[Chem. Pharm. Bull.
29(6)1755—1758(1981)]

Analytical Studies on Pyrimidine Derivatives. VI.[1] Sensitive Spectrophotometric Determination of Gold(III) by Solvent Extraction with 5-*p*-Dimethylaminocinnamylidene-1-phenyl-2-thiobarbituric Acid[2]

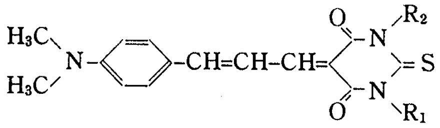
KENICHIRO NAKASHIMA* and SHUZO AKIYAMA

*Faculty of Pharmaceutical Sciences, Nagasaki University,
Bunkyo-machi 1-14, Nagasaki, 852, Japan*

(Received December 11, 1980)

A sensitive spectrophotometric method for the determination of gold(III) with 5-*p*-dimethylaminocinnamylidene-1-phenyl-2-thiobarbituric acid (DACTB) is described. DACTB reacts easily with gold(III), forming a blue complex in diluted hydrochloric acid which can be extracted with chloroform. The chloroform solution of the complex has an absorption maximum at 622 nm against a reagent blank and shows a constant absorbance over the final hydrochloric acid concentration range of 0.02—0.07 M. Beer's law holds over the range of 0.1—0.9 μg/ml of gold(III) at 622 nm. The molar extinction coefficient of the complex is $1.1 \times 10^5 \cdot l \cdot mol^{-1} \cdot cm^{-1}$. The coefficient of variation was 1.34% for 0.5 μg/ml of gold(III). The coexistence of cations such as Pd(II), Fe(II), Ce(IV), and Mn(VII) interfered considerably with the determination.

Keywords——5-*p*-dimethylaminocinnamylidene-1-phenyl-2-thiobarbituric acid; solvent extraction; gold(III); spectrophotometric determination; sensitive method



Ia: $R_1=R_2=H$
Ib: $R_1=CH_3$, $R_2=H$
Ic: $R_1=R_2=CH_3$
Id: $R_1=C_6H_5$, $R_2=H$

Chart 1. 5-*p*-Dimethylaminocinnamylidene-2-thiobarbituric Acid Derivatives

In the previous paper,[1] the use of 5-*p*-dimethylaminobenzylidene-2-thiobarbituric acid (DABTB) for the spectrophotometric determination of silver (I) was described. We have synthesized DABTB analogs, 5-*p*-dimethylaminocinnamylidene-2-thiobarbituric acid derivatives (Chart 1), as chromogenic reagents for gold (III). Among these derivatives, 5-*p*-dimethyl-aminocinnamylidene-1-phenyl-2-thiobarbituric acid (Id), (DACTB), readily reacts with gold (III), forming a blue complex which can be extracted with chloroform, and gives a high sensitivity. Thus, a sensitive method for the determination of the Au(III) ion with DACTB has been established.

Experimental

Apparatus——Absorbances at various wavelengths were recorded with a Hitachi 323 recording spectrophotometer. Absorbances at fixed wavelengths were measured with a Shimadzu 150 double beam spectrophotometer. Matched glass cells of 10 mm path length were used. An Iwaki KM shaker was used to shake glass-stoppered test tubes.

Synthesis of 5-*p*-Dimethylaminocinnamylidene-2-thiobarbituric Acid Derivatives——5-*p*-Dimethylaminocinnamylidene-1-phenyl-2-thiobarbituric Acid (Id): 1-Phenyl-2-thiobarbituric acid (1 g) was dissolved in 50% aqueous ethanol and refluxed with 0.8 g of *p*-dimethylaminocinnamaldehyde dissolved in 30 ml of ethanol. After refluxing for 20 minutes, the resulting violet precipitates were filtered off and recrystallized from ethanol to give violet prisms with a metallic luster.

The other derivatives (Ia, Ib, and Ic in Chart 1) were prepared in the same manner as Id by using the corresponding 2-thiobarbituric acid derivatives in place of 1-phenyl-2-thiobarbituric acid. The elemental analytical data of these compounds and their yields in the syntheses are summarized in Table I.

Reagents——DACTB (Id) Solution (1.32×10^{-4} M): Dimethylformamide (DMF) solution was prepared. This solution should be used within 5 days.

Gold(III) Standard Solution: A gold standard solution (1 mg/ml, $f=1.00$, Wako) was diluted when necessary.

TABLE I. Elemental Analyses of 5-*p*-Dimethylaminocinnamylidene-2-thiobarbituric Acid Derivatives and Their Yields

Compd. No.	mp (°C) (dec.)	Yield (%)	Formula	Analysis (%) Calcd. (Found) C	H	N	S
Ia	264—265	90	$C_{15}H_{15}N_3O_2S$	59.79 (59.89	5.02 5.05	13.95 14.05	10.64 10.67)
Ib	259—260	83	$C_{16}H_{17}N_3O_2S$	60.94 (60.87	5.43 5.36	13.33 13.32	10.17 10.17)
Ic	248—249	78	$C_{17}H_{19}N_3O_2S$	61.99 (61.90	5.82 5.90	12.76 12.79	9.74 9.76)
Id	257	76	$C_{21}H_{19}N_3O_2S$	66.83 (66.87	5.07 5.22	11.14 11.06	8.49 8.45)

All other reagents used were of reagent grade.

Recommended Procedure——An aqueous test solution containing 1—9 μg of gold(III) is pipetted into a 50 ml glass-stoppered test tube and to this is added 1 ml of 0.05 M hydrochloric acid. The solution is diluted with water to 10 ml and then 0.75 ml of DACTB solution is added. The mixture is allowed to stand for 2 min, then shaken with 10 ml of chloroform for 1 min using a shaker. The separated organic phase is filtered and the absorbance is measured at 622 nm against the reagent blank.

Results and Discussion

Synthesis of 5-p-Dimethylaminocinnamylidene-2-thiobarbituric Acid Derivatives and Their Reactions with Gold(III)

5-*p*-Dimethylaminocinnamylidene-2-thiobarbituric acid derivatives were prepared by condensation of 2-thiobarbituric acid derivatives with *p*-dimethylaminocinnamaldehyde in a manner similar to that used for DABTB.[1] All these compounds were easily obtained in good yields. Among these compounds, Ia, Ib, and Ic reacted with gold(III), forming blue complexs in diluted hydrochloric acid solution which could be extracted with chloroform and/or benzene. The absorption maxima of the extracts in chloroform and/or benzene are shown in Table II. The chloroform extract of the gold(III)-Id(DACTB) complex showed the largest molar extinction coefficient. This suggests that an -NH-CS- functional group is required for complex formation with the ion.

TABLE II. Absroption Maxima of Au(III) Complexes of 5-*p*-Dimethylaminocinnamylidene-2-thiobarbituric Acid Derivatives

Comp. No.	λmax, nm Chloroform	Benzene
Ia	619 (2.1×10^4)	------
Ib	618 (8.3×10^4)	602 (7.9×10^3)
Ic	------	------
Id	622 (1.1×10^5)	605 (1.4×10^4)

Numbers in parentheses are apparent molar extinction coefficients.

Absorption Spectrum of the Complex

The absorption spectrum of the DACTB complex in chloroform has an absorption maximum at 622 nm when measured against the reagent blank, and at 600 nm against chloroform (Fig. 1, I and II). The reagent blank has a maximum at 580 nm. The absorption maximum measured against chloroform shows a red shift with increasing concentration of gold(III) and finally shifts to 622 nm. As the absorbance of the reagent blank at 622 nm was relatively large, the absorbance of the complex should be measured at 622 nm against the reagent blank, not against chloroform.

Effect of Concentration of Hydrochloric Acid

Final concentrations of the acid in the range of 0.02—0.07 M give the maximum absorbance.

Effect of Concentration of DACTB

The DACTB concentrations from 1×10^{-6} to 1.6×10^{-5} M were examined. The concentration of DACTB should be more than twice that of gold(III) to obtain the maximum absorbance.

Effect of Time on Complex Formation and Shaking Time for Extraction

The complex formation is completed within a few minutes, and the resulting complex can be extracted with chloroform by shaking for at least 1 min.

Stability of Color Developed

The change of absorbance of the complex in chloroform was recorded at 622 nm. The absorbance did not change for at least 24 hr after the extraction.

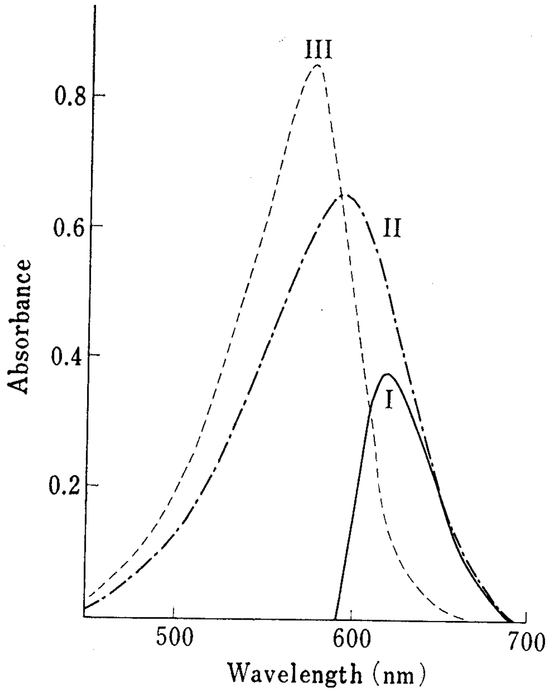


Fig. 1. Absorption Spectra of Au(III)–DACTB Complex

I: Au(III)–DACTB against reagent blank.
II: Au(III)- DACTB against chloroform.
III: Reagent blank against chloroform.
Au(III): 7 μg; DACTB: 9.9×10^{-6} M.

TABLE III. Effects of Various Inos on the Determination of Au(III)

Ions	Added (μg)	Added as	Au(III) Found (μg)
Ag(I)	100	$AgNO_3$	4.9
	200		4.7
Pd(II)	5	$PdCl_2$	5.8
	3		5.2
Hg(II)	50	$HgCl_2$	4.8
Fe(II)	10	$FeSO_4(NH_4)_2SO_4\cdot6H_2O$	0.0
Ca(II)	1000	$CaCl_2$	5.1
Cu(II)	2000	$CuSO_4\cdot5H_2O$	4.9
Mg(II)	1000	$MgSO_4\cdot7H_2O$	5.0
Ni(II)	1000	$NiSO_4\cdot7H_2O$	5.0
Co(II)	1000	$Co(NO_3)_2\cdot6H_2O$	5.0
Zn(II)	1000	$ZnCl_2$	5.0
Pd(II)	1000	$Pd(CH_3COO)_2\cdot3H_2O$	5.1
Fe(III)	20	$Fe(NO_3)_3\cdot9H_2O$	4.7
Al(III)	1000	$AlCl_3\cdot6H_2O$	5.0
Bi(III)	500	$Bi(NO_3)_3\cdot5H_2O$	5.1
Ce(IV)	10	$Ce(SO_4)_2$	0.0
Pt(IV)	50	H_2PtCl_6	5.2
Cr(VI)	50	$K_2Cr_2O_7$	4.8
Mn(VII)	10	$KMnO_4$	0.0

Au(III) taken; 5.0 μg/10 ml.

Calibration Curve

Conformity to Beer's law over a range of 0.1—0.9 μg/ml of gold(III) was obtained by using the procedure recommended. The coefficient of variation was 1.34% (n=6) for 0.5 μg/ml of gold (III). The molar extinction coefficient of the complex at 622 nm was estimated to be $1.1 \times 10^5 \cdot l \cdot mol^{-1} \cdot cm^{-1}$.

Composition of the Gold(III)-DACTB Complex

This was examined by the molar ratio and the continuous variation methods. The ratio of gold(III) to DACTB in the complex was found to be 1: 2.

Effect of Other Ions

As shown in Table III, Fe(II), Mn(VII), Ce(IV), and Pd(II) interfered considerably with the determination. Therefore, these ions should be removed before this method is practically applied. This may be carried out by selective extraction of gold (III) with ethyl acetate or ethyl ether.[3d,4]

Sensitivity of the Method

The sensitivity of this method is higher than those of the *p*-dimethylaminobenzylidenerhodanine,[3] *o*-tolidine,[5] bromoaurate,[6] tin(IV)-chloride,[7] tetron,[8] and 2-pyridyl-2-thienyl-Z-ketoxime[9] methods. It is comparable to that of rhodamine B method[10] and lower than that of the thio-Michler's ketone method.[11] This method may be applicable for the microdetermination of gold(III) in biological materials from subjects given auric preparations.

References and Notes

1) Part V: K. Nakashima and S. Akiyama, *Chem. Pharm. Bull.*, **28**, 2518 (1980).
2) A part of this work was presented at the 29th Annual Meeting of the Japan Society for Analytical Chemistry, Fukuoka, October 1980, p. 512.
3) *a*) E.B. Sandell, *Anal. Chem.*, **20**, 253 (1948); *b*) T.M. Cotton and A.A. Woolf, *Anal. Chim. Acta*, **22**, 192 (1960); *c*) S. Hara, *Bunseki Kagaku*, **7**, 147 (1958); *d*) S. Hirano, A. Mizuike, and K. Yamada, *Bunseki Kagaku*, **9**, 164 (1960); *e*) R. Borissova, *Talanta*, **22**, 797 (1975).
4) Ō. Kammori and K. Kubota, *Bunseki Kagaku*, **15**, 171 (1966).
5) E.B. Sandell, "Colorimetric Determination of Traces of Metals," 3rd ed., Interscience, New York, 1959, p. 494.
6) W.A.E. McBryde and J.H. Yoe, *Anal. Chem.*, **20**, 1094 (1948).
7) C.G. Fink and G.L. Putnam, *Ind. Eng. Chem. Anal. Ed.*, **14**, 468 (1942).
8) N. Jordanov and C. Daiev, *Talanta*, **10**, 163 (1963); *idem, ibid.*, **11**, 501 (1964).
9) P.W. Beaupré and W.J. Holland, *Microchim. Acta* (Wien), **1979**, II, 433.
10) B.J. MacNulty and L.D. Woollard, *Anal. Chim. Acta*, **13**, 154 (1955); H. Onishi, *Microchim. Acta*, **1**, 9 (1959).
11) K.L. Cheng and B.L. Goidish, *Microchem. J.*, **10**, 158 (1966).

Chem. Pharm. Bull.
29(6)1758—1762(1981)

Mutagenicity of Amide Type and Carbamate Type Diazoalkane-generating Agents in *Salmonella*/Microsome Assay

TAMOTSU MORITA*, MASAO SAWAKI and ICHIJI MIFUCHI

Shizuoka College of Pharmacy, 2-2-1 Oshika, Shizuoka, 422, Japan

(Received October 29, 1980)

The mutagenicity of several N-nitroso compounds, $RCONHCH_2N(NO)R'$, which were reported as new diazoalkane-generating agents, was assayed by the *Salmonella*/microsome