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The Interaction of Adrenochrome with Some Thiol-Containing Amino Acids

Isolation of Some Products[1]

W. S. POWELL[2, 3] AND R. A. HEACOCK[4]

Chemistry Department, Dalhousie University, Halifax, Nova Scotia, and *Atlantic Regional Laboratory, National Research Council of Canada, Halifax, Nova Scotia, Canada*

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The interactions of adrenochrome with some thiol-containing amino acid derivatives, namely, *N*-acetylcysteine and *N*-acetylpenicillamine, have been studied. The 5,6-dihydroxy-1-methylindole thioethers which were formed in these reactions were isolated and characterized. The structures of these products were analogous to those of similar compounds which had been obtained from the reactions of aminochromes with some simple thiols. Reactions of this type may be important in the formation of melanoproteins.

Amino acids and peptides containing free —SH groups have been shown to inhibit the polyphenoloxidase-catalyzed formation of melanin from catecholamines (cf. **1**) by combining with some of the intermediates formed during this process. Glutathione and cysteine can add to the open-chain quinone intermediate (cf. **2**) to form addition products which tautomerize to the corresponding catechol derivatives (*1*, *2*). In the case of cysteine the main product is the 5-thiosubstituted catecholamine (cf. **3**) while the 2-thiosubstituted product (cf. **4**) is formed to a lesser extent (*3*, *4*).

If insufficient thiol is present to inhibit the reaction completely at the open-chain quinone stage the thiol may add to the cyclized catecholamine (*1*, *2*, *5*). Bouchilloux and Kodja (*1*, *2*) and later Mason and Peterson (*5*) suggested that the thiol reacted with the indole-5,6-quinone (cf. **5**) to give thiosubstituted 5,6-dihydroxyindoles (cf. **6**). The former authors also found that noradrenochrome (**7**: $R_1 = R_2 = H$; $R_3 = OH$) and dopachrome (**7**: $R_1 = R_3 = H$; $R_2 = CO_2H$), which they had prepared in aqueous solution by oxidation of the corresponding catecholamine, reacted with thiols to give similar products, and they suggested that in such cases the thiol added to the indole-5,6-quinone (*1*, *2*). Recent evidence suggests, however, that the aminochrome is probably the reactive species (*6*).

Adrenochrome (**8**) has been shown to react with cysteine, glutathione, and homocysteine (*7–11*) to give similar products (i.e., 5,6-dihydroxyindole thioethers), along with the reduction product, 5,6-dihydroxy-1-methylindole (**9**).

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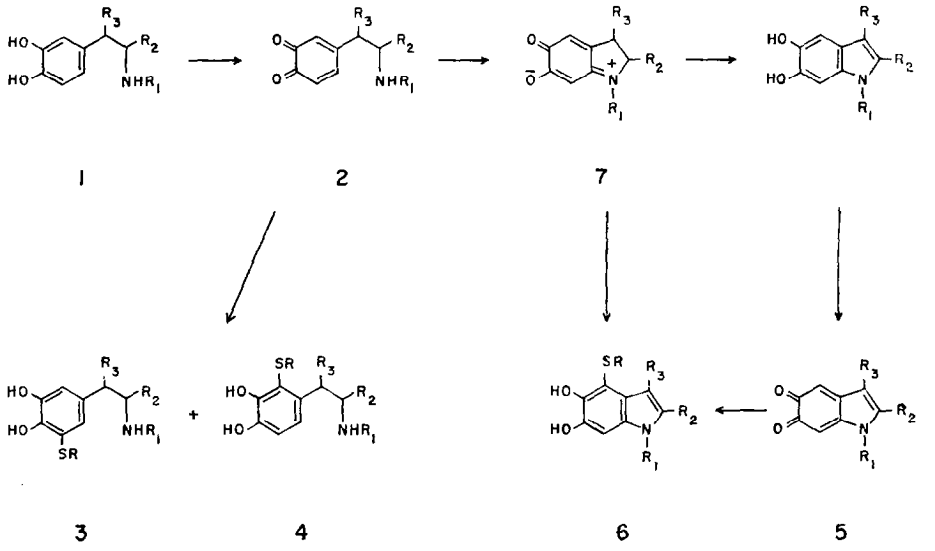
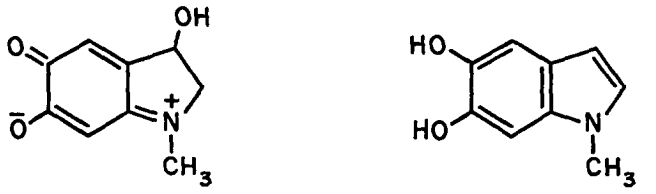
[2] Taken from the Ph.D. thesis of W. S. Powell, Dalhousie University, 1972.

[3] Holder of an NRCC scholarship (1967–1971).

[4] Correspondence and reprint requests should be addressed to this author (R.A.H.) at the Atlantic Regional Laboratory.



The thiosubstituted 5,6-dihydroxyindoles (cf. **6**) produced in the reactions of aminochromes with either thioglycollic acid or β-mercaptopropionic acid have been isolated as their acetyl derivatives (*12*). The thiol substituent has been shown to be present in the 4-position of the indole nucleus in each case (*12, 13*). However, none of the corresponding indole thioethers formed in the reactions of aminochromes with thiol amino acid derivatives have previously been isolated. Compounds of this nature are of considerable interest in melanin formation, since it has been postulated that the melanin–protein



linkages in melanoproteins take the form of bonds between the sulfur of the cysteine units in the protein molecule and the 4-carbon atom of some of the indole subunits of the melanin polymer (*14, 15*).

In view of the relevance of these reactions to melanoprotein formation, an attempt was made to isolate and characterize the indole thioethers formed in this manner.

Homocysteine had been shown to react with adrenochrome (**8**) to give **9**, an adrenochrome–homocysteine addition product (cf. *16*) and a compound which had the properties of a 5,6-dihydroxy-1-methylindole thioether (*11*). Attempts to acetylate this compound during the course of the current investigation, however, resulted in the formation of a complex mixture of unidentifiable products.

The reactions of **8** with some *N*-acetylamino acids were then studied in aqueous solution. The blocking of the amino group was expected to give an ethyl acetate extractable indole thioether and also eliminate secondary reactions involving the amino group. When the reaction between **8** and *N*-acetylcysteine was carried out in an unbuffered solution (pH ca. 2.5), two main products were formed. This was shown by thin-layer chromatography (tlc) on cellulose with 2% aqueous acetic acid as running solvent.[5] One of these products, which had an R_f of 0.45, was identified as **9**[6] and the other, which had an R_f value of 0.68, appeared to be a 5,6-dihydroxy-1-methylindole thioether. The latter substance was soluble in aqueous sodium bicarbonate, but could be extracted from the acidified reaction mixture with ethyl acetate. It exhibited the colour reactions expected for an indole and for a phenol and gave a blue-green colour with aqueous 1 *N* sodium hydroxide.

Acetylation of the indole thioether gave a mixture of indolic products which could not be resolved. Methylation of this compound with diazomethane, however, gave one major indolic product (as determined by tlc) along with several minor ones and *N,N'*-diacetylcystine dimethyl ester.[7] Attempts to purify the indolic compound by column chromatography on silica gel were unsuccessful. After partition chromatography on a column of Sephadex G-25, however, the methylated indole thioether was obtained as a white crystalline solid with a molecular formula of $C_{17}H_{22}N_2O_5S$. The ir spectrum of this compound showed peaks at 1737, 1651, and 1545 cm^{-1} due to ester carbonyl, amide carbonyl and NH groups, respectively. The aromatic region of its pmr spectrum revealed an AB system for the two protons of the pyrrole ring. As in the cases of the acetylated indole thioethers isolated from other aminochrome–thiol reactions (*12*, *13*), the proton in the 3-position was also coupled to the 7-proton, which was observed as a doublet ($J_{3,7} = 0.9$ Hz). There was also a broad doublet in this region due to the NH proton. Signals were observed for four *N*- or *O*-methyl groups and one acetyl methyl group. An ABX system, with the X proton coupled to the proton of the secondary amino group was observed, presumably due to the $>CHCH_2-$ of the cysteine residue. As a result of these observations one can conclude that this compound has the structure **11** (R = H).

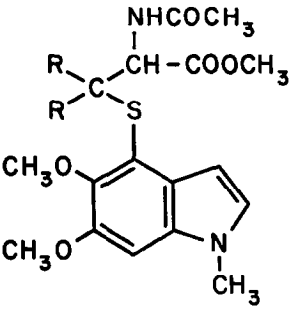
N-Acetylpenicillamine reacted with **8** in a similar manner to *N*-acetylcysteine to give a mixture consisting mainly of 5,6-dihydroxy-1-methylindole (**9**) and an indole thioether along with a considerable amount of dark insoluble material. The relative amount of the indole thioether appeared to be somewhat less in this case, however, probably due to the steric effects of the methyl groups of the *N*-acetylpenicillamine (cf. ref. *11*). After column chromatography, first on Sephadex G-25 and then on silica gel, the

[5] At only slightly acidic pH values (ca. 5), on the other hand, adrenochrome (**8**) has been shown to react with *N*-acetylcysteine to give mainly 3a-[*S*-(*N*-acetylcysteinyl)]-3a,4-dihydroadrenochrome (**10**), which was isolated as its *p*-bromo- and *p*-nitro-phenylhydrazone derivatives. Since **10** was formed reversibly, however, it was gradually replaced in the reaction mixture by the indole thioether and 5,6-dihydroxy-1-methylindole (**9**) (*17*).

[6] The formation of **9** from **8** directly in acid medium has been reported (*18*); however, it is most likely in the present case that the relatively rapid formation of the dihydroxyindole **9** is due to the action of the thiol.

[7] This compound was isolated by column chromatography on silica gel with ethyl acetate as eluant. It was obtained as a white crystalline solid (mp 124–126°) after recrystallization from benzene [lit. mp 125° (*19*)]. Microanalytical data confirmed that it had a molecular formula of $C_{12}H_{20}N_2O_6S_2$.

methylated 5,6-dihydroxyindole thioether was obtained as a pale-yellow oil. Mass spectrometry revealed that the molecular formula of this compound was $C_{19}H_{26}N_2O_5S$. The relevant parts of its pmr spectrum were analogous to those of the compound



11

derived from *N*-acetylcysteine **11** (R = H) so it would appear that the structure of this compound is **11** ($R = CH_3$).

These results indicate that thiol-containing amino acid derivatives react with aminochromes in a manner similar to the simpler thiols which have been studied previously (cf. ref. *12*). It is, therefore, quite possible that similar reactions occur between the thiol groups of proteins and aminochromes formed as intermediates in melanin formation or present as subunits in the melanin macromolecule (cf. refs. *15* and *20*). Reactions analogous to those described above involving quinones and thiol-containing amino acids appear to occur during the biosynthesis of the group of pigments known as the phaeomelanins (cf. refs. *3*, *21–23*).

EXPERIMENTAL

The tlc was carried out using ascending development on Eastman Chromagram cellulose sheets. The chromogenic reagents used were: Ehrlich's reagent, the Folin–Ciocalteu reagent, and 1 *N* aqueous NaOH. The melting points were determined on a Leitz hot-stage polarizing microscope and are uncorrected. The uv, ir, pmr, and mass spectra were measured on Beckman Acta V, Perkin–Elmer model 237, Varian A60-A and Dupont/CEC 21-110B instruments, respectively.

Isolation of 4-S-(N-acetylcysteinyl)-5,6-dimethoxy-1-methylindole Methyl Ester (**11**: *R = H*)

Adrenochrome (500 mg) was added to a stirred two-phase system consisting of ethyl acetate (125 ml) and a solution of *N*-acetylcysteine (1.85 g; 4 equiv) in water (125 ml) (the pH of the reaction mixture was ca. 2.5). Stirring was continued until the red colour of the adrenochrome had been completely discharged (ca. 15 min). After this time the organic layer was removed and the aqueous solution further extracted with ethyl acetate (2 × 125 ml). The ethyl acetate extracts were combined and then extracted with a satu-

rated aqueous $NaHCO_3$ solution (500 ml). The latter solution was acidified to a pH of ca. 2 by the addition of concd HCl and then extracted with ethyl acetate (3 × 500 ml). Diazomethane (ca. 0.75 g) dissolved in ether (40 ml), prepared by the method of DeBoer and Backer (*24*), was added to the combined dried (Na_2SO_4) ethyl acetate extracts. This solution was allowed to stand for 3 hr, after which time it was concentrated *in vacuo* to give a dark-brown oil. This oil was then subjected to partition chromatography on a column (2.5 × 90 cm) of Sephadex G-25 (fine) with the aqueous phase of a water:methanol:ethyl acetate:hexane (2:1:2:2) mixture as the stationary phase and the organic phase as the mobile phase.

The Sephadex was prepared by allowing ca. 150 g of the resin to swell overnight in an excess of the aqueous phase. Excess solvent was then removed by filtration and the swollen Sephadex was slurried with the organic phase and poured, a little at a time, into the column. Each new portion of slurry was packed by compressing the solid with a perforated plunger. The column was equilibrated by prolonged washing with the organic phase until the effluent was free of the aqueous phase.

A series of 5-ml fractions was collected, the flow rate being ca. 10 ml/hr. The fractions were monitored by tlc using Ehrlich's reagent. Fractions 26–33 were combined and the solvent removed *in vacuo* to give a colourless oil. Crystallization from light petroleum (bp 60–80°) gave 4-*S*-(*N*-acetylcysteinyl)-5,6-dimethoxy-1-methylindole methyl ester in clusters of white crystals (82 mg, mp 110–113°). λ_{max} (EtOH) nm (ε): 232 (26,900), 295 (sh), 307 (9270); ν_{max} (KBr): 3295, 1737, 1651, 1614, 1545 cm^{-1}; δ ($CDCl_3$): 6.99 (1 H, d, $J_{2,3} = 3.2$ Hz, H_2); 6.93 (1 H, broad d, $J_{CH,NH} \approx 8$ Hz, NH); 6.82 (1 H, d, $J_{3,7} = 0.9$ Hz, H_7); 6.56 (1 H, dd, $J_{2,3} = 3.2$ Hz, $J_{3,7} = 0.9$ Hz, H_3); 4.78 (1 H, m, X of ABX, $J_{AX} = J_{BX} = 4.5$ Hz, $J_{CH,NH} = 8.2$ Hz, cysteine methine H); 3.92 (6 H, s, 2 *O*- or *N*-CH_3's); 3.72 (3 H, s, *O*- or *N*-CH_3); 3.56 (3 H, s, *O*- or *N*-CH_3); 3.48 (1 H, dd, B of ABX, $J_{AB} = -14.2$ Hz, $J_{BX} = 4.5$ Hz, cysteine methylene H); 3.24 (1 H, dd, A of ABX, $J_{AB} = -14.2$ Hz, $J_{AX} = 4.5$ Hz, cysteine methylene H); 1.75 (3 H, s, *C*-CH_3). Mass spectrum: $M^{+\cdot}$ 366.1254 ± .0010; calcd for $C_{17}H_{22}N_2O_5S$, 366.1250.

Reaction of Adrenochrome with N-Acetylpenicillamine; Isolation of 4-S-(N-Acetylpenicillaminyl-5,6-dimethoxy-1-methylindole Methyl Ester (**11**: $R = CH_3$)

This compound was obtained by the interaction of adrenochrome with *N*-acetylpenicillamine under conditions similar to those which were employed for the preparation of **11** (R = H). The solvent system used for partition chromatography with Sephadex G-25 was water:methanol:ethyl acetate:light petroleum (4:4:3:5). The yellow oil obtained after chromatography on Sephadex was purified by chromatography on a column (17 × 2.2 cm) of silica gel (Davison, 100–200 mesh) with ethyl acetate as eluant. A series of fractions (10 ml) was collected. Fractions 22–28 were combined and concentrated to dryness *in vacuo*, giving **11** ($R = CH_3$) as a colourless oil which could not be induced to crystallize. δ ($CDCl_3$): 7.92 (1 H, broad d, $J_{CH,NH} \approx 9$ Hz, NH); 6.99 (1 H, d, $J_{2,3} = 3.2$ Hz, H_2); 6.89 (1 H, d, $J_{3,7} = 0.8$ Hz, H_7); 6.60 (1H, dd, $J_{2,3} = 3.2$ Hz, $J_{3,7} = 0.8$ Hz, H_3); 4.53 (1 H, d, $J_{CH,NH} = 8.5$ Hz, penicillamine CH); 3.94 (3 H, s, *N*- or *O*-CH_3); 3.92 (3 H, s, *N*- or *O*-CH_3); 3.75 (6 H, s, *N*- or *O*-CH_3's); 1.96 (3 H, s, acetyl CH_3); 1.58 (3 H, s, *C*-CH_3); 1.07 (3 H, s, *C*-CH_3). Mass spectrum: $M^{+\cdot}$ 394.1557 ± .0012; calcd for $C_{19}H_{26}N_2O_5S$, 394.1563.

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