Analysis of in Vitro and ex Vivo Data. Where concentration–inhibition curves were generated, four to ten concentrations of test compound were employed, each done in duplicate. The percent inhibition of control uptake or specific binding was calculated for each concentration of test compound, and the IC_{50} was determined from a linear least-squares analysis of data transformed into a logit vs. log concentration format. Since IC_{50} values are dependent on radioisotope concentration, we obtained an apparent K_i by using the Cheng–Prusoff equation.[82] Confidence limits were calculated by using Fieller's theorem.

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Supplementary Material Available: Table of 95% confidence limits for data in Tables IV; tables of bond distances, bond angles, torsional angles, and positional and thermal parameters for (+)-**24b**·HBr; a stereoview of (+)-**24b**·HBr and a figure showing the atom numbering scheme; experimental procedures for GABA uptake, α_2 receptor binding, and muscarinic cholinergic receptor binding (17 pages). Ordering information is given on any current masthead page.

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Specific Dopamine D-1 and DA_1 Properties of 4-(Mono- and -dihydroxyphenyl)-1,2,3,4-tetrahydroisoquinoline and Its Tetrahydrothieno[2,3-*c*]pyridine Analogue

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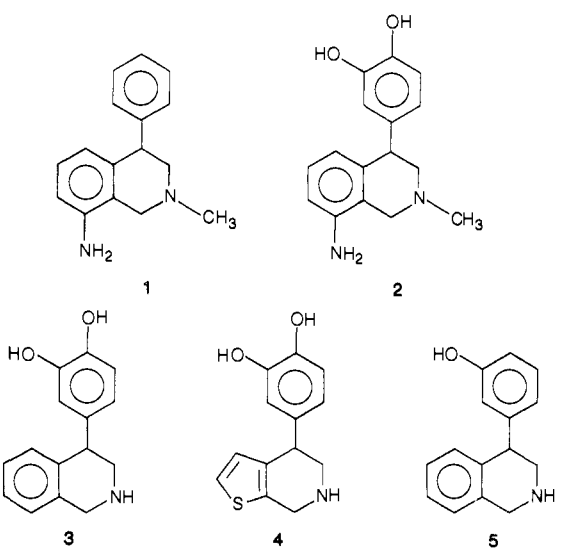
The title compounds were prepared and examined to elucidate further the structure–activity relationships of dopamine agonists related to nomifensine. Two of the compounds, 4-(3,4-dihydroxyphenyl)-1,2,3,4-tetrahydroisoquinoline and 4-(3,4-dihydroxyphenyl)-1,2,3,4-tetrahydrothieno[2,3-*c*]pyridine, have been reported in the patent literature. In stimulation of rat retinal adenylate cyclase, a measure of dopamine D-1 agonist activity, the tetrahydroisoquinoline was about equipotent to dopamine. The thienyl isostere had nearly twice the potency. Both compounds were potent vasodilators in the canine renal artery, producing dilation through stimulation of DA_1 type peripheral dopamine receptors. A monohydroxy analogue, 4-(3-hydroxyphenyl)-1,2,3,4-tetrahydroisoquinoline, had only slight activity in the cyclase assay and was inactive in the canine renal artery. These results, combined with those from an earlier study, demonstrate that N-alkylation decreases both dopamine D-1 and DA-1 agonist potency, with activity ordered as H > methyl > ethyl > propyl. The results also demonstrate the necessity for the catechol function in this series.

Nomifensine (**1**) is an antidepressant agent with weak in vivo dopaminergic activity. This action has been attributed to its ability to inhibit dopamine uptake into neuron terminals.[1] Poat et al.[2] showed that, while nomifensine and its 4-hydroxy derivative were devoid of direct dopamine agonist activity, 3′,4′-dihydroxynomifensine (**2**) exhibited direct dopamine-like agonist properties. Also, **2** shows significant DA_1 agonist activity in the canine renal artery.[3]

It was previously shown[4] that the 8-amino of **2** is not required for dopamine DA_1 agonist activity. The 8-desamino compound (the *N*-methyl derivative of **3**) was equipotent to **2**. Surprisingly, it was discovered that activity decreased in the *N*-ethyl and *N*-*n*-propyl derivatives. Nichols[5] subsequently proposed that the longer *N*-alkyl group might adopt a pseudoequatorial conformation, forcing the nitrogen lone-pair electrons into a pseudoaxial orientation and out of the position proposed to be necessary for optimum receptor activation.

It was decided to complete the 8-desamino series by the synthesis of the nor compound **3**. The corresponding 4-(3′,4′-dihydroxyphenyl)-1,2,3,4-tetrahydrothieno[2,3-*c*]-pyridine (**4**) and the 3′-monohydroxy compound **5** were set as targets to evaluate the bioisosteric effect of the thienyl and to examine the necessity for the catechol function in this series of agonists, respectively. Compounds **3** and **4** have been reported in the patent literature, but no bio-



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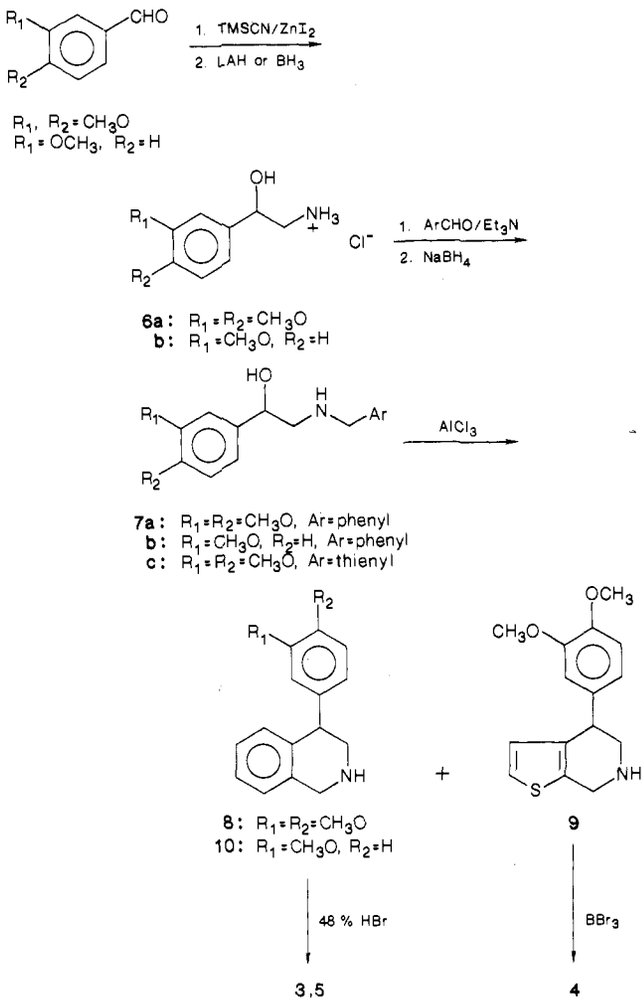
Scheme I



logical data are provided for **3** and only scant data are available for **4**.[6,7]

The compounds were then evaluated for D-1 and DA_1 dopamine agonist activity by using stimulation of rat retinal adenylate cyclase and canine renal vasodilation, respectively. Compounds were compared with previously prepared analogues to develop structure–activity relationships (SAR).

Chemical Discussion

The dimethyl ether **6** of norepinephrine was prepared by a modification of the procedure of Kaiser et al.[8] Veratraldehyde, when treated with trimethylsilyl cyanide in the presence of zinc iodide, readily afforded the trimethylsilyl ether of the cyanohydrin. Reduction with lithium aluminum hydride in refluxing THF afforded **6a**. Compound **6b** was prepared in a analogous manner except that 1 M BH_3/THF was used for reduction of the intermediate cyanohydrin silyl ether.

Following Kaiser's[8] approach, the amine salts of **6a** and **6b** were heated at reflux with 1.1 equiv each of triethylamine and the appropriate aryl aldehyde, followed by reduction with sodium borohydride.

Cyclization was performed on the resulting *N*-benzyl compounds with aluminum chloride in refluxing dichloromethane. The method of Kaiser, 4 equiv of $AlCl_3$ in dichloromethane at 30 °C, led only to recovery of starting material. The desired cyclized product was only obtained by reflux of the dichloromethane solution.

O-Demethylation of the tetrahydroisoquinolines was accomplished by stirring at reflux in 48% HBr, removal of solvent, and recrystallization. Since thiophenes are unstable in concentrated acid, BBr_3 was employed for conversion of **9** to **4**.

Table I. Stimulation of Rat Retinal Adenylate Cyclase by Compounds 3–5[a]

no.	concn, μM	% stimulation
3	0.1	23.6 ± 1.1
	1.0	89.6 ± 7.6
	10.0	152.7 ± 10.7
	100.0	198.8 ± 5.7
4	0.1	70.4 ± 7.7
	1.0	160.2 ± 2.6
	10.0	188.6 ± 2.1
	100.0	207.0 ± 17.7
5	0.1	NT
	1.0	21.0 ± 7.0
	10.0	47.0 ± 4.3
	100.0	81.0 ± 4.7

[a] These assays were run in the same series of experiments and reflect side-by-side comparisons and relative potencies.

Table II. Effect of *N*-Alkyl Length on Adenylate Cyclase Stimulation

compd	concn, μM	% stimulation
3	0.1	11.5 ± 2.5
	1.0	87.7 ± 3.5
	10.0	188.0 ± 5.8
	100.0	288.8 ± 7.3
N-methyl-3	0.1	−1.7 ± 0.72
	1.0	46.5 ± 3.3
	10.0	122.6 ± 2.2
	100.0	144.9 ± 7.1
N-ethyl-3	0.1	−8.8 ± 4.0
	1.0	−1.0 ± 5.7
	10.0	42.7 ± 3.9
	100.0	94.1 ± 3.0
N-*n*-propyl-3	1.0	−7.7 ± 1.9
	10.0	11.8 ± 6.1
	100.0	46.4 ± 5.5

Table III. Comparison of Dopamine, SKF 82526, and Compound 3 on Stimulation of Adenylate Cyclase

drug	concn, μM	% stimulation
DA	0.1	25.7 ± 2.5
	1.0	116.2 ± 5.7
	10.0	209.8 ± 11.4
	100.0	295.3 ± 7.2
3	0.1	36.9 ± 8.8
	1.0	141.9 ± 8.8
	10.0	229.2 ± 16.6
	100.0	252.1 ± 35.2
SKF82526	0.1	165.1 ± 11.1
	1.0	176.0 ± 11.4
	10.0	201.4 ± 13.0
	100.0	246.0 ± 3.9

Results

A direct comparison was conducted between **3**, **4**, and **5** to assess the effect of the thiophene ring and agonist effects, if any, of the *m*-hydroxy compound **5**. In the rat retinal cyclase assay (D-1 activity), **4** was found to be about 1.8 times more potent than **3** at the 1 μM concentration

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(Table I). Compound **5** is significantly less active: $^1/_7$ the activity of **4**, and $^1/_4$ the activity of **3**, respectively (Table I). Compounds **3** and **4** were both inhibited in a competitive manner, when tested in the presence of various concentrations of SCH 23390, a specific D-1 antagonist.[9,10]

Compound **3** was then compared with the *N*-alkyl derivatives previously synthesized by Jacob et al.[4] These data are presented in Table II. The detrimental effect on D-1 activity by progressive lengthening of the *n*-alkyl is clearly evident.

Table III shows a comparison between the dose–response data for **3**, dopamine, and SKF 82526, a specific D-1 agonist.[11] Compound **3** was tested as the racemate and SKF 82526 as the active R enantiomer. While **3** appears capable of inducing a comparable maximal stimulation of cyclase, SKF 82526 is more potent at lower concentrations.

A comparison was made between dopamine, **3**, **4**, and **5** for ability to dilate the canine renal artery. Compound **4** had $^1/_4$ the activity of dopamine, and **3** had $^1/_8$ the activity of dopamine. Compound **5** was inactive in the canine renal artery.

The test compounds **3** and **4** were compared with *N*,*N*-dipropyldopamine (DPDA) for ability to dilate the canine femoral artery. Both compounds had only a weak effect on the femoral artery. For example, DPDA (48 nmol) caused a vasodilation of 59 ± 16 mL/min, whereas compounds **3** and **4** at 760 nmol caused a vasodilation of 28 ± 2 and 39 ± 10 mL/min, respectively. The effect was not blocked by domperidone, indicating the vasodilation was not mediated via the to DA_2 receptor. Blockade of the vasodilation by hexamethonium suggests other neuronal processes are responsible for this.

Discussion

From the data comparing **3** and **5**, it is clear that for optimum stimulation of D-1-receptor-coupled adenylate cyclase, the catechol functionality is required. The *m*-hydroxy compound **5** is nearly 1 order of magnitude less active than **4** and about $^1/_4$ the activity of **3**. This finding parallels the results of Seiler and Markstein[12] in which dihydroxy-2-aminotetralins were more active than monohydroxy-2-aminotetralins.

The test compounds were also evaluated for dopamine DA_1 and DA_2 activity. Compound **4** was twofold more active than **3** in dilating the renal artery. This parallels the results obtained from the D-1 adenylate cyclase assay. However, compound **5** was inactive in the canine renal artery dopamine DA_1 assay. In contrast to the D-1 adenylate cyclase results, the test compounds were less active than dopamine. This suggests a subtle dissimilarity in structure–activity requirements for the central D-1 receptor and the peripheral DA_1 receptor. The femoral artery vasodilation assay results indicate that the test compounds are devoid of DA_2 activity. Therefore, compounds **3** and **4** are selective DA_1 agonists.

N-Alkylation of the 4-phenyl-1,2,3,4-tetrahydroisoquinoline molecule decreased dopamine D-1 agonist activity. The order of D-1 dopamine agonist potency is as follows: H > methyl > ethyl ≫ *N*-*n*-propyl. This result is in sharp contrast to the "*N*-*n*-propyl effect" that is observed in most D-2 and DA_2 dopamine agonists, where greater dopamine agonist potency is conferred by introduction of an *N*-*n*-propyl group.[5] The decreased activity of the *N*-alkyl derivatives can also be compared to the SAR of D-2 agonists, where N-alkylation, specifically an *N*-*n*-propyl group, increases D-2 agonist activity.[5]

Since thiophene is often a good bioisostere of benzene, the activity of **4** is interesting. In the adenylate cyclase assay and in the dog renal artery assay (DA_1 activity), **4** had about twice the potency of **3**. An explanation for the increased activity of **4** is not obvious; various physicochemical constants[13] for thiophene and benzene are similar.

Experimental Section

Chemistry. Melting points were determined with a Fisher-Johns apparatus and are uncorrected. NMR spectra were recorded on a Varian FT-80 or XL-200 instrument; chemical shifts are reported in parts per million, with Me_4Si or DSS as the internal reference. The multiplicities are expressed as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broad. Chemical–ionization (CI) mass spectra were obtained with a Finnigan 4000 quadrupole spectrometer. Elemental analysis was performed by the Purdue Microanalytical Laboratory and results were within ±0.4% of the calculated values unless otherwise noted.

1-(3,4-Dimethoxyphenyl)-2-aminoethanol Hydrochloride (6a). Veratraldehyde (2.5 g, 15 mmol) was stirred for 2 h with 2.4 mL (18 mmol) of trimethylsilyl cyanide and 50 mg of ZnI_2 in 50 mL of dichloromethane. The reaction mixture was washed with H_2O and the organic layer was dried ($MgSO_4$). After removal of the solvent by rotary evaporation, the crude cyanohydrin trimethylsilyl ether was dissolved in 15 mL of THF. The solution was added slowly to a suspension of 1.2 g (32 mmol) of lithium aluminum hydride in 30 mL of THF. The reaction mixture was stirred at 50 °C (reflux) for 1.5 h. The excess lithium aluminum hydride was decomposed with 4 mL of H_2O. The solids were removed by suction filtration and washed with ether (4 × 40 mL). The organic filtrate was dried ($MgSO_4$). After removal of the solvent by rotary evaporation, the crude product was dissolved in 10 mL of absolute ethanol and was acidified with ethanolic HCl. Upon addition of ether, the HCl salt crystallized: yield 2.3 g, 65.5%; mp 166–166.5 °C; (lit.[14] mp 163–65 °C).

1-(3-Methoxyphenyl)-2-aminoethanol (6b). *m*-Anisaldehyde (2.5 g, 18.4 mmol) was stirred overnight with 20.2 mmol of trimethylsilyl cyanide and a catalytic amount of ZnI_2. The dichloromethane was removed by rotary evaporation and the crude trimethylsilyl ether was reduced with 40 mL (40.0 mmol) of 1 M BH_3/THF by stirring at reflux for 4.0 h under a nitrogen atmosphere. Excess THF was removed by rotary evaporation and the borane–amine complex was decomposed by stirring at reflux in methanolic HCl for 1.0 h. Methanol was removed by rotary evaporation and the residue was dissolved in 50 mL of dilute HCl. The aqueous layer was washed with 2 × 50 mL ether and was basified with NH_4OH to pH 10 and extracted with dichloromethane (3 × 30 mL). The organic layer was dried ($MgSO_4$), the dichloromethane was removed by rotary evaporation, and the crude free base was converted to the HCl salt. The salt was recrystallized from ethanol/ether to yield 2.0 g (65%): mp 111–112 °C; NMR (D_2O) δ 7.21 (m, 1, Ar H), 6.82 (m, 2, Ar H), 4.87 (m, 1, CH), 3.70 (s, 3, CH_3O), 3.12 (m, 2, CH_2). Anal. ($C_9H_{14}ClNO_2$) C, H, N.

***N*-Benzyl-1-(3,4-dimethoxyphenyl)-2-aminoethanol Hydrochloride (7a).** Salt **6a** (400 mg, 1.71 mmol), 200 mg of triethylamine (2 mmol), and 200 mg (1.9 mmol) of benzaldehyde were held at reflux in 10 mL of absolute ethanol for 1.0 h. After cooling, 200 mg (5.3 mmol) of sodium borohydride was added. The reaction was then stirred at ambient temperature for 0.5 h. The ethanol was then removed by rotary evaporation and the residue was dissolved in 50 mL of dilute HCl. The aqueous layer was washed with one 25-mL portion of ether, made basic with concentrated NH_4OH to pH 10, and extracted with dichloromethane (2 × 50 mL). The organic layer was dried ($MgSO_4$). After

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solvent removal, 470 mg of free base was obtained: yield 96%; mp (HCl salt) 167–169 °C; NMR (D_2O) δ 7.5 (s, 5, benzyl Ar H), 7.05 (s, 3, Ar H), 3.86 (2 s, 6, CH_3O), 3.29 (m, 2, CH_2). Anal. ($C_{17}H_{22}ClNO_3$) C, H, N.

N-Benzyl-1-(3-methoxyphenyl)-2-aminoethanol Hydrochloride (7b). Compound **6b** (1.5 g, 8.97 mmol) was dissolved in 20 mL of absolute ethanol and 1.05 g (9.9 mmol) of benzaldehyde was added. The solution was held at reflux for 1.0 h under a nitrogen atmosphere. The reaction mixture was then cooled to 25 °C and 0.5 g (13.2 mmol) of $NaBH_4$ was added and the reaction mixture was stirred for 2.0 h. After solvent removal by rotary evaporation, 30 mL of dilute HCl was added. The precipitated salt was collected by filtration and recrystallized from ethanol/ether to yield the *N*-benzyl HCl salt: 1 g (37.9%); mp 159–162 °C; NMR ($CDCl_3$) δ 7.29 (s, 5, Ar H), 6.9 (m, 4, Ar H), 4.74 (dd, 1, CH), 3.81 (s, 2, CH_2), 3.79 (s, 3, CH_3O), 2.86 (m, 2, CH_2), 2.43 (s, 3, NH_2 and OH). Anal. ($C_{16}H_{20}ClNO_2$) C, H, N.

N-(2-Thienylmethyl)-1-(3,4-dimethoxyphenyl)-2-aminoethanol (7c). Compound **6a** (280 mg, 1.2 mmol), 160 mg (1.2 mmol) of thiophene-2-carboxaldehyde, and 150 mg of triethylamine (1.5 mmol) were stirred together at reflux for 45 min in 8 mL of absolute ethanol. After cooling, 100 mg of $NaBH_4$ (2.4 mmol) was added, and the reaction wsa stirred overnight. The ethanol was removed by rotary evaporation, and the residue was dissolved in 50 mL of dilute HCl. The aqueous solution was washed with ether (2 × 20 mL), was adjusted to pH 9 with concentrated NH_4OH, and was then extracted with dichloromethane (3 × 15 mL). The organic layer was dried ($MgSO_4$). Solvent removal afforded the crude free base, which was converted to the HCl salt and crystallized from ethanol/ether to yield 270 mg (67.5%): mp 164–166 °C; NMr (CDC_3) δ 7.19 (d, 1, Ar H), 6.87 (m, 5, Ar H), 4.58 (dd, 1, CH), 3.93 (s, 2, CH_2), 3.74 (2 s, 6, CH_3O), 2.71 (m, 2, CH_2), 1.83 (s, 3, NH_2 and OH). Anal. ($C_{15}H_{20}ClNO_2S$) C, H, N.

4-(3,4-Dimethoxyphenyl)-1,2,3,4-tetrahydroisoquinoline (8). Amine **7a** (300 mg, 0.93 mmol) was converted to the free base and dissolved in 10 mL of dichloromethane. $AlCl_3$ (500 mg, 3.75 mmol) was suspended in 20 mL of dichloromethane and the amine solution was added slowly to the suspension, at 25 °C. The mixture was then held at reflux under a nitrogen atmosphere for 2.5 h.

The reaction mixture was cooled and poured into 100 mL of ice water. The mixture was then adjusted to pH 10 with concentrated NH_4OH, the layers were separated, and the aqueous phase was extracted with ethyl acetate (3 × 40 mL). The combined organic phase was washed with a saturated solution of sodium borate and NaCl and was then dried ($MgSO_4$). After removal of the solvent, 220 mg of the free base, pure by TLC, was obtained: yield 88%. The base was converted to the HCl salt and recrystallized: mp 227–230 °C; CI-MS, M + 1, 270; NMR ($CDCl_3$) δ 6.85 (m, 7, Ar H), 4.6 (dd, 1, CH), 4.45 (s, 2, CH_2), 3.86, 3.8 (2 s, 6, CH_3O), 3.72 (m, 2, CH_2), 3.60 (s, 1 NH). Anal. ($C_{17}H_{20}ClNO_2$) C, H, N.

4-(3,4-Dimethoxyphenyl)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine (9). Compound **7c** (200 mg, 0.6 mmol) was converted into the free base and dissolved in 15 mL of dichloromethane. The amine solution was slowly dripped into a suspension of $AlCl_3$ (240 mg, 1.8 mmol) in 10 mL of dichloromethane. The reaction mixture was stirred at 25 °C for 0.5 h and then held at reflux for 1.0 h. The reaction mixture was then poured into 100 mL of ice water and basified to pH 9 with concentrated NH_4OH. The layers were separated, and the aqueous phase was extracted with ethyl acetate (3 × 30 mL). The combined organic phase was then washed with a solution of sodium borate and sodium chloride and dried ($MgSO_4$). After solvent removal by rotary evaporation, the free base was converted to the HCl salt and recrystallized from ethanol/ether: yield 130 mg, 68%; mp 215–217 °C; CI-MS, M + 1, 278; NMR ($CDCl_3$) δ 7.07 (d, 1, Ar H), 6.67 (m, 4, Ar H), 4.14 (s, 2, CH_2), 3.85 (2 s, 6, CH_3O), 3.58 (m, 1, CH), 3.15 (m, 2, CH_2), 1.87 (s, 1, NH). Anal. ($C_{15}H_{18}ClNO_2S$) C, H, N.

4-(3-Methoxyphenyl)-1,2,3,4-tetrahydroisoquinoline (10). Salt **7b** was converted to the free base, and 800 mg of the amine (3.11 mmol) was dissolved in 20 mL of dichloromethane. The amine solution was dripped slowly into a suspension of 1.35 (10.1 mmol) of $AlCl_3$ in 20 mL of dichloromethane. The mixture was held at reflux for 2.0 h and was then poured over ice and basified to pH 9.5 with concentrated NH_4OH. The layers were separated, and the aqueous phase was extracted with ethyl acetate (4 × 50 mL). The combined organic layers were washed with a concentrated NaCl solution and dried ($MgSO_4$). The solvents were removed by rotary evaporation, and the resulting crude free base (670 mg; 90%) was converted to the HCl salt and recrystallized from ethanol/ether to yield the HCl salt: 665 mg, 77.5%; mp 188–192 °C; CI-MS, M + 1, 240; NMR ($CDCl_3$) δ 6.9 (m, 8, Ar H), 4.11 (br s, 2, CH_2), 4.10 (m, 1, CH), 3.77 (s, 3, CH_3O), 3.29 (m, 2, CH_2). Anal. ($C_{16}H_{18}ClNO$) C, H, N.

4-(3,4-Dihydroxyphenyl)-1,2,3,4-tetrahydroisoquinoline Hydrobromide (3). Compound **8** (154 mg, 0.5 mmol) was dissolved in 2 mL of 48% HBr. The mixture was stirred at reflux under a nitrogen atmosphere for 1.25 h. The 48% HBr was removed by rotary evaporation. Recrystallization from ethanol/ethyl acetate yielded 120 mg (74.5%): mp 253–255 °C; CI-MS, M + 1, 242; NMR (D_2O) δ 7.44–6.56 (m, 7, Ar H), 4.58 (s, 2, CH_2), 4.4 (dd, 1, CH), 3.96–3.24 (m, 2, CH_2). Anal. ($C_{15}H_{16}BrNO_2$) C, H, N.

4-(3,4-Dihydroxyphenyl)-4,5,6,7-tetrahydrothieno[2,3-*c*]pyridine Hydrobromide (4). Compound **9** (250 mg, 0.9 mmol) was dissolved in 20 mL of dry dichloromethane. The solution was cooled in a dry ice/2-propanol bath and a 1 M solution of BBr_3/dichloromethane (0.27 mL, 0.27 mmol) was added, all at once. The reaction mixture was allowed to warm to room temperature, was stirred overnight, and was then quenched by addition of 4 mL of methanol. The solvents were removed by rotary evaporation, and the residue was dried under a high vacuum for 24 h. The crude salt thus obtained was recrystallized from 1-butanol/ethyl acetate to yield 220 mg (75%): mp 237–240 °C; CI-MS, M + 1, 248; NMR (D_2O) δ 7.39 (d, 1, Ar H), 6.7 (m, 4, Ar H), 4.59 (s, 2, CH_2), 4.42 (m, 1, CH), 3.50 (m, 2, CH). Anal. ($C_{13}H_{14}BrNO_2S$) C, H, N.

4-(3-Hydroxyphenyl)-1,2,3,4-tetrahydroisoquinoline Hydrobromide (5). Compound **10** (80 mg, 0.29 mmol) was dissolved in 1.0 mL of 48% HBr and held at reflux for 1.25 h under a nitrogen atmosphere. After removal of HBr by rotary evaporation, residual water was azeotropically removed with absolute ethanol by rotary evaporation. The residue was recrystallized from ethanol/ether to yield 65 mg (65.6%): mp 255–260 °C; CI-MS, M + 1, 226; NMR ($CDCl_3$) δ 6.98 (m, 8, Ar H), 6.45 (s, 1, Ar OH), 4.05 (s, 2, CH_2), 4.00 (m, 1, CH), 3.60 (br, s, 1, NH), 3.30 (m, 2, CH_2). Anal. ($C_{15}H_{16}BrNO$) C, H, N.

Pharmacology. The procedure for the dopamine-sensitive rat retinal adenylate cyclase assay was as follows: Rat retinas were homogenized in 150 vol/wt of 2.0 mM Tris·HCl, pH 7.4, with 2 mM EDTA with a Teflon–glass homogenizer. Each reaction mixture contained the following final concentrations in a volume of 0.2 mL: 2 mM $MgSO_4 \cdot 7H_2O$, 0.5 mM EGTA, 1 mM IBMX, 0.01 mM GTP, 80 mM Tris·HCl (pH 7.4), 0.5 mM ATP with approximately 5 × 10^6 dpm [^{32}P]ATP, and 20–30 μg of retinal homogenate protein. Following an incubation of 20 min at 30 °C, the reaction was terminated by adding 200 μL of a solution containing 1% SDS, 20 mM ATP, 0.7 mM cyclic AMP with 1.0 × 10^4 dpm [^{3}H]cyclic AMP in 80 mM Tris·HCl, pH 7.4, and heating to 85 °C for 2 min. Cyclic AMP was isolated from the mixture by using the column chromatographic technique of Salomon.[15]

The dog renal artery assay was performed according to previously reported procedures.[3,4] Briefly, experiments were carried out in pentobarbital-anesthetized adult male mongrel dogs, 20–25 kg. Following a flank incision and retroperitoneal dissection, the right renal artery was isolated and prepared for measurement of renal blood flow with an electromagnetic flow probe. Drug injection was via a 25-gauge needle bent at about an 80° angle and placed into the renal artery proximal to the flow probe.

Phenoxybenzamine, 5–10 mg/kg, was infused intraarterially over a 15–20-min period. Complete α-adrenergic blockade was confirmed by elimination or reversal of the vasoconstrictor effects of injection of 12 nmol of norepinephrine. Under these conditions, dopamine (DA) in doses of 12–190 nmol (contained in 0.2-mL injection volume) produced dose-related short-lived reversible increases in renal blood flow. After standard responses to DA

(15) Salomon, Y. *Adv. Cyclic Nucleotide Res.* **1979**, *10*, 35.

had been obtained, the test drugs 3–5 (again in 0.2-mL injection volumes) were injected in increasing doses into the renal artery.

Agonists that exhibited vasodilation were also tested with SCH 23390 to determine the degree of DA_1 inhibition. A dose of 2.5 μg/kg of SCH 23390 was given iv and 3 min later a dose of DA was given to ascertain complete blockade, followed by a dose of the test drug. In order to determine whether the dilation was β-adrenergic in origin, propranolol (2.5–5 mg/kg) was infused intraarterially over a 20-min period. β-Adrenergic blockade was established by abolition of the vasodilator effects of isoproterenol (3 nmol).

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Chemistry and Inhibitory Activity of Long Chain Fatty Acid Oxidation of Emeriamine and Its Analogues

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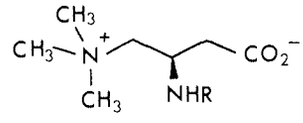
Emericedins A, B, and C, new betaines having inhibitory activity of long chain fatty acid oxidation, were isolated from the culture broth of *Emericella quadrilineata* IFO 5859. Their structures were determined by spectroscopic analyses as (*R*)-3-(acylamino)-4-(trimethylammonio)butyrate (acyl: A, acetyl; B, propionyl; C, *n*-butyryl). Structural confirmation and assignment of absolute configuration were made by chemical synthesis from L-asparagine. Deacylation of emericedin gave a potent derivative, (*R*)-3-amino-4-(trimethylammonio)butyrate, designated as emeriamine. In order to study the structure–activity relations, various analogues of emeriamine, including a stereoisomer, were prepared. Among them, *N*-palmitoyl and *N*-myristoyl derivatives showed much stronger inhibition of fatty acid oxidation than emeriamine.

In the course of searching for new biologically active compounds among microbial metabolites, we found that *Emericella quadrilineata* IFO 5859 produced novel betaines, emericedins A (1), B (2), and C (3). Emericedin



1 : R = CH_3CO
2 : R = CH_3CH_2CO
3 : R = $CH_3CH_2CH_2CO$
4 : R = H

is a new type of inhibitor of long chain fatty acid oxidation, although its activity is very low. Earlier, we reported the biochemical properties of deacylemericedin, designated as emeriamine (4), which showed potent inhibitory activity of fatty acid oxidation and hypoglycemic and antiketogenic activities in fasted rats after oral administration.[1] For the purpose of obtaining more potent analogues and also studying structure–activity relations, various analogues of 4, including a stereoisomer, were prepared. This paper deals with the isolation and structures of 1–3 and includes the synthesis and inhibitory activity of long chain fatty acid oxidation in rat liver mitochondria of 4 and its analogues.

Chemistry. Compound 1 was isolated as colorless crystals from the culture broth of *Emericella quadrilineata* IFO 5859 by column chromatography using cation-exchange resins. The molecular formula of 1 was determined to be $C_9H_{18}N_2O_3$ from elemental analysis and secondary ion mass spectrometry (SI-MS).

Structural elucidation of 1 was carried out by the following spectroscopic analyses. The IR spectrum showed characteristic bands at 1660 (amide C=O) and 1590 (CO-O^-) cm^{-1}. Its ^1H NMR spectrum (100 MHz, CD_3OD) indicated the presence of an acetyl group (δ 1.98), two methylenes (δ 2.42 and 3.56, each d, 4 H), a trimethylammonio group (δ 3.20, s, 9 H), and a methine (δ 4.7, m, 1 H). Two doublet methylene signals were each collapsed into a singlet, by the irradiation of the methine proton at 4.7 ppm, suggesting the structural unit of CH_2CHCH_2. Acid hydrolysis of 1 with 6 N HCl at 100 °C yielded a ninhydrin-positive compound, named emeriamine (4), as the dihydrochloride, $C_7H_{16}N_2O_2 \cdot 2HCl$. The ^1H NMR spectrum of the dihydrochloride of 4 showed two methylenes (δ 3.27 and 4.10, each d, 4 H), a trimethylammonio group (δ 3.50, s, 9 H), and a methine (δ 4.57, m, 1 H), but showed the absence of an acetyl methyl signal. From these results, the structures of 1 and 4 were elucidated to be 3-(acetylamino)-4-(trimethylammonio)butyrate and its deacetyl derivative, respectively. Table I shows the ^{13}C NMR spectral data of 1 and the dihydrochloride of 4. The signal at δ 69.8 (t) in the spectrum of 1 was assigned to the methylene carbon attached to a quarternary amino group on the basis of comparison with the ^{13}C NMR spectrum of L-carnitine (Table I). The minor components, emericedins B ($C_{10}H_{20}N_2O_3$) and C ($C_{11}H_{22}N_2O_3$), were also isolated and found, from their ^1H NMR spectra, to have a propionyl and a *n*-butyryl group, respectively, instead of an acetyl group.

In order to confirm the structure of 1 including the C-3 stereochemistry, synthesis of 1 from L-asparagine was carried out as illustrated in Scheme I. L-2-[(Benzyloxy-

Table I.[a] ^{13}C NMR Spectral Data of 1 and 4 (25 MHz, D_2O)

$(CH_3)_3N^+CH_2CH(R)CH_2COO^-$
4 3 2 1

carbon no.	chemical shift, ppm 1	4[b]	carnitine[c]
C-1	173.9 (s)	172.9 (s)	174.5 (s)
C-2	42.4 (t)	37.6 (t)	40.9 (t)
C-3	43.9 (d)	43.8 (d)	63.6 (d)
C-4	69.8 (t)	67.7 (t)	70.4 (t)
N^+CH_3	54.9 (q)	54.5 (q)	55.2 (q)
	54.4 (q)		55.1 (q)
	54.3 (q)		54.9 (q)
$COCH_3$	177.7 (s)		
$COCH_3$	23.1 (q)		

[a] 1: R = $NHCOCH_3$. 4: R = NH_2. Carnitine: R = OH. [b] Dihydrochloride. [c] Hydrochloride.

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