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.

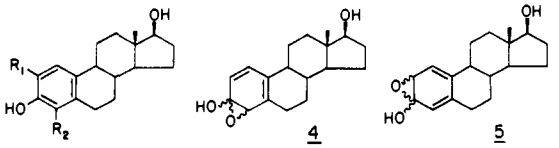
Wendell Wierenga,* Harvey I. Skulnick
Experimental Chemistry Research

Dale A. Stringfellow,* Sheldon D. Weed
Harold E. Renis,* Emerson E. Eidson
Experimental Biology Research, The Upjohn Company
Kalamazoo, Michigan 49001
Received October 10, 1979

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

Sir:

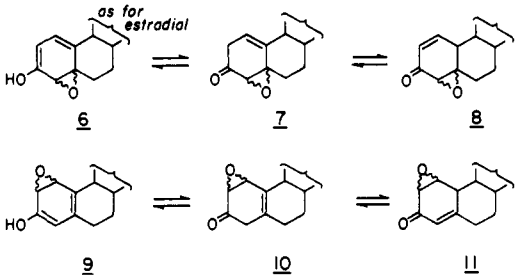
Exogenous estrogens have the clear potential to be a causal factor in the development of neoplasms at certain organ sites where such hormones are metabolized.[2] Though the mechanism for such an initiation remains obscure, it is possible that the pathways in estrogen metabolism generate reactive intermediates 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]

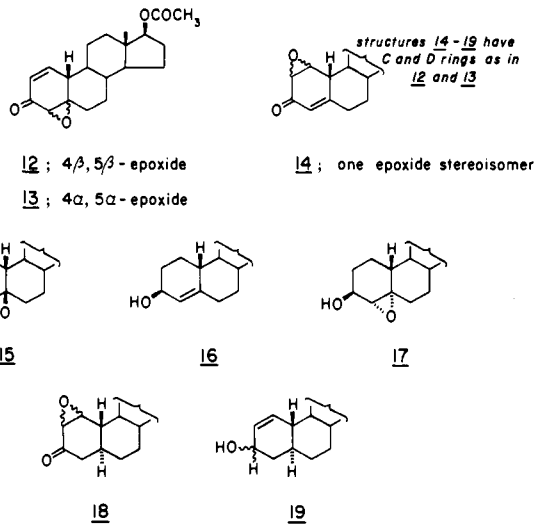
Catechol formation through phenolic arene oxides might, a priori, proceed either via oxirane hemiacetals, such as 4 and 5, or via dienol epoxides and their keto tautomers, e.g., 6–8 and 9–11. The oxirane hemiacetals 4 and 5 are intrinsically interesting but have no known simpler counterparts and, if 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.
(5) Slaga, T. J.; Bracken, W. J.; Gleason, C.; Lewin, W.; Yagi, H.; Jerina, D. M.; Conney, A. H. *Cancer Res.* **1979**, *39*, 67.
(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

drogenation of **15** proceeded cleanly only with selenium dioxide in neutral, strictly anhydrous *tert*-butyl alcohol to give **12**.[10] Compound **13** was prepared by reaction of 17-acetyl-19-nortestosterone with iodine–potassium iodate,[11] followed by selenium dioxide dehydrogenation as above. Alternatively, epoxidation of the known alcohol **16** with *m*-chloroperbenzoic acid gave **17**, although in minor amount compared to the isomeric β-epoxides.[12] Chromium trioxide–pyridine oxidation of **17**, followed by selenium dioxide dehydrogenation, gave **13**.[13]

Dehydrogenation of 17β-hydroxy-19-nor-5α-androstan-3-one was accomplished by sequential treatment with phenylselenyl chloride in ethyl acetate and hydrogen peroxide. Elimination of phenylselenenic acid at room temperature gave the 1-en-3-one, which with alkaline hydrogen peroxide gave one of the 1,2-epoxy-3-ones, **18**, stereospecifically. Reduction of the 1-en-3-one with sodium borohydride gave both stereoisomers of **19**. These were epoxidized with *m*-chloroperbenzoic acid and subsequently oxidized with chromium trioxide–pyridine to generate both stereoisomers of **18**. The stereochemistry of neither isomer of **18** could be assigned unequivocally from the ^{1}H NMR spectra; X-ray work will be utilized to settle these points. The more abundant isomer resulting from these experiments has been dehydrogenated with selenium dioxide in *tert*-butyl alcohol to give **14**.[14]

The three epoxyenones thus obtained are similar and remarkably stable. They do undergo smooth aromatization under acidic conditions; **12** and **13** form the triacetate of **2** by the action of *p*-toluenesulfonic acid in acetic anhydride, and **14** yields, under similar conditions, the known triacetate of **3**.[8] On the other hand, the epoxyenones may be recovered unchanged from methanolic sodium methoxide (20 °C, 12 h) or potassium *tert*-butoxide–*tert*-butyl alcohol (20 °C, 12 h).

The synthesis of these tautomers of dienol epoxides derived from estradiol and their smooth aromatization suggest that they, in equilibrium with their dienol tautomers, may be intermediates in catechol estrogen biosynthesis. The aromatization step may be simply acid catalyzed or may be enzyme mediated.

Compounds **12–14** have been biologically evaluated in two ways. First, their activity on bacteriophage PM2 DNA, as measured by the creation of endonuclease-sensitive sites and subsequent agarose gel electrophoresis, was evaluated.[15] The compounds were inactive by these criteria. Secondly, the compounds were subjected to the foci assay of Kakunaga,[16] which involves determination of the chemical transformation of Balb/c 3T3 mouse fibroblast cells. From the 1-1A-10 line obtained from Dr. Takeo Kakunaga, a subclone (MN-20) was isolated which showed good contact inhibition, flat surface morphology, low spontaneous transformation frequency, and reproducible transformation by 3-methylcholanthrene. Initial findings indicate, interestingly, that in this assay compound **13** (2 μM) is approximately as active as the highly mutagenic 3-methylcholanthrene itself in inducing chemical transformation of the cells and at least two orders of magnitude more effective than estradiol. Compounds **12** and **14** were inactive at the same concentration as used for compound **13**. Extensions of these chemical and biological experiments are in progress.

Acknowledgment. The work at Northeastern University was supported by the Office of Naval Research, Department of the Navy (Contract N00014-76-C-0027). The work at the Southwest Foundation was supported by Grant 1 R01 CA-24629-01 from the National Cancer Institute, DHEW. The original line of Balb/c 3T3 cells used in this work was kindly provided by Dr. Takeo Kakunaga, National Institutes of Health. The authors acknowledge the skillful technical assistance of T. J. Goodwin.

Philip W. Le Quesne,* Adhyam V. Durga Vinayakam Subramanyam
Department of Chemistry and Department of Medicinal Chemistry and Pharmacology, Northeastern University Boston, Massachusetts 02115

Albert H. Soloway,* Ronald W. Hart
College of Pharmacy, The Ohio State University Columbus, Ohio 43210

Robert H. Purdy*
Southwest Foundation for Research and Education San Antonio, Texas 78284

Received May 11, 1979

Synthetic Modulators of the Complement System. 1. Synthesis and Biological Activity of 5,5′,5″-[1,3,6-Naphthalenetriyltris(sulfonylimino)]-tris[1,3-benzenedisulfonic acid] Hexasodium Salt

Sir:

Considerable progress has been made in defining the constituents of the complement system and elaborating their role in the lysis of cells and the inflammatory process.[1] In addition, complement has been implicated in the pathophysiology of a number of diseases, e.g., rheumatoid arthritis, lupus erythematosus, glomerulonephritis and periodontitis.[2]

A possible approach to the treatment of complement-dependent diseases involves the use of a synthetic chemical modulator (activator or inhibitor) to assist the body's

(11) Cornforth, J. W.; Green, D. T. *J. Chem. Soc. C* **1970**, 846.
(12) Cf. Hanson, J. R.; Wilkins, H. J. *J. Chem. Soc, Perkin Trans. 1* **1974**, 1388.
(13) Compound **13** had mp 143–144 °C; $[\alpha]_D$ (EtOH) −55°; IR (film) ν_{max} 3050, 1730, 1675, 1250, 1045, 1020 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.87 (3 H, s, C-18 Me), 2.03 (acetoxy Me), 3.3 (1 H, d, J = 2 Hz, 4-H), 4.67 (1 H, br t, 17-H), 5.93 (1 H, d, J = 10 and 2 Hz, 2-H), 6.70 (1 H dd, J = 10 and 2 Hz, 1-H).
(14) Compound **14** had mp 180–182 °C; $[\alpha]_D$ (EtOH) +149.6°; IR (film) ν_{max} 3020, 1725, 1670, 1620, 1250, 1045, 1025 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 0.90 (3 H, s, C-18 Me), 2.03 (3 H, acetoxy Me), 3.37 (1 H, dd, J = 4 and 2 Hz, 1-H), 3.70 (1 H, m, 2-H), 4.63 (1 H, br t, 17-H), 5.73 (1 H, br s, 4-H).
(15) Compare Mhaskar, D. N.; D'Ambrosio, S. M.; Modak, S. P.; Hart, R. W. *Fed. Proc., Fed. Am. Soc. Exp. Biol.* **1979**, *38*, 484.
(16) Kakunaga, T. *Int. J. Cancer* **1973**, *12*, 463.

(1) (a) S. Ruddy, in "Mediators of Inflammation", G. Weissmann, Ed., Plenum Press, New York, 1974, Chapter 4; (b) P. A. Ward, in "Mechanisms of Immunopathology", S. Cohen, P. A. Ward, and R. T. McClusky, Eds., Wiley, New York, 1979, Chapter 1; (c) H. J. Müller-Eberhard, *Hosp. Practice*, **12**, 33 (1977); (d) M. M. Mayer, *Harvey Lect.*, **72**, 139 (1977).
(2) (a) M. M. Frank and J. P. Atkinson, in "Disease-a-Month", Year Book Medical Publishers, Chicago, Ill., Jan 1975; (b) M. M. Frank, J. A. Gelfand, and J. P. Atkinson, *Ann. Intern. Med.*, **84**, 580 (1976); (c) I. Gigli, in "Comprehensive Immunology", Vol. 2, N. K. Day and R. A. Good, Eds., Plenum Medical Book Co., New York, 1977, Chapter 15; (d) C. Rothback, R. L. Green, M. I. Levine, and P. Fireman, *Am. J. Med.*, **66**, 681 (1979); R. J. Nisengard, *J. Periodontol.*, **48**, 505 (1977); (e) N. J. Zvaifler, *Adv. Immunol.*, **16**, 265 (1973); (f) A. Nicholson, D. T. Fearon, and K. F. Austen, *Handb. Exp. Pharmakol.*, **50**(1), 425-463 (1978).

0022-2623/80/1823-0240$01.00/0