INFRA-RED ABSORPTION SPECTRA OF STEROIDS

II. ESTROGENS

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In this paper, the infra-red absorption spectra of a number of crystalline natural estrogens are presented and considered in relation to chemical structure. As a group, the natural estrogens are distinguished chemically from other steroids by the benzenoid structure of Ring A, which gives rise to certain infra-red absorption bands by which their spectra may be differentiated from those of other steroids. A study has also been made of a synthetic estrogen, stilbestrol (4,4'-dihydroxy-α,β-diethylstilbene).

Methods

Absorption spectra were obtained with the Hardy infra-red spectrophotometer (1), with the same techniques that were employed in the study of androgens (2). The steroids were prepared for analysis by deposition as solid films on rock salt plates from pyridine solutions, as previously described.

EXPERIMENTAL

The absorption spectra of the following compounds are presented: α-estradiol, α-estradiol-3-monobenzoate, β-estradiol, β-estradiol-3-monobenzoate (Fig. 1); equilenin, equilin, estrone, estriol (Fig. 2); and stilbestrol (Fig. 3).

Analysis of Spectra

As in the case of the infra-red absorption spectra of androgens (2), it has been found possible to assign a number of absorption bands in the spectra of estrogens to specific interatomic vibrations. These bands will be discussed first, after which certain other bands will be considered in relation to the structural configurations characterizing the compounds under study.

O—H Absorption—Since all the compounds studied have one or more hydroxyl groups, all their spectra have absorption bands in the region near

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3 μ, attributable to the linear vibration of O—H bonds. The wavelengths of the absorption maxima observed in this region are listed in Table I. All of the wavelengths, except that for stilbestrol, are significantly higher than the 2.75 to 2.77 μ absorption range for unbonded O—H
groups (3). As postulated in the case of androgens containing hydroxyl groups (2), this divergence may be the result of hydrogen bonding of O—H groups in the solid films of these natural estrogens. The wide range over which the absorption maxima occur may be due to the varying strengths...
of hydrogen bonding, since the extent of the shift of O—H absorption varies directly with the strength of hydrogen bonding.

In general, the steroidal estrogens possessing only a phenolic hydroxyl (estrone, equilin, and equilenin) produced an O—H band at a higher wavelength than those containing only an alcoholic hydroxyl (α-estradiol monobenzoate and β-estradiol monobenzoate). It is therefore likely that in the spectra of α-estradiol and β-estradiol the absorption bands at 2.80

![Graph showing absorption bands](image)

**Fig. 3**

**Table I**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Type of O—H</th>
<th>Wavelength (μ)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrone</td>
<td>C4-Phenolic</td>
<td>2.87</td>
</tr>
<tr>
<td>Equilin</td>
<td>&quot;</td>
<td>2.95</td>
</tr>
<tr>
<td>Equilenin</td>
<td>&quot;</td>
<td>2.94</td>
</tr>
<tr>
<td>α-Estradiol</td>
<td>C17-alcoholic</td>
<td>2.80, 2.93</td>
</tr>
<tr>
<td>β-Estradiol</td>
<td>&quot;</td>
<td>2.86, 3.09</td>
</tr>
<tr>
<td>Estradiol-3-monobenzoate</td>
<td>C17- and C18-alcoholic</td>
<td>2.86, 2.98</td>
</tr>
<tr>
<td>α-Estradiol-3-monobenzoate</td>
<td>C17-Alcoholic</td>
<td>2.83</td>
</tr>
<tr>
<td>β-Estradiol-3-monobenzoate</td>
<td>&quot;</td>
<td>2.83</td>
</tr>
<tr>
<td>Stilbestrol</td>
<td>4- and 4′-phenolic</td>
<td>2.78</td>
</tr>
</tbody>
</table>

and 2.86 μ respectively arise from the alcoholic, whereas those at 2.93 and 3.00 μ arise from the phenolic hydroxyl groups.

C—H Absorption—All the steroids in this series give a major band near 3.35 μ. This band, as was pointed out in the case of androgens (2), arises from the linear vibration of hydrogen in aliphatic C—H groups. Other workers (4, 5) have shown that C—H groups of benzene rings absorb nearer 3.25 μ than 3.35 μ. It might therefore be expected that in the
spectra of estrogens containing benzenoid rings a separate band would appear near 3.25 µ in addition to the aliphatic C—H band near 3.35 µ. Such a band, however, does not appear. Hence, it may be concluded that aromatic and aliphatic C—H absorption bands arising from linear hydrogen vibrations have not been resolved in the present study. Likewise in the case of the spectrum of stilbestrol, aromatic and aliphatic C—H bands are not resolved. However, in this compound the aromatic C—H band would appear to predominate, since the absorption maximum occurs around 3.28 µ.

Absorption bands also arise in the region near 7 µ from the angular vibration of hydrogen in C—H groups. In this region, the absorption bands resulting from aromatic and aliphatic C—H groups have been clearly resolved. In the spectra of estrone, α-estradiol, β-estradiol, estriol, and equilin, the aromatic C—H bands occur in the 6.65 to 6.70 µ range. In the case of the monobenzoates of α-estradiol and β-estradiol, in which the C—H groups of the benzoate structure also contribute to the absorption, the band occurs at 6.70 µ. The presence of the two double bonds in Ring B of equilenin apparently causes an upward shift of the band to 6.76 µ, while in the case of stilbestrol, with its para-substituted benzene rings, the band occurs at 6.62 µ.

The bands which may be assigned to aliphatic C—H angular vibrations occur in the 6.85 to 6.90 µ range in estrone, α-estradiol, β-estradiol, estriol, α-estradiol monobenzoate, and β-estradiol monobenzoate. In equilin and equilenin, they are higher, occurring at 6.93 and 6.97 µ respectively. With stilbestrol, as with a number of androgens (2), two aliphatic C—H bands are resolved: one at 6.81 µ and the other at 6.95 µ.

CH₃ Absorption—In the spectra of androgens and related steroids (2), a band consistently occurred in the region between 7.20 and 7.25 µ. On the basis of the work of Barnes et al. on the absorption of methyl group (4), this band was attributed to the angular methyl groups of the androgenic steroids. However, of the estrogens only the following give rise to an absorption band in this specific region: estrone (7.23 µ), α-estradiol (7.23 µ), and β-estradiol-3-monobenzoate (7.23 µ). In the remainder, the bands closest to this region are, for α-estradiol-3-monobenzoate, 7.17 µ; for β-estradiol, 7.28 µ; for estriol, 7.17 µ (shoulder); for equilin, 7.32 µ; and for equilenin, 7.18 µ. The reasons for this variability are not apparent.

In the spectrum of stilbestrol, there is little doubt that the band of medium intensity at 7.23 µ arises from absorption by the two methyl groups of the ethyl side chains.

C=O Absorption—The only carbonyl groups encountered in the present study are C=O ketones and ester carboxyls. The former type is present in
estrone, equilin, and equilenin. In studies on androgens and related steroids (2), the C\textsubscript{17} ketone gave rise to an absorption band at about 5.75 \(\mu\). The C\textsubscript{17} ketone of estrone also absorbs at this wave-length. However, the ketone absorption bands of equilin and equilenin occur at about 5.80 \(\mu\). The increased unsaturation of Ring B of these two compounds may be responsible for this upward shift in the wave-length.

The ester carbonyls of \(\alpha\)-estradiol-3-monobenzoate and \(\beta\)-estradiol-3-monobenzoate both give rise to an absorption band at about 5.77 \(\mu\).

\(\text{C}=\text{C Absorption}\)—Aromatic rings are characterized by bands in the region of 6.20 to 6.30 \(\mu\) (4). Such bands arise from the vibration of conjugated C= C groups in the ring. Since all the compounds under investigation here have one or more aromatic rings, their spectra may be expected to show bands in this region, and such is the case. In most of the spectra a single strong band is found. With equilin and equilenin two bands have been resolved in this region, one of those in equilenin being a small shoulder. The possible origin of these differences is the additional unsaturation of Ring B in these latter compounds.

\(\text{C}–\text{O Absorption}\)—In the compounds under investigation, C–O groups may be divided into two classes: (a) those having a carbon with a double bond linkage, and (b) those having a carbon with only single bond linkages. To the first class belong the C–O groups found in phenolic hydroxyl structures. Because of the double bond linkage to the carbon, such groups may be expected to give an absorption band near 8.0 \(\mu\) (2, 4). Estrone, \(\alpha\)-estradiol, \(\beta\)-estradiol, estriol, equilin, equilenin, and stilbestrol all contain phenolic hydroxyl groups, and all have an absorption band of medium to strong intensity between 7.95 and 8.05 \(\mu\). In the case of \(\alpha\)-estradiol-3-monobenzoate and \(\beta\)-estradiol-3-monobenzoate, the phenolic hydroxyl is esterified. Since each ester linkage has the configuration \(\text{C}–\text{O}–\text{C}\), each possesses two C–O linkages with double bonds attached to the carbons. These C–O groups probably account for the band of strong intensity at 7.82 \(\mu\) in the spectrum of \(\alpha\)-estradiol-3-monobenzoate and at 7.89 \(\mu\) in the spectrum of \(\beta\)-estradiol-3-monobenzoate.

The second class of C–O groups (those having a carbon with only single bond linkages) includes C–O groups found in alcoholic hydroxyl structures. They may be expected to give absorption bands in the 9.0 to 10.0 \(\mu\) region (2, 4). Such groups are present in \(\alpha\)-estradiol, estriol, \(\beta\)-estradiol, \(\alpha\)-estradiol-3-monobenzoate, and \(\beta\)-estradiol-3-monobenzoate.

\(\alpha\)-Estradiol which has a C\textsubscript{17}-hydroxyl trans to a C\textsubscript{18}-methyl, gives a band at 9.41 \(\mu\). This is in accord with the inference made in Paper I (2) that steroids with a C\textsubscript{17}-hydroxyl trans to a C\textsubscript{18}-methyl give a band in the region 9.35 to 9.45 \(\mu\).
In β-estradiol, the alcoholic hydroxyl at C17 is cis to the C18-methyl. This is the same configuration as that of the C17-hydroxyl of androstenediol-3(β),17(β)2 (2). However, in the spectra so far obtained of these two steroids, there is no one outstanding band at a common wave-length among the several small to medium bands in the 9.0 to 10.0 μ region. Because of this circumstance, a present prediction is unwarranted as to which band in the 9.0 to 10.0 μ region of the spectrum of β-estradiol results from the C—O linkage of the C17-hydroxyl.

The spectrum of estriol, which contains a C17(β)-hydroxyl as well as a C18-hydroxyl, contains two major bands at 9.27 and 9.37 μ. These bands probably originate from the hydroxyl groups in question. However, at present, neither band can be assigned to a specific hydroxyl group because of the absence of other reference spectra of steroids containing C17-hydroxyls and the uncertainties of interpretation discussed in the previous paragraph.

Another type of difficulty is encountered in the assignment, in the 9.0 to 10.0 μ region, of absorption bands to the C17-hydroxyls of α- and β-estradiol-3-monobenzoate. In their spectra, the three strong absorption bands with maxima near 9.20, 9.35, and 9.70 μ appear to arise from the benzoate portion of the molecule. Their positions and intensities are such as to mask absorption bands of lesser intensity arising from the C—O vibrations of the C17-hydroxyl groups.

Other Absorption Bands—In the spectra of this group of compounds, there are many absorption bands which cannot as yet be assigned to specific interatomic vibrations. However, as in the case of the androgen series (2), certain of these bands can be attributed to unspecified vibrations within specific polyatomic structural configurations of the molecules. This can be arrived at by cross-comparisons of the spectra, and a correlation of common structural configurations and wave-lengths of absorption.

By such cross-comparisons, it was found that a band of medium to strong...
intensity is present in the range 8.60 to 8.70 \( \mu \) in all those compounds containing the structural configuration A. This configuration is present in 

![Image](A)

estrone, \( \alpha \)-estradiol, \( \beta \)-estradiol, estriol, equilin, \( \alpha \)-estradiol-3-monobenzoate, and \( \beta \)-estradiol-3-monobenzoate. In this connection it is of interest that 4-hydroxy-1,2-dimethylbenzene, which has the same configuration, has been reported to give a strong band at about 8.65 \( \mu \) (4).

Equilenin, which contains structural configuration B, gives a strong band just below the 8.60 to 8.70 \( \mu \) range, at 8.54 \( \mu \). This configuration is the same as is present in \( \beta \)-naphthol, which has been reported to give a strong band at about 8.55 \( \mu \) (4). Stilbestrol gives a strong band at 8.48 \( \mu \). Here the structural configuration involved is probably configuration C, since a number of para-substituted phenols and phenolic ethers having the 

![Image](C)

same configuration give bands in the region 8.45 to 8.52 \( \mu \) (4).

In addition to the bands in the 8.60 to 8.70 \( \mu \) region, the monobenzoates of \( \alpha \)-estradiol and \( \beta \)-estradiol each give a band in the 8.45 to 8.50 \( \mu \) region. It is likely that these bands are attributable to some vibration in the benzoate part of the molecules; however, absorption spectra of other benzoates are necessary to establish this possibility.

A second region in which a band is present in the spectra of all the steroids reported here extends from 11.30 to 11.42 \( \mu \). This band is well defined, though of variable intensity, in all the compounds except \( \alpha \)-estradiol-3-monobenzoate, in which it appears as a shoulder of the strong band preceding it. The bands in the 11.30 to 11.42 \( \mu \) region appear to be attributable to some vibration in structural configuration D, which is common to all of the compounds. In assigning these bands to the configuration
D, their possible origin in configuration E, which is also common to all of these compounds, was considered and excluded by cross-comparisons with the spectra of other steroids containing configuration E. Even though a band occurs in the 11.30 to 11.42 \( \mu \) region in some of the spectra of these other steroids, it is not consistently present as in the case with estrogens.

The fact that some steroids other than estrogens give bands in the 11.30 to 11.42 \( \mu \) region is not unexpected, since the wave-lengths of absorption arising from different atomic groupings may sometimes be very close to one another. For the same reason, it is also not unexpected that some steroids besides estrogens also give a band in the 8.60 to 8.70 \( \mu \) region (see above). This circumstance, however, does not invalidate the usefulness of bands in these two regions in aiding in the identification of estrogens and related steroids. Bands in these two regions, along with a band in the 6.20 to 6.30 \( \mu \) region and a band in the 6.65 to 6.70 \( \mu \) region, might, together, be considered the “characteristic” bands of these compounds. (Equilenin with bands at 6.21, 6.76, 8.45, and 11.38 \( \mu \), is an exception.) Of all the steroids so far investigated, none, other than those considered in this paper, has a spectrum containing bands in all four of these regions. If an unknown steroid under investigation were to give a band of medium to strong intensity in each of these regions, it would be very strong presumptive evidence that it was an estrogen.

In conclusion, it may be pointed out that all four of these “characteristic” bands of estrogens and related steroids result from vibrations of atoms in the benzenoid ring of the molecules. Thus, the structural configuration which chemically distinguishes this group of steroids from other steroids also distinguishes their spectra from those of other steroids.

**SUMMARY**

1. The infra-red absorption spectra, from 2 to 12.4 \( \mu \), of various estrogens and related steroids have been presented.

2. Certain absorption bands in the spectra have been discussed in relation to the chemical structure of these compounds.
3. Four of these bands, all arising from vibrations of atoms in the benzenoid ring of the molecules, may be used to distinguish the spectra of estrogens and related steroids from those of other steroids.

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