Phytochemistry, Vol 23, No 11, pp 2517–2521, 1984
Printed in Great Britain

0031–9422/84 $3 00 + 0 00

POLYPRENOLS FROM CONIFERS: MULTIPLICITY IN CHAIN LENGTH DISTRIBUTION

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(*Received* 9 *March* 1984)

Key Word Index—*Cryptomeria japonica, Metasequoia glyptostroboides*, Taxodiaceae, *Sciadopitys verticillata*, Sciadopitydaceae, *Chamaecyparis obtusa, Juniperus chinensis, J rigida*, Cupressaceae, *Araucaria brasiliana*, Araucariaceae, *Podocarpus macrophylla, P Nagi*, Podocarpaceae, *Cephalotaxus harringtonia*, Cephalotaxaceae, *Taxus cuspidata, Torreya nucifera*, Taxaceae, polyprenols, ^{13}C NMR, characterization

Abstract—Long-chain betulaprenols have been isolated from the leaves of twelve coniferous plants, which can be roughly classified into two categories with respect to the pattern of the chain length distribution A complex distribution pattern with two maxima has been found in six of them for the first time

INTRODUCTION

Betulaprenols are polyprenols in which two *trans*-isoprene residues are linked to an ω-terminal dimethylallyl group and various numbers (j) of *cis*-isoprene residues are successively linked to the *trans*-isoprene units (**1**, $i = 2$) Short chain betulaprenols are found in small amounts in the woody tissue of *Betula verrucosa* (betulaprenols-6 to -9 ($j = 3$ to 6), 0 03% wet wt) [1] and the cells of the bacterium *Lactobacillus plantarum* (bactoprenol-11 ($j = 8$), 0 004% wet wt) [2] Quite recently, however, it has been found that the leaves of *Ginkgo biloba* [3] and the needles of pine trees [4] produce long-chain homologues ($j = 8$ to 20) of betulaprenols in fairly high yield (up to 0 8% of wet wt, or 2% of dry wt) Ficaprenols, in which three *trans*-isoprene residues are linked to the ω-terminal residue (**1**, $i = 3$) [5] have been found predominantly in angiosperms [1, 6–11] The presence of betulaprenols in gymnosperms and ficaprenols in angiosperms is of chemotaxonomic interest and led us to investigate further the occurrence of betulaprenols in other plants in the subdivision Gymnospermae

RESULTS AND DISCUSSION

The leaves (or needles) of twelve plants of the order Coniferales were taken for analysis (Table 1) The plants were identified by Professor S Kuroyanagi, Faculty of Agriculture, Okayama University, who used the classification of Sporne [12]

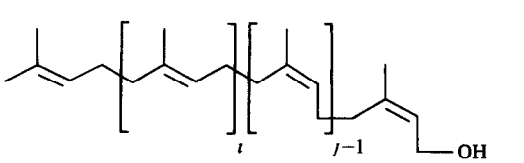

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Table 1 Content of polyprenols and the degree of acetylation

Plants	Total polyprenols (% of dry leaf wt)	Acetylated (% of total polyprenols)
Cryptomeria japonica	1 1	50
Metasequoia glyptostroboides	0 2	~100
Sciadopitys verticillata	1 4	*
Chamaecyparis obtusa	1 0	85
Juniperus chinensis	0 5	40
J rigida	1 1	25
Araucaria brasiliana	1 1	*
Podocarpus macrophylla	2 3	45
P nagi	2 1	90
Cephalotaxus harringtonia subsp *nana*	0 2	~100
Taxus cuspidata	1 6	55
Torreya nucifera	0 5	~100

*Polyprenyl acetate fraction was not separately examined, since the raw lipid extract was immediately subjected to saponification before purification

Structural characterization

The polyprenols were present in the leaves (or needles) of the twelve plants as free alcohols or their acetates (Table 1) Hence, the structure determinations of the polyprenols were done for either form, depending on the relative contents in the leaves and other circumstances The total numbers ($n = i + j + 1$) of isoprene residues were determined by FDMS of the components of each polyprenyl mixture listed in Table 3 The IR spectra of the polyprenols (free or acetylated) were in good agreement with previously reported ones [7]

The ^1H NMR spectra (Table 2) of individual free and acetylated polyprenols were also in good agreement with those reported [3, 4] As the signals at δ1 60, 1 68 and 1 74

Table 2 Relative peak intensities of methyl ^{1}H NMR and C-1 methylene ^{13}C NMR signals in polyprenols (free or acetylated) from conifers

Plants	Free (Fr) or acetylated (Ac)	Number of isoprene units	Me ^{1}H NMR signals			C-1 ^{13}C NMR signals			
			1 60 *trans* and *trans* (ω)	1 68 *cis* and *cis* (ω)	1 74 *cis* (α)	32 01 *trans–cis*	32 24 *cis–cis*	32 41 *cis–cis* (α)	39 74 *trans-trans* and ω-*trans*
Cryptomeria japonica	Ac	17	28	142	11	12	118	12	18
		22	30	190	10				
M glyptostroboides	Ac	18	31	150	10	10	128	14	18
		21	29	180	11				
S verticillata	Fr	17	31	139	11	09	131	*	20
Chamaecyparis obtusa	Ac	17	29	141	11				
		21	30	180	10	12	157	12	19
J chinensis	Fr	18	31	150	09	13	136	*	22
J rigida	Fr	17	31	138	11	09	130	*	21
A brasiliana	Fr	16	33	126	10				
		23	32	198	10	12	190	*	19
P macrophylla	Fr	16	31	129	11				
		23	33	197	10	10	190	*	20
P nagi	Fr	23	32	199	10	14	184	*	22
Cephalotaxus harringtonia subsp *nana*	Ac	18	31	148	11	11	125	12	21
Taxus cuspidata	Ac	18	31	148	12	08	132	12	19
		21	32	177	11				
Torreye nucifera	Ac	18	31	150	09	10	131	10	19

*The signal for *cis–cis* (α) is overlapped on the signal for *cis–cis* in free polyprenols

are due to the methyl protons of isoprene residues in various configurations, then the relative peak areas of these signals can be used for the estimation of the numbers of *cis*- and *trans*-isoprene units in the molecule [3, 4] If a polyprenol (free or acetylated) molecule with a total number of isoprene units of n has two internal *trans* units and a *cis*-α-terminal unit, then the relative peak areas normalized for the total number of methyl groups will be 3 ($n-3$) 1 This was the case for all the polyprenols (free or acetylated) listed in Table 1 within experimental error

The ^{13}C NMR spectra of free and acetylated polyprenols were identical with those previously reported [3, 4], except for the relative intensities of the peaks (Table 2) The peaks around δ32 0–32 5 and 39 7 were assigned to the C-1* atoms and were conveniently used for the determination of the sequence structure of *cis* and *trans*-isoprene units (Table 2) No peak at δ40 0 due to the C-1 atom in *cis–trans* linkage was present The experimental values for the relative peak areas for C-1 were in good agreement with the theoretical ones based on the structure **1** ($i = 2$).

From these results, it was concluded that all the coniferous polyprenols (free or acetylated) examined have the same *cis* and *trans* alignments as those from *G biloba* [3] and pine trees [4], i e long-chain betulaprenols with an average number of isoprene units ($\bar{n}$) ranging from 15 to 24

Chain length distribution

In Table 3 and Fig 1 are shown the chain length distribution patterns of the polyprenyl mixtures from the twelve conifers Figure 1 also includes the patterns of the other gymnosperms reported in the literature The distribution patterns of the native polyprenyl mixtures in the forms of free alcohols and acetates, from the same plant, were in good agreement with each other (Table 3), the latter being unaltered by saponification as exemplified in the case of *Taxus cuspidata* These findings strongly indicate that each of the pairs of alcohols and acetates share a common biogenetic precursor

As shown in Fig 1, two maxima were observed in

*Numbering system is as follows –C(1)–C(2)=C(3)–C(4)–C(5)–

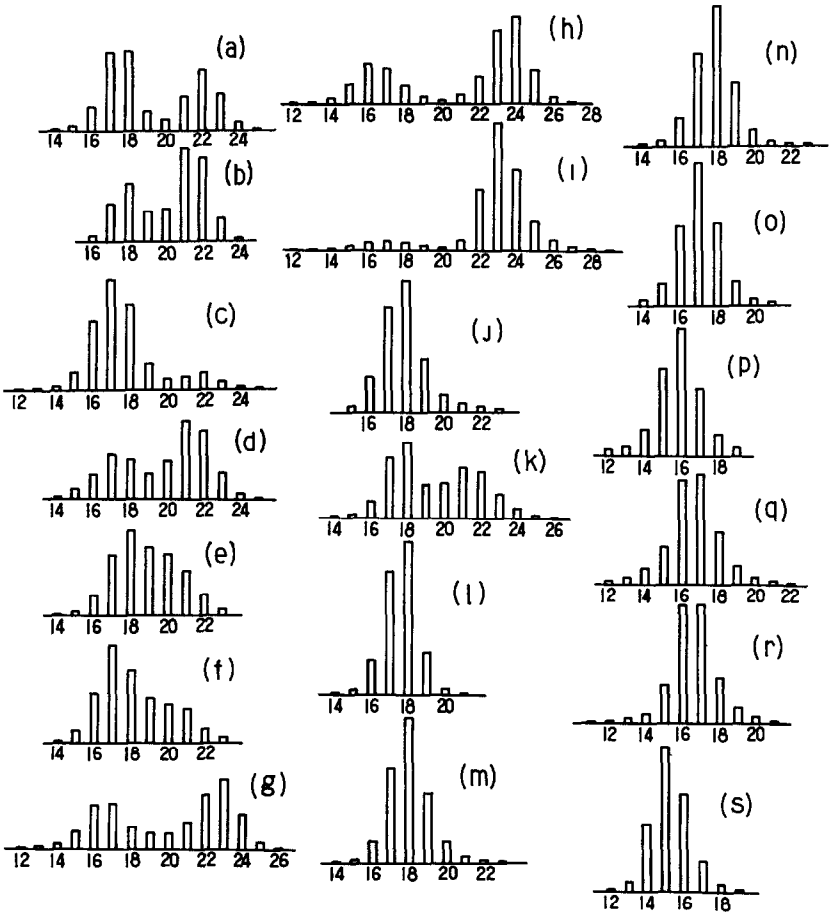

Fig 1 Chain length distribution patterns of polyprenols from (a) *Cryptomeria japonica*, (b) *M glyptostroboides*, (c) *S verticillata*, (d) *Chamaecyparis obtusa*, (e) *J chinensis*, (f) *J rigida*, (g) *A brasiliana*, (h) *P macrophylla*, (i) *P nagi*, (j) *Cephalotaxus harringtonia* subsp *nana*, (k) *Taxus cuspidata*, (l) *Torreya nucifera*, (m) *G biloba* [3], (n) *Cedrus deodara* [4], (o) *Pinus strobus* [4], (p) *P sylvestris* [4], (q) *P densiflora* [4], (r) *P thunbergii* [4], and (s) *Picea abies* [4]

Table 3 Chain length distribution of polyprenols (free or acetylated)

Plants	Free(Fr) or acetylated (Ac)	Composition (% wt)															
		$n=13$	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Cryptomeria japonica	Fr	—	05	11	49	177	219	75	43	100	167	109	35	10	—	—	—
	Ac	—	06	14	66	215	219	56	31	94	165	102	26	05	—	—	—
M glyptostroboides	Ac	—	—	—	17	101	157	83	90	252	226	64	10	—	—	—	—
S verticillata	Fr*	05	11	50	187	301	230	73	29	35	45	22	09	04	—	—	—
Chamaecyparis obtusa	Fr	—	—	15	56	103	98	79	127	225	177	74	30	17	—	—	—
	Ac	—	05	29	67	121	108	70	106	215	188	71	16	04	—	—	—
J chinensis	Fr	—	25	41	72	165	209	168	138	99	49	36	—	—	—	—	—
	Ac	—	02	10	54	163	228	184	165	120	57	17	—	—	—	—	—
J rigida	Fr	—	20	55	152	268	189	111	96	79	25	06	—	—	—	—	—
	Ac	—	06	32	131	265	198	120	102	91	39	16	—	—	—	—	—
A brasiliana	Fr*	05	14	47	120	122	60	45	45	75	150	192	98	21	06	—	—
P macrophylla	Fr†	04	15	52	108	95	49	20	10	23	71	199	237	91	20	06	—
P nagi	Fr†	03	06	12	21	25	21	13	09	27	166	347	221	80	30	12	06
Cephalotaxus harringtonia subsp *nana*	Ac	—	—	17	94	283	356	145	49	26	18	12	—	—	—	—	—
Taxus cuspidata	Ac	—	05	10	46	165	208	93	98	140	127	69	29	10	03	—	—
	Fr†	—	03	08	42	158	206	104	100	137	124	73	30	13	—	—	—
Torreya nucifera	Ac	—	06	14	93	334	415	117	17	05	—	—	—	—	—	—	—

*Total polyprenols obtained from unfractionated mixture by saponification

†Polyprenols obtained from polyprenyl acetate fraction by saponification

respect of the chain length distribution for six plants *Cryptomeria japonica, M glyptostroboides, Chamaecyparis obtusa, A brasiliana, P macrophylla* and *Taxus cuspidata* This is the first time that this type of distribution pattern has been observed for a polyprenyl mixture of plant origin That this distribution pattern is truly composed of a series of polyprenol homologues is substantiated by the fact that the NMR analysis showed that the two typical components in a series have the same generic structure **1** ($i = 2$) (cf Table 2) A typical simple distribution with one maximum as in Pinaceae species [4] and *G biloba* [3] was observed in two cases *Cephalotaxus harringtonia* subsp *nana* and *Torreya nucifera* Deformed patterns were observed in the other four cases either one of the two maxima was very small (*S verticillata* and *P nagi*), or the two maxima overlapped each other (*J chinensis* and *J rigida*)

The above multiplicity in chain length distribution is not directly related with taxonomy, since the distribution patterns are quite different between *Chamaecyparis obtusa* and *J chinensis* or *J rigida*, all of which belong to the family Cupressaceae and between *Taxus cuspidata* and *Torreya nucifera* of the family Taxaceae

Although there is considerable variety in the complex distribution patterns, the maximum positions, i e the number ($\bar{n}$) of isoprene units in each principal component of the two maxima in each complex distribution, are roughly constant $\bar{n} = 16$–18 and $\bar{n} = 21$–24 It is noteworthy that for all of the simple distributions in Fig 1 $\bar{n} = 15$–18, which is nearly equal to that for the maximum with shorter chain lengths in the complex distribution

EXPERIMENTAL

Isolation and purification of polyprenols and polyprenyl acetates The leaves (or needles) of *Cryptomeria japonica* D Don and *M glyptostroboides* Hu et Cheng were collected in June, those of *Chamaecyparis obtusa* Endl in October, those of *Torreya nucifera* Sieb et Zucc in November, those of *J chinensis* L and *J rigida* Sieb et Zucc in February, and those of *Taxus cuspidata* Sieb *et* Zucc, *Cephalotaxus harringtonia* K Koch subsp *nana* (Nakai) Kitagawa, *P macrophylla* D Don, *P nagi* Zoll *et* Moritz *ex* Zoll and *A brasiliana* A Rich in April in Okayama prefecture, Japan The leaves of *S verticillata* Sieb *et* Zucc were collected in April in Wakayama prefecture, Japan The leaves were dried and extracted by maceration with *ca* 10-fold amount (v/w) of Me_2CO–*n*-hexane (1 1) for about a month, after which the residual leaf material was re-extracted by homogenization with *ca* 5-fold amount (v/w) of the same solvent mixture of 5 min The extracts were then purified and separated into individual homologues of polyprenols or polyprenyl acetates by the method described in our previous paper [3] The total polyprenol contents of dry leaves and the percentages of acetylated polyprenols are listed in Table 1 It should be noted that these concentrations do not necessarily correspond to the maximum ones obtainable from the leaves, since it is now known that the amounts of polyprenyl compounds contained in leaves are subject to age-dependent seasonal variations [3, 4, 6]

Spectroscopy MS JEOL JMS D-300 GC/MS, ^1H and ^{13}C NMR spectra 200 MHz and 50 1 MHz, respectively, using a JEOL FX-200 spectrometer, $CDCl_3$ with TMS as an int standard at room temp (about 35°) A pulse repetition time 7–15 sec (45° pulse) was applied for the ^{13}C NMR measurements

Acknowledgement—The authors are grateful to Professor S Kuroyanagi, Okayama University, for the leaf samples

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