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THE NONALKALOIDAL CONSTITUENTS OF *RAUWOLFIA VOMITORIA* AFZUELIA

Abdul Malik and Salimuzzaman Siddiqui

H.E.J. Postgraduate Institute of Chemistry, University of Karachi, Karachi 32

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Sterolic constituents and fatty acids of *Rauwolfia vomitoria* have been isolated and identified. The chemical and spectral studies on serposterol provided evidence for the structure: 28-hydroxy stigmasterol.

INTRODUCTION

As a result of studies on the nonalkaloidal petroleum-ether soluble fraction of the alcoholic extract of *Rauwolfia vomitoria,* serposterol, β-sitosterol, stearic, palmitic, behenic, oleic, linoleic and linoleinic acids, have been isolated and identified. Apart from serposterol [1,2] all other constituents were hitherto unreported from *Rauwolfia vomitoria.* The chemical and spectral studies carried out on serposterol, have provided evidence for the structure: 28-hydroxy stigmasterol.

Serposterol (m.p. 159–60°) was first reported by Siddiqui and Siddiqui in 1931, from the freshly collected and shade dried roots of *Rauwolfia serpentina.* It showed close resemblance to mykosterol (m.p. 159–60°), isolated by Tokeo Ikeguchi, in 1919 from the fungus *Collybia schiitake* [3], but owing to the unaccountable difference in the optical rotatory power, the name serposterol was provisionally assigned to it by them. As a result of analytical data and mass spectrometery, the formula $C_{30}H_{48}O_2$ earlier proposed by Siddiqui and Siddiqui [1], has now been modified to $C_{29}H_{48}O_2$ (M^+428). The two oxygen atoms were shown to be present in alcoholic groups through the formation of crystalline diacetate (m.p. 126°), dibenzoate (m.p. 132–34°), dimethyl ether (m.p. 126°), and diphenyl urethane (m.p. 146°). Perbenzoic acid titration of the diacetate indicated the presence of two double bonds. On hydrogenation over palladium catalyst, serposterol gave saturated tetrahydro derivative–serpostanediol $C_{29}H_{52}O_2$; m.p. 171–2°; $(\alpha)^{25}_D = 62°$ ($CHCl_3$). It also absorbed two moles of bromine in chloroform at 0° to furnish tetrabromo serposterol $C_{29}H_{48}O_2.Br_4$, m.p. 179–80°. This meets the requirement that a doubly unsaturated diol of molecular formula $C_{29}H_{48}O_2$ must have a tetracyclic structure.

The nature of both the alcoholic groups was found to be secondary by selective oxidation of serposterol through Jones' reagent to a diunsaturated nonconjugated diketone which melted at 138–40°, analysed for $C_{29}H_{44}O_2$ (M^+ 424) and showed bands in the IR spectrum at 1720 cm^{-1} (nonconjugated carbonyl groups) and 1650–1660 cm^{-1} (C=C). On Meerwein–Ponndorf reduction it yielded back the parent alcohol.

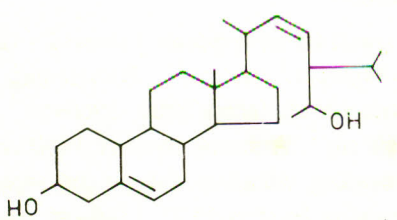
The IR spectrum of serposterol showed –OH stretching at 3380–3400 cm^{-1}, olefinic C–H stretching (3010 cm^{-1}), aliphatic CH stretching 2900–2960 cm^{-1}, and C=C at 1650–1660 cm^{-1}. A strong band at 1460 cm^{-1} represents C–H bending vibration due to the aliphatic side-chain while another band at 1370–1380 cm^{-1} is attributed to C–H bending vibration of terminal isopropyl group. The band at 1070–1080 cm^{-1} is due to C–OH stretching of secondary carbinol in 6-membered ring. Additional peaks at 1200, 1020, 970–72, 960, 840 and 800 cm^{-1}, are fairly in agreement with 3-β-hydroxy-Δ^5-sterolic skeleton [4,5].

Mass spectral studies provided molecular ion peak at *m/e* 428 and other common sterolic peaks at *m/e* 413 $(M-CH_3)^+$ 410 $(M-H_2O)^+$, 395 $(M-H_2O+CH_3)^+$, 392 $(M-2H_2O)^+$, 385 (M-terminal isopropyl group)$^+$, and 367 (M-isopropyl group+H_2O)$^+$. The base peak at *m/e* 273 (M-side chain)$^+$ and intense peaks at 246 (M-side chain-27)$^+$, 228 (M-side chain-27-H_2O)$^+$; 231 (M-side chain-42)$^+$; 231 (M-side chain-42)$^+$; 213 (M-side chain-42-H_2O)$^+$, supported the presence of 1 double bond in the nucleus [6,7]. The prominent peaks at *m/e* 371 $(M-57)^+$ and 57 allowed the placement of double bond in the position Δ^5 [8,9]. The sterolic nucleus is, therefore, similar to β-sitosterol and stigmasterol.

The major peaks at *m/e* 271 (M-side chain-2H)$^+$ and 259 (M-side chain –2H–H_2O)$^+$ are indicative of monounsaturation in the $C_{10}H_{18}OH$ side-chain [6,7]. Another abundant ion at *m/e* 300 represents the cleavage of the

C-20 to C-22 bond with the hydrogen transfer to the $C_8H_{15}OH$ neutral fragment which is a known fragmentation of Δ^{22} sterols [6–8]. The position of double bond at Δ^{22} was further supported by other peaks at *m/e* 285 $(M\text{-}C_8H_{16}O+CH_3)^+$ and 282 $(M\text{-}C_8H_{16}O+H_2O)^+$.

The reactions described for serposterol were closely parallel to those of stigmasterol and the resemblance was fully borne out by the spectral data. The peaks resulting from the sterolic nucleus in the mass spectrum are common to both the sterols, which suggested that serposterol differs from stigmasterol in having an additional OH group in the side-chain. The only position that imparts secondary nature to it is 28 and this was supported by a moderate peak in the mass spectrum at *m/e* 45 resulting from the cleavage of 24–28 bond with charge retention on oxygen atom. Such α cleavages are common for sterols containing alcoholic group in the side chain [5]. On the basis of foregoing evidence the following structure is provisionally assigned to serposterol.



Further support for this structure is obtained from the proton NMR spectrum in deuterated chloroform. A one-proton triplet at δ 5.36–5.4 is due to C_6 olefinic proton split by adjacent protons at C–7. A slightly upfield two-proton signal comes at δ 5.17 and represents C-22 and C-23 olefinic protons of the side chain. The two proton multiplet at δ 3.65 is attributed to *geminal* protons at C-3 and C-28, shifted downfield due to electron withdrawing hydroxylic groups. The sharp three-proton singlets at δ 0.7 and 1.03 represent C-18 and C-19 angular methyl groups [11,12] while C-21 methyl group comes as a distorted doublet at δ 0.94 [6,7]. The six-methyl protons of isopropyl group occur together as undifferentiated signals between δ 0.58–0.89. Another distorted doublet at δ 1.2 C-29 is angular methyl group which is shifted downfield due to the OH group at C-28 [11,12].

EXPERIMENTAL

Methanolic extract of *Rauwolfia vomitoria* (2 kg) was partitioned between ethyl acetate and water. The residue from the ethyl acetate fraction was digested out with petroleum ether and the soluble extract was successively shaken out with acetic acid (30%) and 1:1 methanol–(HCl 10%) to remove the residual basic material. After washing and drying the petroleum ether fraction and removing the solvent 84 g of neutral constituents remained as reddish yellow oily residue. It was saponified by refluxing with alcoholic potash (10%) for 4 hr. On working up the hydrolysate in the usual manner, 38.5 g unsaponifiable constituents and 40.5 g acids were respectively obtained.

From the saturated acid fraction stearic and palmitic acids were obtained through fractional crystallization from alcohol, and behenic acid from the mother liquors by column chromatography over activated neutral alumina. Out of the alcohol-soluble lead salt fraction, oleic acid was obtained through low temperature fractional crystallization from acetone, and its identity confirmed by catalytic reduction to stearic acid and hydroxylation to 9,10-dihydroxy stearic acid, m.p. 131° (lit. m.p. 131°).

GLC of Fatty Acids. Fifty mg of the total acids alongwith 20 mg heptane decanoic acid, were methylated with diazomethane, taken up in heptane and analyzed by GLC using Fischer–Victoreen aerograph instrument at a temperature range of 180–82°. The peak areas were calculated by an automatic ball and disc integrator and the percentage of each acid was determined by comparing its peak area with that of heptane decanoic acid used as internal reference. The relative percentage yields were palmitic (13.2); stearic (33.8); oleic (40.5); linoleic (traces); linolenic (5.4); and behenic acids (7.1%).

The unsaponifiable matter which showed on TLC the major concentration of two spots, was divided up into acetone-soluble and insoluble portions. The latter showed greater concentration of one of the spots and was subjected to fractional crystallization from methanol–acetone to afford from the top fractions a silky white crystalline sterol which melted at 160° and gave no depression on admixture with an authentic sample of serposterol. (Found: C 81.3, H 11.4, O 7.30%, M^+ peak at 428. Calcd. for $C_{29}H_{48}O_2$: C 81.3, H 11.23, O 7.47%; mol. wt. 428).

The residue from the acetone-soluble fraction yielded through fractional crystallization and column chromatography over activated neutral alumina, further harvests of serposterol (total yield 0.5% on the weight of methanolic extract) alongwith β-sitosterol as colourless needles, m.p. 138–39° $(\alpha)^{25°}_{D}$ = 36° ($CHCl_3$). (Found: C 84.1, H 12.02%; M^+ peak 414. Calcd. for $C_{29}H_{50}O$: C 84, H 12.07%; mol. wt. 414). The identity of β-sitosterol was confirmed through optical rotation and superimposable spectral data as well as formation of crystalline acetate, m.p. 132° (lit. m.p. 131–35°) and benzoate, m.p. 142° (lit. m.p. 141–42°).

Characterization of Serposterol

Serposterol Diacetate. To a solution of 50 mg sterol in dry pyridine (1 ml), acetic anhydride (2 ml) was added, refluxed for 1 hr, diluted with water and extracted with ether. On working up the product the residue crystallised out from methanol as colourless rectangular plates of diacetate, m.p. 126°. (Found: C 77.14, H 10.25, O 12.60%, $C_{33}H_{53}O_4$ requires: C 77.34, H 10.16, O 12.5%). The IR spectrum showed carbonyl absorption at 1730 and C=C at 1650 cm^{-1}.

Serposterol Dibenzoate. It was prepared in exactly the same manner as diacetate through benzoyl chloride. It crystallized from methanol as colourless shining needles, m.p. 132–4°. (Found: C 81.20, H 8.80, O 10.10%. $C_{43}H_{56}O_4$ requires: C 81.13, H 8.80, O 10.06%). The IR spectrum showed carbonyl absorption at 1700 cm^{-1} and C=C at 1650–1660 cm^{-1}.

Serposterol Dimethyl Ether. To a solution of 50 mg serposterol in acetone was added two ml dimethyl sulphate and one ml aqueous sodium hydroxide (5%). The mixture was refluxed for 2 hr with constant stirring, poured in cold water and extracted out with ether. On working up the product serposterol dimethyl ether crystallized out from methanol as colourless shining prismatic rods, m.p. 128°. The IR spectrum showed C=C at 1650–1660 and OCH_3 at 1260 cm^{-1}.

Diphenyl Urethane of Serposterol. To a solution of 100 mg serposterol in 3 ml dry benzene was added 5 ml phenyl isocyanate. After keeping overnight the supernatant liquid was decanted from a small quantity of carbanilide formed. The residue left on removal of solvent from the decantate was extracted out with hot petroleum ether, rejecting the insoluble carbanilide. On concentration and cooling in the ice chest diphenylurethane of serposterol was obtained as colourless spike-shaped needles, m.p. 146°.

Perbenzoic Acid Titration of Serposterol Diacetate. To 40 mg. of serposterol diacetate in 10 ml $CHCl_3$ at 0° was added 5 ml 0.3*N* perbenzoic acid in benzene. The reaction mixture was allowed to stand at 0° in the dark. At the end of 48 hr an aliquot was withdrawn and added to 25 ml water containing 1.5 g KI and 1.5 ml glacial acetic acid. The liberated iodine was titrated against a standard solution of sodium thiosulphate. A blank was similarly treated. The sample consumed the equivalent of 2 double bonds.

Serpostanediol. Serposterol was taken up in absolute alcohol and hydrogenated over palladium catalyst at the atomospheric pressure for 24 hr. The solution was filtered, freed of solvent and the residue crystallized from methanol to afford fine needles of serpostanediol, m.p. 171–2°; $(\alpha)^{25}_D = +62°$ ($CHCl_3$) and M^+ peak at 432.

Oxidation of Serposterol. Serposterol (0.4 g) was dissolved in acetone and treated with 6 ml of Jones' reagent. The mixture was stirred for 6 hr checking the completion of the reaction from time to time on TLC. A little sodium bisulphite was then added to discharge the brown colour, the solution was poured into crushed ice and extracted with ether. The ethereal fraction was chromatographed over neutral alumina when diketo- derivative of serposterol crystallized out from methanolic-acetone as leaflets of colourless crystals, m.p. 138–40° (yield 60%). On Meerwein–Ponndorf reduction it furnished back pure serposterol. (Found: C 81.95, H 10.50; M^+ peak 424. Calcd. for $C_{29}H_{44}O_2$: C 82.07 H 10.37; mol. wt. 424).

Bromination of Serposterol. Serposterol (0.2 g) in 2 ml dry chloroform was titrated with 1.5% w/v solution of bromine in chloroform at 0°. The solvent was removed in rotary evaporator at room temperature, the residue was repeatedly washed with petroleum ether when on rubbing with the same solvent tetrabromoserposterol was obtained as a colourless microcrystalline powder giving a single spot on TLC, m.p. 177–78°. (Found: Br 42.59%. $C_{29}H_{48}O_2.Br_4$ requires: Br 42.78%). On debromination with NaI in alcohol it afforded back the parent alcohol.

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