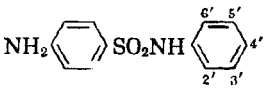
TABLE II

SULFANILANILIDES[a]



Compound	Yield, %	M.P. Corr.	Calcd., % / Found, % C	H	N	F
2'-F	92	190–191	54.1	4.2	10.5	7.1
			54.7	4.4	10.7	6.7
3'-F	98	167–168	54.1	4.2	10.5	7.1
			54.0	4.2	10.9	7.1
4'-F	89	162–163	—	—	—	—
2'-CH_3-5'-F	85	148–149	55.7	4.7	10.0	6.8
			55.9	5.0	10.1	7.0
2',4'-F_2	21	161–162	50.7	3.6	9.9	13.4
			51.1	3.8	10.1	12.6
2',5'-F_2	66	183–185	50.7	3.6	9.9	13.4
			50.9	3.6	10.1	13.7
3',5'-F_2	91	178–178.5	50.7	3.6	9.9	13.4
			50.8	3.8	9.9	12.7
3'-CF_3	55	115–117	49.4	3.5	8.9	18.0
			49.4	3.7	8.9	18.3

[a] The sulfanilanilides in Table II were prepared by the procedure used to reduce 3',5'-difluoro-4-nitrobenzenesulfonamide to the corresponding sulfanilanilide. These sulfanilanilides were crystallized analytically pure from the reduction solution, the melting point being unchanged by further purification.

of 45 p.s.i. of hydrogen. On termination of the reduction, the catalyst was removed, and the filtrate concentrated to obtain 31.9 g. (91%) of white crystalline 3',5'-difluorosulfanilanilide, m.p. 178–178.5° corr.

Acknowledgment. We are indebted to Mr. L. Brancone and his staff for the elemental analyses.

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The Synthesis of 9α-Hydroxy Steroids[1]

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Received April 21, 1961

This paper is concerned with the development of a chemical method for the introduction of a 9α-hydroxyl group into steroids.

One of the mechanisms of steroid degradation by microorganisms involves a 9α-hydroxylation reaction, followed by a 1,2-dehydrogenation (or *vice-versa*) with the formation of a 9,10-seco-steroid.[2,3] In order to study the enzymatic mechanism of the conversion of 9α-hydroxyandrostene-3,17-dione to 9,10-seco-3-hydroxy-1,3,5(10)-androstatriene-9,17-dione, a substantial quantity of 9α-hydroxyandrostene-3,17-dione was needed; it was solely for this reason that this work was undertaken.

The conventional method for the preparation of 9α-hydroxy steroids has been by microbiological methods. The yield of 9α-hydroxy steroids has been low in some cases and usually a number of other major hydroxylated products have been also produced to complicate the isolation processes.[4,5] In cases where the yield of 9α-hydroxy steroids have been relatively efficient.[3,6,7] The organisms used have not been available for general circulation and special equipment is needed for large scale fermentations.

4-Androstene-9α,11β-diol-3,17-dione,[8] 9α-hydroxycortisone acetate, 9α-hydroxyhydrocortisone acetate.[9,10] and 9α-hydroxyhydrocortisone[11] have been prepared by the acid catalysis of their corresponding 9β,11β-epoxides by chemical methods. However, 9α-hydroxy steroids devoid of oxygen functions at the 11- positions in these series have not been prepared to our knowledge. This method describes the synthesis of 9α-hydroxyandrostene-3,17-dione based on the reduction of its corresponding 9α,11α-epoxide with lithium aluminum hydride to the corresponding 9α-(axial) hydroxyl compound. 3β-Acetoxyergostan-9α-ol has been prepared by the reduction of 3β-acetoxy-9α,11α-epoxyergostane with lithium-ethylamine,[12] but apparently lithium aluminum hydride was unable to reduce this epoxide. The method herein described should also be applicable for the synthesis of other 9α-hydroxy steroids such as 9α-hydroxyprogesterone and 9α-hydroxycortexolone. The procedure developed is formulated as follows (I–VIII):

(1) This investigation was supported in part by the American Cancer Society Institutional Reasearch grant.

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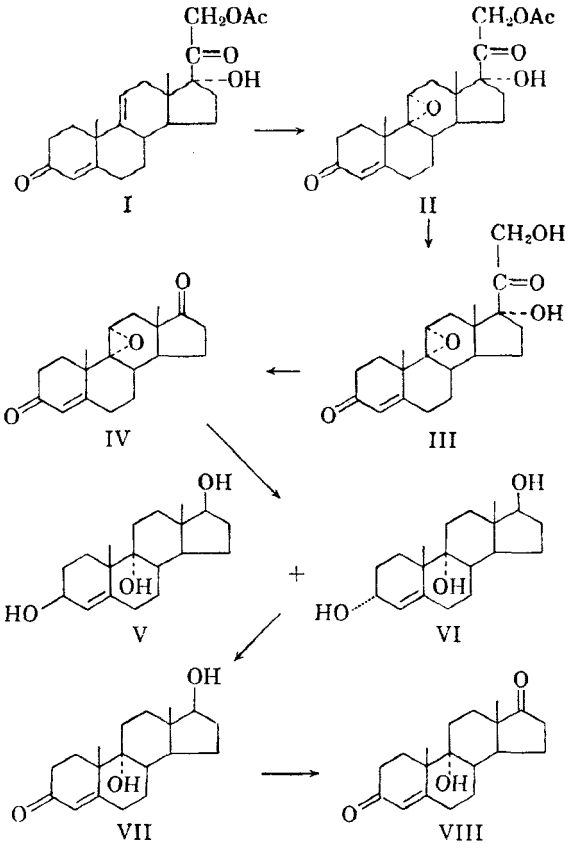

4,9(11)-Pregnadiene-17α,21-diol-3,20-dione 21-acetate (I) was converted to the known 4-pregnene-9α,11α-oxido-17α,21-diol-3,20-dione 21-acetate (II)[9] in 97% yield by reaction with perbenzoic acid in the cold. Treatment of II with potassium carbonate gave 4-pregnene-9α,11α-oxido-17α,21-diol-3,20-dione (III) in 90% yield. Reaction of III with sodium bismuthate afforded 9α,11α-oxidoandrostene-3,17-dione (IV) in 65% yield. Reduction of IV with lithium aluminum hydride gave a mixture of 4-androstene-3β,9α,17β-triol (V) and 4-androstene-3α,9α,17β-triol (VI); this mixture was not isolated as such but was converted directly into 9α-hydroxytestosterone (VII)[13] by treating with manganese dioxide. The conversion of IV to VII was obtained in 26% yield. Oxidation of VII with chromic acid gave 9α-hydroxyandrostene-3,17-dione (VIII) in 68% yield.

EXPERIMENTAL

All melting points were uncorrected and were determined in open soft-glass capillaries. Values of $[\alpha]_D$ have been approximated to the nearest degree. Ultraviolet absorption spectra were determined in methanol on a Cary recording spectrophotometer (Model 11 MS). Infrared spectra were recorded on a Beckman IR 5 double beam infrared recording spectrophotometer. Microanalysis were carried out by Mr. J. Alicino of Metuchen, N. J. Tetrahydrofuran was refluxed with lithium aluminum hydride prior to redistillation. Manganese dioxide was prepared according to the method of Mancera *et al.*[14]

4-Pregnene-9α,11α-oxido-17α,21-diol-3,20-dione, 21-acetate (II). Two grams of 4,9(11)-pregnadiene-17α,21-diol-3,20-dione 21-acetate (I) was dissolved in an ice-cold 0.026*M* solution of perbenzoic acid in chloroform (500 ml.) and the mixture allowed to remain in the refrigerator. Iodometric titration after 20 hr. indicated the consumption of 1.10 mole equivalents of perbenzoic acid. The chloroform solution was extracted with 0.05*M* sodium iodide in 0.01*N* sulfuric acid, 0.05*N* sodium sulfite, 0.5*N* sodium bicarbonate, and water. The chloroform solution was dried over sodium sulfate and concentrated to dryness. The crystalline residue weighed 2.01 g. (97%), m.p. 240–245°. Recrystallization from acetone gave a sample which melted at 248–249°; $[\alpha]_D^{25}$ = +100 (*c*, 1.0 chloroform); λ_{max}^{alc} 238 mμ (ϵ 16,000); $\lambda_{max}^{CHCl_3}$ 2.90, 5.79, 6.00, 6.18 μ.

Anal. Calcd. for $C_{23}H_{30}O_6$ (402.47): C, 68.63; H, 7.51. Found: C, 68.88; H, 7.61.

4-Pregnene-9α,11α-oxido-17α,21-diol-3,20-dione (III). To 1.0 g. of 4-pregnene-9α,11α-oxido-17α,21-diol-3,20-dione 21-acetate (II) in methanol (12 ml.) was added 2.5 ml. of 10% aqueous potassium carbonate (free of oxygen). The mixture was stirred under nitrogen for 1 hr., during which period the acetate dissolved completely and the free alcohol began to crystallize. The reaction was terminated by the addition of glacial acetic acid (0.4 ml.) and ice water (120 ml.). After cooling, the crystalline precipitate was collected and dried *in vacuo*. The crude material weighed 720 mg. (80%); extraction of the filtrate with chloroform yielded an additional 82 mg. (9.0%). Recrystallization from acetone gave a sample, m.p. 213–215°; $[\alpha]_D^{25}$ = +86 (*c*, 1.0 dioxane); λ_{max}^{alc} 238 mμ (ϵ 16,600); λ_{max}^{Nujol} 2.98, 5.85, 6.06 μ.

Anal. Calcd. for $C_{21}H_{28}O_5$ (360.44): C, 70.02; H, 7.77. Found: C, 70.37; H, 8.10.

9α,11α-Oxidoandrostene-3,17-dione (IV). To a solution of 4-pregnene-9α,11α-oxido-17α,21-diol-3,20-dione (III) (5.0 g.) in glacial acetic acid (500 ml.) and water (500 ml.) was added 40 g. of sodium bismuthate and the resulting mixture was shaken vigorously at room temperature in the dark for 4 hr. The solution was then filtered; the precipitate was washed with chloroform and the total filtrate was extracted with chloroform. The chloroform solution was extracted with sodium bicarbonate and exhaustively with water. It was then dried over sodium sulfate and concentrated to dryness. The crystalline residue weighed 3.33 g., and it was chromatographed over 20 g. of acid-washed alumina; elution with hexane-benzene (1:2) yielded 2.72 g. (65%) of a product which melted at 270–272°. Recrystallization from acetone gave a sample, m.p. 272–274°; $[\alpha]_D^{25}$ = + 185 (*c*, 1.0 chloroform); λ_{max}^{alc} 236 mμ (ϵ 16,000); $\lambda_{max}^{CHCl_3}$ 5.76, 6.02, 6.18 μ.

Anal. Calcd. for $C_{19}H_{24}O_3$ (300.38): C, 75.97; H, 8.05. Found: C, 76.13; H, 7.95.

4-Androstene-3β,9α,17β-triol (V) *and 4-androstene-3α,9α,-17β-triol* (VI). One gram of 9α,11α-oxidoandrostene-3,17-dione (IV) was dissolved in 30 ml. of dry tetrahydrofuran and was slowly added with stirring to 2 g. of lithium aluminum hydride in 50 ml. of tetrahydrofuran. The mixture was stirred for 16 hr. after which it was further refluxed for 4 hr. The excess lithium aluminum hydride was decomposed by the cautious addition of water. The mixture was filtered and the precipitate was washed with tetrahydrofuran; the filtrate was dried over sodium sulfate and concentrated to dryness. The residue weighed 910 mg. which represents a mixture of V and VI. This mixture was used directly for the following reaction.

9α-Hydroxytestosterone (VII). To 500 mg. of the mixture containing V and VI in 75 ml. of chloroform was added 5.0

(13) The molecular rotatory contribution of VII [M_D (9α-hydroxytestosterone) $-M_D$ (testosterone) = -23]. This is in agreement with the molecular rotatory contribution of the 9α-hydroxyl group in 3β-acetoxyergostan-9α-ol (-31)[12] and of the 9α-hydroxyl group in 9α-hydroxyandrostene-3,17-dione (-18).[3]

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g. of manganese dioxide. The reaction mixture was stirred for 16 hr. after which it was filtered and the precipitate washed with chloroform. The filtrate was concentrated to dryness and the residue weighed 452 mg. This residue was chromatographed over acid-washed alumina (10 g.); elution with a gradient system consisted of benzene-chloroform afforded 162 mg. of 9α-hydroxytestosterone (VII). Recrystallization from acetone–petroleum ether (b.p. 60–80°) gave 128 mg. of a sample, m.p. 210–211°; $[\alpha]_D^{25} = +104$ (*c*, 1.0 chloroform); λ_{max}^{alc} 242 mμ (ε 15,200); $\lambda_{max}^{CHCl_3}$ 2.92, 6.03, 6.20 μ.

Anal. Calcd. for $C_{19}H_{28}O_3$ (304.41): C, 74.96; H, 9.27. Found: C, 75.32; H, 9.46.

9α-Hydroxyandrostene-3,17-dione (VIII). 9α-Hydroxytestosterone (100 mg.) was dissolved in 10.0 ml. of acetone and treated dropwise with stirring with a solution prepared by dissolving 30 mg. of chromic acid and an equivalent amount of sulfuric acid in 3.0 ml. of acetone. When the reaction was complete, the chromic sulfate was removed by centrifugation and washed with acetone. The combined acetone washings were evaporated to dryness, the residue taken up in chloroform, washed with water, dried over sodium sulfate, and the chloroform solution concentrated. The residue crystallized from acetone-hexane yielding 67 mg. of 9α-hydroxyandrostene-3,17-dione (VIII), m.p. 220–222°; $[\alpha]_D^{25} = +182$ (*c*, 0.9 chloroform); λ_{max}^{alc} 242 mμ (ε 16,000); $\lambda_{max}^{CHCl_3}$ 2.90, 5.75, 6.01, and 6.18 μ.

Anal. Calcd. for $C_{19}H_{26}O_3$ (302.40): C, 75.46; H, 8.67. Found: C, 75.18; H, 8.72.

Acknowledgment. The author wished to express his thanks to Dr. Gunther S. Fonken of The Upjohn Co. for supplying bulk quantities of $\Delta^{9(11)}$-cortexolone acetate used in this work and to Dr. R. M. Dodson of G. D. Searle Co. for an authentic sample of 9α-hydroxyandrostene-3,17-dione.

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Flavonols in Spinach Leaves

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Received April 24, 1961

There has been no previous report of the identification of individual flavonol compounds in spinach leaves, although a colorimetric, quantitative determination of the gross, flavonoid-like compound content of spinach (*Spinacia oleracea*) has been reported by Weatherby and Cheng.[1] Williams[2] has isolated a flavonoid compound in pure form from spinach leaves. Its exact identity, however, was not determined. This note describes the extraction from spinach and identification of the flavonol, patuletin, and also of a quercetagetin dimethyl ether. The latter compound has not been found previously in nature, and the name "spinacetin" is proposed for it. Spinacetin has been tentatively identified as quercetagetin-3′,6-dimethyl ether. Quercetagetin is 3,3′,4′,5,6,7-hexahydroxyflavone. Patuletin was first isolated by Rao and Seshadri from the flowers of *Tagetes patula*,[3] and later was proved to be quercetagetin-6-monomethyl ether.[4]

EXPERIMENTAL

Extraction of spinach leaves. Fifty pounds of packaged, frozen fresh spinach (Safeway Stores, Inc., "Belair" brand) was ground in an ice crusher, then loaded into 4-l. containers with 1.5 l. of water, and pressure-cooked at 115° for 45 min. The juice was squeezed out, and filtered through cotton cloth and then through a bed of Super Cel (Fisher Scientific). The clear, yellow-brown filtrate was adsorbed under pressure on wet Magnesol (Food Machinery and Chemical Corp., New York, N.Y.) packed on three glass funnels (22 cm. diam., 18 cm. deep). A yellow zone, 6 cm. deep, was formed on the Magnesol in each funnel. The adsorbent was washed with 1 l. of water, and the yellow zone was eluted with 70% ethyl alcohol–water. The dark brown eluate (4.5 l.) was concentrated *in vacuo* to 200 ml. and then "freeze-dried" to yield a black-appearing solid. Pulverization and then extraction with hot methyl alcohol (12 × 100 ml.) gave a reddish brown extract which was poured onto an 8-cm. column previously filled to a depth of 50 cm. with Magnesol in methyl alcohol. Development under 5 lb. pressure, with ethyl acetate saturated with water, readily moved a broad, bright yellow zone. The yellow eluate (1600 ml.) was taken to dryness *in vacuo*. Crystallization from acetone-water gave 0.205 g. of a yellow powder. Paper chromatography revealed the presence of at least two compounds. Separation into individual compounds was achieved by silicic acid chromatography. A column (6 cm. diam.) was packed to a depth of 38 cm. with silicic acid (Mallinckrodt No. 2847) in benzene-acetone (84:16 v./v.), under 5 lb. pressure. The yellow powder (0.205 g.) was dissolved in acetone (18 ml.) and diluted with benzene (102 ml.), and then chromatographed. On development with the benzene-acetone, two major zones formed, and they were eluted separately. After removal of the solvent *in vacuo*, the eluate from the faster-moving zone yielded 75 mg. of a yellow powder, called "compound A-2." From the eluate of the slower moving zone, 76 mg. of fine yellow needles called "compound A-1," were obtained.

Identification of compound A-1. The yellow product containing compound A-1 was chromatographed on a silicic acid column using benzene-acetone (84:16 v./v.), and then crystallized from ethyl alcohol–water to give yellow needles. These were dried at 110° *in vacuo*, yield 59 mg., m.p. 261–263° (all melting points are uncorrected). R_f values in 60% acetic acid, *n*-butyl alcohol–acetic acid–water (6:1:2 v./v./v.), and phenol-water (3:1 w./w.), using Whatman No. 1 chromatography paper and descending chromatography, were 0.47, 0.75, and 0.63, respectively. The ultraviolet absorption spectrum showed maxima at 257 and 375 mμ and minima at 240 and 285 mμ.

Anal. Calcd. for $C_{16}H_{12}O_8$: C, 57.83; H, 3.64; OCH_3, 9.34. Found: C, 58.05; H, 3.98; OCH_3, 9.28.

Compound A-1 (10 mg.) was refluxed for 6 hr. with 1 ml. of dimethyl sulfate in 8 ml. of anhydrous acetone and potassium carbonate (2.5 g.) to give colorless needles, m.p. 143–144°. No depression occurred on mixed melting point determination with synthetic quercetagetin hexamethyl ether. The melting point of authentic patuletin was not depressed by the addition of compound A-1. The ultraviolet and infrared spectra of compound A-1 and the reference patuletin were identical, respectively. With spectral measurements by the

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