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THE ABSORPTION SPECTRA OF STEROIDS IN CONCENTRATED SULFURIC ACID. II. STRUCTURAL CORRELATIONS. ANALYSIS OF THE 220–300 mμ REGION[1, 2]

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In the first paper of this series (*loc. cit.*), there was tabulated quantitative data ($E_{1\,cm}^{1\%}$ for λ_{max} and λ_{min}) for the absorption spectra of a large number of steroids in 97% sulfuric acid in the range 220–600 mμ. The compounds studied represented a wide variety of structural features.

One of our objectives in undertaking this investigation was to examine and establish, if possible, certain structural correlations.[3] In this paper, the discussion will be confined principally to the 220–300 mμ region with special emphasis on observations relevant to the selective absorption of the α,β-unsaturated ketone moiety.

The structural correlations to be presented are based on the analysis of 177 compounds. This number does not include derivatives such as acetates when the parent free steroid was available.[4] Moreover "estrogens", steroid alkaloids, and ketals are not included.

Ninety-two compounds exhibited selective absorption (*i.e.*, maximum, plateau or inflection) in the 220–278 mμ (inclusive) region, with the large majority between 230–249 mμ (Table I). These compounds were examined according to structural groups (singly and in combination), and the results are given in Table II. This analysis revealed that if a compound exhibited absorption in this region it almost invariably contained an hydroxyl group. All the exceptions (9 compounds) contained an isolated ketone group. Thus, it appears that absorption in this region may be ascribed primarily to the hydroxyl group, and, to a lesser extent, to the isolated ketone group. The spectra of androstane-17β-ol (No. 38),[5] and ergostane-3-one (No. 177) illustrate this correlation. Eighty-five (57%) out of 147 compounds possessing an hydroxyl group showed selective absorption in the 220–278 mμ region.

Generally, an α,β-unsaturated ketosteroid will exhibit a pronounced maximum

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[1] Presented in part before the Organic Discussion Group at the Fifth Annual Meeting In Miniature of the North Jersey Section, American Chemical Society, Newark, N. J., January 26, 1953.

[2] Paper I, Bernstein and Lenhard, *J. Org. Chem.*, **18**, 1146 (1953).

[3] In this connection the reader is referred to the recent publication of A. Zaffaroni, *Recent Progress in Hormone Research, The Proceedings of the Laurentian Hormone Conference*, Volume VIII, p. 51, Academic Press Inc., Publishers, New York, N. Y., 1953. See also, L. R. Axelrod, *J. Biol. Chem.*, **205**, 173 (1953).

[4] For all practical purposes, free steroids and their acetates may be considered "equivalent" for the purpose of structural analysis. However, this does not imply that their absorption spectra are always identical in all respects. This point will be discussed in detail in a future publication.

[5] The compound numbers throughout correspond to those employed in Paper I (*loc. cit.*).

TABLE I

No. of Compounds with Maximum[a] in Region 220–278 mμ

Region, mμ (Inclusive)	No. of Compounds with Maximum[a, b]
220–229	4
230–239	54
240–249	33
250–259	3
260–269	1
270–278	6

[a] I.e., maximum, plateau or inflection.

[b] Compounds No. 74, 100, 113, and 170 exhibited two or more maxima over the region 220–278 mμ, and were accordingly counted more than once.

TABLE II

Structural Analysis of 220–278 mμ Region

Structural Group(s) in Compound	No. of Compounds
Hydroxyl	85
Hydroxyl + isolated ketone	31
Isolated ketone	37
α,β-Unsaturated ketone	10
α,β-Unsaturated ketone + hydroxyl	10
α,β-Unsaturated ketone + hydroxyl + isolated ketone	7
α,β-Unsaturated ketone + isolated ketone	7
$\Delta^{1,4}$-Unsaturated ketone + hydroxyl	1
Double bond (other than conjugated with ketone)	49
Double bond + hydroxyl	46
Double bond + hydroxyl + isolated ketone	4
Double bond + isolated ketone	7

between 279–302 mμ (inclusive). This correlation may be supported by the following considerations.

One of the simplest α,β-unsaturated ketosteroids is Δ^4-androstene-3-one (No. 16), whose spectrum in concentrated sulfuric acid is shown in Figure I. This compound exhibits a single maximum at 296 mμ which may be ascribed to the Δ^4-3-ketone moiety. All told, 49 α,β-unsaturated ketosteroids have been examined, and these represent five structural types, *e.g.*,

(a) *Δ^1-3-Ketone:* Δ^1-allopregnene-17α,21-diol-3,11,20-trione 21-acetate (No. 103); λ_{max} 279 mμ[6] (Figure II);

(b) *Δ^4-3-Ketone:* Δ^4-androstene-3-one (*vide supra*); also, Δ^4-pregnene-17α,21-diol-3,11,20-trione 21-acetate (cortisone acetate) (No. 104); λ_{max} 283 mμ (Figure II);

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[6] This and other compounds also exhibit maxima outside of the "α,β-unsaturated ketone" region. Unless pertinent to the discussion, these maxima will be omitted.

Fig. I

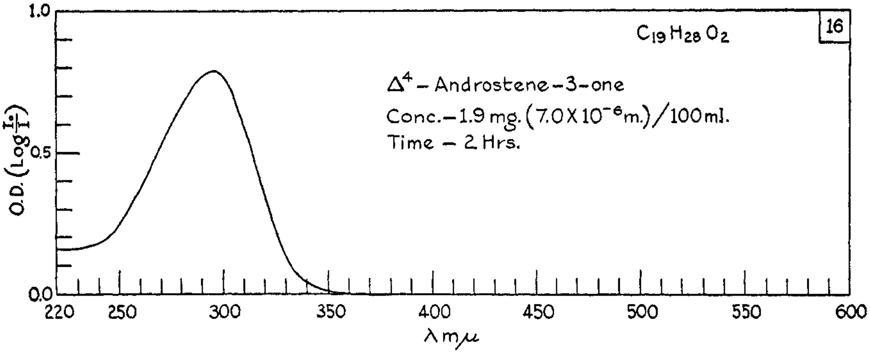


Fig. II

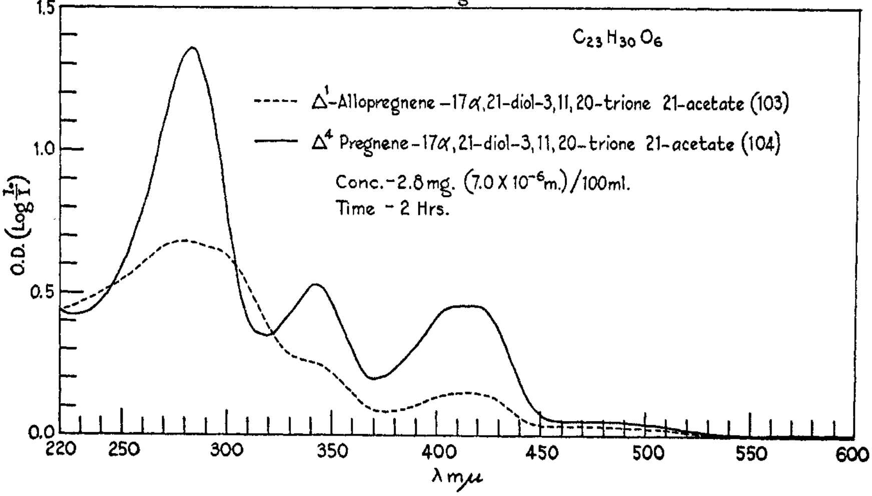


Fig. III

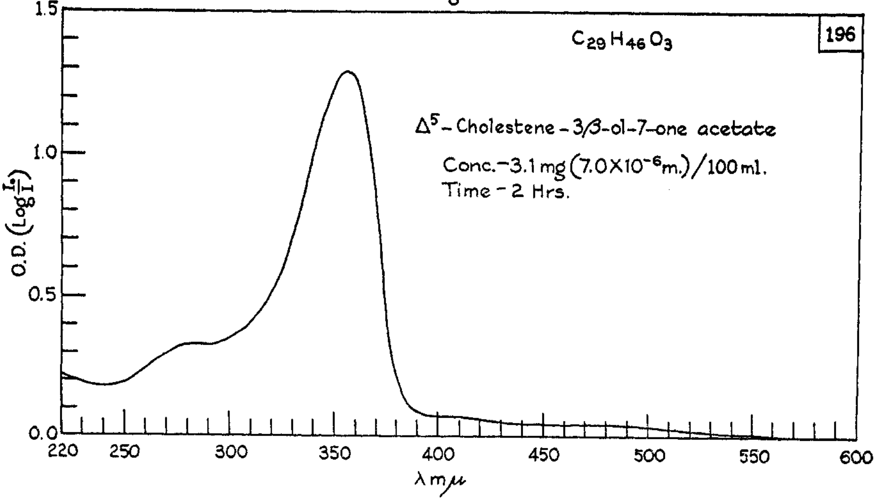


Fig. IV

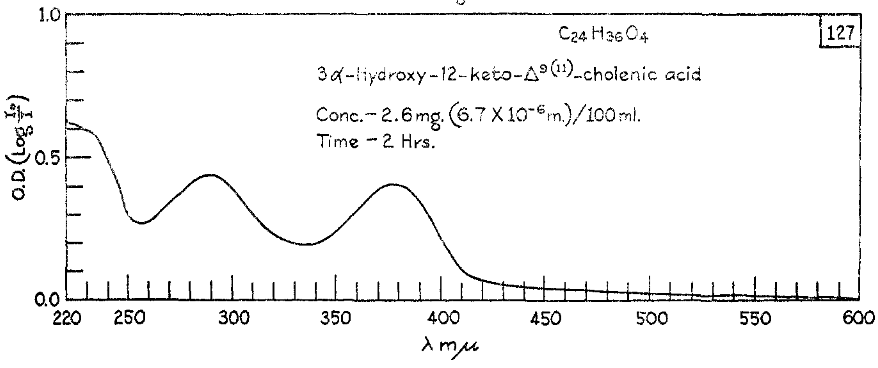
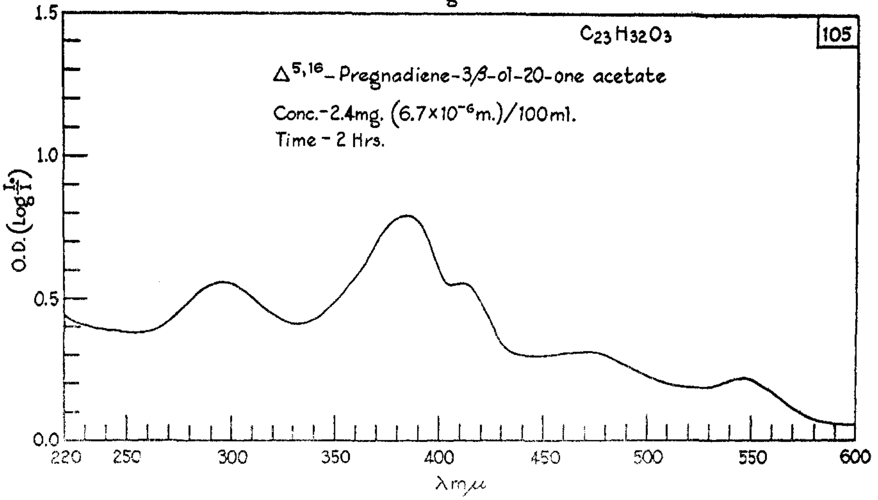


Fig. V



(c) Δ^5-*7-Ketone:* Δ^5-cholestene-3β-ol-7-one acetate (No. 196); λ_{max} 285 mμ[7] (Figure III);

(d) $\Delta^{9(11)}$-*12-Ketone:* 3α-hydroxy-12-keto-$\Delta^{9(11)}$-cholenic acid (No. 127); λ_{max} 290 mμ (Figure IV);

and (e) Δ^{16}-*20-Ketone:* $\Delta^{5,16}$-pregnadiene-3β-ol-20-one acetate (No. 105); λ_{max} 296 mμ ($E_{1\,cm}^{1\%}$ 233) (Figure V).[8]

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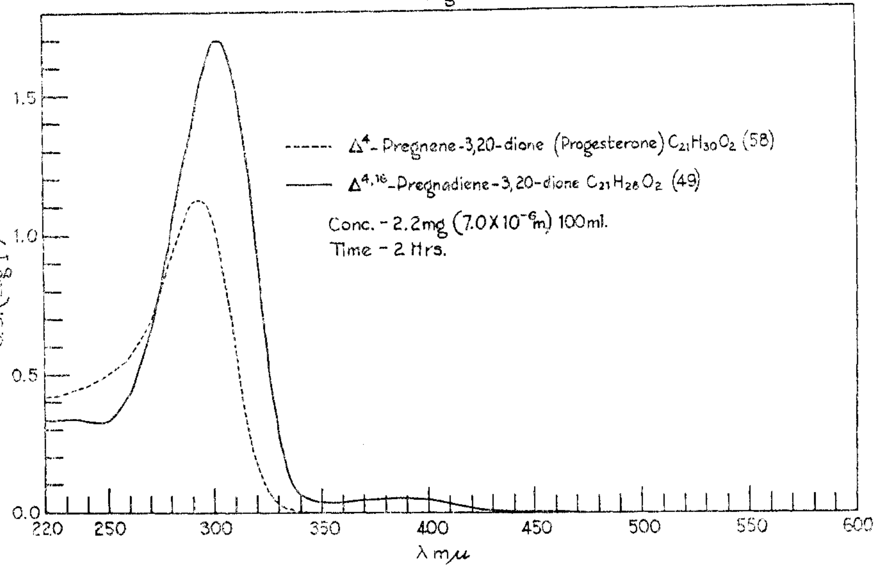
[7] The principal maximum of this compound is at 355 mμ, ascribable to the $\Delta^{3,5}$-7-ketone moiety, which would result from the removal of the C_3 hydroxyl group, see also footnote 11.

[8] This spectrum is to be compared with that of progesterone (No. 58) (λ_{max} 291 mμ, $E_{1cm}^{1\%}$ 504), and with that of $\Delta^{4,16}$-pregnadiene-3,20-dione (No. 49) (λ_{max} 302 mμ, $E_{1cm}^{1\%}$ 773) (Figure VI). The magnitude of the extinction coefficient of the latter indicates a more or less additivity of the extinction coefficients of the two isolated α,β-unsaturated ketone chromophores. In this connection, the spectra of jervine (No. 157) (λ_{max} 282 mμ, $E_{1cm}^{1\%}$ 203), and Δ^4-jervone (No. 153) (λ_{max} 281 mμ, $E_{1cm}^{1\%}$ 471) show conformity with this conclusion. All data are for "2 hour" spectra.

Fig. VI



In Table III are listed the number of compounds which comprise the above five structural types with their respective "α,β-unsaturated ketone" maxima. All 49 of the compounds exhibited a maximum between 279–302 mμ (inclusive). However, if one excludes the "atypical" $\Delta^{4,16}$-pregnadiene-3,20-dione (No. 49) (two chromophores), the region is shortened by 2 mμ to 279–300 mμ which is decidedly more representative of the α,β-unsaturated ketone absorption region, and more useful for structural interpretation work. Thus, if a steroid (benzenoid steroids excluded) in concentrated sulfuric acid exhibits selective absorption between 279–300 mμ, in all probability, it contains an α,β-unsaturated ketone.

On the other hand, there have been examined 129 compounds which do not contain an α,β-unsaturated ketone, and of this group 13 (10%) showed a maximum (maximum, plateau or inflection) between 279–300 mμ. These exceptions are listed in Table IV.

Summarily, in Table V are listed a number of Δ^4-3-ketosteroids which exhibit *only one* maximum. Certain of the compounds may be differentiated from each other not only by the wavelength of the absorption maximum but also by its extinction coefficient. Attention is directed to the observation that testosterone (No. 20) may be differentiated from its C_{17}-α-epimer (No. 19) by extinction coefficient. The pairs of compounds, No. 7,10 and 52,58, are of particular interest in that the hypsochromic effect of a C_{11}-carbonyl group on the Δ^4-3-ketone chromophore is decisively demonstrated.[9] Generally, an 11-keto-Δ^4-3-ketosteroid

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[9] The hypsochromic effect of an 11-carbonyl substituent parallels that observed in conventional U.V. spectroscopy (alcohol as solvent); Antonucci, Bernstein, Heller, Lenhard, Littell, and Williams, *J. Org. Chem.*, **18**, 70 (1953); Dorfman, *Chem. Revs.*, **53**, 47 (1953).

TABLE III

ABSORPTION MAXIMA OF α,β-UNSATURATED KETONES

α,β-Unsaturated Ketone	No. Cpds "Positive"/Tot. No.	Region mμ
(1) Δ^{1}-3-Ketone	2/2	279–299
(2) Δ^{4}-3-Ketone	42/42	281–300
(3) Δ^{5}-7-Ketone	1/1	285
(4) $\Delta^{9(11)}$-12-Ketone	1/1	290
(5) Δ^{16}-20-Ketone	2/2	283–296
(2 and 5) $\Delta^{4,16}$-3,20-Diketone	1/1	302
Total score	49/49	279–302

TABLE IV

COMPOUNDS OTHER THAN α,β-UNSATURATED KETONES WITH ABSORPTION BETWEEN 279–300 mμ

Compound (No.)	λ_{max} mμ
$\Delta^{5,7,9(11)}$-Androstatriene-3β-ol-17-one (6)	291
Androstane-17β-ol-3,16-dione (22)	294
Δ^{7}-Allopregnene-3,20-dione (55)	289 (*I*)[a]
Pregnane-17α-ol-3,20-dione (82)	290
Pregnane-3α,11α,17α-triol-20-one (monohydrate) (90)	300
Pregnane-3α,11β,17α,20β,21-pentol (97)	290 (*I*)
3α,9α-Oxido-11-ketonorcholanic acid (119)	290
Pregnane-11β,17α,21-triol-3,20-dione 21-acetate (122)	291
22a-Allospirostane-3β-ol-11-one (160)	280
$\Delta^{7,9(11)}$-22a-Allospirostadiene-3β-ol acetate (187)	286
$\Delta^{5,7}$-22a-Spirostadiene-3β-ol acetate (188)	285
$\Delta^{3,5,7}$-Cholestatriene-3β-ol acetate (189)	291
$\Delta^{5,7,9(11)}$-Cholestatriene-3β-ol acetate (190)	279

[a] The symbol, *I*, designates an inflection or plateau.

TABLE V

Δ^{4}-3-KETOSTEROIDS WITH SINGLE ABSORPTION MAXIMUM

Compound (No.)	λ_{max} mμ $\left(E^{1\%}_{1\,\text{cm.}}\right)$
Δ^{4}-Androstene-3,11,17-trione (7)	283 (524)
Δ^{4}-Androstene-3,17-dione (10)	294 (730)
Δ^{4}-Androstene-3-one (16)	296 (416)
Δ^{4}-Androstene-17α-ol-3-one (19)	299 (724)
Δ^{4}-Androstene-17β-ol-3-one (20)	299 (837)
Δ^{4}-Pregnene-3,11,20-trione (52)	283 (491)
Δ^{4}-Pregnene-3,20-dione (58)	292 (513)
Methyl Δ^{4}-3-ketoetiocholenate (62)	292 (513)
Δ^{4}-Pregnene-3-one (78)	294 (409)
Δ^{4}-22a-Spirostene-3-one (158)	292 (397)
Δ^{4}-Cholestene-3-one (162)	292 (437)

TABLE VI

HYPSOCHROMIC EFFECT OF C_{11}-CARBONYL GROUP

Compound (No.)	λ_{max} mμ	$\Delta\lambda_{max}$ mμ
Δ^4-Androstene-3,17-dione (10)	294	
Δ^4-Androstene-3,11,17-trione (7)	283	11
Δ^4-Androstene-17β-ol-3-one (20)	298	
Δ^4-Androstene-17β-ol-3,11-dione (14)	285	13
Δ^4-Pregnene-3,20-dione (58)	291	
Δ^4-Pregnene-3,11,20-trione (52)	283	8
Δ^4-Pregnene-17α,21-diol-3,20-dione (73)	289	
Δ^4-Pregnene-17α,21-diol-3,11,20-trione (54)	283	6
Δ^4-Pregnene-21-ol-3,20-dione 21-acetate (109)	288	
Δ^4-Pregnene-21-ol-3,11,20-trione 21-acetate (102)	283	5

will exhibit an absorption maximum in the 279–300 mμ region which will be at a shorter wavelength (hypsochromic effect of 5–13 mμ) than the maximum of the parent Δ^4-3-ketosteroid unsubstituted in the C_{11}-position (see Table VI). The effect of a C_{11}-hydroxyl group (α or β-configuration) is variable, and no definite conclusions may be stated.

It is of some interest to examine the influence of a 6β-hydroxyl group on the absorption of the Δ^4-3-ketone moiety. In Table VII three such examples are given. The 6β-hydroxy-Δ^4-3-ketone moiety has two characteristic maxima, namely, at about 286–290 mμ, and 343–346 mμ. The latter has the higher extinction coefficient. The 286–290 mμ maxima are of lower extinction coefficients than those found with the parent 6-desoxycompounds. The appearance of the 343–346 mμ maximum may be conveniently understood by assuming the re-

TABLE VII

INFLUENCE OF 6β-HYDROXYL GROUP ON Δ^4-3-KETONE ABSORPTION

Compound (No.)	λ_{max} mμ $\left(E_{1\text{ cm.}}^{1\%}\right)$
Δ^4-Pregnene-3,20-dione (58)	291 (504)
Δ^4-Pregnene-6β-ol-3,20-dione (63)	290 (296), 344 (343), 465 (38)
Δ^4-Pregnene-11α-ol-3,20-dione (64)	290 (404), 318 (254) (I), 473 (38)
Δ^4-Pregnene-6β,11α-diol-3,20-dione (69)	288 (174), 346 (532), 470 (96)
Δ^4-Pregnene-17α,21-diol-3,20-dione (73)	240 (254), 288 (537), 338 (142), 486 (50) (I), 535 (67)
Δ^4-Pregnene-6β,17α,21-triol-3,20-dione (75)	237 (182) (I), 286 (208), 343 (450), 398 (65), 430 (76), 475 (84)

TABLE VIII

BATHOCHROMIC EFFECT OF KETAL GROUPING

Compound	Free Ketone		Bis-Ketal		$\Delta m\mu$
	(Cpd. No.)	λ_{max} $m\mu$	(Cpd. No.)	λ_{max} $m\mu$	
Δ^4-Androstene-3,11,17-trione	(7)	283	(111)	293	10
Δ^4-Pregnene-17α,21-diol-3,11,20-trione	(54)	283	(144)	292	9
Δ^4-Pregnene-11α,17α,21-triol-3,20-dione	(76)	282	(148)	291	9
Δ^4-Pregnene-11β,17α,21-triol-3,20-dione	(77)	282	(149)	291	9
Δ^4-Androstene-11α-ol-3,17-dione	(12)	284	(121)	296	12
Δ^4-Pregnene-3,20-dione	(58)	292	(145)	302	10
Δ^4-Pregnene-17α,21-diol-3,20-dione	(73)	289	(146)	295	6

moval of the C_6-hydroxyl group to give the equivalent species of a $\Delta^{4,6}$-3-ketone in conc'd sulfuric acid.[10, 11]

It is noteworthy that Δ^4-pregnene-6β-ol-3,20-dione (No. 63) may be easily differentiated from either Δ^4-pregnene-11α-ol-3,20-dione (No. 64) or Δ^4-pregnene-11β-ol-3,20-dione (No. 65).

Δ^4-Pregnene-3,6,20-trione (No. 51) shows a single maximum, namely at 348 mμ. We believe this selective absorption to be diagnostic for the Δ^4-3,6-diketone chromophore.

$\Delta^{1,4}$-Androstadiene-17β-ol-3-one (No. 8) shows a prominent maximum at 327 mμ which may be characteristic for the $\Delta^{1,4}$-3-ketone chromophore.

Finally, we have examined the effect of ethylene ketal formation on the absorption of the Δ^4-3-ketone chromophore (7 examples). The ketal grouping exerts a bathochromic effect of 6–12 mμ (Table VIII).

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

SUMMARY

Certain structural correlations for the spectra of steroids in concentrated sulfuric acid have been established in the 220–300 mμ region.

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[10] This remark does not necessarily imply that the spectrum of a 6β-hydroxy-Δ^4-3-keto-steroid will be identical in all respects with that of its corresponding $\Delta^{4,6}$-3-ketosteroid.

No information is available to us on the influence of a 6α-hydroxyl group but it may be conjectured that here also the two characteristic maxima will be present.

[11] It is in this respect, among others, that absorption analysis of steroids in concentrated sulfuric acid may find an important use; *i.e.*, the formation of a new chromophore *in situ*, which should offer valuable structural information. It appears that an hydroxyl group either in the α- or β-carbon position to an unsaturated ketone will give rise to a new chromophore; *e.g.*, 6β-hydroxy-Δ^4-3-ketone → $\Delta^{4,6}$-3-ketone, and 3β-hydroxy-Δ^5-7-ketone → $\Delta^{3,5}$-7-ketone.

Little information is available to us in regard to an hydroxyl group on an adjacent carbon (either α or β) to a non-conjugated ketone. However, it is interesting to observe that Δ^4-pregnene-17α-ol-3,20-dione (No. 66) does not give rise to a spectrum "equivalent" to that of $\Delta^{4,16}$-pregnadiene-3,20-dione (No. 49), whereas Δ^4-pregnene-16α-ol-3,20-dione does. Data on the latter compound will be presented later.

Selective absorption between 220–278 mμ may be ascribed primarily to the hydroxyl group, and to a lesser extent, to the isolated ketone group.

If a steroid (benzenoid steroids excluded) exhibits selective absorption between 279–300 mμ, in all probability, it contains an α,β-unsaturated ketone moiety. The absorption maxima of several related chromophores have also been indicated.

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