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Absorption Spectra of Steroids in Concentrated Sulfuric Acid. III. Structural Correlations. Analysis of the 300–600 Mμ Region[1]

SEYMOUR BERNSTEIN AND ROBERT H. LENHARD

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Certain structural correlations for the spectra of steroids in concentrated sulfuric acid are proposed in the 300–600 mμ region. Generally, hydroxylated steroids will exhibit selective absorption in the 300–600 mμ region. An associated hydroxyl absorption region, 220–278 mμ, has been previously established.[1] 3α- or 3β-Monohydroxylated steroids will probably possess a maximum in the 300–350 mμ region. Some evidence is presented for the selective absorption of the corticoid dihydroxyacetone side chain between 450–549 mμ, especially in the region 474–490 mμ. Selective absorption in the 300–399 mμ region may also be attributed to the ketone function, especially in the region 300–325 mμ. An associated ketone absorption region, 220–278 mμ, has been previously established.[1] Steroids which contain isolated double bonds may be correlated with selective absorption in the 400–449 mμ region.

In the previous paper of this series,[1] certain structural correlations for the spectra of steroids in concentrated sulfuric acid were proposed in the 220–300 mμ region. It is our purpose here to suggest the possibility of additional structural correlations, especially in the 300–600 mμ region of the spectrum. As previously, these observations are based on the analysis of the spectra of 177 compounds.[2]

For convenience, the 300–600 mμ region has been divided into five regions: *A*, 300–399 mμ; *B*, 400–549 mμ (three subdivisions); and *C*, 550–600 mμ. The number of compounds which exhibit selective absorption in these regions are listed in Table I. It will be noted that no compound in our catalog exhibited selective absorption in the 550–600 mμ region.[3] The discussion which follows will be according to the above designated regions.

A. *300–399 Mμ Region.*[4] The following considerations support the proposal that selective absorption in the 300–399 mμ region may be attributed primarily to the ketone and hydroxyl groups. In this connection, let us first examine the spectra of saturated and unsaturated ketosteroids which do *not* contain either an α,β-unsaturated ketone or a hydroxyl group. These compounds may be arbitrarily classified as "non-conjugated" ketones. In Table II are listed 11 such compounds with

TABLE I

NUMBER OF COMPOUNDS WITH MAXIMUM[a] IN REGION 300–600 Mμ

	Region, Mμ (inclusive)	No. of Compounds with Maximum[a,b]
A	300–399	151
B	400–549	211
	400–449	117
	450–499	85
	500–549	9
C	550–600	0

[a] *i.e.*, maximum, plateau, or inflection. [b] A large number of compounds exhibited two or more maxima over the region 300–600 mμ, and were counted accordingly more than once.

TABLE II

ABSORPTION MAXIMA OF NONCONJUGATED KETONES

Compound (No.)	λ_{max} Mμ ($E_{1\ cm.}^{1\%}$)	
Androstane-3,11,17-trione (11)		none [321(18)][a]
Etiocholane-3,11,17-trione (15)		none
Androstane-3,17-dione (18)		302 (595)
Etiocholane-3,17-dione (21)		302 (540)
Δ^7-Allopregnene-3,20-dione (55)	289(132) (I),[b]	322 (209)
Pregnane-3,20-dione (79)		237 (159) (I)
Δ^7-Cholestene-3-one (163)	240 (86),	317 (189)
Cholestane-3-one (165)		312 (152)
Coprostane-3-one (167)	235(91)(I),	316 (146), 477(12)
$\Delta^{7,22}$-Ergostadiene-3-one (172)	238(103),	312 (272)
Ergostane-3-one (177)	240(46),	313 (125)

[a] For all practical purposes, this compound is considered as showing no selective absorption. [b] The symbol, *I* designates an inflection or plateau.

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(1) Paper II, S. Bernstein and R. H. Lenhard, *J. Org. Chem.*, **19**, 1269 (1954).

(2) This number does not include derivatives such as acetates, when the parent free steroid was available. Also, estrogens, steroid alkaloids, and ethylene ketals are not included.

(3) It is not inferred that there are no steroids which show selective absorption in this region. Recently, L. L. Smith and W. H. Muller, *J. Org. Chem.*, **23**, 960 (1958), have observed a λ_{max} 568 mμ ($E_{1\ cm}^{1\%}$ 132) for $\Delta^{1,4}$-pregnadiene-9β,11β-epoxy-16α,17α,21-triol-3,20-dione 16,21-diacetate.

(4) It has already been indicated that α,β-unsaturated ketosteroids exhibit selective absorption between 279–302 mμ (inclusive). Thus, there is in this case a minor overlapping in certain aspects of the structural correlations. It should also be borne in mind that the following chromophores exhibit selective absorption in this region, *i.e.*, 6β-hydroxy-Δ^4-3-ketone ($\cong\Delta^{4,6}$-3-ketone) at about 343–346 mμ (based on three examples); 3β-hydroxy-Δ^5-7-ketone ($\cong\Delta^{3,5}$-7-ketone) at about 355 mμ (based on one example); Δ^4-3,6-diketone at about 348 mμ (based on one example); and, $\Delta^{1,4}$-3-ketone at about 327 mμ (based on one example). In regard to the last chromophore, L. L. Smith and W. H. Muller, *loc. cit.*, have suggested that the combination of selective absorption in the 247–267 mμ region and in the 295–318 mμ region is characteristic of the $\Delta^{1,4}$-3-ketone moiety in the corticoid series.

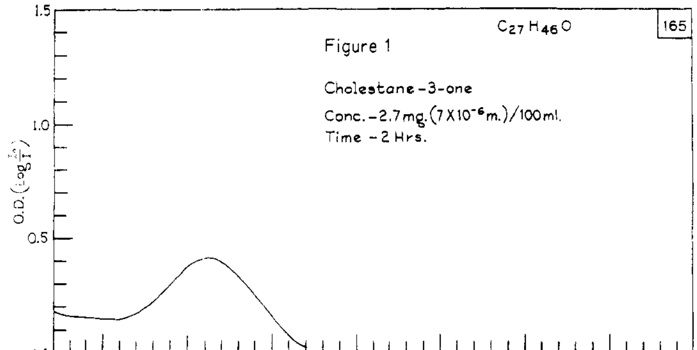

Figure 1

Cholestane-3-one
Conc.-2.7mg.(7X10^{-6}m.)/100ml.
Time -2 Hrs.

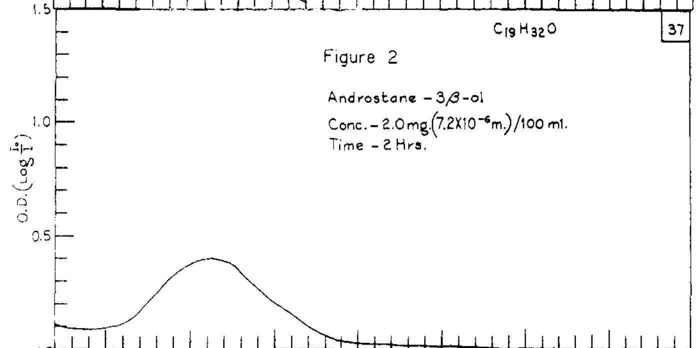

Figure 2

Androstane-3β-ol
Conc.-2.0mg.(7.2X10^{-6}m.)/100 ml.
Time -2 Hrs.

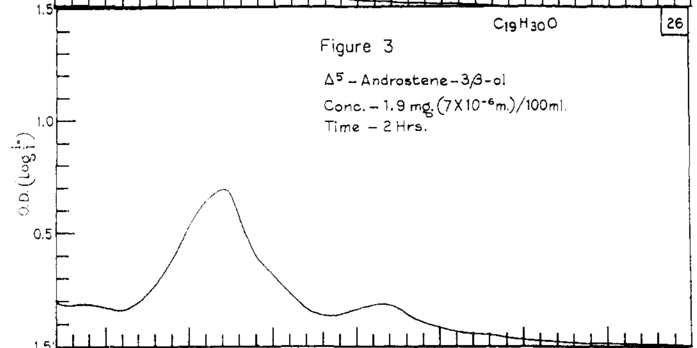

Figure 3

Δ^5-Androstene-3β-ol
Conc.-1.9 mg.(7X10^{-6}m.)/100ml.
Time -2 Hrs.

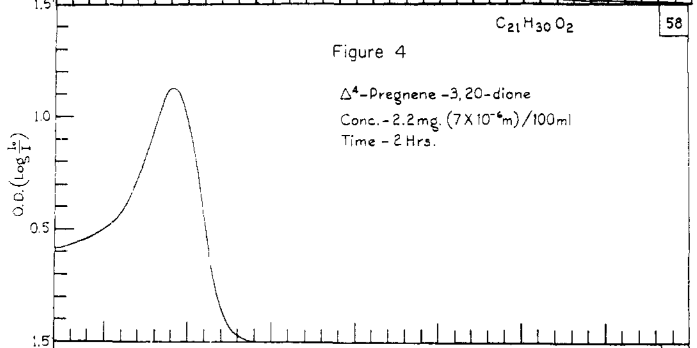

Figure 4

Δ^4-Pregnene-3,20-dione
Conc.-2.2mg.(7X10^{-6}m)/100ml
Time -2 Hrs.

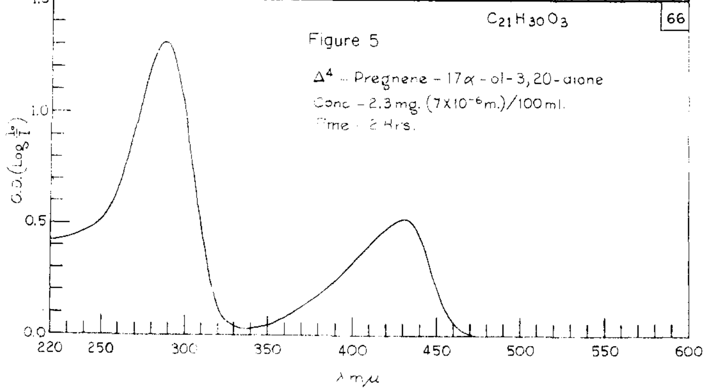

Figure 5

Δ^4-Pregnene-17α-ol-3,20-dione
Conc.-2.3mg.(7X10^{-6}m.)/100ml.
Time -2 Hrs.

their respective maxima and extinction coefficients.[5] Eight of these compounds exhibit a sizeable absorption maximum between 302–322 mμ (inclusive) ($E^{1\%}_{1\,cm}$ 125–595). Thus, the portion of the spectrum between 300–325 mμ generally represents an additional ketone absorption region to the one already observed, *i.e.*, between 230–278 mμ.[1]

In Fig. 1 is presented the spectrogram of cholestane-3-one (No. 165)[6] which possesses a single maximum at 312 mμ ($E^{1\%}_{1\,cm}$ 152). This illustrates the above correlation in a decisive manner.

The three exceptions to this correlation merit further discussion. Pregnane-3,20-dione (No. 79) exhibits no maximum but only an inflection at 237 mμ, which, however, falls in the lower ketone absorption region. Androstane-3,11,17-trione (No. 11), and its 5β-epimer, etiocholane-3,11,17-trione (No. 15) show essentially no selective absorption, which is in striking contrast to their corresponding 11-deoxo-compounds, androstane-3,17-dione (No. 18), and etiocholane-3,17-dione (No. 21). Thus, the 11-carbonyl group has exerted in some manner (transmissional effect?) a very pronounced influence on the absorption of the 3,17-diketone chromophore. This surprising result is of interest in view of the finding already noted[1] that an 11-ketone group produces an hypsochromic effect on the Δ^4-3-ketone chromophore.

In Table II it may be observed that if the nonconjugated ketone showed selective absorption in both of the designated ketone regions, the band at the longer wave length invariably possessed the higher extinction coefficient. Therefore, it is believed that the region at approximately 300–325 mμ most likely represents the principal ketone absorption band.[7]

In Table III, there are presented the results of an examination of the absorption bands of 146 compounds which contain hydroxyl groups. The compounds are tabulated according to the number of hydroxyl groups contained in each. Also by each listing there is given the number of combinations or different positions occupied by these hydroxyl groups. Of the 146 hydroxyl-containing steroids 136 (total score of 93%) exhibited selective absorption in the 300–399 mμ region. Although in our catalog of compounds there are 161 compounds

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(5) Throughout for the purpose of structure correlations, maximum, plateau, and inflection are considered equivalent.

(6) The compound numbers throughout correspond to those employed in Paper I, S. Bernstein and R. H. Lenhard, *J. Org. Chem.*, **18**, 1146 (1953).

(7) In conventional absorption spectroscopy, a nonconjugated ketone will exhibit selective absorption in the 170–200 mμ and 280–300 mμ regions [L. Dorfman, *Chem. Rev.*, **53**, 47 (1953); and, A. Gillam and E. S. Stern, *An Introduction to Electronic Absorption Spectroscopy in Organic Chemistry*, pages 47–51, Edward Arnold (Publishers) Ltd., London, 1954]. Thus, one also finds in sulfuric acid spectroscopy two ketone absorption regions which apparently have resulted by reason of a bathochromic effect of the sulfuric acid solvent.

which display a band in this region, the overall score of 136/161 or 84% offers a high degree of assurance for the correlation of selective absorption for a hydroxylated steroid in this region.

TABLE III

ABSORPTION MAXIMA OF HYDROXYLATED STEROIDS

Type Compound	No. of Combinations	No. of Compounds Positive/ Total No.	Range
Monoöls	8	90/97	302–399 mμ
Diols	18	32/33	300–391 mμ
Triols	11	11/13	300–398 mμ
Tetrols	2	2/2	330–332 mμ
Pentols	1	1/1	360–378 mμ
		Total score: 136/146 (93%)	300–399 mμ
		Overall score: 136/161 (84%)	

In Table IV 97 monohydroxylated steroids are tabulated according to the location and configuration of the hydroxyl group. A *tentative* conclusion may be stated that most 3α- or 3β-monohydroxylated steroids will show selective absorption in the 300–350-mμ region. This correlation is based on the fact that 63 out of 75 such steroids absorb selectively within this range. Ten C3-monohydroxylated steroids do not conform to this correlation; however, these compounds show selective absorption between 351–399 mμ.

TABLE IV

Monoöls	No. Cpds. "Positive"/ Total No.	Range
3α	15/15	11 307–350 mμ 4 351–389 mμ
3β	58/60	52 304–350 mμ 6 351–399 mμ
6β	1/1	344 mμ
11α	3/3	318–381 mμ
11β	2/2	318–380 mμ
17α	1/4	302 mμ
17β	8/10	302–395 mμ
21	2/2	354–372 mμ
Total Score	90/97 (93%)	302–399 mμ

In regard to steroids monohydroxylated at other positions, no such correlation may be stated (or attempted) due to the insufficient number of examples available.

In Fig. 2 is given the spectrogram of androstane-3β-ol (No. 37), with its absorption maximum at 312 mμ, illustrative of the structural correlation of hydroxylated steroids showing selective absorption in the 300–399 mμ region.

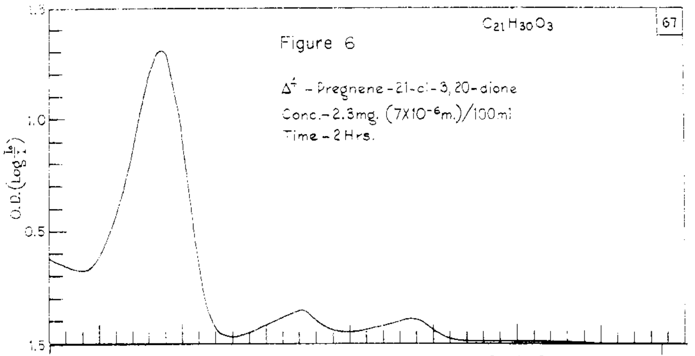

Figure 6

Δ^4 – Pregnene-21-ol-3,20-dione
Conc.–2.3mg. (7×10^{-6}m.)/100 ml
Time – 2 Hrs.

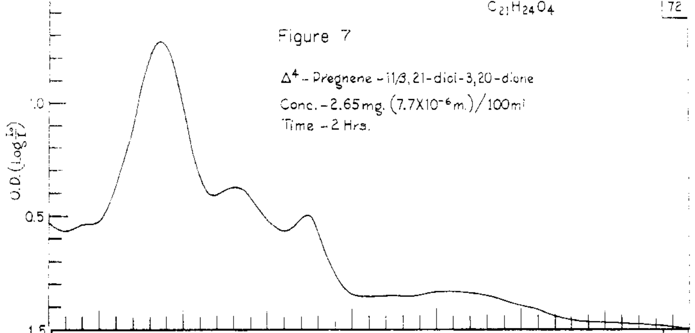

Figure 7

Δ^4 – Pregnene-11β,21-diol-3,20-dione
Conc.–2.65mg. (7.7×10^{-6}m.)/100ml
Time – 2 Hrs.

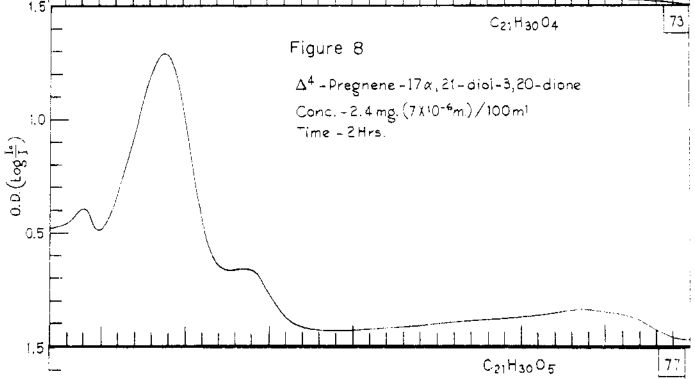

Figure 8

Δ^4 – Pregnene-17α,21-diol-3,20-dione
Conc.–2.4 mg. (7×10^{-6}m.)/100ml
Time – 2 Hrs.

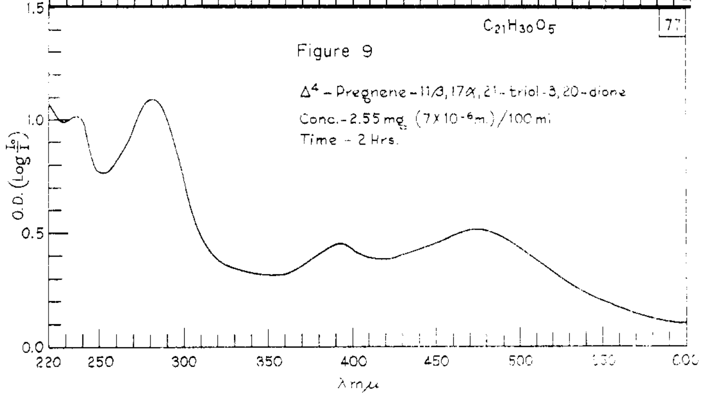

Figure 9

Δ^4 – Pregnene-11β,17α,21-triol-3,20-dione
Conc.–2.55 mg. (7×10^{-6}m.)/100 ml
Time – 2 Hrs.

B. *400-549 mμ region.* Our catalog contains 67 compounds which contain one to four double bonds and which are not conjugated with a ketone function. In Table V these compounds are tabulated according to the degree of unsaturation. Fifty-nine (88%) of these compounds show selective absorption in the 400–449 mμ region. Thus, it appears that unsaturated (non-conjugated) steroids generally will have selective absorption in this region. However, this correlation must be used with considerable caution as all-told our catalog contains

125 steroids which show absorption in this region. Of this number, 56 contain a hydroxyl function, and three (not really exceptions) contain a Δ^4-3-ketone group with Δ^7- or $\Delta^{7,9(11)}$-double bonds present.

TABLE V

ABSORPTION MAXIMA OF UNSATURATED STEROIDS

Unsaturated Cpds.	No. Cpds. "Positive"/Total No.	Range
Monoenes	35/40	402–449 mμ
Dienes	17/18	405–446 mμ
Trienes	6/8	400–438 mμ
Tetraenes	1/1	433 mμ
	Total Score: 59/67 (88%)	400–449 mμ
	Overall Score: 59/125 (47%)	

The selective absorption of double bonds and hydroxyl groups in this area leads one to believe that both types of compounds in concentrated sulfuric acid give rise to a common species, such as an alkyl hydrogen sulfate.[8] In Fig. 3 is presented the spectrogram of Δ^5-androstene-3β-ol (No. 26) with maxima at 236, 321, and 416 mμ. The 236 and 321 mμ bands may be ascribed to the hydroxyl group, and the 416 mμ band to the Δ^5-double bond (with or without the hydroxyl group?).

In Figs. 4–9 there are presented the spectrograms of Δ^4-pregnene-3,20-dione (No. 58), Δ^4-pregnene-17α-ol-3,20-dione (No. 66), Δ^4-pregnene-21-ol-3,20-dione (No. 67), Δ^4-pregnene-11α,21-diol-3,20-dione (No. 72), Δ^4-pregnene-17α,21-diol-3,20-dione (No. 73), and Δ^4-pregnene-11β,17α,21-triol-3,20-dione (No. 77). With increasing number of hydroxyl functions, the spectrograms become increasingly more complicated in the 300–549 mμ region with a definite trend toward increasing absorption at the higher wave lengths. It appears then that the 400–549 mμ region may also be associated with the hydroxyl function. Moreover, generally hydroxylated steroids will display selective absorption in the 300–549 mμ region. Also it should be noted that absorption in the region 220–278 mμ has been ascribed to the hydroxyl function.[1]

In our catalog there are seven steroids (Nos. 66, 70, 82, 88, 89, 90, and 91) with the 17α-hydroxy-17-acetyl side chain. Also there are three steroids (Nos. 67, 71, and 72) which contain a C17-ketol ($COCH_2OH$) side-chain. All 10 compounds show selective absorption in the 400–499 mμ region (see Figs. 5 and 6).

Also, in our catalog there are twelve steroids (Nos. 54, 73, 74, 75, 76, 77, 103, 113, 122, 141, and 147) which contain the dihydroxyacetone side-chain at C17. It was of interest to examine whether this important grouping displayed some characteristic absorption properties. All twelve show selective absorption (maximum or inflection, $E_{1\,cm}^{1\%}$ 11-204) between 474–535 mμ. Of these twelve compounds eleven (one exception, No. 147, pregnane-3α,-11β,17α,21-tetrol-20-one-3,21-diacetate, λ_{max} 506 mμ) show selective absorption between 474–490 mμ ($E_{1\,cm}^{1\%}$ 11-204).[9] It was also interesting to observe that eight out of twelve of the above compounds also show selective absorption in the 338–343 mμ region. ($E_{1\,cm}^{1\%}$ 93-450), and nine out of twelve show selective absorption in the 233–271 mμ region ($E_{1\,cm}^{1\%}$ 109-420).

Acknowledgment. We wish to acknowledge the invaluable assistance of Mr. Walter Muller who determined all the spectra. Also we wish to thank Mr. Walter Hearn for the reproductions of the spectrograms.

PEARL RIVER, N. Y.

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(8) R. J. Gillespie and J. A. Leisten, *Quart. Rev.*, **8**, 40 (1954).

(9) L. L. Smith and W. H. Muller, *loc. cit.*, have recorded the spectra (at 15 min., 2 hr., and 20 hr.) of 26 1-dehydrocorticosteroids (with a dihydroxyacetone side-chain) in concd. sulfuric acid. Of this number 18 contain in addition to the $\Delta^{1,4}$-3-ketone function a 16α-hydroxy or acetoxy group. Of the 8 corticosteroids which do *not* contain a 16-oxygenated function, $^3/_8$ (15 min.); $^5/_8$ (2 hr.) and $^7/_8$ (20 hr.) show selective absorption in the 450–549 mμ region. Of the 18 corticosteroids which contain a 16-oxygenated function $^1/_{18}$ (15 min.); $^2/_{18}$ (2 hr.) and $^8/_{18}$ (20 hr.) show selective absorption in the 450–549 mμ region.