Chemistry of the Aminochromes. Part XVII.[1] Formation of Addition Products with Bisulphite and Thiols †‡

By **W. S. Powell** and **R. A. Heacock,*** Chemistry Department, Dalhousie University, Halifax, Nova Scotia, and Atlantic Regional Laboratory, National Research Council of Canada, Halifax, Nova Scotia, Canada

It has been confirmed that the addition products formed between aminochromes (indoline-5,6-diones) and sodium hydrogen sulphite are 3a,4-dihydroaminochrome-3a-sulphonates. These compounds were found to exist as the keto tautomers in aqueous solution and not as the enol tautomers as postulated earlier. Aminochromes also react with thiols, to give 3a-sulphides analogous to the 3a-sulphonates. Several of these have been isolated as their arylhydrazone derivatives, the structures of which have been determined by physical and chemical methods.

AMINOCHROMES(INDOLINE-5,6-DIONES) with a 3-hydroxy-group, such as adrenochrome (1), have been shown to react with thiols to give, in general, three main types of product, two of which are indoles (5,6-dihydroxyindoles and 5,6-dihydroxyindol-4-yl sulphides), whereas the

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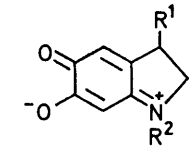
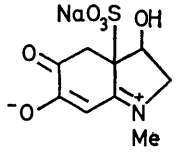
‡ Preliminary reports, *Experientia*, 1972, **28**, 124; *Chem. and Ind.*, 1971, 1021.

[1] Part XVI, W. S. Powell, R. A. Heacock, D. G. Smith, and A. G. McInnes, *Canad. J. Chem.*, in the press.

other is an aminochrome–thiol addition product.[2-5] Adrenochrome methyl ether (2) reacted similarly to give



(1) R^1 = OH, R^2 = Me
(2) R^1 = OMe, R^2 = Me
(3) R^1 = H, R^2 = Me
(7) R^1 = OH, R^2 = Pr^i



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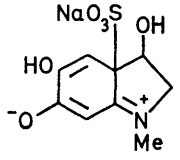

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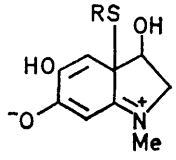


(6)

indolic products,[4] whereas epinochrome (3) reacted with thiols to give indolines rather than indoles.[6]

Some of the properties of the adrenochrome–glutathione addition product, first described in 1965,[2] were similar to those of the addition product [7,8] formed between adrenochrome and sodium hydrogen sulphite, but the former appeared to be less stable. The thiol addition product had $\lambda_{max.}$ *ca.* 350 nm (*cf.* 348 nm for the sulphite addition product) and each compound exhibited yellow fluorescence in u.v. light. They both reacted with ferric chloride and with Ehrlich's reagent, giving similar colours. Both were readily decomposed by alkali with regeneration of adrenochrome.[2]

Tse and Oesterling [8] postulated that the adrenochrome–sodium hydrogen sulphite addition product had structure (4) in the solid state and structure (5) in aqueous solution. This conclusion was based mainly on microanalytical and i.r. spectral data together with the foregoing considerations. Furthermore the sulphite addition product was reported to form a monosemicarbazone and a mono-*p*-nitrophenylhydrazone, which could be converted by either heat or alkali into compounds with properties similar to those of adrenochrome monosemicarbazone and mono-*p*-nitrophenylhydrazone, respectively (*cf.* ref. 7). As a result of the similarity between the properties of the adducts Mattock and Heacock proposed that the thiol addition product had structure (6).[2]

The ^{1}H n.m.r. spectrum of the adrenochrome–sodium hydrogen sulphite addition product in [$^2H_6$]dimethyl sulphoxide and in deuterium oxide has now been measured and is in accord with structure (4). With the former solvent, the spectrum resembles that of adrenochrome (1) in the same solvent (see Table and ref. 1). However the low-field doublet observed for the 4-proton in the latter is replaced by a two-proton singlet at higher field [C(4)H_2]. The ABX system observed for the C(3)H–C(2)H_2 group is modified in the case of the sulphite addition product since one of the coupling constants ($J_{2A,3}$) is now approximately zero.

^{1}H N.m.r. data * for adrenochrome and some of its derivatives and addition products

Compd.:	(1)	(12)	(4)	(11) †
4-H	6·42 (d)	6·92 (d)		
OH	6·02 (d)	5·70 (d)	5·65 (d)	
7-H	5·39 (s)	5·52 (s)	5·41 (s)	5·77 (s)
3-H	4·98 (m)	5·02 (m)	4·48 (t)	4·73 (d)
2B-H	4·02 (dd)	3·91 (dd)	4·12 (dd)	4·31 (dd)
2A-H	3·51 (dd)	3·46 (dd)	3·34 (d)	3·77 (d)
NMe	3·08 (s)	3·02 (s)	2·95 (s)	2·96 (s)
Other		8·36—7·39 (aromatic), 16·45 (s, NH)	2·91 (s, 4-H_2)	8·40—7·76 (aromatic), 4·13 (d, 4A-H), 3·67 (d, 4B-H), 3·03 (m, CH_2·CH_2)
$J_{3,4}$	2·0	1·2		
$J_{3.OH}$	5·5	4·3	5·2	
$J_{2B.3}$	6·8	6·8	4·5	3·4
$J_{2A.3}$	3·4	3·2	*ca.* 0·0	*ca.* 0·0
$J_{2A.2B}$	−12·1	−12·0	−11·9	−11·9

* Solutions in $(CD_3)_2SO$ unless otherwise stated; δ values in p.p.m. from internal Me_4Si; J in Hz. † In C_5D_5N–D_2O (5 : 1).

In solution in D_2O the 4-protons are observed as an AB system (J_{AB} −17·0 Hz). No evidence was found for the existence of the enol tautomer (5) in this solvent and no exchange involving the 4-protons was observed.

The addition products between sodium hydrogen sulphite and both 1-isopropylnoradrenochrome (7) and adrenochrome methyl ether (2) were obtained in pure form. Their spectral characteristics were analogous to those of compound (4). Epinochrome (3), however, reacted with sodium hydrogen sulphite to give an unresolved mixture of products.

The aminochrome–thiol addition products were considerably less stable than the sulphite addition products and were not isolated in the solid state. van Espen [7] had reported that stable monosemicarbazone and mono-*p*-nitrophenylhydrazone derivatives of the adrenochrome–sodium hydrogen sulphite addition product could be prepared. This suggested that similar derivatives might be obtained from the aminochrome–thiol addition products.

The first reaction studied in the current investigation was that between adrenochrome (1) and *N*-acetylcysteine. Preliminary experiments indicated that, under acidic conditions, this reaction gave rise mainly to products absorbing in the region 300—310 nm, presumably 5,6-dihydroxy-1-methylindole ($\lambda_{max.}$ 299 nm) and a 5,6-dihydroxy-1-methylindol-4-yl sulphide, which would be expected to have $\lambda_{max.}$ *ca.* 305 nm.[4] At neutral or only slightly acidic pH, however, a product with $\lambda_{max.}$

[2] G. L. Mattok and R. A. Heacock, *Canad. J. Chem.*, 1965, **43**, 119.
[3] G. L. Mattok, *Arch. Biochem. Biophys.*, 1967, **120**, 170.
[4] W. Powell, R. A. Heacock, G. L. Mattok, and D. L. Wilson, *Canad. J. Chem.*, 1969, **47**, 467.
[5] R. A. Heacock and W. S. Powell, *Progr. Medicin. Chem.*, 1972, **9**, 275.
[6] W. S. Powell and R. A. Heacock, *Canad. J. Chem.*, 1972, **50**, 3360.
[7] J. van Espen, *Pharm. Acta Helv.*, 1958, **33**, 207.
[8] R. L. Tse and M. J. Oesterling, *Clin. Chim. Acta*, 1963, **8**, 393.

358 nm, presumably the adduct, was initially the major product, although it was eventually replaced by the indolic products.

The reaction was then carried out at pH 5·2 and was followed spectroscopically. After *ca.* 1 h, when the concentration of the addition product had reached a maximum (as indicated by its $\lambda_{max.}$ at 358 nm; see Figure 1), aqueous semicarbazide, at the same pH, was added. This gave rise to a much more intense peak at 352 nm, presumably due to a semicarbazone of the adduct. When semicarbazide was not added, however, the peak at 358 nm diminished in intensity and was gradually replaced by another at about 310 nm, due to the indolic products. Addition of alkali to a solution containing the semicarbazone resulted in its gradual

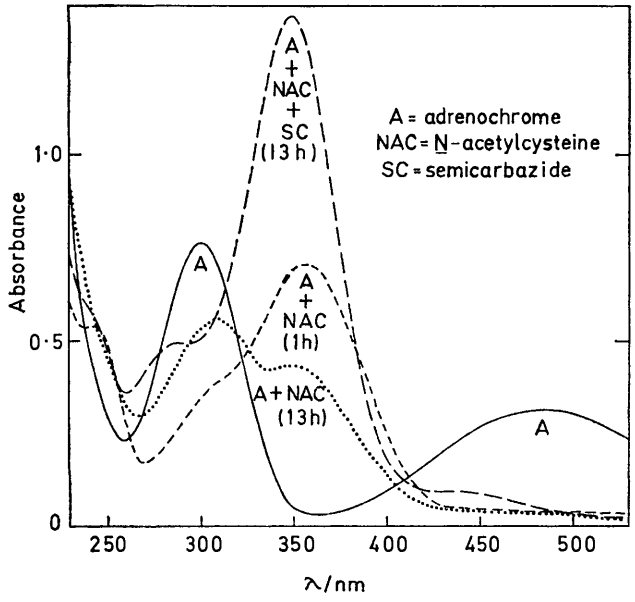


FIGURE 1 U.v.–visible spectra of adrenochrome, the products of the adrenochrome–*N*-acetylcysteine reaction (pH 5·2) and the products of the adrenochrome–*N*-acetylcysteine–semicarbazide reaction (pH 5·2)

conversion into a compound having spectroscopic properties similar to those of adrenochrome monosemicarbazone (8). In alkaline solution this degradation product had $\lambda_{max.}$ 443 nm which shifted to 356 nm (shoulder at 445 nm) in neutral solution and to 374 nm in dilute acid. The corresponding values for compound (8) in alkaline, neutral, and acidic solutions are 444, 355 (a shoulder at 445 nm), and 376 nm, respectively.

The reaction mixture containing the addition product semicarbazone was also examined by two-dimensional t.l.c. on cellulose, with propan-2-ol–water–acetic acid (14 : 5 : 1) as eluant in both directions (see Figure 2). After development in the first direction the major component appeared to be a pale yellow substance (R_F 0·25) exhibiting an intense blue fluorescence in u.v. light. A considerable amount of the semicarbazone (8), observed as an intense yellow spot (R_F 0·64), and several minor products were also present. Upon heating the developed chromatogram at 70° overnight the spot of R_F 0·25 became darker yellow. After cooling to room temperature the chromatogram was rerun in the same solvent at

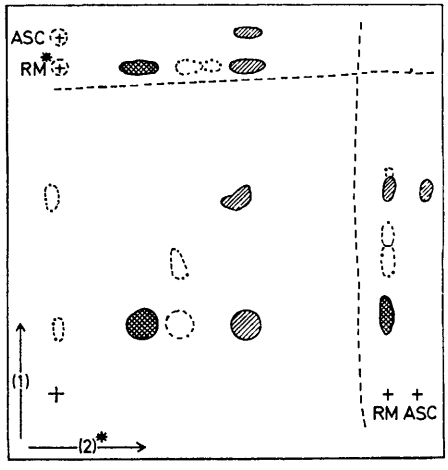
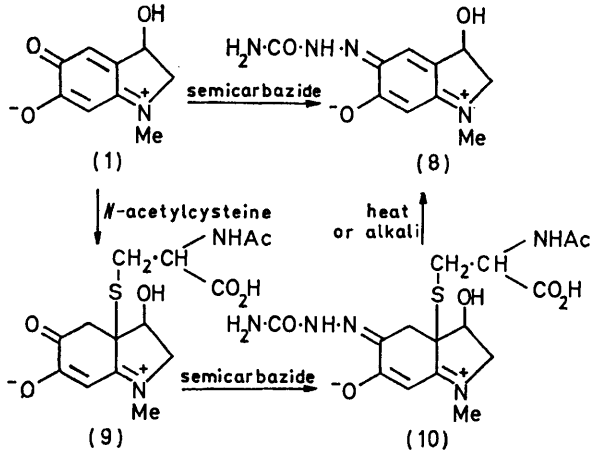


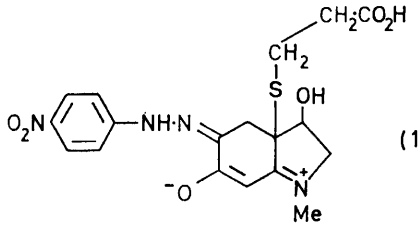
FIGURE 2 Two dimensional t.l.c. of the products of the adrenochrome–*N*-acetylcysteine–semicarbazide reaction (RM = reaction mixture; ASC = adrenochrome semicarbazone; * after heating)

90° to the first direction. The yellow substance (R_F 0·25) gave rise to an additional yellow product identical in R_F value to compound (8). The heating had apparently partially converted the original product (R_F 0·25), presumably the semicarbazone of the addition product, into adrenochrome monosemicarbazone (8).

These experiments indicate that the C-5 carbonyl group of the aminochrome–thiol addition product [see (9)] had reacted with the semicarbazide to give a monosemicarbazone [see (10)], showing that the addition product could not have been a thioacetal or an α-hydroxy-sulphide formed by reaction involving the C-5 carbonyl group. The formation of what appears to be a mono- rather than di-semicarbazone is explicable if the C-6 carbonyl group of the addition product is part of a vinylogous amide system, as it is in adrenochrome. These results are therefore consistent with the proposed attachment of the thiol residue to C-3a of adrenochrome and can be explained as shown below.



Attempts to isolate the semicarbazone of the adrenochrome–*N*-acetylcysteine addition product were unsuccessful owing to its high solubility in water, even at acid pH. The *p*-bromo- and *p*-nitro-phenylhydrazones were obtained in the solid state, however, along with the *p*-nitrophenylhydrazones of the addition products



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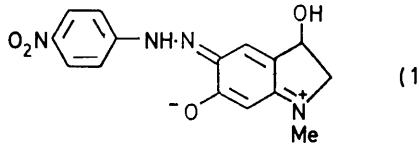


(12)

formed between β-mercaptopropionic acid and adrenochrome (1), 1-isopropylnoradrenochrome (7), and epinochrome (3). These derivatives were soluble in aqueous sodium hydrogen carbonate but insoluble in acid. They were reasonably stable and could be recrystallized in most cases from methanol or methanol–ethyl acetate. Microanalytical data and equivalent weights were compatible with the structures proposed. Mass spectra did not show molecular ions, which was not surprising since the compounds decomposed without melting, over a wide temperature range upon heating.

The i.r. spectra of these compounds were difficult to interpret because of their complexity; only tentative assignments have been made. The spectrum of the *p*-nitrophenylhydrazone of the adrenochrome–β-mercaptopropionic acid addition product in Nujol, for example, exhibits a peak at **3335** with a shoulder at **3290** cm^{-1}, presumably due to the NH and 3-OH groups, respectively. The carboxy OH group would be expected to give a fairly broad peak, which is probably covered by the peaks already mentioned above and the strong Nujol peak. A peak at **1723** cm^{-1} is probably due to the carboxylic acid carbonyl group and peaks at **1621** and **1590** cm^{-1} may be due to the vinylogous amide system. The corresponding system in adrenochrome gives rise to peaks at **1613** and **1570** cm^{-1} and in the adrenochrome–sodium hydrogen sulphite addition product to peaks at **1629** and **1571** cm^{-1}. Peaks at **1522** and **1503** cm^{-1} could be due to NH bending and asymmetric NO_2 stretching vibrations. A peak due to symmetric NO_2 stretching vibrations is seen at **1317** cm^{-1}. In general the other addition product arylhydrazones have similar i.r. spectra.

The structures of these compounds were proved unequivocally with the aid of ^1H n.m.r. spectroscopy.

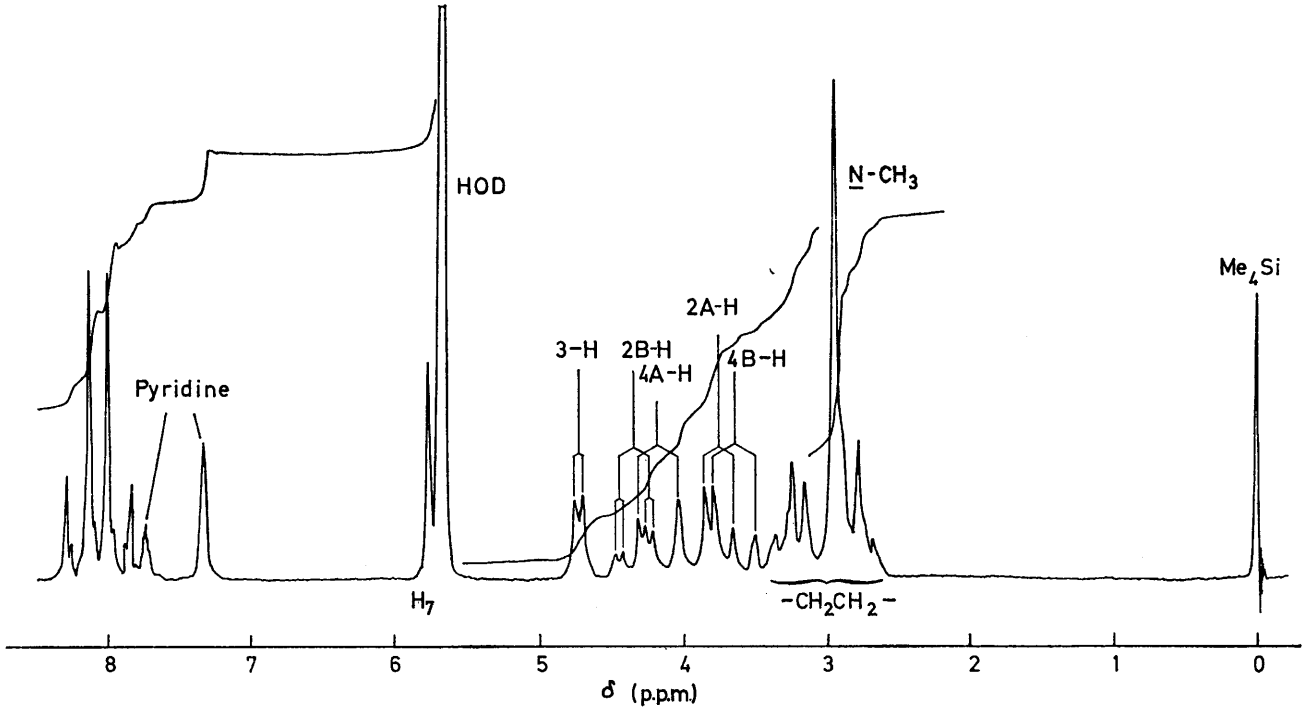


FIGURE 3 ^1H N.m.r. spectrum of the *p*-nitrophenylhydrazone(11) of the adrenochrome–β-mercaptopropionic acid addition product in C_5D_5N–D_2O (5 : 1)

The spectrum of the *p*-nitrophenylhydrazone (**11**) of the addition product between adrenochrome and β-mercaptopropionic acid in [$^2H_5$]pyridine–D_2O (5 : 1) (Figure 3) is in general similar to that of the adrenochrome–sodium hydrogen sulphite addition product (see Table). As in the case of the latter compound, an analogy may be

drawn with the spectrum of adrenochrome [1] as well as that of adrenochrome mono-*p*-nitrophenylhydrazone (12).

As in the case of the sulphite addition product the signal corresponding to that for the 4-proton of adrenochrome (1) or adrenochrome mono-*p*-nitrophenylhydrazone (12) is absent in the spectrum of compound (11). Instead, a two-proton pair of doublets (AB system) is observed at higher field, presumably due to $C(4)H_2$. An analogous AB system was also observed for the adrenochrome–sodium hydrogen sulphite addition product in D_2O.

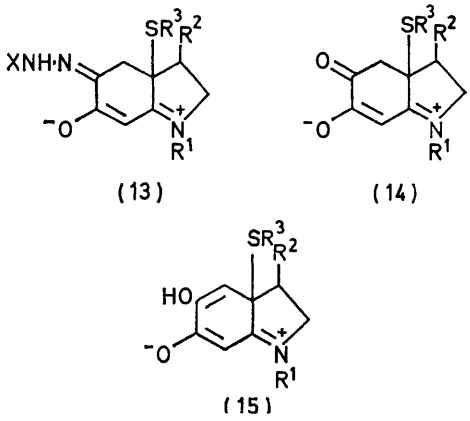
The $C(2)H_2{\cdot}C(3)H$ group gives rise to an ABX system similar to that of the adrenochrome–sodium hydrogen sulphite addition product; $J_{2A,3}$ is again zero. Consequently the C-3 methine signal is observed as a doublet; one of the C-2 methylene protons, H_{2B}, gives a doublet of doublets and the other, H_{2A}, another doublet.

A complex multiplet, centred at δ 3·03, partially obscured by the NMe signal, is observed for the four protons of the carboxylic acid side chain. Signals are also observed for the 7-proton and for the aromatic protons of the arylhydrazone residue; these are similar to those observed in the spectrum of adrenochrome mono-*p*-nitrophenylhydrazone (12).

The ^1H n.m.r. spectra of the other thiol addition product arylhydrazones are similar to that just discussed. No peaks were observed for either the 3-OH or the carboxylic acid OH of any of the compounds investigated, but there was a broad peak which disappeared in the presence of D_2O, probably due to the N·NH of the arylhydrazone residue. In solutions in [$^2H_7$]dimethylformamide–[$^2H_5$]pyridine(3 : 2), for example, this peak was observed at δ 10·12 in the case of the *p*-bromophenylhydrazone of the adrenochrome–*N*-acetylcysteine addition product and at δ *ca.* 10·8 p.p.m. for the *p*-nitrophenylhydrazones of the addition products between adrenochrome and *N*-acetylcysteine and between epinochrome and β-mercaptopropionic acid.

The physical and chemical properties of the substituted arylhydrazone derivatives of the aminochrome–thiol addition products thus confirm that the thiol residue is attached to the 3a-position as previously suggested [2] (*cf.* ref. 8). Most of the evidence obtained in the current investigation concerns the structure of condensation products of aminochrome–thiol adducts and it certainly appears that these compounds exist in the imino form (13). On the basis of the similarity of the properties of the aminochrome–thiol addition products themselves and those of the aminochrome–sodium hydrogen sulphite addition products [2] it appears that the former exist as the keto (14) and not as the enol tautomers (15) as first suggested.[2]

The formation of aminochrome–thiol addition products of this type may have some biological significance. It has been suggested that aminochromes present in the body could be stabilized by the reversible formation of addition products with the thiol groups of naturally occurring amino-acids, peptides, or proteins. These



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substances could then act as ' aminochrome carriers ', regenerating the aminochrome under the appropriate conditions.[2] The formation of aminochrome–thiol addition products may also be important in explaining the mechanism of inhibition of certain enzymes by catecholamine oxidation products.[9-12]

EXPERIMENTAL

General.—The u.v.–visible spectra were obtained with either a Bausch and Lomb Spectronic 505 or a Beckman DK-2 recording spectrophotometer; the i.r. spectra were measured on a Perkin-Elmer 237 recording spectrophotometer and the ^1H n.m.r. spectra were recorded on a Varian A-60A instrument (tetramethylsilane as internal reference).

Adrenochrome,[13] 1-isopropylnoradrenochrome,[14] adrenochrome methyl ether,[14] epinochrome,[6] and sodium 3a,4-dihydroadrenochrome-3a-sulphonate (*i.e.* the adrenochrome–sodium hydrogen sulphite addition product [7]) were prepared as described in the literature.

All the aminochrome–sodium hydrogen sulphite adducts and aminochrome–thiol addition product arylhydrazones isolated during this investigation decomposed over a wide temperature range upon heating.

Aminochrome–Thiol Addition Products.—Preliminary experiments. (a) *Formation of the semicarbazone* (10). A solution of adrenochrome (40 mg) in water (4 ml) was added to a stirred solution of *N*-acetylcysteine (72·8 mg, 2 equiv.) in acetate buffer (4 ml) at pH 5·2. Samples were taken at various times and diluted appropriately with water, and their u.v.–visible spectra in the range 230—530 nm were recorded as rapidly as possible. When the concentration of the adrenochrome–*N*-acetylcysteine addition product ($\lambda_{max.}$ 358 nm) reached its maximum level (*ca.* 65 min), 4 ml of the mixture were added to a stirred solution of semicarbazide hydrochloride (25 mg, 2 equiv.) in acetate buffer (2 ml) at pH 5·2. The reaction was followed spectroscopically as before, the final spectrum being taken 13 h after the

[9] V. M. Denisov, *Ukrain. biokhim. Zhur.*, 1964, **36**, 711 (*Chem. Abs.*, 1965, **62**, 2930).
[10] M. A. Inchiosa, *Biochem. Pharmacol.*, 1967, **16**, 329.
[11] M. A. Inchiosa and I. B. Rodriguez, *Biochem. Pharmacol.*, 1969, **18**, 1883.
[12] A. R. Krall, G. J. Siegel, D. M. Gozansky, and F. L. Wagner, *Biochem. Pharmacol.*, 1964, **13**, 1519.
[13] R. A. Heacock, C. Nerenberg, and A. N. Payza, *Canad. J. Chem.*, 1958, **36**, 853.
[14] R. A. Heacock and B. D. Scott, *Canad. J. Chem.*, 1960, **38**, 516.

start of the experiment. The semicarbazone of the adduct formed in this way had $\lambda_{max.}$ 352 nm. For comparison the spectrum of the original reaction mixture was also measured at this time (see Figure 1).

(b) *Degradation of the semicarbazone* (10). (i) *By alkali.* A solution of the semicarbazone (0·17 ml) prepared as in (a) was diluted to 200 ml with 0·5N-sodium hydroxide and the reaction was followed spectroscopically. When the reaction was complete (*ca.* 7 h) the mixture was made strongly acidic (pH 1·1) with conc. hydrochloric acid and the u.v.–visible spectrum measured. The pH was then adjusted to 5·1 with conc. aqueous sodium acetate and the spectrum was again measured. The $\lambda_{max.}$ values for the degradation product in strongly acid, weakly acid, and alkaline solutions were 374, 356 (with a shoulder at 445), and 443 nm, respectively.

(ii) *By heating.* A sample of the reaction mixture containing the adrenochrome–*N*-acetylcysteine addition product was examined by two-dimensional t.l.c. on cellulose layers (Eastman Chromagram sheets; 20 × 20 cm) with propan-2-ol–water–acetic acid (14 : 5 : 1) as running solvent in both directions (see Figure 2). The reaction mixture and adrenochrome monosemicarbazone were spotted separately near the edges of the chromatograms as indicated for comparison purposes. The chromatogram was heated at 70° overnight between the first and second developments. Adrenochrome monosemicarbazone, R_F 0·64, was detected by its yellow colour, and the addition product semicarbazone, R_F 0·25, by its strong blue fluorescence in u.v. light.

3a-(β-*Carboxyethylthio*)-3a,4-*dihydroadrenochrome Mono*-p-*nitrophenylhydrazone* (11).—Adrenochrome (500 mg) was added to a solution of β-mercaptopropionic acid (593 mg, 2 equiv.) and sodium acetate (458 mg, 2 equiv.) in water (5 ml). Nitrogen was bubbled through for about 10 min and the solution was then added to a stirred suspension of *p*-nitrophenylhydrazine hydrochloride (582 mg, 1·1 equiv.) and sodium acetate (254 mg, 1·1 equiv.) in water (30 ml). The mixture was stirred for 20 min, then sodium hydrogen carbonate (1 g) was added cautiously. The resulting mixture was filtered and acidified, with stirring, dropwise with 2N-hydrochloric acid. The precipitate was filtered off, dried *in vacuo*, and triturated with methanol (4—5 ml). The remainining solid was filtered off and dissolved in boiling methanol (150 ml). The solution was filtered and kept overnight at −20°, and a small quantity of yellow product which had separated out was filtered off and discarded. Water (150 ml) was added to the filtrate and the solution was concentrated *in vacuo* to about 150 ml, giving a solid (320 mg, 27%). Recrystallisation from methanol–ethyl acetate gave the *adduct* p-*nitrophenylhydrazone* (11) as a yellow microcrystalline solid, $\lambda_{max.}$ (0·1M-$NaHCO_3$ aq.) 231 (ε 13,460) and 416 nm (46,400), $\nu_{max.}$ (Nujol) 3335, 3290sh, 1723, 1621, 1607, 1590, 1522, 1503, and 1317 cm^{-1} (see Table for n.m.r. data) (Found: C, 51·5; H, 4·9; N, 13·1; S, 7·55%; Equiv. wt., 416. $C_{18}H_{20}N_4O_6S$ requires C, 51·4; H, 4·8; N, 13·35; S, 7·65%; Equiv. wt., 420).

3a-(2-*Acetamido*-2-*carboxyethylthio*)-3a,4-*dihydroadrenochrome Mono*-p-*nitrophenylhydrazone*.—Adrenochrome (500 mg) was added to a solution of *N*-acetylcysteine (1·37 g, 3 equiv.) and sodium acetate (687 mg, 3 equiv.) in water (5 ml). Nitrogen was bubbled through for about 10 min and the solution was then added to a stirred suspension of *p*-nitrophenylhydrazine hydrochloride (582 mg, 1·1 equiv.) and sodium acetate (254 mg, 1·1 equiv.) in water (30 ml). The mixture was stirred for 20 min and then sodium hydrogen carbonate (2 g) was added cautiously. The resulting mixture was filtered and acidified dropwise with 2N-hydrochloric acid. The precipitate was filtered off, dried *in vacuo*, and triturated with methanol (3—4 ml), and the remaining solid was filtered off and recrystallized from methanol to give the *adduct* p-*nitrophenylhydrazone* as a yellow microcrystalline solid (265 mg, 20%), $\lambda_{max.}$ (0·25M-$NaHCO_3$ aq.) 233 (ε 13,830) and 416 nm (48,300), $\nu_{max.}$ (Nujol) 3615, 3320, 3245, 1690, 1656, 1637, 1625, 1605, 1592, 1573, 1534, 1511, and 1338 cm^{-1}, δ[$(CD_3)_2N{\cdot}CDO$–C_5D_5N (3 : 2)] 10·80br (1H, s, hydrazone NH), 8·55 (1H, d, $J_{NH,CH}$ 7·7 Hz, cysteine NH), 8·39—7·47 (4H, aromatic AA'BB' centred at 7·93), 5·48 (1H, s, 7-H), *ca.* 4·85 (1H, m, cysteine methine H), 4·63 (1H, d, X of ABX, $J_{2A,3}$ *ca.* 0, $J_{2B,3}$ 3·2 Hz, 3-H), 4·29 (1H, dd, B of ABX, $J_{2A,2B}$ −11·8, $J_{2B,3}$ 3·2 Hz, 2-H), 3·85 (1H, d, A of AB, $J_{4A,4B}$ −16·8 Hz, 4-H), 3·62 (1H, d, A of ABX, $J_{2A,2B}$ −11·8, $J_{2A,3}$ *ca.* 0 Hz, 2-H), 3·56 (1H, d, B of AB, $J_{4A,4B}$ −16·8 Hz, 4-H), *ca.* 3·33 (2H, m, cysteine CH_2), 2·95 (3H, s, NMe), and 2·01 (3H, s, CMe) (Found: C, 50·25; H, 4·85; N, 14·75; S, 6·6%; Equiv. wt., 461. $C_{20}H_{23}N_5O_7S$ requires C, 50·3; H, 4·85; N, 14·65; S, 6·7%; Equiv. wt., 477·5).

3a-(2-*Acetamido*-2-*carboxyethylthio*)-3a,4-*dihydroadrenochrome Mono*-p-*bromophenylhydrazone*.—This was similarly prepared as a yellow microcrystalline *solid* (25% after recrystallization from methanol), $\lambda_{max.}$ (0·1M-$NaHCO_3$ aq.) 240 (ε 15,170), 263sh, and 387 nm (33,800), $\nu_{max.}$ (Nujol) 3610, 3325, 3250, 1694, 1661, 1638, 1622, 1601, 1575, and 1510 cm^{-1}, δ[$(CD_3)_2N{\cdot}CDO$–C_5D_5N (2 : 1)] 10·06br (1H, s, hydrazone NH), 8·43 (1H, d, $J_{NH,CH}$ 8·0 Hz, cysteine NH), 7·44 (4H, s, aromatic), 5·37 (1H, s, 7-H), *ca.* 4·77 (1H, m, cysteine methine H), 4·55 (1H, d, X of ABX, $J_{2A,3}$ *ca.* 0, $J_{2B,3}$ 3·4 Hz, 3-H), 4·26 (1H, dd, B of ABX, $J_{2A,2B}$ −11·5, $J_{2B,3}$ 3·4 Hz, 2-H), 3·68 (1H, d, A of AB, $J_{4A,4B}$ −17·0 Hz, 4-H), 3·56 (1H, d, A of ABX, $J_{2A,2B}$ −11·5, $J_{2A,3}$ *ca.* 0 Hz, 2-H), 3·41 (1H, d, B of AB, $J_{4A,4B}$ −17·0 Hz, 4-H), 3·25 (2H, m, cysteine CH_2), 2·92 (3H, s, NMe), and 1·97 (3H, s, CMe) (Found: C, 46·9; H, 4·55; Br, 15·4; N, 10·45; S, 6·35%; Equiv. wt., 494. $C_{20}H_{23}BrN_4O_5S$ requires C, 46·95; H, 4·55; Br, 15·65; N, 10·95; S, 6·25%; Equiv. wt., 511).

3a-(β-*Carboxyethylthio*)-3a,4-*dihydro*-1-*isopropylnoradrenochrome Mono*-p-*nitrophenylhydrazone*.—This compound was obtained in a similar fashion. After recrystallization from methanol it was isolated as a yellow microcrystalline *solid* in a yield of 33%, $\lambda_{max.}$ (0·1M $NaHCO_3$ aq.) 231 (ε 12,580) and 417 nm (47,100), $\nu_{max.}$ (Nujol) 3635, 3300sh, 3265, 1682, 1607sh, 1595sh, 1577, 1512, 1501, and 1324 cm^{-1}, δ[$(CD_3)_2N{\cdot}CDO$] 10·58br (1H, s, hydrazone NH), 8·35—7·39 (4H, aromatic AA'BB' system centred at 7·87), 5·37 (1H, s, 7-H), 4·34 (1H, d, X of ABX, $J_{2A,3}$ *ca.* 0, $J_{2B,3}$ 3·2 Hz, 3-H), 4·01 (1H, dd, B of ABX, $J_{2A,2B}$ −11·8, $J_{2B,3}$ 3·2 Hz, 2-H), 4·00 (1H, m, *J* 6·5 Hz, N·CH<), 3·63 (1H, d, A of AB, $J_{4A,4B}$ −16·7 Hz, 4-H), 3·59 (1H, d, A of ABX, $J_{2A,2B}$ −11·8, $J_{2A,3}$ *ca.* 0 Hz, 2-H), 3·30 (1H, d, B of AB, $J_{4A,4B}$ −16·7 Hz, 4-H), *ca.* 2·71 (m, $CH_2{\cdot}CH_2$ chain, partially obscured by signal from solvent), 1·25 (3H, d, *J* 6·5 Hz, CMe), and 1·20 (3H, d, *J* 6·5 Hz, CMe) (Found: C, 53·55; H, 5·3; N, 12·4; S, 7·0%; Equiv. wt., 453. $C_{20}H_{24}N_4O_6S$ requires C, 53·55; H, 5·4; N, 12·5; S, 7·15%; Equiv. wt., 448·5).

3a-(β-*Carboxyethylthio*)-3a,4-*dihydroepinochrome Mono*-p-*nitrophenylhydrazone*.—This compound was obtained in essentially the same manner. The product obtained after addition of 2N-hydrochloric acid to the reaction mixture was not sufficiently soluble in methanol to be recrystallized but

it was triturated with a large quantity of this solvent. The compound was thus obtained as a yellow microcrystalline *solid* (30%), $\lambda_{max.}$ (0·1M-$NaHCO_3$ aq.) 229 (ε 14,420) and 416 nm (46,700), $\nu_{max.}$ (Nujol) 3520, 3215, 1687, 1620, 1610, 1588, 1510sh, 1502, and 1324 cm^{-1}, δ(M-$NaDCO_3$ in D_2O) 8·20—7·08 (4H, aromatic AA'BB' centered at 7·64), 5·28 (1H, s, 7-H), 3·61br (3H, m, 2-H_2 and 4-H), 2·97 (3H, s, NMe), and 2·83—1·70 [7H, m, CH_2·CH_2 (δ *ca.* 2·52), 3-H_2 and 4-H], δ[$(CD_3)_2N$·CDO–C_5D_5N (2 : 1)] 10·75br (1H, s, hydrazone NH), 8·35—7·45 (4H, aromatic AA'BB' centred at 7·90), 5·34 (1H, s, 7-H), 4·11 (1H, d, *J* −16·5 Hz, 4-H), 3·67 (2H, m, 2-H_2), 2·94 (s, NMe_3, superimposed upon other signals, including those due to the solvent), and 2·35 (2H, m, 3-H_2); signals for the other 4-H and the CH_2·CH_2 group partially obscured by the solvent signals (Found: C, 53·15; H, 5·05; N, 13·7; S, 8·05%; Equiv. wt., 390. $C_{18}H_{20}N_4O_5S$ requires C, 53·45; H, 5·0; N, 13·85; S, 7·95%; Equiv. wt., 404·5.

Sodium 3a,4-*Dihydro*-3-*methoxyadrenochrome*-3a-*sulphonate*.—Adrenochrome methyl ether (400 mg) was added to a stirred solution of sodium disulphite (186 mg, 0·47 equiv.) in water (40 ml) and nitrogen was bubbled through the mixture for 1 h. Methanol (60 ml) was then added and the solution concentrated to dryness *in vacuo* below 25°. The residue was dissolved in water (2 ml). Ethanol (20 ml) was added, followed by ether (20 ml), giving a tarry precipitate. The supernatant liquid was decanted off and ether (20 ml) was added to it, giving an oily precipitate which was filtered off and dissolved in water (2 ml) and ethanol (20 ml). Addition of ether (30 ml) to the filtered solution gave a light yellow-brown precipitate which was dissolved in water (2 ml) and ethanol (20 ml). The solution was stirred with charcoal for a few minutes and then filtered. Addition of ether (30 ml) gave a pale yellow precipitate which was filtered off. Upon repetition of this procedure, the *adduct* was obtained as pale yellow crystals (224 mg, 34%), $\lambda_{max.}$ (H_2O) 245 (ε 7480) and 349 nm (15,400), $\nu_{max.}$ (Nujol) 1712, 1627, 1568, 1242, and 1049 cm^{-1}, δ[C_5D_5N–$(CD_3)_2SO$ (2 : 1)] 5·79 (1H, s, 7-H), 4·65 (1H, d, X of ABX, $J_{2A,3}$ 0, $J_{2B,3}$ 4·4 Hz, 3-H), 4·40 (1H, dd, B of ABX, $J_{2A,2B}$ −11·4, $J_{2B,3}$ 4·4 Hz, 2-H), 3·65 (1H, d, A of ABX, $J_{2A,2B}$ −11·4, $J_{2A,3}$ *ca.* 0 Hz, 2-H), 3·60 (1H, d, A of AB, $J_{4A,4B}$ −17·0 Hz, 4-H), 3·34 (1H, d, A of AB, $J_{4A,4B}$ −17·0 Hz, 4-H), 3·33 (3H, s, OMe), and 2·95 (3H, s, NMe) (Found: C, 40·6; H, 4·0; N, 4·8; Na, 7·65; S, 10·85. $C_{10}H_{12}NaNO_6S$ requires C, 40·4; H, 4·05; N, 4·7; Na, 7·75; S, 10·8%).

Sodium 3a,4-*Dihydro*-1-*isopropylnoradrenochrome*-3a-*sulphonate*.—This was similarly prepared from 1-isopropylnoradrenochrome and sodium disulphite as a yellow crystalline solid (55%), $\lambda_{max.}$ (H_2O) 247 (ε 7220) and 352 nm (16,400), $\nu_{max.}$ (Nujol) 3366, 1717, 1602, 1566, 1245, and 1052 cm^{-1}, δ[$(CD_3)_2SO$] 5·61 (1H, d, $J_{3,OH}$ 5·0 Hz, OH), 5·47 (1H, s, 7-H), 4·49 (1H, t, X of ABX, $J_{2A,3}$ *ca.* 0, $J_{3,OH} \approx J_{2B,3} \approx$ 4·5 Hz, 3-H), 3·98 (1H, m, *J* 6·6 Hz, N·CH), 3·96 (1H, dd, B of ABX, $J_{2A,2B}$ −11·2, $J_{2B,3}$ 4·1 Hz, 2-H), 3·34 (1H, d, A of ABX, $J_{2A,2B}$ −11·2, $J_{2A,3}$ *ca.* 0 Hz, 2-H), 2·91 (2H, s, 4-H_2), 1·17 (3H, d, *J* 6·6 Hz, CMe), and 1·12 (3H, d, *J* 6·6 Hz, CMe). In solutions in D_2O the 4-protons were observed as an AB system with doublets at δ 3·31 and 3·13 ($J_{4A,4B}$ −17·9 Hz) (Found: C, 42·5; H, 4·6; N, 4·4; Na, 7·3; S, 10·2. $C_{11}H_{14}NaNO_6$ requires C, 42·45; H, 4·55; N, 4·5; Na, 7·4; S, 10·3%).

Adrenochrome Mono-p-*nitrophenylhydrazone* (12).—Adrenochrome (200 mg) was added to a rapidly stirred suspension of *p*-nitrophenylhydrazine hydrochloride (212 mg) in a solution of sodium acetate (115 mg) in water (25 ml). The mixture was stirred for a further 15 min and the red precipitate was filtered off and triturated with methanol (2 × 150 ml). Adrenochrome mono-*p*-nitrophenylhydrazone was obtained as a red microcrystalline *solid* (245 mg, 68%), m.p. 255—258°, $\lambda_{max.}$ (N-NaOH) 280 (ε 8100), 298sh, 421sh, and 585 nm (43,400), $\nu_{max.}$ (Nujol) 3400sh, 3185, 1658sh, 1651, 1594, 1515, 1504, and 1338 cm^{-1} (for n.m.r. data see Table) (Found: C, 57·3; H, 4·45; N, 17·35. $C_{15}H_{14}N_4O_4$ requires C, 57·3; H, 4·5; N, 17·85%).

Veer [15] claimed to have prepared the *p*-nitrophenylhydrazone of adrenochrome as a brown crystalline solid [m.p. 200° (decomp.)] by the reaction of adrenochrome with *p*-nitrophenylhydrazine in boiling 30% acetic acid. The compound which he obtained, however, may well be a degradation product of adrenochrome mono-*p*-nitrophenylhydrazone (12) since our attempts to recrystallize (12) led to its decomposition with the formation of an unidentified brown product.

The synthesis of (12) was also reported in a Belgian patent [16] although neither the experimental details nor the physical characteristics of this compound were given. van Espen [7] apparently successfully synthesized (12) and reported that it had $\lambda_{max.}$ *ca.* 580 nm in 1N-NaOH.

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[15] W. L. C. Veer, *Rec. Trav. chim.*, 1942, **61**, 638.

[16] Société Belge de l'Azote et des Produits Chimiques du Marly, Belg.Pat. 453,374/1943 (*Chem. Abs.*, 1947, **41**, 7686).