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Journal of Molecular Structure (Theochem) 624 (2003) 29–36

THEO CHEM

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Carcinogenic activity in estrone and its derivatives: a theoretical study

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Received 23 June 2002; accepted 11 August 2002

Abstract

Estrone and some of its derivatives have been studied in the context of their possible activity as carcinogenic agents. The study has been carried out in a theoretical fashion (B3LYP/6-31G); the features chosen for the study were: (1) the energy gap between HOMO and HOMO-1, (2) the localized contributions of some regions to the relevant wave functions (related to local density of states) and (3) the aromatic character measured by nucleus independent chemical shift and indirectly by molecular electrostatic potential. Estrone does not have the expected carcinogenic activity but its derivatives exhibit some intriguing behavior.

Keywords: Carcinogenic activity; Estrone; Estradiol; Aromaticity; Theoretical calculations

1. Introduction

Estrone (E1) is a basic feminine hormone and estradiol (E2) is the most important of its precursors. There are at least three main natural reactions involved in the synthesis of E1 and all involve a hormone in the reaction. These hormones are sulphatase [1,2], aromatase [3,4] and 17β-hydroxy-steroid deshydrogenase (17β-HSD) [5,6] each of which gives rise to different biochemical pathways.

In the case of postmenopausal women, the ovaries cease the production of estrogen and the peripheral conversion of androgens to estrogens, by aromatization, becomes the major source for the endogenous estrogen pool [7]. It is now well established that E2 is one of the most important risk factors for the genesis and evolution of breast tumors [8,9]. The formation of this substance in young women depends on the pathways mentioned earlier, i.e. the aromatase pathway that transforms the androgens into estrogens, the sulphatase pathway which converts estrone sulfate (E1S) into estrone, and finally the transformation of the estrone in E2 itself by means of the reductive action of 17β-HSD. However, in the mentioned postmenopausal cases, the main production of estradiol comes via sulphatase, instead of the aromatase hormone, because this hormone loses its activity in these cases.

This topic has received much scientific interest from medical, biomedical and biochemical specialists

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PII: S0166-1280(02)00581-X

since cancer is the leading cause of death in many countries [10–20].

However, there is a question without answer in this context. Why is estradiol a carcinogenic agent and estrone is not? The difference between the molecules is only the presence of a carbonyl group (in the estrone case) versus a hydroxyl group (in the case of estradiol) and the interconversion of one to the other is common. Indeed estradiol is the main source of estrone in young women and its occurrence is essential for the development of feminine characteristics in adolescence.

The main aim of this work is to study this intriguing topic by analyzing the molecular and electronic structure of both molecules. In addition, we have included estrone sulfate, and even a synthetic steroid in order to elucidate which is the source of the carcinogenic activity. The study was carried out by means of density functional methods.

2. Methodology

All calculations were performed using the hybrid B3LYP/6-31G method [21], which combines the exact Hartree–Fock exchange with Becke's theory [22] and uses the Lee–Yang–Parr correlation function [23] in order to include the most important correlations effects. The version used was that belonging to the GAUSSIAN98 code [24] included in the Cerius package [25]. Initial geometries were obtained using the molecular mechanics method [26] included in the same package. This choice of calculation was made because recent studies have demonstrated that the DFT-B3LYP method leads to excellent results for the analysis of geometries and energies [27,28].

Nucleus independent chemical shift (NICS) values were evaluated using the technique developed by Schleyer [29–31] and the gauge-independent atomic orbital method GIAO [32] included in the GAUSSIAN98 code. The free energy (ΔG) and zero point corrections were found from frequency calculations carried out on estrone and estradiol by means of the same code and at the same level of theory. The molecules under study are mainly the estrone and the estradiol but estrone sulphate and diethylstilbestrol (see Fig. 1) also were calculated. The latter results were compared with the obtained data corresponding to estrone and estradiol.

3. Results and discussion

The problem of carcinogenicity yielded by polyaromatic hydrocarbons (PAHs) and related compounds has been considered by several groups and there are various propositions about the source of the problem [33,34]. In the case of compounds with two or more fused aromatic rings, the situation is more or

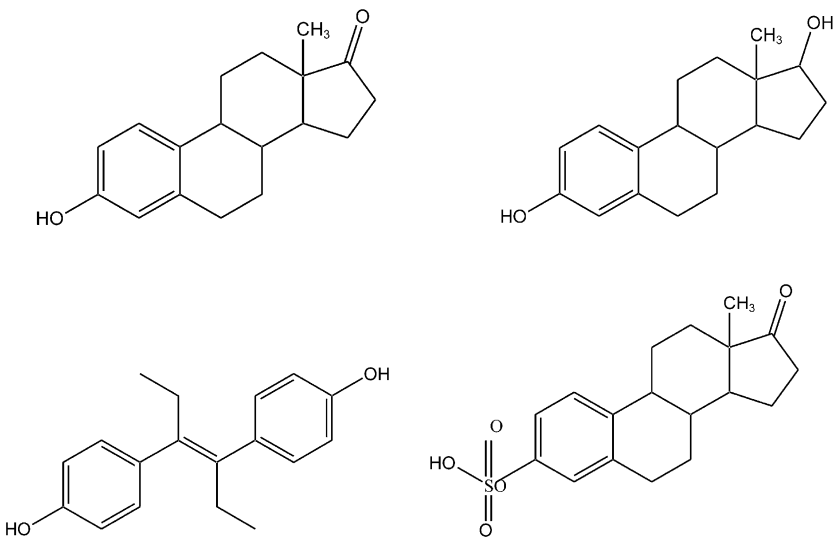

Fig. 1. Molecules under study: estrone, estradiol, diethylestilbestrol and estrone sulphate.

less clear. One terminal ring reacts enzymatically in aqueous media to incorporate hydroxyl group that subsequently reacts also in presence of another enzyme to produce an epoxide [35–39]. These species interact with the purine bases of the DNA chain and change the transmission codes of genetic information (see Fig. 2). The mechanism has been described and studied both experimentally and theoretically [40].

The different regions of the PAHs outlined in Fig. 2 have been studied as potential reactive sites. Indeed the region B in the figure is the so-called 'bay region' and it has been object of many studies [41,42] because it has been suggested that the carcinogenic activity of compounds that contain such a region is larger than that of linear PAHs.

Barone and co-workers suggested a theoretical method for the prediction of the carcinogenic activity [43,44]. Their model is based on three simple rules derived from the energetic difference between the HOMO and HOMO-1 (Δ), the local density of states (LDOS) [45] on the ring and with the highest bond-order (RHBO).

The electronic DOS is defined as the number of electronic states per energy unit. This result can be obtained over a specific molecular region and then arise the concept of LDOS, the concept of ring highest bond-order is simply referred to a ring containing a sextet into the context of a PAH molecule in which we found several fused aromatic rings, following a criterion as that of the Clar's model [46,47].

The rules suggested by these authors may be useful in our case in spite of the fact that we have not a PAH kind of molecules. The basis of the Barone's rules is the reactivity of some aromatic rings into the context of a more complicated molecule. We have in the present case only one aromatic ring, however, the rules will be applicable if the primary reactivity of estrone and estradiol would be focused on their aromatic regions, therefore we will apply a local modification of the rules to analyze the results.

The rules are as follows.

(a) Pyrene-like molecules. If the molecule contains a pyrene-like structure and Δ is greater than 0.25β ($\beta \approx 2.4$ eV), it will be strongly carcinogenic, otherwise the molecule will be inactive in this regard.
(b) Non-pyrene molecules. If the HOMO is the highest contribution to the LDOS over RHBO, the molecule will be completely inactive.
(c) If the HOMO contribution to the LDOS over RHBO is greater than that of HOMO-1 (but not the highest contribution) and $\Delta > 0.15\beta$, the molecule will present a strong or moderate carcinogenic activity. If the HOMO-1 contribution is greater than that of the HOMO, the molecule will present weak or no activity at all.

However, estrone and estradiol are not PAHs. Barone's rules attempt to establish a large separation between HOMO and HOMO-1 in order to have

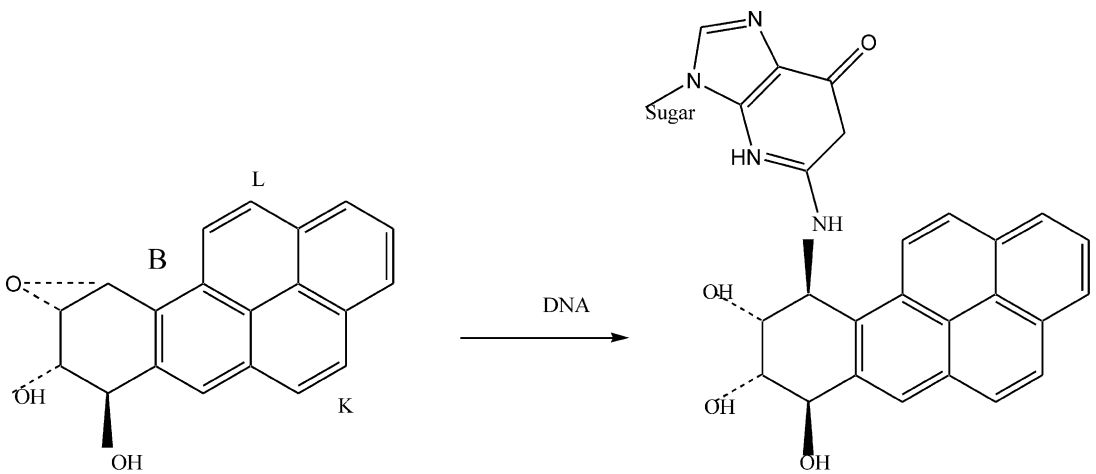

Fig. 2. Generation of a complex from a PAH epoxide and a purine base.

Table 1
Δ(HOMO−HOMO − 1)

Molecule	Δ(eV)
Estrone	0.5703
Estradiol	0.7510
Estrone sulfate	0.2800
Diethylstilbestrol	0.4449

a 'clean' reaction to produce the carcinogenic agent therefore it is important to establish if this kind of criterion can be used in the case of steroid derivatives. It is clear that the reactive pathways followed by these two kinds of organic families must be different in living beings, however, we are going to carry out a comparison only from an organic chemistry point of view considering the estrone derivatives belongs similar ended rings than PAHs.

The first interesting result in our case is related to this proposition. Our calculations show a trend that is similar to the Barone proposition. Table 1 shows the values of Δ for the molecules under study. The analysis made on the basis of the proposition of Barone is very interesting because, although our species are not pyrene-like molecules, the results indicate that estradiol is a carcinogenic agent but estrone and the other compounds are not. This is an intriguing result, for it is correct with respect to the estrone–estradiol pair. However, it is known that diethylstilbestrol is a very carcinogenic agent, whereas our result indicates that it should be practically inactive.

This last result can be rationalized on the basis of the second criterion of Barone, we indirectly obtain the LDOS values by relating them to the shape and nature of the frontier molecular orbitals. We expect that if the HOMO and HOMO-1 are not localized in the same regions, the compound will not be carcinogenic. On the other hand if the localization and nature of both orbitals are similar, we expect the substance to exhibit carcinogenic behavior. The sets of orbitals corresponding to estrone and estradiol are shown in Fig. 3. The difference is obvious for in the case of estrone the orbitals are completely different whereas in the case of estradiol they are localized in the same ring atoms.

The case of diethylstilbestrol is also analyzed under the same criterion and its behavior is similar to that of estradiol. In this case, both orbitals are localized on the same atoms. Therefore, this criterion confirms that this molecule should have carcinogen activity (see Fig. 4). The estrone sulphate has a very similar behavior to the estrone, i.e. the HOMO and HOMO-1 have very different localization properties. Therefore, this molecule is predicted to have no carcinogenic activity on the basis of this criterion (see Fig. 4).

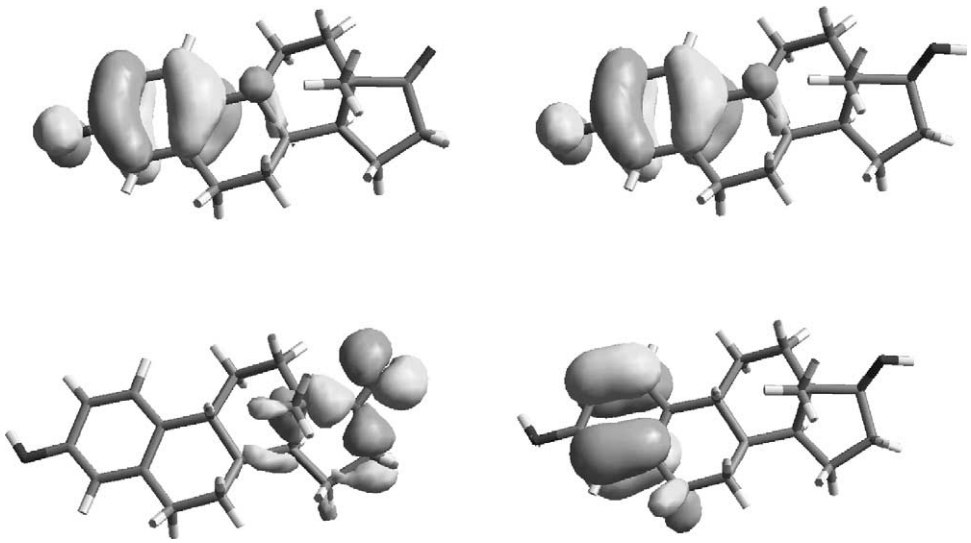

Fig. 3. HOMO and HOMO-1 of estrone and estradiol.

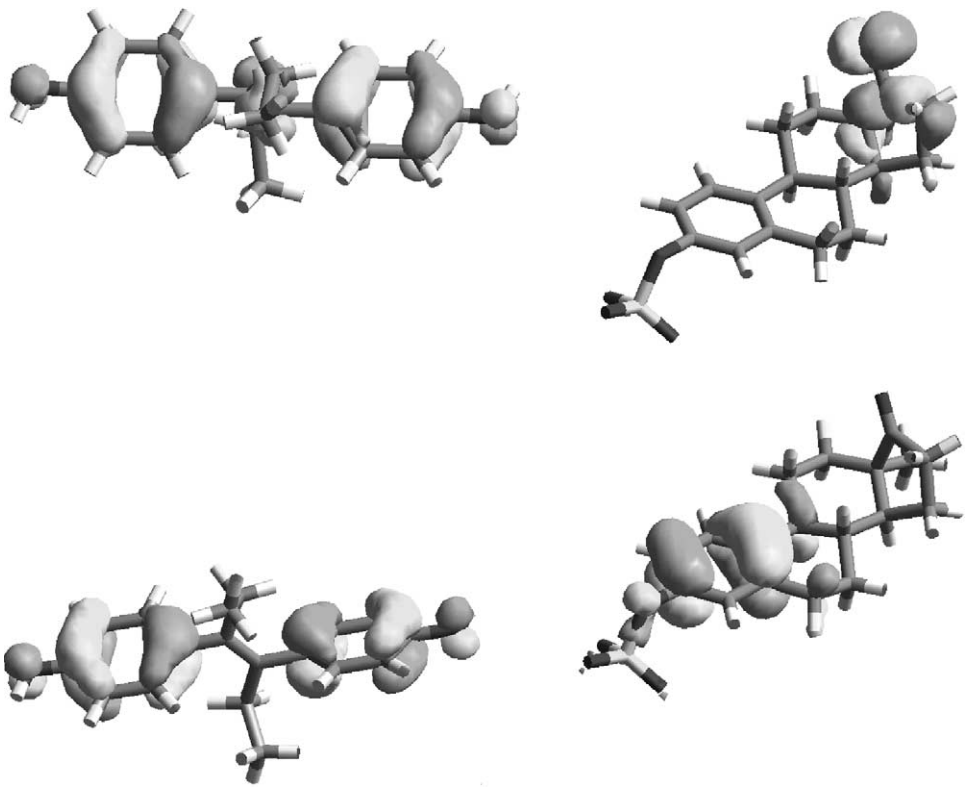

Fig. 4. HOMO and HOMO-1 of diethylstilbestrol and estrone sulphate.

However, the question about the carcinogenic activity of estrone and estradiol has not been answered. By now we have only a confirmation of the experimental behavior, but still there is no information relating to the chemical cause of why estradiol is carcinogenic and estrone is not. In order to tackle this topic, we studied the kind of reaction that should be followed to produce the epoxide species that participate in the reaction with DNA.

Estrone, estradiol and their derivatives are esteroid molecules. Because they cannot be classified as PAHs, it therefore is important to establish if they can participate in reactions that are known to occur for PAHs. The epoxidation reaction is a known procedure in arene molecules [39,48] (see Fig. 5). One or several hydroxyl groups can be substituted in one of the terminal aromatic rings. The process implies the rupture of aromaticity because the main step of

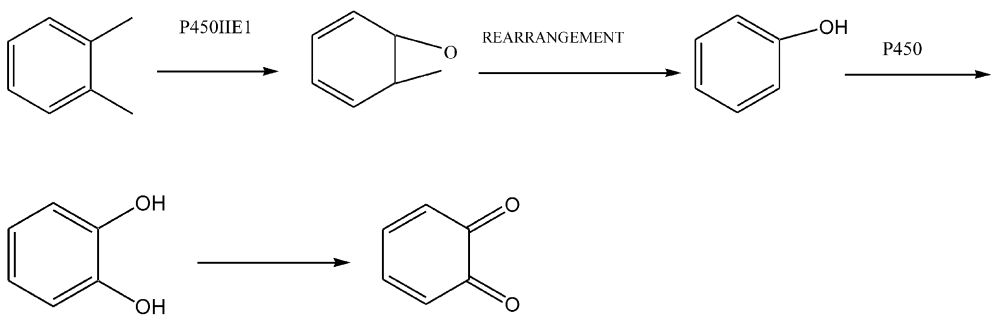

Fig. 5. Epoxidation pathway for the synthesis of ortho-quinone.

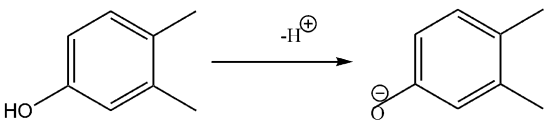

Fig. 6. Ionization of a phenolic ring.

the reaction is the formation of an epoxide that yields an ortho-quinone where both compounds are not aromatic.

The epoxide is susceptible to involvement in a nucleophilic reaction with a terminal amine group coming from a puric base of the DNA chain, for example, a guanine fragment (see Fig. 2). This reaction precludes the genetic transmission and is the cause of mutation that generates cancer.

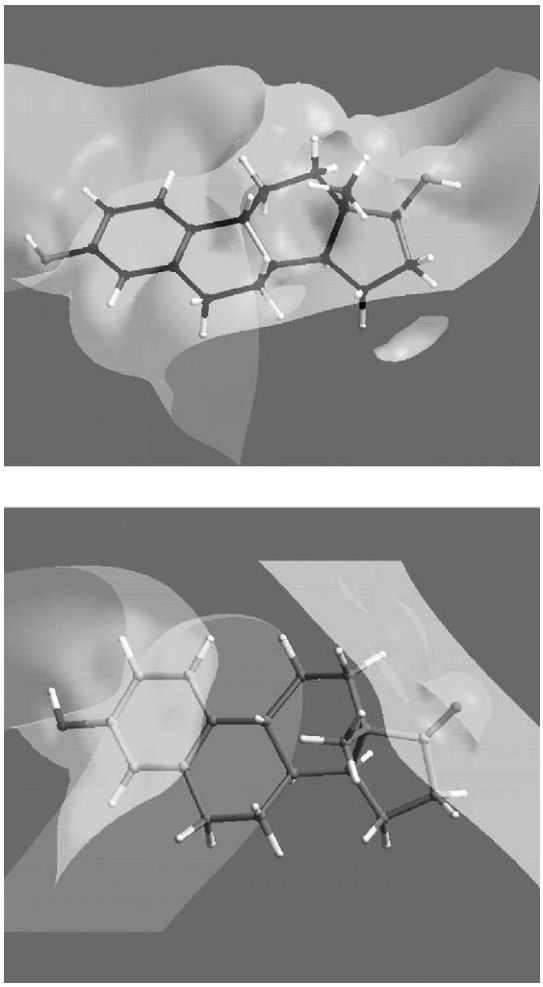

Fig. 7. Electrostatic potential schemes of estradiol and estrone.

The important topic for our study is the epoxidation reaction. Both molecules, estrone as well as estradiol, are susceptible to participation in reactions that involve aromatic rings. It is possible to suggest that the epoxidation reaction can progress in molecules of this kind, as in the case of PAHs, because two positions of the terminal aromatic ring are blocked as well as in the case of PAHs. Therefore, we can start from the idea that the reaction is possible in steroids.

The reaction that we expect is very simple (see Fig. 6). While it is not a typical reaction of an aromatic compound, such as electrophilic aromatic substitution, the aromaticity plays a very important role. The first step consists of the ionization of the terminal hydroxyl group. If we consider the fragment of the molecule where the hydroxyl is supported as a phenolic species, we can see that the hydrogen atom should be easily lost. This therefore is the cause of the acidity of the phenolic substances. Thus, in our case, we observe a process of this kind. Nevertheless, the ionization takes place only if the ring is aromatic. The hydrogen atom is acidic because the hydroxyl group releases electrons to the ring and the electronic density acts as an electronegative center. Therefore, the more aromatic the ring is, the better the ionization of the hydroxyl group will be.

The resulting anion holds a negative charge on the oxygen atom and can start an electronic rearrangement that leads to the rupture of aromaticity and the formation of the epoxide compound. This epoxide can change further, yielding polyalcohols or ortho-quinones, but these steps are not important for our study. The important part is the ionization step.

Aromaticity values of estrone and estradiol were calculated by the NICS technique [29–31]. These values −9.215 and −10.1847, respectively, may be compared with the value for benzene of −11.5 at the same level of theory [29–31]. It is very interesting to note the appreciable difference between both values considering the only change from estradiol to estrone is the hydroxyl group of the former versus the carbonyl group of the latter. What is the reason for this marked difference?

The aromaticity of both molecules was indirectly estimated by the calculation of the electrostatic potential as suggested by De Proft and Geerlings [49]

and also by Suresh and Gadre [50], The result was dramatic, for the electrostatic potential surface of estradiol is extended practically over the entire molecule, although it is mainly generated by the aromatic ring. However, in the case of estrone there are two electrostatic potential surfaces of the same sign, one generated by the aromatic ring and the other by the carbonyl group. Thus, the aromaticity of estrone is diminished by the competitive presence of the second electrostatic potential (see Fig. 7). Therefore, based on these results we can conclude that the estradiol molecule is more aromatic than the estrone molecule as was previously estimated by the NICS technique.

The phenomenon described in the last paragraph is fundamental to explain the carcinogenic activity of both molecules, since the activity of the phenolic fragment is ruled by aromatic character in each case. On this basis, it seems estradiol can easily generate the corresponding anion so as to produce the epoxide whereas estrone has a clear impediment.

4. Conclusions

The carcinogenic activity of estrone and estradiol was analyzed by taking advantage of different models. The criteria developed by Barone and coworkers to analyze PAH were applied even though estrone and its derivatives cannot be classified as PAHs. The first criterion (for pyrene-like molecules) predicts carcinogenic activity for estradiol but failed in predicting the same activity of ethylstilbestrol. The second criterion based on the analysis of LDOS yields results that match very well in all cases.

The original question about the source of carcinogenic activity of estradiol and the lack of the same in estrone was resolved by analysis of the aromaticity of both molecules. NICS results allowed us to see that estradiol is more aromatic than estrone, in that estradiol has only one electrostatic potential surface localized on the aromatic ring, whereas estrone has two, one on the aromatic ring and the other localized on the carbonyl group. The interaction between the two electrostatic potentials diminishes the aromaticity of this molecule.

Acknowledgements

The authors would like to acknowledge Dr Vincent Ortiz for helpful discussion, Mrs Sara Jiménez and Ms Maria Teresa Vázquez for technical support. A.P. thanks to the Instituto Politecnico Nacional for support.

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