[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF SYNTEX, S. A.]

STEROIDAL SAPOGENINS. XXI.[1] IDENTIFICATION BY THE ABSORPTION SPECTRA OF THEIR SULFURIC ACID CHROMOGENS

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Received January 22, 1952

Recently (1) one of us reported on the absorption curves of the sulfuric acid chromogens of a number of corticosteroids which proved useful in the identification of trace amounts of such hormones after paper chromatographic separations (2). In connection with a study of paper chromatography of steroidal sapogenins and saponins under way in this laboratory, it was necessary to devise a method for the characterization of the various spots in paper chromatograms and the present article is concerned with the application of the sulfuric acid method (1) to steroidal sapogenins.

EXPERIMENTAL

After a number of trials it was found that the most stable and reproducible chromogen was produced by heating the sapogenin in conc'd sulfuric acid solution at 40° for one hour. For the determination of the absorption curves, approximately 5 mg. of sapogenin of analytical purity was dissolved in 10 cc. of conc'd sulfuric acid, warmed at 40° for one hour, and the absorption curve was measured with the appropriate dilution using a Beckman model DU ultraviolet spectrophotometer with tungsten lamp. Measurements were carried out at 2 mμ intervals from 320 mμ to 600 mμ. The position of the maxima and minima with log ϵ values are given in Table I and represent the average of triplicate determinations. The position of the maxima remained essentially unchanged for twenty hours.

DISCUSSION

The results are summarized in Table I and, while they are in the main self-explanatory, a number of points merit emphasis. The presence of a hydroxyl or keto group is essential for absorption since the spiroketal side chain *per se*, as in 22-isoallospirostan (No. 1), does not produce any maximum above 320 mμ. Isolated double bonds have practically no effect on the spectra (*cf.* No. 10, 11, and 12) and hydroxyl groups lead to much higher extinction coefficients than keto substituents (*cf.* No. 2 *vs.* 3, No. 8 *vs.* 9) when in the 3-position. However, 12-keto sapogenins (*e.g.* No. 19, 20) and the 1,4-diketo moiety characteristic of "kryptogenin" derivatives (No. 24, 25) exhibit very high extinction.

The effect of configurational changes upon the spectrum is variable. Isomerization at C-22 apparently cannot be detected (*cf.* No. 7 *vs.* 8) while isomerization at C-2 and C-3 of dihydroxysapogenins produces very marked differences (*cf.* No. 13, 14, 15). As was pointed out recently (3) the visual color is at times

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[1] Paper XX, Ringold, Rosenkranz, and Djerassi, *J. Am. Chem. Soc.*, in press.

[2] Taken in part from a thesis presented by Srta. Griselda Diaz to the Facultad de Ciencias Quimicas, Universidad Motolinia.

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TABLE I

Absorption Spectra of Sulfuric Acid Chromogens of Steroidal Sapogenins

SAPOGENIN			COLOR WITH H_2SO_4	ABSORPTION SPECTRUM IN H_2SO_4 (320–600 mμ)	
No.	New Nomenclature (ref. 4)	Old Nomenclature		Max (log ε)	Min (log ε)
1.	22-Isoallospirostan	Desoxytigogenin	Colorless	No max.	
2.	22-Isoallospirostan-3β-ol	Tigogenin	Yellow	394 (3.50)	375 (3.44)
3.	22-Isoallospirostan-3-one	Tigogenone	Colorless	453 (1.98)	438 (1.92)
4.	22-Isospirostan-3α-ol	Epismilagenin	Light yellow	390 (3.52)	383 (3.50)
5.	22-Isospirostan-3-one	Smilagenone	Light yellow	424 (2.78)	390 (2.63)
6.	Spirostan-3β-ol	Sarsasapogenin	Yellow	398 (3.44)	380 (3.39)
7.	Δ^{5}-Spirosten-3β-ol	Yammogenin	Orange	412 (4.13), 512 (3.40)	364 (3.78), 457 (3.28)
8.	Δ^{5}-22-Isospirosten-3β-ol	Diosgenin	Orange	334 (3.86), 412 (4.11), 512 (3.52)	366 (3.77), 458 (3.36)
9.	Δ^{4}-22-Isospirosten-3-one	Δ^{4}-Diosgenone	Yellow	459 (1.93), 568 (1.70)	438 (1.87), 545 (1.66)
10.	Δ^{7}22-Isoallospirosten-3β-ol	—	Yellow	412 (4.23), 470 (3.27)	364 (3.91), 460 (3.26)
11.	$\Delta^{8(14)}$-22-Isoallospirosten-3β-ol	—	Orange	411 (4.21), 469 (3.34)	363 (3.90), 462 (3.32)
12.	$\Delta^{9(11)}$-22-Isoallospirosten-3β-ol	—	Yellow	410 (4.01), 472 (3.22)	376 (3.84), 460 (3.20)
13.	22-Isoallospirostan-2α,3β-diol	Gitogenin	Purple	399 (3.09)	386 (3.07)
14.	22-Isoallospirostan-2α,3α-diol	—	Colorless	493 (2.44)	485 (2.43)
15.	22-Isoallospirostan-2β,3α-diol	—	Colorless	384 (3.07)	382 (3.06)
16.	22-Isoallospirostan-3β,6α-diol	Chlorogenin	Yellow	330 (3.98), 400 (3.56), 470 (3.11)	320 (3.95), 378 (3.50), 456 (3.07)
17.	Δ^{5}-22-Isospirostene-2α,3β-diol	Yuccagenin	Purple	345 (3.67), 404 (3.91)	321 (3.60), 362 (3.55)
18.	22-Isospirostan-2,3-diol (?)	Samogenin	Colorless	342 (3.10), 398 (2.83), 494 (2.14)	320 (3.07), 385 (2.82), 452 (2.06)
19.	22-Isoallospirostan-3β-ol-12-one	Hecogenin	Yellow	351 (3.86), 396 (4.20)	335 (3.83), 358 (3.85)
20.	$\Delta^{9(11)}$-22-Isoallospirosten-3β-ol-12-one	9-Dehydrohecogenin	Pink	388 (4.18), 514 (2.95)	320 (3.81), 478 (2.91)
21.	22-Isospirostan-2,3-diol-12-one (?)	Mexogenin	Colorless	347 (3.62), 394 (3.31), 468 (2.82)	320 (3.34), 378 (3.28), 440 (2.81)
22.	22-Isoallospirostan-2α,3β-diol-12-one	Mannogenin	Light purple	346 (3.77), 470 (2.78)	320 (3.58), 440 (2.72)

23.	Δ^5-22-Isospirostene-2α,3β-diol-12-one	Kammogenin	Purple	346 (3.65), 474 (2.82), 554 (2.81)	320 (3.41), 428 (2.72), 530 (2.75)
24.	Cholestane-3β,26-diol-16,22-dione	Dihydrokryptogenin	Yellow	380 (4.00)	352 (3.75)
25.	Δ^5-Cholestene-3β,26-diol-16,22-dione	Kryptogenin	Orange	384 (4.02), 484 (2.89)	338 (3.88), 462 (2.87)
26.	$\Delta^{5,16(23)}$Fesadien-3β,26-diol-22-one[a]	Fesogenin	Orange	363 (3.95), 414 (4.07)	320 (3.61), 382 (3.80)

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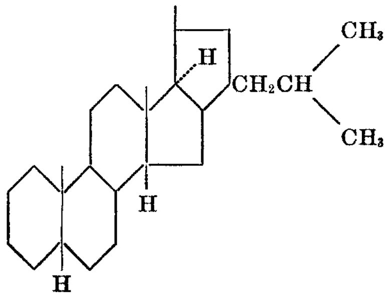
[a] This nomenclature is based on the hypothetical substance "Fesane":



quite characteristic and is particularly useful in the case of 2,3-dihydroxy sapogenins (No. 13–17, 22, 23). For instance "gitogenin" (No. 13) and "chlorogenin" (No. 16) which often occur in the same plant are readily distinguished by the presence or absence of a purple color upon covering a crystal of the substance with a drop of conc'd sulfuric acid.

An inspection of Table I will demonstrate that on the whole the sulfuric acid spectra differ appreciably among each other and they have already proved quite valuable in this laboratory in aiding in the characterization of sapogenins isolated from natural sources. Quantities of the order of 0.03–0.1 mg. are sufficient for this analytical procedure.

SUMMARY

The spectra of twenty-six steroidal sapogenins have been measured in conc'd sulfuric acid and pertinent data are reported which aid appreciably in the identification of trace quantities of such sapogenins.

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