucose, 0.2% peptone, 0.1% meat-extract, 0.1% yeast-extract, and 0.3% corn-steep liquor, IV (1000 mg) was added and the culture process was further continued at 30° with periodic analysis of the mixture by TLC and GLC. After 4 days IV began to change and after 22 days, the starting material disappeared completely on TLC. Then the culture medium was made alkaline with 5% NH_4OH, homogenized, and extracted with

6) S. Okuda, K. Abe, and M. Onda, *Chem. Pharm. Bull.* (Tokyo), **16**, 1124 (1968).

$CHCl_3$. The combined $CHCl_3$ layer was shaken three times with 2% HCl and the water layer was again made alkaline with 5% NH_4OH and extracted with $CHCl_3$. The organic layer was washed with H_2O, dried over anhyd. Na_2SO_4, and evaporated *in vacuo* to give oily substance (670 mg). Thus obtained basic residue contains four products identical respectively to be VII, II, V, and VIII by comparison with the authentic samples on TLC and GLC as shown in Fig. 1.

Microbial Transformation of 14β-Bromocodeine (VI)——VI was transformed by *Trametes sanguinea* with the same procedures as employed for IV and from 100 mg of VI gave 61 mg of oily product which contains three spots on TLC, which was identical with VII, II, and VIII as shown in Fig. 2.

Blank Test——Incubation of IV and/or VI was carired out by means of the standard procedures as employed for microbial transformation described above but without using any microorganisms. From 100 mg of IV gave 89 mg of crystalline substance which was recrystallized from acetone–ethanol to give 80 mg of IV (recovered) and from 100 mg of VI gave 71 mg of oily substance which showed the same result as microbial transformation of VI as above.

Purification and Identification of the Products——The microbial transformation products of IV obtained above was separated on alumina column chromatography (Woelm, grade III, 50 g). The first fractions eluted with benzene were recrystallized from *n*-hexane to give colorless needles of 9α-hydroxyindolinocodeine (VII) (117 mg), mp 194—195°. Next fractions eluted with benzene–ethyl acetate (1:1) were recrystallized from ether–pet. ether to give 14β-hydroxycodeine (II) (190 mg), mp 159—160°, and that from ethyl acetate fractions gave neopine (V) (22 mg), mp 127—128°, and from ethyl acetate–methanol (3:1) fractions gave 7β-hydroxyneopine (VIII) (140 mg), mp 167—168°. These compounds were identical respectively with the authentic samples by the comparison of IR and NMR and mixed melting point test.

Hydrolysis of VI with Buffer Solution——To a stirring solutions of phosphate buffer (Kolthoff–Buffer, consisting of 0.1M potassium phosphate and 0.05M sodium borate, adjusted to pH 5.4, 7.0, 8.0, 9.0) (20 ml), 14β-bromocodeine (VI) (100 mg) in tetrahydrofuran (10 ml) was added and the stirring was continued until the starting material (VI) disappeared on TLC (2—3 hr at room temp.). After stayed overnight, organic solvent was evaporated *in vacuo*, and the solution was made alkaline with 5% NH_4OH, extracted with $CHCl_3$. After usual work up, the reaction product was checked on TLC and GLC and separated with column chromatography. The result was the same as that of blank test of VI as described above.

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