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Some Doping Agents, Relations of Gas Phase Energetics, Thermal Properties and Biosynthesis

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BAZI DOPİNG MOLEKÜLLERİNİN GAZ FAZI ENERJETİĞİ VE ISISAL ÖZELLİKLERİ İLE BİYOSENTEZİ ARASINDAKİ İLİŞKİLER

Özet

Steroid hormonlarının en önemli özelliklerinden biri diğer hormonlardan farklı olarak salgılanmak üzere depolanmayıp, gerektiğinde biyosentez hızıyla düzeylerinin denetlenmesidir. İkinci önemli karakteristikleri ise biyosentez yolunun enzimleri ile ilgilidir. Örneğin 11-HSD çalıştığı organa bağlı olarak hidrogenaz veya dehidrogenaz olarak davranmasıdır.

Bu çalışma çok adımlı hızlı steroid biyosentezi ve steroid moleküllerinin enerjetiği (iyonlaşma ve görünüm gerilimleri ve etkinleşme enerjileri) arasındaki ilişkileri saptamak üzere, düşük enerjili elektronla bombardımanlı iyon kaynağı içeren VARIAN-MAT-112 kütle spektrometresi ve Shimadzu DT-30 Termal Analizör kullanılarak yapılmıştır. Enerjetik çalışmalarının iyonlaşma gerilimleri bulguları biyosentezdeki ara ürünlerinin zincirleme hızlı tepkimeye elverişli olduklarını göstermiştir. Ayrıca biyolojik aktif olarak bilinen C-3 (-OH), C-3 (=0), C-10 ($-CH_3$), C-11 (-OH), C-13 ($-CH_3$), C-17 (-OH), C-17 (=0) ve C-21 ($-CH_2OH$) gruplarının koparılması ile oluşturulan kısımların görünüm gerilimleri ilgili molekülün iyonlaşma geriliminden düşük bulunmuştur. Bu bulgular bu tür moleküllerin gaz fazında kararsız fakat biyolojik koşullarda aktif olduğunu göstermektedir.

Summary

The most important characteristics of steroid hormones unlike other hormones are that they are not stored for subsequent secretion, but when required the steroid levels are controlled by the rate of biosynthesis.

The second important characteristics are related to the enzymes of the pathway, e.g. 11-HSD acts as hydrogenase or dehydrogenase depending on the organ which the enzyme works in it.

This study has been carried out to find the relationships between multistep fast biosynthesis and energetics of steroid molecules. Ionisation (I), Appearence potentials (A) and Activation energies (Ea) by using gas phase low energy electron impact ion source of VARIAN-MAT-112 Mass spectrometer to obtain I and A, and Shimadzu-DT-30 Thermal analyser to obtain thermal data. Results of energetics has shown that the intermediates of biosynthesis are available for fast chain reactions. Furthermore some of the appearance potentials of the residues which were obtained by loosing the well known biologically active sites C-3 (-OH), C-3 (=0), C-10 ($-CH_3$), C-11 (-OH), C-13 ($-CH_3$), C-17 (-OH), C-17 (=0) and C-21 ($-CH_2OH$) were found to be lower than ionisation potentials of the related whole molecules. These findings also confirm that steroids are unstable in the gas phase and but active under biological conditions.

Key words : *Steroids - Gas Phase Energetics- Ionisation and Appearence Potentials - Activation Energy - Thermal Analysis Data - Mass Spectrometry - Relations With Biosynthesis*

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INTRODUCTION

The biological active sites of steroid molecules have been described as C-3-OH, C-3=0, C-10-CH_3 , C-11-OH, C-13-CH_3, C-17-OH, C-17=0 and C-21-OH (1). There are many attemps and studies made to elucidate the steroid biosynthesis in the mitochondria of the cell of the inner and outside of the adrenal cortex. And in some of was shown that the initial biosynthesis of the steroid molecules is in these regions of the adrenal cortex (2-5). Mitochondrial cytochrome(s) P-450 was found to be effective on biosynthesis of pregnenolone from cholesterol by using spectroscopic tracing techniques. In some studies the spectroscopic shift of the λ_{max} of the cytochrome(s) P-450 was related to the quality and quantity of the oxidised substrate (6-8). And in some studies the changes in activities of 11-ß hydroxylase and 11-ß-hydroxystreoid dehydrogenase (11-HSD) were noted to be related to the energetics of steroid biosynthesis. But these data were limited on corversion of 11-deoxycortisol to cortisol and cortisol to cortisone and *vice-versa* (9-10). The most interesting part of these findings was that on the activity of 11-HSD, depending to the organ, it was shown with reducing or oxidating properties or activities (9).

One of the oldest studies on gas phase energetics of steroid molecules has been carried out by *Mayo* and *Reed* (11) using low energy electron impact. After *Mayo* and *Reed* (11) there are many studies in the literature have been elucidated the fragmentation mechanism of steroids; most of them was rewieved by *Budzikiewicz* (12). Another review on ionisation and appearance potentials was made by *Zaretsky* (13).

This work was carried out to elucidate the relations between energetics, biosynthesis and thermal analysis data.

MATERIALS AND METHODS

Ionisation potentials (I) and appearance potentials (A) of the steroid molecules were obtained from Varian-Mat-112 Mass spectrometer with electron impact ion source. Thermal analysis of these compounds was done with Shimadzu-DT-30 thermal analyser with a derivative module and dynamic conditions. Pure steroids were obtained from Merck Darmstadt and Sigma Chemicals Co.

Dynamic thermograms under dry nitrogen stream (25 ml/min) from ambient temparature up to 600°C by increasing 10 deg/min of each water free and pure substance were obtained. And by application of these thermal values to *Freeman-Carrol* method (14) activation energies of each molecule were calculated. By the aids of thermal data a common vaporisation temperature was chosen that 120°C ± 1°C for all compounds under10-7 torr vacum of the EI ion source from the direct insertion probe of the mass spectrometer. After getting a stable pressure in the ion source for each compound the electron energy was reduced 0.2 eV step by step from 20 eV to 6 eV of whose point shows no ion current. This process was done for molecular ion, important fragment ions of each compound and for the references water and molecular ion of cholesterol. For each step the ion current was measured by useing 4 digit milivoltmeter by pluggings it paralel to the potentiometric recorder. To obtain (I) and (A) value electron energy versus

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on current plots (Ionisation Efficiency Curves) were drawn and vanishing current method was applied to these curves (15). By comparing the obtained values by reference values the corrected I and A values were calculated.

Moreover the steroids of different molecular weight following each other on the biochemical pathway were inserted, vaporised and idnised who follow as dual mixtures. So the difference between I values of the molecular ions were obtained directly. Ion monitoring and selections were made by using 30 seconds integration time of the multi ion selector (MIS) of the instrument.

RESULTS and DISCUSSION

The energetic values of the reactants (substractes) and the products of each important step on the pathway of steroid biosynthesis have been printed under their formulas with related elucidations. Cumulative energetic values have been given in Table I dynamic thermograms have been given as Figures 1-4.

Table I. Cumulative thermal and energetic values of some steroid molecules[a).]

Molecules	*I(eV)*	*A(M-18)(eV)*	*Ea(kJ/mol)*	*Inital Degrad. Temp (°C)*	*Max, Degrad. Temp (°C)*
Cholesterol	10.93	11.45	142.77	282	355
Pregnanediol	10.47	10.51	*	*	*
Pregnanolone	10.89	10.34	*	*	*
Androstandione	10.22	11.25	69.77	257	347
Dehydro-epi-androsterone	9.75	10.55	112.35	255	332
Androsterone	9.29	8.889	112.43	270	325
Progesterone	10.22	**	81.14	250	335
Cortisone	11.15	10.95	*	*	*
Cortisol	18.85	10.45	*	*	*
Aetiocholanol	10.08	9.98	66.03	250	323
Testosterone	10.26	10.48	103.23	250	298
Estrone	8.04	**	*	*	*
Estradiol	7.59	**	63.04	270	355
Estriol	7.92	**	78.97	220	280

a) For energetic values of other fragments refer to our previous study (Ref. 16).
* Material not sufficient for analysis.
** Peak not abundant for Ionisation Efficiency Curves.

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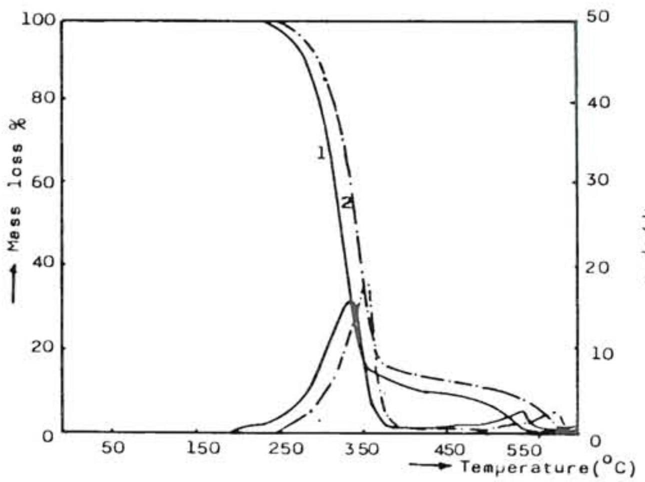

Figure 1. Dynamic thermograms of 1. Progesterone, 2. Cholesterol.

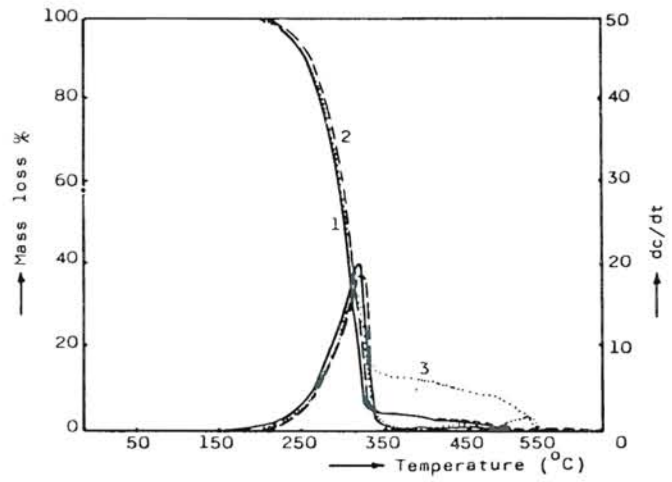

Figure 2. Dynamic thermograms of 1. Aetiocholanol, 2. Androsterone, 3. Androstandione.

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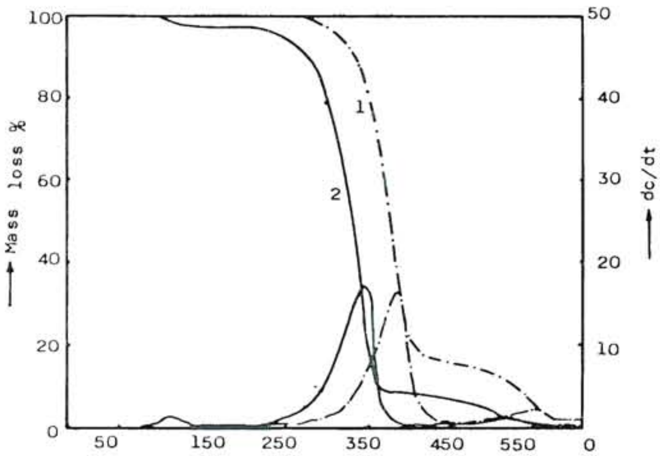

Figure 3. Dynamic thermograms of 1.Eostriol, 2. Eostradiol.

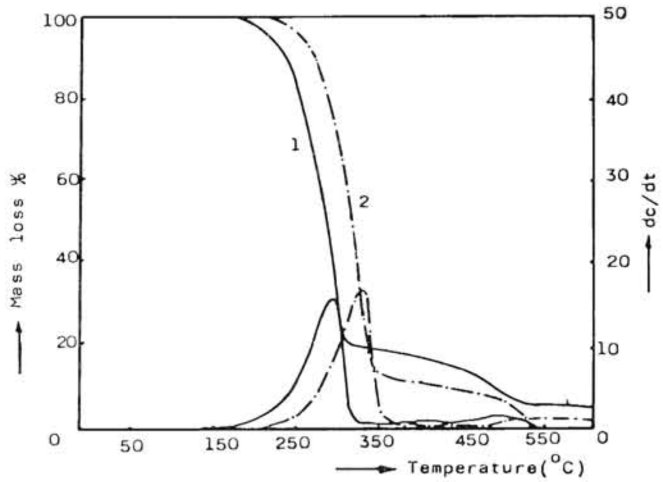

Figure 4. Dynamic thermograms of 1. Testosterone, 2. Dehidroepiandrosterone.

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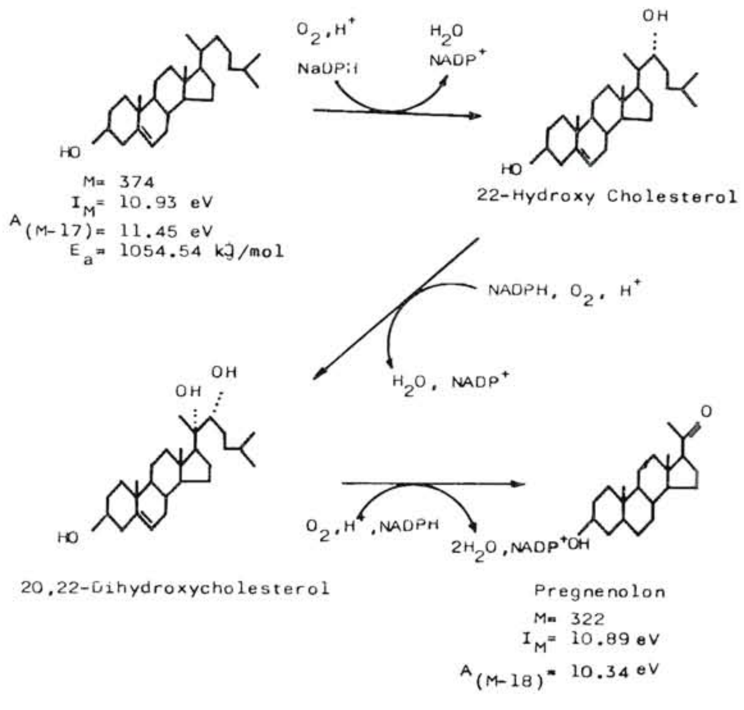
1. Pregnenolone from Cholesterol $\Delta E = -4\ mV$

Biosynthesis of pregnenolone from cholesterol in mitochondrial complex includes three monooxygenases and is followed by lyase reactions. Also the difference between the ionisation potentials of the molecular ions is very small, it has no mean because of I values of hydroxycholesteroles. But these values are important on comparison of the energies of the HOMO's (Highest Occupied Molecular Orbitals) of the related compounds. Moreover data for pregnenolone is I_M-$A_{(M-18)}$=55 meV and A below I, so it can be said that the 3-OH group of pregnenolone is unstable and should be have as an active site on the molecule.



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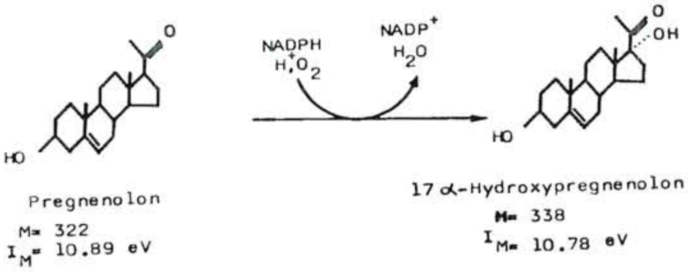
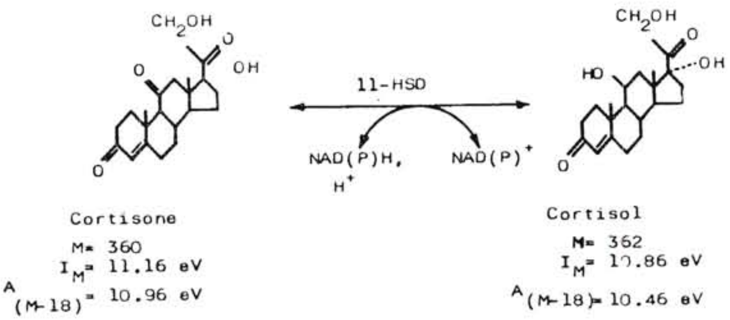
2. 17 α - Hydroxypregnenolon from pregnenolon ΔE = -11 mV



This step is a monooxygenase catalysed hydroxylation, and the difference between I's of the proruct and substrate is 11 mV. This has shown that 17α - Hydroxypregnolone is more unstable than pregnenolone or, expressed otherwise, E_{HOMO} of pregnenolone is 11 mV lower than E_{HOMO} of 17 α - hydroxypregnenolone. This difference with higher quantity was found between Cortison and Cortisol as shown below.

3. Cortisol from Cortisone ΔE = 30 mV (MIS)



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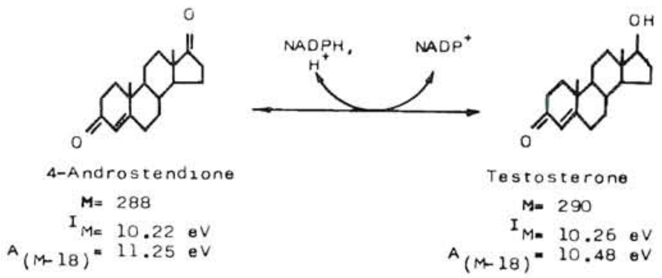
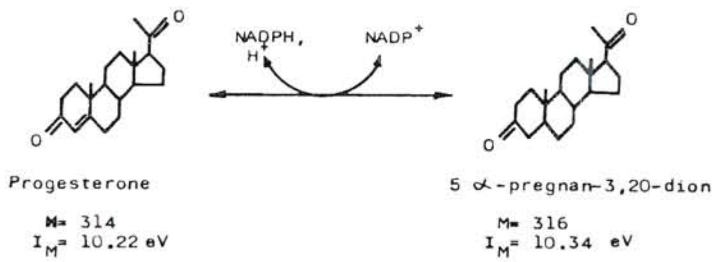
There is only a single difference between cortisone and cortisol, 11-position of being cortisone C=O and of cortisol C-OH. And this difference has made the E_{HOMO} of cortisone 30 mV lower than E_{HOMO} of cortisol. So cortisol should behave more unstable and more reactive than cortisone. There is another evidence showing the reactivity of OH groups oi cortisol and cortison. The appearant potential of M-18 fragments is lower than Ionisation potentials of whole molecules.

4. Testosterone from 4-Androstendione ΔE = 4 mV (MIS)



This step is catalysed by a Hydroxysteroid dehydrogenase. And tha function of this enzyme seems to be reversible because of very low difference ΔE = 4 mV. The same elucation could be made for step-5 which is a Red-Ox reaction of a double bond.

5. 5α-Pregnan-3, 20-dione from Progesterone ΔE = 12 mV (MIS)



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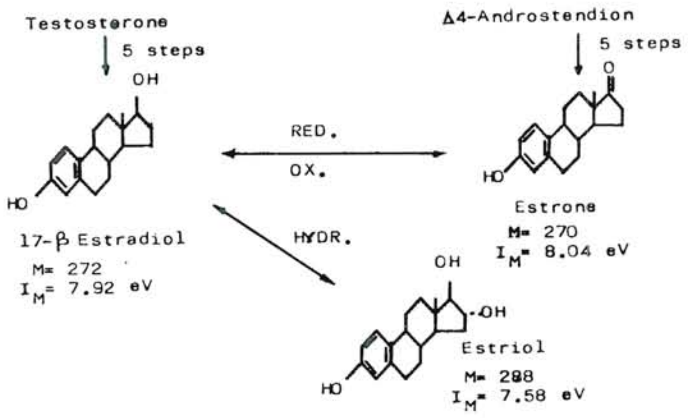
Quantitative results of this study have been shown that the differences of E_{HOMO} of substrates and products of all reactions except reactions catalysed by desmolases and lyases are very small if compared by Red-Ox energies of NAD(P)/NAD(P)H. And because the same relations with activation energies the fast biosynthesis and interconversions of steroid molecules could easily done by mitocondrial and/or microsomal cytochrome(s)-P-450 systems as argued by *Monder* (9) and *Howard* and *Eacho* (10).

Interconversions of estrogens are as shown below. Because we have get no sufficient quantitative data about intermadiates of Testosterone-Estradiol and Androstendione-Estrone we could not present any qualitative elucadation about them. But from the limited thermal and gas phase energetic values of Estrone, Estradiol and Estriol we can only elucidate that the interconversion of these molecules should be appeared easily and rapidly.



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