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The Function of Bandrowski's Base in Hair Dyeing

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Synopsis—Proposals for the structure of Bandrowski's Base (BB) are reviewed, and it is concluded that the structure originally proposed by Green is the correct one. The role of BB in dyeing of human hair was studied by examining hair dyed with *p*-phenylenediamine and hydrogen peroxide. Solvent extraction of dyed hair or decomposition by dilute alkali yielded mixtures which contained little or no BB when they were examined by thin-layer chromatography. Based on these experiments, it is concluded that BB is not the main colorant of hair dyed with *p*-phenylenediamine.

Introduction

Bandrowski's Base (BB) has been the subject of considerable controversy since its discovery in 1889 (1). Its exact structure and its role in hair and fur dyeing by oxidation of *p*-phenylenediamine (PPD) have been vigorously debated (1–11). It is the purpose of this study to review the evidence for a definitive structure of BB and to investigate BB's role in the *in vivo* dyeing of hair.

Structure

Essentially two structures have been proposed for BB. One of these (I) was proposed without any evidence by Bandrowski (1) and has subsequently received the support of other workers (4, 7). A second structure (II) has been proposed by Green (6) and was corroborated experimentally by Sunde and Lauer (8, 9). Finally, a third structure has been

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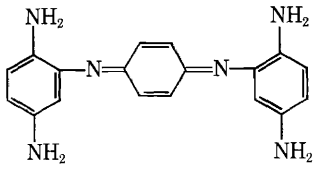
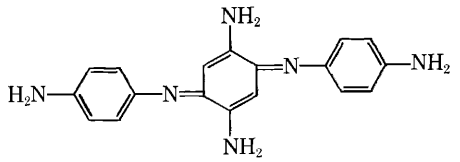
proposed by Austin (10). This structure (III) is tautomeric with II, and the differences between structures II and III appear to be of secondary interest.



(I)



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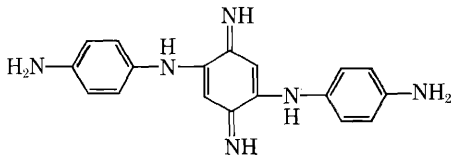

(III)

Green's (6) evidence for structure II was largely conjectural and is based on his work on the structure of Barsilowsky's Base (oxidation product of *p*-toluidine). The evidence of Sunde and Lauer (8, 9) was negative; these workers synthesized I and found it to be different from BB.

During the course of the studies reported below, Greenough and Altman (11) of this laboratory chose one of the modern techniques of structural organic chemistry to obtain a more definitive answer to this problem. Their nuclear magnetic resonance data clearly indicate that structure II is the correct one. The evidence presented by Green and later by Sunde and Lauer is, therefore, confirmed.

Role of Bandrowski's Base in Hair Dyeing

The formation of BB by oxidation of PPD in the presence of hydrogen peroxide is well established. It is generally assumed that the intermediate in the formation of BB is quinone diimine. It is also generally assumed that BB *via* some unknown mechanism is further oxidized or modified to an azine-like compound (12–18). It has even been claimed that this azine complexes or reacts further with hair keratin (19).

Cox (13, 16) indicated that BB is formed during dyeing of keratin fibers. He further believed that the color body is an azine formed

within the fiber. His conclusions were based on the fact that he was able to extract BB from the fiber with hot pyridine without appreciably altering the depth of color of the fiber. He also isolated a material which he claimed to be "an azine combined with the protein of the fur" after decomposition of the fiber. Unfortunately, Cox's work was conducted with copper-mordanted rabbit fur. Any influence by copper on the reactions between hydrogen peroxide and PPD has not been established.*

On the other hand, Austin (10) presented cogent arguments for the fact that BB itself is responsible for the color of dyed hair. He showed that: (a) BB is known to form under conditions of oxidative hair dyeing; (b) hair can be dyed with an aqueous suspension of reduced BB at 100°C; and (c) hair can be dyed from a pyridine solution of BB. Austin also suggested that white hair, dyed rapidly by PPD and hydrogen peroxide, yields resistant reddish-brown dyes upon prolonged treatment with dilute hydrogen peroxide. From these findings he concluded that BB is the main colorant and that it can be further oxidized to an azine type of dye. This latter step, however, is due to over-oxidation and is undesirable.

In a recent paper, Tucker (20) used the formation of BB as a measure of the amount of dye formation from PPD. He indicated that BB is the first stable product formed during the oxidation of PPD and implied that BB is at least a precursor of the dyes formed in hair.

The study reported here was undertaken to shed some light on the question of whether BB is present in or on hair which has been dyed with PPD and hydrogen peroxide

Experimental

Bandrowski's Base—This substance was synthesized by the method of Ritter and Schmitz (7). Five grams of PPD (recrystallized from benzene) was dissolved in 375 ml of water, and 1.5 ml of 28% NH_4OH was added (pH 9.5). To this solution was added 62.5 ml of 3% H_2O_2, and the resulting mixture was maintained for 24 hours at room temperature. About 1 g of fairly pure BB was removed by filtration. The solution yielded an equal quantity of less pure BB 24 hours later. The results of a synthesis under identical conditions and with the same quantities of

* The synthesis of BB in the presence of Cu^{++} by the technique described in the experimental section was attempted here. The reaction proceeded rapidly and yielded a variety of color bodies and considerable quantities of very impure BB.

raw materials but in the presence of an added 10 g of resorcinol are described later.

For purification, BB was dissolved quickly in hot pyridine and then precipitated by the slow addition of water (4). The determination of the molecular weight was considered necessary because no definitive evidence could be found in prior literature which would confirm the generally accepted formation of BB from three moles of PPD. The molecular weight was determined in boiling pyridine. The following analytical data were obtained:

Melting point	236 °C
Elemental analysis for $C_{18}H_{18}N_6$	
Calculated	C 67.9%; H 5.7%; N 26.4%
Found	C 67.3%; H 5.7%; N 27.5%
Molecular weight*	
Calculated	318
Found	339

Dyeing of Hair—White, natural hair obtained from S. Kalinsky & Son† was extracted overnight in a Soxhlet extractor, first with chloroform and then with methanol. Bundles of hair, weighing from 0.3–0.5 g, were dyed with a 0.5% solution of PPD (adjusted to pH 9.5 with ammonia) which was mixed with an equal volume of 6% H_2O_2 just before use. The hair was saturated with the mixture, drained for a few seconds, and allowed to react on a watch glass for 30 minutes. The swatches of hair were then rinsed with tap water, shampooed five times with a detergent shampoo, rinsed well with distilled water, and finally dried at ambient temperature.

Removal of Dye from Hair—After dyeing, the swatches of hair were then treated by one of the following methods:

1. Freshly dyed hair swatches were soaked in about 50 ml each of methanol, ethanol, or pyridine for 1–7 days at 45 °C.

2. Freshly dyed hair swatches were extracted in a Soxhlet extractor with about 200 ml of the above solvents and with dimethyl formamide and dimethyl sulfoxide.

3. Freshly dyed or extracted hair swatches were decomposed with about 100–125 ml of 0.1*N* NaOH.

Detection of Bandrowski's Base—Evidence for the presence of BB was sought by thin-layer chromatography. The above extracts were chroma-

* Determined by Schwarzkopf Microanalytical Laboratory, 56-19 37th Avenue, Woodside, N. Y. 11377.

† 110 West 17th Street, New York, N. Y.

tographed (as quickly as possible after completion of the extraction) on Uniplate Silica Gel G plates of 0.250 mm thickness.* The plate was spotted with a 10λ pipette (more than one charge was used if the extract was dilute) while confining the spot to a diameter of 3–4 mm. After evaporation of the solvent, the plate was developed with a mixture of $CHCl_3$/MeOH (9:1 by volume) for a distance of 10 cm. Fresh solution was needed for each plate. As little as 2.5×10^{-5} mg of BB was visible without any further development or visualization; other spots were made visible by iodine fumes. Because of slight variations of R_f values in this system, samples of authentic BB (and occasionally of PPD) were included on each plate.

The usual R_f value of BB in this system was 0.25, and the spot appeared as a blue or violet spot without need for any visualization. The PPD spot appeared at R_f 0.60 and turned brown after exposure to iodine fumes.

Results and Discussion

Solvent Extraction—Only trace amounts of BB were found in those chromatograms which were made with freshly prepared methanol, ethanol, and pyridine extracts of dyed hair. BB seemed to be a negligible component of the color; its amount did not seem to be commensurate with the considerable loss of color of the hair in these treatments. During extractions, the hair changed from a deep brown-black to a medium red, while the extracts were generally reddish. It is estimated that only about one-half of the color was removed from hair by extraction with solvents. This portion was removed fairly quickly, but the remainder of the dye resisted extraction. Extraction with pyridine appeared to remove more dye than other solvents and yielded an additional red spot of R_f 0.7.

Carbon tetrachloride removed no visible dyes or BB from hair. On the other hand, dimethyl formamide (DMF) completely decolorized dyed hair. The extract was faint yellow-red, but the chromatogram showed no BB. When BB was maintained in DMF at 60 °C for two days, it was completely destroyed. Finally, dimethyl sulfoxide (DMSO) also completely decolorized hair to yield an almost black extract. This extract showed no BB by TLC. As in the case of DMF, BB was destroyed when it was heated in DMSO at 60 °C for two days.

The failure to find BB in appreciable quantities in the chromatograms of the extracts of dyed hair was not due to the technique. In fact, solutions of BB (of about the same color intensity as the extracts) in any

* Analtech, Inc., Wilmington, Del.

of these extractants readily yielded the expected spot of BB on chromatograms.

The major spot on chromatographic plates was a red-brown spot which did not move from the origin. No attempt was made to resolve this spot further or to identify it chemically. The spot has the same appearance and characteristics as a spot formed from a solution of pure BB which has been heated for prolonged periods with the solvents used for extraction. One might infer from this finding that BB is formed on the hair and that it is changed to a colored material during attempts to extract it. Although this interpretation may appear attractive, it must be rejected. In a lengthy series of boiling tests in methanol, it was shown that BB survived the extraction treatments fairly well.* Even the weak spot of BB (which is routinely found in extracts from dyed hair) is not lost after long periods (up to four days) of boiling of methanol and ethanol extracts.† The reactivity of BB in the boiling tests was not affected by the presence of hair which, conceivably, could accelerate the decomposition of BB. In agreement with Cox (13), it is, therefore, concluded that a small amount of BB is formed *on* the fiber and not readily removed during rinsing and shampooing. Alternately, it might be possible that the faint BB spot (R_f 0.25) in the hair extracts is due to trace amounts of PPD (which are always found in chromatograms of these extracts) which was oxidized during chromatographing. Actually, the BB spot appears whenever (even pure) PPD is chromatographed.

Decomposition of Hair—A final attempt was made to remove BB from hair by decomposition with 0.1*N* NaOH. In experiments with unextracted and solvent extracted hair, no filterable amounts of BB could be removed; but this was not surprising in view of the small amounts of BB and other colorant which were actually produced under the described dyeing conditions. The thin-layer chromatogram of the digestion fluid gave a faint test for BB only in samples which had not been previously solvent-extracted. Alkaline treatment of dyed hair immediately freed color, but this color contained only trace quantities of BB. In separate experiments, it was established that BB is sufficiently soluble in dilute alkali to yield a satisfactory thin-layer chromatogram. It was also established that it is relatively stable in dilute sodium hydroxide. This series of experiments was repeated several times on hair which had not

* When a solution of 0.5 mg of BB in 100 ml of methanol was refluxed for 16 hours, BB could still be detected chromatographically in an aliquot of the solution equivalent to 2.5–5.0 $\times 10^{-5}$ mg of BB. If pyridine was used instead of methanol, the lower limit of detection was 5.0–10 $\times 10^{-5}$ mg of BB.

† Admittedly, the spot of BB will disappear completely if boiling is continued long enough.

been solvent-extracted or with hair that was not shampooed after dyeing; in no case were significant quantities of BB found.

Attempts to Modify the Formation of Bandrowski's Base —It may be recalled that BB is generally isolated without difficulty whenever PPD is treated with alkaline H_2O_2. In contrast, little or no BB is found on hair after dyeing. The possibility remains that the protein interferes with the formation of BB. For this reason, preparation of BB was carried out in the presence of glycine at pH 8.7. Under these conditions, the initial yield of crude BB was 3.5 g, i.e., larger than the quantity isolated when the oxidation was conducted in the absence of glycine. The yield dropped slightly when the pH was raised to 9.5 or when 2.5 g of cystine was substituted for the glycine. It was also determined that gelatin* did not influence the formation of BB. These experiments suggest that the presence of free amino acids or of a soluble protein does not interfere with formation of BB. One would reason, therefore, that the insoluble protein, hair, has no significant effect on the formation of BB. However further study of this problem would appear desirable since the model protein (gelatin)—in contrast to hair—contains almost no aromatic amino acids.

In assessing the significance of these experiments for conventional hair dyeing practice, it must be remembered that in dyeing PPD is not used alone but, instead, is oxidized in the presence of "modifiers." In order to determine whether BB is formed during the oxidation of PPD in the presence of a typical modifier, this reaction was carried out in the presence of an excess of resorcinol. After completion of the reaction, there was no precipitate, and the solution yielded only a faint test for BB; in the absence of resorcinol, this reaction should have yielded approximately 0.7–1.0 g of BB (removable by simple filtration). This finding not only confirms that BB is not formed during standard hair dyeing but also yields some insight into the mechanism of the reactions taking place during hair dyeing. Apparently, PPD is oxidized to a reactive intermediate (quinone diimine?) which in the presence of a modifier reacts preferentially with the modifier to form the hair dye but not with PPD to yield BB.

Conclusions and Summary

On the basis of the work described here, BB is not formed to any appreciable extent when hair is dyed by the hydrogen peroxide oxidation of PPD. The formation of BB by this oxidation is a slow process. According to Tucker (20) only about 3% of PPD is converted to BB after

* Pharmagel B, Kind and Knox Gelatin Co., Camden, N. J.

30 minutes in the presence of alkaline hydrogen peroxide. This is the average period required for *in vivo* hair dyeing and the period of hair treatment adopted for this study. It is not surprising, therefore, that little or no BB was found in hair dyed by the technique described above.

Cox (16) showed conclusively that BB is not the coloring species when Cu-mordanted fur is dyed for 24 hours in an alkaline oxidizing dye bath containing PPD. Thus, even if the time is long enough for large amounts of BB to form, this substance does not act as a dye. The authors suggest that BB cannot account for the color of hair dyed with PPD alone or in the presence of a reactive modifier, such as resorcinol. In all likelihood, BB is the end product of an undesirable side reaction during hair dyeing.

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