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 Earlier work on the leaves of *Arum maculatum* by F. W. Hemming & A. R. Wellburn of this Department (unpublished work) had shown an unknown compound that reduced FeCl₃, stained with dianisidine and had an ultraviolet spectrum similar to that of the tocopherols.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.

We thank Dr O. Isler, F. Hoffmann-La Roche, for a gift of plastoquinone and Dr D. R. Threlfall for a gift of plastochromanol (solanachromene). We also thank Professor R. A. Morton for his continuous interest in the work. K. J. W. and P. J. D. were in receipt of Research Scholarships from the Department of Scientific and Industrial Research.

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Biochem. J. (1965) **96**, 19c

Studies on Autobiotics: Retine in Human Urine

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(Received 26 May 1965)

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

'retine', and its biological antagonist 'promine'. Later investigations indicated that the same biological activity could be demonstrated in various animal tissues and in human urine (Szent-Györgyi, Hegyeli & McLaughlin, 1963). These results indicated that cells contain highly active substances which regulate their division. Since these appeared to be closely related substances, produced by cells, and acting on the producing cells themselves, Szent-Györgyi (1963) suggested the term 'autobiotics' (by analogy with antibiotics).

The present communication shows that human urine offers advantages for the isolation of the growth inhibitor. Results on the growth-inhibiting properties of various crude precipitates of urine are presented.

Freshly collected human urine from males of the age group 20–25 years was concentrated on a continuous film evaporator at 45° *in vacuo*. As the urine is concentrated, a precipitate is formed. The precipitates formed between 0- and 10-fold and between 10- and 100-fold concentrated urine were collected by filtration, washed with cold water and dried by suction.

The biological activity of the precipitates was tested by an assay method described by Szent-Györgyi *et al.* (1963). For biological assays the precipitates were suspended in water and the suspension was injected into tumour-bearing mice. The same suspensions were tested for toxicity on normal mice. No general toxic effects were observed on daily injection of 120mg./animal/day, administered in a volume of 0.25 ml., for 7 days. This dosage is twice that used for the study of tumour inhibition. However, mild local irritation at the site of the subcutaneous injection was noticed. The filtrate of the 100-fold concentration was toxic. The toxicity could be eliminated by adding methanol (6 vol.) and discarding the precipitate formed. To the methanol filtrate acetone (17%, v/v) was added and the precipitate again discarded. The clear filtrate was concentrated 10-fold *in vacuo* and kept on ice overnight. An amorphous deposit formed, and this was collected, washed with cold methanol and dried. This solid was soluble in water and was tested for inhibition of tumour growth.

The activity of the precipitates is shown in Table 1, expressed in retine units, 1 unit being equivalent to the quantity that retarded the growth of transplanted Krebs II tumours by 50%. These values do not necessarily correspond to the actual retine content, since, if promine is present, it may have compensated for part of the retine action (Szent-Györgyi *et al.* 1962). Thus the retine activity observed indicates the imbalance of retine and promine rather than the absolute quantity of retine.

As Table 1 shows, human urine is a favourable starting material for retine preparations; 100l. of

Table 1. *Retine activity of various precipitates formed on concentration of urine*

Details are given in the text. The total recovery was 8460 retine units from 100l. of urine.

	Crude ppt. of 10-fold concn.	Crude ppt. of 100-fold concn.	Filtrate of 100-fold concn.
Yield (retine units in 100l. of urine)	2170	3950	2340
Vol. of urine containing 1 retine unit (ml.)	46.2	25.3	42.8
Percentage distribution of total units	25	47.5	27.5

urine contains 8000–9000 units of retine, from which 2000–3000 units (about 25%) are in the precipitate formed between 0- and 10-fold concentration. On further concentration to 100-fold, 4000–5000 units (about 50%) are in the precipitate. The isolated solid from the supernatant of the latter contains 2500–3000 units (about 27%) of retine. It showed an ultraviolet absorption with maximum at 272m μ in water at pH 7.0. The absorption maximum was pH-dependent. In acidic solution (pH 1.0) the peak shifted to 270m μ , but in alkaline solution (pH 10.0) it appeared at 292m μ . The peak at 272m μ is often shown by aliphatic ketones (Ferguson, 1963), and the shift may be due to keto–enol tautomerization. That retine contains a ketonic group was indicated earlier by A. Hegyeli's (personal communication) preparations.

The yield is higher, by about a factor of 8–10-fold, than that obtained by a different method by Hegyeli, McLaughlin & Szent-Györgyi (1963).

The precipitates obtained on concentration of urine can be stored at 2° without loss of activity. They are thus a favourable material for large-scale work.

I am grateful to the Abbott Laboratories, Chicago, Ill., U.S.A., and especially to Dr William M. Mozen, who arranged the large-scale concentration of urine. I thank Dr Albert Szent-Györgyi for his interest in the work. This research was supported by a grant (GM-10383) from the National Institutes of Health, U.S. Public Health Service.

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