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Studies on the Thalleioquine Reaction[1)]

ATSUSHI TAKADA,* HARUO NEGISHI, and TAKEO UEDA

School of Pharmaceutical Sciences, Kitasato University, 5–9–1, Shirokane, Minato-ku, Tokyo 108, Japan

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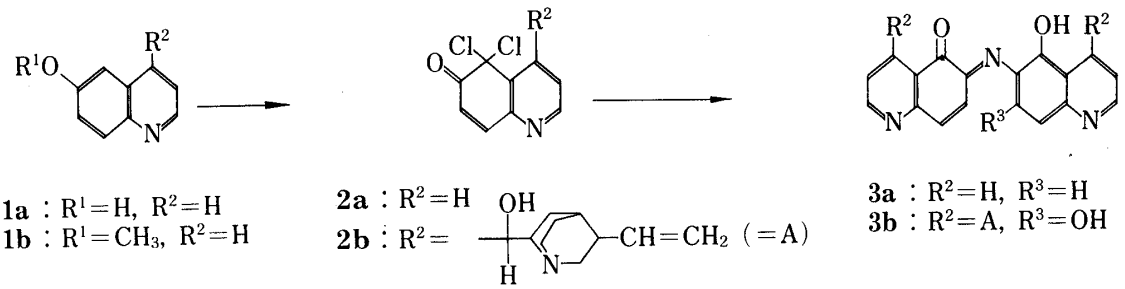
Two colored substances produced in the thalleioquine reaction using 6-methoxyquinoxaline as a model compound were isolated. The red substance was determined to be an 8,8′-biquinolinyl derivative (**6a**), and the blue substance was found to be a super-stable radical compound.

Keywords——thalleioquine reaction; erythroquine reaction; free radical; 6-methoxyquinoline; ESR; NMR; phenolic oxidation

The thalleioquine reaction (T-reaction)[2)] is a well known coloration and identification reaction for quina alkaloids, and is employed as a test for quinine salts in the pharmacopoeias of many countries. This reaction has been studied by several researchers, but the colored substances and the coloration mechanisms have not been sufficiently characterized as yet. The T-test is carried out by addition of bromine test solution (Br-T.S.) to salts of quina alkaloids and addition of ammonia test solution (NH_3-T.S.) to the resulting reaction mixture. In the course of this reaction, a red color is observed at the beginning, changing through reddish-purple, to emerald-green at the end. There is scarcely any information regarding the nature of this red substance. The authors found that this substance was identical with the red substance produced in the erythroquine reaction (E-reaction).[3)] The E-reaction gives a red color on addition of Br-T.S., potassium ferrocyanide-T.S. and NH_3-T.S. to quina alkaloids solutions. This reaction has been studied by some researchers, but without clear-cut results.

In both colorations, 6-hydroxy-, 6-methoxy-, and 5-hydroxyquinoline have often been used in place of quinine, and there seems to be little difference between the quinuclidine or hydroquinuclidine ring.[4)] For example, Führer reacted chlorine with a solution of 6-hydroxyquinoline (**1a**) hydrochloride, isolated 5,5-dichloro-6-oxo-5,6-dihydroquinoline (**2a**) from the resulting solution, and obtained a greenish-blue product by adding NH_3-T.S. to an alcoholic solution of **2a**.[5)] He gave the structural formula **3a** for the product mainly on the basis of elementary analyses (Chart 1). Auterhoff produced **2a** by chlorinating 6-methoxyquinoline (6-MQ; **1b**)[6)] and obtained a green substance by adding NH_3-T.S., just as Führer described. On the basis of this finding, he synthesized 5,5-dichloro-6-oxo-hydroquinine (**2b**) by reacting chlorine with hydroquinine, then obtained a greenish-blue product by adding NH_3-T.S.[6)] He gave the structure **3b** for the product on the basis of the elementary analysis data, ultraviolet (UV) spectrum, infrared (IR) spectrum and measurement of active hydrogen.



1a : $R^1=H$, $R^2=H$
1b : $R^1=CH_3$, $R^2=H$

2a : $R^2=H$
2b : $R^2=$ —CH=CH_2 (=A)

3a : $R^2=H$, $R^3=H$
3b : $R^2=A$, $R^3=OH$

Chart 1

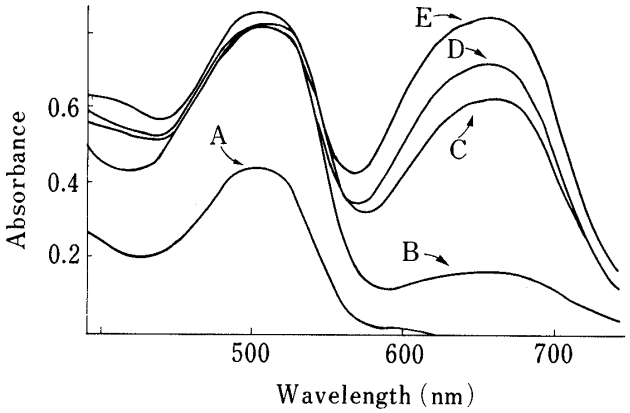


Fig. 1. Effect of Amount of NH_3 Used

One ml of 1 M HCl, 16 ml of Br_2-water and 1 M NH_3 solution were added successively to a solution of 97.8 mg of 6-MQ HCl in 100 ml of water, then the colored solution was extracted with DCM. The volume of the extract was adjusted to 200 ml with DCM. The amount of 1 M NH_3 used was: A, 1.5 ml; B, 2.0 ml; C, 3.0 ml; D, 5.0 ml; E, 10.0 ml.

The authors employed 6-MQ as a model compound of quinine, and reacted it with Br-T.S. and NH_3-T.S. to obtain a blue product. We found that this substance was not a compound of low molecular weight such as Fühner described, but a super-stable free radical of very high molecular weight. This report describes the determination of the structure of the red substance and the mechanism of the blue coloration in the T-reaction.

Preliminary Examination of the Thalleioquine Reaction

In order to examine the colored substance in the T-reaction, 16 ml of 10%-saturated Br-T.S., and 1 M NH_3-T.S. were added to 50 ml of 0.01 M solution of 6-MQ hydrochloride, matching the practical conditions for the identification reaction. When a small amount of NH_3 was added, a red color appeared immediately, and the solution became greenish-blue through reddish-purple as the amount of NH_3 added was increased. At that time, the colored solution was extracted with dichloromethane (DCM), and the extract was subjected to thin layer chromatography (TLC).[7] Hereupon, a red component was observed at *Rf* 0.76, a very faint blue component at *Rf* 0.66 and a blue component at *Rf* 0.48, in addition to unreacted 6-MQ.

To survey the influence of the added amount of NH_3, 50 ml of 0.01 M 6-MQ hydrochloride solution was mixed with 16 ml of 10%-saturated solution of Br_2 and various amounts of 1 M NH_3-T.S. (1.5 to 10.0 ml), then the colored solution was extracted wih DCM. The absorption spectra of the extracts were measured in the visible region. The results are shown in Fig. 1.

When 1.5 ml of NH_3-T.S. was added, a red absorption maximum was observed at 505 nm, but the blue maximum at 660 nm was not observed. When more than 2.0 ml of NH_3-T.S. was added, the maximum at 660 nm was observed, and its intensity increased with the amount of NH_3-T.S. added, while the intensity at 505 nm did not increase further when more than 2.0 ml of NH_3-T.S. was added.

Separation and Identification of Red-Colored Substances in the Thalleioquine Reaction and Erythroquine Reaction

The T-reaction was carried out under conditions where only the red substance was produced (the case of curve A in Fig. 1). The reaction mixture was extracted with DCM. The extract was shaken with NH_3-T.S. No blue color was observed in the extract. The E-reaction is used for detection of quina alkaloids, and is like the T-reaction, except for the usage of potassium ferrocyanide. In this reaction, a solution of 6-MQ salt became red when Br-T.S. and potassium ferrocyanide test solution (P-T.S.), followed by NH_3-T.S., were added, but did not turn blue with further addition of NH_3-T.S. The red-colored solution was extracted with DCM and the absorption maximum of the extract in the visible region was measured; it showed a maximum at 505 nm. A red spot was detected at *Rf* 0.76 when the extract was

subjected to silica gel TLC. Thus, the red substance of the E-reaction might be identical with that of the T-reaction. The red-colored substances of E- and T-reactions were isolated according to the methods described in the experimental section.

Both substances were isolated as blackish-red needles; their DCM solutions showed absorption maxima at 505 nm and they gave identical IR spectra. Therefore, they were considered to be the same substance. They melted at over 300 °C, were soluble in chloroform and DCM, sparingly soluble in methanol, ethanol and dimethylformamide, and almost insoluble in benzene, ethyl acetate, carbon tetrachloride and water. The molecular formula was determined as $C_{20}H_{16}N_2O_4$ by high resolution mass (MS) analysis (Calcd *m/e* 348.110, M^+; found *m/e* 348.108), in accord with the elementary analysis data. We propose the structure **6a** for the red substance on the basis of following data. The IR spectrum revealed the presence of an NH absorption band at 3400 cm^{-1} (see Fig. 2). The proton nuclear magnetic resonance (H-NMR) spectrum showed signals at 3.98 ppm (6H, s) due to methoxy protons, 7.48 (2H, dd, $J_{3,4(3',4')}=8.0$ Hz, $J_{2,3(2',3')}=4.5$ Hz) due to the 3- and 3′-protons of the quinoline ring, 8.66 (2H, dd, $J_{4,3(4',3')}=8.0$ Hz, $J_{4,2(4',3')}=2.0$ Hz) due to the 4- and 4′-protons, and 8.90 (2H, dd, $J_{2,3(2',3')}=4.5$ Hz, $J_{2,4(2',4')}=2.0$ Hz) due to the 2- and 2′-protons, 9.11 (2H, s) due to the 7- and 7′-protons, and 1.58 (2H, s, exchangeable with D_2O) due to two NH hydrogens (Fig. 3).

We next considered the reaction pathway for the formation of 1*H*,5*H*,1′*H*,5′*H*-6,6′-dimethoxy-1,5,1′,5′-tetrahydro-8,8′-biquinolinyl-5,5′-dione, **6a**. On the basis of Fühner's[5] and Zinke's[8] reports regarding the chlorination of 6-hydroxyquinoline, 5-bromo-6-methoxyquinoline might be formed by the reaction of 6-MQ with bromine, then hydrolyzed

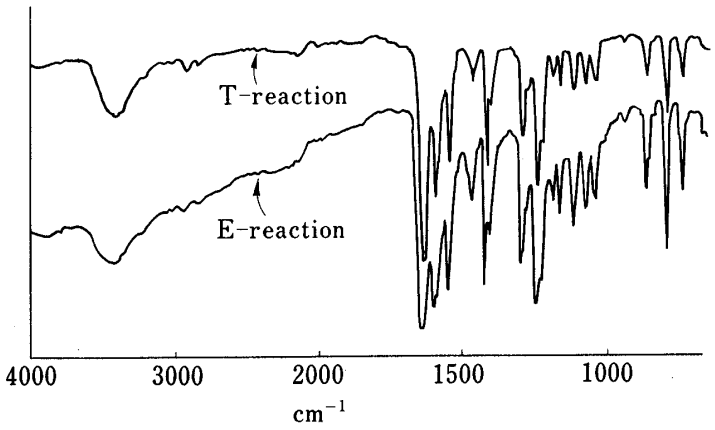


Fig. 2. IR Spectra of Samples of **6a** Formed in the T-Reaction and the E-Reaction (KBr)

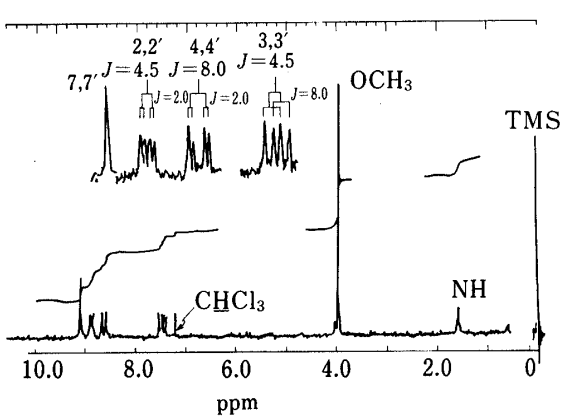


Fig. 3. H-NMR Spectrum of **6a** Formed in the E-Reaction ($CDCl_3$ Solution)

to 5-hydroxy-6-methoxyquinoline (**5a**), followed by oxidative coupling to produce the dimer **6a** through a mechanism resembling phenolic oxidation[9] (see Chart 2). Similarly, when the reaction was conducted with 6-methoxyquinaldine hydrochloride in place of 6-MQ hydrochloride, blackish-red needles of 1*H*,5*H*,1′*H*,5′*H*-6,6′-dimethoxy-1,5,1′,5′-tetrahydro-8,8′-biquinaldinyl-5,5′-dione (**6b**) were obtained; the structure of **6b** was determined on the basis of MS (M^+, *m/e* 376) and NMR analyses as illustrated in Fig. 4. It is noteworthy that the red substance of the T-reaction is identical with that of the E-reaction. This is the first report of such a result.

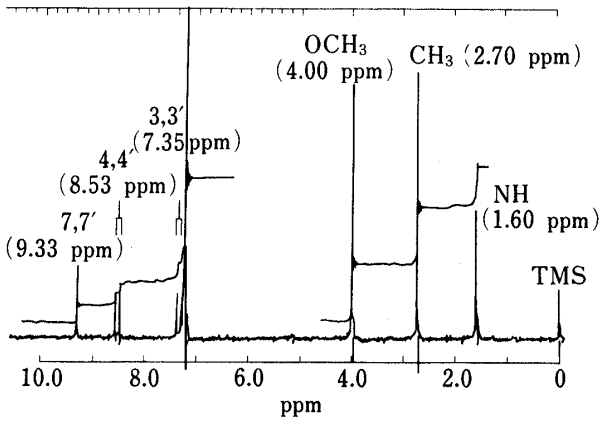


Fig. 4. H-NMR Spectrum of **6b** Formed in the E-Reaction ($CDCl_3$ Solution)

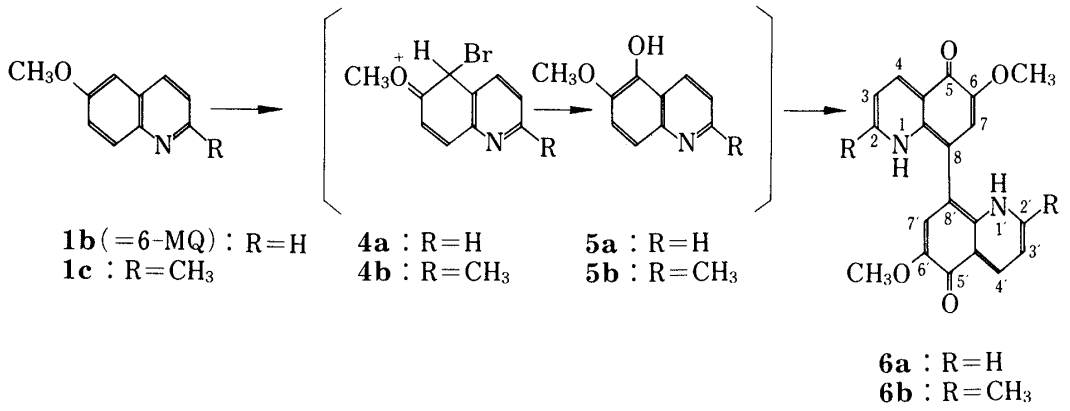


Chart 2

Analyses of the Blue Substance in the Thalleioquine Reaction

The blue coloration in the T-reaction was very stable and durable. This suggested that the colored species could be extracted from the reaction mixture and separated by chromatography. The species, therefore, was produced and purified as follows. The coloration reaction was carried out with 0.9 g of 6-MQ hydrochloride, and the reaction mixture was extracted with DCM. The extract contained colored species and unreacted 6-MQ. TLC showed a spot at *Rf* of 0.76 assigned to the red species, spots of *Rf* 0.66 and 0.48 (blue species), and a spot of 6-MQ. Since the spot of *Rf* 0.66 was very faint, further analysis was not possible. In order to purify the blue substance of *Rf* 0.48, silica gel column chromatography was conducted twice with a mixture of DCM and methanol (9 : 1), as described in the experimental section. After that, the purified blue substance was obtained by gel filtration chromatography on Sephadex LH-20. This substance showed a single blue spot at *Rf* 0.48 on TLC. The substance is a deeply blue powder, does not melt up to 360 °C, is stable at room temperature, and is soluble in organic solvents, such as DCM, chloroform, and methanol. Its DCM solution showed the

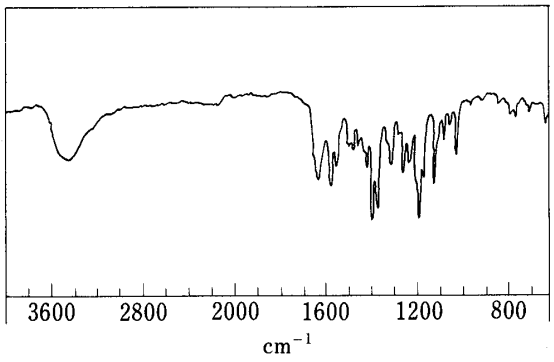


Fig. 5. IR Spectrum of Blue Substance (KBr)

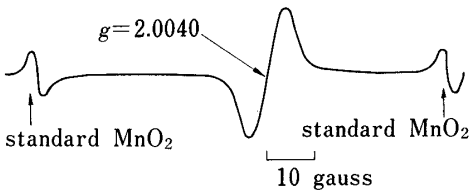


Fig. 6. ESR Spectrum of Blue Substance

absorption maximum of 660 nm, just as seen in the T-reaction. Moreover, no marked peaks were seen in mass spectroscopy almost all the sample remained as a solid residue, and no signals except for a broad peak in the range of 1 to 2 ppm were observed in the NMR spectrum. Figure 5 shows its IR spectrum. These results indicated that the blue substance might be a free radical of high molecular weight. This view was supported by the ESR spectrum (shown in Fig. 6), where a strong signal was observed at $g=2.0040$ although the concentration of the solution was very low. No hyperfine structure was observed. The fact that the blue substance was soluble in chloroform and DCM, and could be separated by chromatography, suggested that it might be a neutral radical. Thus, we concluded that the blue coloration is due to the formation of a stable radical, contrary to the speculations of Führer[5] and Auterhoff[6] that the coloration of the T-reaction might be due to the formation of a compound such as **3a** or **3b**.

The empirical formula $C_{40}H_{29}N_6O_{13}$ was obtained by elementary analysis of the substance, but its molecular weight has not yet been determined. The blue color was lost on reacting the substance with a solution of hydrazine or hydrosulfite. The reduced solution showed no signal in the ESR spectrum, but recovered the blue color when allowed to stand in the air. This product showed the same blue spot as the original substance on TLC. The blue substance dissolved in hydrochloric acid to form a red solution which showed an ESR signal of reduced intensity. This suggests that the radical involves a nitrogen atom(s). At present, several super-stable radicals are known. Among them, *N*-oxylradicals, for example, 2,2,6,6-tetramethyl-4-oxopiperidine-1-oxyl react with acids to form salts, and are reduced to hydroxylamine type compounds with reducing agents.[10] The structure of our blue substance is unknown at present. Further work is in progress, and the results will be published elsewhere.

Experimental

Absorption spectra were measured with a Shimadzu MPS-50L spectrophotometer in a cell of 10 mm optical length, IR spectra with a JASCO IRA-1 spectrophotometer, H-NMR spectra with a JEOL PS-100 spectrometer at 100 MHz with tetramethylsilane as an internal standard, MS with a JMS-D100 mass spectrometer, and high resolution MS with a JMS-01S spectrometer. ESR spectra were obtained on a JEOL JMS-ME-1X spectrometer with manganese dioxide as an external standard. Melting points were determined with a Yamato Scientific stirred liquid apparatus and are uncorrected.

Isolation of 1*H*,5*H*,1′*H*,5′*H*-6,6′-Dimethoxy-1,5,1′,5′-tetrahydro-8,8′-biquinolinyl-5,5′-dione (6a) from E-Reaction——Ten ml of 1 M HCl solution, 16 ml of 10%-saturated Br-T.S., 50 ml of 10% P-T.S., and 20 ml of 1 M NH_3-T.S. were successively added to a solution of 900 mg of 6-MQ hydrochloride in 500 ml of water with stirring. After sufficient time for the coloration to occur (about 5 min), the reaction mixture was extracted with DCM (300 ml × 3 times), and the extract was washed with a small volume of 0.1 M HCl until the water layer was no longer colored. The organic layer was dried over Na_2SO_4, and evaporated to dryness under a reduced pressure. Recrystallization from DCM gave 4.8 mg of blackish-red needles of **6a** (*Rf* 0.76), mp >300 °C. UV λ_{max}^{DCM} 505 nm ($\varepsilon=5.1\times10^4$). MS *m/e*: M^+

348.108. Calcd for $C_{20}H_{16}N_2O_4$: M^+ 348.110, *Anal.* Calcd for $C_{20}H_{16}N_2O_4$: C, 68.96; H, 4.63; N, 8.04. Found: C, 68.67; H, 4.09; N, 7.99.

Isolation of 1*H*,5*H*,1'*H*,5'*H*-6,6'-Dimethoxy-1,5,1',5'-tetrahydro-8,8'-biquinaldinyl-5,5'-dione (6b) from the E-Reaction——By the same method as described above, 5.2 mg of **6b** (*Rf* 0.74) was isolated as blackish-red needles from the E-reaction using 1 g of 6-methoxyquinaldine hydrochloride. mp >300 °C. UV λ_{max}^{DCM} 505 nm. MS: *m/e* 376 (M^+). *Anal.* Calcd for $C_{22}H_{20}N_2O_4$: C, 70.20; H, 5.36; N, 7.44. Found: C, 70.18; H, 5.30; N, 7.47.

Isolation of 6a from T-Reaction——Ten ml of 1 M HCl solution and 110 ml of 10%-saturated Br-T.S. were added to a solution of 900 mg of 6-MQ hydrochloride in 500 ml of water followed immediately by 15 ml of 1 M NH_3-T.S., with stirring. After one minute, the mixture was extracted with DCM (300 ml × 3 times), and the extract was washed with water to remove excess ammonia, dried over Na_2SO_4, and concentrated under reduced pressure to afford an oily red substance. The product was subjected to Sephadex LH-20 column chromatography with DCM as the eluant, and the eluate was washed with a small volume of 0.1 M HCl to remove unreacted 6-MQ. The organic layer was dried over Na_2SO_4, and evaporated to dryness. Recrystallization from DCM gave 2.2 mg of **6a** as blackish-red needles (*Rf* 0.76).

Isolation of Blue Substance from the T-Reaction——A larger volume (20 ml) was used in place of 15 ml of 1 M NH_3-T.S. in the T-reaction described above. After sufficient time for the coloration, the reaction mixture was extracted with DCM (300 ml × 3 times), and the extract was washed with water to remove excess ammonia, then dried over Na_2SO_4, and concentrated under a reduced pressure to afford an oily blue substance, which was chromatographed twice on a silica gel column, and then on a Sephadex LH-20 column using DCM–methanol (9 : 1) as the eluant. A blue powder was obtained in 2.8 mg yield. *Rf* 0.48, mp >300 °C. UV λ_{max}^{DCM} 660 nm. *Anal.* Calcd for ($C_{40}H_{29}N_6O_{13}$): C, 59.92; H, 3.64; N, 10.40. Found: C, 60.46; H, 3.62; N, 10.48.

References and Notes

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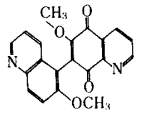

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