A New Series of Coumarin Derivatives Having Monoamine Oxidase Inhibitory Activity from *Monascus anka*[1)]

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Monankarins A—F (1—6), a new series of pigments having conjugated pyrano-coumarin skeleton have been isolated from *Monascus anka*. Their structures have been elucidated by spectroscopic means and chemical modification. Monankarins A—D exhibited monoamine oxidase (MAO) inhibitory activity, while the activity was not observed in monankarins E and F or some other simple coumarins. Monankarin C (3) showed stronger inhibition of MAO-B than MAO-A in mice brain. However, the specificity was not found in mice liver MAO.

Key words monankarins A—F; pyrano-coumarin pigment; *Monascus anka*; monoamine oxidase inhibition

In our continuing search for monoamine oxidase (MAO) inhibitors from fungi, some azaphilones were found to have the MAO inhibitory activity.[2)] *Monascus* species are well known for their azaphilone metabolites, and have been used as natural colorants and also as traditional medicines in China and Japan.[3)] As the MeOH extract of *Monascus anka* indicated the MAO inhibitory activity (38% at 1×10^{-4} g/ml), we isolated the azaphilone components, monascin and ankaflavin. They did not show the activity in tests, but a new series of pigments with MAO inhibitory activity was isolated. This paper deals with the isolation and structure determination of the pigments called monankarins A—F together with their MAO inhibitory activities.

Isolation and Structure Elucidation *Monascus anka* IFO 30878 was stationarily cultured in modified Nishikawa medium[4)] at 31 °C for 30 d. After collecting the mycelia, the culture medium (40 l) was passed through a Diaion HP-20 column; pigmented matter was obtained by elution with MeOH. The dried mycelia were extracted with *n*-hexane and MeOH successively to give the extracts. Details of the isolation of the new pigments from the *n*-hexane extract will be published elsewhere.

The MeOH extract of the mycelia and the pigmented matter of the medium were separated independently by repeated silica gel and/or Sephadex LH-20 column chromatography to get a mixture of monankarins A—D and monankarin E and F. The mixture was further separated to each pigment by octadecyl silica (ODS)-HPLC. These pigments exhibited strong greenish-yellow fluorescence in aqueous and organic solutions and indicated similar NMR spectra, some of which signals were broadening.

Monankarin A (**1**) was obtained as yellow needles, mp 208—209 °C, by crystallization from MeOH–H_2O. Its molecular formula $C_{20}H_{22}O_6$ (obs. *m/z* 358.1419) was determined by high resolution electron impact mass spectrum (HR-EI-MS). The bathocromic shift observed upon addition of NaOH solution in UV spectrum indicated the presence of a phenolic group. Absorptions at 1725 cm^{-1} for an ester carbonyl group and at 1640 cm^{-1} for a conjugated carbonyl group were observed in the IR spectrum.

The ^{1}H- and ^{13}C-NMR spectra indicated the presence of a 1-methyl-2-oxypropyl group at δ_H 1.25 (δ_C 23.39), δ_H *ca.* 4.20 (δ_C 72.23), δ_H *ca.* 3.50 (δ_C 44.84) and δ_H 1.39 (δ_C 19.41), three isolated aromatic methines at δ_H 6.58 (δ_C 100.92), δ_H 6.71 (δ_C 107.11) and δ_H 9.12 (δ_C 144.07), a hydroxy propyl at δ_H 1.52 (δ_C 21.24), δ_H 4.56—4.63 (δ_C 77.05) and δ_H 2.26—2.47 (δ_C 44.50), an aromatic methyl at δ_H 2.26 (δ_C 12.66) and an aliphatic and a phenolic OH proton at δ 3.86 and at δ *ca.* 9.80, respectively. The two dimensional (2D)-NMR experiments of H,H-correlation spectroscopy (H–H-COSY), C–H-COSY, and correlation *via* long range coupling spectroscopy (COLOC) suggested the presence of 3-hydroxy-2-methyl-1-(1-methyl-2-hydroxypropyl)benzene conjugated to other chromophores, although the complete structure of monankarin A was not determined by these experiments.

Methylation of **1** gave a monomethyl ether (**7**), indicating the presence of a phenolic hydroxyl group in **1**. Acetylation of **1** afforded a diacetate derivative (**8**) which could be purified by silica gel column chromatography. However, on silica gel TLC or by addition of silica gel for preparation of TLC to the solution, the diacetate was readily converted to its monoacetate derivative **9**. Methylation of **9** with diazomethane gave monomethyl monoacetate **10**.

In the heteronuclear multiple-bond correlation spectroscopy (HMBC) experiment of **8** shown in Fig. 1, the cross peaks of δ 6.75 (H-16)↔δ 162.79 (C-11)/δ 43.97 (C-14) and δ 4.62—4.68 (H-13)↔δ 162.79 (C-11)/δ 192.50 (C-15) suggested the presence of a dihydro γ-pyrone ring. The cross peaks of δ 6.75 (H-16)↔δ 117.85 (C-3) and δ 8.90 (H-4)↔δ 162.79 (C-11) indicated that this ring was attached to the carbon at δ 117.85 (C-3). The methyl protons at δ 1.51 and δ 2.25 had correlation with the carbon at δ 143.81 (C-6) and the latter methyl also correlated with the carbon at δ 128.17 (C-7) and 154.05 (C-8), both of which had cross peaks with the aromatic proton at δ 7.02 (H-9). Those indicated the substituted A-ring as shown in Fig. 1. The IR spectrum of **1** suggested the presence of an ester carbonyl group by the absorption at 1725 cm^{-1}. Therefore, the signal at δ 156.98 in the ^{13}C-NMR of **8** was assigned to be the ester carbonyl that would be present in a coumarin ring, because of its unusual high field shift.[5)]

In the ^{1}H-NMR of **9**, a phenolic hydroxyl proton at δ 8.52 was observed instead of an acetyl group in **8**. In the

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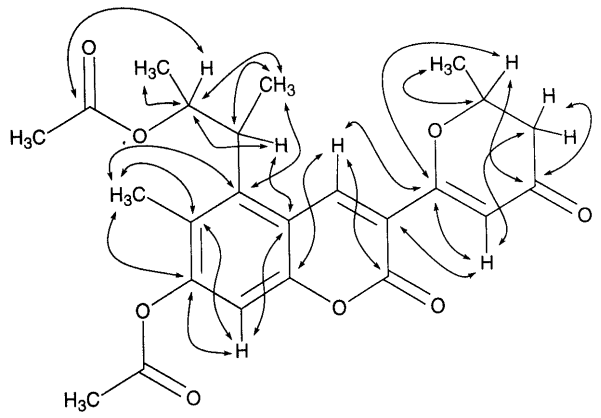


Fig. 1. HMBC Cross Peaks Observed in Monankarin A Diacetate (**8**)

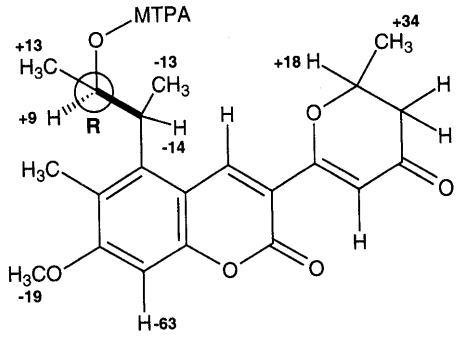
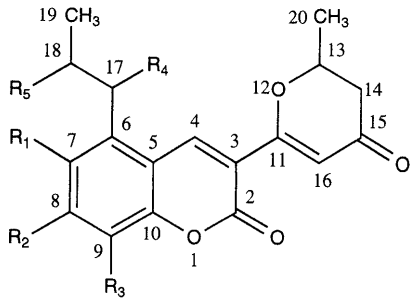


Fig. 2. $\Delta\delta$ Values ($\delta_S-\delta_R$ in Hz) of MTPA Derivatives of Monankarin A



1 and **2**:	R_1=CH_3	R_2=OH	R_3=H	R_4=CH_3	R_5=OH
3 and **4**:	R_1=CH_3	R_2=OH	R_3=CH_3	R_4=CH_3	R_5=OH
5:	R_1=H	R_2=OH	R_3=CH_3	R_4=H	R_5=OH
6:	R_1=CH_3	R_2=OH	R_3=CH_3	R_4=H	R_5=OH
7:	R_1=CH_3	R_2=OMe	R_3=H	R_4=CH_3	R_5=OH
8:	R_1=CH_3	R_2=OAc	R_3=H	R_4=CH_3	R_5=OAc
9:	R_1=CH_3	R_2=OH	R_3=H	R_4=CH_3	R_5=OAc
10:	R_1=CH_3	R_2=OMe	R_3=H	R_4=CH_3	R_5=OAc

Fig. 3. Structures of Monankarins A—F (**1**—**6**) and the Derivatives

^{1}H-NMR of **10**, a methoxy methyl signal at δ 3.91 was observed in place of the phenolic hydroxy proton of **9**. The nuclear Overhauser effect spectroscopy (NOESY) cross peaks between this methoxy methyl at δ 3.91, and an aromatic proton and a methyl group at δ 6.71 (1H, s) and δ 2.29 (3H, s), respectively, supported the position of a phenolic hydroxy group in **1**.

Absolute stereochemistry at C-18 of **1** was estimated by Mosher's method.[6] 8-*O*-Methyl monankarin A (**7**) was treated with *R*-(−)- and *S*-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPA-Cl) to afford the *S*-(−)- and *R*-(+)-esters, respectively. Due to the presence of two conformers at room temperature probably caused by hindered rotation of the bulky substituent at C-6, their NMR spectra showed pairs of signals for most of the protons, but each paired signal became slightly broadened for a single conformer at 55 °C. Assignment of each proton was done by H–H-COSY and NOESY experiments. Calculated $\Delta\delta$ values ($\delta_S-\delta_R$) shown in Fig. 2 from the diastereomeric esters suggested that the stereochemistry at C-18 was *R*.

The stereochemistry at C-17 and C-18 was also analyzed independently by the DADAS90 implemented MolSkop system (JEOL, Ltd., Japan),[7] which generates three dimensional structures of the possible isomers of the molecule satisfying the distance constraints obtained from NOESY experiment. The analysis suggested that the stereochemistry at C-17 and C-18 positions was *R*/*R* by giving the smallest target function values among all possible isomers (*R*/*R*, *R*/*S*, *S*/*R* and *S*/*S*) with the assumption of *R* configuration at C-13. Stereochemistry at C-13 did not affect the result, and was not determined by this method due to insufficient data from the NOESY experiment.

Monankarin B (**2**) was obtained as yellow needles, mp 212—213 °C, having the same molecular formula, $C_{20}H_{22}O_6$ (obs. *m*/*z* 358.1421), as **1** by HR-EI-MS. The spectral data of **2** were very similar to those of **1** except for the circular dichroism (CD) spectrum. These facts suggested that **2** was a diastereomer of **1**.

Monankarin C (**3**) was yellow needles, mp 243—244 °C, which molecular formula was calculated as $C_{21}H_{24}O_6$ (obs. *m*/*z* 372.1573) from HR-EI-MS. The ^{1}H-NMR spectrum of **3** showed the additional methyl singlet at δ 2.17 instead of an aromatic proton in **1**. In the COLOC experiment, the methyl group indicated correlation with the carbons at δ 160.27 (C-8), δ 108.49 (C-9) and δ 153.37 (C-10). Another aromatic methyl at δ 2.22 gave cross peaks with the carbons at δ 141.99 (C-6), δ 123.07 (C-7) and δ 160.27 (C-8). Thus, the methyl groups at δ 2.17 and δ 2.22 were assumed to be bonded to C-9 and C-7, respectively. The position of the attachment of the side chain of 1-methyl-2-oxypropyl was determined by the cross peak which was observed between the methyl signal at δ 1.30 in the side chain and δ 141.99 (C-6) as well. Stereochemistry of **3** is probably the same as monankarin A because of the resemblance in CD and optical rotatory dispersion (ORD) spectra.

Monankarin D (**4**), yellow needles, mp 239—240 °C, gave the same molecular formula of $C_{21}H_{24}O_6$ (obs. *m*/*z* 372.1588) as **3** by HR-EI-MS. The spectral data (UV, IR, ^{1}H-, ^{13}C-NMR) were very similar to those of **3** except for the CD spectrum. This evidence suggested that **4** is a diastereomer of **3**.

Monankarin E (**5**) was yellow needles, mp 200—202 °C. The molecular formula of **5** was estimated to be $C_{19}H_{20}O_6$ (obs. *m*/*z* 344.1259) by HR-EI-MS, which was CH_2 less than that of **1**. The ^{1}H-NMR spectrum showed methylene protons at δ 2.79 (1H, dd, J=13.6, 5.5 Hz) and δ 2.86 (1H, dd, J=13.6, 7.0 Hz) instead of the signals ascribed

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Table 1. ^{1}H-NMR Assignments of Monankarins A—F (**1**—**6**)

	1[a]	**2**[a]	**3**[b]	**4**[b]	**5**[a]	**6**[a]
H-4	9.12 br s	9.16 br s	9.01 br s	9.08 br s	8.56 br s	8.74 br s
H-7					6.77 s	
H-9	6.58 s	6.57 s				
H-13	4.56—4.63 m	4.54—4.59 m	4.65 br s	4.65 br s	*ca.* 4.67 m	4.58—4.63 m
H-14	2.26—2.47[c]	2.38 dd (16.5, 4.2) 2.45 dd (16.5, 12.5)	*ca.* 2.45[c]	2.44 dd (16.8, 3.3) 2.53 dd (16.8, 12.9)	2.45—2.57[c]	2.44[c]
H-16	6.71 s	6.70 s	6.53 s	6.54 br s	6.50 br s	6.72 s
H-17	*ca.* 3.50 m	*ca.* 3.50 m	3.37 br s	*ca.* 3.40[c]	2.79 dd (13.6, 5.5) 2.86 dd (13.6, 7.0)	2.99 dd (14.0, 5.2) 3.06 dd (14.0, 7.4)
H-18	*ca.* 4.20 m	4.16—4.23 m	4.29 br s	4.07 br s	3.81 m	3.88—3.92 m
H_3-19	1.25 br s	1.24 br s	1.18 br s	1.17 br s	1.08 d (5.9)	1.21 d (6.1)
H_3-20	1.52 d (6.3)	1.52 d (6.2)	1.48 br s	1.49 br s	1.50 d (6.2)	1.53 d (6.3)
H_3-R_1	2.26 s	2.26 br s	2.22 s	2.23 s		2.28[d] s
H_3-R_3			2.17 s	2.19 s	2.12 s	2.27[d] s
H_3-R_4	1.39 br d (6.9)	1.40 br d (7.0)	1.48 br s	1.49 br s		

a) In THF-d_8, *b*) in DMSO-d_6, *c*) overlapped with the water signal, *d*) interchangeable.

Table 2. ^{13}C-NMR Assignments of Monankarins A—F (**1**—**6**)

C-	**1**[a]	**2**[a]	**3**[b]	**4**[b]	**5**[a]	**6**[a]
2	158.27	157.82	157.47	157.62	157.71	158.14
3	113.50	112.97	109.50[c]	109.31[d]	111.35	114.24
4	144.07	143.88	143.37	143.69	141.56	142.38
5	111.41	111.02	109.33[c]	109.05[d]	110.34	112.24
6	146.43	145.94	141.99	141.98	138.37	136.90
7	124.25	123.78	123.07	123.28	114.81	122.46
8	162.37	161.96	160.27	160.91	161.36	159.81
9	100.92	100.47	108.49	108.52	108.56	109.47
10	157.33	156.90	153.37	153.50	154.35	153.92
11	164.51	164,00	163.72	163.96	163.62	163.95
13	77.05	76.62	75.36	75.44	75.59	76.71
14	44.50	44.09	42.47	42.59	42.45	44.09
15	192.94	192.39	192.68	192.85	192.84	192.40
16	107.11	106.68	104.62	104.52	105.03	106.91
17	44.84	44.30	42.69	42.59	40.95	38.74
18	72.23	71.71	70.23	70.14	67.29	68.82
19	23.39	22.96	22.51	22.63	23.30	24.16
20	21.24	20.84	20.05	20.14	20.00	20.80
R_1	12.66	12.21	12.75	12.75		12.80
R_3			8.58	8.67	7.64	8.37
R_4	19.41	19.00	18.68	18.77		

a) In THF-d_8, *b*) in DMSO-d_6, *c*, *d*) interchangeable.

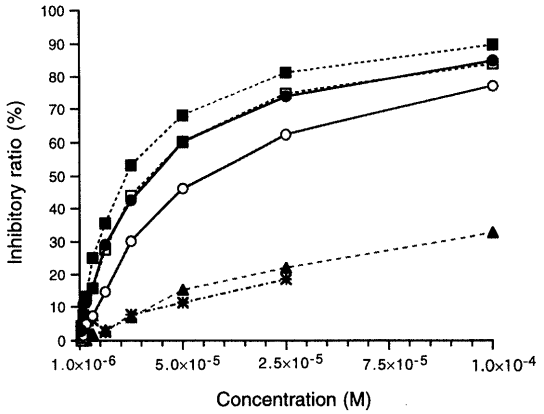

Fig. 4. MAO Inhibitory Activities of Monankarins A—F (**1**—**6**)
—●—, **1** IC_{50} 15.5 μM; —○—, **2** IC_{50} 30.5 μM; ---■---, **3** IC_{50} 10.7 μM; ---□---, **4** IC_{50} 17.2 μM; ---▲---, **5** $IC_{50} > 100$ μM; —·—✱—·—, **6** $IC_{50} > 100$ μM.

to a methyl (δ 1.39) and a methine (*ca.* δ 3.50) at C-17 in **1**. In the COLOC, these methylene protons showed cross peaks with δ 110.34 (C-5), δ 138.87 (C-6) and δ 114.81 (C-7). The data suggested that the side chain at C-6 was 2-hydroxy-propyl in **5**. The methylene carbon at δ 40.95 (C-17) was correlated with an aromatic proton of δ 6.77 (1H, s) at C-7 which also had correlation with the carbons at δ 110.34 (C-5) and δ 108.56 (C-9). From these data and the cross peaks between the methyl signal at δ 2.12 (3H, s) and the carbons at δ 161.36 (C-8), δ 108.96 (C-9) and δ 154.34 (C-10), the position of the aromatic methyl group was assigned at C-9.

Monankarin F (**6**) was obtained as yellow needles, mp 237—238 °C, and its molecular ion of *m/z* 358 was the same as that of **1** by EI-MS. In the NMR of **6**, a 2-hydroxypropyl group was revealed by the assignment of methylene signals at δ 2.99 (1H, dd, J=14.0, 5.2 Hz) and δ 3.06 (1H, dd, J=14.0, 7.4 Hz), hydroxy methine at δ 3.88—3.92 (1H, m) and δ 3.81 (1H, d, J=4.1 Hz, OH) and methyl at δ 1.21 (3H, d, J=6.1 Hz). Two aromatic methyl signals at δ 2.27 and δ 2.28 and two isolated aromatic protons at δ 8.74 and δ 6.72 were also observed. In the NOE difference (NOEDF) experiment, the NOEs of the methyl signals at δ 2.27 and δ 2.28 were observed by irradiation of δ 8.51 (8-OH), and the NOEs of δ 3.87—3.92 (H-18) and δ 2.99 and δ 3.06 (H_2-17) by irradiation of δ 8.74 (H-4). These observations suggested the structure of **6**. However, monankarin F might be a mixture with a small amount of its diastereomer, since some of the peaks were delicately split out to small pair signals.

MAO Inhibitory Activities of the Pigments The new yellow pigments, monankarins A—F, were tested for their MAO inhibitory activity; measurement was made by a modification of Kraml's method.[8] Mouse brain or liver homogenate was used for a crude MAO preparation, and kynuramine was used as the substrate.

As shown in Fig. 4, monankarin C (**3**) exhibited the most potent inhibitory activity (IC_{50} 10.7 μM). Monankarin A (**1**, IC_{50} 15.5 μM), in which the methyl group at C-9 was absent, showed slightly less potency than monankarin C. Marked decrease of the activity was observed in monankarin E (**5**, $IC_{50} > 100$ μM) and monankarin F (**6**,

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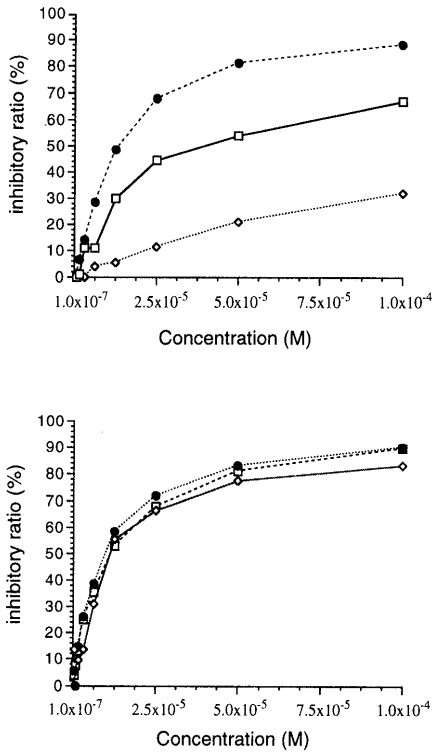

Fig. 5. MAO-A and MAO-B Inhibitory Activities of Monankarin C (**3**) in Mice Brain (Above) and in Mice Liver (Below)

Above: —□—, total MAO; ---◇---, MAO-A; ——●——, MAO-B. Below: ---□---, total MAO; —◇—, MAO-A; ---●---, MAO-B.

$IC_{50} > 100\,\mu M$). Both pigments lack a methyl group at C-17 in the side chain. The stereochemistry of the side chain seems to have only slight influence on the activity by comparing between **1** and **2**, or **3** and **4**. These results indicate that methyl groups, especially at C-17, have some participation in the MAO inhibitory activity in this series of pigments.

Since these pigments possess a coumarin ring, the activities of simple coumarin derivatives were also tested. Umbeliferon ($IC_{50} > 100\,\mu M$), fraxetin ($IC_{50} > 100\,\mu M$) and coumarin ($IC_{50} > 100\,\mu M$) did not exhibit the significant MAO inhibitory activity. Therefore, the coumarin ring by itself cannot demonstrate this activity; other criteria such as ring conjugation or the presence of side chains seems necessary.

Specific inhibition towards MAO-A and MAO-B of monankarin C (**3**) was examined in mice liver and brain preparations. Deprenyl-treated MAO preparation was used for the measurement of MAO-A activity, and a clorgyline-treated one for MAO-B. Monankarin C showed higher specificity towards MAO-B than MAO-A in brain MAO, while no specificity was observed in liver (Fig. 5).

Experimental

All melting points were measured on a Yanagimoto micro-melting point apparatus and are uncorrected. The spectral data were measured on the following instruments: Optical rotation on JASCO DIP-40; UV spectra on Hitachi U-3400; IR spectra on Hitachi EPI-G3 and 260-10; ORD and CD spectra on JASCO J-20 and J-500, respectively; the EI-MS and HR-EI-MS on Hitachi M-60 and JMS-HX110A, respectively; ^1H- and ^{13}C-NMR on JEOL GSX 400 and GSX 500.

Cultivation and Isolation of Monankarins A—F The strain *Monascus anka* IFO 30873 was purchased from the Institute of Fermentation, Osaka, Japan. The fungus was stationarily cultured at 31 °C for 30 d in medium (40 l) consisting of 10 g peptone, 100 g sucrose, 2.0 g KNO_3, 2.0 g $(NH_4)_2HPO_4$, 0.5 g $MgSO_4$, 0.5 g $ZnSO_4$, and 0.14 g $CaCl_2$ per one liter.[4] After sterilization, the mycelium was separated from the medium. The medium (30 l) was passed through Diaion HP-20 (500 ml) column (70 i.d. × 250 mm), which was then eluted with water, MeOH–H_2O (1 : 1) and MeOH. The MeOH eluate, a pigmented fraction (26.3 g), was subjected to silica gel column with a gradient solution of *n*-hexane and AcOEt to give fr. 1a—1g. Fraction 1d (1.22 g) eluted with *n*-hexane–AcOEt (1 : 1) was further chromatographed on silica gel flash column (benzene–AcOEt, 1 : 1 and 1 : 4) to yield yellow colored fractions, fr. 2b (145 mg) and fr. 2e (97 mg), respectively. By washing with AcOEt, the former fraction gave a mixture of **1**—**4** (18 mg) and the latter yielded **5** (5 mg).

The mycelia (887 g) were dried and extracted with *n*-hexane and then with MeOH. The MeOH extract (161 g) was subjected to silica gel column (*n*-hexane–acetone gradient) to get fr. 3a—3d. Fraction **3c** (3.6 g) obtained by elution with *n*-hexane–acetone (5 : 2) was flash chromatographed on silica gel with *n*-hexane–acetone (5 : 1) eluent to get fr. 4a—4g. Further separation of fr. 4c (1.5 g) by silica gel flash chromatography (benzene–AcOEt, 1 : 1), followed by crystallization from benzene–acetone, gave a mixture of **1**—**4** (53 mg). Fraction 4d (0.8 g) was subjected to silica gel flash chromatography, and the eluents of benzene–AcOEt (1 : 1 and 1 : 2) afforded fr. 5b (120 mg) and fr. 5d (90 mg) and fr. 5e (110 mg), respectively. Crystallization of fr. 5b from benzene–acetone yielded the mixture of **1**—**4** (8 mg). Fraction 5e (110 mg) was crystallized from benzene–acetone, and the crude crystals (18 mg) were subjected to HPLC with ODS column (Senshu Pak, 20 i.d. × 300 mm) using MeOH–H_2O (7 : 5) to get **5** (13 mg). Flash column chromatography of fr. 5d with ODS (MeOH–H_2O, 7 : 3) gave fr. 6b (10 mg), which yielded **6** (1.6 mg) by crystallization from EtOH.

The fractions containing **1**—**4** (79 mg) were combined and were separated by repeated HPLC with ODS (Senshu Pak, 20 i.d. × 300 mm, MeOH–H_2O, 7 : 5) to yield **1** (30 mg), **2** (4 mg), **3** (23 mg) and **4** (6 mg).

Monankarin A (**1**): Yellow needles from MeOH–H_2O, mp 208—209 °C. $[\alpha]_{589}^{19}$ −316° (c=0.0096, MeOH). HR-EI-MS m/z: 358.1419 (M^+, err. +0.3 mmu for $C_{20}H_{22}O_6$). EI-MS m/z (%): 358 (M^+, 87), 314 (43), 247 (58), 230 (53), 206 (100), 191 (36), 162 (15). UV λ_{max}^{MeOH} nm ($\log\varepsilon$): 236 (4.02), 265 (3.83), 283 (3.74), 391 (4.50), 406 (sh, 4.43), 459 (2.85). IR ν_{max}^{KBr} cm^{-1}: 3350, 2980, 1725, 1640, 1575, 1418, 1320, 1280. CD (c=0.0096, MeOH) $\Delta\varepsilon^{19}$ (nm): −4.6 (386) (negative maximum), +0.2 (315) (positive maximum), −3.2 (280) (negative maximum).

Monankarin B (**2**): Yellow needles from acetone–H_2O, mp 212—213.5 °C. $[\alpha]_{589}^{24}$ +116° (c=0.0142, MeOH). HR-EI-MS m/z: 358.1421 (M^+, err. +0.5 mmu for $C_{20}H_{22}O_6$). EI-MS m/z (%): 358 (M^+, 74), 314 (42), 272 (25), 244 (58), 230 (53), 244 (21), 230 (100), 206 (61), 191 (23), 162 (27). UV λ_{max}^{MeOH} nm ($\log\varepsilon$): 233 (3.86), 266 (3.57), 279 (3.49), 391 (4.44), 407 (sh, 4.36), 459 (2.52). IR ν_{max}^{KBr} cm^{-1}: 3350, 2980, 1725, 1640, 1575, 1418, 1320, 1280. CD (c=0.0099, MeOH) $\Delta\varepsilon^{24}$ (nm): +2.5 (389) (positive maximum), −1.0 (322) (negative maximum), +1.9 (279) (positive maximum).

Monankarin C (**3**): Yellow needles from MeOH–H_2O, mp 243—244 °C. $[\alpha]_{589}^{19}$ −210° (c=0.0162, MeOH). HR-EI-MS m/z: 372.1573 (M^+, err. +0.1 mmu for $C_{21}H_{24}O_6$). EI-MS m/z (%): 372 (M^+, 100), 328 (39), 244 (45), 220 (38), 202 (6), 176 (7), 153 (5). UV λ_{max}^{MeOH} nm ($\log\varepsilon$): 235 (3.77), 261 (3.52), 279 (3.55), 391 (4.42), 406 (sh, 4.35), 472 (3.52). IR ν_{max}^{KBr} cm^{-1}: 3360, 2980, 1728, 1624, 1570, 1402, 1230. CD (c=0.0095, MeOH) $\Delta\varepsilon^{24}$ (nm): −3.5 (392) (negative maximum), +0.5 (322) (positive maximum), −2.4 (283) (negative maximum).

Monankarin D (**4**): Yellow needles from MeOH–H_2O, mp 239—240 °C. $[\alpha]_{589}^{19}$ −18° (c=0.0274, MeOH). HR-EI-MS m/z: 372.1588 (M^+, err. +1.6 mmu for $C_{20}H_{22}O_6$). EI-MS m/z (%): 372 (M^+, 63), 328 (41), 286 (17), 258 (18), 244 (69), 220 (15), 176 (14). UV λ_{max}^{MeOH} nm ($\log\varepsilon$): 235 (4.01), 261 (3.84), 279 (3.84), 390 (4.49), 409 (sh, 4.40), 469.8 (3.58). IR ν_{max}^{KBr} cm^{-1}: 3370, 2980, 1725, 1625, 1570, 1402, 1225. CD (c=0.0160, MeOH) $\Delta\varepsilon^{24}$ (nm): −0.4 (385) (negative maximum), 0.0 (320) (positive maximum), −0.2 (277) (negative maximum).

Monankarin E (**5**): Yellow needles from MeOH–H_2O, mp 200—202 °C. $[\alpha]_{589}^{19}$ −153° (c=0.0110, MeOH). HR-EI-MS m/z: 344.1259 (M^+, err. 0.0 mmu for $C_{19}H_{20}O_6$). EI-MS m/z (%): 344 (M^+, 100), 329 (9), 300 (17), 274 (33), 258 (29), 230 (21), 216 (94). UV λ_{max}^{MeOH} nm ($\log\varepsilon$): 234 (3.97), 265 (3.84), 277 (3.85), 385 (4.48), 403 (sh, 4.39), 466 (3.13). IR ν_{max}^{KBr} cm^{-1}: 3370, 2980, 1725, 1625, 1570, 1402, 1225. CD (c=0.0110, MeOH) $\Delta\varepsilon^{19}$ (nm): −2.8 (388) (negative maximum), +0.5 (325) (positive maximum), −2.0 (280) (negative maximum).

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Monankarin F (**6**): Yellow needles from MeOH, mp 237—238 °C. EI-MS *m/z* (%): 358 (M^+, 65), 314 (12), 272 (13), 244 (23), 230 (100), 174 (22), 146 (16). UV λ_{max}^{MeOH} nm (log ε): 235 (3.85), 260 (3.68), 279 (3.67), 390 (4.32), 407 (sh, 4.24), 472 (3.28). CD ($c=0.0047$, MeOH) $\Delta\varepsilon^{23}$ (nm): −1.9 (391) (negative maximum), 0.0 (330) (positive maximum), −1.6 (278) (negative maximum).

Preparation of Derivatives 8-*O*-Methyl Monankarin A (**7**)[9]: To a solution of monankarin A (9 mg) in acetonitrile–MeOH (9 : 1, 0.3 ml) were added trimethylsilyl diazomethane (44 μl, 39 μmol) and *N,N*-diisopropyl ethylamine (6.8 μl, 39 μmol). The reaction solution was stirred at room temperature for 16 h. The crude product was chromatographed on ODS (MeOH–H_2O, 7 : 5) to afford 8-*O*-methyl monankarin A (**7**, 5 mg).

^{1}H-NMR (acetone-d_6) δ: 1.33 (d, 5.8 Hz, H_3-19), 1.44 (br s, CH_3-17), 1.58 (d, 6.1 Hz, H_3-20), 2.29 (s, CH_3-7), 2.47 (ddd, 16.6, 4.3, 0.8 Hz, H-14), 2.53 (dd, 16.6, 12.4 Hz, H-14), 3.56—3.61 (m, H-18), 4.00 (s, CH_3O-8), 4.29—4.32 (m, H-17), 4.68—4.72 (m, H-13), 6.70 (s, H-16), 6.86 (s, H-9), 9.18 (s, H-4).

Monankarin A Diacetate (**8**): The solution of monankarin A (18 mg) in pyridine (0.2 ml) and acetic anhydride (0.3 ml) was stirred at room temperature for 27 h. Monankarin A diacetate (16 mg) was obtained after purification of the reaction mixture by HPLC (Senshu Pak, silica-5251-N, 20 i.d. × 300 mm) with *n*-hexane–AcOEt (1 : 1).

^{1}H-NMR (THF-d_8) δ: 1.38 (d, 6.2 Hz, H_3-19), 1.50 (d, 7.0 Hz, CH_3-17), 1.56 (d, 6.2 Hz, H_3-20), 1.67 (s, CH_3COO-18), 2.25 (s, CH_3-7), 2.31 (s, CH_3COO-8), 2.44 (dd, 16.6, 3.7 Hz, H-14), 2.53 (dd, 16.6, 12.1 Hz, H-14), *ca.* 3.75 (m, H-17), 4.62—4.68 (m, H-13), 5.38—5.45 (m, H-18), 6.75 (s, H-16), 7.02 (s, H-9), 8.90 (s, H-4).

^{13}C-NMR (THF-d_8) δ: 13.19 (CH_3-7), 18.27 (CH_3-17), 19.30 (C-19), 20.45 ($\underline{C}H_3COO$-18, $\underline{C}H_3COO$-8), 20.69 (C-20), 41.62 (C-17), 43.98 (C-14), 74.28 (C-18), 77.10 (C-13), 108.01 (C-16), 110.03 (C-9), 116.07 (C-5), 117.85 (C-3), 128.17 (C-7), 140.82 (C-4), 143.81 (C-6), 154.05 (C-8), 154.94 (C-10), 156.98 (C-2), 162.79 (C-11), 168.65 ($CH_3\underline{C}OO$-8), 169.59 ($CH_3\underline{C}OO$-18), 192.53 (C-15).

18-*O*-Acetyl Monankarin A (**9**): Monankarin A diacetate was found to be unstable on silica gel TLC plate to give two spots. Monankarin A diacetate (15 mg) in MeOH (4 ml) was stirred with TLC silica gel (400 mg) at room temperature for 46 h. The monoacetylated product (**9**, 5 mg) was obtained after purification by silica gel column chromatography ($CHCl_3$–MeOH, 20 : 1) and HPLC (Senshu Pak, aquasil 10 i.d. × 200 mm, $CHCl_3$–MeOH, 20 : 1).

^{1}H-NMR (acetone-d_6) δ: 1.42 (d, 6.1 Hz, H_3-19), 1.48 (d, 7.6 Hz, CH_3-17), 1.61 (d, 5.8 Hz, H_3-20), 1.79 (br s, CH_3COO-18), 2.32 (3H, s, CH_3-7), 2.55 (dd, 16.8, 3.3 Hz, H-14), 2.62 (dd, 16.8, 12.8 Hz, H-14), *ca.* 3.69 (m, H-17), 4.69 (br s, H-13), 5.45 (br s, H-18), 6.87 (s, H-16), 6.89 (s, H-9), 8.52 (s, OH-8), 8.86 (s, H-4).

^{13}C-NMR (acetone-d_6) δ: 11.98 (CH_3-7), 18.28 (CH_3-17), 19.29 (C-19), 20.56 ($\underline{C}H_3COO$-18), 20.93 (C-20), 40.48 (C-14), 43.07 (C-17), 74.18 (C-18), 75.91 (C-13), 101.00 (C-16), 106.32 (C-9). 110.35 (C-5), 112.60 (C-3), 123.52 (C-7), 142.26 (C-4), 142.80 (C-6), 155.36 (C-10), 158.53 (C-2), 160.49 (C-8), 163.97 (C-11), 170.23 ($CH_3\underline{C}OO$-18), 192.53 (C-15).

8-*O*-Methyl-18-*O*-acetyl Monankarin A (**10**): A solution of **9** (4 mg) with diazomethane in THF (0.2 ml) was stirred at room temperature for 24 h. The reaction mixture was purified by HPLC (ODS, MeOH–H_2O, 65 : 35) to get 8-*O*-methyl-18-*O*-acetyl monankarin A (**10**, 2.5 mg).

^{1}H-NMR ($CDCl_3$) δ: 1.40 (d, 5.9 Hz, H_3-19), 1.47 (d, 7.5 Hz, CH_3-17), 1.59 (d, 5.8 Hz, H_3-20), 1.77 (br s, CH_3COO-18), 2.29 (s, CH_3-7), 2.53 (dd, 4.3, 16.8 Hz, H-14), 2.61 (dd, 12.5, 16.8 Hz, H-14), *ca.* 3.69 (br s, H-17), 3.91 (s, CH_3O-8), *ca.* 4.67 (br s, H-13), 5.42 (br s, H-18), 6.71 (s, H-9), 6.89 (s, H-16), 8.80 (s, H-4).

(*R*)-(+)-α-Methoxy-α-(trifluoromethyl)phenylacetyl-8-*O*-methyl Monankarin A: (*S*)-(+)-MTPA-Cl (2.3 μl, 12 μmol) was added to a solution of **7** (2 mg) in pyridine (20 μl), and the solution was allowed to stand at room temperature for 13 h. After addition of *N,N*-dimethyl-1,3-propanediamine (1.4 μl, 12 μmol), the reaction mixture was evaporated. The residue was subjected to silica gel flash column chromatography using *n*-hexane–AcOEt (1 : 1) to get 2 mg of (*R*)-(+)-MTPA-8-*O*-methyl monankarin A.

^{1}H-NMR ($CDCl_3$) δ: 1.45 (d, 7.4 Hz, CH_3-17), 1.55 (br s, H_3-19), 1.51 (br d, 6.1, H_3-20), 2.16—2.29 (br s, CH_3-7), *ca.* 2.53 (br m, H_2-14), 3.13 (s, MTPA-OCH_3-18), 3.69 (br s, H-17), 3.90 (s, CH_3O-8), 4.61 (br s, H-13), 5.64 (br s, H-18), 6.69 (s, H-9), 6.71—6.27 (m, MTPA-$H_{5(aromatic)}$-18, H-16), 8.41, 8.69 (total 1H, br s, H-4).

(*S*)-(−)-α-Methoxy-α-(trifluoromethyl)phenylacetyl-8-*O*-methyl Monankarin A: To a solution of **7** (2 mg) in pyridine (20 ml) was added (*R*)-(−)-MTPA-Cl (2.3 μl, 12 μmol), and the solution was allowed to stand at room temperature for 13 h. *N,N*-Dimethyl-1,3-propanediamine (1.4 μl, 12 μmol) was added and the reaction mixture was evaporated. The residue was subjected to silica gel flash column chromatography using AcOEt–*n*-hexane (1 : 1) to get 2 mg of (*S*)-(−)-MTPA-8-*O*-methyl monankarin A.

^{1}H-NMR ($CDCl_3$, 55 °C) δ: 1.42 (d, 7.4 Hz, CH_3-17), 1.57 (d, 5.8 Hz, H_3-19), 1.58 (d, 6.3 Hz, H_3-20), 2.02—2.16 (br, CH_3-7), 2.52—2.61 (m, H_2-14), 3.36 (br s, MTPA-OCH_3-18), 3.66 (br s, H-17), 3.86 (br s, CH_3O-8), 4.64 (br s, H-13), 5.66 (br s, H-18), 6.56 (s, H-9), 6.82 (s, H-16), 7.09—7.27 (br s, MTPA-$H_{5(aromatic)}$-18), *ca.* 7.24 (overlapped, H-MTPA-Ar), 8.37, 8.63 (total 1H, br s, H-4).

Bioassay Preparation of Crude MAO Male ddY mice obtained from Japan SLC Inc. were used. Each mouse liver was homogenized with 4 volumes of 0.15 M KCl, and each mouse brain with 10 volumes of 0.25 M sucrose in a Teflon homogenizer under ice cooling. The homogenates were centrifuged at 1000 × *g* for 10 min. Fat layer was removed by spatula, and the supernatant was used for assay.

Assay of MAO Activity MAO activity was assayed by modified Kraml method with kynuramine as the substrate.[8] Samples dissolved in dimethyl sulfoxide (DMSO) were added to the incubation medium (final concentration of DMSO: 2.8%). Production of fluorescence intensity of the reaction product was measured at 380 nm (emission) with excitation at 315 nm. As a blank test, the reaction was carried out omitting the substrate.

To measure MAO-B inhibitory activity, the crude enzyme preparation of MAO was treated with 1×10^{-6} M of clorgyline (a MAO-A inhibitor) for 20 min. The treatment caused the inhibition of 59% and 39% in total brain MAO and liver MAO, respectively, in our preliminary experiments. Similarly, the crude enzyme preparation of MAO was treated with 1×10^{-8} M of deprenyl (a MAO-B inhibitor) for 20 min to analyze MAO-A inhibitory activities in mouse brain and liver. The treatment resulted in the inhibition of 47% and 67% in total brain and liver MAO, respectively.

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