Spectroscopic Properties of Violacein and Related Compounds: Crystal Structure of Tetramethylviolacein

Hartmut Laatsch and Ronald H. Thomson *
Department of Chemistry, University of Aberdeen, Meston Walk, Old Aberdeen AB9 2UE, Scotland
Philip J. Cox
School of Pharmacy, Robert Gordon's Institute of Technology, Schoolhill, Aberdeen AB9 1FR, Scotland

Violacein and deoxyviolacein have been isolated from cultures of *Alteromonas luteoviolacea*. The violacein-type lactams (**1**) and lactones (**3**) have a merocyanine chromophore which was confirmed by the effects of substituents on the visible spectra and by HMO calculations. Using parameters derived from an *X*-ray crystal structure analysis of tetramethylviolacein, PPP calculations of the electronic spectra of violacein and isoviolacein derivatives showed good agreement with observed values.

During work directed towards the antibiotics produced by *Alteromonas luteoviolacea* (strain NCMB 1893)[1] we isolated two violet pigments. The properties and molecular formulae, $C_{20}H_{13}N_3O_3$ and $C_{20}H_{13}N_3O_2$, suggested that they were violacein (**1**; R^1 = OH, $R^2 = R^3 = R^4$ = H)[2] and deoxyviolacein (**1**; $R^1 = R^2 = R^3 = R^4$ = H), respectively. However, the ^{1}H n.m.r. spectrum [in $(CD_3)_2SO$] of the major component showed three sharp NH signals in the region δ 10.88—12.16 which at first seemed to be at variance with structure (**1**), and an isomeric structure (**2**) was therefore considered. Furthermore, the $\lambda_{max.}$ values for the visible spectra of the two pigments show a significant difference (16 nm in EtOAc) not expected for (**1**; R^1 = OH and H, $R^2 = R^3 = R^4$ = H) which appeared to have an indigoid chromophore. In view of these doubts we degraded[3] the tetramethyl derivative of the major pigment, and repeated the synthesis[2] of deoxyviolacein. Some new observations are reported in the Experimental section but the results left no doubt that our pigments were indeed violacein (**1**; R^1 = OH, $R^2 = R^3 = R^4$ = H) and deoxyviolacein (**1**; $R^1 = R^2 = R^3 = R^4$ = H). The violacein structure was further confirmed, later, by an *X*-ray crystallographic analysis of its tetramethyl derivative. This then led us to examine the spectra of a series of violacein derivatives, and related lactones of types (**3**) and (**4**), and simple isatins (**5**). New compounds were prepared by known methods.

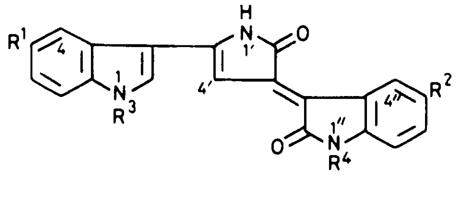
Results and Discussion

^{1}H *N.m.r. Spectra*.—The data are collected in Tables 1—3. Assignments were made by comparison with model compounds and appropriate decoupling. The most striking features of the ^{1}H n.m.r. spectra are the low-field NH signals in $(CD_3)_2SO$ solution. In the violacein series the 'indole NH' (N-1) peaks

Table 1. ^{1}H n.m.r. and i.r. spectra of violacein and related lactams

	Indole nucleus						Lactam ring		Isatin nucleus						
Compound	1-H	2-H	4-H	5-H	6-H	7-H	1′-H	4′-H	1″-H	4″-H	5″-H	6″-H	7″-H	Other	I.r.[e] (cm⁻¹) $\nu_{CO,C=C}$
Violacein[b] (**1**; R^1 = OH, $R^1 = R^3 = R^4$ = H)	12.16 s	8.13 s	7.30 s		6.86 d	7.41 d	10.88 s	7.61 s	11.00 s	8.98 d	7.00 t	7.25 t	6.90 d	OH, 9.41	1 680br, 1 655br, 1 602
-Me_4[c]		7.95 s	7.48 s		6.99 d	7.46 d		7.71 s		9.09 d	7.01 t	7.30 t	6.90 d	OMe, 3.91 NMe, 3.89, 3.42, 3.21	1 671, 1 599
-Ac_4[a]		7.75 s	7.28 d		7.13 dd	8.48 d		7.78 s		9.10 dd	7.25 td	7.43 td	8.32 dd	OAc, 2.33 NAc, 2.66, 2.76, 2.77	1 755sh, 1710, 1 611, 1 595
-*NO*-Ac_2[b]		8.60 s	7.50 s		7.25 d	8.41 d	10.69 s	7.61 s	10.79 s	8.93 d	6.95 t	7.27 t	6.84 d	OAc, 2.33 NAc, 2.66	1 750sh, 1 680sh 1 612sh
Deoxyviolacein[b] (**1**; $R^1 = R^2 = R^3 = R^4$ = H)	12.10	8.15 d	7.84 m	7.29 m	7.29 m	7.56 m	10.62 s	7.62 s	10.80 s	8.91 d	6.95 t	7.21 t	6.82 d		1 690, 1 670, 1 620sh, 1 610
-Me_3[a]		7.67[d] s	7.99 d	7.32 m	7.32 m	7.32 m		7.44[d] s		9.12 d	7.06 t	7.29 t	7.68 d	NMe, 3.87, 3.40, 3.27	1 689w, 1 668, 1 599
-*NN″*-Ac_2-*N′*-Me		7.76 s	7.79 d	7.44 m	7.44 m	8.48 d		7.54 s		9.31 d	7.44 m	7.25 t	8.30 d	NAc, 2.73, 2.73 NMe, 3.32	1 720—1 690, 1 592
Isoviolacein (**1**; $R^1 = R^3 = R^4$ = H, R^2 = OMe)-OMe[b]	12.14 s	8.19 d	7.88 m	7.31 m	7.31 m	7.58 m	10.47 s	7.68 s	10.81 s	8.70 br s		6.86 dd	6.74 d	OMe, 3.78	1 690—1 665, 1 620, 1 609
-Me_4[a]		7.64 s	7.99 d	7.30 m	7.30 m	7.30 m		7.40 s		8.85 d		6.84 dd	6.65 d	OMe, 3.89 NMe, 3.83, 3.48, 3.23	1 665, 1 588, 1 580

220 MHz, δ values in [a] $CDCl_3$, [b] $(CD_3)_2SO$, [c] $(CD_3)_2CO$; if not mentioned m couplings were not resolved. [d] Assignments could be exchanged. [e] In KBr.



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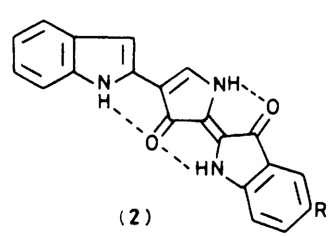

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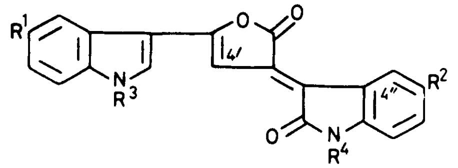

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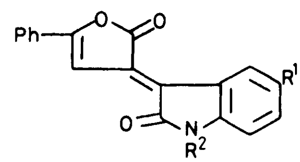

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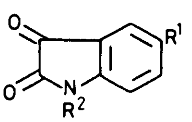

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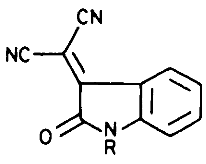

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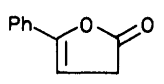

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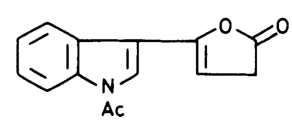

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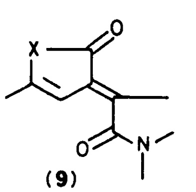

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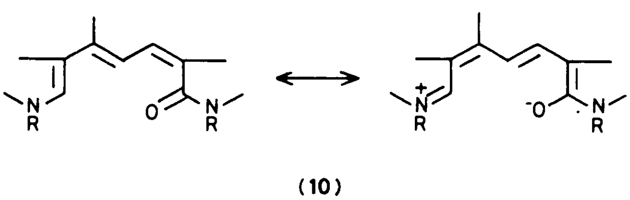

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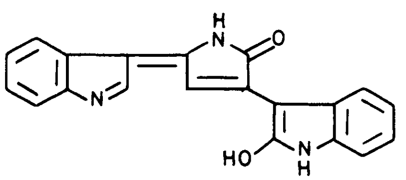

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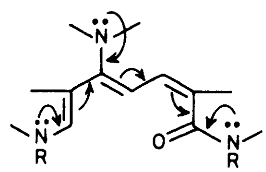

(12)

appear at δ 12.10—12.16 and the 'isatin NH' (N-1″) signals at δ 10.79—11.00, both somewhat lower in the analogous lactones (**3**), while the 'lactam NH' (N-1′) protons resonate between δ 10.47 and 10.88. Low-field signals from indole[4] and pyrrole[5] NH protons have been observed previously in $(CD_3)_2SO$ solution, especially when electron-withdrawing substituents are attached to the pyrrole ring, and attributed[6] to hydrogen-bonding with the solvent. However, in the violacein spectrum the three low-field signals are surprisingly sharp. In the spectra of simple isatins[5] (Table 3) the NH signals are broad (in the spectrum of the parent compound it was not observed) and in the somewhat similar isatin–pyrrole–indophenines[7] they are also broad.

In the 'isatin nucleus' of the lactams (**1**) and lactones (**3**) and (**4**) the aromatic proton signals are easily recognised by the characteristic d-t-t-d coupling pattern as in the spectra of isatin (Table 3). Comparison with the spectra of 5-bromo- and 5-nitro-isatin shows that the doublet at highest field derives from 7″-H (δ *ca.* 6.6—7.0, shifted to *ca.* 8.2—8.3 on *N*″-acetylation), and this is coupled to the triplet from 6″-H at δ *ca.* 7.2—7.3 (lower in the lactone series). It follows that the triplet seen close to δ 7 (shifted downfield by *N*″-acetylation) belongs to 5″-H, and the doublet usually observed at δ *ca.* 8.8—9.1 in the lactam series to 4″-H. In the lactone spectra the 4″-H signal is significantly downfield (1 p.p.m. or more) relative to that of 4-H in the simple isatins. This is attributed to the stereoelectronic effect of the lactone carbonyl group, as extension of the conjugated system of isatin by conversion into the dinitriles (**6**; R = H and Me) shifted the 4-H doublet downfield only by *ca.* 0.4 p.p.m.

In violacein and its derivatives the aromatic proton signals

Table 2. ^{1}H N.m.r. and i.r. spectra of violacein-related lactones of types (**3**) and (**4**)

Compound	Indole nucleus 1-H	2-H	4-H	5-H	6-H	7-H	Lactone ring 4'-H	Isatin nucleus 1''-H	4''-H	5''-H	6''-H	7''-H	Other	I.r.[f] (cm^{-1}) $\nu_{CO,C=C}$
(**3**; R^1 = R^2 = R^3 = R^4 = H)[b]	12.34 s	8.25 d	7.83 m	7.30 m	7.30 m	7.58 m	7.91 s	10.80 s	8.63 d	7.01 t	7.30 m	6.88 d		1 760, 1 689, 1 635, 1 616
-1''-Me[b]	12.35 s	8.21 s	7.79 s	7.30 m	7.30 m	7.56 m	7.90 m		8.62 d	7.03 t	7.30 m	6.96 d	NMe, 3.19	1 768, 1 664, 1 630, 1 608
-1,1''-Me$_2$[a]		8.05 s	8.03 m	7.37 m	7.37 m	6.80 d	7.73 s		8.84 d	7.09 t	7.37 m	6.81 d	NMe, 3.89, 3.30	1 764, 1 678, 1 620, 1 598
-1-Ac[b,d]		8.62 s	7.89 m	7.50 m	7.50 m	8.45 m	8.11 s	10.78 br s	8.49 d	7.03 td	7.34 td	6.88 d	NAc, 2.79	1 760, 1 708, 1 630, 1 615
-1,1''-Ac$_2$[a]		8.18 s	7.98 m	7.50 m	7.50 m	8.55 m	8.08 s		9.09 d	7.30 t	7.50 t	8.34 d	NAc, 2.80, 2.74	1 775, 1 720, 1 705sh, 1 624, 1 595
-1-Ac-1''-Me[a]		8.24 s	7.98 m	7.48 m	7.48 m	8.52 m	8.03 s		8.90 d	7.13 td	7.40 td	6.83 d	NAc, 2.72 NMe, 3.92	1 769, 1 706, 1 686, 1 621, 1 600
-5''-NO$_2$[b,d]	12.47 s	8.42 s	7.86 m	7.36 m	7.36 m	7.60 m	7.94 s	11.36 s	9.56 d		8.20 dd	7.06 d		1 765, 1 686, 1 619
-5''-OMe[b]	12.24 s	8.23[e] d	7.81 m	7.30 m	7.30 m	7.55 m	7.92 s	10.43 s	8.33[e] d		6.89 dd	6.75 d	OMe, 3.74	1 767, 1 698, 1 634
-1-Ac-5''-OMe[b,d]		8.64 s	7.92 m	7.52 m	7.52 m	8.47 m	8.15 s	10.58 s	8.40 d		6.98 dd	6.81 d	NAc, 2.80 OMe, 3.78	1 780, 1 705br, 1 628
-1-Ac-5''-Br[b,d]		8.68 s	7.90 m	7.50 m	7.50 m	8.68 s	8.12 s	10.92 br s	8.84 d		7.50 d	6.85 d	NMe, 2.79	1 768, 1 722, 1 695, 1 630, 1 610
						C_6H_5								
(**4**; R^1 = R^2 = H)[b]						7.88 2 H, br 7.57 3 H, br	8.06 s	10.91 s	8.68 d	7.02 t	7.35 t	6.89 d		1 770, 1 686, 1 655, 1 602
-*N*-Me[a]						7.76 2 H, m 7.42 3 H, m	7.99 s		8.76 d	7.00 t	7.25 t	6.90 t	NMe, 3.18	1 770, 1 690, 1 600
-1-Ac[a]						7.73 2 H, m 7.38 3 H, m	7.90 s		8.90 d	7.12 d	7.30 t	8.16 d	NAc, 2.60	1 786, 1 725, 1 705
-5''-Br[b]						7.86 2 H, m 7.56 3 H, m	8.00 s	11.00 s	8.78 s		7.46 d	6.80 d		1 777, 1 769, 1 690, 1 609
-*N*-Ac-5''-Br[a]						7.90 2 H, m 7.51 3 H, m	8.09 s		9.24 d		7.57 dd	8.23 d	NAc, 2.76	1 788, 1 720, 1 702, 1 608
-*N*-Me-5''-Br[a]						7.83 2 H, m 7.47 3 H, m	8.05 s		8.98 s		7.44 d	6.66 d	NAc, 3.24	1 778, 1 766, 1 690, 1 602
-5''-NO$_2$[b]						7.97 2 H, m 7.60 3 H, m	8.07 s	11.50 br s	9.60 d		8.30 dd	7.07 d		1 782, 1 699, 1 621
-*N*-Me-5''-NO$_2$[a]						7.93 2 H, m 7.54 3 H, m	8.13 s		9.84 d		8.36 dd	6.93 d	NMe, 3.39	1 784, 1 767, 1 702, 1 609
-*N*-Ac-5''-NO$_2$[a]						7.96 2 H, m 7.57 3 H, m	8.14 s		10.00 d		8.37 dd	8.53 d	NAc, 2.82	1 788, 1 725, 1 709, 1 609
(**4**; R^1 = R^2 = H, Me in place of Ph)[b,d,g]							7.22 d	10.60 br s	8.54 d	6.88 td	7.22 td	6.78 d	Me, 2.22	1 770, 1 692, 1 613

220 MHz, δ values in [a] $CDCl_3$, [b] $(CD_3)_2SO$, [c] $(CD_3)_2SO$; if not mentioned m couplings were not resolved, [d] at 50 °C. [e] Assignments could be exchanged. [f] In KBr. [g] 200 MHz Fourier transform.

from the indole nucleus are readily assigned from their chemical shifts and coupling constants (Table 1) but in the spectra of the deoxy- and iso-violaceins and the indolyl-lactones (**3**) the signals overlap and are not informative. 2-H signals were observed as doublets in a few spectra but otherwise they could be distinguished from the 4'-H singlets by slight broadening, or by

Table 3. ^{1}H N.m.r. and i.r. spectra of isatins

Compound	1-H	4-H	5-H	6-H	7-H	Other	I.r.[d] (cm^{-1}) $\nu_{CO,C=C}$
Isatin[b]	[c]	7.50d	7.07t	7.60t	6.92d		1 725, 1 610
-*N*-Ac[a]		7.27d	7.33t	7.70t	8.38d	NAc 2.73	1 780, 1 750, 1 710, 1 610
-*N*-Me[b]		7.59d	7.12t	7.61t	6.91d	NMe 3.26	
-5-Br[b]	11.16s	7.61s		7.72dd	6.86d		1 760sh, 1 750, 1 710, 1 611
-5-Br-*N*-Me[b]		7.73s		7.76dd	6.84d	NMe 3.30	
-5-NO_2[b]	11.70s	8.18s		8.42dd	7.08d		1 782, 1 750, 1 621

220 MHz, δ values in [a] $CDCl_3$, [b] $(CD_3)_2SO$; if not mentioned m couplings were not resolved. [c] Signal not observed. [d] In KBr.

Table 4. Electronic spectra[a] of violacein and related lactams and lactones [$\lambda_{max.}$/nm (log ε)]

Violacein (**1**; R^1 = OH, R^2 = R^3 = R^4 = H)	
-Me$_4$	274(4.37), 378(4.09), 568(4.33)
-Ac$_4$	266(4.32), 302(4.00), 560(4.28)
-*NO*-Ac$_2$[b]	521(4.25)
Deoxyviolacein (**1**; R^1 = R^2 = R^3 = R^4 = H)	548(4.27)
-Me$_3$	276(4.36), 380(4.03), 562(4.32)
-*NN*″-Ac$_2$-*N*′-Me	261(4.26) 546(4.16)
Isoviolacein (**1**; R^1 = R^3 = R^4 = H, R^2 = OH)	
-*O*-Me[b]	282(4.25), 375(3.91), 550(4.33)
-*ONN*′-Me$_3$	283(4.27), 390(3.86), 556(4.20)
-*ONN*″-Me$_3$	283(4.34), 370(3.86), 553(4.30)
-Me$_4$	287(4.31), 385(3.86), 562(4.21)
(**3**; R^1 = R^2 = R^3 = R^4 = H)	265(4.20), 307sh(3.83), 524(4.20)
-1″-Me	257—273(4.17), 315(3.51), 506(4.39)
-1,1″-Me$_2$	263(4.28), 320(3.56), 520(4.47)
-1-Ac	264(4.34), 310(3.89), 480(4.45)
-1,1″-Ac$_2$	260(4.37), 310(3.80), 510(4.51)
-1-Ac-1″-Me	280(4.28), 311(3.72), 480(4.42)
-5″-NO_2	536
-5″-OMe	277(4.29) 508(4.16)
-1-Ac-5″-Br	265(4.26), 303(3.74), 484(4.41)
-1-Ac-5″-NO_2	258(4.23), 305(3.93), 482sh(4.26), 512(4.27), 550sh(4.10)
-1-Me-1″-Ac	262(4.25) 565(4.50)
-1-Ac-5″-OMe	255(4.22), 275sh(4.21), 465(4.37), 487(4.37)
(**4**; R^1 = R^2 = H)	270(4.29), 445sh(4.34), 461(4.34)
-*N*-Me	272(4.33), 447(4.39), 470sh(4.34)
-*N*-Ac	272(4.25), 483(4.38)
-5″-Br	272(4.34), 450(4.39), 466sh(4.37)
-*N*-Ac-5″-Br	270(4.32), 472(4.40), 505sh(4.33)
-*N*-Me-5″-Br	273(4.28), 452(4.35), 476(4.29)
-5″-NO_2	261(4.18), 462(4.29), 500sh(4.19)
-*N*-Me-5″-NO_2	262(4.21), 446sh(4.30), 466(4.32)
-*N*-Ac-5″-NO_2	268(4.25), 340(3.68), 490(4.41)
(**4**; R^1 = R^2 = H, Me in place of phenyl)	264(4.08), 409(4.09)

In [a] $CHCl_3$, [b] $(CD_3)_2SO$.

the chemical shift in $(CD_3)_2SO$, or by sharpening on irradiation of the NH signal.

I.r. Spectra.—The conjugated phenyl-lactones (**4**) show carbonyl absorption (in KBr) at 1 770—1 788 (lactone), 1 686—1 709 (lactam), and 1 720—1 725 cm^{-1} (*N*-acetyl) (Table 2). The lactones (**3**; R^1 = R^3 = R^4 = Me, R^2 = Br and NO_2) and (**3**; R^1 = R^3 = R^4 = H, R^2 = NO_2) show an additional strong band at 1 766—1 769 cm^{-1} indicating that they exist in the solid state in two (rotameric?) forms. The phenyl-lactone (**7**) also shows (in KBr) two lactone carbonyl bands at 1 794 and 1 799 cm^{-1} as does the indolyl-lactone (**8**) at 1 803 and 1 782 cm^{-1}.

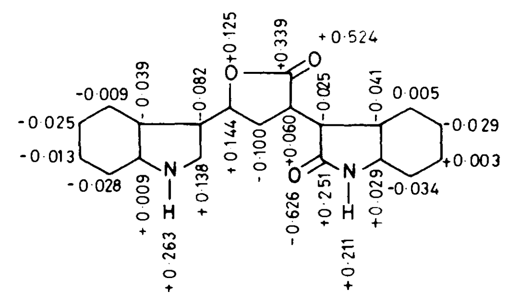

Figure 1. Total charge distribution in (**3**; R^1 = R^2 = R^3 = R^4 = H) (ground state)

Lactone (**8**) exhibits only one carbonyl band in chloroform solution at 1 800 cm^{-1}. Here again two forms are evidently present in the solid state. The lactone carbonyl absorptions of the indolyl-lactones (**3**) are similar to those of (**4**) with lactam absorption shifted to 1 644—1 689 cm^{-1}. Some lactam (**1**) i.r. spectra have been reported previously.[2]

Visible Spectra.—The possibility that the lactones (**3**) and (**4**) might have an indigoid-type chromophore (**9**) is ruled out by the data presented in Table 4. Thus replacing the phenyl group in (**4**; R^1 = R^2 = H) by a methyl leads to a hypsochromic shift of $\lambda_{max.}$ by 52 nm but replacement by an indol-3-yl substituent (**3**; R^1 = R^2 = R^3 = R^4 = H) results in a bathochromic shift of 63 nm, decreasing to 19 nm for the *N*-acetyl derivative (**3**; R^1 = R^2 = R^4 = H, R^3 = Ac). Further, a hypsochromic shift is observed[2] on passing from violacein to deoxyviolacein, and it is clear that the indole nucleus in (**1**) and (**3**) interacts strongly with the conjugated dilactam and lactone-lactam systems. Indeed substituents in the indole nucleus have a greater effect on $\lambda_{max.}$ then substituents in the 'isatin' nucleus. This leads to the conclusion that violacein and the lactones (**3**) have a merocyanine chromophore (**10**).

The merocyanine character of the lactone (**3**; R^1 = R^2 = R^3 = R^4 = H) is also evident from HMO calculations (Figure 1) which show further that the alternative merocyanine structure (**11**), conceivable for violacein although not possible for *N*-substituted derivatives, is less stable in terms of its π-energy. The benzenoid rings have a very small net charge and are not involved in the chromophoric system (as in indigo).[8] Similarly, the lactone bridge (excess of charge 0.059e) seems to be required only to stabilise the *trans*-butadiene unit. (A furan ring operates in a similar way in certain optical brighteners of the stilbene type.)[9]

In the carbon chain the alternating charge distribution and the low net charge are evident. Charge is transferred along the chain from the indole nitrogen (N-1) to the isatin carbonyl group which also receives charge from N-1″ thereby lowering its acceptor strength. Consequently acetylation of N-1″, which reduces its donor ability, results in a bathochromic shift. All the

Table 5. Observed[a] and calculated electronic spectra of methylated violaceins [$\lambda_{max.}$/nm (log ε)]

Tetramethylviolacein obs.	230sh(4.20), 241(4.20), 274(4.20), 297sh(3.83), 315(3.76), 363(3.82), 547(4.18)
calc.	232.9(4.00), 247.6(4.08), 285(4.00), 302(3.99), 317.5(3.39), 363(3.79), 549.3(4.38)
Tetramethylisoviolacein obs.[b]	281, 363, 528
calc.	283, 334, 547
Trimethyldeoxyviolacein obs.[b]	362, 405, 537
calc.	335, 411, 540

[a] In hexane. [b] Too insoluble in hexane to obtain ε values.

substituent effects on the visible spectra are easily understood in terms of a merocyanine system; electron donors which increase the donor strength of the indole nitrogen (N-1) (N-Me, 5-OH, 5-OMe, 7-X) or reduce the electron density at the isatin nitrogen (N-1″) (N″-Ac, 5″-NO_2) should result in a bathochromic shift as observed. The opposite is found, as expected, when electron donors and acceptors are exchanged (Table 4). Although the lactone bridge does not contribute to the chromophore, the lactams (**1**) absorb at longer wavelength than the corresponding lactones showing that the lactam nitrogen (N-1′) enhances the merocyanine character (**12**).

PPP calculations of the electronic spectra of the *N*-methylated lactams using parameters derived from the *X*-ray analysis of tetramethylviolacein gave good [tetramethylisoviolacein (**1**; R^1 = H, R^2 = OMe, R^3 = R^4 = Me, NMe in place of NH)] or excellent [tetramethylviolacein (**1**; R^1 = OMe, R^2 = H, R^3 = R^4 = Me, NMe in place of NH)] agreement with experimental results (Table 5). This is an additional confirmation of the merocyanine system.

Experimental

U.v. spectra were measured in $CHCl_3$ solution, i.r. spectra as KBr discs, and n.m.r. spectra in $CDCl_3$ solution unless otherwise stated. Merck silica gel GF_{254} was used for chromatographic separations.

Cultivation.—*A. luteviolacea* was grown on a sea water–yeast–peptone medium,[10] as described, in 1 l Roux bottles. After 2—3 days at room temperature the bacterial cells were removed and the suspension sterilised with chloroform and stored at 4 °C. The bacterial suspensions from 120 Roux bottles were combined, adjusted to pH 6, filtered through a layer of Celite, and washed with water. The residue was exhaustively extracted with acetone, followed by moist ethyl acetate, until cells and filtrate were colourless. The combined extracts were combined and evaporated leaving an aqueous residue. This was diluted with an equal volume of water and the crude pigments filtered off and washed repeatedly with benzene [to extract the active component(s)]. The pigment mixture (200 mg) was suspended in acetone, transferred to a column (2 × 40 cm) of acidic alumina, and eluted with acetone–methanol (95:5). Deoxyviolacein was eluted first. The eluates were evaporated, the residues redissolved in ethyl acetate, shaken with 0.5M-hydrochloric acid, dried, and evaporated: yields, 1 mg deoxyviolacein and 164 mg violacein. The two samples were identical in all respects with authentic materials as were their tetramethyl derivatives.

Violacein (**1**; R^1 = OH, R^2 = R^3 = R^4 = H) forms blue-black crystals (from acetone–methanol), m.p. >290 °C (Found: M^+, 343.0936. $C_{20}H_{13}N_3O_3$ requires *M*, 343.0956); δ(C_5D_5N) 13.02, 11.87, and 11.66 (each 1 H, NH); *m/z* 343 (M^+, 100%), 315 (8), 172 (13), 133 (50), 129 (10), and 104 (12). The *NO*-diacetyl derivative was obtained by dissolving violacein (60 mg) in acetic anhydride (1 ml) and pyridine (0.5 ml) in an ultrasonic bath. Reaction was complete in 1 min, giving black-brown needles (from chloroform–methanol) (65 mg, 73%), m.p. >290 °C (Found: C, 65.4; H, 4.3. $C_{24}H_{17}N_3O_5$ requires C, 67.4; H, 4.0%); *m/z* 427 (M^+, 55%), 385 (92), 343 (100), 315 (20), and 133 (25).

Hydrolysis of Tetramethylviolacein.—A suspension of tetramethylviolacein (100 mg) in methanol (20 ml) and M-sodium hydroxide (5 ml) was heated to 60 °C until dissolution was complete. The yellow solution was acidified, extracted with ethyl acetate, and the crude acid adsorbed onto acid-washed silica gel (15 g, washed with M-HCl and air dried) which was heated at 100 °C for 15 h. Extraction with chloroform–methanol yielded the *lactone* which was purified by repeated p.l.c. on silica in chloroform–methanol (98:2) to give (**3**; R^1 = OMe, R^2 = H, R^3 = R^4 = Me) as a dark purple solid (2 mg, 2%), m.p. *ca.* 290 °C (decomp.) (Found: M^+, 386.1262. $C_{23}H_{18}N_2O_4$ requires *M*, 386.1265); $\lambda_{max.}$ 265 and 538 nm; $\nu_{max.}$ 1 770, 1 680, 1 629, and 1 605 cm^{-1}; *m/z* 386 (M^+, 100%), 315 (5), 275 (13), 259 (17), 218 (24), 193 (17), 188 (84), 165 (50), and 110 (55).

Degradation of Tetramethylviolacein.—(a) *With zinc.*[3] An intimate mixture of tetramethylviolacein (100 mg) and zinc dust (1 g) was carefully heated over a flame until distillation ceased. The products from three pyrolyses were combined and separated by p.l.c. on silica in benzene into four mobile components, which were each further purified by sublimation in a high vacuum at 150 °C to give (i) impure 1-methylindole (1 mg) as an oil, red-violet with Ehrlich's reagent, *m/z* 131; (ii) 5-methoxy-1-methylindole, leaflets (22 mg), m.p. 101 °C (lit.,[3] 104 °C) (Found: M^+, 161.0830. Calc. for $C_{10}H_{11}NO$: *M*, 161.0840); δ 7.19 (1 H, d, *J* 8 Hz, 7-H), 6.87 (1 H, dd, *J* 8 and 2 Hz, 6-H), 7.08 (1 H, d, *J* 2 Hz, 4-H), 6.39 (1 H, d, *J* 2 Hz, 3-H), 3.82 (3 H, s, OMe), and 3.72 (3 H, s, NMe); *m/z* 161 (M^+, 100%), 146 (70), 131 (7), and 118 (40); (iii) 5-methoxyindole (7 mg), an oil, red-violet with Ehrlich's reagent (Found: M^+, 147.0684. Calc. for C_9H_9NO: *M*, 147.0684); *m/z* 147 (M^+, 100%), 132 (62), 118 (10), and 104 (35); (iv) 1-methyloxindole, needles (45 mg), m.p. 86 °C (lit.,[11] 89 °C) (Found: M^+, 147.0681. Calc. for C_9H_9NO: *M*, 147.0684); δ 7.26 (1 H, t, *J* 8 Hz, 6-H), 7.19 (1 H, d, *J* 8 Hz, 7-H), 7.01 (1 H, t, *J* 8 Hz, 5-H), 6.79 (1 H, d, *J* 8 Hz, 4-H), 3.48 (2 H, s, CH_2), and 3.18 (3 H, s, Me); *m/z* 147 (M^+, 100%), 132 (22), 118 (73), and 104 (10); it gave a very weak reaction with Ehrlich's reagent. Heating tetramethylviolacein without zinc dust gave nearly the same result; 60 mg yielded 6 mg 5-methoxy-*N*-methylindole and 3 mg *N*-methyloxindole.

(b) *With ozone.* A solution of tetramethylviolacein (200 mg) in chloroform (200 ml) was ozonised at −10 °C until it became orange. After evaporation the residue was purified by p.l.c. on silica in chloroform–methanol (99:1); the yellow band, which gave a blue colour with pyrrolidine–acetic acid, was eluted and sublimed at 200 °C and 0.1 Torr to give oily orange needles. Washing with a few drops of carbon tetrachloride yielded *N*-methylisatin (11 mg), m.p. 133 °C (lit.,[12] 134 °C); *m/z* 161 (M^+, 95%), 133 (50), 132 (14), 105 (90), 104 (100), 92 (15), and 78 (50).

Deoxyviolacein (**1**; $R^1 = R^2 = R^3 = R^4 = H$).—This formed blue-black crystals (from ethyl acetate), m.p. >290 °C (Found: M^+, 327.1003. Calc. for $C_{20}H_{13}N_3O_2$: *M*, 327.1008); *m/z* 327 (M^+, 100%), 299 (15), 270 (6), 255 (6), 143 (11), and 91 (10). Identical synthetic material was obtained following refs. 2 and 13 with some modifications, several new compounds were obtained. In the final step, conversion of the lactone (**3**; $R^1 = R^2 = R^4 = H$, $R^3 = Ac$) into deoxyviolacein, the reaction with ammonia must be carried out in boiling ethanol and not in the cold as described.[14]

The crude product from the reaction of indolylmagnesium iodide [from indole (32 g) and γ-methoxycarbonylpropionyl chloride (40 g)], was dissolved in methanol (50 ml). On keeping, crystals deposited, possibly a tetrameric indole (M^+, 464). Evaporation of the mother liquor left a mixture of indole derivatives which were separated on a column of silica, in chloroform into indole (band 1, least polar), the required methyl γ-indol-3-yl-γ-oxobutyrate (band 5), while band 2 yielded *methyl γ-indol-1-yl-γ-oxobutyrate*, prisms or needles, m.p. 82 °C (from benzene) (1.05 g, 1.5%) (Found: C, 67.2; H, 5.7; N, 6.2. $C_{13}H_{13}NO_3$ requires C, 67.5; H, 5.7; N, 6.05%); $\nu_{max.}$(KBr) 1 706 and 1 530 cm^{-1}; δ 8.42 (1 H, d, *J* 8 Hz, 7-H), 7.56 (1 H, d, *J* 8 Hz, 4-H), 7.48 (1 H, d, *J* 2 Hz, 2-H), 7.35 and 7.26 (each 1 H, t, *J* 8 Hz, 5- and 6-H), 6.65 (1 H, d, *J* 2 Hz, 3-H), 3.73 (3 H, s, OMe), 3.25 and 2.84 (each 2 H, t, *J* 7 Hz, 2 × CH_2), and band 3 gave *dimethyl indole-1,3-di-γ-[γ-oxobutyrate)*, needles (1.26 g, 1.2%), m.p. 162 °C (from chloroform–methanol) (Found: C, 62.5; H, 5.6; N, 4.1. $C_{18}H_{19}NO_6$ requires C, 62.5; H, 5.55; N, 4.05%); $\nu_{max.}$ 1 735sh, 1 719, and 1 664 cm^{-1}; δ 8.31 (1 H, m, 4- and 7-H), 8.19 (1 H, s, 2-H), 7.38 (2 H, m, 5- and 6-H), 3.73 and 3.70 (each 3 H, s, OMe), 3.30, 3.23, 2.87, and 2.78 (each 2 H, t, *J* 7 Hz, 4 × CH_2). Band 4 yielded an unidentified compound, m.p. 240 °C.

4-(1-Acetylindol-3-yl)-4-hydroxybut-3-enoic lactone (**8**) was obtained as described;[13] rapid crystallisation from hot methanol gave pale yellow needles, m.p. 162 °C (lit.,[13] 155 °C), δ 8.48 (1 H, d, *J* 8 Hz, 7-H), 7.63 (1 H, s, 2-H), 7.68 (1 H, d, *J* 8 Hz, 4-H), 7.40 and 7.32 (each 1 H, t, *J* 8 Hz, 5- and 6-H), 5.79br (1 H, s, 4′-H), 3.42br (2 H, s, CH_2), and 2.58 (3 H, s, Ac).

Derivatives of 4-(*Indol*-3-*yl*)-4-*hydroxy*-2,3′-*oxindolylidene-but*-3-*enoic Lactone* (**3**; $R^1 = R^2 = R^3 = R^4 = H$).—(*a*) The lactone (**3**; $R^1 = R^2 = R^4 = H$, $R^3 = Ac$)[13] (110 mg) was converted into the *diacetyl derivative* (**3**; $R^1 = R^2 = H$, $R^3 = R^4 = Ac$) by heating with acetic anhydride (5 ml) and pyridine (2 ml) on a steam-bath for 6 h. It crystallised on cooling as black needles, m.p. 280—282 °C (decomp.) (Found: C, 69.6; H, 4.0; N, 6.8. $C_{24}H_{16}N_2O_5$ requires C, 69.9; H, 3.9; N, 6.8%).

(*b*) The lactone (**3**; $R^1 = R^2 = R^4 = H$, $R^3 = Ac$) (1 g) was converted into (**3**; $R^1 = R^2 = R^3 = R^4 = H$) by suspension in 90% ethanol (100 ml) into which ammonia was passed for 1 h, or by warming in methanol (100 ml) at 40 °C with M-sodium hydroxide (5 ml). The bromine-coloured solution was reduced to 50 ml *in vacuo*, diluted with water, and acidified (HCl) to give a dark precipitate (470 mg). This product (300 mg) in ethyl acetate was absorbed onto acid-washed silica gel (10 g) and heated at 100 °C for 15 h. Extraction with chloroform–methanol gave the desired *lactone*, black-brown prisms (79%), m.p. >290 °C (Found: C, 73.0; H, 3.9; N, 8.2%; M^+, 328.0846. $C_{20}H_{12}N_2O_3$ requires C, 73.15; H, 3.7; N, 8.5%; *M*, 328.0848); *m/z* 328 (M^+, 95%), 300 (10), 272 (5), 271 (5), 164 (10), 144 (100), and 116 (15).

(*c*) A suspension of the lactone (**3**; $R^1 = R^2 = R^3 = R^4 = H$) (200 mg) and anhydrous potassium carbonate (5 g) in acetone (50 ml) was boiled under reflux with dimethyl sulphate (1 ml) for 15 h, and filtered. Evaporation of the yellow filtrate left a residue which was boiled in methanol (30 ml) and M-sodium hydroxide (20 ml) for 2 min, cooled, acidified, and extracted with ethyl acetate. The crude product was adsorbed onto acid-washed silica gel (10 g) and heated at 100 °C for 15 h. The silica was then extracted with chloroform–methanol and the extract was further purified by p.l.c. on silica in chloroform. The main violet zone yielded the *dimethyl derivative* (**3**; $R^1 = R^2 = H$, $R^3 = R^4 = Me$), dark brown needles (45 mg, 21%), m.p. >290 °C (from chloroform–methanol) (Found: C, 73.9; H, 4.5; N, 8.25. $C_{22}H_{16}N_2O_3$ requires C, 74.15; H, 4.5; N, 7.9%).

(*d*) A solution of 4-(1-acetylindol-3-yl)-4-hydroxybut-3-enoic lactone (0.5 g) and *N*-methylisatin[12] (0.3 g) in methanol (30 ml) and pyridine (0.3 ml) was refluxed for 30 min. The product was filtered off, washed with methanol, and crystallised from chloroform–methanol to give the *acetyl-methyl derivative* (**3**; $R^1 = R^2 = H$, $R^3 = Ac$, $R^4 = Me$) in brown-red needles (300 mg, 38%), m.p. 275—280 °C (decomp.) (Found: C, 71.9; H, 4.1; N, 7.1. $C_{23}H_{16}N_2O_4$ requires C, 71.9; H, 4.2; N, 7.3%).

(*e*) A suspension of the foregoing acetyl-methyl derivative (200 mg) in methanol (20 ml) and M-sodium hydroxide (1 ml) was warmed to 40 °C until dissolution was complete. The orange solution was diluted with water (50 ml) and acidified with acetic acid. The crude acid was filtered off, suspended in methanol in an ultrasonic bath for 5 min, and again collected to yield greenish yellow needles (130 mg), m.p. 170 °C (decomp.), of a mixture (*cis-trans?*) of 4-(indol-3-yl)-4-oxo-2,3′-(1-methyloxindolylidene)butyric acids (Found: C, 69.8; H, 4.6; N, 7.6. $C_{21}H_{16}N_2O_4$ requires C, 70.1: H, 4.5; N, 7.8%); δ[$(CD_3)_2SO$] (main isomer first) 12.08/12.15 (1 H, s, NH), 8.44/8.49 (1 H, d, *J* 3 Hz, 2-H), 8.11 (1 H, d, *J* 8 Hz, 7-H), 7.6—6.9 (7 H, m, ArH), 4.93/4.51 (2 H, s, CH_2), and 3.66 (3 H, s, Me). This mixture (120 mg) was adsorbed from ethyl acetate onto acid-washed silica gel (20 g), heated at 100 °C for 12 h, and then extracted with chloroform–methanol (98:2). After addition of methanol to the extract, and concentration, it deposited the *monomethyl derivative* (**3**; $R^1 = R^2 = R^3 = H$, $R^4 = Me$) as black-brown needles (108 mg, 95%), m.p. 290 °C (Found: C, 73.6; H, 4.2; N, 8.4. $C_{21}H_{14}N_2O_3$ requires C, 73.7; H, 4.1; N, 8.2%); *m/z* 342 (M^+, 55%), 314 (8), 313 (5), 298 (6), 286 (6), 171 (9), 144 (100), and 116 (20).

(*f*) A suspension of γ-(1-methylindol-3-yl)-γ-oxobutyric acid[14] (200 mg) and isatin (200 mg) in acetic anhydride (30 ml) was heated under reflux for 2 h to give a blue-violet solution. After evaporation *in vacuo*, soluble compounds were removed by washing with methanol (2 ml) and the residue was purified by p.l.c. on silica in chloroform to give the *acetyl-methyl derivative* (**3**; $R^1 = R^2 = H$, $R^3 = Me$, $R^4 = Ac$), black-brown needles (4 mg, 1%), sublimes at *ca.* 250 °C, m.p. >290 °C (Found: M^+, 384.1095. $C_{23}H_{16}N_2O_4$ requires *M*, 384.1110); $\lambda_{max.}$ 262 and 565 nm (log ε 4.25 and 4.50); $\nu_{max.}$ 1 775, 1 705, 1 625, and 1 593 cm^{-1}; *m/z* 384 (M^+, 40%), 342 (55), 325 (5), 314 (7), 313 (10), 286 (8), 285 (8), 158 (100), 130 (20), and 103 (15).

Isoviolacein Methyl Ether (**1**; $R^1 = R^3 = R^4 = H$, $R^2 = OMe$).—4-(1-Acetylindol-3-yl)-4-hydroxybut-3-enoic lactone (1 g) and 5-methoxyisatin[15] (0.9 g) were condensed in methanol–pyridine as above. The *lactone* (**3**; $R^1 = H$, $R^2 = OMe$, $R^3 = Ac$, $R^4 = H$) separated as dark brown needles (1.3 g, 72%), m.p. >290 °C (Found: C, 69.1; H, 4.2; N, 7.2. $C_{23}H_{16}N_2O_5$ requires C, 69.0; H, 4.0; N, 7.0%); *m/z* 400 (M^+, 35%), 358 (55), 144 (95), 116 (10), 83 (8), and 44 (100). Hydrolysis of this acetyl derivative (300 mg) in the usual way and cyclisation on silica gel gave the lactone (**3**; $R^1 = R^3 = R^4 = H$, $R^2 = OMe$). Extraction with ethanol (Soxhlet) gave green, glistening crystals (155 mg, 58%), m.p. >290 °C (Found: C, 70.45; H, 3.85; N, 7.8. $C_{21}H_{14}N_2O_4$ requires C, 70.4; H, 3.95; N, 7.8%). The same acetyl derivative (500 mg) was suspended in boiling 90% ethanol (50 ml) while ammonia was passed through for 4 h. The brown, slimy precipitate was filtered off, washed with water until the filtrate was colourless, then with M-hydrochloric acid, and water. The residue (190 mg) was dissolved in hot pyridine and chloroform was slowly added to precipitate the lactam which was collected and washed with chloroform. *Isoviolacein methyl ether* was obtained as a black, amorphous powder, m.p. >290 °C (Found: C, 70.6; H, 4.4; N, 11.4. $C_{21}H_{15}N_3O_3$ requires C, 70.6; H, 4.2; N, 11.75%); *m/z* 357 (M^+, 100%), 329 (3), 314 (12), 286 (10), 212 (12), 179 (10), 157 (7), 143 (12), 117 (10), and 44 (47).

Methylation of Isoviolacein Methyl Ether.—Crude isoviolacein methyl ether (from 250 mg acetyl-lactone as described above) was dissolved in dimethyl sulphoxide (10 ml) and methyl iodide (0.5 ml) and shaken violently with 40% sodium hydroxide (3 drops) for 30 s. The solution turned from blue-violet to green and then to blue again. The solution was immediately diluted with water (50 ml) and acidified with hydrochloric acid. The violet precipitate was collected and separated by p.l.c. on silica in chloroform–methanol (98:2) into four main zones. Zone 1 (highest R_F) yielded *tetramethylisoviolacein* (**1**; R^1 = H, R^2 = OMe, R^3 = R^4 = Me, NMe in place of NH), violet needles (40 mg), m.p. 235 °C (softening from 180 °C) (from chloroform–methanol) (Found: C, 71.8; H, 5.5; N, 10.7%; M^+, 399.1582. $C_{24}H_{21}N_3O_3$ requires C, 72.15; H, 5.3; N, 10.5%; *M*, 399.1583); *m/z* 399 (M^+, 100%), 384 (6), 370 (5), 356 (38), 225 (16), 199 (11), 178 (8), 171 (13), and 156 (14). Zone 2 did not give useful material but the blue-violet zone 3 yielded the *trimethyl derivative* (**1**; R^1 = R^4 = H, R^2 = OMe, R^3 = Me, NMe in place of NH), dark brown, glistening needles (7 mg), m.p. 268 °C (from chloroform–methanol) (Found: M^+, 385.1428. $C_{23}H_{19}N_3O_3$ requires *M*, 385.1426); $\nu_{max.}$ 1 665 and 1 590 cm^{-1}; *m/z* 385 (M^+, 100%), 356 (5), 342 (17), 225 (8), 192 (12), 171 (15), 158 (10), 156 (15), and 83 (77). The violet zone 4 afforded the *trimethyl derivative* (**1**; R^1 = H, R^2 = OMe, R^3 = R^4 = Me), dark brown needles (8 mg), m.p. 257—260 °C (from chloroform–methanol) (Found: M^+, 385.1432. $C_{23}H_{19}N_3O_3$ requires *M*, 385.1426); $\nu_{max.}$(KBr) 1 665, 1 618, and 1 590 cm^{-1}; *m/z* 385 (M^+, 100%), 342 (20), 229 (13), 192 (16), 171 (15), 158 (12), and 130 (10).

Methyl 4-Methoxy-2,3'-(1-methyloxindolylidene)-4-phenylbut-3-enoate.—This was obtained instead of (**4**; R^1 = H, R^2 = Me) on methylation of the parent lactone (**4**; R^1 = R^2 = H). The lactone (**4**; R^1 = R^2 = H)[14] (500 mg) in acetone (50 ml) was boiled under reflux for 15 h with dimethyl sulphate (1 ml) and anhydrous potassium carbonate (2 g). After filtration and evaporation to dryness, the residue was dissolved in methanol. On keeping, the *ester* separated as pale yellow parallelepipeds (65 mg, 10%), m.p. 132 °C, solidifying, and remelting at 152 °C (Found: C, 72.4; H, 5.4%; M^+, 349.1315. $C_{21}H_{19}NO_4$ requires C, 72.2; H, 5.5%; *M*, 349.1314); δ 8.02 (1 H, s, –CH=), 7.56 and 7.37 (5 H, m, C_6H_5), 7.18 (2 H, m, ArH), 6.92 (1 H, t, ArH), 6.73 (1 H, d, *J* 8 Hz, ArH), 3.95, 3.52, 3.14 (each 3 H, s, Me); *m/z* 349 (M^+, 33%), 318 (100), 230 (37), 289 (40), 275 (20), 274 (14), 243 (25), 105 (50), and 77 (55).

4-Hydroxy-2,3'-(5-nitro-oxindolylidene)-4-phenylbut-3-enoic Lactone (**4**; R^1 = NO_2, R^2 = H).—A suspension of crude phenyl-lactone (**7**) (2.3 g) and 5-nitroisatin (2.0 g) in methanol (30 ml) and pyridine (0.4 ml) was boiled under reflux for 30 min. The product was filtered off, washed with methanol, and crystallised from pyridine as brown-red prisms (after washing with acetone, brick-red needles), m.p. >290 °C (2.7 g, 56%) (Found: C, 64.3; H, 3.1; N, 8.2. $C_{18}H_{10}N_2O_5$ requires C, 64.7; H, 3.0; N, 8.4%); $\lambda_{max.}$ 261, 462, and 500sh nm (log ε 4.18, 4.29, and 4.19). Both crystal forms gave the same i.r. (KBr) spectra. The *N-acetyl derivative*, obtained by heating this lactone with acetic anhydride and pyridine for 12 h, formed deep brown-red needles with a metallic, green sheen, m.p. 283 °C (Found: C, 63.9; H, 3.3; N, 7.4. $C_{20}H_{12}N_2O_6$ requires C, 63.8; H, 3.2; N, 7.45%); $\lambda_{max.}$ 268, 340, and 490 nm (log ε 4.25, 3.68, and 4.41). The *N-methyl derivative* was prepared by methylation of the above lactone (**4**; R^1 = NO_2, R^2 = H) (0.5 g) with Me_2SO_4–K_2CO_3–Me_2CO in the usual way. The filtrate was evaporated to dryness, the residue was boiled for 2 min with methanol (20 ml) and M-sodium hydroxide (5 ml), acidified, and extracted with chloroform. Evaporation gave a residue which was adsorbed onto acid-washed silica gel (20 g) and heated for 12 h at 100 °C. The lactone was eluted with chloroform–methanol (19:1) which, after concentration, yielded rust-brown needles (160 mg, 31%), m.p. 285—288 °C (Found: C, 65.2; H, 3.1; N, 8.0. $C_{19}H_{12}N_2O_5$ requires C, 65.5; H, 3.5; N, 8.05%); $\lambda_{max.}$ 262, 446sh, and 466 nm (log ε 4.21, 4.30, and 4.32).

4-Hydroxy-2,3'-(5-bromo-oxindolylidene)-4-phenylbut-3-enoic Lactone (**4**; R^1 = Br, R^2 = H).—This was prepared as for the above nitro-analogue. It crystallised from pyridine–methanol in black needles (2.9 g, 53%), m.p. >290 °C (Found: C, 58.5; H, 2.6; N, 3.8. $C_{18}H_{10}BrNO_3$ requires C, 58.7; H, 2.75; N, 3.8%); $\lambda_{max.}$ 272, 450, and 466sh nm (log ε 4.34, 4.39, and 4.37). The *N-acetyl derivative* formed bright coppery needles, m.p. 244—246 °C (Found: C, 58.3; H, 3.0; N, 3.4. $C_{20}H_{12}BrNO_4$ requires C, 58.55; H, 2.95; N, 3.4%); $\lambda_{max.}$ 270, 472, and 505 nm (log ε 4.32, 4.40, and 4.33). The *N-methyl derivative*, obtained as above, formed black needles, m.p. 228 °C (from chloroform–methanol) (Found: C, 59.6; H, 3.3; Br, 20.8; N, 3.6. $C_{19}H_{12}BrNO_3$ requires C, 59.7; H, 3.15; Br, 20.9; N, 3.65%); $\lambda_{max.}$ 273, 452, and 476 nm (log ε 4.28, 4.35, and 4.29).

4-Hydroxy-4-methyl-2,3'-oxindolylidenebut-3-enoic Lactone (**4**; R^1 = R^2 = H, Me *in place of* Ph).—A solution of 4-hydroxypent-3-enoic lactone (0.7 g) and isatin (1.0 g) in pyridine (10 ml) was boiled under reflux for 5 min and then diluted with water (50 ml). The precipitate was collected and crystallised from chloroform–methanol to give the *lactone* as ruby-red cubes (120 mg, 8%), m.p. 233 °C (Found: C, 68.6; H, 4.05; N, 6.0. $C_{13}H_9NO_3$ requires C, 68.7; H, 4.0; N, 6.15%).

4-(1-Acetylindol-3-yl)-4-hydroxy-2,3'-(5-nitro-oxindolylidene)but-3-enoic Lactone (**3**; R^1 = R^4 = H, R^2 = NO_2, R^3 = Ac).—4-(1-Acetylindol-3-yl)-4-hydroxybut-3-enoic lactone[13] (0.5 g) was condensed with 5-nitroisatin (0.5 g) in the usual way. The product crystallised from pyridine–methanol in dark red needles (250 mg, 29%), m.p. >290 °C (Found: C, 63.3; H, 3.1; N, 9.8. $C_{22}H_{13}N_3O_6$ requires C, 63.6; H, 3.15; N, 10.1%); $\lambda_{max.}$ 258, 305, 482sh, 512, and 550 nm (log ε 4.23, 3.93, 4.25, 4.27, and 4.10). This was hydrolysed with aqueous methanolic sodium hydroxide and recyclised, as above, to give *4-(indol-3-yl)-4-hydroxy-2,3'-(5-nitro-oxindolylidene)but-3-enoic lactone* (**3**; R^1 = R^3 = R^4 = H, R^2 = NO_2) as black needles (77%), m.p. >290 °C (from chloroform–methanol) (Found: C, 64.1; H, 3.0: N, 10.9. $C_{20}H_{11}N_3O_3$ requires C, 64.3; H, 3.0; N, 11.25%); $\lambda_{max.}$ 536 nm.

4-(1-Acetylindol-3-yl)-4-hydroxy-2,3'-(5-bromo-oxindolylidene)but-3-enoic Lactone (**3**; R^1 = R^4 = H, R^2 = Br, R^3 = Ac).—Prepared as for the nitro-analogue from 5-bromoisatin, this formed black needles (91%), m.p. >290 °C (Found: C, 60.4; H, 3.1; N, 6.2. $C_{22}H_{13}BrN_2O_4$ requires C, 60.7; H, 3.0; N, 6.4%); $\lambda_{max.}$ 265, 303, and 484 nm (log ε 4.26, 3.74, and 4.41).

4-Hydroxy-4-phenylbut-3-enoic Lactone.[16]—This gave δ 7.59 (2 H, m, ArH), 7.37 (3 H, m, ArH), 5.78 (1 H, m, 3'-H), and 3.40 (2 H, d, *J* 2 Hz, CH_2).

3-Dicyanomethyleneoxindole (**6**; R = H).—To a solution of malonodinitrile (1.7 g) in methanol (60 ml) was added isatin (3.7 g) and pyridine (0.3 ml). The crystalline product was collected

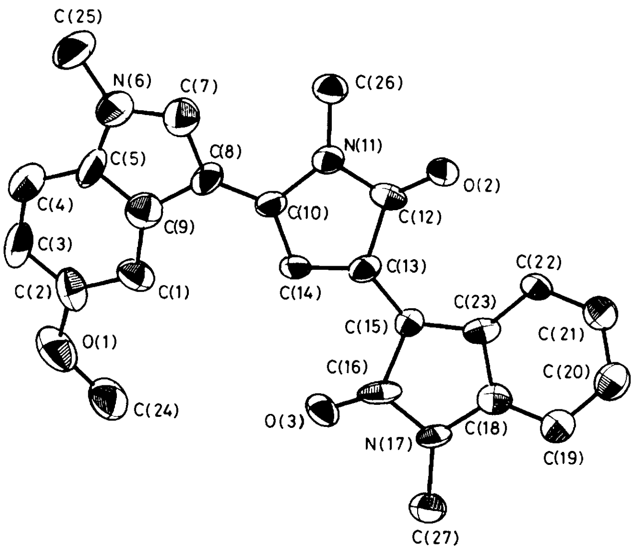

Figure 2. Molecular structure of tetramethylviolacein

after 1 h and more was obtained by concentration as copper-red needles (86%), m.p. 243 °C (Found: C, 67.7; H, 2.6; N, 21.6. $C_{11}H_5N_3O$ requires C, 67.7; H, 2.6; N, 21.5%); $\lambda_{max.}$ 265, 355, and 487 nm (log ε 4.18, 4.06, and 3.18); $\nu_{max.}$ 3 240, 2 215, 1 720, 1 708, and 1 610 cm^{-1}; δ[$(CD_3)_2SO$] 11.24 (1 H, s, NH), 7.87 (1 H, d, *J* 8 Hz, 4-H), 7.59 (1 H, t, *J* 8 Hz, 6-H), 7.12 (1 H, t, *J* 8 Hz, 5-H), and 6.94 (1 H, d, *J* 8 Hz, 7-H). This compound (0.5 g) was suspended in acetic anhydride (10 ml) and pyridine (3 ml) was added. After 5 min the solution was carefully hydrolysed by addition of methanol and water. The *acetyl derivative* crystallised as orange-yellow needles, (86%), m.p. 181 °C (Found: C, 65.9; H, 3.1; N, 17.9. $C_{13}H_7N_3O_2$ requires C, 65.8; H, 3.0; N, 17.7%); $\lambda_{max.}$ 266, 362, and 446 nm (log ε 4.03, 4.07, and 3.30); $\nu_{max.}$ 2 220, 1 758, 1 710, and 1 590 cm^{-1}; δ ($CDCl_3$) 8.36 (1 H, d, *J* 8 Hz, 7-H), 8.29 (1 H, d, *J* 8 Hz, 4-H), 7.70 (1 H, t, *J* 8 Hz, 6-H), 7.38 (1 H, t, *J* 8 Hz, 5-H), and 2.78 (3 H, s, Ac).

*3-Dicyanomethylene-*1-*methyloxindole* (**6**; R = Me).—Prepared from malonodinitrile (0.66 g) and *N*-methylisatin (1.61 g) as above, this gave brown needles (47%), m.p. 235—238 °C (Found: C, 68.7; H, 3.6; N, 19.8. $C_{12}H_7N_3O$ requires C, 68.9; H, 3.4; N, 20.1%); $\lambda_{max.}$ 270, 354, and 502 nm (log ε 4.22, 4.02, and 3.04); $\nu_{max.}$(KBr) 2 210, 1 713, 1 603, and 1 590 cm^{-1}; δ 8.09 (1 H, d, *J* 8 Hz, 4-H), 7.58 (1 H, t, *J* 8 Hz, 6-H), 7.13 (1 H, t, *J* 8 Hz, 5-H), 6.86 (1 H, d, *J* 8 Hz, 7-H), and 3.26 (3 H, s, Me).

HMO calculations were made using the combined programs of Weckherlin and Heilbronner [17] in the modification of Knieriem, University of Göttingen.

PPP calculations were done using the PCSCF program of Wild, ETH, Zürich, in the 3D modification of Knieriem, 1982 version, University of Göttingen.

Crystal Data.—Tetramethylviolacein, $C_{24}H_{21}N_3O_3$, M = 399.4, space group $P2_1/n$, a = 14.253(16), b = 5.625(6), c = 29.658(40) Å, β = 124.71(9)°, U = 1 954.6 $Å^3$, Z = 4, $F(000)$ = 840, D_c = 1.36 g cm^{-3}, μ(Mo-K_α) = 0.53 cm^{-1}.

Crystallographic Measurements.—Intensity measurements were obtained from a Nicolet P3 automated diffractometer using monochromatized Mo-K_α radiation. Integrated relative intensities for 1 566 independent reflexions with $2\theta < 50°$ were measured by the θ—2θ scan method. 894 Reflexions had $I > 3\sigma(I)$.

Table 6. Fractional atomic co-ordinates (× 10^4) with e.s.d.s

	x	y	z
O(1)	−4 151(7)	−4 125(20)	−0 419(3)
O(2)	1 311(6)	0 297(15)	2 908(3)
O(3)	−2 588(7)	3 567(16)	1 132(3)
C(1)	−2 232(9)	−3 346(23)	0 378(5)
C(2)	−2 983(11)	−4 515(26)	−0 110(5)
C(3)	−2 636(14)	−6 226(29)	−0 323(6)
C(4)	−1 504(14)	−6 906(29)	−0 041(6)
C(5)	−0 729(13)	−5 716(25)	0 454(6)
N(6)	0 450(10)	−6 055(21)	0 822(5)
C(7)	0 806(11)	−4 597(27)	1 247(5)
C(8)	−0 075(10)	−3 262(25)	1 206(5)
C(9)	−1 058(11)	−4 033(23)	0 662(5)
C(10)	−0 040(9)	−1 635(21)	1 562(5)
N(11)	0 837(7)	−1 801(18)	2 143(4)
C(12)	0 684(9)	−0 007(23)	2 419(5)
C(13)	−0 396(9)	1 258(21)	1 975(5)
C(14)	−0 752(10)	0 146(21)	1 483(5)
C(15)	−0 876(8)	3 120(21)	2 087(4)
C(16)	−1 987(10)	4 210(23)	1 605(6)
N(17)	−2 199(7)	6 189(18)	1 810(4)
C(18)	−1 354(9)	6 377(23)	2 377(5)
C(19)	−1 290(10)	8 138(23)	2 720(5)
C(20)	−0 393(11)	7 973(25)	3 280(6)
C(21)	0 390(10)	6 147(24)	3 469(5)
C(22)	0 343(8)	4 427(22)	3 120(5)
C(23)	−0 546(9)	4 561(21)	2 561(5)
C(24)	−4 576(11)	−2 313(31)	−0 238(6)
C(25)	1 117(13)	−7 797(28)	0 755(6)
C(26)	1 681(9)	−3 646(22)	2 449(5)
C(27)	−3 155(10)	7 794(23)	1 486(5)
H(1)	−2 492(9)	−2 100(23)	0 525(5)
H(3)	−3 211(14)	−6 974(29)	−0 685(6)
H(4)	−1 248(14)	−8 185(29)	−0 182(6)
H(7)	1 623(11)	−4 468(27)	1 565(5)
H(14)	−1 447(10)	0 608(21)	1 118(5)
H(19)	−1 856(10)	9 467(23)	2 576(5)
H(20)	−0 323(11)	9 190(25)	3 543(6)
H(21)	1 012(10)	6 059(24)	3 869(5)
H(22)	0 926(8)	3 131(22)	3 263(5)
H(24A)	−5 426(11)	−2 224(31)	−0 497(6)
H(24B)	−4 235(11)	−0 746(31)	−0 230(6)
H(24C)	−4 362(11)	−2 707(31)	0 138(6)
H(25A)	0 617(13)	−8 619(28)	0 394(6)
H(25B)	1 440(13)	−8 992(28)	1 058(6)
H(25C)	1 755(13)	−6 976(28)	0 768(6)
H(26A)	1 676(9)	−4 778(22)	2 188(5)
H(26B)	1 494(9)	−4 524(22)	2 681(5)
H(26C)	2 454(9)	−2 908(22)	2 688(5)
H(27A)	−3 121(10)	9 083(23)	1 727(5)
H(27B)	−3 886(10)	6 895(23)	1 319(5)
H(27C)	−3 116(10)	8 513(23)	1 188(5)

Structure Analysis.—The crystal structure was elucidated (with some difficulty due to crystal quality) by direct methods using the MULTAN program.[18] Subsequent calculations were performed with the SHELX suite of programs [19] with anisotropic thermal parameters for the non-H atoms and a common isotropic thermal parameter for the H atoms; refinement converged at R 6.3%. Unit weights were used throughout the least-squares refinement.

Observed and calculated structure amplitudes and the thermal parameters of the atoms are listed in Supplementary Publication No. 23933 (12 pp.).*

* For details of Supplementary Publications see Instructions for Authors (1984) in *J. Chem. Soc., Perkin Trans. 2*, 1984, Issue 1.

Discussion

The molecular structure is shown in Figure 2. Atomic coordinates are listed in Table 6 and the bond lengths, valency angles, and torsion angles are in SUP 23933.

There are no obvious deviations from planarity within each of the five rings. The indole ring systems are displaced from the plane of the pyrrole ring by 25.3 and -5.1°, respectively. Both in-plane and out-of-plane distortions are greater around the C(8)–C(10) than around the C(13)–C(15) bond. As standard deviations associated with the geometric parameters are quite high it is not possible to draw firm conclusions regarding bond orders. However, contributions from the canonical forms are evident and similar π-electron delocalisation is found in other indole derivatives such as 3-acetyl-1-methoxyindole.[20]

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References

1 (*a*) M. J. Gauthier, *Can. J. Microbiol.*, 1976, **22**, 138; (*b*) M. J. Gauthier and G. N. Flatau, *ibid.*, p. 1612; (*c*) M. J. Gauthier, *Int. J. Syst. Bact.*, 1982, **32**, 82.
2 J. A. Ballantine, R. J. S. Beer, D. J. Crutchley, G. M. Dodd, and D. R. Palmer, *J. Chem. Soc.*, 1960, 2292.
3 J. A. Ballantine, C. B. Barrett, R. J. S. Beer, S. Eardley, A. Robertson, B. L. Shaw, and T. H. Simpson, *J. Chem. Soc.*, 1958, 755.
4 R. V. Jardine and R. K. Brown, *Can J. Chem.*, 1963, **41**, 2067.
5 H. H. Wassermann, J. E. McKeon, L. A. Smith, and P. Forgione, *Tetrahedron*, 1966, Suppl. 8, Pt. II, 647.
6 D. M. Porter and W. S. Brey, *J. Phys. Chem.*, 1968, **72**, 650.
7 U. Pindur, *Arch. Pharm.*, (*Weinheim, Ger.*) 1981, **314**, 337.
8 E. Wille and W. Lüttke, *Chem. Ber.*, 1973, **106**, 3240.
9 H. Gold in 'The Chemistry of Synthetic Dyes,' ed. K. Venkataraman, Academic Press, New York, 1971, vol. 5, p. 535; A. Dorlars, C.-W. Schellhammer, and J. Schroeder, *Angew. Chem., Int. Ed. Engl.* 1975, **14**, 665.
10 J. Lyman and R. H. Fleming, *J. Mar. Res.*, 1940, **3**, 134.
11 O. Hinsberg and J. Rosenzweig, *Ber.*, 1894, **27**, 3257.
12 G. Heller, *Ber.*, 1907, **40**, 1291.
13 C. B. Barrett, R. J. S. Beer, G. M. Dodd, and A. Robertson, *J. Chem. Soc.*, 1957, 4810.
14 J. A. Ballantine, C. B. Barrett, R. J. S. Beer, B. G. Boggiano, S. Eardley, B. E. Jennings, and A. Robertson, *J. Chem. Soc.*, 1957, 2227.
15 S. Pietra, *Farmaco, Ed. Sci.*, 1958, **13**, 75 (*Chem Abstr.*, 1958, **52**, 13704).
16 M. Kugel, *Liebig's. Ann. Chem.*, 1898, **299**, 50.
17 E. Heilbronner and H. Bock in 'Das HMO-Modell und seine Anwendung,' Verlag Chemie, Weinheim, 1970, vol. III, p. 191.
18 P. Main, S. E. Hull, L. Lessinger, G. Germain, J. P. Declercq, and M. M. Woolfson, 'A System of Computer Programs for the Automatic Solution of Crystal Structures from *X*-ray Diffraction Data,' University of York, 1978.
19 G. M. Sheldrick, 'A Program for Crystal Structure Determination, University of Cambridge, 1976.
20 R. M. Acheson and J. D. Wallis, *Acta Crystallogr., Sect. B*, 1980, **36**, 3125.

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