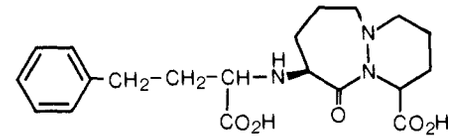
Table III. Geometric Parameters Defining Position of Zinc Atom Relative to Zinc-Binding Functions

compd	bond length Zn–O,S, Å	bond angle, deg	torsion angle, deg
1, 3–5	2.0	124 (Zn, S, C)	180 (Zn, S, C, C)
6, 8	1.8	111 (Zn, O, P)	−96 (Zn, O, P, N)
7	2.0	138 (Zn, O, C)	17 (Zn, O, C, C)
9	1.7	97 (Zn, O, C)	120 (Zn, O, C, C)

lysin and carboxypeptidase A.

These features are summarized in Figure 8, which shows the four structurally distinct ACE inhibitors, 1, 7, 8, and 9, binding to a three-dimensional model of the active site. It should be stressed that this model is not a totally unique interpretation of the data: in particular, the alternative values of ϕ_1 in captopril (60°, 180°) have been excluded on the basis of relatively small energy differences, and the locations of the carboxyl and carbonyl binding groups are not yet tightly defined. We believe, however, that the obvious agreement between the conformational and orientational requirements for the four major binding groups in these different classes of ACE inhibitors, as illustrated in Figure 8, supports the proposed model, which provides a simple template for the design of further conformationally restricted analogues. A similar conclusion has been reached by Hassall and co-workers,[25,26] who have used the activities of a series of bicyclic analogues related to 12 to design the potent new inhibitor 13 ($I_{50} = 6 \times 10^{-10}$ M), and



13

by Tute, who has derived a very similar model[27] by fitting captopril and related inhibitors to the observed crystal structure of carboxypeptidase A.

(25) M. R. Attwood, R. J. Francis, C. H. Hassall, A. Krohn, G. Lawton, I. L. Natoff, J. S. Nixon, S. Redshaw, and W. A. Thomas, *FEBS Lett.*, **165**, 201 (1984).
(26) C. H. Hassall, A. Krohn, C. J. Moody, and W. A. Thomas, *J. Chem. Soc., Perkin Trans. 1*, 155 (1984).
(27) M. S. Tute, private communication.
(28) M. Fujinaga and M. N. G. James, *Acta Crystallogr., Sect. B*, **B36**, 3196 (1980).

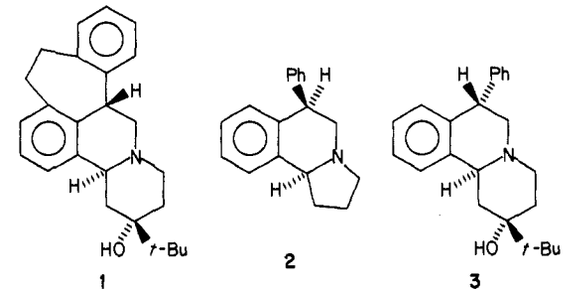
The pK_a of Butaclamol and the Mode of Butaclamol Binding to Central Dopamine Receptors

Frank A. Chrzanowski, Beth A. McGrogan, and Bruce E. Maryanoff*

Department of Chemical Research and Section of Basic Pharmaceutics, McNeil Pharmaceutical, Spring House, Pennsylvania 19477. *Received June 4, 1984*

The pK_a values for butaclamol (1), 1,2,3,5,6,10bβ-hexahydro-6α-phenylpyrrolo[2,1-*a*]isoquinoline (2, McN-4612-Y), and 2-*tert*-butyl-1,3,4,6,7,11bβ-hexahydro-7β-phenyl-2*H*-benzo[*a*]quinolizin-2α-ol (3, McN-4171) were determined to be 7.2, 9.1, and 7.0, respectively. The values for 1 and 3 are anomalous; however, the value for 1 (7.2) is not as low as the one reported in the literature (pK_a = 5.9). We also determined pK_a values for apomorphine, chlorpromazine, and lidocaine, for reference purposes (7.6, 9.2, and 7.9, respectively). The results indicate that 1 would not be predominantly unprotonated under the physiological conditions of receptor binding, rather it would be about 50% protonated. This fact may contravene a suggested binding model used to map the central dopamine receptor (viz., ref 3).

Butaclamol (1) is a dopamine receptor antagonist and a potent antipsychotic agent,[1] which has shown clinical activity.[2] The compound possesses a rather rigid molecular geometry and exhibits high, enantioselective affinity for central dopamine receptors.[1] The limited conformational flexibility of 1 has established it and its analogues as agents for the "mapping" of dopamine receptors in the central nervous system (CNS).[3] In interacting with the dopamine receptor, it is presumed that the nitrogen atom would be a relatively important binding site. Indeed, the nitrogen has been viewed[3] as the primary site for 1 and its congeners. However, one needs to ask a question that can be critical to specification of receptor geometry: Is the nitrogen more likely to be in the free-base or protonated state?



1 2 3

In considering this key question, Philipp et al.[3b] concluded that since butaclamol has a pK_a of 5.9, compared to a pH of 7.3 for homogenized rat caudate nucleus, the butaclamol ligand exists almost exclusively in the free-base (unprotonated) form during the binding event. Thus, they excluded ligand–receptor binding based on ionic interaction via a charged, protonated species in favor of binding via a hydrogen bond between the nitrogen lone pair of electrons and the receptor. This selection was a crucial one in their establishment of geometric parameters for receptor mapping.

More recently, Froimowitz and Matthysse have tried to rationalize the "anomalously low pK_a for butaclamol" on the basis of unfavorable geometries for ion solvation with protonated butaclamol.[4] Specifically, it was suggested that the strong preference of protonated 1 for a cis conformation (i.e., a cis D–E ring fusion) gives rise to its high acidity,

(1) (a) Voith, K.; Herr, F. *Psychopharmacologia* **1975**, *42*, 11. (b) Bruderlein, F. T.; Humber, L. G.; Voith, K. *J. Med. Chem.* **1975**, *18*, 185. (c) Seeman, P. *Pharmacol. Res.* **1980**, *32*, 230. (d) Kukla, M. J.; Bloss, J. L.; Brougham, L. R. *J. Med. Chem.* **1979**, *22*, 401 and ref 5–12 cited therein.
(2) (a) Inaz, F.; Ban, T. A.; Lehmann, H. E. *Psychopharmacol. Bull.* **1976**, *12*, 31. (b) Nestoros, J. N.; Lehmann, H. E.; Ban, T. A. *Int. Pharmacopsychiatry* **1978**, *13*, 138. (c) Clark, M. L.; Paredes, A.; Costiloe, J. P.; Wood, F. *J. Clin. Pharmacol.* **1977**, *17*, 529.
(3) (a) Humber, L. G.; Bruderlein, F. T.; Philipp, A. H.; Götz, M.; Voith, K. *J. Med. Chem.* **1979**, *22*, 761. (b) Philipp, A. H.; Humber, L. G.; Voith, K. *Ibid.* **1979**, *22*, 768.

0022-2623/85/1828-0399$01.50/0

through various, special steric mechanisms.

The anomalous pK_a for butaclamol attracted our attention because of its relation to attempted receptor modeling and its recent theoretical justification. Since we had already determined the pK_a of analogous compound **2** (McN-4612-Y), a reasonable value of 9.1, we became curious about the value reported for butaclamol. We herein report that our independent measurements place the pK_a of butaclamol in the range of 7.0–7.5. With this pK_a butaclamol would be approximately 50% protonated when interacting with the dopamine receptor. Such a result contravenes the premise employed by Philipp et al. and tempers the proposed[3] receptor mapping because of ambiguity introduced with regard to the form adopted by the ligand on binding to the receptor.

Results and Discussion

The pK_a values for **1–3** were determined in water by standard techniques for compounds possessing limited aqueous solubility.[5] Full procedural details are presented in the Experimental Section. The pK_a values, uncorrected for ionic strength, are 7.15, 9.12, and 7.03 for **1–3**, respectively. The true concentration-dependent pK_a for **2** of 9.0 (corrected for ionic strength) is only slightly different from the uncorrected value. We also determined the pK_a values of three amine drugs, lidocaine, apomorphine, and chlorpromazine, for reference purposes (each as an HCl salt). These reference pK_a values were 7.95 (method of ref 5a), 7.58 (method of ref 5a), and 9.22 (method of ref 5b), respectively, which compare favorably with reported values of 7.86,[5a] 7.0–7.2,[6a] and 9.2–9.3,[6b] respectively.

The values for **1** and **3** depart significantly from the pK_a range for a standard benzylamine (8.5–9.5).[7] In fact, model compound **2** has a pK_a of 9.1, within the expected range. The lower pK_a values for **1** and **3** may be attributable partly to the hydroxy substituent, which is expected to exert a field effect on the order of 1 pK_a unit.[8] This still leaves a discrepancy of ca. 1 pK_a unit for **1** and **3**. Whether this is truly anomalous, or related to subtle, unrecognized structural factors, requires careful study of a series of 4-hydroxypiperidines. In any event, since the hydroxy group is not required for biological activity in the butaclamol series,[3a] those compounds lacking it should have higher pK_a values and be predominantly protonated in the biological milieu.

The pK_a of ca. 7.0 for **1** allows ca. 50% protonation under physiological conditions. Thus, both amine forms would need to be considered when describing possible ligand–receptor interactions, which would impede the adoption of only a single model for receptor binding of butaclamol (**1**). However, most ligands for CNS dopamine receptors (e.g., dopamine, spiroperidol, pimozide, apomorphine, deshydroxybutaclamol compounds, and chlorpromazine) should have pK_a values in the range of 7.0–9.5, causing them to be substantially protonated under physiological conditions. Indeed, our measured pK_a values for apomorphine and chlorpromazine are 7.6 and 9.2, respectively. Thus, a model entailing a protonated ligand, such as the one described by Olson et al.,[11] may be more appropriate.

Experimental Section

Materials. A sample of (+)-butaclamol hydrochloride was kindly furnished by Ayerst Research Laboratories, Montreal, Canada. Compound **2**, (–)-isomer hydrochloride (mp 230–238 °C),[12] and **3** (McN-4171; mp 190–192 °C)[1d] were synthesized. The following reagents were employed: reagent-grade KCl; carbonate-free KOH (0.0982 N); 0.1 N HCl; and distilled, deionized water that had been degassed by boiling for 20 min.[13]

Titration Procedures. Forty or fifty milliliters of a dilute solution of **1–3** (1 or 5×10^{-4} M), as hydrochloride salts, were placed in a beaker at 25 ± 1 °C. A presaturated stream of nitrogen was bubbled through the solution for 20 min prior to the titration and during the titration. The ionic strength of the solution of **2** was adjusted to 0.1 with KCl. The hydrochloride salt of **3** was formed in situ with use of 0.1 N HCl. Carbonate-free KOH solution (0.0982 N) was added from a 2-mL microburet, which was calibrated to 0.0002 mL. The ionic strength of the carbonate-free KOH solution was adjusted to 0.1 with KCl. The ionic strengths of the solutions of **1** and **3** were not adjusted since the addition of KCl resulted in precipitation. The references lidocaine, apomorphine, and chlorpromazine (as HCl salts) were measured analogously, with the ionic strength adjusted.

A pH meter calibrated to 0.001 units, standardized against 0.05 M potassium hydrogen phthalate (pH 4.010) and 0.05 M potassium phosphate monobasic and sodium hydroxide (pH 7.000), was used.

The solubilities of **1** and **2** free bases were determined after neutralization with a 20% excess of KOH titrant. The solutions were filtered through 0.45-μm cellulose ester filters and analyzed with a UV spectrophotometer. The solubility value for **1** was measured as $(6.12 \pm 0.55) \times 10^{-5}$ M.

Calculations. The ionization constants (K_a) and pK_a values were calculated by fitting the pH-titration data (at least five determinations for each compound **1–3** and at least four for the references) to model linear equations derived by Benet and Goyan[5a] for soluble conjugate acids or Levy and Rowland[5b] for sparingly soluble compounds. The data were fitted to the respective model equations with use of a regression program, NONLIN.[14]

The curve fitting allowed calculation of the K_a from the slope of the Benet and Goyan equation[5a] or from the solubility and slope of the Levy and Rowland equation.[5b] The y intercept should be equal to the concentration of the test compound (1 or 5×10^{-4} M) originally in solution, which serves as a methodology check. In the case of **2**, a true concentration-dependent pK_a was calculated since the calculated H_3O^+ concentration was corrected for ionic strength (0.1). The pK_a values were calculated from the mean K_a values for each compound.

The method of ref 5a was used for lidocaine, apomorphine, **2**, and **3**; the method of ref 5b was used for chlorpromazine and **1**.

The pK_a mean values with error ranges are as follows: **1**, 7.15 ± 0.3; **2**, 9.12 ± 0.1; **3**, 7.03 ± 0.4; lidocaine, 7.95 ± 0.1; apomorphine, 7.58 ± 0.2; and chlorpromazine, 9.22 ± 0.04.

(4) Froimowitz, M.; Matthysse, S. *Mol. Pharmacol.* **1983**, *24*, 243.
(5) (a) Benet, L. Z.; Goyan, J. E. *J. Pharm. Sci.* **1965**, *54*, 1179. (b) Levy, R. H.; Rowland, M. *J. Pharm. Sci.* **1971**, *60*, 1155.
(6) (a) Tencheva, J.; Velvinov, G.; Budevsky, O. *Arzneim.-Forsch.* **1971**, *60*, 1155. Sober, H. A., Ed. "Handbook of Biochemistry", CRC, Cleveland, 1968, p J–169. (b) Green, A. L. *J. Pharm. Pharmacol.* **1967**, *19*, 10. Chaffen, L. G.; Harris, L. R. *Anal. Chem.* **1962**, *34*, 1495.
(7) (a) Albert, A.; Serjeant, E. P. "Ionization Constants of Acids and Bases"; Wiley: New York, 1962; Chapter 8. (b) Jones, F. M., III; Arnett, E. M. *Prog. Phys. Org. Chem.* **1974**, *11*, 263.
(8) A β-hydroxy substituent lowers amine basicity on the order of 1.5 pK_a units.[7] This field effect[9] also occurs with a δ-hydroxy substituent, as evidenced for the 4-substituted quinuclidinium system.[10] Although a decrease in pK_a of ca. 1.5 units (from the parent) was measured in the quinuclidinium series,[10] it should be noted that there are three bond pathways involved. For a 4-hydroxypiperidine (with two pathways), the pK_a difference from the parent would be about −1 unit.
(9) March, J. "Advanced Organic Chemistry", 2nd ed.; McGraw-Hill: New York, 1977; Chapter 8.
(10) Ceppi, E.; Eckhardt, W.; Grob, C. A. *Tetrahedron Lett.* **1973**, 3627.
(11) Olson, G. L.; Cheung, H. C.; Morgan, K. D.; Blount, J. F.; Todoro, L.; Berger, L.; Davidson, A. B.; Boff, E. *J. Med. Chem.* **1981**, *24*, 1026.
(12) Maryanoff, B. E.; McComsey, D. F.; Costanzo, M. J.; Setler, P. E.; Gardocki, J. F.; Shank, R. P.; Schneider, C. R. *J. Med. Chem.* **1984**, *27*, 943.
(13) Reference 7a, pp 16–42.
(14) Niebergall, P. J.; Schnaare, R. L.; Sugita, E. T. *J. Pharm. Sci.* **1971**, *60*, 1393.