UDC 612.015.3:547.856.1

13. Masuo Akagi,*1 Yoneshiro Oketani,*1 and Masahiko Takada*2 : Studies on Metabolism of 2-Methyl-3-*o*-tolyl-4(3*H*)-quinazolinone. I. The Estimation of 2-Methyl-3-*o*-tolyl-4(3*H*)-quinazolinone in Biological Materials.

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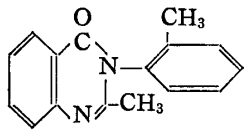
Recently, Gujral, *et al.*[1] found, during the study of synthetic antimalarials, that 2-methyl 3-*o*-tolyl-4(3*H*)-quinazolinone(MTQ)was the most effective hypnotic among the quinazolone derivatives tested, and the effect of the drug was also confirmed by Boissier, *et al.*[2] Ravina[3] reported, from clinical observations, that the action of the drug was rapid and prolonged for 6 to 8 hours after oral administration, and had not unpleasant side effects.



MTQ, having the following structural formula, has white crystalline form of m.p. 114°~116°, which is soluble in acids and alcohols, and insoluble in water.

There is no information concerning the physiological disposition and metabolic fate of the drug. In view of the clinical importance of the drug, the authors have been studying the physiological disposition and metabolic fate of the drug.

In this paper two methods for the determination of this compound in biological materials are described.

Experimental

Methods

(A) Single Point Procedure——The method is suitable for the estimation of MTQ in the plasma and urine. In this procedure the drug is extracted from alkalinized biological materials with hexane, the solvent evaporated to dryness *in vacuo*, and the residue dissolved in 0.1*N* HCl, and measured spectrophotometrically at 234 mμ.

Reagents and biological blanks are run through the same procedure, and 0.1*N* HCl is used for the zero setting. The concentration of the drug is, therefore, obtained by subtracting the blanks from the estimation value.

(B) Triple Point Procedure——The method, suitable for tissues, involves the estimation of the absorbances at three wave lengths, λ_1, λ_2 and λ_3 and the principle of the method is described in the following. Tissue blank is not reproducible even in duplicate determination of the same tissue.[4]

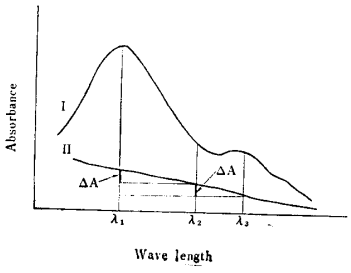


Fig. 1. Ultraviolet Absorption Spectra of Apparent MTQ in the Biological Material and Normal Biological Blank

I : apparent MTQ in 0.1*N* HCl
II : blank in 0.1*N* HCl

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1) M. L. Gujral, *et al.* : Ind. J. Med. Res., **43**, 637 (1955).
2) J. R. Boissier, *et al.* : Therapie, **13**, 30 (1958).
3) A. Ravina : Press Med., **67**, 891 (1959).
4) B. B. Brodie, *et al.* : J. Pharmacol. Exptl. Therap., **98**, 85 (1950).

The correction for this blank depends upon the observations that the blank value decreases for high wave length gradually and that, by geometrical treatment of the blank values at λ_1, λ_2 and λ_3, they become negligible. In comparison with the single point procedure, however, the sensitivity of the drug estimation lowers slightly, as shown in Fig. 3.

If B_{λ_t} is the absorbance of biological blank at wave length (λ_t), λ_1, λ_2 and λ_3 are selected at the wave lengths where B_{λ_2} is equal to $B_{\lambda_1}+B_{\lambda_3}/2$. As shown in Fig. 1, the following equations are derived :

$$A_1'-A_2'=A_1+\Delta A-A_2 \quad (1)$$

$$A_2'-A_3'=A_2+\Delta A-A_3 \quad (2)$$

where ΔA is equal to $(B_{\lambda_1}-B_{\lambda_2})$ and $(B_{\lambda_2}-B_{\lambda_3})$.

Subtracting equation (2) from (1),

$$A_1'+A_3'-2A_2'=A_1+A_3-2A_2=\alpha C \quad (3)$$

If

$$A_1=\alpha_1 C \quad (4)$$

$$A_2=\alpha_2 C \quad (5)$$

$$A_3=\alpha_3 C \quad (6)$$

the drug concentration is expressed as

$$C=A_1'+A_3'-2A_2'/\alpha_1+\alpha_3-2\alpha_2$$

where A_1', A_2' and A_3'=absorbances of apparent drug at λ_1, λ_2 and λ_3, respectively

A_1, A_2 and A_3=absorbances of drug at λ_1, λ_2 and λ_3, respectively

C=unknown concentration of drug being measured (g./L.)

α, α_1, α_2 and α_3=extinction coefficients (sample path length 1 cm.)

In case of the estimation of MTQ, λ_1 and λ_3 were selected at 234 and 285 mμ, respectively.

The two procedures described above, as indicated by a countercurrent distribution technique, possess a high degree of specificity (Fig. 4).

Reagents

1. Standard Solution of MTQ——MTQ (100 mg.; m.p. 116° from EtOH) is dissolved in 1 L. of 0.1*N* HCl. Working standards are made by diluting the stock solution with 0.1*N* HCl. The solutions are very stable.

2. *n*-Hexane——Hexane is purified by distillation (b.p. 68~69°), followed by successive washings with 1*N* HCl, 1*N* NaOH, and two washings with H_2O.

3. 0.1*N* HCl, 5*N* HCl

4. 1*N* NaOH, 30% NaOH

Preparation of Calibration Curves*3——Calibration curves were made by using the standard solutions involving 1, 2, 3 and 5 μg. of MTQ per cc. (Fig. 3).

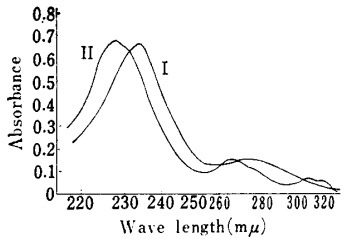

Fig. 2. Ultraviolet Absorption Spectra of MTQ
I : MTQ in 0.1*N* HCl (5 μg./cc.)
II : MTQ in 0.1*N* NaOH (5 μg./cc.)

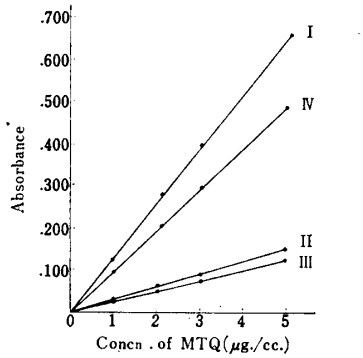

Fig. 3. Calibration Curves of Standard Solution of MTQ
I : at 234 mμ II : at 260 mμ III : at 285 mμ
IV : calibration curve obtained from triple point procedure (λ_1, λ_2 and λ_3 are 234, 260 and 285 mμ, respectively.)

*3 In this work Hitachi Model EPU-2A spectrophotometer and Shimazu Model RS-27 recording spectrophotometer were used.

Procedure for Plasma——To 1 to 2 cc. of the plasma in a 50 cc. glass-stoppered bottle were added 3 cc. of 0.1*N* HCl, 1 cc. of 1*N* NaOH and 30 cc. of hexane, and the mixture was shaken mechanically for 60 min. The bottle was centrifuged at 3000 r.p.m. for 10 min. and 20 cc. of the organic phase was transferred to a 50 cc. distillation flask, evaporated to dryness *in vacuo* at 50°, and the residue was dissolved by adding 5 cc. of 0.1*N* HCl, and measured at 234, 255 and 285 mμ.

Same concentration of HCl was used for the zero setting. An absorbance of about 0.700 was obtained at 234 mμ when 40 μg. of MTQ were carried through the procedure. In the triple point procedure, reagents, and plasma blanks run through the procedure were negligible. In case of the single point procedure, however, the blanks*4 usually gave a reading equivalent to 1～2 μg. of MTQ per cc. of the plasma. MTQ added to the plasma in amounts of 10～40 μg. was recovered with adequate precision (single method : 94±3; triple method : 98±2%) (Table I).

Table I. Recovery of MTQ added to the Plasma

MTQ added	Single point procedure		Triple point procedure	
	MTQ recovered (μg.)	Recovery (%)	MTQ recovered (μg.)	Recovery (%)
1.3[b](10.0)[a]	1.3[b]	100	1.3[b]	100
	1.3	100	1.3	100
	1.2	92	1.3	100
2.0 (15.0)	1.8	90	1.9	95
	1.8	90	1.9	95
	1.8	90	1.9	95
5.3 (40.0)	5.1	96	5.2	98
	5.0	94	5.2	98
	5.1	96	5.2	98

10.0, 15.0, and 40.0 μg. of MTQ were added to 1 cc. of the rat plasma and measured as described in the text.

a) Figures in parentheses show μg. of MTQ added to plasma.

b) Figures show the concentrations of MTQ (μg./cc.) in the final measurement solutions.

Procedure for Urine——To 2～5 cc. of the human urine (centrifuged, if necessary) in a 50 cc. glass-stoppered bottle were added 1 cc. of 1*N* NaOH and 30 cc. of hexane, and the procedure was continued as described above for the estimation of MTQ in the plasma, and the absorbances were determined at 234, 270 and 285 mμ. The drug (10～40 μg.) was recovered from the urine with adequate precision (single method : 97±3; triple method : 99±3%). In case of single point procedure, normal human urine gave a blank reading equivalent to 0.5～1 μg. of the drug per cc.

Procedure for Fat Tissues——To 2 cc. of the homogenate*5 in a 50 cc. glass-stoppered bottle were added 1 cc. of 30% NaOH and 30 cc. of hexane, and the procedure continued as described above, and the measurement was done*6 at 234, 260 and 285 mμ. The drug was recovered from fat homogenate with adequate precision (single method : 101±2; triple method : 100±2%). In the single point procedure, fat blanks were equivalent to 4～8 μg. of the drug per g. of fat tissues.

Procedure for Other Tissues——To 5 cc. of the homogenate*7 in a 50 cc. glass-stoppered bottle were added 1 cc. of 1*N* NaOH and 30 cc. of hexane, and the procedure continued as described above, and the measurement was carried out at 234, 260 and 285 mμ. The drug was recovered from tissues with adequate precision (single method : 102±3; triple method : 99±2%). Normal blanks in single point procedure were equivalent to 3～6 μg. of the drug per g.

Specificity of the Methods——It is important to know the identity of the substance measured, since the inclusion in the measurement of transformation products of MTQ would invalidate the results. The possible interference by metabolites of MTQ was examined by a technique of countercurrent distribution. MTQ was extracted with hexane from the pooled plasma of six rats (300 to 400 g. of body weights), which had each received the drug (200 mg./kg. of body weight) orally and sacrificed 2 hr. later. The countercurrent distribution was performed by means of a mechanized apparatus

*4 Reagent blanks through the procedure, using H_2O instead of the plasma, were negligible.

*5 Fat tissues (1 g.) of the rat was emulsified in 5 cc. of 5*N* HCl to extract MTQ into the aqueous phase, and diluted with H_2O to 10 cc. The emulsion was centrifuged for 10 min., the upper layer (a small amount of oil) was removed, and the clear solution was used for samples.

*6 The solution of final measurement should be filtered through a sintered glass-filter (No. 2), since there is a small amount of insoluble oily residue.

*7 Rat tissues (1 g. liver, kidney and brain) was added to 5 cc. of 0.1*N* HCl and ground to an emulsion in an electrically driven homogenizer, and diluted with H_2O to 10 cc.

involving eight transfers,[5] using equal volume (10 cc.) of hexane and AcONa-HCl buffer (pH 1.55). After countercurrent distribution, the total concentration of apparent MTQ in each tube was determined as follows: MTQ in aqueous phase was transferred to organic phase by adding 0.5 cc. of 30% NaOH to each tube with shaking, and the determination procedure continued as described above, and measurement was done at 234 mμ. Authentic MTQ was also subjected to countercurrent distribution at the same time with the same solvents. The theoretical amounts of the drug that should be present in these tubes and the partition coefficients of authentic and apparent MTQ were calculated by application of the binomial expansion in the manner described by Williamson and Craig.[6] Partition coefficients of the authentic and apparent drug were 0.82 and 0.81, respectively. Theoretical and experimental curves are shown in Fig. 4. Tubes 0, 1, 7 and 8 contained about 15% of the total apparent drug. Theoretically 8% should have been present in these tubes. The non-MTQ in these tubes, therefore, was about 7% of the total MTQ, and about 93% of apparent MTQ in the plasma was shown to be identical with pure MTQ.

Further evidence for the specificity of the methods for the estimation of MTQ in the plasma came from UV spectra of the contents of tubes (Fig. 5). Fig. 5 indicates that UV spectra of the contents of tubes 1 to 6 were similar to that of the standard solution of MTQ.

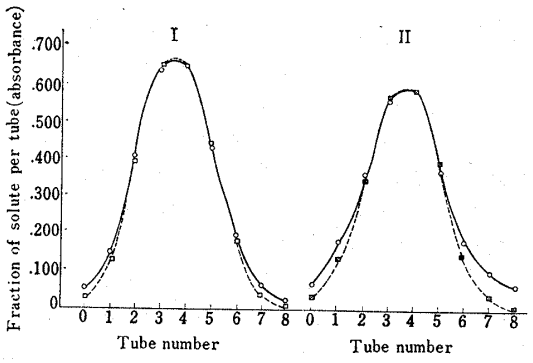

Fig. 4. Patterns of Eight Transfer Distribution
Solvents: hexane and AcONa-HCl buffer (pH 1.55), $t=24\pm1°$
I: MTQ II: apparent MTQ in the plasma
o—o: experimentally found curve
□---□: calculated curve, $K=0.82$
×---×: calculated curve, $K=0.81$

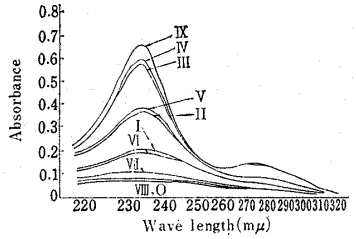

Fig. 5. Ultraviolet Absorption Spectra of the Contents of Tubes after Countercurrent Distribution
0～Ⅷ: contents of tubes (0～8) in 0.1N HCl
Ⅸ: standard soln. of MTQ (5 μg./cc.)

Results and Discussion

Although various estimation methods of MTQ in biological materials may be employed, it was established in this study that ultraviolet spectrophotometry could be applied, with adequate precision and sensitivity, to the estimation of MTQ in biological materials.

MTQ is soluble in ethylene dichloride easily and soluble in hexane, and it was shown that the drug was extracted quantitatively with these solvents from aqueous phase. The absorbances of MTQ were unchanged in 0.01N～1NHCl solution at various wave lengths and the authors used, therefore, 0.1NHCl for the final measurement. MTQ in 0.1NHCl solution possesses absorption maxima at 234 mμ (molar extinction coefficient (ε): 33,000) and at 270 mμ (ε: 7,900), and measurements are, therefore, made at 234 mμ, because the small amounts of MTQ in biological materials can not be estimated at 270 mμ with adequate precision and sensitivity. As shown in Table II, MTQ was not quantitatively recovered with hydrochloric acid from ethylene dichloride solution, but from hexane solution. When hexane used for extraction solvent, however,

5) L.C. Craig, *et al.*: J. Biol. Chem., **161**, 321 (1945).
6) B. Williamson, L.C. Craig: J. Biol. Chem. **168**, 687 (1947).

the lower reproducibility of the estimation will be obtained owing to the considerable reagent blanks at 234 mμ, if the drug returned to hydrochloric acid solution. The organic phases were, therefore, evaporated to dryness *in vacuo* and the residue was dissolved in 0.1*N*HCl for the final measurement, so that reagent blanks were negligible and the drug was recovered quantitatively through the procedure.

Table II. Recovery of MTQ dissolved in Organic Solvents with Hydrochloric Acids (solvents : HCl=2:1)

Concn. of HCl (*N*)	Recovery	
	Ethylene dichloride soln. (%)	Hexane soln. (%)
0.1	3	30
1	5	100
3	35	
5	51	

The adsorption of MTQ from organic solvents, by glass surfaces, would be negligible and the addition of an alcohol[7] (e.g., *iso*-amyl alcohol[4,8]) to the extraction solvents would not be necessary.

There are normal blanks in the estimation of drugs in biological materials and these have been removed with various techniques in ultraviolet spectrophotometry, that is, using the absorbance difference[9] at two pH values, and estimation[4] at two wave lengths over 300 mμ, and *etc.* The authors could not, however, estimate MTQ successfully by means of the two techniques described above. Consequently, the authors employed the single point procedure (generally used) and devised the triple point procedure.

Generally, normal blanks are variable in tissue samples, especially in the liver,[4] and show high values occasionally. To such a case the triple point procedure may be applied successfully. Comparing the single point procedure with the triple one, the values obtained from the absorbances of MTQ standard solutions involving 1, 2, 3, and 5 μg./cc. agreed each other, and it seems to be unnecessary that we should consider estimation error occurring in the measurement at three wave lengths. Comparing with the both procedures, the values obtained did not show significant differences in the urine and plasma. For tissue samples, however, the triple method did show better results. Employing the triple method, the authors sometimes found incidental errors occurred in the single method.

Table III. Normal Tissue Blanks equivalent to Amounts of MTQ in Single Point Procedure

Material	Hexane extraction method (μg./cc. or g.)	Ethylene dichloride extraction method (μg./cc. or g.)
Human urine	0.1～1	1～ 2
Plasma	1～2	2～ 5
Fat tissues	4～8	4～10
Other tissues	3～6	6～30

The data in Table III indicate that the hexane extraction procedure was superior to the ethylene dichloride, and the triple point procedure was better for the hexane procedure.

As shown in Fig. 3 and Table I, the sensitivity and precision of the both procedures are adequate for the amounts of the drug from 1 to 5 μg./cc. of final measure-

7) B.B. Brodie, *et al.* : J. Biol. Chem., **168**, 299 (1947).
8) *Idem* : *Ibid.*, **168**, 311 (1947).
9) L.R. Goldbaum : Anal. Chem., **24**, 1604 (1952).

ment solution. The detailed report concerning to the triple point procedures will be published in near future.

The extent of interference by metabolic products of the drug was assayed by a countercurrent distribution technique (Fig. 4) and ultraviolet absorption spectrum (Fig. 5), and the results indicated that the material was at least 93% pure.

The authors wish to thank Eisai Co., Ltd., for their supply of 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone (Hyminal).

Summary

Two methods are described for the estimation of 2-methyl-3-*o*-tolyl-4(3*H*)-quinazolinone (MTQ) in biological fluids and tissues. In the first, MTQ is extracted from alkalinized biological material with hexane, the solvent is evaporated, and the residue is dissolved in dilute hydrochloric acid, and measured spectrophotometrically at 234 mμ.

The second, suitable for tissues, is the method in which normal biological blank becomes negligible by geometrical treatment of its absorbances at three wave lengths.

These procedures permit the determination of MTQ in amounts as 1 μg./cc. of final measurement solution. The methods are specific for MTQ in that they do not include any transformation products of the compound.

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14. Yasuo Makisumi and Hideo Kanō : Studies on the Azaindolizine Compounds. XII.*2 Alkyl Rearrangement of 5-Methyl-7-alkoxy-*s*-triazolo[1,5-*a*]pyrimidines.

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It has been known that the lactim ethers of configuration I will undergo rearrangement to their isomeric and stable lactam form II. These transformations are irreversible and can be brought about by the application of heat or through the influence of special catalytic agents, and have been observed to take place in both acyclic and cyclic compounds.

I II

This interesting rearrangement has been illustrated by Hilbert and Johnson[1] in the pyrimidine series and this study has been extended by Chi and co-workers.[2] So, a similar rearrangement in the *s*-triazolo[1,5-*a*]pyrimidine series was investigated.

The present paper deals with the synthesis, property, and alkyl rearrangment of 5-methyl-7-alkoxy-*s*-triazolo[1,5-*a*]pyrimidines.

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*1 Fukushima-ku, Osaka (牧角徳夫, 加納日出夫).

*2 Part XI : This Bulletin, **10**, 620 (1962).

1) G. E. Hilbert, T. B. Johnson : J. Am. Chem. Soc., **52**, 2001 (1930).

2) Y. F. Chi, C. Wei, N. S. Pan : J. Am. Chem. Soc., **60**, 1719 (1938).