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The search for polyprenols in dendroflora of Vietnam★☆

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The occurrence of polyprenols in leaves of over 340 species of dendroflora in natural habitats in the regions of Hanoi and Hue in Vietnam was studied. Plant material was collected in the late autumn (October/November) during the end of a vegetation season. Leaves of about 200 plant species did not contain detectable amounts of polyprenols in contrast to few systematic families, e.g. *Moraceae, Euphorbiaceae*, where polyprenols were highly abundant and their pattern could be used as a chemotaxonomic criterion. Most often dominating polyprenols were prenol-11 and prenol-12. In several angiosperm species prenol-13 and detectable amounts of prenol-14 were also found. The incidence of prenol-13 and -14 was not restricted to a specific taxonomic group since species exhibiting domination of such longer chain polyprenols belonged to various systematic families. In some plants (e.g. *Ceiba pentandra*) α-*cis* polyprenols were accompanied by α-*trans* counterparts. This report describes several new plant species that may serve as natural sources of long chain polyprenols.

Keywords: polyprenols, plant chemotaxonomy

INTRODUCTION

Studies on the biodiversity of the flora of South East Asia have often led to deeper understanding of the phytochemical phenomena. Thus the search for long chain polyprenols (Fig. 1a) has been given new impetus and caused distinct qualitative changes in the approach of biochemists to the problem of long chain polyprenols in plants. The earliest findings were focused on the occurrence and accumulation of undecaprenol (prenol-11) in leaves (Stone *et al.*, 1967) and for a long time the name 'plant polyprenol' and 'undecaprenol' were interchangeable. Up to the end of the 20th century a number of other types of polyprenols were discovered (Swiezewska *et al.*, 1994; Rezanka & Votruba, 2001). This was due to studying many various plant species especially of tropical and subtropical regions. The opportunity to study plants of the phytogeographic region of Vietnam has emerged from the well established scientific cooperation between the Polish Academy of Sciences and the similar institutions in Vietnam. The interest of Polish botanists in the flora of South East Asia was marked by such events as the expeditions of Tadeusz Przybylski (1965) in various regions of Vietnam in 1960s and efforts of Wieslaw Gawrys and Katarzyna Goller, Kazimierz Browicz and Tadeusz Chojnacki resulting in acquiring new plants for our local living collections and finding original chemotaxonomic types of plant species (Skoczylas *et*

★This paper is dedicated to Professor Tadeusz Chojnacki from the Institute of Biochemistry and Biophysics, Polish Academy of Sciences in Warsaw on the occasion of the 50th anniversary of his scientific activity and 75th birthday.

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Abbreviations: HPTLC, high performance thin-layer chromatography; P-n, prenol composed of n isoprene units; RP-18, reversed phase octadecyl modified.

Figure 1. Structures of a, polyprenols and b, dolichols; t and c indicate internal *trans* and *cis* isoprenoid residues, respectively.

al., 1994; Jankowski & Chojnacki, 1995). This phytogeographic region has been known to the wide scientific public since it was described many centuries ago by Chi Han (Ji & Li, 1979).

A few plant species recorded in this book known since almost 2000 years, were now subjected to the search for long chain isoprenoids together with a major part of only presently recorded species. It should be noticed that from about 80 plant species described by Chi Han in 304 A.D. we could meet only a few identical plant species on performing "at random" the collection of over 340 various leaf specimens in late autumn of 2003 in the region of Hanoi and Bach Ma National Park. The knowledge of the main record of plant species (Flore generale de l'Indochine) by Lecomte (1965) had to be supplemented on collecting the leaf samples with the actual data and great practice of local Vietnamese botanists.

In the more recent publications and reviews by scientists of the Vietnamese Academy of Science and Technology and Hanoi National University (2001, 2003, 2005), Vietnam was considered as biodiversity-rich with its about 15 000 plant species and a great number of endemites. Specific distribution of different climatic types and diverse soils support very unusual and important habitats for many unique species. It is stated that 350 species are on the Red List of endangered plants. The deep interest in performing phytochemical research on this biological material is thus fully justified. It is also worth mentioning that this most valuable biodiversity is disappearing in an alarming rate, with the loss of landraces and the destruction of natural habitats.

As the results of screening for the presence of long chain polyprenols a large number of unique interesting plant species in their natural habitats were expected to give additional information concerning the metabolism of isoprenoid-secondary substances and provide a new tool for plant chemotaxonomy (Roslinska *et al.*, 2002). These results may be taken as a record of any post-war effect the huge amounts of defoliants that have been used in Vietnam 40 years ago.

The reconnaissance work presented in this report and a record of it may be useful for any future enterprises concerning botanochemicals and protection of the environment.

MATERIALS AND METHODS

Leaves of 340 plant species were collected in natural habitats in the region of Hanoi and in Bach Ma National Park, i.e. in the northern part of Vietnam down to the middle part of the country (Hue region). The collection was performed in October and November of 2003 during the end of a vegetation period of leaves. About 20 of them were performed in duplicate to exclude the possibility of the effect of insolation on the rate of accumulation of polyprenols (Bajda *et al*., 2005).

The leaves were allowed to dry at room temperature for 3 months (the tests performed on about 100 samples stored for 2 years gave similar results) before analyses. Portions (100 mg) of leaves were homogenized in 2 ml of acetone/hexane (1 : 1, v/v) and the extracts (0.01 ml) were subjected to thin-layer chromatography (TLC) on silica gel plates in two solvent systems (A, toluene/ethyl acetate, 9 : 1, v/v, and B, toluene/hexane, 1 : 1, v/v) and on RP-18 plates in acetone (solvent C). Staining of chromatograms was performed in iodine vapors. HPTLC plates were from Merck (Darmstadt, Germany), organic solvents from POCh (Gliwice, Poland). The identity of separated polyprenoids was demonstrated by co-chromatography with known standards (Collection of Polyprenols, Institute of Biochemistry and Biophysics, PAS, Warszawa, Poland; Woldanski *et al.*, 1995).

Extract from leaves of *Magnolia kobus* (prenol-10, -11, -12 and -13, Fig. 2, line 3) and needles of *Picea abies* (prenol-14,-15,-16,-17 and -18, Fig. 2, line 4) were used as standards. Mixtures of polyprenyl esters were from *Ginkgo biloba* and *Sorbus suecica*; they gave rise to a mixture of polyprenols composed of 17–40 isoprene units upon strong alkaline hydrolysis (Wellburn *et al*., 1967). All-*trans*-prenol-9 (solanesol)

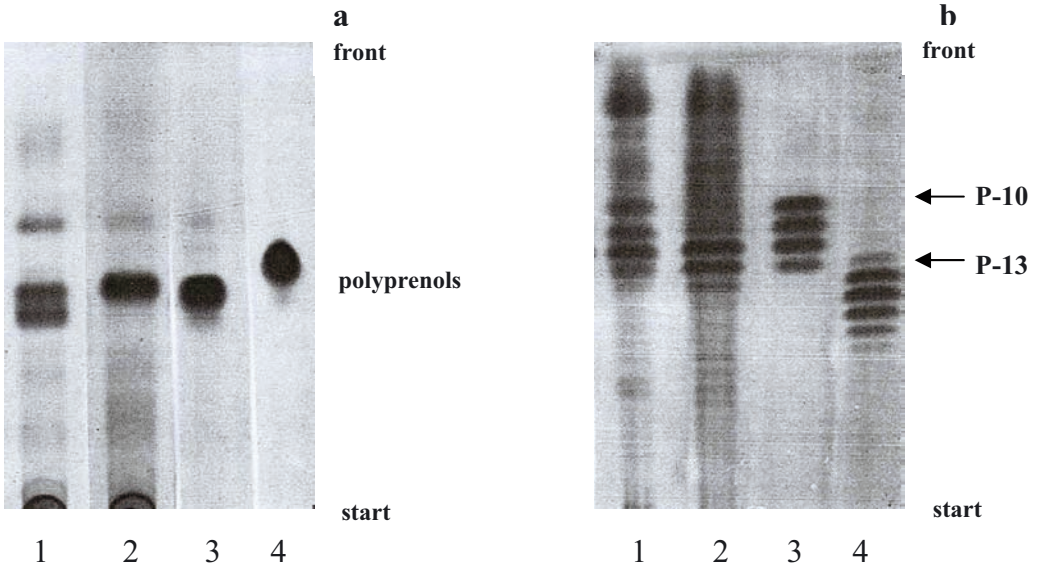

Figure 2. Representative TLC records (a, silica gel, b, RP-18) of polyprenols isolated from *Ceiba pentandra* (lane 1), *Hibiscus tiliaceus* (2), polyprenol standard mixture (prenol-10, -11, -12, -13) (3), *Picea abies* (4).

was prepared from tobacco leaves (Rowland *et al.,* 1956).

Semiquantitative estimation of polyprenols was done by comparing the size and intensity of chromatographic spots with those of known amounts of standard substances (Wellburn & Hemming, 1966).

RESULTS AND DISCUSSION

In over 300 studied plant species belonging to 104 taxonomic families (listed in the Supplementary Table) we found several very high accumulators of long chain polyprenols. The 28 plant species in which, according to semiquantitative assay, the content of polyprenols exceeded 1% of the dry weight are listed in Table 1. In the majority of them the polyprenol family consisted of prenologues composed of 10 to 14 isoprene units. In one of the studied species the presence of not a group but rather one, not fully identified prenologue (prenol-10 in *Disporum trabeculatum*) was observed. In another species, *Smilax perfoliata* a family of relatively shorter prenologues, prenol-9 and -10 was detected. The rich sources of polyprenols listed in Table 1 represent 17 systematic families. The highest content of polyprenols in leaves, exceeding 4% of dry weight, was found in *Euodia lepta* (*Rutaceae*) and in *Commersonia bartramia* (*Sterculiaceae*). These high amounts of polyprenols may not be exceptional in the plant kingdom; on examining leaves of two or more individuals of the same species growing in different places we could find variations of the rate of accumulation of polyprenols that might have been due to ecological conditions e.g. the extent of insolation (cf. Bajda *et al.,* 2005, see also the record of all specimens studied in Supplementary material).

In a number of other plant species (in about 1/3 of the studied species) we found significant amounts of polyprenols though not exceeding 1% of dry weight. Thus in *Annonaceae* considerable amounts of polyprenols were found in 6 of the studied 7 species. Also in the *Euphorbiaceae* family 8 out of the 21 studied species contained relatively high amounts of polyprenols. In *Fabaceae* the proportion was 14 of 26 studied species, in *Lauraceae* 7 of 14, in *Malvaceae* 5 of 7, in *Moraceae* 13 of 17 and in *Sterculiaceae* 4 of 7 species. Relatively high accumulation of polyprenols in representatives of *Euphorbiaceae, Lauraceae, Moraceae, Sapindaceae* etc. is in agreement with our previous observations (Swiezewska *et al.,* 1994; Jankowski *et al*., 1994; Jankowski & Chojnacki, 1995; George *et al*., 2001).

In some systematic families we could find no polyprenols, e.g. 7 species belonging to systematic family *Apocynaceae* were polyprenol negative; so were 15 species of the *Verbenaceae* family and 10 species of *Myrtaceae*. In 6 studied species of *Theaceae* and *Rubiaceae* families we could find traces of polyprenols only in one species. A full record of all studied species is available in the Supplementary material. The present results on the absence of polyprenols from all species of a systematic group are in agreement with the earlier data of Ranjan *et al*. (2001) and Ciepichal who found a complete absence of polyprenols in more than one hundred representatives of genus *Eucalyptus*, family *Myrtaceae* (unpublished).

Table 1. The richest plant sources of polyprenols of dendroflora of North and Central Vietnam

Family	Species	Total number of species tested	Chain length (number of isoprene units)	Approximate content (% dry weight)
Annonaceae		7		
	Annona squamosa		10,11	1.0–2.0
	Cananga odorata		10,11	2.0–4.0
Bombaceae		1		
	Ceiba pentandra		10,11,12,13	2.0–4.0
Euphorbiaceae		16		
	Antidesma ghaesembila		12,13,14	1.0–2.0
Fabaceae		26		
	Acacia auriculariformis		11,12,13	2.0–4.0
	Archidendron sp.		12,13,14	1.0–2.0
Lauraceae		13		
	Litsea glutinosa		10,11,12,13	2.0–4.0
Liliaceae		1		
	Disporum trabeculatum (Gagnep)		10	1.0–2.0
Magnoliaceae		2		
	Michelia alba		10,11,12	2.0–4.0
Malvaceae		7		
	Hibiscus tiliaceus		11,12,13,14	2.0–4.0
	Sida mysorensis		10,11,12	1.0–2.0
Moraceae		17		
	Ficus benjamina		10,11	1.0–2.0
	Ficus elastica		10,11,12	1.0–2.0
Ochnaceae		1		
	Gomphia striata		9,10,11	1.0–2.0
Rutaceae		9		
	Euodia lepta		11,12,13,14	> 4.0
	Zantoxylum scabrum		11,12,13	2.0–4.0
Sapindaceae		3		
	Allophylys caudatus		11,12,13	1.0–2.0
	Cardiospremum calicacabum		11,12,13	2.0–4.0
	Dimocarpus longan		12,13,14	2.0–4.0
Smilacaceae		3		
	Smilax corbularia		10,11,12	2.0–4.0
	Smilax perfoliata (Gagnep.)		9,10	1.0–2.0
Sterculiaceae		8		
	Commersonia bartramia		10,11,12	> 4.0
	Pterospermum diversifolium		11,12,13	1.0–2.0
Tiliaceae		3		
	Muntingia calabura		10,11,12	1.0–2.0
Ulmacease		4		
	Celtis sinnsis		11,12,13	1.0–2.0
	Trema angustifolia		10,11	1.0–2.0
	Trema orientalis		10,11	2.0–4.0
Zingiberaceae		1		
	Alpinia globosa		11,12,13,14	1.0–2.0

Our data show that the phenomenon of accumulation of large amounts of polyprenols may be observed in species of several different botanical families, though in some other families we could not find any (e.g. *Verbenaceae*) or only single (e.g. *Rubiaceae*) examples of polyprenol-positive species. High accumulation of polyprenols in representatives of *Lauraceae*, *Moraceae*, *Sapindaceae* etc. was in agreement with our previous observations (Swiezewska *et al*., 1994). Distinct amount of solanesol, typical for tobacco (Rowland *et al*., 1956) and other *Solanaceae* plants could be observed in only one representative of this family (*Datura metel*, Supplementary Table at: www.actabp.pl).

In the presented studies we do not comment on the occurrence of large molecular weight natural

rubber which in some cases formed a distinct tailing in adsorption chromatography and remained on the start on reversed phase TLC system. In the present study we did not pay special attention to the presence of partially saturated polyprenols (dolichols, Fig. 1b) which could be present in plant material (Jankowski *et al.*, 1994; Wojtas *et al*., 2005; Swiezewska & Danikiewicz 2005).

In a number of plants studied in this report the dominating polyprenol was prenol-13. Domination of this polyprenol and the presence of a longer chain prenol-14 was previously observed only in the species belonging to *Capparidaceae* and *Sapindaceae* families (Jankowski & Chojnacki, 1991; 1995). In the present study we could observe its moderately high content in leaves of plants belonging to at least seven other botanical families (Table 1 and Supplementary material): *Amaranthaceae (Celosia argentea)*, *Araliaceae (Trevesia* sp.*)*, *Euphorbiaceae (Antidesma ghaesembilla)*, *Fabaceae (Archidendron)*, *Malvaceae (Hibiscus tiliaceus)*, *Rutaceae (Euodia lepta)*, *Sapindaceae (Dimocarpus longan)* and *Zingiberaceae (Alpinia globosa)* as observed earlier for *Euphorbiaceae* (George *et al*., 2001). In *Tiliaceae* (*Triumfetta bartramia*) and in another *Alpinia* plant (*Zingiberaceae*) a polyprenol pattern (prenol-16-20), quite unique in these systematic families, was detected.

One can distinguish also a group of plants (both with low and high content of polyprenols covering the range from 10 to 14 isoprene units) in which two closely migrating, not well separated, spots of polyprenols could be observed upon adsorption chromatography in solvent A. These plants were as follows: *Fabaceae* (*Acacia auriculiformis*, *Acacia mangium*, *Archidendron clypera*, *Adenanthera microsperma*, *Archidendron* sp., *Bauhinia lorantha* and *Cassia fistula*), *Lecythidaceae* (*Barringtonia macrostachya*), *Malvaceae* (*Abutilon indicum, Sida mysorensis*), *Sapindaceae* (*Allophylus caudatus*) and *Smilacaceae* (*Smilax corbularia*). Recording of a number of such cases in the present studies drew our attention to the nature and the factor(s) responsible for such a phenomenon that we have not encountered previously on checking several hundreds of plant polyprenol mixtures. According to the NMR spectrum the occurrence of the double spot of polyprenols in some plants is due to the presence of two isomeric forms, i.e. α-*cis*- and α-*trans*-poly-*cis*-prenols, alloprenols (Ciepichal *et al*., 2007). The reason for the presence of two isomeric forms of polyprenols in some plants is not clear. TLC pattern of polyprenols of representative plants with typical α-*cis* and a mixture of α-*cis* and α-*trans* unit, e.g. *Hibiscus tiliaceus* and *Ceiba pentandra*, respectively) is shown in Fig. 2.

In a small group of plants we found accumulation of larger amounts of polyprenyl esters in leaves; they are listed in Supplementary material. Their content varied approximately from 0.1–1.0% of the dry weight and polyprenols in these cases were composed of 16 to 40 isoprene units. The exception was *Dacrydium elatum* (*Podocarpaceae*) containing 2–4% of polyprenyl esters while the size of molecules was from 18, 19 to 24–27 isoprene units. The polyprenyl families in leaves of plants of this group were similar to those already recorded in *Coniferopsida* and in *Rosaceae* (Swiezewska *et al*., 1992).

The present paper points at the possibility of exploiting of a great number of plant species of Vietnamese flora for large scale preparation of unique polyprenyl lipids. A special interest in the use of polyprenols of unique size, like prenol-13 can be fulfilled due to its presence in a number of plant species as stated in the present paper.

It is hoped that the presented record of the occurrence of polyprenols in shrubs and trees of Vietnamese flora may contribute to its better understanding and to practical use.

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