THE MECHANISM IN THE COLORIMETRY OF CAFFEINE AND THEOBROMINE BY HYPOCHLOROUS ACID-PYRIDINE METHOD

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Caffeine (1) and theobromine (2) reacted with pyridine in the presence of hypochlorous acid to give 8-(N-pyridinium)xanthine salts, which were characterised as the triiodides (3 and 5) and monoiodides (4 and 6). Treatment of 8-(N-pyridinium)caffeine iodide (3) with sodium hydroxide afforded sodium 5-(caffeine-8-yl)-imino-1,3-pentadienoxide (7). This experiment made clear the mechanism in the colorimetry of caffeine by hypochlorous acid-pyridine method.

Caffeine (1) is medically used as a stimulant for central nervous system, respirator and heart, and quantitatively analysed by dimethylglyoxime thiosemicarbazide solution[1] or hypochlorous acid-

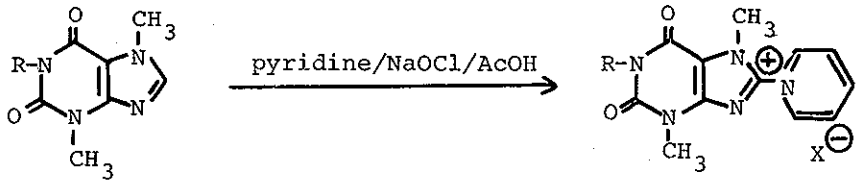
pyridien method.[2,3] The latter method is more widely used. In this communication we wish to report the mechanism of the coloration.

A solution of caffeine (1) and pyridine in aqueous acetic acid at pH 5.36 - 5.71 was treated with sodium hypochlorite at 0 - 3°, and then iodine was added to the resulting aqueous solution to give 8-(N-pyridinium)caffeine triiodide (3),[4] m.p. 228 - 230°, in 25.3 % yield. Reduction of the iodide (3) with sodium bisulphite afforded the monoiodide (4), m.p. 268 - 271°, whose structure was determined as follows. The n.m.r. spectrum (δ in D_2O) of 4 showed five protons due to pyridinium ring at 8.15 - 9.30 in addition to three N-methyl groups at 3.27, 3.45 and 3.91 p.p.m., and the proton at the 8 position of caffeine disappeared. The absorptions due to carbonyl groups were observed at 1705 and 1675 cm^{-1} in the i.r. spectrum (KBr). The u.v. spectra [$\lambda_{max}^{0.01\ \underline{N}\ HCl}$ nm (log ε): 262 (4.07) and 335 (3.66); $\lambda_{max}^{0.01\ \underline{N}\ NaOH}$ nm (log ε): 455 (4.89)] were similar to those of 7-methyl-8-(N-pyridinium)xanthine methylbisulphate.[5] Heating the monoiodide with 0.1 N sodium hydroxide solution yielded 8-aminocaffeine (8).

Theobromine (2) also furnished 8-(N-pyridinium)theobromine triiodide (5), m.p. 216 - 220° (decomp.) and the corresponding monoiodide (6), m.p. 296 - 301° (decomp.) by the same treatment as above.

Treatment of the monoiodide (5) with aqueous sodium hydroxide solution at room temperature afforded quantitatively red prisms, m.p.> 290°, $\lambda_{max}^{0.01\ \underline{N}\ NaOH}$ nm (log ε):455 (4.86), to which the structure (7) was assigned on the basis of the following evidences. The n.m.r. spectrum (δ in D_2O) exhibited five olefinic protons at 8.27 (1H, d, J = 9.5 Hz, C_1-H), 7.65 (1H, d, J = 11.0 Hz, C_5-H), 6.18

Scheme 1



(1) R=CH_3

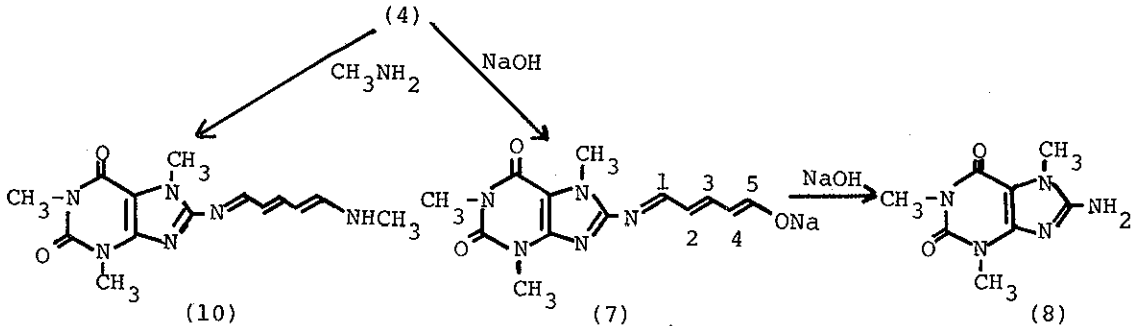
(2) R=H

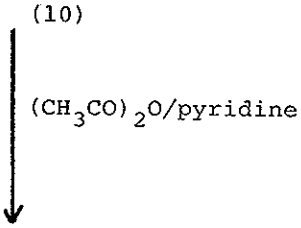
(3) R=CH_3, X=I_3

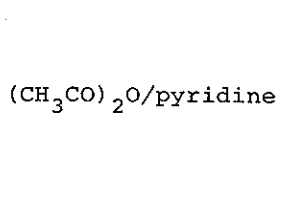
(4) R=CH_3, X=I

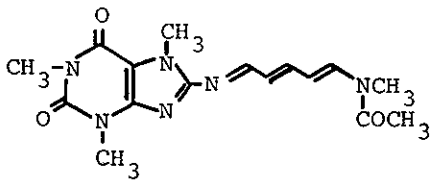
(5) R=H, X=I_3

(6) R=H, X=I

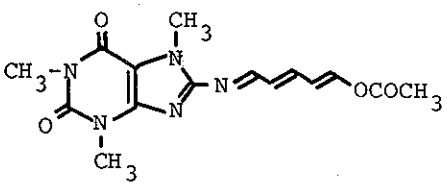








(11)



(9)

(1H, t, J = 13.0 Hz, C_3-H), 5.45 (1H, dd, J = 11.0 and 13.0 Hz, C_4-H), and 5.37 (1H, dd, J = 9.5 and 13.0 Hz, C_2-H) along with three N-methyl signals at 3.04, 3.28 and 3.45 p.p.m. After further treatment of 7 with sodium hydroxide, the resulting solution showed the absorption due to glutaconic dialdehyde anion at 364 nm in the u.v. spectrum.[5] In this case 8-aminocaffeine (8) was isolated.

Acetylation of 7 with acetic anhydride and pyridine gave the acetate (9), m.p. 223 - 225° (decomp.), $\lambda_{max}^{CHCl_3}$ nm (log ε): 320 (4.34), 382 (4.42) and 395 (4.43); λ_{max}^{KBr} cm^{-1}: 1760 (enol acetate), 1680 and 1665 (amide carbonyls) and 965 (trans olefin); m/e 331 (M^+). The coupling constant of the five olefinic protons in the n.m.r. spectrum [(δ in $CDCl_3$) 8.85 (1H, d, J = 9.0 Hz, C_1-H), 7.75 (1H, d, J = 12.0 Hz, C_5-H), 7.08 (1H, dd, J = 11.0 and 15.0 Hz, C_3-H), 6.55 (1H, dd, J = 9.0 and 15.0 Hz, C_2-H) and 6.28 p.p.m. (1H, dd, J = 11.0 and 12.0 Hz, C_4-H)] indicated an all-trans geometric isomer (9).[6]

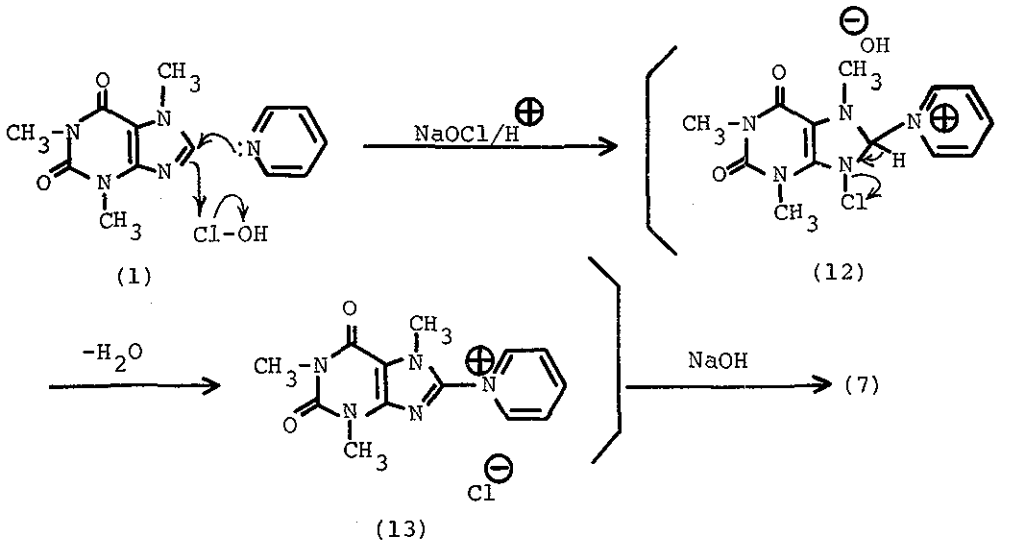
The above salt (7) was also obtained, when a solution of caffeine (1) and pyridine in aqueous acetic acid was treated with sodium hypochlorite followed by addition of sodium hydroxide according to the hypochlorous acid-pyridine method.[3]

Ring-opening of pyridinium salts with some nucleophiles has recently been reported by several workers.[7-9] and the reaction of the monoiodide (4) with methylamine afforded similarly 5-methylamino-N-(caffeine-8-yl)-2,4-pentadienylideneimine (10), m.p.> 290°, $\lambda_{max}^{H_2O}$ nm (log ε): 445 (4.85), which was converted to the acetate (11), m.p. 240°, m/e 344 (M^+). The all-trans structure of 11 was

suggested by the n.m.r. spectrum (δ in CF_3CO_2H), in which five olefinic protons appeared at 8.83 (1H, d, J = 11.0 Hz, C_1-H), 8.58 (1H, d, J = 12.0 Hz, C_5-H), 8.30 (1H, dd, J = 12.0 and 13.0 Hz, C_3-H), 7.08 (1H, dd, J = 11.0 and 13.0 Hz, C_2-H) and 6.63 p.p.m. (1H, t, J = 12.0 Hz, C_4-H).

It was thus clarified that sodium 5-(caffeine-8-yl)imino-1,3-pentadienoxide (7) formed as shown in Scheme 2 is the actual colored material in the colorimetry of caffeine by hypochlorous acid-pyridine method. Application of the substitution of caffeine and theobromine with pyridine in the presence of hypochlorous acid would provide a method for the preparation of many 8-substituted xanthines.

Scheme 2



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