INFRA-RED ABSORPTION OF STEROLS

I. ERGOSTEROL AND RELATED STEROLS

By HARRIS ROSENKRANTZ, ADE T. MILHORAT, AND MILTON FARBER

(From the Departments of Medicine and Psychiatry, Cornell University Medical College, the Russell Sage Institute of Pathology, and The New York Hospital, New York, New York)

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In a recent report from this laboratory (1), the infra-red spectrum of a purified fraction derived from the so called "guinea pig antistiffness" factor was shown to be identical with that of stigmasterol, and chemical and biological studies in another laboratory (2) have since demonstrated that the antistiffness factor is identical with stigmasterol. The infra-red spectral study consisted of the comparison of the spectra of a large number of sterols in the ergosterol and cholesterol families with those of fractions from the counter-current distribution of the crude factor obtained from sugar-cane wax, and with a highly purified preparation isolated by the Armour Laboratories (2). This recording of the spectra of many members of two important sterol families has now made it possible to attempt the analysis of these spectra in terms of structural correlations. It is felt that this information will be a desirable addition to the growing catalogue of infra-red data on sterols, which is proving of value to many investigators. The present paper discusses the ergosterol family, and a subsequent paper will deal with cholesterol and related sterols.

Method

The infra-red absorption spectra were obtained with a Baird Associates infra-red recording spectrophotometer, the spectral range observed extending from 2 to 16 \( \mu \). Since several of the substances studied darkened when heated in air, films for analysis were prepared in an inert atmosphere. The samples were either melted between two salt plates or crystallized from pyridine on a salt plate.

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EXPERIMENTAL

The absorption spectra shown (Figs. 1 to 4) are copies of original automatic recordings, changes in calibration having been made only to align the 3.4 μ absorption band in all the tracings. The absorption characteristics of the following compounds were obtained: stigmasterol, ergosterol, α- and γ-dihydroergosterol (Fig. 1); α-, β-, and γ-ergostenol, and ergostanol (Fig. 2); ergostane, ergostanone, α-spinasterol, and vitamin D₂ (Fig. 3); α- and γ-ergostenyl acetate and ergostanyl acetate (Fig. 4).

Since the vibrations of specific atomic groups are detected in the 3 to 10 μ region, this region will be discussed first.

O—H Absorption—Most of the compounds studied contained a hydroxyl group in the 3 position, the hydroxyl vibrations occurring between 2.85 and 2.93 μ. Absorption by alcoholic hydroxyl groups usually occurs nearer 2.75 μ, but some intermolecular hydrogen bonding in the crystalline state may account for the shift to longer wave-lengths (3). As had been expected, an absorption band near 3 μ was absent in the spectra of ergostane, ergostanone, and the acetate esters.

C—H Absorption—All the compounds studied gave the expected C—H absorption bands near 3.4 and 6.8 μ. The band at 3.4 μ arises from the linear vibrations of hydrogen in saturated C—H groups. It has been shown (4) that the C—H frequency of unsaturated carbons occurs close to 3.25 rather than 3.4 μ. Since the sterols observed in this study con-
tain mainly saturated C—H linkages, it is not surprising that the unsaturated C—H vibration was not manifested. This was also true of the angular vibrations of the C—H groups, since no bands appeared between 6.5 and 6.7 \( \mu \) at which aromatic C—H absorption in steroids usually occurs (Figs. 1 to 4) (3). The angular saturated C—H vibrations were assigned to a doublet near 6.8 and 6.9 \( \mu \), since these also occurred in the completely saturated molecules, ergostanol, ergostane, and ergostanone (Figs. 2 and 3).

C=O and C=C Absorption—Only two types of carbonyl groups were encountered in this study. The ketone carbonyl group in ergostanone (Fig. 3) gave rise to the absorption band near 5.8 \( \mu \), and the vibrations of the ester carbonyl group in the acetylated sterols (Fig. 4) appeared near 5.75 \( \mu \). An absorption band of weak intensity near 5.8 \( \mu \) was obtained with ergosterol, \( \gamma \)-dihydroergosterol, and \( \alpha \)- and \( \beta \)-ergostenol. This unassigned band in the ketone region may have been due to some oxidation impurity.

The spectra of those sterols which contained at least one C=C group had an absorption band of weak intensity near 6 \( \mu \). The highly conjugated double bond system in the ruptured ring B of vitamin D2 (Fig. 3) probably gave rise to the strong absorption band near 6.1 \( \mu \). Similarly, a weak absorption band near 6.2 \( \mu \) in the spectrum of ergosterol (Fig. 1) may possibly be assigned to its conjugated double bond system.

C—CH\(_3\) Absorption—Methyl group vibrations are known to produce absorption near 7.25 \( \mu \) (4). All of the spectra except those of \( \gamma \)-ergostenol...
and its acetate (Figs. 2 and 4) showed either a resolved doublet near 7.2 \( \mu \) or an absorption band with a side inflection near 7.27 \( \mu \). This doublet near 7.2 and 7.27 \( \mu \) probably is due to a combination of the vibrations of the quaternary angular methyl groups and the various tertiary substituted C—CH\(_3\) groups of the side chain. An almost identical doublet is known to be produced by either tertiary butyl or isopropyl groups (4).

**C—O Absorption**—Two types of C—O vibrations in the compounds under investigation are possible. The sterols containing a C—O group in which the carbon has a double bond linkage give rise to bands near 8 \( \mu \). The absorption doublet in \( \alpha \)-ergostenyl acetate and ergostanyl acetate occurring near 7.88 and 8.03 \( \mu \) (Fig. 4) may be assigned to this type of C—O linkage. In the spectrum of \( \gamma \)-ergostenyl acetate, this doublet occurred as a broad single band (Fig. 4). Ergostanone was the only other compound that showed strong absorption in this region of the infra-red spectrum, but the absorption bands were not similar to those seen in the spectra of the acetate derivatives.

The second class of C—O groups (those having the carbon with only single bond linkages) includes C—O groups found in alcoholic hydroxyl and ester structures, having absorption bands in the 9 to 10 \( \mu \) region (4). Of the substances studied, only ergostane and ergostanone do not contain such a group. In the spectra of all the other sterols a moderate to intense absorption band appeared between 9.4 and 9.7 \( \mu \), which can be assigned to the vibrations of the saturated C—O group. Ergosterol and ergostanyl acetate gave two intense bands in this region. The broad and intense band near 9.5 \( \mu \) in the spectrum of vitamin D\(_2\) may possibly be due to interaction of the ruptured ring B with the C—O linkage of the hydroxyl group.

**Other Absorption Bands**—Several absorption bands in the 10 to 13 \( \mu \) region appeared to be characteristic of the ergosterol skeleton. The spectra of all of the ergosterol derivatives had absorption bands near 10.6, 11.8, and 12.5 \( \mu \). Jones (5) recently has demonstrated that an absorption band near 10.3 \( \mu \) occurs in the spectra of ergostenyl compounds containing a \( \Delta^{22} \) double bond. All of the sterols studied here which contained the \( \Delta^{22} \) double bond gave a strong band near 10.3 \( \mu \). However, a weak to moderately intense band near 10.3 \( \mu \) occurred also in the spectra of the ergostenols and of ergostanol, which do not possess a \( \Delta^{22} \) double bond. Several differences in absorption characteristics observed in the 10 to 12 \( \mu \) region permitted differentiation of all the sterols studied. For example, the spectrum of stigmasterol showed characteristic bands near 10.3 and 10.4 \( \mu \), which permitted its differentiation from the spectra of all the ergosterol derivatives studied.

The structures of the sterols presented in this report are similar; yet
significant differences in their infra-red absorption spectra permit rapid
differentiation and identification of each sterol. However, most of the
absorption bands employed for identifying a particular compound cannot
be assigned to a specific structural configuration. This obviously limits
the use of infra-red spectra in establishing molecular structure, but does
not affect the value of infra-red spectroscopic analysis in identifying
specific atomic groupings and in confirming final identifications.

SUMMARY

1. The infra-red absorption spectra, from 2 to 16 μ, of various ergosterol
derivatives and related sterols have been recorded.
2. Absorption bands arising from specific atomic groups have been
discussed in relation to the structure of these compounds.
3. Bands in the 10 to 12 μ region have been discussed for characterizing
the sterols studied.

BIBLIOGRAPHY

4. Barnes, R. B., Gore, R. C., Liddell, U., and Williams, V. Z., Infrared spectros-
copy, New York (1944).
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