(20 ml) was added a solution of Ia (1.19 g) in DMSO (10 ml) and the mixture was heated at 60° for 24 hr. The reaction mixture was poured into ice water and extracted with ether, washed with water and dried over anhydrous potassium carbonate. The solvent was removed *in vacuo* and the residue was recrystallized from *n*-pentane to give 906 mg (75%) of IX, mp 73—74°. *Anal.* Calcd. for $C_{17}H_{17}N$: C, 86.77; H, 7.28; N, 5.95. Found: C, 86.69; H, 7.28; N, 5.91. NMR ($CDCl_3$): δ 3.72 (4H, triplet, $J=2$ cps, CH_2NCH_2), 4.41 (1H, singlet, CH), 4.80 (2H, quartet, $J=2$ cps, $=CH_2$), 7.00—7.50 (10H, multiplet, aromatic).

1-Diphenylmethyl-3-hydroxyiminoazetidine (X)——To a solution of Ia (1.00 g) and hydroxylamine hydrochloride (500 mg) in ethanol (20 ml) was added a solution of sodium hydroxide (500 mg) in water (2 ml) and the mixture was heated at 80° for 15 min. The reaction mixture was poured into water and neutralized with acetic acid. The resulting precipitates were collected and dried. Recrystallization from ether gave 1.03 g (97%) of X, mp 169—170°. *Anal.* Calcd. for $C_{16}H_{16}ON_2$: C, 76.16; H, 6.39; N, 11.10. Found: C, 76.14; H, 6.36; N, 11.22. NMR ($CDCl_3$): δ 3.94 (4H, quartet, $J=4$ cps, CH_2NCH_2), 4.51 (1H, singlet, CH), 7.10—7.45 (10H, multiplet, aromatic), 7.78 (1H, singlet, N-OH).

Spiro-(1-diphenylmethylazetidine-3,5′-hydantoin) (XI)——A mixture of Ia (1.20 g), potassium cyanide (0.65 g) and ammonium carbonate (1.92 g) in 50% ethanol (20 ml) was stirred at 110° in a sealed tube for 15 hr. After cooling the reaction mixture was poured into water and the resulting precipitates were collected and dried. Recrystallization from ethanol gave 465 mg (34%) of (XI), mp 238—240°. *Anal.* Calcd. for $C_{18}H_{17}O_2N_3$: C, 70.34; H, 5.58; N, 13.67. Found: C, 70.31; H, 5.49; N, 13.71. IR ν_{max}^{Nujol} cm^{-1}: 3250 (NH) and 1730 (NHCO). NMR (d_6-DMSO): δ 3.20 and 3.40 (4H, two doublets, $J=8$ cps, CH_2NCH_2), 4.46 (1H, singlet, CH), 7.00—7.50 (10H, multiplet, aromatic), 9.50 and 11.57 (2H, two singlets, NHCONH).

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Studies on Microbial Transformation. XXVI.[1] Microbial Oxidation of (−)-Sparteine

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The successful establishment of the application of microorganisms to the synthesis of biologically active steroids has awaken the interest in the field of microbial conversion of various alkaloids, and hence the systematic investigations of yohimbine-,[3] steroid-,[4] ergot-,[5] and morphine-[6] alkaloids have been carried out in the last decade.

Although lupin alkaloid is one of the large groups in alkaloid kingdom, only one transformation of the compound of this type, *i.e.*, a microbial transformation of (+)-lupanine

1) Part XXV: K. Abe, M. Onda, H. Isaka, and S. Okuda, *Chem. Pharm. Bull.* (Tokyo), **18**, 2070 (1970).
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3) *a*) W.O. Godtfredsen, G. Korsby, H. Lork, and S. Vangedal, *Experientia*, **14**, 88 (1958); *b*) S.C. Pan and F.L. Weisenborn, *J. Am. Chem. Soc.*, **80**, 4749 (1958); *c*) R.E. Hartman, F.F. Krause, W.W. Andrens, and E.L. Patterson, *Appl. Microbiol.*, **12**, 138 (1964).
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5) A. Brack, R. Brunner, and H. Kobel, *Helv. Chim. Acta*, **45**, 276 (1962).
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(XIV) to 17-hydroxylupanine, has been reported.[7] The microbial transformation of (−)-sparteine, which is a typical, important lupin alkaloid and has been used in cardiac arrhythmias and early stage of labour, seemed attractive with the possibility of developing a unique preparation of a new derivative.

Experimental[8]

Substrate——(−)-Sparteine (I) monosulphate ($C_{15}H_{26}N_2 \cdot H_2SO_4 \cdot 5H_2O$), mp 136° (decomp.) supplied by Mochida Pharmaceutical Co., Ltd., was used after recrystallized from EtOH.

Microorganism——The microorganisms used for screening were Basidiomycetes [45 strains from Fermentation Institute, Agency of Industrial Technology and Science. 55 strains from Prof. H. Terakawa's Laboratory, Tokyo Medical and Dental University], Fungi imperfecti (33 strains from Fermentation Institute, Agency of Industrial Technology and Science) and, Acetobacter and Gluconobacter (27 strains from our institute).

Culture Medium——Basidiomycetes and Fungi Imperfecti: Koji extract containing 0.3% corn-steep-liquor.

Acetobacter and Gluconobacter: Glucose (10%), yeast extract (1%), $CaCO_3$ (0.3%). In the case of poor growth, EtOH (5%) was added.

Detection of a Microbial Transformation Product——Ethyl acetate extract of culture broth containing microorganisms, homogenized and adjusted at pH 10—11 with aqueous ammonia, was examined on paper chromatogram. Chromatographic Conditions: Ascending method, Toyo filter paper No. 51, solvent system (A: *n*-butanol, conc. HCl, H_2O (5:1:1:). B: *n*-butanol, AcOH (25:1), saturated with H_2O, color developing reagent (iodoplatinate sol.).

Screening of (−)-Sparteine (I) Transforming Microorganisms——A sterilized medium, prepared as described above, in a test tube (180×20 mm), was inoculated with a test strain and incubated at 28° for the appropriate period (usually 3 or 4 days) on a shaker. To each test tube containing 10 ml of culture was added 5 mg of finely powdered (−)-sparteine monosulphate, and the mixture shaken again under the same conditions to perform microbial transformation. Each culture broth containing cells was homogenized, adjusted basic with aqueous ammonia and extracted twice with each 15 ml of ethyl acetate. The combined extracts were worked up as usual and examined by paper chromatograph.

Microbial Transformation of (−)-Spartein (I) by *Trametes gibbosa*——From the growth test in various media, Koji extract containing 0.3% corn-steep-liquor (pH 4.8—5.0) was chosen in the following experiment. I-Monosulphate (400 mg) was treated in 800 ml of *Trametes gibbosa* culture (8 shake flasks) for a week at 28°. Ethyl acetate extract of culture broth and mycelia was extracted with 3.5% HCl to separate neutral material. Usual work up for recovery of a basic fraction gave about 120 mg of slightly yellow gum which devided into two parts for the following treatments. 1) This half was utilized for characterizing the microbial transformation product. The gum (35 mg) was dissolved in a small amount of $CHCl_3$, allowed to stand at room temperature overnight and after concentration a small amount of EtOH was added. The white crystalline material precipitated was purified by recrystallization from ether to afford 15 mg of fine needles, mp 128—130°. *Anal.* Calcd. for $C_{16}H_{25}N_2Cl_3$: C, 54.63; H, 7.16; Cl, 30.24. Found: C, 54.36; H, 7.26; Cl, 30.18. Mixed melting point test and a comparison of IR spectrum with the authentic sample clearly demonstrated this compound to be 17-trichloromethyl sparteine (III).[9]

The remainder was derived to picrate which after two recrystallizations from EtOH–H_2O gave fine needles, mp 153—155°. Mixed melting point test with authentic sample of 17-hydroxysparteine (II) picrate, mp 153—155°,[9] showed no depression. 2) $K_3Fe(CN)_6$ Oxidation[9] of the Microbial Transformation Product to 17-Oxosparteine (IV): About 60 mg of the gum were dissolved in 1.5 ml of 0.5N H_2SO_4 and then 0.5 g of $K_3Fe(CN)_6$ and 0.5 ml of 16% NaOH were added. After vigorous stirring for 30 min the reaction mixture was repeatedly extracted with ether. Usual work up followed by recrystallization from *n*-pentane gave 45 mg of needles, mp 85—87°. This compound was identified with the authentic IV by comparison of IR spectrum and mixed melting point test.

Result and Discussion

As shown in Table I, 22 strains among 100 tested Basidiomycetes showed transformation of (−)-sparteine (I) to give one product (*Rf*: 0.45 in system A, 0.30 in system B) as a sole

7) M. Mozejko-Tczko, W. Brzeski, and A. Kakolewska-Baniuk, *Bull. Acad. Polon. Sci. Ser. Sci. Biol.*, **11**, 161 (1963).

8) All melting points are uncorrected. IR spectra were taken in $CHCl_3$ solution.

9) M. Rink and K. Grabowski, *Arch. Pharm.*, **61**, 695 (1956).

metabolite. In contrast with this, none of the tested Fungi imperfecti, Acetobacter nor Gluconobacter could transform this substrate.

TABLE I

Microorganisms	Product	Recovered I	*Rf* of product A	*Rf* of product B	Growth
Coprinus comatus	++	+	0.45	0.30	+
Coriolus versicolor	+++	−	0.45	0.30	+
Merulius confluens	++	+	0.45	0.30	++
Pholiota nameko	++	+	0.45	0.30	++
Pleurotus ostreatus	+++	−	0.45	0.30	+++
Pleurotus serotinus	++	+	0.45	0.30	++
Trametes gibbosa	+++	−	0.45	0.30	+++
Strain No. 7[a)]	++	+	0.45	0.30	+
Strain No. 19[a)]	+	++	0.45	0.30	++
Strain No. 73[a)]	+++	±	0.45	0.30	++
Strain No. 85[a)]	+	++	0.45	0.30	++
Strain No. 118[a)]	+	++	0.45	0.30	++
Strain No. 122[a)]	++	+	0.45	0.30	++
Strain No. 195[a)]	+	++	0.45	0.30	+
Strain No. 224[a)]	+++	−	0.45	0.30	++
Strain No. 253[a)]	++	+	0.45	0.30	+++
Strain No. 272[a)]	+	++	0.45	0.30	++
Strain No. 282[a)]	+++	±	0.45	0.30	++
Strain No. 316[a)]	+++	−	0.45	0.30	++
Strain No. 338[a)]	+	++	0.45	0.30	+++
Strain No. 549[a)]	+++	−	0.45	0.30	+++
Strain No. 615[a)]	+	++	0.45	0.30	++

solvent system A: *n*-BuOH: HCl: H_2O=5:1:1
solvent system B: *n*-BuOH: CH_3COOH=100:4 (saturated with H_2O)
a) These strains were kindly offered by Prof. Terasaka and were not identified.

Trametes gibbosa (Japanese name: Oochirimentake) was found most suitable for studying this transformation because of the relative ease in cultivation and the marked transforming ability. The product obtained by transformation with this microorganism was derived to picrate and trichloromethyl derivative (III) and its structure was proved to be 17-hydroxysparteine (II). Although the paper chromatogram of this product showed the existence of only one basic compound and the absence of starting material, several attempts to isolate this as crystalline state were not successful. To determine the transformation yield, potassium ferricyanide oxidation was applied because this procedure was known as a mild and

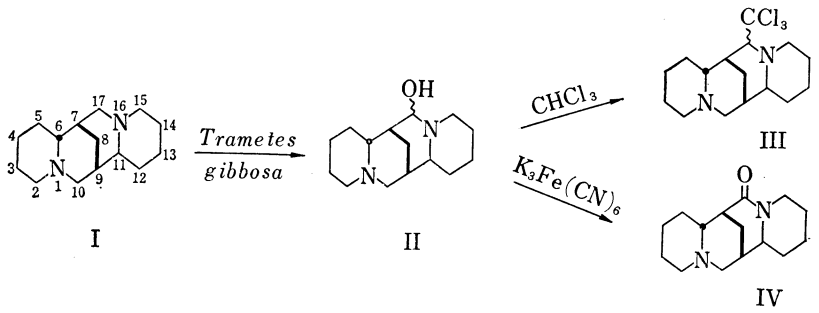

Chart 1

selective one to convert II into crystalline 17-oxosparteine (IV). Thus the microbial transformation yield was shown about 38% based from the weight of crystalline IV. It is noteworthy that this C_{17}-methylene in I, microbiologically oxidized, is also the only position which can be oxidized by potassium ferricyanide in alkaline solution.

The transformations of various quinolizidine type compounds, quinolizidine (V), (−)-lupinine (VI), (+)-epilupinine (VII), (−)-α-isosparteine (VIII), (−)-lupanine (IX), (+)-hydroxylupanine (X), (−)-anagyrine (XI), (+)-matrine (XII) and (+)-matridine (XIII), by this microorganism were preliminarily tested under the same conditions which I was transformed into II. Although VIII was converted into a more polar derivative, the other bases were recovered without any changes. The structure of the product from VIII was not further investigated because of shortage of material.

In regard to the absolute configuration of C_8-methylene bridge,[10] I, VIII and X are the same, but those of IX and XI antipodal. The relationship between methylene bridge and C_{11}–H is *trans* in I, IX, X, and XI, and *cis* in VIII.[10] It is rather interesting that *Tramctes gibbosa* can transform I and VIII but cannot IX, whose framework is the same as that of I excepting the substituents. If the microbial transformation of (+)-lupanine (XIV) is carried out with this microorganism, a role of 13-hydroxyl group will be rationalized.

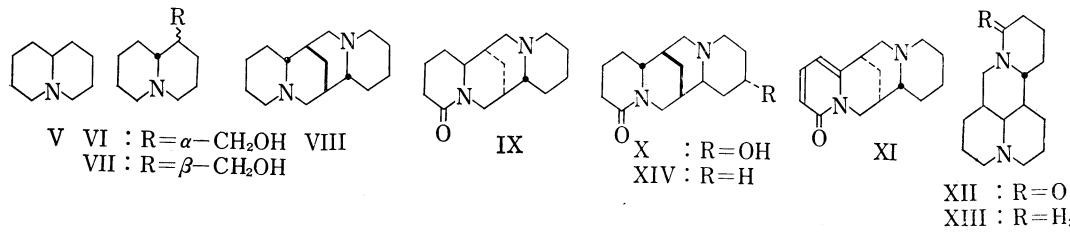

Chart 2

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10) N.J. Leonard, "The Alkaloids: Lupin Alkaloids," Vol. 7, Academic Press, New York-London, 1960, p. 253; F. Bohlmann and D. Schuman, *ibid.*, Vol. 9, 1967, p. 175.