0.74 in 60% acetic acid, and 0.72 in butyl alcohol–acetic acid–water (40–10–50). Approximate yield of pure isoquercitrin was 2.3 g.

Upon methylation, then hydrolysis and recrystallization, 3′,4′,5,7-tetramethoxy-3-hydroxyflavone, m.p. 195–196°, was obtained. The sugar is, therefore, attached to the 3-position of the aglycone.

Hydrolysis of the glycoside with 1% sulfuric acid yielded 1 mole of glucose per 1 mole of quercetin. The quercetin was identified by R_f values, melting point 313–314°, absorption spectrum and pentaacetate, m.p. 194°. The sugar was identified as glucose by its osazone and by paper chromatography.

Fraction V, therefore, is isoquercitrin.

Purification and Identification of Quercetin-3-glucoglucoside (Fraction VI).—Fraction VI was placed on a fresh Magnesol column from an acetone solution. The column was then eluted with ethyl acetate–water solution made slightly acid with acetic acid. This moved the flavonoid off the column as a sharp band. This band was collected and after evaporation to dryness, the solid was recrystallized from alcohol–water solution. The product was dried at 110 for 1 hr., m.p. 182–184°. Approximate yield of pure product was 55 mg.

R_f values were as follows: 0.56 in 15% acetic acid, 0.76 in 60% acetic acid, 0.86 in butyl alcohol–acetic acid–water (40–10–50), 0.71 in isopropyl alcohol–water (1:3.5), and 0.90 in 60% isopropyl alcohol.

The ultraviolet absorption spectrum was identical with one that was run on authentic rutin. Its behavior on Magnesol was also similar to that of rutin and further indicated the probable presence of 2 moles of sugar on the flavonol aglycone.

A sample of pure fraction VI was methylated, then hydrolyzed and recrystallized, giving 3′,4′,5,7-tetramethoxy-3-hydroxy flavone. This compound was identified by its melting point 196°, and by comparison of the absorption spectrum of this tetramethoxyquercetin with that of the known compound as prepared from authentic quercitrin. The sugar is, therefore, attached at position three.

A sample of fraction VI was hydrolyzed by refluxing in 2% sulfuric acid for 2 hr. After cooling for 24 hr., the aglycone was filtered off and weighed. The recrystallized quercetin was identified by its R_f values, melting point 314–315°, absorption spectrum and pentaacetate, m.p. 193°. The hydrolysis filtrate was neutralized with Amberlite IR-4B ion exchange resin, and the sugar identified as glucose by paper chromatography. The ratio of 2 moles of glucose per mole of quercetin was determined by the method of Fisher, Parsons and Morrison.[10] Fraction VI is, therefore, quercetin-3-gluco-glucoside.

Discussion.—The "huckleberry" leaves used were purchased from Meer Corporation, New York. They were authenticated by an official of that corporation as having been obtained from North Carolina, 1951 season, and classified as *Vaccinium myrtillis*.

A corresponding batch of "huckleberry" leaves from the 1950 crop was studied in detail and was found to contain quercetin, quercetin-3-arabinoside, isoquercitrin and quercitrin, but none of the quercetin-3-glucoglucoside and the new quercetin-rhamnoside.

Acknowledgments.—The authors express their appreciation to Dr. Thomas B. Gage, Rayon Technical Research, E. I. du Pont de Nemours and Co., Richmond, Va., who while at Oklahoma, gave valuable advice and help in the early stages of this research, and to Dr. George J. Goodman, director of the University of Oklahoma Herbarium, who aided in the proper classification of the leaves. This research was supported in part by grants from the Atomic Energy Commission and the Office of Naval Research.

NORMAN, OKLAHOMA

[CONTRIBUTION FROM THE ANALYTICAL DIVISION, DEPARTMENT OF CHEMISTRY, AND DEPARTMENT OF BIOCHEMISTRY, COLLEGE OF AGRICULTURE, UNIVERSITY OF WISCONSIN]

Infrared Spectra of Some Cholestanol Derivatives[1]

BY D. R. JOHNSON, D. R. IDLER, V. W. MELOCHE AND C. A. BAUMANN

RECEIVED JULY 21, 1952

The infrared absorption spectra from 2 to 16μ are presented for seven sterols differing only in double bond character. Many of the observed variations in absorption could be attributed to specific functional groups. The 6 and 3.3μ regions have been shown to be the most useful in providing information on double bond location. Some secondary effects of double bond location have also been noted.

Several general studies have indicated that infrared spectrophotometry may be useful in the determination of steroids[2–5] and such spectra were recently used in the identification of the new skin sterol, Δ^7-cholestenol.[6] In contrast to the steroids, data on the infrared spectra of the sterols are relatively meager.[4–10] Jones, *et al.*,[9,11] have studied effects of the location of certain ethylenic double bonds on the spectra of unsaturated steroids in solution. While others have observed the 6 μ C=C stretching band in the spectra of solid films of steroids,[5,8] Bladon, *et al.*, extended these studies to new compounds and included both solid state and solution spectra. In the present study complete solid spectra have been taken of a series of

(1) Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Supported in part by the Wisconsin Alumni Research Foundation.

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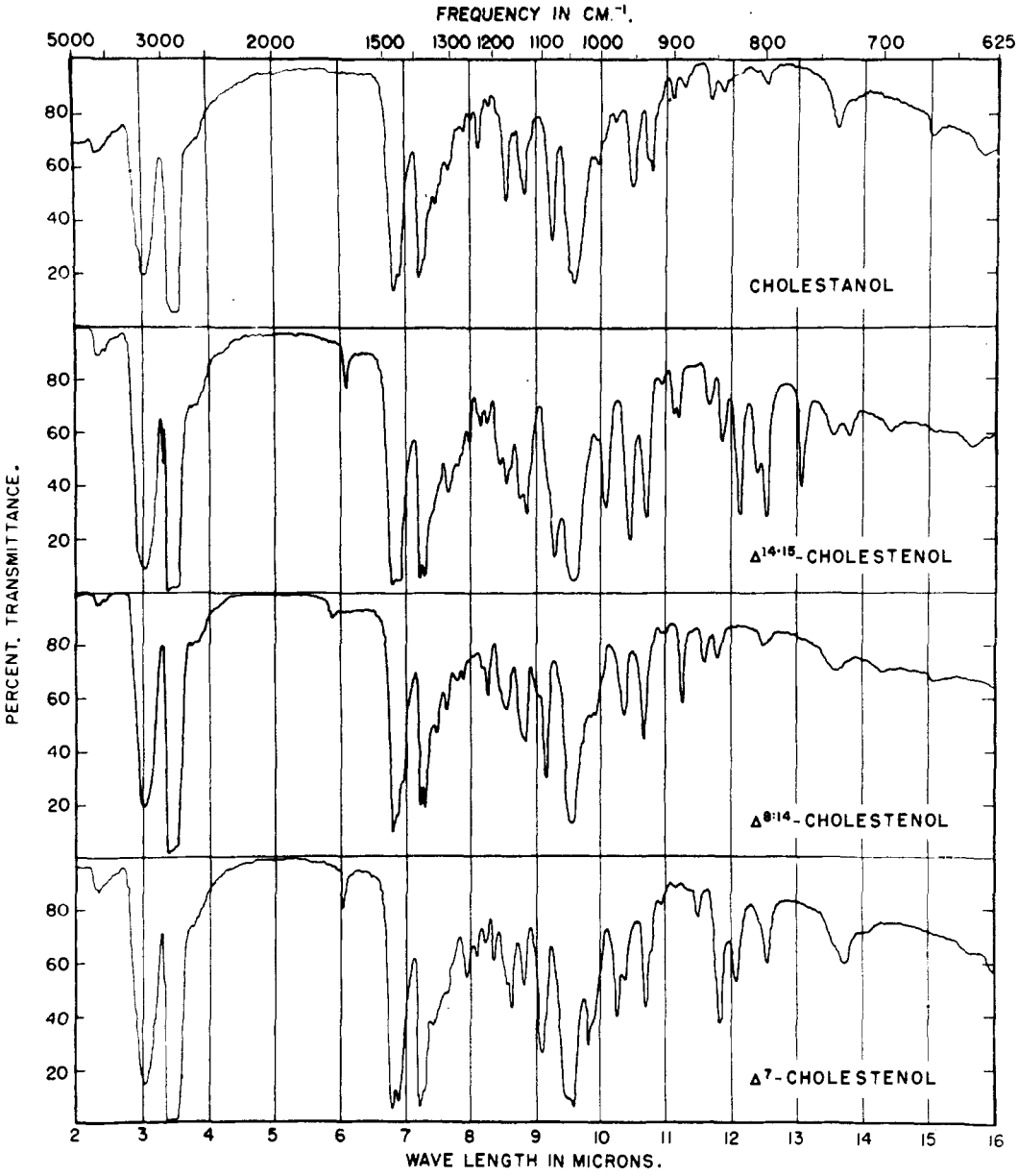

Fig. 1.—Infrared spectra of solid films of cholestanol and unsaturated derivatives; NaCl prism, 25°, effective thickness of sample *ca.* 0.05 mg./mm.².

cholestenols (Δ^5, Δ^6, Δ^7, $\Delta^{8(14)}$ and $\Delta^{14(15)}$) which differ only in the position of the double bond, and these were compared to the saturated sterol, cholestanol and to the doubly unsaturated $\Delta^{5,7}$-cholestadienol. Certain spectra were also taken in solution.

Experimental

The preparation and purification of the compounds have been described elsewhere.[12] The infrared absorption spectra were recorded with a Baird Associates Model B infrared spectrophotometer, a sodium chloride prism being used for the range 2 to 16 μ and a calcium fluoride prism for higher resolution in the 2 to 9 μ region. The crystalline sterols were prepared as solid films by fusing them between rock salt plates under nitrogen and quickly cooling to room temperature, thus forming a film having a thickness of ~0.05 mg./mm.². Solution spectra were run in carbon disulfide in a 1.5-mm. microcell at concentrations of 5 to 10 mg. per ml. All spectra were run at 25°. The solid films proved to be of particular value in this study since they provided maximum effective concentration and sharp, well resolved weak bands with a small sample, while solvent interference was absent. Although certain features of solid state spectra may be affected by the structure of the crystal lattice, X-ray diffraction studies of the crystalline state of sterols[13] suggest that a relatively consistent crystal structure, double layer with interacting hydroxyl groups, may be expected in the present series of compounds.

Results

Each of the cholestenols of the present series showed sufficiently characteristic distribution and variation in the intensities of the bands in the "fingerprint" region beyond 8 μ to permit their identification (Figs. 1 and 2). In addition many of the spectra showed characteristic bands in regions assigned to particular functional groups.

O–H Stretching.—In dilute solution all sterols exhibit a sharp band at 2.76 μ (3620 cm.$^{-1}$) char-

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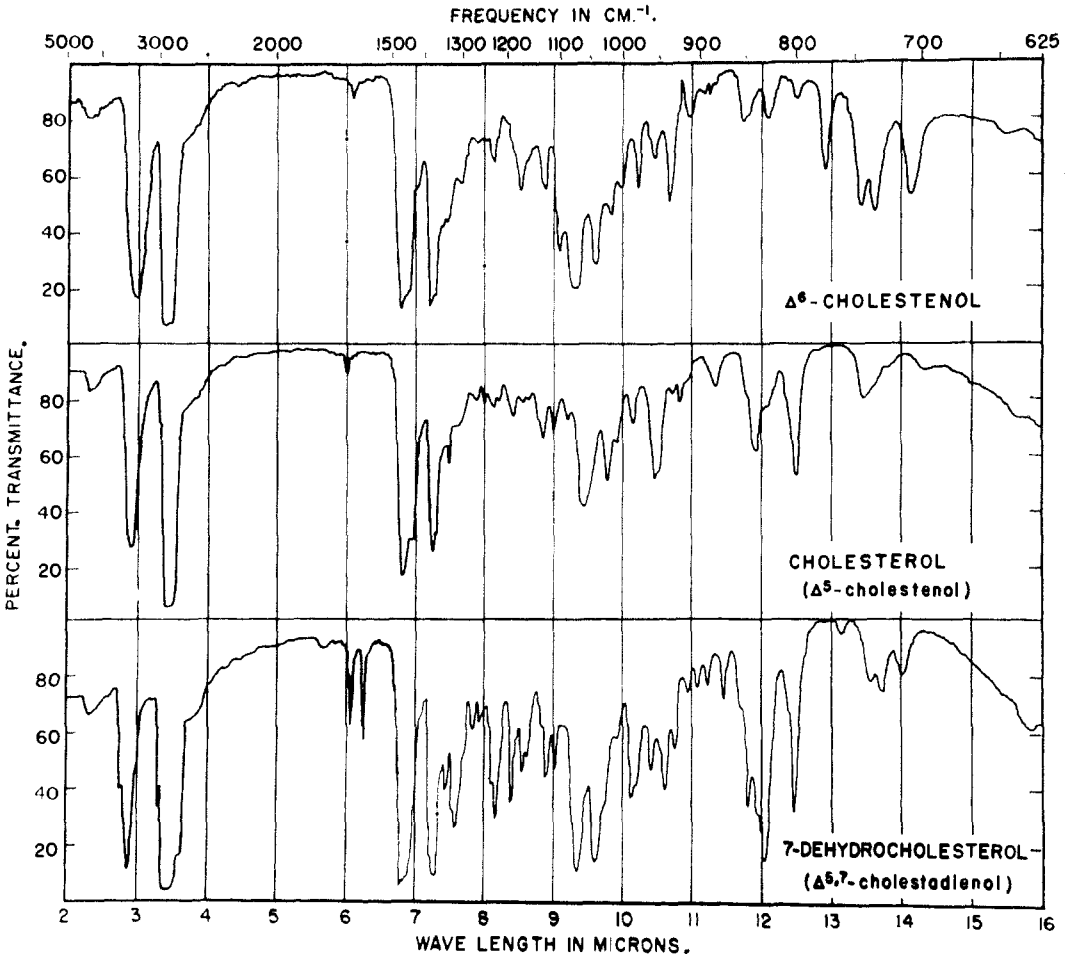

Fig. 2.—Infrared spectra of unsaturated cholestanol derivatives; NaCl prism, 25°, effective thickness of sample *ca.* 0.05 mg./mm.².

acteristic of free O–H stretching. In the solid state, this O–H stretching band becomes broadened, intensified and shifted to longer wave length with increased hydrogen bonding.[5] In the present series there was a shift to the longer wave lengths as the distance between the double bond and the hydroxyl group was increased. In 7-dehydrocholesterol ($\Delta^{5,7}$-cholestadienol) 2 bands appeared in the O–H stretching region, that at 2.87 μ (3482 cm.$^{-1}$) originating in the weakly associated groups, and the one at 2.76 μ (3620 cm.$^{-1}$) indicating free hydroxyl. However, it is not safe to assume from this evidence alone that such a progression holds for other steroids, since changes in the side chain and other parts of the molecule may also affect the location of the O–H stretching band observed in the solid state.

C–H Stretching.—C–H stretching manifests itself in a strong absorption band near 3.4 μ.[4] Under high resolution this band was resolved into a strong band at 3.40 μ (2940 cm.$^{-1}$) and a weaker one at 3.50 μ (2860 cm.$^{-1}$) for all the compounds of the present series. The band assigned to the C–H stretching of the double bond terminal carbons[2,9] appeared prominently at 3.28 μ (3050 cm.$^{-1}$) in the spectra of $\Delta^{14(15)}$-cholestenol and of 7-dehydrocholesterol. With the Δ^5-, Δ^6- and Δ^7-cholestenols this band occurred at about 3.32 μ (3015 cm.$^{-1}$) as an ill-defined shoulder on the 3.4 μ band. It was absent from the spectra of $\Delta^{8(14)}$-cholestenol and from that of cholestanol.

C=C Stretching.—The stretching region of the ethylenic double bond, 5.9–6.3 μ (1590–1700 cm.$^{-1}$), also showed changes that varied with the location of the double bond within the molecule. The data obtained here agree with those of Jones[2,9] and Bladon[10] in showing the effect of double bond position on the location of the band. Study of this region was facilitated by the high effective concentration attainable in the solid state and by the elimination of recorded water vapor absorption in the double-beam instrument. Termination of the double bond of a six-membered ring in a bridgehead carbon shifted the band from the normal 6.1 μ (1640 cm.$^{-1}$) to 6.00 μ (1668 cm.$^{-1}$). The 8(14)-double bond, which has two bridgehead carbons, has been reported to show no absorption in the 6 μ region.[9,10] In the present studies, however $\Delta^{8(14)}$-cholestenol did show a weak absorption at 5.88 μ (1699 cm.$^{-1}$). This band was found to increase in intensity on standing. Thus, although the method of preparation precludes the presence of a carbonyl impurity, the compound is evidently subject to air oxidation. The 14(15)-unsaturation exhibited absorption at a wave length longer than that of the linkage in a six-membered

ring, an effect paralleled in the case of cyclopentene and cyclohexene.[14] This agrees with the findings of Bladon, *et al.*,[10] who attribute this effect to weakening of the endocyclic bonds in a five-membered ring because of the "bending" of σ-bonds. The $\Delta^{5,7}$-conjugated diene system exhibited the characteristic intensification, splitting and shift to a longer wave length.[7]

C–H Bending.—All of the compounds studied showed the strong absorption at 6.8 to 6.9 μ attributed to the methylene deformation. Absorption by C–CH_3 deformation at 7.2 to 7.3 μ was also present in all cases.

C–O Stretching.—Rosenkrantz, *et al.*,[8] have suggested that the strong bands between 9 and 10 μ (1110–1000 cm.$^{-1}$) assigned to C–O stretching in alcohols[5] might serve in determining the steric configuration of the 3- and 5-positions. For the present series of compounds, however, all of which have 3β-hydroxyl groups and five of which have a *trans* C-5 configuration, enough variation existed with varying double bond position to confuse or invalidate other configurational assignment (Figs. 1 and 2). However, spectra of solutions appear to be somewhat less variable in this respect. Among the compounds having the same 3–5 configuration there was a migration of the C–O band to shorter wave lengths in the spectra of the solid films as the double bond approaches the A ring, but the occurrence of a trigonal carbon in the 5-position and the attendant elimination of the 5-hydrogen markedly disrupted this progression.

Spectra of Films *vs.* Solutions.—Although the spectra of solid and dissolved sterols bear sufficient resemblance to permit identification of most sterols by comparison of the fingerprint regions, the spectra often showed significant differences in band shape and location. The shift of bands associated with groups engaged in intermolecular association has already been noted in connection with O–H and C–O stretching. The greater effective concentration and orientation of the side chain in the solid state are manifested in the appearance of weak but distinct bands not resolved in the solution spectra. Also, absence of a solvent in the solid state permits observation of the entire spectral region scanned and eliminates the variable of solvent interaction.

(14) American Petroleum Institute Research Project 44, Catalog of Infrared Spectral Data, No. 696 and 697 (1948).

MADISON, WISCONSIN

[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF THE UPJOHN COMPANY]

Microbiological Transformations of Steroids. II. The Preparation of 11α-Hydroxy-17α-progesterone

BY P. D. MEISTER, D. H. PETERSON, H. C. MURRAY, S. H. EPPSTEIN, L. M. REINEKE, A. WEINTRAUB AND H. MARIAN LEIGH

RECEIVED AUGUST 29, 1952

4,16-Pregnadien-3,20-dione is converted to 11α-hydroxy-17α-progesterone by *Rhizopus nigricans* Ehrb (A.T.C.C. 6227b).

Introduction

The microbiological introduction of an oxygen into position 11 of the steroid nucleus has been reported by us, in particular the formation of 11α-hydroxyprogesterone from progesterone.[1] This paper deals with the microbiological conversion of 4,16-pregnadien-3,20-dione by *Rhizopus nigricans* Ehrb. (A.T.C.C. 6227b). This compound was first prepared by Butenandt and Schmidt-Thomé[2] from 3β-acetoxy-5-androstene-17-one. Today, however, it is readily available from steroidal sapogenins such as Diosgenin.[3]

Methods

The methods used in the microbiological conversion of 4,16-pregnadien-3,20-dione are in all details identical to those reported in the first paper of this series.[1c] For a preliminary orientation fermentations with *Rhizopus nigricans* were run on a small scale (25 mg. of substrate/100 ml.) in shake flasks. For purposes of isolation and identification 1–2 g. of substrate were incubated in a 24–24- or 24–48-hour cycle (24-hour growth and 24- or 48-hour transformation) with a culture of *Rhizopus nigricans*, which had been grown on our medium H at an average *p*H of 4.5 and an aeration of 1 l./hr. Chromatography over alumina was usually necessary to separate the bioconversion product from unchanged starting material.

Results

Paper chromatography of the small scale fermentations using the propylene glycol–toluene system, revealed that a new compound had been formed in 25–40% yield which showed a mobility similar to 11α-hydroxyprogesterone. Chromatography over alumina led to the isolation of a bioconversion product (II) $C_{21}H_{30}O_3$, in 25–30% yield. The comparison of its physical properties (Table I and II) to those of the starting material and 11α-hydroxyprogesterone led to the following interpretation: one hydroxyl group had been formed, as indicated by the microanalysis and the formation of a monoacetate.

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