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Electronic structure and biological activity of steroids

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Abstract

We present the analysis of the electronic structure for 31 steroids by using HeI UV photoelectron spectroscopy (UPS) and MO calculations. The electronic structure of molecules in the gas phase is related directly to steroid–receptor binding measurements. The results indicate that formally 'inert' σ-skeleton plays a crucial role in diversifying the electronic structures of the title compounds ('ribbon-orbital effect'). This is an attempt to rationalize the biological activity of steroids (represented through steroid–receptor binding) by making direct correlation between spectroscopic and biological data.

Keywords: Photoelectron spectroscopy; Steroids

1. Introduction

Steroids are compounds whose biochemical importance has been well established. Their biological activity is based on binding to suitable protein receptors in tissue cells and the formation of the steroid–receptor (SR) complex. The investigation of SR complexes is a difficult problem, which includes both stereochemical and electronic structure aspects. The stereochemical aspect has been reviewed recently [1]. The strength of binding in the SR complex may (besides steric factors) depend on the electronic structure and our aim in this work is to establish how discernible that influence is. The gas-phase UV photoelectron spectroscopy (UPS) is one of the best methods to study electronic structures and has been applied to steroids in the past [2–6]. Concomitantly with UPS studies, the molecular and electronic structure and especially the identity of frontier orbitals in steroids was investigated theoretically by various groups [7–16]. Steroids present a very challenging, but interesting problem for UPS for the reasons outlined below:

1. deconvolution of spectral bands may be difficult due to strong band overlap; and
2. variable photon energy measurements

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(HeI/HeII) are not practical due to involatility of samples. Low HeII photon flux requires higher sample vapor pressure that is difficult to achieve with involatile samples.

2. Experimental and theoretical methods

The choice of sample compounds was limited to that which were available commercially, in quantities necessary for UPS measurements. The samples which were purchased from Fluka Chemie AG, were of $>96\%$ purity which was estimated by GC. HeI photoelectron spectra were recorded on the Vacuum Generators UV-G3 spectrometer and calibrated with Xe gas. Sample temperatures were in the range 150–200°C. Only regions of low ionization potential (IP) could be assigned and hence only these regions are displayed in Figs. 1 and 2. AM1 calculations which included full geometry optimization, were performed with HyperChem 5 program [17] in order to get orbital ordering and define MO character of frontier orbitals and thus aid spectral assignment. The relevant references for MO calculations and UPS data for each steroid molecule are

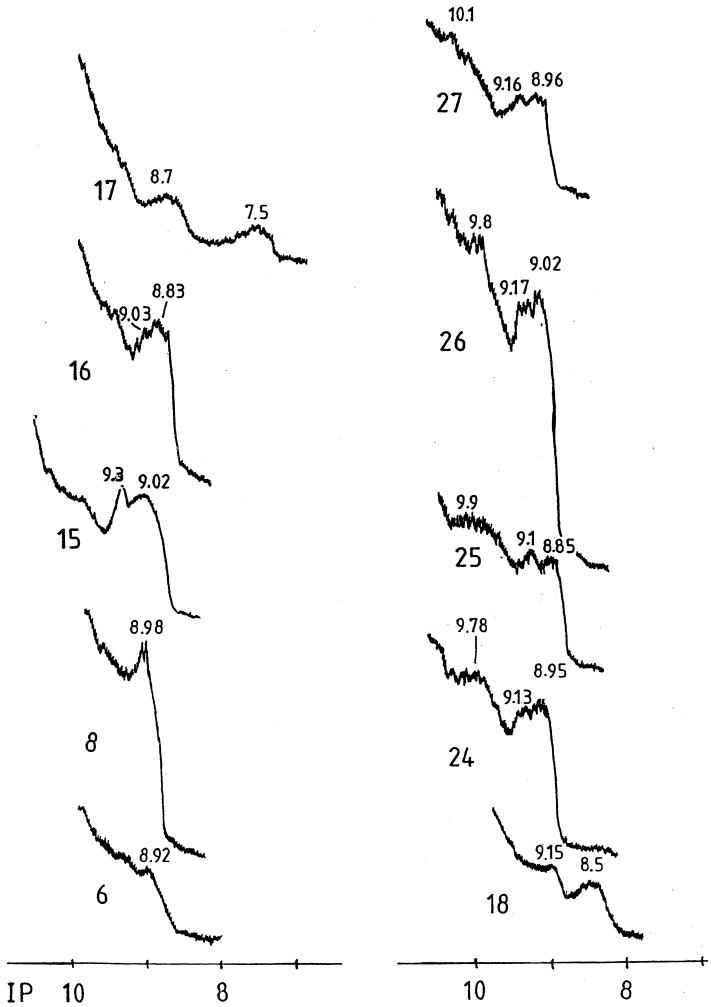


Fig. 1. HeI photoelectron spectra of **6**, **8**, **15**, **16**, **17**, **18**, **24**, **25**, **26**, **27** in the region IP < 11 eV.

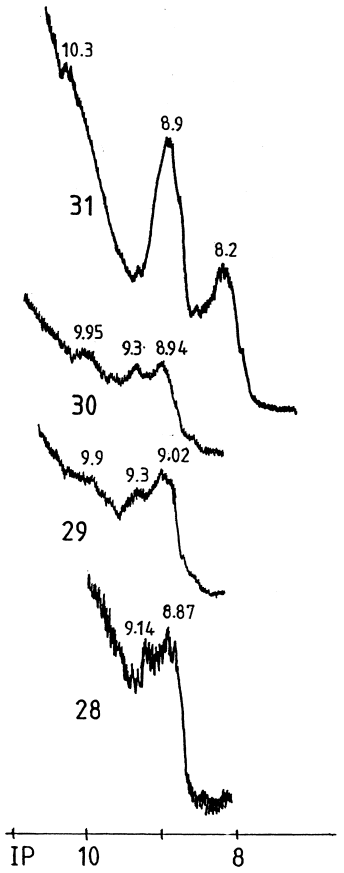


Fig. 2. HeI photoelectron spectra of **28**–**31** in the region IP < 11 eV.

given in Table 1. The results of MO calculations, whether reported previously or obtained in this work (tw), were used to assign bands with lowest ionization energies assuming the validity of Koopmans approximation.

3. Results and discussion

The discussion is divided in two parts. In Section 3.1 we present detailed arguments describing how the spectra were analyzed and what conclusions about the electronic structure were achieved. In Section 3.2 the information concerning electronic structure is combined with the knowledge of steroid–receptor binding with the final aim of elucidating biochemical concepts in the light of spectroscopic evidence.

3.1. Electronic structure analysis

We shall discuss the UPS spectra, according to the number and type of different functional groups bonded to 5α-androstane skeleton (**1**). In order to avoid repetition, only the spectra not previously reported are shown in Figs. 1 and 2. The remaining spectra can be found elsewhere [2–6]. The assignments summarized in Table 1 are based primarily on comparison between spectra of related molecules and relative band intensities (see Section 1). An important common feature of the electronic structure of steroids concerns interactions between σ-orbitals localized on saturated rings with orbitals localized on functional groups. This type of interactions, which we shall call 'ribbon-orbital effect' (ROE), have been well established [2–16] and forms an important tool in our analysis. Such interactions are a special case of the well-known 'through-bond' interactions.

3.1.1. Class A (single type of functional group)

The spectra of **1**–**5** were reported previously [4] while the spectrum of **6** is new (Fig. 1). HOMO ionizations in **2**–**5** belong to oxygen lone pairs (n_o) of the keto group. Inspection of the spectra of isomers **2** and **3** suggests that through-bond interaction between –CO group and androstane skeleton is stronger in 17- than in 3-position. This assertion is based on the IP values, which are 8.75 and 8.92 eV, respectively.

The spectrum of **6** can be analyzed by noting that HOMOs in hydroxyl derivatives of androstane have σ- rather than O2p character [16]. In the spectrum of **6** we observed HOMO energy of 8.97 eV which is lower than in **1** (9.21 eV). This is interesting because it suggests that hydroxyl groups do not affect androstane ring solely in inductive manner. If this were the case, one would expect HOMO IP > 9.21 eV.

3.1.2. Class B (two types of functional groups)

Spectra of isomers **7** and **8** can be regarded as perturbed cases of **3** and **2**, respectively. The perturbation comes from the hydroxyl group whose presence increases slightly (by 0.1 eV) n_o energies. However, n_o ionization energy is lower

Table 1
Vertical ionization potentials (IP ± 0.05 eV) and frontier orbitals for steroids[ab]

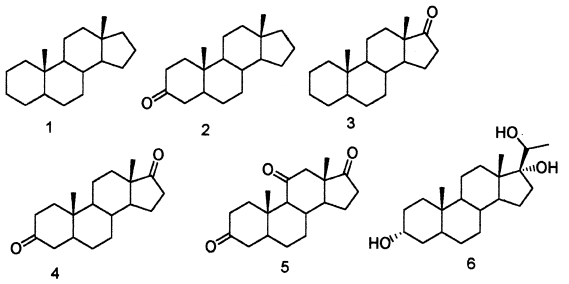
Molecule	Bands	IP/eV	Assignment	MO/UPS
1	X,A	9.21, 9.49	σ, σ	15b/4
2	X	8.92	n_o	15b/4
3	X	8.75	n_o	15b/4
4	X,A	8.94, 9.06	n_o n_o	15b/4
5	X,A,B	8.92, 9.09, 9.50	n_o n_o n_o	4/4
6	X	8.97	σ	tw/tw
7	X	8.87	n_o	15b/5
8	X	8.98	n_o	15b/tw
9	X,A	(8.76, 8.76)	π_{cc}, n_o	8/4
10	X,A,B	(8.90), 9.25	π_{cc}, n_o, n_o	4/4
11	X,A,B	8.28, 8.71, 9.13	π_{cc}, n_o, n_o	tw/3
12	X,A,B	(8.98, 8.98), 9.35	π_{cc}, n_o, n_o	10/4
13	X,A,B,C	8.91, (8.99, 8.99), 9.30	π_{cc}, n_o, n_o, π_{cc}	3/4
14	X,A,B	8.84, 8.99, 9.28	π_{cc}, n_o, n_o	tw/3
15	X,A,B	8.83, 9.02, 9.30	π_{cc}, n_o, n_o	15a/tw
16	X,A	8.87, 9.03	π_{cc}, n_o	tw/tw
17	X,A,B	7.48, 8.73, 9.40	π_{cc}, π_{cc}, π_{cc}	tw/tw
18	X,A	8.50, 9.15	π_{cc}, σ	tw/tw
19	X,A,B,C	(7.55, 7.55), (8.70, 8.70)	π_{cc}, π_{cc}, π_{cc}, π_{cc}	6/6
20	X,A,B	(7.55,7.55), 8.54	π_{cc}, π_{cc}, π_{cc}	6/6
21	X,A	(8.7, 8.7)	π_{cc}, n_o	tw/5
22	X,A	8.94, 9.09	π_{cc}, n_o	tw/3
23	X,A	8.78,9.06,9.68	π_{cc}, n_o, n_o	tw/3
24	X,A	8.95, 9.13	π_{cc}, n_o	14/tw
25	X,A	8.85, 9.10	π_{cc}, n_o	10/tw
26	X,A	9.02,9.17	π_{cc}, n_o	tw/tw
27	X,A,B	8.84, 8.96, 9.16	π_{cc}, n_o, n_o	tw/tw
28	X,A,B	8.77, 8.87, 9.14	π_{cc}, n_o, n_o	tw/tw
29	X,A,B	(9.02,9.02), 9.32	π_{cc}, n_o, n_o	15a/tw
30	X,A,B	(8.94, 8.94), 9.30	π_{cc}, n_o, n_o	tw/tw
31	X,A,B	8.20 (8.90, 8.90)	π_2, π_3, σ	tw/tw

[a] the rightmost column in the table gives references to MO calculations and UPS measurements performed either previously or in this work (tw).
[b] IP values in brackets signify unresolved bands.

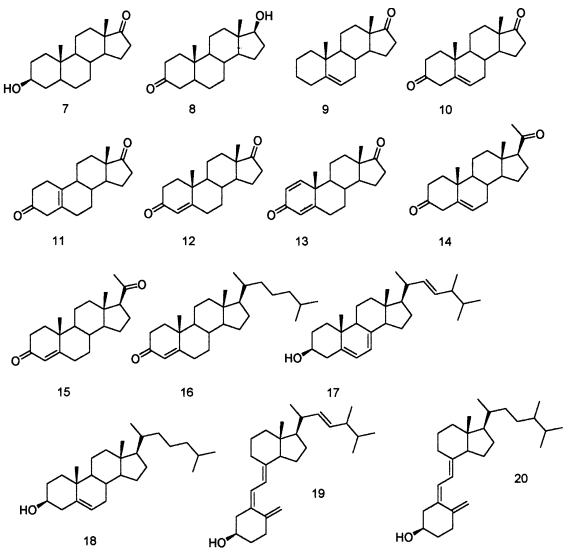
for keto groups in 17- than in 3-position as discussed previously. This leads to the conclusion that 17-position favors substituent–androstane skeleton interactions. The same conclusion applies to spectra of **9** and **16** where the first band comprises two ionizations and appears at lower IP in **9** than in **16**.

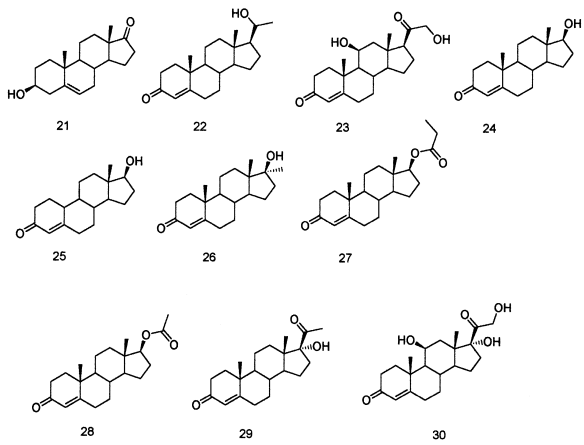
Spectra of **10**–**12** can be expected to show three ionizations in the low energy region. The changing position of the endocyclic double bond affects the energy separation between ionizations from keto groups at 3- and 17-positions, as was noted previously [3]. Adding of another double bond in **13** leads to the pronounced separation of π_{cc} ionizations. Spectra of **14** and **15** are interesting because they show that shifting the endocyclic double bond does not lead to observable changes in the electronic structure when keto group is not bonded directly to androstane system. HOMO ionization in **14** and **15** is again of π type [15,16].

When keto groups are replaced by hydroxyl as in **17**–**20** a very different and interesting electronic structures arise. The most obvious change is the reduction in HOMO ionization potentials



(π_{cc}) when compared to keto derivatives. In cholesterol (**18**) the HOMO ionization of 8.5 eV is slightly destabilized when compared to **16** (8.87 eV). However, in **17**, **19** and **20** the destabilization is pronounced with HOMO IP having a nearly constant value of 7.5 eV. Furthermore, this value seems independent of whether the double bonds are conjugated or if they are endocyclic as in ergosterol (**17**) or exocyclic as in cholecalciferol (**20**). All three compounds (**17**, **19**, **20**) share the common characteristic of being related biochemically to vitamin D class. Ergosterol (also called provitamin D_2) is a precursor which can be converted photochemically to vitamin D_2. The similarity in HOMO energies between **17**, **19** and **20** can be understood if one considers UPS of related polyenes [18]. The HOMO ionization potentials in cyclohexadiene and hexatriene are 8.25 and 8.29 eV, respectively. Our results thus indicate uniform and pronounced destabilization of HOMO energies in members of vitamin D family.





3.1.3. Class C (three or more types of functional groups)

The discernible variations in the electronic structure in Class C can be rationalized by the combination of arguments presented for Classes A–B. Two effects are noticeable in the spectra of **21** and **24**: the lowering of HOMO energy when the keto group is in 17-position and the splitting of ionization energies for orbitals localized at opposite ends of the 5α-androstane skeleton (induced by endocyclic double bond). The broadening of the band with lowest IP in the spectrum of **30** vs. **23** suggests that 17-hydroxy substitution increases π_{cc}–n_o splitting. This demonstrates not only the importance of 17-position, but also of the long range ROE.

The spectra of **24**–**26** are interesting because they probe the influence of methyl substitution at 10- and 17-positions. The methyl substitution is known to cause inductive destabilization of occupied orbitals. However, we observed the opposite trend in **24**–**26**, i.e. Me group slightly increased the HOMO IP. A possible explanation for this 'reverse Me effect' can be sought in the competition between ROE and methyl substituent effects. Esterification of testosterone (**24**) gives **27** and **28.**

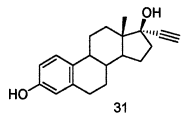
The influence of esterification on the electronic structure is minimal as the PE spectra clearly show (Figs. 1 and 2).

Finally, changes in the electronic structure upon hydroxylation of progesterone shall be examined. Comparison of the spectra of **15** (progesterone) and **29** (17α-hydroxy derivative) indicates that there is a distinct change in shape of the first band which comprises three ionizations (Table 1). Recently reported high-level ab initio calculations [15,16] suggest that HOMO ionization in both molecules is of π_{cc} type. The implication then is that hydroxylation inverts the relative stabilities of oxygen lone pair orbitals n_o(keto) and n_o(acetyl).

3.1.4. Class D

This class contains a single molecule **31** (17α-ethynylestradiol). The molecule is unique amongst steroid derivatives studied here, because it contains an aromatic ring and a triple bond. The rationale for not choosing other estrogens like estrone and estradiol was that we wished initially, to investigate possible orbital interactions between androstane moiety and the aromatic ring. Selection of for example estrone would make spectral assignments more difficult because of the band overlap between n_o ionization from keto group and the ring π-ionizations.

The assignment of the spectrum of **31** (Fig. 2) can be obtained by comparison with the spectra of phenol and isopropylethyne and by taking into account relative band intensities. The main interest lies in possible interactions between aromatic ring's π-orbitals and the remnants of the androstane system. The π-ionization bands in phenol are at 8.7 and 9.39 eV. In **31** they are shifted to lower energies (8.2 and 8.9 eV) while retaining the same energy separation. This indicates that the shift is inductive and that the 'ribbon-orbital effect' is not significant, presumably due to strong aromatic stabilization. The π-ionization potential in isopropylethyne [18] is 10.05 eV and this value makes it unlikely that X–B bands contain ionization from electrons in ethynyl moiety. These arguments together with AM1 calculation lead us to the assignment proposed in Table 1.



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3.2. Correlation between biological activity and electronic structure[19,20]

The electronic structure of steroids had been studied in the past with the aim of establishing a possible correlation with biological activity. However, biological activity is a complex phenomenon which involves steroid transport, stereochemistry and receptor binding. Only the relative binding affinity (RBA) can logically be expected to show any correlation with electronic structure. Unfortunately, RBA depends not only on steroid ligand, but also on the receptor type: androgene receptor (Ar), progesteron receptor (Pr), glucocorticoid receptor (Gr), estrogene receptor (Er) and mineralocorticoid (Cr). The caveat in any correlation is that the numerical RBA values must pertain to the same receptor. This limits the amount of biological data available for electronic structure analysis. We have used the recent data for RBA values [1,19] and summarized the results in Table 2. In order to interpret the data one must recall that the common functional groups on steroid skeleton are double bonds or aromatic rings, –OH and –CO groups. Double bonds and

Table 2
Correlation of relative binding affinity (RBA [19], IC_{50} [1]) and IP for n_o orbital

Molecule	IP (n_o)/eV	RBA(Pr)	RBA(Ar)	RBA(Gr)
24	9.13	1	100	3
8	8.98	1.5	120	1
26	9.17	3	45	2
25	9.1	20	155	4
15	9.02, 9.3	100	5.5	115
23	9.06	5	0.5	100
30	8.94, 9.3	0.1	0.1	45
		IC_{50}		
15	9.02, 9.3	1		
7	8.87	21		
4	8.94, 9.06	8		

aromatic rings usually interact with flat hydrophobic parts of the receptor via van der Waals interactions [21]. Hydroxyl and keto groups are involved in hydrogen bonding (HB) to amino acids on the receptor and this type of interaction is stronger than VDW. The IP of the lone pair of hydroxyl group is higher than of keto and the corresponding n_o(OH) band can not usually be deconvoluted in steroid spectra. For this reason we have used the IP(n_o) of the keto group.

HB is essentially electrostatic in nature [22] which suggests a simple model according to which lower IP(n_o) energy leads to stronger binding (larger RBA). Inspection of Table 2 shows that in some cases the trend mentioned above is broadly followed, e.g. compounds **15, 7, 4.** However, for other compounds n_o-RBA correlation is poor (**24–30**). What is also clear from Table 2 is that the activity depends strongly on receptor type which may be an additional reason for poor correlation (besides the crudity of our model). We shall now discuss binding qualitatively for particular receptor types.

3.2.1. Er-type

The only compound studied from the Er set is **31.** UPS spectrum shows that its electronic structure is significantly different from other steroids (Fig. 2) in terms of HOMO ionization potential and type (aromatic π-orbital). This difference explains why steroids with high affinity for Er have little affinity for other receptors and vice versa. It also raises the possibility that steroid–receptor binding may involve π-complexation of steroid with suitable group on the receptor [20].

3.2.2. Cr-type

The compounds interacting with this receptor are **23** and **30**. UPS data show that their electronic structures are different. The biological data [20] suggest that the presence of 17α-hydroxy group is not essential (does not influence) steroid–receptor binding. The conclusion is that the binding takes place via a mechanism different from HB. Indeed, it is thought that binding depends on the conformation of steroid A ring with hydrophobic interactions playing a major role [20]. This may explain lack of correlation n_o-RBA correlation for **30** in Table 2.

3.2.3. Pr-type

The compounds **15** and **29** exhibit similar UPS spectra (Figs. 1 and 2). Nonetheless, while compound **15** is biologically active, **29** is inactive. The conclusion is that the binding mechanism does not involve HB, but hydrophobic interactions with ring A which is itself in an inverted conformation [19].

3.2.4. Ar-type

Steroids **8** and **24–28** exhibit affinity for Ar receptors. The comparison of UPS data for **8** and **24** (Fig. 1 and Table 1) indicates that n_o(keto) ionization potential is lower in **8** (8.98 eV) than in **24** (9.13 eV) and RBA(Ar) shows the same trend. Note that the trend is reversed for Gr receptor which may indicate a different binding mode. For **24–26** Table 2 exhibits very close values of n_o energies, but different RBA(Ar) activities which highlight the importance of steric factors.

3.2.5. Sterols

Among sterols, **19** and **20** bind to the same receptor and can hence provide meaningful comparison between electronic structure and biological activity. The UPS shows that π-orbital and hence n_o(hydroxy) energies in the two molecules are identical, in spite of the additional double bond in **19**. This is consistent with their similar biological potency [20] which is derived from HB binding (via 3-hydroxyl group) to the protein receptor.

4. Conclusion

We have analyzed variations in the electronic structure of steroids on the basis of UPS spectra and MO calculations. Some variations are interesting (e.g. 'inverse Me effect', 'HOMO destabilization' in vitamin D family) and demonstrate not only the usefulness of the UPS method for solving difficult electronic structure problems, but also the great versatility of steroid molecules when it comes to subtle changes in the electronic

structure. The usefulness of correlation between biological and UPS data is less clear-cut. The difficulties in establishing such correlations stem from the scarcity of quantitative RBA data for a single receptor, band overlap in the spectra and concomitant inaccuracy in IP values. In general, the range of IP values is much smaller than RBA. However, the correlation did provide some interesting insights which need to be further clarified and tested by UPS and RBA measurements as well as X-ray and NMR studies of SR complexes.

Acknowledgements

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