Summary

A simple and sensitive method for extraction and detection of morphine in urine was described.

The continuous extraction method showed a virtually complete recovery of morphine and prevented the formation of emulsion unavoidable in the extraction by shaking.

The hydrolysis condition which led to liberation of most of morphine from the conjugates in urine was found to be that of heating for 30 minutes, in the 15% hydrochloric acid concentration. This procedure was simple, but afforded the comparable result to that of sealed tube hydrolysis in the 5% hydrochloric acid concentration.

Double thin-layer chromatographic method was successfully applied first to the separation of morphine from a large amount of impurities in the urine extract using thicker plate (1 mm.) and then to the final detection of morphine using ordinary one (0.25 mm.).

(Received June 9, 1965)

12. Tetsuji Kametani, Seiichi Takano, Kazuko Masuko, and Fujinori Sasaki: Bisbenzylisoquinoline Alkaloids and Related Compounds. V.** A Total Synthesis of Diastereoisomeric Mixture of Liensinine.*2,*3

(Pharmaceutical Institute, Tohoku University School of Medicine**)

The isolation of a new alkaloid, liensinine, with the composition of C_{42}H_{54}O_{4}N_{4}, m.p. 95°-99°, from the embryo loti of Nelumbo nucifera Gaertn. was described by Chao-yuan, et al.1) and it was then shown that liensinine possessed the structure (I), mainly based on the results of Hoffmann degradation and potassium permanganate oxidation.2)

The purpose of the present investigation was to study the Ullmann reaction between both tetrahydroisoquinoline derivatives, X and XVII, in order to obtain diastereoisomeric mixture of O,O-dibenzylliensinine (II) as a possible intermediate for the synthesis of I', possessing the same planar structure as natural liensinine (I). Benzylolation of 3-bromo-4-hydroxyphenylacetic acid3) (III) with benzyl chloride in the presence of sodium ethoxide in ethanol afforded 3-bromo-4-benzoxoxyphenylacetic acid (IV), which was converted into the acid chloride (V).

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*2 This forms Part CXXXII of “Studies on the Syntheses of Heterocyclic Compounds” by T. Kametani.
*3 This work was reported at the Tohoku Branch Meeting of Pharmaceutical Society of Japan, February 20, 1965.
** Kita–4-bancho, Sendai (巻道香治, 高野誠一, 塚生和子, 佐々木康紀).
Bischler–Napiersalski cyclization of the amide (Ⅶ), which was obtained by Schotten–
Baumann reaction of 3,4-dimethoxyphenethylamine (Ⅵ) with acid chloride (Ⅴ) gave the
1-(3-bromo-4-benzoylbenzyl)-6,7-dimethoxy-3,4-dihydroisoquinoline (Ⅶ) as an oil,
which was characterized as the styrphate, m.p. 200~200.5°. Reduction of the methio-
dide (Ⅷ) of the above dihydroisoquinoline derivative (Ⅶ) with sodium borohydride in
methanol gave one component (X) of the starting materials as a pale brown glass,
which was characterized as the perchlorate, m.p. 180.5~181.5°.

Schotten–Baumann reaction of 3-methoxy-4-tosyloxyphenethylamine4,5 (Ⅲ) with
4-benzoylphenacetyl chloride6 (Ⅳ) afforded the amide (Ⅲ) as colorless needles, m.p.
100~101°. Bischler–Napiersalski reaction of the above amide (Ⅲ) with phosphoryl
chloride in benzene gave the dihydroisoquinoline derivative (XIV), which was converted
into the methiodide (XV). Reduction of the compound (XV) with sodium borohydride
gave the tetrahydroisoquinoline derivative (XVI), whose detosylation yielded the sub-
stance (XVII) on being allowed to stand in ethanolic potassium hydroxide solution
overnight.

Ullmann condensation between X and
XVII was carried out in the presence of
copper powder, potassium carbonate and a small amount of potassium iodide in pyri-
dine.7 During the reaction, the samples from the reaction mixture were taken up,

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<th>Fraction No.</th>
<th>Yield (mg.)</th>
<th>Beilstein test</th>
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<tr>
<td>F₁₁</td>
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<td>F₂₂</td>
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and inspected by thin-layer chromatography, whose data were shown in Fig. 1. Only a new spot (C) was detected and attributed to that of diastereoisomeric mixture of O,O-dibenzzylliensinine (II) by the results described in the experimental section. And then it was also revealed that the debrominated substance of X or the diphenyl derivative, which would be formed by bimolecular Ullmann reaction of X were not formed in this reaction.

Alumina-chromatography of the product as above gave diastereoisomeric mixture of O,O-dibenzzylliensinine (II) in a yield of 23%, which was characterized as the perchlorate. Finally, treatment of the above compound (II) with concentrated hydrochloric acid yielded the synthetic lienisinine (I'), m.p. 95~105°C, with infrared and ultraviolet spectra superimposable on those of natural lienisinine, which was extracted from the embryo loti of *Nelumbo nucifera* GAERTN., according to the procedure reported by Chao Tse-yuan, et al.¹) Both specimens behaved similarly on paper and thin-layer chromatography, but nuclear magnetic resonance spectra of synthetic and natural lienisinine showed a marked difference in a signal of methoxyl radicals as described in experimental section. Since Fujitani, et al.²) recently reported on the difference of nuclear magnetic resonance spectrum between natural product and the diastereoisomeric mixture in case of bisbenzylisoquinoline type alkaloid, which contains only one diphenyl ether group, I must be a diastereoisomeric mixture.

These facts reveal that the total synthesis of a mixture of stereoisomeric liensinine has been accomplished and the planar structure of natural liensinine (I) is correct.²⁶

Experimental²⁶

3-Bromo-4-benzyloxyphenylacetic Acid (IV)—To a solution of 2.4 g of EtONa in 58 ml of ethanol, 30 g of 3-bromo-4-hydroxyphenylacetic acid (II) was added, giving a red clear solution to which 33 g of benzyl chloride was added, and the mixture was heated on a water-bath. After 7 hours' refluxing a red color disappeared and NaCl separated. The mixture was distilled off, admixed with 180 ml of H₂O, and extracted with benzene. The extract was washed with H₂O, dried on Na₂SO₄, and distilled to give an oil, which was heated under reflux with a mixture of 4.4 g of KOH, 4.4 ml of water and 44 ml of EtOH in a current of N₂ for 16 hr., in order to complete the hydrolysis of benzyl 3-bromo-4-benzyloxyphenylacetic acid (V) as colorless needles, m.p. 115~115.5⁰. Anal. Calcd. for C₉H₈O₂Br : C, 56.12; H, 4.02. Found : C, 55.82; H, 4.31.

N-(3,4-Dimethoxyphenethyl)-3-bromo-4-benzyloxyphenylacetamide (VII)—To a suspension of 1.7 g of the preceding acid (V) in 24 ml of ligroin was added 0.76 g of thionyl chloride, and the mixture was heated at 62⁰ on a water-bath for 2 hr. Removal of an excess of thionyl chloride in vacuo gave 3-bromo-4-benzyloxyphenacyl chloride (V) as a crystalline solid, which was extracted with 10 ml of CHCl₃.

A solution of 2.4 g of 3,4-dimethoxyphenethylamine (VI) in 10 ml of CHCl₃ was dropwise added to a cooled and stirred CHCl₃ solution of V as above. After the addition, the mixture was allowed to stand for 1 hr., washed with 3% HCl, saturated aq. NaHCO₃, and H₂O, and dried on K₂CO₃. Removal of the solvent gave a viscous syrup, which was recrystallized from MeOH to give the amide (VII) as colorless long needles (2.3 g, 87.5%), m.p. 129.5~130.5⁰. Anal. Calcd. for C₂₃H₂₃O₂N : C, 61.98; H, 5.14; N, 2.89. Found : C, 61.95; H, 5.36; N, 2.92.

1-(3-Bromo-4-benzyloxybenzyl)-6,7-dimethoxy-3,4-dihydroisoquinoline (VIII)—A mixture of 3 g of the above amide (VII), 25 ml of dry benzene, and 2.5 ml of POCI₃ was refluxed for 20 min. until a gas of HCl no longer evolved. An excess of 250 ml of ligroin was added to the reaction mixture and an upper layer was removed by decantation. The residue was repeatedly washed with ligroin and dissolved in CHCl₃. The resultant CHCl₃ solution was poured into an excess of cooled and stirred ammonium hydroxide solution. The solvent was separated, washed with H₂O, dried on K₂CO₃, and distilled, to give 2.2 g of the dihydroisoquinoline (VIII) as a pale yellow viscous syrup. Recrystallization of the ploceronate from EtOH yielded yellow needles, m.p. 200~200.5⁰ (decomp.). Anal. Calcd. for C₂₃H₂₃O₂NBr-C₂₃H₂₃O₂:: C, 56.15; H, 4.57; N, 9.36. Found : C, 56.50; H, 4.17; N, 9.55. The perchlorate of VIII was characterized by recrystallization from MeOH as colorless feathers, m.p. 179~180.5⁰. Anal. Calcd. for C₂₃H₂₃O₂NBr·HClO₄·½H₂O : C, 52.14; H, 4.50; N, 2.43. Found : C, 51.91; H, 4.56; N, 2.14. IR νmax cm⁻¹ : 3450 (OH) (water of crystallization).

1-(3-Bromo-4-benzyloxybenzyl)-2-methyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (X)—To the preceding dihydroisoquinoline (VIII) was added an excess of methyl iodide, which was enough for dissolving the compound (VIII). The mixture was allowed to stand at room temperature for a brief period during which time crystals separated. Evaporation of an excess of methyl iodide, with a quantitative yield, gave the methiodide (X), m.p. 200~201⁰ (decomp.), which was too easy to become resinosus, and, therefore, used without recrystallization in the following reaction. Only one recrystallization from methanol gave the crude methiodide (X) as yellow plates. Anal. Calcd. for C₂₃H₂₃O₂Br·HNO₃ : C, 50.59; H, 4.41; N, 2.27. Found : C, 51.21; H, 4.56; N, 2.33.

Sodium borohydride (4 g) was added in small portions with shaking to a cooled suspension of 4.1 g of the above crude methiodide (X) in 30 ml of CHCl₃ and 100 ml of EtOH, the yellow solution becoming

²⁶ Recently a total synthesis of liensinine has been reported independently by Hsich, et al.²⁶
²⁷ Infrared and ultraviolet spectra were measured on a Type EPI-2 Hitachi infrared spectrophotometer and Type ETS-3 Hitachi recording spectrophotometer, respectively. Nuclear magnetic resonance spectra were determined on a Varian A-60 spectrophotometer with deuteriochloroform as solvent and tetramethylsilane as internal reference. Melting points were determined on a Kofer block and uncorrected.
²⁸ This was dried over P₂O₅ at room temperature (3 mm Hg) for 36 hr.
colorless. After the reaction the solvent was distilled off in vacuo, the residue was treated with a small amount of 1% NaOH and H₂O, and extracted with CHCl₃. The CHCl₃ extract was washed with H₂O, dried on K₂CO₃, and evaporated, leaving 2.8 g. of the tetrahydroisoquinoline derivative (X) as a colorless viscous syrup. Recrystallization of the perchlorate of X from EtOH afforded colorless scales, m.p. 180–181°. *Anal.* Calcd. for C₉H₆O₃NBr·HClO₄: C, 53.56; H, 5.01; N, 2.40. Found: C, 53.07; H, 5.33; N, 2.34.

N-(3-Methoxy-4-tosoyloxyphenethyl)-2-(4-benzoyloxyphenyl)acetamide (XIII) — A solution of 3.81 g. of 3-methoxy-4-tosoyloxyphenethylamine (XII) in 50 ml. of CHCl₃ was added to a cooled solution of 2.5 ml. of triethylamine in 25 ml. of CHCl₃. To the above mixture was dropwise added a solution of 2.94 g. of 4-benzoyloxyphenylacetic chloride (X) in 25 ml. of CHCl₃. After being allowed to stand at room temperature overnight, the solvent was distilled off at <50°, and the residue was crystallized on being triturated with a small amount of MeOH. Recrystallization from MeOH gave 3.5 g. of the amide (XIII) as colorless scales, m.p. 108–109°. *Anal.* Calcd. for C₁₅H₁₆O₅NS: C, 68.23; H, 5.73; N, 2.57. Found: C, 67.95; H, 5.76; N, 2.67. IR νmax cm⁻¹: 3400 (NH), 1655 (C=O), 1370 and 1140 (SO₄=O).

1-(4-Benzoyloxybenzyl)-2-methyl-6-methoxy-7-tosoyloxy-1,2,3,4-tetrahydroisoquinoline (XVI) — A mixture of 0.5 g. of the above amide (XIII), 3 ml. of dry benzene, and 2 ml. of POCl₃ was heated at 73–75° for 30 min. and then at 80° for 45 min. After the mixture had been cooled, 100 ml. of ligroin was added to it and an upper layer was removed by decantation. The residual oil was dissolved in 30 ml. of CHCl₃ and decomposed with an excess of saturated NaHCO₃. The solvent was separated, washed with H₂O, dried on Na₂SO₄, and distilled off, to give 0.4 g. of 1-(4-benzoyloxybenzyl)-6-methoxy-7-tosoyloxy-3,4-dihydroisoquinoline (XIV) as a reddish-brown syrup, which could not be characterized as crystals.

A mixture of 0.4 g. of the above dihydroisoquinoline (XIV) and 1 ml. of methyl iodide was allowed to stand at room temperature overnight, and removal of an excess of methyl iodide at <30° in vacuo gave the methiodide (XV) as a reddish-brown caramel-like solid.

To a solution of the above methiodide (XV) in 30 ml. of MeOH was added 0.5 g. of sodium borohydride in small portions with shaking. After the reaction, the solvent was distilled off, the residue treated with H₂O and extracted with CHCl₃. The CHCl₃ extract was washed with H₂O, dried on Na₂SO₄, and evaporated, leaving a pale green viscous syrup, which was again extracted with ether. Removal of the solvent gave 0.31 g. of the tetrahydroisoquinoline derivative (XVI) as an oily syrup. Recrystallization of the staphyline from MeOH gave yellow scales, m.p. 92–93°. *Anal.* Calcd. for C₁₅H₁₆O₅NS: C, 57.86; H, 4.60; N, 7.10. Found: C, 58.19; H, 4.69; N, 6.71.

1-(4-Benzoyloxybenzyl)-2-methyl-6-methoxy-7-hydroxy-1,2,3,4-tetrahydroisoquinoline (XVII) — A solution of 0.9 g. of the preceding tetrahydroisoquinoline (XVI) in 3 ml. of tetrahydrofuran was admixed with 2.3 ml. of 5% ethanolic KOH solution. After being allowed to stand at room temperature overnight, the mixture was distilled off at <40°, and 20 ml. of H₂O was added. This was treated with crystalline NH₄Cl until the solution showed pH 8.8, and the yellow precipitate formed was extracted with benzene. The benzene extract was washed with saturated NaCl solution, dried on Na₂SO₄, and distilled off, to give 0.5 g. of the tetrahydroisoquinoline derivative (XVII) as a pale yellow viscous syrup, which was recrystallized from MeOH to afford colorless plates, m.p. 138–139°. *Anal.* Calcd. for C₁₅H₁₄O₃N: C, 77.09; H, 6.99; N, 3.60. Found: C, 77.06; H, 6.99; N, 3.44. The piperate was crystallized from MeOH to afford yellow scales, m.p. 80–81°. *Anal.* Calcd. for C₁₅H₁₄O₃N·C₄H₆O₇·2H₂O: C, 60.18; H, 4.89. Found: C, 59.91; H, 5.00. The chloroplatinate of XVII was characterized by recrystallization from EtOH as a colorless powder, m.p. 160–170°. *Anal.* Calcd. for C₁₅H₁₄O₃N·½H₂PtCl₆·H₂O: C, 49.03; H, 5.43; N, 2.29. Found: C, 48.60; H, 5.05; N, 2.75.

Diastereoisomeric Mixture of O.O-Dibenzyllinesinine (II) — A mixture of 2.5 g. of 1-(4-benzoyloxy-3-bromobenzyl)-2-methyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (X), 2 g. of 1-(4-benzoyloxybenzyl)-2-methyl-6-methoxy-7-hydroxy-1,2,3,4-tetrahydroisoquinoline (XVII), 285 mg. of Cu powder, 1.07 g. of K₂CO₃, 100 mg. of KI, and 9.5 ml. of dry pyridine was gradually heated, with stirring, in an oil-bath in a current of N₂ until the temperature of the mixture reached 150–151°(bath), at which temperature the mixture was kept for 48 hr. The mixture was cooled and admixed with 20 ml. of benzene. Filtra- tion and evaporation of the solvent gave the residue which was thrice extracted with benzene. The extract was washed with H₂O and dried on Na₂SO₄. Removal of the solvent gave the residue, which was chromatographed on alumina column (20×3.5 cm.).

Elution with benzene (3200 ml.), benzene-CHCl₃ (2:1; 1300 ml.), benzene-CHCl₃ (1:1; 1600 ml.) and benzene-CHCl₃ (1:2; 2600 ml.) successively and evaporation of each solvent gave a viscous oily base, showing a positive Beilstein test. This substance was identical with the starting material (X) in IR spectrum and mixed melting point test of their perchlorates. Successive elution with benzene-CHCl₃ (1:5; 23–24 ml.) fractions gave the results shown in Table I. The eluate F₂₃₋₉₁ gave a diastereoisomeric mixture of O.O-dibenzyllinesinine as a pale yellow glass. Nuclear magnetic resonance spectrum of this compound in CDCl₃ showed two signals of methylene proton in benzoyl group at 5.00 × 10⁻¹ and 5.12 × 10⁻¹ independently, excluding symmetrical structure such as biphenyl-derivatives which would be formed by bimolecular condensation of X. Recrystallization of the perchlorate of EtOH gave a colorless
powder, m.p. 113°-117°. *Anal.* Calcd. for C\textsubscript{6}H\textsubscript{12}O\textsubscript{9}N\textsubscript{2}\cdot2HClO\textsubscript{4}\cdotH\textsubscript{2}O: C, 60.65; H, 5.79; N, 2.77. Found: C, 60.83; H, 5.60; N, 2.84. IR ν\textsubscript{OH}\textsubscript{max} cm\textsuperscript{-1}: 3500 (OH) (water of crystallization).

**Diastereoisomeric Mixture of Liensinine (I')** — A solution of 0.5 g. of the above compound (II) in 20 ml. of benzene was shaken with 50 ml. of conc. HCl for 30 min. After being allowed to stand at room temperature overnight, the mixture was heated under reflux in a current of N\textsubscript{2} at 100° for 6 hr. After cooling, the reaction mixture was diluted with an excess of H\textsubscript{2}O, and then, H\textsubscript{2}O was removed by distillation *in vacuo*. After an additional mixture of EtOH and benzene had been added to the above residue, evaporation of the solvent gave the crude HCl salt as a caramel-like substance, which was dissolved in H\textsubscript{2}O and extracted with benzene. The aqueous layer was basified with 10% NaOH and extracted with CHCl\textsubscript{3}. The above alkaline solution was filtered, and crystalline NH\textsubscript{4}Cl was added to the filtrate, giving a pale yellow precipitate, which was thrice extracted with 100 ml. of CHCl\textsubscript{3}. The solvent was washed with saturated NaCl solution, dried on Na\textsubscript{2}SO\textsubscript{4}, and distilled off, to give a caramel-like substance (266 mg.). Recrystallization from ether gave diastereoisomeric mixture of liensinine (I') as colorless plates, m.p. 95°-103°. *Anal.* Calcd. for C\textsubscript{6}H\textsubscript{12}O\textsubscript{9}N\textsubscript{2}\cdot2\frac{1}{2}H\textsubscript{2}O: C, 71.71; H, 6.99; N, 4.52. Found: C, 71.39; H, 6.83; N, 4.07. IR ν\textsubscript{max} cm\textsuperscript{-1}: 3430 (broad) (OH) (water of crystallization and phenolic OH), 2845 (N-Me). UV λ\textsubscript{max}\textsubscript{ethanol} m\textsubscript{p} (log ε): (synthetic) 285 m\textsubscript{p} (log ε 4.06), (natural) 285 m\textsubscript{p} (log ε 4.03), RT (paper chromatography) (synthetic) 0.555, (natural) 0.558 [BuOH–AcOH–H\textsubscript{2}O (5:1:4) as solvent; the spots were detected by their fluorescence under UV light (short wave 2536 Å, cycle 50)]. NMR (4) (in CDCl\textsubscript{3}) spectra: (synthetic), 6.18, 6.25, 6.42, 6.50, 6.57 (9H, 2OMe), and 7.48 (6H, 2NMe); (natural), 6.14 (3H, OMe), 6.20 (3H, OMe), 6.59 (3H, OMe) and 7.48 (6H, 2NMe).

We are grateful to Prof. S. Ito, Department of Chemistry, Tohoku University for determining the nuclear magnetic resonance spectra.

**Summary**

Diastereoisomeric mixture of O,O-dibenzylic-liensinine (II) was successfully obtained by the modified Ullmann reaction of d,l-1-(4-benzxyoxybenzyl)-2-methyl-6-methoxy-7-hydroxy-1,2,3,4-tetrahydroisoquinoline (XVI) with d,l-1-(2-bromo-4-benzyloxybenzyl)-2-methyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (X) which has a large substituent such as benzoxylgroup at the ortho position of bromobenzene nucleus, without any detectable side reaction on the thin-layer chromatography. Removal of benzyl group by treating II with hydrochloric acid gave diastereoisomeric mixture of liensinine (I'), whose infrared and ultraviolet spectra were identical with those of natural product.

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