Chemical Investigation of *Trichodesma indicum* Leaves-I. Non-Steroidal Constituents of the petroleum ether Extract.

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Trichodesma indicum L. syn. Trichodesma hirsutum . and *Borago indica L.*, is a herb with small blue flowers. It belongs to the family Boraginaceae, and is widespread in India and Pakistan. In Pakistan, it is found at low altitudes in Karachi, Sind, Baluchistan, Chitral, Swat, Hazara, Jhelum, Dhamyal, the Rawalpindi hills, Poonch and Kashmir. The plant is reputed[2] to be an antidote for snake poison. Badami et. al. had reported the isolation of some fatty acids from the seeds of the plant, but no chemical work has so far been done on the plant, although several new alkaloids of pharmacological importance have been isolated[4-9] from other species of *Trichodesma* growing indigenously in Pakistan. it was therefore, appropriate to undertake the systematic chemical investigation of the plant which grows abundantly on the capus of this University. The present paper describes the isolation of three crystalline, non-steroidal (Liebermann-Burchard negative) compounds from the petroleum ether extract of the air dried leaves.

Hexacosane (1), a C-26 straight chain saturated hydrocarbon obtained in a yield of 0.01% was isolated by chromatography of the non-steroidal part of the petroleum ether extract on a silica gel column. Hexacosane has previously been isolated[10-12] from the flower of *Chrysanthemum indicum* and from *Chrysalis* oil.

$$CH_3\ (CH_2)_{24}CH_3$$

(1)

The ethyl ester (2) of hexacosanoic acid is the major constitutent of the petroleum ether extract. It crystallized out during the removal of the solvent from the extract, and an additional amount was obtained when the non-steroidal (Libermann-Burchard negative) portion was separated on a alumina and subjected to column chromatography on silica gel for a total yield of 0.14%. On recrystallization from methanol-benzene it melted at 62.3°. It shows two strong bands at 1265 cm^{-1} and 1710 cm^{-1} in the infra red spectrum, pointing to the presence of an ester group. An intense M-28 peak in the mass-spectrum pointed to the elimination of an ethene molecule from the ethyl ester through a six-membered transition state. It was thus identified[13] as the ethyl ester of a straight chain C-26 fatty acid, hexacosanoic (cerotic) acid (2). Hexacosanoic acid had been found to be the constituent of various waxes and oils e.g. bees wax[13-15], Chinese insect wax[16], Gedd wax[17], and sunflower seeds oil[18]. It has also been used as a preservative in H_2O_2[19].

$$CH_3\ (CH_2)_{24}CO_2C_2H_5$$

(2)

Compound 3 was the last non-steroidal component to be eluted from the silica-gel column and was obtained in the form of a colourless crystalline solid, melting at 72°C after recrystallization from benzene-methanol in a yield of 0.01%. The mass-spectral fragmentation pattern (M^+ = m/e 420) of 3 resembles that of (2), and suggests an ethyl ester of an unsatura-

$$CH_3(CH_2)_{19} - CH = CH - CH_2 - CH = CH - CO_2C_2H_5$$

(3)

ted straight chain C-26 acid. It exhibits two strong bands at 1710 cm^{-1} and 1275 cm^{-1} in the infra-red spectrum, showing the presence of an ester group. In addition, a weak band in infra-red spectrum at 1655 cm^{-1} corresponds to an unconjugated C=C stretching. The only C-26 dienoic acid obtained from a natural source is 17,20-hexacosadienoic acid, which was first isolated[20] from the lipids of the sponge Spheciospongiavesperia. Compound (3), however, does not seem to be 17,20-hexacosadienoic acid ethyl ester since it shows a peak m/e 111 in the mass spectrum, which indicates there is a continuous saturated straight chain of twenty carbons starting from the methyl group. Therefore, the compound is 21,24-hexa-cosa-dienoic acid ethyl ester.

Experimental

Extraction and isolation of the non-steroidal components: Air-dried leaves (1500 g) of the plant collected from the campus of this Univeristy in the month of August '80, were powdered and exhaustively extracted with petroleum ether ($40^o - 60^o$) in a soxhlet apparatus. The petroleum ether extract upon concentration under vacuum deposited 2g. of a crystalline non-steroidal, Libermann-Burchard negative solid which was filtered off. A gummy yellowish residue (10 g., 0.66%) was obtained after complete removal of the solvent from the filtrate. This residue was dissolved in small amount (5 ml) of petroleum ether and was subjected to column chromatography over 350 g. (60 x 2.7 cm column) of neutral alumina (70-230 mesh ASTM, E. Merck), and the column was eluted successively with the solvent of increasing polarity. The fraction eluted with 5% benzene-pet-ether afforded a Libermann-Burchard negative solid. Total yield of Liebermann-Burchard negative non-steroidal portion was 2.8 g (0.18% based on the weight of dried leaves).

Separation and identification of (1), (2), and (3):

1: The solid (0.8 g) obtained above was rechromatographed over 100 g. (70 x 1.7 cm. column) of silica-gel (70-230 mesh, ASTM, E. Merck) and eluted succesively with petroleum ether and benzene. The fraction eluted with 1% benzene pet-ether afforded 0.15 g of a white crystalline solid, melting at 55.5^o after recrystallization from ether. R_f = 0.69 (Alumina 150, netural, Type T., E. Merck) cyclohexane.

MS: m/e 366 (M^+), 309 (M-C_4H_9), 281 (M-C_6H_{13}), 253 (M-C_8H_{17}), 239, 225, 211, 197, 183, 169, 155, 141, 127, 113, 99, 85, 71, 57 (100 %), 55.

(2): Fractions eluted with 2% benzene petroleum ether yielded 0.2 g. of white crystalline solid melting at $62\text{-}63^o$ after recrystallization from benzene-ethanol mixture. R_f = 0.33, (0.25 mm. thick, 20 x 20 cm. chromatoplates of silica gel 60 G.E. Merck) /benzene.

MS: m/e 424.4286 (M^+), calculated for $C_{28}H_{56}O_2$ = 424.4280; 396.3975 (M-C_4H_4), calculated for $C_{26}H_{52}O_2$ = 396.3967; 381, 339, 325, 311, 297, 283, 269, 255, 241, 227, 213, 199, 185, 171, 129, 115, 71, 57 (100%), 55.

IR: 1710 cm^{-1}(s); 1265 cm^{-1}(s). (KBr).

The crystalline solid (2g) obtained on concentrating the petroleum ether extract and this fraction were found to be identical in physical as well as spectral properties and were identitified as ethyl ester of hexacosanoic acid.

(3): Fraction eluted with 3% benzene petroleum ether afforded 0.15 g. of white crystalline solid, melting at 72° on recrystallization from mixture of benzene and methanol.

R_f = 0.29, silica gel/benzene.

MS: m/e 420 (M^+), 392 (M-C_2H_4), 379, 365, 279, 265, 251, 237, 223, 209, 195, 181, 167, 139, 125, 111, 57 (100%), 55.

IR: 1710 cm^{-1}(s), 1655 cm^{-1}(s), 1275 cm^{-1}(s),

(KBr). Infra-red spectra were recorded on Pye-Unicam SP-100 spectrophotometer.

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