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STERCULIACEAE

PHYTOCHEMICAL STUDIES OF THE FLOWERS OF *PTEROSPERMUM ACERIFOLIUM*

S. A. I. RIZVI and TAJWAR SULTANA*

Chemical Laboratories, University of Allahabad, Allahabad, India

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Pterospermum acerifolium Willd.,[1] a large tree belonging to the Sterculiaceae, is widely distributed in India particularly in sub-Himalayan tract and outer Himalayan valleys and hills up to 4000 ft. Survey of the literature reveals no work to have been done on this plant except the recent report of kaempferol and kaempferide-7-glucoside[2] from the alcoholic extracts of the flowers. In the present communication we record the isolation and characterization of 24 β-ethylcholest-5-en-3β-*O*-α-cellobioside, 3,7-diethyl-7-methyl-1:5-pentacosanolide, dilignocerate of *n*-hexacosane-1, 26-diol, friedelan-3α-ol and its β-isomer, β-amyrin, β-sitosterol, *n*-triacontanol, *n*-hexacosane-1, 26-diol, a mixture of acids and saturated hydrocarbons from the light petroleum extract of the dried flowers. The acids identified were myristic, palmitic, stearic, arachidic, behenic, lignoceric, oleic, linoleic and linolenic acids. Presence of arachidonic acid in a small quantity could only be recorded by spectrophotometry after alkali isomerization.

EXPERIMENTAL

Melting points are uncorrected. The IR spectra were recorded on Perkin–Elmer–Infracord. Satisfactory elemental analyses were obtained.

The light petroleum (60–80°) extracts of the dried *P. acerifolium* flowers (5 kg) on concentration yielded a semisolid mass (2·32%) which was resolved into ether soluble and insoluble fractions.

Examination of Ether Insoluble Fraction

The light yellow solid on repeated crystallizations from $CHCl_3$–MeOH afforded a white amorphous mass which was taken up in $CHCl_3$. The insoluble portion after necessary purification yielded a crystalline phytosterolin (1·8 g), $C_{41}H_{70}O_{11}$, m.p. 294–295°, $[\alpha]_D^{28}$ +66° (C_5H_5N), mol. wt. (Rast) 746; gave a heptaacetyl derivative, m.p. 170–171°, $[\alpha]_D^{28}$ +14° ($CHCl_3$) and a heptamethyl derivative, m.p. 90–92°, $[\alpha]_D^{28}$ +26° ($CHCl_3$). Hydrolysis of the phytosterolin (600 mg) with boiling 8% MeOH–H_2SO_4 for 6 hr gave glucose which was identified by paper chromatography and through the preparation of osazone, m.p. 202–203°. The aglycone was crystallized from MeOH–$CHCl_3$ as shining white flakes, m.p. 137–138°, $[\alpha]_D^{26}$ −36° ($CHCl_3$), ν_{max}^{KBr} 3400 cm^{-1} (OH); formed a monoacetate, m.p. 127–128°, $[\alpha]_D^{26}$ −42° ($CHCl_3$); a monobenzoate, m.p. 145–146°, $[\alpha]_D^{26}$ −15° ($CHCl_3$); a 3:5-dinitrobenzoate, m.p. 202–204°, $[\alpha]_D^{26}$ −10° ($CHCl_3$) and a digitonide m.p. 226–228°. It was identified as β-sitosterol by mixed m.p. and IR comparison with an authentic sample. Methanolysis[3] of the methyl ether followed by hydrolysis with 5% HCl at 100° gave 2,3,6-tri- and 2,3,4,6-tetra-*O*-methyl-D-glucose in 1:1 molar ratio. Hydrolysis of phytosterolin followed by determination of the sugar revealed the presence of 42·6% glucose as anhydrohexose units ($C_{41}H_{70}O_{11}$

* Requests for reprints should be addressed to Tajwar Sultana, 39-Mohammad Ali Park, Allahabad-3, U.P., India.

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requires for 2 moles of anhydrohexose 43·9%). Partial acid hydrolysis gave glucose and cellobiose which were identified by co-chromatography with authentic samples on paper and TLC. Enzymic hydrolysis of sterolin (400 mg) with emulsin yielded glucose and a degraded sterolin, $C_{35}H_{60}O_6$, m.p. 283–285°, $[\alpha]_D^{27}$ +90° (C_5H_5N); gave a tetraacetate, m.p. 162–163°, $[\alpha]_D^{27}$ +42° ($CHCl_3$) and a methyl ether, m.p. 85–86°, $[\alpha]_D^{27}$ +53° ($CHCl_3$) which upon hydrolysis yielded 2,3,4,6-tetra-*O*-methyl-D-glucose and therefore it was identified as α-glucoside of β-sitosterol[4] by mixed m.p. with an authentic sample. Periodate oxidation studies supported the existence of cellobioside moiety. Thus the phytosterolin was identified as α-cellobioside of β-sitosterol.

The $CHCl_3$ soluble fraction was resolved column chromatographically on alumina into two fractions. Fraction A obtained from C_6H_6–$CHCl_3$ yielded a wax (2·8 g), m.p. 82–84° raised to 89–90° ($C_{74}H_{146}O_4$) on rechromatography and repeated crystallizations from MeOH–$CHCl_3$, ν_{max}^{KBr} 1735 cm^{-1} (ester CO), N.E. 558; contains two C–CH_3 but no O–CH_3 group. Saponification (1·2 g) with boiling 8% ethanolic KOH for 12 hr gave an alcohol and an acid. The alcohol (380 mg), $C_{26}H_{54}O_2$, m.p. 109–110°, ν_{max}^{KBr} 3450 cm^{-1} (OH), diacetate, m.p. 75–76°, was identified as *n*-hexacosane-1,26-diol[5] by mixed m.p. and co-chromatography with an authentic sample. The acid was crystallized as white flakes (600 mg), $C_{24}H_{48}O_2$, m.p. 85–86°, N.E. 363, ν_{max}^{KBr} 1705 cm^{-1} (CO of COOH); formed a methyl ester, m.p. 57–58°. It was identified as lignoceric acid by mixed m.p. and co-chromatography[6,7] with an authentic sample. Thus the pure wax was identified as dilignocerate of *n*-hexacosane-1,26-diol.

Fraction B obtained from $CHCl_3$ eluates yielded an optically active lactone (1·2 g), $C_{30}H_{58}O_2$, m.p. 73–74°, $[\alpha]_D^{27}$ −9·6° ($CHCl_3$), N.E. 462; ν_{max}^{KBr} 2860, 1740 and 1160 cm^{-1} (aliphatic δ or higher lactone)[8]; contains $4CH_3$–C groups; saponification followed by the acidification yielded the original lactone (mixed m.p. 72–73°). Treatment of the lactone with Na/Hg in acid solution produced an acid, $C_{30}H_{60}O_2$, m.p. 47–48°, $[\alpha]_D^{27}$ −6° ($CHCl_3$) whereas reduction with $LiAlH_4$ produced a diol, $C_{30}H_{62}O_2$, m.p. 82–83°, $[\alpha]_D^{27}$ ±0° ($CHCl_3$), diacetate, m.p. 61–62°. Alkaline $KMnO_4$ oxidation of the lactone as well as diol produced the same two acids in major yields. Acid I (H_2O insoluble) $C_{23}H_{46}O_{26}$, m.p. 54–55°, $[\alpha]_D^{27}$ −5·6° ($CHCl_3$), amide, m.p. 57–58°, was identified as (−)-2D-methyl-2L-ethyleicosanoic acid[9] although direct comparison could not be made. Acid II (H_2O soluble), $C_7H_{12}O_4$ (dibasic), m.p. 71–72°, optically inactive, anhydride (liquid), was identified as 2-ethylglutaric acid[10] after surveying the literature. NMR showed a (12H) signal at 9·22 τ for four —CH_3 groups, a large (42H) signal at 8·65 τ for —CH_2— groups, a (1H) signal at 8·30 τ for CH— group, a (2H) signal at 7·38 τ for —CH_2— adjacent to carboalkoxy group and a (1H) signal at 5·28 τ for CH—O—CO—group. Thus the lactone was assigned a tentative structure: 3,7-diethyl-7-methyl-1:5-pentacosanolide.

Examination of Ether Soluble Fraction

The ether soluble portion, after purification with Fuller's earth, was saponified and separated into a mixture of fatty acids and unsaponifiable matter. The mixed acids (86 g, S.V. 190·4, I.V. 76·2, SCN. V. 55·2 were studied for the composition by the combined application of solid–liquid countercurrent distribution of urea inclusion complexes[11,12] of, (a) mixed fatty acids, and (b) solid and liquid acid fractions separated by lead–salt method, reversed phase paper partition chromatography,[6,7,13] TLC[14] and spectrophotometry.[15,16] The fatty acid components in the mixed acids have been found: myristic, 2·62, 2·80; palmitic, 6·10, 5·86; stearic, 15·43, 16·24; arachidic, 18·02, 17·68; behenic, 1·82, 2·16; lignoceric, 2·40, 2·96; oleic, 29·16, 29·60 and 30·38; linoleic, 22·30, 21·02 and 22·9 ($E_{1\,cm}^{1\%}$ in EtOH at 2340 Å 220·0); linolenic, 2·15, 1·68 and 1·98 ($E_{1\,cm}^{1\%}$ in EtOH at 2680 Å 12·5) and arachidonic, 0·6% ($E_{1\,cm}^{1\%}$ in EtOH at 3010 Å 1·55).

The unsaponifiable matter (16 g) was separated into six fractions on alumina column. Fraction I obtained from petroleum (40–60°) eluates gave white flakes (2·6 g), m.p. 39–40°, resistant towards acetylation and addition of Br_2, ν_{max}^{KBr} 2950, 2860, 1470, 1365 and 725 cm^{-1} (saturated hydrocarbon). Mass spectral studies

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showed it to be a mixture of eicosane (*m/e* 282), heneicosane (*m/e* 296), docosane (*m/e* 310), octacosane (*m/e* 394, traces) and triacontane (*m/e* 422, traces).

Fraction II obtained from petroleum (60–80°)-benzene (85:15) was crystallized from MeOH (1·2 g), $C_{30}H_{62}O$, m.p. 85–86°, ν_{max}^{KBr} 3450 cm^{-1}(OH); gave an acetate, m.p. 69–70°. Oxidation of the alcohol (600 mg) with conc. HNO_3 gave a monobasic acid, $C_{30}H_{60}O_2$, m.p. 92–93°. Thus the alcohol was identified as *n*-triacontanol[17] by mixed m.p. and co-chromatography on TLC with an authentic sample.

Fraction III obtained from petroleum–benzene (7:3) gave crystals (800 mg) from benzene, $C_{30}H_{50}O$, m.p. 197–198°, $[\alpha]_D^{30}$ +89° ($CHCl_3$), ν_{max}^{KBr} 3300 cm^{-1} (OH); gave positive tests with Liebermann–Burchardt reagent and tetranitromethane for unsaturated triterpene. It formed an acetate, m.p. 239–240°, $[\alpha]_D^{30}$ +78° ($CHCl_3$), a benzoate, m.p. 232–233°, $[\alpha]_D^{30}$ +105° ($CHCl_3$) and a *p*-nitrobenzoate, m.p. 256–258°, $[\alpha]_D^{30}$ +99° ($CHCl_3$). The triterpene was identified as β-amyrin by mixed m.p. and TLC with an authentic sample.

Fraction IV obtained from petrolcum–benzene (1:1) gave a diol (1·56 g), $C_{26}H_{54}O_2$, m.p. 110° (C_6H_6–Me_2CO), ν_{max}^{KBr} 3450 cm^{-1} (OH), acetyl derivative, m.p. 76–77°, which was identified as *n*-hexacosane-1, 26-diol by mixed m.p. and TLC with an authentic sample.

Fraction V obtained from petroleum-benzene (4:6) (2·2 g), m.p. 136–137° (MeOH–$CHCl_3$), $[\alpha]_D^{28}$ −36° ($CHCl_3$) was found homogeneous by TLC and responded to the colour reactions of sterols. It formed an acetate, m.p. 126–127°, $[\alpha]_D^{28}$ −40° ($CHCl_3$) and was identified as β-sitosterol by mixed m.p. and TLC with an authentic sample.

Fraction VI obtained from C_6H_6–$CHCl_3$ (1:1) and $CHCl_3$ eluates showed two spots on TLC in $CHCl_3$ (R_f 0·18 and 0·30). Efforts to separate them by fractional crystallization of their mixed benzoates were successful. The benzoate (600 mg), m.p. 248–249°, which separated first from $CHCl_3$–MeOH (1:1) on hydrolysis yielded a triterpene, $C_{30}H_{52}O$, m.p. 302–303° (C_6H_6), $[\alpha]_D^{30}$ +22° ($CHCl_3$), acetyl derivative, m.p. 311–312°, $[\alpha]_D^{30}$ −11° ($CHCl_3$). The residual benzoate (500 mg), m.p. 252–253° gave on hydrolysis a triterpene, $C_{30}H_{52}O$, m.p. 290–291° (C_6H_6), $[\alpha]_D^{30}$ +13° ($CHCl_3$), acetyl derivative, m.p. 294–295°, $[\alpha]_D^{30}$ +28° $CHCl_3$). Chromic acid oxidation of these two triterpenes in acetic acid medium at 30° for 4 hr yielded friedelin, $C_{30}H_{50}O$, m.p. 258–260°, $[\alpha]_D^{30}$ −27° ($CHCl_3$), the identity of which was confirmed by mixed m.p. with an authentic sample. Thus the triterpenes were identified as friedelan-3-α-ol and friedelan-3-β-ol respectivcly.

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MONOCOTYLEDONAE

GRAMINEAE

CYANIDIN 3-GLUCOSIDE FROM *OROPETIUM THOMAEUM*

K. M. M. DAKSHINI and R. K. TANDON

Department of Botany, University of Delhi, Delhi 7, India

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CLIFFORD and Harborne[1] showed that cyanidin glucosides were present in 21 species of grasses belonging to some 19 genera, but have indicated that anthocyanins of only 8 grass

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