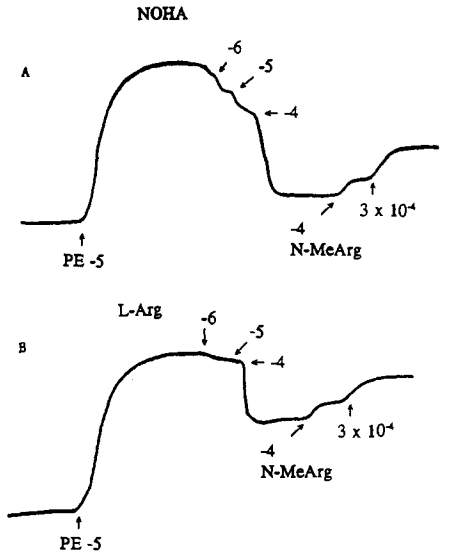

Figure 3. NOHA-elicited vasorelaxation in arginine-depleted bovine pulmonary artery. Isolated rings of endothelium intact bovine pulmonary artery were precontracted with phenylephrine (PE) and challenged with acetylcholine (ACh) added cumulatively (not shown). The tissues were then allowed to stand in oxygenated Krebs solution for 24 h to deplete endogenous arginine levels. To these depleted tissues were then added PE, NOHA (A), and L-arginine (B) as shown. Inhibitor was added after NOHA and arginine addition was completed as indicated. Concentrations are expressed as the base power of 10 and represent final bath concentrations.

NOHA is a relatively stable molecule, now accessible by a simple synthetic route, that can cause vasorelaxation in bovine pulmonary arteries. To our knowledge, it is the only simple N-substituted arginine analogue found to have biological activity similar to that of arginine itself (L-homoarginine can also elicit vasorelaxation[8,9]). All other arginine analogues tested (i.e., N^{ω}-methyl-, N^{ω}-amino-, N^{ω}-nitroarginine)[10,11] in a variety of cells and tissues are inhibitors of NO biosynthesis.

Registry No. 1, 3304-51-6; 2, 53054-01-6; 3, 53054-02-7; 4, 53054-03-8; 5, 133374-42-2; 6, 133374-43-3; NOHA, 53054-07-2; NO, 10102-43-9; H-Arg-OH, 74-79-3.

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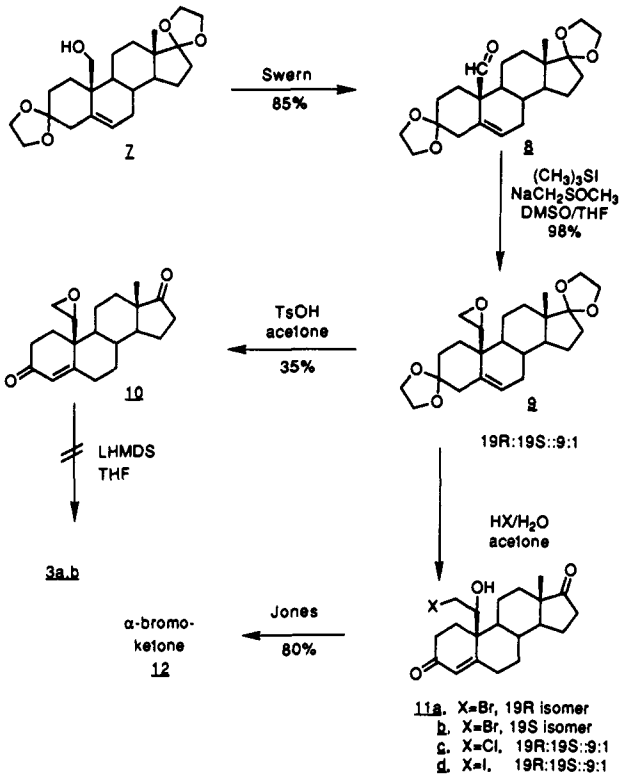
Novel Time-Dependent Inhibitors of Human Placental Aromatase

Aromatase is a cytochrome P_{450} enzyme system that converts androgens to estrogens by oxidative dealkylation of the angular C-19 methyl group and aromatization of the steroidal A ring. This transformation requires 3 equiv of oxygen and NADPH.[1] The initial hydroxylations produce

Scheme I



the 19-hydroxy and 19-oxo androgen derivatives,[2] whereas the site of the third hydroxylation has not yet been determined.[3] A 2β-hydroxylation has been proposed,[4] i.e., to produce 1 from 19-oxoandrostenedione, which would allow the intermediacy of lactol 2, although reports on the aromatization of 1[5,6] and recent labeling experiments and studies on peroxide models for aromatase[7-9] suggest an alternate process. The C-10, C-2 hydroxyethyl bridged species **3a** and **3b** could be viewed as stable, carbon analogues of lactol **2**.[10] This communication describes the

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(10) The synthesis of **3c**, which could be considered as a less rigorous transition state analogue of lactol **2**, has been reported: Lan-Hargest, H.-Y.; Elliot, J. D.; Eggleston, D. S.; Holt, D. A.; Levy, M. A.; Metcalf, B. W. *Tetrahedron Lett.* **1987**, *28*, 6117-6120. Compound **3c** was recently shown to be a competitive inhibitor and not a substrate for human placental aromatase: Greway, A. T.; Menzer, M.; Levy, M. A. *Biochem. Int.* **1990**, *20*, 591-597.

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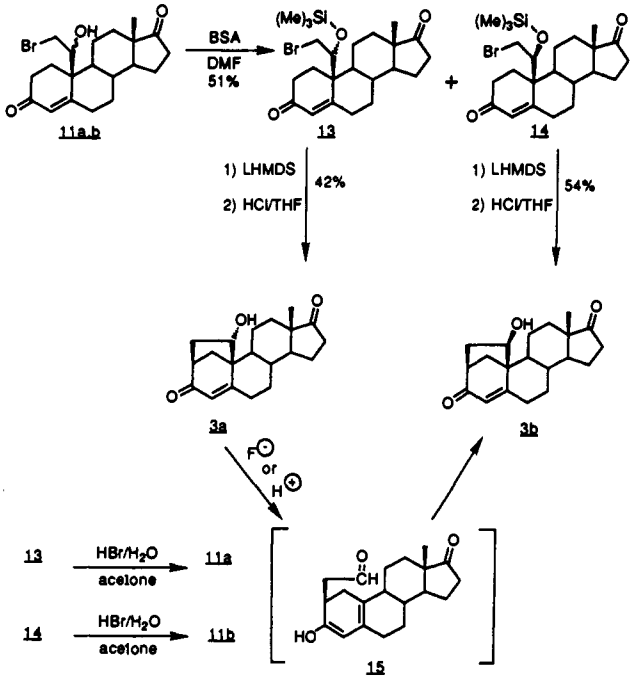
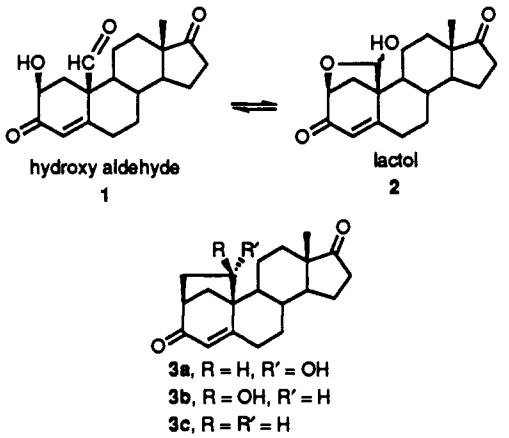
Scheme II



synthesis of **3a** and **3b**, their affinities for human placental aromatase, and the synthesis of related compounds (halohydrins **11a,c,d**) that are potent, time-dependent inhibitors of aromatase.



Epoxides **10**, potential precursors to **3a** and **3b** via intramolecular alkylation,[10-12] were prepared as shown in Scheme I. Swern oxidation of alcohol **7** gave the corresponding aldehyde **8** (85%), which was treated with the ylide generated from trimethylsulfonium iodide and dimsyl anion to afford a 9:1 ratio of 19*R*:19*S* epoxides **9** (98%). Removal of the ketals gave epoxy diones **10** (35%), which failed to undergo intramolecular alkylation to a hydroxyethylene bridged system with lithium hexamethyldisilazide in THF. Inspection of a model of the kinetic enolate of **10** revealed that appropriate orbital overlap for the intramolecular alkylation was probably insufficient. In addition, this cyclization is classified as a 5-endo-trig closure and is not a favored process.[13]

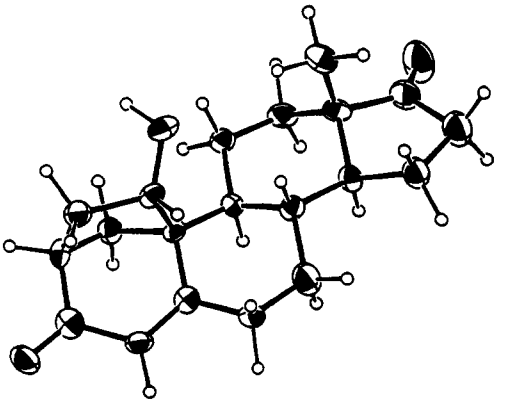


Figure 1. ORTEP drawing for (1α,3α,10α)-1-hydroxy-3,19-cyclo-*A*-homoandrost-5-ene-4,17-dione (**3a**).

Treatment of epoxides **9** with 48% hydrobromic acid in acetone gave bromohydrins **11a** and **11b** (81%) in a ratio of 7:1, respectively. Likewise, treatment of **9** with hydrochloric and hydroiodic acids gave chlorohydrins **11c** (65%) and iodohydrins **11d** (60%), respectively.[14] Swern oxidation of a mixture of bromohydrins **11a** and **11b** afforded the corresponding bromo ketone **12** (80%).

Treatment of a mixture of bromohydrins **11a** and **11b** with bis(trimethylsilyl)acetamide (BSA) in DMF gave a mixture of *O*-trimethylsilyl bromohydrins **13** and **14** (51%), which were chromatographically separable (Scheme II). Epoxides **10**, when treated with trimethylsilyl bromide[15] in chloroform, likewise afforded a mixture of **13** (21%) and **14** as well as bromohydrin **11a** (21%), which may have been an artifact of workup. Pure samples of each bromohydrin isomer could be obtained by treatment of the corresponding *O*-trimethylsilyl bromohydrin with aqueous hydrobromic acid in acetone.

O-Trimethylsilyl bromohydrins **13** and **14** underwent clean intramolecular alkylation at the 2-position when treated with lithium hexamethyldisilazide, to give hydroxyethyl bridged enediones **3a** (42%) and **3b** (54%), respectively, after brief treatment with HCl. Suitable crystals of *S* diastereomer **3a** were grown for X-ray crystallographic studies; the ORTEP drawing for **3a** is shown in Figure 1.

Interestingly, *S* diastereomer **3a** could be converted to **3b** upon prolonged exposure to dilute hydrochloric acid in tetrahydrofuran. In addition, treatment of the *O*-trimethylsilyl derivative of **3a** with tetrabutylammonium fluoride in tetrahydrofuran gave **3b**, presumably via **3a**. A suggested intermediate in this transformation is hydroxy diene aldehyde **15**, which arises from **3a** via a retro vinylogous aldol condensation. Closure of **15** by attack of the dienol at the opposite face of the aldehyde then produces the thermodynamically more stable *R* diastereomer **3b**. Propagation of this rearrangement with either fluoride ion, presumably acting as a base, or dilute acid suggests a push-pull mechanism wherein the retro vinylogous aldol condensation is initiated either by abstraction of the hydroxyl proton or by protonation of the A-ring carbonyl group.

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(14) Epoxide opening in acidic solution usually results in nucleophilic attack at the carbon atom which can best accommodate the positive charge. However, in the case of epoxides **9**, the preferred carbon is substituted with a neopentyl center and steric hindrance does not allow nucleophilic attack. See: Smith, J. G. *Synthesis* **1984**, 629–656.
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Table I. Inhibitory Constants for Human Placental Aromatase[a]

stereochem at C-19	no.	K_i, μM	time dep	τ_{50}, min	k_{cat}/K_i
S	**3a**	0.926[b]			
R	**3b**	0.870[b]			
	3c	0.070[b,c]	NTD		
R	**11a**	0.027[d]	yes	4.85	88220
S	**11b**	1.13[d]	yes	5.33	1900
R[e]	**11c**	0.063[d]	yes	3.60	50700
R[e]	**11d**	0.011[d]	yes	2.22	490000
	12	0.190[d]	yes	32.7	1860

[a] Assay previously described in ref 18 and 19. [b] Apparent K_i estimated from $K_i = IC_{50}/(1 + [S]/K_m)$ when substrate ([1-^{3}H]-androstenedione) concentration was 16.8 nM. The K_m value determined for androstenedione was 39 nM. [c] Prepared as described in ref 10. [d] Determined from Kitz–Wilson plot as shown in Figure 2. [e] Sample contained 10% of the *S* isomer.

Inhibitory constants for hydroxyethyl bridged compounds **3a** and **3b** and halohydrins **11a–d** for human placental aromatase are displayed in Table I. The apparent K_i values for **3a** and **3b** are similar, 0.93 and 0.87 μM, respectively, and higher by 1 order of magnitude than the ethylene-bridged compound **3c** (K_i value of 0.070 μM). Affinity also decreases with hydroxylation for an analogous series of nonbridged compounds, e.g., (*R*)-19-methyl-19-hydroxy-, (*S*)-19-methyl-19-hydroxy-, and 19-methylandrostenediones, whose K_i values are 11, 9.9, and 0.081 μM, respectively.[16] However, K_i values for our rigid alcohols **3a** and **3b** are lower by 1 order of magnitude than for the analogous 19-methyl-19-hydroxyandrostenediones.

Figure 2 shows inhibitory curves for aromatase from human placenta by bromohydrin **11a**. Incubation of aromatase preparations,[17-19] to which an NADPH-generating system had been added, for varying time periods with different concentrations of **11a** gave a time-dependent loss of enzyme activity. The reciprocal of the inhibitor concentration was plotted against the enzyme half-life at these different inhibitor concentrations as described by Kitz and Wilson[20] (Figure 2 inset); the K_i value for **11a** was found to be 27 nM and the calculated enzyme half-life at infinite inhibitor concentration (τ_{50}) was 4.9 min.

At a saturating inhibitor concentration (500 nM) a time-dependent loss of enzyme activity was still observed in the absence of the NADPH-generating system. This finding suggests that oxidation of the inhibitor is not necessary for inhibitor activation. Interestingly, bromoacetyl compound **12**, which is a potential product of enzymatic oxidation of **11a**, which would require the NADPH-generating system for its production, is a time-dependent inhibitor of aromatase but with decidedly less affinity than **11a** (Table I). Another possible product of enzymatic transformation of **11a** is 19*R* epoxide **10**, which was previously shown by Robinson et al.[21,22] to be a potent, competitive inhibitor of human placental aromatase with a K_i value of 7 nM. These authors also found that the 19*S* isomer of **10** had a K_i value of 75 nM;[21,22] our *S* bromohydrin **11b**, which is the potential precursor to the 19*S* isomer of **10** also is less potent than **11a**, having a K_i value of 1.13 μM compared to the K_i value of 27 nM for **11a** (Table I).

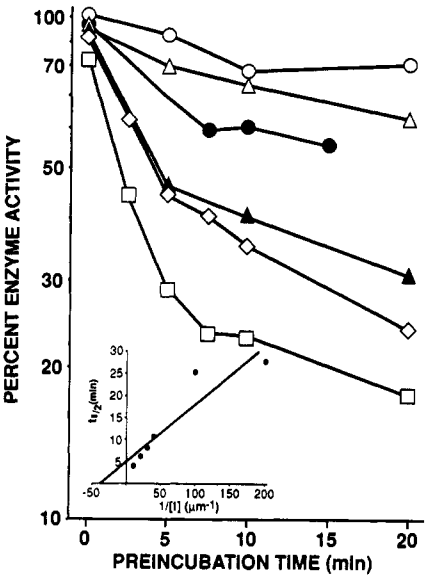

Figure 2. Aromatase inhibition by bromohydrin **11a**. Time-dependent inhibition was determined by incubating 100 μL of compound **11a** (5 nM, ○; 10 nM, △; 25 nM, ●; 30 nM, ▲; 50 nM, ◆; 100 nM, □) at intervals from 0 to 20 min with 700 μL of human placenta microsomal preparation (enzyme activity = 15 ± 2 or 34 ± 7 pmol of estrogen formed per min per mg of protein) and 600 μL of NADPH-generating system at 25 °C. The relative enzyme activity remaining following the preceding incubation intervals were determined by measurement of the ^3H_2O from the stereospecific elimination of 1β-^{3}H (56.7%) from 100 μL of [1-^{3}H]androstenedione (750 pmol, 0.71 μCi) during a 10-min assay. The inherent losses of aromatase activity in buffer controls were 4% from 0 to 15 min and 12% from 15 to 20 min of preincubation. The inhibitor was solubilized in DMSO to provide a 15 mM solution, which was subsequently diluted with assay buffer. Assay methods have been previously described in detail (ref 18, 19). The data represent mean values from 19 assays with three to five assays at each concentration from 10 to 100 nM. The insert represents a linear regression analysis of a Kitz–Wilson plot (ref 20) of the pseudo-first-order inactivation rates ($t_{1/2}$) for preincubation intervals of 0–10 min (5–30 nM) and 0–7.5 min (25–100 nM) vs the reciprocal of inhibitor concentrations. For **11a**, the K_i of inactivation was 27 nM with a τ_{50} value of 4.85 min (r = 0.93).

In summary, we have shown that steroidal 10,2-bridged alcohols **3a** and **3b**, which are stable, carbon analogues of lactol **2**, are competitive inhibitors of human placental aromatase with low micromolar affinity. The halohydrins **11a**, **11c**, and **11d** are potent, time-dependent inhibitors of aromatase with low nanomolar affinity.

Acknowledgment. We thank Dr. John C. Huffman of Indiana University for crystallographic studies on **3a**. Complete crystallographic details are available in microfiche form from the Chemistry Library, Indiana University, Bloomington, Indiana, 47405. Request MSC Report No. 89702.

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