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SHORT COMMUNICATION

EXTRACTIVES FROM THE LEAVES OF *LEUCONOTIS EUGENIFOLIA*

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Abstract—Leuconol, previously reported as a new triterpene alcohol obtained from the leaves of *L. eugenifolia*, has been shown to be a mixture consisting mainly of bauerenol, α- and β-amyrin. The leaves were also found to contain the hydrocarbons *n*-nonacosane and *n*-hentriacontane, the triterpenoids lupenyl acetate, α- and β-amyrin, and the new triterpenoid esters β-amyrenyl behenate and β-amyrenyl eicosanoate.

INTRODUCTION

Leuconotis eugenifolia is a climber which grows luxuriantly in Malaysia and Indonesia and its latex has been used in the treatment of yaws.[1] During investigations on Apocyanaceous plants, Chatterjee *et al.* isolated from the leaves of *L. eugenifolia* lupenyl acetate and an apparently new triterpene alcohol, m.p. 214–215°, which was named as leuconol.[2] Owing to the lack of material no structural work was done on leuconol.

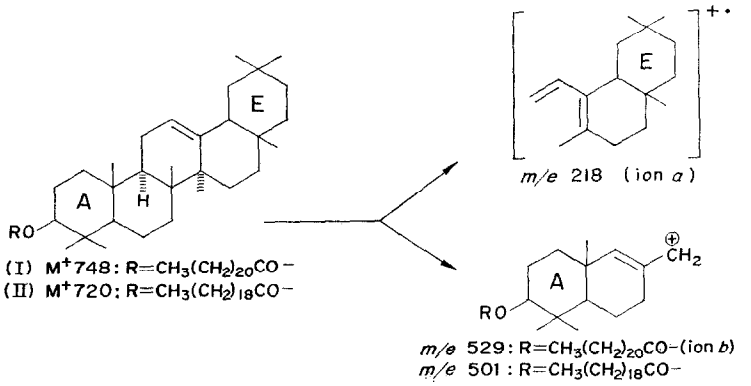
We have recently re-investigated the leaves of *L. eugenifolia* to obtain leuconol for structural studies. Extraction of the plant material with light petroleum and subsequent fractionation of the extract gave a neutral fraction which on recrystallization yielded a colourless substance, m.p. 218–220°. This substance appeared to be homogeneous on TLC in various solvent systems and had the properties ascribed to leuconol. However, careful acetylation of the sample yielded a sparingly soluble acetate which was easily separated and purified. This acetate was characterized as bauerenyl acetate and on saponification it afforded bauerenol, the triterpene previously obtained from *Acronychia baueri*[3] and *Gelonium multiflorum*.[4] The mother liquor of the acetylation mixture was found to consist mainly of a mixture of the acetates of α- and β-amyrin. Hence it seems that the substance leuconol is probably a mixture consisting of bauerenol, α- and β-amyrin.

Chromatography of the light petroleum extract has also yielded a mixture of *n*-nonacosane and *n*-hentriacontane in the ratio of about 3 to 1 respectively as shown by its mass

[1] I. H. BURKILL, *Dictionary of the Economic Products of the Malay Peninsula*, Government Printing Press, Singapore, Vol. 2, p. 1339 (1935).
[2] A. CHATTERJEE, B. DAS and S. K. ROY, *J. Indian Chem. Soc.* **36**, 92 (1959).
[3] F. N. LAHEY and M. V. LEEDING, *Proc. Chem. Soc.* 342 (1958).
[4] P. SENGUPTA and H. N. KHASTGIR, *Tetrahedron* **19**, 123 (1963).

spectrum, a mixture of triterpenoid esters, lupenyl acetate and α- and β-amyrin. β-amyrin has previously been isolated from the stems of this plant.[5]

Rechromatography of the triterpenoid ester mixture followed by recrystallization of the product from ethyl acetate furnished a substance, m.p. 79–83°, which imparted a yellow colour to tetranitromethane and showed strong IR absorptions at 1740 and 1176 cm^{-1}, indicating that it is an aliphatic ester. Analytical data suggested that it has the formula $C_{52}H_{92}O_2$ and this was confirmed by its mass spectrum which showed a molecular ion peak at *m/e* 748. The mass spectrum also showed fragments indicating that it is the ester of α- or β-amyrin with a C_{22} fatty acid, namely behenic acid. Thus, the most intense peak in the spectrum was observed at *m/e* 218 (ion *a*) which is characteristic of the triterpene of the α- or β-amyrin series.[6] The fragment bearing the behenoyl ester group also gave a peak at *m/e* 529 (ion *b*). The loss of the behenoyloxy group and behenic acid from the molecular ion gave rise to peaks at *m/e* 409 and 408, respectively. A peak at *m/e* 340 corresponds to the molecular ion of behenic acid itself formed from the triterpene ester on electron impact. The peaks at *m/e* 748, 529 and 340 were accompanied by peaks of much lower intensity at *m/e* 720, 501 and 312, indicating that the ester group is partially formed of a lower homologous acid, namely, eicosanoic acid (C_{20}).



The above spectroscopic deductions were confirmed by the alkaline hydrolysis of the ester which resulted in the isolation of β-amyrin, characterized as its acetate, and a mixture of fatty acids consisting mainly of behenic acid and eicosanoic acid and trace amounts of stearic acid and palmitic acid which were characterized as their methyl esters. Thus the triterpenoid ester mixture consisted mainly of β-amyrenyl behenate (I) and β-amyrenyl eicosanoate (II).

EXPERIMENTAL

M.ps. were determined on a Hoover capillary apparatus. IR spectra were recorded in Nujol on a Perkin–Elmer 337 instrument and the mass spectra were taken on an A.E.I. MS9 spectrometer. Light petroleum refers to the fraction of b.p. 56–70°.

Extraction. Ground leaf powder of *L. eugenifolia* (258 g) was continuously extracted with light petroleum (3 l.) for 95 hr. Evaporation of the solvent yielded a residue which was dissolved in ether and fractionated into an acidic fraction (2·0 g), and a neutral fraction (40·9 g). Recrystallization of the neutral residue from light petroleum yielded a colourless solid (0·62 g). The light petroleum mother liquor was chromatographed on an alumina column (59 × 3·8 cm). Fractions of 100 ml were collected and worked up as described below.

[5] B. DAS and R. MUKHERJEE, *J. Sci. Ind. Res., India* **21B**, 506 (1962).
[6] H. BUDZIKIEWICZ, J. M. WILSON and C. DJERASSI, *J. Am. Chem. Soc.* **85**, 3688 (1963).

Bauerenol. The colourless solid obtained above was recrystallized thrice from acetone to give colourless needles, m.p. 218–220°, which showed only one spot on various TLC systems. Acetylation of the recrystallized product (0·15 g) with Ac_2O (3 ml) and pyridine (1·5 ml) by heating the mixture on a water-bath for 0·5 hr deposited colourless plates (70 mg), which was filtered off, washed with Ac_2O (2 ml), and recrystallized thrice from $CHCl_3$–acetone as colourless plates, m.p. 288–291°. (Found: C, 81·5; H, 11·2. Calc. for $C_{32}H_{52}O_2$: C, 82·0; H, 11·2%), undepressed on admixture with authentic bauerenyl acetate,[4] and having the IR spectrum identical to that of the latter.

Saponification of the acetate with alcoholic KOH afforded a colourless solid which on recrystallization from acetone gave colourless needles, m.p. 207–208°, $[\alpha]_D$ −24·6° (c 1·87 in $CHCl_3$). Reference 4 gives bauerenol m.p. 206–207°, $[\alpha]_D$ −25° (in $CHCl_3$).

Addition of H_2O to the acetylation mother liquor remaining after the removal of bauerenyl acetate yielded a colourless solid which on recrystallization from acetone–MeOH gave needles, m.p. 200–215°. The IR spectrum of this product showed that it consisted mainly of a mixture of α- and β-amyrin acetates which could not be separated by fractional recrystallization.

Mixture of aliphatic hydrocarbons. Fractions 1–2, eluted by light petroleum from the column described above, yielded a waxy solid (0·35 g) which was recrystallized thrice from EtOAc in colourless plates, m.p. 64–67°. (Found: C, 85·3; H, 14·9. Calc. for $C_{29}H_{60}$: C, 85·2; H, 14·8%; M, 408; and $C_{31}H_{64}$: C, 85·2; H, 14·8%; M, 436.) The mass spectrum of the substance showed the M^+ peaks at *m/e* 408 and 436, in the ratio of about 3 to 1 respectively. The substance gave no colour with tetranitromethane and its IR showed a strong methylene rocking doublet at 730 and 720 cm^{-1}. Reference 7 gives *n*-nonacosane, m.p. 63·4–63·7°, and *n*-hentriacontane, m.p. 67·5–68·5°.

Mixture of triterpenoid esters. Fractions 11–56, eluted by light petroleum, yielded a colourless solid (0·43 g) which was rechromatographed on an alumina column and then recrystallized thrice from EtOAC in colourless granules, m.p. 79–83°. (Found: C, 83·4; H, 12·6. $C_{52}H_{92}O_2$ requires C, 83·4; H, 12·4% and $C_{50}H_{88}O_2$ requires C, 83·3; H, 12·3%.)

The purified product (100 mg) was dissolved in benzene (6 ml) and 12 N KOH added to it and the mixture was refluxed. The reaction was followed by TLC at different intervals of time and the saponification was found to be complete after 5 hr. The usual working up gave a neutral and an acidic fraction.

The neutral portion gave a white solid (54 mg) which was acetylated and purified by chromatography over alumina. Crystallization from light petroleum furnished β-amyrin acetate (20 mg), m.p. 238°, M^+ 468, which was identified by direct comparison (IR and mixed m.p.) with an authentic sample.

The acidic fraction was esterified with diazomethane and the mass spectrum of the methylated product showed molecular ion peaks at *m/e* 354 and 326 indicating it to be a mixture of methyl behenate and methyl eicosanoate. The two esters were identified by comparison, with authentic samples, of their GLC retention times on a column packed with 10% OV-1 on Gas-Chrome Q, operating at 200°. The GLC as well as the mass spectrum also indicated the presence of small amounts of methyl stearate (C_{18}) and methyl palmitate (C_{16}) in the mixture.

β-Amyrin. Fractions 212–225, eluted by benzene-$CHCl_3$ (10:1), gave a colourless solid (0·59 g) which was recrystallized thrice from acetone as colourless needles, m.p. 196–198°. (Found: C, 85·05; H, 12·1. Calc. for $C_{30}H_{50}O$: C, 84·4; H, 11·8%), $[\alpha]_D$ +86·76° (c 2·42 in $CHCl_3$.) This compound formed an acetate, m.p. 237–239°. (Found: C, 82·6; H, 11·3. Calc. for $C_{32}H_{52}O_2$: C, 82·0; H, 11·2%), $[\alpha]_D$ + 93·5° (c 2·45 in $CHCl_3$), undepressed on admixture with authentic β-amyrin acetate and having the same IR spectrum.

Fractions 236–255, eluted by benzene–$CHCl_3$ (7:1), yielded a residue which on crystallization from acetone furnished colourless needles (2·11 g). The IR spectrum indicated that this product was a mixture of α- and β-amyrin, with the former predominating. Attempts to separate the two components by fractional recrystallization, column chromatography, or by conversion and separation of its acetates and benzoates[8] met with no success.

α-Amyrin and lupenyl acetate. In another experiment, ground leaf powder of *L. eugenifolia* (582 g) was continuously extracted with light petroleum (3 l.) for 1 week. The dark green solid (10·50 g) which appeared on the walls of the flask was filtered off. Chromatography of this material on a silica gel column (40 × 2·3 cm) and elution with light petroleum–$CHCl_3$ (7:1) gave a fraction (0·44 g) which was recrystallized thrice from acetone–MeOH in colourless needles, m.p. 187–189°, $[\alpha]_D$ + 86·6° (c 2·18 in $CHCl_3$). (Found: C, 84·9; H, 12·1. Calc. for $C_{30}H_{50}O$: C, 84·4; H, 11·8%), undepressed on admixture with mixture with authentic α-amyrin and having the same IR spectrum. This compound formed an acetate, m.p. 223–224°, $[\alpha]_D$ +77·56° (c 1·97 in $CHCl_3$). (Found: C, 81·8; H, 11·25. Calc. for $C_{32}H_{52}O_2$: C, 81·2; H, 11·2%), and having the same IR spectrum as an authentic sample of α-amyrin acetate. The light petroleum filtrate remaining after the removal of the above solid was concentrated to a small volume (*ca.* 150 ml). On cooling a solid (5·5 g) was filtered off. This solid on preliminary investigation showed that it consisted of a complex mixture, mainly of the triterpenes, bauerenol and α- and β-amyrin, and was not further investigated. The light

[7] W. KARRER, *Konstitution und Vorkommen der organischen Pflanzenstoffe*, 18, 19, Birhauser–Verlag, Basel, (1958).

[8] O. C. MUSGRAVE and H. M. WAGNER, *J. Chem. Soc.* 2937 (1952).

petroleum filtrate was chromatographed on an alumina column (55·3 × 3·5 cm) and eluted with light petroleum. Fractions of 150 ml each were collected. Fractions 1–3 furnished the aliphatic hydrocarbons and fractions 4–20 yielded the mixture of triterpenoid esters.

The fractions 30–40 afforded a yellowish semi-solid (0·64 g) consisting mainly of lupenyl acetate which could not be purified by fractional crystallization. Rechromatography of this crude material in light petroleum on an alumina column (11·3 × 1·4 cm) yielded a fraction (0·36 g) which was recrystallized thrice from acetone in colourless needles, m.p. 216–218°, $[\alpha]_D$ +35·3° (c. 2·28 in $CHCl_3$), identified as lupenyl acetate by direct comparison (IR and mixed m.p.) with an authentic sample.

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