*The Structure of Tanshinone-II**

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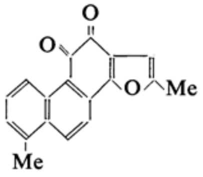
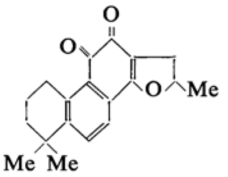
Nakano and Fukushima isolated[1)] three pigments, tanshinone-I, tanshinone-II and tanshinone-III, from the root of *Salvia miltorrhiza* Bge. (Chinese drug, Tan-shen). Among these pigments, tanshinone-III was shown afterwards by Takiura[2)] to be a mixture of tanshinone-II and a new pigment, cryptotanshinone. Von Wessely et al.[3)] proved that tanshinone-I was a condensed phenanthrenequinone of the structure I, and cryptotanshinone was proved by Takiura[4)] to have the diterpenoid naphthoquinone structure II.



I



II

Von Wessely and Lauterbach[5)] worked on the chromophore of tanshinone-II but the whole structure of this pigment was not clarified. Thomson assigned a structure to tanshinone-II in his book[6)], but it has no experimental basis.

Tanshinone-II is an orange-red substance of molecular formula $C_{19}H_{18}O_3$. It is shown to be an *o*-quinone by formation of a quinoxaline derivative on condensation with *o*-phenylenediamine[2)] and from infrared absorption maxima at 1701 and 1650 cm^{-1} attributed to quinone carbonyl absorptions. The ultraviolet absorption spectrum of this substance has four absorption maxima at 223, 268, 344 and 457 mμ and is obviously different from those of tanshinone-I and cryptotanshinone (Fig. 1). Leucotanshinone-II-diacetate and leucotanshinone-II-dimethyl ether (VI) derived from tanshinone-II by reductive acetylation and reductive methylation, respectively, show ultraviolet absorption maxia at 250, 257, 326 and 342 mμ, and their spectra are distinctly different from those of leucotanshinone-I-dimethyl ether (λ_{max}: 270, 316 and 347 mμ) and of 1-ethoxy-3, 4-diacetoxy-naphthalene (λ_{max}: 295, 323 mμ), synthesized as a model compound. The characteristics in the ultraviolet absorption show that tanshinone-II is neither a furanophenanthrenequinone, like tanshinone-I, nor merely an *o*-naphthoquinone derivative, like cryptotanshinone.

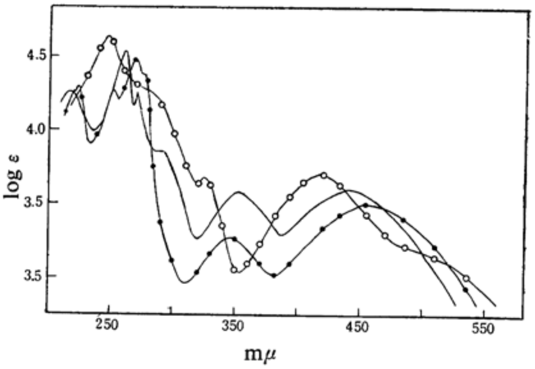


Fig. 1. Ultraviolet absorption spectra of tanshinones;
—○— Tanshinone-I, —●— Tanshinone-II,
—— Cryptotanshinone in ethyl alcohol.

Of the three oxygen atoms of tanshinone-II, $C_{19}H_{18}O_3$, two are attributable to quinone carbonyls, while the third must be in an ether structure, since tanshinone-II and leucotanshinone-II-diacetate show no O–H stretching absorption and leucotanshinone-II-dimethyl ether shows no C=O stretching absorption in their infrared spectra.

The ultraviolet absorption spectrum of the semicrystalline solid which was obtained from leucotanshinone-II-dimethyl ether (VI) by Se-dehydrogenation under a mild condition is almost identical with that of leucotanshinone-I-dimethyl ether (VII), so it is conceivable that tanshinone-II has the same carbon skeleton as tanshinone-I. But this dehydrogenation product is not identical with leucotanshinone-I-dimethyl ether in its infrared spectrum, and it shows two fluorescent spots on paper in chromatography, one of which coincides with leucotanshinone-I-dimethyl ether. Thus, the

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1) M. Nakano and T. Fukushima, *J. Pharm. Soc. Japan* (*Yakugaku Zassi*), **54**, 154 (1934).

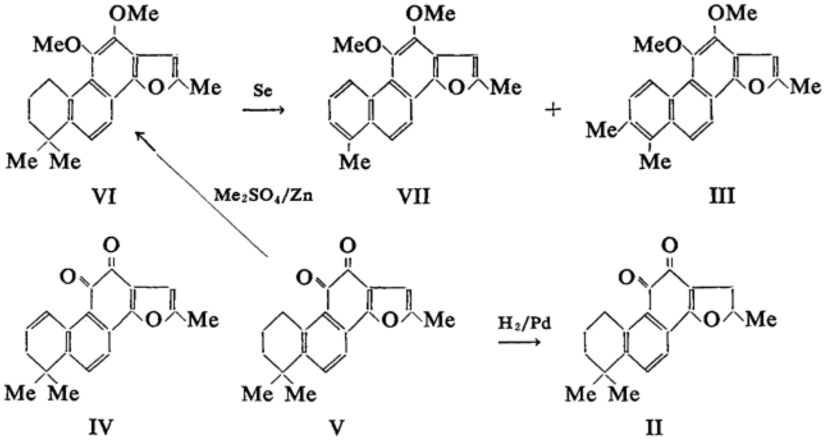
2) K. Takiura, ibid., **61**, 475 (1941).

3) F. von Wessely and S. Wang, *Ber.*, **73**, 19 (1940).

4) K. Takiura, *J. Pharm. Soc. Japan* (*Yakuguku Zassi*), **61**, 482 (1941).

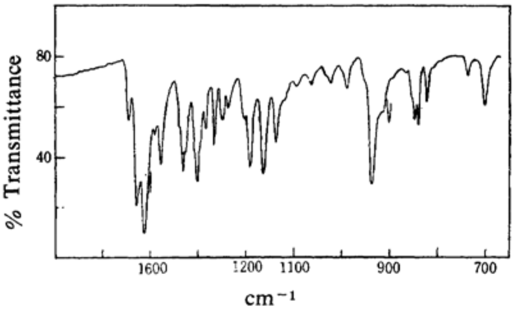
5) F. von Wessely and T. Lauterbach, *Ber.*, **75**, 958 (1942).

6) R. H. Thomson, "Naturally Occurring Quinones", Butterworths Sci. Pub. (1957), p. 263.

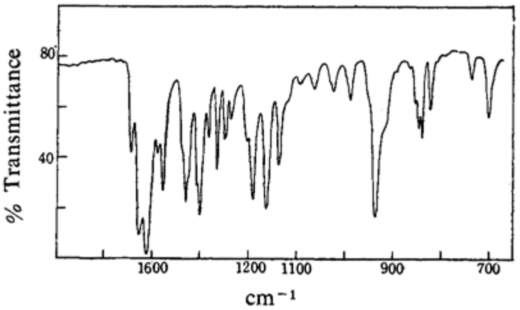


dehydrogenation product is a mixture. Tanshinone-II and its derivatives show absorptions ascribed to a gemdimethyl group at 1381 and 1361 cm^{-1} in their infrared spectra; therefore the second fluorescent spot may be due to the substance III, which can be produced by the migration of one methyl group during the Se-dehydrogenation process⁷⁾.

On catalytic hydrogenation with Pd-charcoal in methanol, tanshinone-II absorbs two moles of hydrogen and becomes colorless, but this colorless substance is readily autoxidized to an orange substance. When this hydrogenation product is treated with ferric chloride-acetic acid solution and the separated ferric chloride-complex is decomposed with water, orange needles of melting point 187°C are obtained. The infrared spectrum of this orange substance is identical with that of cryptotanshinone (Fig. 2). Since tanshinone-I, tanshinone-II, and their leuco-derivatives show absorptions characteristic of the furan ring⁸⁾ at 3155 and 860 cm^{-1} in their infrared spectra, tanshinone-II must be represented by structure V. If tanshinone-II has structure IV, it must be converted into an alkali-soluble substance by cleavage of the dihydrofuran ring with alkali in the same fashion as cryptotanshinone⁴⁾ and β-lapachone⁹⁾. But tanshinone-II does not undergo this transformation, and this fact also supports the structure V¹⁰⁾.



Infrared Spectrum of the hydrogenated tanshinone-II (KBr).



Infrared spectrum of the cryptotanshinone (KBr).

Fig. 2.

Experimental

All melting points are uncorrected. Infrared spectra were obtained with a Hilger H-800 double beam spectrophotometer and ultraviolet absorption spectra were obtained with Beckman DK-2 spectrophotometer.

Tanshinone-II.—The ether extract of roots of *Salvia miltorrhiza* was fractionated according to the method of Takiura, and it gave tanshinone-I (m.p. 231~232°C), cryptotanshinone (m. p. 191°C), and tanshinone-II, (m. p. 211°C).

Ultraviolet absorption maxima in ethanol, log in parentheses: 457 (3.45), 344 (3.23), 268 (4.37), 250 (4.24) and 223 mμ (4.31). Infrared absorptions (KBr): 3157, 1701, 1650, 1584, 1539 and 1505 cm^{-1}.

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7) H. H. Zeiss and F. W. Grant, Jr., *J. Am. Chem. Soc.*, **79**, 1201 (1957); E. Lederer, F. Marx, D. Mercier and G. Perot, *Helv. Chim. Acta*, **29**, 1354 (1946).

8) "Kagaku no Ryoiki (IR Tokushiu)", **3**, Nankodo (1954), p. 101.

9) S. C. Hooker, *J. Chem. Soc.*, **61**, 611 (1892); **69**, 1355 (1896).

10) A private communication from K. Takiura told us that he had reached the same conclusion as ours by other methods.

Found: C, 77.58, 77.71; H, 6.000, 6.033. Calcd· for $C_{19}H_{18}O_3$: C, 77.55; H, 6.12%.

Leucotanshinone - II - diacetate.—One hundred milligrams of tanshinone-II was heated for five minutes with 0.6 ml. acetic anhydride, 20 mg. fused sodium acetate, and 200 mg. zinc dust. Following removal of the zinc dust, the filtrate was concentrated under reduced pressure in a stream of nitrogen. The residue gave purple-fluorescent white crystals melting at 174~175°C on recrystallization from ethanol.

Ultraviolet absorption maxima in ethanol: 340 (4.23), 324 (3.24), 310 (3.08), 285 (3.72), 257 (shoulder 3.81), 254 (4.81) and 240 mμ (4.74).

Found: C, 72.70, 72.40; H, 6.30, 6.27. Calcd. for $C_{23}H_{24}O_3$: C, 72.61; H, 6.36%.

Leucotanshinone-II-dimethyl Ether.—Tanshinone-II (100 mg.) was dissolved in 10 ml. ethanol, and 15 ml. of 10% sodium hydroxide solution was added. On boiling gently in the atmosphere of nitrogen, the mixture turned pale yellow. Then, 6 ml. of dimethyl sulfate was dropped gradually into this boiling mixture. After cooling, the reaction mixture was extracted with ether, and the ether layer was washed with 1 N sodium hydroxide, then with water, and dried with anhydrous sodium sulfate. A pale yellow resinous substance was obtained on evaporation of the solvent under reduced pressure. This was dissolved in hexane and submitted to chromatography on a column of alumina. The fraction eluted by hexane gave white crystals, m. p. 103~104°C, on recrystallization from ethanol.

Ultraviolet absorption maxima in ethanol 343 (3.28), 327 (3.35), 258 (4.95) and 250 mμ (4.84).

Infrared spectrum (KBr), 3157, 1635, 1567, 1520 and 1508 cm^{-1}.

Found: C, 77.60, 77.86; H, 7.47, 7.40. Calcd. for $C_{21}H_{22}O_3$: C, 77.75; H, 7.46%.

Dehydrogenation of Leucotanshinone-II-dimethyl Ether.—A mixture of 100 mg. leucotanshinone-II-dimethyl ether and 100 mg. selenium powder was heated in a metal bath at 230°C for 24 hr. After cooling, the mixture was extracted three times with 20 ml. ether, and the extracts were combined and washed with dilute sodium hydroxide solution and then with water. On evaporation of ether after drying with anhydrous sodium sulfate, a yellow viscous oil was obtained. This oil was dissolved in hexane and submitted to chromatography on a column of alumina. The fraction eluted by a (9:1)-mixture of hexane and benzene gave a semi-crystalline substance, which on crystallization from ethanol melted at 131~140°C.

Ultraviolet absorption maxima in ethanol: 315, 295 and 272 mμ.

Catalytic Hydrogenation of Tanshinone-II.—Tanshinone-II (100 mg.) dissolved in 50 ml. of methanol was stirred with 100 mg. of 10% Pd-charcoal in the atmosphere of hydrogen at room temperature. After absorption of 26 ml. of hydrogen the solution became colorless. The catalyst was filtered off, and the filtrate was concentrated under reduced pressure in a stream of nitrogen. The residue was dissolved in 3 ml. of acetic acid, and then treated with ferric chloride-acetic acid solution. Black crystals of ferric chloride-complex were separated on the funnel, washed with glacial acetic acid, and then dried in a vacuum dessicator. This complex was decomposed with water and extracted with ether. The ether extract gave an orange solid on evaporation. This was recrystallized from methanol as orange crystals, m. p. 187°C, the infrared spectra was identical with that of cryptotanshinone (Fig. 2).

1-Ethoxy-3, 4-diacetoxynaphthalene.—A mixture of 500 mg. 4-ethoxy-β-naphtoquinone, 1 g. zinc dust, 6 ml. acetic anhydride, and one drop of triethylamine was boiled for 5 min. The cooled mixture was filtered, and the filtrate was evaporated under reduced pressure and resulted in a pale yellow viscous oil. This was recrystallized from ethanol-water.

White prisms, m. p. 106°C. Ultraviolet maxima in ethanol: 323 (3.43) and 295 mμ (3.83).

Found: C, 66.72, 66.54; H, 5.64, 5.71. Calcd. for $C_{16}H_{16}O_5$: C, 66.66; H, 5.59%.

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