[CONTRIBUTION FROM THE NATIONAL INSTITUTE OF ARTHRITIS AND METABOLIC DISEASES, NATIONAL INSTITUTES OF HEALTH, PUBLIC HEALTH SERVICE, FEDERAL SECURITY AGENCY]

Degradation of Tomatidine[1]

BY YOSHIO SATO, ALFRED KATZ[2] AND ERICH MOSETTIG

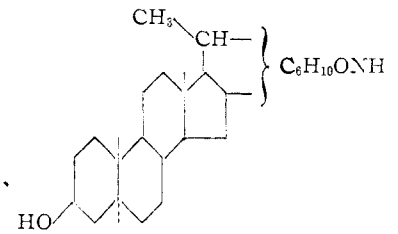
The degradation of tomatidine to Δ^{16}-allopregnen-3(β)-ol-20-one is described.

Tomatin, a new glycosidal alkaloid, was first isolated and obtained in pure crystalline form by Fontaine, *et al.*[3] It was found to consist of an aglycone portion, tomatidine, and a tetrasaccharide moiety composed of xylose, galactose and two glucose units.[4] Fontaine, Ard and Ma[5] established for the glycoside the empirical formula $C_{50}H_{83}NO_2$, and for the crystalline aglycone tomatidine the formula $C_{27}H_{45}NO_2$. Of the latter, these authors prepared a crystalline hydrochloride, a crystalline N,O-diacetyl derivative (acetic anhydride, pyridine, room temperature), and with $LiAlH_4$ a crystalline dihydrotomatidine which gave an amorphous triacetyl derivative (acetic anhydride, pyridine). No evidence of a methoxyl group was found, and infrared spectra indicated the absence of an ethylenic double bond. The authors speculated that in the $LiAlH_4$ reduction an oxygen linkage may have opened as Marker and his co-workers[6] had found in the catalytic reduction of a number of steroidal sapogenins.[7]

Very recently Kuhn, *et al.*,[8] obtained from a number of *Lycopersicum* species, a glycosidal alkaloid and aglycone which they recognized to be tomatine and tomatidine by the agreement in melting points and in the nature of the sugar components. In the catalytic hydrogenation (Pt, glacial acetic acid) of the glycoside, as well as of the aglycone, approximately one mole of hydrogen was absorbed. The authors assumed the presence of an olefinic double bond and suggested tentatively $C_{27}H_{43}NO_2$ for tomatidine.

Our analytical figures for tomatidine, the hydrochloride and the diacetyl compound, agree rather with $C_{27}H_{45}NO_2$ than with $C_{27}H_{43}NO_2$. Tomatidine forms a sparingly soluble digitonide and gives readily an N-nitroso compound.[9] When tomatidine was refluxed in acetic anhydride an unsaturated compound of m.p. 105–107°, presumably a triacetyl derivative[10] (A) was formed, which in turn can be hydrogenated (platinum, glacial acetic acid) to the corresponding dihydro compound. Oxidation of A with chromic acid in acetic acid solution, and hydrolysis of the resulting oily oxidation product gave in good yield Δ^{16}-allopregnen-3(β)-ol-20-one. The identity of this compound and its acetate has been established by direct comparison with authentic samples.[11]

The isolation of the allopregnenolone establishes the structure of the steroidal moiety of tomatidine I, and the attachment of the portion containing the secondary nitrogen, at C-20. The second point of attachment is most likely at position 16.



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Acknowledgment.—We are greatly indebted to Dr. Thomas D. Fontaine of the Bureau of Agricultural and Industrial Chemistry for samples of tomatidine, and considerable quantities of tomatine. Microanalyses are from the Institutes' service analytical laboratory under the direction of Dr. William C. Alford.

Experimental[12]

Tomatidine.—To 6.0 g. of crystalline, slightly greenish tomatine in 360 cc. of ethanol was added 230 cc. of 2.5 *N* hydrochloric acid. Gradually a clear green solution resulted which was heated on the steam-bath for 3 hours. Most of the alcohol was removed, the crystalline hydrochloride was collected, washed with a little cold methanol and ether and redissolved in methanol. By addition of excess ether to the filtered and cooled methanol solution a copious amount of fine crystalline hexagonal platelets of tomatidine hydrochloride was obtained; yield 2.0 g., m.p. 255–260° (with previous browning).

Anal. Calcd. for $C_{27}H_{46}ClNO_2$: C, 71.73; H, 10.26. $C_{27}H_{44}ClNO_2$: C, 72.05; H, 9.85. Found: C, 71.83; H, 10.35.

The hydrochloride was dissolved in 75% hot methanol, and dilute ammonium hydroxide was added until the solution became alkaline to litmus. Tomatidine crystallized as fine plates and melted at 205–208.5°; yield 1.55 g. A small second crop was obtained by concentration of the mother liquor.

Anal. Calcd. for $C_{27}H_{45}NO_2$: C, 78.02; H, 10.91; N, 3.37. $C_{27}H_{43}NO_2$: C, 78.40; H, 10.48; N, 3.39. Found: C, 78.02; H, 10.96; N, 3.43.

To a solution of 15.9 mg. of tomatidine in 10 cc. of ethanol was added 10 cc. of a 1% ethanolic solution of digitonin. After standing at room temperature overnight the copious

(1) A preliminary account was given in THIS JOURNAL, **73**, 880 (1951).

(2) Organisch-chemische Anstalt, University of Basel.

(3) Fontaine, Irving, Ma, Poole and Doolittle, *Arch. Biochem.*, **18**, 467 (1948).

(4) Ma and Fontaine, *ibid.*, **27**, 461 (1950).

(5) Fontaine, Ard and Ma, THIS JOURNAL, **73**, 878 (1951).

(6) Fieser and Fieser, "Natural Products Related to Phenanthrene," 3rd ed., Reinhold Publishing Corp., New York, N. Y., 1949, p. 586.

(7) Various chemical and phytogenetic considerations led Fontaine and his co-workers to assume for tomatidine tentatively a structure similar to that of the steroidal sapogenins. Private communication, see also, Fontaine, Ard, Ma, Ogg and Willits, *Federation Proc.*, **9**, 171 (1950).

(8) (a) Kuhn and Löw, *Chem. Ber.*, **81**, 552 (1948); (b) Kuhn, Löw and Gauhe, *ibid.*, **83**, 448 (1950).

(9) The secondary character of tomatidine was postulated by Fontaine, *et al.*, on the basis of infrared spectra.[5]

(10) The hydrolysis of this derivative proceeds in a complicated manner to give products which have not as yet been completely identified.

(11) Samples of the acetate were generously supplied to us by Dr. R. B. Wagner of Pennsylvania State College and by Dr. George Rosenkranz of Syntex, S. A.

(12) All melting points were taken on the Kofler block and are uncorrected.

precipitate was filtered and dried in a vacuum at 100°; recovered 60.0 mg. of digitonide, calcd. 62.9 mg.

N-Nitrosotomatidine.—To 56 mg. of tomatidine dissolved in 1 cc. of ethanol and 0.1 cc. of acetic acid was added 28.5 mg. of sodium nitrite dissolved in a few drops of water. After standing for a short time, addition of several drops of water and scratching induced the formation of hexagonally shaped crystals. The compound gradually changed to flat rods before melting at 234–237°. When the substance was heated beyond its melting point, evolution of gas was observed; λ_{max} 233 mμ, log ϵ 3.87; λ_{max} 360, log ϵ 1.83 (ethanol).

Anal. Calcd. for $C_{27}H_{44}N_2O_3$: N, 6.30. Found: N, 6.36.

N,O-Diacetyltomatidine.—This derivative was prepared in the usual manner with acetic anhydride and pyridine. After recrystallization from acetone and ether, the material melted unsharply at 192° (sintering at 185°), lit. 193–194°, evac. tube,[5] 192–194° uncor.[3b] Chromatographic purification (benzene–petroleum ether) did not improve the melting point.

Anal. Calcd. for $C_{31}H_{49}NO_4$: C, 74.50; H, 9.88. $C_{31}H_{47}NO_4$: C, 74.81; H, 9.52. Found: C, 74.66; H, 9.92.

Compound A.—A mixture of 616 mg. of tomatidine and 12 cc. of acetic anhydride was refluxed vigorously in an oil-bath for two hours. The solvent was removed *in vacuo*, and the oily residue dissolved in ethanol. Water was added to the point of slight turbidity and the compound allowed to crystallize. It formed shiny plates melting at 97–105°. After one recrystallization from ethanol–water 450 mg. of a compound melting at 103.5–107° was obtained. An additional 50 mg. was recovered upon concentration. For analysis a sample was recrystallized twice, m.p. 105–107°, $[\alpha]^{20}D$ −11.6° (*c* 1.25, chloroform).

Anal. Calcd. for $C_{33}H_{51}NO_5$: C, 73.16; H, 9.49; N, 2.59; $COCH_3$ (three), 23.8. Found: C, 73.12; H, 9.78; N, 2.66; $COCH_3$, 21.8 (alkaline hydrolysis).

Catalytic Hydrogenation of Compound A.—A mixture of 50.5 mg. of A and 51 mg. of platinum oxide (prereduced) in 8.0 cc. of glacial acetic acid absorbed in approximately 2 hours a slight excess above the calculated amount (1 mole) of hydrogen, when the absorption ceased. After filtration of the catalyst, the solvent was evaporated *in vacuo*, the residue dissolved in ethanol and water added. The reaction product seemed to be contaminated slightly with starting material, but after several recrystallizations from ethanol–water flat rods of melting point 119–122° were obtained. No hydrogenation took place in an ethanolic solution of A.

Anal. Calcd. for $C_{33}H_{53}NO_5$: C, 72.89; H, 9.83. Found: C, 72.98; H, 9.82.

Chromic Acid Oxidation of Compound A.—To a solution of 0.600 g. of A in 20 cc. of glacial acetic acid was added dropwise with stirring 0.500 g. of chromic acid anhydride in 10 cc. of 80% acetic acid. The reaction mixture was kept at *ca.* 10° by cooling in ice-water. After standing at room temperature (20–23°) for 1.5 hours, the dark-brown solution was poured into ice-water and extracted with ether. The ethereal solution was washed with water, dilute sodium carbonate solution and water again, dried over sodium sulfate and evaporated. The resulting oily residue was dissolved in 20 cc. of 2% ethanolic potassium hydroxide and refluxed on the steam-bath for 30 minutes. The reaction mixture was poured into ice-water and the flocculent precipitate extracted with ether. After removal of solvents a semi-crystalline product (155 mg.) was obtained and recrystallized from dilute methanol. Another recrystallization from the same solvent gave a compound with the constant m.p. 207–208°. The mixture with an authentic sample of Δ^{16}-allopregnen-3(β)-ol-20-one of m.p. 209–209.5° melted at 208–208.5°, $[\alpha]^{20}D$ +50.4° (*c* 0.91, ethanol), lit. $[\alpha]^{17}D$ +50.2° ± 4.2° (*c*, 1.4, ethanol).[13]

Anal. Calcd. for $C_{21}H_{32}O$: C, 79.70; H, 10.19. Found: C, 79.55; H, 10.30.

A mixture of 33 mg. of the above alcohol and 1.5 cc. of acetic anhydride was gently refluxed for 20 minutes. After removing the solvent *in vacuo*, the residue was recrystallized from dilute methanol: glistening, hexagonally shaped platelets; m.p. 165–168°. After sublimation in a high vacuum and recrystallization, the compound melted at 167–169°; λ_{max} 240 mμ, log ϵ 3.98; λ_{max} 320 mμ, log ϵ, 1.86; $[\alpha]^{20}D$ +36.7° (*c*, 1.04, $CHCl_3$).[14]

Anal. Calcd. for $C_{23}H_{34}O_3$: C, 77.05; H, 9.56. Found: C, 77.02; H, 9.67.

The melting points of mixtures with two authentic samples[11] gave no depression. The infrared spectra of the samples compared were identical.

(13) Klyne, Schachter and Marrian, *Biochem. J.*, **43**, 231 (1948).

(14) Klyne and Marrian[13] report: m.p. 165–167°; λ_{max} 240 mμ, log ϵ 3.93; λ_{max} 320 mμ, log ϵ 1.92; $[\alpha]^{22}D$ +36.3° ± 0.7° (*c* 1.4, $CHCl_3$). Plattner, *et al.*, *Helv. Chim. Acta*, **30**, 385 (1947), report: m.p. 166–167°; λ_{max} 240 mμ, log ϵ 4.2; λ_{max} 320 mμ, log ϵ 2.1, $[\alpha]^{20}D$ +42.2° (*c* 1.42, $CHCl_3$).

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[CONTRIBUTION FROM THE DEPARTMENT OF MEDICINE, WESTERN RESERVE UNIVERSITY, AND THE LAKESIDE HOSPITAL]

A Partial Synthesis of Δ^5-Pregnenetriol-3β,16α, 20α and of Other 16α-Hydroxysteroids[1]

BY H. HIRSCHMANN, FRIEDA B. HIRSCHMANN AND MARGARET A. DAUS

A procedure for the introduction of the 16α-hydroxy group into the steroid molecule is described. 16α-Benzyloxy-Δ^5-pregnenol-3β-one-20 acetate prepared from $\Delta^{5,16}$-pregnadienol-3β-one-20 and benzyl alcohol gave on successive reductions with lithium aluminum hydride and with sodium and alcohol two isomeric triols. One of these was identical with the Δ^5-pregnenetriol-3β,16α,20α isolated from adrenal tumor urine, while the other, Δ^5-pregnenetriol-3β,16α,20β, upon reduction of its acetate gave the triacetate of allopregnanetriol-3β,16α,20β of the urine of pregnant mares. The latter was epimerized at C-3 by treating the 3-tosylate 16,20-diacetate with sodium acetate to give allopregnanetriol-3α,16α,20β triacetate.

Several years ago a new steroid was isolated[2a] from the urine of a boy with an adrenal tumor and characterized[2b] as Δ^5-pregnenetriol-3β,16α,20α. This observation demonstrated that pregnane derivatives oxygenated in the 16 position can be derived from adrenal steroids, but failed to disclose whether the oxygen atom is introduced into the molecule by adrenal tissue or subsequently while the adrenal steroid is metabolized by other organs of the body. In order to investigate this question and to test the effect of 16-oxygenation on the biological activity of potent adrenal steroids methods for the synthesis of 16α-hydroxysteroids were required. 16-Hydroxysteroids with a configuration[3] opposite to that found in urinary metabolites are readily available from sapogenins[4] but their inversion to

(1) This investigation was supported by grants from the Hanna Research Fund and from the American Cancer Society on the recommendation of the Committee on Growth.

(2) (a) H. Hirschmann and F. B. Hirschmann, *J. Biol. Chem.*, **157**, 601 (1945); (b) *ibid.*, **184**, 259 (1950).

(3) H. Hirschmann, F. B. Hirschmann and M. A. Daus, *ibid.*, **178**, 751 (1949).

(4) R. E. Marker, D. L. Turner, R. B. Wagner, P. R. Ulshafer, H. M. Crooks, Jr., and E. L. Wittle, THIS JOURNAL, **63**, 774 (1941); R. E. Marker, D. L. Turner, R. B. Wagner and P. R. Ulshafer, *ibid.*, **63**, 772 (1941). The reactions described in these papers were also applied to other sapogenins.