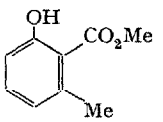
selected ethanol as standard since the effects of intramolecular chelation are minimised in this medium for the particular absorption band under review[7].

We illustrate the use and scope of the method by some examples of varying complexity.

Example 1. Methyl 6-methylsalicylate (I)

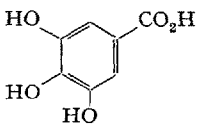


(I)

$\lambda^{*8}_{calc} = 230 + 7(ortho\text{-OH}) + 3(ortho\text{-Alk}) = 240$ mμ

$\lambda^{*}_{obs} = 244$ mμ (ε 10000)[9]

Example 2. Gallic acid (II)

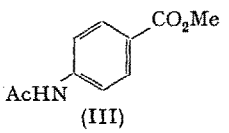


(II)

$\lambda^{*}_{calc} = 230 + 2\times7(meta\text{-OH's}) + 25(para\text{-OH}) = 269$ mμ

$\lambda^{*}_{obs} = 270$ mμ (ε 8000)[10]

Example 3. p-Acetylamino methyl benzoate (III)

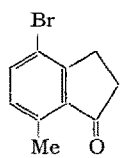


(III)

$\lambda^{*}_{calc} = 275$ mμ

$\lambda^{*}_{obs} = 274$ mμ (ε 15000)[11]

Example 4. 4-Bromo-7-methylindanone (IV)

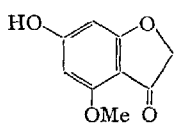


(IV)

$\lambda^{*}_{calc} = 246 + 3(ortho\text{-Me}) + 3(\text{ortho-ring residue}) + 2(meta\text{-Br}) = 254$ mμ

$\lambda^{*}_{obs} = 254$ mμ (ε 10000)[12]

Example 5. 6-Hydroxy-4-methoxycoumaranone (V)

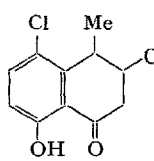


(V)

$\lambda^{*}_{calc} = 246 + 14(2\times ortho\text{-OR}) + 25(para\text{-OH}) = 285$ mμ

$\lambda^{*}_{obs} = 284$ mμ (ε 22500)[13]

Example 6. Ethyl 4-methyl-5-chloro-8-hydroxy-tetralone-3-carboxylate (VI)

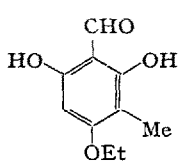


(VI)

$\lambda^{*}_{calc} = 256$ mμ

$\lambda^{*}_{obs} = 257$ mμ (ε 8000)[14]

Example 7. 2,6-Dihydroxy-3-methyl-4-ethoxybenzaldehyde (VII)



(VII)

$\lambda^{*}_{calc} = 292$ mμ

$\lambda^{*}_{obs} = 295$ mμ (ε 21950)[15]

Extension to benzonitrile by using the basic value of 224 mμ for this system reveals that the increments in Table II may be transposed to certain other series, e.g. p-aminobenzonitrile has λ^{*}_{calc} 279 mμ and absorbs at 277.5 mμ (ε 24000)[16]. Complete details of this survey will appear elsewhere.

Zusammenfassung. Eine allgemeine Methode für die Berechnung der Hauptabsorptionsbande benzolischer Carbonylverbindungen wird zur Diskussion gestellt.

A. I. Scott

Chemistry Department, The University, Glasgow (Great Britain), October 17, 1960.

[7] Correlation involving the *less intense* E.T. band of the *ortho*-disubstituted series on changing from hydrocarbon to hydroxylic solvent have already provided useful information in natural product studies [e.g. L. H. Conover, Chem. Soc. Special Publications *5*, 48 (1956). – R. A. Morton and A. L. Stubbs, J. chem. Soc. *1940*, 1347].

[8] $\lambda^{*} = \lambda_{max}$ in EtOH.

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Inhibition of Tyrosinase and Uricase Activity by Ultraviolet Radiation

Recent studies from this laboratory have shown that ultraviolet (UV) radiation at 2537 Å was capable of destroying both the *in vitro* oxidase activity of serum as well as the oxidative activity of the copper protein ceruloplasmin[1,2]. The latter is the component of serum thought to be responsible for the oxidase activity. Furthermore, the logarithm of the relative enzyme activity was found to be proportional to the incident energy of the UV radiation within the range studied. It was also found that as the oxidase activity fell with increasing dose of UV radiation, the bound copper content[3,4] of the ceruloplasmin and serum samples decreased while the direct-reading copper content[4] of the samples increased. Several other copper enzymes were studied to see a) what, if any, is the effect of UV irradiation on them and b) if there was an effect on the activity, did a relationship exist between loss of enzymatic activity and 'bound' copper content of the samples. The three copper enzymes chosen for study were uricase, plant tyrosinase and mammalian tyrosinase.

Plant tyrosinase used in this study was a Worthington lyophilized preparation from mushrooms. Uricase was the Worthington powder preparation. Mammalian tyrosinase was prepared as a crude extract[5] from Harding-Passey

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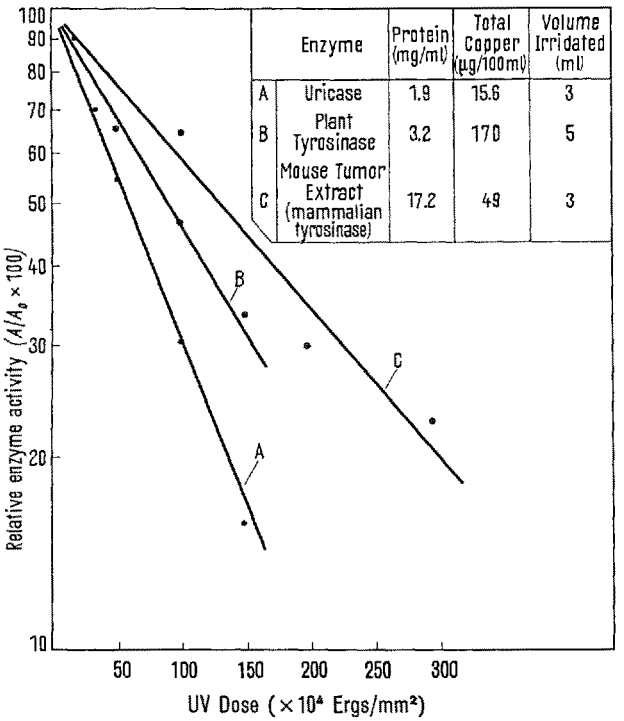

Fig. 1. Effect of UV light (2537 Å) upon the activity of uricase, plant tyrosinase and mouse melanoma extract (mammalian tyrosinase) exposed to irradiation at 0°C. Ordinate: Relative activity ($A\% = A/A_0$); abscissa: UV dose expressed as ergs/mm².

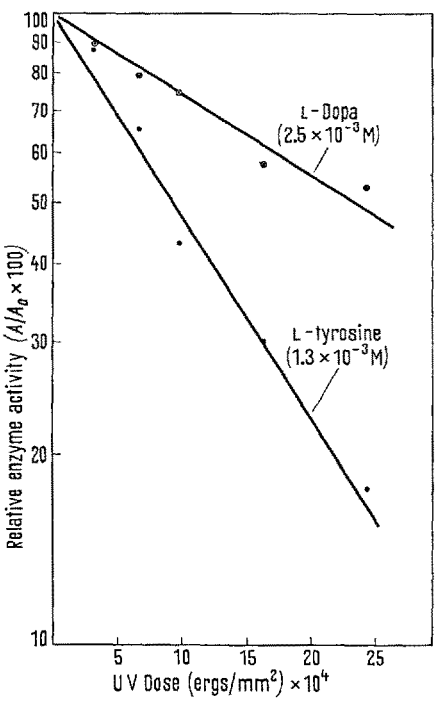

Fig. 2. Effect of UV light (2537 Å) on the activity of plant tyrosinase as assayed against L-DOPA and L-tyrosine. Optimum substrates concentrations were employed. Coordinates are the same as in Fig. 1.

mouse melanomas grown in BALB/c strain mice obtained from the Roscoe B. Jackson Memorial Laboratory.

Tyrosinase was assayed against L-tyrosine and L-dihydroxyphenylalanine (DOPA), while uric acid was the substrate for uricase. All assays were carried out at constant temperature (37°C) in a Beckman Spectrophotometer equipped with thermospacers, following the methods outlined by WORTHINGTON[6] with slight modifications. These involved running the assays at conditions of optimum substrate and pH. Tyrosinasè oxidizes its substrates to an o-quinone which can be measured by an increase in optical density at 280 mμ. Uricase converts uric acid to allantoin with a resulting decrease in optical density at 290 mμ, a wavelength at which allantoin shows no absorption.

The enzyme preparations were irradiated in a Petri dish 5 cm in diameter and set in a stainless steel water bath. Ice water at 0°C and not varying more than 0.1°C was continually circulating through the water bath. After temperature equilibration was established, the sample was exposed to the UV radiation for a definite length of time. The UV source was a Mineralight ultraviolet lamp, Model V-41 (Ultraviolet Products, San Gabriel, California), and was mounted above the enzyme preparations in such a way that it could be moved in the vertical or horizontal direction. The intensity of radiation striking the solution was $(1.63 \pm 0.09) \times 10^4$ ergs/mm² as determined by the uranyloxalate actinometric method[7]. The value of the quantum yield used in the calculations was 0.60[8].

Total copper and direct-reading copper determinations were made by the method of GUBLER et al.[3,4]. Bound copper content was determined as the difference between total copper and direct-reading copper.

The effect of UV irradiation on uricase, plant tyrosinase and mammalian tyrosinase is shown in Figure 1. The logarithm of the relative enzymatic activity (ordinate) is plotted against the corresponding dose of UV irradiation (abscissa). The activity of each enzyme was reduced when subjected to varying amounts of UV radiation at 2537 Å. The logarithm of the relative activity was proportional to the incident energy within the experimental error. Mathematically, the results may be represented by equation (1):

$$A = A_0 e^{-kD} \qquad (1)$$

where A is the enzymatic activity after a UV dose D (ergs/mm²), A_0 is the enzymatic activity of the control or nonirradiated sample, and k is a constant. The ratio A/A_0 is the relative activity.

In Figure 2, the effect of increasing doses of UV irradiation 2537 Å on plant tyrosinase activity as assayed with two different substrates is shown. The data in the upper curve were obtained using L-DOPA (2.5×10^{-3}M) while the lower curve gives results using L-tyrosine (1.3×10^{-3}M). These substrate concentrations were found to be optimal with the former being about twice the latter. The regression coefficient of the L-tyrosine curve is 0.063, approximately twice that for the L-DOPA curve which is 0.027. Thus the inhibition of the tyrosinase activity as assayed against L-DOPA is only about one half that seen when L-tyrosine is used. This may be due to the

[6] C. C. WORTHINGTON, *Worthington Enzymes*, Descriptive Manual No. 9, p. 36 (1957).

[7] F. DANIELS, J. H. MATHEWS, and J. W. WILLIAMS, *Experimental Physical Chemistry* (McGraw-Hill, New York 1941), p. 274.

[8] W. G. LEIGHTON and G. S. FORBES, J. Amer. chem. Soc. *52*, 3139 (1930).

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fact that since both steps in the reaction, tyrosine to DOPA and DOPA to o-quinone, are under the influence of the single enzyme, tyrosinase, there is apparently double inhibition when the primary substrate, tyrosine, is used.

When the two copper methods used previously with serum and purified ceruloplasmin (50%) were employed on the samples containing the tyrosinase and uricase preparations to determine if copper to protein linkages were ruptured, clear cut results were not obtained. This is now understandable since MAHLER et al.[9] have shown that in the case of uricase, the complexing or color reagent diethyldithiocarbamate will react with copper in this system regardless of its state in the protein molecule. This is probably also true for tyrosinase. Therefore, the irradiated uricase and tyrosinase samples were dialyzed in order to see if any 'unbound' copper would pass through the membrane. As seen from the data on Table I, copper ions could pass through the membrane used in the dialysis procedure. However, the uricase activity was lost when the preparation was subjected to this treatment. Further attempts to correlate the loss of enzymatic activity with any change in copper content or state were unsuccessful.

In the case of plant tyrosinase, the dilemma could be circumvented since the enzyme was only slightly inactivated after dialysis. Therefore, after irradiation, samples subjected to different amounts of UV radiation, as well as a control, were dialyzed against copper-free distilled water at 4°C. If copper was removed or released from the bound site in the protein molecule during UV irradiation, this amount of copper would pass through the membrane and into the copper-free water on the outside. Therefore, after 24 h, the dialyzed material in the bags was removed and again assayed for oxidase activity and total copper content. These data are given in Table II and are corrected for the slight increase in volume due to dialysis. It was found that the copper concentration did not decrease proportionately as the UV dose increased. There was a difference of approximately 40 μg/100 ml of copper between the non-dialyzed sample and all the dialyzed samples. These data suggested that there must have been 40 μg of free copper/100 ml present in the original material and this was removed in each sample regardless of the amount of UV irradiation. The fall in enzymatic activity was characteristic of non-dialyzed samples subjected to similar amounts of UV irradiation.

Furthermore, it was not possible to demonstrate a change in the absorption curves (240–900 mμ) of the irradiated plant tyrosinase samples as was the case with the copper protein, ceruloplasmin. The latter had an absorption peak at 600 mμ which decreased as the oxidase activity fell and as the UV irradiation dose increased[1].

Finally, in the case of the mouse tumor extract, the difficulties encountered were such that again a direct conclusion was impossible. The optical density of this crude extract was too large to enable us to determine if slight changes in the direct-reading copper occurred after irradiation.

Tab. I. Effect of dialysis on a solution of $CuSO_4$[a] (unbound copper)

Time of dialysis (h)	Copper concentration (μg/100 ml)	% lost due to dialysis
0	200	0
5.5	14	93
24.0	3	99

[a] 5 ml samples of copper sulfate solution were used. Dialysis was done at 4°C against copper free water.

Tab. II. Effect of dialysis on copper content of UV irradiated plant tyrosinase solutions[a]

UV dose (ergs/mm²)	Dialysis time (h)	Enzymatic activity (OD/min)	Copper content (μg/100 ml)
0	0	0.0876	170
0	24	0.0825	125
48.9×10^4	24	0.0535	136
97.8×10^4	24	0.0381	122
146.7×10^4	24	0.0274	136

[a] 5 ml samples of plant tyrosinase (4 mg/ml protein) were irradiated and subsequently dialyzed against copper-free distilled water (4°C). Approximately 40 μg/100 ml of direct reading or 'unbound' copper is present; copper content of the samples does not increase with UV irradiation.

Résumé. L'activité de l'uricase et des tyrosinases d'origine végétale et animale est diminuée par irradiation UV (2537 Å). Il existe une relation exponentielle entre l'activité enzymatique relative et la quantité de rayonnement. Il n'a pas été possible de mettre en évidence une relation entre la perte d'activité de la tyrosinase végétale des échantillons irradiés et la teneur en cuivre de ces derniers.

M. H. APRISON and K. M. HANSON

The Institute of Psychiatric Research and Departments of Biochemistry and Psychiatry, Indiana University Medical Center, Indianapolis, October 24, 1960.

Specific Precipitins for Type XIV Pneumococcus Polysaccharide from *Abrus precatorius* Seeds

Several seed haemagglutinins, specific for certain human blood group characters, have been described[1]. However, many others fail to differentiate human erythrocytes[2]; the precise basis of action of these non-specific agglutinins is generally obscure. Attempts to ascribe specificity for certain 'high-incidence' human blood group antigens to some non-specific seed agglutinins were unsuccessful[3]. However, many seed agglutinins, which fail to make individual distinctions among human erythrocytes, are not altogether devoid of specificity in that they can make species distinctions among the bloods of various animals[4]. Thus various non-specific seed agglutinins can be divided into distinct classes, each class having a characteristic pattern of reactions; for example, the agglutinins of *Lens esculenta* and *Vicia faba* agglutinate rabbit and guinea pig erythrocytes but not those of several other species.

[1] G. W. G. BIRD, Brit. Med. Bull. *15*, 165 (1959).
[2] M. KRÜPE, *Blutgruppenspezifische pflanzliche Eiweisskörper (Phytagglutinine)* (Ferdinand Enke Verlag, Stuttgart 1956).
[3] G. W. G. BIRD, Vox Sanguinis *4*, 318 (1959).
[4] G. W. G. BIRD, Brit. J. exp. Path. *35*, 252 (1954).