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MASS SPECTROMETRY OF STEROID SYSTEMS—XXII[a]

APPEARANCE-IONIZATION POTENTIALS DIFFERENCES AS A GUIDE TO THE RELATIVE STABILITIES OF THE *CIS*- AND *TRANS*-A/B-STEROIDS

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Abstract—The ionization potentials of *cis*- and *trans*-A/B-steroids of the androstane and pregnane series, as well as the appearance potentials of the M^+-Me ions have been determined.

The approximate activation energies (obtained as the AP-IP differences) for the loss of an angular Me from the molecular ion were found to be higher in the case of the more stable *trans*-A/B (5α)-steroids as compared to their *cis* (5β)-isomers.

Recently, using as examples two pairs of stereoisomeric steroid compounds of D-homoequilenin and D-homoestrone series, we have shown that less energy is necessary to produce the same ions in the case of the more strained *cis*-C/D or B/C-structures as compared with the more stable *trans*-isomers.[1] These results are in agreement with the conclusion[2] that the thermodynamic stability of a stereoisomer molecular ion is reflected in the decomposition of this ion and consequently, the excess energy bound by steric interaction is released in the fragmentation process.

It may be that reliable information, on the relative stabilities of *cis*- and *trans*-fused steroids could be obtained by comparison of the activation energies of the same simple cleavage reactions occuring in the molecular ions of the stereoisomers.

For this type of fragmentation the kinetic and competitive shifts as well as the reverse activation energy are likely to be small, or at least very similar for both stereoisomers, and can then be neglected, hence the appearance (AP)—ionization potentials (IP) differences can be regarded as the practical measure of the activation energy (E_0) for such reactions[3] (i.e. AP-IP ~ E_0).

In the present communication the ionization potentials and appearance potentials of the M^+-Me ions have been determined for the *cis*- and *trans*-A/B-steroids of the androstane and pregnane series (**1–14**) (Table 1).

RESULTS AND DISCUSSION

trans- *and* cis-*A/B Androstanes* (**1–4**) *and pregnanes* (**5–6**). The activation energy for the expulsion of the Me radical was found to be greater in case of 5α-isomers (**1, 3**, Table 1), which agrees with the higher stability of these steroids as compared to their respective 5β-isomers (**2, 4**).[4] The same regularity was obtained also in isomeric pregnanes (**5, 6**). It was shown earlier that in case of 5α-androstane (**1**) and 5α-pregnane (**5**) the expelled Me radical originates exclusively from the C-18 and C-19 Me groups in ratios of 3:2 and 1:4 respectively.[5,6] The fact that less activation energy is necessary for this process in 5β-isomers (**2, 4, 6**), may be due to enhanced loss of the C-19 Me group thus relieving the increased strain of the A/B-*cis* ring junction. Interestingly, the different modes of fusion of rings C and D in 14α-(**1, 2**) and 14β-androstanes (**3, 4**) respectively were not reflected in the activation energy for the elimination of the Me radical. On the other hand, just like the 14α-compounds (**1, 2**), the E_0 values for the same reaction in 14β-androstanes (**3, 4**) were increased on passing from the *cis*-A/B(5β)- to the *trans*-A/B(5α)-isomer (Table 1).

5α- *and* 5β-*Androstane*-3-*one* (**7, 8**). The elimination of the Me radical from the molecular ion of the *cis*-A/B ketone (**8**), as in the case of the respective hydrocarbons, required less energy than that of the *trans*-ketone (**7**). The M^+-Me ion is formed from the loss of a Me radical from the two angular positions: C-10 and C-13 in the ratio 1:1,[7] and the difference in the E_0 value is probably due to the increased loss of the Me group from C-10 in case of more strained 5β-ketone (**8**).

5α- *and* 5β-*Androstane*-17-*one* (**9, 10**). The presence of the C-17 CO function was found to affect the mechanisms of the Me-elimination from the molecular ions of the keto-steroids:[8] there is a substantial decrease in the loss of the 18-Me (25%) compared with that (66 and 50%) in the 5α-androstane (**1**) and 5α-androstane-3-one (**7**) respectively. However, the stereochemical differences between the *trans*-A/B (5α)- and *cis*-A/B (5β)-ketones (**9, 10**) reflected in the same way, as it was noted for the hydrocarbons (**1–4**) and 3-ketones (**7, 8**), in the activation energy of the process of Me loss, the largest E_0 being exhibited by the 5α-isomer (**9**) (Table 1).

5α- *and* 5β-*Androstane*-3,17-*dione* (**11, 12**) *and* 5α- *and* 5β-*androstane*-3,11,17-*trione* (**13, 14**). It is apparent from the comparison of the E_0 values (Table 1) that in the 3,17-diketones (**11, 12**) the configuration at C-5 has, practically, no effect on the activation energy of the Me elimination. It may be that, while the 5α-isomer (**11**) is known to be more stable than the 5β-one (**12**),[9] under the

[a] For Part XXI, see Ref. 1.

Table 1. IP, AP and activation energy data (eV) for (M^+-Me) reactions in steroids **1–14**

Compound	IP	AP	E_0[a]
5α,14α-Androstane (**1**)	9·13 ± 0·07	10·10 ± 0·04	0·97 ± 0·06
5β,14α-Androstane (**2**)	9·20 ± 0·02	9·87 ± 0·04	0·67 ± 0·03
5α,14β-Androstane (**3**)	9·18 ± 0·05	10·18 ± 0·04	1·00 ± 0·05
5β,14β-Androstane (**4**)	9·10 ± 0·09	9·78 ± 0·06	0·68 ± 0·08
5α-Pregnane (**5**)	9·15 ± 0·06	10·16 ± 0·02	1·01 ± 0·04
5β-Pregnane (**6**)	9·25 ± 0·06	10·05 ± 0·06	0·80 ± 0·06
5α-Androstane-3-one (**7**)	9·10 ± 0·08	10·15 ± 0·03	1·05 ± 0·06
5β-Androstane-3-one (**8**)	9·11 ± 0·09	9·93 ± 0·03	0·82 ± 0·06
5α-Androstane-17-one (**9**)	9·04 ± 0·06	10·00 ± 0·04	0·96 ± 0·05
5β-Androstane-17-one (**10**)	8·99 ± 0·07	9·82 ± 0·05	0·83 ± 0·06
5α-Androstane-3,17-dione (**11**)	9·02 ± 0·05	10·19 ± 0·08	1·17 ± 0·07
5β-Androstane-3,17-dione (**12**)	9·00 ± 0·04	10·28 ± 0·09	1·28 ± 0·07
5α-Androstane-3,11,17-trione (**13**)	8·89 ± 0·08	10·33 ± 0·08	1·44 ± 0·08
5β-Androstane-3,11,17-trione (**14**)	8·87 ± 0·08	9·48 ± 0·06	0·61 ± 0·07

[a] $E_0 \sim$ AP-IP.

mass spectrometric conditions, owing to the deformation present in the ring D[10] and/or "flipping" of the ring C into its more flexible boat conformation,[6] the latter was changed into an energetically more favorable 5β,14β-conformation, and this fact was reflected in the E_0 values for the Me elimination (Table 1). Anyway, practically the same E_0 values for the Me loss in both isomeric 3,17-diketones (**11, 12**) suggest that the energy differences between these steroids are very small. On the other hand, in the 5α- and 5β-androstane-3,11,17-tri-ketones (**13, 14**), with a more rigid ring C, the same regularity, as in the corresponding hydrocarbons (**1–4**) and monoketones (**7–10**), was obtained: the activation energy for the reaction $M^+ \rightarrow M^+$-Me was greater in case of the *trans*-A/B-fused isomer (**13**) (Table 1).

CONCLUSION

The comparison of the AP-IP differences showed that less activation energy is required for the $M^- \rightarrow M^+$-Me reaction in the more strained *cis*-A/B-fused (5β) steroids as against that of the respective *trans*-A/B (5α) isomers.

These results reveal that a comparison of the activation energy values for simple cleavage reactions makes it possible, as a rule, to determine reliably the relative stabilities of the stereoisomeric steroids.

Another problem is, of course, the suitability of the mass spectrometric AP measurements for the quantitative determinations of the energy differences between stereoisomeric steroids. Earlier several attempts have been made with variable success to relate differences in the strain energies in the neutral molecules and the appearance potentials of the "similar" ions.[11] Our results suggest that the use of the AP differences for this purpose must be based on the knowledge of the structure of the ions formed. Indeed, the differences in the AP (M^+-Me) values between *trans*- and *cis*-A/B-androstanes (**1–2, 3–4**; Table 1) are more than a factor of five higher than the respective relative ΔG^0 values.[4] This lack of quantitative correlation is presumably due to different structures of the M^+-Me ions being formed in each case (e.g., even though the expelled Me radicals originated from the same angular Me, in both 5α- and 5β-steroids, the complete loss of the steric differences between the M^+-Me ions seems to be hardly possible). It has been pointed out recently[12] that enthalpy differences between stereoisomeric molecules and those for the isomeric ions are not equal.

It is clear, therefore, that, while the qualitative correlation of the activation energy values (E_0) for the M^+-Me reactions and the relative stabilities of the steroid stereoisomers (**1–14**) were remarkably high, it is unreasonable at the present time to use appearance potentials for the accurate estimation of the energetic differences between the neutral steroid molecules.

EXPERIMENTAL

Ionization and appearance potentials were measured by the method of Lossing,[13] using an automatic electronic scanner for the rapid recording of the ionization efficiency curves,[14] Ar being used as calibrating gas. The compound under investigation and the argon were admitted simultaneously to the TO-4 ion source of the Atlas CH-4 Mass Spectrometer via the High Temperature Inlet (200°) and the Double Inlet System (120°) respectively. The source temp. was 245–250°. Each ionization efficiency curve, both of the sample ion and that of Ar, were repeatedly scanned at least 10 times. Four-five series of replicate IP and AP determination were carried out on different days with each compound.

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