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Reaction of Aspirin with Fecapentaene-12: A Possibility for Aspirin to Make Fecapentaene-12 Lose its Mutagenecity

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While the controversy continues over the carcinogenicity of fecapentaene-12 (Fp-12), as a potent colorectal mutagen, and while reports on reducing the risk of colon cancer by consumption of aspirin are still widespread, our HF and B3LYP calculations indicate the possibility of the loss of mutagenecity of Fp-12 through its reaction with aspirin, where an acetyl transfer from aspirin to a primary or secondary hydroxyl group of Fp-12 appears exothermic.

Key Words: Fp-12, Fecapentaene-12, Mutagen, Aspirin, Acetyl, DFT, *ab initio*, HF, B3LYP, Carcinogenicity, Colon cancer.

INTRODUCTION

Diet is important in the ethiology of colon cancer[1,2]. Several hypotheses on the causative agents and their mechanisms are proposed, including a group of fecal mutagens named fecapentaenes[3,4]. These are found in human feces and may play a role in the pathogenesis of colon carcinoma[2]. Among these mutagens, the most important is fecapentaene-12 (Fp-12), **1**, which has a (S)-configuration (Fig. 1)[5,6]. Fecapentaenes are formed in the colon by certain bacteroides from polyunsaturated ether phospholipids. They exhibit a specific UV-triplet absorbance spectrum. They are highly unstable and undergo degradation when exposed to light, oxygen and acidic pH[4,7,8]. The genetoxic effects of fecapentaenes are previously studied in mammalian cells and a prototype for these compounds, causes DNA single strand breaks, sister chromatid exchanges and mutations in cultured human fibroblasts[4,9]. These results indicate that **1** is a potent genetoxic agent in human cells and a potent colon cancer mutagen.

Recently, we reported[10,11] the kinetics of interactions between **1** and DNA. In order to perform kinetic investigations on the stability of **1** in the absence and presence of DNA at different pHs, Fp-12 concentrations lower than 1 μM, at room temperature are employed in each case. Data have been analyzed by spectrofluometry as well as UV-Vis spectroscopy.

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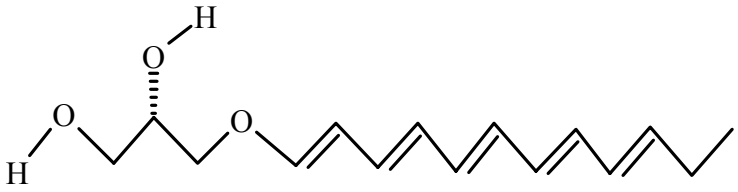


Fig.1. Structure of fecapentaene-12 (Fp-12), **1**

Absorption spectrum of Fp-12 in standard buffer shows three peaks at 324, 338 and 356 nm, which decrease in intensity as a function of time. In various pHs, the interaction between **1** and calf thymus DNA under similar conditions is clearly observed. As increasing amounts of DNA are added to the standard buffer solution of **1**, the original λ_{max} at 338 nm shows progressive hypochromic red-shift to 346 nm and then to 359 nm. This spectral shift is related to binding interaction of **1** with DNA, which gives three first-order rate constants whose values are pH-dependent. These are possibly related to the DNA-interstrand cross-links, DNA-single-strand breaks and DNA-protein cross-links reported by previous investigators[10,11].

Again, the controversial **1** is a fecal mutagen whose occurrence is correlated with population at risk for colon cancer[11-13]. Some call it a carcinogen[2,12], while others challenge this hypothesis[14]. On the other hand, substantial experimental, clinical and epidemiological studies indicate a 30-50 % reduction in the risk of colorectal neoplasia among regular users of aspirin, **2**[15].

Here, we discuss the possibility of interaction between **1** and **2** through five alternatives routes (**a-e**) (**Scheme-I**), *ab initio* and DFT calculations are carried out at HF/6-31G* and B3LYP/6-31G* levels of theory.

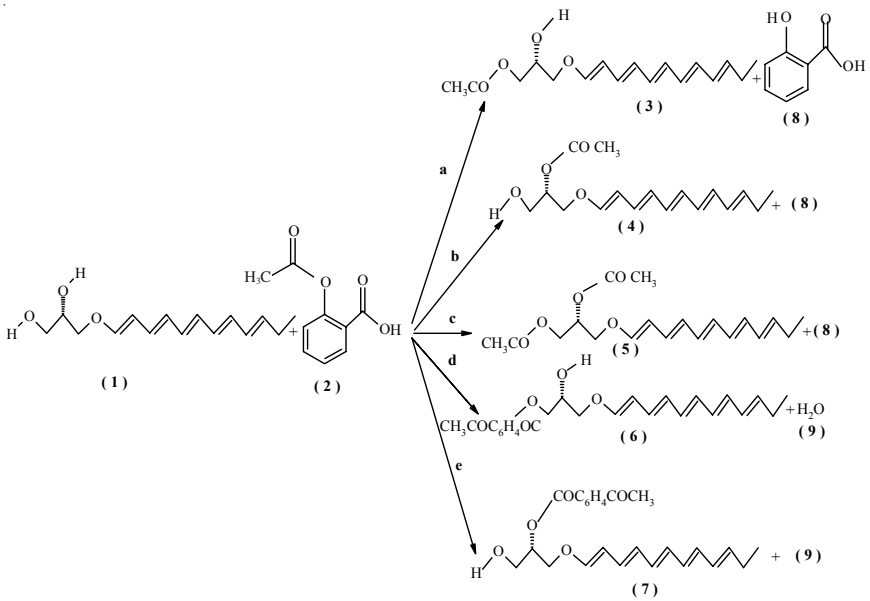
EXPERIMENTAL

All calculations are preformed using the Gaussian 98 program packages[16]. To assess the performance of this approach, all species are computed at higher theoretical levels; in a way that HF/STO-3G outputs are used as inputs for the HF/6-31G*. Also, HF/6-31G* outputs are used as inputs for the B3LYP/6-31G*. These are done in order to obtain rather highly accurate values for changes in thermal energies (ΔE_{rxn}), enthalpies (ΔH_{rxn}) and Gibbs free energies (ΔG_{rxn}) of five alternative routes (**a-e**). For calculating energy minima, the keyword FOPT is used. In order to confirm the nature of the stationary points frequency calculations are carried out at both *ab initio* (HF/6-31G*) and DFT (B3LYP/6-31G*) levels of theory, with the keyword FREQ=NORAMAN. Thermodynamic functions obtained through frequency calculations are corrected by the scaling factor of 0.99 and 0.89 for B3LYP/6-31G* and HF/6-31G*, respectively. This is to

account for differences between the harmonic vibrations and the harmonic oscillations of the actual bonds. For minimum state species, only real frequency values (with a positive sign) are accepted.

RESULTS AND DISCUSSION

The routes **a**, **b** and **c** are analogous to the reaction of **2** with PGH synthase[17-19]. In these routes, **2** transfers its acetyl moiety to primary and/or secondary hydroxyl groups of **1**, forming primary monoacetate **3** (route **a**), secondary monoacetate **4** (route **b**) and/or diacetate **5** (route **c**, **Scheme-I**). The HF/6-31G* calculated changes in thermal Gibbs free energy for forming **3**, **4** and **5** are -14.9, -12.5 and -11.7 kcal/mol, respectively (Tables 1 and 2, **Scheme-I**).



Scheme-I: Possible routes (**a-e**) for the reaction of Fp-12 (**1**) with aspirin (**2**)

However at B3LYP/6-31G* level of theory, changes in thermal Gibbs free energy of forming **3**, **4** and **5** are -6.2, -3.6 and 3.7 kcal/mol, respectively (Tables 1 and 2, **Scheme-I**). Hence, both methods of calculations indicate that the formation of mono protected fecapentaene-12, **3** and/or **4**, is more thermodynamically feasible than the formation of diprotected fecapentaene-12 (**5**).

Considering the esterification of carboxylic acids with alcohols, which are known to occur when a driving force is available, to shift the equilibrium to the right[20,21], one may expect **2** to react with **1** to form fecapentaenyl-12 primary monosalicylate (**6**) (**Scheme-I**, route **d**), and/or fecapentaenyl-12 secondary monosalicylate (**7**) (route **e**). At HF/6-31G* level of theory,

TABLE-1
HF/6-31G* AND B3LYP/6-31G* OPTIMIZED THE CORRECTED THERMAL ENERGIES (E), THERMAL ENTHALPIES (H) AND THERMAL FREE ENERGIES (G), IN kcal mol^{-1}, for Fp-12, ASPIRIN AND OTHER COMPOUNDS ARE SHOWN IN **SCHEME-I**

Compd.	HF/6-31G* (kcalmol^{-1})			B3LYP/6-31G* (kcalmol^{-1})		
	E	H	G	E	H	G
1	-504873	-504871	-504960	-508081	-508080	-508176
2	-404495	-404494	-404549	-406843	-406842	-406906
3	-600074	-600073	-600172	-603830	-603828	-603936
4	-600071	-600070	-600170	-603827	-603826	-603933
5	-695257	-695256	-695366	-699563	-699562	-699680
6	-861709	-861708	-861828	-867009	-867008	-867138
7	-861693	-861692	-861809	-866997	-866995	-867123
8	-309303	-309302	-309352	-311100	-311098	-311152
9	-47666	-47665	-47690	-47917	-47916	-47943

TABLE-2
HF/6-31G* AND B3LYP/6-31G* CALCULATED CHANGES IN THERMAL ENERGY, (ΔE_{rxn}), THERMAL ENTHALPIES (ΔH_{rxn}) AND THERMAL FREE ENERGIES (ΔG_{rxn}), IN kcal mol^{-1}, FOR FIVE REACTION ROUTS, SHOWN IN **SCHEME-I**

Routs	HF/6-31G* (kcalmol^{-1})			B3LYP/6-31G* (kcalmol^{-1})		
	ΔE_{rxn}	ΔH_{rxn}	ΔG_{rxn}	ΔE_{rxn}	ΔH_{rxn}	ΔG_{rxn}
a	-8.7521	-8.7521	-14.9885	-5.7584	-5.7584	-6.2129
b	-6.0304	-6.0304	-12.5231	-3.0300	-3.0306	-3.5669
c	0.5486	0.54864	-11.6539	3.5696	3.5683	3.6582
d	-7.4557	-7.4551	9.1658	-2.4968	-2.4975	0.8067
e	8.2032	8.2032	9.4555	10.2146	10.2141	15.2033

the changes in thermal Gibbs free energy of 9.2 and 9.5 kcal/mol are anticipated for formation **6** and **7**, respectively. Whereas, at B3LYP/6-31G* level of theory, one observes the changes in thermal Gibbs free energy of 0.8 and 15.2 kcal/mol, respectively (Tables 1 and 2, **Scheme-I**). Hence, both *ab initio* and DFT methods show the formation of **6** and/or **7** to be thermodynamically not feasible.

Therefore, upon reaction of **2** with **1** the formation of mono-protected Fp-12 (**3**) is more exothermic than **4**. On the other hand, formation of esters **6** and/or **7** is not energetically feasible.

Conclusion

Since protection of hydroxyl groups of **1**, in the form of the known precursor of fecapentaene-12, has been shown to translate into the loss of mutagenicity of Fp-12[22], it is reasonable to expect similar effects *via* pro-

tection of **1** and formation of **3** upon reaction with **2**. On the basis, one may suspect that reducing the risk of colon cancer by consumption of aspirin may be related to a possible reaction of Fp-12 with aspirin and formation a protected Fp-12, **3** with no mutagenecity.

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