Phytochemistry, Vol. 23, No. 4, pp. 783–786, 1984.
Printed in Great Britain.

0031–9422/84 $3.00 + 0.00
Pergamon Press Ltd.

LONG-CHAIN POLYPRENOLS IN THE FAMILY PINACEAE

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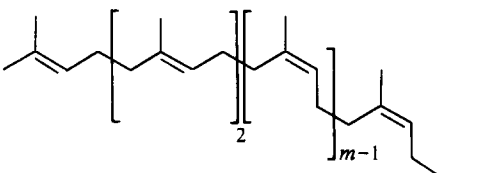
(*Revised received* 1 *August* 1983)

Key Word Index—*Pinus strobus*; *P. sylvestris*; *P. densiflora*; *P. thunbergii*; *Picea abies*; *Cedrus deodara*; Pinaceae; ^{13}C NMR; polyprenols; characterization.

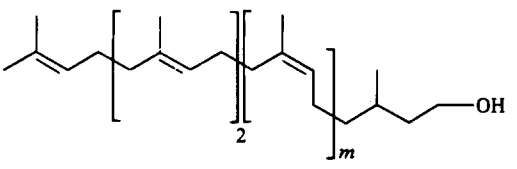
Abstract—Polyprenols with an average number of isoprene residues of 15 to 18 were isolated from the needles of six plants in the Pinaceae, the content being 0.2–2.0% of the dry wt. ^{1}H and ^{13}C NMR, and FDMS spectroscopy revealed that all of the polyprenols were long-chain homologues of betulaprenols with the following sequence of isoprene residues: *ω-trans-trans*-7 to 19 *cis-cis α*.

INTRODUCTION

The dominant vegetation zones of gymnosperms occupy about 8% of the land surface of the earth. Indigenous and planted forests in subarctic and temperate zones are often composed of a single species of gymnosperm, especially pine trees, and this gives these species the advantage over other higher plants as potential resources of some useful materials. In a previous paper [1], we reported that the leaves of *Ginkgo biloba* in late autumn contain a high concentration (*ca* 2.0% of the dry wt) of polyprenols mainly as acetates with the structures **1a** and **1b**, respectively, where $m = 11$–19. The arrangement of the two *trans* (*ω-trans-trans*) and *cis* residues indicates that the polyprenols are long-chain homologues of betulaprenols [2]. These polyprenols have been used as starting material for the synthesis of mammalian dolichols (**2**, $m = 12$–19, mainly 14, 15 and 16) [3–5], compounds which are now recognized as one of the most important materials in the biosynthesis of glycoprotein [6, 7].



R
1a H
1b Ac



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In a search for a resource of dolichols, we have investigated the polyprenols in the family Pinaceae. The presence of polyprenols in pine trees had been reported for the needles of *Pinus strobus* L., *P. sylvestris* L. and *Picea abies* (L.) Karst. [8–10]. However, the structures and chain lengths of these polyprenols had not been determined in detail. The structural characterization of the polyprenols from the Pinaceae was carried out for these plants in addition to *P. densiflora* Sieb. et Zucc, *P. thunbergii* Parl. and *Cedrus deodara* Loud. The former three plants were identified by Professor T. Akai, University Forest, Kyoto University, and the latter three by Professor S. Kuroyanagi, Faculty of Agriculture, Okayama University. The results showed that all the polyprenols are homologues of the polyprenols from *G. biloba* having the same alignment of *ω-trans-trans* residues followed by poly *cis* residues.

RESULTS AND DISCUSSION

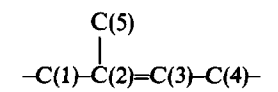
Structural characterization

FDMS of the mixture of polyprenyl acetates from the needles of *C. deodara* gave molecular ions of mass numbers 1012, 1080, 1148, 1216, 1284, 1352, 1420, 1488, 1556 and 1624, which corresponded to polyprenyl-14 through to −23 acetates of the general formula **1b** ($m = 11$–20), respectively. On saponification, this mixture gave a mixture of polyprenols which gave molecular ions of mass numbers 970, 1038, 1106, 1174, 1242, 1310, 1378, 1446, 1514 and 1582, corresponding to polyprenols-14 through to -23 of the general formula **1a** ($m = 11$–20), respectively. The mass spectrum (EIMS) of the polyprenol-18 from this mixture gave the typical mass fragmentation pattern of polyprenols [11]. The native polyprenols from *C. deodara* gave molecular ions of mass numbers 1174, 1242 and 1310, corresponding to polyprenols-17, -18 and -19 (**1a**, $m = 14$, 15 and 16), respectively. From mass numbers, the main components

of the other five plants were found to be polyprenyl-17 acetate for *P. strobus* (mass number 1216), polyprenyl-16 acetate for *P. sylvestris*, *P. densiflora* and *P. thunbergii* (1148), polyprenyl-15 acetate for *Picea abies* (1080) and polyprenol-16 for *P. densiflora* and *P. thunbergii* (1106).

The ^{1}H NMR spectrum of the polyprenyl-17 acetate from *P. strobus* isolated by HPLC from the polyprenyl acetate mixture was similar to that of the polyprenols from *G. biloba* [1], except that the =CH and $-C\underline{H}_2OAc$ protons in the α-terminal residue exhibited signals at δ5.34 (*t*) and 4.55 (*d*), respectively; these signals shifted to δ5.44 and 4.08 after saponification of the polyprenyl acetate. The relative intensities of the signals are listed in Table 1. The observed values were consistent with the theoretical ones for the structure **1b** ($m = 14$), showing the presence of two *trans* residues and 14 *cis* residues. The polyprenyl-16 acetate from *P. sylvestris*, *P. densiflora* and *P. thunbergii*, polyprenyl-15 acetate from *Picea abies* and polyprenyl-18 acetate from *C. deodara* showed similar ^{1}H NMR spectra as the polyprenyl-17 acetate from *P. strobus*. The relative intensities of signals in these polyprenyl acetates were in good agreement with theoretical ones for structure **1b** (Table 1). These facts indicated that the principal polyprenyl acetates from these six plants were composed of two internal *trans* residues, 11 to 14 internal *cis* residues and a *cis* α-terminal residue.

The ^{13}C NMR spectrum of the polyprenyl-17 acetate from *P. strobus* was identical with those of polyprenols [1] except for the signals of the carbon atoms in the α-terminal residue at δ32.52 (C-1), 142.40 (C-2), 119.48 (C-3), 61.10 (C-4), 170.82 (Me$\underline{C}$OO–) and 20.96 (Me$\underline{C}$OO–). The C-1 methylene carbon atoms resonated at δ32.10, 32.34 and 39.81 and were assigned to the internal isoprene residues in the *trans-cis*, *cis-cis* and *trans-trans* + ω-*trans* linkages, respectively [12]. The carbon atoms in the isoprene residues were designated as follows:



The signal at δ32.52 was assigned to the C-1 methylene carbon atom in the *cis* α-terminal residue, since it shifted

Table 1. Relative intensities of ^{1}H NMR signals in polyprenyl acetates

Origin of polyprenyl acetates	No. of isoprene residues	Chemical shift [δ-values (ppm)] and assignment					
		1.59 Me *trans*, ω (*trans*)	1.67 Me *cis*, ω(*cis*)	1.75 Me α(*cis*)	4.53 4.57 $C\underline{H}_2OH$	5.12 =CH–	5.31 5.34 5.38 $=C\underline{H}CH_2OH$
P. strobus	17	3.21* (3)	13.8* (14)	0.96* (1)	1.99 (2)	16.1 (16)	0.93 (1)
P. sylvestris	16	3.23* (3)	12.7* (13)	1.07* (1)	1.99 (2)	14.8 (15)	1.23 (1)
P. densiflora	16	3.19* (3)	12.8* (13)	0.97* (1)	2.03 (2)	14.7 (15)	1.27 (1)
P. thunbergii	16	3.17* (3)	12.8* (13)	1.07* (1)	2.06 (2)	14.9 (15)	1.00 (1)
Picea abies	15	2.99* (3)	12.1* (12)	0.95* (1)	2.01 (2)	13.8 (14)	1.18 (1)
C. deodara	18	3.13* (3)	14.9* (15)	1.01* (1)	1.98 (2)	17.0 (17)	0.99 (1)

Theoretical values are in parentheses.
*The observed and theoretical values for methyl protons are the number of methyl groups.

Table 2. Relative intensities of C-1 methylene carbon signals in polyprenyl acetates

Origin of polyprenyl acetates	No. of isoprene residues	Chemical shift [δ-values (ppm)] and assignment			
		32.10 *trans-cis*	32.34 *cis-cis*	32.52 *cis-cis*(α)	39.81 *trans-trans* ω-*trans*
P. strobus	17	1.14 (1)	11.9 (12)	0.94 (1)	1.99 (2)
P. sylvestris	16	1.02 (1)	11.0 (11)	0.95 (1)	2.05 (2)
P. densiflora	16	1.08 (1)	11.0 (11)	0.83 (1)	2.11 (2)
P. thunbergii	16	0.95 (1)	10.9 (11)	1.12 (1)	2.09 (2)
Picea abies	15	1.16 (1)	9.9 (10)	0.97 (1)	1.98 (2)
C. deodara	18	0.97 (1)	12.8 (13)	1.05 (1)	2.15 (2)

Theoretical values are in parentheses.

to δ32.34 on saponification of the polyprenyl acetate. The absence of the signal around δ40.0 which is characteristic of the C-1 methylene carbon atom in *trans-trans* + *ω-trans* linkages [12] clearly demonstrated that the *trans* residues were in the *ω-trans-trans* sequence. The presence of the *ω-trans* linkage was also confirmed by the C-2 carbon signal at δ131.14, which is characteristic of the *ω*-terminal in the *ω-trans* linkage, while in the *ω-cis* linkage it resonates at δ131.5–131.6 [13]. The polyprenyl acetates from the other five plants showed the same splitting pattern as the polyprenyl-17 acetate from *P. strobus*. The relative intensities of the C-1 methylene signals of these polyprenyl acetates were in good agreement with theoretical values obtained according to the general structure **1b** (Table 2). This indicates that the *ω*-terminal, two *trans* residues, 11–14 *cis* residues and *cis* *α*-terminal are aligned in that order.

The native polyprenols from *C. deodara*, *P. densiflora* and *P. thunbergii* showed the same ^{1}H and ^{13}C NMR spectra as those obtained by saponification of the corresponding polyprenyl acetates. Therefore, it can be concluded that the polyprenols and polyprenyl acetates are a series of polyprenyl homologues having the same alignment of the isoprene residues.

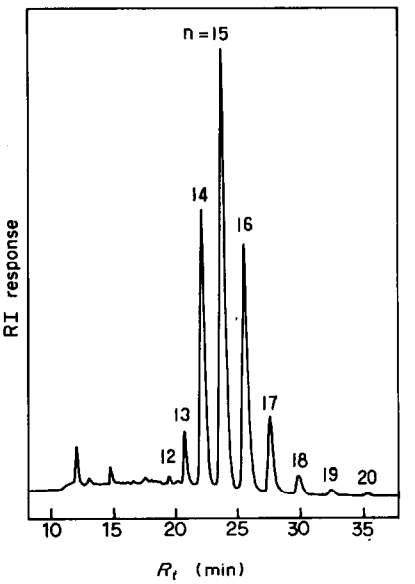


Fig. 1. Reversed-phase HPLC of polyprenyl acetate mixture from the needles of *Picea abies*.

Chain length distribution

The chain length distribution of the polyprenyl acetates was determined by reversed-phase HPLC. A typical separation is shown in Fig. 1. The number of isoprene residues was determined by FDMS. The chain length and percentage composition of the polyprenyl acetates from the six plants are listed in Table 3. The chain length distribution for *Picea abies* was reported by Sasak *et al.* [10] from which the average number of isoprene residues *n* can be calculated to be 14.3. On the other hand, our result gives an $\bar{n}$ value of 15.2 for the polyprenyl acetates from *Picea abies*. Also, the average chain length of 18 for *P. strobus* estimated by Zinkel *et al.* [8] is different from our value of $\bar{n}$ = 17.0. In the case of *P. sylvestris*, however, our value for $\bar{n}$ of 15.7 is in good agreement with the value of 15.4 calculated from the distribution data reported by Hannus *et al.* [9]. The average numbers of isoprene residues was found to be 16.4 ± 0.7 for the four plants in the genus *Pinus*, while *C. deodara* and *Picea abies* deviated by + 1.4 and − 1.2 from the average value, respectively. It is noteworthy that almost the identical distribution was observed for both *C. deodara* in the family Pinaceae and *G. biloba* in the family Ginkgoaceae.

Thus, all the polyprenols from the needles or leaves of gymnosperms investigated until now are long-chain homologues of the betulaprenol-type (**1a**, average $m \geqslant 12$). On the other hand, no such polyprenol has been found in the leaves of angiosperms in which the dominant polyprenols are relatively short chain homologues (average $m \leqslant 8$) of the ficaprenol-type in which the internal three *trans* residues are aligned *ω-trans-trans-trans-* [14].

In the previous paper, we reported that polyprenols from *G. biloba* can be used as a starting material for the preparation of synthetic dolichols. The present study shows that the needles of *C. deodara* can be also used as a good source of polyprenols (*ca* 2% of the dry wt) for the preparation of synthetic dolichols.

EXPERIMENTAL

Isolation and purification of polyprenyl acetates and polyprenols. The needles of *P. strobus*, *P. sylvestris* and *Picea abies* were collected on late December in Kyoto city, Japan, and those

Table 3. Chain length distribution of polyprenyl acetates

Origin of polyprenyl acetates	Composition (% wt)													
	11	12	13	14	15	16	17	18	19	20	21	22	23	$\bar{n}$*
P. strobus				1.8	6.4	21.5	38.8	22.3	6.7	1.8	0.9			17.0
P. sylvestris		2.0	3.0	7.8	24.2	35.1	18.8	6.4	2.8					15.7
P. densiflora		1.1	2.0	4.4	10.2	28.3	30.0	14.3	5.2	2.3	1.3	0.8		16.5
P. thunbergii	0.5	0.8	1.5	2.6	10.6	32.3	32.3	12.4	4.3	2.0	0.8			16.4
Picea abies		0.8	2.8	18.4	39.6	26.9	8.6	2.2	0.8					15.2
C. deodara May			0.6	0.8	2.1	7.0	24.3	38.5	19.2	5.2	1.4	0.5	0.4	17.8
C. deodara Nov.				0.9	1.9	8.0	25.7	38.4	17.7	4.8	1.4	0.7	0.4	17.8
G. biloba [1]				0.3	1.1	5.9	25.6	39.2	19.0	5.8	1.8	0.8	0.5	17.8

*Average number of isoprene residues.

Table 4. Content of polyprenyl acetates and polyprenols in dry needles

Origin		Content (% dry wt)	
		Polyprenyl acetates	Polyprenols
P. strobus		0.2	—
P. sylvestris		0.3	—
P. densiflora		0.3	1.0
P. thunbergii		0.2	0.8
Picea abies		0.8	—
C. deodara	May	0.39	0.02
	Nov.	1.92	0.05

of *P. densiflora* and *P. thunbergii* on mid-May in Kurashiki city, Japan. The needles of *C. deodara* were collected twice in mid-May and mid-November in Kurashiki city, Japan. The polyprenyl alcohol and acetate fractions were isolated from the needles by the method described by Ibata *et al.* [1]. The fractions were then separated into each homologue by reversed-phase partition HPLC: Scortex-OT (JASCO Co. 600 × 10.7 mm), Me_2CO–MeOH (9:1) 3 ml/min. The concn of polyprenols and polyprenyl acetates is tabulated in Table 4.

Spectroscopy. MS: JEOL JMS D-300; GC/MS; ^1H and ^{13}C NMR: 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°) or 45°. A pulse repetition time of 7 or 3 sec (45° pulse) was applied for the ^{13}C NMR measurements.

Acknowledgements—The authors are grateful to Professor S. Kuroyanagi, Okayama University, and Professor T. Akai, Kyoto University, for the needle samples.

REFERENCES

1. Ibata, K., Mizuno, M., Takigawa, T. and Tanaka, Y. (1983) *Biochem. J.* **213**, 305.
2. Wellburn, A. R. and Hemming, F. W. (1966) *Nature* **212**, 1364.
3. Burgos, J., Hemming, F. W., Pennock, J. F. and Morton, R. A. (1963) *Biochem. J.* **88**, 470.
4. Gough, D. P. and Hemming, F. W. (1970) *Biochem. J.* **118**, 163.
5. Freeman, D. J., Rupar, C. A. and Carroll, K. K. (1980) *Lipids* **15**, 191.
6. Hemming, F. W. (1977) *Biochem. Soc. Trans.* **5**, 1223.
7. Waechter, C. J. and Lennarz, W. J. (1976) *Ann. Rev. Biochem.* **45**, 95.
8. Zinkel, D. F. and Evans, B. B. (1972) *Phytochemistry* **11**, 3387.
9. Hannus, K. and Pensar, G. (1974) *Phytochemistry* **13**, 2563.
10. Sasak, W., Mankowski, T., Chojnacki, T. and Daniewski, W. M. (1976) *FEBS Letters* **64**, 55.
11. Wellburn, A. R., Stevenson, J., Hemming, F. W. and Morton, R. A. (1967) *Biochem. J.* **102**, 313.
12. Tanaka, Y., Sato, H. and Kageyu, A. (1982) *Polymer* **23**, 1087.
13. Tanaka, Y., Sato, H. and Kageyu, A. (1983) *Rubber Chem. Technol.* **56**(2), 299.
14. Tanaka, Y. and Takagi, M. (1979) *Biochem. J.* **183**, 163.