tissues, it follows that the ratio 3:5 obtained for ergosterol (Table 1) indicates the presence of ^3H on C-24. This same carbon atom carries an extra methyl group in ergosterol, and Lederer (1964) has considered a number of schemes for the alkylation step. These involve intermediates with the following arrangements in the side chain:

which are then converted into ergosterol carrying the methyl group on C-24 thus:

Our results deny any scheme that involves intermediates containing the groups (i) and (ii) in the side chain, for these would require the absence of ^3H on C-24 in ergosterol derived from 4*R*- ^3H -labelled mevalonate. However, intermediates of types (iii)

and (iv) are allowed, as schemes involving these do not necessarily require loss of the ^3H on C-24. This argument holds whether the compound that is alkylated is desmosterol (Lederer, 1964) or, as seems more likely, some compound closely related to lanosterol (Goad & Goodwin, 1965).

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Plastochromanol in the Leaves of *Hevea brasiliensis*

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The latex of the rubber tree, *Hevea brasiliensis*, has been examined and shown to contain α -, γ - and δ -tocotrienol in relatively large amounts (P. J. Dunphy, K. J. Whittle, J. F. Pennock & R. A. Morton, unpublished work). The toco-

trienols were present both free and esterified, γ -tocotrienol being the predominant form in both cases. Since α -tocopherol is normally the main tocopherol-type compound in green leaves, it was decided to investigate leaves of *Hevea*.

Fig. 1. Two-dimensional thin-layer chromatography of total lipid from the leaves of *Hevea brasiliensis*. Spot 1, α -tocopherol; spot 2, plastochochromanol; spot 3, γ -tocopherol. Spots 1, 2 and 3 were stained with Emmerie-Engel reagent, spots 2 and 3 with dianisidine and all others with phosphomolybdic acid (the other constituents do not concern this communication).

A supply of fresh *Hevea brasiliensis* leaves was made available by Dr E. G. Cockbain of the Natural Rubber Producers Research Association. A small sample of leaf (6g.) was macerated in acetone, and the acetone solution was diluted with water and extracted with light petroleum to yield 143mg. of lipid. Small samples of this lipid (2–7.5mg.) were chromatographed on thin layers (500 μ thickness) of silica gel by using the two-dimensional system of Pennock, Hemming & Kerr (1964). The chromatograms were sprayed with Emmerie-Engel reagent or with diazotized *o*-dianisidine. Three reducing spots were found in the tocopherol region (see Fig. 1), two of which stained with the dianisidine reagent. By adding authentic tocopherols before chromatography, it was possible to show that two of the reducing compounds corresponded to α - and γ -tocopherol, the latter staining with the dianisidine reagent.

The third spot did not correspond to any known tocopherol and it was decided to isolate a small amount for spectrophotometric analysis. The lipid from 50g. of leaves was extracted by the method of Folch, Ascoli, Lees, Meath & LeBaron (1951) to yield 1.895g., which was chromatographed on 150g. of Brockmann grade III alumina, and fractions eluted by increasing concentrations of diethyl ether (E) in light petroleum (P) were collected. Plastoquinone was eluted by 2% and 4% E/P whereas α -

and γ -tocopherol, together with the unidentified reducing compound, were found in the fractions eluted by 4% and 6% E/P. A small amount of δ -tocopherol (which had not been detected by the two-dimensional system) was found in the 10% E/P fraction, and α -tocopherolquinone was present in the 25% E/P fraction. The 6% E/P fraction (45.9mg.) was fractionated on preparative thin-layer chromatograms, and after development with 20% (v/v) isopropyl ether in light petroleum the chromatograms were sprayed with 0.01% fluorescein in ethanol, and the u.v.-quenching band corresponding to the unknown was cut out and the material eluted with diethyl ether. This fraction weighed 9.1mg.

The sample showed ultraviolet absorption maxima in cyclohexane at 294 and 300.5 $m\mu$ (of equal intensities) with a minimum at 257 $m\mu$, and after further purification on ether-extracted silicic acid thin-layer chromatograms the $E_{1\text{cm}}^{1\%}$ at 294 $m\mu$ was about 55. Thus the ultraviolet spectrum was qualitatively identical with that of γ -tocopherol or γ -tocotrienol but the $E_{1\text{cm}}^{1\%}$ value at 294 $m\mu$ was much lower (the value for γ -tocopherol is 95). The infrared spectrum was very similar to that of γ -tocotrienol but there were some differences in the relative intensities of the peaks. The unknown also reduced FeCl_3 , stained with dianisidine as does γ -tocotrienol, and its position on two-dimensional thin-layer chromatograms together with the low $E_{1\text{cm}}^{1\%}$ value suggested a longer-chain isoprenologue of γ -tocotrienol. Such a compound might be a cyclic compound related to plastoquinone. If the cyclic compound is called plastochochromanol-8 (related to plastoquinone-9) then γ -tocotrienol could be called plastochochromanol-3.

The conversion of plastoquinone into plastochochromanol was carried out by the method of Schudel, Mayer, Metzger, Rüegg & Isler (1963) that was used for the preparation of α - and β -tocotrienol. Plastoquinone (600mg.) was refluxed for 3hr. in freshly redistilled pyridine (10ml.), the mixture was diluted with saturated NaCl solution and extracted with light petroleum. After chromatography on alumina a product (440mg.) was obtained that had absorption maxima in cyclohexane at 234, 268 ($E_{1\text{cm}}^{1\%}$ 75) and 333 $m\mu$ with a shoulder at 276 $m\mu$. The spectrum of solanachromene (plastochochromenol) reported by Rowland (1958) had the same maxima with $E_{1\text{cm}}^{1\%}$ 76 at 267 $m\mu$. The plastochochromenol (400mg.) was refluxed with sodium in ethanol for 3hr. in a nitrogen atmosphere and, after extraction, chromatography on alumina yielded fractions eluted by 4% and 6% E/P that contained plastochochromanol (approx. 270mg.). The 6% E/P fraction (197mg.) gave only one spot on thin-layer chromatography and had λ_{max} 294 and 300.5 $m\mu$ with a minimum at 257 $m\mu$. The $E_{1\text{cm}}^{1\%}$ at 294 $m\mu$ was 56.

Plastoquinone-9

Plastochromanol-8

Examination of the infrared spectrum of the synthetic plastochromanol showed it to be identical with that of the unknown fraction from *Hevea* leaves. The main absorption bands were 3440 (O-H), 2855 and 2928 (C-H), 1660 (unconjugated C=C), 1612 and 1452 (aromatic), 1452 and 1384 (C-CH₃ and C-CH₂), 1224 and 1080 (chroman ring) and 840 cm.⁻¹ (trisubstituted ethylene). The two compounds were inseparable on thin-layer chromatography with chloroform (*R_F* 0.58) or 20% isopropyl ether in light petroleum (*R_F* 0.56), and on reversed-phase (paraffin) thin-layer chromatography with 90% acetone in water (*R_F* 0.27). On reversed-phase paper chromatography (paraffin as stationary phase) the compounds had *R_F* 0.64 with 95% acetone in water as solvent and *R_F* 0.59 with 3% water in dimethylformamide as solvent. The two compounds gave the same slate-blue colour with dianisidine. It is clear that the unknown compound from *Hevea brasiliensis* leaves is plastochromanol-8.

The concentrations of several isoprenoid compounds in the leaves were estimated on chromatographic fractions, or with plastochromanol and the

tocopherols, by Emmerie-Engel reaction after two-dimensional thin-layer chromatography of the total lipid. The following values (per g. wet wt. of leaf) were found; plastoquinone, 195 µg.; β-carotene, 177 µg.; α-tocopherolquinone, 23 µg.; α-tocopherol, 115 µg.; γ-tocopherol, 26 µg.; plastochromanol, 318 µg. The plastochromanol was estimated by the Emmerie-Engel method by using a conversion factor of 162 calculated from the conversion factor for γ-tocopherol (90).

The existence of plastochromanol in Nature has not been described previously. Earlier work on the leaves of *Arum maculatum* by F. W. Hemming & A. R. Wellburn of this Department (unpublished work) had shown an unknown compound that reduced FeCl₃, stained with dianisidine and had an ultraviolet spectrum similar to that of the tocopherols. From its position on thin-layer chromatograms it is clear that this compound must be very similar to, or identical with, plastochromanol.

It was found possible to oxidize plastochromanol with AuCl₃ by the method of Scudi & Buhs (1942). Two products were obtained both showing absorption maxima at 257 mµ with shoulders at 262 mµ and both could be reduced with NaBH₄. They could be separated by thin-layer chromatography, the *R_F* values being 0.42 and 0.68 with 15% ethyl acetate in benzene as solvent.

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Studies on Autobiotics: Retine in Human Urine

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Workers in this Laboratory have been engaged in the isolation of an as yet unknown biological substance, first observed in thymus extracts (Szent-

Györgyi, Hegyeli & McLaughlin, 1962), that showed an inhibitory action on transplanted malignant growth in mice. This substance has been called