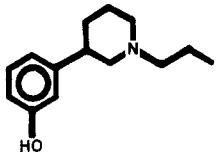
Synthesis and Dopamine Autoreceptor Activity of a 5-(Methylmercapto)methyl-Substituted Derivative of (±)-3-PPP (3-(3-Hydroxyphenyl)-1-*n*-propylpiperidine)

T. Ross Kelly,* Harry R. Howard, B. Kenneth Koe, and Reinhard Sarges

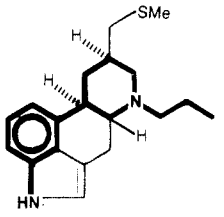
Pfizer Central Research, Groton, Connecticut 06340. *Received December 26, 1984*

In an attempt to enhance the potency of the dopamine autoreceptor agonist 3-PPP (1), racemic *cis*-3-(3-hydroxyphenyl)-5-[(methylmercapto)methyl]-*N*-*n*-propylpiperidine (3) has been prepared in a stereoselective synthesis. NMR studies of 3 show a diequatorial conformation for the 3- and 5-substituents, which gives compound 3 an intriguing overlap with the ergoline derivative pergolide (2). Pharmacological testing revealed that 3, which is a 5-(methylmercapto)methyl derivative of racemic 3-PPP (1) does not show the anticipated potency increase as a dopamine autoreceptor agonist. In vitro (inhibition of tyrosine hydroxylation) 3 and 1 have similar potency, and the in vivo potency (inhibition of GBL accelerated dopamine synthesis) of 3 is inferior to that of 1.

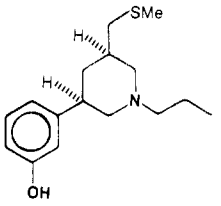
The discovery of 3-(3-hydroxyphenyl)-*N*-*n*-propylpiperidine (1; (±)-3-PPP), a centrally acting dopamine receptor agonist with selectivity for dopamine autoreceptors,[1] offers a potential alternative to neuroleptics in the



1 (3-PPP)



2 (Pergolide)



3

treatment of schizophrenia. The structure of 1 also has an intriguing overlap with parts of the ergoline ring system. Since the potency of ergoline type dopamine agonists can be enhanced by certain substituents in the D-ring, e.g., with the (methylmercapto)methyl group in the 8-position of pergolide (2),[2] we investigated whether a similar potency enhancement could be achieved by incorporating a (methylmercapto)methyl group in the corresponding position of 1, as in compound 3.

Chemistry

The synthesis of (±)-3 is outlined in Scheme I. Bromination[3] of 4 and reduction of the crude product (5) with $NaBH_4$ gave 6 which was protected as its benzyl ether (7). Palladium-catalyzed coupling[4] of the latter with *m*-anisylmagnesium bromide afforded 8 in good yield. Oxidation of the side chain followed by esterification provided 9. Selective saturation of the pyridine ring was achieved by hydrogenation over PtO_2 in methanolic HCl[5] to give a 2:1 mixture of the cis and trans piperidines 10 and 11, respectively. The stereochemical assignment was initially made from epimerization studies since exposure of this mixture to methanolic methoxide converts it to a ca. 8:1 mixture of epimers. As the cis isomer is the more stable of the two isomers by virtue of the diequatorial disposition of the substituents on the piperidine ring, the epimer that predominates under equilibrating conditions was concluded to possess the desired cis stereochemistry. Separation of 10 and 11 proved difficult, and the mixture of isomers was therefore carried through to 13 at which stage the minor isomer was removed by chromatography. Demethylation of 13 than gave (±)-3. NMR analyses (decoupling and NOE) of 13 and 3 confirmed the cis stereochemistry and the diequatorial conformation of the phenyl and (methylmercapto)methyl substituents.

Pharmacology

Dopmaine autoreceptor agonist activity was measured as previously described[6] by inhibition of γ-butyrolactone (GBL) induced acceleration of dopamine synthesis in mouse striatum[7] (i.e., Dopa accumulation in Dopa decarboxylase inhibited mice) and by measuring dopamine synthesis (tyrosine hydroxylation) in rat striatal synaptosomes in vitro.[8]

Results

The anticipated potency enhancement by incorporation of the (methylmercapto)methyl group in (±)-3-PPP (1) was not found. Whereas 1 caused a dose-responsive decrease of GBL-induced Dopa accumulation over a dose range of 10–320 μmol/kg, sc,[6] the 5-(methylmercapto)methyl derivative of 3-PPP (compound 3) elicited no significant effect at 32 μmol/kg, sc (Dopa accumulation 89 ± 7% SE of GBL; $p > 0.05$, $N = 4$). In contrast, 1 at the same dose significantly reduced Dopa accumulation after GBL (60 ± 4% of GBL; $p < 0.01$, $N = 5$). Convulsions were seen in one mouse at an initial test dose of 100 μmol/kg sc, of compound 3. In vitro, the 5-(methylmercapto)methyl derivative 3 had potency similar (IC_{50} = 45 μM, mean of two determination) to 1 (IC_{50} = 55 ± 13 μM, $N = 3$) in

* On sabbatical leave from the Department of Chemistry, Boston College, Chestnut Hill, MA 02167, where inquiries should be addressed.

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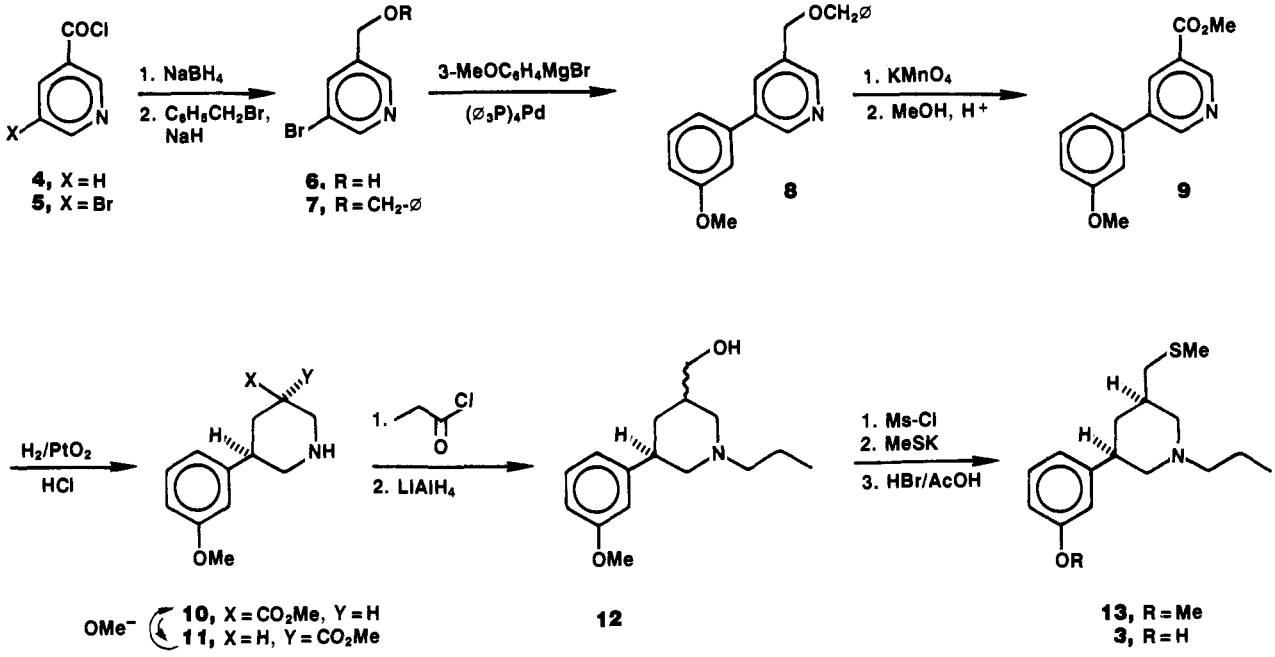
0022-2623/85/1828-1368$01.50/0

Scheme I



inhibiting dopamine synthesis.

Conclusion

Incorporation of a *cis*-(methylmercapto)methyl group into the 5-position of racemic 3-PPP (**1**) has resulted in a compound (**3**) with a configuration equivalent to that of pergolide (**2**). However, although the (methylmercapto)-methyl group confers enhanced postsynaptic dopamine agonist activity to pergolide (**2**), this group does not lead to enhanced dopamine autoreceptor agonist activity in **1**. The in vitro effects (inhibition of tyrosine hydroxylation) of **3** and **1** are essentially the same, whereas the in vivo effects (inhibition of dopamine synthesis) are clearly diminished in **3**.

Experimental Section

Melting points were determined in Pyrex capillaries on a Thomas-Hoover melting point apparatus and are uncorrected. Microanalyses were performed by the Pfizer Central Research Microanalysis Laboratory. ^{1}H NMR spectra were recorded on Varian T-60 and Bruker WM-250 spectrometers; chemical shifts are reported with reference to internal tetramethylsilane. Analytical thin-layer chromatography (TLC) was conducted on 0.25-mm-thick silica gel 60 F-254 plates manufactured by E. Merck and Co.; plates were visualized with UV light and iodoplatinic acid.

5-Bromo-3-pyridinemethanol (6). A mixture of 100 g (0.56 mol) of nicotinoyl chloride hydrochloride (Fairfield Chemical Co.) and 40 mL (0.78 mol) of Br_2 was heated in a good hood under reflux in an oil bath held at 170 °C.[3] Progress of the reaction was monitored by quenching aliquots into absolute EtOH and examining by TLC ($CHCl_3$).[9] After 18 h, conversion to the 5-bromonicotinoyl halide hydrohalide salt (**5**) was deemed essentially quantitative. The crude product was used without purification (most of the excess Br_2 escapes through the condenser during the course of the reaction).

Reduction of crude **5** was run in 25-g batches. Thus, to 250 mL of absolute EtOH cooled to −10 to 0 °C (internal temperature; dry ice–acetone bath) was added 12.5 g of $NaBH_4$. After most of the $NaBH_4$ had dissolved (ca. 5 min), crude **5** was added in small (ca. 1-g) portions with stirring over the course of 1.5 h (Caution! The reaction is very exothermic at the beginning.) while maintaining the internal temperature at −10 to −0 °C. When addition of **5** was complete, the reaction mixture was diluted with H_2O and extracted twice with Et_2O. The Et_2O extracts were combined, washed with brine, dried over anhydrous K_2CO_3, and saturated with HCl gas. An oil (which subsequently crystallized) separated; the Et_2O phase was decanted and the crude hydrochloride salt of **6** was dissolved in 100 mL of hot absolute EtOH. Approximately 100 mL of Et_2O was added and the mixture allowed to stand overnight. Filtration gave 4.84 g of **6**, mp 154–155 °C (lit.[10] mp 158–160 °C).

3-[(Benzyloxy)methyl]-5-bromopyridine (7). To a stirred mixture of 8.02 g (0.167 mol) of 50% dispersion of NaH in oil in 110 mL of HMPA was added (Caution! vigorous H_2 evolution) 12.5 g (0.056 mol) of **6** in portions over 1 h, with cooling effected with a room-temperature water bath. When addition of **6** was complete and gas evolution had subsided, 7.36 mL (0.062 mol) of benzyl bromide was added portionwise over 0.5 h, the internal temperature being always kept below 35 °C. After the addition of benzyl bromide was complete, the reaction mixture was stirred at 25 °C for 1 h, at which time TLC ($CHCl_3$)[9] indicated conversion of **6** to **7** was complete. The reaction mixture was then carefully (Caution! H_2 evolution) poured over ice and thrice extracted with Et_2O. The Et_2O extracts were combined, washed with brine, and dried over anhydrous K_2CO_3, and HCl gas was passed in. The oil that precipitated was separated from the Et_2O layer by decantation and partitioned between NaOH and Et_2O. The Et_2O layer was separated and the aqueous layer twice extracted with Et_2O. The combined Et_2O phases were washed with brine, dried (K_2CO_3), and concentrated to give 26.9 g (87%) of **7** as an oil homogeneous by NMR [(CD_3OD) δ 4.72 (s, 2 H), 4.77 (s, 2 H), 7.35 (m, 5 H), 8.72 (bs, 1 H), 8.80 (s, 1 H), 9.01 (bs, 1 H)] and TLC ($CHCl_3$);[9] mass spectrum *m/e* 277, 279.

3-[(Benzyloxy)methyl]-5-(3-methoxyphenyl)pyridine (8). In a dry 250-mL round-bottomed flask fitted with an addition funnel, condenser, and magnetic stirrer under a N_2 atmosphere were placed 26.9 g (0.097 mol) of **7**, 40 mL of dry THF, and 2.0 g (1.7 mmol) tetrakis(triphenylphosphine)palladium ($[C_6H_5)_3P]_4Pd$, Alfa). The mixture was heated to reflux, and a solution of (3-methoxyphenyl)magnesium bromide prepared[5] under N_2 from 12.7 mL (0.1 mol)[11] of *m*-bromoanisole and 2.43 g of magnesium turnings in 120 mL of THF was added.

(9) After spotting, but before eluting, the spot was exposed to concentrated NH_4OH vapors for 1–2 min to neutralize amine salts.

(10) Hedbom, C.; Helgstrand, E.; Misiorny, A.; Stjernstrom, N. E.; Westin, G. *Acta. Pharm. Suec.* **1972**, *9*, 259.

(11) When excess Grignard reagent is used, the initially formed **8** suffers displacement of the benzyl ether grouping by a second mole of Grignard to give 3-(3-methoxyphenyl)-5-(3-methoxybenzyl)pyridine.

After the addition was complete, the reaction was maintained under reflux for 1.5 h at which time it was judged complete by TLC[9] ($CHCl_3$). The reaction mixture was then cooled, poured into a mixture of 125 mL of concentrated HCl and 3 L of H_2O, and washed twice with Et_2O. The aqueous layer was carefully (Caution! foaming) basified with a saturated aqueous solution of $NaHCO_3$ until further addition of aqueous $NaHCO_3$ no longer resulted in gas evolution and then extracted with Et_2O (3×). The Et_2O extracts were combined, washed with brine, dried over K_2CO_3, and concentrated to give 19.2 g (65%; a 78% yield was realized on a $^1/_4$-scale procedure) of **8** as a liquid that was converted to **9** without further purification.

Methyl 5-(3-Methoxyphenyl)nicotinate (9). In a 3-L flask fitted with a mechanical stirrer were placed 25.0 g (0.0982 mol) of **8** and 1.5 L of H_2O. The stirred mixture was maintained at 95 ± 2 °C on a steam bath, and 100 g of powdered $KMnO_4$ was added in five portions over the course of 1 h. After an additional 1 h, all the $KMnO_4$ had been consumed and the MnO_2 was removed by filtration while the reaction mixture was still hot. The MnO_2 was washed with hot water, and the filtrate and wash were combined and allowed to cool. The solution was acidified (Caution! foaming) with 10% HCl to pH 2.8 (pH meter) whereupon a white solid precipitated.[12] Air drying gave 7.38 g of a snow white solid that was a mixture of the desired nicotinic acid and benzoic acid. Pure 5-(3-methoxyphenyl)nicotinic acid: mp 225–226 °C; NMR (Me_2SO-d_6) δ 3.85 (s, 3 H), 7.0–7.5 (m, 4 H), 8.45 (m, 1 H), 9.00 (d, 1 H), 9.13 (d, 1 H), 13.5 (br s, 1 H); mass spectrum *m/e* 229. Anal. ($C_{13}H_{11}NO_3$) C, H, N.

The white solid was mixed with 250 mL of MeOH, and HCl gas was passed in (internal temperature rose to 50 °C but the solution was not saturated with HCl). After standing overnight, volatiles were removed in vacuo and the residue partitioned between CH_2Cl_2 and aqueous KOH. Evaporation of the CH_2Cl_2 layer gave 3.5 g of a mixture of **9** and methyl benzoate; acidification of the aqueous layer to pH 2 afforded a solid that was collected and esterified with ethereal diazomethane to give a second crop of **9** contaminated with methyl benzoate.

The crude material from both esterification operations was partitioned between dilute aqueous HCl(A) and CH_2Cl_2(B) to remove the methyl benzoate. Basification of the HCl layer (A) gave after an extractive workup 2.30 g of **9** as an oil. B was evaporated and the residue taken up in Et_2O and exposed to gaseous HCl. The precipitate that separated was partioned between dilute aqueous NaOH and CH_2Cl_2 to give an additional 0.77 g of **9** for a total yield of 3.07 g (16.2%). After standing, **9** crystallized to a solid: mp 92–93 °C dec; NMR ($CDCl_3$) δ 3.9 (s, 3 H), 4.0 (s, 3 H), 6.95–7.5 (m, 4 H), 8.5 (m, 1 H), 9.0 (d, 1 H), 9.2 (d, 1 H); mass spectrum *m/e* 243. Anal. ($C_{14}H_{13}NO_3$) C, H, N.

***cis*- and *trans*-Methyl 5-(3-Methoxyphenyl)piperidine-3-carboxylate (10/11).** A mixture of 3.02 g (0.013 mmol) of crude **9**, 45.5 mL of a solution prepared by mixing 63 mL of MeOH and 7.5 mL of concentrated HCl, and 550 mg of PtO_2 was hydrogenated at approximately 50 psi on a Parr apparatus with occasional monitoring by TLC[9] (Et_2O or 9:1 $CHCl_3$/MeOH). When the hydrogenation was complete (ca. 6 h), the catalyst was removed by filtration and washed with MeOH, and the combined filtrate and wash were concentrated in vacuo. The residue was partitioned between CH_2Cl_2 and dilute NaOH, the layers were separated, and the aqueous layer extracted with CH_2Cl_2. The combined CH_2Cl_2 layers were dried (K_2CO_3) and concentrated to give 2.24 g of an oil that was approximately a 2:1 mixture of stereoisomers as judged by NMR, but otherwise quite pure.

The mixture of stereoisomers (2.22 g) was epimerized by heating at 60 °C under N_2 for 7 h with 6.6 g of potassium *tert*-butoxide in 300 mL of dry MeOH. The reaction mixture was cooled and poured into a mixture of ice and CH_2Cl_2, the layers were separated, and the aqueous layer was twice extracted with CH_2Cl_2. The combined CH_2Cl_2 solutions were dried (K_2CO_3) and concentrated to give 1.64 g (54%) of an oil that was quite pure as judged by NMR and TLC[9] and was estimated on the basis of the areas of the carbomethoxy resonances in the ^1H NMR to be an approximately 8:1 mixture of the cis and trans epimers; mass spectrum *m/e* 249.1353 (calcd 249.1364 for $C_{14}H_{19}NO_3$). The two epimers were not readily separable by chromatography, and separation was derived until later in the synthesis (see preparation of **13**).

***cis*-3-(3-Methoxyphenyl)-5-[(methylmercapto)methyl]-*N*-*n*-propylpiperidine (13).** The crude mixture of esters **10**/11 from above (after epimerization, 1.64 g, 6.92 mmol) was dissolved in 20 mL of CCl_4, the solution was placed in an ice bath, and 2 mL of Et_3N and 1 mL of propionyl chloride were added. The reaction mixture was stirred 3 h (during which time it warmed to room temperature) at which point TLC[9] (Et_2O) showed no starting material. The reaction mixture was poured into 3% aqueous HCl and the resulting mixture extracted twice with CH_2Cl_2. The combined CH_2Cl_2 layers were dried (K_2CO_3) and concentrated in vacuo to give 2.27 g of a brown oil. This oil was dissolved in 100 mL of THF and added to a stirred slurry of 2.25 g of $LiAlH_4$ in 50 mL of THF under N_2. The mixture was stirred at room temperature overnight (18 h) at which time TLC[9] (9:1 $CHCl_3$/MeOH) indicated the absence of starting material. The product was isolated by dropwise (Caution! gas evolution) addition of 11.25 mL of 3% aqueous NaOH, dilution with Et_2O, removal of insolubles by filtration, and evaporation to give 1.58 g (87%) of crude **12** as a colorless oil (mass spectrum *m/e* 263) that was used directly in the next reaction.

Crude **12** (1.47 g, 5.59 mmol) and 3 mL of Et_3N were dissolved in 45 mL of dry THF under N_2 and cooled in ice, and 1.5 mL of CH_3SO_2Cl was added dropwise over the course of ca. 1 min. TLC[9] (9:1 $CHCl_3$/MeOH) 5 min later showed that all **12** had reacted. This reaction mixture was then combined with an ethanolic solution of $CH_3S^-K^+$ (prepared by bubbling methanethiol through a mixture of 90 mL of absolute EtOH and 7 mL of 40% aqueous, KOH until no more CH_3SH dissolved) in a stoppered flask and heated at 55 °C for 1 h. After cooling, the reaction mixture was diluted with 1200 mL of H_2O and thrice extracted with CH_2Cl_2. The combined CH_2Cl_2 extracts were dried and concentrated to give 1.67 g of an oil containing some suspended solid. This oil was purified by preparative TLC on 32 10 cm × 20 cm × 0.5 mm E. Merck silica gel 60 F-254 plates, eluting with 10:10:0.2 EtOAc/hexane/Et_3N. The major band (R_f 0.5) was collected; extraction of the band with 9:1 CH_2Cl_2/MeOH gave 0.93 g (57%) of **13** as a yellow oil in a high state of purity as judged by ^1H NMR and two-dimensional TLC (50:1 Et_2O/Et_3N; 50:1 EtOAc/Et_3N. As judged by TLC's of crude **13** these two solvent systems are complementary in their ability to detect impurities): NMR ($CDCl_3$) δ 0.9 (t, 3 H), 1.2 (q, 1 H; J = 12 Hz; axial 4-H), 1.6 (m, 3 H), 1.95 (t, 1 H), 2.1 (s, 3 H; m, ~2 H; equatorial 4-H, axial 5-H), 2.35 (m, 2 H), 2.45 (d, 2 H), 2.9 (bt, 1 H; axial 3-H), 3.1 (d, 1 H), 3.2 (d, 1 H), 3.85 (s, 3 H), 6.8 (m, 3 H), 7.3 (m, 1 H); mass spectrum *m/e* 293.1814 (calcd for $C_{17}H_{27}NOS$ 293.1807).

***cis*-3-(3-Hydroxyphenyl)-5-[(methylmercapto)methyl]-*N*-*n*-propylpiperidine (3).** A mixture of 500 mg (1.71 mmol) of **13** and 20 mL of a freshly prepared[13] saturated solution of HBr gas in AcOH was heated at 130 °C in a flask fitted with a condenser for 1.5 h. After cooling, the reaction was poured into 500 mL of aqueous K_2HPO_4 (pH of aqueous phase after mixing was ca. 7) and extracted 3× with CH_2Cl_2. The combined CH_2Cl_2 extracts were dried and converted to give 320 mg of an oil that was purified by preparative layer chromatography on four 20 cm × 20 cm × 1 mm Analtech Silica GF plates, eluting with 50:1 Et_2O/Et_3N. The UV-active band (R_f 0.5) was extracted with 9:1 CH_2Cl_2/MeOH. The extract was concentrated and the residue dried by addition of CCl_4 and evaporation to give 195 mg of pure **3** as an oil that crystallized on standing. Recrystallization from benzene/hexane gave material melting at 115–116 °C; NMR ($CDCl_3$) δ 0.9 (t, 3 H), 1.3 (q, 1 H; J = 12 Hz; axial 4-H), 1.6 (m, 2 H), 1.8 (t, 1 H; J = 12 Hz; axial 6-H), 2.0 (t, 1 H; J = 11 Hz, axial 2-H), 2.1 (s, 3 H; m, ~2 H), 2.4 (m, ~3 H), 2.45 (d, 2 H), 2.95 (bt, 1 H; J = 11 Hz; axial 3-H), 3.25 (bt, 2 H), 6.75 (m, 3 H), 7.2 (m, 1 H); mass spectrum *m/e* 279. Anal. ($C_{16}H_{25}NOS$) C, H, N.

Acknowledgment. We are grateful to Diane M. Sweet, Dr. E. B. Whipple, and Mark R. Seger for NMR deter-

(12) A portion of the filtrate was lyophilized and treated with methanolic HCl. No **9** was detected.

(13) A commercial sample of HBr in HOAc which was brown in color contained sufficient Br_2 to give partially brominated **3**.

minations and to Richard S. Ware for mass spectra analyses. We thank Dr. U. Hacksell for a preprint of ref 5.

Registry No. (±)-3, 97000-20-9; 4·HCl, 20260-53-1; 5·HCl, 59105-51-0; 5·HBr, 97000-21-0; 6·HCl, 22620-36-6; 7, 97000-22-1; 8, 97000-23-2; 9, 97000-24-3; 9 (acid), 97000-30-1; (±)-10, 97000-25-4; (±)-11, 97000-26-5; (±)-12 (isomer 1), 97000-27-6; (±)-12 (ketone) (isomer 1), 97000-31-2; (±)-12 (mesylate) (isomer 1), 97000-33-4; (±)-12 (isomer 2), 97000-28-7; (±)-12 (ketone) (isomer 2), 97000-32-3; (±)-12 (mesylate) (isomer 2), 97000-34-5; (±)-13, 97000-29-8; 3-(3-methoxyphenyl)-5-(3-methoxybenzyl)pyridine, 97000-35-6.

Preparation of Biologically Active Ristocetin Derivatives: Replacements of the 1′-Amino Group

Thomas R. Herrin, Alford M. Thomas,* Thomas J. Perun, James C. Mao, and Stephen W. Fesik

Pharmaceutical Discovery Division, Abbott Laboratories, Abbott Park, Illinois 60064. *Received January 16, 1985*

A series of ristocetin analogues with modifications (OH, C=O, C=NOH, $NCOCH_3$) at the C-1′ amino group was synthesized and found to possess antibacterial activity against gram-positive bacteria and to bind to Ac_2-Lys-D-Ala-D-Ala, a model for the antibiotic's site of action. Due to the lack of a positively charged amino group, the active analogues could not form a salt bridge, indicating that an electrostatic interaction between the positively charged 1′-amino group of ristocetin and the carboxylate anion of the peptide is not required for complex formation. The only compound that did not exhibit good antibacterial activity was epiristocetin aglycone (an analogue with the 1′-amino group in the opposite configuration (*S*) as ristocetin). On the basis of NMR studies of epiristocetin aglycone in solution, the 1′-amino group is located in the proposed carboxylate binding pocket and may sterically block complex formation.

The glycopeptide antibiotics (ristocetin, vancomycin, avoparcin) have been the subject of intense investigation in recent years, especially with respect to structure and mode of action. It is now well established that the glycopeptide antibiotics exert their antibiotic activity by binding specifically to cell wall precursors terminating with the peptide D-alanyl-D-alanine.[1] The remarkable specificity of this complexation was demonstrated by Perkins using synthetic analogues of the cell wall peptide.[2] The binding of the glycopeptide antibiotics was found to be dependent on the chirality of either D-alanine residue of the synthetic analogue, the length of the amino acid side chains, and the presence of a free carboxyl group.

On the basis of extensive NMR studies of the complex formed between Ac-D-Ala-D-Ala and ristocetin, Williams and co-workers[3] have proposed a structure for the complex. The interactions postulated to stabilize the complex include three hydrogen bonds between three amide NH groups (2′, 3′, 4′) and the carboxylate ion of the peptide, two additional hydrogen bonds, hydrophobic interactions, and an electrostatic interaction between the protonated 1′-amine of ristocetin and the peptide carboxylate anion. For the vancomycin/Ac-D-Ala-D-Ala complex, a similar structure was proposed.[4] It has been shown that vancomycin undergoes a large conformational change on binding that orients the NH groups for hydrogen bonding and positions the amino group to allow the formation of a bent salt bridge with the carboxylate anion. Unlike vancomycin, however, ristocetin is more rigid due to the diphenyl ether linkage between the F and G rings and cannot orient the protonated amino group for an ideal electrostatic interaction. In fact, in the proposed structure of the complex the amino group is about 5 Å from the carboxylate and pointing in the wrong direction for salt bridge formation.[3] Nevertheless, an interaction between the peptide carboxylate and protonated amine of ristocetin has been postulated[5] to be important for the initial formation of the complex, resulting in fast on-rates and in the final structure of the complex. Although the peptide carboxylate anion and antibiotic cation are separated by a considerable distance (5 Å) in the final structure of the complex, the ristocetin salt bridge is proposed to be more important than anticipated due to its location in a hydrophobic environment formed by the C, F, and G rings of ristocetin.[5]

In order to assess the relative importance of an electrostatic interaction for complex formation, we have synthesized a series of ristocetin analogues (Figure 1) that cannot form a salt bridge. These compounds were tested for their antibacterial activity and ability for binding to Ac_2-L-Lys-D-Ala-D-Ala. Since ristocetin ψ-aglycone (II) has greater antimicrobial potency than the parent molecule and should be more amenable to selective modification, II was chosen as the starting point for our synthetic modifications. Due to the structural complexity of the target compounds, high magnetic fields and two-dimensional NMR methods were required to interpret their complicated ^1H NMR spectra. The NMR data were useful for the structural elucidation of the compounds as well as for providing information on the molecular conformations that might be related to the biological activity of these molecules.

Results and Discussion

Chemistry. Ristocetin ψ-aglycone (II, Scheme I) was obtained by mild acid hydrolysis of ristocetin.[6] Attempts to prepare aglycone IV by acidolysis, as reported by Bognar,[7] resulted in concomitant ester cleavage to give the aglycone acid III (same structure as IV with the ester hydrolyzed). The aglycone was prepared cleanly by re-

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