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THE INTERACTION OF RETINAL (VITAMIN A ALDEHYDE) WITH INDOLES IN ACIDIC MEDIA

D.S. JOHNSTON , J.H.P. TYMAN* and D. CHAPMAN

Department of Biochemistry and Chemistry, Royal Free Hospital School of Medicine, London NW3 and *Department of Chemistry, Brunel University, Uxbridge, Middlesex UB8 3PH

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Abstract - The interaction of equimolar proportions of retinal with indoles under acidic conditions yields primarily 1:1 blue purple pigments which are retinylidene indolenines or retinylidene (3H) indolium salts reducible to retinylidene indoles with sodium borohydride. With 3-substituted indoles, reaction is considered to occur at the 2-position and at the 3-position with indole and 2-substituted indoles, the latter series being more stable and readily formed.

Intensive work[1] has been carried out and is still continuing on the structure of bacteriorhodopsin[2] and its photocycle to unravel unknown features. Similarities in the absorption spectra of products from retinal and tryptophan analogues with the main forms of the bacteriorhodopsin photocyle have suggested[3] that an aromatic amino acid may be involved as well as a protonated Schiff's base. It seemed of value to examine the organic chemical role of the indole nucleus and the interaction in acidic conditions of retinal and indoles including tryptophan has been studied.[4,5]

The product from retinal and tryptophan was shown[5] not to be a charge-transfer complex as originally thought.[6] In the case of other indoles enamine structures[7] and other possibilities[8] all appear to be contradictory. The present work with a variety of indoles has shown the blue purple products to be primarily 1:1 compounds resulting from condensation, together with some of the 2:1 (indole/retinal) compound, the extent of reaction being dependent upon the molar ratio of reactants, the solvent and the type of acid used.

EXPERIMENTAL

Ethanol (A.R. grade) was used. Dichloromethane (A.R. grade) was placed over calcium hydride (one week) and then distilled. Hydrogen chloride saturated ethanol and dichloromethane were obtained by passing the dry gas through the solvents. Trifluoroacetic acid, trifluoromethanesulphonic acid, triethyloxonium hexachloroantiomonate, methyl trifluoromethanesulphonate, indole, methylindoles (Aldrich) and all-trans retinal type III (Sigma) were used. Tryptamine, (3-(2-aminoethyl)indole) was obtained from the hydrochloride (Sigma) with excess ethylamine. Tryptamine in pyridine was reacted with acetic anhydride to obtain N_b-acetyltryptamine. The butylamine Schiff's base of all-trans retinal was synthesized[9] to examine its possible involvement with indole systems under acidic conditions.

'Crystal violet' (Aldrich), tris(3-methylindolyl-2)methane and bis(3-methylindolyl)methene were used as reference compounds, the last two being synthesized by standard procedures.[10] Sodium borohydride reductions were carried out on all the retinal/indole condensation products, 'crystal violet' and bis(3-methylindolyl)methene following the procedure adopted for certain related reactions.[11]

Thin layer chromatography was carried out on silica gel G with Merck commercial plates and purification attempted by preparative TLC with a Chromatotron unit. The instability of reaction products in chromatographic experiments made it necessary to examine reaction mixtures directly preferably at high conversion. HPLC analyses were carried out by the reverse phase partition method and by the normal mode with acetonitrile/water and iso-octane/isopropanol respectively on a

5 µm Spherisorb ODS column. Gradient elution was effected with an Altex programmable unit and separations were monitored by a variable wavelength Perkin Elmer LC55 spectrophotometer. Ultraviolet and visible spectra were recorded on a Pye-Unicam SP8-100 and Perkin Elmer Lambda-3 spectrophotometers. Infrared spectroscopy was carried out with potassium bromide discs (3% w/w) or in dichloromethane solution. The solution cell used had sodium chloride windows and a path length of 125 µm. The infrared spectrometer was a Perkin Elmer 457 unit. ^{1}H NMR spectra were recorded on JEOL PMX 60MHz and HA100 (PCMU Harwell) spectrometers in deuterated chloroform with TMS as an internal standard.

Elemental analyses were carried out at the School of Pharmacy, London University and by BMAC Ltd.

Mass spectra were determined by the PCMU, Harwell and by Dr E. Waight, Imperial College (FAB and EI in both cases). Accurate mass determinations were made by Dr M. Mruzek, University College, London. For FAB spectra samples were dissolved in either thioglycerol, 2,4-diamylphenol or dimethylsulphoxide/glycerol. The mass spectrometer was a VG instrument equipped with an Ion-Tech Ltd ion gun (8kV and 1ma). It was not possible to examine all products and those in the indole and 3-methylindole series were examined as typical members.

Reaction of Retinals and Other Carbonyl Compounds with Indoles in the Presence of Alkylating Agents and Acids

In the first place all trans retinal, 13-cis and 11-cis were examined and the first two were found to be identical in their reaction with indole in HCl/ethanol whereas 11-cis did not react. With all three in HCl/dichloromethane reaction in the case of indole occurred but the spectrum of the 11-cis product was different. Subsequently all trans retinal was used in all experiments.

Indole, 2-methylindole, 3-methylindole, 1,2-dimethylindole, 2,3-dimethylindole and N_b acetyltryptamine were used in experiments with alkylating agents, strong and weak acids.

(a) Reactions in the presence of alkylating agents

Triethyl oxonium hexachloroantimonate:

To all-trans retinal (8.6 mg; .03 millimole) in anhydrous dichlormethane (5 cm^3) the indole (0.03 millimoles) was added at 0-10^o and then triethyloxonium hexachloroantimonate (13.3 mg; .03 millimoles). Reaction caused the solution to turn deep blue and proceeded slowly at ambient temperature. It was monitored on aliquots diluted with dichloromethane by measuring the relative intensities of absorptions for retinal at 388 nm and the product at 660 nm. When reaction had ceased, in all cases in under three hours, the dichloromethane was evaporated in a stream of dry nitrogen. The product was obtained as a black brittle film.

A limited number of experiments were carried out in the case of 1,2-dimethylindole with β-ionone, carotenal and 2,4-hexadienal as well as with the retinals in the presence of triethyloxonium hexachloroantimonate. β-ionone and carotenal gave relatively stable products with absorption at 325 nm and 860 nm respectively while the coloured product from 2,4-hexadienal had a limited lifetime.

Methyl trifluoromethane sulphonate:

The procedure was essentially the same as the preceding except that the reagent was added at 0-10^o as a 3 millimolar solution (1 cm^3) in dichloromethane.

(b) Reactions in the presence of strong acids:

The procedure was essentially the same and the reagent, trifluoromethane sulphonic acid, was added at 0-10^o as a 3 millimolar solution (1 cm^3) in dichloromethane. In the case of alkylating agents and strong acids, TLC (CCl_4, $CHCl_3$) indicated a polar product (R_f, 0) and traces of the starting indole. The products did not prove amenable to purification treatments.

(c) Reaction in the presence of weak acids:

All-trans retinal was dissolved in either hydrogen chloride-saturated ethanol or hydrogen chloride- saturated dichloromethane to produce a solution which in a 1 cm path length cell had unit absorbance at 388 nm. An excess of the indole (1 mg) was then added to the cell and the ensuing reactions monitored by recording the changes in the spectrum of the solution.

Reduction of Indole/Retinal Products with Sodium Borohydride

The respective indole/retinal product (5 mg) dissolved in methanol cooled to 0^o was reduced by the addition of excess sodium borohydride. Following the disappearance of the initial blue colour of the reactant, the reaction mixtures were diluted with water and ethereally extracted. TLC examination indicated that polar substances had disappeared and the products resembled the reference compounds, 3-methylindole and tris-(3-methylindolyl)methane. The products from ethereal extraction were examined by both EI and FAB/MS.

Silylation Treatment of Reaction Products

In an attempt to obtain more volatile substances suitable for EI/MS, reaction products were

treated with bis-trimethylsilylacetamide in anhydrous pyridine solution by warming at 70°. Products were recovered by removal of pyridine and excess silylating agent under vacuum in a rotary evaporator.

RESULTS AND DISCUSSION

Indole, 1-methyl, 2-methyl, 3-methylindole, 1,2-dimethylindole, N_b-acetyltryptamine and the methyl ester of tryptophan reacted with all trans-retinal under a variety of acidic conditions. Weak acids did not effect reaction and in methanol or ethanol containing hydrogen chloride the corresponding retinal acetal tended to form (found: C, 81.52; H, 10.59, $C_{24}H_{38}O_2$ requires C, 80.45; H, 10.61%). In dichloromethane the yields of blue purple products increased with increasing acidity and to improve the yield, trifluoroacetic acid, trifluoromethane sulphonic acid and the powerful alkylating agents triethyloxonium hexachloroantimonate and methyl trifluoromethane-sulphonate were found valuable giving conversions which appeared to be substantially complete. Some results in different solvents with various acids are shown in Table 1. Reactions were monitored by U.V. spectrophotometry (Figs. 1 and 2 show typical spectra) by TLC and by IR

Table 1. U.V. absorption of indole/retinal products with different acids and solvents

	Retinal	Solvent	Acid	Indole	(max) of products (nm)
1	All-trans and 13-cis	EtOH	HCl	Indole	630
2	"	CH_2Cl_2	HCl	"	630
3	"	EtOH	CF_3CO_2H	"	496
4	"	CH_2Cl_2	"	"	518 equimolar, 576 excess indole
5	"	DMSO	"	"	500
6	11-cis	EtOH	HCl	"	no product
7	"	CH_2Cl_2	"	"	514
8	All-trans and 13-cis	EtOH	"	2-methylindole	600
9	"	CH_2Cl_2	"	"	604
10	"	"	CF_3CO_2H	"	636
11	"	"	HCl	3-methylindole	-
12	"	EtOH	"	"	598
13	"	CH_2Cl_2	CF_3CO_2H	"	740
14	"	CH_2Cl_2	HCl	N_b-acetyltryptamine	680
15	"	EtOH	"	"	540

In expt. 1 no coloured product was formed with acetic acid in place HCl. When irradiated with light from a 150 watt projector bulb the complex was bleached. $Bu_4N^{\oplus}$ $ClO_4^{\ominus}$ increased the rate of formation of the complex.

examination in dichloromethane solution. Reaction in dichloromethane (Fig. 1) was considerably slower than in ethanol (Fig. 2) and maximum absorption was attained later. In the same solvent indole, 1-methylindole, 2-methylindole, and 1,2-dimethylindole reacted more rapidly, while 3-methylindole was slower to react. In all cases the UV max. of retinal at 388 nm was replaced by bands in the region 580-740 nm. In the infrared spectra of the compounds formed by indole, 1,2-dimethylindole and 3-methylindole with retinal in the presence of triethyloxonium hexachloroantimonate (solution measurements in CH_2Cl_2) tne N-H stretch of indoles at 3500 cm^{-1} was shifted to 3300 cm^{-1}. In KBr discs, C=O (1660 cm^{-1}) and C=C (1575 cm^{-1}) were replaced by absorptions at 1610 cm^{-1} (weak) and 1520 cm^{-1} (strong).

In the ^1H NMR spectrum ($CDCl_3$) the product from indole and retinal with trifluoromethane-sulphonic acid showed no CHO and an unresolved distribution of protons into two groups, aromatic and olefinic at low field with δ, 6.18-7.60 (-CH= conjugated, HAr), 5.35-5.80 (-CH=) and at high field, 1.10-2.49 (CH_3,CH_3-C=, $-CH_2$-C=). The characteristic 2- and 3- protons of the indole nucleus at 6.76δ and 6.38δ (Sadtler 473) were no longer present .

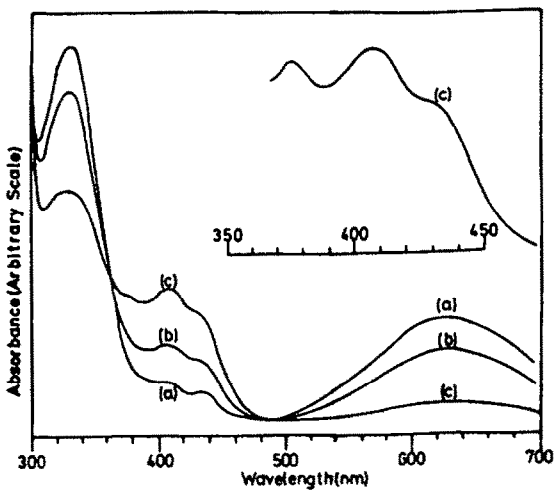


Fig. 1 Visible spectra of an acidified (0.05M HCl) ethanol solution of all-trans retinal (2.5×10^{-5}M) and indole (0.1M) (a) 2 minutes, (b) 10 minutes and (c) 30 minutes after mixing.

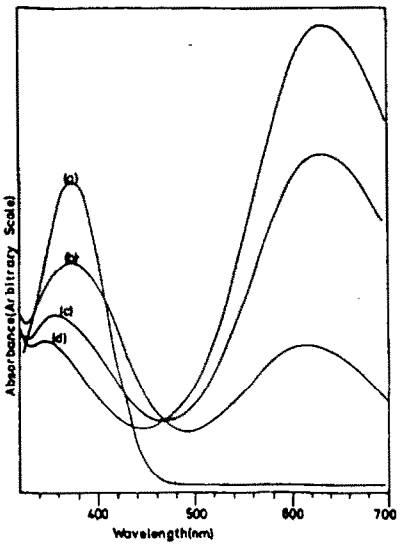


Fig. 2 Visible spectra of an acidified (0.05M CF_3COOH) dichloromethane solution of all-trans retinal (2.5×10^{-5}M) and 2-methylindole (0.1M) (b) 2 minutes, (c) 10 minutes and (d) 30 minutes after mixing.
(a) Spectrum of retinal before addition of acid and indole. $E = 3.8 \times 10^4$ litre.mol^{-1}.cm^{-1}.

Table 2. Spectra of indole and 3-methylindole retinal products

Indole/retinal (1:1), CF_3SO_3H : mass (% of base peak)

768(6.1), 673(9.60), 650(11.6), 501(8.8), 499(11.1), 384(87.7), 382(37.9), 381(32.7), 370(22.5), 292(46.0), 258(39.2), 244(39.0), 218(52.8), 217(45.2), 194(100), 193(40.6), 170(77.9), 167(67.0), 165(44.0), 158(23.9).

Indole/retinal (1:1), $Et_3O^{\oplus}$ $SbCl_6^{\ominus}$

501(20.5), 384.1(24.4), 383.1(10.8), 382(18.70), 381.8(8.49), 282.1(10.3), 286.1(10.8), 269.0(10.4), 268.1(11.61), 217.1(16.02), 194.1(24.3), 167.0(25.1), 165.1(27.1), 130(100).

3-Methylindole /retinal (1:1), $Et_3O^{\oplus}$, $SbCl_6^{\ominus}$

527.0(10.5), 525(13.4), 432.0(18.0), 430.0(10.4), 414(10.9), 412(13.4), 410.0(18.3), 399(10.4), 397.1(53.3), 396(100), 395.1(39.2), 394(86.3), 378.0(11.7), 366(10.2), 338(10.1), 335(11.2), 273(25.1), 272(24.6), 265.1(26.4), 260.0(24.9), 258(25.9), 248(25.2), 219.9(24.6), 217.9(27.9), 194(26.9), 105(18.6).

3-Methylindole/retinal (2:1), CF_3SO_3H

529.1(10.7), 415.0(13.6), 412(21.8), 398(16.0), 397(22.3), 396(10.1), 375(11.2), 325(11.4), 323(10.3), 294(17.2), 257(26.7), 218(21.8), 208.0(25.4), 197(16.2), 196(31.2), 194(32.6), 184(28.6), 169.9(26.3), 167(33.3), 144.0(100).

Tris-(3-methylindolyl-2)methane (reference compound)

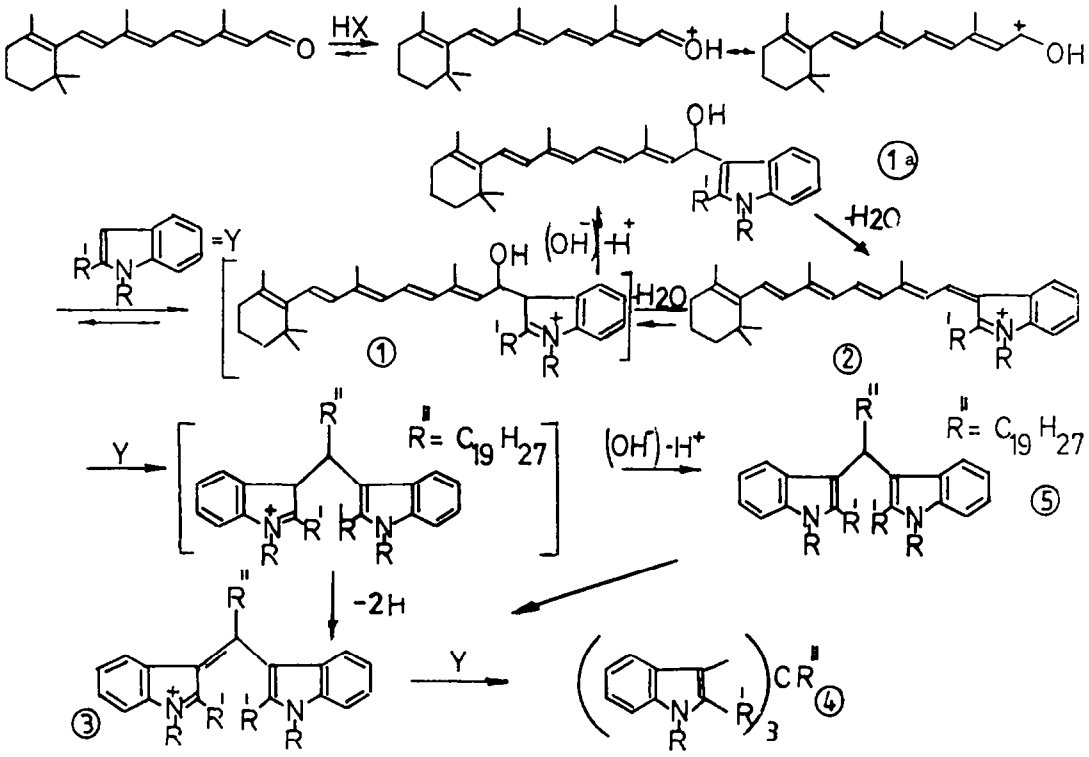
402(100), 403(83.3), 404(22.0), 274(26.9), 273(100), 269(17.8), 257(55.6), 256(22.4), 130(20.7).

Information on the molecular ions present was obtainable only with FAB/MS in this series of compounds. Molecular ions, and fragments ions together with their relative abundance (compared with the base peak) are listed in Table 2. With the strong acids and alkylating agents the percentage conversions of the reactants to the four products listed were considered to be the highest in the series of indole/retinal reactions and they were selected for MS study. For the indole/retinal product the observed molecular ion m/z 384 agreed with that calculated for $C_{28}H_{34}N$ and similarly for the 3-methyl compound (m/z 398 for $C_{29}H_{36}N$). (The reference compound, tris-(3-methylindolyl-2) methane possessed the expected molecular ion at m/z 402.) These masses indicate there is primarily 1:1-substitution. Ions observed at m/z 501, and m/z 529 could be ascribed to 2:1 (indole/retinal)substances, $C_{36}H_{41}N_2$ and $C_{38}H_{45}N_2$ respectively although both the

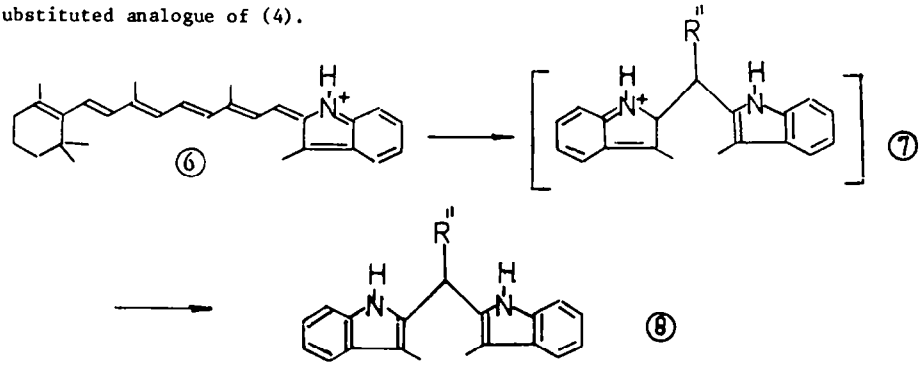
nature of this relatively new technique and a lack of data for related compounds would suggest that higher masses are due to a 'clustering effect'. With the FAB technique normal fragmentation tends to be diminished and it seems probable that 2:1 products are present to a minor extent in most of the reactions studied. Other evidence indicates that unless the molar ratio of the indole/retinal is at least 2:1, 1:1-substitution is predominant. Structures involving di-indole[3] and enamines[7] are inconsistent with the present extensive spectroscopic evidence.

Mechanism of Formation

For indole, 2-methylindole and 1,2-dimethylindole the proposed mechanism would involve the formation of the 1:1-products, (2; R=R^1=H), (2; R=H, R^1=Me) and (2; R=R^1=Me) respectively, all being aza benzofulvenium salts, by way of a carbocation and the hydroxy intermediates (1) and (1a). Thence by further stages the diindolylmethane (5) would result and the methene (3) by oxidative loss. This through reaction with Y could give finally the trisubstituted compound (4) accounting for very small masses in the region of m/z 615 in the mass spectrum. Formation of (5) and (1a) by proton loss would explain the loss of colour upon basification and equally the rapid development of colour by the strong acid dehydration of (1a). There is a parallel with the leuco and dye structures in the triphenylmethane series. No evidence could be found for Michael-type adducts resulting from reaction of indole at the 3-position and the β-carbon of retinal.



From 3-methylindole, the less stable structure (6) would form resulting finally in the 2:1 compound, the diindolymethane (8) by way of the very transient intermediate (7) which would be less likely to form a trisubstituted analogue of (4).



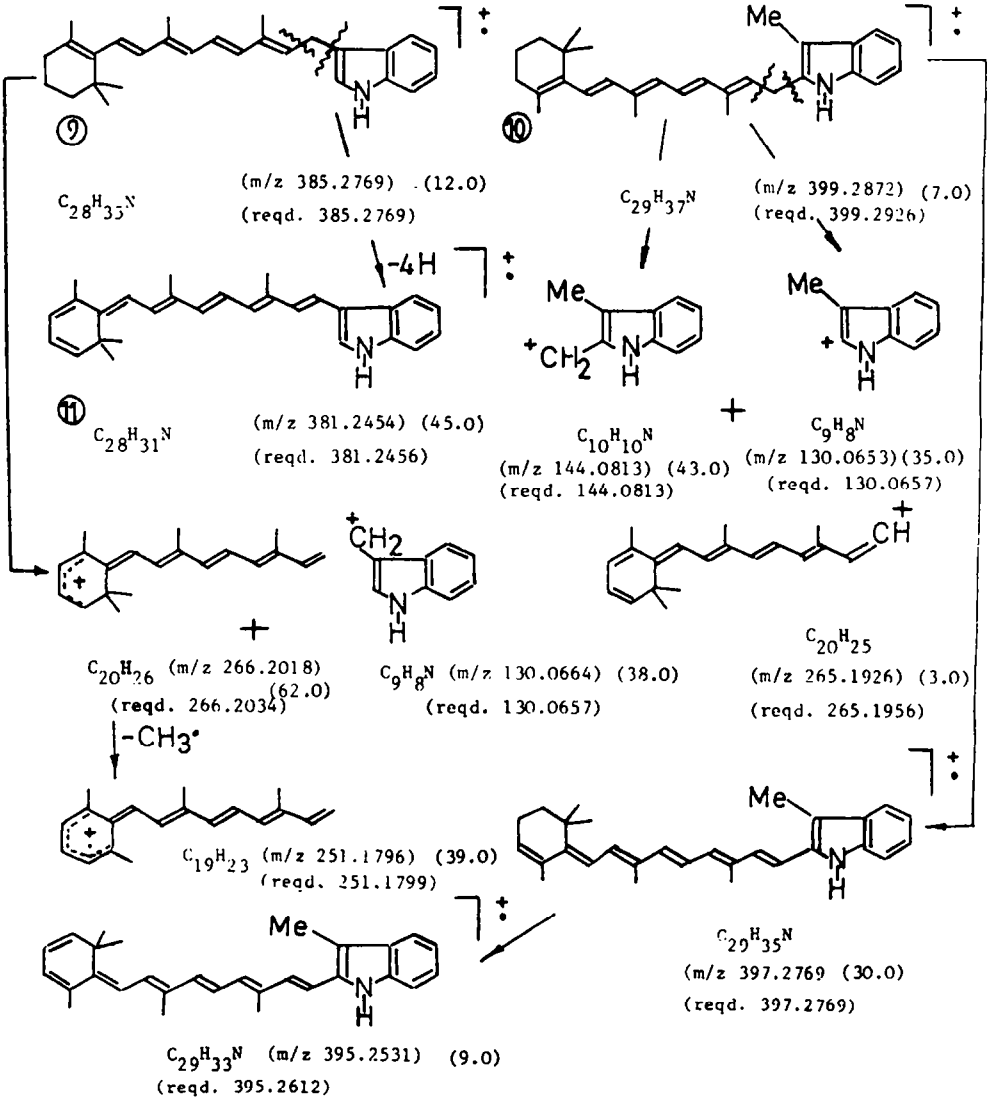
The relative failure of 2,3-dimethylindole to react comparably is due to the unlikely formation of a 'betanidin'-type structure and the absence of enamines. 2,3,4,5-Tetramethylpyrrole interacts slowly with 4-dimethylaminobenzaldehyde in the Ehrlich reaction through displacement of the α-methyl group.[17]

Reduction Products of Reginylidene Indolenine Salts

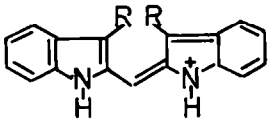
Although mass spectra on the indolenine salts could only be obtained by the FAB technique the sodium borohydride reduction products enabled EI/MS and accurate mass determinations to be made. Evidence for the predominant presence of the 1:1 (indole/retinal) compound in the reactions came from the EI/MS of their reduction products with sodium borohydride giving (9) with the indole/retinal reaction and the substance (10) from the 3-methylindole/retinal analogue. The molecular ions and their abundance together with fragment ions are listed in Scheme 1.

Scheme 1

MS FRAGMENTATION (AND ACCURATE MASSES) OF SODIUM BOROHYDRIDE REDUCED INDOLE/RETINAL PRODUCTS



A strong ion at m/z 381 was present formed by the loss of 3H and 1H from the cyclohexenyl ring and the 3-methylene group respectively followed by isomerisation to give structure (11). The presence of a strong ion for $C_{20}H_{26}$ suggests the existence of an intact retinal chain in both indole and 3-methylindole products. The reference compound (12), a blue methene obtained[10] from 3-methylindole and formaldehyde, and (13) the triphenylmethene, 'crystal violet' under similar



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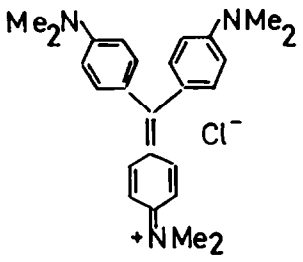

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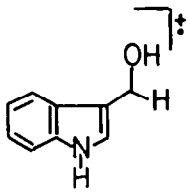

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borohydride reduction conditions underwent the same loss of colour and afforded the expected products $C_{19}H_{18}N_2$ and $C_{25}H_{31}N_3$ having the correct accurate molecular masses.

EI/MS rather than FAB/MS examination of the sodium borohydride reduction product from the 3-methylindole/retinal and the indole/retinal reactions afforded information on the presence of 2:1 substances such as (5). For the 3-methylindole/retinal product an appreciable fragmentation product (12; R=Me) (21%) was found with m/z 273.1384 ($C_{19}H_{17}N_2$ requires 273.1391) which was absent in products from reactions carried out with a 1:1 molar proportion of reactant

Products of basification from attempted trimethylsilylation corroborated the results from reduction treatment. From the indole/retinal reaction the basified product exhibited ion (11), m/z 381.2487, (required for $C_{28}H_3N$ 381,2456), with others at m/z 266.2031, (required for $C_{20}H_{26}$ 266,2018), m/z 117.0574, (required for C_8H_7N, indole 117.0578) and m/z 147.0666, (required for C_9H_9NO 147,0684), probably the fragment (14) indicative of some of the original intermediate (1a) from the retinal/indole reaction. In reaction mixtures which had proceeded partially, and were then basified followed by recovery, this same fragment was found in greater abundance by EI/MS.

Earlier work:

In previous investigations[12,13,14,15,16] on the interaction of carbonyl compounds with indoles (and pyrroles),[17] diindolylmethanes have been isolated which are readily oxidizable to the corresponding coloured methenes. In the case of o-chlorobenzaldehyde* a monosubstituted compound from 2-methylindole has been isolated[11] although the reversible transformation of a diindolylmethane to a monosubstituted compound with loss of an aldehyde molecule has been observed.[18] Little work has been carried out with aliphatic aldehydes because of the formation[18] of materials "difficult to purify". The formation[19] of a 1,3 di-substituted product from propenal and 2-methylindole must have involved an oxidative step by way presumably of the carbocation of 3-allyl-2-methylindole. The products in the present work have been obtained essentially under non-oxidative conditions. Although a family of 2:1-products could conceivably arise from a series of carbocations derived from (1), the spectroscopic and other evidence indicates the main product to be a 1:1 compound confined to reaction at the terminal aldehyde group of retinal. Their independent synthesis in the perhydro form is under examination. In the case of tryptophan protected at the HN_2 and CO_2H groups a product similar to (6) would be expected and similarly the substituent at the 3-position would be an amino acid sequence replacing one of the hydrogen atoms of the methyl group in 3-methylindole.

In the case of tryptophan and its derivatives the substances described in this work may be of interest as models for the bacteriorhodopsin photocycle.

*the Ehrlich reaction test may be included also.

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