tained at 552 mμ and 510 mμ correspond to the optical density of the ribose.

In order rapidly to convert the values of the optical densities into μg of sugar, it is advisable to prepare a calibration curve.

The difference in sensitivity of the original DISCHE-BORENFREUND method and of that proposed here is quite obvious from the data reported in Table I, which clearly demonstrates the higher sensitivity (almost double) of our method over that of the above-mentioned research workers. Quite naturally, we also studied the possibility of interference of other substances, among which we examined fructose, mannose, galactose, glucuronic acid and glucosamine, substances frequently mixed with ribose in biological material. We did not consider glucose, as it is already present to a large extent in the phloroglucinol reagent where it has the function of stabilizing the colour. This test was carried out in parallel, using our modified procedure and the original DISCHE-BORENFREUND method. We first of all tested the above substances singly mixed with ribose (Tab. II). It can be seen that the substances tested interfer to a negligible or only slight extent. Only glucuronic acid interfers both in our method and in that of DISCHE-BORENFREUND.

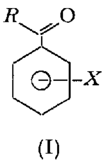
Riassunto. Si descrive una modificazione del metodo di DISCHE e BORENFREUND per la determinazione dei pentosi, che raddoppia all'incirca la sensibilità. La modifica più importante consiste nel trattamento iniziale della soluzione zuccherina con HCl concentrato e nella riduzione a soli 5 min del tempo di immersione in bagno maria bollente dopo l'aggiunta del reattivo al floroglucinolo.

L. BOLOGNANI, G. COPPI, and V. ZAMBOTTI

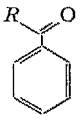
Istituto di Chimica biologica dell'Università di Pavia (Italy), October 5, 1960.

The Electron-Transfer Absorption of Substituted Benzenes

The electronic spectrum of the composite molecule containing unlike conjugated chromophores has been interpreted in terms of wave functions for the separated fragments[1,2]. These functions describe electronic transitions involving locally excited (L.E.) states and electron transfer (E.T.) states. Consideration of the principal E.T. bands of acetophenone, benzaldehyde, benzoic acid and their mono-substituted derivatives reveals a marked distinction between the *ortho-* and *meta*-disubstituted benzenes and their *para*-isomers. Thus, not only do the *para*-isomers of (I) (where X = alkyl, ring residue, –OH, –OMe, $-O^-$, –Hal, $-NR_2$) absorb at longer wavelengths than the *ortho-*[3] or *meta*-isomers, but further, the spectra of the latter class are characterised by the appearance of *two* E.T. bands, whereas the *para*-compounds display a single band due to such a transition. These observations are in accord with a wave-mechanical treatment of electron-transfer absorption[4].



(I)



(II)

The fundamental nature of the these effects inclines us to the view that the principal E.T. band of the basic chromophore (I) should be affected in a predictable manner by the nature and position of X[5]. We have therefore selected parent values for the chromophore (II) listed in Table I, which, in conjunction with the increments in Table II allow of calculation of the position of the principal E.T. band of a variety of *poly*-substituted aromatic ketones, aldehydes, and carboxylic acids to within 5 mμ. The choice of solvent is most important and we have

Tab. I. Parent values for chromophore (II)[a] [EtOH solution]

R.	λ*[a](mμ)
Alkyl or ring residue	246
OH or OAlk	230
H	250

[a] λ* = λ_{max} in EtOH.

Tab. II. Calculation of the principal E.T. band of the *poly*-substituted benzenes, Ar.CO*R*. (EtOH solution)

R.		λ*[a](mμ)
Alkyl or ring residue	*o*-, *m*-	3
	p-	10
–OH, –OAlk	*o*-, *m*-	7
	p-	25
$-O^-$	*o*-,	11
	m-	20
	p-	78
–Cl	*o*-, *m*-	0
	p-	10
–Br	*o*-, *m*-	2
	p-	15
$-NH_2$	*o*-, *m*-	15
	p-	58
–NHAc	*o*-, *m*-	20
	p-	45
$-NMe_2$	*o*-, *m*-	20
	p-	85

[a] λ* = λ_{max} in EtOH.

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[1] H. C. LONGUET-HIGGINS and J. N. MURRELL, Proc. Phys. Soc. *68A*, 601 (1955).

[2] J. N. MURRELL, J. chem. Soc. *1956*, 3779.

[3] The presence of bulky *ortho*-substituent(s) may lower the *intensity* of the E.T. band to a considerable extent, or even remove the band completely from the spectrum. This effect may be accompanied by a wavelength shift (blue *or* red) and has been studied in a unified molecular orbital treatment by E. HEILBRONNER and R. GERDIL [Helv. chim. Acta *39*, 1996 (1956)]. The present treatment allows examples of severe steric repulsion to be recognised only with respect to wavelength shift, but does not appear to be unduly limited by such considerations.

[4] J. TANAKA and S. NAGAKURA, J. chem. Phys. *24*, 1274 (1956).

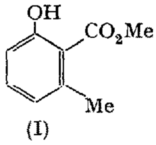
[5] Indeed, such a correlation was sought in the valuable study of substituted benzenes by DOUB and VANDENBELT[6]. However, these authors used a hypothetical base line of 180 mμ as a means of deriving substitutional parameters, implying no fundamental difference between benzenoid L.E. and E.T. absorption. Further, no allowance was made for the varying effect of the disposition of substituents in the *ortho-* or *para*-position with respect to a $-M$ group.

[6] L. DOUB and J. M. VANDENBELT, J. Amer. chem. Soc. *69*, 2714 (1947); *71*, 2414 (1949); *77*, 4535 (1955).

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)

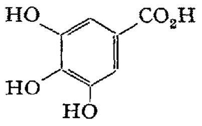


(I)

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

$\lambda^{*}_{\mathrm{obs}} = 244\ \mathrm{m}\mu\ (\varepsilon\ 10000)$[9]

Example 2. Gallic acid (II)

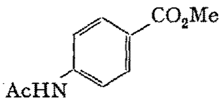


(II)

$\lambda^{*}_{\mathrm{calc}} = 230 + 2\times 7(\mathit{meta}\text{-OH's}) + 25(\mathit{para}\text{-OH}) = 269\ \mathrm{m}\mu$

$\lambda^{*}_{\mathrm{obs}} = 270\ \mathrm{m}\mu\ (\varepsilon\ 8000)$[10]

Example 3. p-Acetylamino methyl benzoate (III)

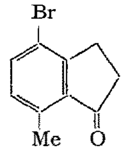


(III)

$\lambda^{*}_{\mathrm{calc}} = 275\ \mathrm{m}\mu$

$\lambda^{*}_{\mathrm{obs}} = 274\ \mathrm{m}\mu\ (\varepsilon\ 15000)$[11]

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

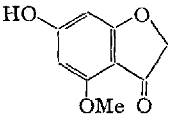


(IV)

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

$\lambda^{*}_{\mathrm{obs}} = 254\ \mathrm{m}\mu\ (\varepsilon\ 10000)$[12]

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

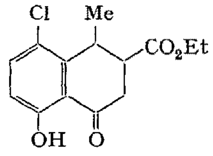


(V)

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

$\lambda^{*}_{\mathrm{obs}} = 284\ \mathrm{m}\mu\ (\varepsilon\ 22500)$[13]

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

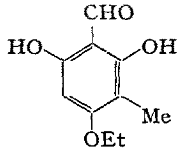


(VI)

$\lambda^{*}_{\mathrm{calc}} = 256\ \mathrm{m}\mu$

$\lambda^{*}_{\mathrm{obs}} = 257\ \mathrm{m}\mu\ (\varepsilon\ 8000)$[14]

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



(VII)

$\lambda^{*}_{\mathrm{calc}} = 292\ \mathrm{m}\mu$

$\lambda^{*}_{\mathrm{obs}} = 295\ \mathrm{m}\mu\ (\varepsilon\ 21950)$[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 $\lambda^{*}_{\mathrm{calc}}$ 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.

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

[9] C. J. W. Brooks, personal communication.

[10] J. E. Hay and L. J. Haynes, J. Chem. Soc. *1958*, 2231.

[11] P. Grammaticakis, Bull. Soc. Chim. Fr. Docum. *18*, 220 (1951).

[12] R. A. Friedel and M. Orchin, *Ultraviolet Spectra of Aromatic Compounds* (Wiley, New York 1951).

[13] L. A. Duncanson, J. P. Grove, J. MacMillan, and T. P. C. Mulholland, J. chem. Soc. *1957*, 3555.

[14] H. Muxfeldt, Chem. Ber. *92*, 3122 (1959).

[15] R. A. Morton and Z. Sawires, J. chem. Soc. *1940*, 1052.

[16] A. Burawoy and J. P. Critchley, Tetrahedron *5*, 340 (1959).

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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[1] M. H. Aprison and K. M. Hanson, Proc. Soc. exp. Biol., N.Y., *100*, 643 (1959).

[2] M. H. Aprison and K. M. Hanson, Program and Abstracts of the Biophysical Society, L 11 (1959).

[3] C. J. Gubler, M. E. Lahey, H. Ashenbrucker, G. E. Cartwright, and M. M. Wintrobe, J. biol. Chem. *196*, 209 (1952).

[4] C. J. Gubler, M. E. Lahey, G. E. Cartwright, and M. M. Wintrobe, J. clin. Invest. *32*, 405 (1953).

[5] G. H. Hogeboom and M. H. Adams, J. biol. Chem. *145*, 273 (1942).