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ISOLATION OF A NOVEL STEROL: STIGMASTA-8(14),22-DIEN-3β-OL.

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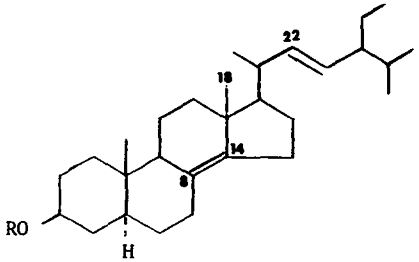
The toxic plant "rayless goldenrod" (Aplopappus heterophyllus) has been previously reported[1] to contain two members of the small group of naturally occurring simple benzofuran derivatives: toxol (2S-isopropenyl-3S-hydroxy-5-acetyl-2,3-dihydrobenzofuran)[2] and dehydrotremetone (2-isopropenyl-5-acetylbenzofuran). Using modified isolation procedures we have since found tremetone (2S-isopropenyl-5-acetyl-2,3-dihydrobenzofuran)[2], previously isolated[3] from "white snakeroot", and the previously unreported 2,5-diacetylbenzofuran.[4]

Of even greater interest was the isolation of 5α-androstane-3β, 16α, 17α-triol from "rayless goldenrod", since this represented the first androstane derivative related to the urinary steroids to be isolated in the plant kingdom.[5] We now wish to report the occurrence in "rayless goldenrod" of another unusual sterol; namely, stigmasta-8(14),22-dien-3β-ol (I).



I R = H

II R = $COCH_3$

Sterol I was isolated by crystallization of the non-saponifiable methanolic plant extract or by chromatography (alumina) of the non-ketonic fraction of the methanolic plant extract. However, a persistent contaminant (glc), identified tentatively from the mass spectrum of slightly impure I as stigmasta-8(14)-en-3β-ol (Calc. for $C_{29}H_{50}O$: M.W. 414.386. Found M.W. by mass spec.

414.387) was only removed by repeated conversion of I to its acetate (II), recrystallization of the acetate and saponification to give back I. The infrared spectrum of I (m.p. 165-166°; Calc. for $C_{29}H_{48}O$: M.W. 412.371, found M.W. by mass spec. 412.376) in $CHCl_3$ showed an O-H band at $3600cm^{-1}$ and the presence of a trans disubstituted double bond by a weak band at $1597cm^{-1}$ and a stronger band at $974cm^{-1}$. The presence of a side chain double bond was also indicated by the appearance of a signal for one methyl group (C-18) at high field (δ0.53) in the n.m.r. spectra of I and II and by the presence of a vinylic multiplet centered at δ5.06 integrating for two protons. The presence of a nuclear, tetrasubstituted double bond was indicated by the mass spectrum, absence of other vinylic proton signals in the n.m.r. and by a positive Tortelli-Jaffe test.[6] Ozonolysis of I, followed by steam distillation, gave a volatile aldehyde which on Tollens oxidation gave an acid whose amide was found to have the same R_f by tlc as 2-ethyl-3-methylbutyramide and to be different from 2,3-dimethylbutyramide. The mass spectrum of I provided further evidence for the presence of a $C_{10}H_{19}$ side chain by showing intense peaks at m/e 273.223 ($M^+-C_{10}H_{19}$) and m/e 255.210 ($M^+-C_{10}H_{19}-H_2O$). The carbon skeleton of I and the nature of the side chain were firmly established by hydrogenation of II in chloroform in the presence of platinum oxide to give the dihydroacetate, which still gave a positive Tortelli-Jaffe test but no longer showed the $974cm^{-1}$ band in the I.R. Further hydrogenation in acetic acid containing perchloric acid over PtO_2 gave the tetrahydroacetate which no longer gave a positive Tortelli-Jaffe test. Saponification of the tetrahydroacetate followed by chromic acid oxidation and Huang-Minlon reduction gave stigmastane, identical by glc with an authentic sample but different from authentic samples of ergostane and cholestane.

The exact position of the nuclear double bond was determined by the method of Jones et al.[7] which involved conversion of the non-volatile acid, from the above mentioned ozonolysis, to its methyl ester followed by osmylation, reduction with lithium aluminum hydride and finally oxidation with lead tetraacetate to give a colorless oil, the I.R. spectrum of which showed carbonyl bands at $1734cm^{-1}$ and $1725cm^{-1}$ indicating the presence of a five-membered ketone. In addition, the U.V. spectra of I and II showed the characteristic strong end absorption reported by Henbest et al.[8] for $\Delta^{8(14)}$ double bonds (for I, ε_{205}^{MeOH} 9542, ε_{210} 6274, ε_{220} 3137; for II ε_{205}^{MeOH} 9765, ε_{210}6909, ε_{220} 2856). Likewise, on the basis of molecular rotation differences[9] the nuclear double in I can be assigned as $\Delta^{8(14)}$; thus for I; $\Delta_{acetate}$ = -48, $\Delta_{benzoate}$ -67, Δ_{ketone} +82.

Finally, dihydro-I, prepared by hydrogenation of I under neutral conditions, and the corresponding acetate, showed melting points and optical rotations similar to those previously reported[10] for α-spinastenol (stigmast-8(14)-ene-3β-ol) and its acetate respectively.

Sterol I is, as far as we are aware, the first authenticated example of a naturally occurring 5α-stigmast-8(14)-ene and its method of isolation precludes its formation by rearrangement from a naturally occurring $\Delta^{8(9)}$ or $\Delta^{7(8)}$ precursor. It is interesting that nature has provided so many of the thermodynamically less stable stenols as compared to the more stable $\Delta^{8(14)}$ stenol and this may provide a clue to the biosynthetic introduction of the nuclear double bond. 5β-Stigmas-8(14)-en-3β-ol has been reported to occur in Daemia extensa.[11] Recently[12] the isolation of another new sterol, stigmasta-7,24(28)-dien-3β-ol, isomeric with I was reported.

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