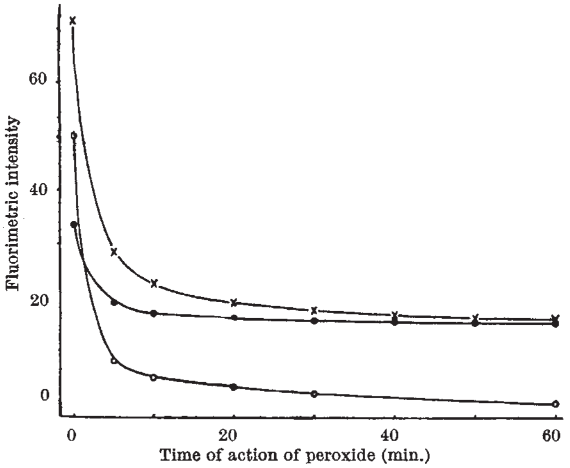


Destruction of the fluorescence of œstrogens by 20-vol. hydrogen peroxide: ○—○—, œstrone; ●—●—, urinary extract; ×—×—, urinary extract and œstrone

of the fluorescence. This fact is due to the oxidization of substances producing in the solution an absorption of the non-specific fluorescence.

The procedure outlined can be carried out with most of the fluorimetric methods described in the literature. However, with Engel's method[1] we observed the development of a brown colour after the addition of hydrogen peroxide, which made measurement impossible.

Consequently, it seems that the subtraction of the residual fluorescence of urinary solutions after action for 1 hour of 5 per cent by volume of 20 per cent hydrogen peroxide, from the initial fluorimetric intensity figures, gives more specific values in the estimation of urinary œstrogens. This procedure, very suitable for routine determinations, must be considered more as an approach than as a definitive answer to the problem of the specific fluorimetric estimation of urinary œstrogens. The method previously described[3], when modified as described here, gives, during the menstrual cycle, elimination-rates for œstrone that correspond even better with the values given by biological tests.

C. Heusghem
(Associate of the Belgian F.N.R.S.)
Chemical Research Department,
University of Liège.
Jan. 15.

[1] Engel, L. L., Slaunwhite, W. R., Carter, P., and Nathanson, I. T., *J. Biol. Chem.*, **185**, 255 (1950).

[2] Swyer, G. I. M., and Braunsberg, H., *Proc. Soc. Endocrin.*, **7**, 60 (1951).

[3] Heusghem, C., *Annales d'Endocrin.*, **13**, No. 3, 479 (1952).

[4] Kober, S., *Biochem. Z.*, **239**, 209 (1931).

[5] Marrian, G. F., *Biochem. J.*, **24**, 435 (1930).

[6] Veldhuis, A. H., *J. Biol. Chem.*, **202**, 107 (1953).

[7] Bates, R. W., and Cohen, H., *Endocrin.*, **47**, 166 and 182 (1950).

Action of Ionizing Radiation on Aqueous Solutions of Carbohydrates

Very little work has been reported in the literature on the effects of ionizing radiations on aqueous solutions of carbohydrates[1,2]. Attention appears to have been focused on some of the physical changes observable during irradiation, for example, *p*H, optical rotation and reducing power, and no study has been made of the products formed in these reactions. Because of the wide occurrence of carbohydrates in Nature and their important biological functions, a study of their decomposition by ionizing radiations was undertaken, and a summary of the results is presented here.

Three naturally occurring hexoses, D-glucose, D-galactose and D-mannose, were irradiated in dilute aqueous solution ($\leqslant 5 \times 10^{-2}\,M$) with 1-MV. electrons from the Chemistry Division Van de Graaff generator at Harwell. Contrary to expectations, the radiation produced highly specific changes in the molecule. From the hexoses, the only product formed was the corresponding uronic acid, attack by the radicals formed being confined to the primary alcohol group at C6 in the sugar. The yield of uronic acid was independent of the initial hexose concentration within the range $5 \times 10^{-2}\,M$ to $5 \times 10^{-5}\,M$, providing adequate demonstration of the 'indirect' nature of the radiation action. The energy of the electron beam is absorbed almost exclusively by the water, and the chemical change is due to the secondary effect of the radicals produced during the radiolysis of the water. There is exact coincidence between the behaviour of the three hexoses, the G value (yield of uronic acid in molecules/100 eV. energy input) being the same in each case and equal to 0·4 for evacuated solutions. Under these conditions, there is also a continuous evolution of gas during irradiation, the pressure rise above the liquid surface showing a linear dependence on irradiation dose. The gas was mainly hydrogen (87 per cent), carbon dioxide (10 per cent) and a small amount of carbon monoxide.

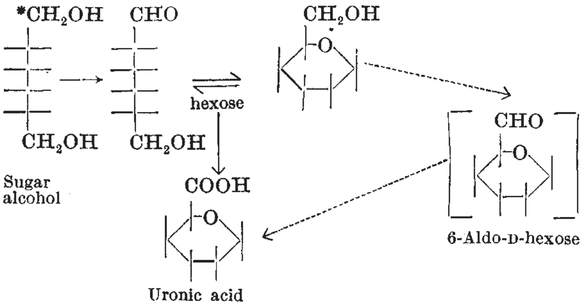
The presence of oxygen had a marked enhancing effect on the rate of production of uronic acid, and under such conditions hydrogen peroxide becomes one of the major products. In an oxygenated solution, the uronic acid and hydrogen peroxide are, in fact, produced in equimolecular proportions ($G = 1{\cdot}9$). It was observed both for evacuated and oxygenated solutions that a slow reaction continued after the irradiation had ceased. This slow post-irradiation process can be identified with a slow thermal reaction which is possible between uronic acids and hydrogen peroxide (cf. Everett and Sheppard[3]).

Reactions very similar in form to the production of uronic acids from hexoses have been encountered outside the carbohydrate field. For example, Weiss[4] found that when an aqueous solution of lactic acid is irradiated with 200-kV. X-rays, pyruvic acid is produced; ethyl alcohol under similar conditions gives acetaldehyde[5,6]. The general form of this series of reactions appears then to be:

$$R.\mathrm{CH_2OH} \rightsquigarrow R.\mathrm{CHO}.$$

It would be extremely difficult to obtain direct evidence of this step from the irradiation of hexose solutions, because under these conditions the intermediate D-hexodialdoses are unstable. The sugar alcohols, however, present a better comparison because the aldehyde, if formed, would be stable, being in fact an aldose sugar. For this reason the irradiation of aqueous D-mannitol solutions with 1-MV. electrons was studied. The product from this reaction is D-mannose, and this is not converted into mannuronic acid until quite appreciable concentrations have been built up in the solution. This provides a highly specific and efficient method of preparing aldose sugars from sugar alcohols. Oxygen again has a marked effect on the reaction, but here it was interesting to observe that the stoichiometric balance between hydrogen peroxide and mannose is different, the amount of hydrogen peroxide being double that of mannose [G(mannose) = 1·5; G(hydrogen peroxide) = 3·0].

Production of mannose from mannitol gives strong support to the view that uronic acids are produced from the hexoses by way of the intermediate aldehyde, 6-aldo-D-hexose. The complete reaction may then be represented as follows:



*Configuration according to sugar

Through the kindness of Dr. M. Ebert and his colleagues at the Medical Research Council Radiotherapeutic Research Unit, Hammersmith Hospital, it was possible to study the behaviour of aqueous mannitol solutions when irradiated with 190-kV. X-rays and obtain a comparison with the electron irradiations described above. The results were of interest in that the G value for mannose formation was about 25 per cent higher than the electron value. Dosimetry of the X-rays was with ferrous sulphate solutions, a value of G(ferric iron) = 15·5 being used in the calculations. Using G(ferric iron) = 19·9, the divergence would, of course, increase still further. Further work on a comparison between X-rays and electrons is in progress; the difference due to the changed quality of radiation must obviously be regarded as quite significant.

The results will be presented in full and the detailed mechanism of the reactions considered elsewhere.

G. O. PHILLIPS
Atomic Energy Research Establishment,
Harwell. Feb. 9.

---

[1] Holtz and Becker, *Arch. Exp. Path. Pharmakol.*, **182**, 160 (1936).

[2] Slosse, *C.R. Soc. Biol.*, **89**, 96 (1923).

[3] Everett and Sheppard, Univ. Oklahoma Med. School, Dept. Biochem. (1944).

[4] Johnson, Scholes and Weiss, *J. Chem. Soc.*, 3094 (1953).

[5] Weiss (private communication).

[6] Swallow, *Biochem. J.*, **54**, 253 (1953).

Spectroscopic Examination of Punicic Acid

PUNICIC acid was first isolated from pomegranate (*Punica granatum*) seed oil by Toyama and Tsuchiya[1]. This fact was later confirmed by a re-investigation of the oil by Toyama and Hozaki[2] and also by other workers[3].

Following the results of ozonolysis and identification of the decomposition products, punicic acid has been accorded a 9 : 11 : 13 octadecatrienoic structure. However, no identity has been attached to the geometric configuration of the unsaturation other than to indicate that it is presumably a stereoisomer of elaeostearic acid which itself is known to exist in the α- and β-forms. It is therefore of interest to attempt to establish the true configuration of the double bonds present.

Absorption spectroscopy is ideally suited to the determination of *cis* and *trans* isomers, and a previous publication[4] has been concerned with the identification of the orientation of some C_{18} unsaturated fatty acids. These included both α- and β-elæostearic acids, which have been accorded conjugated *cis–trans–trans* and *trans–trans–trans* configurations respectively. A further publication[5] dealt with the spectroscopic examination of pomegranate seed oil; but at the time no sample of punicic acid was available and it was only possible to postulate the *cis–trans* orientation of the component acid from measurements on the absorption spectrum of the oil. Since that time a sample of punicic acid has been prepared, and the present communication is concerned with its ultra-violet and infra-red absorption spectra and their interpretation in terms of the corresponding data recorded for the oil.

Dried and crushed pomegranate seeds were placed in a Soxhlet apparatus and extracted with 40–60 petroleum ether for several hours, following which the solvent was distilled off under nitrogen and the last traces removed by blowing nitrogen through the oil at 100° C. In this manner, about a 15 per cent yield of oil was obtained calculated on the weight of seed taken. About 5 gm. of the oil was then saponified by refluxing with 2 gm. potassium hydroxide dissolved in 2 ml. water and 18 ml. ethyl alcohol for two hours under nitrogen. The mixture was diluted with water, acidified with hydrochloric acid and the fatty acids extracted with ether. This extract was then washed with water until neutral, dried over anhydrous sodium sulphate and the ether removed. The fatty acids thus obtained were recrystallized three times from methanol, the final product being a white crystalline solid, melting point 43·5–44·5° C. The latter figures are in agreement with published data.

The ultra-violet spectrum of the acid has been recorded over the wave-length range 220–300 mμ in cyclohexane solution with a Beckman spectrophotometer[6]. Three sharp bands are noted in the 270-mμ region characteristic of the conjugated triene system, and the wave-lengths are in good agreement with those reported for the oil. A comparison of the wave-lengths and specific extinction coefficients (k) of the principal bands for both acid and oil are noted below:

Punicic acid	265·5 mμ	275·0 mμ (k = 171·9)	287·0 mμ
Pomegranate seed oil	265·0 mμ	274·5 mμ (k = 122·1)	285·0 mμ

The infra-red spectrum of the acid has been recorded over the wave-length range 2·5–15 μ in carbon tetrachloride and carbon disulphide solutions as appropriate with a Perkin–Elmer spectrometer[7]. In addition, quantitative measurements have been made on the absorption bands in the 10-μ region characteristic of the *cis–trans* vibrations. The absorption characteristics for the acid and those for the oil previously reported are noted below:

Punicic acid	10·67 μ (k = 0·700)	—	10·12 μ (k = 0·632)
Pomegranate seed oil	10·67 μ (k = 0·366)	10·36 μ (k = 0·159)	10·12 μ (k = 0·611)

The absence of an absorption band at 10·36 μ, in the spectrum of the acid, which is known to arise from the presence of non-conjugated *trans* unsaturation in the oil, corroborates the purity of the acid.

The difference in the specific extinction coefficients of the principal absorption bands at 10·12 μ arises from the necessarily lower punicic acid character of