6-Hydroxy-Δ^4-3-ketones. A 6β-hydroxy substituent exerts a hypsochromic effect of -3 to -6 mμ while a 6α-hydroxy substituent exhibits essentially no shift (0 to -1 mμ). Only inductive effects appear to be operative since it is apparent from these results that the 6β-hydroxyl group cannot be entering into a significant degree of participation.

The difference between hydroxyl group behaviour and halogen (e.g. chlorine or bromine) with respect to neighbouring group participation in the excited state is readily explained on the basis of the smaller size of the oxygen atom as well as its greater reluctance to accept a positive charge.

Zusammenfassung. Es werden die Hauptabsorptionsbanden (N→V Übergang) für eine Serie von Δ^4-3-Steroidketonen mit C-6-Substituenten diskutiert. Dabei wird versucht, die Lage der Hauptbanden dieser Verbindungen zu erklären. Besonders wird der Einfluss des induktiven Effekts und der von benachbarten Gruppen auf den angeregten Zustand berücksichtigt.

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Determination of Pentoses

Many methods have been suggested for assaying the pentoses and particularly for ribose[1–7]. The number of procedures is an obvious indication of the difficulties of such a determination.

The method now used, with marked advantage over the other, is that of DISCHE and BORENFREUND[8], based on a modification of the phloroglucinol reaction of TOLLENS[9].

Even the investigators and originators of this method themselves admit that it is less sensitive (about half) than the orcinol method suggested by BIAL[1], but it has the enormous advantage of greater specificity. In consideration of the latter, we decided to see if it was possible to increase the sensitivity of the DISCHE-BORENFREUND method.

Our aim was reached after many attempts, by suitably modifying the original method of DISCHE and BORENFREUND. The modified method is of advantage for its increased sensitivity and consists in treating the sugar solution with concentrated HCl which doubles the intensity of the colour developed by pentose and phloroglucinol with respect to that obtained in the experimental conditions suggested by DISCHE and BORENFREUND.

New method. Reagents and technique. The new method requires the following reagents: a) Hydrochloric acid (d = 1.19); b) Glacial acetic acid; c) 0.8% aqueous glucose solution; d) Alcoholic solution of phloroglucinol, prepared by dissolving 5 g of phloroglucinol in 100 ml of ethanol 95°.

At the moment of use, 2 ml of hydrochloric acid (d = 1.19), 110 ml of glacial acetic acid, 1 ml of 0.8% glucose solution and 5 ml of alcoholic phloroglucinol solution are mixed. The mixture forms the phloroglucinol reagent and may be stored in a refrigerator at 0°C.

After preparing the reagent, 0.5 ml of a ribose solution containing from 5–25 μg of ribose are placed in a test-tube; 0.5 ml of HCl (d = 1.19) are added and the mixture shaken. Then 4.5 ml of the phloroglucinol reagent are added and the tube immersed in a boiling water bath for exactly 5 min. At the end of this period, the tube is taken out of the water-bath and immediately cooled in ice, remaining in ice until the spectrophotometric reading, which must be performed at 552 and 510 mμ, using a blank where the pentose has been replaced by 0.5 ml of distilled water. The differences between the values ob-

Tab. I. Comparison between the optical density obtained from D-ribose with the original DISCHE-BORENFREUND method and that as here modified

D-ribose in 5.5 ml final volume	DISCHE-BORENFREUND method			Modified DISCHE-BORENFREUND method		
	Optical density		Difference	Optical density		Difference
	552 mμ	510 mμ		552 mμ	510 mμ	
5 μg	0.089	0.026	0.063	0.134	0.035	0.099
10 μg	0.164	0.044	0.120	0.285	0.074	0.211
15 μg	0.238	0.064	0.174	0.435	0.116	0.319
20 μg	0.320	0.080	0.240	0.585	0.165	0.420
25 μg	—	—	—	0.705	0.190	0.515

Tab. II. Optical density (O.D.) obtained in treating 10 μg of ribose alone and in the presence of 30 μg of fructose, galactose, glucosamine, mannose and glucuronic acid, respectively, according to the phloroglucinol reaction

Quantity of sugar in 5.5 ml final volume	DISCHE-BORENFREUND method		Difference	Modified DISCHE-BORENFREUND method		Difference
	O.D. at 552 mμ	O.D. at 510 mμ		O.D. at 552 mμ	O.D. at 510 mμ	
Ribose 10 μg	0.145	0.035	0.110	0.280	0.065	0.215
Ribose 10 μg + fructose 30 μg	0.150	0.044	0.116	0.325	0.102	0.223
Ribose 10 μg + galactose 30 μg	0.150	0.037	0.113	0.305	0.087	0.218
Ribose 10 μg + glucosamine 30 μg	0.144	0.034	0.110	0.282	0.067	0.215
Ribose 10 μg + mannose 30 μg	0.155	0.040	0.115	0.325	0.095	0.230
Ribose 10 μg + Gluc. acid 30 μg	0.180	0.042	0.138	0.340	0.089	0.251

[1] M. BIAL, Dtsch. Med. Wschr. *28*, 253 (1902).
[2] G. EMBDEN and M. LEHNARTZ, Z. physiol. Chem. *201*, 149 (1931).
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[6] W. MEJBAUM, Z. physiol. Chem. *258*, 117 (1939).
[7] A. BONSIGNORE, G. CONTE, and M. ORUNESU, G. Biochim. *1*, 383 (1951–52).
[8] Z. DISCHE and E. BORENFREUND, Bioch. biophys. Acta *23*, 639 (1957).
[9] H. J. WHEELER and B. TOLLENS, Liebigs Ann. Chem. *254*, 329 (1889).

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.

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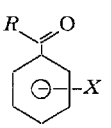
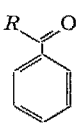
The Electron-Transfer Absorption of Substituted Benzenes

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) [EtOH solution]

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

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

Tab. II. Calculation of the principal E.T. band of the *poly*-substituted benzenes, Ar.COR. (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] $\lambda^* = \lambda_{max}$ in EtOH.

[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).