Structure and Biosynthesis of Cleomeprenols from the Leaves of *Cleome spinosa*

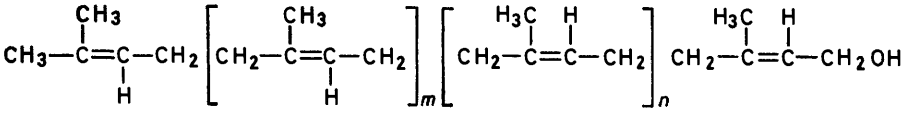
By **Takayuki Suga*** and **Tsuyoshi Shishibori**, Department of Chemistry, Faculty of Science, Hiroshima University, Higashisenda-machi, Hiroshima 730, Japan

Cleomeprenols isolated from *Cleome spinosa* L. (Capparidaceae) have been identified as nonaprenol (1), decaprenol (2), and undecaprenol (3), which are composed of an ω-terminal isoprene, three internal *E*-isoprene, and the remaining *Z*-isoprene residues, respectively. Feeding experiments using stereospecifically double-labelled radioactive mevalonate showed that all the cleomeprenols are composed of four biogenetically *E*- and the remaining biogenetically *Z*-isoprene residues. Occurrence of the successive *cis*-condensation of isoprene residues with (2*E*, 6*E*, 10*E*)-geranylgeranyl pyrophosphate was demonstrated by comparing the incorporation of a homologue of all-*E*-prenyl pyrophosphates with that of the corresponding 2*Z*-isomer.

It is well known that larvae of the butterfly *Pieris rapae crucivora* Boisduval feed on the leaves of *Cleome spinosa* L. (Capparidaceae) and some plants belonging to the Cruciferae.[1] In connection with a biochemical study of the relationship between the diet and the growth of insects, we examined the chemical constituents of the leaves of *C. spinosa* and communicated, in preliminary form, the isolation and structural elucidation of a homologous series of polyprenols.[2] We also studied the biotransformation of the polyprenols by the larvae of *P. rapae crucivora*.[3] Polyprenol phosphates have been shown to act as carriers of carbohydrate components in the formation of bacterial wall polymers.[4-6] The biological functions of polyprenols in mammalian tissues has been described,[6-8] but there have been relatively few investigations of the polyprenols of higher plants. We report here details of the structural elucidation and the biosynthetic study of cleomeprenols isolated from *C. spinosa*.

RESULTS AND DISCUSSION

Structure of Cleomeprenols.—A neutral fraction of the hexane-soluble part obtained from the methanol extract of the leaves of *C. spinosa* was chromatographed on a silica-gel column to give a mixture of polyprenols. The polyprenol content of the plant increased with growth and weighed 25 and 509 mg per kg of the fresh leaves at the end of May and July, respectively. Reversed-phase t.l.c. analysis [9] of the polyprenols showed spots at R_F 0.61 (20%), 0.53 (41), 0.45 (33), and 0.40 (6). Each component was then separated by reversed-phase preparative t.l.c., and named cleomeprenol-9 (1), -10 (2), -11 (3), and -12 (4). Their i.r., n.m.r., and mass spectra showed a similar pattern, indicating a homologous series of polyprenols.

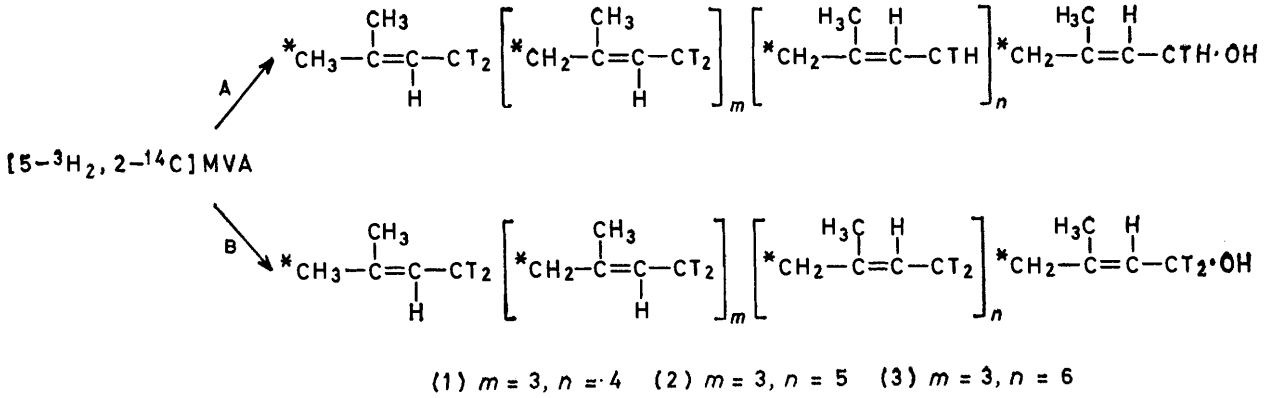


(1) $m = 3, n = 4$ (2) $m = 3, n = 5$ (3) $m = 3, n = 6$ (4) $m = 3, n = 7$

Cleomeprenol-10 (2), giving a spot with R_F 0.53 on reversed-phase t.l.c., exhibited a M^+ peak corresponding to $C_{50}H_{82}O$ in the mass spectrum. The i.r. and u.v. spectra indicated the presence of a hydroxy-group and isolated double bonds. Acetylation of (2) with acetic anhydride and pyridine gave an acetate, $C_{52}H_{84}O_2$, which showed no hydroxy-band in the i.r. spectrum. Oxidation of (2) with manganese dioxide gave an α,β-unsaturated aldehyde, indicating the presence of an allylic primary hydroxy-group. The mass spectrum 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. Loss of the 69 mass units from the *m/e* 680 peak, followed by successive loss of 68 mass units corresponding to an isoprene residue results in occurrence of a prominent peak of *m/e* 135. The n.m.r. spectrum of (2) indicated the presence of 11 allylic methyls, 18 methylenes, an allylic hydroxymethylene, 9 vinyl protons, and a proton of $=CHCH_2OH$. These facts suggest that (2) is a polyprenol similar to heveaprenols,[9,10] castaprenols,[11] and ficaprenols,[12] and is composed of 10 isoprene units. The allylic methyl signals at δ 1.59, 1.66, and 1.73 in the n.m.r. spectrum are assignable [13] 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, cleomeprenol-10 (2) has been established to involve an α-terminal *Z*, 5 internal *Z*, 3 internal *E*, and ω-terminal isoprene residues.

Cleomeprenols-9 (1) and -11 (3) gave spots with R_F 0.61 and 0.45, respectively, on reversed-phase t.l.c., and M^+ peaks at *m/e* 630 corresponding to $C_{45}H_{74}O$ and at *m/e* 766 to $C_{55}H_{90}O$, respectively, in the mass spectra. On

the basis of the M^+ 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 being 4 : 5 : 1 and 4 : 7 : 1 in the n.m.r. spectra, cleomeprenols-9 (1) and -11 (3) were found to have 4 and 6 internal *Z*-isoprene residues, respectively, in addition to the other isoprene residues found in cleomeprenol-10 (2). On the other hand, no sample of the compound giving a spot with R_F 0.40 could be isolated because of the small amount present, but its chromatographic behaviour on reversed-phase t.l.c. indicated the compound to be dodecaprenol as shown by (4).



SCHEME 1 Possible labelling of ^3H and ^{14}C in cleomeprenols-9 (1), -10 (2), and -11 (3) biosynthesized from [5-$^3H_2$, 2-^{14}C]mevalonate. T and *C in figure denote ^3H and ^{14}C, respectively

Biosynthesis of Cleomeprenols.—Several polyprenols composed of *Z*- and *E*-isoprene residues have been isolated.[14,15] Betulaprenols have been described to be formed in *Betula verrucosa* by *cis*-condensation of isoprene residues with (2*E*, 6*E*)-farnesyl pyrophosphate.[16-19] Castaprenols isolated from *Aesculus hippocastanum*,[11] heveaprenols from *Hevea brasiliensis*,[9,10] ficaprenols from *Ficus elastica*,[12] and aglaprenols from *Aglaonema robelinii* [10] are composed of 9 to 13 isoprene residues containing 3 internal *E*-residues, similar to cleomeprenols-9 (1) to -11 (3), but there has been no experimental proof regarding the sequence of the internal *Z*- and *E*-isoprene residues. In order to clarify the sequence, the biological formation of *Z*- and *E*-isoprene residues in cleomeprenols has been examined.

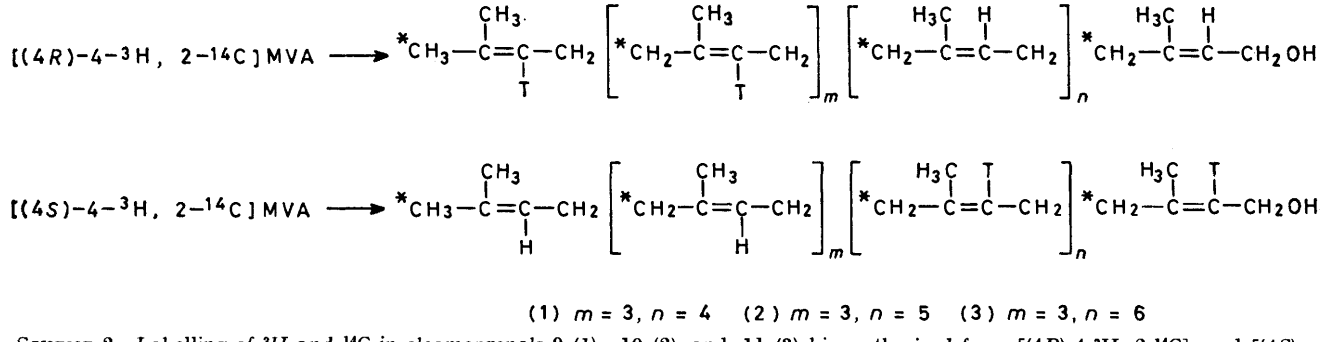
TABLE 1

Incorporation of [5-$^3H_2$, 2-^{14}C]mevalonate into cleomeprenols-10 (2) and -11 (3) of leaves of *Cleome spinosa*

	Observed				Expected	
Compd.	^3H (d.p.m.)	^{14}C (d.p.m.)	$^3H/^{14}C$	Atom ratio [a] (^3H : ^{14}C)	*via* route A (^3H : ^{14}C)	*via* route B (^3H : ^{14}C)
(2)	1 843	210	8.78	20.0 : 10	14 : 10	20 : 10
(3)	790	89	8.88	22.3 : 11	15 : 11	22 : 11

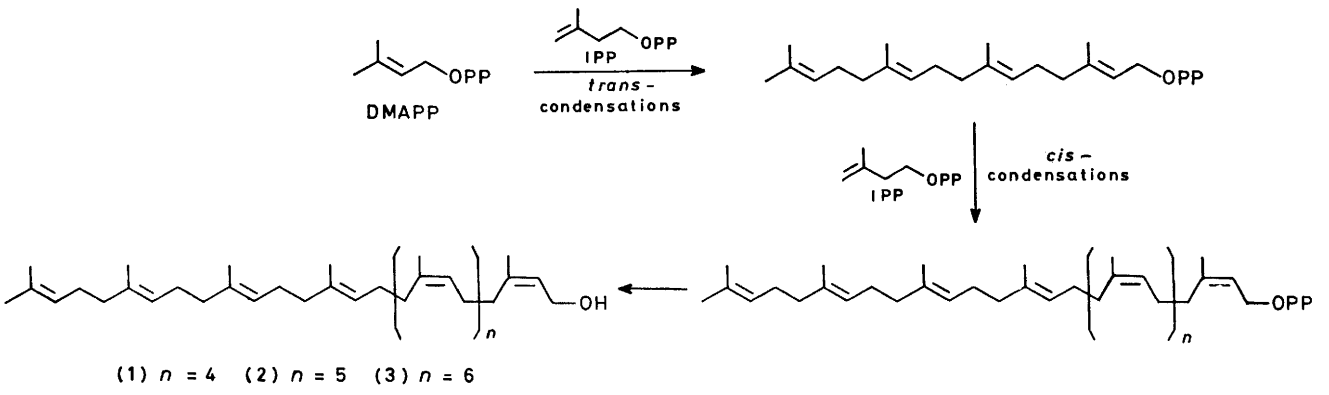
[a] This is the ratio obtained by dividing the observed $^3H/^{14}C$ ratio by the $^3H/^{14}C$ ratio of the original mevalonate and multiplying the answer by the number of C-atoms from C-2 of mevalonate expected to be presented in each molecule of the compounds. The $^3H/^{14}C$ ratio of the original [5-$^3H_2$, 2-^{14}C]mevalonate was 8.76.

It has been reported that the *Z*-isoprene residue in (2*Z*, 6*E*)-farnesol is formed from (2*E*, 6*E*)-farnesol by isomerization *via* a corresponding aldehyde intermediate in fungi and plants.[20-24] Whether such a *Z*–*E* isomerization is involved in the biosynthesis of cleomeprenols or



SCHEME 2 Labelling of ^3H and ^{14}C in cleomeprenols-9 (1), -10 (2), and -11 (3) biosynthesized from [(4*R*)-4-^3H, 2-^{14}C]- and [(4*S*)-4-^3H, 2-^{14}C]-mevalonates. T and *C in figure denote ^3H and ^{14}C, respectively

not has been examined first by means of administration of [5-$^3H_2$, 2-^{14}C]mevalonic acid (MVA) to the leaves of *C. spinosa*. Provided the *Z*-isoprene residues of cleomeprenols are formed by isomerization *via* an aldehyde, an IPP with 3,3-dimethylallyl pyrophosphate or its higher homologues.[25-27] In feeding experiments of [(4*R*)-4-^{3}H, 2-^{14}C] and [(4*S*)-4-^{3}H, 2-^{14}C]MVA into cleomeprenols, the observed ^{3}H : ^{14}C atom ratios for cleomeprenols-9 to



SCHEME 3

atom of ^{3}H would be lost from each *Z*-isoprene residue, and cleomeprenols are expected to be labelled as shown in route A (Scheme 1). Feeding experiments (Table 1) indicated that the ^{3}H/^{14}C ratio of the original MVA was retained in the prenols; such a labelling is shown in route B (Scheme 1). Accordingly, it was found that the *Z*–*E* isomerization is not involved in the biosynthesis of cleomeprenols.

-11 (Table 2) agreed with the ratios calculated by assuming these prenols to be composed of 4 biogenetically *E*-residues, the remaining being *Z*-residues. A combination of this finding and the n.m.r. spectroscopic evidence revealed that the ω-terminal and 3 internal *E*-residues are biogenetically *E*, and the α-terminal and the remaining residues are *Z* (Scheme 2).

It is thus suggested that cleomeprenols are formed

TABLE 2

Incorporation of [(4*R*)-4-^{3}H, 2-^{14}C]- and [(4*S*)-4-^{3}H, 2-^{14}C]-mevalonates into cleomeprenols-9 (1), -10 (2), and -11 (3) of the leaves of *Cleome spinosa*

		From [(4*R*)-4-^{3}H, 2-^{14}C]mevalonate [a]			
		Observed			Expected
Expt.	Compd.	^{3}H (d.p.m.)	^{14}C (d.p.m.)	^{3}H/^{14}C	Atom ratio (^{3}H : ^{14}C)	Atom ratio (^{3}H : ^{14}C)
1	(1)	59 795	19 212	3.11	4.34 : 9	4 : 9
	(2)	32 194	13 062	2.46	3.81 : 10	4 : 10
	(3)	7 157	3 197	2.24	3.82 : 11	4 : 11
2	(2)	549	322	1.70	3.97 : 10	4 : 10
	(3)	991	635	1.56	4.01 : 11	4 : 11
3	(2)	872	479	1.82	4.25 : 10	4 : 10
	(3)	2 479	1 580	1.57	4.04 : 11	4 : 11
4	(2)	744	427	1.74	4.07 : 10	4 : 10
	(3)	615	383	1.61	4.14 : 11	4 : 11
		From [(4*S*)-4-^{3}H, 2-^{14}C]mevalonate [a]			
		Observed			Expected
Expt.	Compd.	^{3}H (d.p.m.)	^{13}C (d.p.m.)	^{3}H/^{14}C	Atom ratio (^{3}H : ^{14}C)	Atom ratio (^{3}H : ^{14}C)
5	(1)	12 518	2 597	4.82	4.99 : 9	5 : 9
	(2)	38 457	7 727	4.98	5.72 : 10	6 : 10
	(3)	4 857	838	5.80	7.33 : 11	7 : 11

[a] The ^{3}H/^{14}C ratio of the [(4*R*)-4-^{3}H, 2-^{14}C]mevalonate used was 6.45 (expt. 1) and 4.28 (expt. 2, 3, and 4). The ratio of [(4*S*)-4-^{3}H, 2-^{14}C]mevalonate was 8.70 (expt. 5).

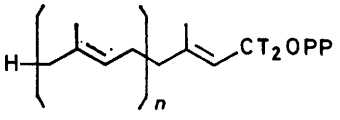
The *Z*-isoprene residues of cleomeprenols are thus presumed to be formed by another process such as that operative in the biosynthesis of natural rubber which is composed of all-*Z*-isoprene residues.[25] The process involves loss of a hydrogen atom from C-2 of isopentenyl pyrophosphate (IPP), which corresponds to a pro-4*R* hydrogen atom of mevalonoid, in the condensation of by successive *cis*-condensation of isoprene residues with (2*E*, 6*E*, 10*E*)-geranylgeranyl pyrophosphate (GGPP) (Scheme 3). Such a mechanism requires all 3 internal *E*-isoprene residues to be adjacent to the terminal C_5 unit. The location of the internal *E*-isoprene residues in cleomeprenols was biosynthetically pursued by incorporation of all-(*E*)-[1,1-$^3H_2$]di-, tri-, tetra-, and penta-prenyl

pyrophosphates, (5), (7), (9), and (11), and their (2*Z*) isomers, (6), (8), (10), and (12), into cleomeprenols. The incorporation of (2*E*, 6*E*, 10*E*)-GGPP (9) into cleomeprenols-10 (2) and -11 (3) was higher than that of its (2*Z*)-isomer (10) (Table 3). The same tendency was observed for incorporations of a pair of the lower homologues of all-(*E*) isomers, (5) and (7), and their (2*Z*)-isomers, (6) and (8). In contrast the incorporation of the all-(*E*) homologue (11) was lower than that of its (2*Z*)-isomer (12). These results imply that three internal *E*-isoprene residues are adjacent to the terminal C_5 unit and that the remaining residues are in the *Z*-configuration. This finding indicates clearly that each cleomeprenol is biosynthesized by successive *cis*-condensation of IPP with (2*E*, 6*E*, 10*E*)-GGPP (9) (Scheme 3).

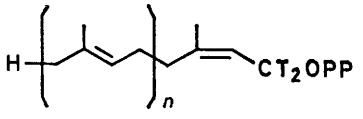
TABLE 3

Incorporation of a homologous series of (2*E*)-[1-^{3}H] and (2*Z*)-[1-^{3}H]prenyl pyrophosphates [(5), (7), (9), and (11) and (6), (8), (10), and (12), respectively] into cleomeprenols -10 (2) and -11 (3) of the leaves of *Cleome spinosa*

		Cleomeprenol-10 (2)			Cleomeprenol-11 (3)		
[1,1-$^3H_2$]Prenyl pyrophosphates	Radioactivity of substrates used (d.p.m.)	d.p.m.	Incorporation (%)	Incorporation ratio of (2*E*) to (2*Z*) isomers	d.p.m	Incorporation (%)	Incorporation ratio of (2*E*) to (2*Z*) isomers
(5)	1.49×10^7	2 567	0.018	3.6	2 554	0.017	5.7
(6)	3.19×10^7	1 667	0.005		874	0.003	
(7)	4.73×10^6	3 300	0.087	2.4	3 164	0.072	2.7
(8)	2.85×10^6	1 067	0.037		779	0.027	
(9)	1.58×10^6	4 456	0.28	5.3	3 339	0.21	1.6
(10)	1.56×10^6	829	0.053		2 086	0.13	
(11)	1.84×10^6	638	0.035	0.14	1 202	0.065	0.31
(12)	8.25×10^5	2 043	0.25		1 726	0.21	



(5) *n* = 1
(7) *n* = 2
(9) *n* = 3
(11) *n* = 4



(6) *n* = 1
(8) *n* = 2
(10) *n* = 3
(12) *n* = 4

The above results suggest strongly that such *Z*, *E*-polyprenols as castaprenols,[11] heveaprenols,[9,10] ficaprenols,[12] and aglaprenols,[10] all of which contain 3 internal *E*-isoprene residues, are biosynthesized in a manner similar to cleomeprenols.

EXPERIMENTAL

N.m.r. spectra were determined at 100 MHz in $CDCl_3$ with Me_4Si as an internal standard, i.r. spectra for thin films, u.v. spectra in iso-octane, and mass spectra at 70 eV using a direct-inlet system. G.l.c. was performed on 2% SE-30 and 15% DEGS columns with an oven temperature of 150—300 °C. Reversed-phase partition t.l.c. was performed on paraffin–Kieselguhr G plates, which were prepared by saturating the Kieselguhr G (Merck) plates (0.25 or 0.75 mm thick) with a solution of liquid paraffin in hexane (5%, v/v) and then evaporating the solvent.[9] Densitometric measurement of spots on the reversed-phase Kieselguhr G plate (0.25 mm) was made by a t.l.c. photodensitometer (Tōyō DENSITROL DMU-2) at 500 nm-light. T.l.c. radiochromatographic measurements were performed on Silica gel GF_{254} plates by an Aloka radiochromato-scanner. Radioactivities of [^{3}H, ^{14}C]-labelled samples were assayed with a Packard Tri-Carb liquid scintillation spectrometer (model 3 330) by the spill-over method [28] in Bray's scintillant.[29] In this system, counting efficiencies of ^{3}H and ^{14}C were 35 and 57%, respectively. Radioactivity of [^{3}H]-labelled samples was measured by the same spectrometer with counting efficiency of 45% in the same scintillant. The radioactivities shown in Tables 3, 4, and 5 have standard deviations of ±1.5% for ^{3}H and ±4% for ^{14}C.

Isolation of Cleomeprenols from C. spinosa.—Leaves (1.1 kg) were collected from *C. spinosa* cultivated in a suburb of Hiroshima city at the end of July and air-dried to 0.5 kg. The half-dried leaves were immersed in methanol (7 l) for 1 month at room temperature. Removal of the solvent from the methanol solution *in vacuo* gave a residue, which was extracted with hexane. The hexane solution was washed successively with 5% sodium hydrogen carbonate, 5% potassium hydroxide, and 7% hydrochloric acid to give a neutral oily material (3.6 g). This was chromatographed on a silica gel column (400 g) using a mixed solvent of hexane and ethyl acetate with successively increasing amounts of the latter (0 to 15%, v/v) to give a mixture of polyprenols (560 mg) as a main component. The mixture was subjected to reversed-phase t.l.c. on a paraffin-Kieselguhr G plate with acetone–water (9 : 1, v/v) to separate each of the polyprenols. The plate, on spraying with a *p*-anisaldehyde–sulphuric acid reagent [9] and heating to 100 °C for 5 min

gave four spots at R_F 0.61, 0.53, 0.45, and 0.40; the polyprenols giving these spots were named cleomeprenols-9 (1), -10 (2), -11 (3), and -12 (4), respectively. The composition of the cleomeprenols, (1)—(4), was determined by means of a t.l.c. densitometer to be 20, 41, 33, and 6%, respectively. Preparative-scale separation of each cleomeprenol was carried out on the same type of t.l.c. plate as that described above. Cleomeprenols on the plate were located by spraying with 0.01% ethanolic solution of 2′, 7′-dichlorofluorescein and then by irradiation with 254-nm u.v. light. The fluorescent areas were scraped off and extracted separately with ether. Each of cleomeprenols was then subjected to separation from liquid paraffin and 2′,7′-dichlorofluorescein by t.l.c. using Silica gel G and hexane–ethyl acetate (9 : 1).

Cleomeprenol-10 (2).—This prenol, giving a spot at R_F 0.53, was obtained as an oil; $\nu_{max.}$ (neat) 3 327, 1 005 (OH) and 1 660, 840 cm^{-1} (isolated C=C); no u.v. absorption of wavelengths greater than 210 nm; *m/e* 698 (M^+, 4%), 680 ($M - H_2O$, 11), 611 ($R_9OH - H_2O$, where R = CH_2-CMe=$CHCH_2$, 2), 543 ($R_8OH - H_2O$, 3), 475 ($R_7OH - H_2O$, 3), 407 ($R_6OH - H_2O$, 4), 339 ($R_5OH - H_2O$, 4), 271 ($R_4OH - H_2O$, 7), 203 ($R_3OH - H_2O$, 14), 135 ($R_2OH - H_2O$, 33), 121 (48), 95 (67), 81 (97), 69 (Me_2C=$CHCH_2$, 100), and 55 (Me_2C=CH, 54); δ 1.59 (12 H, s, *E*-C*Me*=CH), 1.66 (18 H, s, *Z*-C*Me*=CH), 1.73 (3 H, s, *Z*-C*Me*=CH-CH_2OH), 2.01, 2.03 (36 H, each s, =CCH_2CH_2C=), 4.09 (2 H, d, *J* 7 Hz, =CHCH_2OH), 5.10 (9 H, m, =CH), and 5.43 (1 H, t, *J* 7 Hz, =C*H*CH_2OH).

Acetylation of compound (2). A mixture of the prenol (2) (14 mg), acetic anhydride (0.1 ml), and pyridine (0.1 ml) was allowed to stand overnight, and the mixture, after addition of a large amount of water, was extracted with ether. A product obtained from the ether extract was purified by t.l.c. using Silica gel G and hexane–ethyl acetate (9 : 1) to give the acetate of (2) (10 mg) as a colourless oil; $C_{52}H_{84}O_2$; *m/e* 740 (M^+); $\nu_{max.}$ 1 736, 1 235 (OCOMe) and 1 660, 835 cm^{-1} (C=C); δ 2.02 (3 H, s, OCOMe) and 4.56 (2 H, t, *J* 7 Hz, =C*H*CH_2OAc).

Oxidation of compound (2). Active manganese dioxide (50 mg) was added to a solution of the prenol (2) (10 mg) in hexane (3 ml), and the mixture was stirred for 2 h at room temperature in a stream of nitrogen. Removal of the solvent, after filtration, afforded an oily residue, which was then purified by t.l.c. on Silica gel G with a hexane–ethyl acetate (19 : 1) solvent to give an aldehyde (8 mg) as an oil: $\nu_{max.}$ 2 728, 1 676, and 1 631 cm^{-1} (C=CCHO); $\lambda_{max.}$ 243 nm (ε 14 100); δ 5.86 (1 H, d, *J* 8 Hz, C=C*H*CHO) and 9.93 (1 H, d, *J* 8 Hz, C=CHC*H*O).

Cleomeprenol-9 (1).—The prenol (1) (R_F 0.61) was obtained as a colourless oil; *m/e* 630 (M^+, 4%), 612 ($M - H_2O$), 543 (4), 475 (6), 407 (6), 339 (6), 271 (8), 203 (18), 135 (28), 121 (38), 95 (59), 81 (82), 69 (100), and 55 (60); δ 1.60 (12 H, s), 1.69 (15 H, s), 1.75 (3 H, s), 2.02, 2.06 (32 H, each s), 4.09 (2 H, d, *J* 7 Hz), 5.12 (8 H, m), and 5.42 (1 H, t, *J* 7 Hz). The mass and n.m.r. spectral assignments for (2) were as for (1).

Cleomeprenol-11 (3).—The prenol (3) (R_F 0.45) was obtained as a colourless oil; *m/e* 766 (M^+, 4%), 748 ($M - H_2O$, 9), 679 (2), 611 (2), 543 (2), 475 (3), 407 (3), 339 (3), 271 (5), 203 (8), 135 (21), 121 (33), 95 (46), 81 (88), 69 (100), and 55 (23); δ 1.59 (12 H, s), 1.66 (21 H, s), 1.73 (3 H, s), 2.01, 2.05 (40 H, each s), 4.08 (2 H, d, *J* 7 Hz), 5.10 (10 H, m), and 5.42 (1 H, t, *J* 7 Hz). The mass and n.m.r. spectral assignments for (3) were as for (1).

Administration of [5-$^3H_2$,2-^{14}C]*Mevalonate to the Leaves of* C. spinosa.—A solution of potassium [5-$^3H_2$, 2-^{14}C]mevalonate (0.7 μCi of ^{14}C, ^{3}H/^{14}C=8.76) in water (0.3 ml) was fed through the cut stem to the leaves (130 g), which were cut off from the mother plant just before the feeding. After uptake of the tracer over 1 h at 25 °C, water was taken up over 70 h in a similar way. The plant materials were cut into small pieces and extracted with methanol (1 l × 4 times). The methanol solution was concentrated *in vacuo* and the residue obtained subjected to preparative t.l.c. on Silica gel G with hexane–ethyl acetate (9 : 1). A fraction composed of cleomeprenols was further purified by t.l.c. on a plate of the same type with benzene–ethyl acetate (9 : 1). Cleomeprenols thus purified were again subjected to reversed-phase t.l.c. to separate cleomeprenols-10 (2) and -11 (3), which were further purified separately to constant specific activity. The results of radioassay are shown in Table 1.

Administration of [(4*R*)-4-^{3}H, 2-^{14}C] *and* [(4*S*)-4-^{3}H, 2-^{14}C]*Mevalonates to Leaves of* C. spinosa.—*Experiments* 1 *and* 5. A solution of potassium [(4*R*)-4-^{3}H, 2-^{14}C]mevalonate (15 μCi of ^{14}C, 46.18 Ci mol^{-1} for ^{3}H and 7.16 Ci mol^{-1} for ^{14}C) in a phosphate-buffered solution (pH 7.3, 1 ml) was fed to the leaves (20 g) through their cut stems over 4 h at 25 °C, and water was taken up over 20 h. On the other hand, potassium [(4*S*)-4-^{3}H, 2-^{14}C]mevalonate (6.5 μCi of ^{14}C, 62.30 Ci mol^{-1} for ^{3}H and 7.16 Ci mol^{-1} for ^{14}C) was fed to the leaves through their cut stems in the same manner as above. Separation and purification of cleomeprenols were performed in the same manner as above.

Experiments 2, 3, *and* 4. Aliquot portions of a solution of potassium [(4*R*)-4-^{3}H, 2-^{14}C]mevalonate (1.74 μCi mol^{-1} of ^{14}C, 30.67 Ci mol^{-1} for ^{3}H and 7.16 Ci mol^{-1} for ^{14}C) in a phosphate-buffered solution (pH 7.3, 1 ml) were fed to the leaves (120 g) through their cut stems over 4 h at 25 °C. After completion of the uptake of tracer, water was taken up over 20 h (experiment 2), 30 h (experiment 3), and 70 h (experiment 4) in a similar way. The results are shown in Table 2.

Preparation of [1-^{3}H]*Prenols*.—[1-^{3}H]*Geraniol* (5a) *and* [1-^{3}H]*nerol* (6a). To a solution of geraniol (1.0 g) in hexane (150 ml) active manganese dioxide (3 g) was added, and the mixture was stirred overnight. After filtration and evaporation, the residue was subjected to t.l.c. on Silica gel GF_{254} with hexane–ethyl acetate (9 : 1) to give (2*E*)-citral (0.8 g). To a solution of citral (10 mg) in dry ether (1 ml) lithium aluminium [^{3}H]hydride (1 mCi, 157.8 Ci mol^{-1}) was added, and the mixture was stirred for 2 h. The reaction was completed by addition of an excess of cold lithium aluminium hydride. After addition of moistened ether and extraction with ether, the product obtained was subjected to preparative t.l.c. (Silica gel G; hexane–ethyl acetate, 9 : 1) to give [1-^{3}H]geraniol (5a) (6 mg) as an oil. In a similar manner, [1-^{3}H]nerol (6a) was prepared from (2*Z*)-citral (derived from nerol by oxidation) by reduction with lithium aluminium [^{3}H]hydride.

(2E, 6E)- *and* (2Z, 6E)-[1-$^3H_2$]*Farnesols*, (7a) *and* (8a). Freshly distilled phosphorus tribromide (13.5 g) was added dropwise to a solution of geraniol (24.8 g) in dry ether (50 ml) the temperature being kept below 0 °C with stirring. The mixture, after 4 h, was poured into ice–water and extracted with ether. Removal of the solvent from the ether solution gave geranyl bromide (33.6 g, b.p. 92—93.5 °C at 6 mmHg). The bromide (23.8 g) was added dropwise to ethyl acetoacetate (12.9 g) dissolved in a solution made by

dissolving sodium (2.3 g) in absolute ethanol (50 ml). The mixture, after being filtered free from sodium bromide and concentration to a small volume *in vacuo*, was stirred with 5% sodium hydroxide overnight. The oily layer was extracted with hexane and the aqueous layer, after acidification with 50% sulphuric acid, with ether. The combined extract was chromatographed on a silica-gel column using a mixture of hexane and ethyl acetate with successively increasing amounts of the latter (0 to 10%, v/v) to give geranylacetone as an oil (56% yield based on geraniol): *m/e* 194 (M^+), 43 (base); δ 1.60 (6 H, s, *E*-C*Me*=CH), 1.66 (3 H, s, *Z*-C*Me*=CH), and 2.02 (3 H, s, COMe); $\nu_{max.}$ 1 725 and 1 160 cm^{-1} ($COCH_3$).

Geranylacetone (321 mg) was added to a clear solution made by adding methyl diethylphosphonoacetate (347 mg) to a slurry of sodium hydride (79 mg) in dimethoxyethane (10 ml) in a stream of nitrogen. The mixture was allowed to stand for 2 days and then extracted, after addition of water, with ether. Removal of the solvent from the ether solution gave a 2*Z*- and 2*E*-mixture of methyl farnesoates (350 mg), which was subjected to multiple development t.l.c.[30] on Silica gel GF_{25} with hexane–ether (97 : 3). Three developments resulted in separation of methyl (2*E*,6*E*)-farnesoate (7b) (131 mg) and the (2*Z*,6*E*)-isomer (8b) (49 mg) as colourless oils, which were located at R_F 0.62 and 0.72 on the plate by irradiation with 254-nm u.v. light, and exhibited spectral data as follows: (7b), *m/e* 250 (M^+), 69 (base); δ 1.60 (6 H, s, *E*-C*Me*=CH), 1.66 (3 H, s, =CMe), 2.16 (3 H, s, *E*-C*Me*=CHCOOMe),[31] 3.60 (3 H, s, COOMe), and 5.62 (1 H, s, C-2 H); (8b), *m/e* 250 (M^+), 69 (base); δ 1.60 and 1.66 (6 H and 3 H, each s, =CMe), 1.90 (3 H, s, *Z*-C*Me*=CHCOOMe),[31] 3.60 (3 H, s, COOMe), and 5.61 (1 H, s, C-2 H).

To a solution of methyl (2*E*, 6*E*)-farnesoate (7b) (50 μmol) in dry ether (1 ml) lithium aluminium [^{3}H]hydride (1 mCi, 157.8 Ci mol^{-1}) was added, and the mixture was refluxed for 2 h. The unchanged methyl ester was reduced by addition of an excess of cold lithium aluminium hydride. After work-up, the product was purified by preparative t.l.c. on Silica gel GF_{254} with hexane–ethyl acetate (4 : 1). The radioactivity was located with a t.l.c. radiochromato-scanner, and (2*E*, 6*E*)-[1-$^3H_2$]farnesol (7a) was extracted from the plate with ether as an oil.

(2*Z*, 6*E*)-[1-$^3H_2$]Farnesol (8a) was prepared from methyl (2*Z*, 6*E*)-farnesoate (8b) in the same manner as (7a).

(2E, 6E, 10E)- *and* (2Z, 6E, 10E)-[1-$^3H_2$]*Geranylgeraniols*, (9a) *and* (10a). Methyl (2*E*, 6*E*)-farnesoate (7b) (2.38 g) dissolved in dry ether (35 ml) was added to a suspension of lithium aluminium hydride (368 mg) in dry ether (35 ml) with stirring. The mixture was refluxed for 2 h and then worked up in the usual manner; purification of the product by t.l.c. gave (2*E*, 6*E*)-farnesol (1.68 g) as an oil: δ 1.60 and 1.67 (9 H and 3 H, each s, =CMe), 4.02 (2 H, d, *J* 6 Hz, CH_2OH), and 5.35 (1 H, t, *J* 6 Hz, =$CH$$CH_2OH$).

(2*E*, 6*E*)-Farnesol (1.68 g) was converted into a mixture of methyl (2*E*, 6*E*, 10*E*)- and (2*Z*, 6*E*, 10*E*)-geranylgeranoates, (9b) and (10b), *via* farnesylacetone in the same manner as the farnesoates, (7b) and (8b), from geraniol. Separation of the *Z*–*E* mixture in the same manner as above gave (9b) (288 mg) as an oil: δ 1.60 and 1.67 (9 H and 3 H, each s, =CMe), 2.13 (3 H, s, *E*-C*Me*=CHCOOMe),[31] 3.60 (3 H, s, COOMe), and 5.60 (1 H, s, C-2 H); and (10b) (76 mg) as an oil: δ 1.60 and 1.67 (9 H, and 3 H, each s, =CMe), 1.87 (3 H, s, *Z*-C*Me*=CHCOOMe),[31] 3.60 (2 H, s, COOMe), and 5.60 (1 H, s, C-2 H).

The esters, (9b) and (10b), upon reduction with lithium aluminium [^{3}H]hydride afforded [1-$^3H_2$]geranylgeraniols, (9a) and (10a), as oils.

(2E, 6E, 10E, 14E)- *and* (2Z, 6E, 10E, 14E)-[1-$^3H_2$]-*Geranylfarnesols*, (11a) *and* (12a). In the same manner, a *Z*–*E* mixture of methyl geranylfarnesoates, (11b) and (12b), was prepared from a sample of (2*E*, 6*E*, 10*E*)-geranylgeraniol (9a) (100 mg), which was prepared from methyl (2*E*, 6*E*, 10*E*)-geranylgeranoate (9b) by reduction with lithium aluminium hydride. The mixture of (11b) and (12b) was subjected to preparative t.l.c. to give (11b) (12 mg) as an oil: δ 1.60 and 1.66 (12 H and 3 H, each s, =CMe), 2.13 (3 H, s, *E*-C*Me*=CHCOOMe),[31] 3.60 (3 H, s, COOMe), and 5.60 (1 H, s, C-2 H); and (12b) (3 mg) as an oil: δ 1.60 and 1.67 (12 H and 3 H, s, =CMe), 1.87 (3 H, s, *Z*-C*Me*=CHCOOMe), 3.60 (3 H, s, COMe), and 5.60 (1 H, s, C-2 H). Each of the esters was converted into [1-$^3H_2$]geranylfarnesols, (11a) and (12a), by reduction with lithium aluminium [^{3}H]hydride.

The purity of all samples of the [1-^{3}H]prenols prepared as above was more than 97% by g.l.c. on a 2% SE-30 column and t.l.c. radiochromatography on a Silica gel GF_{254} plate.

Preparation of [1,1-$^3H_2$]*Prenyl Pyrophosphates*, (5)—(12). —[1,1-$^3H_2$]*Geranyl and* [1,1-$^3H_2$]*neryl pyrophosphates*, (5) *and* (6). A solution of bis(triethylammonium) phosphate (60 mg) in trichloroacetonitrile (2 ml) was added to [1-^{3}H]-geraniol (5a) (10 mg) dissolved in trichloroacetonitrile (70 mg) during a 3 h period. The mixture was stirred for an additional 2 h at room temperature[32] and extracted, after dilution with ether (10 ml), 3 times with 0.1M-ammonium hydroxide (10 ml). The ammonium hydroxide solution, after treatment with ether, was concentrated *in vacuo* to 0.5 ml and subjected to preparative t.l.c. on Silica gel H with propanol–ammonium hydroxide–water (6 : 3 : 1). Examination of the plate by a t.l.c. radiochromato-scanner located the radioactivity at R_F 0.8 and 0.5, which correspond to geranyl monophosphate and pyrophosphate, respectively. The radioactive area at R_F 0.5 was scraped off from the plate and extracted with the same solvent as the developing one. Removal of the solvent *in vacuo* gave [1,1-$^3H_2$]geranyl pyrophosphate (5) as an amorphous solid with a specific activity of 7.5 Ci mol^{-1}. [1,1-$^3H_2$]Neryl pyrophosphate (6) (4.7 Ci mol^{-1}) was prepared from [1,1-$^3H_2$]nerol (6a) in the same manner as (5).

Other [1-$^3H_2$]*prenyl pyrophosphates*,(7)—(12). (2*E*, 6*E*)-[1-$^3H_2$]Farnesol (7a) was phosphorylated in the same manner as (5). Purification of the product by XAD-2 resin followed by column chromatography on DEAE-cellulose[26] gave (2*E*, 6*E*)-[1-$^3H_2$]farnesyl pyrophosphate as an amorphous solid with a specific activity of 3.7 Ci mol^{-1}. Other homologues of prenyl pyrophosphates, (8)—(12), were prepared from the corresponding alcohols, (8a)—(12a), in the same manner as (7), and showed specific activities of 1.6, 13.8, 10.0, 3.8, and 1.8 Ci mol^{-1}, respectively.

Administration of [1,1-$^3H_2$]*Prenyl Pyrophosphates*, (5)—(12), *to the Leaves of* C. spinosa.—A solution of [1,1-$^3H_2$]-prenyl pyrophosphates, (5)—(12), in water (1 ml) was fed to the leaves (20 g) through their cut stalks over 2 h at 25 °C, and then water was taken up over 24 h. Cleomeprenols isolated from the leaves were subjected to separation in the same manner as for the mevalonate. Radioactivities of each substrate administered and cleomeprenols isolated are shown in Table 3.

The present work was partially supported by a Grant-in-

Aid for Scientific Research from the Ministry of Education, Science, and Culture and a Scholarship from the Saneyoshi Scholarship Foundation and the Kudō Science Foundation.

[9/1302 *Received*, 15*th August*, 1979]

REFERENCES

[1] T. Shirōzu, ' Butterflies of Japan in Colour,' Hokuryukan, Tokyo, 1965, p. 84.
[2] T. Suga, T. Shishibori, S. Kosela, Y. Tanaka, and M. Itoh, *Chem. Letters*, 1975, 771.
[3] T. Suga and T. Shishibori, *Experientia*, 1979, **35**, 1423.
[4] Y. Higashi, J. L. Strominger, and C. C. Sweeley, *Proc. Nat Acad. Sci. USA*, 1967, **57**, 1878.
[5] M. Scher and W. J. Lennarz, *J. Biol. Chem.*, 1969, **244**, 2777.
[6] F. W. Hemming, in ' Biochemistry of Lipids,' ed. T. W. Goodwin, Butterworth, London, Biochemistry Series 1, vol. 4, p. 39.
[7] J. F. Caccam, J. J. Jackson, and E. H. Eylar, *Biochem. Biophys. Res. Comm.*, 1969, **35**, 505.
[8] N. H. Behrens, A. J. Parodi, and L. F. Leloir, *Proc. Nat. Acad. Sci. USA*, 1971, **68**, 2857.
[9] P. J. Dunphy, J. D. Kerr, J. F. Pennock, and K. J. Whittle, *Chem. and Ind.*, 1966, 1549.
[10] P. J. Dunphy, J. D. Kerr, J. F. Pennock, K. J. Whittle, and J. Feeney, *Biochim. Biophys. Acta*, 1967, **136**, 136.
[11] A. R. Wellburn, J. Stevenson, F. W. Hemming, and R. A. Morton, *Biochem. J.*, 1967, **102**, 313.
[12] K. J. Stone, A. R. Wellburn, F. W. Hemming, and J. F. Pennock, *Biochem. J.*, 1967, **102**, 325.
[13] R. B. Bates and D. M. Gale, *J. Amer. Chem. Soc.*, 1960, **82**, 5749.
[14] F. W. Hemming, in ' Natural Substances Formed Biologically from Mevalonic Acid,' ed. T. W. Goodwin, Academic Press, London, 1970, Biochemical Society Symposium No. 29, p. 105.
[15] T. Noda, T. Take, T. Watanabe, and J. Abe, *Bull. Chem. Soc. Japan*, 1970, **43**, 2174.
[16] B. O. Lindgren, *Acta Chem. Scand.*, 1965, **19**, 1317.
[17] A. R. Wellburn and F. W. Hemming, *Nature*, 1966, **212**, 1364.
[18] D. P. Gough and F. W. Hemming, *Biochem. J.*, 1967, **105**, 10C.
[19] D. P. Gough and F. W. Hemming, *Biochem. J.*, 1970, **117**, 309.
[20] G. Jacob, E. Cardenil, L. Chayet, R. Téllez, R. Pont-Lezica, and O. Cori, *Phytochemistry*, 1972, **11**, 1683.
[21] K. Imai and S. Marumo, *Tetrahedron Letters*, 1972, 4401.
[22] R. Evans, A. M. Holtom, and J. R. Hanson, *J.C.S. Chem. Comm.*, 1973, 465.
[23] K. H. Overton and F. M. Roberts, *J.C.S. Chem. Comm.*, 1974, 385.
[24] W. E. Shine and W. D. Loomis, *Phytochemistry*, 1974, **13**, 2095.
[25] B. L. Archer, D. Barnard, E. G. Cookbain, J. W. Cornforth, R. H. Cornforth, and G. Popják, *Proc. Roy. Soc.*, 1965, **B163**, 519.
[26] J. W. Cornforth, R. H. Cornforth, C. Donninger, and G. Popják, *Proc. Roy. Soc.*, 1965, **B163**, 492.
[27] T. W. Goodwin and J. H. Williams, *Proc. Roy. Soc.*, 1965, **B163**, 515.
[28] G. Hentenji and J. Reynolds, *Internat. J. Appl. Radiation Isotopes*, 1967, **18**, 331.
[29] G. A. Bray, *Analyt. Biochem.*, 1960, **1**, 279.
[30] E. Stahl, in ' Thin-Layer Chromatography,' ed. E. Stahl, Springer-Verlag, Berlin, New York, 1969, p. 86.
[31] F. A. L. Anet and A. J. R. Bourn, *J. Amer. Chem. Soc.*, 1965, **87**, 5250.
[32] R. H. Cornforth and G. Popják, in ' Methods in Enzymology,' ed. R. B. Clayton, Academic Press, New York, 1969, vol. 15, p. 359.