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The occurrence of long-chain polyprenols 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 polyprenols of this type were found in half of the 20 species studied. In most cases the polyprenols occurred in the form of fatty acid esters. Only in one species — *Combretum molle*, the polyprenols were found in the form of free alcohols. The amount of long-chain polyprenols varied with the plant species; the richest source was *C. molle* (about 4% of dry mass of leaves). Polyprenol 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 polyisoprene 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 polyprenols 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 (Świeżewska *et al.*, 1994). In *Lumnitzera racemosa* belonging to *Combretaceae* family we detected polyprenols 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 Haiewa 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 polyprenols was studied in leaves of species belonging to *Combretaceae* family (Table 1). Long-chain polyprenols were detected in about half of the studied plants. Their presence was confirmed upon examining plants from other regions of Hawaii. The content of polyprenols in the two groups of plants was not identical as exemplified by *Bucida buceras*. In six plant species polyprenols were undetectable by thin-layer chromatography

Table 1
Polyprenols in leaves of plant species of Combretaceae family

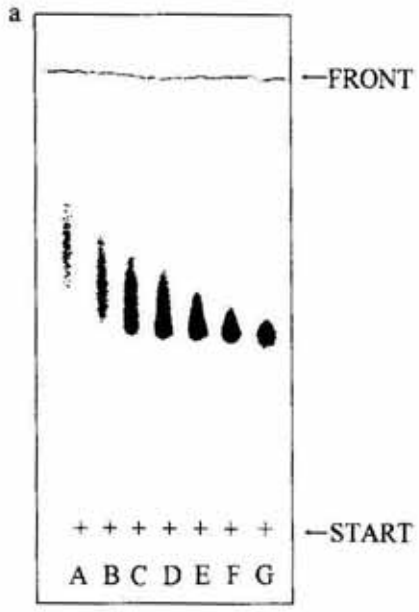
Plant species and origin	Content of polyprenols (% dry wt)	
	Free alcohols	Esterified
Bucida buceras L. (W.A.)	–	0.1–0.5
Bucida buceras L. (Hon)	–	< 0.1
Combretum bracteosum Brandis (W.A.)	–	< 0.1
Combretum celastroides Welw. ex C. Laws. (W.A.)	–	< 0.1
Combretum farinosum H.B.K. (W.A.)	–	0.1–0.5
Combretum molle R. Br. ex G. Don (W.A.)	2.0–4.0	< 0.1
Conocarpus erectus L. var. sericeus Fors ex Dc. (W.A.)	–	1.0–2.0
Conocarpus erectus L. var. sericeus Fors ex Dc (Hon)	–	0.1–0.5
Terminalia arjuna (Roxb.) (W.A.)	–	0.1–0.5
Terminalia bentzoe Pers.	–	0.5–1.0
Terminalia bentzoe ssp. bentzoe (L.) L.f. (W.A.)	–	1.0–2.0
Terminalia calamansana (Hon)	–	< 0.1
Terminalia catappa (Hon)	–	< 0.1
Terminalia ivorensis (Hon)	–	0.1–0.5
Terminalia kaernbachii Warb. (W.A.)	–	2.0–4.0
Terminalia littoralis var. littioralis Seem. (W.A.)	–	0.5–1.0
Terminalia sambesiaca (Hon)	–	< 0.1
Terminalia samoensis Rech. (W.A.) (Guam)	–	0.1–0.5
Terminalia samoensis Rech. (W.A.) (Atol Taka)	–	1.0–2.0
Terminalia sp. (Fiji)	–	< 0.1

The semiquantitative estimation of the polyprenol content was performed by comparing the size and intensity of the TLC spot with that of the known amount of standard polyprenols and polyprenol esters.
The origin of leaves is indicated in parentheses: W.A., Waimea Arboretum and Botanical Gardens; Hon, Honolulu Botanical Gardens; Guam, Guam Botanical Garden; Atol Taka, Botanical Garden at Taka; Fiji, Fiji Botanical Garden.

(*Combretum bracteosum, Combretum celastroides, Terminalia calamansana, Terminalia catappa, Terminalia sambesiaca* and a local *Terminalia* sp. originating from Fiji).

In only one of the studied plants, *C. molle*, a high content of polyprenols could be detected upon thin-layer chromatography in ethyl acetate/benzene (1:19, v/v). These polyprenols formed a long not separable spot migrating (R_F = 0.30–0.55) ahead of polyprenols 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 polyprenyl esters was observed in the extracts of *C. molle*.

The fraction of free polyprenols 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 polyprenol fraction was divided into seven



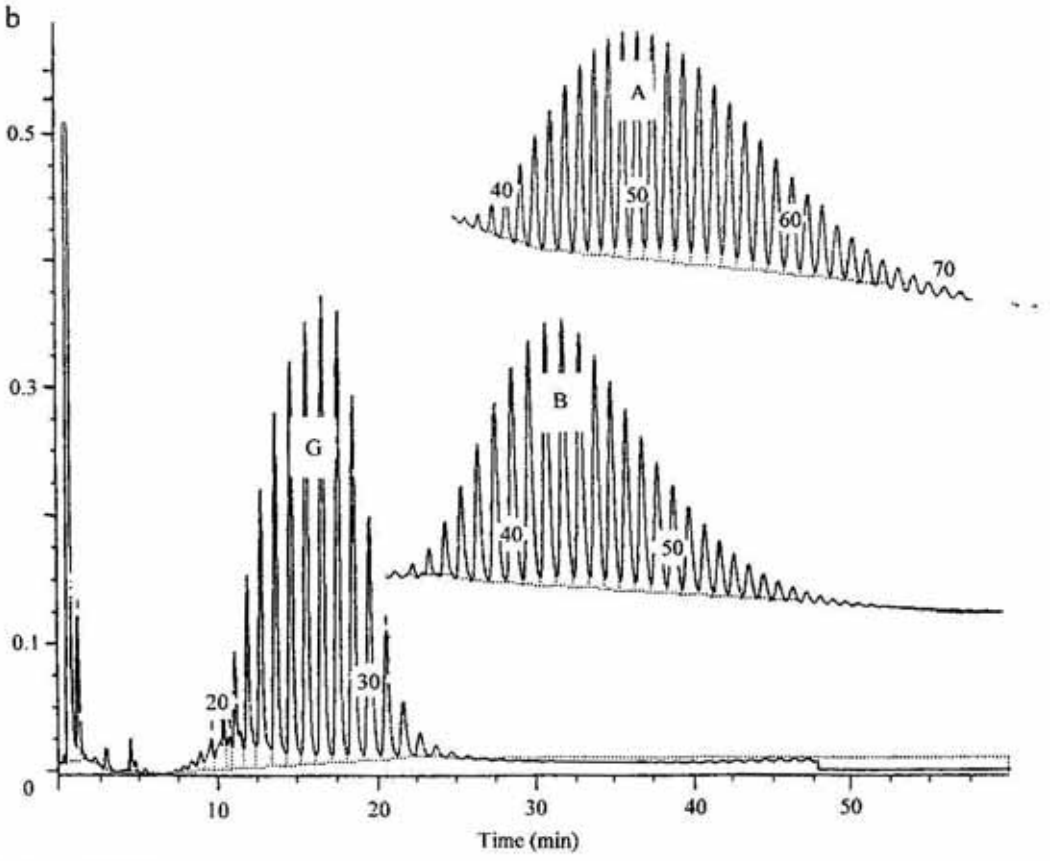


Fig. 1. TLC and HPLC records of the polyprenols of C. molle.

a. The polyprenol 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 polyprenol 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 polyprenols 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. aurea*.

subfractions (A–G), representing faster and slower moving polyprenols (R_F = 0.6, 0.57, 0.55, 0.53, 0.51, 0.50 and 0.49, respectively; Fig. 1a). These polyprenols 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 polyprenols 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-esterified polyprenols 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 polyprenols found previously in leaves of *L. racemosa* (up to 100 isoprene residues) were in the form of esters (Skoczylas *et al.*, 1994) and the prenologues composed of about 20 isoprene units were the dominating ones.

In half of the studied species we have detected considerable amounts of polyprenols. In all polyprenol positive plants the polyprenol spectrum was similar in that it began with prenologues composed of about 20 isoprene units and contained dozens of longer chain polyprenols up to prenologues composed of about 70 (cf. Fig. 1) and more isoprene units. It seems that the polyprenol 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 polyprenols which are common in various groups of plants typical of tropical and subtropical regions (Sasak & 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 polyprenol fraction from leaves of *C. molle* by 200 MHz ^{1}H NMR spectrometry revealed the characteristic domination 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 demonstrated in the case of polyprenols of *C. molle* because the isolated polyprenol mixture (1–2 mg) was not sufficiently pure. The identity of the HPLC record of the studied polyprenols with those of the fully identified di-*trans*-polyprenols of *P. aurea* (Świeżewska *et al.*, 1992) and of *L. racemosa* may suggest that polyprenols 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 polyprenols deserves further investigations. The 16 species of this family investigated in this study provide data suggesting that the observed type of polyprenol 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 550 (Szwejkowska & Szwejkowski, 1992) and chemotaxonomic studies on larger material are required before definite conclusions can be reached.

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