[CONTRIBUTION FROM THE CHEMICAL PROCESS IMPROVEMENT DEPARTMENT, LEDERLE LABORATORIES DIVISION, AMERICAN CYANAMID COMPANY]

Absorption Spectra of Some 1-Dehydro Corticosteroids in Concentrated Sulfuric Acid

LELAND L. SMITH AND WALTER H. MULLER

Received December 26, 1957

A correlation between selective absorption in concentrated sulfuric acid in the 247–267 mμ region and the $\Delta^{1,4}$-3-ketone functional group of several corticosteroids has been made. An associated band in the 295–318 mμ region is also found. The presence of absorption in both the 247–267 mμ and 295–318 mμ region suggests the $\Delta^{1,4}$-3-ketone feature of the corticosteroid molecules.

The use of absorption spectra in concentrated sulfuric acid for characterization and for estimation has become a powerful aid in the study of many classes of steroids. The initial work of Zaffaroni and co-workers[1–3] together with the extensive studies of Bernstein and Lenhard[4,5] provides a comprehensive catalog of selective absorption in concentrated sulfuric acid for several hundred steroids. Selective absorption of steroids in several strong acids has recently been reviewed by Linford.[6]

Certain structural features of the steroid molecule have been related to selective absorption in concentrated sulfuric acid: the α,β-unsaturated carbonyl feature having been correlated with absorption in the 279–300 mμ region,[5] and the isolated carbonyl and hydroxyl group with absorption in the 239–249 mμ region.[5] Some correlations between structure and selective absorption have been made in other strong acid systems.[7–10] The correlation of selective absorption in the 247–267 mμ region and the 295–318 mμ region with the $\Delta^{1,4}$-3-ketone system of the new 1-dehydro corticosteroids is the subject of this report.

None of the simple Δ^4-3-ketones included by Bernstein and Lenhard[4] absorbs in the 247–267 mμ region. Indeed, only three steroids of the 220 listed by Bernstein and Lenhard[4] absorb in the 250–259 mμ region, and these are pregnane and allopregnane derivatives. Only one compound was listed as absorbing in the 260–269 mμ region, 3β-acetoxy-5,7-pregnadien-20-one maleic anhydride adduct (max. 261 mμ). Cevine was also listed as having absorption at 253 mμ and at 263 mμ. Several steroidal sapogenins also have selective absorption in the region 247–267 mμ. under slightly different conditions (94% sulfuric acid). Desoxyhecogenin (268 mμ), hecogenone (269 mμ), sarsasapogenone (267 mμ), smilagenone (268 mμ), and yuccagenin (268 mμ) exhibit bands in this region.[8] In fuming sulfuric acid methoxydoisynolic acid absorbs at 265 mμ.[11] Thus of over 250 steroids described to date only about ten not having the $\Delta^{1,4}$-3-ketone feature absorb in the region of 247–267 mμ. Only one $\Delta^{1,4}$-3-ketone is cited by Bernstein and Lenhard, namely 17β-hydroxy-1,4-androstadiene-3-one (max. 327 mμ), but this steroid does not have selective absorption in the 247–267 mμ region.

An associated band in the region 295–318 mμ is found where the $\Delta^{1,4}$-3-ketone structure is present and selective absorption in the 247–267 mμ region is exhibited. This characteristic absorption, while occurring in most of the cases of $\Delta^{1,4}$-3-ketones cited here (except for No. 8), is not as convincing a correlation as is the case of the 247–267 mμ region correlation, for many steroids absorb in the 295–318 mμ region, and the region overlaps that assigned by Bernstein and Lenhard to the simple α,β-unsaturated ketones.[5] However, Bernstein and Lenhard list only three compounds which absorb in both ranges: veratramine (249 mμ inflection, 311 mμ max., etc.), 3β-acetoxy-5,7-pregnadiene-20-one maleic anhydride adduct (261 mμ inflection, 309 mμ maximum, etc.), and 3β-acetoxy-16α,17α-epoxy-5,7,9(11)-pregnatrien-20-one (259 mμ inflection, 309 mμ inflection). The combination of selective absorption in the 247–267 mμ region and in the 295–318 mμ region is thus suggested as characteristic of the $\Delta^{1,4}$-3-ketone system in the cortical steroid series. Recently maxima for several 1-dehydrocorticosteroids have been reported: 17α,21-dihydroxy-1,4-pregnadiene-

(1) A. Zaffaroni, *J. Am. Chem. Soc.*, **72**, 3828 (1950).
(2) G. Diaz, A. Zaffaroni, G. Rosenkranz, and C. Djerassi, *J. Org. Chem.*, **17**, 747 (1952).
(3) A. Zaffaroni, *Recent Progress in Hormone Research*, **8**, 51 (1953).
(4) S. Bernstein and R. Lenhard, *J. Org. Chem.*, **18**, 1146 (1953).
(5) S. Bernstein and R. Lenhard, *J. Org. Chem.*, **19**, 1269 (1954).
(6) J. H. Linford, *Can. J. Biochem. Physiol.*, **35**, 299 (1957).
(7) J. H. Linford and C. J. Fleming, *Can. J. Med. Sci.*, **31**, 182 (1953).
(8) H. A. Walens, A. Turner, Jr., and M. E. Wall, *Anal. Chem.*, **26**, 325 (1954).
(9) W. J. Nowaczynski and P. R. Steyermark, *Arch. Biochem. Biophys.*, **58**, 453 (1955).
(10) W. J. Nowaczynski and P. R. Steyermark, *Can. J. Biochem. Physiol.*, **34**, 592 (1956).
(11) L. R. Axelrod, *J. Am. Chem. Soc.*, **75**, 6301 (1953).

TABLE I

ABSORPTION SPECTRA IN CONCENTRATED SULFURIC ACID OF SOME STEROIDS

No.	Empirical Formula	Compound	λ_{Max}, Mμ ($E^{1\%}_{1\ cm.}$)[a] 15 Min.	2 Hr.	20 Hr.	λ_{Min}, Mμ ($E^{1\%}_{1\ cm.}$) 15 Min.	2 Hr.	20 Hr.
			C_{21} Steroids					
1	$C_{21}H_{26}O_4$	17α,21-Dihydroxy-1,4,9(11)-pregnatriene-3,20-dione	265(344) 280(256)I 308(190)I 359(259)	267(275)I 278(289) 358(480)	242(482)I 278(377) 285(364)I 354(311) 460(134)	235(292) 325(178)	260(257) 305(177)	262(358) 311(243) 430(128)
2	$C_{21}H_{26}O_5$	17α,21-Dihydroxy-1,4-pregnadiene-3,11,20-trione	264(419) 283(345)I 310(234)I 332–345(141)I 410–428(153)	265(428) 280(393)I 310(243)I 345(179) 410–428(158)	265(430) 280(400)I 347(192) 410(200)I 425(205)	220(142) 372(51)	220(173) 330(170) 371(77)	220(222) 330(176) 370(108)
3	$C_{21}H_{27}O_5F$	9α-Fluoro-11β,17α,21-trihydroxy-1,4-pregnadiene-3,20-dione	263(364) 308(223)I 375(115) 451(123)	262(341) 308(233)I 372(92)I 451(141)	263(359) 308(259)I 450(167)	228(241) 350(106) 400(89)	237(279) 400(85)	240(311) 385(97)
4	$C_{21}H_{27}O_6Cl$	9α-Chloro-11β,16α,-17α,21-tetrahydroxy-1,4-pregnadiene-3,20-dione	258(244) 262(242)I 315(105) 395(36)	258–262(250) 315(110) 381(65)	258(270)I 264(276) 312(118) 375(82)	220(59) 286(58) 350(13)	220(82) 289(73) 345(29)	220(123) 298(107) 345(49)
5	$C_{21}H_{27}O_6F$	9α-Fluoro-11β,16α,-17α,21-tetrahydroxy-1,4-pregnadiene-3,20-dione	260(334) 310(172) 390(42)	260(346) 310(176) 380(86)	263(386) 310(176) 375(94)	220(63) 287(128) 345(16)	218(71) 288(142) 342(34)	220(80) 300(166) 340(40)
6	$C_{21}H_{28}O_4$	17α,21-Dihydroxy-1,4-pregnadiene-3,20-dione	267(305) 276(286)I 305(239) 345(121)I 426(94)	234(271)I 267(376) 275(370)I 298–307(268)I 343(129)I 385(81)I 420(99) 520(62)	233(317)I 267–274(418) 285(370)I 308(304)I 342(169)I 385(103) 395–415(97)I 500(69)	225(202) 292(235) 373(57)	221(255) 370(66) 465(58)	221(306) 372(88) 460(66)
7	$C_{21}H_{28}O_5$	11β,17α,21-Trihydroxy-1,4-pregnadiene-3,20-dione	240(298)I 262(348) 303(204) 375(239)	240(312)I 265(263) 278(250) 359(353) 390(222)I 470(89)	240(424)I 270–278(300) 355(254) 390(195)I 470(132)	222(250) 289(210) 333(129)	310(183)	321(210)
8	$C_{21}H_{28}O_6$	11β,16α,17α,21-Tetrahydroxy-1,4-pregnadiene-3,20-dione	262(184) 288(124)I 358(256)	235(163)I 244(152)I 280(143) 359(404)	241(308)I 268(272)I 280(260)I 359(285) 500(85)I	227(107) 317(92)	258(133) 308(83)	310(157)
9	$C_{21}H_{29}O_5F$	9α-Fluoro-11β,17α,21-trihydroxy-4-pregnene-3,20-dione	283(478) 319–332(110)I 400(163)I 408(170) 503(68)	283(488) 318–333(138)I 370(139)I 400(167)I 408(174) 503(63)	283(493) 333(147)I 370(147)I 400(181)I 408(184) 502(55) 530(53)	231(158) 353(96) 450(35)	231(162) 352(117) 450(39)	230(173) 353(123) 450(42) 517(50)
10	$C_{21}H_{29}O_6F$	9α-Fluoro-11β,16α,-17α,21-tetrahydroxy-4-pregnene-3,20-dione	284(440) 390(57)	283(462) 380(78)	283(565) 375(78)	227(59) 330(10)	228(65) 325(16)	227(76) 325(25)
11	$C_{21}H_{30}O_5$	17α,21-Dihydroxy-pregnane-3,11,20-trione	340(158) 410–420(158)	232(142)I 250–270(101) 341(206) 410–420(158)	250–270(119) 340(186) 410–420(142) 475(37)I	373(50)	290(83) 370(78)	290(87) 370(87)
			C_{23} Steroids					
12	$C_{23}H_{28}O_4$	21-Acetoxy-4,9(11),16-pregnatriene-3,20-dione	288(438) 387(648)	288(419) 387(708)	288(404) 385(740)	240(155) 326(46)	240(148) 325(42)	239(129) 325(29)
13	$C_{23}H_{28}O_5$	21-Acetoxy-4,16-pregnadiene-3,11,20-trione	285(513) 380(19)	285(550) 380(37)	285(518) 364(125)	335(7)	335(19)	325(46)

TABLE I (*Continued*)

No.	Empirical Formula	Compound	λ_{Max}, Mμ ($E^{1\%}_{1\,cm.}$)[a] 15 Min.	2 Hr.	20 Hr.	λ_{Min}, Mμ ($E^{1\%}_{1\,cm.}$) 15 Min.	2 Hr.	20 Hr.
14	$C_{23}H_{29}O_6F$	21-Acetoxy-9α-fluoro-11β,17α-dihydroxy-1,4-pregnediene-3,20-dione	263(370) 308(228)I 380(141) 410(117)I 452(148)	263(342) 290(275)I 310(247)I 373(114)I 450(171)	264(345) 290(285)I 310(252)I 373(114)I 450(193)	235(268) 350(123) 400(110)	242(291) 390(104)	245(304) 383(107)
			C_{25} Steroids					
15	$C_{25}H_{29}O_8Cl$	16α,21-Diacetoxy-9α-chloro-17α-hydroxy-1,4-pregnadiene-3,11,20-trione	257(285) 311(98)	257(283) 311(98) 390(28)	258(295) 312(107) 390(96)	221(66) 280(59)	221(66) 280(61) 350(12)	220(84) 294(92) 345(30)
16	$C_{25}H_{29}O_8F$	16α,21-Diacetoxy-9α-fluoro-17α-hydroxy-1,4-pregnadiene-3,11,20-trione	258(354) 305(149)	258(354) 305(151) 385(41)	258(354) 307(158) 381(96)	220(74) 279(100)	220(74) 279(100) 345(22)	220(91) 279(144) 342(46)
17	$C_{25}H_{30}O_7$	16α,21-Diacetoxy-17α-hydroxy-1,4,9(11)-pregnatriene-3,20-dione	262(260) 308(115) 355(156)	250(152) 283(101)I 355(423) 520(57)	241(330)I 281(183)I 355(195) 515(196)	230(103) 283(90) 325(102)	238(149) 300(79) 460(52)	310(132) 430(129)
18	$C_{25}H_{30}O_8$	16α,21-Diacetoxy-9β,-11β-epoxy-17α-hydroxy-1,4-pregnadiene-3,20-dione	230(271)I 247–253(208)I 318(163) 415(84) 500(113)I 570(133)	220–230(305) 248–251(259)I 319(200) 405(104) 505(129)I 568(132)	232(339) 252(317)I 316(244) 400(149) 490(149)	290(129) 350(52) 435(81)	285(158) 350(81) 435(91)	221(333) 290(200) 345(105) 435(123)
19	$C_{25}H_{30}O_8$	16α,21-Diacetoxy-17α-hydroxy-1,4-pregnadiene-3,11,20-trione	259(310) 295(142)	259(310) 295(140) 385(27)	259(312) 310(137)I 380(137)	221(63) 281(135)	221(67) 281(138) 345(9)	221(104) 337(43)
20	$C_{25}H_{31}O_8Cl$	16α,21-Diacetoxy-9α-chloro-11β,17α-dihydroxy-1,4-pregnadiene-3,20-dione	258–261(280) 313(109)	258–261(280) 313(112) 382(32)	258–261(299) 313(130) 378(143)	220(66) 285(50)	220(73) 285(57) 345(17)	220(124) 289(100) 342(60)
21	$C_{25}H_{31}O_8F$	16α,21-Diacetoxy-9α-fluoro-11β,17α-dihydroxy-1,4-pregnadiene-3,20-dione	261(297) 308(136)	261(297) 308(136) 380(35)	260(302) 308(133) 375(187) 477(10)I	220(59) 283(88)	220(59) 283(88) 345(17)	217(82) 285(109) 335(50)
22	$C_{25}H_{31}O_8F$	16α,21-Diacetoxy-9α-fluoro-17α-hydroxy-4-pregnene-3,11,20-trione	280(338)	280(338) 390(36)	280(353) 382(90)	230(63)	230(63) 333(14)	230(63) 323(23)
23	$C_{25}H_{32}O_7$	16α,21-Diacetoxy-17α-hydroxy-4,9(11)-pregnadiene-3,20-dione	285(320)	285(313) 380(23)	250(132)I 284(292) 475(146)	233(46)	233(46) 340(16)	230(91) 345(34)
24	$C_{25}H_{32}O_8$	16α,21-Diacetoxy-9β,-11β-epoxy-17α-hydroxy-4-pregnene-3,20-dione	283(317)	282(331) 390(44)	282(350) 380(93) 483(146)	235(111)	239(137) 350(36)	240(173) 340(62) 410(84)
25	$C_{25}H_{32}O_8$	16α,21-Diacetoxy-17α-hydroxy-4-pregnene-3,11,20-trione	283(336)	283(338) 385(30)	281(345) 381(133)	231(57)	231(60) 333(13)	233(77) 322(25)
26	$C_{25}H_{32}O_8$	16α,21-Diacetoxy-11β,-17α-dihydroxy-1,4-pregnadiene-3,20-dione	263(261) 315(125) 338–355(25)	261(148) 353(296)	243(286)I 353(185) 520(164)	221(69) 285(100)	230(109) 300(77)	308(109) 430(108)
27	$C_{25}H_{33}O_8Br$	16α,21-Diacetoxy-9α-bromo-11β,17α-dihydroxy-4-pregnene-3,20-dione	289(277)	289(183)	235(151)I 288(172) 385(64)I	228(68)	239(62)	245(136) 345(47)
28	$C_{25}H_{33}O_8F$	16α,21-Diacetoxy-9α-fluoro-11β,17α-dihydroxy-4-pregnene-3,20-dione	283(373)	283(373) 380(32)	281(379) 377(163)	230(67)	230(67) 324(12)	233(79) 317(19)

[a] I denotes an inflection or plateau.

3,11,20-trione, 263 mμ, 340 mμ (shoulder), 420 mμ;[12] 11β,17α,21-trihydroxy-1,4-pregnadiene-3,20-dione, 267 mμ, 359 mμ;[12] 21-acetoxy-9α-fluoro-11β,17α - dihydroxy - 1,4 - pregnadiene - 3,20 - dione, 262.5 mμ, 310 mμ;[13] 16α,21-diacetoxy-9α-fluoro - 11β,17α - dihydroxy - 1,4 - pregnadiene - 3,20-dione, 261 mμ 308 mμ, 387 mμ.[14]

The time course of selective absorption is of particular value in qualitative identity of several steroids, the characteristic alteration of spectra with time being unique for the several compounds listed. In certain instances the characteristic absorption assigned to the $\Delta^{1,4}$-3-ketone system disappears rapidly, and for this reason measurements must be made at short times by the correlation of spectra and structure of use. The recognition of the $\Delta^{1,4}$-3-ketone system in steroids eluted from paper chromatograms is facilitated with this new correlation.

Comparison of absorption spectra in concentrated sulfuric acid with that in the "100%" phosphoric acid of Nowaczynski and Steyermark[9,10] is made in Table II. Similarities in the 260 mμ region are apparent. A correlation between absorption at 260 mμ in "100%" phosphoric acid and the $\Delta^{1,4}$-3-ketone system was suggested by Nowaczynski and Steyermark.[9,10] From their data there are ten steroids out of some 101 steroids listed that have selective absorption in the 260 mμ region and which do not bear the $\Delta^{1,4}$-3-ketone system; among these are both pregnane and allopregnane derivatives, but there is also one Δ^{4}-3-ketone (6β,17α,21-trihydroxy-4-pregnene-3,20-dione, max 260 mμ). The associated band at 295–318 mμ (concentrated sulfuric acid) is not found for all the $\Delta^{1,4}$-3-ketones in "100%" phosphoric acid. Also this region is correlated with 17-hydroxyl and/or 17-carbonyl groups by Nowaczynski and Steyermark.

TABLE II

COMPARISON OF SELECTIVE ABSORPTION OF $\Delta^{1,4}$-3 KETOSTEROIDS IN CONCENTRATED SULFURIC ACID AND IN "100%" PHOSPHORIC ACID

(I = inflection, plateau)

Compound	Conc. Sulfuric Acid, λ_{max} ($E^{1\%}_{1\ cm.}$)[a]	"100%" Phosphoric Acid, λ_{max} ($E^{1\%}_{1\ cm.}$)
16α,21-Diacetoxy-9α-fluoro-11β,-17α-dihydroxy-1,4-pregnadiene-3,20-dione	260 mμ (302) 308 mμ (133) 375 mμ (187) 475–480 mμ (10) I	260 mμ (299)[b] 290 mμ (213) I 310 mμ (205) I 375 mμ (144)
17α,21-Dihydroxy-1,4-pregnadiene-3,11,20-trione	265 mμ (430) 280 mμ (400) I 347 mμ (192) 410 mμ (200) I 425 mμ (225)	257 mμ (512)[b] 290 mμ (232) I 360 mμ (64) I
11β,17α,21-Trihydroxy-1,4-pregnadiene-3,20-dione	240 mμ (424) I[c] 270–278 mμ (300) 355 mμ (254) 390 mμ (195) I 470 mμ (132)	260 mμ (265)[b] 355 mμ (155) 460 mμ (94) I

[a] Sulfuric acid spectra at 20 hours; 20 hour spectra are thought to be most nearly comparable with the "100%" phosphoric acid spectra as described by Nowaczynski and Steyermark.[9] [b] Calculated from the optical density data for 25 μg./ml. solutions given by Nowaczynski and Steyermark.[10] [c] The data for 20 hours is lacking in absorption in the regions of interest. Both 15 minute and 2 hour data (Table I) show absorption in the 262–265 mμ region.

EXPERIMENTAL

Absorption spectra were determined on a Cary Recording Spectrophotometer Model 11S in essentially the same manner described by Bernstein and Lenhard.[4] Steroid concentration ranged between 25 μg./ml. and 35 μg./ml.; determinations were made at 22°. All steroids used were of high purity as evidenced by combinations of melting point, infrared spectra, ultraviolet spectra, and papergram mobility. The several $\Delta^{1,4}$-3-ketones exhibited absorption in the 6.1–6.25 μ region of the infrared characteristic of the $\Delta^{1,4}$ unsaturated ring A.

Acknowledgment. The capable assistance of Mrs. Irene Palestro is gratefully acknowledged. Our appreciation is extended to Dr. Seymour Bernstein, Mr. William Allen, and Mr. Robert Lenhard, Research Division, American Cyanamid Company, for the gift of several 1-dehydro steroids.

PEARL RIVER, N. Y.

(12) W. R. Slaunwhite and A. A. Sandberg, *J. Clin. Endocrinology & Metabolism*, **17**, 395 (1957).

(13) R. F. Hirschmann, R. Miller, R. E. Beyler, L. H. Sarett, and M. Tishler, *J. Am. Chem. Soc.*, **77**, 3166 (1955).

(14) S. Bernstein, R. Lenhard, and W. S. Allen, U. S. Patent No. **2,789,118**, April 16, 1957.