Beta-Carboline Alkaloids in Crude Drugs

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Beta-carboline alkaloids in crude drugs were quantified by a reversed-phase HPLC method without interference from their artifactual formation during analysis and with fluorometric detection specific to each individual analyte. 1-Methyl-β-carboline, β-carboline, 7-hydroxy-1-methyl-β-carboline, 7-methoxy-1-methyl-β-carboline, 1-methyl-1,2,3,4-tetrahydro-β-carboline and 1,2,3,4-tetrahydro-β-carboline showed a wide distribution in the crude drugs and the former two β-carbolines were detected in all those tested. Schisandrace Fructus, Pinelliae Tuber, Evodiae Fructus and Passiflora incarnata contained relatively large amounts of β-carbolines at ng—μg/g dry weight levels. Beta-carboline alkaloids may be responsible for the pharmacological effects of crude drugs as the potent active substances.

Key words β-carboline alkaloids; crude drugs; HPLC analysis

A series of β-carbolines with the 9H-pyrido[3,4-b]indole structure have been considered as pharmacologically active mammalian alkaloids since findings of their presence in humans and their various biological activities.1,2,3 Beta-carbolines are produced by a condensation reaction between indoleamines (tryptamine, serotonin and/or tryptophan) and carbonyl compounds (aldehydes and/or α-keto acids).3 Although there is a possibility that β-carbolines are endogenously produced (partly biosynthesized) in humans,1,3,4 their overwhelmingly large amounts are more likely to be supplied exogenously.5 Beta-carbolines are originally alkaloidal components in plants,1,5,6 therefore they would be present in crude drugs as detected in limited kinds of medicinal plants recently.6,7 If crude drugs contain significant amounts of β-carbolines, such alkaloids are speculated to participate in their pharmacological effects.

The representative β-carboline alkaloids originating from plants are 1-methyl-1,2,3,4-tetrahydro-β-carboline (MTBC) and 1,2,3,4-tetrahydro-β-carboline (TBC) which are oxidized to 1-methyl-β-carboline (MBC) and β-carboline (BC), respectively. MBC is also structurally modified to 7-hydroxy-1-methyl-β-carboline (7-OH-MBC) and 7-methoxy-1-methyl-β-carboline (7-M-MBC). Since the condensation reaction for β-carbolines occurs under experimental conditions to form β-carbolines as the artifacts,8 the analytical method without interference from such an artifactual formation is essential to determine the original content of β-carbolines in crude drugs. The artifactual formation was successfully suppressed by treating samples with fluorescamine prior to HPLC analysis.9,10 In the present study, that method was applied to crude drugs for the compositional assessment of β-carboline alkaloids as the potent active substances.

Materials and Methods

Chemicals MTBC, TBC and 2-ethyl-1,2,3,4-tetrahydro-β-carboline (ETBC) were synthesized by the method of Tsuchiya et al.11 MBC, BC, 7-OH-MBC, 7-M-MBC and 1-hydroxymethyl-β-carboline (HMBC) were purchased from Funakoshi (Tokyo, Japan). Fluorescamine was obtained from Fluka (Buchs, Switzerland). Acetonitrile of HPLC grade (Kishida, Osaka, Japan) was used for preparing the mobile phase and fluorescamine solution. All other reagents were of the highest grade available. Water was redistilled using an all-glass apparatus after purifying by a Milli-RO water purification system (Nihon Millipore, Tokyo, Japan).

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Sample Preparation All the dried crude drugs approved by Japanese Pharmacopoeia XIII12 were purchased from Nakarai Koshindo (Kobe, Japan) on April 15, 1997. Passiflora incarnata and Tribulus terrestris were obtained from Matsuura Yakugyo (Nagoya, Japan) on May 16, 1997 and June 6, 1997, respectively. The pulverized samples were homogenized in 0.1 M HCl containing 10 mM semicarbazide and the homogenates were centrifuged at 10000 × g for 15 min. The supernatants were diluted with 0.1 M HCl containing 10 mM semicarbazide as required (0.5—50 mg/ml) according to the alkaloid concentrations. To 0.5 ml of each diluent, 50 μl of an aqueous solution of ETBC (200.0 ng/ml) and HMBC (25.0 ng/ml) was added. The mixture was vortex-mixed with 0.5 ml of 2 M potassium phosphate buffer (pH 8.5) and 0.5 ml of a fluorescamine solution in acetonitrile (5 mg/ml) for 30 s, and then with 0.5 ml of 2 M potassium phosphate buffer (pH 8.5) containing glycine (100 mg/ml) for 30 s, followed by three-step extractions.9,10 The finally obtained extract was evaporated to dryness and the residue was dissolved in 200 μl of 0.2% (v/v) trifluoroacetic acid solution. An aliquot (50—100 μl) of the resulting solution was loaded onto an HPLC column.

HPLC Analysis The HPLC system consisted of an LC-10AD liquid chromatograph (Shimadzu, Kyoto, Japan) connected to an SCL-10A system controller (Shimadzu) and a DGU-4A degasser (Shimadzu), a 7125 sample injector (Rheodyne, Cotati, U.S.A.) and a Shim-pack CLC-C8 (M) column (25 cm × 4.6 mm i.d., particle size of 5 μm, Shimadzu) placed in a CTO-6A column oven (Shimadzu). The separation was performed by delivering the mobile phase, acetonitrile/trifluoroacetic acid/water (18.0: 0.2: 81.8, v/v/v), at a flow rate of 1.0 ml/min and at a column temperature of 50 °C. Based on spectral measurement and retention time of each analyte,7,13 7-OH-MBC, MTBC, TBC and ETBC at 275 nm for excitation and 350 nm for emission. An RF-550 spectrofluorometric detector (Shimadzu) which was time-programmed as follows: excitation/emission wavelengths (nm) were 325/417 at 0—7.50 min for 7-OH-MBC, 300/474 at 7.50—10.00 min for HMBC and BC, 300/430 at 10.00—14.50 min and 330/417 at 14.50—18.00 min for 7-M-MBC. An RF-535 spectrofluorometric detector (Shimadzu) was connected in series to detect MTBC, TBC and ETBC at 275 nm for excitation and 350 nm for emission. Beta-carbolines in oxidized- and reduced-form were quantified based on the peak area ratios to HMBC and ETBC, respectively. Their contents in crude drugs were corrected by the recoveries obtained from spiking experiments and the dilution factors.

Analytical Evaluation To evaluate the recovery and analytical precision, a mixture of 7-OH-MBC (0.4—4.0 ng/ml), BC (0.2—2.0 ng/ml), MBC (0.2—2.0 ng/ml), 7-M-MBC (0.4—4.0 ng/ml), MTBC (2.0—20.0 ng/ml) and TBC (2.0—20.0 ng/ml) was added to crude drug diluent samples. Repli- cate spiked samples were analyzed on the same day (n = 6 for recovery and intra-assay precision) and on different days (n = 4 for inter-assay precision) as described above.

Results and Discussion All analytes including two internal standards were simultaneously analyzed by a single run of HPLC with fluorometric

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detection specific to each β-carboline as shown by the representative chromatograms (Fig. 1). The obtained peaks reflect the original contents in crude drugs, not including the artifacts because occurrence of the condensation reaction during analysis was completely suppressed by the fluorescamine-pretreatment, in which the samples were reacted with fluorescamine in the first step of an extraction procedure to remove the precursor indoleamines from the analytical system and only the purified original analytes were subjected to HPLC separation.9,10)

Both recovery and reproducibility were so high that the present method was applicable to the quantitation of β-carbolines in crude drugs as shown by the typical results (Table 1). More than 80% of the spiked β-carbolines were recovered from the homogenate samples, and intra- and inter-assay CVs were within 8% except for a part of the samples. The quantitative range was 0.01—20.0 ng/ml for MBC and BC, 0.02—20.0 ng/ml for 7-OH-MBC, 0.03—20.0 ng/ml for 7-M-MBC and 0.1—50.0 ng/ml for MTBC and TBC.

Quantitative analyses revealed that β-carboline alkaloids were contained in various crude drugs (Table 2). BC and MBC were detected in all those tested. 7-OH-MBC showed relatively wide distribution. However, the presence and content of MTBC, TBC and 7-M-MBC depended on the kind of crude drugs analyzed. Some β-carbolines were previously detected in Rutaceae, Leguminosae, Passifloraceae, Loganiaceae, Plamae, etc.5) MBC, BC, MTBC and/or TBC were also found in plants belonging to Solanaceae and their products such as tomato, tomato juice, tabasco and tobacco.4,10,13) The present quantitation has proven that the crude drugs con-
responding to that classification uniformly contain β-carboline alkaloids. In particular, Schisandrae Fructus and Pinelliae Tuber contained MTBC at μg/g dry weight levels. Evodiae Fructus was superior in content of BC, MBC and 7-OH-MBC to the other crude drugs. The relatively large amounts of β-carbolines in oxidized-form were present in Scopoliae Rhizoma, Puerariae Radix, Polygalae Radix, Zingiberis Rhizoma, Bupleuri Radix, Passiflora incarnata and Tribulus terrestris.

Beta-carbolines have a variety of neuropharmacological activities.\(^1\)\(^2\)\(^3\)\(^4\) They include benzodiazepine antagonism (IC\(_{50}\) for inhibition of flunitrazepam; 5—7 μM for MBC, 6—8 μM for BC, 64 μM for 7-OH-MBC, 920 μM for TBC and 1450 μM for MTBC),\(^15\)\(^16\) inhibition of monoamine oxidase (IC\(_{50}\) for inhibition of calf liver and mouse brain monoamine oxidase: 0.75 nm—20 μM for BC, 5 nm—3.3 μM for MBC, 15—80 nm for M7-MTB, 27.5 nm—5.8 nm for 7-OH-MBC, 42.5 nm—120 μM for MTBC and 58 μM for TBC),\(^17\)\(^18\) inhibition of biogenic amine uptake (IC\(_{50}\) for inhibition of serotonin, dopamine and epinephrine uptake: 1.0—6.4 μM for MTBC, 3.0—6.2 μM for BC and 3.2 μM for 7-M-MBC).\(^3\) Since Schisandrae Fructus and Pinelliae Tuber influence the central nervous system, their major component, MTBC may act as a neuroactive substance. Evodiae Fructus, Passiflora incarnata and Tribulus terrestris have been used as a sedative agent, which may be attributed to BC, MBC, 7-OH-MBC and 7-M-MBC. While β-carbolines have the affinity for benzodiazepine receptors, both peripheral- and central-type benzodiazepine receptors exist in parotid and submandibular glands of rats.\(^19\)\(^20\) Rat xerostomia induced by diazepam was restored by Byakko-ninjin-to (45—90 mg/kg, p.o.) and Bakumondo-to (90 mg/kg, p.o.)\(^21\) which contain Pinelliae Tuber, Ginseng Radix and Ophiopogonis Tuber. As an active substance in these crude drugs, β-carbolines may promote salivary secretion by binding to benzodiazepine receptors in salivary glands. Several β-carbolines also show antimicrobial activity against various bacteria and fungi, and their minimal inhibitory concentrations range 0.9—15 μg/disc for BC, 1.9—120 μg/disc for MBC, 100—500 μg/ml for 7-OH-MBC and 50—500 μg/ml for 7-M-MBC.\(^22\)\(^23\) Therefore, the β-carbolines detected in certain crude drugs traditionally used for intestinal diseases potentially contribute to their therapeutic effects by influencing intestinal microflora. Beta-carboline alkaloids have different activities in addition to their neuroactive and antimicrobial effects,\(^2\)\(^3\)\(^4\)\(^10\) suggesting their potent pharmacological significance in crude drugs.

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### References


### Table 2. Beta-Carboline Alkaloids in Crude Drugs

<table>
<thead>
<tr>
<th>Crude drug</th>
<th>Content (ng/g dry weight)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>7-OH-MBC</td>
</tr>
<tr>
<td>Schisandrae Fructus</td>
<td>125±9.1</td>
</tr>
<tr>
<td>Pinelliae Tuber</td>
<td>ND</td>
</tr>
<tr>
<td>Scopoliae Rhizoma</td>
<td>10.3±5.9</td>
</tr>
<tr>
<td>Evodiae Fructus</td>
<td>400±75</td>
</tr>
<tr>
<td>Ophiopogonis Tuber</td>
<td>2.4±1.2</td>
</tr>
<tr>
<td>Ginseng Radix</td>
<td>ND</td>
</tr>
<tr>
<td>Bupleuri Radix</td>
<td>32.7±0.8</td>
</tr>
<tr>
<td>Puerariae Radix</td>
<td>ND</td>
</tr>
<tr>
<td>Polygalae Radix</td>
<td>127±45</td>
</tr>
<tr>
<td>Gentianae Radix</td>
<td>9.1±3.2</td>
</tr>
<tr>
<td>Arecae Semen</td>
<td>ND</td>
</tr>
<tr>
<td>Zingiberis Rhizoma</td>
<td>172±11</td>
</tr>
<tr>
<td>Caryophylli Flos</td>
<td>ND</td>
</tr>
<tr>
<td>Forsythiae Fructus</td>
<td>30.2±1.1</td>
</tr>
<tr>
<td>Plantaginis Semen</td>
<td>ND</td>
</tr>
<tr>
<td>Passiflora incarnata</td>
<td>410±201</td>
</tr>
<tr>
<td>Tribulus terrestris</td>
<td>232±112</td>
</tr>
</tbody>
</table>

Each value represents mean±S.E. (n=3—5). ND, not detected.


