°C; R_f (C) 0.38. Anal. ($C_{29}H_{30}N_6O_5S$) C, H, N.

6-(*N*-Benzoyl-DL-arginylamido)quinoline (13). To 33 mg (0.06 mmol) of 12 was added 850 μL of methanesulfonic acid containing 15 μL of anisole. After standing at room temperature for 40 min, the product was precipitated by addition of 50 mL of ether, washed several times by decantation, and dried under reduced pressure. Purification was effected by ion-exchange chromatography conducted on Amberlite CG-50 resin (carboxylate form), using a stepwise gradient of ammonium carbonate from 0.1 to 1.0 M. The blue fluorescent fractions were combined and lyophilized to produce 20 mg, 77% yield: mp 230 °C dec; R_f (F) 0.41; positive Sakaguchi test. Anal. ($C_{22}H_{24}N_6O_2 \cdot H_2CO_3$) C, H, N.

1-Methyl-6-(*N*-benzoyl-DL-arginylamido)quinolinium Diiodide (9). A solution consisting of 25 mg (0.05 mmol) of 13, 2 mL of methyl iodide, and 0.5 mL of dimethylformamide was allowed to stand at room temperature in the dark for 48 h. The solvents were removed under reduced pressure and ether was added to solidify the oily residue. Recrystallization from ethanol–ether produced 22 mg, 62% yield: mp 220 °C dec; R_f (E) 0.06; positive Sakaguchi test. Anal. ($C_{23}H_{28}N_6O_2I_2$) C, H, N.

Enzyme Assays. Kinetic constants were determined from initial rates of hydrolysis by the Lineweaver–Burk method. Correlation coefficients for all experiments were 0.998 or higher. Assays using pancreatic elastase were conducted in 67 mM Tris-HCl buffer (pH 8.8) at 37 °C, using a thermostatically controlled cuvette. The specific activity of elastase was determined using orcein-elastin[30] prior to each experiment. Substrates for chymotrypsin were evaluated in 50 mM Tris-HCl buffer (pH 8.0) containing 10 mM $CaCl_2$ at 25 °C. The specific activity of the enzyme was determined with use of the chromogenic inactivator 2-nitro-4-carboxyphenyl diphenylcarbamate.[31] Assays utilizing trypsin were performed in 46 mM Tris-HCl buffer (pH 8.1) containing 11 mM $CaCl_2$ at 25 °C. For the titration of trypsin, *p*-nitrophenyl *p*-guanidinobenzoate was used following the method of Chase and Shaw.[32] The concentrations of enzymes and the ranges at which substrates were employed are listed in Table I. Excitation and emission wavelengths used to monitor the appearance of MAQ^+ were 410 and 550 nm, respectively, and for 6-AQ they were 355 and 550 nm, respectively. All of the substrates prepared in this study were stable to buffer in the absence of enzymes: after they were exposed for 24 h at 25 °C to the assay media alone, no increase in fluorescence due to the appearance of the leaving group was detected.

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Registry No. 1, 580-15-4; 2, 84614-59-5; 3·2HBr, 90605-99-5; 4, 84614-60-8; 5, 90606-00-1; 6, 90606-01-2; 7, 84614-61-9; 8, 90606-02-3; 9, 90606-03-4; 10, 154-92-7; 11, 90606-04-5; 11 dicyclohexylammonium, 90606-06-7; 12, 90693-47-3; 13, 84680-45-5; 15, 90606-05-6; Cbz-Ala, 1142-20-7; Cbz-Ala-Ala, 16012-70-7; Cbz-Phe-6AQ, 80115-53-3; *p*-methoxybenzenesulfonyl chloride, 98-68-0; elastase, 9004-06-2; chymotrypsin, 9004-07-3; trypsin, 9002-07-7.

Supplementary Material Available: The fast atom mass spectra for compounds 5–8 (4 pages). Ordering information is given on any current masthead page.

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Luteinizing Hormone-Releasing Hormone Antagonists Containing Very Hydrophobic Amino Acids[1]

John J. Nestor, Jr.,*† Ram Tahilramani,† Teresa L. Ho,† Georgia I. McRae,‡ and Brian H. Vickery‡

Institute of Bio-Organic Chemistry and Institute of Biological Sciences, Syntex Research, Palo Alto, California 94304.
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In a continuation of our studies on the effects of hydrophobic substitutions in analogues of luteinizing hormone-releasing hormone (LH-RH), we have synthesized LH-RH antagonists containing the very hydrophobic amino acid 3-(2-naphthyl)-D-alanine (D-Nal(2)). The D-Nal(2) substitution was found to be effective when incorporated in positions 3 and 6. The most potent analogue containing two D-Nal(2) residues was [N-Ac-Pro^1,D-pF-Phe^2,D-Nal(2)^{3,6}]LH-RH (ED_{50} = 2.2 μg, rat antiovulatory assay, propylene glycol–saline vehicle). This analogue also demonstrates that the N-Ac-Pro^1 substitution is as effective as the more costly N-Ac-Δ^3-Pro^1 modification. Analogues containing D-Nal(2) in combination with the hydrophilic D-Arg residue in position 6 were prepared. Neither N-Ac-Pro at position 1 nor D-Nal(2) at position 3 was effective in combination with D-Arg. N-Ac-D-Nal(2) at position 1 gave a highly potent antagonist ([N-Ac-D-Nal(2)^1,D-pF-Phe^2,D-Trp^3,D-Arg^6]LH-RH; ED_{50} = 2.4 μg) that exhibited a prolonged duration of action (ED_{50} = 9.0 μg, corn oil vehicle, dosing on diestrus II).

The possibility that synthetic analogues of polypeptide hormones might retain their receptor binding ability without appreciable agonistic activity was recognized early in the study of peptide hormone analogues.[2,3] The first competitive inhibitors[4] of luteinizing hormone-releasing hormone (<Glu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH_2,LH-RH) were discovered soon after the disclosure of the structure of the native hormone.[5] Although the initial inhibitors ([Gly^2]LHRH, des-His^2-LH-RH) required high doses in order to be effective[6] (in vitro), they identified position 2 as the crucial site for modification to obtain antagonists. Later substitutions in the des-His^2-LH-RH

† Institute of Bio-Organic Chemistry.
‡ Institute of Biological Sciences.

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structure by D-Ala at position 6 or by Pro-NHEt in position 9, modifications that increased potency in the agonist series,[7,8] also gave more effective antagonists.[7,9] However, the Pro^9-NHEt modification led to decreased antagonist potency in other analogues,[10] reportedly due to increased residual agonistic potency associated with this substitution.[11] Incorporation of a D-Phe or a *p*-halo-D-Phe residue in position 2 led to substantial increases in potency.[12,13] Workers in several groups then effected stepwise potency increases by further substitutions in positions 1, 2, 3, and 6.[14-17] At the time the presently reported studies began, the most potent LH-RH antagonist reported was [N-Ac-D-Phe^1,D-pCl-Phe^2,D-Trp^{3,6}]LH-RH with a reported 60% inhibition of ovulation at a 62-μg dose in the rat antiovulatory assay.[18]

During our studies of LH-RH agonists,[19-22] we investigated the effect of substitution by very hydrophobic, unnatural D-amino acids in position 6. Although previous studies of analogues containing the D isomers of native amino acids showed increasing receptor binding affinities[23-25] with increasing hydrophobicity (D-Ala to D-Trp), we expected that substitution with even more hydrophobic amino acids would yield analogues exhibiting prolonged pharmacokinetics due to their ability to partition into hydrophobic compartments (hydrophobic carrier proteins, lipid bilayers, lipoprotein assemblies, etc.) and be later released to the circulation.[22] Detailed metabolic studies have shown that the extremely high potency (200 × LH-RH) of the hydrophobic [D-Nal(2)^6]LH-RH[19] (nafarelin acetate) is, in part, related to its prolonged biological half-life ($t_{1/2}$ > 2 h in women).[26]

We have adopted a similar approach in our studies in the LH-RH antagonist series. Since a competitive antagonist probably must be present continuously to block the receptor site from intermittent pulses of endogenous LH-RH, prolonged pharmacokinetics may be especially important for such a compound. We therefore replaced the D-Trp residues in the standard, [N-Ac-D-Phe^1,D-pCl-Phe^2,D-Trp^{3,6}]LH-RH, in a stepwise fashion with D-Nal(2) residues. The N-Ac-D-Phe residue was replaced by N-Ac-Pro because of the report that this less costly substitution was as effective as substitution by N-Ac-D-amino acids.[27] The initial series that served as a probe for this type of modification was as follows: [D-Phe^2,D-Nal(2)^6]-LH-RH; [D-Phe^2,D-Trp^3,D-Nal(2)^6]LH-RH; [D-Phe^2,D-Nal(2)^{3,6}]LH-RH; [N-Ac-Pro^1,D-Phe^2,D-Trp^3,D-Nal(2)^6]-LH-RH; [N-Ac-Pro^1,D-pCl-Phe^2,D-Nal(2)^{3,6}]LH-RH; [N-Ac-Pro^1,D-pF-Phe^2,D-Nal(2)^{3,6}]LH-RH.[22,28] Further substitutions, which were designed to examine a broad range of global hydrophobicities in the analogue series, were incorporated.

Recent work has demonstrated that a D-Arg substitution in position 6 can also be effective. Thus [N-Ac-D-pCl-Phe^1,D-pCl-Phe^2,D-Trp^3,D-Arg^6,D-Ala^{10}]LH-RH has been reported to have an ED_{50} of 3 μg (rat antiovulatory assay, noon of proestrus, saline vehicle).[29] Significantly, this more hydrophilic analogue was found to be more potent when administered in oil, suggesting that higher intrinsic hydrophobicity in this class of analogue structure may give better pharmacokinetics and greater potency. We have also investigated the effect of coupling this hydrophilic modification with our very hydrophobic amino acid substitutions.

Chemistry. All of the peptides were synthesized by the solid-phase method.[30] Decapeptide analogues were prepared on (benzhydrylamino)polystyrene–1% divinylbenzene resin,[31] and nonapeptide ethylamide analogues

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Table I. Hydrophobic LH-RH Antagonists

no.	LH-RH analogue[a]	$[\alpha]^{25}_D$ (c, HOAc), deg	HPLC:[b] k'	TLC[c] BAW	TLC[c] BEAW	rat antiovulatory assay: ED_{50},[d] μg/inj PG/saline[e]	rat antiovulatory assay: ED_{50},[d] μg/inj corn oil
1	[N-Ac-Pro^1,D-pF-Phe^2,D-Nal(2)^3,D-pNH$_2$-Phe^6]LH-RH	−49.0 (0.4)	0.4	0.42	0.64	72	
2	[N-Ac-D-Ser^1,D-pF-Phe^2,D-Nal(2)^{3,6}]LH-RH	−35.2 (0.5)	0.5	0.52	0.72	4.3	1.4
3	[N-Ac-D-Thr^1,D-pF-Phe^2,D-Nal(2)^{3,6}]LH-RH	−33.2 (0.5)	0.6	0.52	0.72	7.0	4.5
4	[N-Ac-D-Phe^1,D-pCl-Phe^2,D-Trp^{3,6}]LH-RH[f]		0.7			160	74
5	[N-Ac-Pro^1,D-pF-Phe^2,D-Phe^3,D-Nal(2)^6]LH-RH	−53.7 (0.5)	0.7	0.48	0.69	12.2	
6	[N-Ac-Pro^1,D-pCl-Phe^2,D-Phe^3,D-Nal(2)^6]LH-RH	−54.6 (0.7)	0.9	0.48	0.69	5.6	
7	[N-Ac-Pro^1,D-pF-Phe^2,D-Nal(2)^{3,6}]LH-RH	−50.0 (0.8)	1.03	0.50	0.70	2.2	3.3
8	[N-Ac-Δ^3-Pro^1,D-pF-Phe^2,D-Nal(2)^{3,6}]LH-RH[g]		1.05				2.8
9	[N-formyl-Pro^1,D-pF-Phe^2,D-Nal(2)^{3,6}]LH-RH	−49.5 (0.3)	1.06	0.50	0.70	3.0	4.4
10	[N-Ac-Pro^1,D-pCl-Phe^2,D-Nal(2)^{3,6}]LH-RH	−62.7 (0.8)	1.6	0.52	0.71	4.8	3.6
11	[N-Ac-Pro^1,D-pF-Phe^2,D-Nal(2)^{3,6},Pro^9-NHEt]LH-RH	−60.0 (0.6)	2.2	0.59	0.72	3.4	
12	[N-Ac-D-pCl-Phe^1,D-pCl-Phe^2,D-Nal(2)^{3,6}]LH-RH	−35.2 (0.3)	2.8	0.59	0.79	400	
13	[Boc-Ser(OBzl)^1,D-pF-Phe^2,D-Nal(2)^{3,6}]LH-RH	−38.4 (0.3)	5.8	0.59	0.79	400	

[a] Acceptable amino acid analyses were obtained for all LH-RH analogues (see Experimental Section). [b] k' = (retention volume − void volume)/void volume; conditions in the Experimental Section. [c] BAW = 1-BuOH/HOAc/H_2O, 4:1:5 (upper phase); BEAW = 1-BuOH/EtOAc/HOAc/H_2O, 1:1:1:1. [d] Based on 10 animals per dose group. [e] Compound administered in 50% propylene glycol/0.9% saline vehicle. [f] Reference 18. [g] Compound supplied by Dr. J. Rivier (ref 41).

were synthesized on (chloromethyl)polystyrene–1% divinylbenzene resin[32] followed by cleavage from the resin with Et_2NH_2. N^{α}-*tert*-Butoxycarbonyl (Boc) protection[33] was used on all amino acids. Amino acid side-chain protection was as follows: Arg (Tos),[34] Tyr (2,6-Cl_2Bzl),[35] Ser (Bzl). Anhydrous liquid HF was used for the final deprotection.[36] The crude peptides were purified by preparative high-performance liquid chromatography (prep-HPLC). Boc-D-Nal(2)[19] and Boc-D-pNH$_2$-Phe[37] were prepared as described. N-Ac-D-pCl-Phe-OMe and N-Ac-D-pF-Phe-OMe were resolved with subtilisin Carlsberg.[19,38]

Biological Results and Discussion

These data demonstrate that the substitution of positions 3 and 6 by D-Nal(2) is very effective and confers extremely high potency on these analogues. Of the initial series studied, the most hydrophobic compounds, **7** and **10**, were by far the most potent, and more detailed biological studies have been performed with both.[39,40] Thus, the N-Ac-Pro modification is compatible with very high antagonistic potency, as was reported earlier in a different analogue series ([N-Ac-Pro^1,D-Phe^2,D-Trp^{3,6}]LH-RH, ED_{100} = 200 μg).[27] This result is interesting also in view of a series of analogues that have been prepared with use of N-Ac-Δ^3-Pro in position 1 because of its superiority to N-Ac-D-Pro in this position.[17] These studies also yielded extremely potent antagonists. However, the most potent member of that series, [N-Ac-Δ^4-Pro^1,D-pF-Phe^2,D-Nal(2)^{3,6}]LH-RH (**8**),[41] is not significantly different from [N-Ac-Pro^1,D-pF-Phe^2,D-Nal(2)^{3,6}]LH-RH (**7**) in our studies, and the N-Ac-Δ^3-Pro modification offers no advantage over the less expensive N-Ac-Pro substitution.

The use of a corn oil vehicle has potentiated the activity of some earlier, more hydrophilic analogues,[18] possibly by prolonging the release of the compounds into the circulation. Both **7** and **10** have been tested with use of a 40% propylene glycol/saline vehicle. The use of a propylene glycol/saline vehicle enhanced the potency of **7**. This compound therefore exhibits prolonged biological activity without an added depot agent. Since human clinical applications are not compatible with continued administration of an oil-based vehicle, this property may offer distinct advantages.

After settling on the [N-Ac-Pro^1,D-p-halo-Phe^2,D-Nal(2)^{3,6}]LH-RH substitution pattern as the model for very potent LH-RH antagonists, we proceeded to make modifications to this structure that were designed to alter the hydrophobicity and pharmacokinetics of the analogues. The compounds are arranged in Table I in order of their hydrophobicities as determined by their relative retention on a reversed-phase HPLC column.

One of these modifications, which imparts a significantly greater hydrophobicity as well as presumed protection from post-proline cleaving enzyme, is the Pro-NHEt substitution in position 9. Although the incorporation of Pro-NHEt caused large decreases in potency in the earlier studies in the antagonist series, we have shown that it does not decrease potency in the tetrasubstituted analogue series. Thus, the potencies of **7** and **11** are not substantially different in this assay. The previously reported decreases in potency for this substitution were said to be due to a retention of intrinsic agonistic activity caused by the Pro-NHEt structure.[11] This mechanism may no longer operate in analogues that incorporate a D-amino acid in position 3 since this modification was said to remove residual agonistic activity.[16] As was shown previously in the Δ^3-Pro series, the N-formyl group is also compatible with high potency (**9**).

It appears that a region of optimum hydrophobicity may exist for this analogue series. In the series reported here, compounds with a k' greater than 2.2 exhibit a rapid falloff in potency. Quantitative structure/activity relationships have been derived for mono-[42] or disubstituted[43] LH-RH agonists that suggest a strong correlation between potency

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Table II. Hydrophobic Antagonists Containing D-Arg[6]

no.	LH-RH antagonist[a]	$[\alpha]^{25}_D$, (c, HOAc), deg	HPLC:[b] k'	TLC[c] BAW	TLC[c] BEAW	antiovulatory assay: ED_{50},[d,e] μg/inj
14	[N-Ac-Pro[1],D-pF-Phe[2],D-Nal(2)[3],D-Arg[6]]LH-RH	−41.1 (0.2)	0.25	0.35	0.61	21
15	[N-Ac-D-Nal(2)[1],D-pF-Phe[2],D-Trp[3],D-Arg[6]]LH-RH[f]	−31.1 (0.8)	0.39	0.35	0.66	2.4
16	[N-Ac-D-pCl-Phe[1],D-pCl-Phe[2],D-Trp[3],D-Arg[6],D-Ala[10]]LH-RH[g]		0.43			1.8
17	[N-Ac-Pro[1],D-pF-Phe[2],D-Nal(2)[3],D-Arg[6],Pro[9]-NHEt]LH-RH	−45.5 (0.9)	0.45	0.35	0.66	32
18	[N-Ac-D-Nal(2)[1],D-pF-Phe[2],D-Nal(2)[3],D-Arg[6]]LH-RH	−27.2 (0.7)	0.57	0.36	0.67	16
19	[N-Ac-Pro[1],D-pCl-Phe[2],D-Nal(2)[3],D-Arg[6],Pro[9]-NHEt]LH-RH	−46.6 (0.4)	0.6	0.35	0.66	14

[a-e] See footnotes to Table I. [f] See also Rivier, J.; et al., 1984.[46] [g] Reference 29.

Table III. Duration of Antiovulatory Activity

no.	ED_{50}, μg, time of dosing[a] (vehicle)[b] P (p.g.)	DII (p.g.)	P (c.o.)	DII (c.o.)
7	2.2	35	4.0	140
15	2.4		1.1	9.0
16[c]	1.7	5	1.2	5

[a] Dosing was either at noon on proestrus (P) or noon on diestrus II (DII), i.e., 24 h. prior to P. [b] The vehicle was either 50% propylene glycol/0.9% saline (p.g.) or Mazola corn oil (c.o.). [c] Reference 29.

and the calculated hydrophobicity of the substituent in position 6. Although such a clear relationship is not obvious for the global hydrophobicity parameter (k') measured for these multisubstituted LH-RH antagonists,[41] these data may be helpful for planning analogue syntheses within closely related LH-RH antagonist structural groups.

Although the replacement of a D-Trp residue in [N-Ac-D-pCl-Phe[1],D-pCl-Phe[2],D-Trp[3],D-Phe[6],D-Ala[10]]LH-RH with a D-Arg residue surprisingly led to a highly potent analogue (**16**),[29] the same replacement in **7** resulted in a significantly less potent compound, **14** (Table II). Increasing the global hydrophobicity of the molecule by incorporating the Pro-NHEt modification gave decreased potency (**17**). Increasing the hydrophobicity further by using D-pCl-Phe in position 2 (**19**) increased potency, but all of the N-Ac-Pro analogues containing D-Arg were substantially less potent than **7**.

The use of an aromatic amino acid in position 1 in combination with the D-Arg residue, as was used in the standard (**16**),[29] resulted in **15** and **18**. The more hydrophobic analogue, **18**, still exhibits decreased potency. The D-Nal(2) residue in position 3 appears not to be effective in conjunction with D-Nal(2) in position 1. Compound **15** was the most potent analogue in this series. When the relatively hydrophilic analogue **15** is administered in a corn oil vehicle, it exhibits even higher potency (ED_{50} = 1.1 μg, noon of proestrus) as also observed with the standard, **16** (ED_{50} = 1.2 μg, noon of proestrus).

When the most potent compounds are administered 24 h before the usual noon of proestrus injection time, i.e., on diestrus II, the duration of antiovulatory effect can be assessed (Table III). It is apparent that the Arg-containing compounds require a lower multiple of the proestrus dosage to maintain effectiveness than the compound containing D-Nal(2) in position 6 (e.g., dosage multiple for **7** is 16, for **16** is 2.9). Similar results have been reported recently by other workers.[44-46]

In agreement with our results in the LH-RH agonist series,[19] the use of the very hydrophobic D-Nal(2) residue in positions 3 and 6 of LH-RH antagonists provides substantial potency increases compared with earlier analogues containing D-Trp in these positions. We have also shown that the N-Ac-Pro substitution in position 1 is as effective as the less accessible N-Ac-Δ^3-Pro modification. In the antagonist series containing D-Arg in position 6, the N-Ac-Pro substitution is not effective, but the N-Ac-D-Nal(2) residue again is able to generate highly potent analogues. The D-Arg-containing analogues exhibit a prolonged duration of action. Whether this is due to higher receptor binding affinities or due to altered pharmacokinetics caused by interaction of this positively charged residue with phospholipid membranes is not yet known. We have undertaken analogue studies with a class of amino acids designed to enhance this latter type of interaction.[22,47]

Experimental Section

General Methods. Melting points were obtained on a Thomas-Hoover apparatus, and optical rotations were measured on a Perkin-Elmer Model 141 polarimeter in a 1-dm microcell at 25 °C at the concentration indicated (w/v %). Thin-layer chromatography (TLC) was performed in a solvent vapor saturated chamber on 5 × 20 cm glass plates coated with a 250-μm layer of silica gel GF (Analtech). The plates were visualized by UV absorption followed by chlorination (Cl_2) and 1% KI/starch spray.

Analytical HPLC was performed under isocratic conditions on a Spectra-Physics Model 8700 equipped with a 20-μL loop injector (Rheodyne) and an Altex Ultrasphere 5-μm C-18 reversed-phase column (4.6 × 250 mm). The eluent contained 40% CH_3CN (Burdick and Jackson, UV) and was 0.03 M in NH_4OAc (Tridom/Fluka, puriss) at pH 7. The column effluent was monitored at 214 nm with a Schoeffel SF770 spectroflow detector.

Amino Acid Analysis. Amino acid analyses were performed on a Beckman 119CL analyzer in the single column mode after 18–24-h hydrolysis in 4 N $MeSO_3H$–0.2% 3-(2-aminoethyl)indole reagent[48] (Pierce). The buffer sequence pH 3.25 (50 min), pH 4.12 (67 min), pH 6.25 (100 min) was used. Satisfactory amino acid analyses (±10%) were obtained for compounds 1–19.

Peptide Synthesis. Protected peptides were prepared on a Beckman 990 synthesizer using a standard program, employing 50% CF_3CO_2H/CH_2Cl_2 and 10% Et_3N/CH_2Cl_2 as deprotection and neutralization reagents, respectively. Final deprotection/cleavage was performed with anhydrous (CoF_3), redistilled liquid HF containing 10% anisole as scavenger for 1 h at 0 °C. The crude product was converted to the AcO form by passage through a column of the weakly basic anion exchanger AG3 (AcO form) in H_2O and was lyophilized. This material was purified by re-

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versed-phase preparative HPLC as described,[19] using a 2.5 × 100 cm column (Altex) packed with 25–40 μm Lichroprep RP-18 (E. Merck). The eluent contained various proportions of CH_3CN and H_2O, depending on the hydrophobicity of the compound (k'), but in each case the eluent was 0.03 M in NH_4OAc (pH 7).

The fractions containing the product (analytical HPLC) were pooled and concentrated to dryness. The bulk of the NH_4OAc was sublimed under vacuum from the flask into a Kjeldahl head by use of a 40 °C water bath. The residual traces were removed by lyophilization three times from H_2O to yield the pure product as a fluffy white powder.

Rat Antiovulatory Assay.[49] Adult female Simonsen Albino rats (Sprague-Dawley strain; >180 g) were acclimatized to laboratory conditions (14:10, light:dark with lights on at 5 a.m.) for at least 10 days. Daily vaginal lavages were taken from each rat between 7:30 and 9:00 a.m. for at least 12 days. Cytology of vaginal lavages was examined microscopically to determine the stage of the estrous cycle. Rats with at least two normal 4-day cycles preceding the test cycle were selected. The rats were injected subcutaneously with a solution of the analogue in vehicle (50% propylene glycol/0.9% saline or Mazola corn oil).

On the morning of expected estrus, the rats were sacrificed, and the oviducts were removed and examined under a dissecting microscope for the presence of freshly ovulated eggs. The eggs were teased out of the oviducts and counted. The percent of females ovulating was plotted against the log-dose to calculate the ED_{50} for antiovulatory activity. Usually three or more dose groups (data from a total of 10 animals per dose group) were used to determine the ED_{50}, but occasionally two dose groups were found to be sufficient.

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Registry No. 1, 90531-06-9; 2, 90552-82-2; 3, 90531-07-0; 4, 75851-13-7; 5, 90531-08-1; 6, 90531-09-2; 7, 82778-58-3; 8, 85466-65-5; 9, 90531-10-5; 10, 82778-57-2; 11, 90531-11-6; 12, 90531-12-7; 13, 90531-13-8; 14, 90531-14-9; 15, 86855-16-5; 16, 81608-49-3; 17, 90531-15-0; 18, 90531-16-1; 19, 90531-17-2.

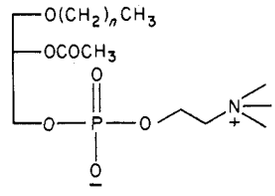
Analogues of Platelet Activating Factor (PAF). 1. Some Modifications of the Alkoxy Chain

A. Wissner,* P-E. Sum, R. E. Schaub, C. A. Kohler, and B. M. Goldstein

Metabolic Disease Research and Cardiovascular Biological Research Sections, Medical Research Division, American Cyanamid Company, Lederle Laboratories, Pearl River, New York 10965. *Received January 23, 1984*

Analogues of platelet activating factor (PAF) in which the ether oxygen has been removed (**6**) and in which the alkoxy chain at C_1 has been replaced with a *o*-, *p*-, or *m*-alkylphenoxy group (**30**, **31**, and **35**, respectively) have been prepared. Compound **6** shows reduced platelet aggregation and hypotensive activity in comparison with C_{16} and C_{18} PAF. The results obtained for compounds **30**, **31**, and **35** indicate that the hypotensive and platelet aggregating responses are sensitive to structural modification of the ether chain. The ortho analogue **30** shows no platelet aggregating activity and only a weak hypotensive response. The para analogue **31** exhibits a moderate decrease in activity in both assays. The meta analogue **35** is the most active of the three.

1-*O*-Alkyl-2-*O*-acetyl-*sn*-glyceryl-3-phosphorylcholine in which the alkyl component is comprised largely of the C_{18} and C_{16} homologues (**1a** and **1b**) has been identified with platelet activating factor (PAF) by two independent research groups.[1] Coincidently, the same substance was



1a, $n = 17$
b, $n = 15$

prepared by Muirhead and colleagues and was found to have similar biological properties to those of a phospholipid isolated from the renal medulla and designated as the antihypertensive polar renomedullary lipid (APRL).[2] The identification of this structure has stimulated a vast amount of research on the biological properties of this substance in recent years. It is increasingly becoming apparent that PAF is not only one of the most potent aggregators of platelets known but is also one of the important mediators of anaphylaxis and inflammation promoting the activation of neutrophils, basophils, and other inflammatory cells.[4] In addition, Muirhead and coworkers have shown that this substance is exceedingly potent in its ability to lower blood pressure,[2] while our laboratory has presented evidence that this hypotensive effect is the result of a nonspecific vasodilation.[3] It is the latter property of PAF that has stimulated our interest.

We are proposing the hypothesis that the multiple biological activities of PAF need not be mediated by a common receptor and that the hypotensive effect is not a consequence of mediator cell (e.g., platelet) activation and release. If this indeed be the case, it should be possible to identify a structure in which these two properties have been separated sufficiently to allow the development of a therapeutically useful hypotensive agent.

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