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Unsaponifiable Matter of the Fixed Oil from the Seeds of *Caesalpinia sappan* Linn.

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The unsaponifiable matter (1.12%) of the fixed oil of *Caesalpinia sappan* Linn. has been studied for its chemical analysis and found to consist of n-triacontane, lupeol, β-amyrin, stigmasterol, β-sitosterol and an unidentified triterpenoidal alcoholic compound.

INTRODUCTION

Caesalpinia sappan Linn.,[1] family Leguminosae, is a shrub or small tree abundant in South India, Bengal and Burma. The decoction of the wood stops bleeding, cures vata, ulcers and improves complexion[2]. The decoction of leaves is useful in rheumatic pains[3]. Looking to its wild growth and its medicinal importance, the seeds of the plant were taken up for chemical analysis. The present communication reports the study of the unsaponifiable matter from the fixed oil[4] of the seeds collected from Hoshangabad (Madhya Pradesh, India).

EXPERIMENTAL

The seeds of *C. sappan* (1 kg) were extracted with petroleum ether (60–80°C) in a Soxhlet apparatus for 18 hrs. On filtration and removal of the solvent, an orange coloured fixed oil was obtained in an yield of 18.10%. The saponification of the oil (100 gm) with N/2 KOH and subsequently working up gave a waxy unsaponifiable matter (1.12 gm). It was investigated by chromatography. The different constituents were characterised and identified by derivatisation, co-TLC and spectroscopy. Investigation on TLC plate revealed six spots (A, B, C, D, E and F) when sprayed with conc. sulphuric acid. Spots A (green) and F (violet) were sharp and markedly separated. Spots B, C, D and E were diffused and overlapping.

The material (1.2 gm) was subjected to column chromatography over alumina grade II using solvents in the order of increasing polarity.

The petroleum ether (60–80°C) fractions on removal of the solvent gave a component A (250 mg). It did not give test for any functional group. It was crystallised from acetone giving m.pt. 60°C. The compound was identified as

n-triacontane by mixed m.pt. and mixed R_f value with the authentic sample of *n*-triacontane.

The petroleum ether : benzene (90 : 10) fractions on removal of the solvent yielded a component B (250 mg) which gave a single pinkish orange spot on TLC when sprayed with conc. sulphuric acid. It responded to all the colour reactions of triterpenoids. On crystallisation from acetone it gave m.pt. 213–14° and $(\alpha)_D^{26}$ + 28° ($CHCl_3$). This was found to be identical with lupeol by direct comparison (m.m.pt., co-TLC and IR).

Evaporation of petroleum ether (60–80°C): Benzene (80 : 20) eluate fractions gave a component C (250 mg) which gave a single pink spot on TLC plate. The component responded with the usual colour reactions of triterpenoids. Crystallisation from methanol afforded a crystalline colourless compound, m.pt. 200°C and $(\alpha)_D^{25}$ + 86°($CHCl_3$) characterised as β-amyrin by direct comparison (m.m.pt., mixed R_f and superimposable IR spectra with an authentic sample).

Elution with benzene gave a mixture of two components which were isolated by repeated column chromatography using 3% and 5% ethyl acetate in benzene as the solvents.

Removal of the 3% ethyl acetate in benzene eluate fractions by evaporation yielded a residue C (200 mg) giving a single bluish spot on TLC. The residue gave characteristic colour reactions of steroids. On crystallising from methanol it gave colourless needles, m.pt. 170° and $(\alpha)_D^{26}$ – 41° ($CHCl_3$). This was confirmed as stigmasterol by direct comparison (m.m.pt., mixed R_f and I.R.).

Evaporation of 5% ethyl acetate in benzene fractions yielded a component D (200 mg) giving a single violet blue spot on TLC plate. It gave usual tests for steroids. Crystallising from methanol gave shining white flakes, m.pt. 136–137°C, $(\alpha)_D^{26}$ – 31° ($CHCl_3$). These physical constants tallied with β-sitosterol. The identity of the component as β-sitosterol was established by m.m.pt., co-TLC and co-IR with an authentic specimen of β-sitosterol.

Further elution of the main column with 10% ethyl acetate in benzene led to the isolation of a component F (250 mg) giving a single greenish blue spot on TLC. On crystallisation from methanol the colourless compound gave m.pt. 198–99°C and $(\alpha)_D^{26}$ + 5.6°($CHCl_3$). The compound gave usual test of triterpenoids but no test for steroids. IR (KBr pellet) gave peaks broad 3320, sharp 2920, 2820, weak 2000, 1720, 1440, 1360, 1250, 1010 and strong 800 cm^{-1}. IR studies revealed the presence of tertiary alcoholic group (3320, 1440, 1360 cm^{-1}) and saturated nature (absence of peaks 1680, 1620 cm^{-1}). Further investigation could not be done due to the paucity of the material.

RESULTS AND DISCUSSION

The unsaponifiable matter (1.12%) of the fixed oil of *C. sappan* has been

studied and found to consist of *n*-triacontane, lupeol, β-amyrin, stigmasterol, β-sitosterol and an unidentified triterpenoidal alcoholic compound.

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