# Structure and hydrogen-bonding ability of estrogens studied in the gas phase

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## Abstract

The structures of estrogens (estrone, β-estradiol, estriol) and their 1:1 hydrogen-bonded (hydrated) clusters with water formed in supersonic jets have been investigated by various laser spectroscopic methods and quantum chemical calculations. In the $S_1$-$S_0$ electronic spectra, all the three species exhibit the band origin in the 35050–35200 $cm^{-1}$ region. By applying ultraviolet-ultraviolet hole-burning (UV-UV HB) spectroscopy, two conformers, four conformers and eight conformers, arising from different orientation of OH group(s) in the *A*-ring and *D*-ring, were are identified for estrone, β-estradiol, and estriol, respectively. The Infrared-ultraviolet double resonance (IR-UV DR) spectra in the OH stretching vibration were observed to discriminate different conformers of the *D*-ring OH for β-estradiol and estriol, and it is suggested that in estriol only the intramolecular hydrogen bonded conformer exists in the jet. For the 1:1 hydrated cluster of estrogens, the $S_1$-$S_0$ electronic transition energy is quite different depending on whether the water molecule is bound to *A*-ring OH or *D*-ring OH. It is found that the water molecule prefers to form an H-bond to the *A*-ring OH for estrone and β-estradiol due to the higher acidity of phenolic OH than that of the alcoholic OH. On the other hand, in estriol the water molecule prefers to be bound to the *D*-ring OH due to the formation of a stable ring-structure H-bonding network with two OH groups. From these results, we conclude that estriol has a hydrogen bonding ability quite different from that of β-estradiol although their difference is just only one substituent.

## I. INTRODUCTION

Estrogens (estrone, β-estradiol, and estriol) are important endogenous female hormones. They have a common steroidal backbone (scheme 1) with a phenolic OH group at position 3C of the *A*-ring. The differences among them are the substituents at 16C and 17C in the *D*-ring. Estrone has a carbonyl group at 17C, β-estradiol has an OH group at β side of 17C, and estriol has two OH groups, one is at α side of 16C and the other is at β side of 17C. Estrogens exert several physiological activities such as regulating differentiation, growth and homeostasis in various tissues of human body[1,2]. However, estrogens play a negative role as well as a positive role on human body, such as inducing breast or endometrial cancer[3,4]. Thus, up to now, endogenous and exogenous estrogens have been investigated by variety of studies to reveal the origin of the activities[5-21]. From these studies, it is known that the physiological effect of estrogen is initiated by binding with some receptors, such as estrogen-receptor. In these receptors, estrogens are fixed to the ligand binding domains (LBDs) via the hydrogen-bonding (H-bonding) at the *A*-ring OH, and the H-bond at the *D*-ring OH (CO) regulates structural alternations of LBDs for physiological activity[5-8, 19]. It has been revealed that β-estradiol has the highest physiological activity, which is followed by estrone and estriol. However it is not well understood how the difference of the substituents is related to the difference of their activities. Since estrogens have many possible conformations at room temperature in solution, it will be very difficult to investigate how the substituents as well as the conformation are related to the inherent property of each estrogen. To solve these problems, we have been studying the structures of gas phase cold estrogens and their hydrated clusters formed in supersonic free jet expansion.

In our previous study[22] we reported the conformation of β-estradiol and the structure of its mono-hydrated cluster. We showed that *A*-ring OH is more acidic than the *D*-ring OH so that water prefers to form H-bonding to the *A*-ring OH as an acceptor. On the other hand, *D*-ring OH plays both a donor and an acceptor depending on it orientation. In the present study, we extend our investigation to the conformation of estrone and estriol, and the structures of the hydrated clusters. The molecule

and the clusters are cooled in a supersonic free jet expansion, and we measure the electronic and IR spectra by applying various laser spectroscopic techniques; laser induced fluorescence (LIF), fluorescence-detected UV-UV HB, and IR-UV DR spectroscopy. We determine their structures by the comparison of the observed spectra with those obtained by theoretical calculations with density functional theory (DFT). On the basis of the analysis of the conformation and H-bonding structure for each estrogen, we discuss their H-bonding ability and its relationship with a physiological activity.

## II. EXPERIMENTAL AND COMPUTATIONAL METHODS

The details of the experimental approach were described in a previous paper[22]. Briefly, jet-cooled estrogen and its hydrated clusters were produced by a supersonic expansion of gaseous samples using a homemade heated pulsed nozzle. Solid powder of estrogen was put in a housing attached at the head of the pulsed nozzle and was heated at 150 ˚C for estrone, 130 ˚C for β-estradiol and 190 ˚C for estriol. A gas mixture of estrogen and He carrier gas at total pressure of 3-5 bar was expanded into a vacuum chamber through a 1 mm orifice of the nozzle. Hydrated clusters of estrogen were obtained by introducing small amount of water vapor into He carrier gas and expanding the mixture into vacuum. The $S_1$–$S_0$ electronic spectra were measured by LIF spectroscopy. A tunable UV light obtained by second harmonics generation (SHG) of an output of the pulsed dye laser (Lambda Physik Scanmate) pumped by $Nd^{3+}$:YAG laser (Continuum Surelite II) crossed the supersonic jet of estrogen at 30 mm downstream of the nozzle. Fluorescence from the sample was collected on a photomultiplier tube after passing through a band pass filter, and the current from the photomultiplier tube was fed into a boxcar integrator (Stanford Research systems SR250). Averaged signals were processed by a PC via an analog/digital converter. We also carried out UV-UV hole-burning (HB) spectroscopy[23] to distinguish a peak belonging to a different conformer. In this spectroscopy, the frequency of the probe UV laser was fixed to a vibronic band of a specific species and the fluorescence intensity was monitored. Under this condition, another tunable UV laser light

(hole laser) obtained by SHG of a pulsed dye laser (Continuum ND6000 /Surelite II) was introduced at 10 mm upstream of the jet with a timing of ~4 μs prior to the probe laser pulse. The frequency of the UV hole laser was scanned and depletion of the fluorescence intensity induced by the hole laser was observed. Thus, the UV-UV HB spectrum is observed as a fluorescence-dip spectrum. IR-UV DR spectroscopy[24-26] is very similar to UV-UV HB spectroscopy. An output of a pulsed tunable IR laser (LaserVision/Quanta-Ray GCR250) was introduced 90 ns prior to the probe UV pulse. The frequency of the IR laser was scanned and depletion of the fluorescence induced by the IR laser was observed, giving IR-dip spectra of the UV monitored species. Estrogens were purchased from SIGMA-ALDRICH and used without further purification.

Geometry optimization and vibrational analysis were done by DFT calculations at the M05-2X/6-311++G** level of theory. The oscillator strengths and the transition energies were calculated with time-dependent DFT (TD-DFT) using the same calculation level. This calculation level was also used in our previous work on β-estradiol and its mono-hydrated clusters.[22] All the calculations were performed by using GAUSSIAN 09 program package.[27] For comparison of the observed IR spectra with the calculated ones, scaling factors of 0.9360, 0.9383 and 0.9359 were employed to the calculated vibrational frequencies of estrone, estriol and its monohydrated cluster, respectively. These factors were determined so as to reproduce the frequency of the phenolic OH stretch vibration for each species. For the electronic transition energies, we used a scaling factor of 0.8432 for all estrogens, which was determined so that an averaged value of the calculated transition energies of different conformers of bare estrogen is equal to that of the observed transitions.

## III. RESULTS AND DISCUSSION

### III. 1 UV and IR spectra of estrogens

Figure1 show the $S_1$-$S_0$ LIF spectra of jet-cooled (a) estrone, (b) β-estradiol, and (c) estriol, in the frequency region of 34960-35250 $cm^{-1}$. Since they have a common phenol chromophore, they show the absorption spectra in a similar region. As seen in Fig.1, spectral features become

complicated in the order of complexity of the *D*-ring substituent. In following sections, we show the experimental results of bare estrogens in the order of estrone, β-estradiol and estriol, and determine their structures by comparison of computational results.

### III.1.1 estrone

Figure 2(a) shows the $S_1$-$S_0$ LIF spectrum of bare estrone. There are two strong bands at 35065 (band **1**) and 35164 $cm^{-1}$ (band **2)**. The UV-UV HB spectra obtained by monitoring bands **1** and **2** shown in Figure 2(b) indicate that bands **1** and **2** are the origin bands of different conformers, that is *cis* and *trans*-estrone. The interval of the two bands, 99 $cm^{-1}$, is almost the same as the interval of the transition energies of *cis*- and *trans*-conformers of 3-methyl phenol (118.5 $cm^{-1}$).[28] The IR-UV spectra obtained by monitoring these two bands in Figure 2(d) show almost identical *A*-ring OH stretch band. These results indicate that bands **1** and **2** are *cis*- and *trans*-estrone, respectively. All the three estrogens investigated in the present study exhibit the electronic transitions with similar interval due to the conformers originating from the orientation of the *A* ring OH group. We tried to generate the hydrated clusters of estrone by adding water vapor to He carrier gas. However, we could not generate them because estrone easily changes to β-estradiol upon the heating of the estrone/water mixture at high temperature.

### III.1.2 β-estradiol and its hydrated clusters

Figure 3(a) displays the LIF spectrum of bare β-estradiol in the 34600-35200 $cm^{-1}$ region. In this region, we found four prominent bands labeled **a** (35048 $cm^{-1}$), **b** (35051 $cm^{-1}$), **c** (35148 $cm^{-1}$), and **d** (35151 $cm^{-1}$). The assignments of these bands were already reported in our previous paper[22] on the basis of the comparison of the electronic transition energies and IR spectra of the OH stretching bands with those of the calculated ones. Though the calculation predicts six stable conformers, as shown in Figure 3(d), only four bands are observed under the jet-cooled condition. The bottom panel of Figure 3(d) displays the three configurations of the *D*-ring OH group with respect to the 17C–H

bond (see Scheme 1). The configuration with the H–O–17C–H dihedral angle of ~180 degrees is called *anti*, and those with the angle of about +60 and about -60 degrees are called *gauche*(+) and *gauche*(–), respectively.

Figures 3(b) and 3(c) show the LIF spectra of hydrated β-estradiol. Two types of monohydrated clusters of β-estradiol were observed by adding water vapor. One is the clusters labeled **A**-**D**, in which a water molecule forms H-bond with *A*-ring OH. This type of the cluster exhibit large spectral red-shift as seen in Figure 3(b). The other is the one in which the water molecule is bound to the *D*-ring OH, as seen with asterisks in Figure 3(c). These clusters exhibit very small shifts of the electronic transition, because the effect of the H-bond on the electronic structure is very small. The former clusters are much more abundant than the latter because the *A*-ring (phenolic) OH is more acidic than the *D*-ring (alcoholic) OH. This experimental result that shows the difference of hydrophilicity between *A*-ring and *D*-ring is consistent with the results of solid-state NMR given by Scheidt et al.[16]

**III.1.3 estriol and its hydrated clusters**

Figures 4(a) and 4(b) show the LIF spectrum of bare estriol and the UV-UV HB spectra obtained by monitoring bands **1**-**7** and **9**-**11** in Fig. 4(a). The UV-UV HB indicates that all of them are the band origins belonging to different species from each other; hereafter we use the numbers **1**-**12** also for calling the species of the corresponding bands. Bands **1**-**4**, **9**, and **10** are located on the red side of bands **5**-**8**, **11** and **12** by ~100 $cm^{-1}$. Similar to the case of estrone and β-estradiol, this interval is attributed to the difference of *cis* ant *trans*-isomers of estriol, where the former and the latter species are the *cis*- and *trans*-isomers, respectively. On the other hand, the bands with small splitting, 3-7 $cm^{-1}$, in each isomer are attributed to different conformers originating from the different conformation of the OH groups in the *D*-ring. The IR-UV DR spectra in the OH stretching region for bands **1**-**3** and **5**-**7** and **9**-**11** are shown in Figures 5(a)-(i). Among them, bands **1**-**3**, **5**-**7** exhibit two bands at 3644-3664 $cm^{-1}$, which are assigned to the *A*-ring (phenolic) OH and *D*-ring OH stretch

bands. Since estriol has three OH groups, three OH peaks are expected to exist in this region. The reason of the disappearance of the third band is due to the accidental overlap of the OH bands. A similar situation was observed in β-estradiol,[22] where one or two OH bands appear(s) depending on the conformation of *D*-ring OH. In all the spectra of Figures 5(a)-(i), one band is observed at ~3656 $cm^{-1}$. Since this frequency is almost the same as the stretch frequency of the *A*-ring OH of estrone (Figure 2(b)) and β-estradiol[22], the bands around 3656 $cm^{-1}$ can be assigned to the *A*-ring OH of estriol. The bands appearing at 3644 and 3663 $cm^{-1}$ are assigned to the *D*-ring OH groups. Species **1** and **5** have a similar *D*-ring conformation with each other, because these species show similar IR spectra (Figs. 5(a) and 5(d)). Similarly, species **2** and **6, as** well as species **3** and **7** have a similar *D*-ring OH configuration with each other.

The IR spectra for bands **9-11** show different spectral features from those of bands **1-7**. The IR bands at ~3657 $cm^{-1}$ are narrower than the corresponding bands of species **1-7**. In addition, new bands are found at ~3536 and ~3722 $cm^{-1}$, and the former bands are quite broad. Since this spectral feature is typical for the H-bonded OH and free OH of water, bands **9-11** are due to hydrated cluster.[22,29] The UV-UV HB spectra of estriol in Fig. 6(a) provide additional support for the assignments of species **9-11** to the hydrated clusters. In the figure, two bands are commonly observed at 31 and 47 $cm^{-1}$ for bands **1-7**, corresponding to estriol monomer. On the other hand, the spectra of species **9-11** show a new band at 20-30 $cm^{-1}$, assignable to intermolecular vibrational mode. In addition, the positions of the monomer bands at 32 $cm^{-1}$ is shifted to 37 $cm^{-1}$. These results also support that the species **1-3**, **5-7** and **4**, **8** are assigned to bare estriol, and species **9-11** are its hydrated clusters. Therefore, from these results and those of the IR spectra, we observed eight conformers of estriol monomer(band **1-8**); four each cis and trans, and four isomer of hydrated cluster of estriol(band **9-12**); two each cis and trans. In the later section, we will give further discussion by comparing the observe spectra with the calculated ones, figures 6(b) and 6(c).

**III. 2 Structure of bare estrogens**

### III. 2. 1. Estrone

In Figure 2(f), we show two stable conformations of bare estrone obtained by the DFT calculation. At the M05-2X/6-311++G** level, *trans*-conformer is 0.41 kJ/mol more stable than the *cis*-conformer. TD-DFT calculated $S_1$- $S_0$ electronic transition energies in Figure 2(c) indicates that bands **1** and **2** are assigned to the *cis*- and *trans*-conformer, respectively. The lower transition energy for the *cis*-conformer than that for the *trans* one is due to the difference of spatial distribution of molecular orbitals (MOs) between them, as explained in our previous paper of β-estradiol.[22] That is, TD-DFT calcualted results show that the spatial distribution of the highest occupied MO (HOMO) is almost the same between the *cis*- and *trans*-confomers, while that of the lowest unoccupied MO (LUMO) of the *cis*-conformer is more delocalized and stabilized than that of *trans*-coformer, resulting in energy gap between HOMO and LUMO of *cis*-conformer is less than that of *trans*-coformer. The calculated OH stretch band for the two conformers in Figure 2(e) shows almost identical spectra similar to the observed ones of Figure 2(d). From these results, we conclude that bands **1** and **2** are assigned to *cis*- and *trans*-estrone, respectively. Table 1 lists the electronic transition energies of estrone and their assignments.

### III. 2. 2. β-estradiol

Since the structure of bare β-estradiol was already determined in our previous paper, the conclusion is briefly explained here.[22] The upper panel of Figure 3(d) shows the six lowest stable conformers. We assigned bands **a**-**d** to *cis*-*anti*, *cis*-*gauche*(+), *trans*-*anti*, and *trans*-*gauche*(+), from the comparison of the observed electronic transition energies and IR spectra with those of the calculated ones. Two *gauche*(-) conformers, *cis*-*gauche*(-) and *trans*-*gauche*(-), are predicted to have higher energies than the others. In addition, the energy barrier between the *gauche*(-) and the *anti* configurations are quite low (260 $cm^{-1}$), compared to the other barriers. Therefore, the *gauche*(-) conformers are considered to relax to the *anti* conformers during the cooling process and are not seen in the experiment. Table 1 also lists the observed electronic transition energies of β-estradiol and

their assignments.

### III. 2. 3. Estriol

Figure 7 show sixteen lowest energy stable conformers of estriol optimized by the DFT calculations at the M05-2X/6-311++G** level. These sixteen conformers come from different orientations of the OH groups in the *A*- and *D*-rings. In the labels of the conformers (3*c*_16*g*-_17*g*+, for instance), the numbers 3, 16 and 17 indicate the position of the carbon atoms in Scheme 1. The alphabets *c*, *t*, *a*, *g*- and *g*+ stand for *cis*, *trans*, *anti*, *gauche*(-) and *gauche*(+) configuration, respectively. For example, "3*c_16g-_17g*+" represents a configuration of estriol with the dihedral angles "H–O–3C–4C" to be ~0 degree, "H–O–16C–H" to be ~-60 degrees and "H–O–17C–H" to be ~+60 degrees, respectively. The ZPE corrected total energies relative to the most stable "3*c_16g-_17g*+" conformer are also shown. In Figure 7, the conformers are first classified into two groups, *cis* and *trans*, where the energy difference between them is very small. Each group can be further classified into two groups; intramolecular H-bonded structure and non-H-bonded structure. There is energy difference of 2 kJ/mol between the former and the latter groups. The right panel in the figure summarizes the eight configurations of the OH groups around 16C–17C bond (see Scheme 1). There are nine possible conformations with the 16OH and 17OH groups, because these OH groups have three possible configurations each (*anti*, *gauche*(-), and *gauche*(+)). However, "16*g*-_17*g*-" configuration is not obtained as a stable one in the calculation. This configuration has a huge steric hindrance between the hydrogen atoms of 16 and 17 OH groups.

Figure 8 shows the potential energy curves of estriol along the conformation coordinate in the *D*-ring. In Figure 8(a), the configuration of 16OH is fixed to "*gauche*(+)" and the energy is calculated as a function of the dihedral angle of H–17C–O–H. Similarly, in Figures 8(b) and (c), 16OH is fixed to "*anti*" and "*gauche*(-)", respectively, and the dihedral angle of 17 OH is changed. In Figures 8(a) and 8(b), the intramolecular H-bonded conformer is most stable, and there is very small barrier (2.47 kJ/mol at most) between the lowest conformer and higher ones. Therefore, only the

intramolecular H-bonded conformer will be generated in these configurations. On the other hand, in Figure 8(c), both "*16g-_17g-*" and "*16g-_17a*" are stable and there is a large barrier (~6kJ/mol) between them. Thus, both conformers will be generated in the jet. Thus, four conformers are expected to coexist for *trans* and *cis*-conformers of estriol in the supersonic jet, which is in good agreement with the experiment. From these results, we conclude that all bare estriol observed in LIF spectra have the intramolecular H-bonded structure.

We then assign the bands of estriol in the LIF spectrum to the conformers in Fig. 7, based on the calculated total energies and IR spectra. In the LIF spectrum of Fig. 4(a), the band intensity is in the order **3** > **1** > **2** > **4** for the *cis*-conformer, and **7** > **5** > **6** > **8** for the *trans*-conformer. If the fluorescence quantum yield is same for all the conformers, the relative band intensity will correlate to the relative stability of the conformers. In the calculation, the relative stability of the *cis*- and *trans*-conformer is in the order 16*g*-_17*g*+ > 16*g*+_17*g*- > 16*g*-_17*a* > 16a_17g-. Thus, the bands **3**, **1**, **2**, and **4** can be assigned to 3*c*_16*g*-_17*g*+, 3*c*_16*g*+_17*g*-, 3*c*_16*g*-_17*a* and 3c_16a_17g-, respectively. In the same manner, the bands **7**, **5**, **6** to **8** can be assigned to the corresponding *trans* conformers, that is, 3*t*_16*g*-_17*g*+, 3*t*_16*g*+_17*g*-, 3*t*_16*g*-_17*a* and 3t_16a_17g-, respectively. These assignments are further supported by the comparison of the observed IR spectra with those of calculated ones in Figs. 5(a)-(f), where the calculated IR spectra of the eight intramolecular H-bonded conformers are compared with the observed IR-UV DR spectra. In all the calculated spectra, the *A*-ring (phenolic) OH stretching vibration appears at the same position for different conformers. In the observed IR spectra, this vibration is considered to correspond to band appeared at 3656-3658 $cm^{-1}$ for all the complexes. On the other hand, in the calculated spectra the positions of the two OH stretching vibrations in the *D*-ring sensitively differ for different conformers, which gives an appearance of the two OH bands in overall with different energy interval for different conformers. Among the possible conformers, the calculated 3*c*_16*g*+_17*g*- and 3*t*_16*g*+_17*g*-conformers show a small interval (~10 $cm^{-1}$) of the two OH bands compared to other conformers. These conformers correspond to the species of bands **1** and **5**, respectively, showing 6-7

cm$^{-1}$ intervals in their IR spectra. The assignment of bands **1** and **5** to 3*c*_16*g*+_17*g*- and 3*t*_16*g*+_17*g* , respectively, is in good agreement with that determined from the relative stability as discussed above. For other conformers, the observed spectra are also compared with those of the plausible conformers based on the relative stability. In these species, two OH bands are observed with the interval of 12-14 cm$^{-1}$, which correspond to 20 cm$^{-1}$ in the calculated spectra. We see that the calculated IR spectrum of each conformer well reproduces the observed IR spectrum of each band. Tables 1 and 2 show the assignments of the structures for the observed bands and the relative energies.

### III.3 Structures of hydrated clusters of estrogens

#### III.3.1 Hydrated clusters of β-estradiol

In Fig. 3(a), weak bands are seen in the 34700-34900 cm$^{-1}$ region, which are 300-400 cm$^{-1}$ red-shifted from the monomer bands **a**-**d**. The intensities of these bands, labeled as bands **A**-**D**, become very stronger by adding the water vapor so that they are attributed to hydrated clusters of β-estradiol. In our previous study[22], we assigned them to the mono-hydrated cluster of β-estradiol in which a water molecules is H-bonded to the *A*-ring OH, that is β-estradiol-(*A*)-$H_2O$. Figure 9 exhibit the stable structures of β-estradiol-(*A*)-$H_2O$ obtained by DFT calculation. Among the calculated six isomers, we assigned bands **A** and **B** to *cis*-*anti*-(*A*)-$H_2O$ and *cis*-*gauche*(+)-(*A*)-$H_2O$, respectively, and bands **C** and **D** to *trans*-*anti*-(*A*)-$H_2O$, and *trans*-*gauche*(+)-(*A*)-$H_2O$, respectively. The reason of the missing of the other two higher energy isomers can be explained by the low energy barrier between *cis*-*gauche*(-)-(*A*)-$H_2O$ to *cis*-*anti*-(*A*)-$H_2O$, that is essentially the same as the case of monomer.

The bands of other isomers of the mono-hydrated cluster are observed near the band origins of the monomer. They are marked by asterisks in the LIF spectrum of Fig. 3(c). These bands are also observed by adding water vapor to the sample gas. The UV-UV HB spectroscopic measurement indicates that they are different isomers of species **A**-**D**. By comparing the electronic transition using

TD-DFT calculation, we concluded that they are the hydrated cluster of β-estradiol in which a water molecule is bound to the *D*-ring OH, β-estradiol-(*D*)-$H_2O$, as shown in Fig. 9(b). In these isomers, the *D*-ring OH acts either as an H-donor or acceptor depending on its conformation. Such the different role is a typical behavior of a secondary alcohol. For example, the H-bond donor and acceptor parameters ($\log K_\alpha$ and $\log K_\beta$)[30] of 2-propanol are reported to be 0.91 and 1.36, respectively, while those of phenol are 2.1 and 0.2, respectively.[7, 30] Thus, in β-estradiol, the *A*-ring OH works mainly as an H-donor, while the *D*-ring OH works as an H-donor and an acceptor, which is consistent with the computational results by Gao et al[19]. As seen in Figs. 3(b) and 3(c), the relative intensity of the bands attributed to β-estradiol-(*A*)-$H_2O$ is much stronger than those of β-estradiol-(*D*)-$H_2O$, because of higher acidity of *A*-ring OH than the *D*-ring OH. The calculated energies of the clusters also support larger stabilization energy of β-estradiol-(*A*)-$H_2O$ than β-estradiol-(*D*)-$H_2O$; the latter isomers are 3-6 kJ/mol higher than the former isomers. The *A*-ring is more hydrophilic than the *D*-ring in β-estradiol.

**III.3.2 Hydrated clusters of estriol**

In estriol, an additional OH group is substituted to β-estradiol at 16α position of the *D*-ring. As mentioned in bare estriol, the additional OH group largely controls the conformation of the *D*-ring OHs. That is only the intramolecular H-bonded conformers are generated. This situation will also affect the H-bond formation with water molecule(s). Actually, we did not observe the $S_1$-$S_0$ bands attributed to the clusters H-bonded at the *A*-ring OH as shown in Fig. 4(c). In this spectrum, we measured LIF spectrum by adding water vapor to He carrier gas. The bands attributed to estriol-(*A*)-$H_2O$ are expected to appear in the 34700-34800 $cm^{-1}$ region, which are red-shifted by 300-400 $cm^{-1}$ from the monomer bands. Instead, the relative intensities of bands **1**-**12** change by adding water vapor. Actually, bands **9**-**11** are attributed to the hydrated clusters as seen in the IR-UV DR spectra of Figs. 5(g)-(i). Though we did not measure the IR spectrum of band **12**, this band is also attributed to the hydrated cluster from the relative position of bands **12** and **10**. In these IR

spectra, a sharp band appears at 3720 $cm^{-1}$ and a broad one at 3535 $cm^{-1}$, which are assigned to the free OH stretch and the H-bonded OH of water, respectively. Since their $S_1$-$S_0$ band positions are close to those of monomer, they are assigned to the hydrated clusters in which water is H-bonded to the *D*-ring OHs.

Figure 10(a) exhibits stable isomers of estriol-(*D*)-$H_2O$ obtained by DFT calculation. The ZPE corrected energies relative to the most stable isomer, (3*t*_16*g*+_17*g*-)-(*D*)-$H_2O$, are also shown in the figure. In each *trans*- and *cis*-conformer, there are four most stable isomers within 2 kJ/mol. They have a ring-type H-bonded structure, in which the 17OH acts as a donor to the water molecule and the 16OH acts as an acceptor or vice versa. Another point that should be noted is that the number of possible conformers for estriol is reduced upon the H-bonding with a water molecule on the *D*-ring site. In bare estriol, the number of possible conformers is sixteen, while it is reduced to twelve as seen in Fig. 10(a). This reduction is experimentally supported from the number of $S_1$-$S_0$ bands of estriol monomer and estriol-(*D*)-$H_2O$; the number of estriol is eight (bands **1**-**8**) while it is reduced to four (bands **9**-**12**) in the cluster. Such the reduction of the possible conformation or conformation preference was observed in hydrated clusters of amino acids[31], crown ethers[32] and other molecules[33]. Figure 5(j) shows the calculated IR spectra of the four lowest energy conformers of *cis*-estriol-(*D*)-$H_2O$, which can be compared with the observed IR-UV DR spectra of bands **9**-**11**. The IR spectra of *trans*-conformer are essentially the same with those of *cis*-conformer, so that they are not shown here. All of them show very similar spectral feature. The bands at 3500-3520 $cm^{-1}$ are the mixed vibrations of H-bonded OH group of estriol (17OH or 16OH) and $H_2O$ and the band at 3710 $cm^{-1}$ is assigned to $\nu_3$ of $H_2O$ free from the H-bond. The band at 3567 $cm^{-1}$, which appears in all the clusters are assigned to *A*-ring OH.

In addition to the comparison of the IR spectra, we also compared the electronic transition energies for the additional support of the structure determination. Figure 4 shows the $S_1$-$S_0$ electronic transition energies of (d) estriol, (e) estriol-(*D*)-$H_2O$ and (f) estriol-(*A*)-$H_2O$ obtained by TD-DFT calculation. The band positions of both estriol monomer and estriol-(*D*)-$H_2O$ are well reproduced in

the calculation. The $S_1$-$S_0$ band origins of the estriol-(*A*)-$H_2O$ isomer are predicted to appear at 34700-34800 $cm^{-1}$, but no band appears in this region as seen in Figure 4(c). Thus, it is concluded that the estriol-(*A*)-$H_2O$ isomer is not generated in the present condition. Figures 6(b) and 6(c) show the position of low frequency bands of estriol-(*D*)-$H_2O$ in $S_0$, which can be compared to the UV-UV HB spectra of Figure 6(a). In the UV-UV HB spectra of the monomer (band **1**-**7**), two bands are observed at 31 and 47 $cm^{-1}$, and in the cluster (bands **9**-**11)**, three bands are observed at 25, 37 and 45 $cm^{-1}$. In the calculated spectra of monomer two bands appear at 30 and 57 $cm^{-1}$, and three bands appear at 24, 37 and 54 $cm^{-1}$ in the clusters. The results also support the validity of the assignments of the hydrated cluster of estriol.

We then compare the stability of the H-bonding at *A*-ring OH of estriol. Figure 10(b) shows the stable isomers of estriol-(*A*)-$H_2O$ calculated at the same level of calculation. Very interestingly, the energies of the *A*-ring hydrated clusters are more than 11 kJ/mol higher than the *D*-ring hydrated clusters, estriol-(*A*)-$H_2O$. This is opposite to the case β-estradiol-$H_2O$, where β-estradiol-(*A*)-$H_2O$ are 4-6 kJ/mol more stable than β-estradiol-(*D*)-$H_2O$ as seen in Fig. 9. The results strongly indicate that the H-bonding ability of *D*-ring of estriol is much larger than that of β-estradiol, or *D*-ring of estriol is much more hydrophilic than that of estriol.

This result sounds very strange since physiological activity of β-estradiol is known to be stronger than estriol, and we simply consider that the activity will be proportional to the H-bonding ability of the *D*-ring. However, the present results show opposite relationship. That is the physiological activity of estriol having stronger H-bonding ability is weaker than β-estradiol with weaker H-bonding ability. Though we do not have clear answer to explain this contradiction, we can point out a few possibilities. In the biological system, the phenolic OH of the estrogen receptor (ER) first bind to the ligand binding domain (LBD) and the *D*-ring OH group(s) bind with histidine residue (His 524) either as a H-donor or an acceptor depending on the protonation state of the associated His 524. Thus, one possibility to explain lower physiological activity of estriol is that *D*-ring OHs of estriol first incorporate a water molecule by forming the stable ring-type H-bonding

and this water cannot be easily replaced with His 524. Another possibility is the functionality of the *D*-ring OH for the H-bonding. That is the aliphatic OH group has high deprotonation energy so that it will also act as a hydrogen acceptor. In this sence, the *D*-ring OH of β-estradiol can be H-donor or an acceptor depending on the orientation of the OH group so that it can more easily bind to His 524 compared to estriol.

## IV CONCLUSION

In this study, the conformation of bare estrone, β-estradiol, estriol and their H-bonded clusters with water molecule have been investigated by measuring the electronic spectra and IR spectra under jet-cooled conditions and by quantum chemical calculations. These species exhibit the electronic transitions in the same energy region, but the number of the conformers drastically increases in the order of estrone < β-estradiol < estriol. By comparing the structures of the conformers and isomers of β-estradiol and estriol, we found a considerable difference of the conformation stability and hydrogen-bonding ability between them. First in bare estriol, the intramolecular H-bonding formed by the two OH groups at the *D*-ring leads a severe conformational restriction of *D*-ring OHs. This restriction will result in lower receptor binding affinity[7] than that of β-estradiol, because the *D*-ring OH of β-estradiol is more flexible to bind with the receptor compared to estriol[19]. Secondly, β-estradiol and estriol show quite different H-bonding ability of their *D*-ring between them. In β-estradiol, the *A*-ring OH-$H_2O$ isomer is much more stable than the *D*-ring OH-$H_2O$ isomer, while it becomes opposite in estriol. In other words, *A*-ring is more hydrophilic than *D*-ring in β-estradiol, while in estriol *D*-ring is more hydrophilic than *A*-ring. It is not clear how the conformational stability and the opposite H-bonding ability will reflect in the difference in their physiological activities, and further study on the structures on the clusters with histidine residue will be necessary.

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**Table 1.** Positions ($cm^{-1}$) of the $S_1$-$S_0$ band origin and assignments for estrone, β-estradiol, estriol and and their 1:1 hydrated clusters.

| species | Position / $cm^{-1}$ | Label | Assignment | Species | Position / $cm^{-1}$ | Label | Assignment |
|---|---|---|---|---|---|---|---|
| estrone | 35065 | **1** | *cis*-estrone | estriol | 35046 | **1** | 3*c*_16*g*+_17*g*- |
| | 35164 | **2** | *trans*-estrone | | 35053 | **2** | 3*c*_16*g*-_17*a* |
| | | | | | 35059 | **3** | 3*c*_16*g*-_17*g*+ |
| β-estradiol | 35048 | **a** | *cis-anti* | | 35039 | **4** | 3c_16a_17g- |
| | 35051 | **b** | *cis-gauche*(+) | | 35147 | **5** | 3*t*_16*g*+_17*g*- |
| | 35148 | **c** | *trans-anti* | | 35153 | **6** | 3*t*_16*g*-_17*a* |
| | 35151 | **d** | *trans-gauche*(+) | | 35159 | **7** | 3*t*_16*g*-_17*g*+ |
| β-estradiol-$H_2O$ | 34710 | **A** | *cis-anti*-(*A*)-$H_2O$[a] | | 35142 | **8** | 3t_16a_17g- |
| | 34713 | **B** | *cis-gauche*(+)-(*A*)-$H_2O$[a] | estriol-$H_2O$ | 35043 | **9** | *cis*-estriol-(*D*)-$H_2O$[a] |
| | 34805 | **C** | *trans-anti*-(*A*)-$H_2O$[a] | | 35062 | **10** | *cis*-estriol-(*D*)-$H_2O$[a] |
| | 34810 | **D** | *trans-gauche*(+)-(*A*)-$H_2O$[a] | | 35144 | **11** | *trans*-estriol-(*D*)-$H_2O$[a] |
| | ~35031 | * | β-estradiol-(*D*)-($H_2O$) | | 35163 | **12** | *trans*-estriol-(*D*)-$H_2O$[a] |
| | ~35057 | * | β-estradiol-(*D*)-($H_2O$) | | | | |
| | 35157 | * | β-estradiol-(*D*)-($H_2O$) | | | | |

[a] (*A* or *D*)-$H_2O$ represents the 1:1 hydrated cluster in which $H_2O$ is H-bonded to the *A- or D*-ring OH. See text.

**Table 2** Relative energies (kJ/mol) of possible conformers of bare estriol, estriol(*D*)-$H_2O$ and estriol(*A*)-$H_2O$ obtained by DFT calculation at the M05-2X/6-311++G** level.

| | bare-estriol[a] | Estriol-(*D*)-$H_2O$[b] | Estriol-(*A*)-$H_2O$[b] |
|---|---|---|---|
| 3*c*_16*g*-_17*g*+ | **0.00** | 1.66 | 11.7 |
| 3*c*_16*g*+_17*g*- | 0.554 | 0.168 | 12.4 |
| 3*c*_16*g*-_17*a* | 2.49 | 1.81 | 14.0 |
| 3*c*_16*a*_17*g*- | 2.94 | 1.58 | 14.5 |
| 3*c*_16*g*+_17*g*+ | 5.57 | 15.7 | 17.4 |
| 3*c*16*g*+_17*a* | 8.85 | - | 20.4 |
| 3*c*16*a*_17*g*+ | 9.52 | 17.4 | 20.5 |
| 3*c*_16*a*_17*a* | 9.96 | - | 21.3 |
| 3*t*_16*g*-_17*g*+ | 0.268 | 1.78 | 11.6 |
| 3*t*_16*g*+_17*g*- | 0.554 | **0.00** | 11.8 |
| 3*t*_16*g*-_17*a* | 2.48 | 1.83 | 13.3 |
| 3*t*_16*a*_17*g*- | 2.94 | 1.37 | 14.1 |
| 3*t*_16*g*+_17*g*+ | 5.55 | 15.6 | 17.0 |
| 3*t*_16*g*+_17*a* | 8.73 | - | 19.6 |
| 3*t*_16*a*_17*a* | 9.52 | - | 20.6 |
| 3*t*_16*a*_17*g*+ | 9.63 | 17.5 | 20.7 |

[a] relative to the most stable 3*c*_16*g*-_17*g*+ conformer of bare estriol

[b] relative to the most stable 3*t*_16*g*+_17*g*- conformer of estriol-(*D*)-$H_2O$

**Figure caption**

Figure 1. The LIF spectra of jet-cooled (a) estrone, (b) β-estradiol and (c) estriol in the $S_1$-$S_0$ band origin region.

Figure 2. (a) The $S_1$-$S_0$ LIF spectrum of jet-cooled estrone. (b) The UV-UV HB spectra of estrone obtained by monitoring bands **1** and **2**. (c) The calculated $S_1$–$S_0$ transition energy and oscillator strengths of bare estrone, where a scaling factor of 0.8432 is multiplied to the transition energies. (d) IR-UV DR spectra of the OH stretching vibration of estrone observed by monitoring bands **1** and **2**. (e) The calculated IR spectra of *cis* and *trans*-estrone. A scaling factor of 0.9360 is multiplied. (f) *Cis* and *trans*-conformers of estrone calculated at the M05-2X/6-311++G** level. The numbers represent the ZPE corrected total energy relative to that of the most stable one(*trans*-estrone).

Figure 3. (a) The $S_1$–$S_0$ LIF spectrum of jet-cooled β-estradiol. (b) The $S_1$–$S_0$ LIF spectrum of β-estradiol obtained by adding large amount water vapor to He carrier gas. Bands **A**–**D** are assigned to the origin band of β-estradiol(*A*)-$H_2O$. See text. (c) The LIF spectrum of the β-estradiol-water clusters obtained with a trace of water vapor in the sample gas. The bands marked by asterisks are assigned to β-estradiol(*D*)-$H_2O$. (d) (Upper) Stable conformers of bare β-estradiol calculated at the M05-2X/6-311++G** level. The numbers in the figure represent the total energy relative to that of the most stable one (*trans*-*gauche*(+)). (Bottom) Newman-projections of *anti*, *gauche*(+), and *gauche*(–) configurations at the OH group of the *D*-ring.

Figure 4. (a) The $S_1$–$S_0$ LIF spectrum of jet-cooled estriol. (b) The UV-UV HB spectra of estriol obtained by monitoring bands **1**-**7** and **9**-**11**. (c) The $S_1$–$S_0$ LIF spectrum of estriol measured by adding water vapor to He carrier gas. The calculated $S_1$–$S_0$ transition energy and oscillator strengths of (f) bare estriol (g) estriol-(*D*)-$H_2O$, and (h) estriol-(*A*)-$H_2O$ calculated at the M05-2X/6-311++G** level. A scaling factor of 0.8432 is used.

Figure 5. (a)-(f) IR-UV DR spectra obtained by monitoring monomer bands of estriol **1**-**3**, **5**-**7** and its 1:1 hydrated cluster bands **9**-**11.** In figures (a)-(f), also shown are the calculated IR spectra of possible conformers having intramolecular H-bond in *D*-ring. See text. A scaling factor of 0.9383 is used. (j) The calculated IR spectra of the possible four most stable *cis*-estriol(*D*)-$H_2O$ isomers. A scaling factor of 0.9359 is used.

Figure 6. (a) The UV-UV HB spectra of estriol. The horizontal axis represents the energy relative to the origin bands and only the bands less than 90 $cm^{-1}$ are shown. (b), (c) The DFT calculated vibrations for the most stable eight conformers of bare estriol and estriol(*D*)-$H_2O$ in the 0-60 $cm^{-1}$ region of $S_0$.

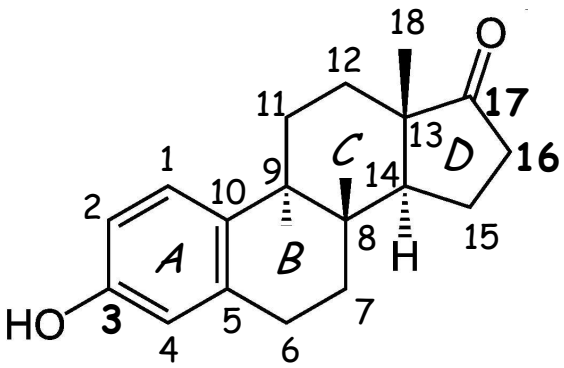
Figure 7. Stable conformers of bare estriol calculated at the M05-2X/6-311++G** level. The numbers in each structure represent the energy relative to that of the most stable one (3*c*_16*g*-_17*g*+). (Right panel) The enlarged portion of *D*-ring conformation of estriol. The dashed line represents the intramolecular H-bond.

Figure 8. The one-dimensional potential energy curves of estriol vs the dihedral angle of the 17OH group of the *D*-ring with the 16OH conformation fixed at (a)*g*+, (b)*a* and (c)*g*-. ZEP corrections are not included.

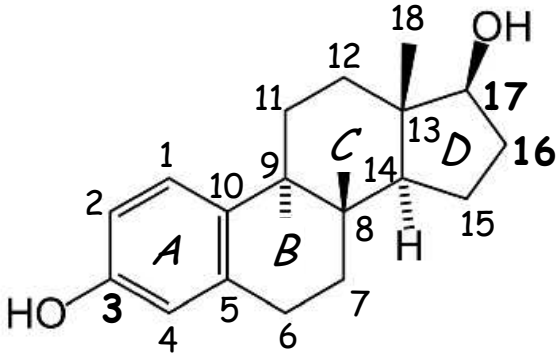
Figure 9. Stable isomers of (a) β-estradiol-(*A*)-$H_2O$ and (b) β-estradiol-(*D*)-$H_2O$ calculated at the M05-2X/6-311++G** level. The numbers in each structure represent the energy relative to that of the most stable one (*cis*-*gauche*(+)-β-estradiol-(*A*)-$H_2O$). The dashed line represents the H-bond.

Figure 10. Stable isomers of (a) estriol-(*D*)-$H_2O$ and (b) estriol-(*A*)-$H_2O$ calculated at the M05-2X/6-311++G** level. The numbers in each structure represent the energy relative to that of the most stable one ((3*t*_16*g*+_17*g*-)-estriol-(*D*)-$H_2O$). The dashed line represents the H-bond.

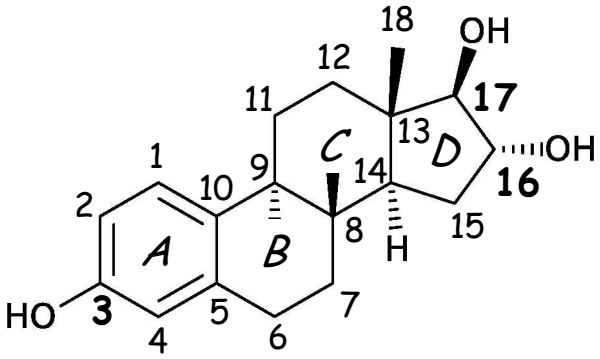
**scheme.1** estrogen




estrone($E_1$)




β-estradiol($E_2$)




estriol($E_3$)

**Fig.1**

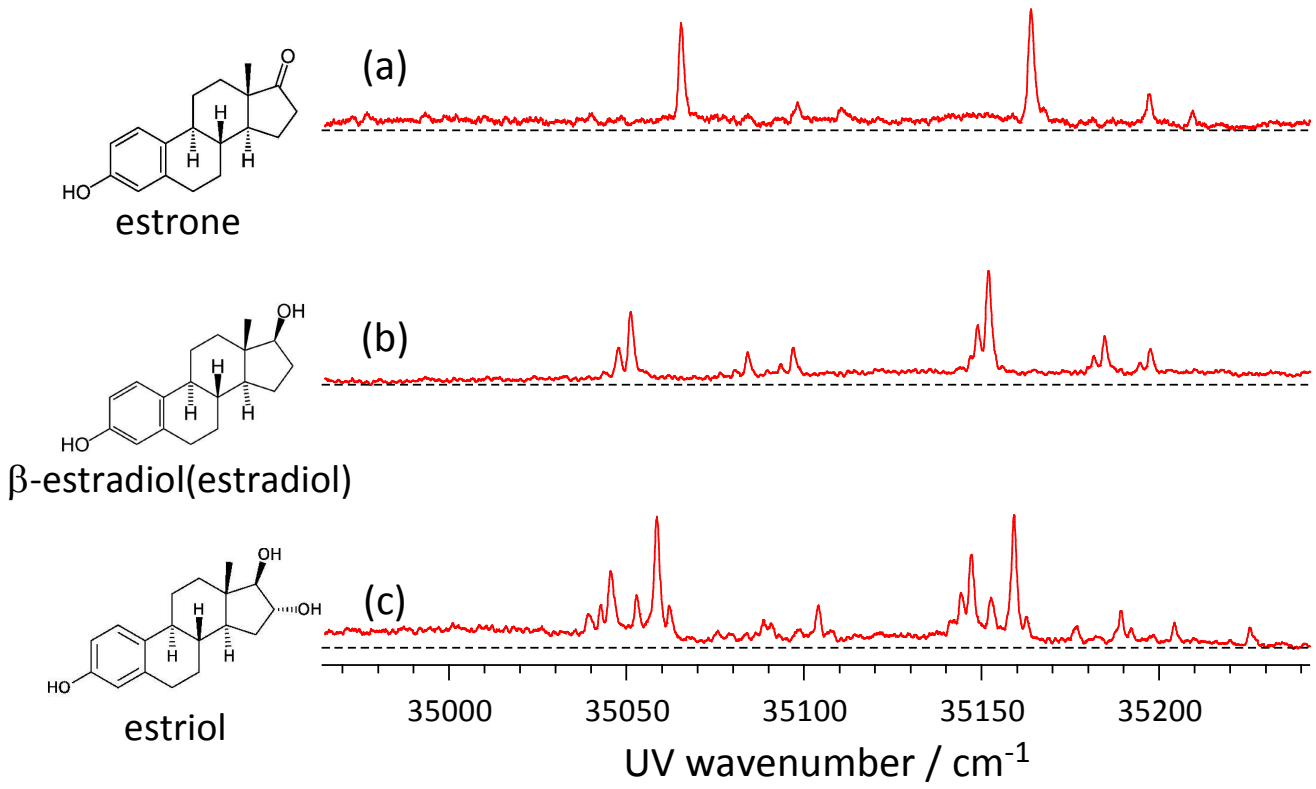

(a)
estrone
(b)
β-estradiol(estradiol)
(c)
estriol
35000
35050
35100
35150
35200
UV wavenumber / cm-1

**Fig.2**

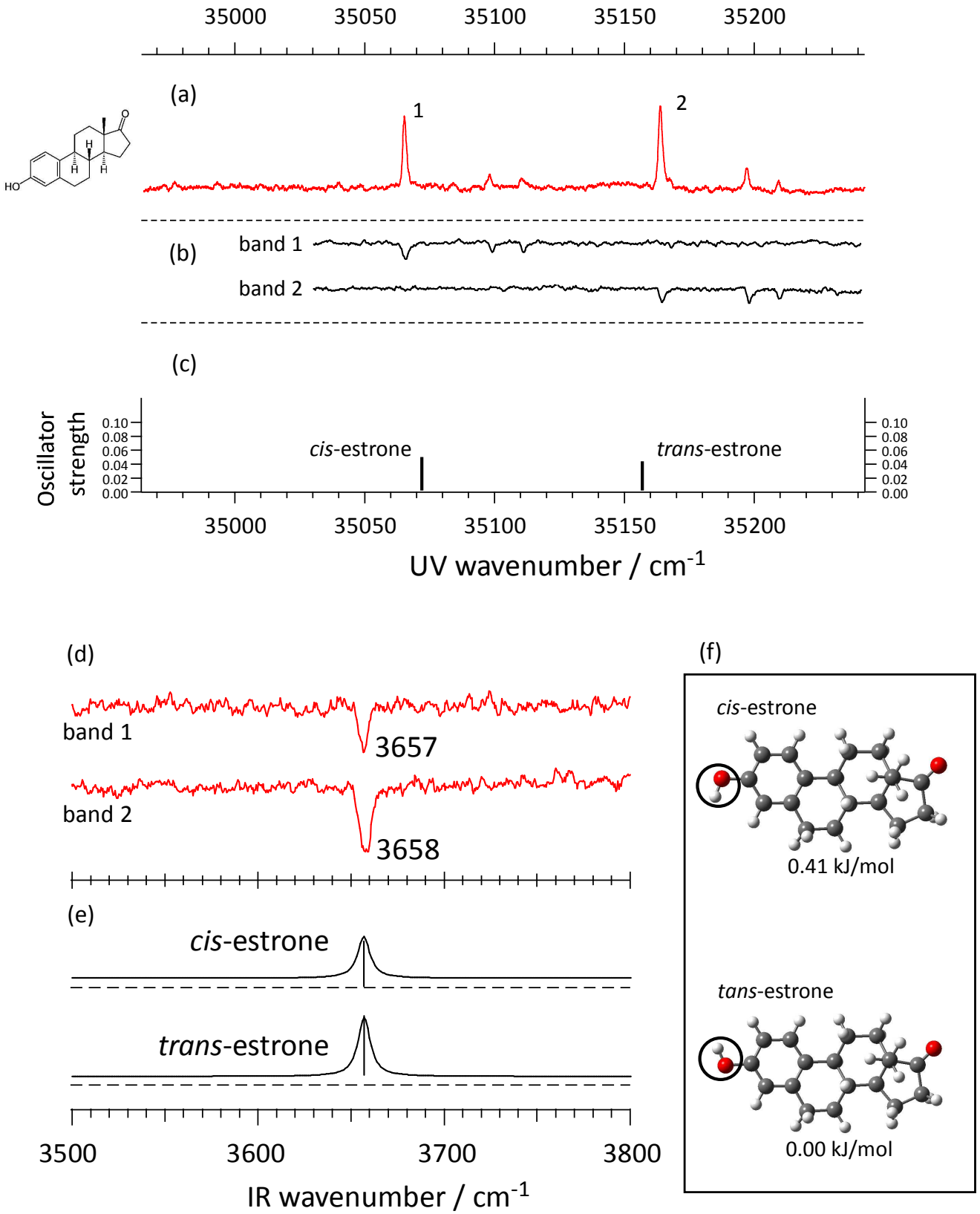

35000
35050
35100
35150
35200
(a)
1
2
HO
H
H
H
O
(b)
band 1
band 2
(c)
Oscillator strength
0.10
0.08
0.06
0.04
0.02
0.00
cis-estrone
trans-estrone
35000
35050
35100
35150
35200
UV wavenumber / cm-1
(d)
band 1
3657
band 2
3658
(e)
cis-estrone
trans-estrone
3500
3600
3700
3800
IR wavenumber / cm-1
(f)
cis-estrone
0.41 kJ/mol
tans-estrone
0.00 kJ/mol

Fig.3

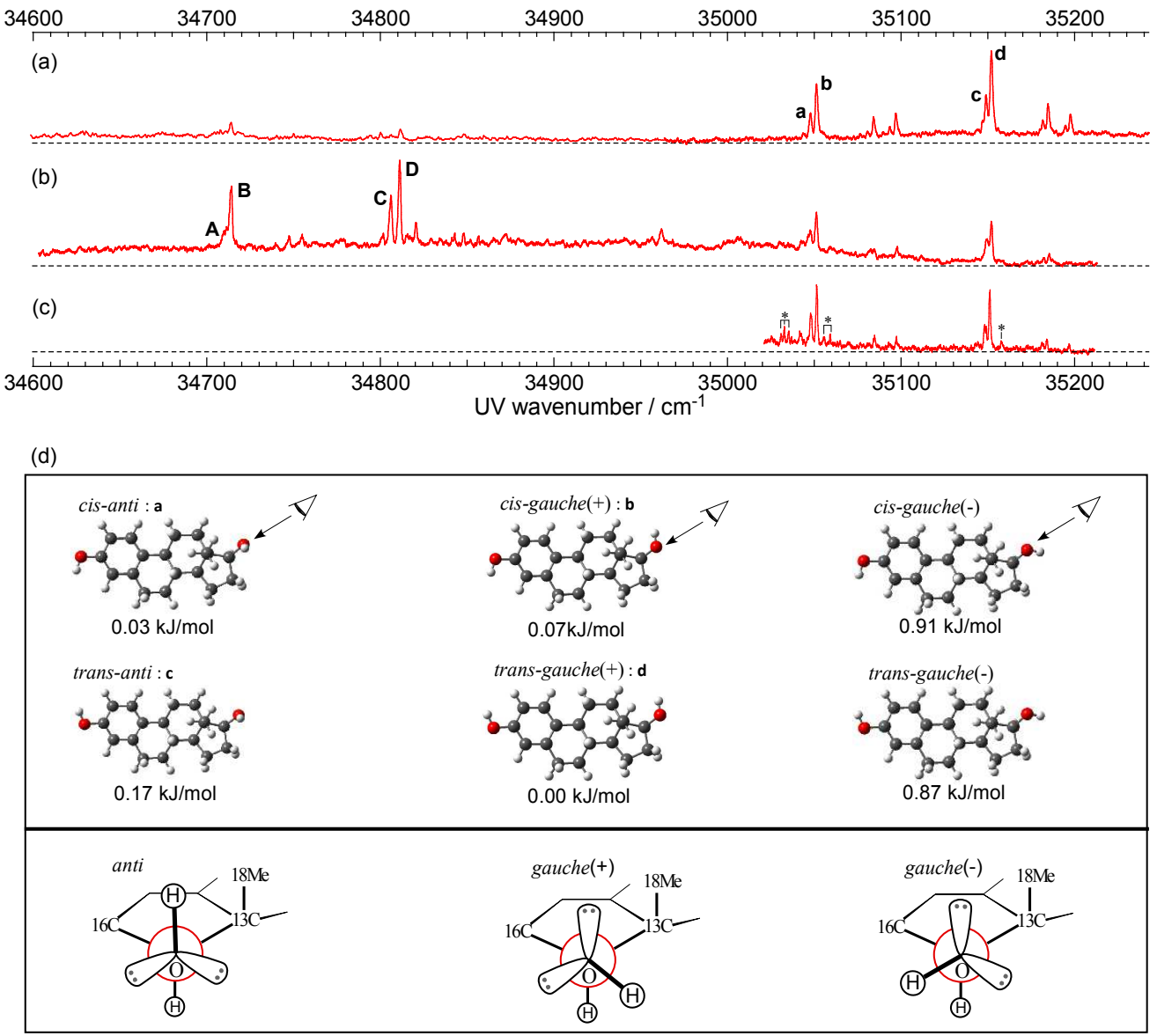

34600
34700
34800
34900
35000
35100
35200
(a)
a
b
c
d
(b)
A
B
C
D
(c)
UV wavenumber / cm-1
(d)
cis-anti : a
0.03 kJ/mol
cis-gauche(+) : b
0.07kJ/mol
cis-gauche(-)
0.91 kJ/mol
trans-anti : c
0.17 kJ/mol
trans-gauche(+) : d
0.00 kJ/mol
trans-gauche(-)
0.87 kJ/mol
anti
gauche(+)
gauche(-)
18Me
16C
13C
O
H

Fig.4

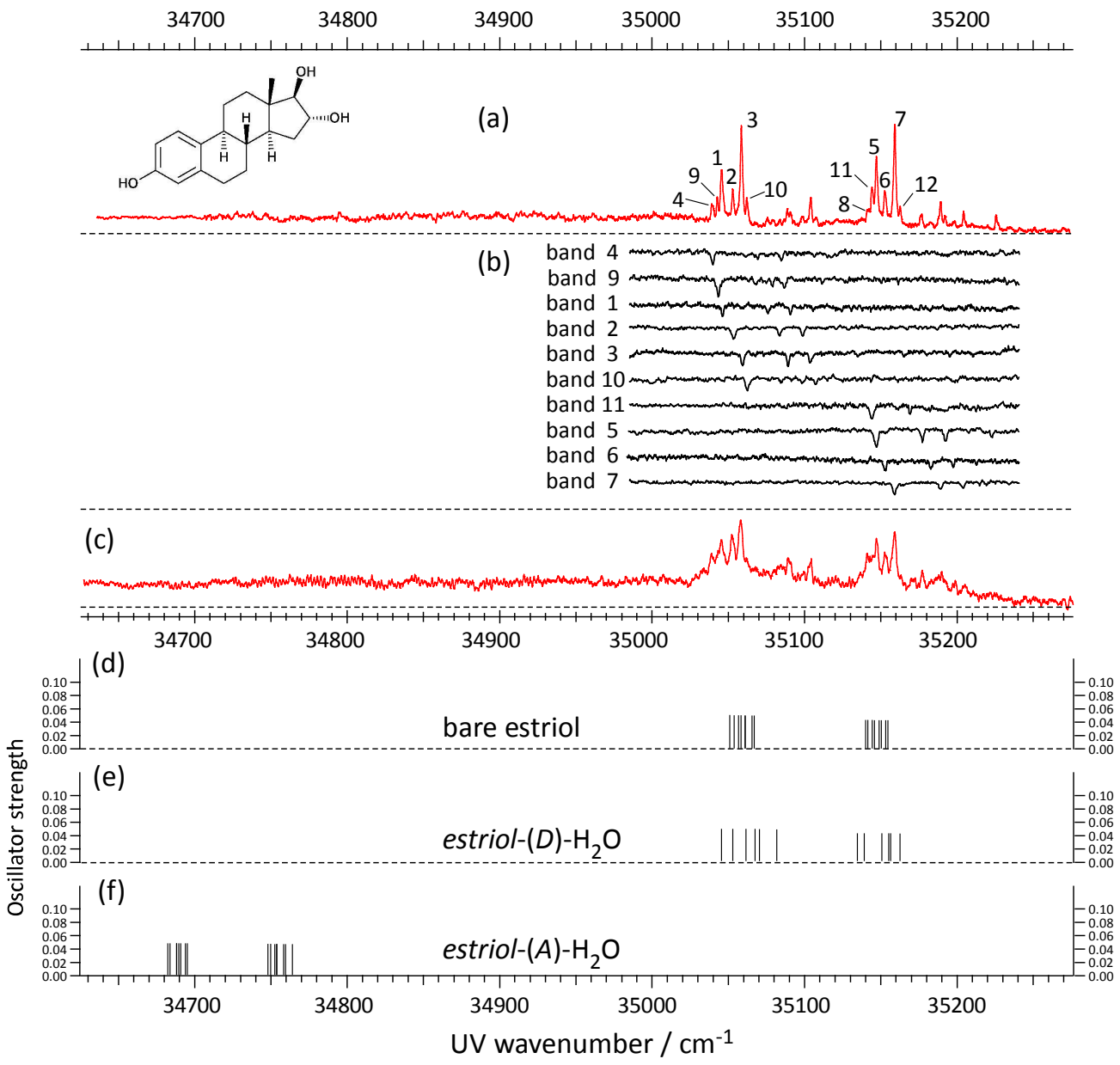

34700
34800
34900
35000
35100
35200
OH
OH
HO
H
H
H
(a)
1
2
3
4
5
6
7
8
9
10
11
12
(b)
band 4
band 9
band 1
band 2
band 3
band 10
band 11
band 5
band 6
band 7
(c)
(d)
bare estriol
(e)
estriol-(D)-H2O
(f)
estriol-(A)-H2O
Oscillator strength
0.00
0.02
0.04
0.06
0.08
0.10
UV wavenumber / cm-1

Fig.5

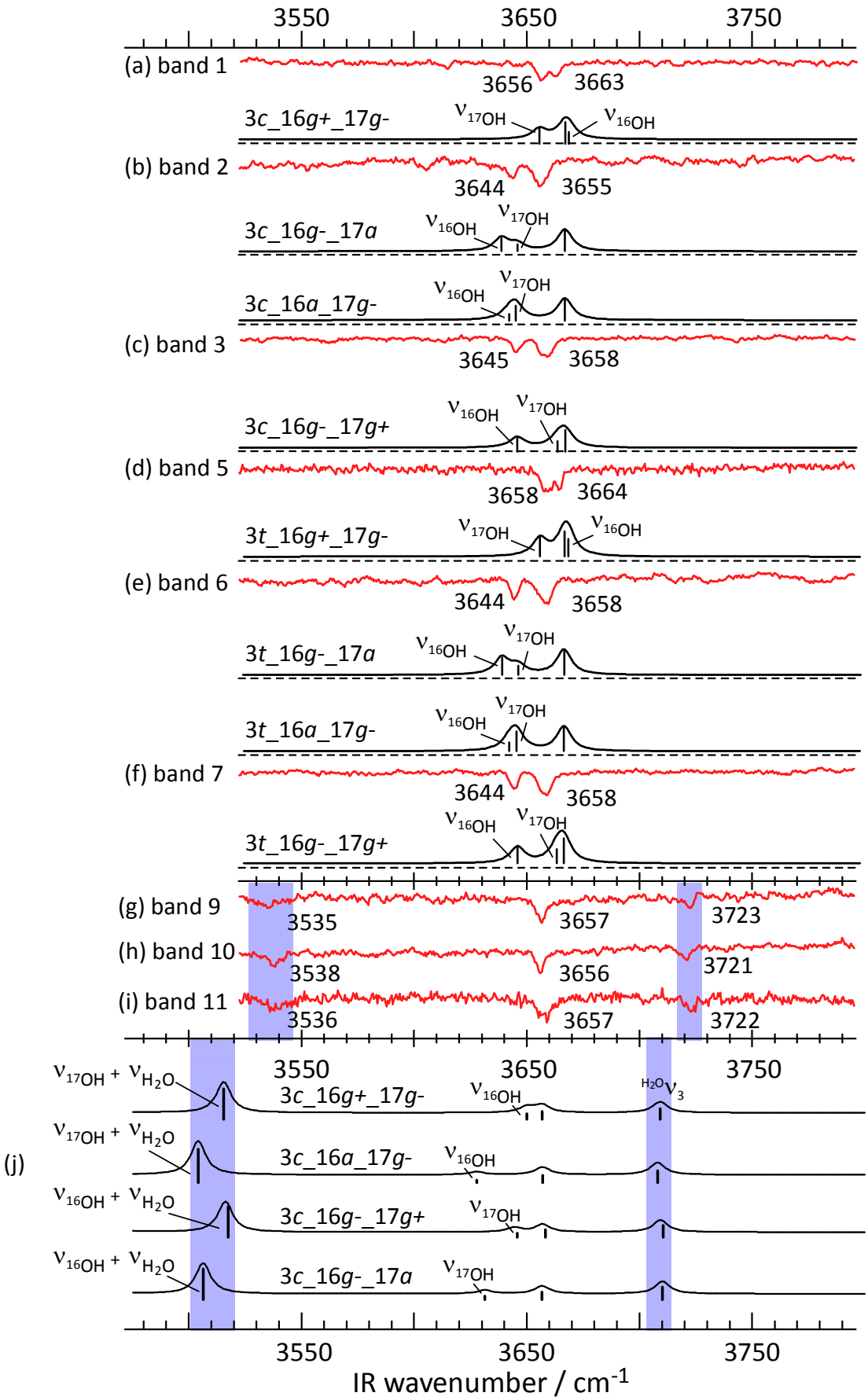

3550
3650
3750
(a) band 1
3656
3663
3c_16g+_17g-
ν17OH
ν16OH
(b) band 2
3644
3655
3c_16g-_17a
3c_16a_17g-
(c) band 3
3645
3658
3c_16g-_17g+
(d) band 5
3658
3664
3t_16g+_17g-
(e) band 6
3644
3658
3t_16g-_17a
3t_16a_17g-
(f) band 7
3644
3658
3t_16g-_17g+
(g) band 9
3535
3657
3723
(h) band 10
3538
3656
3721
(i) band 11
3536
3657
3722
(j)
ν17OH + νH2O
ν16OH + νH2O
3c_16g+_17g-
3c_16a_17g-
3c_16g-_17g+
3c_16g-_17a
H2Oν3
IR wavenumber / cm-1

Fig.6

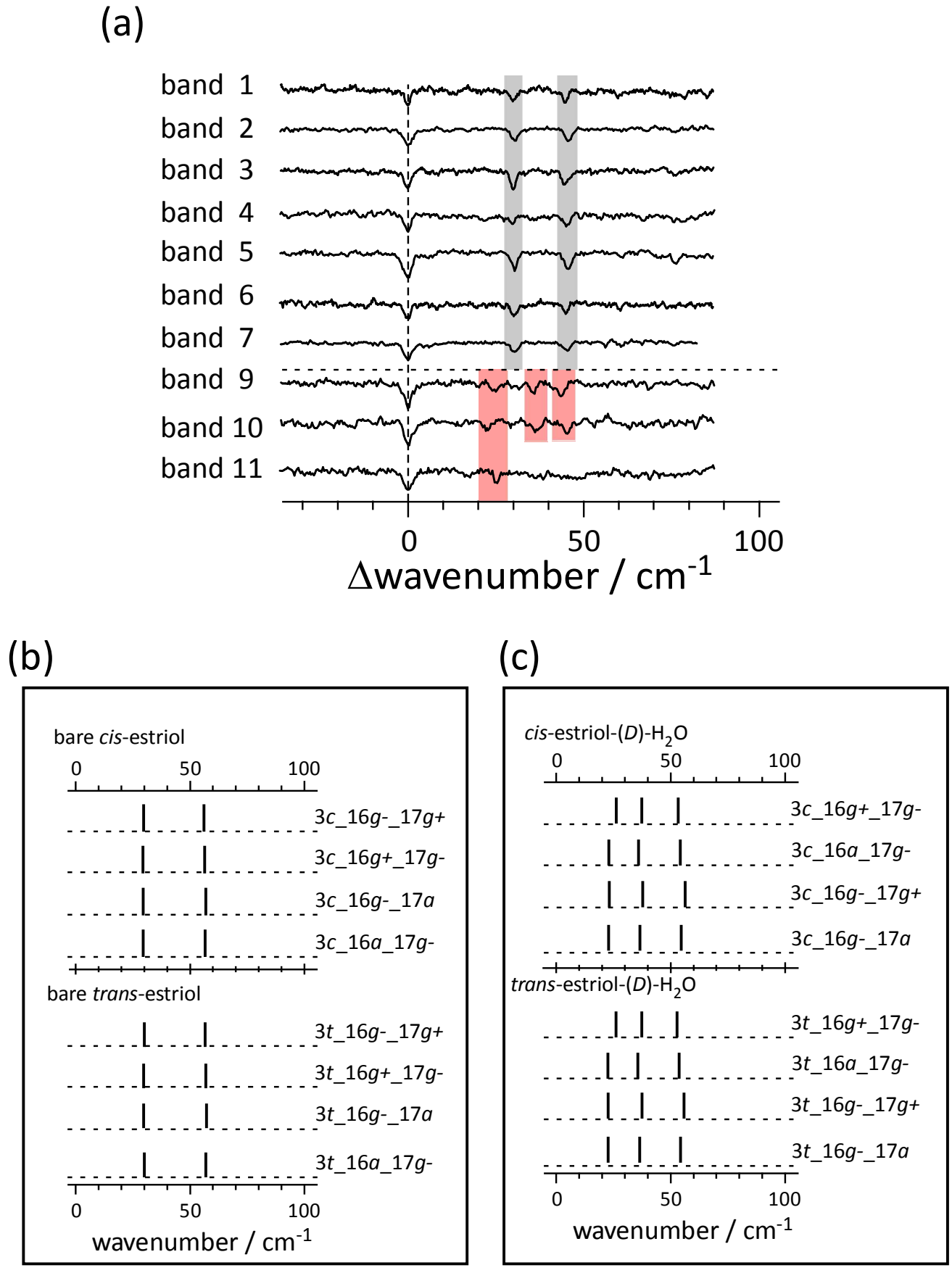

(a)
band 1
band 2
band 3
band 4
band 5
band 6
band 7
band 9
band 10
band 11
0
50
100
Δwavenumber / cm-1
(b)
bare cis-estriol
0
50
100
3c_16g-_17g+
3c_16g+_17g-
3c_16g-_17a
3c_16a_17g-
bare trans-estriol
3t_16g-_17g+
3t_16g+_17g-
3t_16g-_17a
3t_16a_17g-
0
50
100
wavenumber / cm-1
(c)
cis-estriol-(D)-H2O
0
50
100
3c_16g+_17g-
3c_16a_17g-
3c_16g-_17g+
3c_16g-_17a
trans-estriol-(D)-H2O
3t_16g+_17g-
3t_16a_17g-
3t_16g-_17g+
3t_16g-_17a
0
50
100
wavenumber / cm-1

Fig.7

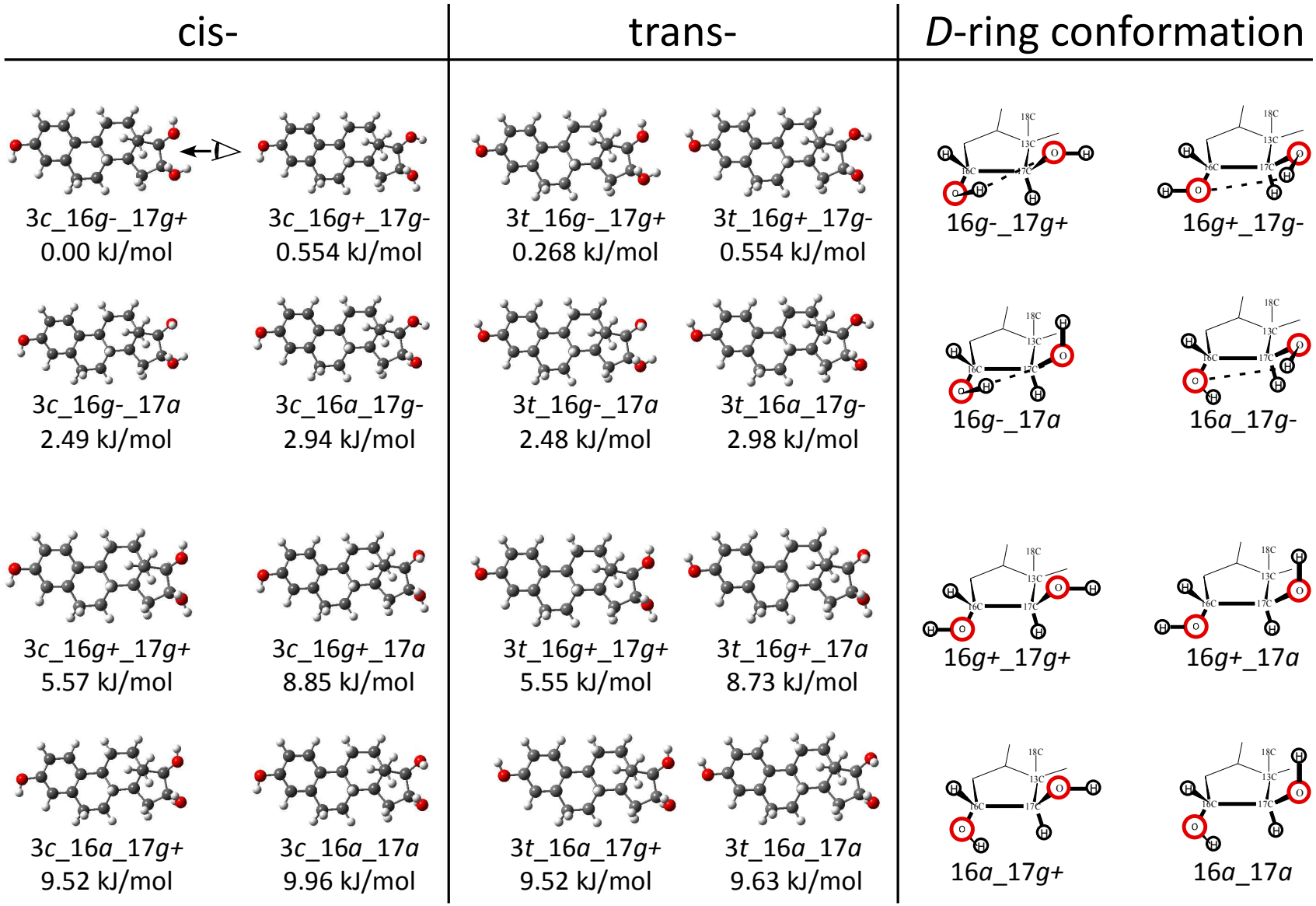

cis-
trans-
D-ring conformation
3c_16g-_17g+
0.00 kJ/mol
3c_16g+_17g-
0.554 kJ/mol
3t_16g-_17g+
0.268 kJ/mol
3t_16g+_17g-
0.554 kJ/mol
16g-_17g+
16g+_17g-
3c_16g-_17a
2.49 kJ/mol
3c_16a_17g-
2.94 kJ/mol
3t_16g-_17a
2.48 kJ/mol
3t_16a_17g-
2.98 kJ/mol
16g-_17a
16a_17g-
3c_16g+_17g+
5.57 kJ/mol
3c_16g+_17a
8.85 kJ/mol
3t_16g+_17g+
5.55 kJ/mol
3t_16g+_17a
8.73 kJ/mol
16g+_17g+
16g+_17a
3c_16a_17g+
9.52 kJ/mol
3c_16a_17a
9.96 kJ/mol
3t_16a_17g+
9.52 kJ/mol
3t_16a_17a
9.63 kJ/mol
16a_17g+
16a_17a

Fig.8

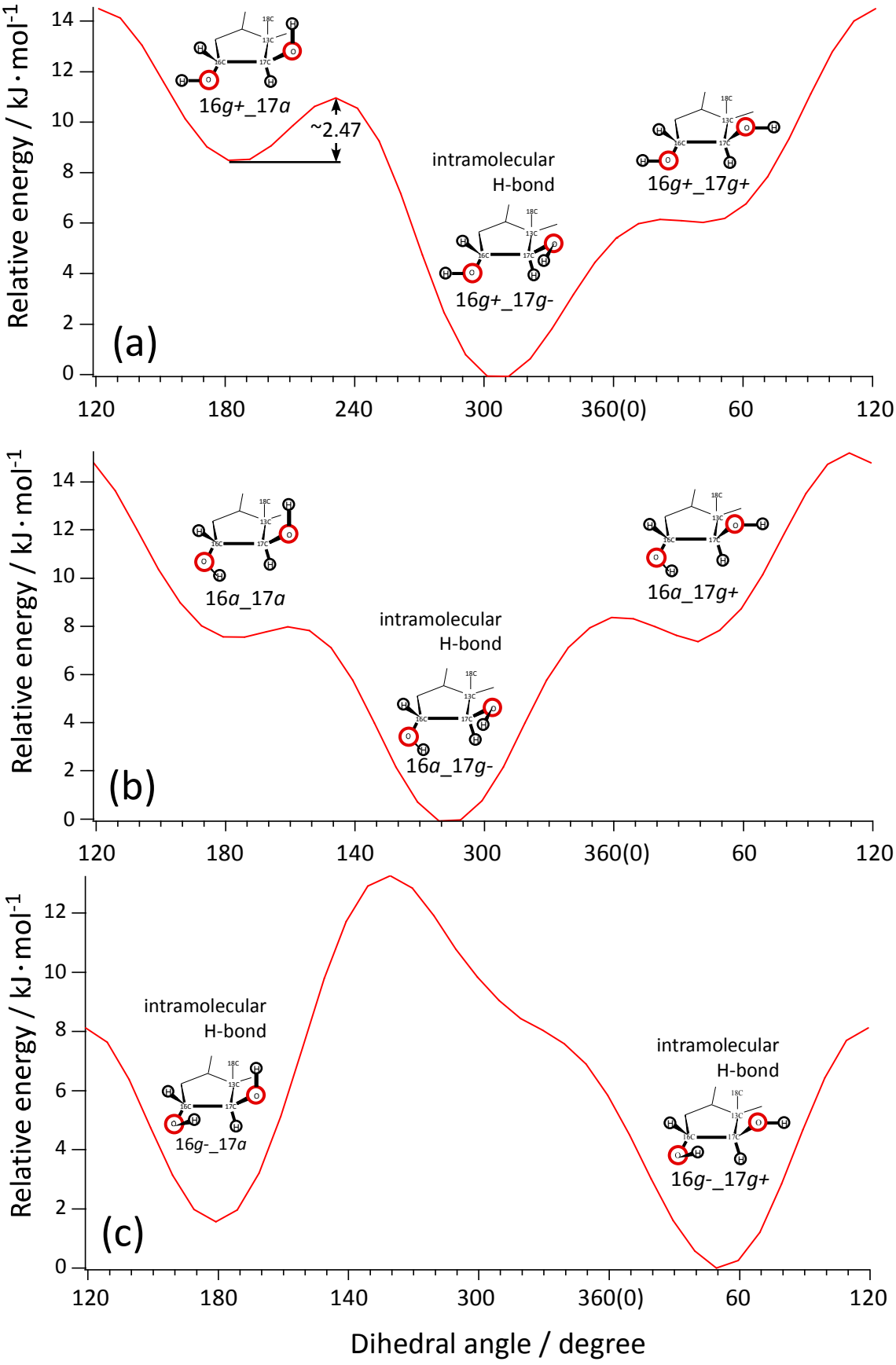

Relative energy / kJ·mol-1
16g+_17a
~2.47
intramolecular
H-bond
16g+_17g+
16g+_17g-
(a)
120
180
240
300
360(0)
60
120
Relative energy / kJ·mol-1
16a_17a
intramolecular
H-bond
16a_17g+
16a_17g-
(b)
120
180
140
300
360(0)
60
120
Relative energy / kJ·mol-1
intramolecular
H-bond
16g-_17a
intramolecular
H-bond
16g-_17g+
(c)
120
180
140
300
360(0)
60
120
Dihedral angle / degree

Fig.9

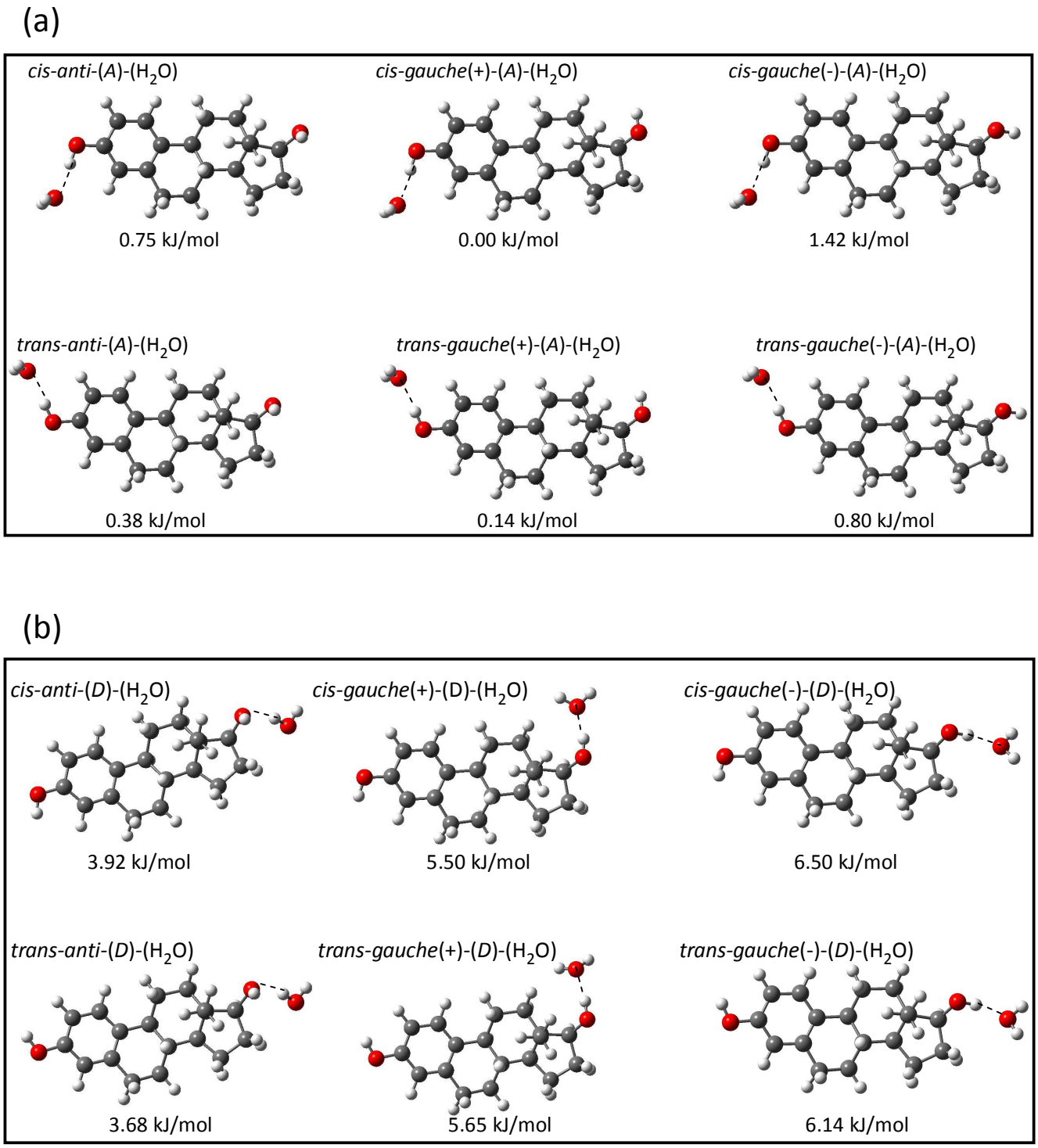
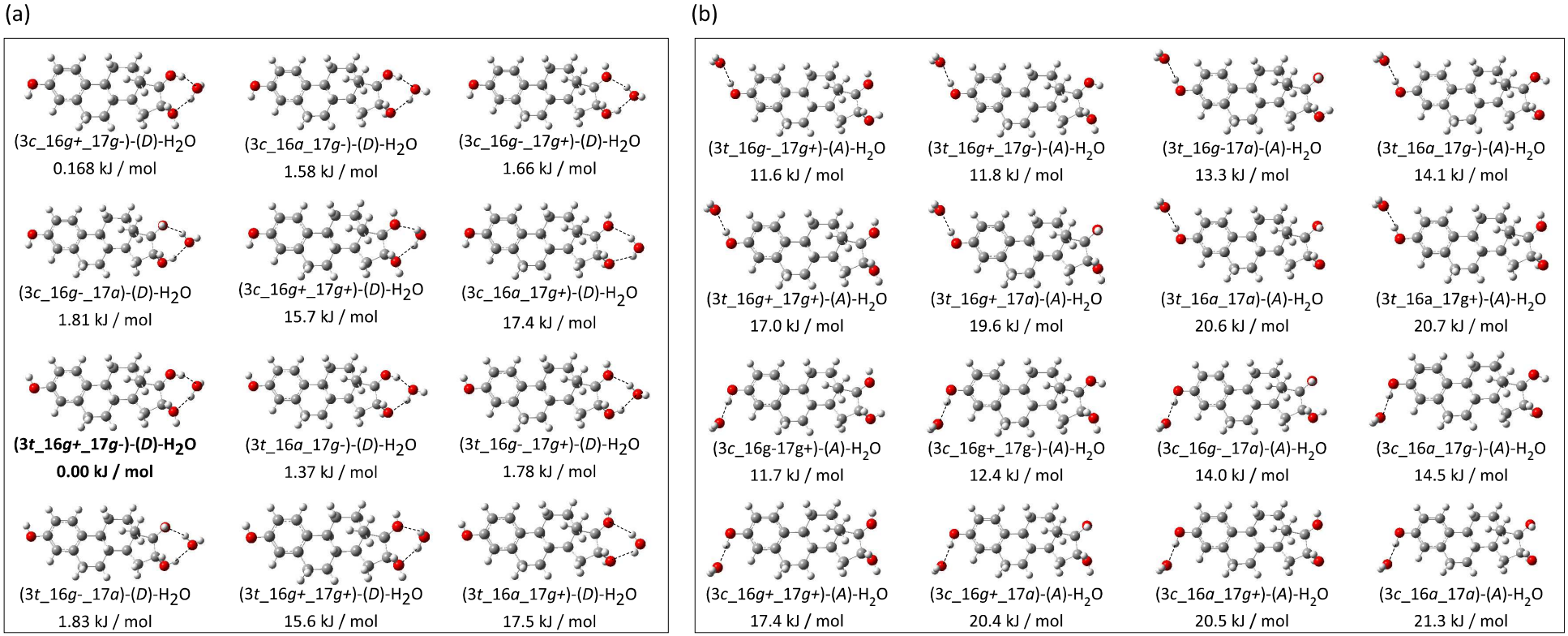

(a)
cis-anti-(A)-(H2O)
0.75 kJ/mol
cis-gauche(+)-(A)-(H2O)
0.00 kJ/mol
cis-gauche(-)-(A)-(H2O)
1.42 kJ/mol
trans-anti-(A)-(H2O)
0.38 kJ/mol
trans-gauche(+)-(A)-(H2O)
0.14 kJ/mol
trans-gauche(-)-(A)-(H2O)
0.80 kJ/mol
(b)
cis-anti-(D)-(H2O)
3.92 kJ/mol
cis-gauche(+)-(D)-(H2O)
5.50 kJ/mol
cis-gauche(-)-(D)-(H2O)
6.50 kJ/mol
trans-anti-(D)-(H2O)
3.68 kJ/mol
trans-gauche(+)-(D)-(H2O)
5.65 kJ/mol
trans-gauche(-)-(D)-(H2O)
6.14 kJ/mol

Figure 10


(a)
(3c_16g+_17g-)-(D)-H2O
0.168 kJ / mol
(3c_16a_17g-)-(D)-H2O
1.58 kJ / mol
(3c_16g-_17g+)-(D)-H2O
1.66 kJ / mol
(3c_16g-_17a)-(D)-H2O
1.81 kJ / mol
(3c_16g+_17g+)-(D)-H2O
15.7 kJ / mol
(3c_16a_17g+)-(D)-H2O
17.4 kJ / mol
(3t_16g+_17g-)-(D)-H2O
0.00 kJ / mol
(3t_16a_17g-)-(D)-H2O
1.37 kJ / mol
(3t_16g-_17g+)-(D)-H2O
1.78 kJ / mol
(3t_16g-_17a)-(D)-H2O
1.83 kJ / mol
(3t_16g+_17g+)-(D)-H2O
15.6 kJ / mol
(3t_16a_17g+)-(D)-H2O
17.5 kJ / mol
(b)
(3t_16g-_17g+)-(A)-H2O
11.6 kJ / mol
(3t_16g+_17g-)-(A)-H2O
11.8 kJ / mol
(3t_16g-17a)-(A)-H2O
13.3 kJ / mol
(3t_16a_17g-)-(A)-H2O
14.1 kJ / mol
(3t_16g+_17g+)-(A)-H2O
17.0 kJ / mol
(3t_16g+_17a)-(A)-H2O
19.6 kJ / mol
(3t_16a_17a)-(A)-H2O
20.6 kJ / mol
(3t_16a_17g+)-(A)-H2O
20.7 kJ / mol
(3c_16g-17g+)-(A)-H2O
11.7 kJ / mol
(3c_16g+_17g-)-(A)-H2O
12.4 kJ / mol
(3c_16g-_17a)-(A)-H2O
14.0 kJ / mol
(3c_16a_17g-)-(A)-H2O
14.5 kJ / mol
(3c_16g+_17g+)-(A)-H2O
17.4 kJ / mol
(3c_16g+_17a)-(A)-H2O
20.4 kJ / mol
(3c_16a_17g+)-(A)-H2O
20.5 kJ / mol
(3c_16a_17a)-(A)-H2O
21.3 kJ / mol