Laser Spectroscopic Study of β-Estradiol and Its Monohydrated Clusters in a Supersonic Jet

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Abstract

The structure of 17β-estradiol (estradiol) and its monohydrated clusters is studied in a supersonic jet. The laser induced fluorescence (LIF) spectrum of estradiol shows several sharp bands in the 35050–35200 cm^{-1} region. Ultraviolet-ultraviolet hole-burning (UV-UV HB) and infrared-ultraviolet double-resonance (IR-UV DR) spectra of these bands indicate that they are due to four different conformers of estradiol originating from the orientation of two OH groups in the *A*- and *D*-rings. Attachment of one H_2O molecule to estradiol shifts the monomer origin bands to red by ~350 cm^{-1} with keeping the interval between the four bands, suggesting that estradiol-H_2O 1:1 complexes have conformations similar to those of the four bare estradiol conformers, and that the H_2O molecule is bonded to the OH group of the *A*-ring (phenyl ring). In addition, very weak bands are also found near the origin bands of bare estradiol. These bands are attributed to isomers of estradiol-H_2O 1:1 having a hydrogen-bond at the *D*-ring OH. We determine the conformation of bare estradiol and the structures of its monohydrated complexes with the aid of density functional theory, and discuss the relation between the stability of hydrated clusters and the conformation of estradiol.

I. INTRODUCTION

Estradiol is one of female hormones called estrogen (estrone, estradiol, and estriol); it has a steroidal backbone and one OH group in each of *A*- and *D*-ring (Scheme 1). Estradiol is the most physiologically active molecule among the three estrogens, which regulate the differentiation and maintenance of neural, skeletal, cardiovascular, and reproductive tissues.[1–3] In crystallographic and computational studies, it t has been reported that estrogen forms extended hydrogen bond networks with estrogen-receptors to exert their psychological effects.[4–7] Estrogen has been extensively investigated also in solution to elucidate its physiological activity by observing the interaction with bio-membranes.[8–11] In ligand binding domains (LBDs) of estrogen-receptors, estrogen is fixed principally by hydrogen-bonds (H-bonds) at the *A*-ring OH group. In addition, the H-bond at the *D*-ring regulates structural alternations of LBDs for physiological activity. Another key in the activity of estrogen with bio-membranes is a balance of the interactions at the *A*- and *D*-rings. *A*-ring has a higher hydrophilicity than *D*-ring. As a result, *A*-ring interacts with carbonyl groups by H-bond, and *D*-ring is attached to carbon chains of bio-membranes, giving conformations effective to physiological activity.[1,12,13] In addition estrogen has several conformers owing to the orientation of the OH groups, which have not been revealed so far either in condensed phase or under biological environments.

In this study, we examine the structure of estradiol and its hydrated clusters formed in a supersonic jet by various laser spectroscopic methods; laser induced fluorescence (LIF), fluorescence-detected UV-UV hole-burning (HB), and IR-UV double-resonance (DR) spectroscopy, and theoretical calculation by density functional theory (DFT). On the basis of the conformations and isomers determined in this study, we discuss the relation between the conformation of estradiol and its hydration stability.

II. EXPERIMENTAL AND COMPUTATIONAL METHODS

The details of our experimental approach have been given elsewhere.[14] Briefly, jet-cooled

estradiol and its hydrated clusters were produced with a homemade pulsed nozzle. Solid estradiol was put in a housing attached at the head of a commercial pulsed nozzle and was heated at 130 ˚C. A gas mixture of estradiol and He carrier gas was expanded into a vacuum chamber through a 1 mm orifice. Hydrated clusters of estradiol were obtained by adding water vapor to the sample gas. The S_1–S_0 electronic spectra of bare estradiol and its hydrated clusters were measured by LIF spectroscopy. A tunable UV light obtained by second harmonics generation (SHG) of a tunable pulsed dye laser output (Lambda Physik Scanmate) pumped by Nd^{3+}:YAG laser (Continuum Surelite II) crossed the supersonic jet of estradiol. Fluorescence from the sample was collected on a photomultiplier tube, and the current from the photomultiplier tube was fed into a boxcar integrator. Averaged signals were processed by PC via an analog/digital converter. We also performed UV-UV HB spectroscopy[15] to distinguish peaks in the LIF spectra that belong to different conformers. The frequency of the probe UV laser was fixed to a vibronic band of specific species and its fluorescence intensity was monitored. Another tunable UV light obtained by SHG of a pulsed dye laser (Continuum ND6000 and Surelite II) was introduced to the jet at ~4 μs prior to the probe pulse. The frequency of the UV hole laser was scanned and depletion of the fluorescence intnensity induced by the pump laser excitation was observed. Thus, the UV-UV HB is observed as a fluorescence-dip spectrum. IR-UV DR spectroscopy[16 – 18] is very similar to UV-UV HB spectroscopy. An output of a tunable IR laser (LaserVision and Quanta-Ray GCR250) was introduced 50 ns prior to the probe UV pulse. The frequency of the IR laser was scanned and depletion of the fluorescence was observed, giving IR-dip spectra in the S_0 state. Estradiol was 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 strength and the transition energy were calculated with time-dependent DFT (TD-DFT) using the same calculation level. All the calculations were performed by using GAUSSIAN 09 program package.[19] For comparison of the observed IR and UV spectra with the calculated ones, scaling factors 0.950 and 0.850 were

employed to the calculated vibrational frequencies and the S_1–S_0 transition energies.

III. RESULTS

III.1. S_1–S_0 electronic spectra

Figure 1(a) shows the S_1–S_0 LIF spectrum of jet-cooled estradiol. There are several sharp bands in the 35000–35250 cm^{-1} region. Figure 1(b) displays the UV-UV HB spectra obtained by monitoring bands **a** (35048 cm^{-1}), **b** (35051 cm^{-1}), **c** (35148 cm^{-1}), and **d** (35151 cm^{-1}). These HB spectra indicate that the vibronic bands in the 35000–35250 cm^{-1} region are classified into four different conformers and that bands **a**–**d** are their origin bands; hereafter we use the band labels **a**–**d** also for distinguishing conformers that give these bands. Bands **a** and **b** are ~100 cm^{-1} red-shifted from **c** and **d**. Since this interval of the origin band is almost the same as that for *cis* and *trans* conformers of 3-methylphenol (*m*-cresol),[20] conformers **a** and **b** of bare estradiol have an different OH orientation in the *A*-ring from that of **c** and **d**. In contrast, the separation between bands **a** and **b** or between **c** and **d** is only 3 cm^{-1}. The structural difference between **a** and **b,** and between **c** and **d** would originate in the *D*-ring conformation.

In addition to the strong bands **a**–**d**, the LIF spectrum in Fig. 1(a) exhibits weak bands in the 34700–34900 cm^{-1} region. These bands become very strong with adding water vapor, as shown in Fig. 1(c). Therefore, the sharp bands in the 34700–34900 cm^{-1} region are attributed to estradiol-H_2O clusters. Three strong bands appear at 34713 cm^{-1} (band **B**), 34805 cm^{-1} (**C**), and 34810 cm^{-1} (**D**). In addition, band **B** has a shoulder at 3 cm^{-1} lower frequency side, which is marked by **A**. We confirmed that **A**–**D** are due to three different species by UV-UV HB spectroscopy. As seen in Figs. 1(a) and 1(c), three monomer bands (bands **a**–**d**) seem to shift in parallel to the low frequency by ~340 cm^{-1}, giving bands **A**–**D**. Since the value of the red-shift due to the complexation is close to that of phenol-H_2O (~354 cm^{-1}),[21] bands **A**–**D** can be assigned to estradiol-H_2O 1:1 clusters with the *A*-ring OH is H-bonded with H_2O, and the estradiol part similar to bare forms **b**–**d**, respectively.

Figure 1(d) displays the LIF spectrum of estradiol observed at lower partial pressure of water vapor than that of Fig. 1(c). Several weak bands highlighted by asterisks are found near the band origin of bare estradiol. A careful control of the partial pressure of water in the sample gas suggests these bands are assignable to different isomers of the estradiol-H_2O 1:1 complex. The intensities of these bands decease even the intensities of bands A-D continue to increase with an increase of water vapor pressure. On the basis of the TD-DFT calculations shown later, these bands can be ascribed to isomers of estradiol-H_2O in which an H_2O molecule is bonded to *D*-ring OH. Hereafter we call estradiol-H_2O 1:1 complex in which the H_2O molecule is bonded to the OH group of the *A*-ring as estradiol(*A*)-H_2O and *D*-ring as estradiol(*D*)-H_2O. The bands in 34700–34900 cm^{-1} are attributed to estradiol(*A*)-H_2O, and the bands with asterisks around the origin band of bare estradiol in Fig. 1(d) are due to estradiol(*D*)-H_2O.

III.2. IR spectra in the OH stretching region

The top panel in each of Figs. 2(a–d) shows the IR-UV DR spectra in the OH stretching region (red curves) measured by monitoring the origin bands of bare estradiol (bands **a**–**d** in Fig. 1(a)). In the IR spectra of conformers **a** and **c**, two OH stretch bands appear with an interval of ~15 cm^{-1}, while there is only one band in the spectra of **b** and **d**. Estradiol has an OH group in each of the *A*- and *D*-rings. Since phenol and cyclopentanol have the OH stretching vibration at almost the same frequency (~3657 cm^{-1}),[16,22] we expected that the two OH bands of estradiol may appear at almost the same position such as the isomers **b** and **d**, giving one band maximum at ~3657 cm^{-1}. However, for isomers **a** and **c**, the OH stretching bands appear at different frequencies between the *A*- and *D*-rings. As mentioned later, the vibrational analysis of bare estradiol predicts that the low- and high-frequency bands are attributed to the OH group in the *D*-ring and the *A*-ring, respectively.

In Figs. 3(A–D), the IR-UV DR spectra obtained by monitoring bands **A**–**D** are compared with those of bare estradiol (bands **a**–**d**). As described above, all species **A**–**D** are thought to be estradiol(*A*)-H_2O 1:1 complex. The phenolic OH group in the *A*-ring is H-bonded to the H_2O

molecule as a donor, so that its stretching frequency will shift to the lower frequency as in the case of phenol-H_2O.[16] Thus, the bands around 3526 cm^{-1} are attributed to the stretching vibration of the H-bonded OH group in the *A*-ring. The IR bands of **A**–**D** in the 3640–3655 cm^{-1} region can be assigned to the stretching vibration of the free OH group in the *D*-ring. For conformers **A** and **C**, the band position of the OH group in the *D*-ring (~3642 cm^{-1}) is almost the same as that of the lower-frequency band of bare conformers **a** and **c** (~3643 cm^{-1}), which confirms the assignment of the lower-frequency band of **a** and **c** to the OH group in the *D*-ring. For estradiol(*A*)-H_2O 1:1 complex, the OH stretching bands of the H_2O component are also detected in the IR spectra. For species **C** and **D**, a band is found at 3747 cm^{-1}, which is assigned to ν_3 the H_2O component because phenol-H_2O complex shows the ν_3 band at 3748 cm^{-1}.[16] The ν_1 band is not clearly seen in the same spectrum because of weak intensity of ν_1 compared to ν_3. In the IR spectra of **A** and **B**, the ν_3 band cannot be detected. The reason for the absence of ν_3 of H_2O in the IR spectra of **A**, **B** is not clear in the present stage. However, since the frequency of the H-bonded OH stretch (~3526 cm^{-1}) of the *A*-ring is almost the same in the IR spectra of **A**-**D**, the species for bands **A**-**D** are thought to have a same H-bonding structure. Figure 3 also shows the calculated IR spectra (black bars and curves) of stable 1:1 complexes whose structures are described later. For the bands of estradiol(*D*)-H_2O complex observed in the LIF spectrum of Fig. 2(d), we could not obtain their IR spectra because of very weak intensity of the fluorescence.

IV. DISCUSSION

IV.1. Conformation of bare estradiol

Figure 4 displays six stable conformers for bare estradiol optimized by DFT calculations at the M05-2X/6-311++G** level. The difference in the structure among these six conformers comes from the orientation of the OH group in the *A*- and *D*-rings; since the steroid backbone (hydrocarbon backbone) of estradiol is very rigid, no substantial difference can be seen in the backbone between these conformers. These conformers can be identified by two structural

descriptions. Firstly, *cis* and *trans* are used for the conformation of the OH group in the *A*-ring; the conformation in which the dihedral angle of H–O–3C–4C (see Scheme 1) is ~0 and ~180 degree is called *cis* and *trans*, respectively. Secondly, it is necessary to distinguish three *gauche* configurations for the OH group in the *D*-ring. The right panel of Fig. 4 displays the three configuration of the OH group with respect to the 17C–H bond (see Scheme 1). The configuration in which the dihedral angle of H–O–17C–H is ~180 degree is called *anti*; in *gauche*(+) and *gauche*(–) configurations the dihedral angle is about 60 and –60 degree, respectively. The six conformers of ~~bare~~ estradiol in Fig. 4 are described with the combination of (*cis* or *trans*) and (*anti*, *gauche*(+), or *gauche*(–)). Among the six forms, *trans-gauche*(+) conformer is ~~the~~ most stable. The total energy of these conformers relative to that of *trans-gauche*(+) is shown in Fig. 4 and Table 1 in cm^{-1} unit.

The direction of the OH group in the *A*-ring can be determined on the basis of the S_1–S_0 transition energy of these conformers. Figure 1(e) shows the S_1–S_0 transition energies of the six conformers obtained by TD-DFT calculation. The transition energies of the *trans* conformers are ~85 cm^{-1} higher than that of the *cis* forms. The interval is in reasonable agreement with the observed one of 100 cm^{-1} between bands **a,b** and **c,d**. Therefore, conformers **a** and **b** of estradiol have *cis* conformation in the *A*-ring, and **c** and **d** have *trans* one. Next we compare the IR-UV spectra of bands **a**–**d** with the calculated IR spectra of the six isomers in Fig. 2. Conformers **a** and **c** show the two resolved OH bands, whereas **b** and **d** has only one band maximum in each of the IR spectra. As seen in Fig. 2, the conformers having an *anti* configuration in the *D*-ring show two band maxima corresponding to the OH stretching vibrations of the *A*- and *D*-rings. For the four isomers with *gauche* configuration the OH stretching frequency of the *A*- and *D*-rings is very close to each other, giving only one band around 3650 cm^{-1}. Therefore, we attribute bands **a** and **c** to *cis-anti* and *trans-anti* conformers, respectively. For bands **b** and **d**, it is difficult to determine their structures definitely because *gauche*(+) and *gauche*(–) configurations have similar IR and UV spectra shown in Figs. 1(e) and 2(b, d), and the total energy is not so substantially different between them (Fig. 4).

However, we can derive a reasonable conclusion that band **b** is assigned to *cis-gauche*(+) and band **d** to *trans-gauche*(+) conformers, from the following two reasons; (1) the *gauche*(+) conformers are more stable than the *gauche*(–) ones by ~75 cm^{-1} as seen in Fig. 4; (2) the S_1–S_0 transition energy of the *gauche*(+) conformers is slightly higher than that of the *anti* conformers, which is similar to the band position of **b** and **d** with respect to **a** and **c**.

IV.2. Structure of estradiol-H_2O 1:1 clusters

Estradiol(A)-H_2O

The parallel red-shift of bands **a**–**d** to **A**–**D** in the LIF spectra (Figs. 1(a) and 1(c)) suggests that conformers **A**–**D** of 1:1 complex have estradiol forms similar to monomer conformers **a**–**d**. Figure 5 shows stable structures of estradiol(*A*)-H_2O optimized by DFT calculations at the M05-2X/6-311++G** level. In these forms, the direction of the estradiol OH groups is described with the combination of (*cis* or *trans*) and (*anti* or *gauche*(+)), the same as conformers **a**–**d**. The S_1-S_0 transition energies of these forms are displayed in Fig. 1(f). Comparing the calculated spectra in Figs. 1(e) and 1(f), one can find that the order of the band position for the monomer conformers (*cis-anti*, *cis-gauche*(+), *trans-anti*, and *trans-gauche*(+), from low to high UV wavenumber) is kept for estradiol(*A*)-H_2O. Therefore, we assign bands **A**–**D** of 1:1 complex to *cis-anti*-estradiol(*A*)-H_2O, *cis-gauche*(+)-estradiol(*A*)-H_2O, *trans-anti*-estradiol(*A*)-H_2O, and *trans-gauche*(+)-estradiol(*A*)-H_2O in Fig. 5, respectively. The IR spectra of these forms are shown in Fig. 3. The calculated IR spectra reproduce the appearance of a strong band of the H-bonded OH group of *A*-ring around 3500 cm^{-1} and the free OH group of D-ring around 3650 cm^{-1}. They also predict the OH bands (ν_1 and ν_3) of the H_2O part in the 3600–3800 cm^{-1} region. In the IR-UV spectra, however, only isomers **C** and **D** show these bands as described previously and the reason for the absence of the ν_3 bands of H_2O for **A** and **B** is still unclear in the present stage.

Estradiol(D)-H_2O

Figure 6 shows the stable structures of estradiol(*D*)-H_2O complex. The numbers in the figure represent the total energy of these isomers relative to that of the most stable complex, *trans-anti*-estradiol(*A*)-H_2O in Fig. 5. These estradiol(*D*)-H_2O complexes are less stable than those of estradiol(*A*)-H_2O by more than 200 cm^{-1}. Since the *D*-ring OH is less acidic than the *A*-ring OH, it works as both of the H-bonding donor and acceptor. Our calculations show that in *anti*-estradiol(*D*)-H_2O conformers the *D*-ring OH preferentially acts as the acceptor, whereas *gauche*(+)-estradiol(*D*)-H_2O conformers donate the OH group of the *D*-ring to H_2O. The calculated S_1–S_0 transition energies of the estradiol(*D*)-H_2O isomers ~~is~~ are displayed in Fig. 1(g). These isomers have the transitions at the UV frequency very close to that of monomer conformers, since the conformation in the D-ring affects very small to the S_1–S_0 transition of benzene chromophore of A-ring. Therefore, we assign the weak bands highlighted by asterisks in Fig. 1(d) to estradiol(*D*)-H_2O isomers.

V. CONCLUSION

In this study, the conformation of ~~bare~~ estradiol and the structure of estradiol-H_2O cluster have been investigated by using LIF, UV-UV HB and IR-UV DR spectroscopy, under jet-cooled conditions with the aid of quantum chemical calculations. We found four conformers for bare estradiol, which are assigned to *cis-anti*, *trans-anti*, *cis-gauche*(+) and *trans-gauche*(+)-conformers. For estradiol-H_2O complex, two types of the clusters are found: estradiol(*A*)-H_2O and estradiol(*D*)-H_2O. In the case of estradiol(*A*)-H_2O complex, the OH group of the *A*-ring acts as the H-donor, showing a strong IR band at ~3526 cm^{-1}. In contrast, the OH group of the *D*-ring in estradiol(*D*)-H_2O complex works both as the donor and acceptor, depending on the OH orientation. As was described in the introduction, in ligand binding domains (LBDs) of estrogen-receptors, estrogen is fixed principally by hydrogen-bonds (H-bonds) at the *A*-ring OH group, while the H-bond at the *D*-ring is thought to regulate structural alternations of LBDs for physiological activity.

The present results clearly showed such a difference of the activity for the two OH groups in forming the H-bonding.

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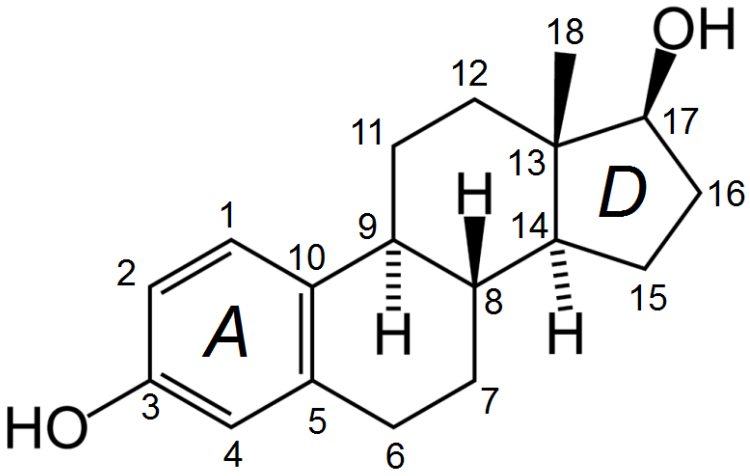


Scheme 1. 17β-estradiol (estradiol)

Table 1 Positions (cm^{-1}) of the S_1-S_0 band origin and assignments for bare β-estradiol and its hydrated cluster.

Position / cm^{-1}	Label	Assignment
35048	**a**	*cis-anti*
35051	**b**	*cis-gauche*(+)
35148	**c**	*trans-anti*
35151	**d**	*trans-gauche*(+)
34710	**A**	*cis-anti*-estradiol(*A*)-H_2O[a]
34713	**B**	*cis-gauche*(+)-estradiol(*A*)-H_2O[a]
34805	**C**	*trans-anti*-estradiol(*A*)-H_2O[a]
34810	**D**	*trans-gauche*(+)-estradiol(*A*)-H_2O[a]

[a]Estradiol(*A*)-H_2O represents estradiol-H_2O 1:1 complex in which the H_2O molecule is H-bonded to the OH group of the *A*-ring.

Table.2 Relative energies (cm^{-1}) of possible conformers of bare estradiol, estradiol(*A*)-H_2O and estradiol(*D*)-H_2O obtained by DFT calculation at the M05-2X/6-311++G** level of theory.

	cis-anti	*cis-gauche*(+)	*cis-gauche*(-)	*trans-anti*	*trans-gauche*(+)	*trans-gauche*(-)
bare estradiol[a]	2.2	5.9	76.2	13.8	0.0	73.1
estradiol(*A*)-H_2O[b]	62.6	0.0	118.3	31.4	11.6	66.9
estradiol(*D*)-H_2O[b]	328	460	543	308	472	513

[a]With respect to the most stable conformer, *trans-gauche*(+).
[b]With respect to the most stable hydrated isomer, *cis-gauche*(+)-estradiol(*A*)-H_2O.

Figure caption

Figure 1 (a) The S_1–S_0 LIF spectrum of estradiol. (b) The UV-UV HB spectra of estradiol obtained by probing bands **a**–**d**. (c) The S_1–S_0 LIF spectrum of estradiol measured by adding water vapor to the sample gas. Bands **A**–**D** are assigned to the origin band of estradiol(*A*)-H_2O. (d) The LIF spectrum of the estradiol-water clusters measured with a trace of water vapor in the sample gas. The bands marked by asterisks are assigned to estradiol(*D*)-H_2O. The calculated oscillator strength of the S_1–S_0 transition of (e) bare estradiol (f) estradiol(*A*)-H_2O, and (g) estradiol(*D*)-H_2O. A scaling factor of 0.850 was applied to the transition energy calculated for comparison with the LIF spectra.

Figure 2 The IR-UV DR spectra of estradiol observed by monitoring bands **a**–**d** (red curves). The calculated IR spectra of stable estradiol conformers (black bars and curves). A scaling factor of 0.950 was applied to the vibrational frequency calculated for comparison with the IR-UV spectra.

Figure 3 The IR-UV DR spectra obtained by monitoring bands **A**–**D** with the IR-UV spectra of monomer conformers **a**–**d**. The Calculated IR spectra of stable estradiol(*A*)-H_2O isomers. A scaling factor of 0.950 was applied to the vibrational frequency calculated for comparison with the IR-UV spectra.

Figure 4 (Left) Stable conformers of bare estradiol. The numbers in the figure represent the total energy relative to that of the most stable one (*trans-gauche*(+)). (Right) Newman-projections of *anti*, *gauche*(+), and *gauche*(–) configurations at the OH group of the *D*-ring.

Figure 5 Stable isomers of estradiol(*A*)-H_2O complex. The numbers in the figure represent the total energy relative to that of the most stable one (*cis-gauche*(+)-estradiol(*A*)-H_2O).

Figure 6 Stable isomers of estradiol(*D*)-H_2O complex. The numbers in the figure represent the total energy relative to that of the most stable one (*cis-gauche*(+)-estradiol(*A*)-H_2O in Fig. 5).

Fig. 1

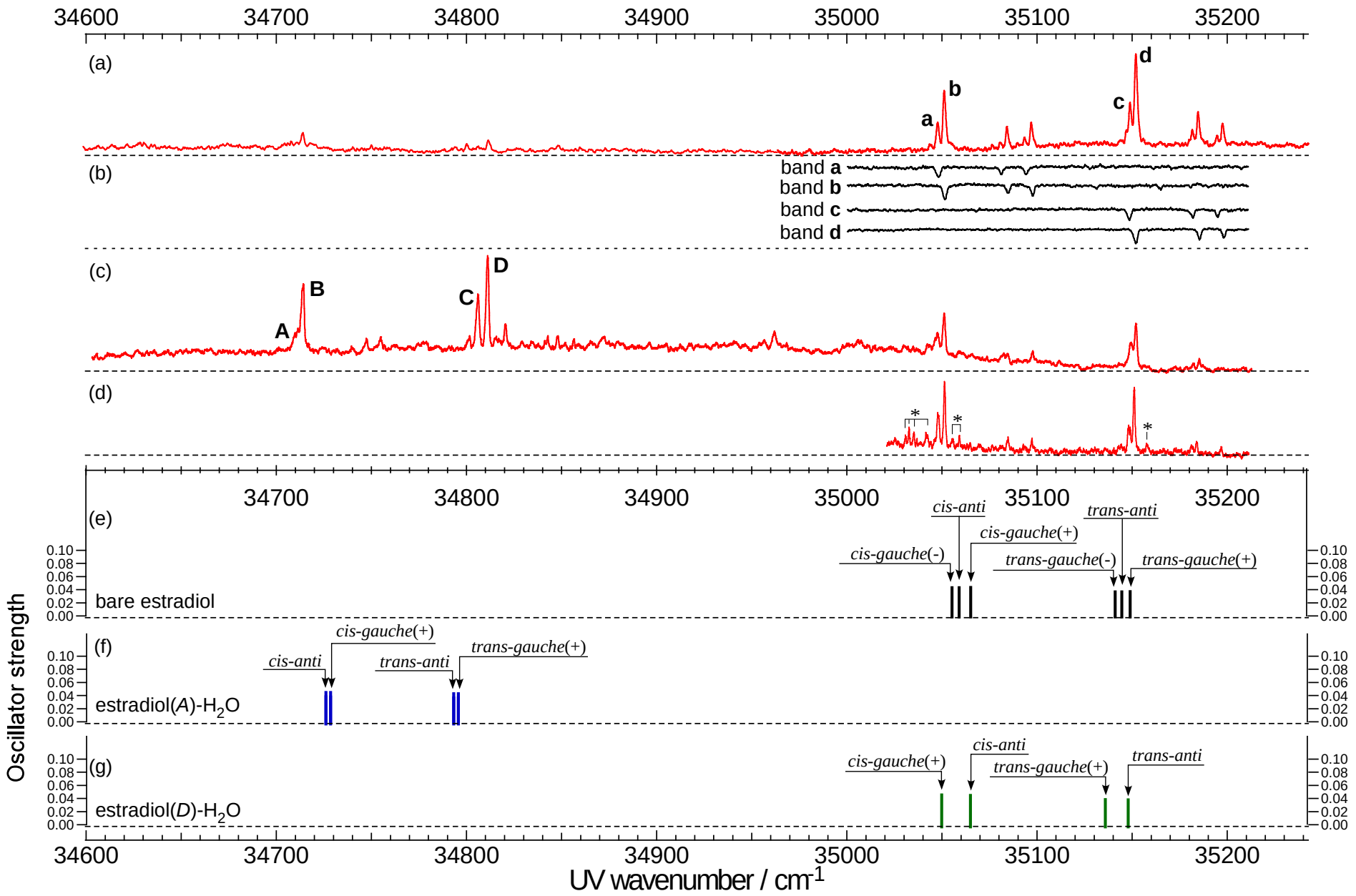

34600
34700
34800
34900
35000
35100
35200
(a)
a
b
c
d
(b)
band a
band b
band c
band d
(c)
A
B
C
D
(d)
*
*
*
(e)
bare estradiol
cis-anti
cis-gauche(+)
cis-gauche(-)
trans-anti
trans-gauche(-)
trans-gauche(+)
(f)
estradiol(A)-H2O
cis-anti
cis-gauche(+)
trans-anti
trans-gauche(+)
(g)
estradiol(D)-H2O
cis-gauche(+)
cis-anti
trans-gauche(+)
trans-anti
Oscillator strength
0.00
0.02
0.04
0.06
0.08
0.10
UV wavenumber / cm-1

Fig. 2

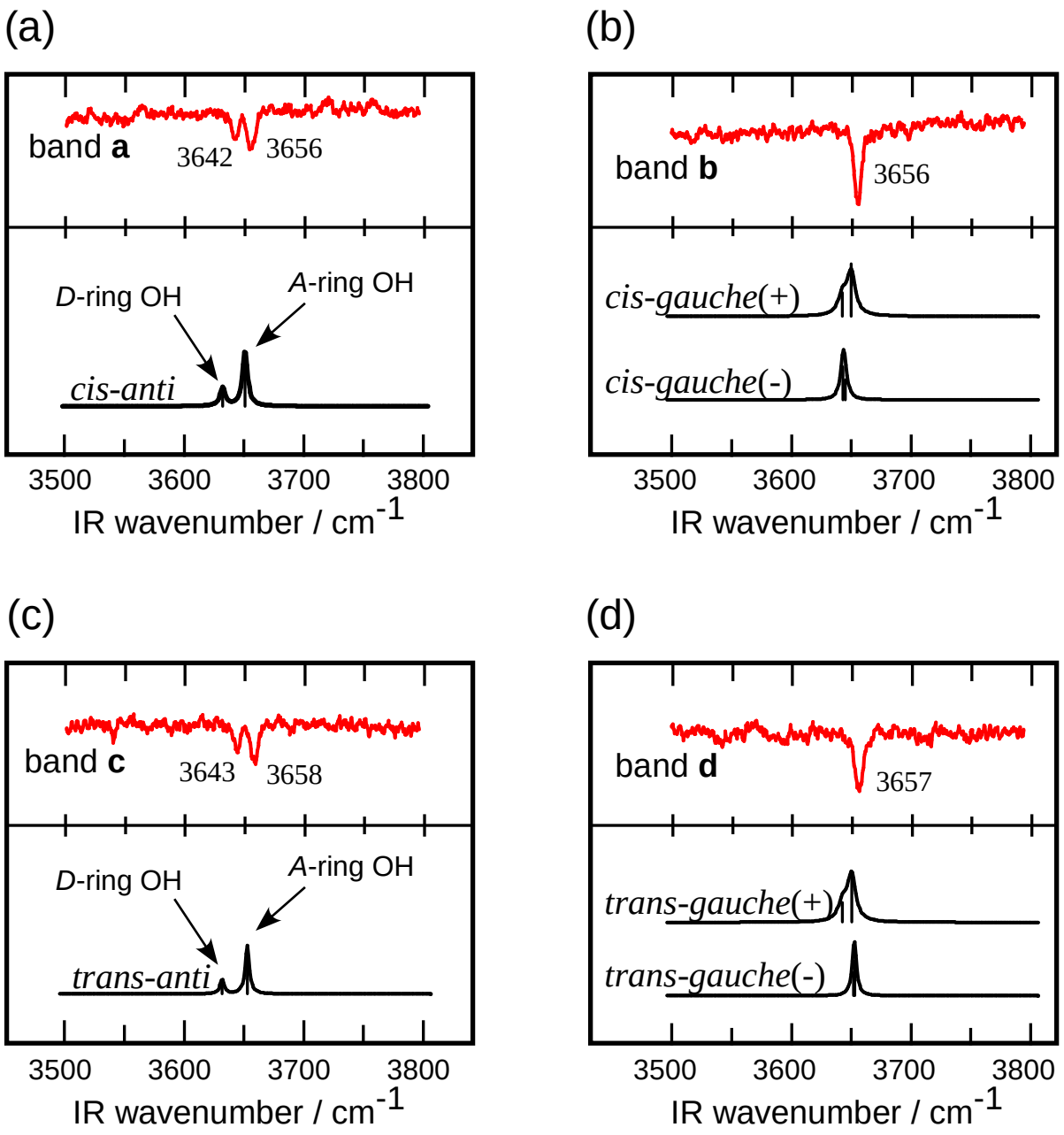

(a)
band a
3642
3656
D-ring OH
A-ring OH
cis-anti
3500
3600
3700
3800
IR wavenumber / cm-1
(b)
band b
3656
cis-gauche(+)
cis-gauche(-)
3500
3600
3700
3800
IR wavenumber / cm-1
(c)
band c
3643
3658
D-ring OH
A-ring OH
trans-anti
3500
3600
3700
3800
IR wavenumber / cm-1
(d)
band d
3657
trans-gauche(+)
trans-gauche(-)
3500
3600
3700
3800
IR wavenumber / cm-1

Fig. 3

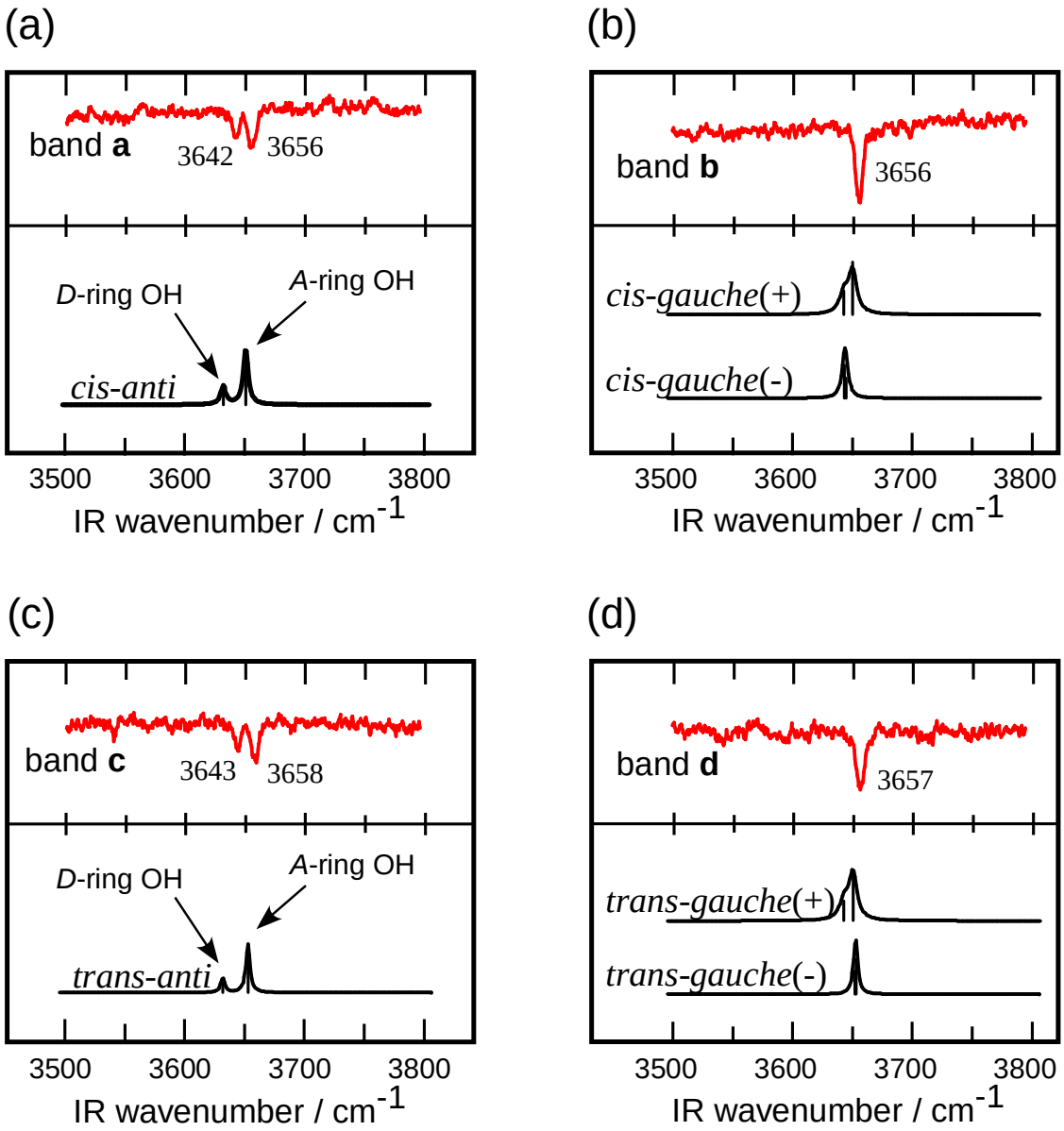

(a)
band **a**
3642
3656
D-ring OH
A-ring OH
cis-anti
3500
3600
3700
3800
IR wavenumber / cm^{-1}
(b)
band **b**
3656
cis-gauche(+)
cis-gauche(-)
3500
3600
3700
3800
IR wavenumber / cm^{-1}
(c)
band **c**
3643
3658
D-ring OH
A-ring OH
trans-anti
3500
3600
3700
3800
IR wavenumber / cm^{-1}
(d)
band **d**
3657
trans-gauche(+)
trans-gauche(-)
3500
3600
3700
3800
IR wavenumber / cm^{-1}

Fig. 4

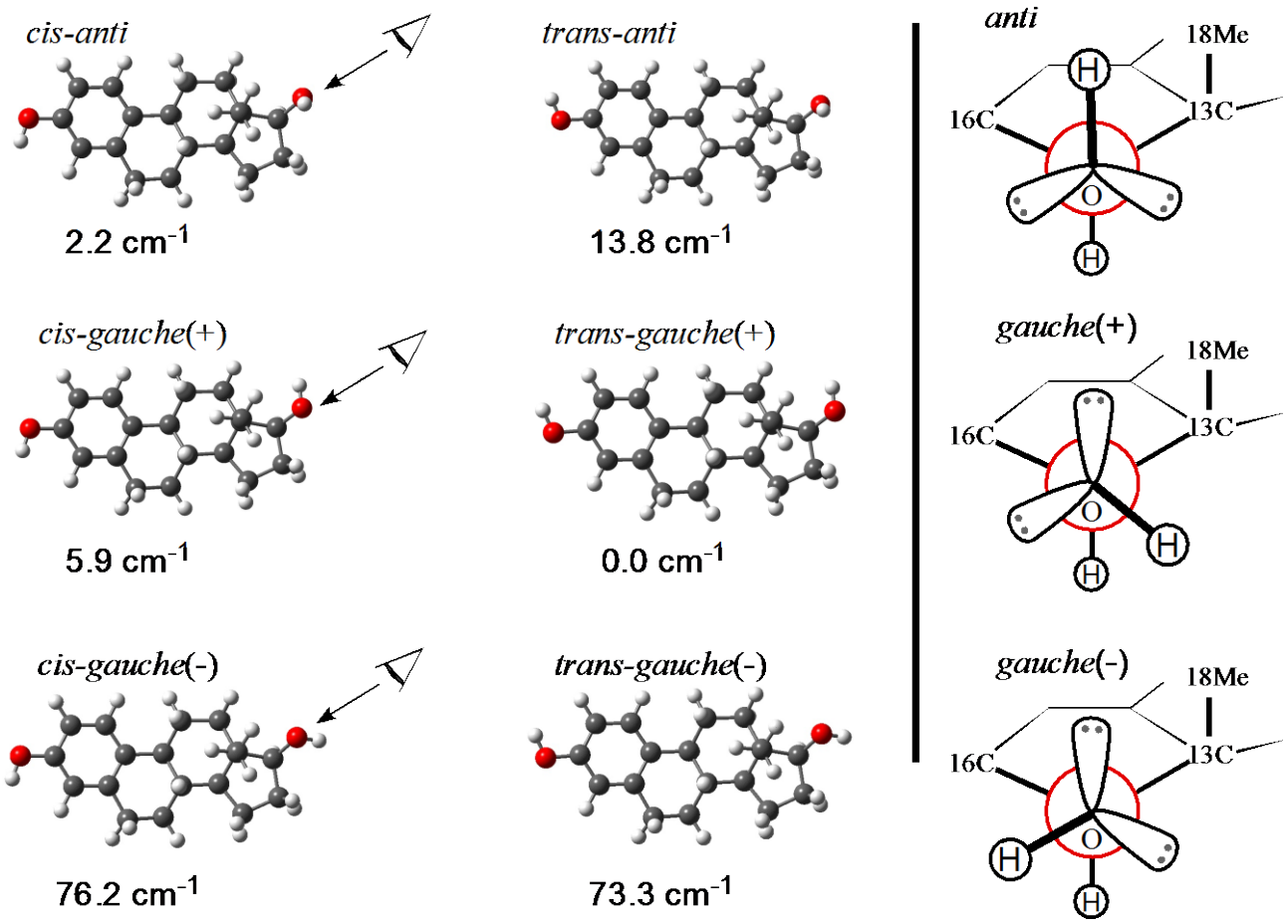

cis-anti
2.2 cm-1
trans-anti
13.8 cm-1
cis-gauche(+)
5.9 cm-1
trans-gauche(+)
0.0 cm-1
cis-gauche(-)
76.2 cm-1
trans-gauche(-)
73.3 cm-1
anti
18Me
16C
13C
O
H
gauche(+)
gauche(-)

Fig. 5

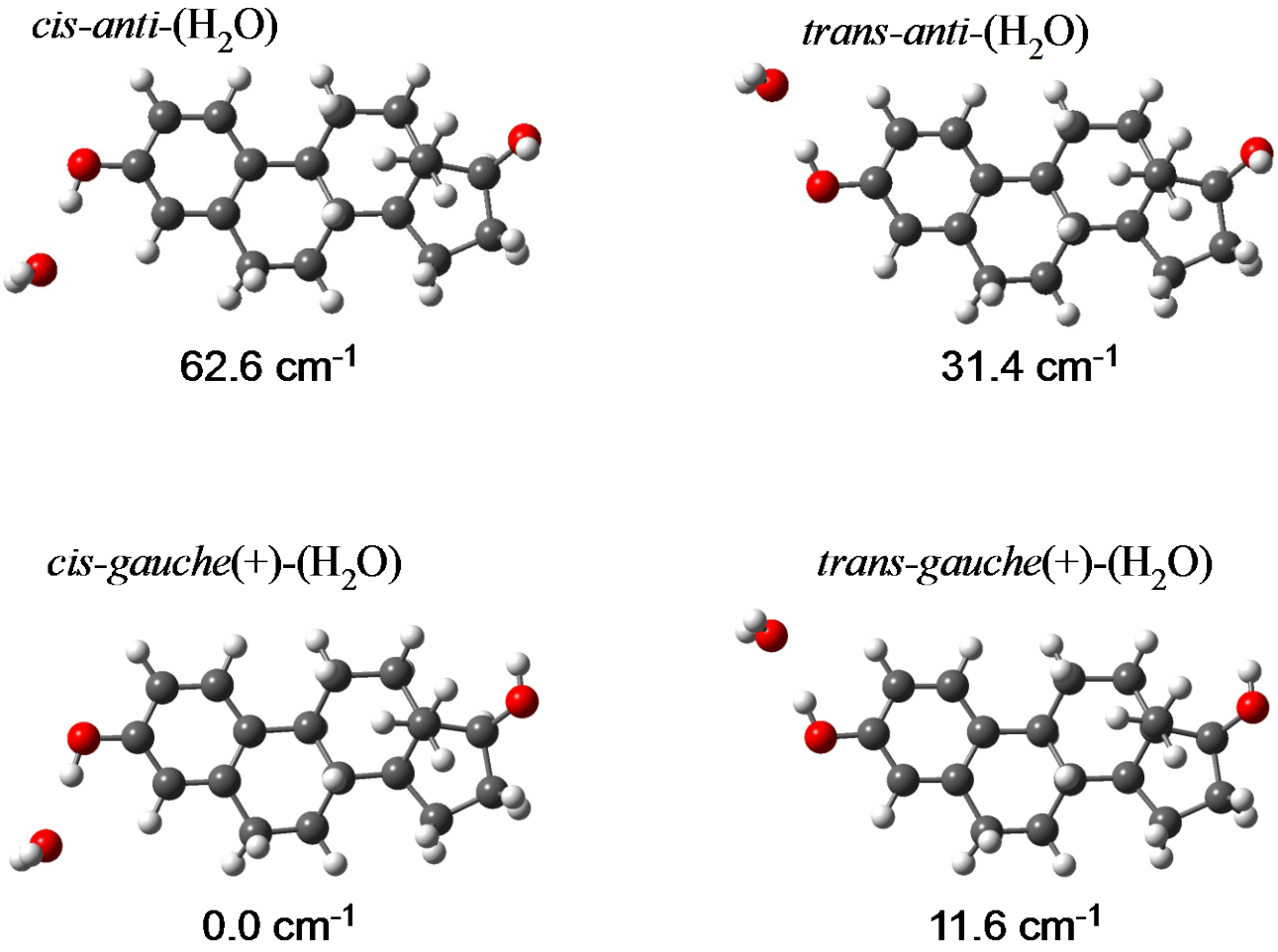
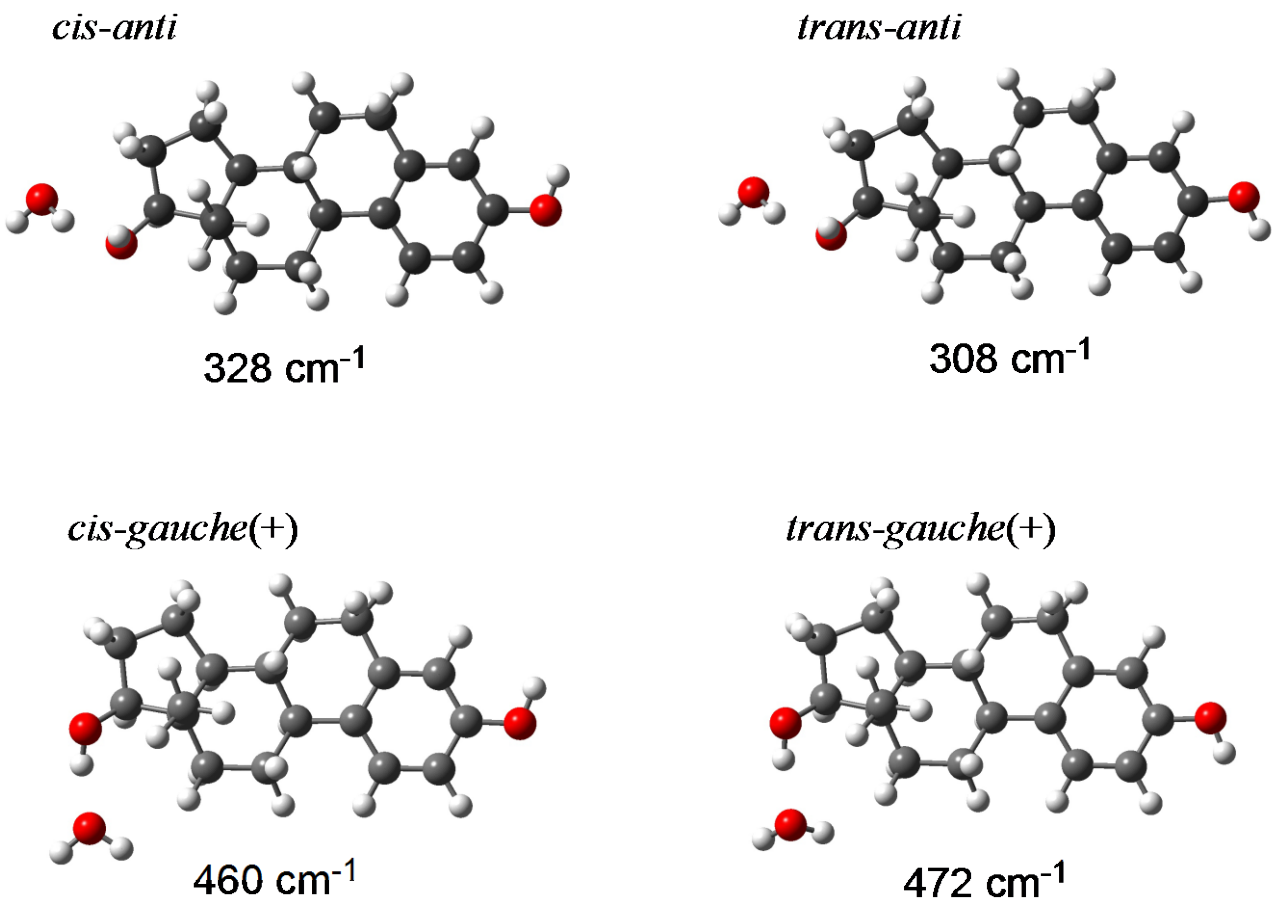

cis-anti-(H_2O)
62.6 cm^{-1}
trans-anti-(H_2O)
31.4 cm^{-1}
cis-gauche(+)-(H_2O)
0.0 cm^{-1}
trans-gauche(+)-(H_2O)
11.6 cm^{-1}

Fig. 6


cis-anti
328 cm^{-1}
trans-anti
308 cm^{-1}
cis-gauche(+)
460 cm^{-1}
trans-gauche(+)
472 cm^{-1}