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OXIDATION OF THIOL WITH 5-ARYLIDENE-1,3-DIMETHYLBARBITURIC ACID: APPLICATION TO SYNTHESIS OF UNSYMMETRICAL DISULFIDE[1]

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Abstract ---- 5-Arylidene-1,3-dimethylbarbituric acid derivatives, such as **1a** and **1b**, effectively oxidized both alkane- and benzene-thiols to disulfides under neutral condition with concomitant formation of the dihydro compounds (**2a**) and (**2b**).

Thiol adduct of the dihydro compound was prepared as a stable compound and successfully applied to the synthesis of unsymmetrical disulfide under mild condition in excellent yield.

Mechanistic consideration for the oxidation was also described briefly.

Introduction

Formation and cleavage of disulfide bond are essentially concerned not only in acyl group transfer in energy metabolism but also in the secondary and tertiary structures of polypeptide and protein in connection with physiological function in organisms. It is well known that flavin adenine dinucleotide (FAD) plays a leading role in these enzymatic redox transformations.

There have been many reported methods of oxidation of thiols into disulfides.[2] Of these, flavin (isoalloxazine) and analogs are characteristic oxidants toward thiols under certain conditions.[3-6] In the course of our investigation for development of more efficient, selective and simpler mimic compounds having redox potentials, we have prepared and used 5-arylidene-1,3-dimethylbarbituric acid derivatives for the oxidation of alcohols.[7] It has been found that some of these model compounds of coenzymes oxidize allylic and benzylic alcohols to the corresponding carbonyl compounds effectively and selectively under neutral condition in organic solvent.

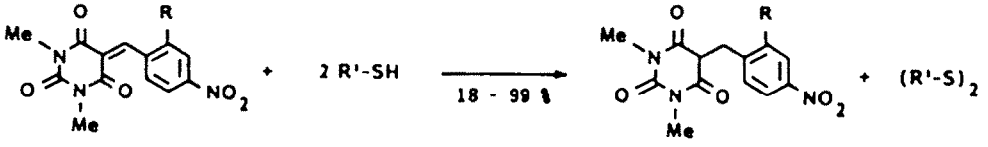
This article is dedicated to Professor E.C.Taylor on his sixty-fifth birthday.

In this paper, we now describe the oxidation of thiols with 5-arylidene-barbituric acid derivatives and its use in the synthesis of unsymmetrical disulfides.[1] In addition, mechanistic consideration of the oxidation will be described briefly.

5-Arylidene-1,3-dimethylbarbituric acid preserves inherent electron-deficient double bond surrounded by carbonyl groups found in some redox coenzymes. Of these derivatives, especially the compounds substituted by nitro group on the benzene ring have potential oxidation ability toward alcohols.[7] Therefore, we selected these compounds, **1a** and **1b**, for the oxidation of thiols.

Results and Discussion

The oxidation was carried out in a sealed tube because of the volatility of the starting thiol and for keeping the reaction safe from air-oxidation. Using the compound (**1a**) and appropriate thiol in the molar ratio of 1 : 2, the



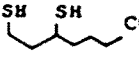
1a : R = NO_2
1b : R = H

2a : R = NO_2
2b : R = H

Scheme 1. Oxidation of Thiol

oxidation was undertaken in dioxane at 120 - 150° for two days (Scheme 1). The amount of disulfide produced was determined by gas-liquid chromatography and the disulfide was isolated together with the corresponding dihydro compound.

Table I. Oxidation[a] of Thiols with Compound (1a)

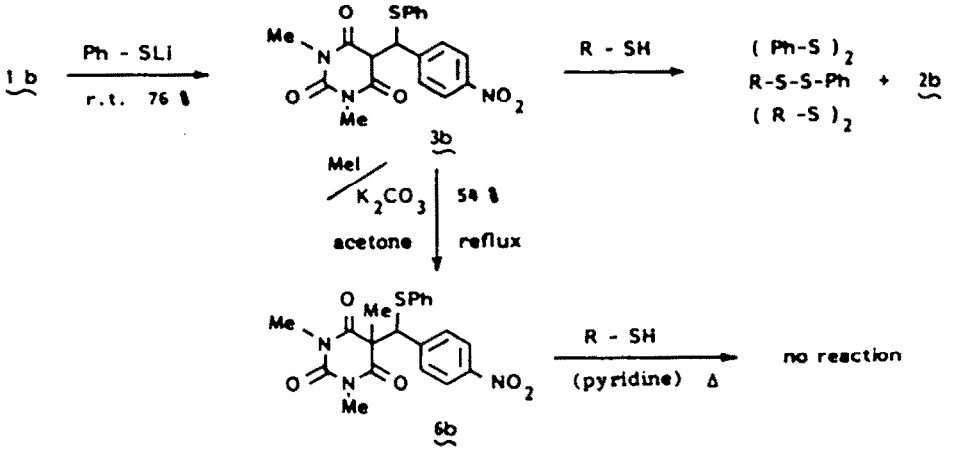
Thiol & Additive	Isolated Yield (%) of Disulfide
Ph-SH	43 (70)[b]
$PhCH_2$-SH	31
n-Bu-SH	39
t-Bu-SH	18
	97
n-BU-SH + Et_3N	88
Ph-SH + Ph_3P	99

a) In every run, 0.1 - 10 % yield of disulfide was observed when the oxidation was performed without 1a.
b) Oxidation was carried out with refluxing in dioxane.

5-(2',4'-dinitrobenzyl)-1,3-dimethylbarbituric acid (**2a**), and then identified. These results are listed in Table I. Contrary to the reported fact that alkanethiols are slowly oxidized and benzenethiol is not oxidized by flavins under ambient conditions, it is worthy to note that in our procedure benzenethiol is much more easily oxidized than alkanethiols. In addition, if triethylamine (0.1 eq) or triphenylphosphine (0.1-1.0 eq) was added into the reaction mixture, both the rate and yield of the reaction were increased to a great extent. Thus, even p-nitrobenzylidene derivative (**1b**) displayed a strong oxidizing ability toward benzenethiol in the presence of triphenylphosphine to give the corresponding disulfide in 88 % yield, whereas the disulfide was obtained in only 5 % yield without the additive.

Next, we tried to deduce a possible mechanism of the oxidation. First we examined the oxidation in the presence of free radical, galvinoxyl, and found no influence upon both the rate and yield of the reaction. So ,we left the radical mechanism out of consideration. In the ionic mechanism , there might be two possibilities, one of them is the mechanism involving thiol addition on α-benzyl site of 5-arylidene-1,3-dimethylbarbituric acid similar to that of flavin,[8] and the other is hydride shift as observed in the oxidation of alcohols reported previously.[7]

In order to clarify the mechanism, we first synthesized compound (**3b**) as a tentatively proposed intermediate in a stable crystalline form, and let it



Scheme 2.

react with a second thiol under the same conditions described above. The products thus obtained were a mixture of three kinds of disulfides. This might come from elimination of thiol group from the adduct under the conditions employed. Consequently, the compound (**6b**), an analogue of **3b** except for possessing methyl group at 5 position of barbituric acid, was synthesized to

escape from the equilibrium. However, the compound (**6b**) was too stable to react with any thiols under the same conditions remaining intact (Scheme 2). From these results, it seems unlikely that addition of the thiol at α-benzyl position of 5-arylidene-barbituric acid derivatives is crucial step in oxidation.

On the other hand, the observed facts that the reaction is accelerated by the addition of triphenylphosphine and triethylamine and that oxidation of dihydro lipoic ester in intramolecular fashion is very facile process, suggest the transition state involving hydride transfer in each case as described in Fig. 2. One of the thiol groups of dihydro lipoic ester might act as intramolecular

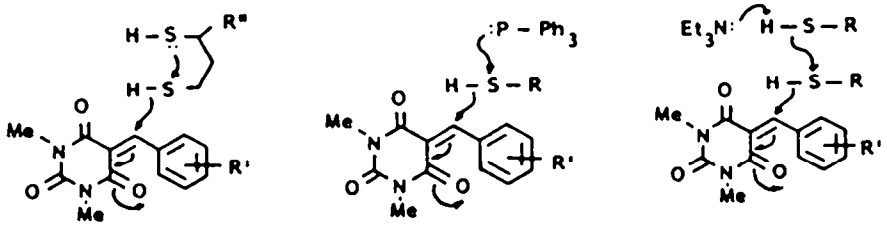


Fig. 2

nucleophilic catalysis and the lone pair electrons on the sulfur and phosphorus atom participate to drive the hydride transfer as intermolecular general base catalysis.

Therefore it may be concluded that the mechanism involving hydride transfer in the transition state appears to be reasonable in the oxidation of thiols as well as alcohols with 5-arylidene-1,3-dimethylbarbituric acid.[7]

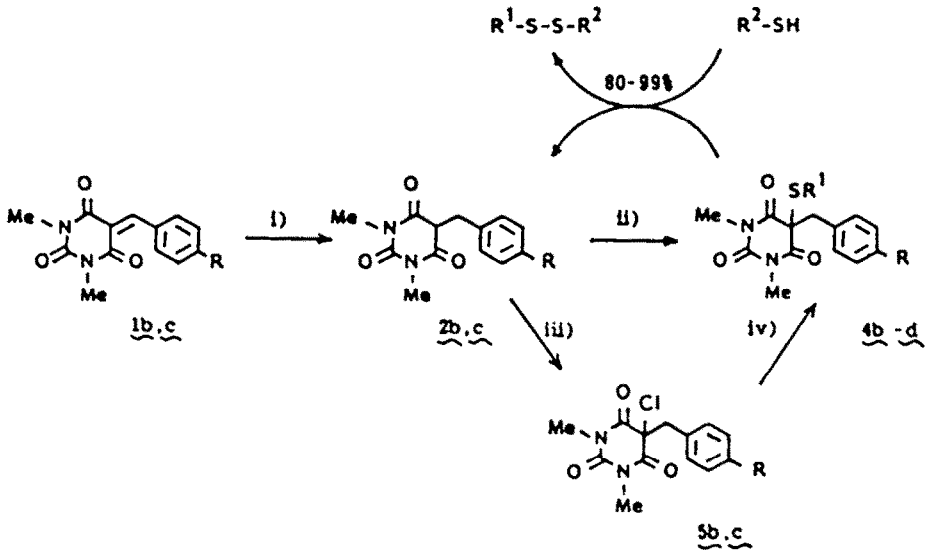
Cross coupling reaction of the two different thiols belongs to one of the basic and important synthetic transformation. Despite much effort toward this subject,[2] there seems to remain room for further improvement. As described by Bruice in 1975,[8] the postulated inseparable intermediates, such as N(5)-adduct and C(4a)-adduct, are involved in the reduction of flavin by thiol(Fig.1),

N(5)-adduct C(4a)-adduct

Fig. 1

therefore it is expected that if this type of thiol adduct intermediate could be isolated, subsequent attack of a second thiol on the adduct will result in the formation of an unsymmetrical disulfide.

Thus, addition of lithium mercaptide to the compound (**1b**) afforded the



b : R = NO_2, R^1 = Ph, c : R = OMe, R^1 = Ph, d : R = OMe, R^1 = n-Bu

i) $NaBH_4$, MeOH , r.temp., ~100% ; ii) R^1SCl , CH_2Cl_2 , r.temp., ~100% ;
iii) NCS , CH_2Cl_2 ,r.temp., ~100% ; iv) R^1SH , Et_3N , CH_2Cl_2 , r.temp., 70-90% .

Scheme 3. Oxidation of Thiol and Synthesis of Unsymmetrical Disulfide

adduct (**3b**), which corresponds to the N(5)-adduct of flavin in good yield as a stable crystalline compound. As mentioned above, however, the reaction of this adduct (**3b**) with a second different thiol gave a mixture of three kinds of disulfides unselectively. A retro-Michael type reaction of the adduct under the condition employed may cause this nondiscrimination (vide supra). Namely, the regenerated compound (**1b**) oxidizes the two kinds of liberated thiol existing in the media unselectively to afford a mixture of disulfides.

We next tried to synthesize another type of compound (**4**) corresponding to the C(4a)-adduct of flavin. The dihydro compound (**2**) formed in the thiol oxidation was also derived from **1** quantitatively by reduction with sodium borohydride (Scheme 3).

Transformation of the dihydro compound (**2**) into the thiol adduct (**4**) was successfully achieved by two ways, direct and indirect methods. Treatment with alkyl or aryl sulfenyl chloride gave the adduct (**4**) directly in good yield, while chlorination with N-chlorosuccinimide furnished the chloride (**5**), which was then converted into the thiol adduct (**4**) by substitution with a thiol in the presence of triethylamine in 70 - 80 % overall yield. The thiol adduct in hand was reacted with a second different thiol in the presence of weak base (triethylamine or pyridine) at room temperature to give desired unsymmetrical disulfide in high yield (80 - 99%) and with high selectivity. These results are summarized in Table II. In this reaction, the regenerated crystalline

Table II. Isolated Yields of Unsymmetrical Disulfides

[a,b,c] R	OMe						NO_2		
Thiol: R^1	*n*-Bu			Ph			Ph		
Thiol: R^2	*p*-ClC_6H_4	$PhCH_2$	Ph	*p*-Cl-C_6H_4	$PhCH_2$	*n*-Bu	*p*-ClC_6H_4	$PhCH_2$	*n*-Bu
R^1-S-S-R^2 (%)	91	97	99	89	94	80	86	80	89*
$(R^1\text{-S})_2$* (%)	0	0	0	3	5	6	3	0	6
$(R^2\text{-S})_2$* (%)	0	1	0	1	0	0	10	0	3

*) determined by g.l.c.

dihydro compound (**2**) can be easily isolated and repeatedly used for the next cycle.

Concluding Remarks

In summary, it is noted that 5-arylidene-1,3-dimethylbarbituric acid fulfills not only nicotinamide adenine dinucleotide (NAD)-like function of oxidation of alcohol but also FAD-like function of thiol oxidation as described here.

Furthermore, it is worthy to note that cross coupling reaction of thiols proceeds conveniently and selectively under mild conditions giving unsymmetrical disulfides in excellent yields. We consider the present study on oxidation of thiols possesses a synthetic utility as well as some suggestive information about the enzymatic redox reaction mechanism.

Experimental

Melting points were determined on a Yanagimoto melting point apparatus and are uncorrected. Infrared spectra (IR) were recorded on a Shimadzu IR-400 spectrophotometer. The proton nuclear magnetic resonance (^{1}H-NMR) spectra were obtained in chloroform-d at 200 MHz on a JEOL FX 200 instrument with chemical shifts being reported in δ units from tetramethylsilane as an internal standard and couplings in hertz. Mass spectra (MS) were taken in a JEOL JMS 01SG-2 instrument by direct insertion at 75 eV. Gas-liquid chromatography (glc) was taken on Shimadzu GC-7AG with capillary column (FQ type, OV-101, 25m x 0.2mm). Preparative thin-layer chromatography (p-TLC) was run on 20 x 20cm plates coated with a 0.1-1.5mm layer of Merck silica gel PF_{254} and/or GF_{254}

General method for oxidation of thiol

A mixture of compound (1) (1.0 mM), thiol (2.0 mM) and dioxane (8 ml) was heated at 120-150° in a sealed tube (ϕ10 × 300 mm) for 2 days. Quantitative analysis was carried out by gas-liquid chromatography using authentic standard of disulfide. After concentration of the reaction mixture, followed by addition of methanol, dihydro compound (2) was separated in crystalline form and isolation of the disulfide was undertaken by p-TLC of the mother liquor with n-hexane.

1,3-Dimethyl-5-(4'-nitrophenyl)phenylthiomethylbarbituric acid (**3b**).

A solution of lithium phenylmercaptide in benzene (1.54 M solution, 31 ml), prepared from n-butyl lithium and benzenethiol in benzene at 25° for 2 hr, was added dropwise to a stirred solution of **1b** (4.0 g, 14 mM) in 50 ml of tetrahydrofuran. The mixture was stirred for 3 hr at 25° under argon. Aqueous solution (5%) of hydrochloric acid (50 ml) was added to the mixture, which was extracted with chloroform. The chloroform layer was dried over $MgSO_4$ and concentrated under reduced pressure to leave the residue which was crystallized from benzene (5.52g, 76%, prisms), mp 125-128°. IR ν_{max} cm^{-1}: 1685, 1520, 1380, 1350. ^1H-NMR δ: 2.19(3H,s), 2.22(3H,s), 4.09(1H,d,J=3), 4.24(1H,d,J=3), 7.25(5H, m), 7.62(2H,d,J=9), 8.13(2H,d,J=9). (Found: C 57.42, H 4.22, N 10.47. Calc for $C_{19}H_{17}N_3O_5S$: C 57.13, H 4.29, N 10.52 %).

1,3,5-Trimethyl-5-(4'-nitrophenyl)phenylthiomethylbarbituric acid (**4b**).

A mixture of compound (**3b**)(610 mg), methyl iodide (3 ml), potassium carbonate (700 mg) and 50 ml of acetone was refluxed for 1 hr. The mixture was poured into water (100 ml) and extracted with chloroform, and the layer of chloroform was concentrated to give an oily residue, which was purified by short column chromatography on silica gel (hexane : chloroform = 1 : 1)to give **4b** (340 mg, 54%), mp 79-82° (n-hexane, powder), IR ν_{max} cm^{-1}: 1685, 1520, 1380, 1348, 1290. ^1H-NMR δ:1.68(3H,s), 3.20(3H,s), 3.27(3H,s), 4.76(1H,s), 7.21(5H,s), 7.49(2H,d, J=9), 8.11(2H,d,J=9). MS (Found: C 58.10, H 4.63, N 10.16. Calc for $C_{20}H_{19}N_3O_5S$: C 58.28, H 5.03, N 9.79 %). m/s: 413 (M^+), 244.

1,3-Dimethyl-5-(4'-methoxybenzyl)barbituric acid (**2c**).

Sodium borohydride (1.0 g, 26 mM) was added portionwise to a stirred solution of **1c** (4.0 g, 15 mM) in methanol (50 ml) and the mixture was stirred for 10 min at room temperature. Water (100 ml) was added to the mixture and the mixture was washed with chloroform. After acidification of the aqueous layer with 5 % hydrochloric acid, the layer was extracted with chloroform. The chloroform extract was dried over $MgSO_4$ and then concentrated to give crystalline residue, which was recrystallized from methanol. The yield was 4.0 g (99 %). mp 90-92° (MeOH, prisms). IR ν_{max} cm^{-1}: 1695, 1510, 1440, 1380. ^1H-NMR δ: 3.14(6H,s), 3.41(2H,d,J=5), 3.72(1H,t,J=5), 3.76(3H,s), 6.75(2H,d,J=9), 6.94(2H,d,J=9). (Found: C 60.74, H 5.84, N 10.14. Calc for $C_{14}H_{16}N_2O_4$: C 60.86, H 5.84, N 10.14 %).

1,3-Dimethyl-5-(4'-nitrobenzyl)-5-phenylthiobarbituric acid (**4b**).

Phenylsulfenyl chloride (3.0 g, 21 mM) was added to a solution of **2b** (4.0 g, 14 mM) in 40 ml of dichloromethane at 25° and the mixture was stirred for 12 hr at the same temperature. Concentration of the mixture gave a residue which was then crystallized from a mixture of benzene and ether to furnish 3.0 g of **4b**. Concentration of the mother liquor gave another crop of crystal (1.0 g). The yield is 4.0 g (73 %). A further crop of the product (**4b**) was obtained by column chromatography of the mother liquor on silica gel. mp 217-218° (benzene and ether, plates). IR ν_{max}^{KBr} cm^{-1}: 1680, 1520, 1375, 1345. ^{1}H-NMR δ: 2.99(6H,s), 3.75(2H,s), 7.42(7H,m), 8.06(2H,d,J=9). (Found: C 56.96, H 4.11, N 10.36. Calc for $C_{19}H_{17}N_3O_5S$: C 57.13, H 4.29, N 10.52 %).

5-Chloro-1,3-dimethyl-5-(4'-methoxybenzyl)barbituric acid (**5c**).

A mixture of **2c** (4.0 g,14.5 mM), N-chlorosuccinimide(2.5 g,18.6 mM) and dichloromethane (80 ml) was stirred for 4 hr at room temperature and then the mixture was washed with water (50 ml×3). The layer of dichloromethane was dried over $MgSO_4$ and concentrated under reduced pressure. The residue was crystallized from MeOH to afford 4.5 g (99 %) of **5c**. mp 78-79° (MeOH, prisms). IR ν_{max}^{KBr} cm^{-1}: 1695, 1510,1440, 1380. ^{1}H-NMR δ: 3.20(6H,s), 3.68(2H,s), 3.77(3H,s), 6.75(2H,d, J=9), 7.00(2H,d,J=9). (Found: C 54.02, H 4.77, N 9.06. Calc for $C_{14}H_{15}N_2O_4Cl$: C 54.11, H 4.87, N 9.02 %).

5-Chloro-1,3-dimethyl-5-(4'-nitrobenzyl)barbituric acid (**5b**).

This compound was synthesized in the same manner as that of **5c** in 99% yield. mp 171-172° (MeOH, needles). IR ν_{max}^{KBr} cm^{-1}: 1695, 1510, 1440, 1380. ^{1}H-NMR δ: 3.27(6H, s), 3.89(2H,s), 7.40(2H,d,J=9), 8.10(2H,d,J=9). (Found: C 47.75, H 3.50, N 12. 82.Calc For $C_{13}H_{12}N_3O_5Cl$: C 47.94, H 3.71, N 12.90 %).

1,3-Dimethyl-5-(4'-methoxybenzyl)-5-phenylthiobarbituric acid (**4c**).

Triethylamine (0.90 g,8.91 mM) was added to a solution of **5b**(2.78 g, 8.91mM) and benzenethiol(0.98 g,8.91 mM) in 50 ml of dichloromethane at room temperature for 12h, and then poured into 5 % hydrochloric acid (50 ml). Extraction with chloroform, drying over $MgSO_4$, following evaporation of chloroform gave a residue. Crystallization from ether and column chromatography of the mother liquor furnished 2.4 g (77 %)of crystalline **4c**. mp 112-113°C (benzene and ether, powder). IR ν_{max}^{KBr} cm^{-1}: 1680, 1610, 1375. ^{1}H-NMR δ: 2. 96(6H,s), 3.58(2H,s), 3.72(3H,s), 6.72(2H,d,J=8.5), 7.12(2H,d,J=8.5), 7.40(5H,m). (Found: C 62.23, H 5.02, N 7.11. Calc for $C_{20}H_{20}N_2O_4S$: C 62.48, H 5.24, N 7.29 %).

5-(n-Butylthio)-1,3-dimethyl-5-(4'-methoxybenzyl)barbituric acid (**4d**).

This compound was prepared in the same manner as **4c** above. The yield was 74 %. IR ν_{max}^{KBr} cm^{-1}: 1685, 1510, 1440, 1380. ^{1}H-NMR δ: 0.91(3H,t,J=8), 1.48(4H,m), 2.74(2H, t,J=7.5), 3.23(6H,s), 3.51(2H,s), 3.74(3H,s), 6.73(2H,d,J=9), 7.06(2H,d,J=9), MS m/z : 364(M$^+$), 121.

General method for synthesis of unsymmetrical disulfide

Triethylamine (1.0 mM, in the case of alkanethiol) or pyridine (1.0 mM, in the case of benzenethiol) was added to a solution of compound (4)(1.0 mM) and a thiol (1.0 mM) in 50 ml of dichloromethane and the solution was stirred for 2-4 hr at room temperature under argon. Concentration of the mixture gave the dihydro compound (2) as a crystal and the mother liquor was subjected to p-TLC with n-hexane to yield the mixture of disulfides. Quantitative analysis of the mixture was carried out by gas-liquid chromatography using standard sample of symmetrical disulfides. If necessary, the unsymmetrical disulfide was isolated by p-TLC.

Acknowledgements

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