Chemistry of the aminochromes. Part X. Some further observations on the reactions of aminochromes with thiols[1,2,3]

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The reactions between aminochromes and thiols have been reinvestigated. In general aminochromes react with thiols to give mixtures of products including: 5,6-dihydroxyindoles; thio-substituted 5,6-dihydroxyindoles (the mercapto residue is now considered to be in the 4-position of the indole nucleus and not the 7-position as previously reported); and relatively unstable addition products formed between the aminochrome and the thiol. The mechanisms by which these compounds are formed are discussed. The characterization of three thio-substituted 5,6-dihydroxyindoles by nuclear magnetic resonance spectroscopy is described. The nuclear magnetic resonance spectra of a number of 5,6-diacetoxyindole derivatives are reported.

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Solutions of adrenochrome (**1**) are readily decolorized by a wide variety of thiols (1–9). The nature of the products obtained from aminochromes in reactions of this type and the mechanisms by which they were formed have been the subjects of several recent investigations (6–13). As a result of these studies it was concluded that (6–9) aminochromes react with thiols to form three different types of product: (**A**) 5,6-dihydroxyindoles; (**B**) thio-substituted 5,6-dihydroxyindoles; and (**C**) addition products of the aminochrome with the thiol which appear to be structurally similar to the adrenochrome–sodium bisulfite addition product (cf. 14). It has been reported that in the case of the reaction between adrenochrome (**1**) and glutathione the type **A**, **B**, and **C** products are respectively: (**A**) 5,6-dihydroxy-1-methylindole (**2**); (**B**) 7-*S*-glutathionyl-5,6-dihydroxy-1-methylindole (**3**); and (**C**) 9-*S*-glutathionyl-2,3,6,9-tetrahydro-3,5-dihydroxy-1-methyl-6-oxoindole (**4**).

Reactions of this type may be of biological importance since the attachment of melanin pigment molecules to proteins could involve interaction of the thiol groups of the protein molecule with aminochrome units, which were either formed as intermediates in the melanization process or were present as integral parts of the melanin macromolecule. The presence of sulfide linkages in melanoproteins was first suggested in 1950 (15), however the manner by which such linkages could form has only recently been systematically investigated (cf. 10–12, 16, 17). Bouchilloux and Kodja studied the reactions that occurred between several oxidized catechol derivatives and the thiol-substituted aminoacids glutathione and cystein (10–12), and observed that a number of products were formed when glutathione reacts with dopachrome (**5**) including 5,6-dihydroxyindole-2-carboxylic acid (**6**), 5,6-dihydroxyindole (**7**), and a compound described as 4-*S*-glutathionyl-5,6-dihydroxyindole (**8**) (12). Mason and Peterson also reported the formation of thio-substituted 5,6-dihydroxyindoles by the interaction of suitable thiols with oxidized 5,6-dihydroxyindole (or 3,4-dihydroxyphenylalanine), (16).

The possibility that aminochrome–thiol addition products (cf. 4) may play a different significant physiological role has been considered. Inchiosa suggested that the inhibition of cardiac actomyosin ATPase by an adrenaline derivative involves the formation of a specific addition product between an oxidation product of adrenaline,

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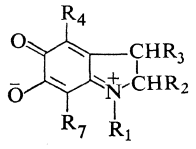
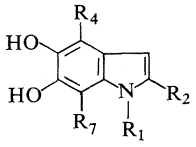
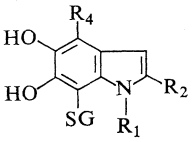


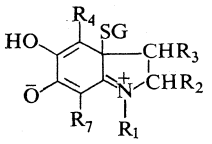
1 : $R_1 = CH_3$; $R_2 = R_4 = R_7 = H$; $R_3 = OH$
5 : $R_1 = R_3 = R_4 = R_7 = H$; $R_2 = COOH$
9 : $R_1 = R_2 = R_7 = H$; $R_3 = OH$; $R_4 = CH_3$
10 : $R_1 = R_2 = R_4 = H$; $R_3 = OH$; $R_7 = CH_3$
16 : $R_1 = R_2 = R_4 = H$; $R_3 = OH$; $R_7 = I$
23 : $R_1 = C_2H_5$; $R_2 = R_4 = R_7 = H$; $R_3 = OH$
24 : $R_1 = i\text{-}C_3H_7$; $R_2 = R_4 = R_7 = H$; $R_3 = OH$



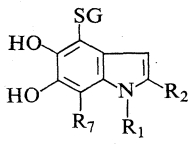
2 : $R_1 = CH_3$; $R_2 = R_4 = R_7 = H$
6 : $R_1 = R_4 = R_7 = H$; $R_2 = COOH$
7 : $R_1 = R_2 = R_4 = R_7 = H$
11 : $R_1 = R_4 = CH_3$; $R_2 = R_7 = H$
12 : $R_1 = R_7 = CH_3$; $R_2 = R_4 = H$



3 : $R_1 = CH_3$; $R_2 = R_4 = H$
14 : $R_1 = R_4 = CH_3$; $R_2 = H$



4 : $R_1 = CH_3$; $R_2 = R_4 = R_7 = H$; $R_3 = OH$
17 : $R_1 = R_7 = CH_3$; $R_2 = R_4 = H$; $R_3 = OH$



8 : $R_1 = R_2 = R_7 = H$
13 : $R_1 = CH_3$; $R_2 = R_7 = H$
15 : $R_1 = CH_3$; $R_2 = H$; $R_7 = I$

SG = glutathione residue

(isomeric with adrenochrome) and the thiol groups in the myosin molecules (13). Mattok and Heacock had previously suggested that the formation of adrenochrome–thiol adducts (such as **4**) offered the possibility that suitable thiols could act as "carriers" for the highly reactive aminochromes in physiological systems (6, 7).

The structures of the thio-substituted 5,6-dihydroxyindole derivatives (cf. **3** or **8**) were proposed on the basis of chromatographic and spectroscopic evidence (cf. 6–12) and on mechanistic considerations (6–8). Whilst structures of the type **3** or **8** were not incompatible with the evidence available, the question of the precise position of the thiol residue was not satisfactorily resolved. It was therefore decided, firstly to investigate the influence of substituents in the 4- and 7-positions of the aminochrome nucleus on their reactions with thiols in general, and secondly to attempt the isolation and unambiguous characterization of one of the thio-substituted 5,6-dihydroxyindole derivatives.

Aqueous solutions of adrenochrome (**1**); 4-methyladrenochrome (**9**); and 7-methyladrenochrome (**10**) were treated, in turn, with a slight excess of each of several different thiols. After filtration the reaction mixtures were examined spectroscopically and paper chromatographically (cf. 5–9). 4-Methyladrenochrome (**9**) reacts with these thiols to give essentially two products. The main product (R_f, 0.34)[8] appeared to be 5,6-dihydroxy-1,4-dimethylindole (**11**). A second, minor, product (R_f, ca. 0.49–0.55) was also detected in most cases. However, no products

[8]Unless otherwise stated the R_f values quoted in this paper were obtained by means of descending paper chromatography, using 2% acetic acid in water as the running solvent, and Ehrlich's reagent for visualization.

with R_f values > 0.8 were observed in any of the reaction mixtures derived from **9**. The chromatographic pictures obtained from the reaction mixtures derived from 7-methyladrenochrome (**10**) were somewhat different. Two main products were observed in most cases, neither of which appeared to be 5,6-dihydroxy-1,7-dimethylindole (**12**); one had an R_f value of ca. 0.55 whilst a second product (R_f, ca. 0.8), which reacted quite slowly with Ehrlich's reagent, was detected.

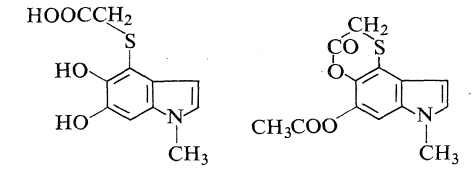
All the reaction mixtures absorbed maximally in the 275–300 mμ region. The reaction mixtures prepared from **1** or **10** had absorption maxima at 300 mμ, whilst those from **9** absorbed maximally at 276 mμ (shoulder at 290 mμ). The adrenochrome (**1**) reaction mixtures also showed absorption maxima at 350 mμ, whilst those from its 7-methyl derivative (**10**) absorbed at 380 mμ; however, no significant absorption was detected in the 300–400 mμ region in the products derived from 4-methyladrenochrome (**9**). For comparison purposes solutions of 5,6-dihydroxy-1-methylindole (**2**), 5,6-dihydroxy-1,4-dimethylindole (**11**), and 5,6-dihydroxy-1,7-dimethylindole (**12**) were prepared by the reduction of **1**, **9**, and **10** respectively by sodium hydrosulfite. The R_f values of authentic samples of **11** and **12** were 0.36 and 0.31 respectively.

The above-mentioned results suggest that the type and distribution of products obtained by the action of thiols on the aminochromes **9** and **10** are different both from each other and from the corresponding products obtained from **1**. Only traces of 5,6-dihydroxy-1,7-dimethylindole (**12**) were formed by the action of glutathione on **10**. The major product (R_f, 0.56) which contained the thiol residue could not, in view of the substituent originally present in the 7-position, be a 7-thio derivative; this fact alone indicated that the previous formulation of this type of product as a 7-thio derivative (cf. **3**) (cf. 6–9) was incorrect. An examination of the products obtained by the action of glutathione on 4-methyladrenochrome (**9**) tended to confirm this thesis. 5,6-Dihydroxy-1,4-dimethylindole (**11**) (R_f, 0.33) was the major product formed in this case and only traces of a second product (R_f, 0.50) were detected. Substituents in the 4-position of the aminochrome nucleus therefore prevent the formation of thio-substituted 5,6-dihydroxyindoles, similar to those which can be obtained from **1** and **10** (cf. **3** or **8**). These facts suggest that the thiol residue is attached to position-4 of the indole nucleus in the type **B** products and that the product obtained from adrenochrome (**1**) by the action of glutathione, previously described as 7-*S*-glutathionyl-5,6-dihydroxy-1-methylindole (**3**) (cf. 6, 7), is actually the isomeric product 4-*S*-glutathionyl-5,6-dihydroxy-1-methylindole (**13**). The minor product (R_f, 0.50) obtained from **9** may be the 7-*S*-glutathionyl-5,6-dihydroxyindole derivative **14**.

4-*S*-Glutathionyl-5,6-dihydroxy-7-iodo-1-methylindole (**15**) was reported to be one of the major products obtained by the action of glutathione on 7-iodoadrenochrome (**16**) (8); the current results suggest that this structure is the correct one for this compound. Reaction mixtures derived from **1** by the action of thiols always showed strong absorption at ca. 350 mμ; however, this absorption was observed at ca. 380 mμ in the case of the products derived from **10**. This latter value is close to that previously reported for one of the 7-iodoadrenochrome–glutathione reaction products (8). In general 7-substituted aminochromes exhibit absorption maxima at longer wavelengths than the parent compounds [e.g. **1**: λ_{max}, 487 mμ; **16**, λ_{max}, 535 mμ; **10**: λ_{max}, 534 mμ (cf. 18, 19)]. An absorption maximum of ca. 380 mμ would therefore be expected for the 7-methyladrenochrome-glutathione addition product (**17**). However, this type of compound is not formed from 4-methyladrenochrome (**9**), since reaction mixtures derived from **9** by the action of thiols show no absorption at ca. 350 mμ. The fact that this type of compound is not formed in this case may be due to steric hindrance by the 4-methyl substituent to reaction by the thiol at the bridgehead 9-position.

Preliminary attempts to isolate one of the thio-substituted 5,6-dihydroxyindole derivatives, obtained from **1** by the action of thiol-containing aminoacids, were not successful. However, a derivative of a compound of this type has been isolated from the products obtained by the action of thioglycollic acid on adrenochrome (**1**). The reduction of adrenochrome by thioglycollic acid was first described in 1945 (2), and the nature of the products formed was subsequently investigated by Heacock and Scott (5). It appeared that two major products (R_f values: 0.42 and 0.48) and one minor product (R_f, 0.18) were formed. One of the major products (R_f, 0.42) was 5,6-dihydroxy-1-methylindole (**2**) and could be

separated from the other major product (R_f, 0.48) since the latter was soluble in aqueous sodium bicarbonate, whilst **2** was not. The product (R_f, 0.48) exhibited color reactions expected for the indole ring system, the *o*-diphenol group, the carboxy group, and divalent sulfur [alkaline sodium nitroprusside spray reagent (20)]. This substance, presumably **18** or **19**, and in view of some of the evidence presented above, probably the latter, could only be obtained as a yellow oil. A crystalline acetyl derivative was obtained on treatment of the crude product with acetic anhydride and pyridine. The acetyl derivative was not acidic and its infrared spectrum showed no absorption in the NH/OH stretching region, but did show absorption in the carbonyl region at 1765 and 1775 cm^{-1}. The ultraviolet spectrum confirmed that the indole ring system was still intact. Microanalytical data and mass spectrometry indicated an empirical formula of $C_{13}H_{11}NO_4S$. These facts suggested that an intramolecular cyclization reaction had occurred at the same time as the acetylation, to give a structure such as **20** or **21**. An analogous internal cyclization reaction had been observed earlier when the corresponding product obtained by the action of thioglycollic acid on *p*-benzoquinone (21, 22) was allowed to stand in a vacuum desiccator in the presence of phosphorus pentoxide (21).



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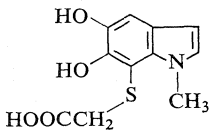

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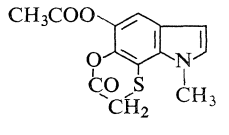

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Nuclear magnetic resonance (n.m.r.) spectroscopy has been widely used for the elucidation of structural problems in the indole series and it appeared that this technique should provide a convenient method for determining which of the structures **20** or **21** was correct for the acetyl derivative, since the problem was essentially one of distinguishing between the presence or absence of the 4- and 7-protons in the indole ring system. The signals due to the protons in the 2- and 3-positions of the indole nucleus are easily recognized (cf. 23–25). Recently, long range coupling has been observed between the protons in the 3- and 7-positions in the indole nucleus (26–31); this coupling (ca. 0.8–1.0 Hz) is of greater magnitude than any of the other possible long range couplings, ignoring couplings involving the proton at position-1, which are not relevant in the present case. The majority of the other couplings are $\leqslant$ 0.3 Hz and are scarcely detectable (30). The exceptions [$J_{2,6} \sim$ 0.4–0.5 Hz and $J_{4,7} \sim$ 0.5–0.8 Hz (30)] would not be observable in compounds such as **20** and **21**.

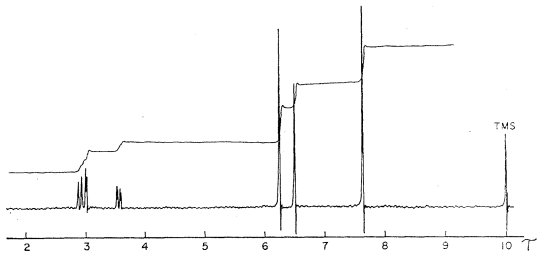


FIG. 1. The nuclear magnetic resonance spectrum of 6-acetoxy-4-*S*-carboxymethylthio-5-hydroxy-1-methylindole lactone (**21**) in $CDCl_3$ with tetramethylsilane as an internal standard.

The n.m.r. spectrum of the acetyl derivative which is shown in Fig. 1 shows that formula **21** is the correct structure for this compound. The signals due to the *C*—CH_3 (τ, 7.65), *N*—CH_3 (τ, 6.5), and *S*—CH_2— (τ, 6.25) (cf. 32) can clearly be seen in the spectrum. In the aromatic proton region the signal due to the 3-proton appears as a doublet of doublets centered at τ, 3.55 with splittings of 3.0 Hz ($J_{2,3}$) and 0.8 Hz ($J_{3,7}$). The 2-proton is observed as a doublet centered at τ, 2.9 ($J_{2,3}$ = 3.0 Hz) whilst the 7-proton appears as a doublet centered at τ, 3.0 ($J_{3,7}$ = 0.8 Hz).

The n.m.r. spectra of a number of related indole compounds with two or more substituents in the benzene ring have been recorded and the relevant τ values and coupling constants for the protons in the 2-, 3-, 4-, and 7-positions are given in Table I. The aromatic proton regions of the n.m.r. spectra of four of these compounds are shown in Fig. 2 and it can clearly be seen that the assignments made for compound **21** are probably correct. In general the signal due to the 4-proton in the

TABLE I

Nuclear magnetic resonance spectral* data for some 5,6-diacetoxyindole derivatives

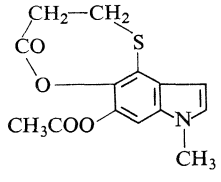
Indole compound	τ-Values†				Coupling constants (Hz)	
	2-H	3-H	4-H	7-H	$J_{2,3}$	$J_{3,7}$
5,6-Diacetoxy-1-methylindole	3.01 (d)‡	3.61 (dd)‡	2.63 (s)‡	2.9 (d)	3.1	1.0
5,6-Diacetoxy-1,4-dimethylindole	3.02 (d)	3.55 (dd)	—	3.05 (d)	3.0	0.8
5,6-Diacetoxy-1,7-dimethylindole	3.05 (d)	3.61 (d)	2.78 (s)	—	3.0	—
5,6-Diacetoxy-7-iodo-1-methylindole	3.05 (d)	3.69 (d)	2.67 (s)	—	3.1	—
5,6-Diacetoxy-7-iodo-1,4-dimethylindole	3.03 (d)	3.65 (d)	—	—	3.0	—
3,5,6-Triacetoxy-7-iodo-1-methylindole	2.73 (s)	—	2.67 (s)	—	—	—
5,6-Diacetoxy-1-isopropylindole§	2.79 (d)	3.55 (dd)	2.63 (s)	2.82 (d)	3.2	1.0
5,6-Diacetoxy-7-iodo-1-isopropylindole§	2.67 (d)	3.58 (d)	2.63 (s)	—	3.3	—
6-Acetoxy-4-carboxymethylthio-5-hydroxy-1-methylindole lactone	2.9 (d)	3.55 (dd)	—	3.0 (d)	3.0	0.8
6-Acetoxy-4-(β-carboxyethyl)thio-5-hydroxy-1-methylindole lactone	2.85 (d)	3.32 (dd)	—	2.88 (d)	3.2	0.9
6-Acetoxy-4-carboxymethylthio-5-hydroxy-1-isopropylindole lactone§	2.73 (d)	3.53 (dd)	—	2.94 (d)	3.0	0.9

*The spectra were recorded in $CDCl_3$ with tetramethylsilane as an internal reference.
†The N-CH_3 signal was always observed as a well defined singlet at τ, 5.8–6.4.
‡(d) = doublet; (dd) = doublet of doublets; (s) = singlet.
§The methine proton was observed as a complex multiplet centered at τ, 5.4 and 5.5 respectively in the cases of 5,6-diacetoxy-1-isopropylindole and 6-acetoxy-4-carboxymethylthio-5-hydroxy-1-isopropylindole lactone, whereas in the case of the 7-iodo compound, 5,6-diacetoxy-7-iodo-1-isopropylindole the methine proton was observed as a complex multiplet centered at τ, 4.00, presumably due to the deshielding effect of the 7-iodo substituent.

spectra of 5,6-disubstituted indole derivatives is observed as a well defined singlet downfield from the other aromatic proton signals (see Fig. 2, spectra of 5,6-diacetoxy-1,7-dimethylindole and 5,6-diacetoxy-7-iodo-1-methylindole), whereas the signal due to the 7-proton is usually seen as a doublet (provided the 3-position is free) with a splitting ($J_{3,7}$) of ca. 0.8–1.0 Hz (see Fig. 2, spectra of 5,6-diacetoxy-1-methylindole and 5,6-diacetoxy-1,4-dimethylindole).

The ultraviolet absorption patterns of solutions of **21** change on alkalinization in the manner that would be expected if the lactone ring opened (see Fig. 3). The long wavelength absorption maximum shifts from 298 mμ (shoulder at 307 mμ) in neutral solution to 337 mμ in alkaline solution; on acidification of the alkaline solution the peak is observed at 320 mμ, suggesting that the lactone ring does not reform. This latter shift (i.e. 337 mμ → 320 mμ) is probably due to the change in absorption associated with the ArO^- → ArOH change. The ultraviolet spectrum of **21** did not change appreciably with time, in alkaline solution (see Fig. 3). In view of the known instability of 6-hydroxyindoles, relative to the corresponding 5-hydroxy isomers, in alkaline solution, this observation tends to confirm that the unacetylated phenolic group is in the 5-position, (after the lactone ring has been opened), rather than in the 6-position, of the indole nucleus.

β-Mercaptopropionic acid readily reduces adrenochrome (**1**) in solution; in this case the major products are 5,6-dihydroxy-1-methylindole (**2**) and 4-*S*-(β-carboxyethylthio)-5,6-dihydroxy-1-methylindole. On treatment with an acetic anhydride – pyridine mixture the latter compound also undergoes an intramolecular cyclization to form a cyclic lactone (i.e. **22**), analogous to **21**, which in this case, contains a seven-membered ring. The structure of this lactone was confirmed by the micronalytical data and by n.m.r. and mass spectroscopy.



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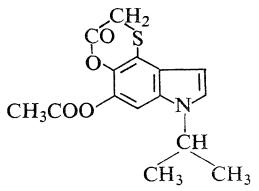

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Preliminary paper chromatographic studies indicated that *N*-ethylnoradrenochrome (**23**) and *N*-isopropylnoradrenochrome (**24**) react analogously to adrenochrome (**1**) with thioglycollic

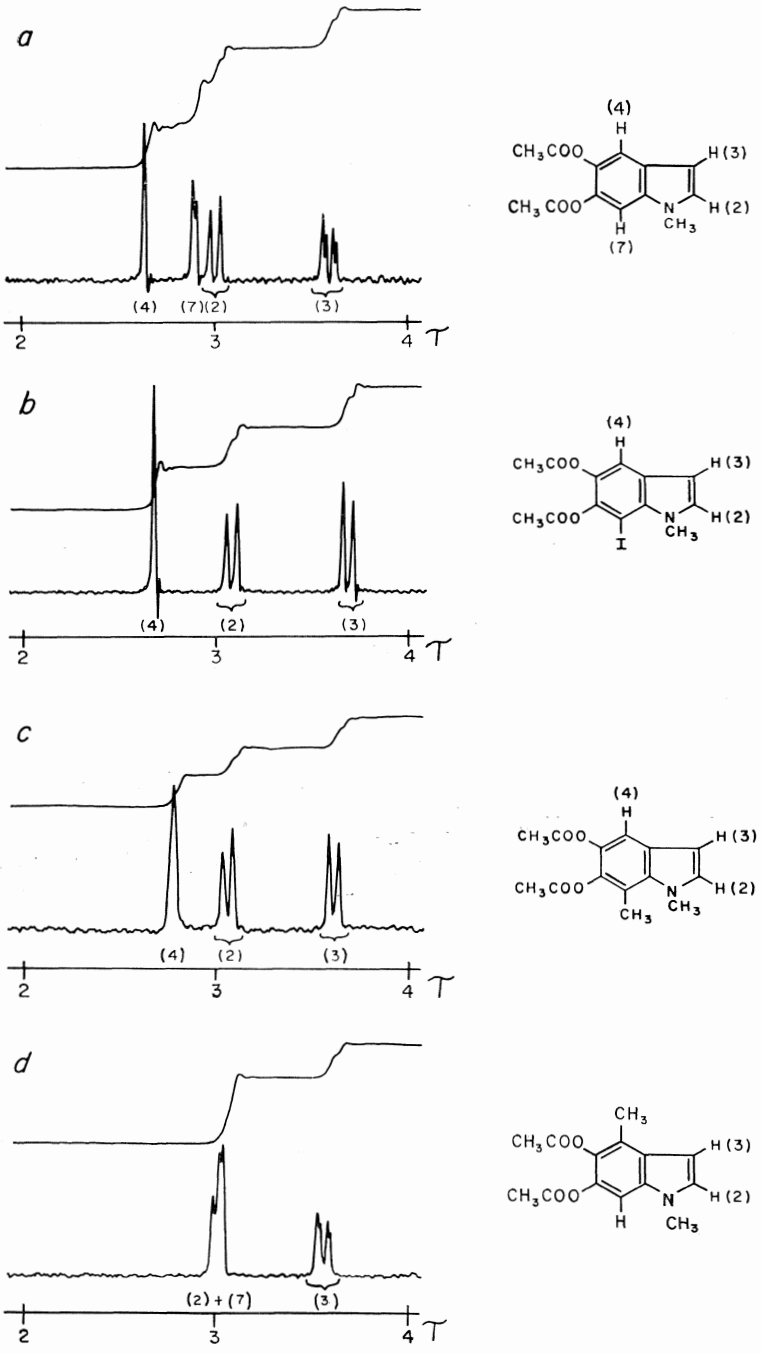


FIG. 2. The nuclear magnetic resonance spectra (in the region τ, 2.00 to τ, 4.00) of: 5,6-diacetoxy-1-methylindole (*a*); 5,6-diacetoxy-7-iodo-1-methylindole (*b*); 5,6-diacetoxy-1,7-dimethylindole (*c*); 5,6-diacetoxy-1,4-dimethylindole (*d*). The spectra were recorded in $CDCl_3$ with tetramethylsilane as an internal standard.

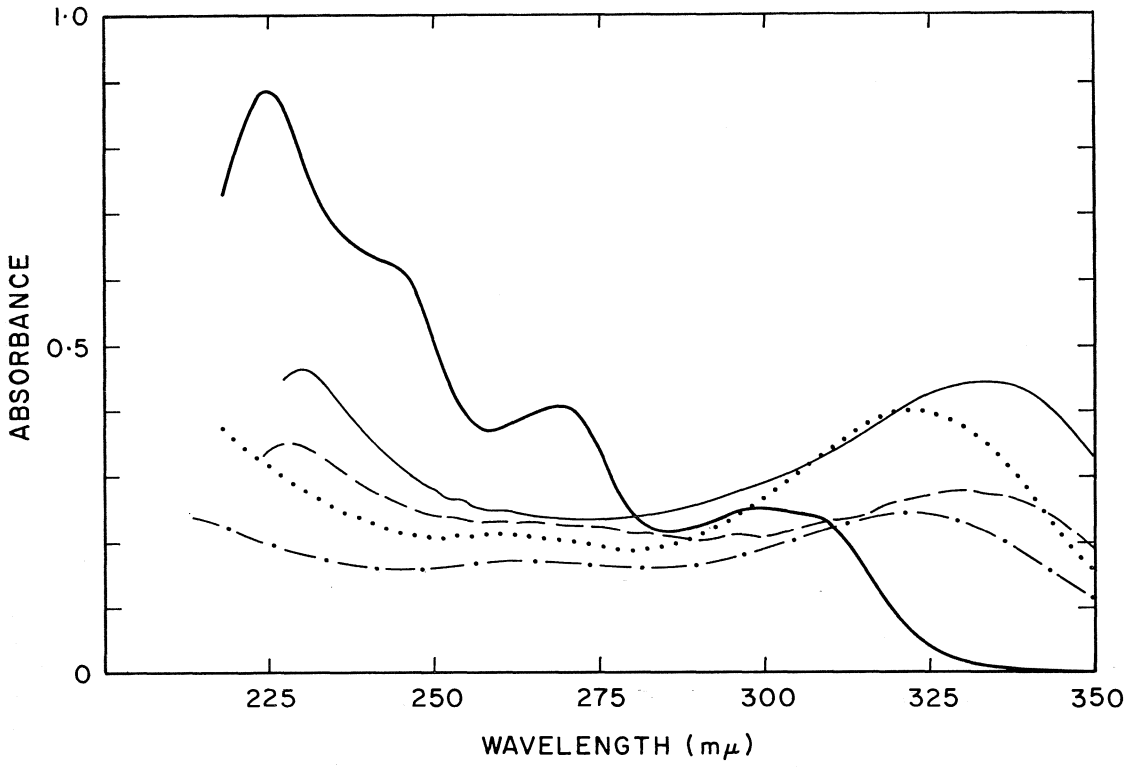
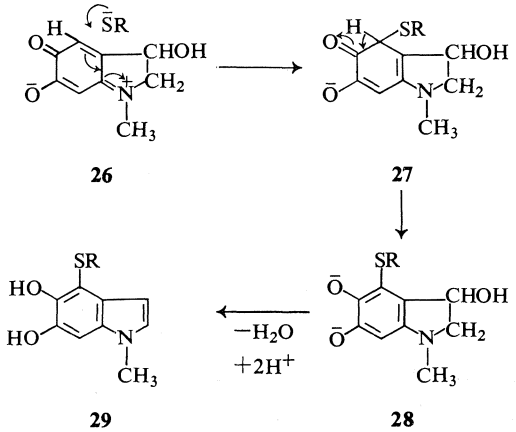
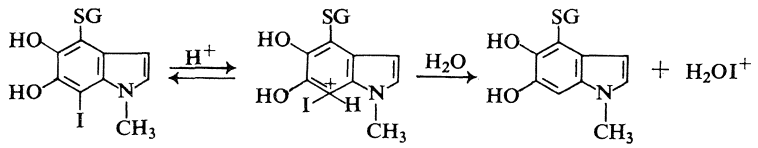


FIG. 3. Absorption spectra of: (*a*) **21** in ethanol (———); (*b*) **21** in ethanol directly after the addition of a few drops of aqueous NaOH (———); (*c*) **21** in ethanol 18 h after addition of the alkali (— — —); (*d*) solution (*b*) acidified (. . . . .); (*e*) solution (*c*) acidified (—·—·—).

acid to form the appropriate 1-alkyl-5,6-dihydroxyindole; 1-alkyl-4-*S*-carboxymethylthio-5,6-dihydroxyindole; and an unknown product, in each case. In the latter case the lactone analogous to **21** (i.e. **25**) was isolated and characterized unambiguously.

It was suggested that the main products obtained by the interaction of thiols with aminochromes were formed either by direct reduction, or by 1,4-addition of the thiol to the two α,β-unsaturated carbonyl systems apparently present in the aminochrome molecule. These were the systems involving the C-5 carbonyl group and either the C_6—C_7 or the C_4—C_9 double bond (6–8) and gave, in the case of the adrenochrome–glutathione reaction, the products **3** and **4** respectively. It would now appear that whilst the second of these alternatives satisfactorily accounts for the formation of **4**, the former reaction probably does not occur. A reaction sequence involving an initial nucleophilic attack at the adrenochrome C-4 carbon by the mercaptide anion, as shown below (i.e. **26** → **27** → **28** → **29**) would, however, satisfactorily account for the formation of 4-thio-substituted 5,6-dihydroxyindole derivatives such as **8** or **13**. The formation of quinone thioethers by nucleophilic substitution reactions has





15 **30** **13**

previously been described (34). It has also been shown that, like glutathione (cf. 7), thioglycollic acid does not react with 5,6-dihydroxy-1-methylindole (**2**) under the conditions employed in this investigation; thus the initial nucleophilic attack by the mercaptide anion must be on the aminochrome molecule.

It would now appear that some of the mechanisms previously proposed for the formation of the products that are observed in 7-iodoadrenochrome–glutathione reaction mixtures (8) require revision. 4-*S*-Glutathionyl-5,6-dihydroxy-7-iodo-1-methylindole (**15**), one of the major products, could be formed from 7-iodoadrenochrome (**16**) by a mechanism analogous to that described above (cf. Scheme 4). The product previously described (8) as 7-*S*-glutathionyl-5,6-dihydroxy-1-methylindole (**3**) is in all probability the isomeric 4-*S*-glutathionyl derivative (i.e. **13**), formed by the acid-catalyzed deiodination of **15** [cf. acid-catalyzed deiodination of some aromatic iodo compounds by the mechanism suggested by Batts and Gold (35)]. Such a mechanism (i.e. **15** → **30** → **13**), would account for the preponderance of deiodinated products observed among the products obtained from 7-iodoadrenochrome (**16**) by the action of glutathione as the free acid as compared to those produced by glutathione as the monosodium salt (8).

Experimental

The infrared spectra were recorded on a Perkin–Elmer model 237 recording spectrophotometer; the ultraviolet (u.v.) absorption spectra were obtained on either a Beckman DK-2 or a Bausch and Lomb "Spectronic 505" recording spectrophotometer and the nuclear magnetic resonance spectra were recorded on a Varian A-60-A instrument.

*Reactions of Adrenochrome (**1**), 4-Methyladrenochrome (**9**), and 7-Methyladrenochrome (**10**) with Some Thiols*

Materials

Adrenochrome (36), 4- and 7-methyladrenochrome (19), *N*-ethylnoradrenochrome (33), *N*-isopropylnoradrenochrome (33), and adrenochrome methyl ether (33) were prepared by methods described in the literature. Glutathione, its monosodium salt, and homocysteine were obtained from the Nutritional Biochemicals Corporation; thioglycollic acid and β-mercaptopropionic acid from the Eastman Kodak Company.

Reaction Mixtures

The reactions were all carried out at room temperature. Aqueous solutions (1 ml) of each of the aminochromes (2 mg/ml) were treated, in turn, with a slight excess (i.e. until the red color of the solution was permanently discharged) of some of the thiols mentioned above. The reaction mixtures were filtered and examined by paper chromatography and by ultraviolet–visible spectroscopy.

Paper Chromatography

Descending chromatography was carried out on Whatman No. 1 paper with 2% acetic acid in water as the running solvent. In all cases the solvent was allowed to descend ca. 35–40 cm, which required 2.5–3.0 h. The developed chromatograms were allowed to dry in air at room temperature; they were then examined for fluorescence in u.v. light and then sprayed with one of the following chromogenic reagents:[9] Ehrlich's reagent, cinnamaldehyde, *p*-dimethylaminocinnamaldehyde, ninhydrin, and Gibb's reagent.

Spectroscopy

Spectra were obtained from suitably diluted solutions of the reaction mixtures or ether extracts, and were recorded on a Beckman DK-2 spectrophotometer.

The results of the chromatographic and spectroscopic examination of the reaction mixtures are shown in Tables II and III.

*6-Acetoxy-4-S-carboxymethylthio-5-hydroxy-1-methylindole Lactone (**21**)*

Freshly prepared silver oxide (30 g) was added portionwise, during a period of about 3–4 min to a stirred solution of adrenaline bitartrate (7.0 g) in water (150 ml). The temperature of the reaction mixture was not allowed to exceed 30° during the addition of the oxidant. The resulting deep red suspension was filtered through a Dowex-1 (×10) (Cl^-) resin bed[10] (diameter = 4.5 cm; height = 2.5 cm) and thioglycollic acid (4 ml) added dropwise, with stirring, to the filtrate. The resulting opaque and brownish-green solution was acidified with conc. hydrochloric acid (3 ml) and extracted with ether[11] (6 × 100 ml). The combined ether extracts, which contained both 5,6-dihydroxy-1-methylindole and 4-*S*-car-

[9]The chromogenic reagents were prepared by the usual methods described in the literature (cf. ref. 37).

[10]The resin was prepared by extensive washing with: (*1*) 3 *N* hydrochloric acid and (*2*) water, until neutral to litmus.

[11]Peroxide-free ether was used throughout this investigation.

TABLE II

Paper chromatography* and ultraviolet absorption characteristics of some aminochrome–glutathione reaction mixtures

Aminochrome	λ_{max} (mμ)	R_f values and colors †of products§ with Ehrlich's reagent		
Adrenochrome	350^m‡; 300^m	0.42 (b.v.)^m	0.65 (b.v.)^m	0.85 (b)^m
4-Methyladrenochrome	290^m; 276^m	0.35 (r.b.)^s	0.49 (b)^{vw}	
7-Methyladrenochrome	295^s		0.56 (r.b.)^s	

*Descending development with 2% acetic acid in water on Whatman No. 1 paper.
†Colors: b = blue; b.v. = blue-violet; r.b. = royal blue. Spots due to excess glutathione and oxidized glutathione are not reported.
‡Intensities of spots and absorptions: ^{s} = strong; ^{m} = medium; ^{vw} = very weak.
§The R_f values of authentic samples of 5,6-dihydroxy-1-methylindole, 5,6-dihydroxy-1,4-dimethylindole, and 5,6-dihydroxy-1,7-dimethylindole under these conditions are 0.41, 0.36, and 0.31 respectively.

TABLE III

Paper chromatography* of the products obtained by the action of thioglycollic acid on some aminochromes

Aminochrome	R_f values† and intensities of main spots	Colors with chromogenic reagents‡			
		Ehr.	Cinn.	DMAC	NaNP
Adrenochrome	0.18 (m)§	B	P→GyV	B→BGy	G→Gy
	0.44 (s)	BV	OrBr→VBr	BG→B	—
	0.51 (s)	BV	OrR→GyV	BG→BGy	BG→Bg
	0.80 (m)‖				
N-Ethylnoradrenochrome	0.24 (m)	B	P→GyV	B→BGy	G→Gy
	0.52 (s)	BV	OrBr→VBr	BG→B	—
	0.55 (s)	BV	OrR→GyV	BG→BGy	BG→Bg
	0.80 (m)‖				
N-Isopropylnoradrenochrome	0.28 (m)	B	P→GyV	B→BGy	G→Gy
	0.57 (s)	BV→V	OrBr→VBr	BG→BGy	—
	0.60 (s)	V→BV	OrR→GyV	BG→BGy	BG→Bg
	0.80 (m)‖				
Adrenochrome methyl ether	0.16 (m)	B	P→GyV	B→BGy	G→Gy
	0.44 (s)	BV	OrBr→VBr	BG→B	—
	0.48 (s)	BV	OrR→GyV	BG→BGy	BG→Bg
	0.80 (m)‖				

*Descending development with 2% acetic acid in water on Whatman No. 1 paper.
†The R_f values of the first three spots using radial development are 0.27, 0.56, and 0.61 respectively.
‡Reagents: Ehr. = Ehrlich's reagent; Cinn. = cinnamaldehyde; DMAC = *p*-dimethylaminocinnamaldehyde; NaNP = sodium nitroprusside. Colors: B = blue; BV = blue–violet; BG = blue–green; BGy = blue–grey; V = violet; VBr = violet–brown; GyV = grey–violet; OrBr = orange–brown; OrR = orange–red; Gy = grey; Bg = beige.
§s = strong; m = medium; w = weak.
‖Excess thioglycollic acid (no color reactions given).

boxymethylthio-5,6-dihydroxy-1-methylindole, were extracted with saturated aqueous sodium bicarbonate (6 × 100 ml). Concentrated hydrochloric acid (50 ml) was added slowly with stirring to a two-phase system composed of the combined sodium bicarbonate extracts and ether (100 ml). Small quantities of ether were added from time to time to replace losses due to evaporation. After addition of the acid was complete, the ether layer was separated and the aqueous mother liquors further extracted with ether (5 × 100 ml). The original ether layer and extracts were combined, dried (Na_2SO_4), and filtered. A mixture of acetic anhydride (7.5 ml) and dry pyridine (7.5 ml) was added to the filtrate and the reaction mixture allowed to stand overnight at room temperature, after which time the ether was removed *in vacuo*, below 30°, and the resulting solution added, dropwise, with stirring to an ice–water mixture (500 ml). The crude acetyl derivative, which was obtained as a pale-yellow gummy solid, was dissolved in a benzene–light petroleum[12] mixture (3:1), and adsorbed on a silica gel[13] column (diameter = 2 cm; height = 15 cm). The column was eluted with the same solvent and a series of fractions (50 ml) collected until the eluant gave a positive reaction with Ehrlich's reagent. Concentration, *in vacuo*, below 30°, of the first four Ehrlich negative fractions merely gave traces of unidentifiable gummy products which were discarded. Further elution of the column with the same solvent (300 ml) and (700 ml) gave crude **21** as white crystalline solids (137 mg;

[12]British Drug Houses Analar grade (b.p, 60–80°).
[13]Obtained from the Koch–Light laboratories (200/300 mesh size).

m.p. 159–161°) and (119 mg; m.p. 136–157°). The latter product was a somewhat impure version of the former. The presence of **21** in the eluant was determined by radial chromatography on formamide treated paper using benzene – light petroleum mixtures [either (1:1) or (1:2)] as running solvent. The R_f values of **21** in these systems are 0.86 and 0.65 respectively. The compound **21** could easily be located on the developed chromatograms by spraying with the modified Ehrlich's reagent (*p-N,N*-bis(2-chloroethyl)aminobenzaldehyde) (mauve color) or the sodium nitroprusside reagent (blue–green color) (see Table II).

Pure 6-acetoxy-4-*S*-carboxymethylthio-5-hydroxy-1-methylindole lactone (**21**) (53.6 mg; m.p. 161–163°) was obtained as colorless needles on repeated careful recrystallization of the solid product obtained from the column from light petroleum (b.p. 80–100°): λ_{max} (cyclohexane): 225, 244 (sh), 268, 300, 310 mμ; λ_{max} (EtOH)[14]: 225, 245 (sh), 268, 299, 308 (sh) mμ; ν_{max} (Nujol): 1765, 1775 (sh) cm^{-1}.

Anal. Calcd. for $C_{13}H_{11}NO_4S$ (mol. wt., 277): C, 56.30; H, 4.00; N, 5.05; S, 11.57. Found (mol. wt., 277): C, 56.06; H, 4.02; N, 5.12; S, 11.73.

6-Acetoxy-4-S-(β-carboxyethyl)thio-5-hydroxy-1-methylindole Lactone (*22*)

This was prepared from adrenaline by essentially the procedure described above, using β-mercaptopropionic acid in place of thioglycollic acid and gave colorless plates, m.p. 165–168°.

Anal. Calcd. for $C_{14}H_{13}NO_4S$: C, 57.73; H, 4.50; N, 4.82; S, 11.01. Found: C, 57.45; H, 4.25; N, 4.81; S, 11.28.

6-Acetoxy-4-S-carboxymethylthio-5-hydroxy-1-isopropylindole Lactone (*25*)

This was prepared in an analogous manner from *N*-isopropylnoradrenaline hydrochloride and thioglycollic acid and gave colorless plates, m.p. 154–157°.

Anal. Calcd. for $C_{15}H_{15}NO_4S$: C, 58.98; H, 4.95; N, 4.59; S, 10.51. Found: C, 58.85; H, 4.62; N, 4.73, S, 10.17.

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[14]The u.v. spectrum of **21** in methanol solution is initially similar to those obtained in ethanol or cyclohexane; however, within 30 min at room temperature the above maxima disappear and, are replaced by new maxima at 312 and 240 mμ.