The 1,3-Dihydroxy-5-oxonia-11-azabenzo[b]fluorene Cation; a Synthetic Nitrogenous Anthocyanidin
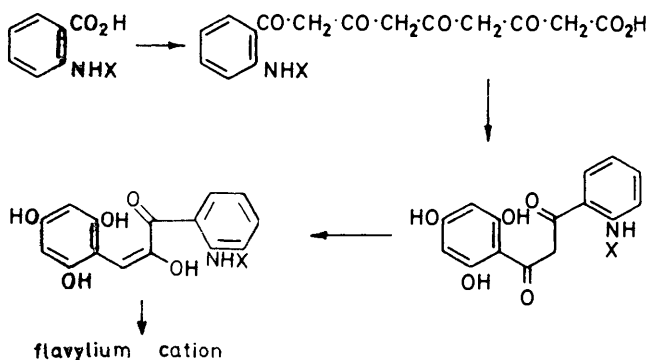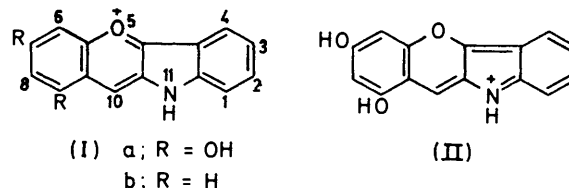
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The chalcone formed from salicylaldehyde and indoxyl cyclises in warm trifluoroacetic acid to yield the 5-oxonia-11-azabenzo[b]fluorene cation (Ib). The constitution of this has been confirmed by oxidation with hydrogen peroxide to the related flavone, [1]benzopyrano[3,2-b]indol-11-one (XI), also accessible by heating 2'-nitroflavone with ethyl phosphite.

The chalcone from indoxyl and 2,4,6-triacetoxybenzaldehyde is converted by trifluoroacetic acid into the 7,9-dihydroxy-5-oxonia-11-azabenzo[b]fluorene cation (Ia). Formally, this cation can be regarded as a nitrogenous anthocyanidin, and the possibility of its occurrence in plants is discussed briefly.

CERTAIN plant pigments such as betanin were once thought to possess anthocyanidin nuclei bearing nitrogen (amino) functions,[1] but the idea lapsed when betanin was eventually shown to have an entirely different type of constitution.[2] We have taken up the idea afresh, however, because of the possibility that a nitrogenous anthocyanidin of structure (Ia) might occur naturally. In contrast to the aminoflavylium derivatives considered earlier, the cation (Ia) fits easily into the biogenetic sequences now known to underlie anthocyanidin formation in plants,[3] and Scheme 1 shows in outline how

(I) a; R = OH
    b; R = H

(II)

elaborated through the acetate/malonate pathway. Accordingly, we have synthesised the cation (Ia) with a view to facilitating a search for this type of compound in plants.

Some flavone derivatives related to (Ia) have already been made accessible by a previous study.[5] Sodium hydroxide converts 2'-hydroxy-5'-methyl-2-nitrochalcone (III) into the N-hydroxyindole (IVa), and ethyl phosphite converts 2'-nitro-6-methylflavone (V) into the corresponding indole (IVa). Although flavones are normally reducible to 4-hydroxyflavenes which, with acids, afford flavylium salts,[6] these indole derivatives resisted all attempts at modification along such lines. For example, compounds (IVa) and (IVc) were not affected by lithium aluminium hydride, sodium borohydride, or diborane, and their methyl derivatives (IVb) and (IVd) were similarly unreactive. Methyl-lithium did appear to react with compound (IVd) but no alcohol could be isolated nor any salt after treatment with acid. In order to obtain a more reactive system, compound (IVd) was converted into the thione (VI) by means of

SCHEME 1

X = Enzymic protective device

anthranilic acid (itself a plant constituent and the source of a major group of alkaloids [4]) might be suitably

[1] F. M. Dean, ' Naturally Occurring Oxygen Ring Compounds,' Butterworths, London, 1963, p. 417.
[2] H. Wyler, T. J. Mabry, and A. S. Dreiding, Helv. Chim. Acta, 1963, 46, 1745.
[3] J. B. Harborne, ' Comparative Biochemistry of the Flavonoids,' Academic Press, London, 1967, ch. 8.

[4] A. R. Battersby, Quart. Rev., 1961, 15, 259; H.-G. Boit, ' Fortschritte in der Alkaloid-Chemie bis 1960,' Akademie-Verlag, Berlin, 1961.
[5] F. M. Dean, C. Patampongse, and V. Podimuang, J.C.S. Perkin I, 1974, 583.
[6] T. R. Seshadri, in ' The Chemistry of Flavonoid Compounds,' ed. T. A. Geissman, Pergamon, London, 1962, ch. 6.

boron sulphide; [7] but although the thione reacted with iodomethane, presumably to give the salt (VII), the product was very unstable and rapidly reverted to the

(III)

(IV) a; R = OH
b; R = OMe
c; R = H
d; R = Me

(V)

(VI)

(VII)
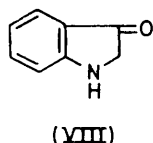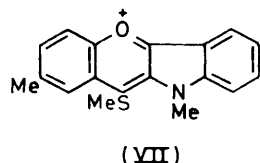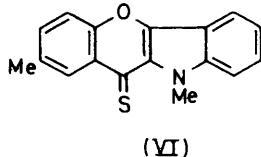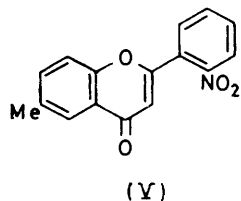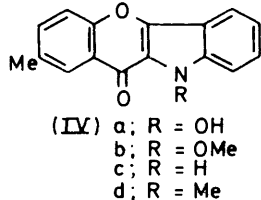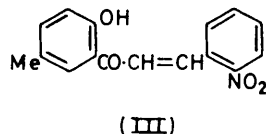
(VIII)

original ketone (IVd) whatever manipulation was attempted.

Successful syntheses were based upon the use of indoxyl (VIII) or of 'indoxyl melt', the commercial crude form of the sodium enolate. Direct preparations of the desired cation (Ib) by condensing indoxyl with salicylaldehyde in strongly acidic media were frustrated by the ready self-condensation of indoxyl, but under milder conditions 2-methoxybenzaldehyde readily gave the chalcone (IXa) and 2-acetoxybenzaldehyde gave the chalcone (IXb). As neither chalcone could be converted into the requisite phenol (IXc) this had to be obtained by the less satisfactory condensation of indoxyl with salicylaldehyde.

The phenol (IXc) resisted the conventional methods of cyclisation to the flavylium cation (Ib). This resistance is most easily explained by assuming the compound to have the *trans* structure shown, and in order to make possible the necessary rotation the compound was hydrogenated to the dihydro-derivative (Xa). In contrast to its acetate (Xb), this phenol was so rapidly dehydrogenated by air that it could not be fully characterised. However, the product was the original phenol (IXc) and no flavylium salt resulted when the dehydrogenation was conducted in acidic media.

The flavylium cation (Ib) was then procured by treating the phenol (IXc) with trifluoroacetic acid. No change occurred in the cold, but at 80° the transformation was smooth and the product was isolated as the trifluoroacetate trifluoroacetic acid monosolvate. The electronic absorption spectrum is consistent with the presence of the flavylium nucleus; it shows maxima at 283 and 406 nm collapsing to a single band at 287 upon

[7] F. M. Dean, J. Goodchild, and A. W. Hill, *J. Chem. Soc. (C)*, 1969, 2192.

addition of sodium acetate, hydrochloric acid reversing the change. The mass spectrum confirmed the existence of a cation (m/e 220), but the i.r. spectrum was not useful since it was dominated by the characteristics of the anion and the solvating acid. Though this last could not be removed without destroying the compound, treatment with warm perchloric acid supplied the less soluble perchlorate which was not solvated and the i.r. spectrum of which was devoid of hydroxy or carbonyl bands above 1600 cm⁻¹. The mass spectrum of this salt was nearly the same as that of the trifluoroacetate except that the chief ion had m/e 219; evidently the cation can be dehydrogenated by the perchlorate residue.

To demonstrate that the flavylium salts do contain the fused heterocyclic system attributed to them we undertook to convert the trifluoroacetate into the corresponding flavone derivative [5] (XI). Alkaline hydrogen peroxide accomplished this oxidation fairly well, despite the fact that it attacks most flavylium salts in a different fashion [8,9] and usually effects fission between positions 2 and 3. Here, however, this area is protected by fusion with the indole nucleus while position 4 remains open to

(IX) a; R = Me
b; R = Ac
c; R = H

(X) c; R = H
b; R = Ac

(XI)

(XII)
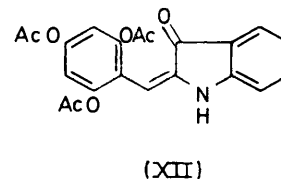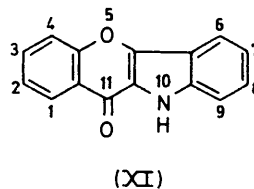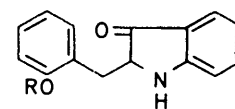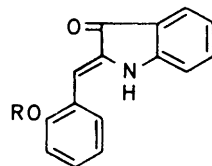
attack. Scheme 2 suggests a radical mechanism for the dehydration, but ionic mechanisms are at least as likely.

Finally, the triacetate of phloroglucinaldehyde was condensed with indoxyl to give the chalcone derivative (XII). Treatment of this with trifluoroacetic acid removed the ester groups and cyclised the compound giving the cation (Ia), which was isolated as a solvate. Attempted conversion into a perchlorate was unsuccessful: no salt separated from the solution and the cation was not stable enough to withstand much manipulation; it also slowly deteriorated in solution and the products have not yet been identified.

The cation (Ia) has the general characteristics associated with the anthocyanidins. Of most importance is

[8] L. Jurd, *Tetrahedron*, 1966, 2913; *J. Org. Chem.*, 1964, **37**, 1887.

[9] W. Dilthey and W. Hoschen, *J. prakt. Chem.*, 1933, **246**, 42.

the electronic spectrum, which shows two chief maxima with two subsidiaries in between, their positions and intensities all falling within the ranges usually found.[10] Thus, although the spectrum does not tally exactly with any of the examples known to us, it seems that neither
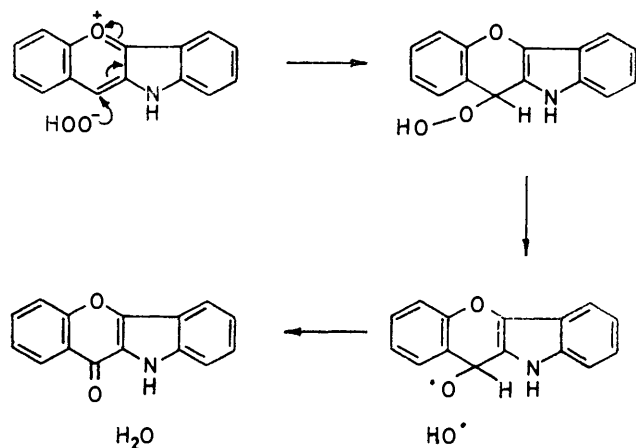
SCHEME 2

the introduction of NH instead of OH at (flavylium) position 3 nor the presence of an extra ring has any marked effect. Moreover, the NH group could serve for the attachment of a sugar residue at (flavylium) position 3, a feature of nearly all known anthocyanins.[11] Since it is not the common practice to search anthocyanin pigments for nitrogen either analytically or mass spectroscopically, it is evident that the occurrence of cations related to (I) might easily be overlooked in general searches.

EXPERIMENTAL

Molecular weights were determined mass spectroscopically. Light petroleum refers to the fraction b.p. 60—80°. U.v. spectra were obtained on ca. 10^{-4}M-solutions in ethanol, and i.r. spectra were determined for mulls in paraffin.

2,10-Dimethyl[1]benzopyrano[3,2-b]indole-11-thione (VI). —The benzopyranoindolone [5] (IVd) (0·20 g) was heated in xylene with boron sulphide [7] (0·14 g) during 8 h, and the product was chromatographed on a silica column. Light petroleum eluted sulphur, then benzene–light petroleum eluted fractions containing the thione, which crystallised from benzene as red needles (0·031 g), m.p. 197—198°, $v_{max.}$ 1618w, 1520, 1290, and 750 cm^{-1} (Found: C, 73·4; H, 4·7. $C_{17}H_{13}NOS$ requires C, 73·1; H, 4·7%), m/e 279 (M^+) and 278 (ca. 1 : 1).

The colour of a solution of the thione (10 mg) in acetone (2 ml) faded at once when mercury(II) acetate (40 mg) in acetic acid (1 ml) was added; isolated by addition of water and extraction into chloroform, the product crystallised from benzene–light petroleum to give the benzopyranoindolone (IVd), m.p. and mixed m.p. 205—207°.

1,2-Dihydro-2-(2-methoxybenzylidene)indol-3-one (IXa).— A mixture of 2-methoxybenzaldehyde (1·3 g) and ' indoxyl

melt ' (4·15 g) in acetic acid (30 ml) under nitrogen was stirred for 12 h. The dark brown precipitate crystallised from benzene giving the indolone as tiny orange needles (0·76 g), m.p. 214—215°, $v_{max.}$ 3320, 1675, 1628, 1585, 1142, and 758 cm^{-1}, τ 6·06 (3H, s, OCH$_3$) and 3·18—2·17 (9H, mm, ArH and C:CH) (Found: C, 76·2; H, 5·14; N, 5·55%; M, 251. $C_{16}H_{13}NO_2$ requires C, 76·5; H, 5·2; N, 5·6%; M, 251).

2-(2-Acetoxybenzylidene)-1,2-dihydroindol-3-one (IXb).— To 2-acetoxybenzaldehyde (0·8 g) in acetic acid (30 ml) under nitrogen was added ' indoxyl melt ' (4·15 g) and the mixture was stirred overnight. Concentration of the solution under reduced pressure and addition of water gave a black tar which was chromatographed on silica. Elution with benzene–chloroform (3 : 1) gave orange fluorescent fractions which were combined and yielded dark red crystals when concentrated. Purified from benzene, this solid supplied the indolone as orange needles (0·69 g), m.p. 169—171°, $v_{max.}$ 3380, 1750, 1690, 1635, 1595, 1215, 1138, and 770 cm^{-1}, τ 7·70 (3H, s, O$_2$C·CH$_3$) and 3·24—2·28 (10H, mm, ArH, NH, and C:CH) (Found: C, 73·2; H, 4·6%; M, 279.* $C_{17}H_{13}NO_3$ requires C, 73·1; H, 4·7%; M, 279).

Shaken with palladium–charcoal (10%; 40 mg) under hydrogen for 8 h, a solution of this compound (200 mg) in ethanol (60 ml) gradually lost its colour. Isolated by evaporation of the filtered solution, the product crystallised from ether giving 2-(2-acetoxybenzyl)-1,2-dihydroindol-3-one (Xb) as faintly greenish prisms (40 mg), m.p. 177—179°, $v_{max.}$ 3370 (NH), 1755 (OAc), 1662 (ArCO), 1610, 1202, and 755 cm^{-1}, τ 7·61 (3H, s, OCO·CH$_3$) and 6·75 and 7·29 (2H, two d, J 12 Hz, CH$_2$) (Found: C, 72·8; H, 5·5%; M, 281. $C_{17}H_{15}NO_3$ requires C, 72·6; H, 5·3%; M, 281).

1,2-Dihydro-2-(2-hydroxybenzylidene)indol-3-one (IXc).— To salicylaldehyde (8 g) in acetic acid (200 ml) under nitrogen was added ' indoxyl melt ' (41·4 g). The mixture was stirred for 12 h and then concentrated under reduced pressure. The precipitate was chromatographed from chloroform on a silica column and the bright red fractions were combined and their contents crystallised from chloroform to give the indolone as red prisms (4·1 g), m.p. 189—191°, $v_{max.}$ 3420 and 3150br (OH, NH), 1618 (ArCO), 1628, 1578, 1255, 1142, and 755 cm^{-1} (Found: C, 75·9; H, 4·7; N, 5·9%; M, 237. $C_{15}H_{11}NO_2$ requires C, 75·9; H, 4·7; N, 5·9%; M, 237).

When shaken with palladium–charcoal (5%; 10 mg) under hydrogen, a red solution of this compound (50 mg) in ethanol (30 ml) slowly became faintly green as 1 mol. equiv. of hydrogen was absorbed. Removal of the catalyst and solvent left pale cream crystals which rapidly became red in air and which regenerated the red benzylideneindolone quantitatively when kept in an exposed solution. The cream substance is considered to be 1,2-dihydro-2-(2-hydroxybenzyl)indol-3-one (Xa) since the mass spectrum showed the appropriate molecular ion at m/e 239; however, the value of other spectroscopic and analytic results was vitiated by the ready oxidation.

5-Oxonia-11-azabenzo[b]fluorene Salts.—1,2-Dihydro-2-(2-hydroxybenzylidene)indol-3-one (0·80 g) in trifluoroacetic acid (150 ml) was heated on a steam-bath for 3 h; then the solution was concentrated under reduced pressure and ether was added until red crystals appeared. These proved difficult to recrystallise, so they were purified by several

* At 20 eV; at 70 eV there was no signal.

[10] L. Jurd, ' The Chemistry of Flavonoid Compounds,' ed. T. A. Geissman, Pergamon, Oxford, 1962, ch. 5.

[11] J. B. Harborne, ' Chemical Plant Taxonomy,' ed. T. Swain, Academic Press, London, 1963, ch. 13.

reprecipitations in the same manner. Thus obtained, 5-oxonia-11-azabenzo[b]fluorene trifluoroacetate formed a *solvate* with trifluoroacetic acid consisting of red prisms (1·5 g), m.p. (rapid heating) 125° (chars) (Found: C, 51·9; H, 2·5. $C_{17}H_{10}F_3NO_3,C_2HF_3O_2$ requires C, 51·0; H, 2·5%). In the mass spectrometer, the solvate gave no ion of importance higher than m/e 220, corresponding to the cation (Ib) ($C_{15}H_{10}NO$ requires M^+, 220). In ethanol, the solvate had $\lambda_{max.}$ 283 and 406 nm (log ε 4·11 and 4·20) changed by sodium acetate to 287 nm (log ε 3·95) and restored by acidification with hydrochloric acid.

The solvate (50 mg) was warmed with perchloric acid (70%; 5 ml) until dissolution was complete; water was added, and the solution kept until no more crystals separated. This solid was purified from ethanol giving 5-*oxonia-*11-*azabenzo*[b]*fluorene perchlorate* as golden orange plates, m.p. 142—144°, $\lambda_{max.}$ 234, 286, and 405 nm (log ε 4·38, 4·155, and 3·60), $\nu_{max.}$ 3220, 1645, 1350, 1115, 785, and 767 cm⁻¹ (Found C, 56·5; H, 3·3; N, 4·6. $C_{15}H_{10}ClNO_5$ requires C, 56·3; H, 3·1; N, 4·4%). The mass spectrum again gave a parent ion at m/e 220 corresponding to the cation (Ib) but a peak at m/e 219 (M^{2+} 109·5) was much stronger.

[1]*Benzopyrano*[3,2-b]*indol*-11-*one* (XI).—(i) 2′-Nitroflavone (1·15 g) was reduced by refluxing ethyl phosphite (100 ml) under nitrogen during 5 h and the product which separated upon cooling was purified from methanol giving the *pyranoindolone* as prisms (0·6 g), m.p. 192—194°, $\nu_{max.}$ 3165 (NH), 1645 (chromone), 1630, 775, and 760 cm⁻¹ (Found: C, 76·6; H, 4·2; N, 5·9%; M, 235. $C_{15}H_9NO_2$ requires C, 76·6; H, 3·9; N, 6·0%; M, 235).

(ii) 5-Oxonia-11-azabenzo[b]fluorene trifluoroacetate solvate (0·10 g) in methanol (20 ml) at 0° was treated with aqueous hydrogen peroxide (30%; 1 ml) and aqueous sodium hydroxide (10%; 10 ml). The red colour faded and a precipitate formed during about 35 min; this was washed with water and dried *in vacuo* before being crystallised from water to give the pyranoindolone as needles (0·02 g), m.p. and mixed m.p. 192°, further identified spectroscopically.

1,2-*Dihydro*-2-(2,4,6-*triacetoxybenzylidene*)*indol*-3-*one* (XII).—For this preparation 'indoxyl melt' proved unsuitable so pure indoxyl was secured from it as follows.

The melt (50 g) was dissolved in dilute acetic acid (25%; 300 ml) and filtered, and the filtrate was cooled in ice until crystallisation was complete. The indoxyl was collected, washed with boiled water, and dried *in vacuo*. All operations were conducted under nitrogen.

2,4,6-Triacetoxybenzaldehyde was prepared according to Malkin and Nierenstein [12] and its constitution checked spectroscopically. Freshly prepared indoxyl (0·35 g) and sodium acetate (1·0 g) were added to 2,4,6-triacetoxybenzaldehyde (0·75 g) in acetic acid (30 ml) under nitrogen and the mixture stirred at 25° for 10 h. The red precipitate (0·3 g) was washed with water, dried *in vacuo*, and recrystallised from acetic acid giving the *indolone* as red-orange platelets, m.p. 193—195°, $\nu_{max.}$ 3410 and 3100br (NH), 1755 (OAc), 1670 (ArCO), 1600, 1200, and 1150 cm⁻¹, τ [(CD₃)₂CO] 7·75 (9H, s, OCO·CH₃) and 3·5—2·1 (8H, mm, ArH, C:CH, and NH) (Found: C, 64·3; H, 4·3%; M, 395. $C_{21}H_{17}NO_7$ requires C, 63·9; H, 4·4%; M, 395).

7,9-*Dihydroxy*-5-*oxonia*-11-*azabenzo*[b]*fluorene Trifluoroacetate*.—The foregoing indolone (200 mg) was warmed on a steam-bath for 2 h with trifluoroacetic acid (25 ml) and the solution was concentrated and left to stand while the trifluoroacetate separated as the trifluoroacetic acid *solvate* in the form of bright red prisms (80 mg), m.p. 133° (decomp.), $\lambda_{max.}$ (EtOH) 282, 310, 434, and 510 nm (log ε 4·04, 3·63, 3·95, and 3·95), $\lambda_{max.}$ (EtOH–NaOAc) 263, 282, 316, 325, 442, 464, and 520 nm (log ε 4·07, 3·83, 3·74, 3·74, 4·16, 4·14, and 3·86), $\nu_{max.}$ 3370, 3220, 1745, 1648, 1628, 1590, 1190, 872, and 718 cm⁻¹ (Found: C, 47·4; H, 2·3; N, 3·0. $C_{17}H_{10}F_3NO_5,C_2HF_3O_2$ requires C, 47·6; H, 2·35; N, 3·0%). The mass spectrum showed a parent ion at m/e 252 corresponding to cation (Ia) ($C_{15}H_{10}NO_3$ requires M^+, 252); remarkably, this was the only strong peak in the entire spectrum.

Attempts to recrystallise the solvate or to remove the solvating species led only to extensive decomposition.

We thank I.C.I. Limited for a gift of indoxyl melt.

[12] T. Malkin and M. Nierenstein, *J. Amer. Chem. Soc.*, 1931, **53**, 241.