Reaction of β -Naphthol with l - α - and l - β -Phellandrene

SOME years ago, Goodway and West¹ reported that low yields of the maleic anhydride adduct corresponding to α -phellandrene were obtainable from β -phellandrene, and it was considered probable that the l - α -phellandrene adduct isolated was derived by the thermal decomposition of the primary resinous material obtained from the l - β -phellandrene.

In attempts to obtain another derivative characteristic for β -phellandrene, we have studied the reaction of this terpene with β -naphthol and obtained a product which melted at 137–138°, whereas Salfeld² obtained a product melting at 139–140° from a sample of α -phellandrene of which the constants were not recorded. The l - β -phellandrene utilized in our experiments was derived from Canada balsam oil which has been shown to be free from the α -isomeride³, and the l - α -phellandrene used for comparison was prepared by fractionation from the oil of *Eucalyptus dives*. From the melting point, mixed melting point, and optical rotation of the reaction products of the two terpenes with β -naphthol, the adducts appear to be identical. Further evidence of the identity and structure of the adducts is being sought, and in view of the fact that the infra-red absorption spectra⁴ of α - and β -phellandrene offer the best possibility of distinguishing readily between the two terpenes, it is hoped that it may be possible to establish the mechanism of the reaction of the β -isomeride with β -naphthol.

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¹ Goodway, N. F., and West, T. F., *Nature*, **140**, 934 (1937); *J. Chem. Soc.*, 2028 (1938).

² Salfeld, J. C., *Ber.*, **73**, 376 (1940).

³ Macbeth, A. K., Smith, G. E., and West, T. F., *J. Chem. Soc.*, 119 (1938). Goodway, N. F., and West, T. F., *J. Soc. Chem. Ind.*, 56, 472T (1937); 57, 37 (1938).

⁴ Acheson, G. G., and West, T. F. (unpublished observation).

Inhibition of Hexosediphosphatase by Sulphydryl Reagents and by Ascorbic Acid

DURING the course of an investigation of the action of vitamin K and its biological antagonists (dicoumarol, salicylate) on various phosphatases, details of which will be published elsewhere, it was observed that liver hexosediphosphatase¹ exhibited a much greater sensitivity towards quinones than did the other phosphatases. Purified bone phosphomonoesterase², for example, required a concentration of 10^{-3} M benzoquinone for complete inactivation, whereas 10^{-5} M benzoquinone was sufficient completely to inhibit the hexosediphosphatase.

It is well known that enzymes the activity of which is dependent on free —SH groups are inhibited by quinones in very low concentrations, and it has been suggested that the antibiotic activity of quinones is a result of this effect³. With the exception of some pyrophosphatases and transphosphorylases, the phosphatases, the activity of which is probably dependent on free amino-groups⁴, are, on the other hand, not affected by —SH reagents⁵. The comparatively high quinone sensitivity of hexosediphosphatase, however,

led us to examine the behaviour of this enzyme towards other substances which are known to react with thiol groups.

Our experiments show that hexosediphosphatase is inhibited by iodoacetate, by phenyl mercuric nitrate and by alloxan. Cysteine, which itself inhibits in sufficiently high concentration but strongly activates at lower concentrations, completely reverses the iodoacetate and phenyl mercuric nitrate inhibitions and partly reverses that by alloxan. Thus inhibition by iodoacetate (10^{-2} M) or by alloxan (2.5×10^{-3} M) is 40 per cent. Cysteine (10^{-3} M) reactivates the iodoacetate-inhibited enzyme 100 per cent and the alloxan-inhibited enzyme 37 per cent (that is, inhibition is reduced from 40 to 25 per cent). Inhibition by alloxan (5×10^{-3} M) is complete and there is no reactivation by cysteine. Phenyl mercuric nitrate (10^{-4} M) inhibits 100 per cent, with complete reactivation by cysteine (2.5×10^{-4} M). Cysteine (5×10^{-4} M and 1×10^{-3} M) alone activates 98 and 96 per cent respectively, while an increase in concentration to 5×10^{-3} M results in complete inactivation.

The hexosediphosphatase used was prepared as described by Gomori¹ and was free from non-specific phosphatase activity. Mixtures (10.0 ml.) of the enzyme solution (0.1 ml.), borate buffer (2.0 ml.), 0.05 M magnesium sulphate (2.0 ml.), sodium hexosediphosphate solution (2.0 ml. $\equiv$ 0.2 mgm. phosphorus) and inhibitor were incubated for 30 min. at 37° and pH 9.1 in separate tubes, and the inorganic phosphate in each was determined colorimetrically. The quantity of enzyme used was such that half the substrate was hydrolysed in the controls (that is, no inhibitor) during the period of incubation. No phosphate was liberated in the absence of enzyme. When iodoacetate was used, it was incubated with the diluted enzyme-magnesium-buffer mixture for 15 min. before addition of the substrate and of cysteine.

Experiments with ascorbic acid, which was expected to activate the enzyme by virtue of its reducing properties, showed it to be a very powerful inhibitor. Concentrations below 5×10^{-7} M resulted in slight activation (up to 5 per cent), but concentrations greater than 10^{-6} M inhibited, with complete inactivation in the presence of 2×10^{-3} M ascorbic acid. This inactivation by ascorbic acid is undoubtedly due to competition with the hexosediphosphate, as indicated by the results of experiments with varied substrate concentrations. Results of a typical experiment are given in the accompanying table.

Ascorbic acid, molar concentration $\times 10^4$	Substrate solution (ml.)	Inorganic phosphorus (μ gm.)	Inhibition (per cent)
0	1.5	573	0
2.5	1.5	371	35
5	1.5	317	45
5	3.0	245	32
10	3.0	200	45
0	3.0	360	0

These results also confirm Gomori's observation that the substrate itself inhibits the enzyme as the concentration is raised and, for this reason, the amount of enzyme solution used was increased to 0.15 ml. in the above experiment.

Inhibition of β -malt amylase by ascorbic acid and related compounds was attributed by Hanes⁶ to the presence in the molecule of the di-enol grouping. Giri⁷ has shown that several plant and animal phosphatases, which were not affected by ascorbic acid, were inhibited by an ascorbic acid-copper complex. Our experiments demonstrate that ascorbic acid may