Hexadecenal Dimethyl Acetal (7a). This compound was prepared from hexadecanal as described for acetal **5** to give 18.7 g of **7a** (84%, bp 126–130 °C/0.5 mm) as a colorless oil.[15]

9-*cis*-Octadecenal Dimethyl Acetal (7b). This compound was prepared from 9-*cis*-octadecenal as described for acetal **5** to give 19.16 g of **7c** (84%, bp 123–135 °C/0.3 mm) as a colorless oil.

9-*cis*-Hexadecenal Dimethyl Acetal (7c). A solution of **6** (1.129 g, 4 mmol) in 50 mL of anhydrous methanol was added to a suspension of prereduced 5% $Pd/BaSO_4$ (120 mg) and quinoline (120 mg) in 5 mL of anhydrous methanol. After 2 h at room temperature (H_2, 1 atm), the mixture was filtered and evaporated and the residue was distilled to give 0.93 g of **7c** (81%) as a colorless oil (bp 120–121 °C/0.05 mm). Analysis by GLC (DB-5 column) indicated a single product and proton NMR (5.34 t, 2 H, J = 4.5 Hz) confirmed the presence of a cis double bond. Anal. ($C_{16}H_{36}O_2$) C, H. EIMS: 253 (M − 31), 75 (base).

2-(8-*cis*-Heptadecenyl)-4-(hydroxymethyl)-1,3-dioxolane (8b). This compound was prepared from **7b** as described for **8c** to give 19.5 g of **8b** (78%) after chromatography. Anal. ($C_{21}H_{40}O_3$) C, H.

2-(8-*cis*-Pentadecenyl)-4-(hydroxymethyl)-1,3-dioxolane (8c). A mixture of **7c** (0.468 g, 1.65 mmol), glycerol (0.303 g, 3.29 mmol), and 25 mg of 5-sulfosalicyclic acid was stirred vigorously at 140 °C for 2 h and then at 170 °C for 15 min to ensure the complete removal of methanol. After cooling, 50 mL of 1 N NaOH was added and the mixture was stirred for 1 h. This was diluted with 50 mL of water and extracted with 3 × 75 mL of hexane. The combined extracts were washed with 25 mL of H_2O and 25 mL of saturated NaCl, dried (Na_2SO_4), treated with activated carbon, filtered, and evaporated. Chromatography (silica gel, hexane/EtOAc 4:1) gave 0.381 g of **8c** (78%) as a colorless oil. ^1H NMR: δ 5.40 (t, 2 H, HC=CH), 4.89 (m, 1 H, acetal H), 4.00–3.50 (m, 6 H, glycerol H), 2.00 (s, 6 H, OCH_2–H_2 aliph, CH_2HC=$CHCH_2$). IR: 3426, 3003, 2924, 1458, 1142, 1043 cm^{-1}. Anal. ($C_{19}H_{36}O_3$) C, H. EIMS: 312 (M^+), 311, 103 (base), 57.

[(2-Pentadecyl-1,3-dioxolan-4-yl)methyl]phosphocholine (3a). To a cooled (0 °C) solution of 2-bromoethyl phosphorodichloridate[17] (0.288 g, 1.19 mmol) in 5 mL of anhydrous trichloroethylene (TCE) was added a solution of **8a**[15,16] (0.250 g, 0.795 mmol) and NEt_3 (0.483 g, 4.77 mmol) in 10 mL of TCE. After 4 h, 10 mL of toluene was added, and the mixture was filtered and evaporated. The residue was stirred in a mixture of 1 mL of H_2O and 0.1 mL of NEt_3 in 25 mL of Et_2O for 2 h at room temperature. This was diluted with 25 mL of water and extracted with 3 × 20 mL of Et_2O. The ether extracts were washed with 50 mL of 1 N Na_2CO_3, dried ($MgSO_4$), and evaported. The residue was dissolved in 2 mL of $CHCl_3$ and precipitated with 15 mL of anhydrous acetone. The crude intermediate was dissolved in 20 mL of a mixture of 25% trimethylamine (aqueous) in $CHCl_3$/DMF/isopropanol (7:3:5:5) and stirred for 8 h at 50 °C. The solvents were removed, and the residue was refluxed for 1 h in 25 mL of MeOH containing 100 mg of Ag_2O_3. The mixture was filtered and evaporated. The residue was purified by chromatography (silica gel, $CHCl_3$/MeOH/H_2O 65:30:4) to give 0.201 g of **3a** (27%) as a hygroscopic solid. ^1H NMR: δ 4.80 (m, 1 H, acetal H), 4.30 (m, 5 H, glycerol H), 3.81 (m, 4 H, $POCH_2CH_2N$), 3.35 (s, 9 H, $N(CH_3)_3$), 2.76 (s, 2 H, H_2O). IR: 3200 (H_2O), 2900, 2820, 1450, 1200, 1050, 940 cm^{-1}. Anal. ($C_{24}H_{50}NO_6P{\cdot}2H_2O$) C, H, N. CIMS: 537 ($MH^+$ + 57), 480 (MH^+), 421, 209, 181, 103, 72 (100), 60.

[[2-(*cis*-8-Heptadecenyl)-1,3-dioxolan-4-yl]methyl]phosphocholine (3b). This compound was prepared as described for **3a** from **8b** (0.250 g, 0.734 mmol), NEt_3 (0.446 g, 4.40 mmol), and the phosphorus reagent (0.226 g, 1.01 mmol) to give, after chromatography, 0.113 g of **3b** (31%) as a hygroscopic solid. Anal. ($C_{26}H_{52}NO_6P{\cdot}2H_2O$) C, H, N. CIMS: 563 ($MH^+$ + 57), 506 (MH^+), 474, 209, 181, 103, 72, 60 (base).

[[2-(*cis*-8-Pentadecenyl)-1,3-dioxolan-4-yl]methyl]phosphocholine (3c). This compound was prepared as described for **3a** from **8c** (0.381 g, 1.22 mmol), NEt_3 (0.740 g, 7.32 mmol), and the phosphorus reagent (0.442 g, 1.83 mmol) to give, after chromatography, 0.168 g of **3c** (29%) as a hygroscopic solid. Anal. ($C_{24}H_{48}NO_6P{\cdot}2H_2O$) C, H, N. CIMS: 535 ($MH^+$ + 57), 478 (MH^+), 209, 181, 103, 90, 72, 60 (base).

Pharmacology. Taenia coli smooth muscle strips were obtained from guinea pigs of either sex weighing 500–700 g. Strips 2–3 cm in length were mounted in 3-mL organ baths in a modified Krebs solution (mM: NaCl 133, KCl 4.5, $CaCl_2$ 2.6, $MgSO_4$ 1.2, $NaHCO_3$, 16.3, NaH_2PO_4 1.5, and dextrose 7.8) containing 1.5 μM hyoscine. Three hundred milligrams of tension was applied to each preparation and muscle activity was measured isometrically by means of Grass FT:03 force transducers and recorded on a Grass polygraph. Dosing of PAF was performed with increasing concentrations each followed by washout. Dosing of other phospholipids was either random with each dose followed by washout or was cumulative. Trachea were dissected out and mounted in organ baths in Krebs' solution according to the method of Farmer and Coleman.[19] Intraluminal pressure was recorded on a Grass polygraph via Gould Statham P23ID pressure transducers. Preparations were gassed with 95% O_2 and 5% CO_2 and maintained at 37 °C.

Acknowledgment. The financial support of the Kansas Heart Foundation and the National Institutes of Health (Training Grant GM-1341) is gratefully acknowledged.

Cyclic Carbamate Analogues of Pilocarpine

Per Sauerberg, June Chen,† Elizabeth WoldeMussie,† and Henry Rapoport*

Department of Chemistry, University of California, Berkeley, California 94720, and Department of Pharmacology, Allergan, Inc., Irvine, California 92715. *Received October 17, 1988*

A number of pilocarpine analogues containing the (*S*)-3-ethyl-4-[(4′-imidazolyl)methyl]-2-oxazolidinone (**9**) structural feature were synthesized from L-histidine. With 1-benzyl-L-histidine as the key intermediate, a regiospecific synthetic route was developed to the N^{π}-methyl derivative **8**. The regiochemistry of the alkylation of the imidazole nucleus was determined by measuring proton cross-ring coupling constants in the high-field ^1H NMR. The effects on muscarinic receptors of these variously alkylated derivatives **6**–**10** were studied on isolated guinea pig ileum. The derivatives in which the imidazole nitrogen was unsubstituted (**9**), N^{τ}-methylated (**10**), and N^{π}-methylated (**8**) were cholinergic muscarinic agonists with an increasing order of potency; compounds **6** and **7** were inactive. Analogue **8** with the same substitution pattern as pilocarpine was equipotent with pilocarpine, making these hydrolytically stable carbamate derivatives potentially useful drugs.

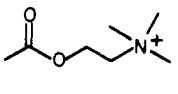
Pilocarpine, the only cholinergic muscarinic agonist in clinical use, is widely employed as a topical miotic for controlling the elevated intraocular pressure associated with glaucoma. In spite of its disadvantage of a short duration of action, pilocarpine has enjoyed widespread use. The duration of lowering of the intraocular pressure caused by pilocarpine lasts only for about 3 h, and consequently the frequency of administration is 3–6 times a day.[1] This

† Department of Pharmacology, Allergan.

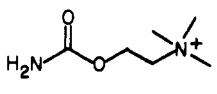
0022-2623/89/1832-1322$01.50/0

short duration of action is mainly due to (a) hydrolytic cleavage of the lactone ring, resulting in the formation of pilocarpic acid and rapid elimination, and/or (b) epimerization to form isopilocarpine.[2] Both pilocarpic acid and isopilocarpine are essentially pharmacologically inactive.[3] Several pilocarpine analogues and prodrug derivatives have been synthesized in order to improve the duration of action and to determine the pharmacophoric activity.[1,4]

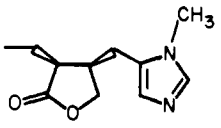
Carbamates in general have a long record of pharmacological acceptability,[5] and carbachol, a carbamate analogue of acetylcholine, in particular is known to be a potent muscarinic agonist. Using pilocarpine as a lead structure we therefore designed and synthesized some analogues incorporating the (*S*)-3-ethyl-4-[(4′-imidazolyl)methyl]-2-oxazolidinone structure as in **9**. Carbamates are more stable toward hydrolysis than lactones, which should give the carbamate analogues the desired longer duration of action. In attempting to elucidate the interactions of the pharmacophoric groups, we decided not only to synthesize the direct pilocarpine analogue with an N^{π}-methyl, **8**, but



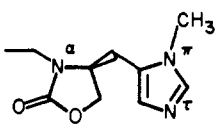
Acetylcholine



Carbochol



Pilocarpine



8

also the N^{τ}-methyl isomer **10** and the unsubstituted NH derivative **9**. By comparing the muscarinic effect of these carbamate analogues with the effect of the corresponding pilocarpine analogues, we would be able to determine how much the carbamate nitrogen altered the physiological properties of the molecule. This in turn would reflect on how different the pharmacological profile of **8** was from that of pilocarpine.

Chemistry

The synthetic route of choice to the three desired products, N^{π}-methyl **8**, N^{τ}-methyl **10**, and NH **9**, is outlined in Scheme I. Histidine was chosen as the closely related chiral educt and benzyl was chosen as the imidazole ring protecting group because it would survive reduction of the ester and amide to the alcohol and secondary amine, respectively. Regardless of the position of the imidazole benzyl group,[6] the NH derivative **9** could be obtained at some stage of the sequence by debenzylation under standard conditions. Methylation of NH compound **9** would give a mixture of N^{π}- and N^{τ}-methyl isomers **8** and **10**, which, hopefully, would be separable by chromatography. Assignment of the regiochemistry of **8** and **10** could then be done through NMR studies measuring the cross-ring coupling constants.[7,8]

1-Benzyl-L-histidine (**1**)[9] was synthesized from L-histidine, as described earlier,[10] and purified by fractional crystallization. The 1-benzyl-L-histidine benzyl ester (**2**)[11] was the next compound of the sequence and was chosen because the additional benzyl group would increase the lipophilicity of the compounds, and because **2** was reported to be more stable than the corresponding methyl ester.[12] Acetylation of benzyl ester **2** to *N*-acetyl-1-benzyl-L-histidine benzyl ester (**3**) was carried out with acetic anhydride in glacial acetic acid, and reduction of **3** with $LiAlH_4$ gave 1-benzyl-4-[2′-(ethylamino)-3′-hydroxypropyl]imidazole (**4**). The histidinol **4** either was converted to the corresponding *N*-benzyloxycarbonyl derivative **5** with benzyl chloroformate and then cyclized to (*S*)-3-ethyl-4-[(1′-benzyl-4′-imidazolyl)methyl]-2-oxazolidinone (**6**) or was cyclized directly to compound **6** with phosgene.

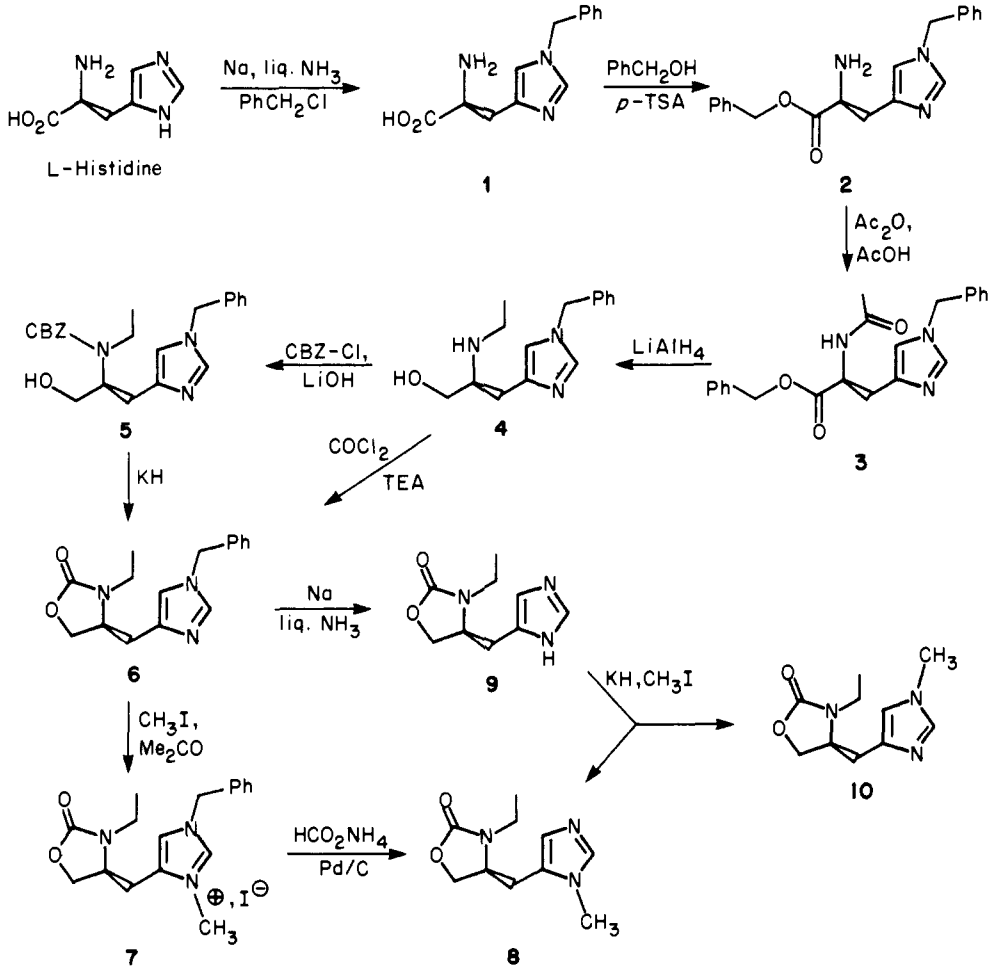
At this point proof of the regiochemistry of the imidazole benzyl group was provided by ^1H NMR studies at 500 MHz. The cross-ring coupling constant between the imidazole ring protons in 1-alkyl histidines (N^{τ}) lies in the range 1.1–1.5 Hz, whereas that for the 3-alkyl isomers (N^{π}) lies in the range 0.9–1.0 Hz.[7] Subsequent crystal structures of histidine analogues have complemented these assignments.[8] The cross-ring coupling constant in molecule **6** was 1.44 Hz. This clearly shows that **6** is the 1-benzyl isomer, as drawn in Scheme I.

Methylation of (*S*)-3-ethyl-4-[(1′-benzyl-4′-imidazolyl)-methyl]-2-oxazolidinone (**6**) with methyl iodide in acetone gave the imidazolium salt **7** as a crystalline precipitate. Debenzylation of this quaternary derivative **7** to the target molecule, (*S*)-3-ethyl-5-[(1′-methyl-4′-imidazolyl)-methyl]-2-oxazolidinone (**8**), was achieved with ammonium formate and palladium on charcoal in methanol. A large excess of ammonium formate was needed to make the reaction go to completion. Sodium in liquid ammonia decomposed the imidazolium ion **7**, but the same conditions debenzylated compound **6** to the NH analogue **9** in good yield. Methylation of **9** with methyl iodide and potassium hydride then gave a 2/1 mixture of N^{τ}-methyl isomer **10** and N^{π}-methyl isomer **8**. Separation by preparative TLC gave each pure target compound, and they were crystallized as fumarate salts.

^1H NMR studies of the 1,5 and 1,4 methyl isomers **8** and **10**, respectively, showed that the two isomers have significantly different chemical shifts for the *N*-methyl and the imidazole protons. This fact can be used to identify and assign regiochemistry to each isomer. As a further test of the regiochemistry of isomers **8** and **10**, cross-ring couplings constants for both were measured at 500 MHz. The 1,5-isomer **8** had a cross-ring coupling constant of 1.03 Hz while the 1,4-isomer **10** had a coupling constant of 1.50 Hz,

(1) Bundgaard, H.; Falch, E.; Larsen, C.; Mosher, G. L.; Mikkelson, T. J. *J. Med. Chem.* **1985**, *28*, 979.
(2) Dunn, D. L.; Scott, B. S.; Dorsey, E. D. *J. Pharm. Sci.* **1981**, *70*, 446.
(3) Anderson, R. A.; Cowle, J. B. *Br. J. Ophthal.* **1968**, *52*, 607.
(4) Aboul-Enein, H. Y.; Al-Badr, A. A. *Meth. Find. Exptl. Clin. Pharmacol.* **1982**, *4*, 321.
(5) Parameswaran, K. N.; Shi, G.-Y.; Klotz, I. M. *J. Med. Chem.* **1987**, *30*, 936.
(6) At this point, the imidazole nitrogen on which the benzyl group resided was not known with confidence. However, our subsequent data established it unambiguously as the N^{τ} regioisomer and we shall thus refer to it. Although both isomers are formed, crystallization leads to the single N^{τ} derivative in 56% yield (see the Experimental Section).
(7) Matthews, H. R.; Rapoport, H. *J. Am. Chem. Soc.* **1973**, *95*, 2297.
(8) Brown, T.; Jones, J. H.; Wallis, J. D. *J. Chem. Soc., Perkin Trans 1* **1982**, 3045.
(9) Nomenclature for histidine derivatives **1**–**3** follows the *Chemical Abstracts* recommendation in which the histidine side chain is arbitrarily assigned to the 4-position of the imidazole ring. Although this rule may violate standard imidazole numbering, it has been adopted by *Chemical Abstracts* only for the special case of histidines. For compounds **4** and after, we have applied standard imidazole nomenclature.
(10) du Vigneaud, V.; Behrens, O. K. *J. Biol. Chem.* **1937**, *117*, 27.
(11) Theodoropoulos, D. *Acta Chem. Scand.* **1958**, *12*, 2043.
(12) Theodoropoulos, D.; Fölsch, G. *Acta Chem. Scand.* **1958**, *12*, 1955.

Scheme I. Synthesis of Carbamate Analogues of Pilocarpine from L-Histidine



in complete agreement with our earlier assignment.

Agonist Effects on Guinea Pig Ileum

The guinea pig ileum was used as a primary screening model for studies of the affinities of the compounds at muscarinic receptors.[13] In this in vitro biological system, which is particularly rich in cholinergic muscarinic receptors, N^{π}-methyl isomer **8** was equipotent with pilocarpine (ED_{50} = 1 μM, Figure 1). The N^{τ}-methyl isomer **10** and the NH parent **9** were both weaker muscarinic agonists (ED_{50} = 14 and 180 μM, respectively), whereas the imidazolium salt N^{τ}-benzyl-N^{π}-methyl derivative **7** and the N^{τ}-benzyl analogue **6** were inactive.

Discussion and Summary

Due to the desire to have muscarinic agonist with a longer duration of action, we designed and synthesized three pilocarpine analogues containing the (*S*)-3-ethyl-4-[(4′-imidazolyl)methyl]-2-oxazolidinone skeleton. L-Histidine was used as starting material to set the correct configuration at the C-4 position. The use of benzyl as a protecting group for the imidazole ring nitrogen gave a regioselective synthetic pathway to the direct pilocarpine analogue, N^{π}-methyl isomer **8** (Scheme I). The regiochemistry of the 1-benzyl-L-histidine (**1**) was established by measuring the cross-ring coupling constant in the key intermediate **6**. The coupling constant of 1.44 Hz clearly placed the benzyl group on the N^{τ} atom.[7,8] The position of the benzyl group was consistent with the resulting

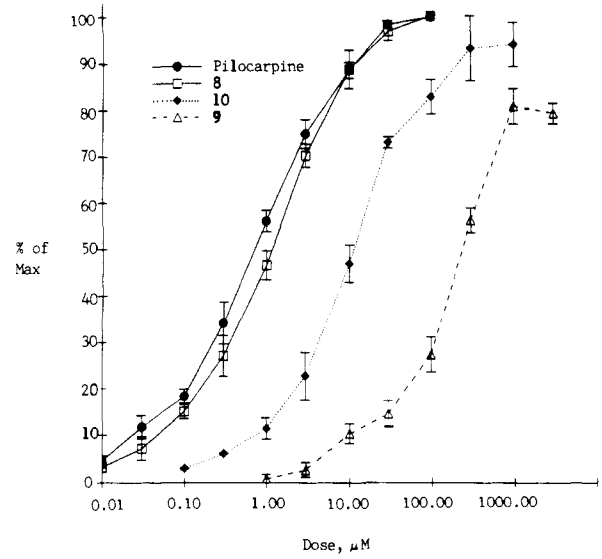


Figure 1. Effect of pilocarpine and its analogues on guinea pig ileum contractions: (●) pilocarpine, (□) **8**, (◆) **10**, (△) **9**.

N^{π}-methyl isomer obtained from methylation and debenzylation of compound **6**. Both imidazolylmethyl isomers **8** and **10** had cross-ring coupling constants (0.99 and 1.50 Hz, respectively) within the range limit indicated by the correlative rule.[7]

Considering the difficulty in obtaining high yield and regioselective alkylation procedures for histidine, proceeding via the 1-benzyl-L-histidine (**1**) intermediate might be the route of choice. This process thus provides con-

(13) Edinburgh Staff *Pharmacological Experiments on Isolated Preparations*, 2nd ed.; E & S Livingston: Edinburgh, 1974; pp 2, 65.

venient regiospecific protection in 56% yield while retaining the basicity of the imidazole ring.[14-17]

The preliminary biological data show the N^{π}-methyl isomer **8** is a muscarinic agonist equipotent with pilocarpine. Both N^{τ}-methyl isomer **10** and NH compound **9** are, like the corresponding N^{τ}-methyl isomer of pilocarpine (neopilocarpine) and *N*-demethylpilocarpine (pilocarpidine), less active. This indicates that the carbamate nitrogen does not interact with the imidazole pharmacophoric group. Pilocarpine and N^{π}-methyl isomer **8** have parallel log concentration–response curves (Figure 1), suggesting that they probably activate the same receptor population.

The fact that **8**, at least in the guinea pig ileum test, is equipotent with pilocarpine shows that the oxazolidinone 3-ethyl group has the flexibility to adopt the necessary receptor-active configuration. In pilocarpine the chiral C-3 atom has the *S* configuration. Isopilocarpine, which has the opposite stereochemistry at C-3, is a much less active muscarinic agonist.[3]

Further in vitro and in vivo pharmacological tests are needed before the therapeutic relevance of the cyclic carbamate analogues **8–10** can be evaluated.

Experimental Section

Chemistry. Melting points were determined in capillary tubes and are uncorrected. 1H NMR were recorded at 200 or 250 MHz in either $CDCl_3$ or D_2O with Me_4Si or 3-(trimethylsilyl)-propanesulfonate, respectively, as internal standards. Column chromatography (CC) was performed on 230–400-mesh SiO_2 (Merck). Preparative thin-layer chromatography (TLC) was done on precoated silica gel GF (20 × 20 cm, 1000 μm) glass plates (Analtech). Elemental analyses were performed by the Analytical Laboratory, College of Chemistry, University of California, Berkeley, and were within ±0.4% of the calculated values. Tetrahydrofuran (THF) was distilled from sodium/benzophenone and methylene chloride (CH_2Cl_2) was distilled from P_2O_5 immediately prior to use.

1-Benzyl-L-histidine (1). The product 1 was synthesized as described[10] by treating a solution of L-histidine (80 g, 138 mmol) in liquid ammonia (600 mL) at −70 °C with sodium (36 g) and benzyl chloride (48 mL, 416 mmol). The desired product crystallized from a water solution at pH 8 and, after recrystallization from ethanol/water, 7/3, was obtained in a 56% (52.2 g) yield: mp 234–238 °C (lit.[10] mp 248–249 °C corr).

1-Benzyl-L-histidine Benzyl Ester (2) Di-*p*-toluenesulfonate. The compound was synthesized as described.[10,11] A mixture of 1 (12.20 g, 49.8 mmol), *p*-toluenesulfonic acid monohydrate (20.84 g, 109.6 mmol), and benzyl alcohol (50 mL, 483 mmol) in carbon tetrachloride (100 mL) was heated under reflux, and the liberated water was removed azeotropically. When water no longer distilled off, the reaction mixture was cooled to room temperature and ether was added to complete the precipitation. Recrystallization from 2-propanol gave **2** as the di-*p*-toluenesulfonate salt in 83% yield (13.94 g): mp 168–170 °C (lit.[11] mp 176–177 °C).

***N*-Acetyl-1-benzyl-L-histidine Benzyl Ester (3).** A solution of **2** (23.70 g, 29.6 mmol) in saturated sodium bicarbonate (100 mL), adjusted to pH 8 with sodium carbonate, was extracted with chloroform/2-propanol (4:1, 3 × 200 mL). The combined organic phase was dried ($MgSO_4$), filtered, and evaporated. The residue was refluxed in a mixture of glacial acetic acid (150 mL) and acetic anhydride (5.64 mL, 60 mmol) for 45 min. The reaction mixture was evaporated. The residue was dissolved in water (100 mL) and evaporated until a white solid was obtained. The residue was dissolved in chloroform (300 mL) and washed with a saturated sodium bicarbonate solution (50 mL). The dried ($MgSO_4$) and filtered organic phase was evaporated to give the desired product as a white solid (10.17 g, 96%). Recrystallization from toluene/petroleum ether gave white crystals in a yield of 86%, 9.71 g: mp 113–114 °C; ^1H NMR ($CDCl_3$) δ 7.4 (m, 10 H), 7.15 (s, 1 H), 6.35 (s, 1 H), 5.10 (dd, 2 H), 4.90 (s, 2 H), 4.80 (m, 1 H), 3.00 (m, 2 H), 2.00 (s, 3 H); $[\alpha]^{22}_D$ −55.3° (*c* 1.5, chloroform). Anal. ($C_{22}H_{23}N_3O_3$) C, H, N.

1-Benzyl-4-[2′-(ethylamino)-3′-hydroxypropyl]imidazole (4). To a solution of **3** (9.70 g, 25.7 mmol) in THF (200 mL) was added a solution of $LiAlH_4$ (25 mL, 62.5 mmol) in THF. After 16 h at reflux, the reaction mixture was cooled and water (20 mL) was added slowly. The mixture was stirred for 15 min at room temperature and filtered through Celite. Sodium chloride was added to separate the aqueous phase, which was extracted with THF (3 × 200 mL). The combined organic phases were dried (Na_2SO_4), filtered, and evaporated, giving a brown oil (7.15, 107%). Crystallization of the residue from acetonitrile and ether gave **4** (2.84 g, 43%) as yellow crystals: mp 109–110 °C; ^1H NMR ($CDCl_3$) δ 7.45 (s, 1 H), 7.4–7.1 (m, 5 H), 6.68 (s, 1 H), 5.05 (s, 2 H), 3.7–3.4 (m, 2 H), 2.95 (m, 1 H), 2.8–2.6 (m, 4 H), 1.06 (t, 3 H); $[\alpha]^{22}_D$ 10.2° (*c* 1.5, methanol). Anal. ($C_{15}H_{21}N_3O$) C, H, N.

1-Benzyl-4-[2′-[*N*-(benzyloxycarbonyl)-*N*-ethylamino]-3′-hydroxypropyl]imidazole (5). Benzyl chloroformate (5.0 mL, 35 mmol) was added dropwise to an ice-cooled solution of crude **4** (6.5 g, 25 mmol) in dioxane (70 mL) and aqueous LiOH (20 mL, 0.5 N). During the addition of benzyl chloroformate, LiOH (0.5 N) was added incrementally to keep the reaction mixture at pH 8. The reaction mixture was stirred at room temperature for 45 min and evaporated. The residue was dissolved in water (50 mL) and extracted with chloroform (3 × 200 mL). The combined organic phases were dried ($MgSO_4$), filtered, and evaporated. The residue was purified by column chromatography using ethyl acetate as eluent to give **5** (3.13 g, 32%) as a yellow oil: ^1H NMR ($CDCl_3$) δ 7.4 (m, 10 H), 7.1 (s, 1 H), 6.62 (s, 1 H), 5.2–4.9 (m, 4 H), 4.55 (m, 1 H), 4.4–4.0 (m, 2 H), 3.3–3.0 (m, 2 H), 3.0–2.8 (m, 2 H), 0.95 (t, 3 H).

(*S*)-3-Ethyl-4-[(1′-benzyl-4′-imidazolyl)methyl]-2-oxazolidinone (6). Method A. To a suspension of potassium hydride (9.2 mmol) in THF (10 mL) was added a solution of **5** (3.12 g, 7.96 mmol) in THF (30 mL). The reaction mixture was stirred at room temperature for 1 h. Water (5 mL) was added followed by sodium chloride to separate the phases. The water phase was extracted with THF (3 × 60 mL), and the combined organic phases were dried ($MgSO_4$), filtered, and evaporated. Column chromatography of the residue using ethyl acetate/methanol (9:1) as eluent gave the desired product as an oil (1.3 g, 59%): ^1H NMR ($CDCl_3$) δ 7.55 (s, 1 H), 7.5–7.3 (m, 5 H), 6.78 (s, 1 H), 5.16 (s, 2 H), 4.4–4.1 (m, 3 H), 3.7–3.5 (m, 1 H), 3.3–3.0 (m, 2 H), 2.8–2.6 (m, 1 H), 1.20 (t, 3 H).

Method B. To a solution of phosgene in toluene (7.85 mL, 10.4 mmol) and triethylamine (3.76 mL, 27 mmol) was added a solution of **4** (2.60 g, 10.04 mmol) in methylene chloride (50 mL) at −60 °C. The reaction mixture was stirred at −60 °C for 2 h and allowed to warm to room temperature. Water (20 mL) and potassium carbonate were added to pH 9. The water was extracted with methylene chloride (3 × 100 mL), and the combined methylene chloride phases were dried ($MgSO_4$) and evaporated. The residue was purified by column chromatography to give **6** (1.29 g, 45%). Anal. ($C_{16}H_{19}N_3O_2 \cdot H_2O$) C, H, N.

Imidazolium Iodide 7. A solution of compound **6** (300 mg, 1.1 mmol) and methyl iodide (314 μL, 5 mmol) in acetone (6 mL) was stirred at 60 °C for 48 h. The product **7** crystallized from the solution in a yield of 400 mg (89%): mp 171–179 °C; ^1H NMR (D_2O) δ 8.78 (s, 1 H), 7.5–7.3 (m, 5 H), 7.28 (s, 1 H), 5.30 (s, 2 H), 4.5–4.3 (m, 2 H), 4.05 (m, 1 H), 3.80 (s, 3 H), 3.5–3.4 (m, 1 H), 3.3–3.0 (m, 3 H); $[\alpha]^{22}_D$ 18.7° (*c* 1.36, 10% methanol). Anal. ($C_{17}H_{22}N_3O_2I$) C, H, N.

(*S*)-3-Ethyl-4-[(1′-methyl-5′-imidazolyl)methyl]-2-oxazolidinone (8) Fumarate. A suspension of **7** (277 mg, 0.65 mmol), ammonium formate (20 g, 316 mmol), and Pd/C (10%, 130 mg) in methanol (50 mL) was refluxed for 4 h. After cooling to room temperature, the reaction mixture was filtered and evaporated. The residue was dissolved in a saturated sodium bicarbonate solution (30 mL) and the pH adjusted to 8.5 with potassium

(14) Brown, T.; Jones, J. H. *J. Chem. Soc., Chem. Commun.* **1981**, 648.
(15) Brown, T.; Jones, J. H.; Richards, J. D. *J. Chem. Soc., Perkin Trans. 1* **1982**, 1553.
(16) Hodges, J. C. *Synthesis* **1987**, 20.
(17) Jones, J. H.; Rathbone, D. L.; Wyatt, P. B. *Synthesis* **1987**, 1110.

carbonate. The aqueous solution was extracted with chloroform/2-propanol (2/1, 2 × 200 mL). The organic phases were washed with sodium thiosulfate (10%, 30 mL), dried (Na_2SO_4), filtered, and evaporated to give the **8** as an oil in 80% yield (110 mg): ^1H NMR ($CDCl_3$) δ 7.49 (s, 1 H), 6.82 (s, 1 H), 4.4–4.3 (m, 1 H), 4.2–3.9 (m, 2 H), 3.62 (s, 3 H), 3.7–3.5 (m, 1 H), 3.1–2.9 (m, 2 H), 2.78 (m, 1 H), 1.20 (t, 3 H).

The free base **8** was crystallized as the hemifumarate salt by adding a solution of fumaric acid (55 mg, 0.48 mmol) to a solution of **8** (100 mg, 0.48 mmol) in 2-propanol. The salt crystallized upon addition of ether: yield, 109 mg, 70%; mp 129–131 °C; ^1H NMR (D_2O) δ 8.70 (s, 1 H), 7.32 (s, 1 H), 6.60 (s, 2 H), 4.6–4.3 (m, 2 H), 4.18 (m, 1 H), 3.86 (s, 3 H), 3.6–3.4 (m, 1 H), 3.3–3.1 (m, 3 H), 1.20 (t, 3 H); $[\alpha]^{22}_D$ 40.3° (*c* 1.0, H_2O). Anal. ($C_{14}H_{19}N_3O_6$) C, H. N.

(*S*)-3-Ethyl-4-[(4′-imidazolyl)methyl]-2-oxazolidinone (9) Fumarate. Compound **6** (1.00 g, 3.50 mmol) was dissolved in liquid ammonia (50 mL) at −70 °C, and sodium pieces were added to a permanent blue color. The reaction mixture was stirred at −70 °C for 2 min, and ammonium chloride was added to quench excess sodium. The ammonia was allowed to evaporate spontaneously at room temperature. The residue was dissolved in water (20 mL) and extracted with chloroform/2-propanol (2/1, 2 × 70 mL). The organic phase was dried ($MgSO_4$), filtered, and evaporated. Crystallization from toluene gave **9** (483 mg, 71% yield) as yellow crystals: mp 92–93 °C; ^1H NMR ($CDCl_3$) δ 7.60 (s, 1 H), 6.90 (s, 1 H), 4.4–4.1 (m, 3 H), 3.7–3.5 (m, 1 H), 3.3–3.0 (m, 2 H), 2.9–2.7 (m, 1 H), 1.20 (t, 3 H). Anal. ($C_9H_{13}N_3O_2$) C, H, N.

A sample of **9** (70 mg, 0.36 mmol) was crystallized as the fumarate salt by adding a solution of fumaric acid (42 mg, 0.36 mmol) in 2-propanol to a solution of **9** in 2-propanol. The **9** fumarate crystallized upon addition of ether (78 mg, 70% yield): mp 157–160 °C; ^1H NM (D_2O) δ 8.65 (s, 1 H), 7.34 (s, 1 H), 6.68 (s, 2 H), 4.4–4.2 (m, 2 H), 4.18 (m, 1 H), 3.5–3.4 (m, 1 H), 3.3–3.1 (m, 3 H), 1.20 (t, 3 H); $[\alpha]^{22}_D$ 23.3° (*c* 0.46, H_2O). Anal. ($C_{13}H_{17}N_3O_6$) C, H, N.

(*S*)-3-Ethyl-4-[(1′-methyl-4′-imidazolyl)methyl]-2-oxazolidinone (10) Fumarate and (*S*)-3-Ethyl-4-[(1′-methyl-5′-imidazolyl)methyl]-2-oxazolidinone (8) Fumarate. A solution of **9** (382 mg, 1.96 mmol) in THF (40 mL) was added to a suspension of potassium hydride (2.1 mmol) in THF (10 mL). The suspension was stirred for 15 min at room temperature and methyl iodide (141 μL, 2.1 mmol) was added. The reaction mixture was stirred at room temperature for 16 h, filtered, and evaporated. The residue (365 mg, 98%) contained **8** and **10** in a 1/2 mixture. Separation by preparative TLC gave pure **10** (145 mg, 35% yield) as the upper spot and pure **8** (60 mg, 15% yield) as the lower spot; both compounds were obtained as oils. **10**: ^1H NMR ($CDCl_3$) δ 7.39 (s, 1 H), 6.72 (s, 1 H), 4.4–4.1 (m, 3 H), 3.67 (s, 3 H), 3.71–3.5 (m, 1 H), 3.3–3.1 (m, 1 H), 3.00 (dd, 1 H), 2.70 (dd, 1 H), 1.20 (t, 3 H). The ^1H NMR spectra of compound **8** was identical with the product from debenzylation of compound **7**.

Crystallization of **10** as the fumarate salt from 2-propanol gave either the fumarate salt, mp 100–104 °C [Anal. ($C_{14}H_{19}N_3O_6$)] or the hemifumarate salt: mp 133–135 °C; $[\alpha]^{22}_D$ 38.3° (*c* 0.75, H_2O); ^1H NMR (D_2O) δ 8.59 (s, 1 H), 7.31 (s, 1 H), 6.53 (s, 1 H), 4.5–4.3 (m, 2 H), 4.15 (m, 1 H), 3.86 (s, 3 H), 3.6–3.4 (m, 1 H), 3.3–3.1 (m, 3 H), 1.20 (t, 3 H). Anal. ($C_{12}H_{17}N_3O_4$) C, H, N.

Cross-Ring Coupling Constants. The cross-ring coupling constants of compounds **6**, **8**, and **10** were measured on a Bruker AM500 spectrometer operating at 500.13 MHz. The experiments were done in $CDCl_3$ at room temperature, using the imidazole 2-H resonance. We employed the resolution enhancement techniques of single zero filling and a squared sine bell apodization in addition to homonuclear decoupling of the *N*-methylene group of **6** or the *N*-methyl group of **8** and **10**.

Guinea Pig Bioassay. We used the bioassay method described by the Edinburgh staff.[13] Briefly, a distal portion of guinea pig ileum was cut and a segment (1–1.5 cm) was tied at both ends. One end was connected to a force displacement transducer and the other end to a muscle holder in a 5-mL organ bath. The tissue was suspended with 1 g tension in Tyrode solution (composition as follows in mM: NaCl 137, KCl 2.7, $CaCl_2$ 1.8, $MgCl_2$ 1.0, $NaHCO_3$ 11.9, Na_2HPO_4 0.4, glucose 5.6; pH 7.4) which was aerated with 95% O_2 and 5% CO_2 and maintained at 37 °C. After the tissue was allowed to equilibrate for 45–60 min, single doses of agonists were administered into the bath and isotonic contractions were recorded on a Grass polygraph.

Acknowledgment. We thank John F. O'Connell for assistance in determining the cross-ring coupling constants. Per Sauerberg was a Danish Research Council/Fulbright Scholar.

Registry No. 1, 16832-24-9; **2**·2 *p*-$CH_3C_6H_4SO_3H$, 116438-47-2; **3**, 119998-67-3; **4**, 119998-68-4; **5**, 119998-69-5; **6**, 119998-70-8; **7**, 119998-71-9; **8**, 119998-72-0; **8**·fumarate, 119998-78-6; **9**, 119998-73-1; **9**·fumarate, 119998-74-2; **10**, 119998-75-3; **10**·fumarate, 119998-76-4; **10**·$^1/_2$fumarate, 119998-77-5; L-histidine, 71-00-1.

Thromboxane A_2 Synthetase Inhibitors. 2. Syntheses and Activities of Tetrahydronaphthalene and Indan Derivatives

Munefumi Kanao,* Yoshifumi Watanabe, Youichi Kimura, Junji Saegusa, Kenjiro Yamamoto, Hideyuki Kanno, Naoaki Kanaya, Hideo Kubo, Shin-ichiro Ashida, and Fumiyoshi Ishikawa

Research Institute, Daiichi Seiyaku Co., Ltd., 13 Kita-kasai 1-16, Edogawa-ku, Tokyo 134, Japan.
Received September 12, 1988

A series of 1-imidazolylalkyl-substituted or 5-thiazolylalkyl-substituted tetrahydronaphthalenecarboxylic acid and indancarboxylic acid derivatives were prepared and tested for the inhibitory activities of thromboxane A_2 (TXA_2) production in vitro and ex vivo. Most of the compounds showed potent TXA_2 synthetase inhibitory activities in vitro and had long duration of inhibition of TXA_2 production in rats when orally or intravenously administrated. The imidazole analogues had slightly less potency in vitro than the thiazole analogues, but the activities of the imidazole analogues in ex vivo models were equal or superior to the activities of the thiazole analogues. 6-(1-Imidazolylmethyl)-5,6,7,8-tetrahydronaphthalene-2-carboxylic acid hydrochloride hemihydrate (**47a**, DP-1904) was chosen for clinical studies.

Thromboxane A_2 (TXA_2) and prostacyclin (PGI_2) are natural bioactive compounds and are produced from prostaglandin G_2 (PGG_2) and/or prostaglandin H_2 (PGH_2) by TXA_2 synthetase and PGI_2 synthetase, respectively. TXA_2 has potent vasoconstricting and platelet-aggregating activities.[1]

Selective TXA_2 synthetase inhibitors that do not inhibit PGI_2 synthetase and cyclooxygenase were noted as ther-

(1) (a) Moncada, S.; Vane, J. R. *Pharmacol. Rev.* **1979**, *30*, 293. (b) Needleman, P.; Wyche, A.; Raz, A. *J. Clin. Invest.* **1979**, *63*, 345.

0022-2623/89/1832-1326$01.50/0