termination of radioactivity. The remaining ethyl acetate was removed; the water phase was made alkaline with solid sodium carbonate and 1.0 mL of 0.5 M borate buffer, pH 10. The amine was then extracted into 6.0 mL of ethyl acetate and 4.0 mL of the organic phase was taken for the assay. The deamination of [$^3$H]tyramine and [$^{14}$C]-5-HT was determined simultaneously by utilizing the double-labeling technique.[21] Inhibition of the MAO activity in the brain slices was determined 1 h after the intraperitoneal injection of the test compound. The inhibition was expressed in percent and calculated on the total formation of acid and neutral products formed in the brain slices of animals treated with the test compounds or with saline.

**Inhibition of the active uptake of [$^{14}$C]-5-HT** in the brain slices was determined simultaneously from the values obtained in the MAO determination. The active uptake was defined as the uptake sensitive to $3 \times 10^{-4}$ M cocaine and the inhibition was expressed in percent of the corresponding uptake of [$^{14}$C]-5-HT in brain slices of the control animals.[22]

**Potentiation of the 5-HTP syndrome** in mice was determined as previously described.[23] The test compounds were injected 1 h prior to *dl*-5-HTP, 90 mg/kg iv.

**Potentiation of tryptamine** tremor and abduction of hind legs was recorded with the same technique as used for the 5-HTP syndrome. The test compounds were injected ip 1 h before tryptamine, 50 mg/kg iv, and the number of animals with tremor or abduction were noted within 0.5 h after the tryptamine injection. The dose producing this effect in 50% of the animals ($ED_{50}$) was determined by probit analysis based on at least four dose levels including five animals per dose level.

**Potentiation of Phenethylamine.** Phenethylamine (10 mg/kg ip) was given to mice pretreated with reserpine, 2.5 mg/kg ip, 16 h before the experiment. In combination with inhibitors of the B form of MAO, phenethylamine causes a rapid reversal of the reserpine sedation. The test compound was injected 1 h prior to phenethylamine and the animals were observed for reserpine reversal for a period of 30 min.

**Motor Activity.** The threshhold dose producing central stimulation within 2 h after ip injection was determined by observation in groups of four mice.

**Reserpine Antagonism.** The decrease in motor activity in mice 1 h after the injection of 2.5 mg/kg ip of reserpine was determined in a locomotion cage. The test compounds were injected ip 1 h prior to reserpine. The dose preventing the decrease in motor activity with 50% ($ED_{50}$) 1 h after reserpine was estimated from dose–response curves based on at least four doses with 10–12 animals per dose level.

**Antiaggressive effect** in isolated male mice was determined as described previously.[24] The $ED_{50}$ values are based on at least three doses with five groups per dose level and determined from log dose–response curves.

**Acute toxicity** was assessed in mice observed for 24 h after ip injection. The $LD_{50}$ values were determined from log dose–response curves based on at least five doses with five animals per dose level.

**Statistical Methods.** Correlations between MAO inhibition and the various behavioral tests employed were calculated by the Spearman rank correlation test.[25]

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# Conformational Analysis of the Molecule Luteinizing Hormone-Releasing Hormone. 3. Analogue Inhibitors and Antagonists


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Conformational energy calculations have been carried out on analogues of luteinizing hormone-releasing hormone which have been shown to be potent inhibitors of the release of luteinizing hormone and follicle-stimulating hormone. The analogues included in this study have D-amino acid substitutions in the 2 and/or 3 positions, such as [D-X$^2$]-LH-RH, [D-X$^2$,D-Y$_3$]-LH-RH, [D-X$^2$,Pro$^3$]-LH-RH, and [D-X$^2$,Leu$^3$]-LH-RH. A configurational property which was common to the low-energy conformers of all the analogues is the directional change of the *cis*-peptide bond of the pyroglutamate ring. Further, there was no overall structural change in the analogues relative to the conformation of native LH-RH, and the orientation of the aromatic side chains relative to one another remained the same throughout this series of analogues.


In two previous papers[1,2] (referred to as papers 1 and 2) of this series, low-energy conformations of the molecule luteinizing hormone-releasing hormone (LH-RH), ∠Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH$_2$, were obtained using "empirical" energy calculations. The low-energy structures found[1,2] were compared to available experimental data, and the effects of various analogues on the molecular conformation were discussed.[2] In this paper,

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the conformational properties of analogues of LH-RH, which have been shown experimentally to exhibit potent inhibition of the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) by LH-RH,[3-8] are considered. It is felt that by looking for structural similarities in known antagonists of LH-RH one may be more successful in designing new potentially important contraceptive agents.

The potent inhibitors found to date generally have several primary structural features in common, such as a D residue in the 2 position (e.g., D-Phe$^2$)[3-5] or with D-X$^2$ and either proline,[6,7] leucine,[6,7] or a D residue (e.g., D-Trp$^3$)[8] in the 3 position. In most cases studied, a D residue in the 6 position was found to enhance inhibition. Less potent inhibitors with sequences such as [Leu$^2$,Leu$^3$,D-Ala$^6$]-LH-RH,[9] [Thr$^2$,Leu$^3$]-LH-RH,[10] and [des-His$^2$]-LH-RH[11-13] will not be considered. The emphasis here will be on analogue changes which include a D residue at the 2 position and L or D residues at the 3 position, since it appears that changes of these types, at these positions, produce the most potent inhibitors.

The molecules studied here are (i) [D-His$^2$,D-Trp$^3$]-LH-RH, (ii) [D-His$^2$,Pro$^3$]-LH-RH, and (iii) [D-His$^2$,-Leu$^3$]-LH-RH and [D-His$^2$]-LH-RH. The use of D-His in the 2 position to find the lowest energy structures is tested against the D-Phe$^2$ analogues and no major structural differences are found between the low-energy conformations. It is not expected that the experimental analogues, such as [D-Phe$^2$,D-Ala$^6$]-LH-RH, [D-Phe$^2$,Pro$^3$,D-Trp$^6$]-LH-RH, and [D-Phe$^2$,D-Trp$^3$,D-Trp$^6$]-LH-RH, will differ in conformation significantly from the analogues noted above. Although the D$^6$-substitution is not considered in this work, the low-energy conformations found previously[1,2] for LH-RH are compatible with a D-substitution at the 6 position.

**Methods.** The nomenclature and conventions adopted for amino acids by an IUPAC–IUB Commission were used throughout,[14] and all energy calculations were carried out using ECEPP (Empirical Conformational Energy Program for Peptides).[15] The empirical potential energy functions and parameters, as well as the energy minimization procedure, have been described previously.[1,16] The energy minimization was terminated when the energy change between cycles became less than 0.1 kcal/mol. Each energy minimization was carried out on the complete molecule with 38–42 dihedral angles (depending upon the analogue) being allowed to vary. The dihedral angles allowed to vary are the same as those described in paper 1. A minimum of eight starting conformers were examined for each analogue, not including different side-chain combinations.

**Choice of Starting Conformations.** LH-RH and its analogues are not small polypeptides from the standpoint of conformational energy calculations (see Figure 1 of paper 1). For this reason, it becomes necessary to limit the search for low-energy structures of the inhibitor analogues to conformational changes at the analogue residue position, starting from the low-energy structures found for LH-RH.[1,2] The procedure adopted was to choose an allowed set of backbone dihedral angles for the 2 and 3 residue positions from the starting sets given in Table I of paper 1. The D residues were treated by exchanging the signs of the dihedral angles from the $\phi$ and $\psi$ values allowed for L residues to those allowed for D residues. Side-chain dihedral angles were also appropriately changed. Each starting set of dihedral angles for the 2 and 3 positions was then used with the remaining residue's dihedral angles taken from the low-energy structures found for LH-RH. Conformers AA–CC of paper 1 were used for

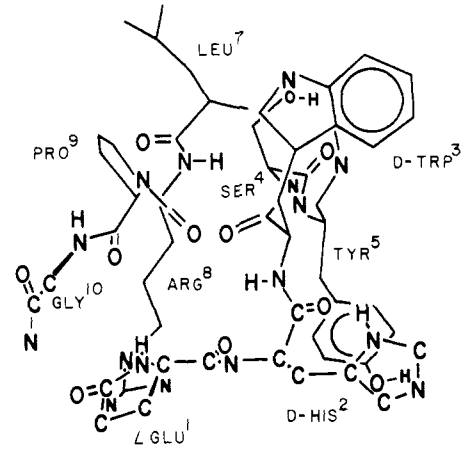


**Figure 1.** Conformer A1 of [D-His$^2$,D-Trp$^3$]-LH-RH. For clarity some carbon and hydrogen atoms have been omitted.

each set of different starting values for the dihedral angles in positions 2 and 3. Energy minimization was then carried out over all the variable dihedral angles described in paper 1, plus any new side-chain variables for the new residues in the 2 and 3 positions.

## Results

The results of the calculations on the molecules [D-His$^2$,D-Trp$^3$]-LH-RH, [D-His$^2$,Pro$^3$]-LH-RH, [D-His$^2$,-Leu$^3$]-LH-RH, and [D-His$^2$]-LH-RH are given in Table I. The dihedral angles of the lowest energy conformers are given for the residues ∠Glu$^1$, D-His$^2$, D-Trp$^3$ (Pro$^3$, Leu$^3$, or Trp$^3$), and Ser$^4$, while the remaining residues retained dihedral angles close to those found for conformers AA or CC of paper 1. Energies are expressed as $\Delta E$ values, where $\Delta E = E - E_0$, and $E_0$ is the energy of the lowest energy conformer for the particular analogue. Energies between analogues cannot be compared, since each is a different molecule.

**Low-Energy Conformations of [D-His$^2$,D-Trp$^3$]-LH-RH.** Figure 1 shows the lowest energy conformation found for the [D$^2$,D$^3$] analogue (conformer A1). The $\phi$ and $\psi$ angles for the D-His$^2$ residue (see Table I) are in the $\alpha_R$ conformation for a D residue, similar to those found for the L isomer in the CC conformation of paper 1 (i.e., $\phi$ = −73°, $\psi$ = −47° for L-His$^2$ of conformer CC). The D-Trp$^3$ backbone dihedral angles are also sign reversed from those found for the Trp residue of the CC conformer (i.e., for L-Trp$^3$, $\phi$ = −127°, $\psi$ = 163°), but the favored side-chain position denoted by $\chi_1^3$ has changed from $\chi_1^3$ = −63° in conformer CC to the extended value, $\chi_1^3$ = −179°, in the D$^2$ analogue. The serine backbone dihedral angles have changed from $\phi$ = −75°, $\psi$ = 95° in conformer CC to those given in Table I for the [D$^2$,D$^3$] analogue. The most noticeable overall result is that the molecule still resembles the native structure in almost every respect, except in the direction that the ∠Glu$^1$ *cis*-peptide bond takes with respect to the rest of the molecule. In the CC structure (see Figure 2) the ∠Glu$^1$ amide bond of the ring points away from the molecule, while in the [D$^2$,D$^3$] analogue (Figure 1), this group points into the molecule. This result will be shown to occur for the lowest energy conformers of all the potent inhibitor analogues studied here. Further, for the next lowest energy conformer found for [D-His$^2$,D-Trp$^3$]-LH-RH (i.e., conformer A2), the ∠Glu$^1$ ring also sits in a similar position as found for conformer A1. The lowest energy conformer of the [D$^2$,D$^3$] analogue which has the D-His$^2$ ring rather than the ∠Glu$^1$ ring pointed into the molecule, similarly to the AA conformer of paper 1, is conformer A3, and it has an energy 3.6 kcal/mol higher

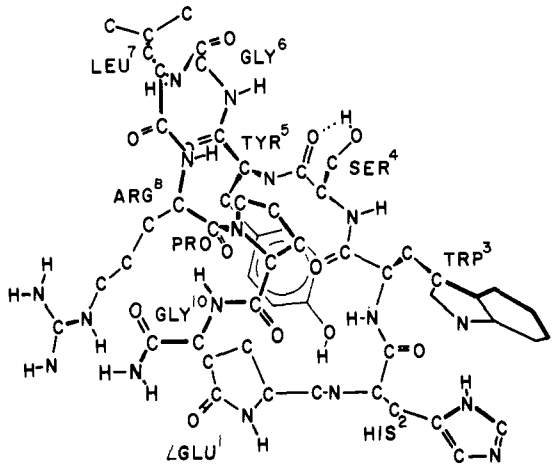


**Figure 2.** Conformer CC of LH-RH found in papers 1 and 2. For clarity some hydrogen atoms have been omitted.

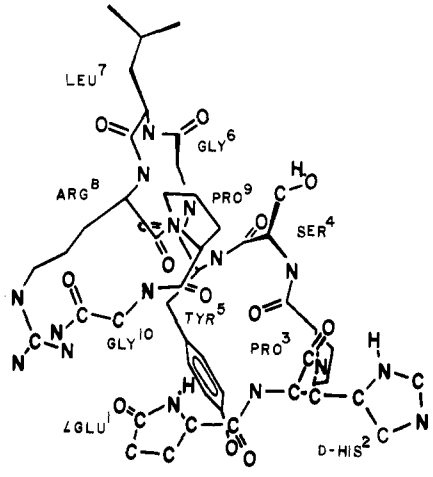


**Figure 3.** Conformer B1 of [D-His$^2$,Pro$^3$]-LH-RH. For clarity some carbon and hydrogen atoms have been omitted.

than $E_0$ for this analogue. This result is just the opposite of that found for native LH-RH where the AA conformer was ~3 kcal/mol lower in energy than the CC conformer.

**Low-Energy Conformations of [D-His$^2$,Pro$^3$]-LH-RH.** The lowest energy conformation of the [D$^2$-Pro$^3$] analogue (B1 of Table I) is shown in Figure 3. The $\phi$ and $\psi$ angles for the D-His$^2$ residue (see Table I) are nearly inversions of the angles found in the AA conformer of paper 1 (i.e., $\phi = -83°$, $\psi = 86°$ for conformer AA). The Pro$^3$ backbone dihedral angles are nearly identical with the angles found for L-Trp in the native LH-RH (i.e., $\phi = -79°$, $\psi = 168°$ for L-Trp in conformer AA). It appears that proline simply retains the AA conformation at the 3 position, while the D$^2$ analogue flips the ∠Glu$^1$ into the same position found in conformer CC for the native LH-RH.[1] The difference between this conformation and that of conformer CC is that the *cis*-peptide bond in the ∠Glu$^1$ ring is now pointed into the molecule, similarly to that found for the [D$^2$,D$^3$] analogue. The remaining amino acids of the molecule retain the overall configuration found for conformer CC (see Figure 2). The second low-energy conformer found (B2 of Table I) also points the ∠Glu$^1$ ring into the molecule, but the resulting structure looks very similar to that found for the [D-His$^2$,Leu$^3$]-LH-RH analogue (see Figure 4). No other conformers with $\Delta E < 10$ kcal/mol for variation in the backbone dihedral angles of the 2 and 3 positions were found.

**Low-Energy Conformations of [D-His$^2$,Leu$^3$]-LH-RH.** The lowest energy conformation of the [D$^2$-Leu$^3$]

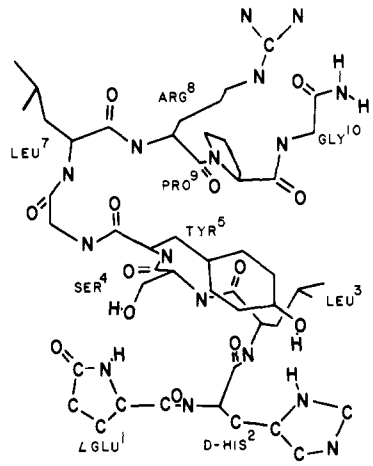


**Figure 4.** Conformer C1 of [D-His$^2$,Leu$^3$]-LH-RH. For clarity some carbon and hydrogen atoms have been omitted.

analogue (C1 of Table I), shown in Figure 4, is not like either the AA or CC conformers of paper 1. The reason is that the backbone dihedral angles in the 3 position have taken on $\alpha_R$ values ($\phi = -70°$, $\psi = -48°$) in conformer C1. The overall result of this change is the S-shaped backbone conformation shown in Figure 4. In this case, the relationship between the Arg$^8$ side chain and Gly$^{10}$-NH$_2$ terminus to the ∠Glu$^1$ ring has been completely changed. Thus, if this conformer binds into a receptor site by a mechanism dictated by the ∠Glu$^1$ and His$^2$ rings, the positively charged Arg$^8$ side chain would be flipped into a completely different location as shown in Figure 4. Clearly, in this case, conformers C2 or C3, both of which are closely similar to conformer B1 of [D-His$^2$,Pro$^3$]-LH-RH (see Figure 3), could be the active inhibiting conformations. Since a smaller population of molecules would have the C2 or C3 conformations, it could explain the greater amount of this analogue needed to inhibit LH release.[6,7] Interestingly, conformer C2 has backbone $\phi_3$ and $\psi_3$ values, which are very similar to those found for L-Trp$^3$ in the native LH-RH conformation.

Conformer C$_3$ also has the ∠Glu$^1$ ring pointing into the molecule while conformer C4, which is 5.0 kcal/mol higher in energy than conformer C1, is the lowest energy conformer in which the D-His$^2$ ring points into the molecule with the ∠Glu$^1$ ring pointing out, similarly to conformer AA of paper 1.

**Low-Energy Conformations of [D-His$^2$]-LH-RH.** The low-energy conformers of the analogue, [D-His$^2$]-LH-RH, are given in Table I as conformers D1–D4. Conformer D1, which is of lowest energy, is very similar to that shown in Figure 3 for [D-His$^2$,Pro$^3$]-LH-RH and is not shown here. As found for all the previous analogues, the ∠Glu$^1$ *cis*-peptide bond in the ring points into the molecule, while the rest of the molecule looks nearly identical with conformer CC of the native LH-RH molecule. The second lowest energy conformer, D2, is found to be closely similar to the S-shaped structure of conformer C1 of [D-His$^2$,Leu$^3$]-LH-RH (see Figure 4). Conformer D3 is similar in structure to conformer D1, while conformer D4, which is ~8.2 kcal/mol higher energy than D1, is the lowest energy conformer in which the His$^2$ ring is pointing into the molecule, similarly to conformer AA of the native molecule.

In Table I, the $\Delta E$ values in parentheses for the D1–D4 conformers are those of the [D-Phe$^2$]-LH-RH analogue. The lowest energy conformer for [D-Phe$^2$]-LH-RH is still that of D1, and the order of higher energy conformers remains the same as that found for the D-His$^2$ analogue.

Table I. Low-Energy Conformers of LH-RH Inhibitors[a]

| Residue | Dihedral angles, deg | | | | | | | $\Delta E$, kcal/mol |
|---|---|---|---|---|---|---|---|---|
| | $\phi$ | $\psi$ | $\omega$ | $\chi_1$ | $\chi_2$ | $\chi_3$ | $\chi_4$ | |
| | | | [D-His², D-Trp³]-LH-RH, Conformer A1 | | | | | |
| Pyroglutamate | | 179 | 176 | | | | | 0.0[b] |
| D-Histidine | 73 | 44 | 180 | −179 | 105 | | | |
| D-Tryptophan | 82 | −153 | 180 | −179 | 108 | | | |
| Serine | −123 | 96 | 180 | 66 | 50 | | | |
| | | | Conformer A2 | | | | | |
| Pyroglutamate | | 151 | 173 | | | | | 1.2 |
| D-Histidine | 71 | 51 | 180 | −178 | 100 | | | |
| D-Tryptophan | 157 | −150 | 180 | −179 | 111 | | | |
| Serine | −165 | 91 | 180 | 66 | 51 | | | |
| | | | Conformer A3 | | | | | |
| Pyroglutamate | | 173 | 180 | | | | | 3.6 |
| D-Histidine | 69 | −133 | 180 | −179 | 113 | | | |
| D-Tryptophan | 82 | −157 | 180 | 180 | 108 | | | |
| Serine | −123 | 101 | 180 | 66 | 48 | | | |
| | | | [D-His², Pro³]-LH-RH, Conformer B1 | | | | | |
| Pyroglutamate | | 71 | 177 | | | | | 0.0[c] |
| D-Histidine | 147 | −83 | 171 | 176 | 92 | | | |
| Proline | −75 | 166 | 180 | | | | | |
| Serine | −93 | 99 | 180 | 66 | 49 | | | |
| | | | Conformer B2 | | | | | |
| Pyroglutamate | | 98 | 180 | | | | | 3.9 |
| D-Histidine | 151 | −77 | 164 | 177 | 100 | | | |
| Proline | −75 | −47 | 180 | | | | | |
| Serine | −100 | 97 | 180 | 66 | 52 | | | |
| | | | [D-His², Leu³]-LH-RH, Conformer C1 | | | | | |
| Pyroglutamate | | 93 | 175 | | | | | 0.0[d] |
| D-Histidine | 80 | −97 | −174 | 175 | 82 | | | |
| Leucine | −70 | −48 | 180 | −178 | 68 | 174 | 180 | |
| Serine | −83 | 90 | 180 | 68 | 55 | | | |
| | | | Conformer C2 | | | | | |
| Pyroglutamate | | 70 | 180 | | | | | 3.5 |
| D-Histidine | 106 | −88 | 175 | 176 | 88 | | | |
| Leucine | −74 | 157 | 180 | −154 | 73 | 175 | −179 | |
| Serine | −86 | 93 | 180 | 66 | 51 | | | |
| | | | Conformer C3 | | | | | |
| Pyroglutamate | | 76 | 180 | | | | | 3.6 |
| D-Histidine | 156 | −84 | −177 | −174 | 131 | | | |
| Leucine | −153 | 152 | 180 | −171 | 68 | 174 | −179 | |
| Serine | −80 | 89 | 180 | 66 | 53 | | | |
| | | | Conformer C4 | | | | | |
| Pyroglutamate | | 60 | −178 | | | | | 5.0 |
| D-Histidine | 72 | 68 | 180 | 165 | 68 | | | |
| Leucine | −65 | 145 | 180 | −176 | 70 | 177 | 180 | |
| Serine | −73 | 92 | 180 | 66 | 51 | | | |
| | | | [D-His²]-LH-RH, Conformer D1 | | | | | |
| Pyroglutamate | | 65 | −178 | | | | | 0.0[e] |
| D-Histidine | 92 | −77 | 180 | 174 | −90 | | | (0.0)[f] |
| Tryptophan | −69 | 149 | 180 | 177 | −98 | | | |
| Serine | −83 | 87 | 180 | 67 | 53 | | | |
| | | | Conformer D2 | | | | | |
| Pyroglutamate | | 70 | 172 | | | | | 2.5 |
| D-Histidine | 83 | −126 | 180 | −173 | 92 | | | (1.1)[f] |
| Tryptophan | −60 | −45 | 180 | −56 | 114 | | | |
| Serine | −83 | 83 | 180 | 67 | 54 | | | |
| | | | Conformer D3 | | | | | |
| Pyroglutamate | | 70 | 178 | | | | | 3.7 |
| D-Histidine | 153 | −91 | 180 | 178 | 91 | | | (1.8)[f] |
| Tryptophan | −153 | 156 | 180 | 177 | −103 | | | |
| Serine | −79 | 85 | 180 | 67 | 53 | | | |
| | | | Conformer D4 | | | | | |
| Pyroglutamate | | 60 | −177 | | | | | 8.2 |
| D-Histidine | 68[g] | 67 | 180 | 174 | 86 | | | (8.2)[f] |
| Tryptophan | −58 | 142 | 180 | −176 | −85 | | | |
| Serine | −81 | 88 | 180 | 67 | 53 | | | |

[a] The dihedral angles of first four residues are given here. The remaining backbone dihedral angles ($\phi$ and $\psi$) are Tyr⁵, −81 ± 3, 97 ± 4; Gly⁶, 80 ± 2, −90 ± 3; Leu⁷, −141 ± 2, 60 ± 2; Arg⁸, −156 ± 4, 87 ± 2; Pro⁹, −75, −29 ± 5; Gly¹⁰, −144 ± 15, 139 ± 15, for all the conformers reported. [b] The $E_0$ value for [D-His², D-Trp³]-LH-RH is −42.2 kcal/mol. [c] The $E_0$ value for [D-His², Pro³]-LH-RH is −53.4 kcal/mol. [d] The $E_0$ value for [D-His², Leu³]-LH-RH is −42.4 kcal/mol. [e] The $E_0$ value for [D-His²]-LH-RH is −49.6 kcal/mol. [f] Values of $\Delta E$ for the [D-Phe²]-LH-RH analogue; $E_0 = -48.8$ kcal/mol. [g] The dihedral angles for the energy minimized D-Phe² analogue were generally within ±10° of those shown, except for $\phi_2$ of conformer D4, where $\phi_2$ (D-Phe²) = 148°.

The only difference between the two analogues is in the magnitude of the $\Delta E$ values, with the first three D-Phe$^2$ conformers being closer together in energy than are the equivalent D-His$^2$ analogues.

## Conclusions

Many analogues of LH-RH have now been tested for inhibitory or antagonist activity. However, only a few have shown truly potent or complete inhibition without some agonist activity. For example, [Leu$^2$,Leu$^3$]-LH-RH did not release but did inhibit release of LH and FSH,[9] but the ratio of inhibitor to LH-RH was ~300000:1. [Thr$^2$,-Leu$^3$]-LH-RH inhibited[10] at a ratio of ~30000:1 and [Leu$^2$,Leu$^3$,D-Ala$^6$,des-Gly$^{10}$]-LH-RH ethylamide[6] and [Leu$^2$,Nva$^3$,D-Ala$^6$,des-Gly$^{10}$]-LH-RH ethylamide[6] were found to inhibit, in vitro, with a ratio of ~3000:1. The analogues equivalent to those studied here have much lower ratios; for example, [D-Phe$^2$,Pro$^3$,D-Trp$^6$]-LH-RH[6,7] and [D-Phe$^2$,Leu$^3$,D-Phe$^6$]-LH-RH[6] inhibit release of LH by LH-RH at ratios of ~50:1, as does the analogue [D-Phe$^2$,D-Trp$^3$,D-Trp$^6$]-LH-RH.[8] The analogue [D-Phe$^2$,D-Ala$^6$]-LH-RH was described[4] to be active at an antagonist to agonist ratio of 20:1, while [des-Gly$^{10}$,D-Phe$^2$,D-Ala$^6$]-LH-RH ethylamide[4] was found to have a 1:5 ratio. This latter ethylamide analogue showed no antiovulatory activity in rats while the [D-Phe$^2$,D-Ala$^6$]-LH-RH was effective in blocking ovulation.

These experimental results clearly show that the histidine ring is necessary for agonist activity and that, by changing to a phenylalanine ring, agonist activity is reduced. This exchange of rings does not change the conformational preferences calculated here (see D1–D4), except in those conformers in which the His$^2$ ring points into the molecule (e.g., conformer AA of paper 1). These AA type conformers simply become higher in energy than the CC type conformers (i.e., those with ∠Glu$^1$ in and the His$^2$ ring out), thus enhancing the conclusions reached in paper 2, which implicated conformer CC as the active agonist structure.

The most obvious conclusion that one can reach concerns the orientation of the *cis*-peptide bond of the ∠Glu$^1$ ring. One can think of this result as being equivalent to changing L-∠Glu$^1$ to D-∠Glu$^1$ in the native LH-RH molecule. The effect of this change would be the same as that found here as far as the conformations of the inhibitors are concerned. Evidence that this isomer change is important has been shown experimentally. The resulting D-∠Glu$^1$ isomer has ~8% agonist activity,[17,18] indicating greatly reduced activity. It is not clear at this time whether the 8% agonist activity found[17,18] is due to a small percentage of L-∠Glu$^1$ as an impurity or whether the ∠Glu$^1$ ring can rotate about the $\psi$ bond to give a small population of conformer with the *cis*-peptide bond pointing toward the receptor. To test the energy difference between the ∠Glu$^1$ *cis*-peptide bond pointing in, and rotated to point out, an energy minimization was carried out on the [D-His$^2$,-Pro$^3$]-LH-RH conformer B1, in which the ∠Glu$^1$ $\psi$, dihedral angle was started at −60°. Total molecular energy minimization was repeated and it was found that the conformer with the ∠Glu$^1$ *cis*-peptide pointing away from the molecule was ~1.5 kcal/mol higher in energy than conformer B1. If no other forces operate on rotating the ∠Glu$^1$ ring, this $\Delta E$ value of 1.5 kcal/mol would produce a population of conformers with ~8% of them pointing the ∠Glu$^1$ *cis*-peptide bond toward the receptor. Thus, the population analysis could explain the slight agonist activity of the D-∠Glu$^1$ analogue.

Using the conformational data found here, one can now postulate new analogues which should show superior antagonist activity. For example, the analogue [D-∠Glu$^1$,Phe$^2$,D-X$^6$]-LH-RH should exhibit all the necessary requirements for inhibition of LH/FSH release. Perhaps an even better antagonist would be [cyclopentanecarboxylic acid$^1$,Phe$^2$,D-X$^6$]-LH-RH. It was recently shown[19] that [cyclopentanecarboxylic acid$^1$]-LH-RH had only ~1.4% agonist activity, while various pyrrolidone substitutions at the 1 position retained significant agonist activity,[19] as long as a carbonyl oxygen was present. The cyclopentane ring should effectively mimic the aliphatic side of the ∠Glu$^1$ ring, but the rotamer population problem described above for D-∠Glu$^1$ is no longer bothersome. We might project several other modifications which could act to stabilize the inhibitor conformations found here. For example, N-methylation of the D-Phe$^2$ residue to give [*N*-Me-D-Phe$^2$,D-Trp$^3$,D-X$^6$]-LH-RH would appear to be favorable for stabilizing the ∠Glu$^1$ ring in the correct position for inhibitory action. A second, and more interesting, possibility is to covalently link the D-∠Glu$^1$ ring (through the ring carbonyl oxygen position by an appropriate active group) to the Arg$^8$ guanidinium group for the analogue [D-∠Glu$^1$,Phe$^2$]-LH-RH. The result of this covalent linkage would be a much more rigid backbone for the whole molecule.

Although the calculated conformation of LH-RH has not as yet been verified experimentally, it seems clear that the CC conformation proposed in papers 1 and 2 is able to incorporate the inhibitor analogues described here and gives an overall similarity of structural feature for the potent antagonists found from activity tests.

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# Adrenoceptor Blocking Agents. 2.[1] 2-($\alpha$-Hydroxyarylmethyl)-3,3-dimethylaziridines, a New Class of Selective $\beta_2$-Adrenoceptor Antagonists[2]

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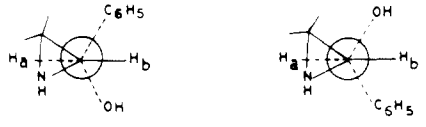
*threo*- and *erythro*-2-($\alpha$-hydroxybenzyl)-3,3-dimethylaziridines (**1a** and **1b**) and *threo*-2-[$\alpha$-hydroxy(2-naphthyl)methyl]- and 2-[$\alpha$-hydroxy(3,4-dichlorobenzyl)]-3,3-dimethylaziridines (**1d** and **1c**) have been prepared as conformationally restricted analogues of $\beta$-adrenoceptor blocking agents like dichloroisoproterenol (DCI) and pronethalol. The aziridine analogues **1** except possibly **1c** are competitive antagonists of isoproterenol-induced response on a guinea pig tracheal chain preparation and the order of potency is **1d** > **1a** ≥ **1b** > propranolol > **1c**. Unlike propranolol, these compounds have no effect on the isoproterenol-induced response on guinea pig auricles and no significant local anesthetic and antiarrhythmic activity. The aziridine analogues **1** represent the first of a new class of selective $\beta_2$-adrenoceptor blocking agents.

A number of 2-($\alpha$-hydroxyarylmethyl)-3,3-dimethylaziridines (**1**) have been synthesized and evaluated for $\beta$-adrenoceptor blocking activity in various pharmacological test models as these incorporate $\beta$-aryl-*N*-isopropylethanolamine[3]—a side chain present in $\beta$-sympathomimetics and $\beta$-adrenoceptor blocking agents—into a more rigid conformation having an additional chiral center and a reactive ethylenimine function.

**Chemistry.** The general method for the synthesis of **1** is outlined in Scheme I. Thus, aryl bromides **2** were converted to diarylcadmium **3** either via magnesium Grignard reagents or lithio compounds. Reaction of **3** with $\beta,\beta$-dimethylacryloyl chloride gave $\beta,\beta$-dimethyl acryloaranones **4**, which were converted to 2-aroyl-3,3-dimethylaziridines **7** either directly[4] by treatment with methanolic iodine and $NH_3$ or via the dibromo derivative **5**, which on condensation with methanolic $NH_3$ at room temperature gave **7**. If this reaction were carried out at 0 °C the dehydrohalogenated product **6** could be isolated, which on further treatment with methanolic $NH_3$ at room temperature gave **7**. Compounds **7** were difficult to purify because of their unstable nature and were used as such in the next step.

Reduction of 2-aroyl-3,3-dimethylaziridines **7** either with sodium borohydride in absolute methanol or with $LiAlH_4$ in dry ether gave one of the isomers of **1** in major amount. In the case of **1** (R = Ph), along with the major isomer **1a** (91.1%, mp 114 °C), a minor isomer **1b** (8.9%, mp 123 °C) was also isolated from the reaction mixture. The stereochemistry and major contributing rotamers for **1a** and



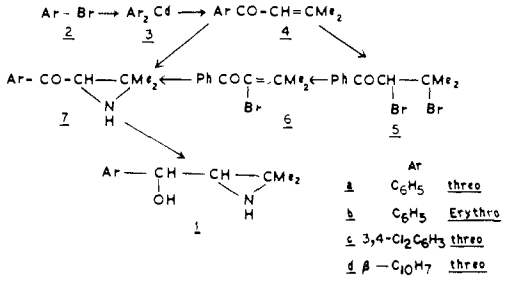
**1a** **1b**

**1b** were estimated on the basis of NMR, pyridine-induced NMR shifts, and dilution IR studies.

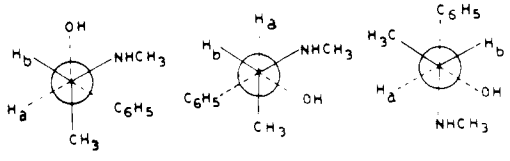
The NMR spectral data of **1a** and **1b** in solvents of diverse polarity are shown in Table I.

Thus both the isomers have similar $J_{ab}$ values of ca. 8–9 Hz, which would indicate major contribution of conformations **1a** and **1b** having trans $H_a$ and $H_b$ with a dihedral

Scheme I

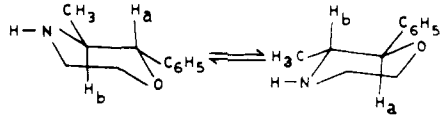


angle of ca. 160°. These conformations[5] are also favored on the basis of the minimum number of gauche interactions as compared to corresponding rotamers wth $H_a$ and $H_b$ gauche to each other. It is pertinent that in ephedrine (**8**) the rotamers **8a** and **8b** contribute significantly.[6] Thus in ephedrine the rotamers with larger gauche interactions



**8a** **8b** **9**

are stabilized by intramolecular hydrogen bonding. On the other hand, **1b** has minimum gauche interactions but no intramolecular hydrogen bonding. However, in the threo isomer **1a** the intramolecular hydrogen bonding stabilizes the favored rotamer having minimum gauche interactions. This situation is similar to $\psi$-ephedrine (**9**) and *trans*-2-phenyl-3-methylmorpholine (**10**). Indeed a comparison of $\delta_{H_a}$ and $J_{ab}$ of **1a** and **1b** with $\psi$-ephedrine (**9**) and ephedrine (**8**) and *trans*- and *cis*-2-phenyl-3-



**10**

methylmorpholines (**10** and **11**) shows a remarkable similarity between **1**, **9**, and **10**, which have similar conformations (Table II).

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