Monoamine Oxidase Inhibitors from Cinchonae Cortex[1)]

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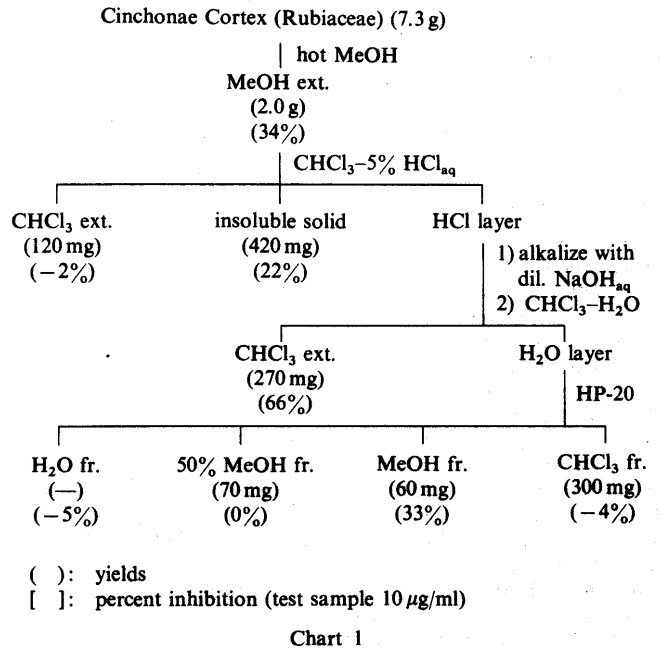
Three strong alkaloidal monoamine oxidase (MAO) inhibitors, quinine (1), cinchonicinol ([1*S*,3'*R*,4'*R*]-3-(3-ethenyl-4-piperidinyl)-1-(4-quinolinyl)-1-propanol) (2) and cinchonaminone ([3'*R*,4'*S*]-2-[2-(3-ethenyl-4-piperidinyl)-acetyl]-1*H*-indole-3-ethanol) (3), were isolated from Cinchonae Cortex (*Cinchona succirubra* PAV., Rubiaceae). The structures of 2 and 3 were elucidated on the bases of spectral data and chemical evidence, and 3 is a new alkaloid.

The inhibitory effects on MAO of 1, 2, 3 and related alkaloids were assayed. The type of inhibition by 1 with respect to benzylamine as a substrate was competitive.

Keywords monoamine oxidase; inhibitor; competitive inhibition; benzylamine; *Cinchona succirubra*; Rubiaceae; quinine; cinchonicinol; cinchonaminone; alkaloid

In screening tests *in vitro* aiming to find monoamine oxidase (MAO, EC 1.4.3.4) inhibitors from many crude oriental drugs, ethyl *p*-methoxy-*trans*-cinnamate was found in the rhizomes of *Kaempferia galanga* L.[2)] A crude methanol extract of Cinchonae Cortex also showed inhibitory activity against MAO, so this study was done. Cinchonae Cortex has been used as an antimalarial, a stomachic and so on, and the many constituents of the cortex have been reported, for instance, alkaloids,[3)] tannins,[4)] triterpenes[5)] and so on. Many biological activities of Chinchona alkaloids are known: antimalarial, antifebrile, oxytocic, antiarrhythmic, stomachic, inhibition of polymorphonuclear leukocyte cytotoxicity[6)] and so on. In this paper, we report the isolation and structural elucidation of alkaloidal MAO inhibitors from Cinchonae Cortex, and their inhibitory effects on MAO.

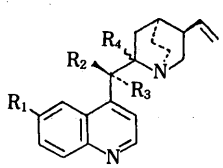
The MAO was obtained from bovine plasma. The activity of MAO *in vitro* towards benzylamine as a substrate was assayed spectrophotometrically at 250 nm by the method described in the previous paper[2)] with a slight modification. The Japanese commercial Cinchonae Cortex (the cortex of *Cinchona succirubra* PAV.,[7)] Rubiaceae) was extracted with methanol, and the extract was fractionated as shown in Chart 1. The yield and inhibitory activity of each fraction are shown in Chart 1. The most potent alkaloid fraction was separated by the method of Cromwell,[3)] and by chromatography on a silica gel column or high-performance liquid chromatography (HPLC), with monitoring by measurment of inhibitory activity against MAO. Three strong and two weak inhibitors were isolated. Quinine (**1**), compound I (**2**) and compound II (**3**) were stronger inhibitors, and cinchonidine (**4**) and cinchonine (**5**) were weaker than the former. The activities of the inhibitors **1**—**5** are listed in Table I.



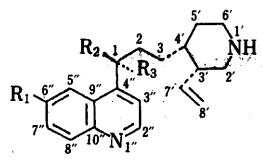
( ): yields
[ ]: percent inhibition (test sample 10 μg/ml)

Chart 1

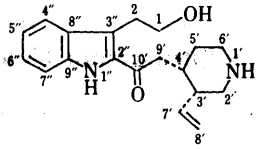
Compound I (**2**) was a pale yellow amorphous powder. $[\alpha]_D^{21}$ +5.6° (EtOH). $C_{19}H_{24}N_2O$. *m/z* 296.188 (M^+). The infrared (IR) spectrum of **2** showed the presence of hydroxyl and amine groups (3360 cm^{-1}). The ultraviolet (UV) spectrum of **2** in ethanol (224, 281, 300 and 314 nm) suggested the presence of a quinoline ring moiety.[8)] The proton nuclear magnetic resonance (^{1}H-NMR) spectrum of **2** showed the signals of an ethenyl group (δ: 4.96, 5.05 and 6.00), a hydroxyl-bearing methine (δ: 5.32) and a 4-substituted quinoline ring[9)] (δ: 7.54, 7.57, 7.66, 7.95, 8.07 and 8.77). The carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectrum of **2** showed the signals of two methylene (δ: 29.0 and 35.8), a *cis*-4-alkyl-3-ethenylpiperidine[10)] (δ: 30.1, 38.4, 42.7, 46.1, 51.6, 116.2 and 137.2), a hydroxyl-bearing methine (δ: 69.8) and 4-substituted quinoline moiety[11)] (δ: 117.3, 123.0, 125.5, 126.1, 128.8, 129.9, 147.9, 150.0 and 151.6). These spectral data suggested that **2** is cinchonicinol.[12)] Cinchonicinol was derived from cinchonidine (**4**) *via* cinchotoxine (**7**)[13)] according to a modification of Grethe *et al.*'s[14)] and ACF's[12)] method, and **2** was confirmed to be identical with synthesized cinchonicinol (**8**) by spectral comparison and chromatography. The synthesized product (**8**) was concluded to be a mixture of 1*R*- and 1*S*-epimers from the ^{13}C-NMR spectrum. The signals of C-1, C-2 and C-4'' of **8** were duplicated, but in the case of the natural product (**2**), they were single signals. The absolute



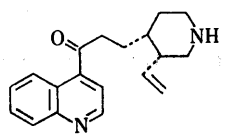
	R_1	R_2	R_3	R_4
1 : quinine	CH_3O	OH	H	◄H
4 : cinchonidine	H	OH	H	◄H
5 : cinchonine	H	H	OH	···H
6 : quinidine	CH_3O	H	OH	···H



	R_1	R_2	R_3
2 : compound I (cinchonicinol)	H	OH	H
8 :	H	OH, H	H, OH



3 : compound II (cinchonaminone)



7 : cinchotoxine

Chart 2

configuration of the hydroxy-bearing methine carbon, C-1, was determined by application of the modified Horeau's method,[15] as described in Experimental. The stereochemistry of the C-1 position was found to be *S*. Absolute configurations at C-3′ and C-4′ of the *cis*-4-alkyl-3-ethenylpiperidine moiety are most probably 3*R* and 4*R*, because they are thought to retain the configurations of the quinuclidine moiety of Cinchona alkaloids. Based on the above results, **2** was assigned to be [1*S*,3′*R*,4′*R*]-3-(3-ethenyl-4-piperidinyl)-1-(4-quinolinyl)-1-propanol (cinchonicinol). This is the first resport of isolation of **2** as a natural product.

Compound II (**3**) was a pale yellow amorphous powder. $[\alpha]_D^{22}$ +10.0° (EtOH). $C_{19}H_{24}N_2O_2$. *m/z* 312.187 (M^+). The IR spectrum of **3** showed the presence of hydroxyl and amine groups (3330 cm^{-1}) and an α,β-unsaturated ketone (1640 cm^{-1}). The UV spectrum of **3** in ethanol (237 and 312 nm) suggested the presence of an indole ring moiety.[16] The ^{1}H-NMR spectrum of **3** showed the signals of an ethenyl group (δ: 5.00, 5.10 and 6.10), 2-substituted ethanol (δ: 3.36 and 3.94) and an indole moiety[17] (δ: 7.12, 7.25, 7.32 and 7.70). The ^{13}C-NMR spectrum of **3** showed the signals of a *cis*-4-alkyl-3-ethenylpiperidine[10] (δ: 28.8, 34.0, 42.7, 45.6, 50.7, 116.8 and 137.1), a hydroxyl-bearing methylene (δ: 62.7), two methylenes (δ: 29.0 and 43.4), a 2-acyl-3-alkylindole moiety[18] (δ: 112.0, 119.8, 120.1, 120.9, 126.0, 128.2, 132.8 and 136.3) and a carbonyl group (δ: 192.9). Some Cinchona alkaloids such as cinchonamine[19] and quinamine[20] have a 3-ethenyl-2-quinuclidylindole moiety in the molecule. Based on the above spectral results and a consideration of the biosynthesis of Cinchona alkaloids, **3** was concluded to be [3′*R*,4′*S*]-2-[2-(3-ethenyl-4-piperidinyl)acetyl]-1*H*-indole-3-ethanol. It is a new compound, and was named cinchonaminone.

Satake,[21] Iida[22] and Hagiwara *et al.*[23] reported studies of the inhibitory effect on MAO of quinine (**1**) in the concentration range of 10^{-2}—10^{-3} M. Satake's assay conditions were not given. For MAO, Iida used the liver homogenate of guinea pigs, rats or cats, and Hagiwara *et al.* used the liver homogenate of pigs. In this paper, MAO was purified from bovine plasma and the substrate was benzylamine. The inhibitory activities of **1**—**8** on MAO are shown in Table I. The most active compound is **2**. The concentration of **2** required under our assay conditions to give 50% inhibition (IC_{50}) was 1.20×10^{-5} M. The IC_{50} of **1** and **3** were 1.63×10^{-5} M and 3.17×10^{-5} M, respectively. Kinetic studies were done on the effect of **1** on the oxidation of benzylamine by MAO under the assay conditions. The results are shown as Lineweaver–Burk plots[24] in Fig. 1.

TABLE I. Inhibitory Activities of Constituents of the Cinchonae Cortex and Related Alkaloids on Monoamine Oxidase

Compound	IC_{50} (M)
Quinine (**1**)	1.63×10^{-5}
Cinchonicinol (**2**)	1.20×10^{-5}
Cinchonaminone (**3**)	3.17×10^{-5}
Cinchonidine (**4**)	$>10^{-4}$
Cinchonine (**5**)	$>10^{-4}$
Quinidine (**6**)	$>10^{-4}$
Cinchotoxine (**7**)	$>10^{-4}$
Synthesized cinchonicinol (**8**)[a]	2.91×10^{-5}

a) Mixture of 1*R*- and 1*S*-epimers.

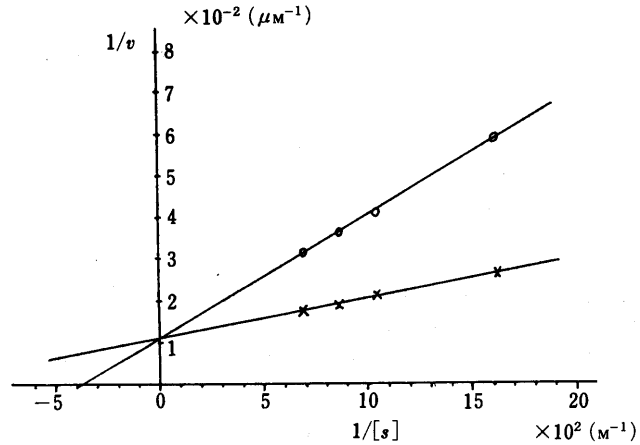


Fig. 1. Inhibitory Effects of **1** on Monoamine Oxidase

Lineweaver–Burk plots in the absence (0 M, ×—×) and in the presence (3.1×10^{-5} M, ○—○) of **1** with benzylamine as the substrate. *v*: μmol substrate metabolized/g enzyme/min. *s*: substrate.

The type of inhibition by **1** is competitive.

Experimental

The following instruments were used to obtain physical data. Melting points were determined on a Yanagimoto melting point apparatus and are uncorrected. The optical rotations were determined with a JASCO DIP-140 digital polarimeter. The IR spectra were recorded on a JASCO IRA-202 infrared spectrophotometer. The UV spectra were recorded on a Shimadzu UV-360 recording spectrophotometer. The ^{1}H- and ^{13}C-NMR spectra were recorded on a JEOL FX-90Q spectrometer (89.55 and 22.50 MHz, respectively). Chemical shifts are given on the δ (ppm) scale with tetramethylsilane (TMS) as an internal standard (s, singlet; d, doublet; t, triplet; m, multiplet; br, broad). Mass spectra (MS) were recorded on a JEOL JMS-D 100 for EI-MS and a JEOL JMS-D 300 for High-MS. Gas chromatography (GC) was run on a Hitachi K53 gas chromatograph. HPLC was carried out on a JASCO 880-PU instrument with Develosil 60-5 or Asahipack ODP-50. Silica gel 60GF_{254} (Merck) was used for thin layer chromatography (TLC) and detection was achieved by

illumination with an ultraviolet lamp, by spraying K_2PtCl_6–KI aqueous solution, or by spraying 20% H_2SO_4 aqueous solution followed by heating. For column chromatography, Silica gel 60 (Merck) was used. The spectrophotometric measurements were carried out with a Hitachi U-1100 spectrophotometer.

Enzyme and Chemicals Monoamine oxidase (EC 1.4.3.4) from bovine plasma was obtained from Sigma Chemical Co. Benzylamine, Tween 80, quinidine, cinchonine, K_2PtCl_6 and KI were obtained from Wako Pure Chemical Industries Ltd. Quinine, cinchonidine and α-phenylbutyric acid were obtained from Tokyo Kasei Kogyo Co., Ltd. Sodium phosphate dibasic 12 hydrate and potassium phosphate monobasic and [*R*]-(+)-α-phenylethylamine were obtained from Kanto Chemical Co., Inc. The buffer and the enzyme solution were prepared as described previously.[2] The substrate, 0.01 M benzylamine sulfate, was prepared immediately before use as follows. Benzylamine (0.54 g), which had been purified by redistillation, and 2 N H_2SO_4 (2.5 ml) were dissolved in H_2O to make 500 ml of solution.

Test Solution The test solutions were prepared as described previously.[2]

Assay of Monoamine Oxidase Activity (MAO) The MAO activities with benzylamine as a substrate were measured spectrophotometrically by the method as described previously[2] with a slight modification. Test solution (1.0 ml), 0.2 M potassium phosphate buffer pH 7.4 (3.9 ml) and enzyme solution (0.1 ml) were mixed and preincubated at 25 °C for 15 min. After this, the substrate (1.0 ml) was added to the assay mixture and the whole was incubated at 25 °C for 120 min. Then 1 N HCl (1.0 ml) was added to stop the reaction. The absorbance of the reaction mixture was measured spectrophotometrically at 250 nm. A blank was prepared in the same way, but the enzyme solution was added to the assay mixture after addition of 1 N HCl.

Estimation of MAO Inhibitory Activity MAO inhibitory activity was expressed as the percentage inhibition of MAO in the above assay system, calculated as $(1 - B/A) \times 100$, where A is the activity of the enzyme without test material and B is the activity of the enzyme with test material. The inhibitory activities of Cinchona alkaloids against MAO are shown in Table I.

Extraction and Isolation For the preliminary experiment the dried Cinchonae Cortex (the cortex of *Cinchona succirubra* PAV., 7.3 g, from Niiya in Shimizu) was extracted with hot MeOH, and the MeOH extract was partitioned with $CHCl_3$ and 5% HCl aqueous solution. The HCl layer was alkalized with dilute NaOH aqueous solution and was partitioned with $CHCl_3$ and H_2O. The H_2O layer was chromatographed on a Diaion HP-20 (Mitsubishi Chemical Industries Ltd.) column with H_2O, 50% MeOH in H_2O, MeOH and $CHCl_3$, successively. The yields and inhibitory activities are shown in Chart 1. From the most active alkaloid-containing fraction, alkaloids were isolated by Cromwell's method[3] and purified by silica gel column chromatography (hexane : CH_2Cl_2 : MeOH : $(C_2H_5)_2NH$ = 66 : 33—18 : 0—18 : 0.65) Develosil 60-5 HPLC (hexane : CH_2Cl_2 : MeOH : $(C_2H_5)_2NH$ = 66 : 31—18 : 2—15 : 0.65) or Asahipack ODP-50 HPLC (MeOH : CH_3CN : 10 mM sodium phosphate buffer (pH 9) = 60—0 : 0—60 : 40). The yields of alkaloids from 500 g of the cortex were **1** (480 mg), **2** (70 mg), **3** (95 mg), **4** (180 mg) and **5** (210 mg); **6** could be detected in the extract of the cortex by TLC and HPLC.

Quinine (1), Cinchonidine (4) and Cinchonine (5) Compounds **1**, **4** and **5** were identical with authentic samples on the bases of melting points, spectral comparison, elemental analysis and chromatographies.

[1*S*,3′*R*,4′*R*]-3-(3-Ethenyl-4-piperidinyl)-1-(4-quinolinyl)-1-propanol (Cinchonicinol) (2) A pale yellow amorphous powder. $[\alpha]_D^{21}$ +5.6° (c = 0.09, EtOH). High-MS m/z: 296.188 (M^+, Calcd $C_{19}H_{24}N_2O$: 296.189). EI-MS m/z (rel. int. %): 296 (M^+, 63), 268 (18), 240 (17), 172 (55), 159 (68), 143 (77), 132 (31), 130 (100), 103 (10), 82 (20), 81 (20), 79 (15), 77 (20). IR (KBr) cm^{-1}: 3360, 2920, 2820, 1590, 1510, 1450, 1350, 1240, 1070, 1000, 910, 850, 760. UV λ_{max}^{EtOH} nm (log ε): 224 (4.10), 281 (2.92), 300 (2.79), 314 (2.72). NMR: Tables II and III. *Anal.* Calcd for $C_{19}H_{24}N_2O \cdot H_2O$: C, 72.58; H, 8.34, N, 8.91. Found: C, 72.37; H, 8.10; N, 8.90. **2** was identical with synthetic cinchonicinol[12] (**8**) on the bases of spectral comparisons and chromatography.

Modified Horeau's Method[15] for 2 (Determination of Absolute Configuration of 2) A solution of **2** (1.5 mg) and 60 μl of (±)-α-phenylbutyric acid anhydride in pyridine was allowed to stand in a sealed microtube at room temperature for 20 h, then 60 μl of [*R*]-(+)-α-phenylethylamine was added. After standing for 30 min, the mixture was concentrated to dryness by blowing N_2 gas over it. The residue was dissolved with a small amount of MeOH and the solution was subjected to GLC analysis (carrier gas N_2, 10 ml/min, column packed with SPB-50, 1 mm × 5 m, column temperature 220 °C, injection temperature 280 °C, FID detector). The relative proportions of the amides of [*R*]-(−)- and [*S*]-(+)-α-phenylbutyric acid were calculated from areas of their peaks. Subtraction of the corresponding value from the reaction for cyclohexanol gave the increment of the percentage area representing the [*R*]-(−)-acid: +5.6%.

Synthesis of Cinchotoxine (7) Cinchotoxine[13] (**7**) was synthesized from cinchonidine (**4**) according to a modification of Grethe *et al.*'s method.[14] A pale yellow amorphous powder. $[\alpha]_D^{21}$ +48.0° (c = 0.13, EtOH). High-MS m/z: 294.173 (M^+, Calcd $C_{19}H_{22}N_2O$: 294.173). EI-MS m/z (rel. int. %): 294 (M^+, 29), 266 (13), 238 (16), 222 (18), 210 (13), 184 (25), 172 (63), 156 (81), 143 (100), 130 (71), 129 (55), 128 (57), 122 (56), 111 (33), 101 (31), 82 (50), 57 (58), 45 (57). IR (KBr) cm^{-1}: 3400, 2920, 2830, 1690, 1640, 1580, 1505, 1460, 1440, 1360, 1270, 1230, 1160, 1130, 1000, 910, 840, 760. NMR: Tables II and III. *Anal.* Calcd for $C_{19}H_{22}N_2O$: C, 77.52; H, 7.53; N,

TABLE II. ^{1}H-NMR Chemical Shifts[a]

Proton No.	2	3	7	8
1	5.32 (1H, m)	3.36 (2H, t, J=6 Hz)		5.33 (1H, m)
2	—	3.94 (2H, t, J=6 Hz)	—	—
7′	6.00 (1H, m)	6.10 (1H, m)	6.13 (1H, m)	5.94 (1H, m)
8′ *trans*	4.96 (1H, dd, J=2, 18 Hz)	5.00 (1H, dd, J=2, 18 Hz)	5.11 (1H, dd, J=2, 17 Hz)	4.88 (1H, dd, J=2, 18 Hz)
8′ *cis*	5.05 (1H, dd, J=2, 10 Hz)	5.10 (1H, dd, J=2, 9 Hz)	5.14 (1H, dd, J=2, 10 Hz)	4.94 (1H, dd, J=2, 9 Hz)
2″	8.77 (1H, d, J=4 Hz)		9.00 (1H, d, J=4 Hz)	8.72 (1H, d, J=4 Hz)
3″	7.54 (1H, d, J=4 Hz)		7.52 (1H, d, J=4 Hz)	7.53 (1H, d, J=4 Hz)
4″		7.70 (1H, d, J=8 Hz)		
5″	8.07 (1H, dd, J=2, 8 Hz)	7.12 (1H, dd, J=7, 8 Hz)	8.28 (1H, dd, J=2, 8 Hz)	8.06 (1H, br d, J=8 Hz)
6″	7.57 (1H, dt, J=2, 8 Hz)	7.25 (1H, dd, J=7, 8 Hz)	7.69 (1H, dt, J=2, 8 Hz)	7.55 (1H, br t, J=8 Hz)
7″	7.66 (1H, dt, J=2, 8 Hz)	7.32 (1H, d, J=8 Hz)	7.78 (1H, dt, J=2, 8 Hz)	7.66 (1H, br t, J=8 Hz)
8″	7.95 (1H, dd, J=2, 8 Hz)		8.18 (1H, dd, J=2, 8 Hz)	7.97 (1H, br d, J=8 Hz)

a) Measured at 89.55 MHz in $CDCl_3$ with TMS (0.00 ppm). —, not assigned.

TABLE III. ^{13}C-NMR Chemical Shifts[a]

Carbon No.	2	3	7	8
1	69.8 (d)	62.7 (t)	203.4 (s)	69.2, 69.4 (d)
2	35.8 (t)	29.0 (t)[b]	39.2 (t)	35.4, 35.6 (t)
3	29.0 (t)		27.2 (t)	28.8 (t)
2′	51.6 (t)	50.7 (t)	51.5 (t)	51.2 (t)
3′	42.7 (d)	42.7 (d)	42.5 (d)	42.5 (d)
4′	38.4 (d)	34.0 (d)	37.8 (d)	38.2 (d)
5′	30.1 (t)	28.8 (t)[b]	28.6 (t)	29.9 (t)
6′	46.1 (t)	45.6 (t)	45.8 (t)	45.7 (t)
7′	137.2 (d)	137.1 (d)	136.9 (d)	136.6, 136.9 (d)
8′	116.2 (t)	116.8 (t)	115.9 (t)	117.2, 117.4 (t)
9′		43.4 (t)		
10′		192.9 (s)		
2″	150.0 (d)	132.8 (s)	149.3 (d)	149.7 (d)
3″	123.0 (d)	119.8 (s)	124.8 (d)	122.9 (d)
4″	151.6 (s)	120.9 (d)	148.6 (s)	151.5, 151.8 (s)
5″	117.3 (d)	120.1 (d)	118.4 (d)	115.9 (d)
6″	126.1 (d)	126.0 (d)	127.5 (d)	125.9 (d)
7″	128.8 (d)	112.0 (d)	129.3 (d)	128.6 (d)
8″	129.9 (d)	128.2 (s)	129.5 (d)	129.5 (d)
9″	125.5 (s)	136.3 (s)	125.8 (s)	125.3 (s)
10″	147.9 (s)		143.0 (s)	147.6 (s)

a) Measured at 22.5 MHz in $CDCl_3$ with TMS (0.00 ppm). *b*) Exchangeable.

9.52. Found: C, 77.46; H, 7.35; N, 9.39.

Synthesis of Cinchonicinol (Mixture of [1*R*]- and [1*S*]-Epimers) (8) A mixture (**8**) of [1*R*]- and [1*S*]-epimers of cinchonicinol was synthesized from **7** according to ACF's method.[12] A pale yellow amorphous powder. $[\alpha]_D^{21}$ +53.4° (c=1.31, EtOH). High-MS m/z 296.190 (M^+, Calcd $C_{19}H_{24}N_2O$: 296.189). EI-MS m/z (rel. int. %): 296 (M^+, 20), 268 (8), 240 (9), 184 (8), 172 (34), 159 (48), 156 (19), 143 (57), 130 (100), 82 (13), 81 (13), 77 (14), 57 (21), 45 (25). IR (KBr) cm^{-1}: 3300, 2920, 2850, 1590, 1510, 1450, 1350, 1240, 1070, 1000, 910, 850, 760. NMR: Tables II and III. *Anal.* Calcd for $C_{19}H_{24}N_2O \cdot H_2O$: C, 72.58; H, 8.34, N, 8.91. Found: C, 72.59, H, 8.25, N, 8.66.

[3′*R*,4′*S*]-2-[2-(3-Ethenyl-4-piperidinyl)acetyl]-1*H*-indole-3-ethanol (Cinchonaminone) (3) A pale yellow amorphous powder, $[\alpha]_D^{22}$ +10.0° (c=0.35, EtOH). High-MS m/z: 312.187 (M^+, Calcd $C_{19}H_{24}N_2O_2$: 312.184). EI-MS m/z (rel. int. %): 312 (M^+, 4), 294 (5), 192 (6), 185 (6), 172 (13), 160 (7), 149 (8), 122 (7), 115 (17), 83 (13), 82 (11), 63 (20), 62 (20), 58 (100), 45 (44). IR (KBr) cm^{-1}: 3330, 2920, 1640, 1530, 1430, 1335, 1260, 1040, 915, 740. UV λ_{max}^{EtOH} nm (log ε): 237 (4.14), 312 (4.23). NMR: Tables II and III. *Anal.* Calcd for $C_{19}H_{24}N_2O_2 \cdot H_2O$: C, 69.06; H, 7.93; N, 8.48. Found: C, 68.91; H, 7.90; N, 8.26.

Lineweaver–Burk Plots The Lineweaver–Burk plots[24] for MAO with benzylamine as a substrate under our assay conditions in the absence and in the presence of **1** are shown in Fig. 1.

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References and Notes

1) This forms part VI of "Studies of Enzyme Inhibitors," Part V, T. Noro, T. Sekiya, M. Katoh, Y. Oda, T. Miyase, M. Kuroyanagi, A. Ueno and S. Fukushima, *Chem. Pharm. Bull.*, **36**, 244 (1988). A part of this work was presented previously, Abstracts of Papers, the 107th Annual Meeting of the Pharmaceutical Society of Japan, Kyoto, April 1987, p. 304, and the 34th Annual Meeting of the Japanese Society of Pharmacognosy, Osaka, October 1987, p. 14.
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