A Dichotomy in the Lipophilicity of Natural Estrogens, Xenoestrogens, and Phytoestrogens

Albert R. Cunningham,[1] Gilles Klopman,[2] and Herbert S. Rosenkranz[1]

[1]Department of Environmental and Occupational Health, University of Pittsburgh, Pittsburgh, Pennsylvania; [2]Department of Chemistry, Case Western Reserve University, Cleveland, Ohio

Using two independent analyses, it is demonstrated that natural (e.g., estradiol) and some xenoestrogens (e.g., methoxychlor metabolite) are characterized by a lipophilic region that is absent in nonestrogens as well as in phytoestrogens. It is suggested that this lipophilic region affects binding to specific receptors and may, in fact, differentiate harmful from beneficial estrogens. — *Environ Health Perspect* 105(Suppl 3):665–668 (1997)

Key words: CASE/MULTICASE, cancer, estrogens, lipophilicity

Introduction

Recently, we identified a 6Å 2-dimensional distance (2D) descriptor associated with the carcinogenicity in mice of estrogens (e.g., diethylstilbestrol and 17β-estradiol) (*1*). This descriptor (biophore) was originally recognized during structure-activity relationship (SAR) studies of diethylstilbestrol (*2*) and tamoxifen and toremifene (*3*), using the SAR expert systems computer automated structure evaluator (CASE) and multiple case (MULTICASE). This biophore was derived from the CASE/MULTICASE learning set of murine carcinogens (*4–8*). Based on its presence in carcinogenic estrogens, we suggested that the 2D biophore represented a ligand binding site on an estrogen receptor (*1*). This hypothesis was supported by the realization that the biophore was derived from estrogens in the carcinogenicity database and the fact that CASE/MULTICASE had been programed to recognize 2D biophores that possess lipophilic centers as well as moieties capable of hydrogen bonding. These characteristics are associated with ligands that bind to cellular receptors. Hence this finding is consistent with an estrogen possessing a hydrogen-bonding moiety at one end and a lipophilic moiety on the other. In fact, CASE/MULTICASE identified it as a lipophilic, anchored, *p*-substituted phenol moiety (Figure 1). This 2D descriptor is absent from the vast majority of nonestrogens.

Using CASE/MULTICASE, we identified a number of chemicals, including many estrogens and xenoestrogens, that possess this 2D moiety (Table 1), as well as a number of estrogens that lack it. However, some of the estrogens devoid of this moiety acquire it following metabolic activation, e.g., tamoxifen metabolism to 4-hydroxytamoxifen; the latter is the metabolite thought to be responsible for the estrogenicity of the parent molecule (*9*). On the other hand, phytoestrogens, as a group, lack this descriptor (Table 1). These findings suggest that the presence of the 2D descriptor could be used to classify estrogens with respect to possible risk to humans and to the ecological biota, or even to distinguish between harmful (xenoestrogens) and potentially beneficial estrogens (e.g., phytoestrogens).

While we do not expect this 2D biophore to provide a unifying principle that accounts for the action of estrogens, it might provide further insight into their mechanism of action. In the present study we expand the definition of the 2D biophore, especially with respect to its putative lipophilicity.

Methods

The CASE/MULTICASE methodologies have been described on a number of occasions (*10,11*). The 6Å moiety (above) identified by CASE/MULTICASE involves phenol substitution at the *p*-position with a carbon atom. The specific lipophilicity of the *p*-substituent is specified by CASE/MULTICASE to include carbon atoms that are four bonds away from heteroatoms. By this criteria 17β-estradiol was identified as possessing the appropriate lipophilic moiety while the carbon para to the phenol in genistein was found to lack it. To clarify the lipophilicity of the 2D biophore, we analyzed a group of molecules with Molecular Modeling Pro (MMP) (*12*) for the presence, location, and characteristics of their lipophilic regions.

Briefly, MMP assigns values for the lipophilicity of each atom of a molecule using the procedure of Hansch and Leo (*13*). For example, a value of 0.23 is assigned to hydrogens, 0.13 to carbons with one hydrogen, 0.22 to carbons with two or more hydrogens, –1.14 to hydroxyl groups, and –2.24 to keto oxygens. Each atom is also modified by its neighbors. The value of atoms α are multiplied by 0.5, β by 0.25, γ by 0.125, and δ by 0.0625. These values are totaled and added to the value of the atom of interest. After all calculations are completed, atoms with negative values are designated "hydrophilic" and those with positive values as "lipophilic." MMP then colors each atom to denote its degree of lipophilicity or hydrophilicity (Figure 1).

This paper was presented in part at the Workshop on Hormones, Hormone Metabolism, Environment, and Breast Cancer held 28–29 September 1995 in New Orleans, Louisiana. Manuscript received at *EHP* 6 June 1996; manuscript accepted 21 August 1996.

This investigation was supported by the U.S. Department of Defense (contract DAAA21-93-C-0046) and the Predoctoral Training in Breast Cancer Biology and Therapy Fellowship awarded by the U.S. Army Medical Research and Acquisition Activity. Additionally, the authors thank the reviewer for the insightful comments and suggestions.

Address correspondence to Dr. H.S. Rosenkranz, Department of Environmental and Occupational Health, University of Pittsburgh, Pittsburgh, PA 15261. Telephone: (412) 967-6510. Fax: (412) 624-1289. E-mail: rsnkranz@vms.cis.pitt.edu

Abbreviations used: CASE, computer atomated structure evaluator; 2D, 2-dimensional distance; DES, diethylstilbestrol; MMP, Molecular Modeling Pro; MULTICASE, multiple case.

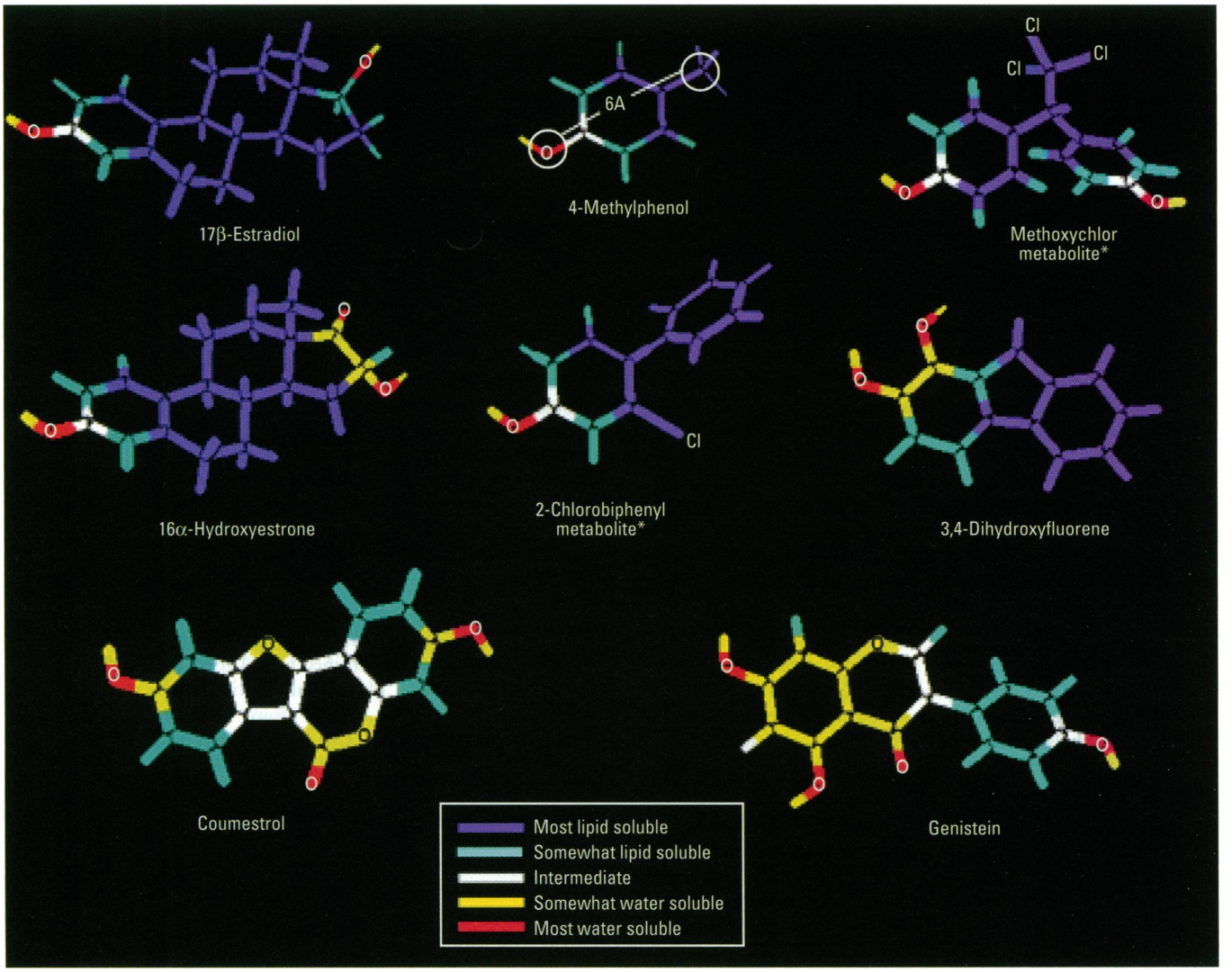

Figure 1. Estrogenic chemicals painted according to lipophilicity. The 6Å 2D biophore is illustrated in 4-methylphenol. All chemicals shown possess the physical distance requirements of the biophore. *Methoxychlor metabolite = 2,2-bis(*p*-hydroxyphenyl)-1,1,1-trichloroethane; 2-chlorobiphenyl metabolite = 2-chloro-4-hydroxybiphenyl.

Results and Discussion

Not all estrogens contain the 2D biophore (above) (Table 1). The simplest molecule that contains this biophore, 4-methylphenol (Figure 1), can illustrate the biophore. The 1-position of 4-methylphenol contains the hydroxyl group that is both hydrophilic and capable of hydrogen bonding. The 4-position is occupied by a benzylic methyl group that is in a lipophilic environment. In general, the benzylic carbon can be methyl, methylene, methine, or quaternary. Between the *p*-hydroxyl group and the lipophilic moiety is a conjugated six-membered ring system that may be substituted at some positions (*1*). The structure of 4-methylphenol can be superimposed on other molecules for easy identification of the 2D biophore.

The major aim of this investigation was to visualize and confirm, using MMP, that in fact the MULTICASE biophore is indeed anchored in a lipophilic region. This is readily demonstrated (Figure 1). All the chemicals shown in Figure 1 possess the physical distance requirements of the biophore (i.e., 6Å from phenol to benzylic carbon); however, the chemicals that lack the biophore have a benzylic carbon atom located in a region that is either hydrophilic or only somewhat lipophilic. For example, diethylstilbestrol (DES) and 17β-estradiol, which possess the 2D biophore, have a large lipophilic region that encompasses the *p*-substituted carbon. On the other hand, dietary estrogens such as coumestrol and genistein, which lack the biophore, have the corresponding carbon embedded in a region intermediate between lipophilic and hydrophilic (Figure 1).

For chemicals to have a lipophilic area at the alkyl end of the 2D biophore, heteroatoms (e.g., oxygen atoms) must be sufficiently distant from the *p*-carbon. Thus chemicals such as the dietary estrogens with their intra- and extracyclic oxygens produce an environment that is not very lipophilic and hence the biophore is absent.

The 2D biophore was originally identified from a carcinogenicity database

Table 1. Distribution of the 2D biophore among selected estrogenic and antiestrogenic chemicals.

Chemical	Type	2D
Phytoestrogens		
2′,4,4′,6′-Tetrahydroxydihydrochalcone (phloretin)	Phytoestrogen	–
5,7-Dihydroxyflavone (chrysin)	Phytoestrogen	–
3,5,7-Rihydroxyflavone (galangin)	Phytoestrogen	–
4′,5,7-Trihydroxyflavone (apigenin)	Phytoestrogen	–
3,3′,4′,7-Tetrahydroxyflavone (fisetin)	Phytoestrogen	–
3′,4′,5,7-Tetrahydroxyflavone (luteolin)	Phytoestrogen	–
3,4′,5,7-Tetrahydroxyflavone (kaempferol)	Phytoestrogen	–
3,5,7-Trihydroxy-4′-methoxyflavone (kaempferide)	Phytoestrogen	–
3,3′,4′,5,7-Pentahydroxyflavone (quercetin)	Phytoestrogen	–
2′,3,4′,5,7-Pentahydroxyflavone (morin)	Phytoestrogen	–
4′,5,7-Trihydroxyflavanone (naringenin)	Phytoestrogen	–
3′,5,7-Trihydroxy-4′-methoxyflavanone (hesperetin)	Phytoestrogen	–
3,3′,4′,5,7-Pentahydroxyflavanone (taxifolin)	Phytoestrogen	–
4′,7-Dihydroxyisoflavone (diadzein)	Phytoestrogen	–
4′,5,7-Trihydroxyisoflavone (genistein)	Phytoestrogen	–
5,7-Dihydroxy-4′-methoxyisoflavone (biochanin A)	Phytoestrogen	–
Coumestrol	Phytoestrogen	–
4,4′-Dihydroxystilbene	Phytoestrogen	+
α-Sitosterol	Phytoestrogen	–
Zearalenone	Phytoestrogen	–
Tetrahydrocannabinol	Phytoestrogen	–
Xenoestrogens and therapeutics		
o,p′-DDE	Xenoestrogen	–
Chlordecone	Xenoestrogen	–
4-Nonylphenol	Xenoestrogen	+
4-*tert*-Butylphenol	Xenoestrogen	+
DES	Estrogen	+
Indenestrol A	DES metabolite	+
4′,4″-Diethylstilbestrol quinone	DES metabolite	–
TMX	Antiestrogen	–
4-Hydroxytamoxifen acid	TMX metabolite	+
TRM	Antiestrogen	–
4-Hydroxydeaminohydroxytoremifene	TRM metabolite	+
ICI 164,384	Antiestrogen	+
ICI 182,780	Antiestrogen	+
LY 117018	Antiestrogen	–
MER 25	Antiestrogen	–
17β-Estradiol	Estrogen	+
17α-Ethinyl estradiol	Estrogen	+
Benzestrol	Estrogen	+
Dienestrol	Estrogen	+
Estriol	Estrogen	+
Estrone	Estrogen	+
Hexestrol	Estrogen	+
Megestrol	Estrogen	–
Norgestrol	Estrogen	–
Norlestrin	Estrogen	+
Phenol red	pH indicator	–
Bis(4-hydroxyphenyl)(2-(phenoxy-sulfonyl)phenyl)methane*	Xenoestrogen	+

Abbreviations: TMX, tamoxifen; TRM, toremifene. *This chemical has been identified as the estrogenic impurity of commercial phenol red preparations (*14*).

(above). The dichotomy between estrogens that display a lipophilic center and those that do not may in fact separate harmful (e.g., carcinogenic) from beneficial (or at least benign) estrogens. Thus carcinogenic estrogens or their metabolites (e.g., DES, tamoxifen, and 17β-estradiol) possess the 2D biophore and have independently been shown, using MMP, to be lipophilic. In addition, xenoestrogens such as the metabolites of methoxychlor, polychlorinated biphenyls, and polycyclic aromatic hydrocarbons also possess this biophore and in fact have a lipophilic region at which to anchor the biophore (Figure 1). On the other hand, dietary phytoestrogens (e.g., genistein, coumestrol, etc.), some of which are thought to be cancer chemopreventive agents, lack this biophore and have been shown herein to lack the lipophilic region (Figure 1).

The 2D biophore and associated lipophilic region appear to have biological significance and are not random occurrences among estrogenic chemicals. Indeed, the lipophilic region associated with the 2D biophore may modulate the binding affinities for these estrogens at different ligand-binding sites (e.g., estrogen receptor or estrogen-metabolizing enzymes).

The current report confirms that the 2D biophore describes a lipophilic center. This biophore is able to distinguish between some beneficial (e.g., genistein and other phytoestrogens) and some harmful (e.g., DES) estrogens. The ability of this biophore to differentiate estrogens suggests that estrogens elicit their responses through various mechanisms. Moreover, this dichotomy suggests that some estrogenic responses may be distinguishable from carcinogenic responses resulting from the action of the estrogens, since not all estrogens are carcinogens. The lipophilic moiety described herein may be involved in this dichotomy.

REFERENCES

1. Rosenkranz HS, Cunningham A, Klopman G. Identification of a 2-D geometric descriptor associated with non-genotoxic carcinogens and some estrogens and antiestrogens. Mutagenesis 11:95–100 (1996).
2. Cunningham A, Klopman G, Rosenkranz HS. The carcinogenicity of diethylstilbestrol: structural evidence for a non-genotoxic mechanism. Arch Toxicol 70:356–361 (1996).
3. Cunningham A, Klopman G, Rosenkranz HS. A study of the structural basis of the carcinogenicity of tamoxifen, toremifene and their metabolites. Mutat Res 349:85–94 (1996).
4. Gold LS, Sawyer CB, Magaw R, Backman GM, deVeciana M, Levinson R, Hooper NK, Havender WR, Bernstein L, Peto R, et al. A carcinogenic potency database of the standardized results of animal bioassays. Environ Health Perspect 58:9–319 (1984).
5. Gold LS, deVeciana M, Backman GM, Lopipero M, Smith M, Blumenthal R, Levinson R, Bernstein L, Ames BN. Chronological supplement to the carcinogenic potency database: standardized results of animal bioassays published through December 1982. Environ Health Perspect 67:161–200 (1986).
6. Gold LS, Slone TH, Backman GM, Magaw R, DaCosta M, Lopipero P, Blumenthal M, Ames BN. Second chronological supplement to the carcinogenic potency database: standardized

results of animal bioassays published through December 1984 and by the National Toxicology Program through May 1986. Environ Health Perspect 74:237–329 (1987).
7. Gold LS, Slone TH, Backman GM, Eisenberg S, DaCosta M, Wong M, Manley NB, Rohrbach L, Ames BN. Third chronological supplement to the carcinogenic potency database: standardized results of animal bioassays published through December 1986 and by the National Toxicology Program through June 1987. Environ Health Perspect 84:215–286 (1990).
8. Gold LS, Manley NB, Slone TH, Garfinkle TH, Rohrbach L, Ames BN. Fifth plot of the carcinogenic potency database: results of animal bioassays published in the general literature through 1986 and by the National Toxicology Program through 1989. Environ Health Perspect 100:65–135 (1993).
9. Lerner LJ, Jordan VC. Development of antiestrogens and their use in breast cancer: Eighth Cain Memorial Award Lecture. Cancer Res 50:4177–4189 (1990).
10. Klopman G. MULTICASE 1. A hierarchical computer automated structure evaluation program. Quant Struct Act Relat 11:176–184 (1992).
11. Klopman G, Rosenkranz HS. Prediction of carcinogenicity/mutagenicity using MULTICASE. Mutat Res 305:33–46 (1994).
12. Molecular Modeling Pro, Version 1.44. WindowChem Software, Fairchild, California, 1995.
13. Hansch C, Leo A. Calculation of octanol-water partition coefficients by fragments. In: Exploring QSAR: Fundamentals and Applications in Chemistry and Biology (Heller SR, ed). Washington:American Chemical Society, 1995;125–168.
14. Bindal RD, Katzenellenbogen JA. Bis(4-hydroxyphenyl)(2-(phenoxy-sulfonyl)phenyl)methane: isolation and structure elucidation of a novel estrogen from commercial preparations of phenox red (phenolsulfonphthalein). J Med Chem 31:1978–1983 (1988).