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# Metabolic Products of *Aspergillus terreus*. VII.[1] Astechrome: an Iron-containing Metabolite of the Strain IFO 6123

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An iron complex, "astechrome," was isolated from *Aspergillus terreus* IFO 6123, and its structure was determined.

**Keywords**——astechrome; *Aspergillus terreus*; IFO 6123; iron complex; indolyl

As reported in the previous paper (Part I[2]), an antitumor metabolite named "eaterriquinone" was isolated from *Aspergillus terreus* IFO 6123 under stationary culture conditions on a malt extract medium. When the fungus was cultivated with shaking, an iron-containing metabolite named "astechrome" (I) was obtained instead of asterriquinone. In this paper, the isolation and the structure of astechrome (I) are described. This compound was also obtained from the strain IFO 8835 on cultivation with shaking.

Although little astechrome was produced under stationary conditions, the yield was greatly increased on cultivation with shaking. For the production of astechrome, Czapek–Dox medium containing polypeptone (3 g/l) and ferrous ion (0.1—0.15 g/l as $FeSO_4 \cdot 7H_2O$) was found to be the best culture medium. When ferrous ion was absent, neither astechrome nor the corresponding iron-free compound was produced, but (−)-3,4-dihydro-6,8-dihydroxy-3-methylisocoumarin, mp 214—215° (II) (lit.[3] mp 214—217°), was formed. Excess ferrous ion (above 0.2 g/l as $FeSO_4 \cdot 7H_2O$) decreased the yield of astechrome.

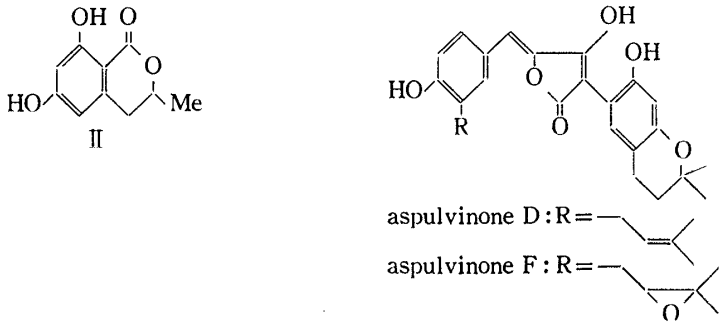



Fig. 1

The fungus was pre-cultivated on the modified Czapek-Dox medium without addition of ferrous ion by shaking at 27° for 2 days. An aliquot of the pre-culture was inoculated into modified Czapek–Dox medium containing ferrous ion, and cultivated under shaking for a further 5 days. The pellet of dark-reddish mycelia was harvested by filtration, and dried. The mycelia contained 81% of the added iron. The mycelia were powdered and treated with petroleum ether to remove oily compounds, and then extracted with chloroform and methanol, successively. The iron in the mycelia was extracted with chloroform in 31.6% yield.

The chloroform extract was chromatographed on acid-washed silica gel with benzene–AcOEt (20: 1). The isolated pigment was crystallized from hexane–chloroform as dark-red needles (I), mp 188—189° (decomp.) (yield, 80 mg from 1 l culture).

From the next fractions, aspulvinone D, mp 256° (Ref.[4] 257—258°), and aspulvinone F, mp 262° (Ref.[4] 234—235°),[5] were isolated. They were identified by comparison with authentic samples.

Astechrome (I) was neutral and optically inactive. It had no characteristic absorption in the infrared (IR) spectrum except for an NH band at 3400 $cm^{-1}$. The proton nuclear magnetic resonance (PMR) spectrum could not be measured.

The presence of iron in I was confirmed qualitatively by coloration with potassium ferrocyanate (red), and the quantity was assayed by a colorimetric method with *o*-phenanthroline (5.56%), by atomic absorption spectrometry after ashing with nitric acid (5.36%), and by weight measurement after combustion (5.15%). The molecular weight of I obtained by field desorption mass spectroscopy was 1112, though the usual mass spectrum (MS) showed *m/e* 351 as the highest mass number. Elementary analysis and MS suggested the empirical formula $C_{60}H_{66}FeN_9O_9$ for astechrome.

The iron in astechrome was very tightly bound to the ligand, and could not be removed by treatment with a chelating agent such as 8-hydroxyquinoline.[6] Elimination of the iron in aqueous alkali was incomplete owing to insolubility in water, and catalytic reduction with palladium gave only hexahydro-astechrome (III), mp 222—223°, without elimination of the iron.

The removal of iron from astechrome was first performed by catalytic reduction with platinum oxide, and colorless needles (IV), mp 141—142°, $C_{20}H_{25}N_3O_2$, were obtained in 77% yield. Positive Ehrlich reaction (red) and ultraviolet (UV) spectral peaks (273 (sh), 282, and 292 nm) suggested the presence of an indole ring in IV. The PMR spectrum showed the presence of one isopentyl, one methyl, one methoxyl, one methylene, and four aromatic protons. Three of the aromatic protons showed a coupling pattern which indicated a vicinal arrangement, and the other was located at the 2-position of the indole ring, coupling with NH ($J$=2.5 Hz; the coupling disappeared with $D_2O$). Thus, this indole ring had two substituents at the 3 and 7 (or 4) positions.

Compound IV was oxidized with hydrogen peroxide in alkali to 3-isopentylanthranilic acid (V), mp 123°, which had previously been obtained as a degradation product of asterriquinone C-2.[7] Therefore, the position of the isopentyl group in IV was identified as position 7 of the indole ring. The presence of an –NH–CO– group in IV was suggested by the IR spectrum (3480, 1640, and 1510 $cm^{-1}$) and the shift of UV absorption from 325 nm (at neutral pH) to 347 nm (at alkaline pH). The IR bands of NH and CO in IV both disappeared in the acetate (VI), mp 149—151°, or in the dimethyl derivative (VII), mp 70—71° (prepared by Hakomori's method[8]). The UV absorptions of these derivatives were not changed by pH. These results also support the existence of an –NH–CO– group in IV. 2-$^{14}$C-Tryptophan and 1-$^{14}$C-alanine were well incorporated into astechrome (14% and 5.6%, respectively).

From these results, the structure of IV was tentatively proposed to be IVa or IVb.

When astechrome (I) was treated with phosphorus tribromide, colorless needles (VIII), mp 167—169°, were obtained. The molecular formula $C_{20}H_{23}N_3O_2$ was assigned for VIII, and the presence of 3,3-dimethylallyl and methylene groups was indicated by the PMR spec-

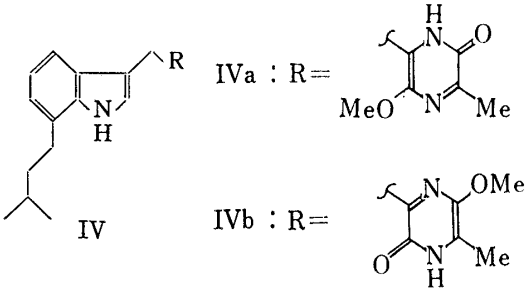



Fig. 2

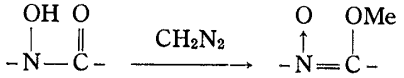



Fig. 3

trum. Compound VIII absorbed one mole of hydrogen on catalytic hydrogenation with Pd/C to afford compound IV.

When astechrome (I) was treated with aqueous sodium hydroxide in acetone, an iron-free colorless powder (IX), mp 45—55° (not sharp) was obtained (yield, 90%), This compound (IX) was hygroscopic and gradually decomposed, and could not be obtained in crystalline form. The molecular formula was determined by high-resolution MS to be $C_{20}H_{23}N_3O_3$. Compound IX regenerated astechrome on treatment with ferric chloride. IX also gave a copper complex on treatment with cupric sulfate as green micro-crystals (X), mp 162°. The molecular formula of X was determined to be $(C_{20}H_{22}N_3O_3)_2Cu$ by elementary analysis and MS ($M^+$, *m/e*: 767).

The change of the absorption spectrum of astechrome as a function of pH (450 nm in pH 7.0 to 520 nm in pH 2) resembled those of iron complexes of hydroxamate such as ferric tribenzohydroxamate[9] (440 nm at pH 8 and 510 nm at pH 1), fusarinine iron-complex[10] (440 nm at pH 8 and 500 nm at pH 2.5), and ferrichrome[9] (425 nm at pH 8 and 470 nm at pH 0).

The iron atom of astechrome was shown to exist as paramagnetic trivalent iron by measurement of its magnetic moment: 5.60 Bohr magnetons (*cf.* ferrichrome A, 5.68[11]).

These results showed that astechrome is an $Fe^{3+}$ complex, in which one iron atom is linked with three identical ligands (IX) containing a hydroxamic acid moiety, and the molecular formula of I was concluded to be $(C_{20}H_{22}N_3O_3)_3Fe$.

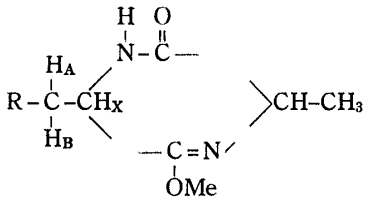
Hexahydro-astechrome (III), mp 222—223°, also lost the iron atom on treatment with sodium hydroxide in acetone to give an amorphous compound (XI), mp 48—55°, $C_{20}H_{25}N_3O_3$. The properties of XI were very similar to those of IX. XI gave 3-isopentylanthranilic acid (V) on oxidation with hydrogen peroxide in an alkaline medium.

Compound IX was treated with trimethoxyphosphine to give a deoxy-compound which was identical with VIII.

Compound IX was methylated with diazomethane to give a methyl ether (XII), mp 129—130°, $C_{21}H_{25}N_3O_3$. XII showed two methoxyl groups in its PMR spectrum, and the UV spectrum (323 nm) was not shifted by change of pH. In the IR spectrum of XII, the presence of an N→O group (1212 and 1230 $cm^{-1}$ in KBr) was apparent, but a CO group was not recognized. The OH band of methanol in chloroform solution was shifted from 3610 to 3320 $cm^{-1}$ by addition of XII, which also supported the presence of an N→O group in XII.[12] The course of the reaction is considered to be as shown in Fig. 3.

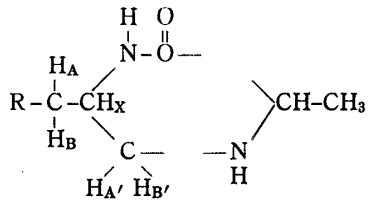
Compound XII was hydrogenated with Pd/C at 3,3-dimethylallyl group to afford colorless crystals (XIII), mp 139—140.5°, $C_{21}H_{27}N_3O_3$, which still had the N→O group. However, when XII was hydrogenated with platinum oxide, the N→O group was reduced, and the methyl ether of IV (XIV) was obtained.

When compound IV was further hydrogenated with platinum oxide in ethanol, a dihydro-compound (XV), mp 148°, $C_{20}H_{27}N_3O_2$, was obtained. In its PMR spectrum, signals of a methylene group ($\delta$, 3.19, AB part of an ABX signal) and a methyl group ($\delta$, 1.03, d) were seen at higher field than in IV. These were coupled with two newly appeared CH groups ($\delta$, 4.03 and 4.26, respectively). The NH signal ($\delta$, 6.53) was coupled with one of the CH groups ($\delta$, 4.26). The UV absorptions at 325 and 365 nm (pyrazine ring) in IV disappeared,




R=7-isopentylindolyl

Fig. 4




7-isopentylindolyl

Fig. 5

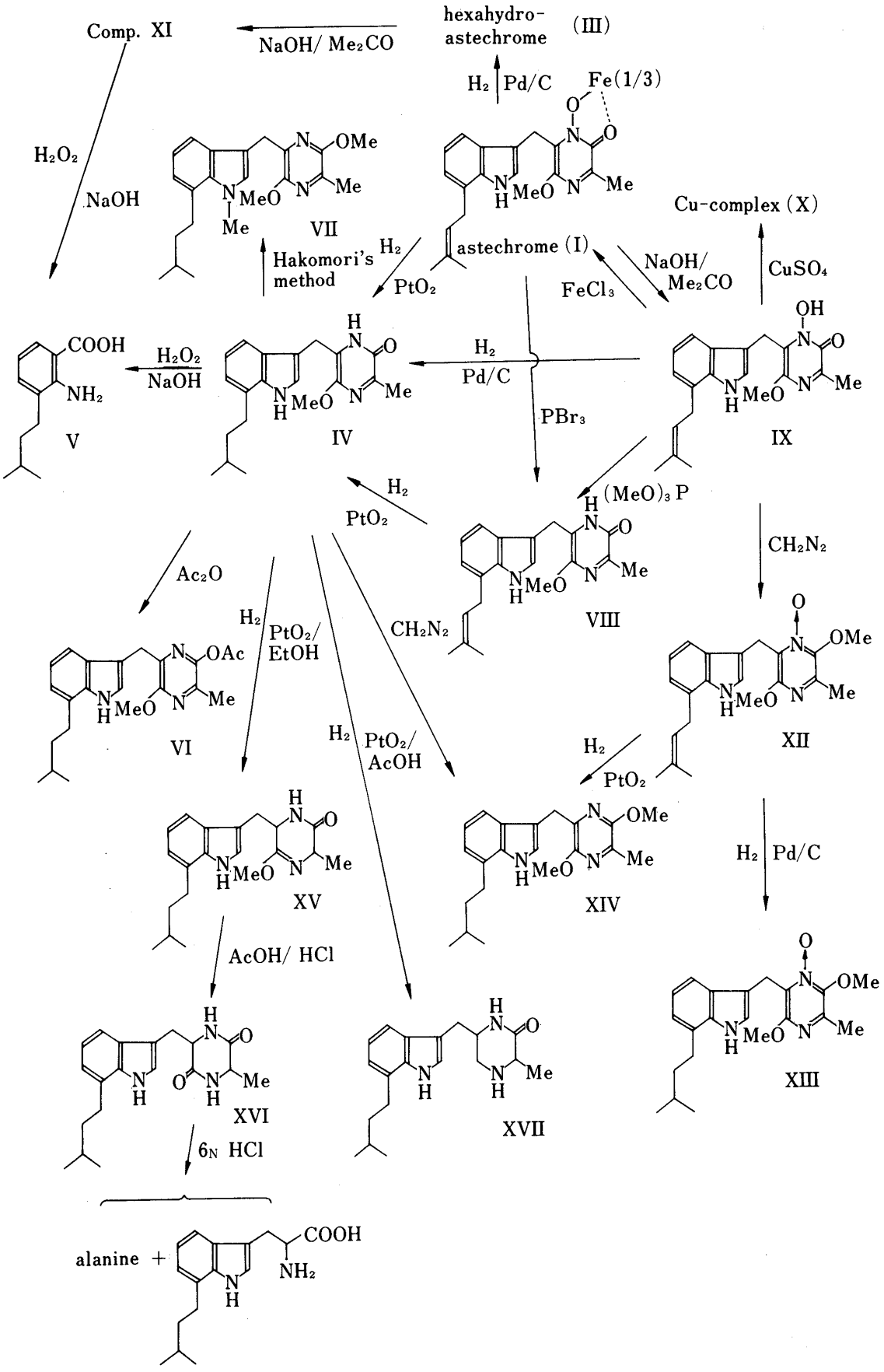



Fig. 6

but the IR band of –NH–CO– (3540, 1650 $cm^{-1}$ in $CHCl_3$) was still observed. The partial structure of XV suggested by these results is shown in Fig. 4.

Compound XV was demethylated with hydrochloric acid in acetic acid to give the diketopiperazine derivative (XVI), mp 235—237°, $C_{19}H_{25}N_3O_2$. The IR spectrum of this compound was very similar to that of echinulin.[13] Compound XVI was hydrolyzed with 6 N hydrochloric acid at 130°, and two ninhydrin-positive compounds were isolated by preparative thin-layer chromatography (TLC). One of them was identical with alanine and the other was presumed to be 7-isopentyltryptophan on the basis of TLC and amino acid analysis.

When compound IV was further hydrogenated with platinum oxide in acetic acid at *ca.* 60°, a colorless compound (XVII), mp 149°, $C_{19}H_{27}N_3O$, was obtained. The PMR spectrum of XVII showed loss of the methoxyl group and the presence of new methylene and NH groups. The partial structure which was suggested by the PMR spectrum is shown in Fig. 5. The presence of an –NH–CO– group was confirmed by the IR spectrum (3450, 1645, and 1550 $cm^{-1}$) and the CMR signal at $\delta$, 171.88 (in $CDCl_3$, C=O).

From these results, the structure of IV was concluded to be IVa and the hydroxamate-iron complex—astechrome— was concluded to have the structure I. The reactions described here are summarized in Fig. 6. Astechrome had a weak antibiotic activity, but no antitumor activity against Ehrlich asites carcinoma was observed.

## Experimental[14]

**Culture Conditions**——Culture medium for pre-cultivation was as follows: glucose, 30 g; $NaNO_3$, 3.0 g; $KH_2PO_4$, 1.0 g; KCl, 0.5 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; polypeptone (Daigo Eiyo), 3.0 g; distilled water, 1 l. For the main cultivation, 0.1 g of $FeSO_4 \cdot 7H_2O$ was added to the above medium. The fungus IFO 6123 was pre-cultivated on 100 ml of the medium in 500 ml Sakaguchi flasks at 27° for 2 days under shaking (reciprocal, 120 rpm). An aliquot of the pre-culture (5 ml) was inoculated into 1 l of the medium for the main cultivation in 3 l Sakaguchi flasks, and cultivated under the same conditions for 5 days. The mycelia were harvested by filtration and warm air-dried.

**Isolation of Astechrome (I) from the Mycelia**——The dried mycelia (46 g from 7 l culture) were extracted with petr. ether and then $CHCl_3$ in Soxhlet apparatus. Ergosterol and oils were removed by petr. ether extraction. The $CHCl_3$ extract was dissolved in benzene, and chromatographed on acid-washed silica gel (Silicic acid AR, Mallinckrodt) with benzene–AcOEt (20:1). The dark-red eluate was evaporated to dryness and the residue was crystallized from hexane–$CHCl_3$ as dark-red fine needles (I), mp 188—189° (dec.), yield, 330 mg. It was insoluble in $H_2O$ but soluble in many organic solvents. *Anal.* Calcd for $C_{60}H_{66}FeN_9O_9$: C, 64.74; H, 5.98; N, 11.33; Fe, 5.02; MW, 1113.1. Found: C, 64.61; H, 5.96; N, 11.05; Fe, 5.56 (colorimetric method with *o*-phenanthroline), 5.36 (atomic absorption), 5.15 (weight method); MS (FD) *m/e*: 1111 (24.5%), 1112 (16.3%), 1113 (13.7%). UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 223 (5.18), 282 (4.39), 292 (4.34), 347 (4.42), 450 (3.68). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3420, 1470, 1405.

**Catalytic Hydrogenation of Astechrome (I) with Pd/C**——Astechrome (100 mg) in AcOEt (30 ml) was hydrogenated with 10% Pd/C (50 mg) for 1 hr. The filtered solution was evaporated to dryness, and the residue was crystallized from hexane–$CHCl_3$ to give dark-red micro-crystals (III), mp 222—223° (yield, 70 mg). *Anal.* Calcd for $C_{60}H_{72}FeN_9O_9$: C, 64.39; H, 6.49; N, 11.26. Found: C, 64.05; H, 6.52; N, 11.49. UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 223 (5.14), 282 (4.41), 292 (4.31), 347 (4.42), 450 (3.68). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3420, 1470, 1405, 1350.

**Catalytic Hydrogenation of Astechrome (I) with $PtO_2$**——Astechrome (300 mg) in AcOEt (100 ml) was hydrogenated with $PtO_2$ (150 mg) and charcoal powder (100 mg) under an IR lamp at *ca.* 60°. After hydrogenation for 2 hr, the filtrate was evaporated to dryness, and the residue was crystallized from hexane–$CHCl_3$ as colorless needles (IV), mp 141—142° (210 mg). *Anal.* Calcd for $C_{20}H_{25}N_3O_2$: C, 70.77; H, 7.43; N, 12.38. Found: C, 70.84; H, 7.52; N, 12.52. MS *m/e*: 339 ($M^+$), 268. UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 223 (4.63), 273 (sh, 3.82), 282 (3.85), 292 (3.81), 325 (3.90), 365 (3.67). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3480, 1640, 1610, 1510, 1460. PMR ($CDCl_3$) $\delta$: 0.95 (6H, d, $J$=6.0 Hz, $2CH_3$), 1.55 (3H, CH and $CH_2$), 2.37 (3H, s, $CH_3$), 2.72 (2H, t, $J$=7 Hz, $CH_2$), 3.95 (3H, s, $OCH_3$), 4.07 (2H, s, $CH_2$), 6.92—7.07 (2H, 5- and 6-CH of indole ring), 7.10 (1H, d, $J$=2.5 Hz, 2-CH of indole), 7.55 (1H, dd, $J$=6, 3 Hz, 4-CH of indole), 7.81 (1H, d, $J$=2.5 Hz, NH). This compound (IV) was also obtained from VIII and IX by catalytic hydrogenation.

**Acetylation of Compound IV**——Compound IV (120 mg) was dissolved in pyridine (5 ml) and acetylated with $Ac_2O$ (10 ml). After standing at room temperature for 6 hr, the reaction mixture was poured into $H_2O$. The resulting precipitate was crystallized from MeOH to give colorless needles (VI), mp 149—151° (yield,

100 mg). *Anal.* Calcd for $C_{22}H_{27}N_3O_3$: C, 69.27; H, 7.13; N, 11.02. Found: C, 69.28; H, 7.00; N, 10.80. MS *m/e*: 381 ($M^+$). UV $\lambda_{max}^{MeOH}$ nm: 223, 292. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3370, 1750 ($CH_3CO$–). PMR ($CDCl_3$) $\delta$: 0.95 (6H, d, $J=6$ Hz, $2CH_3$), 1.57 (3H, CH and $CH_2$), 2.28 and 2.30 (each 3H, s, $CH_3$ and $CH_3CO$–), 2.72 (2H, t, $J=7$ Hz, $CH_2$), 3.93 (3H, s, $OCH_3$), 4.17 (2H, s, $CH_2$), 6.90—7.10 (3H, m, 2-, 5-, and 6-CH of indole), 7.55 (1H, dd, $J=7$, 2.5 Hz, 4-CH of indole), 7.90 (1H, NH).

**Methylation of IV with $CH_2N_2$**——Compound IV (67 mg) was dissolved in ether, and a few drops of MeOH and an excess of ethereal $CH_2N_2$ were added. The solution was kept overnight, then the solvent was evaporated off, and the residue was purified by preparative TLC (acid-washed silica gel[15]) with $CHCl_3$–AcOEt (9: 1). The main spot gave an oily compound (XIV). MS *m/e*: 353 ($M^+$). UV $\lambda_{max}^{MeOH}$ nm: 224, 283, 293, 322. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3400, 1460, 1435, 1370, 1360, 1255. PMR ($CDCl_3$) $\delta$: 0.95 (6H, d, $J=7$ Hz, $2CH_3$), 1.58 (3H, CH and $CH_2$), 2.30 (3H, s, $CH_3$), 2.73 (2H, t, $J=7.5$ Hz, $CH_2$), 3.88 (6H, s, $2OCH_3$), 4.10 (2H, s, $CH_2$), 6.88—7.20 (3H, m, 2-, 5-, and 6-CH of indole), 7.62 (1H, dd, $J=7$, 3.5 Hz, 4-CH of indole), 7.78 (NH).

This compound (XIV) was also obtained from XII by catalytic hydrogenation with $PtO_2$.

**Dimethyl Derivative of IV (by Hakomori's Method)**——Compound IV (50 mg) was dissolved in dry DMSO (2 ml) by warming under an $N_2$ atmosphere, and methylsulfinyl carbanion (2.3 equivalents, prepared from NaH and DMSO, 2 ml) was added. The mixture was stirred at room temperature for 4 hr under an $N_2$ atmosphere. To this solution, $CH_3I$ (1.5 ml) was added dropwise under stirring below 25°. The reaction mixture was treated with $H_2O$ and extracted with $CHCl_3$. The $CHCl_3$ extract was dissolved in petr. ether–ether mixture and washed with $H_2O$. The organic layer was evaporated to dryness and the residue was purified by preparative TLC with benzene as the solvent. The main fraction was extracted with MeOH and crystallized from MeOH as colorless plates (VII), mp 70—71° (yield, 20 mg). *Anal.* Calcd for $C_{22}H_{29}N_3O_2$: C, 71.90; H, 7.95; N, 11.44. Found: C, 71.82; H, 7.78; N, 11.53. MS *m/e*: 367 ($M^+$), 352, 310. UV $\lambda_{max}^{MeOH}$ nm: 227, 303, 323. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 2950, 1470, 1360, 1260. PMR ($CDCl_3$) $\delta$: 0.95 (6H, d, $J=6$ Hz, $2CH_3$), 1.55 (3H, CH and $CH_2$), 2.30 (3H, s, $CH_3$), 2.98 (2H, t, $J=7$ Hz, $CH_2$), 3.90 (6H, s, $2OCH_3$), 3.92 (3H, s, $NCH_3$), 4.05 (2H, s, $CH_2$), 6.77 (1H, s, 2-CH of indole), 6.8—7.1 (2H, 5- and 6-CH of indole), 7.65 (1H, dd, $J=6.5$, 2.5 Hz, 4-CH of indole).

**Oxidative Cleavage of IV with $H_2O_2$ in Alkaline Medium**——A solution of IV (300 mg) in EtOH (70 ml) was mixed with 0.2 N NaOH (9 ml) and 30% $H_2O_2$ (9 ml). The mixture was refluxed for 4 hr, then the organic solvent was evaporated off under reduced pressure. The reaction mixture was acidified and extracted with AcOEt. The AcOEt solution was extracted with 10% $NaHCO_3$, and the acidic fraction was crystallized from petr. ether as colorless needles (V), mp 123° (yield, 7 mg). It was identical with 3-isopentylanthranilic acid as judged by IR and TLC. *Anal.* Calcd for $C_{12}H_{17}NO_2$: C, 69.54; H, 8.27; N, 6.76. Found: C, 69.57; H, 8.02; N, 6.36. MS *m/e*: 207 ($M^+$).

**Removal of Iron from Astechrome (I) by Treatment with Alkali in Acetone**——To a solution of astechrome (100 mg) in acetone (20 ml), 0.2 N NaOH (3 ml) was added dropwise under stirring, and the resulting dark brown precipitate was filtered off. After removal of the acetone by evaporation under reduced pressure, the reaction mixture was acidified with HCl. The resulting precipitate (IX) was collected by filtration, washed with $H_2O$, and dried in a desiccator (yield, 85 mg, 90%). This compound was so unstable and hygroscopic that it could not be obtained in crystalline form. The melting point was not sharp (45—55°). The molecular formula was determined by high-resolution MS to be $C_{20}H_{23}N_3O_3$ (Calcd: 353.174. Found: 353.174). UV $\lambda_{max}^{EtOH}$ nm: 225, 265, 279 (sh), 291, 347 (sh), 364. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3400, 1620, 1530, 1485, 1400. PMR ($CDCl_3$) $\delta$: 1.75 (6H, s, $2CH_3$), 2.38 (3H, s, $CH_3$), 3.47 (2H, d, $J=7$ Hz, $CH_2$), 3.94 (3H, s, $OCH_3$), 4.29 (2H, s, $CH_2$), 5.30 (1H, t, $J=7$ Hz, –CH=), 6.9—7.1 (2H, 5- and 6-CH of indole), 7.36 (1H, s, 2-CH of indole), 7.62 (1H, dd, $J=8$, 3 Hz, 4-CH of indole), 7.90 and 8.39 (NH, OH).

**Removal of Iron from Hexahydro-astechrome (III)**——Compound III (70 mg) was treated with 0.2 N NaOH in acetone under the same conditions as in the case of astechrome (I). A colorless amorphous compound (XI), mp 48—55°, was obtained (yield, 64 mg), but it could not be crystallized. *Anal.* Calcd for $C_{20}H_{25}N_3O_3$: C, 67.58; H, 7.09; N, 11.82, MS, 355.190. Found: C, 67.28; H, 7.00; N, 11.41, MS, 355.189. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3400, 1700, 1615, 1530, 1480, 1225. PMR ($CDCl_3$) $\delta$: 0.94 (6H, d, $J=6$ Hz, $2CH_3$), 1.59 (3H, CH and $CH_2$), 2.44 (3H, s, $CH_3$), 2.74 (2H, t, $J=7$ Hz, $CH_2$), 3.93 (3H, s, $OCH_3$), 4.28 (2H, s, $CH_2$), 6.9—7.15 (3H, 2-, 5-, and 6-CH of indole), 7.59 (1H, dd, $J=7.5$, 3.5 Hz, 4 CH of indole), 7.9 (NH).

**Recovery of Astechrome from IX on Treatment with Ferric Ion**——Compound IX (15 mg) in ether (20 ml) was shaken with aq. 1% $FeCl_3$ (10 ml). The reddish ether layer was washed with $H_2O$ and the solvent was evaporated off. The residue was crystallized from hexane–$CHCl_3$ mixture as dark-red micro-needles, mp 188° (dec.). This compound was identical with astechrome (I) as judged by IR, UV, TLC, *etc.*

**Copper Complex of IX**——Compound IX (50 mg) in ether (50 ml) was shaken with a large excess of aq. 12.5% $CuSO_4 \cdot 5H_2O$. The ether layer was washed with $H_2O$ and evaporated to dryness. The residue (40 mg) was crystallized from acetone as green micro-crystals (X), mp 162°. *Anal.* Calcd for $(C_{20}H_{22}N_3O_3)_2Cu$: C, 62.52; H, 5.77; N, 10.94, MW, 768.3. Found: C, 62.38, 62.30; H, 5.69, 5.46; N, 11.02, 11.04. MS *m/e*: 767 ($M^+$, 100), 769 (54). UV $\lambda_{max}^{MeOH}$ nm: 223, 282, 292, 355, 640. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3420, 1470, 1405, 1200.

**Reaction of Astechrome (I) with $PBr_3$**——$PBr_3$ (0.4 ml) was added to a solution of astechrome (350 mg) in AcOEt (100 ml) under ice cooling. After standing for 2 hr, the reaction mixture was washed with $H_2O$ and the solvent was evaporated off under reduced pressure. The residue was purified by chromatography

on acid-washed silica gel (Silicic acid AR) with $CHCl_3$–MeOH (20: 1). The first eluate was collected and crystallized from MeOH as colorless needles (VIII), mp 167—169° (67 mg). *Anal.* Calcd for $C_{20}H_{23}N_3O_2$: C, 71.19; H, 6.87; N, 12.45. Found: C, 70.88; H, 6.94; N, 12.32. MS *m/e*: 337 ($M^+$), 322, 282, 198. UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 223 (4.46), 282 (3.88), 292 (3.84), 327 (3.91), 365 (3.68). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3420, 1640, 1615, 1520. PMR ($CDCl_3$) $\delta$: 1.70 (6H, s, $2CH_3$), 2.32 (3H, s, $CH_3$), 3.43 (2H, d, $J=7$ Hz, $CH_2$), 3.91 (3H, s, $OCH_3$), 4.02 (2H, s, $CH_2$), 5.25 (1H, t, $J=7$ Hz, –CH=), 6.8—7.1 (3H, 2-, 5-, and 6-CH of indole), 7.50 (1H, dd, $J=$ 6.5, 3 Hz, 4-CH of indole), 7.85 (NH).

**Reaction of IX with $(CH_3O)_3P$**——Compound IX (90 mg) was dissolved in $(CH_3O)_3P$ (0.5 ml) and heated on a water bath for 2.5 hr. The reaction mixture was extracted with AcOEt, and the organic layer was washed with $H_2O$ and evaporated to dryness. The residue was purified by preparative TLC with $CHCl_3$–MeOH (5: 1) as the solvent. The main spot was extracted with MeOH and crystallized from MeOH as colorless needles, mp 167—169° (10 mg). This compound was identical with VIII as judged by IR spectroscopy and mixed melting point determination.

**Catalytic Hydrogenation of IV**——A suspension of IV (100 mg) and $PtO_2$ (30 mg) in EtOH (30 ml) was shaken with $H_2$ at room temperature for 3 hr. The reaction mixture was evaporated to dryness after removal of the catalyst, and the residue was fractionated by preparative TLC with $CHCl_3$–acetone (1: 1) as the solvent. The main band (*Rf*, 0.75) was extracted with MeOH, and the extract was crystallized from petr. ether as colorless needles (XV), mp 148° (yield, 40 mg). *Anal.* Calcd for $C_{20}H_{27}N_3O_2$: C, 70.35; H, 7.97; N, 12.31. Found: C, 70.21; H, 8.03; N, 12.17. MS *m/e*: 341 ($M^+$), 201, 143. UV $\lambda_{max}^{MeOH}$ nm: 223, 282, 292. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3420, 3330, 1700, 1650, 1435. PMR ($CDCl_3$) $\delta$: 0.97 (6H, d, $J=6.5$ Hz, $2CH_3$), 1.03 (3H, d, $J=7.5$ Hz, $CH_3$), 1.60 (3H, CH and $CH_2$), 2.80 (2H, t, $J=7.5$ Hz, $CH_2$), 3.19 (2H, AB part of ABX type, $J=14$, 7.5, 4 Hz, $CH_2$), 3.80 (3H, s, $OCH_3$), 4.03 (1H, qd, $J=7.5$, 2.5 Hz, CH), 4.26 (1H, ddd, $J=7.5$, 4, 2.5 Hz, CH), 6.53 (NH), 6.95—7.10 (3H, 2-, 5-, and 6-CH of indole), 7.40 (1H, dd, $J=7$, 3 Hz, 4-CH of indole), 8.34 (1H, NH of indole).

Another crop of colorless needles (7 mg), mp 141° was obtained from the spot at *Rf* 0.8, which was presumed to be a steric isomer of XV. A small amount of XVI was also obtained by this reaction.

**Demethylation of XV (Formation of the Diketopiperazine Derivative, XVI)**——Compound XV (50 mg) was dissolved in a mixture of AcOH (20 ml) and 10% HCl (1 ml), and the solution was boiled for 10 min. The reaction mixture was neutralized with $NaHCO_3$ and extracted with AcOEt. The AcOEt solution was evaporated to dryness and the residue was purified by preparative TLC with AcOEt as the solvent. From the upper band of the plate, a colorless powder (XVI), mp 235—237° was obtained, which was crystallized from MeOH (yield, 24 mg). *Anal.* Calcd for $C_{19}H_{25}N_3O_2$: C, 69.70; H, 7.70; N, 12.84. Found: C, 69.70; H, 7.64; N, 13.04. UV $\lambda_{max}^{MeOH}$ nm: 222, 275, 281.5, 292. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 1677, 1670. PMR (AcOH-$d_4$) $\delta$: 0.44 (3H, d, $J=7$ Hz, $CH_3$), 0.94 (6H, d, $J=6$ Hz, $2CH_3$), 1.57 (3H, CH and $CH_2$), 2.84 (2H, t, $J=7$ Hz, $CH_2$), 3.35 (2H, AB part of ABX type, $J=14.5$, 4, 4 Hz, $CH_2$), 3.94 (1H, qd, $J=7$, 1.5 Hz, CH), 4.49 (1H, ddd, $J=4$, 4, 1.5 Hz, CH), 6.68—6.92 (2H, 5- and 6-CH of indole), 7.11 (1H, 2-CH of indole), 7.41 (1H, dd, $J=6$, 3.5 Hz, 4-CH of indole).

This compound (XVI) was hydrolyzed with 6 N HCl at 130° for 2 hr. The reaction mixture was neutralized and analyzed with an amino acid analyzer and by TLC: alanine and 7-isopentyltryptophan were identified.

**Hydrogenation of IV with $PtO_2$ in AcOH**——Compound IV (190 mg) was hydrogenated with $PtO_2$ (30 mg) in AcOH (30 ml) as a catalyst under an IR lamp at *ca.* 60° for 5 hr. The catalyst was filtered off and the solvent was removed by evaporation. The residue was purified by preparative TLC with $CHCl_3$–acetone as the solvent. The main band at *Rf* 0.2 was extracted with MeOH, and the extract was crystallized from hexane–$CHCl_3$ as colorless needles (XVII), mp 149° (yield, 70 mg). *Anal.* Calcd for $C_{19}H_{27}N_3O$: C, 72.80; H, 8.68; N, 13.41. Found: C, 72.85; H, 8.80; N, 13.21. MS *m/e*: 313 ($M^+$), 201, 200. UV $\lambda_{max}^{MeOH}$ nm (log $\varepsilon$): 282 (3.84), 292 (3.75). IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3450, 3345, 1645. PMR ($CDCl_3$) $\delta$: 0.95 (6H, d, $J=6$ Hz, $2CH_3$), 1.37 (3H, d, $J=7$ Hz, $CH_3$), 1.65 (3H, CH and $CH_2$), 2.81 (2H, t, $J=7$ Hz, $CH_2$), 2.98 (2H, AB part of ABX type, $J=10$, 6, 3.5 Hz, $CH_2$), 3.08 (2H, AB part of ABX type, $J=13$, 5, 3 Hz, $CH_2$), 3.51 (1H, q, $J=7$ Hz, CH), 3.68 (1H, m, CH), 5.84 (1H, NH), 7.0—7.15 (3H, 2-, 5-, and 6-CH of indole), 7.04 (1H, dd, $J=6.5$, 3 Hz, 4-CH of indole), 8.36 (1H, NH of indole).

**Methylation of IX with $CH_2N_2$**——Compound IX (21 mg) was treated with excess ethereal $CH_2N_2$ for 15 min. The solvent was evaporated off and the residue was purified by preparative TLC with $CHCl_3$–AcOEt (2: 1) as the solvent. The main band was extracted with MeOH and crystallized from petr. ether–ether mixture as colorless needles (XII), mp 129—130° (yield, 6.3 mg). *Anal.* Calcd for $C_{21}H_{25}N_3O_3$: C, 68.64; H, 6.86; N, 11.44. Found: C, 68.34; H, 6.87; N, 11.23. MS *m/e*: 367 ($M^+$), 350, 292, 198. UV $\lambda_{max}^{MeOH}$ nm: 225, 292, 325. The UV spectrum did not change when the pH was changed. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3340, 2840, 1600, 1470, 1430, 1225, 1210. PMR ($CDCl_3$) $\delta$: 1.67 (6H, s, $2CH_3$), 2.33 (3H, s, $CH_3$), 3.46 (2H, d, $J=8$ Hz, $CH_2$), 3.92 (3H, s, $OCH_3$), 3.98 (3H, s, $OCH_3$), 4.30 (2H, s, $CH_2$), 5.24 (1H, t, $J=8$ Hz, –CH=), 6.8—7.0 (2H, 5- and 6-CH of indole), 7.32 (1H, d, $J=3$ Hz, 2-CH of indole), 7.67 (1H, dd, $J=8$, 3.5 Hz, 4-CH of indole), 7.9 (NH).

**Catalytic Hydrogenation of XII with Pd/C**——Compound XII (42 mg) was hydrogenated in AcOEt with Pd/C as a catalyst for 1 hr under an IR lamp at *ca.* 60°. The product was crystallized from hexane

as colorless micro-crystals (XIII), mp 139—140.5° (yield, 21 mg). *Anal.* Calcd for $C_{21}H_{27}N_3O_3$: C, 68.27; H, 7.37; N, 11.37. Found: C, 68.62; H, 7.57; N, 11.46. MS *m/e*: 369 ($M^+$). UV $\lambda_{max}^{MeOH}$ nm: 220, 264, 291, 322. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 3300, 2850, 1600, 1475, 1435, 1225, 1210. PMR ($CDCl_3$) $\delta$: 0.94 (6H, d, $J=7$ Hz, $2CH_3$), 1.57 (3H, CH and $CH_2$), 2.33 (3H, s, $CH_3$), 2.75 (2H, t, $J=8$ Hz, $CH_2$), 3.94 (3H, s, $OCH_3$), 3.96 (3H, s, $OCH_3$), 4.29 (2H, s, $CH_2$), 6.85—7.02 (2H, 5- and 6-CH of indole), 7.30 (1H, d, $J=3$ Hz, 2-CH of indole), 7.63 (1H, dd, $J=8$, 3.5 Hz, 4-CH of indole), 7.96 (1H, NH).

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