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Chemical and Enzymic Phenol Oxidation of (*R*)(−)-N-Methylcoclaurine and (*S*)(+)-Reticuline (Studies on the Syntheses of Heterocyclic Compounds. CMIII[1])

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Phenol oxidation of (*R*)(−)-N-methylcoclaurine (V) and (*S*)(+)-reticuline (VI) with peroxidase was examined in order to get the bisbenzylisoquinoline type compounds, but only the cleaved products were obtained. (±)-O-Benzyl-N-methylcoclaurine (X) also afforded the cleaved one in the same reaction. However, ferricyanide oxidation of VI gave isoboldine (IX) and pallidine (VIII) together with the cleaved products, isovanillin (XIV) and thalifoline (VII). Moreover, phenol oxidation of cuspidaline (XVII) was also described.

Bisbenzylisoquinoline type alkaloids are biosynthesized by phenol oxidation of coclaurine (I) or its biogenetic equivalents.[3] Thus, Barton[4] biosynthesized epistephanine (II) by a feeding experiment of T-labelled coclaurine to *Stephania japonica*. Several attempts to synthesize the bisbenzylisoquinoline alkaloids were carried out by chemical methods in laboratory, but gave the products having biphenyl ether bond which could not be observed in Nature.[5-8] On the other hand, since enzymic oxidation[9] of (±)-N-methylcoclaurine (III) gave liensinine type compound (IV)[10], the phenol oxidation of optically active phenolic isoquinolines (V and VI) with peroxidase was investigated. We here wish to report the results on this type of reaction together with the biogenetic type synthesis of thalifoline (VII). Moreover we wish to report the total synthesis of optically active pallidine (VIII) and isoboldine (IX) by phenol oxidation of (*S*)(+)-reticuline (VI) along the biogenetic theory.[3]

Firstly, we investigated the conversion of (±)-O-benzyl-N-methylcoclaurine[11] (X) into dauricine type compound (XI). Thus, X was oxidized with peroxidase[12] and hydrogen peroxide at pH 6.6 with stirring at 25—28° for 30 hr and the crude product was reduced by sodium borohydride to give O-benzylcorypalline[13] (XII) after separation by silica gel column chromatography. In this reaction, we could not detect the dimeric product by a mass spectrometry.

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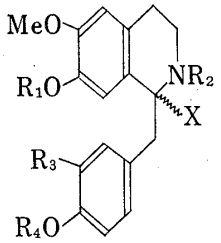
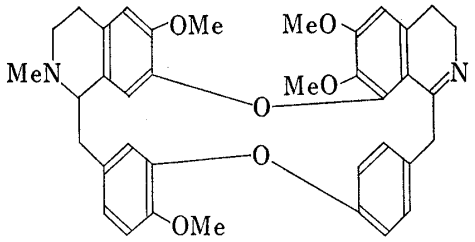
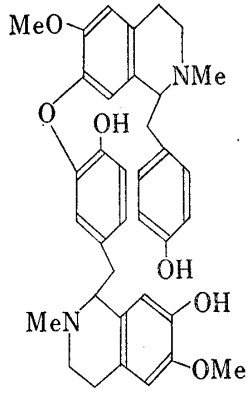




II

I : R_1=H, R_2=H, R_3=H, R_4=H, X=〰H
III : R_1=H, R_2=Me, R_3=H, R_4=H, X=〰H
V : R_1=H, R_2=Me, R_3=H, R_4=H, X=◄H
VI : R_1=H, R_2=Me, R_3=OH, R_4=Me, X=----H
X : R_1=$CH_2C_6H_5$, R_2=Me, R_3=H, R_4=H, X=〰H



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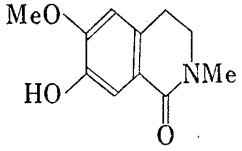
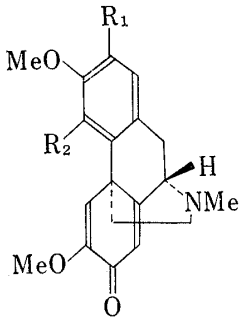
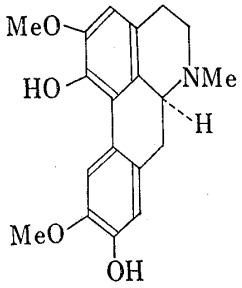


VII



VIII : R_1=OH, R_2=H
XVIII : R_1=H, R_2=OH



IX

Chart 1

The same oxidation of (*R*)(−)-N-methylcoclaurine (V)[14] with peroxidase and hydrogen peroxide, followed by reduction with sodium borohydride, afforded corypalline (XIII)[15] in addition to two unidentified bases.

(*S*)(+)-Reticuline (VI) was also treated with peroxidase and hydrogen peroxide at pH 7.5 for 36 hr to give isovanillin (XIV) and thalifoline (VII),[16] a natural product from *Thalictrum minus* var. *adianitifolium*, as the cleaved products. The structure assignment of the latter compound (VII) was achieved by the mixed melting point test and spectral comparisons with the authentic sample.[17] As thalifoline would be biosynthesized by the oxidative cleavage of 1-benzylisoquinoline,[16] this work constituted the biogenetic type synthesis of thalifoline (VII).

Limacusine (XV) and limacine (XVI) were isolated together with cuspidaline (XVII) from *Limacia cuspidata*,[18] and so the phenol oxidation of cuspidaline (XVII) would biosynthesize limacusine and limacine.[18] Therefore, we examined the oxidation of (±)-cuspidaline[19,20] by peroxidase and hydrogen peroxide at pH 6.5, but recovered the starting material. Moreover, (−)-cuspidaline and its dimethiodide were treated with potassium ferricyanide and sodium

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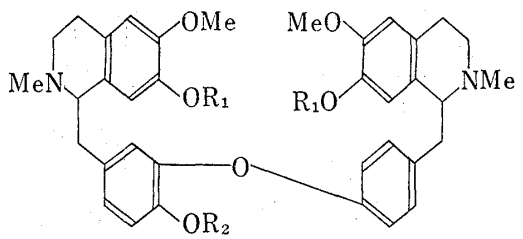
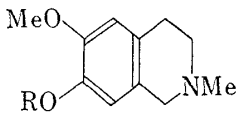
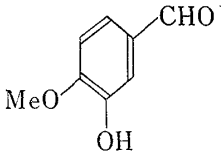


XI : $R_1=CH_2C_6H_5$, $R_2=H$
XVII : $R_1=H$, $R_2=Me$

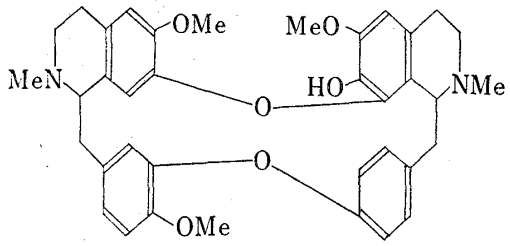


XII : $R=CH_2C_6H_5$
XIII : R=H

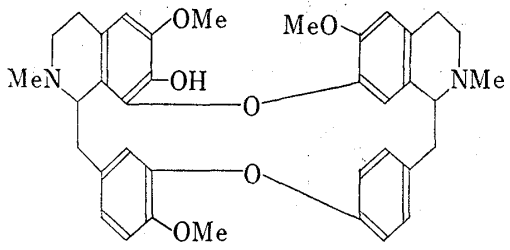


XIV

Chart 2



XV



XVI

Chart 3

bicarbonate to give the unidentified products. In both cases, we could not obtain the expected compounds (XV and XVI).

Finally, (*S*)(+)-reticuline (VI) was oxidized with potassium ferricyanide in the presence of 5% sodium bicarbonate in chloroform[21] to give five compounds after separation by silica gel column chromatography. The first one was assigned to isovanillin (XIV), and the second to thalifoline (VII). The third component, obtained in 0.06% yield, showed the cyclohexadienone system in its infrared (IR) spectrum, which was closely similar to that of the sinoacutine (XVIII), but a further investigation could not be done because of lack of sample. The fourth one was isoboldine (IX), and the fifth component was assigned to pallidine (VIII). Both compounds (VIII and IX) were the same in all aspects with the samples obtained in Nature.[22]

It is interesting that the treatment of the phenolic benzylisoquinoline under the condition of phenol oxidation afforded the cleaved products, isocarbostyril and benzaldehyde derivatives. We are now investigating the enzymic oxidation of the phenolic isoquinoline.

Experimental

The IR spectra were measured with Shimazu IR-27 or Hitachi EPI-3 recording spectrophotometer and ultraviolet (UV) spectra were taken with a Hitachi recording spectrophotometer. Nuclear magnetic resonance (NMR) spectra were measured with Hitachi R-20 or JNM-MH-60 with tetramethylsilane as internal standard, and mass spectra were determined on Hitachi RMU-7 mass spectrometer. Optical rotations were measured with JASCO-PIP-SL automatic polarimeter.

Phenol Oxidation of (±)-O-Benzyl-N-methylcoclaurine (X)——To a solution of 1.5 g of (±)-O-benzyl-N-methylcoclaurine[11] (X) in a small amount of AcOH was added 100 ml of 3% aq. $AcONH_4$ solution and the mixture was adjusted to pH 6.6 by an addition of 10% NH_4OH. To this solution was added 10 mg of peroxidase[12] and 6 ml of 3% H_2O_2, and the mixture was stirred for 20 hr at 25—28°. After further addition of 10 mg of peroxidase and 6 ml of H_2O_2, the mixture was stirred for 10 hr at the same temperature, and then basified with 28% NH_4OH. This solution was extracted with $CHCl_3$ and butanol, and the combined extracts were washed with H_2O, dried over Na_2SO_4, and evaporated to leave 0.85 g of a reddish brown oil, whose solution in 7 ml of $CHCl_3$ and 3 ml of MeOH was reduced with 0.6 g of $NaBH_4$ under stirring for 0.5 hr at room temperature and then under reflux for 0.5 hr. The usual work up gave 0.7 g of a pale brown viscous

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syrup, which was chromatographed on 15 g of silica gel. The first eluant with $CHCl_3$ gave 38 mg of O-benzylcorypalline (XII) as colorless needles, mp 109—110° (lit,[13] mp 110—111°), after recrystallization from ether–petr. ether. The IR (KBr) and NMR spectra of XII were superimposable upon those of the authentic sample prepared by the standard method,[13] and the mixed mp test showed no depression. The second eluant by $CHCl_3$–MeOH (100:1 and 100:2) afforded 520 mg of the starting material as colorless prisms, mp 138—140°, after recrystallization from C_6H_6–hexane.

Phenol Oxidation of (*R*)(−)-N-Methylcoclaurine(V)——A solution of 1.3 g of (*R*)(−)-N-methylcoclaurine[14] (V) in a small amount of AcOH was added to 100 ml of 3% $AcONH_4$, and this solvent was adjusted to pH 6.5 by 10% NH_4OH. After addition of 10 mg of peroxidase and 3 ml of 10% H_2O_2, the mixture was stirred for 3 days at 23—26° in a current of N_2. During this reaction, 10 mg of peroxidase and 1 ml of 10% H_2O_2 were added every 24 hr. After the reaction, the mixture was basified with 28% NH_4OH and extracted with $CHCl_3$. The extract was washed with H_2O, dried over Na_2SO_4, and the solvent was distilled off to leave 0.7 g of a reddish brown syrup, which was stirred with 1 ml of Ac_2O, 2 g of anhydrous Na_2CO_3, and 10 ml of $CHCl_3$ for 4 hr and then set aside overnight at room temperature. After decomposition of the excess of Ac_2O with H_2O, the organic layer was separated, washed with H_2O, dried over Na_2SO_4, and evaporated to give 0.78 g of a yellowish brown syrup, which was chromatographed on 12 g of silica gel. The first $CHCl_3$ eluant afforded 450 mg of O,O-diacetyl-N-methylcoclaurine as a pale yellow syrup. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1762 (CH_3CO). NMR δ (ppm): 2.22 (6H, s, $CH_3CO\times2$), 2.45 (3H, s, NCH_3), 3.73 (3H, s, OCH_3). This was refluxed with 5% methanolic KOH for 30 min to give (*R*)(−)-N-methylcoclaurine (V). The second eluant by $CHCl_3$ and $CHCl_3$–MeOH (100:1—3) afforded 85 mg of a viscous syrup, which was further subjected to column chromatography on 2 g of silicic acid. The first $CHCl_3$ eluant gave 5 mg of unknown base (A) (IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1740—1750) and 8 mg of unknown base (B) (IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1765) as a pale brown viscous syrup.

To the mother liquor in the first extraction by $CHCl_3$ was added 2 g of sodium borohydride within 45 min, and the mixture was allowed to stand for 1 hr. The separated oil was extracted with butanol, and the extract was washed with H_2O, dried over Na_2SO_4, and evaporated to leave 0.45 g of a brown syrup, which was chromatographed on 4.5 g of silica gel. The $CHCl_3$–MeOH (100:1) eluant gave 110 mg of corypalline (XIII) as colorless needles, mp 168—169°, whose IR spectrum (KBr) and mp were identical with those of the authentic sample prepared by the standard route.[15]

Enzymic Oxidation of (*S*)(+)-Reticuline(VI)——To a solution of 1.5 g of (*S*)(+)-reticuline (VI) in 6 ml of AcOH and 30 ml of H_2O was added 200 ml of 3% $AcONH_4$, and the mixture was adjusted at pH 7.5 by addition of 10% NH_4OH. To this solution was added 20.5 mg of peroxidase and 15 ml of 3% H_2O_2, and the mixture was stirred for 16 hr in a current of N_2. After further addition of 32 mg of peroxidase and 15 ml of 3% H_2O_2, the mixture was stirred for 20 hr in a current of N_2 and then basified with 10% NH_4OH and extracted with $CHCl_3$. The extract was washed with H_2O, dried over Na_2SO_4, and evaporated to leave 1.1 g of a dark brown gum, which was chromatographed on 50 g of silica gel. The $CHCl_3$ eluant gave 8 mg of isovanillin (XIV), identical with an authentic sample by IR spectral comparison. The second eluant wih $CHCl_3$–MeOH (99.5:0.5) gave 15 mg of thalifoline (VII) as colorless rods, mp 211—212° (lit.[16] 210—211°) after recrystallization from MeOH. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3500 (OH) and 1640(C=O). UV λ_{max}^{MeOH} mμ: 261 and 302. NMR δ (ppm): 2.88 (2H, t, J=7.5Hz, $ArCH_2CH_2N\langle$), 3.10 (3H, s, NCH_3), 3.52 (2H, t, J=7.5 Hz, $ArCH_2CH_2N\langle$), 3.88 (3H, s, OCH_3), 6.57 (1H, s, C_5-H) and 7.68 (1H, s, C_8-H). Mass Spectrum *m/e*: 207 (M^+), 164, and 136. The spectral data were identical with the authentic sample[17] prepared by the standard method.[16] The fractions with $CHCl_3$–MeOH (99:1, 98:2 and 95:5) recovered the starting material.

Phenol Oxidation of VI with Potassium Ferricyanide——To a mixture of 5 g of (*S*)(+)-reticuline (VI) in 250 ml of $CHCl_3$ was added a solution of 20 g of $NaHCO_3$ in 350 ml of H_2O, to which was added dropwise a solution of 11.5 g of $K_3[Fe(CN)_6]$ in 400 ml of H_2O during 30 min with stirring. Stirring was continued under nitrogen at 15—20° for a further 30 min and the organic layer was separated. The aqueous layer was extracted with chloroform. The combined extracts were washed with H_2O, dried over Na_2SO_4, and distilled off to leave a dark gum, which was chromatographed on 100 g of silica gel. The $CHCl_3$ eluant gave 10 mg of isovanillin (XIV), which was identical with the authentic sample. The second eluant by $CHCl_3$–MeOH (99.5:0.5) afforded 13 mg of thalifoline (VII), whose IR spectrum was identical with that of an authentic sample.[16,17] The $CHCl_3$–MeOH (98:2) eluant gave 82 mg of a brown syrup having the carbonyl band in its IR spectrum, which was subjected to chromatography on 10 g of silica gel. The first $CHCl_3$ eluant furnished 3 mg of sinoacutine-like compound, which was not confirmed. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3560 (OH), 1670, 1645 and 1625 (cyclohexadienone system). Mass Spectrum *m/e*: 327 (M^+). The second $CHCl_3$ eluant gave 23 mg of (+)-isoboldine (IX), mp 122—124°, $[\alpha]_D^{26}$ +42.5° (*c*, 0.093 in $CHCl_3$) [lit.[22] +62° (*c*, 0.114 in $CHCl_3$)], as a pale brown powder after recrystallization from ethanol, whose spectral data were superimposable upon those of a natural isoboldine.[22] The third $CHCl_3$ eluant afforded 21 mg of pallidine (VIII) as a pale yellow syrup, $[\alpha]_D^{26°}$ −33.9° (*c*, 0.560 in EtOH) [lit.[22] −32° (*c*, 0.160 in EtOH)] which was identical in all aspects with a natural pallidine.[22]

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