Phytochemistry, 1980, Vol. 19, pp. 2327–2330. © Pergamon Press Ltd. Printed in England. 0031-9422/80/1101-2327 $02.00/0

STRUCTURE AND BIOSYNTHESIS OF MALLOPRENOLS FROM *MALLOTUS JAPONICUS*

TAKAYUKI SUGA, TSUYOSHI SHISHIBORI and KEIKO NAKAYA

Department of Chemistry, Faculty of Science, Hiroshima University, Higashisenda-machi, Hiroshima 730, Japan

(*Received* 18 *December* 1979)

Key Word Index—*Mallotus japonicus*; Euphorbiaceae; structure elucidation; biosynthesis; polyprenols.

Abstract—The malloprenol isolated from the leaves of *Mallotus japonicus* was elucidated to be a mixture of (2*Z*, 6*Z*, 10*Z*, 14*Z*, 18*Z*, 22*Z*, 26*E*, 30*E*, 34*E*)-3,7,11,15,19,23,27,31,35,39-decamethyl-2,6,10,14,18,22,26,30,34,38-tetracontadecaen-1-ol and its C_{45}- and C_{55}-homologues and not the previously reported structure. The malloprenols were demonstrated to be biosynthesized by successive *cis* condensation of isoprene residues with (2*E*, 6*E*, 10*E*)-geranylgeranyl pyrophosphate.

INTRODUCTION

During recent years polyprenols have received much attention owing to their biological functions as carriers of sugars in the biosynthesis of bacterial wall polysaccharides [1–3] and eucaryotic glycoprotein [3–5]. We recently isolated a homologous series of polyprenols, trivial name cleomeprenols, with 9–12 isoprene units from the leaves of *Cleome spinosa*, elucidated the structures as a geranylgeraniol-derived moiety linked with *Z*-isoprene residues and investigated the biosynthetic pathway [6, 7]. Previously, malloprenol isolated from the leaves of *Mallotus japonicus* was reported to be composed of a nonaprenol with an internal *E*-isoprene unit and the remainder consisting of *Z*-isoprene residues [8]. Our interest in studying the biosynthesis of malloprenol led us to re-investigate its structure, and malloprenol was found to consist of a homologous series of polyprenols, differing from the previously described structure of malloprenol [8]. We now report evidence for the revision of the structure and the establishment of the biosynthetic pathway.

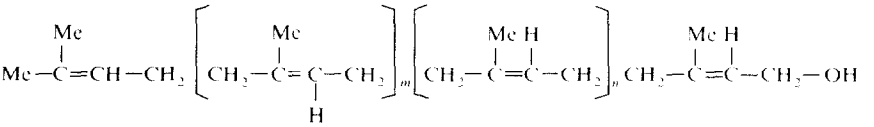
RESULTS AND DISCUSSION

A hexane-soluble fraction of the leaves of *M. japonicus* was chromatographed on Si gel to give a polyprenol fraction. Reversed-phase TLC analysis of the polyprenol fraction on paraffin-impregnated kieselgel (Si gel) showed a single spot, as reported previously [8]. However, the fraction on reversed-phase TLC analysis using a paraffin-impregnated kieselguhr plate [9] showed spots at R_f 0.67, 0.54, 0.44 and 0.35. Each of the components was preparatively separated by reversed-phase TLC and named malloprenol-9 (**1**), -10 (**2**), -11 (**3**) and -12 (**4**), respectively. The proportions of malloprenols **1**–**4** were 7, 43, 42 and 8%, respectively. Their IR, ^{1}H NMR and mass spectra showed a similar pattern, indicating a homologous series of polyprenols.

Malloprenol-10 (**2**), the major component, exhibited a M^+ peak corresponding to $C_{50}H_{82}O$ in the MS. The IR spectrum showed the presence of a hydroxyl group and isolated double bonds. Acetylation of **2** with Ac_2O–pyridine gave an acetate, $C_{52}H_{84}O_2$, which showed no hydroxyl band in the IR spectrum. Oxidation of **2** with MnO_2 gave an α,β-unsaturated aldehyde, indicating the presence of an allylic primary hydroxyl group. The MS of **2** exhibited a base peak at *m/e* 69 due to a 3,3-dimethylallyl ion and an $M^+ - H_2O$ ion peak at *m/e* 680. The loss of 69 mu from the *m/e* 680 peak, followed by successive loss of 68 mu of an isoprene residue resulted in the occurrence of a prominent peak of *m/e* 135. The ^{1}H NMR spectrum of **2** indicated the presence of 11 allylic methyls, 18 methylenes, 9 vinyl protons and a $=CHCH_2OH$ moiety. These facts suggested that **2** was a polyprenol composed of ten isoprene units. The allylic methyl signals at δ 1.59 (12 H), 1.67 (18 H) and 1.73 (3 H) were assignable [10] to a methyl group of the internal *E*-isoprene residue and a terminal methyl group *Z* to the main carbon chain, a methyl group of the internal *Z*-isoprene residue and a terminal methyl group *E* to the main carbon chain, and a methyl group of the α-terminal *Z*-isoprene residue, respectively. Consequently, malloprenol-10 (**2**) was elucidated to involve an α-terminal *Z*, five internal *Z*, three internal *E* and an ω-terminal isoprene residues.

Malloprenol-9 (**1**) and -11 (**3**) showed a M^+ at *m/e* 630 ($C_{45}H_{74}O$) and at 766 ($C_{55}H_{90}O$), respectively. On the basis of the M^+ peak and the characteristic fragmentation pattern in the MS and the relative intensity of the allylic methyl signals in the ^{1}H NMR spectra, malloprenol-9 (**1**) and -11 (**3**) were found, respectively, to have four and six internal *Z* isoprene residues, in addition to the same three internal *E* isoprene residues and an ω-terminal isoprene residue as malloprenol-10 (**2**). No sample of the compound giving a spot with R_f 0.35 on the reversed-phase TLC could be isolated because of its small amount, but the chromatographic behaviour indicated the compound to be dodecaprenol (**4**).

The 'malloprenol' was thus found not to have the structure described previously [8] but to be composed of a series of polyprenologues, i.e. nonaprenol (**1**), decaprenol (**2**), undecaprenol (**3**) and dodecaprenol (**4**), all of which involve an ω-terminal isoprene unit, three internal *E*-isoprene residues and the remainder as *Z*-isoprene



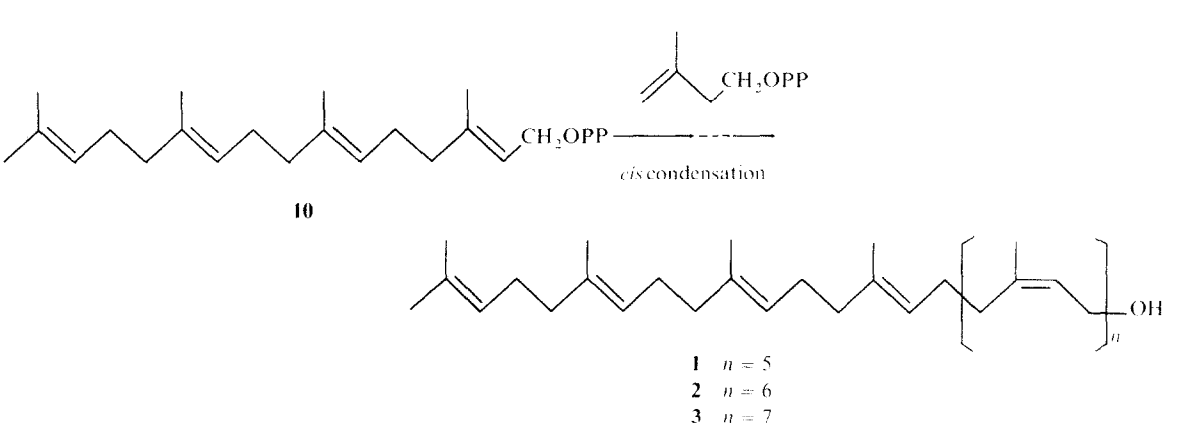
1 $m = 3, n = 4$
2 $m = 3, n = 5$
3 $m = 3, n = 6$
4 $m = 3, n = 7$

residues. Reversed-phase TLC on a paraffin-impregnated kieselguhr plate resulted in complete separation of the homologous series of malloprenols. The failure in separation of the homologous malloprenols in the previous work [8] could be due to the use of paraffin-impregnated kieselgel (Si gel) for the reversed-phase TLC.

The alignment of the *E*- and *Z*-isoprene residues in the malloprenols has not been deduced yet, but co-occurrence of malloprenols with phytol in the leaves of *M. japonicus* suggests the possible formation of malloprenols by successive *cis* condensations of isoprene residues with (2*E*, 6*E*, 10*E*)-geranylgeranyl pyrophosphate, similar to cleomeprenols [6, 7]. The alignment of three internal *E*-isoprene residues adjacent to the ω-terminal isoprene unit in malloprenols was biosynthetically pursued by comparison of incorporations of all-(*E*)-di, tri, tetra- and pentaprenyl-[1-$^3H_2$]-pyrophosphates **6**, **8**, **10** and **12** with those of their (2*Z*)-isomers, **5**, **7**, **9** and **11**. The

EXPERIMENTAL

Extraction and isolation of malloprenols **1**, **2** *and* **3**. The leaves (1.67 kg) of *Mallotus japonicus* Muell. Arg. (Euphorbiaceae), grown widely in the suburbs of Hiroshima city, were collected at the beginning of June, air-dried for 1 day and immersed in MeOH (5 l.) at room temp. for 2 weeks. The MeOH extract was concd to 300 ml. The soln was diluted with H_2O (300 ml) and extracted with hexane. Removal of the solvent from the hexane soln gave a tarry residue (15.2 g), which was subjected to CC on Si gel (700 g) using a hexane–C_6H_6 gradient and then to PLC (Si gel, 0.75 mm, hexane–EtOAc, 9:1) to give a mixture of polyprenols (154 mg). The mixture was subjected to reversed-phase TLC on a paraffin-impregnated kieselguhr (prepared by dipping a kieselguhr plate, 0.25 mm, into a soln of 5% liquid paraffin in hexane) with Me_2CO–H_2O (8:1) satd with paraffin. The plate, on spraying with a *p*-anisaldehyde–H_2SO_4 reagent, gave four spots at R_f 0.67, 0.53, 0.45 and 0.35; the polyprenols giving these spots were named malloprenol-9 (**1**), -10 (**2**), -11 (**3**) and -12 (**4**), respectively.



10

1 $n = 5$
2 $n = 6$
3 $n = 7$

Scheme 1.

incorporation of (2*E*,6*E*,10*E*)-geranylgeranyl pyrophosphate (**10**) into malloprenols **1**, **2** and **3** was higher than that of its (2*Z*)-isomer **9** as shown in Table 1. The same tendency was observed for incorporations of a pair of the lower homologues of all-(*E*)-isomers **6** and **8** and their (2*Z*)-isomers **5** and **7**. On the contrary the incorporation of an all-(*E*) unnatural higher homologue **12** was lower than that of the (2*Z*)-isomer **11**, which should be a natural substrate resulting from the *cis* condensation of an isoprene unit with **10**. These results indicate that (2*E*,6*E*,10*E*)-geranylgeranyl pyrophosphate (**10**) is a better precursor for the occurrence of successive *cis* condensations of *Z*-isoprene units, as shown in Scheme 1. Thus, the structure of malloprenol-10(**2**) was elucidated to be (2*Z*,6*Z*,10*Z*,14*Z*,18*Z*,22*Z*,26*E*,30*E*,34*E*)-3,7,11,15,19,23,27,31,35,39-decamethyl-2,6,10,14,18,22,26,30,34,38-tetracontadecaen-1-ol- and **1** and **3** were its C_{45}- and C_{55}-polyprenologues, respectively.

The separation of each malloprenol was carried out on the same PLC system as above and bands were detected by spraying with 2′,7′-dichlorofluorescein–EtOH (0.01%) followed by irradiation with 254 nm UV light. The fluorescent areas were scraped off and extracted separately with Et_2O. Liquid paraffin and the fluorescein were removed from malloprenol by PLC (Si gel, hexane–EtOAc, 9:1). The composition of malloprenols **1**–**4** was 7, 43, 42 and 8%, respectively. The content of malloprenols varied with growth of the plant; seasons (wt% of malloprenol in the fresh leaves): end of April (0.002), beginning of June (0.01), end of June (0.07), end of July (0.16), end of August (0.17) and end of September (0.20), respectively.

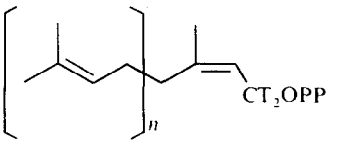
Malloprenol-10 (**2**). Colourless oil. IR ν_{max}^{neat} cm^{-1}: 3330, 1005 (OH), 1660, 840 (C=C); ^{1}H NMR (90 MHz, $CDCl_3$): δ 1.59 (12 H, *s*, *E* C<u>Me</u>=CH), 1.67 (18 H, *s*; *Z* C<u>Me</u>=CH), 1.73 (3 H, *s*, *Z* C<u>Me</u>=CHCH$_2$OH), 2.0–2.1 (36H, =CHC<u>H$_2$</u>C<u>H$_2$</u>C=), 4.06 (2 H, *d*, J = 7 Hz, =CHC<u>H$_2$</u>OH), 5.1 (9 H, *m*, $W_{1/2}$ = 12 Hz, =CH), 5.42 (1 H, *t*, J = 7 Hz, =C<u>H</u>CH$_2$OH); MS (70 eV, direct

Table 1. Incorporation of a homologous series of (2*Z*)- and (2*E*)-prenyl-[1-$^3H_2$]-pyrophosphates into malloprenol-9 (**1**), -10 (**2**) and -11 (**3**) by the leaves of *Mallotus japonicus*

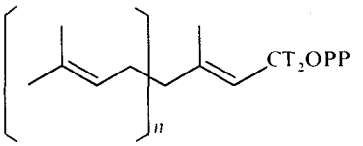
		1			**2**			**3**		
	Precursors (dpm)*	Total act. (dpm)	Incorp. (%)	Ratio†	Total act. (dpm)	Incorp. (%)	Ratio†	Total act. (dpm)	Incorp. (%)	Ratio†
5	9.83×10^6	2.95×10^2	0.003	0.006	6.88×10^2	0.007	0.01			
6	7.70×10^6	4.00×10^4	0.519		4.00×10^4	0.519				
7	4.53×10^6	4.98×10^2	0.011	0.19	1.49×10^3	0.033	0.50	5.06×10^2	0.011	0.06
8	6.04×10^6	3.50×10^3	0.058		4.01×10^3	0.066		1.20×10^4	0.199	
9	2.44×10^6	1.69×10^4	0.693	0.86	1.93×10^3	0.079	0.06	1.80×10^3	0.074	0.22
10	5.25×10^4	4.21×10^3	0.802		7.33×10^3	1.396		1.73×10^3	0.330	
11	4.81×10^4	3.20×10^2	0.665	4.55	2.94×10^2	0.611	2.91	6.85×10^2	1.424	3.82
12	1.23×10^5	1.80×10^2	0.146		2.58×10^2	0.210		4.59×10^2	0.373	

* Radioactivity of precursors used.

† Ratio of % incorporation of (2*Z*)-prenyl-[1-$^3H_2$]-pyrophosphate to that of its (2*E*)-isomer.



5 $n = 1$
7 $n = 2$
9 $n = 3$
11 $n = 4$



6 $n = 1$
8 $n = 2$
10 $n = 3$
12 $n = 4$

inlet) *m/e* (rel. int.): 698 (M^+, 3), 680 ($M^+ - H_2O$, 5), 611 (1), 543 (1.5), 475 (2), 407 (2), 339 (3), 271 (4), 203 (8), 135 (16), 69 (100).

Acetylation of **2**. A mixture of **2** (15 mg), dry pyridine (0.1 ml) and Ac_2O (0.1 ml) was allowed to stand for 24 hr at 25°. After usual work-up, the product was purified by Si gel PLC (hexane–EtOAc, 9:1) to give an acetate (14 mg) as an oil, IR ν_{max}^{neat} cm^{-1}: 1734, 1235 (OCOMe); ^1H NMR (60 MHz), $CDCl_3$): δ 2.02 (3 H, *s*, OCOMe), 4.55 (2 H, *d*, =CHC$\underline{H}_2$OAc); MS (70 eV) *m/e* (rel. int.): 740 (M^+, 2), 680 (M^+ – HOAc, 5), 611 (1), 543 (2), 475 (2), 407 (4), 339 (4), 271 (6), 203 (14), 135 (18), 69 (100).

Oxidation of **2**. A soln of **2** (10 mg) in hexane (3 ml) was stirred with MnO_2 (50 mg) at 25° for 8 hr. Removal of the solvent, after filtration, afforded an oil, which was purified by PLC (Si gel; hexane–EtOAc, 19:1) to give an aldehyde (8 mg), IR ν_{max}^{neat} cm^{-1}: 2728, 1676, 1631 (C=CCHO); UV $\lambda_{max}^{isooctane}$ nm: 243, ε 8400.

Malloprenol-11 (**3**). Oil. ^1H NMR (90 MHz, $CDCl_3$): δ 1.59 (12 H, *s*, *E* C$\underline{Me}$=CH), 1.67 (21 H, *s*, *Z* C$\underline{Me}$=CH), 1.72 (3 H, *s*, *Z* C$\underline{Me}$=CHCH$_2$OH), 2.0–2.1 (40 H, =CHC$\underline{H}_2$C$\underline{H}_2$C=), 4.06 (2 H, *d*, J = 7 Hz, =CHC$\underline{H}_2$OH), 5.1 (10 H, *m*, $W_{1/2}$ = 13 Hz, =CH), 5.42 (1 H, *t*, J = 7 Hz, =C$\underline{H}$CH$_2$OH); MS (70 eV) *m/e* (rel. int.): 766 (M^+, 1), 748 ($M^+ - H_2O$, 2.5), 679 (0.5), 611 (1), 475 (1), 407 (1.5), 339 (2), 271 (4), 203 (11), 135 (27), 69 (100).

Malloprenol-9 (**1**). Oil. MS (70 eV) *m/e* (rel. int.): 630 (M^+, 1), 612 ($M^+ - H_2O$, 1.5), 543 (1), 475 (1.5), 407 (2), 339 (4), 271 (9), 203 (32), 135 (33), 69 (100).

Administration of prenyl-[1-$^3H_2$]*-pyrophosphates* **5**–**12** *to the leaves of* M. japonicus. Labelled pyrophosphates **5**–**12** were prepared as previously described [7]. A soln of **5**–**12** in Tris–HCl buffer (pH 7.3, 0.1 M, 5 ml) was fed to the leaves of *M. japonicus* (200 g) through their cut-stalks over 4 hr at 25°, and then H_2O was taken up over 20 hr. The plant materials were cut into small pieces and extracted with MeOH (500 ml × 2). The MeOH soln was concd under red. pres. to 5 ml. The soln was extracted with hexane (50 ml × 4) and the hexane extract was subjected to the multiple development PLC (Si gel, C_6H_6 × 2) [11]. A polyprenol fraction was rechromatographed on the same system (hexane–EtOAc, 4:1) and further subjected to reversed-phase PLC in the same manner as in the case of the isolation of unlabelled malloprenols. The radioactivity of **1**, **2** and **3** was assayed as described previously [7]. The results are shown in Table 1.

Acknowledgements—The present work was partially supported by a Grant-in-Aid for Scientific Research Nos. 247027 and 347024 from the Ministry of Education, Science and Culture and scholarships from the Saneyoshi Scholarship Foundation, the Kudo Science Foundation and the Ito Science Foundation.

REFERENCES

1. Higashi, Y., Strominger, J. L. and Sweeley, C. C. (1967) *Proc. Natl. Acad. Sci. U.S.A.* **57**, 1878.
2. Scher, M. and Lennarz, W. J. (1969) *J. Biol. Chem.* **244**, 2777.
3. Hemming, F. W. (1974) in *MTP International Review of Science* (Goodwin, T. W., ed.) Vol. **4**, p. 39. Butterworth, London.
4. Caccam, J. F., Jackson, J. J. and Eylar, E. H. (1969) *Biochem. Biophys. Res. Commun.* **35**, 505.

5. Behrens, N. H., Parodi, A. J. and Leloir, L. F. (1971) *Proc. Natl. Acad. Sci. U.S.A.* **68,** 2857.
6. Suga, T., Shishibori, T., Kosela, S., Tanaka, Y. and Itoh, M. (1975) *Chem. Letters* 771.
7. Suga, T. and Shishibori, T. (1980) *J. Chem. Soc. Perkin Trans.* 1, (in press).
8. Noda, T., Take, T., Watanabe, J. and Abe, J. (1970) *Bull. Chem. Soc. Jpn.* **43,** 2174.
9. Stone, K. J., Wellburn, A. R., Hemming, F. W. and Pennock, J. F. (1967) *Biochem. J.* **102,** 325.
10. Bates, R. B. and Gale, D. M. (1960) *J. Am. Chem. Soc.* **82,** 5749.
11. Stahl, E. (1969) in *Thin-Layer Chromatography*, p. 86. Springer, Berlin.