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The Infrared Spectrum of Ostreasterol (Chalinasterol)

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The identity of ostreasterol ($\Delta^{5,22}$-campestadien-3β-ol) with chalinasterol has been confirmed by infrared spectroscopy. A prominent band at 974–965 cm.$^{-1}$, considered to be a characteristic feature of the infrared spectra of all Δ^{22}-*trans* steroids, is not present in the spectrum of ostreasterol (chalinasterol). This anomaly is discussed.

In the course of an investigation dealing with the identification of aromatic hydrocarbons in oysters[1] a sterol was isolated from oysters (*Crassostrea virginica*). When the non-saponifiable material obtained from an oyster extract was chromatographed, and a rather strongly absorbed chromatographic fraction was dissolved in *n*-pentane, the sterol crystallized upon standing in the cold. After purification white crystals were obtained; m.p. 141.5–143°; $[\alpha]_D^{21}$ −41.2° (*c*,1; chloroform). These physical constants as well as the crystal form suggested that the sterol was ostreasterol, previously isolated by Bergmann[2] from the same species and from several other mollusks.[3] The chemical and physical properties of ostreasterol have been described by the same author[2,4]

A small sample of ostreasteryl acetate was obtained from Prof. Bergmann who indicated that it was well over 10 years old and consequently had suffered considerable deterioration. After alkaline hydrolysis, followed by chromatographic fractionation and one recrystallization 1.1 mg. of ostreasterol was obtained which, however, was not sufficiently pure for the purpose of comparison with the sterol isolated in this laboratory. Prof. Bergmann was kind enough to supply also a sample of chalinasterol which upon chromatographic fractionation and recrystallization yielded pure chalinasterol; m.p. 140–142°. Chalinasterol, isolated from various sponges,[5] and ostreasterol appear to be identical, *viz.* $\Delta^{5,22}$-campestadien-3β-ol.[6]

The melting point of the sterol isolated in this laboratory was not depressed by admixture with chalinasterol, and both sterols showed the same behavior when crystallized from a melt (flat needles). However, neither the similarity of melting points and crystal forms nor the lack of melting point depression is sufficient proof for the identity and purity of two compounds, particularly in the field of steroids. The infrared spectra of both sterols were therefore determined (Figure 1). Except for a hardly perceptible and apparently insignificant difference at about 698 cm.$^{-1}$ they are absolutely identical. They indicate therefore the identity of the two compounds and permit the conclusion that the sterol isolated in this laboratory is actually ostreasterol. (The infrared spectrum of the impure authentic sample of ostreasterol was also determined. It shows all the bands of the spectrum of the sterol isolated in this laboratory, and in addition two bands at 1717 and 1262 cm.$^{-1}$. Also the band at 800 cm.$^{-1}$ is more pronounced in the spectrum of the impure ostreasterol sample. As none of the bands present in the spectrum of the sterol isolated in this laboratory is missing, the additional bands are attributed to impurities.[7])

The infrared spectra of chalinasterol and ostreasterol show an interesting feature, *viz.* the absence of a strong band at 974–965 cm.$^{-1}$ which has been shown to be typical for a *trans* configuration around the Δ^{22}-double bond of steroids.[8,9] The Δ^{22} *trans* configuration has been assigned to calciferol, ergosterol, ergosteryl acetate, stigmasterol, stigmasteryl acetate, lumisteryl acetate, and Δ^{22}-ergostene on the basis of the presence of a strong band in this region. An inspection of the infrared spectra of a number of other Δ^{22}- steroids[10–13] also revealed the presence of a very strong band in the same region in each case. In contrast, the spectra of ostreasterol and chalinasterol show only a very weak band at 970 cm.$^{-1}$. When the infrared spectrum of ostreasterol was determined in carbon disulfide at a concentration of 47 mg./ml. in a 0.5-mm. cell, all bands were more pronounced than in

(1) Cahnmann and Kuratsune, *Proc. Am. Assoc. Cancer Research*, 2, 99 (1956).

(2) Bergmann, *J. Biol. Chem.*, 104, 317 (1934).

(3) The genus name *Ostrea* was formerly used for the genus *Crassostrea*.

(4) Bergmann, *J. Biol. Chem.*, 104, 553 (1934).

(5) Bergmann, Schedl, and Low, *J. Org. Chem.*, 10, 587 (1945).

(6) Bergmann and Low, *J. Org. Chem.*, 12, 67 (1947).

(7) A larger sample of impure ostreasteryl acetate was obtained from Prof. Bergmann after this paper had been submitted for publication. In the spectrum of the ostreasterol obtained from it (m.p. 138.5–141°) the bands at 1717 and 1262 cm.$^{-1}$ were missing. Except for minor differences at 1669, 1641, and 885 cm.$^{-1}$ the spectra in KBr and in CS_2 were identical with the corresponding ones of the sterol isolated in this laboratory.

(8) Turnbull, Whiffen, and Wilson, *Chemistry & Industry*, 33, 626 (1950).

(9) Jones, *J. Am. Chem. Soc.*, 72, 5322 (1950).

(10) Jones, *Chemistry in Canada*, 2, 26 (94) (1950).

(11) Rosenkrantz, Milhorat, and Farber, *J. Biol. Chem.*, 195, 503 (1952).

(12) Dobriner, Katzenellenbogen, and Jones, *Infrared Absorption Spectra of Steroids*, Interscience, New York, 1953.

(13) Mosettig and Nes, unpublished data.

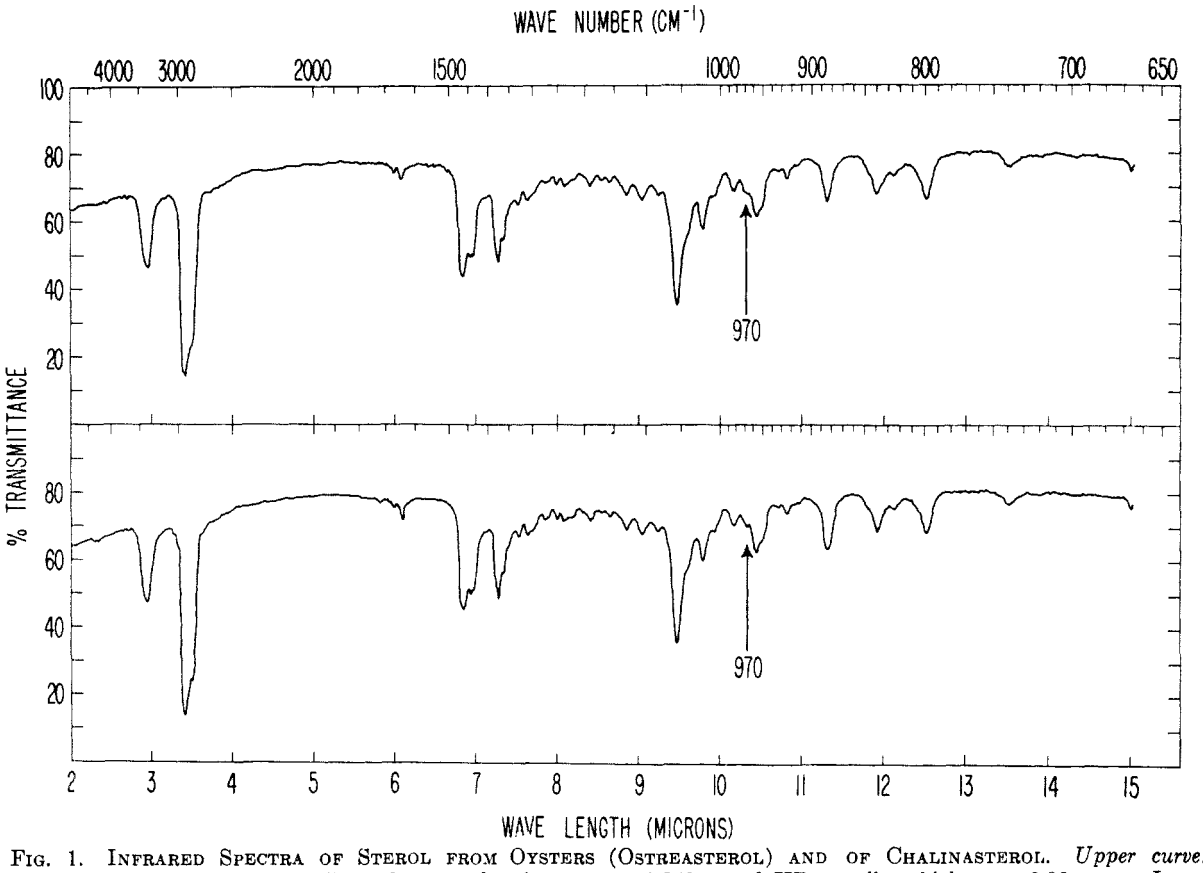

FIG. 1. INFRARED SPECTRA OF STEROL FROM OYSTERS (OSTREASTEROL) AND OF CHALINASTEROL. *Upper curve:* Sterol from oysters (ostreasterol). 1.0 mg. of substance + 0.343 g. of KBr; pellet thickness: 0.90 mm. *Lower curve:* Chalinasterol. 1.1 mg. of substance + 0.365 g. of KBr; pellet thickness: 1.01 mm.

the spectrum shown in Figure 1, but the band at 968 cm.$^{-1}$ (corresponding to the band at 970 cm.$^{-1}$ in potassium bromide) was still a weak one. The somewhat stronger band at 985 cm.$^{-1}$ falls considerably outside the wave number range assigned to a Δ^{22}- *trans* double bond and so does the relatively strong band at 958 cm.$^{-1}$. The latter is known not to arise from a double bond.[9]

A *prominent* band at 974–965 cm.$^{-1}$ is not confined to the spectra of Δ^{22}- *trans* steroids, but is a characteristic feature of the spectra of all *trans* olefinic compounds of the type CHR=CHR′ (R and R′ = alkyl).[14–29] It is due to the out of plane bending vibrations (deformational or flapping vibrations) of C=C—H carbon—hydrogen bonds. It is generally assumed that the absence of a prominent band at 974–965 cm.$^{-1}$ indicates the absence of a CHR=CHR′ *trans* configuration in the molecule. The presence of a weak or even strong band, however, does not yet prove the presence of this grouping and configuration.[25] In the case of steroids this was shown by Rosenkrantz, *et al.*[11] and confirmed by an inspection of a large number of published spectra of steroids.[12] Thus a rather strong band is present in the spectra of $\Delta^{16(17)}$-steroids. On the other hand it has been concluded from the absence of a prominent band around 970 cm.$^{-1}$ in the spectra of certain steroids that these steroids do not contain a Δ^{22}- *trans* double bond.[30]

In view of the absence of a strong band between

(14) Thompson and Torkington, *Trans. Faraday Soc.*, **41**, 246 (1945).
(15) Kilpatrick and Pitzer, *J. Research Natl. Bur. Standards*, **38**, 191 (1947).
(16) Rasmussen and Brattain, *J. Chem. Phys.*, **15**, 120, 131 (1947).
(17) Rasmussen, Brattain, and Zucco, *J. Chem. Phys.*, **15**, 135 (1947).
(18) Anderson, Jr. and Seyfried, *Anal. Chem.*, **20**, 998 (1948).
(19) Rao and Daubert, *J. Am. Chem. Soc.*, **70**, 1102 (1948).
(20) Sheppard and Sutherland, *Proc. Roy. Soc. (London)*, **196 A**, 195 (1949).
(21) Lemon and Cross, *Can. J. Research*, **27 B**, 610 (1949).
(22) Hall and Mikos, *Anal. Chem.*, **21**, 422 (1949).
(23) Hampton, *Anal. Chem.*, **21**, 923 (1949).
(24) American Petroleum Institute, Research Project 44, Carnegie Institute of Technology, *Catalog of Infrared Spectral Data*, 1943–1955.
(25) Barnard, Bateman, Harding, Koch, Sheppard, and Sutherland, *J. Chem. Soc.*, **1950**, 915 (1950).
(26) Crombie and Harper, *J. Chem. Soc.*, 873 (1950).
(27) Sondheimer, *J. Chem. Soc.*, 877 (1950).
(28) Shreve, Heether, Knight, and Swern, *Anal. Chem.*, **22**, 1498 (1950).
(29) Sinclair, McKay, Myers, and Jones, *J. Am. Chem. Soc.*, **74**, 2578 (1952).
(30) Idler and Fagerlund, *J. Am. Chem. Soc.*, **77**, 4142 (1955).

974 and 965 cm.$^{-1}$ in the infrared spectrum of ostreasterol (chalinasterol) a *cis* configuration around the Δ^{22}- double bond in these compounds is therefore a distinct possibility. A band in the general neighborhood of 700 cm.$^{-1}$ has been associated with the out of plane bending vibrations of the carbon—hydrogen bonds in *cis* olefins of the type CHR=CHR'.[15,17,18,21-23,25-27] However, assignment of an absorption band in this region to *cis* ethylenic hydrogen vibrations is subject to doubt,[28,29] and not all *cis* olefins of the type just mentioned yield spectra with a prominent band at about 700 cm.$^{-1}$, at least not when the spectrum is determined at room temperature.[9] The absence of a band between 720 and 680 cm.$^{-1}$ in the spectrum of ostreasterol and the presence of an extremely weak band at 698 cm.$^{-1}$ in the spectrum of chalinasterol do therefore not offer sufficient proof for the absence of a Δ^{22}- *cis* double bond in these sterols.

The infrared spectra of ostreasterol and chalinasterol do not permit at present the assignment of a *cis* or *trans* configuration to the Δ^{22}- double bond. A *cis* configuration is thermodynamically and sterically less likely, but not impossible. Should a *cis* configuration be proven, then ostreasterol (chalinasterol) would be the first known Δ^{22}- *cis* steroid. If on the other hand a *trans* configuration can be ascertained, our present conception that a prominent band at 974–965 cm.$^{-1}$ is a characteristic feature of the infrared spectra of *trans* olefins of the type CHR=CHR' in general and of Δ^{22}- *trans* steroids in particular, must be revised.

EXPERIMENTAL

Ostreasterol from oysters.[31] Freshly shucked oysters (*Crassostrea virginica*) (5 kg.) in 4 liters of methanol were ground in a Waring blendor. The methanolic extracts obtained after centrifugation and repeated washings of the residue with absolute methanol were partitioned between 80% methanol and cyclohexane. The hypophase was extracted five times with cyclohexane and the combined epiphases were evaporated to 0.5 liter. The concentrate was saponified with 0.25 liter of 7 *N* methanolic potassium hydroxide for 4 hours at room temperature. After working up, a non-saponifiable fraction (11 g. of solids) was obtained which was chromatographed on activated alumina.[32] The chromatogram was developed with cyclohexane containing increasing amounts of acetone (from 0 to 2 vol.-%). After development the column was cut into several segments which then were extracted with acetone. The material extracted from the second segment from the top of the column (immediately below a red pigment zone) was transferred to *n*-pentane by means of the method of LeRosen.[33] After concentration to a few milliliters and standing in the cold (3°), the solution solidified to form a crystalline mass. After several recrystallizations from ethanol and from methanol 170 mg. of white crystals were obtained; m.p. 141.5–143° (first droplets at 141°)[34]; $[\alpha]_D^{21}$ −41.2° (*c*, 1; chloroform; 4 dm.).[35] From a cooled melt the sterol crystallized in the form of flat needles.

Ostreasterol from ostreasteryl acetate. A solution of 16 mg. of crude ostreasteryl acetate[36] in 5 ml. of a 5% solution of potassium hydroxide in 80% methanol was refluxed for 75 minutes. After working the hydrolyzate up in the usual manner 12 mg. of crude sterol was obtained. These were chromatographed on activated alumina (3% water). The chromatogram was developed with *n*-pentane containing increasing amounts of dichloromethane (from 50 to 100 vol.-%). 50% CH_2Cl_2 eluted a minimal trace, 60–90% CH_2Cl_2 a few milligrams, and 100% CH_2Cl_2 again a minimal trace of material. The bulk of the eluate obtained with 60–90% CH_2Cl_2 was recrystallized from methanol and 1.1 mg. of white crystals was obtained; m.p. 138–140°.

Chalinasterol. Crude chalinasterol[36] (52 mg.) was purified by chromatography as described in the preceding paragraph for ostreasterol. After two recrystallizations from methanol 5.3 mg. of white crystals was obtained; m.p. 140–142°. From a cooled melt the sterol crystallized in the form of flat needles.

Infrared spectra. A Perkin-Elmer recording spectrophotometer, Model 21, equipped with sodium chloride optics was used. The sterols were mixed with potassium bromide (pellets) or dissolved in carbon disulfide.

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(31) The described procedure was chosen in view of the intended identification of aromatic hydrocarbons and pigments in oysters. For the sole purpose of the isolation of oyster sterols a method similar to the one described by Bergmann, *et al.*[24,25] appears to be simpler.

(32) Harshaw Chemical Co., grade Al 0109-P, slightly deactivated with 3% water [Müller, *Helv. Chim. Acta*, **26**, 1945 (1943)].

(33) LeRosen, *Ind. Eng. Chem., Anal. Ed.*, **14**, 165 (1942).

(34) Kofler block with recalibrated thermometer.

(35) Rudolph precision polarimeter.

(36) Kindly supplied by Prof. Bergmann.