ester was the sole product. Pure **23** was obtained by hydrolysis with 0.01 N HCl in THF at 25 °C for 15 min.

The half-life of **1** was determined by incubating a 10^{-8} M solution in Krebs bicarbonate buffer saturated with CO_2 (pH 7.4) at 37 °C and measuring the capacity of this solution at 4-h intervals to cause relaxation of renal mesenteric artery previously contracted by $PGF_{2\alpha}$. After 24 h, relaxation had decreased by 50%, while PGI_2 under identical conditions required only 10 min.[29] In these experiments, **1** and PGI_2 showed EC_{50} values of $2.3 \pm 0.5 \times 10^{-9}$ and $3.3 \pm 0.5 \times 10^{-9}$ M, respectively.[30] Relaxation of bovine coronary artery is uniquely characteristic for PGI_2 among all the prostaglandins.[31] In this assay, **1** and PGI_2 showed $EC_{50} = 8.5 \pm 1.6 \times 10^{-9}$ and $2.8 \pm 0.6 \times 10^{-8}$, respectively.[29] Comparison of the potency of **1** and PGI_2 in causing complete inhibition of ADP and arachidonic acid induced aggregation of human platelets showed **1** ($ED_{100} = 10^{-8}$ M) to be 70% as active as PGI_2.[1,32]

Intravenous administration of **1** and PGI_2 in doses of 1 to 2×10^{-8} mol/kg as a bolus to an anesthetized dog showed the difluoro derivative to be equal in potency to the natural product in lowering blood pressure and decreasing peripheral and increasing renal blood flow.[33] Only at the highest levels of **1** was a two- to threefold prolongation of action observed when **1** was compared with equipotent levels of PGI_2. Since **1** was shown to be completely resistant to 15-hydroxyprostaglandin dehydrogenase,[34] rapid excretion either unchanged or after β- and/or P-450 catalyzed oxidation is probable.

10,10-Difluoro-13-dehydro-$PGF_{2\alpha}$ (**19**) possesses luteolytic activity equal to that of $PGF_{2\alpha}$ in a hamster antifertility assay.[35]

In summary, the total synthesis of the prostacyclin analogue **1** is described, which mimicks natural PGI_2 in all respects so far examined, except for its 150 times greater half-life and its failure to be inactivated by the 15-hydroxyprostaglandin dehydrogenase.

Acknowledgment. This work was supported by NIH Research Career Award AM 21846 to J. F. and NIH Research Grant AM 11499 and NIH Contract HD-7-2816. Funds provided by the NSF (GP 33116), the NIH (Cancer Center Grant CA 14999), and the Louis Block Fund to purchase the NMR equipment used in this work are gratefully acknowledged.

(29) Hatano, Y. Department of Pharmacological and Physiological Sciences, University of Chicago, unpublished results.
(30) The material prepared and tested in this work represents a 1:1 mixture of diastereomers consisting of structure **1** and its ent-15-epi derivative. To assess the potency of the latter, we prepared and examined its nonfluorinated analogue for its effect on the renal mesenteric artery. In contrast to **1**, this substance caused *contraction* at the 10^{-6} M level, too high to substantially influence the effects of **1**. Our potency estimates are therefore reported in terms of a 50% content of **1**.
(31) Dusting, G. J.; Moncada, S.; Vane, J. R. *Prostaglandins* **1977**, *13*, 3.
(32) Needleman, P. Department of Pharmacology, Washington University, St. Louis, Mo., unpublished results.
(33) Kohli, J. D. Department of Pharmacological and Physiological Sciences, University of Chicago, unpublished results.
(34) Jarabak, J. Department of Medicine, University of Chicago, unpublished results.
(35) Performed through the courtesy of the National Institute for Child Health and Development.

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5-Substituted 2-Amino-6-phenyl-4(3*H*)-pyrimidinones. Antiviral- and Interferon-Inducing Agents

Sir:

A survey of the variety of agents capable of stimulating interferon (IF) production demonstrates that most range in size from viruses and bacterial cell walls to synthetic or biopolymers [e.g., dextran and poly(I:C)].[1] However, there is also a small group of low-molecular-weight compounds which induce IF. Included among these compounds which have been reported to induce IF either in vivo or in vitro are diamines such as *N,N*-dioctadecyl-*N',N'*-bis(2-hydroxyethyl)propanediamine,[2] *N,N'*-dihexadecyl-*m*-xylylenediamine,[3] bis(diethylamino)fluorenone (tilorone),[4] several acridines represented by quinacrine,[5] a 1,5-bis[[(diethylamino)ethyl]amino]-9,10-anthraquinone,[6] 4-[[3-(dimethylamino)propyl]amino]-1,3-dimethyl-1*H*-pyrazolo[3,4-*b*]quinoline,[7] and such inhibitors of cellular proliferation as cycloheximide, actinomycin D, and 5,6-dichloro-1-(β-D-ribofuranosyl)benzimidazole.[8,9] Also part of this class of IF inducers is the recently reported 2-amino-5-bromo-6-methyl-4(3*H*)-pyrimidinone (**II**, R_1 = Br; R_2 = CH_3).[10] We wish to report that the corresponding 6-phenylpyrimidinones (**II**, R_2 = C_6H_5) exhibit substantially enhanced IF induction and antiviral activity.[11]

The synthesis of the pyrimidinones of interest proceeds from the requisite β-keto ester (**I**), itself available in a one-step acylation of the dianion of monoethyl malonate (Scheme I).[12] Condensation of the β-keto ester with guanidine afforded the 2-amino-6-phenyl-4(3*H*)-pyrimidinones (**II**) in 50–70% overall yield.

The 5-halogen-substituted analogues were prepared from **II**, R_1 = H, by halogenation in acetic acid for chlorination (*N*-chlorosuccinimide) and bromination (*N*-bromosuccinimide or Br_2) or in a basic, mixed solvent system ($CHCl_3$, 1 N NaOH, I_2) for iodination (60–90% yield).[13] Halogenation could also be carried out by heating in DMF with

(1) See "Interferons and Their Actions", W. E. Stewart II, Ed., CRC Press, Cleveland, Ohio, 1977, and "Interferon and Interferon Inducers: Clinical Application", D. A. Stringfellow, Ed., Marcel-Dekker, 1979, in press.
(2) B. G. Gatmaitan, E. D. Stanley, and G. G. Jackson, *J. Infect. Dis.*, **127**, 401 (1973); W. W. Hoffman, J. J. Korst, J. F. Niblack, and T. H. Cronin, *Antimicrob. Agents Chemother.*, **3**, 498 (1973). See also R. H. Waldman and R. Ganguly, *J. Infect. Dis.*, **138**, 531 (1978).
(3) R. G. Douglas, Jr., R. H. Waldman, R. F. Betts, and R. Ganguly, *Antimicrob. Agents Chemother.*, **15**, 269 (1979).
(4) R. F. Krueger and G. D. Mayer, *Science*, **169**, 1213, 1214 (1970); E. R. Andrews, R. W. Fleming, J. M. Grisar, J. C. Kihm, D. L. Wenstrup, and G. D. Mayer, *J. Med. Chem.*, **17**, 882 (1974).
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(11) Presented in part at *Int. Congr. Chemother., Proc., 11th*, and *Intersci. Conf. Antimicrob. Agent. Chemother., 19th*, Boston, Mass., Oct 1–5, 1979.
(12) W. Wierenga and H. I. Skulnick, *J. Org. Chem.*, **44**, 310 (1979).
(13) All compounds exhibited satisfactory ^1H and ^{13}C NMR and elemental analyses.

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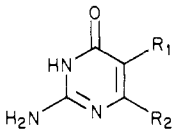
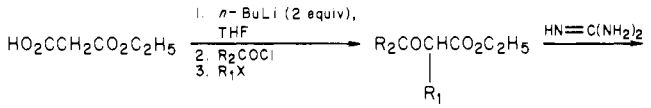
Table I. Antiviral- and IF-Inducing Activities of 2-Amino-4(3*H*)-pyrimidinones



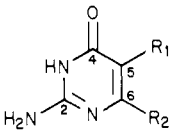
R_1	R_2	SFV ED_{50}, mg/kg[a] ip	po	sc	IF[b]	HSV-1[c] protection index	dose
Br	CH_3	250			++	0.40	150
I	CH_3	200			++	0.16	200
Cl	C_6H_5	100	90	105	+++	0.62	200
Br	C_6H_5[d]	100	100	50	+++	0.60	200
I	C_6H_5	50	>400	25	+	0.86	100
CH_3	C_6H_5[e]	185			+++	0.35	200
CH_3CH_2	C_6H_5[e]	45	280	>400	++	0.57	200
$CH_2=CH_2CH_2$	C_6H_5[e]	95	>400	>400	+	0.43	200
$HC\equiv CCH_2$	C_6H_5	130	>400				
$CH_3CH_2CH_2$	C_6H_5[e]	>400			-	0.13	100
$CH_3CH_2OCH_2CH_2$	C_6H_5	>800	>400	>400		0.15	100

[a] Single dose administered 18 h prior to a lethal infection of Semliki Forest virus (ip); minimum dose needed to protect 50% of the mice calculated using a probit analysis of variance, 24 mice per group. [b] Serum interferon (IF) response from a single ip administration of 800 mg/kg employing a plaque-reduction assay (VSV on L_{929} cells) where +++ ≥ 2000, ++ ≈ 500-1000, + ≈ 50-500, ± ≈ 10-50 units/mL peak IF response within 24 h. [c] Compound administered ip in saline at the dose noted [(mg/kg)/day)] at -40, -28, -20, -3, and +3 h to mice inoculated ip with 5.6×10^4 pfu herpes simplex virus type 1 [H. E. Renis, D. T. Gish, B. A. Court, E. E. Eidson, and W. J. Wechter, *J. Med. Chem.*, 16, 754 (1973)]. Protection index = control mortality minus treated mortality/control mortality, >0.25 considered active [L. W. Catalano and S. Baron, *Proc. Soc. Exp. Biol. Med.*, 133, 684 (1970)]; experiment conducted for 22 days with mean survival time (MST) of control ~8 days, 15 mice/group, and MST of II, R_1 = I, R_2 = C_6H_5, was >21 days. [d] T. B. Brown and H. F. G. Stevens, *J. Chem. Soc., Perkin Trans. 1*, 1023 (1975). [e] K. J. Rorig, U.S. Patent 2 723 977 (1955); G. H. Hitchings and P. B. Russel, U.S. Patent 2 691 655 (1954).

Scheme I



I



II

the appropriate *N*-halosuccinimide. Alternatively, when R_1 was alkyl, the corresponding alkyl β-keto ester was available via in situ alkylation of the intermediate β-keto ester anion with R_1X (X = Br, I) in yields superior to those involving standard conditions for alkylations of β-keto esters.[14]

Of initial interest was the demonstration that not only were the 6-phenyl analogues more effective antiviral and IF inducing agents than the 6-methylpyrimidinone, but relatively modest structural changes achieved dramatic apportions in antiviral- vs. IF-inducing activities.

In Table I is given the in vivo antiviral activity in mice against the RNA virus, Semliki Forest virus (SFV), and the Herpes simplex type-1 DNA virus (HSV-1), as well as the relative capabilities for in vivo IF induction of various pyrimidinones. Besides the two- to fivefold increase in antiviral activity against SFV by II (R_2 = C_6H_5), there was an enhancement of efficacy against HSV-1 in mice. There was, however, no parallel relationship with IF induction, since the more antivirally effective 5-iodo-6-phenylpyrimidinone was a relatively poor IF inducer. Cognizant that IF is an effective agent against such RNA viruses as SFV but not as effective against the HSV-1 DNA virus,[1] the data suggest that an alternate and/or additional mechanism of action from IF induction is required to explain the antiviral activity.[15] Table I also indicates that antiviral efficacy is a function of the route of administration. These pyrimidinones compare favorably in IF induction with such known inducers as tilorone;[4] however, they incorporate advantages over the latter in spectrum of antiviral activity, toxicity, and maintenance of IF responsiveness.[10,15,16]

Changes in the substituent at the 5 position appear to correlate somewhat with the size of the substituent. Activity diminishes rapidly when any group larger than propyl or smaller than chloro is present. Not listed in the table but found inactive were the 5-benzyl- and 5-(methylenetrimethylsilyl)-6-phenylpyrimidinones. The corresponding 5-nitro analogue was toxic, the 5-hydro analogue was completely inactive, and the 5-fluoro analogue was only weakly active.

Although the earlier 6-methylpyrimidinone analogue exhibited useful antiviral activity and IF induction at acceptable doses, a disadvantage manifested by intratubular crystal deposition in the renal papillae was apparent upon chronic administration in several animal species. This drawback is not observed with the 6-phenyl analogues.[17] Thus, the combination of substantially enhanced antiviral activity and IF induction coupled with reduced toxic manifestations provides an incentive for future investigations with these agents.

The generality of the IF induction has been demonstrated in other animal species, such as cats, dogs, and

(14) See H. O. House, "Modern Synthetic Reactions", W. A. Benjamin, Menlo Park, Calif., 1972, pp 514-519.

(15) H. E. Renis, E. E. Eidson, S. D. Weed, and D. A. Stringfellow, manuscript in preparation.

(16) W. L. Albrecht, R. W. Fleming, S. W. Horgan, J. C. Kihm, and G. D. Mayer, *J. Med. Chem.*, 17, 884 (1974).

(17) J. E. Gray and E. R. Larsen, unpublished results, The Upjohn Co.

calves. Similarly, the expression of IF induction and antiviral activity with other analogues, such as substituted 6-aryl and 6-heterocyclic derivatives, has established a diverse structure–activity relationship. Efforts in both of these areas of interest are in progress and will be reported in due course.

Acknowledgment. We thank B. D. Tiffany and R. L. Johnson for scale-up preparations of several of the pyrimidinones.

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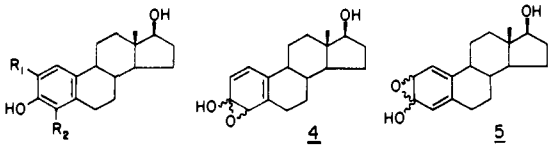
Biomimetic Synthesis of Catechol Estrogens: Potentially Mutagenic Arene Oxide Intermediates in Estrogen Metabolism[1]

Sir:

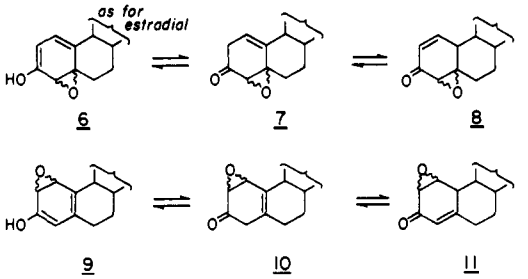
Exogenous estrogens have the clear potential to be a causal factor in the development of neoplasms at certain organ sites where such hormones are metabolized.[2] Though the mechanism for such an initiation remains obscure, it is possible that the pathways in estrogen metabolism generate reactive intermediates capable of binding to the observed cytoplasmic and nuclear receptors.[3] Metabolism of estradiol (1), for example, leads to the



1; $R_1 = R_2 = H$
2 $R_1 = H$, $R_2 = OH$
3 $R_1 = OH$, $R_2 = H$

catechols 2 and 3. Arene oxide derivatives of 1 may be intermediates in this metabolism, in analogy to the arene oxides of polycyclic aromatic hydrocarbons, which have been implicated in the carcinogenic effects of these compounds.[4,5]

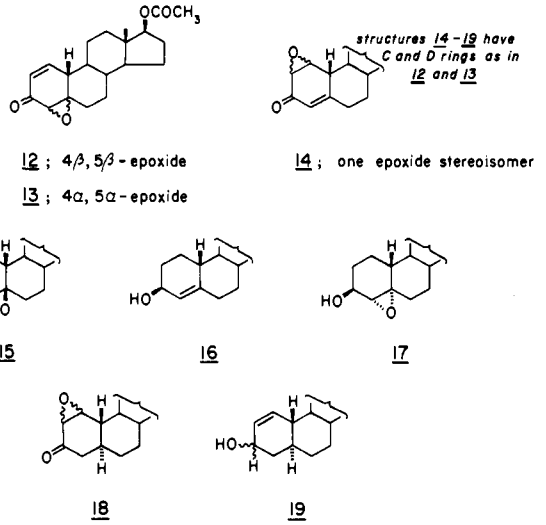
Catechol formation through phenolic arene oxides might, a priori, proceed either via oxirane hemiacetals, such as 4 and 5, or via dienol epoxides and their keto tautomers, e.g., 6–8 and 9–11. The oxirane hemiacetals 4 and 5 are intrinsically interesting but have no known simpler counterparts and, if formed, would be expected to be of such



6 7 8

9 10 11

a highly reactive and transient nature that proof of their existence may be difficult and equivocal. Intermediates 6 and 9, however, are capable of stabilization as the keto tautomers 7, 8, 10, and 11. Several workers[6] have suggested the involvement of dienol epoxides in the formation of catechols from estrone and have demonstrated covalent binding of such estrogens to nucleic acids in the presence of cytochrome P-448 monooxygenase. As an initial investigation of the intermediates in catechol estrogen formation, we have focused on compounds of the conjugated enone series (8 and 11). We now report the synthesis and aromatization of the enone expoxides 12–14.[7]



12; 4β,5β-epoxide
13; 4α,5α-epoxide
14; one epoxide stereoisomer

15 16 17

18 19

Compound 12 was synthesized from 19-nortestosterone essentially according to a procedure developed by Mihailovic and his co-workers.[8] Treatment of 19-nortestosterone with alkaline hydrogen peroxide, followed by acetylation, gave the epoxide 15. The β-epoxide stereochemistry is in accord with expectations based upon work by Burgess[9] and by Jeger and his co-workers.[10] Dehy-

(1) Part of this work has been presented, see: Subramanyam, V.; Durga, A. V.; Le Quesne, P. W.; and Soloway, A. H.; "Abstracts of Papers", 176th National Meeting of the American Chemical Society, Miami, Fla., Sept 11–14, 1978; American Chemical Society: Washington, D.C.; Abstr MEDI 45.
(2) Thomas, D. B. *Cancer Res.* **1978**, *38*, 3991.
(3) Brooks, S. C.; Locke, E. R.; Horn, L. *Cancer Res.* **1978**, *38*, 4238; see also Weinberger, M. J.; Aten, R. F.; Eisenfeld, A. J. *Biochem. Pharmacol.* **1978**, *27*, 2469.
(4) Jerina, D. M.; Lehr, R.; Schaefer-Ridder, M.; Yagi, H.; Karle, J. M.; Thakker, D. R.; Wood, A. W.; Lu, A. Y. H.; Ryan, D.; Levin, W.; Conney, A. H. "Origins of Human Cancer", Hiatt, H.; Watson, J. D.; Winsten, I., Ed.; Cold Spring Harbor Laboratory: Cold Spring Harbor, N.Y., 1977; pp 639–658.
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(6) Tsibris, J. C. M.; McGuire, P. M. *Biochem. Biophys. Res. Commun.* **1977**, *78*, 411; Numazawa, M.; Nambara, T. *J. Steroid Biochem.* **1977**, *8*, 835.
(7) The influence of stereochemistry in the mechanistic details of epoxide ring opening has been noted by Whalen, D. L.; Ross, A. M.; Yagi, H.; Karle, J. M.; Jerina, D. M. *J. Am. Chem. Soc.* **1978**, *100*, 5218, for the dihydrodiol epoxides derived from polycyclic aromatic hydrocarbons. We view the steroidal enone epoxides as substrates for future, analogous, detailed studies.
(8) Mihailovic, M. Lj.; Forsek, J.; Lorenc, Lj. *Tetrahedron* **1977**, *33*, 235.
(9) Wehrli, H.; Lehmann, C.; Keller, P.; Bonet, J. J.; Schaffner, K.; Jeger, O. *Helv. Chim. Acta*, **1966**, *49*, 2218.
(10) Compounds 12 had mp 114–115 °C; $[\alpha]_D$ (EtOH) +261.3°; IR (film) ν_{max} 3050, 2950, 1730, 1680, 1620, 1250, 1050, 1030 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.87 (3 H, s, C-18 Me), 2.03 (acetoxy Me), 3.23 (1 H, m, 4-H), 4.6 (1 H, br t, 17-H), 5.93 (1 H, br d, 2-H), 6.76 (1 H, dd, J = 10 and 6 Hz, 1-H). Structure 12 was assigned earlier (ref 9) to a compound reported as an oil. This compound and all other new compounds gave satisfactory elemental and spectroscopic analyses.

0022-2623/80/1823-0239$01.00/0