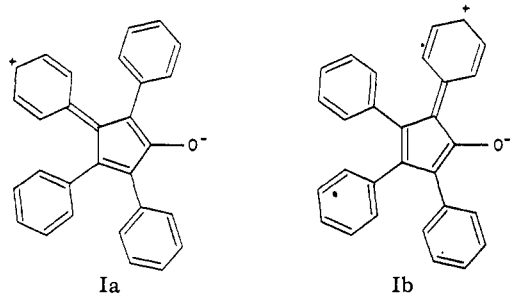
shown shifts in absorption maximum and for molar absorbancy in accord with the electronic effect of the substituent. Thus, the effect on molar absorbancy of the groups is H < Cl < CH_3 = Br < F < OCH_3. As expected OCH_3 effects an appreciable bathochromic shift of the λ_2 peak.

With the substituents in R_2, it is again seen that only absorption peak is shifted appreciably—the λ_1-peak (Fig. 2). Here, the λ_2-peak is shifted only with $N(CH_3)_2$; now the λ_2-peak is overpowered by the large bathochromic effect of $N(CH_3)_2$ on λ_1, and is manifest only as an inflection on the bathochromic slope of the λ_1-peak. The order of the bathochromic effect of the substituents is H = Br < Cl < CH_3 < OCH_3 < $N(CH_3)_2$.

The results are consistent with the assignment of Ia to represent the excited path for λ_1, and of Ib for λ_2. This choice is consonant with the assignment

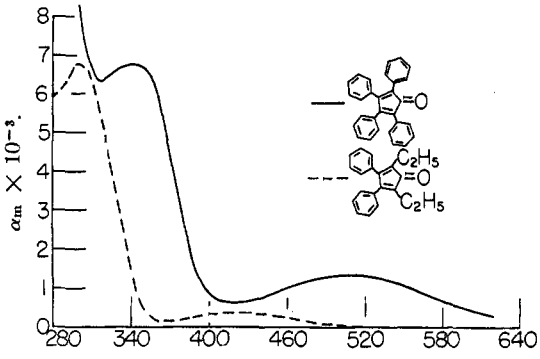


Ia Ib

of the longer path to the longest wave length. The interaction of the substituent groups through the molecule with the carbonyl group is also in agreement with the dipole moment measurements of tetracyclone and certain of its chloro derivatives carried out by Professor C. P. Smyth.[16]

(16) A. Di Giacomo and C. P. Smyth, THIS JOURNAL, **74**, 4411 (1952).

It was of interest to see what effect there would be on the absorption spectrum of tetracyclone if two of the phenyl groups were removed and replaced by alkyl. Accordingly, the absorption spectrum of 2,5-diethyl-3,4-diphenylcyclopentadienone (XII) was taken.[17] As expected there is a large hypsochromic shift of the λ_2-peak along with a somewhat smaller hypsochromic shift of the λ_1-peak (Fig. 4). This is in accord with the fact that the ethyl groups cannot conjugate with the double bonds as well as phenyl, except by hyperconjugation which is not measurable in these experiments.



Wave length (mμ).

Fig. 4.—Absorption spectra of tetracyclone and 2,5-diethyl-3,4-diphenylcyclopentadienone.

It seems possible, therefore, to correlate the two major absorption peaks in tetracyclone with two paths in the molecule corresponding to the structures shown in Ia and Ib.

(17) We are indebted to Dr. J. A. VanAllan of the Eastman Kodak Laboratories for a generous sample.

BROOKLYN, NEW YORK

[CONTRIBUTION FROM THE WORCESTER FOUNDATION FOR EXPERIMENTAL BIOLOGY, AND THE NATIONAL INSTITUTE OF MENTAL HEALTH COÖPERATIVE RESEARCH STATION AT THE WORCESTER FOUNDATION, PUBLIC HEALTH SERVICE, FEDERAL SECURITY AGENCY]

The 9–10 μ Region of Infrared Absorption Spectra of Steroids in Relation to Chemical Structure[1]

BY HARRIS ROSENKRANTZ AND LEONARD ZABLOW

RECEIVED AUGUST 20, 1952

The infrared fingerprint region of seven simple steroids studied in carbon disulfide has been presented. A comparison of the absorption characteristics in the 9–10 μ region of these steroids was made with spectra published in the literature. It has been suggested tentatively that steroid structures containing a C_3-α-hydroxyl group *cis* to a C_5-hydrogen give rise to an absorption band near 10 μ. This is in contrast to the band near 9.6 μ arising from any of the other three possible configurations involving the C_3- and C_5-centers. Steroids containing a Δ^5-double bond with a 3β-hydroxyl group at C_3 appeared to give rise to an appreciable band near 9.4–9.5 μ.

Many infrared spectroscopic studies have attempted to relate particular absorption bands to specific structural arrangements.[2–7] An extensive catalog of steroid spectra has permitted Jones and co-workers to characterize carbonyl groups and ethylene double bonds.[7,8] A band near 10.3 μ has been utilized by Jones[9] for detecting the pres-

(1) The investigations described in this paper were aided by a grant from the U. S. Public Health (C-321) Service and supported in part by contract No. DA-49-007-MD-184 Medical Research and Development Board, Office of the Surgeon, Department of the Army.

(2) R. F. Furchgott, H. Rosenkrantz and E. Shorr, *J. Biol. Chem.*, **171**, 523 (1947).

(3) R. N. Jones and K. Dobriner, in R. S. Harris and K. V. Thimann's "Vitamins and Hormones," Vol. 7, 1949, p. 293.

(4) M. L. Josien, N. Fuson and A. S. Cary, THIS JOURNAL, **73**, 4445 (1951).

(5) H. Rosenkrantz, A. T. Milhorat and M. Farber, *J. Biol. Chem.*, **195**, 503 (1952).

(6) H. Rosenkrantz, A. T. Milhorat and M. Farber, *ibid.*, **195**, 509 (1952).

(7) R. N. Jones, V. Z. Williams, M. J. Whalen and K. Dobriner, THIS JOURNAL, **70**, 2024 (1948).

(8) R. N. Jones, P. Humphries, E. Packard and K. Dobriner, *ibid.*, **72**, 86 (1950).

(9) R. N. Jones, *ibid.*, **72**, 5322 (1950).

ence of a Δ^{22}-double bond in ergostenyl compounds while Bladon, *et al.*,[10] has reported the effects of substitution on ethylene centers in sterols. More recently Hirschmann[11] has utilized bands near 12 μ for differentiating trisubstituted steroidal olefins. Earlier investigations by Furchgott, Rosenkrantz and Shorr[12] attempted to relate absorption bands between 9–10 μ to *cis/trans* configurations in rings A and B. A more definite relationship was established by Jones, Humphries, Herling and Dobriner[13] when the region near 8 μ in the spectra of 3-acetates were compared. A new type of steroid has been examined by Josien, Fuson and Cary[4] who have made a comparison of normal and *i*-steroids (3,5-cyclosteroids). Hydroxy and acetoxy epimers at C_2, C_3 and C_4, respectively, have been studied by Fürst, *et al.*,[14] in the cholestane series.

In the present paper emphasis has been placed on steroids which contain only one substitutent on the nucleus. It was reasoned that since these molecules are relatively simple they might reveal some basic relationships of the infrared absorption spectrum to structural configurations. It was hoped that such relationships would aid in the structural analysis of unknown steroids.

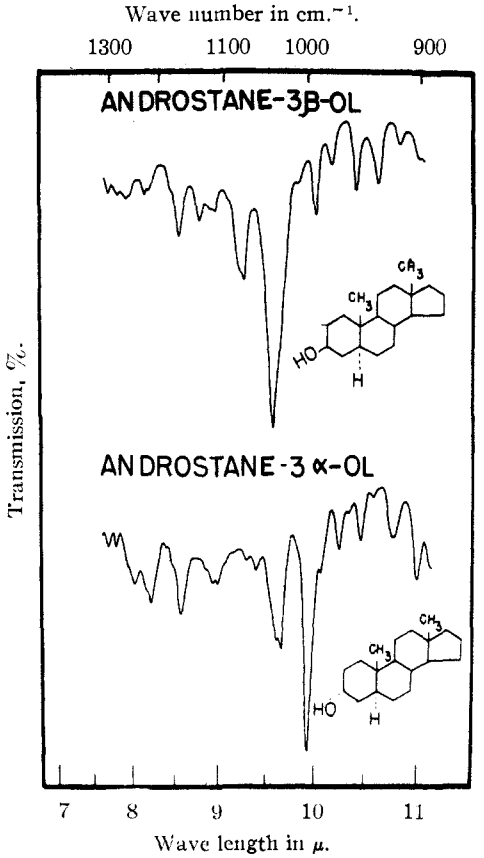


Fig. 1.—Infrared absorption curves of androstane-3β-ol and androstane-3α-ol in carbon disulfide solution.

Method.—The spectra were recorded on a Perkin–Elmer 12C infrared spectrometer between 2.5 to 13 μ (4000–770 cm.$^{-1}$). The parts of the spectra shown in Figs. 1–4 are tracings of the actual recordings, the curves being aligned at 3.4 μ (2940 cm.$^{-1}$). All compounds were studied in carbon disulfide solution, the fingerprint region being of prominent concern. A one-mm. cell was used, concentration being approximately 7 mg. per ml. Spectra of androstane,[15] Δ^5-androstene-3β-ol[15] and $\Delta^{2(\text{or }3)}$-androstene-17-one[3] have been published previously but their interpretation will be included in the present discussion. The following additional compounds were studied: androstane-3β-ol, androstane-3α-ol, androstane-17β-ol, androstane-3-one, androstane-17-one, Δ^4-androstene-3-one, androstane-17β-ol-3-one,[16] epi-testosterone, Δ^{16}-androstene-3α-ol, $\Delta^{4,16}$-androstadiene-3-one and Δ^{16}-androstene-3α-ol, 3-acetate.[17]

Epiandrosterone, androsterone, androstane-3α-ol-11, 17-dione, Δ^9-androstene-3α-ol-17-one, androstane-3α, 11β-diol-17-one, etiocholane-3α-ol-11, 17-dione and Δ^9-etiocholene-3α-ol-17-one also were studied in carbon disulfide solution in the present investigation. Since these spectra were identical with those published by Jones and Dobriner,[3] reference is made to the latter authors for discussion of these compounds. The published spectra of other steroids have been consulted frequently in order to interpret between 9–10 μ (1100–1000 cm.$^{-1}$) in as large a number of spectra as possible. In a number of instances spectra were used which were obtained on samples in the solid state. Only cholestane,[6] ergostane[5] and androstanediol[12] derivatives were studied as such. Rosenkrantz and Zablow[15] recently have demonstrated the extent of comparison of steroid absorption spectra obtained on compounds in different physical states.

Analysis of Spectra.—Absorption bands arising from specific interatomic vibrations will be discussed first.

O–H Groups.—Only those steroids containing a hydroxyl group gave an absorption band near 2.72 μ (3679 cm.$^{-1}$) in carbon disulfide solution. It has been well established that this band shifts toward longer wave lengths in mineral oil mulls and crystalline films because of hydrogen bonding.[4,12,15] This phenomenon also can occur in relatively concentrated solutions and this was the case for epitestosterone whose hydroxyl vibrations occurred near 2.9 μ (3445 cm.$^{-1}$).

C–H Groups.—All compounds contained an intense band near 3.4 μ (2940 cm.$^{-1}$) which arose from the linear C–H vibrations of the different carbon–hydrogen groups. In those substances containing an isolated ethylenic linkage, the band related to the olefinic C–H vibrations in this region was resolved only in the case of the three Δ^{16}-steroids.

C=O Groups.—Five steroids in this series contained single carbonyl groups. Androstane-3-one and androstane-17β-ol-3-one gave their absorption bands near the expected 5.82 μ (1721 cm.$^{-1}$) wave length.[7] The spectra of the conjugated C_3-keto compounds, Δ^4-androstene-3-one, epitestosterone

(10) P. Bladon, J. M. Fabian, H. B. Henbest, H. P. Koch and G. W. Wood, *J. Chem. Soc.*, 2402 (1951).

(11) H. Hirschmann, *Federation Proc.*, **11**, 230 (1952).

(12) R. F. Furchgott, H. Rosenkrantz and E. Shorr, *J. Biol. Chem.*, **163**, 375 (1946).

(13) R. N. Jones, P. Humphries, F. Herling and K. Dobriner, THIS JOURNAL, **73**, 3215 (1951).

(14) A. Fürst, H. H. Kuhn, R. Scotoni, Jr., and Hs. H. Gunthard, *Helv. Chim. Acta*, **35**, 951 (1952).

(15) H. Rosenkrantz and L. Zablow, submitted to *Anal. Chem.*

(16) This compound was studied again because of some misunderstanding in nomenclature. Its spectrum was identified with that of etioallocholanol-17-one-3 which appeared in ref. 6. Since in the present work it was studied in carbon disulfide solution, its spectrum is included here.

(17) We gratefully acknowledge the coöperation of the following in donating crystalline samples of steroids: The Ciba Pharmaceutical Company, Summit, N. J., for androstane-3β-ol, androstane-3α-ol, androstane-3-one, androstane-17-one, Δ^4-androstene-3-one, androstane-17β-ol and androstane-17β-ol-3-one; Dr. Ralph I. Dorfman, Worcester Foundation for Experimental Biology, Shrewsbury, Mass., for Δ^{16}-androstene-3α-ol and its acetate; and to Dr. V. Prelog, Laboratorium für organische Chemie, Zürich, Switzerland, for $\Delta^{4,16}$-androstadiene-3-one.

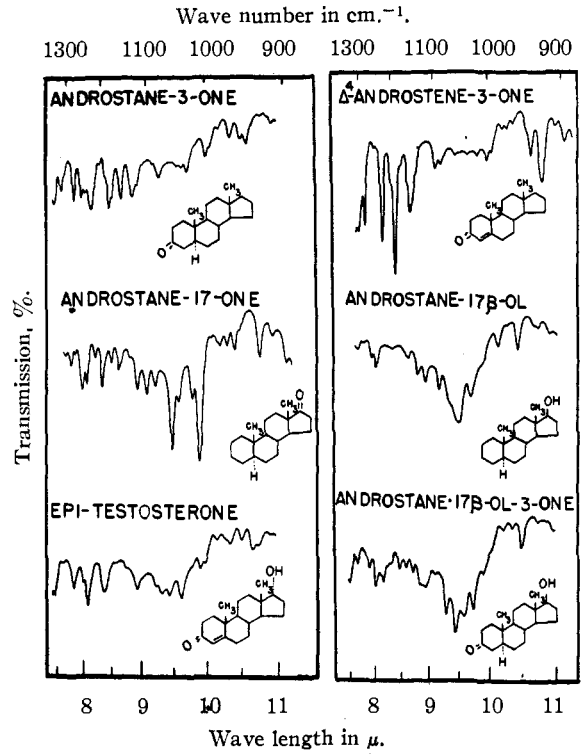


Fig. 2.—Infrared absorption curves of androstane-3-one, androstane-17-one and epi-testosterone in carbon disulfide solution.

Fig. 3.—Infrared absorption curves of Δ^4-androstene-3-one, androstane-17β-ol and androstane-17β-ol-3-one in carbon disulfide solution.

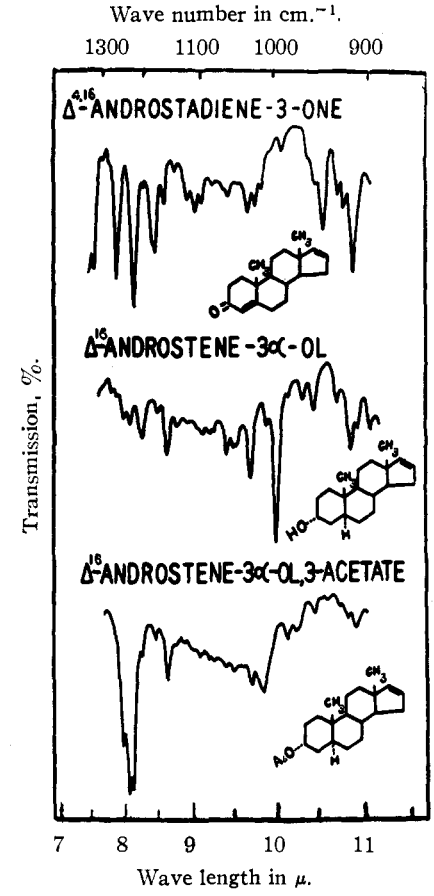


Fig. 4.—Infrared absorption curves of Δ^{16}-androstene-3α-ol, $\Delta^{4,16}$-androstadiene-3-one and Δ^{16}-androstene-3α-ol, 3-acetate in carbon disulfide solution.

and $\Delta^{4,16}$-androstadiene-3-one, showed the usual shift of the carbonyl group to longer wave lengths (5.98 μ or 1670 cm.$^{-1}$). Androstane-17-one gave a carbonyl band near 5.73 μ (1745 cm.$^{-1}$) the wave length for C_{17}-keto groups.[7]

C=C Groups.—Ethylenic groups occurred in five of the steroids, four being non-conjugated (Δ^5-androstene-3β-ol and the Δ^{16}-compounds). Conjugated double bonds are present in Δ^4-androstene-3-one, $\Delta^{4,16}$-androstadiene-3-one and epitestosterone. In all cases solvent interference obliterated the band that may arise from such linkages.[8]

C–O Linkages.—Inspection of spectra of three basic nuclei, androstane,[15] cholestane[6] and ergostane,[5] disclosed a few bands that were of weak intensity between 9 to 10 μ. This was generally true of steroid compounds containing carbonyl groups (excluding ester keto groupings) and no hydroxyl substituents.[3] The spectra of androstane-3-one and Δ^4-androstene-3-one (Figs. 2–3) confirmed this observation but on the other hand the spectra of androstane-17-one (Fig. 2) and Δ^2(or ^{3})-androstene-17-one[3] were contrary. Both the latter substances had a significant absorption near 9.47 μ (1057 cm.$^{-1}$). In those cases where hydroxyl-lacking steroids had notable absorption in the 9–10 μ region, relationships to structure were not apparent.[3] However, compounds which have a hydroxyl group at C_3 revealed some structure–absorption relationships.

It can be seen in Table I that the spectra of steroids with a C_3-α-hydroxyl group *cis* to a C_5-hydrogen contained an intense infrared near 10 μ. This band was observed in the basic structures androstane-3α-ol (Fig. 1), Δ^{16}-androstene-3α-ol (Fig. 4) allopregnane-3α-ol-20-one[3] and epicholestanol.[6] Originally it was pointed out for androstane-3α,-17β-diol[18] by Furchgott, Rosenkrantz and Shorr.[12] Substitution by another hydroxyl group (allopregnane-3α,6α-diol-20-one[3] did not influence this band.

Introduction of a carbonyl group at C_{17} in the androstane compounds did not cause the disappearance of the 10 μ band but an additional intense band was observed near 9.75 μ (1025 cm.$^{-1}$). The addition of a second carbonyl group (androstane-3α-ol-11,17-dione[3]) resulted in a shift of the 9.75 μ band to shorter wave lengths. Acetylation of androsterone and Δ^{16}-androstene-3α-ol (Fig. 4) caused the disappearance of the 10 μ band and an intense absorption occurred near 9.8 μ (1021 cm.$^{-1}$).

Examination of the 9 to 10 μ region in the spectra of compounds with a C_3-α-hydroxyl group *trans* to a C_5-hydrogen is summarized in Table II. Although etiocholane-3α-ol was not available, the other

(18) According to the third edition of Fieser and Fieser's, "Natural Products Related to Phenanthrene," Reinhold Publ. Corp., New York, N. Y., 1949, the orientation of C_{17}-hydroxyl groups have been reversed. Two steroids studied earlier by one of the present authors (H. R.)[12] were designated as androstanediol-3α,17α and androstanediol-3β,17α, respectively. Herewithin they are referred to as androstane-3α,17β-diol and androstane-3β,17β-diol, respectively.

TABLE I

INFRARED ABSORPTION RELATIONSHIPS TO STEROIDS WITH A C_3-α-HYDROXYL GROUP *cis* TO A C_5-HYDROGEN

Steroid[a]	Wave length, μ		Wave number, cm.$^{-1}$	
Androstane-3α-ol	9.95		1006	
Δ^{16}-Androstene-3α-ol	9.99		1001	
Epicholestanol	9.96		1003	
Allopregnane-3α-ol-20-one	9.99		1001	
Androstane-3α,17β-diol	9.90		1010	
Allopregnane-3α,6α-diol-20-one	9.94		1007	
Androsterone	10.02	9.74	998	1027
Δ^{9}-Androstene-3α-ol-17-one	10.03	9.76	996	1023
Androstane-3α,11β-diol-17-one	10.02	9.73	998	1028
Androstane-3α-ol-11,17-dione	10.04	9.76	996	1023
Androsterone acetate	9.86		1013	
Δ^{16}-Androstene-3α-ol,3-acetate	9.81		1020	
$\Delta^{11(?)}$-Androstene-3α-ol-17-one, 3-acetate	9.78		1022	

[a] Absorption curves can be found in references[3,6] and [12] for those spectra which are not recorded in the present paper.

structurally related steroids, epicoprostanol[6] and pregnane-3α-ol-20-one,[3] were reported to give rise to maxima near 9.65 μ (1037 cm.$^{-1}$). Neither addition of a hydroxyl group (etiocholane-3α,11β-diol-17-one nor carbonyl groups (etiocholane-3α-ol-11,17-dione)[3] resulted in significant alterations of this band. Four acetates in this group[3] gave a band near 9.8 μ similar to that seen for the α/*cis*-acetates.

TABLE II

INFRARED ABSORPTION RELATIONSHIPS TO STEROIDS WITH A C_3-α-HYDROXYL GROUP *trans* TO A C_5-HYDROGEN

Steroid[a]	Wave length, μ	Wave number, cm.$^{-1}$
Epicoprostanol	9.62	1040
Pregnane-3α-ol-20-one	9.63	1039
Etiocholane-3α-ol-17-one	9.65	1037
Δ^{9}-Etiocholene-3α-ol-17-one	9.63	1039
17-Isopregnane-3α-ol-20-one	9.63	1039
Etiocholane-3α,11β-diol-17-one	9.68	1032
Pregnane-3α,17α-diol-20-one	9.69	1031
Pregnane-3α,6α-diol-20-one	9.64	1038
Etiocholane-3α-ol-11,17-dione	9.63	1039
Pregnane-3α-ol-11,20-dione	9.64	1038
Etiocholane-3α-ol-17-one-3-acetate	9.72	1029
Etiocholane-3α,17β-diol-11-one-3,17-diacetate	9.73	1028
Pregnane-3α,20α-diol-11-one-3,20-diacetate	9.74	1027
Pregnane-3α,17α-diol-20-one-3-acetate[b]	9.75	1026

[a] Absorption curves can be found in references [3] and [6] for those spectra which are not recorded in the present paper. [b] Unpublished spectrum.

A number of steroids whose infrared absorption spectra have been published contain a C_3-β-hydroxyl group *trans* to a C_5-hydrogen. Table III lists a prominent band occurring between 9 and 10 μ in the spectra of these compounds. The relationship here was similar to that of the C_3-α-hydroxyl *trans* to a C_5-hydrogen. Each of the mother compounds androstane-3β-ol (Fig. 1) cholestanol-3β,[6] ergostanol-3β,[5] and allopregnane-3β-ol-20-one[3] gave rise to a band between 9.56 (1046 cm.-1) and 9.61 μ (1040 cm.$^{-1}$). There was no opportunity to observe the effects of carbonyl substitution but the addition of another hydroxyl group (androstane-3β,17β-diol[18])[12] failed to influence the band near 9.6 μ. Acetylation resulted in the appearance of an absorption band near 9.7 μ (1031 cm.$^{-1}$). It would appear from Table II and III that β/*trans* and α/*trans* configuration behave very similarly. Apparently, the presence of a double bond in ring C (*e.g.*, α-ergostenol) which does not eliminate the C_5-hydrogen, had little effect on the location of the 9.6 μ band.

TABLE III

INFRARED ABSORPTION RELATIONSHIPS TO STEROIDS WITH A C_3-β-HYDROXYL GROUP *trans* TO A C_5-HYDROGEN

Steroid[a]	Wave length, μ	Wave number, cm.$^{-1}$
Androstane-3β-ol	9.59	1042
Cholestanol-3β	9.61	1041
Allopregnane-3β-ol-20-one	9.62	1040
Ergostanol-3β	9.56	1047
α-Ergostenol	9.64	1038
β-Ergostenol	9.64	1038
γ-Ergostenol	9.56	1047
α-Dihydroergosterol	9.62	1040
γ-Dihydroergosterol	9.59	1042
α-Spinasterol	9.63	1039
Androstane-3β,17β-diol	9.61	1041
Epiandrosterone	9.62	1040
Ergostanyl acetate	9.69	1031
α-Ergostenyl acetate	9.73	1028
γ-Ergostenyl acetate	9.75	1026

[a] Absorption curves may be found in references[3], [5], [6] and [12] for those spectra which are not recorded in the present paper.

Only two steroids (etiocholane-3β-ol-17-one[3] and coprostanol)[6] in the final possible steric orientation, a C_3-β-hydroxyl group *cis* to a C_5-hydrogen, have been studied. Obviously no pattern of similarity could be observed in the spectra of just two compounds.

Furchgott, Rosenkrantz and Shorr[12] have postulated a relation between a band near 9.45 μ (1058 cm.$^{-1}$) and a steroid structure containing a C_3-β-hydroxyl group with a double bond between C_5–C_6. This suggestion was seen to apply to steroids in the cholestane and ergostane series,[5,6] the band near 9.5 μ (1052 cm.$^{-1}$) in the spectrum of Δ^5-androstene-3β-ol[15] also conforming to this interpretation. It would appear that replacement of the C_5-hydrogen by a Δ^5-double bond in steroids of the 3β-type results in prominent absorption between 9.45 to 9.5 μ. On the other hand steroids with a C_5-hydrogen and a C_3-β-hydroxyl group absorb nearer 9.6 μ. Although androstane-17β-ol was included in the present study, there were not a sufficient number of spectra of comparable structures which would permit specific band assignment. The spectrum of androstane-17β-ol (Fig. 3) contained an absorption band of marked intensity near 9.5 μ. In the spectrum of androstane-17β-ol-3-one (Fig. 3) the band was closer to 9.45 μ.

Other Absorption Bands.—Insofar as those relatively non-complex steroids are concerned, four bands seemed to occur with reasonable consistency

in their spectra. These absorptions occurred between 7.8–7.9 (1288-1266 cm.$^{-1}$), 8.1–8.2 (1236-1220 cm.$^{-1}$), 8.87–8.97 (1128-1116 cm.$^{-1}$) and 10.4–10.5 μ (962-953 cm.$^{-1}$). The presence of a similar foursome was not observed in the published spectra of other steroids. Therefore these four bands cannot be utilized for identifying a substance as being a steroid. Many of these bands are reflections of the combined vibrations of the molecule (and thus of structural complexity). They may not appear consistently as one goes from relatively simple to more complex molecules.

Conclusions

At the moment the use of the 9–10 μ region for prediction of steric arrangements is not clearly established. The evaluation of spectra originating from structures containing hydroxyl groups at neighboring positions to C_3 is not complete. Fürst, *et al.*,[14] have demonstrated that cholestanol-2α gave rise to an intense doublet near 9.7 μ (1031 cm.$^{-1}$) while a weaker band occurred near 10 μ. The spectrum of cholestanol-2β had one band near 9.85 μ (1015 cm.$^{-1}$). Cholestanol-4α caused absorption near 9.6 μ and cholestanol-4β near 10 μ.

Since too many assumptions must be made in order to apply the 9–10 μ relationships to unknown steroid structures, it is best at present to postpone serious interpretation until additional pertinent compounds are investigated.[19] The assignment of an α/cis orientation would appear to be more selective than the other three possible arrangements.

We wish to express our sincerest gratitude to Dr. R. N. Jones for his suggestions.

(19) After this work was completed, A. R. H. Cole, R. N. Jones and K. Dobriner, THIS JOURNAL, **74**, 5571 (1952), confirmed the essential features of the present investigation.

SHREWSBURY, MASS.

[CONTRIBUTION FROM THE DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY AND THE DEPARTMENT OF MEDICINE, SCHOOL OF MEDICINE, UNIVERSITY OF CALIFORNIA AT LOS ANGELES]

Photoöxidation of Crystalline Estrogens in the Presence of Flavins[1]

BY JOSEPH F. NYC, HARRY B. FRIEDGOOD, JOSEPHINE B. GARST AND DOROTHY M. MARON

RECEIVED FEBRUARY 16, 1952

Spectrophotometric data have been presented as evidence of the fact that estrone, estradiol and estriol in alcoholic solution are destroyed at a rapid rate when exposed to light in the presence of riboflavin or lumichrome. This destruction of the three naturally occurring estrogens is inhibited by the addition of manganous chloride in a molar concentration of 10^{-2}. Experimental evidence indicates that the foregoing photoöxidation action causes the formation from estrone of several products. Zimmermann determinations indicate that the 17-ketone group of estrone is not affected during the photoöxidative process. Photoöxidation of estrone in the presence of riboflavin results in a large loss of its biological activity as determined by a modification of the Doisy vaginal smear method.

It is known that in the presence of visible light, riboflavin, lumichrome and various synthetic dyes such as eosine, methylol riboflavins and fluorescein initiate the oxidation of histidine, methionine, compounds containing an indole ring and a variety of proteins and enzymes.[2,3]

These observations suggested the desirability of investigating the photoöxidative destruction of estrogens by flavins for the purpose of determining whether or not it is a factor which can affect significantly the accuracy of current quantitative methods for the isolation and subsequent analysis of these steroids.

Part I. The Effect of Riboflavin on Estrogens in the Presence of Visible Light.—Five-tenths of a milligram of estrone, estradiol and estriol were dissolved, respectively, in 10 ml. of 95% ethanol.[4] Each tube of ethanol contained 80 micrograms of freshly dissolved riboflavin which had been protected from light. These solutions were exposed to visible light[5] for 90 hours. During this exposure ultraviolet spectral curves were determined at timed intervals on these solutions and also on a simultaneously irradiated riboflavin control solution which contained only the flavin at the same concentration used in the estrogen irradiation tubes. The results on estrone, corrected by subtracting the corresponding values for the flavin control, are recorded in Fig. 1.

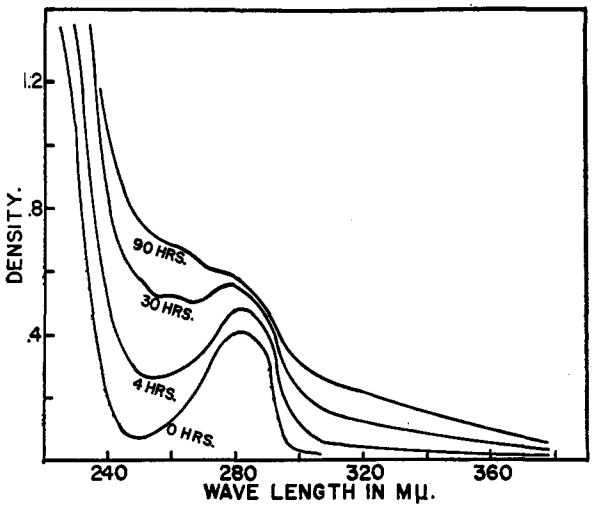


Fig. 1.—Change in ultraviolet spectrum of estrone following exposure to visible light at the time intervals noted. The data were obtained by subtracting the spectral curves of irradiated riboflavin controls from the additive spectra of the solutions containing both estrogen and riboflavin. The control and experimental solutions were irradiated simultaneously: density = log (I_0/I_x).

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(2) A. W. Galston, *Proc. Nat. Acad. Sci.*, **35**, 10 (1939).

(3) (a) A. W. Galston and R. S. Baker, *Science*, **109**, 485 (1949); (b) **111**, 619 (1950).

(4) All solvents were redistilled prior to use.

(5) All of the solutions in these experiments were exposed to about 100 foot candles of light from "daylight" fluorescent bulbs.