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The Characterization of Ficaprenol-10, -11 and -12 from the Leaves of *Ficus elastica* (Decorative Rubber Plant)

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Evidence from mass, nuclear-magnetic-resonance and infrared spectrometry and from gas–liquid and thin-layer chromatography is presented in favour of the presence of *cis–trans*-decaprenol, -undecaprenol and -dodecaprenol in the mixture of polyprenols (2·6mg./g.) isolated from leaf tissue of *Ficus elastica*. The trivial names ficaprenol-10, -11 and -12 are proposed. Nuclear-magnetic-resonance studies showed that each of these prenols contains three *trans* internal isoprene residues and a *cis* 'OH-terminal' isoprene residue. Ficaprenol-11 is the major component of the mixture. Chromatographic evidence suggests the presence also of small amounts of ficaprenol-9 and -13. The precise position of the three *trans* internal isoprene residues was not determined but it is suggested that these are adjacent to the ω-terminal isoprene residue and that the ficaprenols are formed from all-*trans*-geranylgeranyl pyrophosphate. It is also suggested that ficaprenol-10, -11, -12 and -13 are probably the same compounds as castaprenol-10, -11, -12 and -13.

In the preceding paper (Wellburn, Stevenson, Hemming & Morton, 1967) the characterization of the *cis–trans*-polyprenols (castaprenols) isolated from the leaves of *Aesculus hippocastanum* (the horse chestnut) was described. While this work was in progress, preliminary studies being carried out on the minor lipid constituents of leaves of *Ficus elastica* indicated the presence of a polyprenol preparation similar to that isolated from horse-chestnut leaves. The crude preparation (a mixture of polyprenols) gave infrared and nuclear-magnetic-resonance spectra which resembled those of the mixture of castaprenols (J. F. Pennock & F. W. Hemming, unpublished work).

In this paper the characterization of the individual polyprenols of leaves of a mature *Ficus elastica* tree is reported. These alcohols have been given the trivial name ficaprenols.

METHODS

Isolation and purification of ficaprenols. The leaves, of average length 11in., were obtained from a single *Ficus elastica* tree approx. 20ft. tall and grown in a greenhouse of Liverpool Corporation, Parks and Gardens section. They were washed, stripped of their main veins and were then cut into small pieces. Batches (seven × 200g.) of leaf tissue were each macerated for 2min. with acetone–diethyl ether (3:1, v/v; 400ml.) in an Ultra-Turrax homogenizer (Junke und Kunkel K.G., Staufen im Breslau, Germany). The resulting suspension was filtered through muslin. The precipitate was macerated again as before; the suspension was again filtered and the precipitate was washed with light petroleum (400ml.). The bulked filtrates were added to water (600ml.) in a separating funnel. After shaking vigorously, the mixture was allowed to separate and the lower of the 2 layers was discarded. The diethyl ether–light petroleum extract was washed well with water (five × 400 ml.) and was dried over anhydrous Na_2SO_4 before evaporating to dryness.

The bulked lipid extracts (approx. 40g.) from the seven batches of leaves were dissolved in 2% (v/v) diethyl ether in light petroleum (500ml.) and the solution was chromatographed on columns of alumina (four: 250g. of acid-washed, Brockmann grade 3; columns 15cm. × 4·8cm., fitted with a centre rod). The next eluent (20%, v/v, diethyl ether in light petroleum) eluted ficaprenols and relatively non-polar lipids from the alumina but chlorophyll and other polar lipids remained adsorbed on the alumina. This separation of ficaprenols from chlorophyll ensured that in subsequent treatment the former did not become contaminated with phytol. Infrared spectroscopy indicated that the 20% (v/v) diethyl ether in light petroleum fraction contained esters. It was therefore dissolved in benzene (500ml.) and the solution was refluxed for 30min. with a solution of KOH (15%, w/v) in ethanol–water (17:3, v/v; 500ml.). The unsaponifiable lipid was extracted with diethyl ether and weighed 24·46g.

A portion of the unsaponifiable lipid (1g.) was then chromatographed on alumina (100g., acid-washed, Brockmann grade 3; column 14cm. × 3·8cm., fitted with a centre rod). Relatively non-polar lipids were eluted from the column with 4% (v/v) diethyl ether in light petroleum (1l.). The next eluent (12%, v/v, diethyl ether in light petroleum) contained the ficaprenols contaminated principally with tocopherols and sterols. Preparative chromatography of

this fraction on thin layers of silica gel G (seven layers, 20cm. × 20cm., 700μ thick) with isopropyl ether–light petroleum (1:4, v/v) as developing solvent yielded 150mg. of ficaprenols travelling with R_F 0·3–0·4. This corresponds to a yield of 2·6g. of ficaprenols/kg. of leaf tissue. This is the highest yield of polyprenols yet reported.

Reversed-phase partition thin-layer chromatography of a small portion (20μg.) of this preparation on paraffin-impregnated kieselguhr (200μ thick, impregnated with 5%, v/v, liquid paraffin in light petroleum) with acetone–water (23:2, v/v) saturated with paraffin as mobile phase showed the ficaprenol preparation to contain ficaprenol-10, -11, -12 and -13 corresponding in R_F values to castaprenol-10, -11, -12 and -13 (Wellburn *et al.* 1967). This mixture was separated into its components by preparative reversed-phase partition thin-layer chromatography. The same system as described above and 80 chromatoplates (20cm. × 20cm.) were employed. The bands of ficaprenols on the developed plates were detected by spraying with fluorescein (e.g. see Dunphy, Kerr, Pennock & Whittle, 1966). In an attempt to isolate very pure specimens of the prenols, only the central portion of each band was removed from each plate and extracted. After removing the paraffin from each sample, the yield of ficaprenol-11 was 64mg. Rather less of ficaprenol-10 and -12 was isolated and only traces (< 1mg.) of ficaprenol-9 and -13 were recovered.

The prenols were all colourless, odourless, viscous oils at room temperature and they ran as single spots on adsorption (silica gel G, 1%, v/v, methanol in benzene, all R_F 0·38) and on reversed-phase partition (details as above) thin-layer chromatography.

Spectroscopy. Mass spectra were determined by Dr W. Vetter and P. Meyer of the physicochemical laboratories of F. Hoffmann–La Roche and Co. Ltd., Basle, Switzerland. An MS9 spectrometer (A.E.I., Manchester) was used. All other spectra were determined as described in the previous paper (Wellburn *et al.* 1967).

Gas–liquid chromatography. The derivatives of the ficaprenols were prepared and their gas–liquid chromatographic behaviour was examined as described by Wellburn & Hemming (1966*a*) (see also Wellburn *et al.* 1967).

RESULTS

Thin-layer chromatography. Reversed-phase partition thin-layer chromatography showed that the three isolated ficaprenols had R_F values 0·67, 0·53 and 0·39, which were identical with those of castaprenol-10, -11 and -12 respectively. For this reason they were termed ficaprenol-10, -11 and -12. Chromatography of the mixture of ficaprenols indicated the presence of small amounts of ficaprenol-9 (R_F 0·80) and ficaprenol-13 (R_F 0·27).

Gas–liquid chromatography. A sample of the mixture of ficaprenols was acetylated and a portion of the acetates was subjected to microhydrogenation. The saturated hydrocarbons and perhydroacetates so derived and the unsaturated acetates behaved on gas chromatography in a manner identical to corresponding derivatives of another preparation of ficaprenols described by Wellburn & Hemming (1966*a*). The retention times were as listed in Table 1 and when the logarithm of these was plotted against the number of isoprene units present in each compound, a good straight line was produced as expected for a mixture of isoprenologues (Wellburn & Hemming, 1966*a*). The derivatives of ficaprenol-10, -11, -12 and -13 had retention times very close to those of the corresponding derivatives of castaprenol-10, -11, -12 and -13 (Wellburn *et al.* 1967). The perhydroacetate of ficaprenol-9 and the derived saturated hydrocarbon had the same retention times as the corresponding derivatives of solanesol. These figures support the assigned chain lengths of each ficaprenol.

Table 1. *Retention times of derivatives of the mixture of ficaprenols on gas–liquid chromatography*

N.R., Not recorded.

Source	Retention time (min.) of derivatives			
	Saturated hydrocarbons	Perhydroacetates		Acetates
	340°	300°	340°	300°
Ficaprenol-9	0·75	6·0	1·48	*
Ficaprenol-10	1·42	13·6	2·68	10·0
Ficaprenol-11	2·81	29·7	5·44	22·4
Ficaprenol-12	5·33	62·1	9·94	N.R.
Ficaprenol-13	*	*	17·8	N.R.

* Insufficient sample was applied to record this peak.

The areas under the peaks of the chromatograms showed ficaprenol-11 to be the major component (61%) with smaller amounts of ficaprenol-12 (28%) and ficaprenol-10 (8%) and with much smaller amounts of ficaprenol-9 (2%) and ficaprenol-13 (1%). The sensitivity of the detector of the gas chromatograph to these different compounds was not determined but these figures give an approximate guide to the composition of the mixture. Essentially similar figures were obtained on the basis of weights recovered from preparative reversed-phase partition thin-layer chromatography of the mixture of ficaprenols. This was confirmed by the colour and intensity of the stain taken up by each band of material on staining such a chromatogram with anisaldehyde (e.g. see Dunphy *et al.* 1966).

Mass spectrometry. The relative intensities of the most prominent peaks in the mass spectra of the three major ficaprenols are listed in Table 2. The mass number (m/e) of the molecular ions of each alcohol (698, 766 and 834 respectively) are consistent with the proposed structures. Important confirmation is gained from the cracking patterns as indicated by the assignments in Table 2; each

Table 2. *Relative intensities and probable assignments of the most prominent peaks in the mass spectra of ficaprenol-10, -11 and -12*

m/e	Relative intensities*			Assignment†
	Ficaprenol-10	Ficaprenol-11	Ficaprenol-12	
69	N.R.	100	N.R.	$(CH_3)_2C{=}CH{-}C^+H_2$
135	N.R.	6·5	570	$^+b_2$—OH less H_2O
203	100	1·5	100	$^+b_3$—OH less H_2O
271	35	0·9	31	$^+b_4$—OH less H_2O
339	17	1·1	17	$^+b_5$—OH less H_2O
407	11	2·0	10	$^+b_6$—OH less H_2O
475	8·5	3·3	6·7	$^+b_7$—OH less H_2O
543	5·6	3·4	4·1	$^+b_8$—OH less H_2O
611	3·2	2·5	3·0	$^+b_9$—OH less H_2O
679	—	1·5	2·0	$^+b_{10}$—OH less H_2O
680	2·3	—	—	$^+H{-}[{-}CH_2{-}C(CH_3){=}CH{-}CH_2{-}]_{10}{-}$OH less H_2O
698	0·9	—	—	$^+H{-}[{-}CH_2{-}C(CH_3){=}CH{-}CH_2{-}]_{10}{-}$OH
747	—	—	1·2	$^+b_{11}$—OH less H_2O
748	—	2·4	—	$^+H[CH_2{-}C(CH_3){=}CH{-}CH_2{-}]_{11}{-}$OH less H_2O
766		1·3	—	$^+H{-}[{-}CH_2{-}C(CH_3){=}CH{-}CH_2{-}]_{11}{-}$OH
816	—	—	2·3	$^+H{-}[{-}CH_2{-}C(CH_3){=}CH{-}CH_2]_{12}{-}$OH less H_2O
834	—	—	1·1	$^+H{-}[{-}CH_2{-}C(CH_3){=}CH{-}CH_2]_{12}{-}$OH

* Ficaprenol-11: relative to 100 for *m/e* 69. Ficaprenol-10 and -12: relative to 100 for *m/e* 203.

† b=[$CH_2{-}C(CH_3){=}CH{-}CH_2$].

molecular ion first loses water to give $(M-18)^+$ and then loses the ω-terminal isoprene residue to give $(M-18\text{-}69)^+$. This is followed by the loss of further isoprene residues (units of 68) until a prominent ion at *m/e* 135 is reached. The same cracking pattern is shown by the castaprenols and by solanesol and is that expected for a polyisoprenoid alcohol (Wellburn *et al.* 1967). The relative intensities of the main peaks in the spectra of ficaprenol-10 and -12 are very similar and follow quite well the gradual changes shown in the spectra of solanesol and the castaprenols. The spectrum of ficaprenol-11 had the same prominent peaks as the other spectra but below *m/e* 543 the relative intensities of these peaks differed from those in other spectra. The reason for this is not clear: possibly the conditions prevailing when the spectrum was recorded differed in some way from the normal.

The cracking pattern of each compound gave no information regarding the distribution of or the number of *cis* and *trans* isoprene residues in the molecule.

Nuclear magnetic resonance. A photograph of the nuclear-magnetic-resonance spectrum of a solution of ficaprenol-11 in carbon tetrachloride at 100 Mcyc./sec. is reproduced in Fig. 1. Also shown on this Figure are the chemical shifts (τ) of each peak and their assignments to protons (encircled with a broken line) in different chemical environments.

The positions and assignments of resonance peaks of benzene solutions of ficaprenol-10, -11 and -12 are recorded in Table 3. The relative areas under

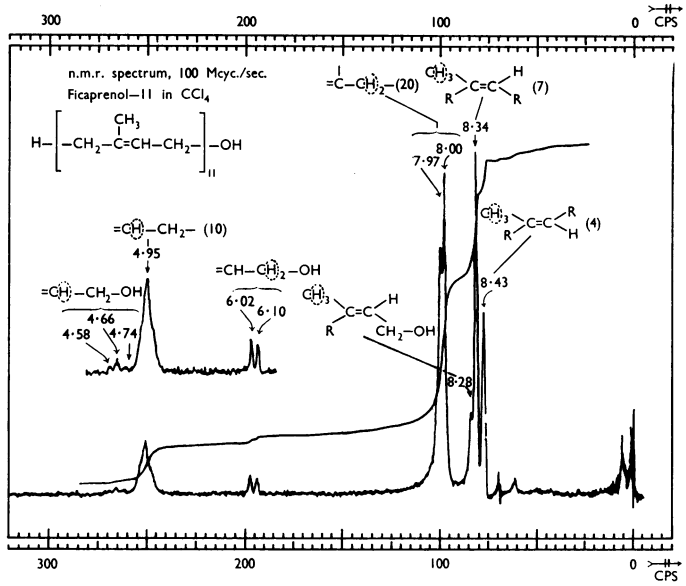

Fig. 1. Photograph of the nuclear-magnetic-resonance spectrum at 100 Mcyc./sec. of a solution of ficaprenol-11 in CCl_4. The chemical shift (in τ) has been written in above each peak and the assignments of these peaks to resonating protons (encircled with a broken line) in different chemical environments are also indicated.

Table 3. *Data for the nuclear-magnetic-resonance spectra at 100 Mcyc./sec. of solutions of ficaprenol-10, -11 and -12 in benzene*

W, Weak.

Peak position τ	Relative areas (theoretical in parentheses) Ficaprenol-10 (11·7 mg.)	Ficaprenol-11 (16·7 mg.)	Ficaprenol-12 (6·7 mg.)	Assignments*
8·43	2·9 (3)	2·9 (3)	2·8 (3)	*trans* H_3C, R, C=C, H_3C, H
8·38	12·2 (12)	11·4 (12)	11·2 (12)	*trans* H_3C, R, C=C, R, H
8·355				plus *cis* H_3C, H, C=C, R, CH_2OH
8·32	2·8 (3)	3·0 (3)	2·8 (3)	*cis* H_3C, H, C=C, H_3C, R
8·25	15·6 (15)	18·6 (18)	21 (21)	*cis* H_3C, H, C=C, R, R
7·95W 7·83	35·4 (36)	40 (40)	45 (44)	$-CH_2-C=$
6·055 5·985	1·6 (2)	1·6 (2)	2·2 (2)	$=CH-CH_2-OH$
4·73 4·52 4·59 4·66	10 (10)	10·4 (11)	13 (12)	$-C=CH-CH_2$

* Resonating protons are in italics. R, Isoprene unit.

the peaks (integrated by the instrument) are also given, together with those expected by theory. These theoretical figures are based upon each compound containing three *trans* internal isoprene residues, a *cis* 'OH-terminal' isoprene residue, and a *cis*, *trans* ω-terminal isoprene residue. It follows that ficaprenol-10, -11 and -12 will contain five, six and seven *cis* internal isoprene residues respectively. In each of the spectra of benzene solutions there is a peak at 8·355τ on the side of the larger peak at 8·38τ. The peak at 8·38τ can be assigned to protons of methyl groups of the internal *trans* isoprene residues and it is probable that the protons of the methyl group in the *cis* 'OH-terminal' isoprene residues resonate at 8·355τ. These peaks were too close to each other for their areas to be measured separately. Bearing this in mind, it is clear that there is good agreement between the measured areas and those expected from the proposed structures.

Spectra in deuterochloroform at 60Mcyc./sec. were also determined. These studies confirmed the above conclusions, giving spectra qualitatively very similar to that of ficaprenol-11 in carbon tetrachloride. The areas of peaks in the different spectra were consistent with the structures assigned to the particular prenol under investigation.

The assignments of the spectrum of a solution in carbon tetrachloride are based in part on work by Bates & Gale (1960) and, in part, on values given by Jackman (1959) and Tiers (1958). The assignments of spectra of benzene solutions are based upon unpublished work (J. Feeney and F. W. Hemming, 1966).

Infrared spectroscopy. The infrared spectra of ficaprenol-10, -11 and -12 are reproduced in Fig. 2. The only significant differences between the spectra are the intensities of the O–H stretching bands at 3575 and 3310cm.$^{-1}$ and the C–O stretching bands at 1000cm.$^{-1}$ relative to the intensities of the other bands in each spectrum. These bands get progressively less intense, relatively, as the size of the molecules increases.

The spectra of ficaprenol-11 and -12 are identical with those of castaprenol-11 and -12 (Wellburn *et al.* 1966). The strong band at 1000cm.$^{-1}$ confirms that each compound is a primary allylic alcohol and the bands at 835cm.$^{-1}$ (C–H deformation of a trisubstituted olefin), at 1660cm.$^{-1}$ (C=C stretching) and at 3024cm.$^{-1}$ (C–H stretching of =CH) together with the relative intensities of the bands at 1450cm.$^{-1}$ (C–H deformation of $-CH_2$ and $-CH_3$) and at 1365cm.$^{-1}$(C–H deformation of $-CH_3$) are in keeping with a polyisoprenoid structure (see Bellamy, 1958). It is also clear from Fig. 2 that each spectrum has a small peak at 888cm.$^{-1}$, at 1089cm.$^{-1}$, at 1130cm.$^{-1}$ (with a smaller peak at 1149cm.$^{-1}$), at 1238cm.$^{-1}$ (with a shoulder at 1221cm.$^{-1}$) and at 1307cm.$^{-1}$ (with a shoulder at 1330cm.$^{-1}$). This pattern, together with the absence of a shoulder at 795cm.$^{-1}$ on the side of the intense peak at 835cm.$^{-1}$ is consistent with each polyprenol containing more *cis* than *trans* isoprene residues (Wellburn *et al.* 1966).

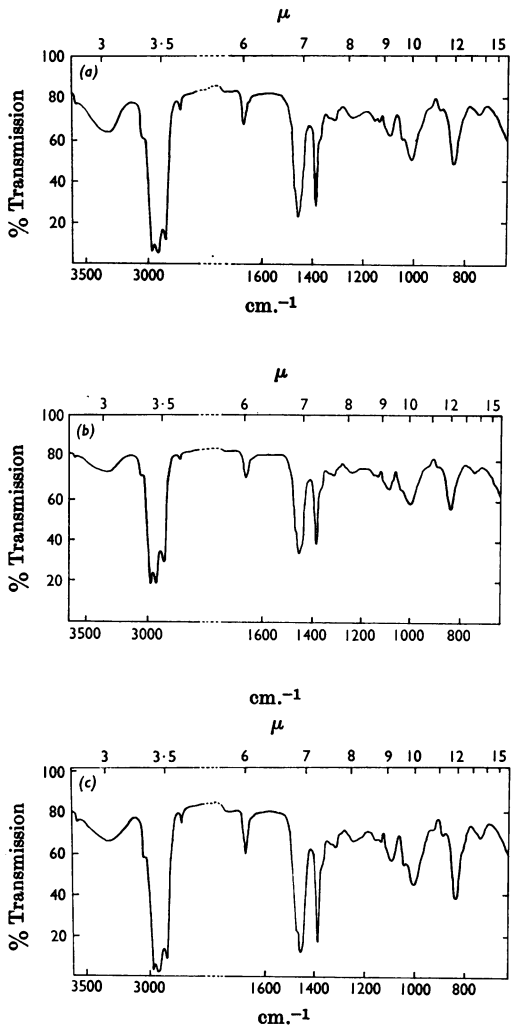

Fig. 2. Infrared-absorption spectra of fiecaprnols as solvent-free films between rock salt prisms. (*a*) Ficaprenol-10; (*b*) ficaprenol-11; (*c*) ficaprenol-12. The films of the different prenols differed in thickness.

DISCUSSION

The evidence presented in the Results section is strongly in favour of the mixture of ficaprenols containing *cis*–*trans*-decaprenol, undecaprenol and

dodecaprenol. Chromatographic evidence suggests the presence also of nonaprenol and tridecaprenol as members of the same family of compounds. The predominance of *cis* over *trans* isoprene residues in each molecule suggested by infrared spectroscopy is confirmed in a quantitative manner by nuclear magnetic resonance. Each of the alcohols contains a *cis* 'OH-terminal' isoprene residue and three *trans* internal isoprene residues. Only the number of *cis* internal isoprene residues is different in each alcohol.

By all of the experimental techniques so far applied, ficaprenol-11 and -12 have appeared identical with castaprenol-11 and -12. Also, chromatographically, ficaprenol-10 and -13 are indistinguishable from castaprenol-10 and -13. As yet, there is no direct evidence available regarding the precise location in each molecule of the internal *trans* isoprene residues. As with the castaprenols, it is tempting to suggest that all three internal *trans* isoprene residues are adjacent to the ω-terminal isoprene residue, each molecule having been formed by addition of *cis* isoprene residues to all-*trans*-geranylgeranyl pyrophosphate. This would also mean that ficaprenol-10, -11, -12 and -13 are the same compounds as castaprenol-10, -11, -12 and -13 respectively. It would also follow that the same compounds occur in the leaves of *Hevea brasiliensis* (J. F. Pennock, P. J. Dunphy & K. J. Whittle, unpublished work), *Betula verrucosa* (A. R. Wellburn & F. W. Hemming, unpublished work) and *Beta vulgaris* (F. W. Hemming & J. F. Pennock, unpublished work). Indeed, chromatographic evidence indicates that all higher plants contain these same prenols (Wellburn & Hemming, 1966*b*). Should the *cis–trans*-polyprenols of leaves of different plants prove to be the same compounds, it is suggested that the trivial name castaprenol be used to describe all of them.

While the percentage composition of the mixtures of *cis* and *trans* polyprenols varies from one source to another, the range in chain lengths of the component polyprenols consistently centres around C_{50}—C_{60}, with small amounts of C_{45} and C_{65} components often occurring. What factors decide the chain lengths of prenols in Nature is not known. An answer to this problem is relevant to all isoprenoid compounds, especially to the side chains of many physiologically active quinones, to the tocotrienols, tocopherols, the carotenoids and chlorophyll and to the many cyclic di- and tetra-terpenoids. It seems that the biosynthesis of all of these compounds is linked to that of the *cis–trans*-polyprenols through the common intermediate all-*trans*-geranylgeranyl pyrophosphate. One of the questions raised by this work is the nature of the factors that dictate the fate of geranylgeranyl pyrophosphate. What dictates whether this compound will cyclize or condense with some aromatic compound, become partially saturated or undergo chain lengthening, and, if the latter, the eventual chain length and the configuration of the residues in the polyisoprenoid chain? The diverse nature of polyisoprenoid compounds and their derivatives offers an important and interesting challenge to students of biochemical control mechanisms.

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