The Crystal Structure, Absolute Configuration, and Phosphodiesterase Inhibitory Activity of (+)-1-(4-Bromobenzyl)-4-(3-(cyclopentyloxy)-4-methoxyphenyl)-pyrrolidin-2-one

Paul W. Baures,† Drake S. Eggleston,† Karl F. Erhard,‡ Lenora B. Cieslinski,§ Theodore J. Torphy,§ and Siegfried B. Christensen,*,‡

Departments of Physical and Structural Chemistry, Medicinal Chemistry, and Inflammation and Respiratory Pharmacology, SmithKline Beecham Pharmaceuticals, P.O. Box 1539, King of Prussia, Pennsylvania 19406-0939

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Chiral HPLC resolution of the phosphodiesterase IV (PDE IV) inhibitor rolipram (1) provided (−)-1, and this enantiomer was converted into its 1-(4-bromobenzyl) derivative, (+)-2. X-ray structural analysis of (+)-2 established the absolute configuration as *R*, which provides the first direct evidence for a previously assumed assignment of configuration. The crystal structure of (+)-2 and the PDE inhibitory activity of both enantiomers of 2 are discussed in the context of a previously proposed topological model.

Introduction

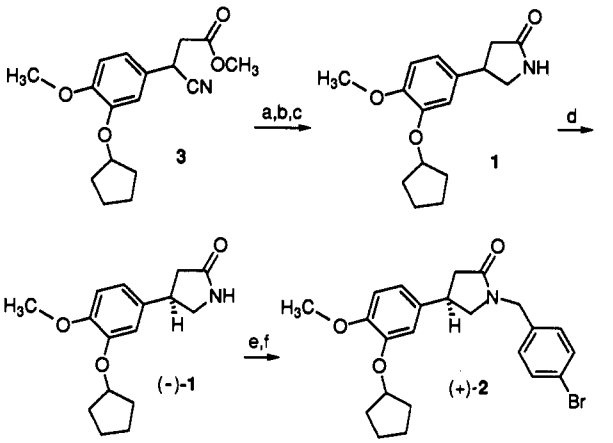
Rolipram (1), (±)-4-(3-(cyclopentyloxy)-4-methoxyphenyl)pyrrolidin-2-one (ZK 62711), is the selective, prototypical inhibitor of the calcium-independent, low K_m cyclic adenosine monophosphate (cAMP)-specific phosphodiesterase (PDE)[1-3] designated PDE IV.[4,5] In addition, rolipram is known to bind saturably, stereoselectively, and with very high affinity to binding sites in brain tissue[6,7] and on human recombinant PDE IV,[8] and in a number of animal models considered predictive of potential antidepressant activity in humans, it is the (−)-enantiomer of 1 [(−)-1] which is the antipode primarily responsible for the observed pharmacological effects.[9-11] In two publications detailing the pharmacokinetic profiles of rolipram in humans,[12,13] and in the report of a recent asymmetric synthesis,[14] the two enantiomers were described as "(*R*)-(−)-rolipram" and "(S)-(+)-rolipram" although no proof of assignment of absolute configuration has appeared in the literature. In the present study, the crystal structure of the 1-(4-bromobenzyl) derivative of (−)-rolipram [(+)-2] is revealed, allowing the absolute configuration of (−)-1 to be deduced. The PDE inhibitory activity of both enantiomers of 2 is discussed in the context of a previously proposed topological model.[21]

Chemistry

Rolipram was synthesized by a modification of literature methods.[15,16] In this modification, reduction of the nitrile of the intermediate methyl 3-cyano-3-(3-(cyclopentyloxy)-4-methoxyphenyl)propionate (3) was accomplished by catalytic hydrogenation in the presence of 10% palladium on carbon and 1 equiv of perchloric acid in methanol, followed by basic workup and cyanide-catalyzed cyclization[17] to the lactam (Scheme I). Semipreparative HPLC separation of the optical isomers of 1 was accomplished on cellulose triacetate,[18] eluting with 95% ethanol, to provide both (−)-1 (>99% ee as determined by analytical HPLC analysis on a cellulose triacetate column) and (+)-1 (>98% ee) with excellent recoveries.

Treatment of (−)-1 with sodium hydride in DMF, followed by reaction with 4-bromobenzyl bromide, provided (+)-2 in reasonable yield (76%); (−)-2 was synthesized from (+)-1 by the same procedure. Single crystals of (+)-2 suitable for X-ray structural analysis were obtained by slow evaporation of a 1:1 toluene/methanol solution.

Scheme I



[a] (a) H_2, Pd/C, $HClO_4$, MeOH; (b) aqueous $NaHCO_3$ workup; (c) NaCN (cat.), toluene, reflux; (d) HPLC (cellulose triacetate); (e) NaH, DMF; (f) 4-bromobenzyl bromide.

Results and Discussion

Figure 1 displays an ORTEPII drawing of the X-ray structure of (+)-2. The absolute configuration of (+)-2 as defined by the Cahn-Ingold-Prelog notation[19] is *R*.[20] Thus, the configuration of (−)-1, the pharmacologically active isomer in animal models of depression, is also *R*. This is the first direct evidence for such an assignment and confirms previous assumptions appearing in the literature.

The structure reveals that the pyrrolidinone ring adopts an envelope conformation with atom C4 displaced by 0.449(6) Å from the plane defined by the other four ring atoms. The central phenyl ring is in a synclinal orientation relative to the pyrrolidinone ring with a dihedral angle between this phenyl ring plane and the mean plane through the pyrrolidinone ring atoms of 85.2(2)°. The carbonyl group lies on the side of the C6–C9 vector adjacent to (or "syn" to) the cyclopentyloxy group as evidenced by the C7–C6–C4–C3 torsion angle of −55°. In this conformation, the carbonyl dipole orientation would be nearly perpendicular to the edge of the phenyl ring with a carbonyl

† Department of Physical and Structural Chemistry.
‡ Department of Medicinal Chemistry.
§ Department of Inflammation and Respiratory Pharmacology.
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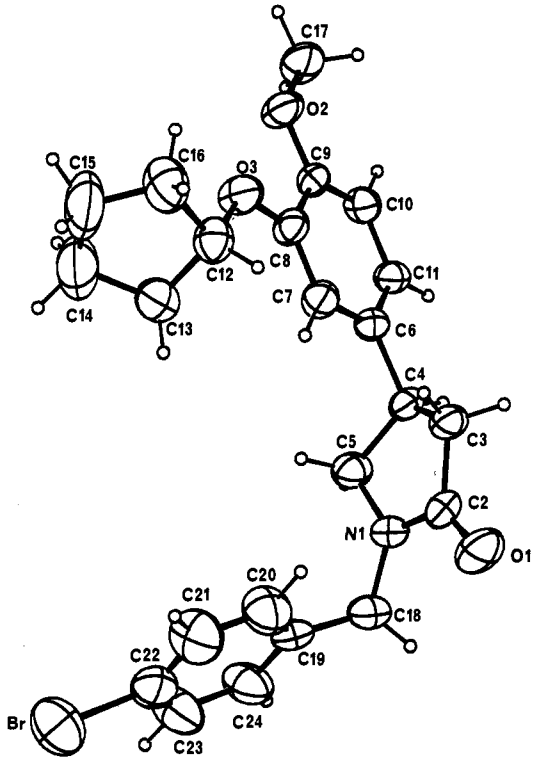

Figure 1. ORTEPII drawing of (+)-2; principal ellipses are drawn at the 50% probability level, H atoms as spheres of arbitrary size.

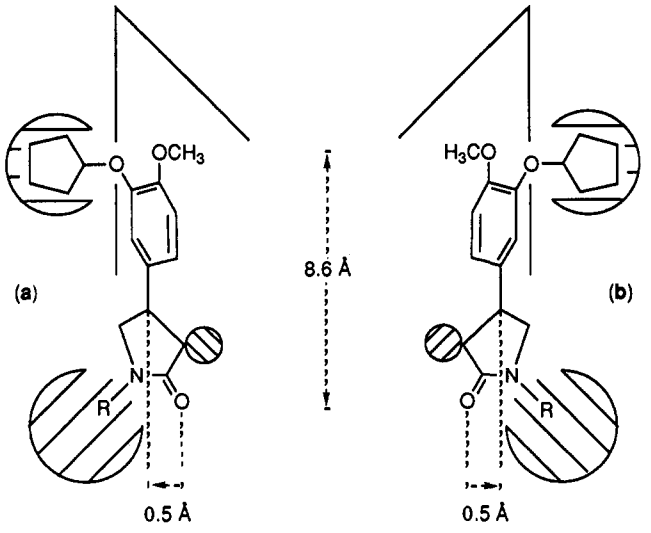

Figure 2. (a) Structural and topological requirements for PDE IV inhibition derived from (*R*)-rolipram (adapted with permission from Marivet et al.[21]); in this model, the dihedral angle between the phenyl ring plane and the mean plane of the pyrrolidinone ring is 26.5°, placing the carbonyl 0.5 Å above the phenyl ring plane. (b) The same model revised to the *S* absolute configuration.

oxygen to methoxyl oxygen distance of 8.78 Å. Thus, the solid state conformation of *R*-(+)-2 contrasts sharply with the relatively flat topology proposed for the active conformation of *R*-1 derived from overlays of *R*-1 and other PDE IV inhibitors (Figure 2a).[21] In that proposed model, the dihedral angle between the central phenyl ring plane and the mean plane through the pyrrolidinone ring atoms is 26.5°, placing the carbonyl dipole nearly coplanar with the phenyl ring plane.

Though necessarily rigid in the crystalline state, the considerable conformational flexibility expected for *R*-(+)-2 in solution should allow it to adopt a conformation appropriate for PDE IV inhibition provided that the *N*-(4-bromobenzyl) moiety is tolerated sterically. Such steric tolerance would be expected based on previous data indicating that both the racemic *N*-benzoyl and *N*-benzyl derivatives of 4-(3,4-dimethoxyphenyl)-1-pyrrolidinone (4, the 3-methoxy analog of 1) are equipotent to or more potent than 4 as inhibitors of bovine smooth muscle PDE IV.[21] However, in our assay system, *R*-(+)-2 was found to be a poor inhibitor (IC_{50} = 74 μM) of partially-purified PDE IV from isolated human monocytes (HMPDE IV),[22] while *S*-(−)-2 was a potent (IC_{50} = 0.17 μM) and selective inhibitor (no significant inhibition of PDEs I, III, or V was observed at 10 μM).[5] Thus, *N*-(4-bromobenzylation) of *R*-1 and *S*-1, which are equipotent, competitive inhibitors of HMPDE IV (IC_{50}s = 1.4 and 1.6 μM, respectively),[8] produces a profound discrimination by this enzyme (>400-fold) between the enantiomers of 2.

In the derivation of the topological model of the bovine smooth muscle PDE IV pharmacophore described previously (Figure 2a), the *R* configuration at the benzylic carbon of rolipram analogues was assumed.[21] The present data demonstrate that for HMPDE IV, the *S* configuration is required for potent inhibition by the rolipram analog with a sterically-demanding, lipophilic 4-bromobenzyl group at N-1. Further studies with additonal enantiomeric pairs of compounds are required to confirm that stereospecific inhibition by the *S* isomers of N-substituted rolipram derivatives is a general property of PDE IV enzymes derived from various tissue sources, as illustrated in the revised topological model of Figure 2b.

Though 1 was originally developed as an antidepressant, recent demonstrations that PDE IV appears to be the predominant functional cAMP PDE in a variety of human inflammatory cells have focused interest upon the potential of rolipram and other selective PDE IV inhibitors as a new class of antiinflammatory, particularly antiasthmatic, agents.[23-25] Details of the stereoselectivity of PDE IV inhibition by 1 and other PDE IV inhibitors, of their activity in inflammatory cells and in animal models of inflammation, will be reported in due course.

Experimental Section

(1) Crystallographic Studies. A block-shaped crystal of (+)-2 with approximate dimensions 0.30 mm on each side was mounted on a glass fiber with epoxy for examination. Lattice parameters were determined at 293 K on an Enraf Nonius CAD-4 diffractometer equipped with graphite monochromated molybdenum radiation (λ(Mo Kα) = 0.710 73 Å) from the setting angles of 25 high-order reflections. The only systematic absence, $0k0$ for k odd, indicated space group $P2_1$. Intensity data were collected on the diffractometer using variable-speed θ–2θ scans where the final scan speed was determined for each reflection from a prescan of its intensity. Scan speeds varied from 2.5 to 6.7 deg min^{-1}. Background estimates were made by extending the scan 25% on each side of the peak. Three intensity standards, monitored at regular intervals, showed a negligible variation. Data were corrected for background, Lorentz, and polarization effects. An absorption correction based on ψ scans[26] of nine reflections with high χ settings was applied; correction factors were 0.999 (max), 0.899 (min), 0.956 (av). A total of 3532 data was collected to $2\theta \leq 60°$ ($0 \leq h \leq 16$; $0 \leq k \leq 7$; $-23 \leq l \leq 23$) which gave 3385 unique data after averaging of symmetry equivalents (R_{int} = 0.020). The structure was solved by direct methods.[27] Atomic positions for non-hydrogen atoms were refined with anisotropic displacement parameters employing full matrix least-squares procedures. The y coordinate of atom Br was held fixed to define the origin. The function minimized was $\Sigma w(|F_o| - |F_c|)^2$, and the weights, w, were defined as $w = 4F_o^2[\sigma^2(I_o) + (0.04I_o)^2]^{-1}$. Positions for the hydrogen atoms were calculated from idealized geometry and

were held fixed in the final cycles along with temperature factors assigned as 1.3 (B_{iso}) of the attached carbon atom. The refinement converged (max $\Delta/\sigma = 0.005$) to the conventional crystallographic agreement values of $R = 0.0422$, $R_w = 0.0412$, GOF = 0.844. The enantiomeric structure was subsequently refined from the same starting point. The ratio of weighted residuals (1.07) for the two refinements gave a clear statistical indication (99.99% confidence level) based on Hamilton's R factor ratio test[28] for a refinement of 252 variables with 1414 observations ($I \geq 3\sigma(I)$). Additionally, Roger's η parameter[29] refined to a value of +1.00(2) for the R configuration. A final difference Fourier map showed maximum features of ±0.306 e Å^{-3}. Values of the neutral atom scattering factors, including $\Delta f'$ and $\Delta f''$ for all non-hydrogen atoms, were taken from reference 30. Crystal data: $C_{23}H_{26}BrNO_3$, Mr 444.38, monoclinic, $P2_1$, a 11.774(3) Å, b 5.462(4) Å, c 16.475(3) Å, β 91.45(2)°, V 1059.1(8) Å^3, Z 2, ρ_{calc} 1.393 g cm^{-3}, μ 19.415 cm^{-1}, $F(000)$ 460.

(2) Chemistry. Melting points were determined on a Thomas-Hoover Unimelt capillary apparatus and are uncorrected. ^{1}H NMR were recorded at ambient temperature on a Bruker AM-250 spectrometer in $CDCl_3$, with chemical shifts reported as apparent centers of multiplets in ppm (δ) downfield from internal TMS and apparent first-order coupling constants reported in hertz. Elemental analyses were determined using a Perkin-Elmer 240C. Optical rotations were measured on a Perkin-Elmer Model 241 polarimeter in a 1-dm cell. Flash chromatography was conducted using silica gel 60 (70–230 mesh ASTM) from E. Merck.

4-(3-(Cyclopentyloxy)-4-methoxyphenyl)pyrrolidin-2-one (1). Methyl 3-cyano-3-(3-(cyclopentyloxy)-4-methoxyphenyl)propionate (6.0 g, 19.8 mmol) and 70% perchloric acid (1.95 mL) were added to a suspension of 10% palladium on carbon (0.9 g) in methanol (100 mL). The mixture was hydrogenated at 50 psi for 1.5 h, diluted with methylene chloride, filtered through Celite, and evaporated. The residue was partitioned between methylene chloride and dilute aqueous sodium bicarbonate and extracted three times. The organic layer was dried (potassium carbonate), and the solvent was evaporated. The resultant yellow oil was dissolved in toluene (100 mL), a catalytic amount of sodium cyanide was added, and the mixture was heated at reflux under an argon atmosphere for 20 h. The solvent was removed *in vacuo* and the residue was partitioned between methylene chloride and water and was extracted twice. The organic layer was dried (potassium carbonate) and evaporated. Purification by flash chromatography, eluting with 95:5 chloroform/methanol, provided a solid (3.7 g, 67%): mp 130 °C (lit.[15] mp 132 °C).

(*R*)-(−)- and (*S*)-(+)-4-(3-(Cyclopentyloxy)-4-methoxyphenyl)pyrrolidin-2-one [*R*-(−)-1 and *S*-(+)-1]. Chiral separation was accomplished using preparative HPLC conditions with an 8-cm × 55-cm column packed with 1.0 kg of cellulose triacetate (15–25 m) from E. Merck. The mobile phase of 95:5 ethanol/water eluted at a flow rate of 20 mL/min with injection of 1 g/30 mL at ambient temperature. Ultraviolet detection of the eluting product was employed at 254 nm.

***R*-(−)-1:** mp 131–133 °C; t_R 68 min; recovery 88% (>99% ee); $[\alpha]^{24}_D$ (c 0.5, methanol) = −31.0°. Anal. ($C_{16}H_{21}NO_3$) C, H, N.

***S*-(+)-1:** mp 129–131°C; t_R 86 min; recovery 87% (>98% ee); $[\alpha]^{24}_D$ (c 0.5, methanol) = +30.9°. Anal. ($C_{16}H_{21}NO_3$) C, H, N.

(*R*)-(+)-1-(4-Bromobenzyl)-4-(3-(cyclopentyloxy)-4-methoxyphenyl)pyrrolidin-2-one [*R*-(+)-2]. A solution of *R*-(−)-1 (0.51 g, 1.85 mmol) in dry DMF (10 mL) under an argon atmosphere at room temperature was treated with NaH (0.062 g of 80% dispersion, 2.04 mmol) for 45 min. A solution of 4-bromobenzyl bromide (0.51 g, 2.04 mmol) in DMF (1 mL) was added, and the mixture was stirred for 3 h. Water was added, and the mixture was extracted three times with ether. The combined extracts were dried (K_2CO_3), and the solvent was removed *in vacuo*. The residue was purified by flash chromatography, eluting with 9:1 ether/methylene chloride, followed by trituration with ether/methylene chloride, to provide a solid (0.63 g, 76.5%): mp 100–102°C. $[\alpha]^{25}_D$ (c 1.0, methanol) = +50.4°; ^{1}H NMR ($CDCl_3$) δ 7.45 (2H, d, J = 8), 7.15 (2H, d, J = 8), 6.78 (1H, d, J = 8), 6.78 (1H, dd, J = 2, 8), 6.64 (1H, d, J = 2), 4.69 (1H, m), 4.49 (2H, AB system, J = 15), 4.40 (3H, s), 3.61 (1H, H_e of five-spin system, dd, J = 8, 9), 3.47 (1H, H_c of five-spin system, dddd, J = 8), 3.23 (1H, H_d of five-spin system, dd, J = 8, 9), 2.85 (1H, H_b of five-spin system, dd, J = 8, 17), 2.58 (1H, H_a of five-spin system, dd, J = 8, 17), 1.84 (6H, br m), 1.60 (2H, br m). Anal. ($C_{23}H_{26}BrNO_3$) C, H, N.

(*S*)-(−)-1-(4-Bromobenzyl)-4-(3-(cyclopentyloxy)-4-methoxyphenyl)-2-pyrrolidinone [*S*-(−)-2]. This compound was prepared from *S*-(+)-1 as described above for synthesis of *R*-(+)-2 and was isolated as a solid: mp 99–100°C. $[\alpha]^{25}_D$ (c 1.0, methanol) = −48.1°. Anal. ($C_{23}H_{26}BrNO_3$) C, H, N.

(3) Phosphodiesterase Assay. Phosphodiesterase activity was assayed as described previously[31] using PDE IV isolated from human monocytes.[22]

Supplementary Material Available: Tables of bond distances, bond angles, positional parameters, and anisotropic displacement parameters (4 pages). Ordering information is given on any current masthead page.

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