Central Drug Research Institute, Lucknow, India

STUDIES ON THE CHEMICAL CONSTITUENTS OF *SPONDIAS PINNATA*

By Sheela TANDON and R. P. RASTOGI

Abstract

The isolation of 24-methylene cycloartanone, stigma-4en-3one, lignoceric acid, β-sitosterol and its β-D-glucoside from Spondias pinnata have been described.

Spondias pinnata (*Anacardiaceae;* local name AMRA or AMRATAKA) is commonly distributed over the sub-Himalayan tract upto 3000' and Western Peninsula. The aerial parts of the plant are reported to have medicinal value [1]. *S. lutea*, *S. purpurea* and other South American species have been investigated for their amino acids, Vitamin and carotene content and pharmacological screening of *S. lutea* was found to exhibit depressor cardio-respiratory activity [2]. In another biological screening report the Indian species, *S. pinnata*, was found to possess CNS depressent activity [3].

The ethanolic extract of the plant material was fractionated into hexane, chloroform and aqueous fractions. Column chromatography of hexane and chloroform fractions over alumina and silica gel respectively, and subsequent chromatographic purification procedures yielded 24-methylene cycloartanone, stigma-4en-3one, β-sitosterol, lignoceric acid, and β-sitosterol β-D-glucoside. It may be mentioned that stigma-4en-3one (Δ^4β-sitostenone) has been reported so far from three plants only i.e. *Quassia amara* [4], *Pinus silvestris* [5] and *Tabebuia rosea* [6]. The identity of the compounds was achieved by spectroscopic data, derivative formation and mixed melting point determination.

Experimental

The melting points are uncorrected. The Rf values pertain to TLC plates prepared with silica G and sprayed with 1% ceric sulphate solution in 2 N H_2SO_4. The PMR spectra were recorded on varian A-60 spectrometer in $CDCl_3$ and chemical shifts are quoted as ppm on δ scale. Mass spectra were run on Hitachi-Perkin Elmer model RMU-6. The infrared spectra were taken in KBr pellet on Perkin Elmer Spectrometer.

Extraction of the plant material

The aerial parts of the plant were collected from Darjeeling area, West Bengal (India), dried

under shade and the powdered plant material (14 kg) was exhaustively extracted with ethanol. Concentration of extract at 40°/vacuo left a dark brown viscous mass which was successively macerated with solvents leading to the isolation of hexane-soluble (103 g), chloroform (15 g) and aqueous fractions. The major hexane fraction was chromatographed on alumina column whose elution with chloroform and ethanol-acetic acid (98:2) led to the separation of neutral (53 g) and acidic (50 g) components respectively. The neutral fraction was subjected to silica gel chromatography (from benzene up to methanol, 100 ml each 64 fractions).

Isolation and Identification of 24-methylene-cycloartanone

From fraction 6–17 (Benzene), a residue (2.47 g) was obtained by chromatographic purification over silica gel column which after crystallization from alcohol yielded colourless plates (1.55 g) m.p. 103–105°, $(\alpha)_D$ + 24° (c, 1.2, $CHCl_3$). It gave Rf 0.5 in benzene and analysed for $C_{31}H_{50}O$ (M+ 438). IR: 1720 (C=O in six-membered ring). 1005 (cyclopropane), 1635 and 884 cm^{-1} (C=CH_2). PMR: 0.34, 0.61 (1 H each d. J = 5 Hz, cyclopropane) 0.89–1.18 (21 H, 7 CH_3), 4.72 (2 H, m, C=CH_2). MS: m/e 438 (M+) 423, 300, 313, 175.

On $NaBH_4$ reduction, it yielded an alcohol, $C_{31}H_{52}O$, which crystallized from alcohol, m.p. 115–116°, $(\alpha)_D$ + 52° (c, 1.0, $CHCl_3$), Rf 0.40 in benzene. Its IR spectrum did not show the carbonyl absorption but band for hydroxyl group appeared at 3400 cm^{-1}. Similarly its PMR spectrum showed, in addition to the signals of original product, a carbinol (–*CH*OH) proton as multiplet at 3.3 ppm. MS: m/e 440 (M+), 424, 422, 407, 379, 353, 300 (base peak). It was identified as 24-methylene cycloartanol (mmp, IR, PMR, MS).

Isolation and Identification of Stigma-4en-3-one

The fraction 26–46 (benzene-methanol 99:1) was rechromatographed over alumina which afforded crystalline material from acetone-CH_2Cl_2 (2.25 g) m.p. 85–86°, $(\alpha)_D$ + 84° (c, 0.65, $CHCl_3$), Rf 0.51 (benzene-methanol 99:1), analyzed for $C_{29}H_{48}O$ (M+ 412) and show uv maxima at 241 mμ (log ε, 4.227). IR: 2940, 2850 (CH_2, CH_3), 1675 (α, β unsaturated C=O), 1615, 868 cm^{-1}

(trisubstituted double bond). PMR: 0.7–1.2 (18 H, 6 x CH_3), 2.3 (2 H, m, –CH_2–C–CH), 5.7 (1 H, br s, –COCH=C). MS: m/e 412 (M_+), 370, 327, 289, 271, 229, 124.

It formed an oxime with hydroxylamine hydrochloride, which crystallized from alcohol, m.p. 181°, $C_{29}H_{49}ON$, (M_+ 427), IR: 3226 (OH) 1639 cm^{-1} (>C=N).

On $NaBH_4$ reduction, it formed two epimeric alcohols (Rf 0.36 and 0.45 in benzene-methanol 99:1). The major spot (Rf 0.36) could be isolated only through benzoyl derivative and crystallized from acetone, m.p. 154–5°, $C_{29}H_{50}O$ (M+ 414), IR: 3344 (OH), 2941 (CH_2, CH_3), 1650 (C=CH), 1471, 1389, 1020, 962, 806 cm^{-1}. PWR: 0.7–1.2 (18 H, 6 x CH_3), 4.15 (1 H, m, CHOH), 5.3 (br s, –CH=C<) MS: m/e 414 (M_+) 396, 382, 344, 288, 255, 229, 203, 149. It was identified as allositosterol (7) which confirmed the original substance as Δ^4 β-sitostenone.

Isolation and Identification of β-Sitosterol

From fraction 47–59 a material was obtained which crystallized from alcohol as colourless needles (2.5 g), m.p. 138°, $(\alpha)_D$ – 35°. It showed a positive Libermann-Burchard reaction and formed an acetyl derivative, m.p. 128°.

Isolation and Identification of lignoceric acid

A portion of the acidic fraction (5 g) was chromatographed over silica gel column, when chloroform eluate yielded a fraction (1.69 g) which crystallized from methanol containing a little acetic acid, m.p. 75–77°, $C_{24}H_{48}O_2$ (M_+ 368). IR: 2900, 2850 (CH_2, CH_3), 1700 (C=O), 1450,

1390, 1300, 720 cm^{-1}. PMR: 0.88 (3 H, t, CH_2CH_3), 1.26 (42 H, s, $[CH_2]_{21}$), 2.2 (2 H, t, $-CH_2CO$). MS: m/e 368 (M_+), 340, 325, 311, 297 upto 171 each sharing a loss of 14 mass units.

Its methylation with diazomethane gave an oily ester, Rf 0.7 (benzene-hexane 4:1). IR showed 1742 cm^{-1} (ester) and PMR exhibited the carbomethoxy methyl at 3.68. MS: m/e 382 (M_+).

Isolation and identification of -sitosterol- -D-glucoside

A portion of the (1.4 g) of chloroform fraction was chromatographed over silica gel when ethylacetate-methanol (98:2) fraction gave colourless needles from methanol (180 mg), m.p. 277–8°, $(\alpha)_D - 46°$. A positive Fiegal test indicated it to be a glycoside. The acid hydrolysis, in usual manner, yielded an aglycone which was identified as β-Sitosterol and the glucose was confirmed in the aqueous fraction by paper chromatography.

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Address: Dr. R. P. Rastogi, Central Drug Research Institute, Lucknow, India