CHEMISTRY LETTERS, pp. 1467-1470, 1983.

STEREOCHEMISTRY OF THE C-4 PROCHIRAL HYDROGEN ATOM ELIMINATION OF MEVALONATE IN THE BIOSYNTHESIS OF POLYPRENOLS IN HIGHER PLANTS

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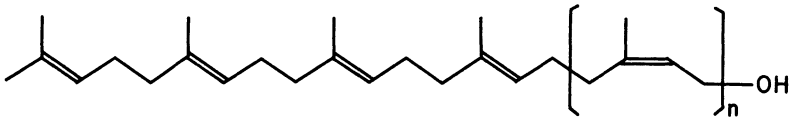
In the biosynthesis of the polyprenols in the five higher plants examined hitherto, it was found that stereochemistry of the C-4 prochiral hydrogen atom elimination of mevalonate in the formation of their E-isoprene chain follows Cornforth's basic principle for mevalonoid biosynthesis, but the formation of their Z-isoprene chain involves, contrary to this basic principle, the elimination of the pro-4S hydrogen atom of mevalonate.

Cornforth's proposal for the formation of prenyl pyrophosphates in mammalian liver and microorganism has been generally accepted as a basic principle; this involves the stereochemical picture that the pro-4S hydrogen atom of mevalonic acid (MVA) is lost in the formation of E-isoprene residues, while the pro-4R hydrogen atom of the acid is eliminated in that of Z-isoprene residues.[1-3] However, we have recently found that the unusual elimination of the pro-4S hydrogen atom of MVA occurs in the formation of the Z-isoprene chain of the polyprenols, malloprenols, by successive addition of isopentenyl pyrophosphate (IPP) to geranylgeranyl pyrophosphate (GGPP) in Mallotus japonicus MUELL. ARG. (Euphorbiaceae).[4] This prompted us to investigate the stereochemistry of the hydrogen atom elimination in the



1—5 (n=5—9)

formation of polyprenols in other higher plants, such as Aleurites cordata MUELL. ARG. and Triadica sebifera SMALL (Euphorbiaceae), Alnus serrulatoides CALL. and Betula platyphylla SUKATCHEV var. japonica HARA (Betulaceae), and Aesculus turbinate BLUME (Hippocastanaceae), and the biosynthesis of polyprenols in all these plants was found to involve the elimination of the pro-4S hydrogen atom of MVA during the formation of their Z-isoprene residues. We here wish to communicate the findings.

The leaves of these five plants contained C_{45} (n=5), C_{50} (n=6), C_{55} (n=7), and C_{60} (n=8) homologs of polyprenols as in the case of M. japonicus. The structures **1—4** shown above for these polyprenols were confirmed by comparison of their IR, ^{1}H- and ^{13}C-NMR, and MS spectra, HPLC, and reversed phase TLC with those of the malloprenols.[5] The leaves of A. turbinate contained C_{65} (n=9) homolog (**5**) in addition to a series of C_{45}, C_{50}, C_{55}, and C_{60} homologs.[6]

The labeling pattern in the E- and Z-isoprene units of each polyprenol was examined by incorporation of (4R)- and (4S)-[2-^{14}C,4-^{3}H]MVAs. The potassium salt of these MVAs dissolved in water was fed to the leaves of each plant through their cut-stalks for 72 h. Polyprenols-9 — -13 (**1—5**) were separated from each other in the same manner as described.[5,7] Radioactivity and $^{3}H/^{14}C$ atom ratio in each of the polyprenols are given in Table 1.[8] Each polyprenol gave one peak on HPLC (Radial Pak C_{18}, MeOH) and one spot on reversed phase TLC.[5] If these polyprenols are formed from the double labeled MVA following Cornforth's basic principle for isoprenoid biosynthesis,[1-3] the $^{3}H/^{14}C$ atom ratios in the polyprenols are expected as given in column (A) of Table 1. However, the observed atom ratios were not coincident with the expected atom ratios, but the observed atom ratios were in good agreement with those expected for the case of the unusual loss of the pro-4S hydrogen atom of MVA during the formation of the Z-isoprene units, as shown in column (B). This agreement indicates that the pro-4S hydrogen atom of MVA is eliminated, in the same way as the biosynthesis of the malloprenols, during the formation of the Z-isoprene chain of the polyprenols in all the higher plants examined.

These findings, together with the previous finding for the malloprenols, obviously indicate that the formation of the E-isoprene chain of GGPP follows

Table 1. Radioactivity and $^{3}H/^{14}C$ atom ratio in polyprenols biosynthesized from (4R)-[2-^{14}C,4-^{3}H]MVA and (4S)-[2-^{14}C,4-^{3}H]MVA

MVA used ($^{3}H/^{14}C$ ratio)	Compd[a]	Observed			Expected atom ratio	
		^{3}H	^{14}C	Atom ratio[b]	(A)[c]	(B)[d]
		dpm	dpm	^{3}H:^{14}C	^{3}H:^{14}C	^{3}H:^{14}C
(4R)-[2-^{14}C,4-^{3}H]MVA						
(3.03)	ACPL-10	456	159	9.5±0.1:10	4:10	10:10
	ACPL-11	900	306	10.7±0.1:11	4:11	11:11
	ACPL-12	1061	348	12.1±0.1:12	4:12	12:12
(4.13)	TSPL-10	11617	2970	9.5±0.02:10	4:10	10:10
	TSPL-11	1073	270	10.6±0.08:11	4:11	11:11
	TSPL-12	594	142	12.1±0.13:12	4:12	12:12
(3.72)	ASPL-11	2400	678	10.5±0.05:11	4:11	11:11
(4.38)	BPPL-10	9520	2107	10.3±0.03:10	4:10	10:10
	BPPL-11	4572	1041	11.0±0.04:11	4:11	11:11
(4.55)	ATPL-13	1372	303	12.9±0.09:13	4:13	13:13
(4S)-[2-^{14}C,4-^{3}H]MVA						
(13.5)	ACPL-10	563	2359	0.18±0.06:10	6:10	0:10
	ACPL-11	502	1477	0.28±0.08:11	7:11	0:11
	ACPL-12	824	1925	0.38±0.07:12	8:12	0:12
(12.7)	TSPL-10	165	1137	0.11±0.001:10	6:10	0:10
	TSPL-11	86	1187	0.06±0.001:11	7:11	0:11
	TSPL-12	15	476	0.03±0.003:12	8:12	0:12
(13.6)	ASPL-11	198	255	0.63±0.11:11	7:11	0:11
(12.8)	BPPL-10	105	350	0.23±0.003:10	6:10	0:10
	BPPL-11	132	156	0.73±0.011:11	7:11	0:11
(12.1)	ATPL-13	92	181	0.55±0.01:13	9:13	0:13

a) ACPL, TSPL, ASPL, BPPL, and ATPL, denote polyprenols obtained from A. cordata, T. sebifera, A. serrulatoides, B. platyphylla, and A. turbinate, respectively.

b) Normalized atom ratio. The deviations were calculated from the standard deviation in the radioactivity of each sample.

c) Calculated from the expectation that the E- and the Z-isoprene residues are formed by loss of the pro-4S and pro-4R hydrogen atoms of MVA, respectively.

d) Calculated from the expectation that both the E- and the Z-isoprene residues are formed by loss of the pro-4S hydrogen atom of MVA.

Cornforth's basic principle, but the formation of Z-isoprene chain by successive cis-addition of IPP to the GGPP involves, contrary to the basic principle, the elimination of the pro-4S hydrogen atom of MVA, and the reversed hydrogen atom elimination is common to the formation of the Z-isoprene chain of the polyprenols in higher plants. The spatial arrangement of the active sites in the enzyme participating in the biosynthesis of the Z-isoprene chain of the polyprenols in the higher plants may be in a diastereomeric relationship with that of the active sites in the enzyme in mammalian and microorganism, and such a relationship may cause the reversed hydrogen atom elimination.

The present work was supported in part by Grant-in-Aids for Scientific Research Nos. 247027 and 347024, Special Project Research No. 56209019, and Co-operative Research No. 57340031 from the Ministry of Education, Science and Culture.

References

1) J. W. Cornforth, R. H. Cornforth, C. Donninger, and G. Popják, Proc. R. Soc. London, Ser. B, **163**, 492 (1965).

2) T. W. Goodwin and J. H. Williams, Proc. R. Soc. London, Ser. B, **163**, 515 (1965).

3) B. L. Archer, D. Barnard, E. G. Cookbain, J. W. Cornforth, R. H. Cornforth, and G. Popják, Proc. R. Soc. London, Ser. B, **163**, 519 (1965).

4) T. Suga, T. Hirata, T. Aoki, and T. Shishibori, J. Am. Chem. Soc., accepted for publication.

5) T. Suga, T. Shishibori, and K. Nakaya, Phytochemistry, **19**, 2327 (1980).

6) A. R. Wellbrum, J. Stevenson, F. W. Hemming, and R. A. Morton, Biochem. J., **102**, 313 (1967).

7) P. J. Dunphy, J. F. Kerr, J. F. Pennock, and K. Whittle, Chem. Ind. (London), **1966**, 1549.

8) The radioactivity was measured on a liquid scintillation spectrometer using Bray's scintillation solvent.[9] The standard deviations were ±2.0% for ^{3}H and ±3.5% for ^{14}C.

9) G. A. Bray, Anal. Biochem., **1**, 279 (1960).

(Received July 7, 1983)