# Hydrocarbon Distribution in Epicuticular Waxes of Five *Euphorbia* Species

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Z. Naturforsch. **51c**, 291–295 (1996); received October 30, 1995/January 2, 1996

Euphorbiaceae, Hydrocarbons, Epicuticular Wax

The distribution pattern of hydrocarbons in the surface waxes of five species of *Euphorbiaceae, E.caducifolia, E.helioscopia, E.milii, E.royleana* and *E.tirucalli* was studied. In addition to homologous series of *n*-alkanes, minor quantities of unsaturated and branched hydrocarbons were also detected. Some of the branched chain hydrocarbons could be explained as having originated from isoprene units and the substituents corresponding to diterpenes and triterpenes.

## Introduction

The higher plants of *Euphorbia* genera are widely distributed in Pakistan (Nasir *et al.*,1986 a). They possess surface wax which contains appreciable amounts of hydrocarbons (Hemmers and Gülz, 1986). Literature survey shows that emphasis has been on the major hydrocarbon components (Tulloch, 1976) and very little attention has been paid to the minor constituents. In order to have a better insight the following five species were selected for the present study. *E.caducifolia* a tall, armed, dense, erect and stout bush with thick cylindrical leafless green branches. A large plantation exists on rocky hillocks and grounds at Drigh Road, Manghopir and Bund Murad Area in Karachi (Jafri, 1966). The latex and root extracts of the plant are used in the indigenous system of medicine (Nadkarni, 1976). *E.helioscopia*, an erect sparingly pilose to glabrescent bright green fleshy annual herb upto 50 cm tall, is a common weed in winter crops throughout Pakistan (Nasir *et al.*, 1986 b). Certain medicinal properties e.g. anthelmintic, hydragogue, warts remover etc. have been attributed to different parts of plant (Kirtikar and Basu, 1984 a). *E.milii* des Moul is a suberect or tailing glabrous much branched, spiny, semisucculent shrub or subshurb upto 60 cm tall. It is planted as an ornamental plant (Nasir *et al.*, 1986 c). *E.royleana* Boiss (*E.pentagona* Royle) is a dense-crowned, succulent, branched, spiny, glabrous, readily-deciduous shrub or small tree usually upto 5 m tall. It is cultivated as a hedge plant in the plains (Nasir *et al.*, 1986 d). The plant reportedly possesses certain medicinal properties (Chopra *et al.*, 1956 and Kirtikar and Basu, 1984 b). *E.tirucalli* L. is an open crowned succulent branched unarmed glabrous shrub or small tree 2–6 m tall and 50–80 cm in girth (Nasir *et al.*, 1986 e). Numerous medicinal properties have been attributed to the plant (Kirtikar and Basu, 1984 c). The hydrocarbon chemistry profile of the surface waxes is reported here.

## Material and Methods

### Collection of plants

All plants were collected from PCSIR Labs. Lahore Campus and its neighborhood. *E.caducifolia* which grows in Karachi area was cultivated in laboratory campus. It was harvested in February. Mature leaves of *E.helioscopia* were collected in February and March. *E.milii* was collected during the month of May, and separated into leaves and stems. *E.royleana* stems were harvested in April. *E.tirucalli* was collected from Government F.C.College neighboring the PCSIR campus during the month of March.

### Extraction of surface waxes

Weighed quantity of fresh leaves or stems was dipped in commercial, locally available, freshly

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distilled hexane (1L) for three minutes and then taken out. After allowing hexane to evaporate from the plant material at room temperature the process was repeated twice. The combined hexane extract (3L) was dried over anhydrous sodium sulphate, filtered and then freed from solvent. The percentage yields are reported in Table I.

### *Isolation of hydrocarbons*

The hydrocarbons were isolated from the waxes by column chromatography on Silica gel (Kieselgel 60, 0.063–0.200 mm, Merck, Germany) and eluted with hexane. Further purification was carried out on Plates coated with silica gel 60 GF$_{254}$ (20×20×.1 cm) using hexane as the mobile phase. The yields are given in Table I.

### *Separation of saturated and unsaturated hydrocarbons*

The silica gel coated glass plate (20×20×0.1 cm) was developed in a 10% silver nitrate solution in acetonitrile, dried and activated at 105 °C for one hour (Ghosh and Thakur, 1982). Hydrocarbon mixture (40 mg) was charged onto the plate and developed in hexane. The plate was allowed to dry and sprayed with 0.1% ethanolic solution of 2,7-dichlorofluorescein. The zones were marked under UV-light, scratched and extracted with chloroform. After evaporation of solvent, saturated and unsaturated hydrocarbons, based on the hydrocarbon fraction (R$_f$ 0.78,30.9 mg, 77.25 %) and (R$_f$ 0.60 7.6 mg, 19.0 %) respectively were obtained.

### *GC-MS analysis of hydrocarbons*

GC-MS analysis of hydrocarbons was carried out on a JEOL JMS-AX505H mass spectrometer coupled with a Hewlett Packard 5890 series II gas chromatograph and JMA-DA5000 data processor. A BP-5 column (SGE), 0.25 mm×25 m, was used for the separation. Operating conditions were; separator temp. 230 °C, injector temp. 230 °C, ion source temp 150 °C, helium as carrier gas, column programming 150 °C to 280 °C with a rise of 5 °C/min. initial hold for 2 min. and final hold at 280 °C ionizing voltage 70 eV and split ratio 1:60. The program was run for 40 minutes 1 µl of a concentrated solution of the sample in chloroform was injected.

## Results and Discussion

The aerial portions of the five species investigated were procured, weighed and then dipped in hexane to recover the surface wax which formed 0.12 to 0.64% of the fresh plant. The leaves, perhaps due to their surface area, contain more wax than stems. The hydrocarbon portions were isolated by column chromatography and separated into saturated and unsaturated hydrocarbons by argentation layer chromatography. The yield from each species is given in Table I. Out of these species only two *E.caducifolia* and *E.tirucalli* contain unsaturated hydrocarbons. The presence of minor unexplained components in the gas chromatogram showed the complexity of the hydrocarbon fractions. Hence the mixtures were subjected to the GC-MS analysis. The individual peaks in TIC were identified by simulating the fragmentation pattern with known compounds available in the library or by examining the fragmentation pattern. The M+ peaks were usually weak but quite discernible. The *n*-alkanes showed first a loss of 29 atomic mass units (A.M.U) due to ethyl group and then progressive loss of 14 A.M.U. due to methylenes. The branched chain hydrocarbons suffered the cleav-

Table I. Yield of surface waxes and hydrocarbons of five *Euphorbia* species.

| Species | Part of species | Weight taken [g] | Weight of wax obtained [g] | % age of surface wax | Hydrocarbons Total | Saturated | Unsaturated |
|---|---|---|---|---|---|---|---|
| *E.caducifolia* | stems | 80 | 0.505 | 0.64 | 6.66 | 5.36 | 1.04 |
| *E.helioscopia* | leaves | 620 | 2.046 | 0.33 | 9.50 | 9.50 | N. D. |
| *E.milii* | leaves | 50 | 0.280 | 0.56 | 2.05 | 2.05 | N. D. |
| *E.milii* | stems | 300 | 0.420 | 0.14 | 2.05 | 2.05 | N. D. |
| *E.royleana* | stems | 1500 | 7.202 | 0.48 | 1.98 | 1.96 | 0.02 |
| *E.tirucalli* | stems | 2800 | 3.360 | 0.12 | 2.08 | 2.08 | N. D. |

N. D. = nondetected.

Table II. Hydrocarbon distribution in surface waxes of five *Euphorbia* species.

| Mole Peak M/Z | Molecular Formula | %age contribution of hydrocarbon fraction in species | | | | | | Identification |
|---|---|---|---|---|---|---|---|---|
| | | *E.caducifolia* | *E.helioscopia* | *E.milii* (leaves) | *E.milii* (stems) | *E.royleana* | *E.tirucalli* | |
| 198 | $C_{14}H_{30}$ | – | – | – | – | – | <0.1 | *n*-tetradecane |
| 212 | $C_{15}H_{32}$ | – | – | – | – | – | 0.1 | *n*-pentadecane |
| 226 | $C_{16}H_{34}$ | – | <0.1 | – | – | <0.1 | <0.1 | *n*-hexadecane |
| 240 | $C_{17}H_{36}$ | <0.1 | 0.1 | – | – | 0.1 | 0.1 | *n*-heptadecane |
| 252 | $C_{18}H_{36}$ | <0.1 | – | – | – | 0.1 | – | *n*-octadecene |
| 254 | $C_{18}H_{38}$ | 0.2 | 0.1 | – | <0.1 | 0.1 | <0.1 | *n*-octadecane |
| 268 | $C_{19}H_{40}$ | <0.1 | 0.1 | – | – | <0.1 | <0.1 | *n*-nonadecane |
| 280 | $C_{20}H_{40}$ | <0.1 | – | – | – | 0.1 | – | 1-eicosene |
| 282 | $C_{20}H_{42}$ | – | – | – | <0.1 | – | – | 2,7,9,12-tetramethylhexadecane |
| 282 | $C_{20}H_{42}$ | – | – | <0.1 | – | – | – | 2,6,10-trimethylheptadecane |
| 282 | $C_{20}H_{42}$ | – | – | – | <0.1 | – | – | 2,7,9-trimethylheptadecane |
| 282 | $C_{20}H_{42}$ | – | – | – | – | – | <0.1 | 9-methylnonadecane |
| 282 | $C_{20}H_{42}$ | <0.1 | 0.1 | – | 0.1 | <0.1 | 0.2 | *n*-eicosane |
| 294 | $C_{21}H_{42}$ | <0.1 | – | – | – | 0.1 | – | 1-heneicosene |
| 296 | $C_{21}H_{44}$ | – | – | <0.1 | – | – | – | 4,8-dimethylnonacosane |
| 296 | $C_{21}H_{44}$ | <0.1 | 0.2 | – | 2.1 | <0.1 | 0.3 | *n*-heneicosane |
| 308 | $C_{22}H_{44}$ | 0.1 | – | – | – | 0.2 | – | 1-docosene |
| 310 | $C_{22}H_{46}$ | 0.2 | 0.2 | <0.1 | 0.5 | 0.2 | 0.3 | *n*-docosane |
| 322 | $C_{23}H_{46}$ | 0.2 | – | – | – | 0.2 | – | 1-tricosene |
| 324 | $C_{23}H_{48}$ | – | – | – | – | – | <0.1 | 10-methyltricosane |
| 324 | $C_{23}H_{48}$ | 0.2 | 0.2 | 0.2 | 1.2 | 0.8 | 0.5 | *n*-tricosane |
| 336 | $C_{24}H_{48}$ | 0.2 | – | – | – | – | – | 1-tetracosene |
| 338 | $C_{24}H_{50}$ | – | – | – | – | – | <0.1 | 11-methyltricosane |
| 338 | $C_{24}H_{50}$ | 0.4 | 0.2 | 0.2 | 1.6 | 0.6 | 0.4 | *n*-tetracosane |
| 350 | $C_{25}H_{50}$ | 0.7 | – | – | – | – | – | 1-pentacosene |
| 352 | $C_{25}H_{52}$ | 0.7 | 0.7 | 0.9 | 3.8 | 1.0 | 1.9 | *n*-pentacosane |
| 364 | $C_{26}H_{52}$ | 3.1 | – | – | – | – | – | 1-hexacosene |
| 366 | $C_{26}H_{54}$ | – | – | – | – | – | <0.1 | 3,7,11-trimethyltricosane |
| 366 | $C_{26}H_{54}$ | 1.2 | 0.9 | 0.8 | 3.6 | 0.7 | 1.7 | *n*-hexacosane |
| 378 | $C_{27}H_{54}$ | 1.3 | – | – | – | – | – | 1-heptacosene |
| 380 | $C_{27}H_{56}$ | 5.4 | 15.5 | 5.4 | 10.8 | 6.2 | 11.0 | *n*-heptacosane |
| 392 | $C_{28}H_{56}$ | 6.8 | – | – | – | – | – | 1-octacosene |
| 394 | $C_{28}H_{58}$ | – | – | <0.1 | – | – | – | 2,12-dimethylhexacosane |
| 394 | $C_{28}H_{58}$ | – | – | 0.1 | – | – | – | 2-methylheptacosane |
| 394 | $C_{28}H_{58}$ | 2.2 | 2.9 | 1.4 | 4.5 | 2.9 | 4.0 | *n*-octacosane |
| 408 | $C_{29}H_{60}$ | – | – | – | <0.1 | – | – | 2-methyloctacosane |
| 408 | $C_{29}H_{60}$ | 17.8 | 29.5 | 11.3 | 18.9 | 9.8 | 33.2 | *n*-nonacosane |
| 420 | $C_{30}H_{60}$ | 2.3 | – | – | – | – | – | 1-triacontene |
| 422 | $C_{30}H_{62}$ | – | – | <0.1 | – | – | – | 2,6,10,14,18,22-hexamethyltetracosane |
| 422 | $C_{30}H_{62}$ | – | – | – | 0.2 | – | – | 3,7,11,19,23-pentamethylpentacosane |
| 422 | $C_{30}H_{62}$ | – | – | – | 0.3 | – | – | 2-methylnonacosane |
| 422 | $C_{30}H_{62}$ | 1.8 | 1.7 | 1.8 | 3.0 | <0.1 | 4.5 | *n*-triacontane |
| 436 | $C_{31}H_{64}$ | – | – | – | 0.2 | – | – | 2-methyltriacontane |
| 436 | $C_{31}H_{64}$ | 19.5 | 23.0 | 28.9 | 23.4 | 36.3 | 25.4 | *n*-hentriacontane |
| 448 | $C_{32}H_{64}$ | 0.6 | – | – | – | – | – | 1-dotriacontene |
| 450 | $C_{32}H_{66}$ | – | 0.1 | – | – | – | – | 6,16-dimethyltriacontane |
| 450 | $C_{32}H_{66}$ | 2.5 | 0.1 | 0.1 | 0.1 | <0.1 | 3.6 | *n*-dotriacontane |
| 464 | $C_{33}H_{68}$ | – | – | <0.1 | – | – | – | 3,12,17,21,25-pentamethyloctacosane |
| 464 | $C_{33}H_{68}$ | – | – | – | <0.1 | – | – | 3,10,14,19,26-pentamethyloctacosane |
| 464 | $C_{33}H_{68}$ | – | – | – | <0.1 | – | – | 3,7,11,18,23-pentamethyloctacosane |
| 464 | $C_{33}H_{68}$ | 31.3 | 16.4 | 27.1 | 15.5 | 27.3 | 3.6 | *n*-Tritriacontane |
| 476 | $C_{34}H_{68}$ | 0.1 | – | – | – | – | – | 1-tetratriacontene |
| 478 | $C_{34}H_{70}$ | 1.3 | 0.4 | 2.1 | 0.9 | 1.5 | 10.0 | *n*-tetratriacontane |
| 492 | $C_{35}H_{72}$ | – | – | <0.1 | – | – | – | 3,7,11,27-tetramethylhentriacontane |
| 492 | $C_{35}H_{72}$ | – | – | <0.1 | – | – | – | 3,7,11-trimethyldotriacontane |
| 492 | $C_{35}H_{72}$ | 3.8 | 6.7 | – | <0.1 | 1.9 | – | *n*-pentatriacontane |
| 506 | $C_{36}H_{74}$ | 0.2 | – | – | – | – | – | *n*-hexatriacontane |
| 520 | $C_{37}H_{76}$ | 0.3 | – | – | – | – | – | *n*-heptatriacontane |

age at the C-C linkage having maximum substitution i.e. a loss of 43 units (isopropyl group) in case of 2-methyl alkanes. The cleavage at the most substituted carbon led to the identification of branching position/positions in the molecules. The terminal alkenes lost first 28 A.M.U. due to certain rearrangements, then 13 A.M.U. and later followed the pattern of *n*-alkanes. In addition to these each prominent peak was surrounded by plus and minus peaks. The saturated alkanes showed single prominent peak for 43, 57, 71, 85, and 99 (A.M.Us) etc., whereas in unsaturated alkenes prominent peaks appeared at 43, 57, 69, 83, 97, 111, 125,(A.M.Us) etc.

The hydrocarbon chemistry profile of each species is given in Table II. The major components are *n*-alkanes/alkenes and the chain length varies from 14 to 37 carbons, a range for several Euphorbia species (Stransky *et al.*, 1967). The homologues with odd no. of carbon atoms predominate as is the normal distribution in angiosperms (Maria *et al.*, 1989). The hydrocarbon mixture from the leaves of *E.helioscopia* is the simplest and contains only *n*-alkanes ranging from hexadecane ($C_{16}$) to hexatriacontane ($C_{36}$) and traces of a branched alkane 6,16-dimethyltriacontane. *E.caducifolia*, shows in addition to the saturated hydrocarbons a considerable portion (1.04% of the surface wax) of unsaturated hydrocarbons which range from 1-heneicosene ($C_{21}$) to 1-tetratriacontene ($C_{34}$). Like *n*-alkanes both odd and even numbered alkenes are present with the highest contribution of 1-octacosene (6.8%). Contrary to the preponderance of odd to even *n*-alkanes, the even numbered alkenes predominate over the odd ones. Prasad and Gülz (Prasad and Gülz, 1990) observed the presence of unsaturated hydrocarbons and variation in their quantities was observed during a study of the development and seasonal variations in epicuticular waxes of beach leaves (*Fagus sylvatica* L). They proposed that the unsaturated compounds may be characteristic for the waxes in folded leaves in the buds and may be destroyed or polymerised by a radical mechanism in the presence of light and after unfolding the leaves. The alkenes in epicuticular waxes of *Quercus robur* leaves as observed by Gülz and Müller (Gülz and Müller, 1992) showed a pattern with even carbon numbers predominating over the odd ones. Among the species selected in the present study *E.caducifolia* which does not have leaves shows a reasonable quantity of alkenes and displays the same order as *Quercus robur*.

The hydrocarbon chemistry profile of the two species *E.milii*, a pot herb and *E.tirucalli*, shows several branched alkanes. The hydrocarbons from *E.milli* leaves contain eight branched hydrocarbons each less than 0.1%. Most of the branching can be explained by head to tail linkage of isoprene units e.g. 2,6,10,14,18,22-hexamethyltetracosane. However no squalane, a hydrogenated product of the most widely accepted precursor squalene (Benveniste *et al.*, 1966) could be detected. Another $C_{20}$ hydrocarbon 2,6,10-trimethylheptadecane has same substituent positions as tiglane, a hypothetical tricyclic skeleton found in phorbol (Crombie *et al.*, 1968), prostratin (Cashmore *et al.*, 1976) milliamine H (Morston and Hecker, 1976), all being possible carcinogens. The stems contain nine branched hydrocarbons altogether different from those found in leaves. *E.tirucalli*, free of unsaturated hydrocarbons comprises *n*-alkane $C_{14}$ to $C_{34}$ and four branched hydrocarbons in concentration of less than 0.1%. As the quantities are small no definite correlation with *n*-alkanes or the 1-alkenes could be established. Among the branched hydrocarbons, three alkanes have only one methyl group as substituent and the fourth one, 3,7,11-trimethyltricosane has a partial structure based on isoprene units. *E.royleana*, which could very easily be mixed up with *E.caducifolia*, contains only a minor amount of 1-alkenes spreading over a very small range 1-octadecene ($C_{18}$ < 0.1%) to 1-tridocosene ($C_{23}$ traces). This taxonomic factor may be utilised to distinguish these two species.

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