**Tetrahedron Letters No.4, pp. 217-221, 1964. Pergamon Press Ltd.
Printed in Great Britain.**

THE OCCURRENCE OF 5α-ANDROSTANE-3β,16α,17α-TRIOL IN "RAYLESS GOLDENROD" (APLOPAPPUS HETEROPHYLLUS BLAKE)

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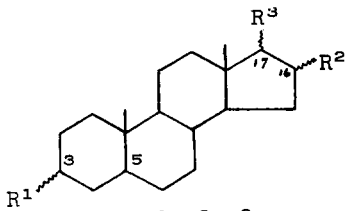
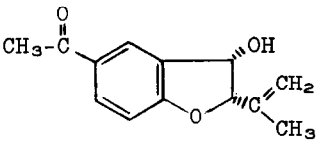
(Received 20 November 1963)

The naturally occurring male hormones or androgens have been isolated from urine and from testicular extracts and are based on the androstane skeleton I (1). We wish to report here the



I $R^1=R^2=R^3=H$
II $R^1=\beta$-OH, $R^2=R^3=\alpha$-OH



III

rather surprising occurrence of 5α-androstane-3β,16α,17α-triol, II, in the plant "rayless goldenrod." "Rayless goldenrod", indigenous to the southwestern United States, has been known for many years to be responsible for a disease of higher animals known as "trembles" or "milksickness" (2,3,4). Recent work has led to the isolation and structure elucidation of a dihydrobenzofuran III, toxol, from the crude plant toxin (5,6). Although toxol was found to inhibit the growth of several bacteria, it has not been shown to be responsible for the plant's toxicity to higher animals.

The sterol II was isolated as follows. Saponification of the methanolic extract of the whole dried plant (10% of weight of plant) with 5% potassium hydroxide gave the non-saponifiable crude toxin ("tremetol", "red-oil") which comprised approximately 1% of the plant. After steam distillation the residual crude toxin was separated into a ketone fraction (25%) and a non-ketone fraction (65%) by the use of Girard's T reagent. Chromatography of the non-ketone fraction on alumina gave II ($C_{19}H_{32}O_3$, m.p. 288° with previous melting at 265°, $[\alpha]_D$ - 16.5° $CHCl_3$) in 0.2% yield based on the crude toxin. The infrared spectrum of II showed strong hydroxyl absorption; unsaturation was not indicated in the infrared spectrum or by the tetranitromethane test.

II readily gave an acetate ($C_{19}H_{32}O_3$, m.p. 168-169°, $[\alpha]_D$ + 8.1 $CHCl_3$) giving an n.m.r. spectrum that indicated a ratio of three acetate groups to two bridgehead methyl groups. That II was an androstane derivative was shown by its conversion to 5α-androstane, I (R=H) by preparation of the tritosylate followed by hydrogenolysis with lithium aluminum hydride. An authentic sample of 5α-androstane was prepared by Huang-Minlon (7) reduction of 5α-androstane-3,17-dione. The two samples of 5α-androstane gave identical melting points (47-49°) alone and on admixture, and identical gas chromatograms (using a 5% SE-30 column) were obtained for each and on admixture. Ruzicka, Prelog and Wieland (8) had previously reported the preparation of 5α-androstane-3β,16α,17α-triol (m.p. 265-266°, $[\alpha]_D$ - 19 ± 4° C_2H_5OH) from 5α-androst-16-en-3β-ol and their triol likewise gave a triacetate (m.p. 165°, $[\alpha]_D$ + 10 ± 4° C_2H_5OH). Owing to the discrepancy observed in the melting points of the reported 5α-androstane-3β,16α,17α-triol and

that isolated from "rayless goldenrod", Ruzicka's synthesis (8) was repeated. 17α-Hydroxy-5α-androstan-3-one benzoate was hydrogenated and reoxidized to give 17α-hydroxy-5α-androstan-3-one hexahydrobenzoate (m.p. 138-139°, $[\alpha]_D$ + 25° $CHCl_3$; reported (9): m.p. 137.5-138°) which on pyrolysis gave 5α-androst-16-en-3-one (m.p. 140-141°, $[\alpha]_D$ + 35° $CHCl_3$; reported (10): m.p. 140-141°, $[\alpha]_D$ + 38° $CHCl_3$). Reduction of the latter compound with lithium aluminum hydride gave 5α-androst-16-en-3β-ol (m.p. 126-127°, $[\alpha]_D$ + 16.1° $CHCl_3$; reported (10): m.p. 125-127°, $[\alpha]_D$ + 11.2 ± 2.5° $CHCl_3$) which on treatment with osmium tetroxide gave 5α-androstane-3β,16α,17α-triol ($[\alpha]_D$ - 17.1 $CHCl_3$) identical in infrared spectrum and melting point with that isolated from "rayless goldenrod". It was found that 5α-androstane-3β,16α,17α-triol exhibits two melting points, one at 265-270° and another at 280° if the material is allowed to resolidify after first melting.

Huffman and Lott (11) suggested that the product was obtained by hydroxylation of 5α-andros-16-en-3β-ol with osmium tetroxide was 5α-androstane-3β,16β,17β-triol since it was not identical with the product (m.p. 251-253°, $[\alpha]_D$ + 18°) they obtained on reduction of Butenandt's triol (12) (androst-5-ene-3β,16α, 17α-triol ?). This conclusion is most probably incorrect in view of the recent studies of Brutcher and Bauer (13) on the conformations of the D rings in steroidal 16,17-cis glycols. The product of hydroxylation of andros-16-en-3β-ol acetate with osmium tetroxide was found to possess a D ring half-chair conformation containing 16α,17α hydroxyl groups. In addition, the closely related estra-1,3,5 (10)-trieme-3, 16α,17α-triol is prepared in an analogous manner from the corresponding C_{16} olefin.

5α-Androstane-3β,16α,17α-triol may have been isolated previously by Butler (14) from "rayless goldenrod" since he reported the presence of an unidentified sterol of m.p.258 in the residue left after distillation of the crude toxin. We have isolated a second sterol ($C_{29}H_{48}O$, m.p. 152-156, α_D - 9 $CHCl_3$) which appears to be isomeric with "α" spinasterol (15) from the non-ketone fraction of the crude toxin. "White snakeroot", a plant which produces a syndrome in higher animals similar to that produced by "rayless goldenrod", contains several benzofurans related to toxol and, in addition, has been reported to contain two unidentified sterols: Sterol I ($C_{30}H_{50}O$, m.p. 184.5-185.5°, $[\alpha]_D$ + 57.2° $CHCl_3$) and sterol II ($C_{21}H_{34}O$, m.p. 147-148°, $[\alpha]_D$ - 32.8 $CHCl_3$) (16).

This is believed to be the first report of the isolation of 5α-androstane-3β,16α,17α-triol from any natural source and its presence in the plant kingdom is particularly intriguing because of its close relationship to the urinary sterols such as 5α-androstane-3α,16α,17β-triol, 5β-androstane-3α,16α,17β-triol and andros-5-ene-3β, 16α,17β-triol.

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