Notes

Electron Spin Resonance Spectra of the Basic Indigoid Dye Radicals

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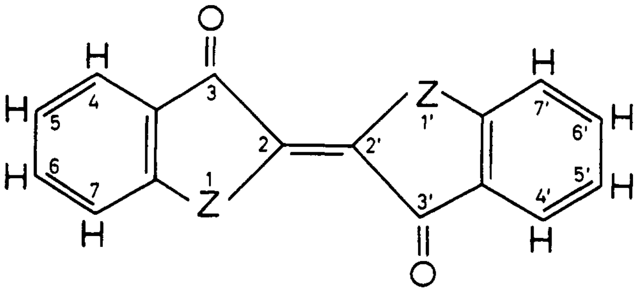
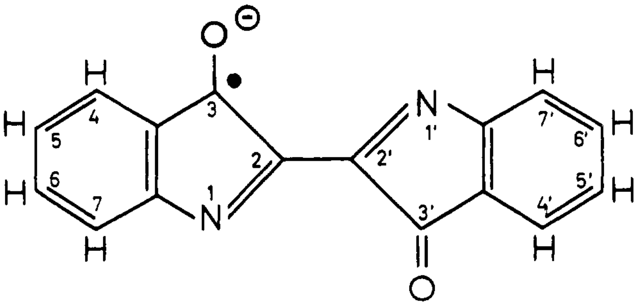
We have recorded and analyzed the electron spin resonance spectra of radicals derived from a large number of indigoid dyes. In this paper we report on four molecules which are basic structures for this group, having the configuration



The hetero-units Z are, respectively, selenium, sulfur, oxygen, or the imino group, NH. The present investigation is meant to collect experimental data on the odd-electron wave function of the blue dye indigo, as an aid to the theoretical interpretation of its color.

Nature of the Radicals.—The radicals of most indigoid dyes investigated could be easily produced in acetone or ethanol by adding sodium hydroxide pellets. The radicals react with the oxygen molecules dissolved in the liquid and a constant radical concentration is reached only after the oxygen is exhausted. As a rule the e.s.r. spectra of these radicals are identical with those obtained by means of a sodium mirror under vacuum, and it can therefore be assumed that the radicals are of the negative ion type.

The e.s.r. spectrum obtained when indigo is dissolved in strongly alkaline acetone, or when Indigo Carmine[1] is dissolved in alkaline water, can be fully understood if it is assumed that no observable spins are attached to the nitrogen atoms. Although neither from the present investigation nor from previous work,[1-3] can it unambiguously be deduced that the protons are really stripped from the nitrogen atoms, for our purpose it seems justified to consider the radicals observed as the negative ions of molecules similar to dehydroindigo. The structure of the indigo radical would thus be of the form



For seleno-, thio-, and oxindigo the negative ion is the same as the semiquinone of the redox system. If, during the formation of the radical, hydrogen is available, one or more protons may be bonded. This seems to occur if, *e.g.*, the thioindigo radical is formed by reduction in glacial acetic acid. The e.s.r. spectrum would then exhibit a large increase of fine structure lines. Indeed the observed spectrum is almost smooth and cannot be analyzed any more.

Results

Selenoindigo.—Of the four molecules discussed here, the reddish violet selenoindigo produces the narrowest spectrum. This spectrum could not be fully resolved. If the selenoindigo spectrum is compared to the one of thioindigo,[3] it is found that the two spectra are very similar. In the selenoindigo spectrum the corresponding lines of thioindigo can be recognized as diffused groups of overlapping lines. In total there are seventeen of these groups. If the calibration of the magnetic field axis is ignored, it is difficult to distinguish the selenoindigo spectrum from the incompletely resolved spectrum of thioindigo. If the magnetic scale is taken into account, the splittings of the thioindigo are found to be 13% larger than the corresponding ones of the selenoindigo (see Table I).

TABLE I

SPLITTING CONSTANTS IN GAUSS FOR NUCLEAR SPINS IN VARIOUS POSITIONS OF INDIGOID DYE RADICALS

						1,1′	
Z-Atom	Total width	4,4′	5,5′	6,6′	7,7′	NN′	pp′
Se	6.0	1.06	0.29	1.26	0.40		
S	6.80	1.20	.33	1.42	.45		
O	10.1	1.78	.59	2.04	.68		
N	12	1.52	.50	1.98	.67	0.67	0.2

Oxindigo.—The spectrum of the bright lemon-yellow oxindigo, as obtained, *e.g.*, with the alkali-mirror technique, is about 5% wider than the one of thioindigo. The spectrum can easily be resolved in lines of about 70 milligauss width. The observed fine structure must be attributed[3] to the four pairs of aromatic ring protons, and to other bonded protons, or to the action of the nuclear spin of the complexing alkali metal. Using a potassium mirror, all lines are split into narrow quartets of equal intensity which are to be assigned to the K-spin of 3/2. In Fig. 1 a recording is shown of the e.s.r. spectrum of the oxindigo semiquinone

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[1] F. Bruin, F. W. Heineken, and M. Bruin, *J. Chem. Phys.*, **37**, 682 (1962).

[2] F. W. Heineken, M. Bruin, and F. Bruin, *ibid.*, **37**, 452 (1962).

[3] M. Bruin, F. Bruin, and F. W. Heineken, *ibid.*, **37**, 135 (1962).

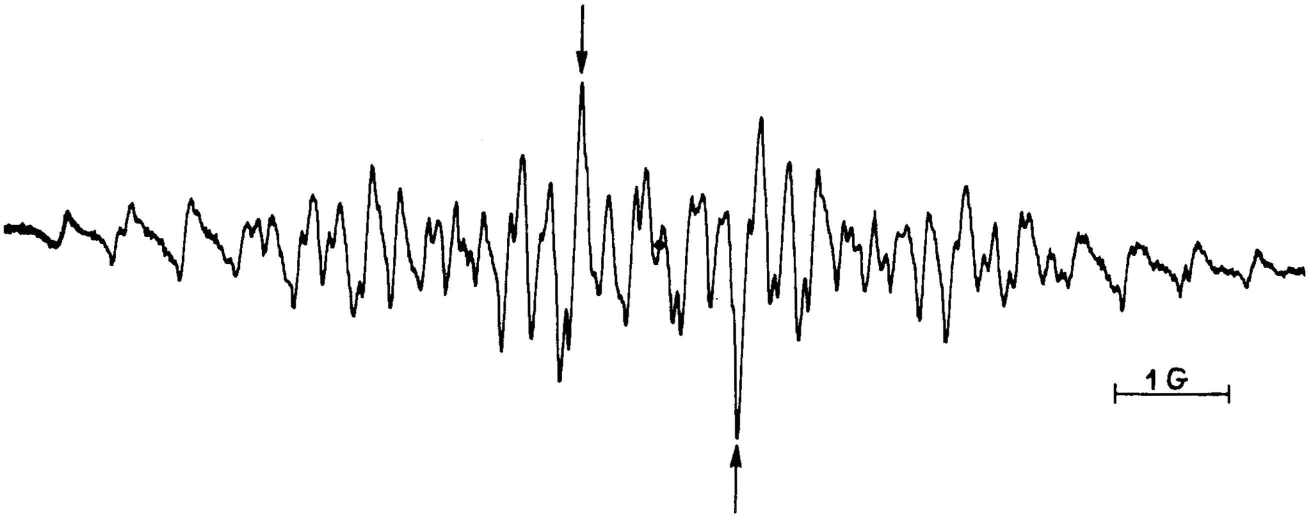

Fig. 1.—Spin resonance spectrum of oxindigo in glacial acetic acid with zinc dust. The arrows indicate a doublet splitting of 1.64 gauss.

as prepared in glacial acetic acid with zinc dust. The large doublet splitting of 1.6 gauss should probably be attributed to a proton bonded to one of the oxygen atoms of the keto groups. The data collected in Table I give the splitting constants for oxindigo in glacial acetic acid. For the total width of 10.1 gauss, as given in the table, the doublet splitting is not taken into account.

Indigo.—If prepared in strongly alkaline ethanol the cherry-red radical of indigo shows a regular e.s.r. spectrum in which the largest triplet splitting of about 2 gauss can be easily recognized. Cooling the sample slightly below room temperature reveals a more detailed spectrum of very narrow lines (Fig. 2).

If the spectrum of dehydroindigo, as it is obtained in alkaline acetone is compared to the one of thioindigo, it is found that each individual line is further split into a quintet, due to the nuclear spins I = 1 of the nitrogen pair. The splitting constant of these quintets is 0.67 gauss. An almost smooth spectrum is obtained for the green indigo radical in 1,2-dimethoxyethane in which the nitrogen protons are still present. The splitting due to these protons may be estimated from comparison of the smooth indigo spectrum with the unresolved spectrum of dehydroindigo. The latter spectrum, between points of maximum slope, is only 4.0 gauss wide, whereas the width for the green indigo radical in 1,2-dimethoxyethane is 4.4 gauss. Assuming in first approximation that this difference is completely due to the pair of nitrogen protons, one finds that the splitting constant of the imino proton triplet should be of the order of 0.2 gauss. If such triplets are added to the theoretical spectrum of dehydroindigo, indeed an almost smooth spectrum results.

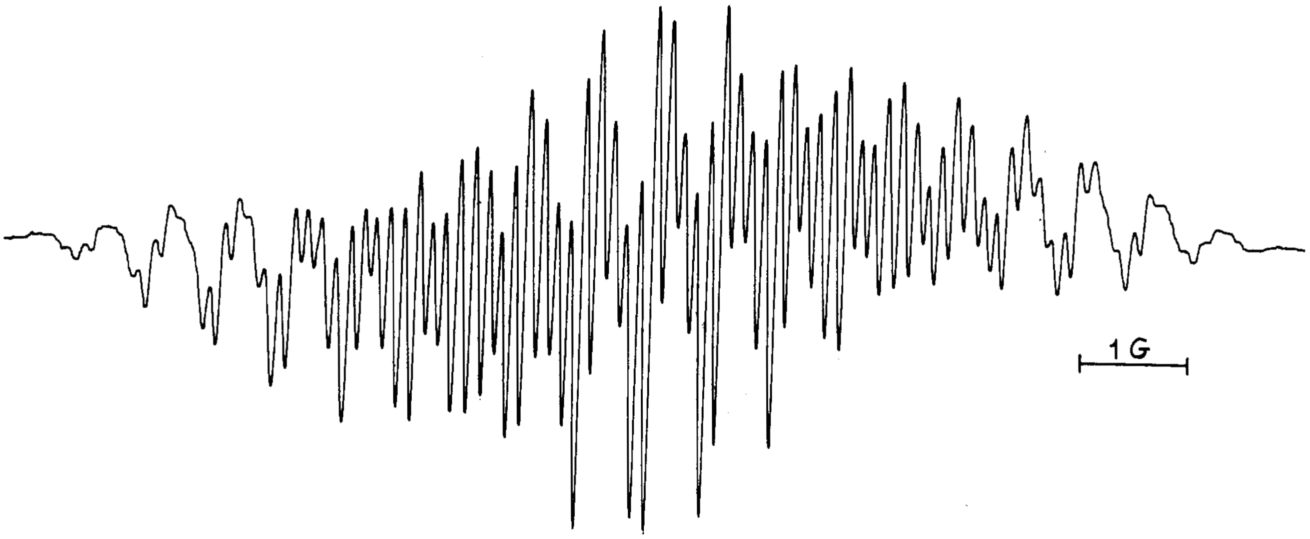

Fig. 2.—Spin resonance spectrum of the [red] indigo radical in strongly alkaline ethanol.

Related Work.—In addition to the spectra discussed above, we recorded and analyzed the e.s.r. spectra of some forty derivatives of indigo, mostly chlorinated and/or brominated compounds, marketed as commercial vat dyes. Unlike the thioindigo derivatives these dyes as a rule yielded broad line spectra of little interest for the present investigation. In this paper we will only mention work done on the 6,6′-dibromo derivative of indigo, generally known as Tyrian Purple. On isomers and some other indigoids of special interest we hope to report in due time.

Ancient crushed shells, excavated from a former Phoenician dye industry near Sidon at the Lebanese coast, were identified as belonging to Murex Trunculus, a mollusk quite common at the rocky shores of Lebanon. Natural Tyrian Purple was extracted from freshly caught animals, following instructions given by Aristotle[4] and Pliny.[5] The e.s.r. spectrum obtained is typical for indigo and was found to be identical to the one of 6,6′-dibromoindigo, kindly synthesized for us by Dr. Floyd Tyson of Philadelphia. Earlier it was shown by Friedlaender[6] that juices extracted from Murex Brandaris, a species found mainly in the northern Mediterranean, contain 6,6′-dibromoindigo as the principal pigment. Our analysis gives final proof that also the old Phoenician dye consisted mainly of 6,6′-dibromoindigo and that it is justified to call the latter compound "Tyrian Purple."

Conclusions

The e.s.r. spectra of the basic indigoid dyes could all be fully explained by assuming that there are four successive triplet splittings, with splitting constants ranging from about 2 gauss to about 0.5 gauss, due to the four pairs of aromatic ring protons, and additional quintets if the heteroatoms are nitrogens. In the earlier communication on thioindigo[3] it was shown how each of these triplets should be assigned to a particular proton pair. The similarity of the spectra justifies the adoption of the same assignment for the cases that the hetero-atoms are selenium, oxygen, or nitrogen. The effect of the heteroatom substitution is mainly one of over-all increase of odd electron density in the outer rings of the molecule, about proportional to the electronegativity[7] of the Z-atoms. As a result the splitting constants in the molecule with Z-atoms of large electron affinity will be larger and produce wider e.s.r. spectra. The splitting constants in gauss for the various aromatic ring proton pairs, as resulting from our analysis, are collected in Table I. The error in these data is about 5%. Note that the splitting constants for selenium, sulfur, and nitrogen are obtained with acetone solutions, whereas for oxygen glacial acetic acid had to be used.

In the series $Z = Se, S, O, N$ the increase in the observable over-all odd-electron density must be at the expense of the density in part (namely the chain $O{=}C{-}C{-}C{-}C{-}O^-$) of the central H-shaped chromophore.[8]

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[4] Aristoteles, "Historia animalium," Book 4, Athens, -0340.

[5] C. Plinius, "Historia naturalis," Book 9, chap. 60–64, Roma, 0075.

[6] P. Friedlaender, *Monatsh. Chem.*, **28**, 991 (1907); **30**, 247 (1909).

[7] C. A. Coulson, "Valence," Oxford University Press, New York, N. Y., 1951, chap. 5, Table 10.

[8] W. Luettke, *Angew. Chem.*, **69**, 645 (1957); **70**, 570 (1958); M. Klessinger and W. Luettke, *Tetrahedron* (1962), in press.

An N.m.r. Study of the Glycoside Link in Glycosides of Glucose and Galactose[1a,b]

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Whether the glycosidic link in disaccharides is α or β can be determined from Hudson's rules[2,3] or enzymatic hydrolysis studies.[4] In this paper we will show how proton n.m.r. spectroscopy may be used to determine link configurations in glycosides of glucose and galactose.

Fig. 1 shows the n.m.r. spectra of glucose (I), cellobiose (II), and maltose (III) at 60 Mc. in deuterium oxide solution. The abscissa is in τ values[5] with the reference being the $(CH_3)_3Si$— line of sodium 2,2-

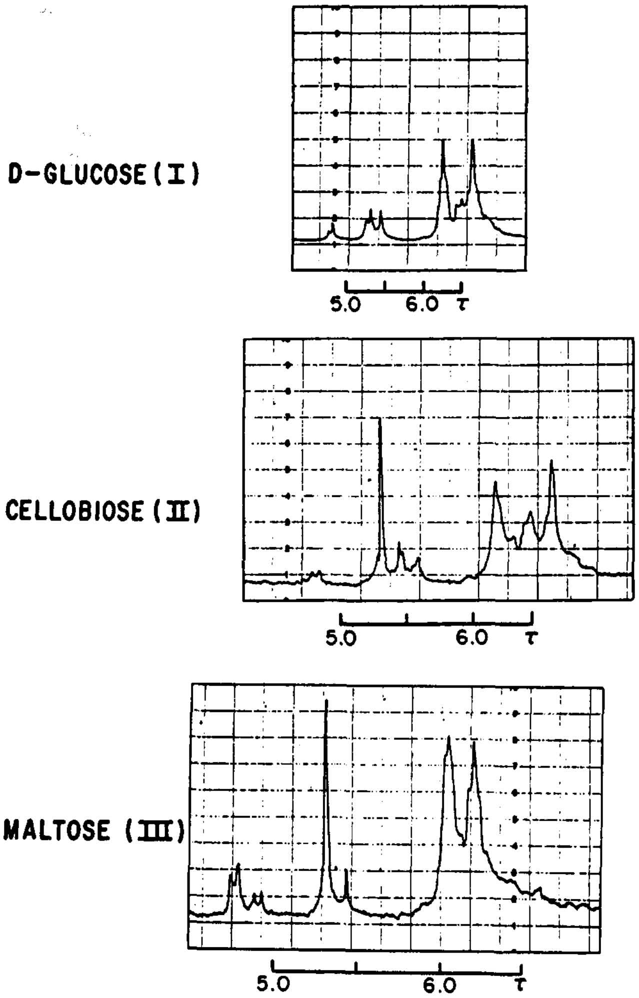

Fig. 1.—N.m.r. spectra of glucose derivatives at 60 Mc./sec. (ref. DSS).

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[1] (a) This investigation was supported by Public Health Service research grants B-904 and B-3370 from the National Institute of Neurological Diseases and Blindness, U. S. Public Health Service. (b) Present address: Stevens Institute of Technology, Hoboken, N. J.

[2] C. S. Hudson, *J. Am. Chem. Soc.*, **31**, 66 (1909).

[3] C. S. Hudson, *ibid.*, **60**, 1537 (1938).

[4] H. Baumann and W. Pigman, "The Carbohydrates," W. Pigman, ed., Academic Press, Inc., New York, N. Y., 1957, chap. X.

[5] G. V. D. Tiers, *J. Phys. Chem.*, **62**, 1151 (1958).