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HYDROCARBON AND STEROIDAL CONSTITUENTS OF *GYMNOCLADUS DIOICA*

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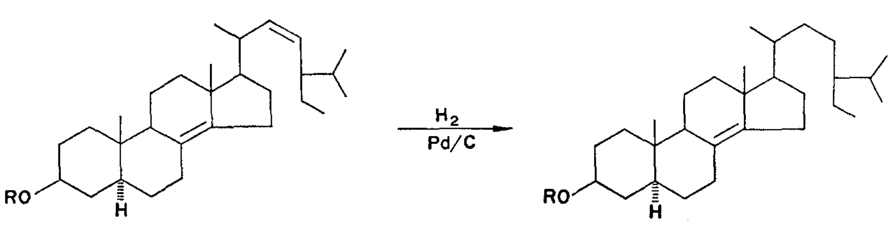
Key Word Index—*Gymnocladus dioica*; Leguminosae; hydrocarbons; sterols; 5α-stigmast-8(14), 22-dien-3β-ol.

Whole leaves of *Gymnocladus dioica* L. (Wayne State University, Department of Biology herbarium, voucher accession No. 15783) were collected in September, 1968 on the flood plain of the Grand River near Grand Ledge, Michigan. The ground leaf material was extracted with light petrol. (b.p. 60–90°) followed by MeOH. The petrol extract was found to contain *n*-nonacosane (m.p. 63–63·5°). The MeOH extract was stirred in hot 10% aq. HCl for 30 min. The acidic mixture was extracted with $CHCl_3$, and the $CHCl_3$ extract washed with 10% NaOH prior to concentration to a thick green oil which was chromatographed on silica gel. Elution with C_6H_6 gave an oil containing methyl oleate and methyl linoleate, identified by NMR and GLC (R_t). Other constituents were not identified. The fraction obtained an elution with C_6H_6–$CHCl_3$ (19:1) contained 1-heptacosanol (m.p. 81–83°), identified by conversion to the acetate (m.p. 57–58°). IR, NMR, and elemental analysis data were consistent for both alcohol and acetate.

Fractions obtained on elution with C_6H_6–$CHCl_3$ (3:1–1:3) contained a crystalline substance which after purification by preparative TLC had m.p. 156–157·5° and a parent ion at *m/e* 412 in the MS. The elemental analysis was in good agreement with a molecular formula ($C_{29}H_{48}O$ (Found: C, 82·43, H, 11·16. Required: C, 81·86; H, 11·13). This substance gave a precipitate with digitonin and its IR spectrum showed OH stretching at 2·76 μ. The presence of unsaturation in the compound was indicated by bands at 6·10 and 10·30 μ in the IR and was confirmed by Br_2 decoloration. In addition, it gave a positive Tortelli–Jaffe test,[1] characteristic of sterols with a tetra-substituted nuclear double bond. This data together with the NMR and MS appear consistent with the formulation, 5α-stigmast-8(14),22-dien-3β-ol (I).



(I) R = H
(II) R = Ac

(III) R = Ac
(IV) R = H

The positions of the side chain methyl and olefinic signals in the NMR spectrum of I were superimposable with the same signals in an NMR spectrum of authentic stigmasterol. The positions of the C-18 and C-19 methyl singlets (52·0 and 41·0 Hz, resp.) agreed well

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[1] FIESER, L. F. and FIESER, M. (1959) *Steroids*, p. 114, Reinhold, New York.

with the calculated[2] values (50·5 and 42·0 Hz, resp.) for structure I. The possibility that the tetra-substituted nuclear double bond might be in the 8(9) position rather than 8(14) was ruled out by the calculated[2] values for the C-18 and C-19 methyl singlets. The value calculated for C-18 in the 8(9) isomer was 35 Hz and that for the C-19 singlet was 55·5 Hz. The low downfield position of the C-19 methyl resonance in I was confirmed in the NMR spectrum of its acetate (II). As expected, the acetyl group caused an upfield shift of the C-19 resonance to 43·0 Hz and had no effect on the C-18 resonance.

A strong peak at *m/e* 271 in the MS of I can be explained by a McLafferty rearrangement from C-22 to C-15 or from C-22 to C-12 following methyl migration (from C-13 to C-17) in the molecular ion.[3] A strong peak at *m/e* 273 in the MS of I is most likely due to cleavage of the 17–20 bond.

Hydrogenation of II at atmospheric pressure using palladium on charcoal in ethyl acetate afforded 3β-acetoxy-5α-stigmast-8(14)-ene (III), also known as α-spinastenol acetate. Both the acetate (III) and the parent sterol (IV), obtained by alkaline hydrolysis of III, had m.ps in close agreement with lit. values. An NMR of III was practically identical to the spectrum[4] of an authentic sample of α-spinastenol acetate.

The MS of IV showed a parent ion at *m/e* 414 and a strong peak at *m/e* 273. No significant peak was noted at *m/e* 271. Clark-Lewis and Dainis[5] have stated that ions at *m/e* 248 and 234 appear to be characteristic of both α-spinastenol and its acetate but these peaks did not appear especially prominent in our MS of III.

5α-Stigmast-8(14),22-dien-3β-ol (I) has been reported by Zalkow *et al.*[6] as occurring in *Aplopappus heterophyllus*. Although these workers based their structural assignment on certain chemical and physical data, they neglected the fact that their reported position (32·0 Hz) of the C-18 methyl resonance in the NMR of I was considerably upfield from its calculated position (52·0 Hz).

The possibility yet exists that compound I may actually be an artifact obtained in the fractionation of the acidic, basic and neutral substances from *Gymnocladus dioica*. Such a $\Delta^{8(14),22}$ diene could conceivably arise by isomerization of a thermodynamically less stable $\Delta^{7,22}$ diene during treatment with acid to remove the basic materials in the leaf extract.

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[2] BHACCA, N. S. and WILLIAMS, D. H. (1964) *Applications of NMR Spectroscopy in Organic Chemistry*, p. 19, Holden-Day, San Francisco.

[3] For an interpretation of the MS of some Δ^{22} sterols see, WYLLIE, S. G. and DJERASSI, C. (1968) *J. Org. Chem.* **33,** 305.

[4] The spectrum was kindly supplied by Prof. J. W. CLARK-LEWIS, The Flinders University of South Australia. The only exception appeared in the position of the acetyl methyl singlet which appeared at 115 Hz in the authentic sample and at 121 Hz in our sample. This may be due to solvent differences since the spectrum supplied by Prof. Clark-Lewis was taken in CCl_4 while our spectrum was run in $CDCl_3$.

[5] CLARK-LEWIS, J. W. and DAINIS, I. (1967) *Australian J. Chem.* **20,** 1971.

[6] ZALKOW, L. H., CABAT, G. A., CHETTY, G. L., GHOSUL, M. and KEEN, G. (1968) *Tetrahedron Letters* 5727.