The occurrence of long-chain polyisoprenols in leaves of plants of Combretaceae family*

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The presence of poly-cis-prenols of chain length 20–60 isoprene units or longer in leaves of plants belonging to Combretaceae family was shown to be a common feature in this group of plants. The polyisoprenols of this type were found in half of the 20 species studied. In most cases the polyisoprenols occurred in the form of fatty acid esters. Only in one species — Combretum molle, the polyisoprenols were found in the form of free alcohols. The amount of long-chain polyisoprenols varied with the plant species; the richest source was C. molle (about 4% of dry mass of leaves). Polyisoprenol groups characteristic of other systematic families of plants were not found in the Combretaceae studied.

Poly-cis-prenols with the longest chain are those known as natural rubber (Tanaka, 1989). The length of these polyisoprenol molecules varies depending on the plant source. The smallest molecules of a natural poly-cis-isoprene which is classified as a rubber-like polymer, occur in leaves of sunflower (Helianthus annuus); this polymer is composed of about 320–360 cis-isoprene units. Polyisoprenes occurring in fungi (representatives of genus Lactarius) are of a slightly smaller length (160–300 cis-isoprene units) (Tanaka et al., 1994). The commonly known rubber polymer present in the latex of Hevea brasiliensis is composed of more than 800–1500 cis-isoprene units, that in goldenrod (Solidago altissima) of 1000–2000, and that in chickle (Achras sapota) of about 2000 cis-isoprene units (cited after Y. Tanaka, 1989).

The studies of the Liverpool group investigators revealed in late 1960-ies the occurrence, in leaves of plants of mainly — cis-prenols composed of 11–12 isoprene units (Wellburn et al., 1967; Stone et al., 1967). This group of plant lipids has been found in a large number of plants. The length of these polyisoprenols varied from 9–10 isoprene units in the majority of plants species to about 15–20 in different conifers and about 20 and more in various species of Rosaceae family (Świężewska et al., 1994). In Lumnitzera racemosa belonging to Combretaceae family we detected polyisoprenols composed of up to 100 cis-isoprene units (E. Skoczylas et al., 1994).

In the present paper we report on the occurrence of the longest-chain free polyisoprenoid alcohols in C. molle and on the presence of this

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group of compounds in other representatives of this systematic group.

MATERIALS AND METHODS

Leaves were collected in the Botanic Garden in Waimea Arboretum and Botanical Garden in Hālawa and in Botanical Gardens in Honolulu (Hawaii, U.S.A.) in March, 1995 and air-mailed to Poland.

Dry leaves (0.5 g) were homogenized in an Ultra-Turrax homogenizer with 5 ml of acetone/hexane (1:1, v/v) and the suspension left in the dark for 2 days at room temperature with occasional shaking. The extract was subjected to thin-layer and column chromatography and to the HPLC procedure as described in the accompanying paper (Świeżewska & Chojnacki, 1996).

RESULTS AND DISCUSSION

The content of polyrenols was studied in leaves of species belonging to Combretaceae family (Table 1). Long-chain polyrenols were detected in about half of the studied plants. Their presence was confirmed upon examining plants from other regions of Hawaii. The content of polyrenols in these two groups of plants was not identical as exemplified by Bucida buceras. In six plant species polyrenols were undetectable by thin-layer chromatography.

<table>
<thead>
<tr>
<th>Plant species and origin</th>
<th>Content of polyrenols (% dry wt)</th>
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<tbody>
<tr>
<td></td>
<td>Free alcohols</td>
</tr>
<tr>
<td>Bucida buceras L. (W.A.)</td>
<td>–</td>
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<tr>
<td>Bucida buceras L. (Hon)</td>
<td>–</td>
</tr>
<tr>
<td>Combretum braeckeiroides Brandis (W.A.)</td>
<td>–</td>
</tr>
<tr>
<td>Combretum celastroides Welw. ex C. Laws. (W.A.)</td>
<td>–</td>
</tr>
<tr>
<td>Combretum farinosum H.B.K. (W.A.)</td>
<td>–</td>
</tr>
<tr>
<td>Combretum molle R. Br. ex G. Don (W.A.)</td>
<td>2.0–4.0</td>
</tr>
<tr>
<td>Conocarpus erectus L. var. sericeus Fors ex Dc. (W.A.)</td>
<td>–</td>
</tr>
<tr>
<td>Conocarpus erectus L. var. sericeus Fors ex Dc (Hon)</td>
<td>–</td>
</tr>
<tr>
<td>Terminalia arjuna (Roxb.) (W.A.)</td>
<td>–</td>
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<tr>
<td>Terminalia bentzoe Pers.</td>
<td>–</td>
</tr>
<tr>
<td>Terminalia bentzoe spp. bentzoe (L.) L.f. (W.A.)</td>
<td>–</td>
</tr>
<tr>
<td>Terminalia calamansana (Hon)</td>
<td>–</td>
</tr>
<tr>
<td>Terminalia catappa (Hon)</td>
<td>–</td>
</tr>
<tr>
<td>Terminalia hookensis (Hon)</td>
<td>–</td>
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<tr>
<td>Terminalia kaemprichii Warb. (W.A.)</td>
<td>–</td>
</tr>
<tr>
<td>Terminalia littoralis var. littoralis Seem. (W.A.)</td>
<td>–</td>
</tr>
<tr>
<td>Terminalia sambesiaca (Hon)</td>
<td>–</td>
</tr>
<tr>
<td>Terminalia samoensis Rech. (W.A.) (Guam)</td>
<td>–</td>
</tr>
<tr>
<td>Terminalia samoensis Rech. (W.A.) (Atol Taka)</td>
<td>–</td>
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<tr>
<td>Terminalia sp. (Fiji)</td>
<td>–</td>
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</tbody>
</table>

The semi-quantitative estimation of the polyrenol content was performed by comparing the size and intensity of the TLC spot with that of the known amount of standard polyrenols and polyrenol esters.

The origin of leaves is indicated in parentheses: W.A., Waimea Arboretum and Botanical Garden; Hon, Honolulu Botanical Gardens; Guam, Guam Botanical Garden; Atol Taka, Botanical Garden at Taka; Fiji, Fiji Botanical Garden.
In only one of the studied plants, *C. molle*, a high content of polyrenols could be detected upon thin-layer chromatography in ethyl acetate/benzene (1:19, v/v). These polyrenols formed a long not separable spot migrating \( R_F = 0.30 - 0.55 \) ahead of polyrenols isolated from various species of *Potentilla* (Świeżewska et al., 1994) and those from leaves of *Prunus incisa* and *Sorbus suecica* (Świeżewska & Chojnacki, 1996). Only a trace amount of material resembling polyrenyl esters was observed in the extracts of *C. molle*.

The fraction of free polyrenols isolated from *C. molle* by column chromatography was subjected to subfractionation on the same column using eluents containing increasing concentrations of ethyl ether in hexane. Thus, the original polyrenol fraction was divided into seven

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**Fig. 1. TLC and HPLC records of the polyrenols of *C. molle*.**

a. The polyrenol mixture isolated from leaves was subfractionated by column chromatography on Silica Gel as described in Materials and Methods, into seven subfractions (A–G) using hexane which contained increasing concentrations of ethyl ether. Each fraction was examined by TLC on Silica gel plates developed with ethyl acetate/benzene (1:19, v/v). Spots were stained with iodine. b. HPLC records of polyrenol fractions isolated as described in Fig. 1a. Only the records of fractions A, B and G are shown. HPLC was performed as described in Methods. Peaks of polyrenols were detected with the UV detector set at 210 nm. The numbers 20, 30, 40, 50, 60 and 70 mark the positions of prenol-20, -30, etc. The position of elution of the studied prenologues was mapped using standard individual prenologues isolated from *P. suecica*. 

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*Combretum bracteosum*, *Combretum cælasteroides*, *Terminalia calamansana*, *Terminalia catappa*, *Terminalia sambesiaca* and a local *Terminalia* sp. originating from Fiji.
subfractions (A–G), representing faster and
slower moving polyisoprenols (Rf = 0.6, 0.57, 0.55,
0.53, 0.51, 0.50 and 0.49, respectively; Fig. 1a).
These polyisoprenols were further examined by
HPLC (Fig. 1b). Each fraction contained a wide
range of polyisoprenoid alcohols, of which the
shortest chain substances were composed of
about 20–25 isoprene units, and the longest of
60–70 isoprene units. There were no polyisen-
rols exceeding the length of 70–75 isoprene
units as it can clearly be seen on the HPLC
record presented in Fig. 1b, subfraction A. The
occurrence of large amounts of free, non-esteri-
fied polyisoprenols of the length exceeding that of
the prenologues previously found in leaves of
various plants, is reported for the first time in
the present paper. The longest chain polyisopre-
nols found previously in leaves of L. racemosa
(up to 100 isoprene residues) were in the form of
esters (Skoczylas et al., 1994) and the prenolo-
gues composed of about 20 isoprene units
were the dominating ones.

In half of the studied species we have detected
considerable amounts of polyisoprenols. In all
polyisoprenol positive plants the polyisoprenol
spectrum was similar in that it began with preno-
logues composed of about 20 isoprene units and
contained dozens of longer chain polyisopren-
ols up to prenologues composed of about 70
(cf. Fig. 1) and more isoprene units. It seems
that the polyisoprenol spectrum in Combretaceae
is not as broad as previously reported for L.
racemosa (Skoczylas et al., 1994). In C. molle it
does not exceed 70–75 isoprene units.

In none of the studied species of Combretaceae
the presence of short chain polyisoprenols which
are common in various groups of plants typical
of tropical and subtropical regions (Sasaki &
Chojnacki, 1973; Jankowski & Chojnacki, 1991;
1995) was detected. This was also true for the
species investigated in the present study as well
as in the previous report (Skoczylas et al., 1994).

The examination of the polyisoprenol fraction
from leaves of C. molle by 200 MHz 1H NMR
spectrometry revealed the characteristic domi-
nation in its structure of cis-isoprene units over
the trans-residues, and the presence of other
characteristic features previously reported for
fully unsaturated poly-cis-isoprenes from
leaves of another representative of Combretaceae
— L. racemosa (Skoczylas et al., 1994). The exact
number of trans-isoprene units in a molecule
could not be estimated and the presence of
allylic structure could not be clearly demon-
strated in the case of polyisoprenols of C. molle
because the isolated polyisoprenol mixture (1–2
mg) was not sufficiently pure. The identity of
the HPLC record of the studied polyisoprenols
with those of the fully identified di-trans-poly-
isoprenols of P. aurea (Świeżewska et al., 1992)
and of L. racemosa may suggest that polyisoprenols
of C. molle have identical structure.

Cytological studies (Stace, 1989) evidenced
the unique character of plants belonging to
Combretaceae family. The relation between
the specific anatomical features of leaves of these
plants and the occurrence of the polyisoprenols
deserves further investigations. The 16 species
of this family investigated in this study provide
data suggesting that the observed type of poly-
isenol may serve as a chemotaxonomic marker
for particular species of this systematic family,
similarly as it was found in the case of other
systematic groups (Świeżewska et al., 1994).
The number of species in Combretaceae family
exceeds 350 (Zwejkowska & Szwajkowski, 1992)
and chemotaxonomic studies on larger
material are required before definite conclu-
sions can be reached.

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