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The Phenol Dyestuff of Liebermann as an Acridan Derivative

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An acridan derivative (III), 7-hydroxyspiro[acridine-9,1'-cyclohexa-2',5'-diene]-2(9H),4'-dione, has been identified by spectroscopic techniques, as being the ' phenol dyestuff ' of the Liebermann reaction.

BAEYER and Caro ${\tt^1}$ were the first to describe the preparation from nitrosophenols of indophenols, which have since been used as oxidation-reduction indicators.² They concluded that the production of a blue colour from phenol and nitrite ion in sulphuric acid, known

¹ A. Baeyer and H. Caro, *Ber.*, 1874, 7, 963. ² H. G. Gibbs, W. L. Hall, and W. Mansfield Clark, Supplement No. 69 to the Public Health reports, Washington D.C., 1928.

as Liebermann's reaction, was due to the formation of indophenol (I), C₁₂H₉NO₂. Liebermann³ did not agree with the conclusions of Bayer and Caro and considered that his product, giving the blue colour described by him as a 'phenol dyestuff', was not $C_{12}H_{11}NO_3$ (*i.e.* $C_{12}H_9NO_2 + H_2O$) but $C_{18}H_{15}NO_3$. This product was

³ C. Liebermann, Ber., 1874, 7, 1098.

reinvestigated by Kraemer ⁴ and by Brunner and Chuit,⁵ who supported Liebermann's empirical formula (formerly included in Beilstein⁶), but in no case was a well defined substance obtained. The material was mentioned by Nietzki⁷ as an example of the oxazine class of dyestuffs.



The present work started with the isolation of a relatively stable crystalline substance which was considered to correspond to Liebermann's phenol dyestuff; by the demonstration of its acridan structure (III) we can now show the analogy, originally suggested by Nietzki,8 with the phenoxazinone structure of resorufin (II).

The crystalline substance was obtained in small yield (10%) by a mixed solvent extraction of a mixture of sulphuric acid, sodium nitrite, and phenol. The remaining indophenol together with other phenolic by-products were removed by treatment of the partly purified product with potassium ferricyanide.

Evidence for structure (III) for the product is as follows. The same compound could be prepared by treatment of indophenol (I) with phenol in sulphuric acid (90% w/w). This suggests that the indophenol is an intermediate in the formation of the Liebermann phenol dyestuff'. This idea was further supported by the fact that a related series of compounds could be formed if indophenol or a substituted indophenol was treated with a substituted phenol in sulphuric acid

7 R. Nietzki, 'Chemistry of the Organic Dyestuffs,' Tr. A. Collin and W. Richardson, Gurney and Jackson, London, 1892, p. 165.

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(90% w/w). Compound (V) was obtained from indophenol and durenol (2,3,5,6-tetramethylphenol). In other cases, the presence of compounds similar to (III) was shown by the characteristic absorption spectrum in alkaline solution after partial purification. For a compound related to compound (III) to be obtained, the phenol used in the reaction with the indophenol should be unsubstituted in the *para*-position, and should not possess electron-withdrawing substituents (e.g. SO₃H, Cl, etc.). This implies that the formation of a bond between the indophenol and the para-position of the added phenol is necessary. Analytical figures for the crystalline substance corresponded to an empirical formula of C₁₈H₁₁NO₃, which has four hydrogen atoms less than the Liebermann product. The mass spectrum showed strong ions at both m/e 289 (C₁₈H₁₁NO₃) and 291. The presence of the latter was taken as evidence for the presence of the leuco-base derived from (III). However, the substance and its leuco-base would be expected to possess different vapour pressures, and hence no assessment of the relative proportions of the two compounds present can be given. It seems unlikely that (III) could be reduced to the leuco-compound in the mass spectrometer, since compounds (VI) and (VII) (discussed later) showed strong parent ions at m/e 363 and 239 and no ions at two mass units higher; the analogous compound (V) showed only the expected molecular ion $(m/e \ 354)$ in its mass spectrum. The spectrum of the related compound (II) similarly exhibited only the expected molecular ion (m/e 213). Compound (III) could be reduced with nickel-aluminium alloy in alkaline solution to give a substance (IV) which showed a similar type of absorption spectrum in alkaline solution but with the maximum shifted towards higher frequency. This suggests that the main chromophoric group was unaffected by the reduction. In structure (III), the plane of the dienone ring generated from the phenol is perpendicular to the rest of the molecule. The mass spectrum confirmed the uptake of six hydrogen atoms, as required by structure (IV), although, as with compound (III), an ion of two mass units higher (m/e 297) was also observed.

Strong evidence for the structure of the compound we consider to be Liebermann's 'phenol dyestuff' was provided by spectroscopic comparison with known compounds of related structure.

If structure (III) represents the C_{18} ' phenol dyestuff ' of Liebermann, the latter may be regarded as being derived from a 9.9-disubstituted 2,7-dihydroxyacridan by removal of two hydrogen atoms. It would thus belong to the relatively little known class of colouring matters discovered by Kehrmann, Goldstein, and Tschudi; 9 compound (VI) was described by Kehrmann and Tschui¹⁰ and compound (VII) by Goldstein and

⁴ C. Kraemer, Ber., 1884, 17, 1878.
⁵ H. Brunner and P. Chuit, Ber., 1888, 21, 249.
⁶ Beilstein's Handbuch, 4 Aufl., 1923, 6, 137.

⁸ R. Nietzki in 'Handwörterbuch der Chemie,' ed. A. Ladenburg, 1887, 4, 81.
F. Kehrmann, H. Goldstein, and P. Tschudi, *Helv. Chim.*

Acta, 1919, 2, 315.

¹⁰ F. Kehrmann and J. Tschui, Helv. Chim. Acta, 1925, 8, 23.

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Kopp.¹¹ These two acridan derivatives were prepared by us and were found to resemble compounds (III) and (IV) closely.

The visible absorption spectra of the various compounds are shown in Figure 1. The absorption maxima of the spiro-compounds are sharp compared with the absorption of the indophenol itself; the absorption





spectra of the acridans (III)—(VII) are similar to that of the phenoxazinone (II), though the maxima occur at longer wavelength.

One marked difference was that compound (VII) in alkaline solution showed a strong red fluorescence, which was absent in solutions of compound (IV). If the latter is left in alkaline solution with excess of an ammonium salt the product resembles that described by Goldstein and Kopp¹¹ as the '2-amino-C-dimethyl-carbazone (7)' which they isolated as an intermediate in their preparation of compound (VII) from the 2,7-diaminoacridan.

The i.r. spectra of compounds (III)—(VII) had many features in common. Notable were two absorption maxima in the range 1640—1680ms and 1615—1605s cm⁻¹. The carbonyl absorptions are similar to those of a β -diketone. In the case of compound (III), an additional carbonyl absorption at 1662s and in that of (V) at 1670s could be assigned to the dienone carbonyl of the spiro-ring. In the spectrum of resorufin (II), similar absorption maxima were observed at 1656ms and 1610s cm⁻¹.

The n.m.r. spectra of compounds (II)—(VII) (Table) show a clear similarity. Signals were assigned by their splitting patterns and by the shifts upon addition of sodium deuteroxide. The spectra could best be interpreted in terms of structure (VIII) or of a rapid equilibrium between the tautomers (A) and (B).

¹¹ H. Goldstein and W. Kopp, *Helv. Chim. Acta*, 1928, **11**, 478.

Single resonances were observed for H(1) and H(8), for H(3) and H(6), and for H(4) and H(5). Because of the low solubility of the compounds in suitable solvents, no attempt was made to obtain low-temperature spectra in order to decrease the rate of exchange between the two tautomers, and thus to observe separate signals for H(1) and H(8), *etc.* Two resonances were observed for each of these pairs of protons in the case of compound (IV). This 'doubling' of the spectral pattern is caused by the dissymmetry in (IV) induced



by the spiro-cyclohexane ring; the two carbocyclic rings of the acridan residue are not then equivalent despite the rapid equilibrium between tautomers (A) and (B). The assignment of this pattern was confirmed by obtaining spectra at two different field strengths (*i.e.* 23.5 and 14 kG) and by spin-decoupling experiments.

We have also included in the Table, for reference, the n.m.r. spectral results for the potassium salt of

N.m.r. ^a	spectra	of th	le	acrid	an	s	and	re	lated	com	pounds
								-			

	Chemical shifts b (δ)										
	Η(1)	H(8)	$H^{(3)}$	H(6)	$H^{(4)}$	$\mathbf{H}^{(5)}$	OH				
(VII)	6.18	6.18	6.75	6.75	7.49	7.49	10.5				
`(VI) °	6.70	6.70	6.90	6.90	7.45	7.45	10.5				
(III) d	6.34	6.34	6.70	6.70	7.49	7.49	10.5				
(V) •	6.17	6.17	6.72	6.72	7.53	7.53	j				
$(IV)^{f}$	6.64	6.65	6.91	7.01	7.39	7.40	10.5				
(II) 9	6.41	6.41	6.74	6.74	7.50	7.50	j				
(I) h	6.90	6.90	6.90	6 ∙90	$7 \cdot 52$	7.52	j				

^a Solutions in $[{}^{2}H_{e}]$ dimethyl sulphoxide. ^b In p.p.m. from internal tetramethylsilane. ^e δ 6·9—7·4 (m, Ph₂). ^d δ 6·95 and 6·70 (A₂B₂, 4 cyclohexadienone protons). ^e δ 1·39(s) and 1·81(s) (4 × Me). ^f δ 3·65 (1H, m, CH·OH) and 1·70 (8H, m, 4 × CH₂). ^e Spectrum obtained at 80° because of low solubility of this compound. ^h Potassium salt. Spectrum was an A₂B₂ pattern (δ_{A} 6·90, δ_{B} 7·52). ^e Broad signal. ^f No signal detected.

indophenol. Since it was not possible to get a suitable concentration of the compound in hexadeuteriodimethyl sulphoxide, a mixture of deuterium oxide and hexadeuteriodimethyl sulphoxide was used. Again, only a single (A_2B_2) pattern for the aromatic protons was observed, indicating either that rapid exchange between tautomeric forms was taking place or that the negative charge of the anion is equally distributed between the two aromatic rings, as shown in structure (VIII).

Formation of Liebermann's dyestuff (III) by treatment of indophenol (I) with phenol in sulphuric acid necessitates the oxidative removal of two hydrogen atoms, either by the indophenol itself or by the sulphuric acid. This may account for the low yields, even apart from the occurrence of side reactions such as sulphonation and oxidation of phenol, and is consistent with the higher yields of compounds (III) and (V) which may

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be obtained by the use of nitrosylsulphuric acid, as originally recommended. The yield of (III) from the indophenol (I) seems to vary with the conditions used, showing in fact that both Liebermann and Baeyer were correct, either the C_{12} compound (I), or the C_{18} compound (III) being formed. Formation of the C_{18} product requires sulphuric acid containing from 7 to 12% (w/w) of water.

The acridan derivatives (III)—(VII) give blue solutions in sulphuric acid which turn red on dilution; this colour is due to protonation of the free base. The neutral forms give yellow solutions in most solvents, while the indophenols are red. This is shown for compound (IV) in Figure 2 and for compound (VII) in



FIGURE 2 Visible absorption spectra for compound (IV) 2.5×10^{-5} M in aqueous solutions: A in 0.1% sodium carbonate; B in N-hydrochloric acid; and C in dilute acetate buffer



FIGURE 3 Visible absorption spectra for compound (VII) $2\cdot11 \times 10^{-5}$ M in aqueous solutions under the same conditions as given for Figure 2

Figure 3. Compared to indophenol, however, the stability of the acridans is greater, and the redox potential is less shifted towards that of the hydrogen electrode than it is in the case of azines, oxazines, and thiazines. This last consideration motivated the present investigation.

EXPERIMENTAL

The elemental analyses were carried out by single runs with Technicon CHN apparatus. Absorption spectra were measured with a Unicam SP 500 instrument and were examined visually with a Zeiss direct vision spectroscope. I.r. spectra were obtained with Perkin-Elmer 21 and 457 spectrometers. The n.m.r. spectra were obtained for solutions of the compounds in $[{}^{2}\mathrm{H}_{6}]$ dimethyl sulphoxide with Varian models A60 and HA-100D spectrometers. Mass spectra were obtained with a Perkin-Elmer-Hitachi RMU-6E mass spectrometer.

7-Hydroxyspiro[acridine-9,1'-cyclohexa-2',5'-diene]-2(9H), 4'-dione (III) .--- Sodium nitrite (250 mg) in sulphuric acid (25 ml; 90% w/w) was placed into a dry Erlenmeyer flask (500 ml) containing glass balls (150 g; 0.5 cm diameter). The flask and contents were maintained at 40° while powdered phenol (1 g) was added, in four portions during 2 min, with thorough shaking. The neck of the flask was sealed with wax film and the shaking was continued for a further 10 min. After a further 20 min, the mixture was poured into ice-water; the final volume was ca. 2 l. The glass balls were removed (Buchner funnel) and the turbid red-brown fluid was extracted with toluene-ether (800 ml, 1:1 v/v). The orange-yellow extract was filtered and then extracted with numerous small volumes of aqueous sodium carbonate solution (3% w/w) to give a dark blue solution (400 ml). After removal of traces of ether with a current of air, potassium ferricyanide (3.5 g) was slowly added until a spot on a filter paper showed a permanent yellow outer ring. After 48 h at room temperature, a black precipitate was filtered off, and the filtrate was treated with sufficient calcium chloride to complete the precipitation of carbonate and was then extracted with ether. The extract was treated as previously with aqueous sodium carbonate solution. The blue aqueous solution was acidified with acetic acid, whereupon the product was obtained as orange-yellow plates (120 mg) sintering with decomposition at 248° . Purification was effected by (a) redissolving the crystals in aqueous sodium carbonate solution (3%) and precipitating with acetic acid (Found: C, 75.0; H, 3.9; N, 4.4. C₁₈H₁₁NO₃ requires C, 74.7; H, 3.85; N, 4.85%), or (b) recrystallising from boiling water (1 g from 6.5 l. Found: C, 75.0; H, 3.9; N, 4.3%). When precipitated from more concentrated solutions, particularly in the presence of sodium chloride, a violet-red solid with green reflex was produced which was converted into the other form by reprecipitation from its more dilute solution in aqueous sodium carbonate. The treatment with ferricyanide destroys any indophenol (which gives a red solution in ether) and also destroys a product derived from the action of aqueous mineral acid on compound (III)this by-product was non-crystalline and gave a violet colour in aqueous sodium carbonate solution and an orangeyellow colour in ether.

4',7-Dihydroxyspiro[acridine-9,1'-cyclohexane]-2(9H)-one (IV).—A Keilin-Thumberg vacuum tube (30 ml) with a hollow stopper and side arm was used. Nickel-aluminium (500 mg; 50% powdered alloy) was added to a suspension of compound (III) (140 mg) in water (15 ml). Aqueous sodium hydroxide solution (0.3 ml; 40%) was placed in the hollow stopper and the tube was evacuated (waterpump) with cooling in iced water to check frothing. The tap was then closed and the tube shaken thoroughly at the ambient temperature. After ca. 4 min the blue colour disappeared and only then were bubbles of hydrogen evolved. After a few min the tube was opened and the contents were rapidly washed into water (500 ml) containing sodium hydrogen carbonate (250 mg). To remove the aluminium hydroxide and the remains of the alloy, the mixture was filtered through a layer of Kieselguhr and talc on a large Buchner funnel. The precipitate was washed with dilute aqueous carbonate solution until the washings were no longer blue, whereupon a current of air was passed through the filtrate and washings until the leuco-compound was completely oxidised. This solution was then saturated with sodium chloride, and sufficient sodium dihydrogen phosphate was added to give a greenish-yellow liquid which was extracted with ether. The yellow ethereal solution was extracted with several small volumes of aqueous sodium carbonate solution (3%), dissolved ether was removed from the extract by a current of air, and the blue aqueous solution was then acidified with acetic acid. After 3 days at 5°, compound (IV) (110 mg) was obtained as orange crystals with a bluish reflex, softening at 218°, m.p. 225°. For analysis two samples were recrystallised (a) from hot water (Found: C, 72.8; H, 5.8; N, 4.3. C₁₈H₁₇NO₃ requires C, 73.2; H, 5.8; N, 4.7%) and (b) by dissolution in dilute sodium hydroxide solution followed by acidification with acetic acid, filtration, and leaving in the cold for 3 days (Found: C, 73.1; H, 5.8; N, 4.3%).

Homologues of Compound (III).—The indephenols were prepared by the literature method from a chosen phenol and p-benzoquinone chloroimine.¹ They were purified by salting out with potassium carbonate. Indephenol, as the potassium salt (100 mg), was dissolved in sulphuric acid (5 ml, 90% w/w) and the chosen phenol or phenol derivative was added. When the blue colour due to the indephenol had become brown, the mixture was poured into cold water and extracted with ether, as described for compound (III). The product, in sodium carbonate solution, was treated with potassium ferricyanide and a direct vision spectroscope was used to look for the characteristic absorption spectrum.

With phenol indophenol the following phenols gave positive results: thymol, 2,6- and 3,5-xylenol, carvacrol, o-phenylphenol, o- and m-cresol, and 2,3,4,6-tetramethylphenol. Negative results were obtained with p-cresol, p-chlorophenol, and 2,6-dichlorophenol. Negative results were also obtained from the following indophenols: thymol indophenol, carvacrol indophenol, and 3-isopropylphenol indophenol.

7-Hydroxy-2',3',5',6'-tetramethylspiro[acridine-9,1'-cyclohexa-2',5'-diene]-2(9H),4'-dione (V).--The potassium salt

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of phenol indophenol (1 g) was mixed with 2,3,4,6-tetramethylphenol (1.5 g). This mixture was added, with thorough mixing, to sulphuric acid (10 ml; 90% w/w) containing sodium nitrite (600 mg) in an Erlenmeyer flask at 40°. A current of dry carbon dioxide was continuously passed over the mixture to remove nitric oxide and atmospheric oxygen. After 15 min more sodium nitrite (600 mg) in sulphuric acid (10 ml; 90% w/w) was added, followed by, after a further 15 min, more 2,3,4,6-tetramethylphenol (1 g). About 30 min later the mixture was poured into ice-water. Compound (V) was isolated in a similar way to compound (III) except that ether was used for the extraction instead of ether-toluene. The purified product (160 mg) was obtained as yellow prisms, m.p. 270-272°. The substance showed a more marked red fluorescence in alkaline solution than did compound (III).

9,9-Diphenyl- and 9,9-Dimethyl-7-hydroxyacridin-2(9H)one [(VI) and (VII)].-These acridan derivatives were for the present purposes prepared by a more direct method than that used by Kehrmann et al.^{10,11} The appropriate 3-hydroxyphenylcarbinols were prepared by Grignard reactions, on methyl 3-hydroxybenzoate with phenylmagnesium bromide and on 3-hydroxyacetophone with methylmagnesium iodide. The tris-(3-hydroxyphenyl)methanol and the 3-hydroxyphenyldimethylmethanol thus obtained were converted into the corresponding indophenols by addition of p-benzoquinone chloroimine as described by Clark.¹ The indophenols were reduced in solution with tin(II) chloride and heated at 90° with 2Nhydrochloric acid for 1 h. After atmospheric oxidation of their leuco-compounds in aqueous sodium carbonate solution, the acridan derivatives (VI) and (VII) were isolated as described in refs. 10 and 11.

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