τ_r. In the case of benzene, dioxane and butanone there is no possibility of hydrogen bonding and hence the values of τ_r are small. Thus the variation of τ_r gives some insight into the competing internal radiationless transition processes. The values of τ_r obtained in the present case show an interesting correlation with the percent polarization of fluorescence obtained in these solvents. The details of these are under evaluation.

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PRESENCE OF INGENOL AND A NEW DITERPENE 4–DEOXY INGENOL IN THE LATEX OF *EUPHORBIA MEGALANTHA* (BOISS)

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THE plants of *Euphorbraceae* family are distributed from tropical to temperate regions of the world and are known to excude a white milky caustic, skin-irritant latex, when the stems or the leaves are cut or broken[1]. These plants or parts thereof are used medicinally, despite being poisonous and toxic to animals and human beings[2,3]. From the plants of this family various tumour-promoting saturated and/or unsaturated fatty acid esters (mono[4], di[5] and sometimes tri[5]; called cryptic irritant and cryptic cocarcinogens[5]) of diterpene ingenol, phorbol and of their various derivatives, have been isolated[6]. In this communication, the occurrence of ingenol and a new diterpene 4–deoxy ingenol in the latex of *E. megalantha*, which grows in abundance at Osku, Iran, is reported[7].

The methanolic latex preparation of the latex on solvent removal under reduced pressure at 40°C gave dry mass (2 g). This was defatted with *n*-hexane, partitioned between methanol and water (3:1) system. The material obtained after working up was transesterified[5,8], which on acetylation yielded 0.40 g of diterpene-rich acetylated product[1,8].

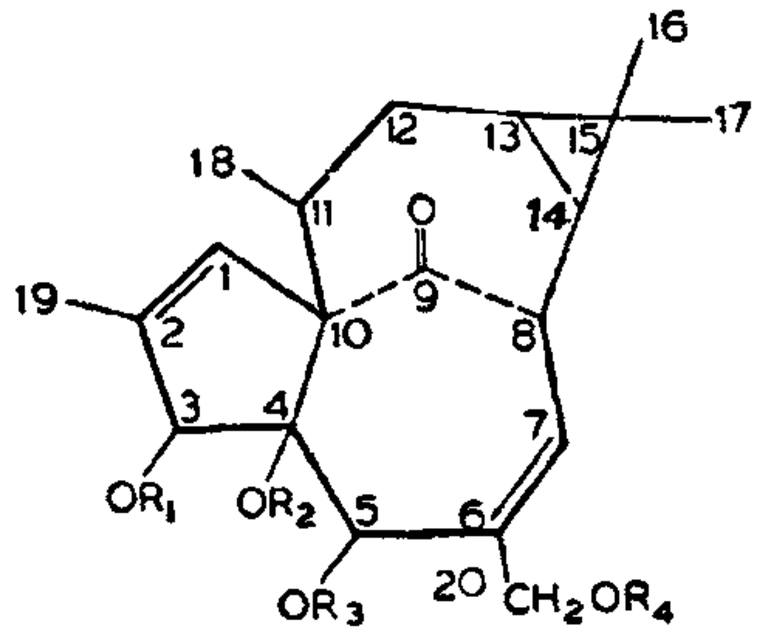

Figure 1. $OR_1 = OR_2 = OR_3 = OR_4 = OH$: Ingenol; OR_1, OR_3, OR_4 = acetate, $OR_2 = OH$: Ingenol, 3, 5, 20 triacetate; OR_1, OR_3, $OR_4 = OH$, $OR_2 = H$: 4-deoxy ingenol; OR_1, OR_3, OR_4 = acetate, $OR_2 = H$: 4-deoxy ingenol, 3, 5, 20 triacetate.

Preparative TLC (silica gel G, 1 mm thick) in hexane, ether, ethylacetate system (1:1:1) gave two zones reacting positive to vanillin/H_2SO_4 reagent[7,8]. The upper zone having R_f 0.33 (blackish brown) and the other with R_f 0.31 (black), were scrapped out, and on repeated purification gave factors EM_1, R_f 0.33 (28 mg) and EM_2, R_f 0.30 (18 mg).

The *factor* EM_1 has been identified as ingenol 3, 5, 20 triacetate from its physical data[1,6–8] recorded below:

UV: λmax (MeOH): 202, 285 nm, ε16300, 200,
IR: (CH_2Cl_2):1740, 1705, 1640 cm^{-1},
Mass: ($C_{26}H_{34}O_8$): m/e 474, 456, 414, 312, 121, 43,
NMR: (CCl_4, TMS δ =0): 1.00, 1.09, 1.28, (9H, H_3-18, H_3-17, H_3-16), 1.9 (3H, H_3-19) 2.00, 2.01, 2.10, (3 CH_3CO), 3.28 (OH–4), 4.00, 4.08, 4.20 (dd, H_2-20:J_{AB} = 12 Hz), 4.32–4.41 (H–8, J = 6 Hz), 5.0 (H_3-3, 5) 5.29 (H–5, S), 5.15 (H–1), 5.15 (H–1), 5.25 (H–7, 1 Hz).

The *factor* EM_2 has been assigned the structure as shown in figure 1 from the following considerations:
UV: λmax (MeOH): 202, 275 nm, ε16300, 200,
IR: (CH_2Cl_2): 1740, 1705, 1640 cm^{-1},
Mass: ($C_{26}H_{34}O_7$): m/e 458, 440, 414, 312, 121, 43,
NMR: (CCl_4, TMS δ = 0): 1.00, 1.09, 1.27, (9H, H_3-18, H_3-17, H_3-16), 1.9 (3H, H_3-19) 2.00, 2.08, 2.10, (3 CH_3CO), 4.00, 4.08, 4.22 (dd, H_2-20: J_{AB} = 12, Hz), 4.32, 4.41 (H–8, J = 6.0 Hz), 5.0

(H_3–3, 5) 5.30 (H–5), 5.18 (H–1), 5.28 (H–7, 1.1 Hz).

The UV and IR spectral data of the factor EM_1 and EM_2 show common skeleton and functionalities, except for mass values, where the difference of one hydroxyl group is indicated clearly in factor EM_2. When we compare the NMR data and count the positions of various hydroxyls including that of the one at 4–position in ingenol moiety, (free in acetylated ingenol)[6], the signals of 4–OH group are present in ingenol 3, 5, 20–triacetate (factor EM_1) but not in EM_2 at 3.28 position. It appears that EM_2 is devoid of a hydroxyl function and hence is called 4–deoxy ingenol 3, 5, 20 triacetate and the parent diterpene will be similarly known as 4–deoxy ingenol[7].

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A PRELIMINARY STUDY ON ROOT ASSOCIATED DIAZOTROPHS OF COTTON

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THE rhizosphere studies conducted with field grown cotton showed a greater number of total (11×10^7 cells/g) and diazotrophic (2×10^5 cells/g) bacteria associated with roots of indigenous cotton. The counts of such bacteria were highest in the rhizoplane and lowest in root macerate samples. Cotton var. H777 and G27 had *Klebsiella* sp. and *Enterobacter* sp. as their root associated diazotrophs. However, more work is required to understand the contribution of these organisms for the improvement of cotton.

Among the rhizosphere organisms, diazotrophic bacteria are of major interest because of their active involvement in crops productivity[1,2]. However, the root associated diazotrophs of cotton have not been studied so far. Therefore, an attempt was made to find out the number and types of these bacteria with cotton roots.

Cotton varieties H777 (*Gossypium hirsutum*) and G27 (*Gosaypium arboreum*) were grown at the university farm following recommended agronomical practices. The plants were uprooted at the preflowering stage. Rhizosphere samples along with roots were collected from 3 different locations for each variety. These were used for total (dilution plating) and most probable number (MPN) of bacteria (acetylene reduction method) from rhizosphere (RS), rhizoplane (RP) and root macerate (RM). Nitrogen-deficient malate medium[3] was used for counts and nitrogenase activity. The MPN was estimated by comparing the results with standard tables[4].

The malate medium plates used for total counts and streaked from MPN positive tubes were taken for isolating the root associated diazotrophs based on colony morphology. A total of 21 isolates (11 from H777 and 10 from G27) were checked for acetylene reduction activity (ARA) on solid as well as semisolid media. The two isolates, 8C (var. H777) and DC8 (var. G27) showing highest ARA were selected for subsequent studies. These were identified by standard procedures[5].

The samples analysed for total and diazotrophic bacteria associated with roots showed greater number with G27 than H777 (table 1). The counts were greater in the rhizoplane compared to rhizosphere, may be due to the availability of more photosynthates near the root surface. The surface-sterilized roots showed very few bacteria. These results show that indigenous cotton has greater potential to harbour nitrogen-fixing and other bacteria to meet the nutritional requirements. The other possible explanation may be the long association of rhizosphere microflora and indigenous cotton which helped in developing a mechanism to live together.