684. *The Search for New Trypanocides. Part VIII.*[1] *Coupling of* m-*Amidinobenzenediazonium Chloride with* 3,8-*Diamino-5-ethyl-6-phenylphenanthridinium Chloride.*

By S. S. Berg.

Structural evidence is presented to show that *m*-amidinobenzenediazonium chloride couples with 3,8-diamino-5-ethyl-6-phenylphenanthridinium salts to give a mixture of a red diazoamino- (II; R = *m*-amidino) and a purple aminoazo-compound (V; R = *m*-amidino), in proportions which vary with the pH of the reaction medium. The diazoamino-compound is formed by reaction of the diazonium salt with the 8-amino-group of the phenanthridinium salt, and the aminoazo-compound by coupling in the *ortho*-position to either the 3- or the 8-amino-group, probably the latter.

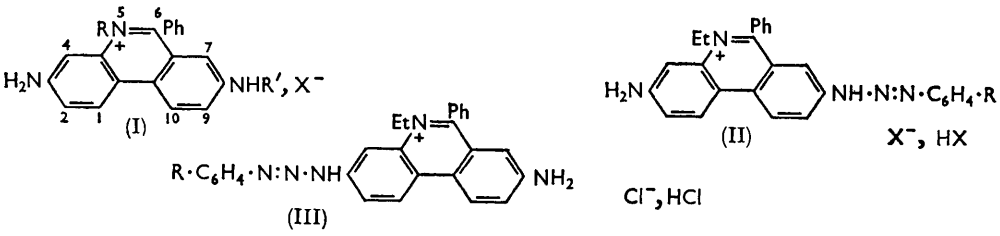
The coupling of *p*-amidinobenzenediazonium chloride with 3,8-diamino-5-ethyl-6-phenylphenanthridinium chloride (homidium chloride) (I; R = Et, R′ = H, X = Cl) was first described by Wragg, Washbourn, Brown, and Hill.[2] When the reaction was carried out in acetic acid–sodium acetate solution a mixture of two isomers, one red and one purple, was obtained, and these could be separated by fractional crystallisation. They were considered to be different diazoamino-derivatives of homidium chloride because of the method of preparation, and because of the evolution of nitrogen when each was heated with sulphuric acid. Structure (II; R = *p*-amidino, X = Cl) was tentatively assigned to the predominant purple isomer and structure (III; R = *p*-amidino) to the red isomer. Considerable differences in the infrared spectra of the two compounds rendered the existence of stable tautomers unlikely.

Similar results were obtained by coupling *m*-amidinobenzenediazonium chloride with homidium chloride, the mixed isomers being given the name metamidium chloride. The high trypanocidal activity of metamidium chloride,[2,3] due largely to its red component, isometamidium,[2,3] prompted the present studies aimed at elucidating the structures of

[1] Part VII, Berg, *J.*, 1962, 677.
[2] Wragg, Washbourn, Brown, and Hill, *Nature*, 1958, **182**, 1005.
[3] Brown, Hill, and Holland, *Brit. J. Pharmacol.*, 1961, **17**, 396.

the isomers and determining optimum conditions for the coupling in order to obtain a product enriched in the more active red isomer.

The coupling of a diazonium salt with an aromatic primary amine to give a diazoamino-compound is an electrophilic substitution involving the diazonium ion and the non-ionised amine.[4] The benzenediazonium ion is a weak electrophile, so that enhancement



of its reactivity by electronegative substitution greatly extends the range of the coupling reaction.[5] If the nucleophilic reactivity of the aryl residue of the primary amine is increased by substitution as in *m*-toluidine [6] or by fusion of rings, as in α- and β-naphthylamine, *C*-coupling occurs preferentially.[6] The behaviour of aromatic primary amines as coupling components is similar to that of phenols, their reactivity being due to the relatively high electron density of the carbon atoms in the *ortho*- and *para*-positions.[7]

The formation of aminoazo-compounds can also occur by rearrangement of diazoamino-compounds at 60—100°, the reaction being catalysed by addition of acid, and even more by addition of the appropriate free amine together with one of its salts.[8]

The reaction of diazonium salts with *Bz*-aminophenanthridinium salts has not been previously studied. The coupling of benzenediazonium chloride with *Bz*-aminoquinolines [9] has been shown to give aminoazo-derivatives, the orientations of which were in accord with theoretical predictions. The reactions were very rapid in acetic acid–sodium acetate solution, but slow in hydrochloric acid. Recently diazoamino-compounds have been prepared by the coupling of the more reactive *m*- and *p*-amidinobenzenediazonium salts with 6-amino-quinolinium and -quinazolinium salts.[10]

Potentiometric titrations with homidium have shown it to be a very weak base, and in the investigations reported in this series of papers it was found that coupling in aqueous media would occur only with the more reactive diazonium salts, the use of benzenediazonium chloride being completely unsuccessful.

Campbell investigated the spectra of aqueous solutions of 3,8-diamino-5-methyl-6-phenylphenanthridinium bromide (dimidium bromide) (I; R = Me, R' = H, X = Br) and showed that the intense red colour over the range pH 4·0—9·0 was due to the absorption band at 483 mμ, which he suggested was associated with resonance involving a change in positive charge between the quaternary nitrogen and the 3-amino-group.[11] The 8-amino-group is unable to participate and should thus be preferentially attacked by electrophilic reagents. This has been demonstrated by Walls,[12] who obtained almost quantitative yields of the

[4] Hauser and Breslow, *J. Amer. Chem. Soc.*, 1941, **63**, 418; Wistar and Bartlett, *ibid.*, p. 413; Dewar, *Research*, 1950, **3**, 154; Zollinger, *Chem. Rev.*, 1962, **51**, 360.

[5] Conant and Peterson, *J. Amer. Chem. Soc.*, 1930, **52**, 1220; Hammett, "Physical Organic Chemistry," McGraw-Hill, New York, 1940, p. 314.

[6] Bamberger, *Ber.*, 1895, **28**, 839; Mehner, *J. prakt. chem.*, 1901, **65**, 401.

[7] Zollinger, "Diazo and Azo Chemistry, Aliphatic and Aromatic Compounds," Interscience Publ., Inc., New York, 1961, p. 211.

[8] Ingold, "Structure and Mechanism in Organic Chemistry," G. Bell and Sons, London, 1953, p. 610.

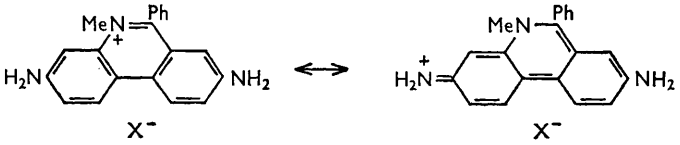
[9] Renshaw, Friedman, and Gagewski, *J. Amer. Chem. Soc.*, 1939, **61**, 3322.

[10] Berg, *J.*, 1961, 4041.

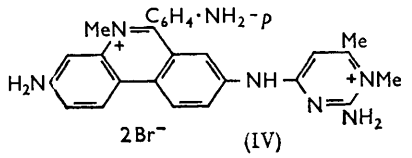
[11] Campbell, *J. Soc. Chem. Ind.*, 1950, **69**, 94; and personal communication.

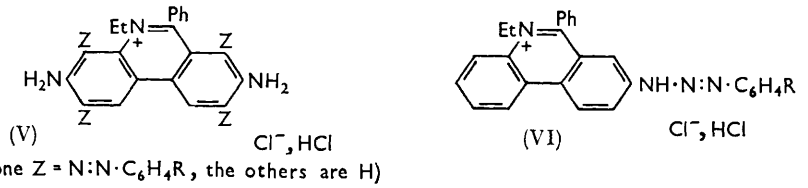
[12] Walls, (*a*) *J.*, 1950, 3514; (*b*) B.P. 746,027.

8-acyl derivatives when dimidium was treated with a number of acylating agents. Similar results were obtained with homidium chloride.[12b] It was thus reasonable to assume that

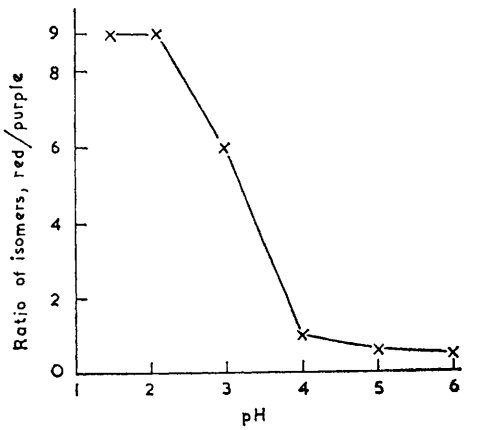


N-coupling of a diazonium salt with homidium would involve the non-ionised 8-amino-group and that *C*-coupling if it took place would most likely occur on the 7- or 9-carbon atom of the phenanthridine ring. Moreover, as homidium is a very weak base, *N*-coupling could occur in strongly acid media, which might be expected to reduce seriously the possibility of *C*-coupling. Confirmatory evidence for the reactivity of the 8-amino-group in strongly acid solution has been provided by Lowe, Dickenson, and Nicholson [13] who condensed 3,8-diamino-6-(*p*-aminophenyl)-5-methylphenanthridinium bromide (trimidium bromide) with 2-amino-6-chloro-3,4-dimethylpyrimidinium iodide in aqueous solution at pH 0·8—1·5 to give mainly the 8-substituted derivative, prothidium bromide (IV).





In order to examine the validity of the foregoing speculations, a series of coupling reactions between *m*-amidinobenzenediazonium chloride and homidium chloride was carried out in aqueous media at controlled pH's from 1·5 to 6. The crude products were



Effect of pH on the coupling of *m*-amidinophenyldiazonium chloride with homidium chloride.

separated from inorganic contaminants, and the mixtures were assayed polarographically in McIlvaine buffer of pH 3 containing ~0·01M-chloride ion. The red and the purple isomer gave half-wave potentials of —0·74 and 0·30 v, respectively, against a mercury-pool anode, and diffusion currents were proportional to concentration. The results are shown in the Figure.

[13] Lowe, Dickinson, and Nicholson, B.P. 828,962.

In the light of these results the structure (II; R = *m*-amidino) was assigned to the red isomer, isometamidium, the principal product at low pH, and one of the aminoazo-structures represented by (V; R = *m*-amidino) to the purple isomer (although *C*-coupling at position 2 or 4 is considered unlikely).

The structure of isometamidium was confirmed by deamination to the diazoamino-compound (VI; R = *m*-amidino) which was unambiguously synthesised by the coupling of diazotised 8-amino-5-ethyl-6-phenylphenanthridinium chloride with *m*-aminobenzamidine, the former being prepared by the deamination of the monoacetyl compound (I; R = Et, R′ = Ac, X = Cl).

The *o*-aminazo-structure for the purple isomer was confirmed by reductive fission with stannous chloride to a highly coloured *o*-phenylenediamine derivative, which was readily converted into a benzimidazole and a quinoxaline. Under similar conditions isometamidium was reduced to homidium. An almost quantitative yield of *p*-chloroaniline was obtained on reductive fission of the purple isomer obtained by coupling *p*-chlorophenyldiazonium chloride and homidium chloride.

Stability studies showed that the purple isomer was more stable than the red, and was unaffected when heated at 150° and in boiling 0·1N-sodium hydroxide or N-acetic acid. The evolution of about 0·65 mol. of nitrogen in boiling 6N-sulphuric acid, previously observed by Wragg *et al.*,[2] was confirmed, but none was evolved on heating with cuprous chloride–3N-hydrochloric acid, a method [14] which gave theoretical yields of nitrogen from the isomeric red isomer.

Attempts to rearrange a red isomer to its corresponding purple isomer have been unsuccessful.

Experimental

Water of crystallisation was determined by the Karl Fischer method.

Coupling at Controlled pH. *General Procedure.*—*m*-Aminobenzamidine monohydrochloride [10] (6·9 g., 0·04 mole) in water (36 ml.) and concentrated hydrochloric acid (10 ml.) was diazotised at 5—8° by addition of sodium nitrite (3·2 g., 0·0465 mole). The excess of nitrous acid was removed by addition of sulphamic acid, and the diazonium solution was brought to the required pH by the careful addition of sodium hydrogen carbonate. Sodium acetate trihydrate (5·44 g., 0·04 mole) in water (20 ml.) was added, and the diazonium solution was added from an ice-cooled burette to a stirred solution of homidium chloride (15·92 g., 0·04 mole) in water (96 ml.) which had previously been adjusted to the required pH by the addition of N-isethionic acid. The reaction was carried out at 10—11° (the lowest temperature at which the pH meter could be accurately used), and the addition was regulated so that the solution was kept at the required pH (reaction time 20—40 min.). After a further 40 min. a solution of sodium acetate (13·6 g.) and sodium chloride (10 g.) in water (100 ml.) was added; the tar which separated quickly solidified on addition of saturated aqueous sodium chloride (80 ml.). The solid was filtered off, washed with saturated aqueous sodium chloride, dissolved in water (200 ml.), and reprecipitated by the addition of sodium chloride (20 g.). Addition of saturated aqueous sodium bromide (100 ml.) to a filtered aqueous solution (200 ml.) of the product precipitated the *bromide hydrobromide* which was filtered off, dried over sulphuric acid, washed free from sodium bromide with 96% w/v aqueous acetone (3 × 400 ml.), and dried over silica gel. After stabilisation in air the products were examined by paper electrophoresis in 3N-acetic acid, to confirm the presence of the two isomers and the absence of significant amounts of homidium chloride, and were then submitted for polarographic examination.

Isolation of the Pure Isomers.—(*a*) The mixed isomers (dark red) (22·9 g., 92·5%), decomp. 245—247° (Found: C, 51·2; H, 4·9; Br, 24·2; N, 14·9; H_2O, 5·6. Calc. for $C_{28}H_{26}BrN_7,HBr,2H_2O$: C, 51·25; H, 4·75; Br, 24·35; N, 15·0; H_2O, 5·65%), obtained by coupling at pH 1·5 were suspended in boiling methanol (115 ml.), and the suspension was allowed to cool overnight. The pure red isomer (II; R = *m*-amidino, X = Br) (18·4 g.) separated as red granules decomposing at 245—247°.[15] Metathesis by passing a methanolic

[14] Lohr, *Analyt. Chem.*, 1953, **25**, 1117.

solution down a column containing Amberlite I.R.A. 400 (Cl^-) resin gave the corresponding chloride hydrochloride as red needles (from methanol), decomp. 244—245°.[15]

(*b*) The damp *chloride hydrochloride* obtained by coupling at pH 5·0 was crystallised from water (75 ml.), the pure purple isomer (V; R = *m*-amidino) separating as purple prisms (7·8 g., 35%), decomp. 258—260° [15] (Found: C, 60·3; H, 5·25; Cl, 12·5; N, 17·7; H_2O, 4·3. $C_{28}H_{26}ClN_7,HCl,1·5H_2O$ requires C, 60·1; H, 5·4; Cl, 12·7; N, 17·5; H_2O, 4·8%).

8-*Acetamido-5-ethyl-6-phenylphenanthridinium Chloride*.—Acetic anhydride (30 ml.) was added to a solution of homidium chloride (45 g.) in water (450 ml.), and the mixture was shaken at room temperature for 1 hr. The finely divided orange solid which had separated was filtered off, washed with ice–water, and resuspended in water (405 ml.) containing 2N-sulphuric acid (190 ml.). To the stirred suspension, at 0—5°, was added sodium nitrite (9·9 g.) in water (30 ml.). After being stirred at 5° for 2 hr., the solution was filtered and the cold red filtrate added in ¼ hr. to boiling ethanol (4·5 l.). Acetaldehyde was evolved, and the pale red reaction mixture was refluxed for a further 1 hr. The inorganic material was filtered off, and the solution was concentrated under reduced pressure to give an orange residue, which on trituration with acetone formed a granular yellow solid. This was filtered off, and crystallised from 0·1N-hydrochloric acid as yellow needles (26·0 g.; 53%), m. p. 241—242° (decomp.) (Found: C, 64·4; H, 5·9; Cl, 8·4; N, 6·55; H_2O, 12·4. $C_{23}H_{22}ClN_2O,3H_2O$ requires C, 64·1; H, 6·5; Cl, 8·3; N, 6·5; H_2O, 12·5%).

8-*Amino-5-ethyl-6-phenylphenanthridinium Chloride*.—A suspension of 8-acetamido-5-ethyl-6-phenylphenanthridinium chloride (4·5 g.) in 2N-hydrochloric acid (45 ml.) was refluxed overnight, a solution being obtained after 1·5 hr. An excess of sodium acetate was added; crystallisation then occurred. The solid was filtered off, washed with brine, and crystallised from ethanol. The *product* (3·2 g., 78·5%) separated as red needles, decomp. 260° (Found: C, 70·3; H, 6·95; Cl, 9·0; N, 6·8; H_2O, 3·4. $C_{21}H_{19}ClN_2,C_2H_5·OH,0·75H_2O$ requires C, 70·3; H, 6·75; Cl, 9·0; N, 7·1; H_2O, 3·45%).

8-(m-*Amidinophenyldiazoamino*)-5-*ethyl*-6-*phenylphenanthridinium Chloride Hydrochloride* (VI; R = *m*-amidino).—A suspension of 8-acetamido-5-ethyl-6-phenylphenanthridinium chloride trihydrate (7·3 g.) in 2N-hydrochloric acid (73 ml.) was refluxed for 1·5 hr. The red solution was cooled to 5°, and N-hydrochloric acid (55 ml.) was added. The solution was diazotised at 0—5° by the addition of sodium nitrite (1·9 g.), and the excess of nitrous acid was removed by the addition of sulphamic acid. *m*-Aminobenzamidine monohydrochloride (4·6 g.) in a mixture of water (14 ml.) and 2N-hydrochloric acid (9 ml.) was added to the diazonium solution, followed by anhydrous sodium acetate (23 g.) in water (69 ml.). The solution was stirred at 5—15° for 2 hr., and addition of sodium chloride (20 g.) then precipitated an orange solid. This was filtered off, washed with brine, and crystallised from aqueous ethanol. The product (7·1 g., 73%) separated as orange needles which were filtered off, washed with ethanol and with acetone, and dried over silica gel. The *salt* decomposed at 260° (Found: Cl, 12·3; N, 14·65; H_2O, 5·65; OEt, 3·8. $C_{28}H_{25}ClN_6,HCl,0·5C_2H_5·OH,1·75H_2O$ requires Cl, 12·45; N, 14·7; H_2O, 5·5; OEt, 3·9%).

Deamination of Isometamidium (II; R = *m*-amidino, X = Cl).—A stirred, fine suspension of the red isomer (15 g.) in 2N-sulphuric acid (150 ml.) was diazotised at 0—2° by sodium nitrite (1·75 g.). The solid dissolved, and after being stirred at 0—2° for 0·5 hr. the solution was added as quickly as possible to boiling ethanol (1500 ml.). The red solution was refluxed for a further 0·25 hr., filtered, and cooled to 20°, and ether (2 volumes) was added. The red precipitate was filtered off, washed with ether and then acetone, and dried over silica gel. Examination of the orange solid (7·2 g.; decomp. 237—246°) by paper electrophoresis showed it to contain an appreciable amount of a substance with a mobility identical with that of substance (VI). Three crystallisations from aqueous ethanol followed by trituration with ethanol, and a final recrystallisation from methanol–acetone gave orange needles (1·7 g.) of the preceding *salt* $(VI)_2$ (different solvation) decomp. 255—258° (Found: C, 56·5; H, 5·25; Cl, 11·95; N, 14·0; H_2O, 12·6. $C_{28}H_{25}ClN_6,HCl,4·25H_2O$ requires C, 56·6; H, 5·8; Cl, 11·9; N, 14·15; H_2O, 12·85%). Comparison of infrared spectra proved the identity.

Reductive Fission of Isometamidium.—The red isomer (1 g.) was added to a solution of stannous chloride (1·3 g.) in concentrated hydrochloric acid (13 ml.). A mildly exothermic reaction occurred, and after 7 min., a clear yellow solution was obtained. After being kept

[15] Berg, Bretherick, Washbourn, and Wragg, *J.*, 1963, in the press.

for 0·5 hr., the solution was diluted with water (20 ml.) and basified to phenolphthalein at 0—10° by the addition of 50% w/v aqueous sodium hydroxide. The pink pseudo-base was filtered off, washed with ice–water, and dried over silica gel. The solid (0·5 g.) was dissolved in 2N-acetic acid (5 ml.), and acetic anhydride (1 ml.) was added. The red solution was shaken for 0·25 hr.; 8-acetylhomidium [1] separated as red crystals which were filtered off, washed with brine and a little ice–water, and dried at 90°. The red solid (0·49 g., 70%), which decomposed at 288—290°, did not depress the decomposition point of an authentic sample.[12b]

Reductive Fission of the Purple Isomer (V; R = *m-amidino*).—A mixture of the purple isomer (1 g.), stannous chloride (1·3 g.), and concentrated hydrochloric acid (13 ml.) was refluxed for 0·5 hr., then cooled. The crystalline stannichlorides were filtered off and dissolved in water (60 ml.). Hydrogen sulphide was passed through the solution until all the tin had been precipitated as sulphide and, after filtration, the red solution was basified at 0—10° with 2N-aqueous sodium hydroxide. The mauve pseudo-base was quickly filtered off, washed with ice–water, and treated with boiling methanol (10 ml.). After being filtered the solution was quickly cooled in ice, to give x,3,8-*triamino*-5-ethyl-5,6-*dihydro*-6-*methoxy*-6-*phenylphenanthridine* as mauve needles (0·4 g., 60%), m. p. 156—157° (decomp.) (Found: C, 71·3; H, 7·05; N, 14·7; OMe, 13·65. $C_{22}H_{24}N_4O,\frac{2}{3}MeOH$ requires C, 71·5; H, 7·0; N, 14·8; OMe, 13·6%). Weakly acid solutions had an intense blue colour which changed to red as the pH was lowered. This product readily oxidised in neutral and weakly acid solutions but was stable in strong acid. The solid can be stored for long periods in the absence of air. Addition of acetic anhydride (1 ml.) to a freshly prepared solution of the triamine (1 g.) in water (15 ml.) containing 2N-aqueous acetic acid (1 ml.) gave a red solution from which a *diacetyl derivative* was precipitated by the addition of sodium chloride. It crystallised as red needles (0·67 g., 58%), m. p. 248° (decomp.), from methanol (Found: C, 66·2; H, 5·7; Cl, 8·1; N, 12·3; *N*-Ac, 10·1; *C*-Me, 9·7. $C_{25}H_{25}ClN_4O_2$ requires C, 66·6; H, 5·6; Cl, 7·9; N, 12·4; *N*-Ac, 9·6; *C*-Me, 10·0%).

Ring Closure of the Diacetyl Compound to a Benzimidazole.—The diacetyl compound (1 g.) in concentrated hydrochloric acid (10 ml.) was heated for 2 hr. on the steam-bath. The solution was diluted with water (20 ml.) and neutralised (Congo Red) by addition of sodium acetate. The red *benzimidazole derivative* was filtered off; it crystallised from aqueous ethanol as bright red prismatic needles (0·45 g.; 52%), m. p. 254° (decomp.) (Found: C, 71·0; H, 5·2; C, 9·1; N, 14·6. $C_{23}H_{21}ClN_4$ requires C, 71·05; H, 5·4; Cl, 9·15; N, 14·4%).

Reaction of the Triamine with Glyoxal.—The triamine (1 g.) in 2N-aqueous sulphuric acid (20 ml.) was treated with a filtered solution of glyoxal bisulphite (1 g.) in water (15 ml.). The solution was heated on the steam-bath for 0·5 hr., a brick-red solid separating. The *quinoxaline* (0·78 g., 66%) was filtered off, washed with water, and dried at 100°/10 mm.; it did not melt below 360° (Found: C, 62·2; H, 4·4; N, 12·6; S, 7·4. $C_{23}H_{20}N_4O_4S$ requires C, 62·0; H, 4·4; N, 12·6; S, 7·2%).

Reductive Fission of the Purple Isomer (V; R = *p*-Cl).—A suspension of the purple isomer [15] (5 g.) in a solution of stannous chloride (6·5 g.) in concentrated hydrochloric acid (65 ml.) was refluxed for 0·5 hr. A red solution was obtained after 10 min., after which crystallisation began. After being cooled overnight, the solution was filtered, and the mixture of stannichlorides was dried over potassium hydroxide. The solid (11·6 g.) was dissolved in water (500 ml.) and treated with hydrogen sulphide. After the removal of the tin sulphide the red solution was neutralised with sodium hydrogen carbonate to pH 6·5, and the intense blue solution was continuously extracted with ether. The ethereal solution was separated, dried (Na_2SO_4), and evaporated to dryness. *p*-Chloroaniline (1·0 g., 82%) was obtained as white crystals, m. p. and mixed m. p. 70—72°.

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