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THE STRUCTURAL ELUCIDATION AND THE BIOSYNTHETIC STUDY OF CLEOMEPRENOLS-9, -10, AND -11 FROM THE LEAVES OF *CLEOME SPINOSA*

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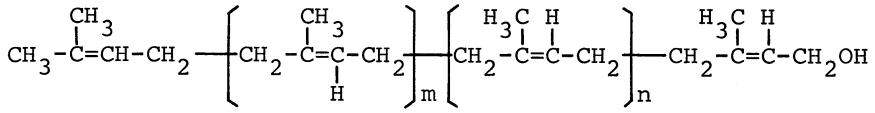
A homologous series of polyprenols isolated from *Cleome spinosa* L. of *Capparidaceae* were established to be nonaprenol (C_{45}), decaprenol (C_{50}), and undecaprenol (C_{55}) composed of an ω-terminal isoprene residues, three internal *trans*-isoprene residues, and the remaining *cis*-isoprene residues, respectively. The prenols were demonstrated to be synthesized biologically by successive *cis*-additions of isoprene residues to all-*trans*-geranylgeranyl pyrophosphate.

It is well known that the larvae of *Pieris rapae crucivora* Boisduval (Japanese name: Monshiro-cho) feed the leaves of *Cleome spinosa* L. (Japanese name: Seiyo-fuchoso) and some plants belonging to *Cruciferae*.[1] In connection with a biochemical study of the relationship between chemical constituents of the diet and the growth of the insect, we isolated a homologous series of polyprenols, named cleomeprenols-9, -10, and -11, from the leaves of *C. spinosa*, and here report the structural elucidation and the biosynthetic study of the cleomeprenols.

The nonpolar fraction separated from a methanol extract of the half-dried leaves of the plant (500 g) were chromatographed on a silica gel column with a mixture of *n*-hexane and ethyl acetate to give a mixture of polyprenols (560 mg) as a main component. Reversed phase TLC analysis[2] of the mixture on a Kieselguhr G plate impregnated with liquid paraffin by a mixture of acetone and water (9:1) showed upon spraying with 2',7'-dichlorofluorescein in ethanol four spots at Rf 0.61, 0.53, 0.45, and 0.40. The composition was determined by densitometric analysis of the spots on the plate to be 20, 41, 33, and 6%. Each component was then separated as a colorless oil by reversed phase TLC. Their spectra (IR, NMR, and mass spectra) showed a similar pattern and indicated the cleomeprenols to be a series of homologs.

Cleomeprenol-10 (II) appeared at Rf 0.53 and showed IR bands at 3327, 1005 (*prim*-OH), 1660, and 840 cm^{-1} (isolated C=C) and no absorption longer than 210 nm. The NMR spectrum in $CDCl_3$ indicated eleven allylic methyls (33H, δ 1.6~1.75 ppm), eighteen methylenes (36H, 2.0~2.1 ppm), an allylic methylene bearing oxygen (2H, 4.09 ppm, *d*, J=7Hz), vinyl protons (9H, 5.10 ppm), and a proton of $=C\underline{H}-CH_2OH$ (1H, 5.43 ppm, *t*, J=7Hz). Acetylation of II with acetic anhydride and pyridine gave an acetate [$C_{52}H_{84}O_2$ (m/e 740, M^+); IR 1736, 1235 ($OCOCH_3$), 1660, and 835 cm^{-1} (C=C); NMR($CDCl_3$) δ 2.02 (3H, *s*, $OCOCH_3$) and 4.56 ppm (2H, *t*, J=7Hz, $=CH-C\underline{H}_2-OAc$)]. These facts suggest the prenol (II) to be such polyprenols as heveaprenols,[2]

castaprenols,[3] and ficaprenols.[4] The mass spectrum of II showed a molecular ion peak at m/e 698 (4.4%) corresponding to $C_{50}H_{82}O$, a base peak at m/e 69 due to a dimethyl allyl ion, and an M^+-H_2O ion peak at m/e 680 (11%). After loss of 69 mass units corresponding to a dimethyl allyl radical from the m/e 680 peak, the occurrence of sequential losses of 68 mass units corresponding to an isoprene residue gave peaks at m/e 611 (2.0%), 543 (2.8), 475 (3.3), 407 (3.5), 339 (4.1), 271 (6.5), 203 (13.9), and 135 (32.9). These fragmentation patterns of the mass spectrum are characteristic of polyprenols.[3] These findings indicate that cleomeprenol-10 (II) is a polyprenol composed of ten isoprene residues. The NMR spectrum in $CDCl_3$ showed allylic methyl signals at δ 1.59 (12H), 1.66 (18H), and 1.73 (3H) ppm, which are assignable[5] to three methyl groups of the internal *trans*-isoprene residues and a "*trans*-methyl"[6] of the ω-terminal, five methyl groups of the internal *cis*-isoprene residues and a "*cis*-methyl"[6] of the ω-terminal, and a methyl group of the α-terminal *cis*-isoprene residue, respectively. Consequently, the cleomeprenol-10 (II) has been elucidated to be composed of an α-terminal *cis*, five internal *cis*, three internal *trans*, and an ω-terminal isoprene residues.



ω-terminal *trans* *cis* α-terminal

I: m=3, n=4 II: m=3, n=5 III: m=3, n=6 IV: m=3, n=7

Cleomeprenol-9 (I) and -11 (III) showed a spot at Rf 0.61 and 0.45 on the TLC plate and an molecular ion peak at m/e 630 (3.5%) corresponding to $C_{45}H_{74}O$ and at m/e 766 (4.0%) to $C_{55}H_{90}O$, respectively. On the basis of the molecular ion peak and the characteristic fragmentation patterns due to sequential losses of 68 mass units corresponding to an isoprene residue in the mass spectra and the relative intensity of the allylic methyl signals at δ 1.59, 1.66, and 1.73 ppm by 4:5:1 and 4:7:1 in the NMR spectra, the prenols-9 (I) and -11 (III) were found to have four and six internal *cis* isoprene residues, respectively, in addition to the same other isoprene residues as in the prenol-10 (II). On the other hand, the compound showing a spot at Rf 0.40 on the TLC plate could not be isolated because of its small amount, but the chromatographic behavior on the reversed phase TLC indicated the compound to be dodecaprenol ($C_{60}H_{98}O$) as structural formula IV.

It has now been established by NMR spectroscopy that in each cleomeprenol three of the internal isoprene residues are *trans* and the other internal isoprene residues and the α-residue are *cis*. However, the sequence of the isoprene residues in the cleomeprenols remains unsolved, and the location of *cis*- and *trans*-isoprene residues was examined biosynthetically. The biosynthesis of polyprenols would involve the isomerization of isopentenyl pyrophosphate (IPP) to dimethylallyl pyrophosphate (DMAPP) and the condensation of IPP with DMAPP or its higher homologs. At each step there would be the loss of a hydrogen atom from C-2 of IPP, which corresponds to C-4 of mevalonic acid (MVA). The hydrogen atom eliminated is known to be 4-*pro-S* and 4-*pro-R* hydrogens of MVA when all-*trans*- and all-*cis*-isoprenoids are

formed biologically, respectively.[7~9] In order to clarify whether each isoprene residue in cleomeprenols having mixed configurations is biogenetically *cis* or *trans*, the biosynthesis of the cleomeprenols was undertaken by means of uptake of stereospecifically labeled mevalonates into the plant. Each of four leaves having stalks was allowed to take up either [2-^{14}C,(4*R*)-4-$^{3}H_1$]- or [2-^{14}C,(4*S*)-4-$^{3}H_1$]-mevalonate [15 (^{3}H/^{14}C=6.45) and 6.5 (^{3}H/^{14}C=8.70) μCi of ^{14}C, respectively, in a phosphate buffered solution, pH 7.3] by transpiration over 4 hr at 25°C and water was then taken up in a similar way over 20 hr. Cleomeprenols were extracted from the leaves with methanol and separated in the same manner as described above. The ^{3}H and ^{14}C contents of the sample were assayed with the Packard 3320 Tri-Carb liquid-scintillation spectrometer and the corrected ^{3}H/^{14}C ratios were determined (Table 1). The corrected ^{3}H/^{14}C ratios for phytol were as expected, being consistent with that it is biogenetically all-*trans*.[10] The corrected ^{3}H/^{14}C ratios for cleomeprenols-9 to -11 agreed with the ratios calculated by assuming these prenols to be composed of four biogenetically *trans*-isoprene residues and remaining *cis*-isoprene residues. On the basis of these findings and the NMR spectroscopic evidence, it was found that the ω-terminal and three internal *trans*-isoprene residues are biogenetically *trans* and the α-terminal and the remaining isoprene residues biogenetically *cis*, as shown in Fig. 1.

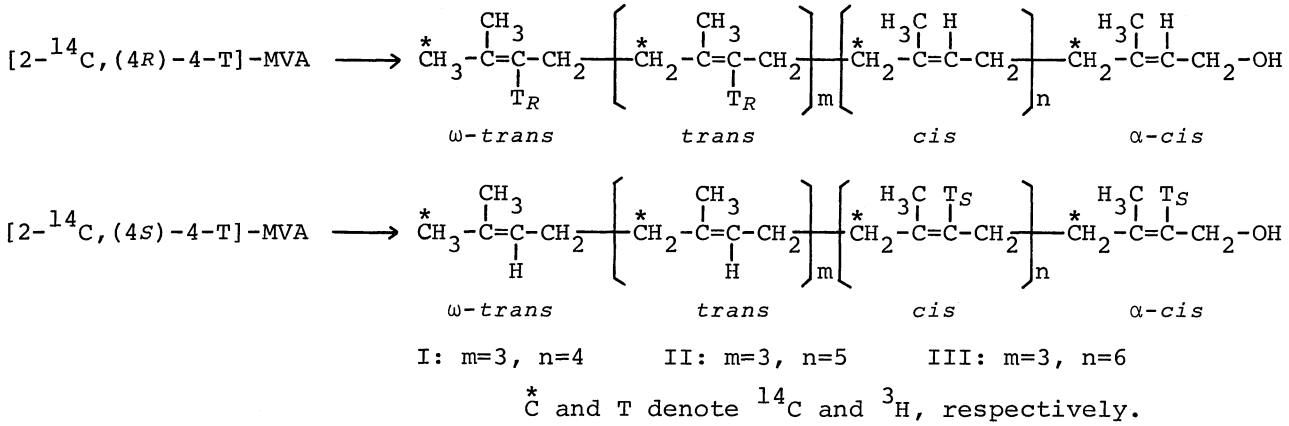


Fig. 1. Labeling of ^{3}H and ^{14}C in cleomeprenols-9 (I), -10 (II), and -11 (III) biosynthesized from [2-^{14}C,(4*R*)-4-$^{3}H_1$]- and [2-^{14}C,(4*S*)-4-$^{3}H_1$]-MVA.

This suggests the cleomeprenols to be probably formed by successive *cis*-additions of isoprene residues to all-*trans*-geranylgeranyl pyrophosphate (GGPP), as shown in Fig. 2. Such a mechanism would require all three internal *trans*-isoprene residues to be adjacent to the ω-terminal isoprene residue. Then, the location of the internal *trans*-isoprene residues in the cleomeprenols was examined biosynthetically by means of incorporation of [1-^{3}H]-2-*trans*- and [1-^{3}H]-2-*cis*-isomers of di-, tri-, tetra-, and penta-prenyl pyrophosphates (V-XII) into the cleomeprenols. The radioactive substrates (V-XII) were prepared from the methyl ester of the corresponding acids by reduction with lithium aluminum hydride-^{3}H followed by phosphorylation.[11] The specific activities of the substrates prepared were as follows: V, 7.5; VI, 4.7; VII, 3.7; VIII, 1.6; IX, 13.8; X, 10.0; XI, 3.8; XII, 1.8 Ci/mol. The substrates

Table 1. Incorporation of [2-^{14}C,(4*R*)-4-$^{3}H_1$]- and [2-^{14}C,(4*S*)-4-$^{3}H_1$]-MVA into cleomeprenols-9 (I), -10 (II), and -11 (III) and phytol of the leaves of *Cleome spinosa*

Compounds	From [2-^{14}C,(4*R*)-4-$^{3}H_1$]-MVA			From [2-^{14}C,(4*S*)-4-$^{3}H_1$]-MVA		
	Incorporation[a] (%)	Corrected $^{3}H/^{14}C$ ratio[b]	Calculated $^{3}H/^{14}C$ ratio[c]	Incorporation[a] (%)	Corrected $^{3}H/^{14}C$ ratio[b]	Calculated $^{3}H/^{14}C$ ratio[c]
I	0.115	4.34 : 9	4 : 9	0.038	4.99 : 9	5 : 9
II	0.078	3.82 : 10	4 : 10	0.107	5.72 : 10	6 : 10
III	0.019	3.82 : 11	4 : 11	0.012	7.33 : 11	7 : 11
Phytol	1.437	4.00 : 4	4 : 4	1.736	0.52 : 4	0 : 4

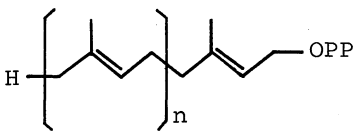
a) The incorporation is based on the amount of ^{14}C present in the natural (3*R*)-isomer in (3*RS*)-MVA.

b) This is the ratio obtained by dividing the observed $^{3}H/^{14}C$ ratio by the $^{3}H/^{14}C$ ratio of the original MVA and multiplying the answer by the number of C-atoms from C-2 of MVA expected to be presented in each molecule of the compounds. The $^{3}H/^{14}C$ ratio of the original [2-^{14}C,(4*R*)-4-$^{3}H_1$]- and [2-^{14}C,(4*S*)-4-$^{3}H_1$]-MVA was 6.45 : 1 and 8.70 : 1, respectively.

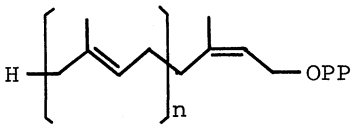
c) This is the ratio calculated by assuming the prenols to possess four biogenetically *trans* isoprene residues and remaing *cis* isoprene residues.

Table 2. Incorporation of a homologous series of [1-^{3}H]-2-*trans*- and [1-^{3}H]-2-*cis*-prenyl pyrophosphates [(V, VII, IX, and XI) and (VI, VIII, X, and XII), respectively] into cleomeprenols-10 (II) and -11 (III) of the leaves of *Cleome spinosa*

[1-^{3}H]-Prenyl pyrophosphates	Cleomeprenol-10 (II)		Cleomeprenol-11 (III)	
	Incorporation (%)	Incorporation ratio of 2-*trans* to 2-*cis*	Incorporation (%)	Incorporation ratio of 2-*trans* to 2-*cis*
V	0.0178	3.4	0.0171	6.1
VI	0.0053		0.0028	
VII	0.0874	2.3	0.0724	2.7
VIII	0.0374		0.0273	
IX	0.2824	5.3	0.2116	1.6
X	0.0532		0.1340	
XI	0.0347	0.14	0.0654	0.31
XII	0.2477		0.2093	



V: n=1
VII: n=2
IX: n=3
XI: n=4



VI: n=1
VIII: n=2
X: n=3
XII: n=4

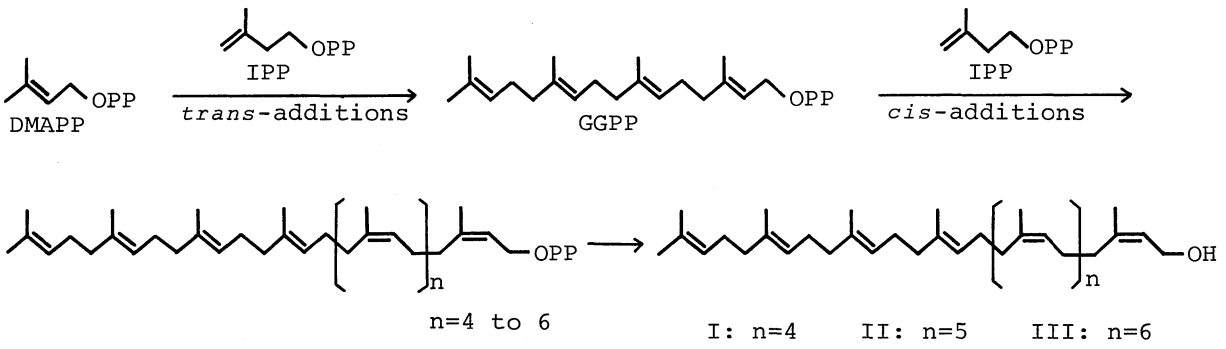


Fig. 2. Biosynthetic pathway of cleomeprenols-9 (I), -10 (II), and -11 (III).

(5×10^6 dpm of ^3H in 1 ml of water) were each taken up by transpiration and the cleomeprenols were separated, in the same manner as in the case of mevalonate. The incorporation of all-*trans*-GGPP (IX) into cleomeprenols-10 (II) and -11 (III) was higher than that of its 2-*cis* isomer (X), as shown in Table 2. The same tendency of the incorporation was also observed for a pair of the lower homologs of all-*trans*- (V and VII) and their 2-*cis*-isomers (VI and VIII), but the all-*trans*- and 2-*cis*-pentaprenyl pyrophosphates (XI and XIII). These results imply that three internal *trans*-isoprene residues are successively adjacent to the ω-terminal residue and all remaining isoprene residues far from the fifth from the ω-terminal have *cis*-configuration. This finding indicates each prenol to be biosynthesized by successive *cis*-additions of isoprene residues to all-*trans*-GGPP, as shown in Fig. 2.

Several polyprenols have been isolated from higher plants.[2~4,12~14] Of the polyprenols, heveaprenols,[2] castaprenols,[3] and ficaprenols[4] are composed of 10 to 13 isoprene residues containing three internal *trans*-isoprene residues similarly to cleomeprenols-9 to -11, but there has been no evidence regarding the sequence of the *cis*- and *trans*-isoprene residues. We have elucidated the cleomeprenols to have structure I, II, and III, respectively, with such a sequence of isoprene residues as shown in Fig. 2 and to be biosynthesized by successive *cis*-additions of isoprene residues to all-*trans*-GGPP.

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* The Japanese Government-sponsored foreign student sent from Department of Chemistry, Faculty of Science, University of Indonesia, Salemba 4, Jakarta, Indonesia.

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