The Biosynthesis of Phenols. Part XI.[1] Oxidation of a Model Carboxyamide Related to 6-Methylpretetramid

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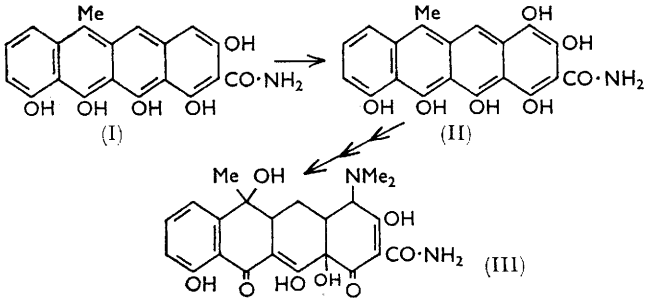
The oxidation of 3-acetyl-2,6-dihydroxy-4-methylbenzamide, a model compound related to pretetramid, has been studied, using oxygen in alkaline solution and Frémy's salt.

ELEGANT studies [2,3] utilising mutants of *Streptomyces aureofaciens* have established that 6-methylpretetramid (I) is converted, *in vivo*, into tetracycline (III) and closely related antibiotics such as 7-chlorotetracycline. The initial step involves hydroxylation at the 4-position of 6-methylpretetramid with the formation of the intermediate (II).[3] It seemed possible that this stage in the biosynthesis might be simulated *in vitro*. In this connection we have investigated the behaviour, in oxidising media, of 3-acetyl-2,6-dihydroxy-4-methylbenzamide (IV), which is related in structure to ring A of 6-methylpretetramid. It was prepared by *C*-acetylation of *p*-orsellinamide [4] using Friedel–Crafts conditions. This amide (IV) was relatively stable to oxidation. There was no significant reaction with mild reagents such as lead dioxide and potassium ferricyanide, whereas in the case of lead tetra-acetate and peroxytrifluoroacetic acid, under various conditions, the complexity of the mixture of products discouraged further study.



Two major products (V) and (VI) were formed when an alkaline solution of the model amide was treated with oxygen. The quinone (V) gave a crystalline acetone-solvate, $C_{18}H_{16}N_2O_8,0{\cdot}5C_3H_6O$. High resolution mass spectrometry established the formula $C_{18}H_{16}N_2O_8^+$ of the molecular ion. The n.m.r. spectrum included three sharp singlets, τ 7·97, 7·47, and 7·14, integrating equally; these signals were attributed to methyl groups in different environments. Two broad bands at lower field, τ 0·8 and 1·7, both integrating for the same number of protons, were assigned to amide functions. The acidic properties (pK_a approx. 5·0), positive iodoform reaction, and formation of a colourless, crystalline quinol, $C_{18}H_{18}N_2O_8$, through reduction with sulphur dioxide, were in accord with the structure (V). The alternative formulation as a diphenoquinone (VII) has been excluded. The u.v. spectrum differs from that of related diphenoquinones [5] but it is very similar to that of "Heinrich's quinone" (VIII) [6] and to the summation spectrum of the quinone (IX) and two equivalents of the phenol (IV).

The second product of oxidation of the model phenol (IV) was identified as 4-carbamoyl-3,6-dihydroxytoluquinone (VI). The mass spectrum confirmed the molecular formula, $C_8H_7NO_5$; the fragmentation pattern, including, in particular, evidence of the ion corresponding to M-CO($C_7H_7NO_4^+$), was in accord with the formulation as a substituted toluquinone. The n.m.r. spectrum included signals at τ 7·97 (CH_3, singlet) and 0·8 ($CO{\cdot}NH_2$, broad band). Acid-catalysed hydrolysis gave a small yield of 3,6-dihydroxytoluquinone which was identified by comparison with authentic material.[7]

We attribute the formation of the quinones (V) and (VI) to initial oxidation of the model compound (IV) to 4-carbamoyl-3-hydroxytoluquinone (X) through a Dakin reaction brought about by oxygen in alkali. This relatively slow reaction allowed the intermediate (X) either to couple with starting material to give (V) or to undergo hydroxylation to (VI). When the oxidation was carried out with hydrogen peroxide in alkali, only traces of (V) and (VI) were found in the reaction mixture; another quinone, $C_{16}H_{12}N_2O_8$, was formed almost exclusively. Mass spectrometry established the molecular weight of this compound, and the ion bombardment fragmentation pattern was in accord with structure (IX). The n.m.r. spectrum of both this quinone and the quinol derived from it by reduction with sulphurous acid included signals for two methyl and two amide groups. The formation of the quinone (IX) when hydrogen peroxide was used for the oxidation of (IV) is attributed to a rapid Dakin reaction leading to the formation of the intermediate (X) in high concentration; this would favour self-coupling to give the quinone (IX).

Evidently, oxygenation in alkali has achieved hydroxylation in the unsubstituted position of the phenol (IV) but the process is complicated by side-reactions. We have found that more specific hydroxylation occurs using Frémy's salt. For satisfactory yields, the reaction was carried out in phosphate buffer (pH 10) rather than the more usual acidic conditions. The

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[2] J. R. D. McCormick, S. Johnson, and N. O. Sjolander, *J. Amer. Chem. Soc.*, 1963, **85**, 1693.
[3] J. R. D. McCormick, "Biogenesis of Antibiotic Substances," ed. Z. Vanek and Z. Hostalek, Academic Press, New York, 1965, p. 78.
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[5] L. Homer and K. H. Weber, *Chem. Ber.*, 1963, **96**, 1568.
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structure of the quinone, $C_{10}H_9NO_5$ (XI), which was formed was defined by the n.m.r. spectrum (resonances at τ 7·91, 7·36 due to CH_3 groups; broad band at 0·8 due to $CO{\cdot}NH_2$) and by the mass spectrum. This included peaks for the molecular ion (*m/e* 223) and two metastable ions corresponding to the transitions 223 ⟶ 206 ⟶ 178; these fragmentations are attributed to consecutive loss of ammonia and carbon monoxide.

The oxidation of 6-methylpretetramid to a compound with an oxygen substituent at the 4-position is being investigated.

EXPERIMENTAL

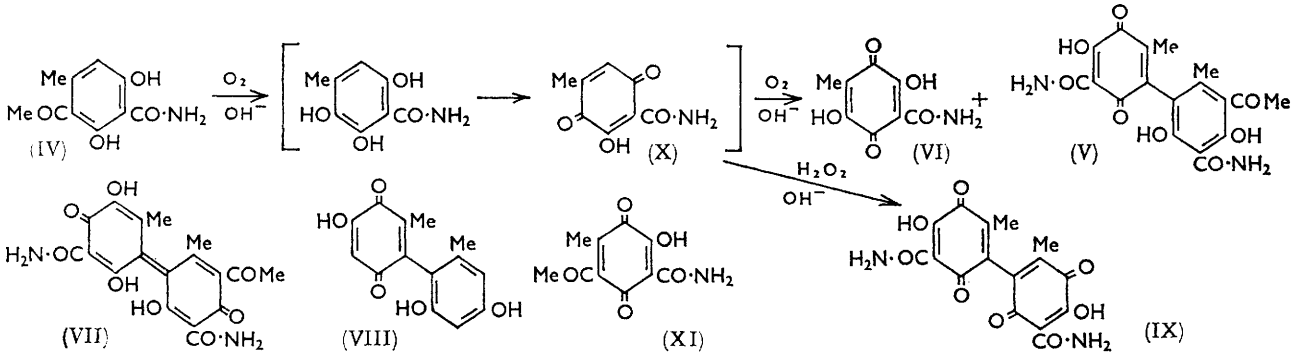
Melting points (corrected) were determined on a Kofler hot-stage apparatus. Infrared spectra were recorded on Perkin-Elmer 237 and 257 spectrometers as potassium bromide discs. Ultraviolet and visible spectra were determined with a Unicam SP 800 spectrophotometer using solutions in methanol to which 5N-hydrochloric acid (0·1%) had been added; log ε values have an accuracy of $\pm 5\%$. N.m.r. spectra were measured on a Perkin-Elmer R.10, 60 Mc./sec. spectrometer. The sparing solubility of the compounds necessitated the use of trifluoroacetic acid (range τ 2·0—10·0) and dimethyl sulphoxide (below τ 2·0). Mass spectra were recorded on A.E.I. M.S.9 spectrometers at Imperial Chemical Industries Limited, Dyestuffs Division (by courtesy of Dr. J. Beynon) and in Swansea. Samples were introduced by means of a direct insertion probe at range 200—300°.

3-Acetyl-2,6-dihydroxy-4-methylbenzamide (IV).—When acetic anhydride (10 ml.) was added to *p*-orsellinamide [4] (10 g.) and anhydrous aluminium chloride (24 g., 3 mol.) in nitrobenzene (100 ml.) a vigorous reaction occurred. After cooling at 0° to control the reaction, the mixture was heated at 100—110° for 10 hr. The solution was cooled, poured on to a mixture of crushed ice (100 g.) and 2N-hydrochloric acid (150 ml.), and steam-distilled to remove nitrobenzene. After cooling, the product was filtered off and washed with ethanol (100 ml.), to give a pink solid (10·5 g.) which yielded the *product* as needles (9 g., 72%) (from acetone), m. p. 260—270° (decomp.) (Found: C, 57·75; H, 5·2; N, 6·8. $C_{10}H_{11}NO_4$ requires C, 57·4; H, 5·3; N, 6·7%), $\nu_{max.}$ 3410, 1650, 1625, 1600 cm.$^{-1}$, $\lambda_{max.}$ 242, 322 mμ (log ε 4·28, 3·78). N.m.r.: τ 7·20, 7·33 (CH_3, singlets, 6H), 3·42 (Ar–H, singlet, 1H), 1·75 ($CO{\cdot}NH_2$, broad band).

The compound was virtually insoluble in the common solvents but dissolved readily in both dimethylformamide and trifluoroacetic acid. Vigorous alkaline hydrolysis gave *p*-orsellinamide,[4] orcinol, and 2,4-dihydroxy-6-methylacetophenone. It gave a positive indophenol test and a wine-red colour with ferric chloride in ethanolic solution.

Refluxing with dimethyl sulphate and potassium carbonate in acetone gave the *O-methyl derivative*, m. p. 233° (decomp.) (Found: C, 59·5; H, 6·1; N, 6·0; *O*-Me, 14·1. $C_{11}H_{13}NO_4$ requires C, 59·2; H, 5·85; N, 6·3; *O*-Me, 14·0%), $\nu_{max.}$ 3395, 1675, 1655, 1610 cm.$^{-1}$, $\lambda_{max.}$ 237, 322 mμ (log ε 4·23, 3·71).

2-(3-Acetyl-5-carbamoyl-4,6-dihydroxy-2-methylphenyl)-6-carbamoyl-5-hydroxy-3-methyl-1,4-benzoquinone (V).—In a typical reaction, oxygen was bubbled through a solution of 3-acetyl-2,6-dihydroxy-4-methylbenzamide (IV) (4 g.) in aqueous potassium hydroxide (2%; 250 ml.) at 20°. The light yellow solution deepened in colour progressively to deep red-brown. After 2 days, the solution was adjusted to pH 7·0 and, when unreacted starting material (2·1 g.) had been removed by filtration and extraction with dichloromethane (3 × 250 ml.), the solution was extracted with additional dichloromethane (4 × 250 ml.) at pH 5·5



Removal of solvent yielded the *product* (V) (1·2 g., 33%), m. p. 240—270° (decomp.), which crystallised from acetone as orange plates (Found: C, 56·25; H, 4·7; N, 7·05. $C_{18}H_{16}N_2O_8{\cdot}0{\cdot}5C_3H_6O$ requires C, 56·05; H, 4·6; N, 6·7%). Spectral measurements were carried out on non-solvated, microcrystalline material obtained by crystallisation from dichloromethane, $\nu_{max.}$ 3460, 3400, 3350, 1600br cm.$^{-1}$, $\lambda_{max.}$ 247, 265, 329 mμ (log ε 4·42, 4·46, 3·86). The summation spectrum (methanol) of the benzamide (IV) and the dibenzoquinone (IX) (0·5 equiv.) showed $\lambda_{max.}$ 250, 323 mμ (log ε 4·42, 3·89) and was very similar to that of the dimer (V) in methanol, $\lambda_{max.}$ 250, 335 mμ (log ε 4·42, 3·89). N.m.r.: τ 7·97, 7·47, 7·14 (3 CH_3 singlets), 0·8 (quinone $CO{\cdot}NH_2$, broad band), 1·7 ($ArCO{\cdot}NH_2$, broad band). The mass spectrum included peaks for the parent ion (*m/e* 388) and abundant ions at *m/e* 356, 345, 326, 311, 298, 270, 115, 91, 86, 84 (base peak). Accurate mass determinations and observation of metastable ion decompositions established the following fragmentation paths:

$$345 \xleftarrow{-CH_3} 360 \xleftarrow{-CO} 388 \xrightarrow{-NH_3} 371 \xrightarrow{-NH_3} 354$$

It gave an orange solution in alkali which changed, reversibly, to yellow in acid. The iodoform reaction was positive but there was no coloration with indophenol.

Reduction with sulphur dioxide in methanol–water at *ca.* pH 2 gave *3-acetyl-5,5′-dicarbamoyl-2,2′-dimethyl-3′,4,4′,6,6′-pentahydroxybiphenyl*, m. p. 260—280° (decomp.) (Found: C, 55·1; H, 4·4; N, 6·8. $C_{18}H_{18}N_2O_8$ requires C, 55·4; H, 4·65; N, 7·2%), $\nu_{max.}$ 3420, 1600br cm.$^{-1}$, $\lambda_{max.}$ 211, 246, 328 mμ (log ε 4·44, 4·37, 3·93). N.m.r.: τ 7·91,

7·62, 7·15 (3 CH_3, singlets), 1·8, 1·7 (2 $ArCO{\cdot}NH_2$, broad bands). It was immediately re-oxidised to the quinone (V) in alkali in the presence of air.

4-Carbamoyl-3,6-dihydroxytoluquinone (VI).—After the quinone (V) had been extracted from the reaction mixture at pH 5·5, the remaining solution was adjusted to *ca.* pH 2 and extracted with dichloromethane (4 × 250 ml.). Removal of solvent yielded the *product* (VI) as an orange solid (0·38 g., 10%), m. p. 232° (decomp.). A sample was sublimed at 130°/0·05 mm. (Found: C, 49·4; H, 3·6; N, 6·6; O, 40·35; *C*-Me, 8·0. $C_8H_7NO_5$ requires C, 48·75; H, 3·6; N, 7·1; O, 40·6; *C*-Me, 7·6%), $\nu_{max.}$ 3375, 3310, 3180, 1640, 1580 cm.$^{-1}$, $\lambda_{max.}$ 278, 293, 420 mμ (log ε 4·35, 4·25, 2·66). Ultraviolet spectra in approx. 10^{-4}M-solutions of various pH (B.D.H. Universal Buffer, Prideaux and Ward) showed two isosbestic points at 285 and 301 mμ (log ε 4·14 and 4·23). This indicated that pK_a values were approx. 3·5 and 6·2. N.m.r. τ 7·97 (CH_3, singlet), 0·8 ($CO{\cdot}NH_2$, broad band). The mass spectrum included peaks for the parent ion (*m/e* 197) and abundant ions at *m/e* 170, 169 (base peak), 152, 124, 96, 86, 83, 69, 68.

Accurate mass determinations and observation of metastable ion decompositions established the fragmentation path:

$$197 \xrightarrow{-CO} 169 \xrightarrow{-NH_3} 152 \xrightarrow{-CO} 124 \xrightarrow{-CO} 96 \xrightarrow{-28} 68$$

Crystallisation from a wide range of solvents yielded only microcrystalline material. It gave a deep red solution in alkali, which changed, reversibly, to yellow in acid. This colour was destroyed by sodium dithionite but slowly returned on exposure to air.

Acid hydrolysis yielded a small quantity of 3,6-dihydroxytoluquinone, which was identified chromatographically [8] by comparison with an authentic sample synthesised by the method of Fichter.[7]

The yields of the quinones (V) and (VI) were dependent on the concentration of starting material in solution, and the ratio of these products could be altered by working at different concentrations. Dilution favoured the monomer (VI) but more concentrated solutions favoured the formation of the dimer (V).

5,5′-Dicarbamoyl-4,4′-dihydroxy-2,2′-dimethyldibenzoquinone (IX).—Hydrogen peroxide (3·2%; 100 ml.) was added, during 5 min., to a solution of 3-acetyl-2,6-dihydroxy-4-methylbenzamide (IV) (5 g.) in aqueous potassium hydroxide (0·5N; 1·5 l.), under an atmosphere of nitrogen. After 4 hr. the solution was adjusted to pH 8·0 with 5N-hydrochloric acid; then the nitrogen flow was discontinued and oxygen was passed for 10 min. The orange solution was extracted with dichloromethane (3 × 250 ml.) to remove any starting material and then with additional dichloromethane (6 × 250 ml.) at pH 2·0. On reducing the volume of the latter extract, a black solid, presumably a quinhydrone, was deposited. More quinhydrone, from the aqueous solution, was collected, and the combined yield was oxidised with silver oxide (1 g.) in acetone (300 ml.) for 1 hr. The resulting orange solid was crystallised from dichloromethane to yield the *product* (IX) (1·1 g., 25%) as orange plates, m. p. 280—330° (decomp.) (Found: C, 53·1; H, 3·55; N, 8·0. $C_{16}H_{12}N_2O_8$ requires C, 53·35; H, 3·35; N, 7·8%), $\nu_{max.}$ 3400, 1650br cm.$^{-1}$, $\lambda_{max.}$ 270, 404 mμ (log ε 4·56, 3·33). N.m.r.: τ 7·91 ($2CH_3$, singlet), 0·7 ($2CO{\cdot}NH_2$, broad band). The mass spectrum included peaks for the parent ion (*m/e* 360) and abundant ions at *m/e* 343, 315, 300, 270 (base peak), 230, 204, 202, 174, 84, 78. The first two fragmentations probably involve successive loss of NH_3 and CO from the parent ion. It gave an orange-coloured solution in alkali which changed, reversibly, to yellow in acid.

Reduction with sulphur-dioxide in methanol–water at approx. pH 2 gave *5,5′-dicarbamoyl-2,2′-dimethyl-3,3′,4,4′,6,6′-hexahydroxybiphenyl* (Found: C, 52·55; H, 4·5; N, 7·6. $C_{16}H_{16}N_2O_8$ requires C, 52·75; H, 4·45; N, 7·7%), $\nu_{max.}$ 3440, 3380br, 3290, 1620br cm.$^{-1}$, $\lambda_{max.}$ 213, 255, 339 mμ (log ε 4·52, 4·29, 3·92). N.m.r.: τ 7·91 ($2CH_3$, singlet), 1·8 ($2CO{\cdot}NH_2$, broad band). It was immediately re-oxidised to the quinone (IX) in alkali in the presence of air.

6-Acetyl-4-carbamoyl-3-hydroxy-2,5-toluquinone (XI).—3-Acetyl-2,6-dihydroxy-4-methylbenzamide (IV) (100 mg.) was dissolved in phosphate buffer (0·1M-Na_2HPO_4 and 0·1M-Na_3PO_4, 5 : 1 by vol.; 60 ml.; *ca.* pH 10·5) and mixed with a solution of Frémy's salt (750 mg.) in the same buffer (40 ml.). After shaking for 2·5 hr. the red-brown solution was adjusted to *ca.* pH 7. Unreacted starting material (63 mg.) was recovered both by filtration and by extraction with dichloromethane (3 × 100 ml.). Further extraction with dichloromethane at *ca.* pH 2 gave a yellow solution which, on removal of solvent, yielded the *product* (XI) (37 mg., 35%), orange plates, m. p. 163—168° (decomp.) [from benzene–light petroleum (b. p. 60—80°)] (Found: C, 54·15; H, 4·1; N, 5·9. $C_{10}H_9NO_5$ requires C, 53·8; H, 4·05; N, 6·3%), $\nu_{max.}$ 3370, 1700, 1650 (all broad) cm.$^{-1}$, $\lambda_{max.}$ 267, 402 mμ (log ε 4·22, 3·04). N.m.r.: τ 7·36, 7·91 ($2CH_3$, singlets), 0·8 ($CO{\cdot}NH_2$, broad band). The mass spectrum included peaks for the parent ion (*m/e* 223) and abundant ions at *m/e* 208, 206, 190, 163, 150, 108, 78 (base peak), 77, 69, 67.

The compound (XI) gave an orange solution in alkali, changing, reversibly, to yellow in acid. It decomposed slowly in alkali, although it was fairly stable up to pH 11.

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