and the resulting mixture extracted well with chloroform. After drying over anhydrous sodium sulfate and filtering from the drying agent, the chloroform extract was saturated with gaseous hydrogen chloride. The hydrochloride was collected on a suction filter and recrystallized from a mixture of water and ethanol. The yield was 0.27 g. (40%). The salt did not melt below 250°. *Anal.* Calcd. for $C_{14}H_{21}ClN_2O_4S$: S, 9.20. Found: S, 9.36.

LEXINGTON, KENTUCKY

[CONTRIBUTION FROM THE FULMER CHEMICAL LABORATORY, STATE COLLEGE OF WASHINGTON]

Schiff Bases and Related Substances. II. Reactions of Thiols with N-Benzylideneaniline and N-Benzylideneanthranilic Acid[1]

BY GARDNER W. STACY, RICHARD I. DAY AND RICHARD J. MORATH[2]

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No substituent effect has been observed in the addition of thiols to *m*- and *p*-benzylideneaminobenzoic acids or *m*- and *p*-benzylideneaminoacetophenones. Under appropriate conditions, N-benzylideneaniline (III) has been found to form addition products with thiols as readily as N-benzylideneanthranilic acid (I). Schiff base-thiol adducts are decomposed readily by dilute sodium hydroxide solution to yield constituent thiols and Schiff bases. Reduction of the *p*-toluenethiol adduct of N-benzylideneaniline (VI) occurs as readily as direct reduction of N-benzylideneaniline (III) with *p*-toluenethiol. Cleavage of Schiff bases by thiols in the presence of small amounts of water has been observed to yield corresponding amines and mercaptals.

In a recent publication[1] from this Laboratory, it was demonstrated that N-benzylideneanthranilic acid (I) would form crystalline adducts (II) with a variety of thiols. Earlier, Gilman and Dickey[3] had investigated the possibility of conjugate,

$$\underset{\text{I}}{C_6H_5CH{=}NC_6H_4COOH\text{-}o} + RSH \longrightarrow \underset{\text{II}}{C_6H_5CH(SR)-NHC_6H_4COOH\text{-}o}$$

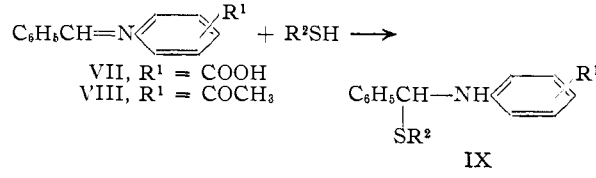
nuclear addition of *p*-toluenethiol to N-benzylideneaniline (III). They observed no addition products, however, but instead found that III was reduced to N-benzylaniline (IV). More recently, some examples of reduction of conjugated azomethine systems have been reported.[4]

$$\underset{\text{III}}{C_6H_5CH{=}NC_6H_5} \xrightarrow{p\text{-}CH_3C_6H_4SH} \underset{\text{VI}}{p\text{-}CH_3C_6H_4SCH(C_6H_5)NHC_6H_5}$$

$$\text{III} + 2\ p\text{-}CH_3C_6H_4SH \longrightarrow \underset{\text{IV}}{C_6H_5CH_2NHC_6H_5} + \underset{\text{V}}{(p\text{-}CH_3C_6H_4S\text{-})_2} \longleftarrow \text{VI} + p\text{-}CH_3C_6H_4SH$$

Because the Schiff bases I and III had been observed to react differently with thiols, it was of obvious interest to study the different factors involved in the two cases. Initially, we wished to investigate the possibility of a substituent effect as a basis for adduct formation. An electron-attracting substituent (as in the case of the carboxyl group in I) *ortho* or *para* to the Schiff base nitrogen might be expected to enhance the polarization of the carbon–nitrogen double bond, and hence adduct formation might be expected to occur readily. The same electron displacement, of course, would not obtain in the case of *meta* isomers, and therefore it might be thought that these would form adducts less readily or not at all, as also would be the case with the unsubstituted Schiff base (III).[5]

Such considerations were studied relative to the *m*- and *p*-benzylideneaminobenzoic acids (VII) and the *m*- and *p*-benzylideneaminoacetophenones (VIII). However, as indicated by the yields (Table I), there was no indication that *meta* isomers underwent adduct formation any less readily than *para* isomers. And certainly there were no striking differences in reactivity, as had been observed with the isomeric nitrostyrenes.[5]



VII, R^1 = COOH
VIII, R^1 = $COCH_3$

IX

The above results suggested that the difference in the reactions that had been observed when I and III were treated with thiols must be due to differences in conditions under which the reactions had been carried out. Gilman and Dickey[3] had run their reduction (III → IV) in refluxing *p*-xylene with a threefold excess of thiol, whereas our additions (I → II and VII, VIII → IX) were carried out equally well at room temperature or in refluxing benzene with one to two equivalents of thiol. Therefore, the formation of adducts of N-benzylideneaniline (III) was attempted employing the conditions that had led to addition in our previous experience. And, indeed, it was found that adducts of III were obtained in excellent yield (Table I). On the other hand, when the conditions that Gilman and Dickey[3] had reported were em-

(1) Presented in part before the Division of Organic Chemistry at the 125th Meeting of the American Chemical Society, Kansas City, Mo., March 24, 1954, and in part before the Montana Section of the American Chemical Society, Missoula, Mont., May, 1953. Paper I, G. W. Stacy and R. J. Morath, THIS JOURNAL, **74**, 3885 (1952).

(2) Abstracted from theses submitted by Richard I. Day and Richard J. Morath in partial fulfillment of the requirements for degrees of Master of Science and Doctor of Philosophy, respectively, State College of Washington, February, 1955, 1954.

(3) H. Gilman and J. B. Dickey, THIS JOURNAL, **52**, 4573 (1930).

(4) H. Gilman, J. L. Towle and R. K. Ingham, *ibid.*, **76**, 2920 (1954).

(5) Systems of comparative interest involve substituted styrenes. Recently, it has been reported that *o*- or *p*-nitrostyrenes will add active methylene compounds in the presence of sodium alkoxide, whereas *m*-nitrostyrene and styrene itself will not; W. J. Dale and C. W. Strobel, *ibid.*, **76**, 6172 (1954).

TABLE I

SCHIFF BASE-THIOL ADDUCTS (IX)

R^2	R^1	Yield, %[a]	Recryst.-solvent[b]	M.p., °C.[c]	Formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Sulfur, % Calcd.	Sulfur, % Found
p-$CH_3C_6H_4$	*p*-COOH	97	I	161-176[d]	$C_{21}H_{19}NO_2S$	72.18	71.91	5.48	5.53	9.18	9.03
2-$C_{10}H_7$	*p*-COOH	90	B	146–182[d]	$C_{24}H_{19}NO_2S$	74.77	74.58	4.97	5.02	8.32	8.16
p-$CH_3C_6H_4$	*m*-COOH	82	B-P	86–88	$C_{21}H_{19}NO_2S$	72.18	72.27	5.48	5.52	9.18	9.11
$C_6H_5CH_2$	*m*-COOH	99	EA–P	102.5–104.5	$C_{21}H_{19}NO_2S$	72.18	72.28	5.48	5.54	9.18	8.95
$HOOCCH_2$	*p*-CH_3CO	69	I	125.6–126.5	$C_{17}H_{17}NO_3S$	64.73	64.66	5.43	5.52	10.17	10.13
C_6H_5	*p*-CH_3CO	81	I	102.5–104	$C_{21}H_{19}NOS$	75.65	75.64	5.74	5.64	9.62	9.66
$C_6H_5CH_2$	*p*-CH_3CO	71	I	96.6- 98.5	$C_{22}H_{21}NOS$	76.04	76.07	5.09	6.27	9.23	9.20
$C_6H_5CH_2$	*m*-CH_3CO	69	I	88.5- 89.5	$C_{22}H_{21}NOS$	76.04	76.08	6.09	6.18	9.23	9.10
p-$CH_3C_6H_4$	*m*-CH_3CO	89	E	72.7- 73.9	$C_{22}H_{21}NOS$	76.04	76.13	6.09	6.15	9.23	9.06
C_6H_5	H	73	E	55.5- 56.5	$C_{19}H_{17}NS$	78.31	78.00	5.88	5.52	11.01	11.29
$C_6H_5CH_2$	H	80	I	61.8- 62.8	$C_{20}H_{19}NS$	78.65	78.80	6.27	6.48	10.50	10.51
p-$CH_3C_6H_4$	H	99	I	72.5- 73	$C_{20}H_{19}NS$	78.65	78.64	6.27	6.48	10.50	10.39
2-$C_{10}H_7$	H	85	E	66.1- 68.1	$C_{23}H_{19}NS$	80.90	80.90	5.61	5.78	9.39	9.31

[a] Yields were based on the weight of the crude product. [b] Analytical samples were recrystallized several times in each instance. Solvents employed: B, benzene; E, ethanol; EA, ethyl acetate; I, isopropyl alcohol; P, petroleum ether (b.p. 30–50°). Combinations denote recrystallizations from mixed solvent. [c] M.p. of analytical sample. [d] Melted with decomposition.

ployed, confirmation of the reduction was obtained. N-Benzylaniline (IV) was isolated as a benzenesulfonamide in a yield of 34%, and no addition product was observed.[6]

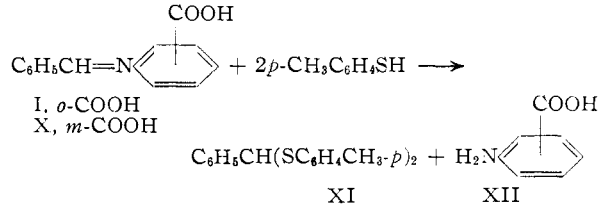
It was of further interest to observe that when the adduct VI was heated with an excess of *p*-toluenethiol in *p*-xylene, then N-benzylaniline IV and the disulfide V were formed in about the same yield as in the direct reduction of the Schiff base III. This indicated that any adduct that might be formed in the presence of excess thiol would be subject to reduction, although it did not demonstrate conclusively that the adduct is necessarily an intermediate in the reduction.

The demonstration of adduct formation with N-benzylideneaniline (III) was of considerable interest, for an earlier attempt to bring about adduct formation between III and 2-naphthalenethiol had appeared to fail.[1] This conclusion had been based on the fact that 2-naphthalenethiol, one of the starting materials, had been obtained in an 86% recovery. The recovery had been effected by extracting the reaction mixture with dilute aqueous alkali; this operation originally had been contemplated as a means of removing traces of unreacted thiol. It now appeared that in all likelihood adduct formation had occurred with the 2-naphthalenethiol, but the adduct formed then had been decomposed by the action of dilute sodium hydroxide solution. This was proved to be the case, for when the alkaline extraction was omitted, the 2-naphthalenethiol adduct was obtained in an 85% yield. When extraction with 10% sodium hydroxide solution was included, our previous result was confirmed in that no adduct was isolated, and a substantial portion of the starting thiol was recovered. Further, when the pure adduct VI, dissolved in *p*-xylene, was washed with 10% sodium hydroxide solution, a decomposition of the adduct was observed, and the constituent N-benzylideneaniline (III) and *p*-toluenethiol were obtained in good yield.

(6) Of related interest is the fact that both reduction and addition have been found to occur when thiols react with *p*-quinonedibenzenesulfonimide; R. Adams, E. F. Elslager and T. E. Young, THIS JOURNAL, **75**, 663 (1953).

Schiff base-thiol adducts are analogous in structure to hemimercaptals[7] and hemiacetals so that the behavior of these substances under alkaline conditions is of interest in relation to the presently observed instability of Schiff base-thiol adducts. Although information in the literature concerning the alkaline instability of simple, acyclic compounds of this type is meager, Baumann[7b] observed the decomposition of the hemimercaptal of chloral and thiophenol when this substance was treated with cold, aqueous alkali. On the other hand, the catalysis of the mutarotation of glucose and derivatives, which are cyclic hemiacetals, by aqueous basic conditions is well known and of related interest.[8]

Having demonstrated that N-benzylideneaniline (III) would undergo adduct formation as well as reduction with thiols under the proper conditions, we now wished to study the possibility that N-benzylideneanthranilic acid (I) might undergo reduction as well as adduct formation. It was found that reduction of I and its *meta* isomer X did occur in yields of 13 and 15%, respectively. However, this reduction occurred only under rigorously anhydrous conditions. When moisture was present either inadvertently or by deliberate addition to the reaction mixture, cleavage of the Schiff base occurred to yield the corresponding aminobenzoic acid (XII) and a mercaptal XI. The identity of XI was established by comparison with a sample of



mercaptal prepared from benzaldehyde and *p*-toluenethiol in the presence of hydrogen chloride and by oxidation of XI to a disulfone. Although

(7) (a) F. Kipnis and J. Ornfelt, *ibid.*, **74**, 1068 (1952); (b) E. Baumann, *Ber.*, **18**, 883 (1885).

(8) C. G. Swain and J. F. Brown, Jr., THIS JOURNAL, **74**, 2534 (1952).

the cleavage of Schiff bases by thiols apparently has not been described prior to this, a similar cleavage by hydrogen sulfide has been reported wherein thio ketones were obtained.[9]

Experimental[10]

Schiff Bases. (a) ***m*-Benzylideneaminobenzoic Acid (X).**—A suspension of 34.3 g. (0.25 mole) of *m*-aminobenzoic acid in 250 ml. of benzene was distilled for a short time to remove moisture from the system. Redistilled benzaldehyde (26.5 g., 0.25 mole) then was added. The mixture was heated under reflux for four hours, during which time 4.2 ml. (93% of the theoretical amount) of water was collected in a Dean–Stark trap. After the reaction mixture had been cooled in a refrigerator, the product was removed by filtration and washed with 20 ml. of cold benzene; yield 47.8 g. (85%), m.p. 131–132°. Two recrystallizations from benzene gave a pale yellow product, yield 42.2 g. (75%), m.p. 131–132°.[11]

(b) ***p*-Benzylideneaminobenzoic Acid.**—Under conditions similar to those described above, 0.25 mole of *p*-aminobenzoic acid and benzaldehyde gave 53.8 g. (96%) of the Schiff base, m.p. 195–196°. Two recrystallizations from 95% ethanol yielded 39.5 g. (70%), m.p. 194–196°.[12]

Anal. Calcd. for $C_{14}H_{11}NO_2$: C, 74.66; H, 4.92; N, 6.22. Found: C, 74.57; H, 4.91; N, 6.34.

(c) ***m*-Benzylideneaminoacetophenone.**—A mixture of 13.5 g. (0.10 mole) of *m*-aminoacetophenone and 10.6 g. (0.10 mole) of redistilled benzaldehyde in 75 ml. of benzene gave 14.0 g. (63%) of the desired product, m.p. 45–47°. An analytical sample was prepared by several recrystallizations from propanol-2, m.p. 47.5–48°.

Anal. Calcd. for $C_{15}H_{13}NO$: C, 80.68; H, 5.88; N, 6.27. Found: C, 80.67; H, 5.81; N, 6.27.

(d) ***p*-Benzylideneaminoacetophenone.**—This was prepared by reaction of 13.5 g. (0.10 mole) of *p*-aminoacetophenone with 10.6 g. (0.10 mole) of redistilled benzaldehyde in 50 ml. of ethanol. After the reaction mixture had been heated under reflux for one-half hour, it was cooled in an ice-bath. The pale yellow needles which formed were removed by filtration; yield 13.5 g. (60%), m.p. 96–99°. Two recrystallizations from propanol-2 afforded colorless needles; yield 11.6 g. (52%), m.p. 99.5–100.5°.[13]

Anal. Calcd. for $C_{15}H_{13}NO$: C, 80.68; H, 5.88; N, 6.27. Found: C, 80.43; H, 5.93; N, 6.47.

Preparation of Schiff Base–Thiol Adducts (IX, *cf.* Table I).—A solution of 0.01 mole of the Schiff base and 0.01 or 0.02 mole of the thiol in 50–75 ml. of benzene was heated under reflux for periods ranging from 12–18 hours. Heating, however, did not appear to be necessary, for excellent yields also were obtained by merely stirring the reactants in benzene at room temperature for one hour.[14] In some instances, the product crystallized from the reaction mixture and was separated by filtration (*e.g.*, *p*-toluenethiol adduct of *p*-benzylideneaminobenzoic acid). When crystallization did not occur spontaneously or on moderate cooling, it was sometimes possible to obtain a good yield of crystalline product by freezing the reaction mixture and by subsequent thawing of the benzene (2-naphthalenethiol adduct of *p*-benzylideneaminobenzoic acid). If neither of the two preceding operations could be employed, the benzene was removed under reduced pressure, and the residue was recrystallized from an appropriate solvent. When the residue failed to solidify on cooling, seed crystals were prepared by triturating a small amount of residue in ether and cooling the mixture in a Dry Ice-bath (2-naphthalenethiol adduct of N-benzylideneaniline).

Reduction of N-Benzylideneaniline (III) **by *p*-Toluenethiol.**—Because difficulties were encountered in applying the procedure reported by Gilman and Dickey,[3] the following alternate procedure was devised. A solution of 9.05 g. (0.05 mole) of N-benzylideneaniline and 24.8 g. (0.20 mole) of *p*-toluenethiol in 100 ml. of *p*-xylene was heated under reflux in an atmosphere of nitrogen for 24 hours. The cooled reaction mixture then was extracted repeatedly with 10% sodium hydroxide solution and washed with water until the washings were neutral to give a *p*-xylene layer (A) devoid of *p*-toluenethiol. After the cooled, aqueous extracts had been acidified with dilute hydrochloric acid, the resulting *p*-toluenethiol was collected by filtration, washed with water, and dried; recovery 19.2 g. Since on occasion this material may not crystallize, it can be converted readily to a 2,4-dinitrophenyl sulfide by reaction with 2,4-dinitrochlorobenzene.[15]

The *p*-xylene (A) then was heated under reflux in a nitrogen atmosphere for one hour with 50 ml. of 10% hydrochloric acid to hydrolyze unreduced III. After the mixture had cooled, the *p*-xylene phase was separated, was extracted with 10% hydrochloric acid, and was washed with water until the washings were neutral to give *p*-xylene (B) now containing only neutral substances. The combined hydrochloric acid extracts and washings were made basic (*p*H 10) with dilute sodium hydroxide and treated with benzenesulfonyl chloride. The alkali-insoluble benzenesulfonamide of N-benzylaniline (IV) was collected by filtration and was washed repeatedly with dilute sodium hydroxide solution and finally with water. The combined filtrate and washings (C) contained N-benzenesulfonylaniline as the sodium salt. The benzenesulfonamide of the reduction product IV was recrystallized from ethanol to yield 5.0 g. (34%), m.p. 119–120°; a mixed melting point determination with an authentic sample showed no depression, m.p. 119–121°. The combined filtrate and washings (C) were acidified with dilute hydrochloric acid to give 4.49 g. of N-benzenesulfonylaniline, m.p. 111–112°; a mixed melting point determination with an authentic sample confirmed the identity of this substance.

The *p*-xylene phase (B) was dried over anhydrous sodium sulfate, and then the *p*-xylene was removed by distillation under reduced pressure. Bis-(*p*-tolyl) disulfide (V), crystallized from solution and after recrystallization from ethanol, amounted to 3.98 g., m.p. 45–46°. This proved to be identical with an authentic specimen, as determined by a mixed melting point. The extent of reduction of III based on recovered thiol was 45%. Although only 34% reduction was indicated by the amount of reduction product isolated as the benzenesulfonamide, the higher yield as based on thiol recovery appears valid because of loss in the conversion of the amine to the sulfonamide. Repeated attempts at preparing this sulfonamide from authentic N-benzylaniline gave yields of 60–65%.

Reduction of the Adduct VI by *p*-Toluenethiol.—A solution of 15.2 g. (0.05 mole) of the adduct VI and 18.6 g. (0.15 mole) of *p*-toluenethiol in 100 ml. of *p*-xylene was heated under reflux under nitrogen for 24 hours. The reaction mixture was worked up as described in the preceding experiment. The substances isolated were: *p*-toluenethiol (16.0 g.), N-benzenesulfonyl-N-benzylaniline (4.60 g., 29%), N-benzenesulfonylaniline (4.58 g.), and bis-(*p*-tolyl) disulfide (5.90 g.). The extent of reduction of VI based on thiol recovery was 42% and based on the benzenesulfonamide was 29%.

N-Benzylideneaniline Adduct Formation with 2-Naphthalenethiol and Subsequent Alkaline Decomposition.—The adduct was prepared by the general method described above in a yield of 85% (Table I). The reaction was repeated in the same way except that instead of removing the benzene after the heating period, the reaction mixture was washed with cold 10% sodium hydroxide solution. The benzene phase then was washed further with water and dried over anhydrous sodium sulfate. The aqueous extract was acidified with 2 *N* hydrochloric acid; the resulting precipitate of 2-naphthalenethiol was removed by filtration and was washed with water; recovery 1.40 g. (88%), m.p. 75–77°.

(9) G. Reddelien and H. Danilof, *Ber.*, **54**, 3132 (1921); C. M. Rosser and J. J. Ritter, THIS JOURNAL, **59**, 2179 (1937).

(10) All melting points are corrected. The microanalytical work was performed by Galbraith Laboratories, Knoxville, Tenn.

(11) A. Roe and J. A. Montgomery, THIS JOURNAL, **75**, 910 (1953), reported m.p. 131–132°.

(12) Although an analysis has not been reported previously for this compound, the following melting points are recorded in the literature: 193.5°, W. Manchot and J. R. Furlong, *Ber.*, **42**, 4383 (1909); 190–192°, B. A. Poraĭ-Koshits, *et al.*, *J. Gen. Chem.* (*U.S.S.R.*), **17**, 1774 (1947); *C. A.*, **42**, 5863 (1948); 190–191° (ref. 11).

(13) Since M. Giua and E. Bagiella, *Gazz. chim. ital.*, **51**, II, 116 (1921), had reported m.p. 95–96°, a sample was submitted for analysis.

(14) Recently, a similar formation of Schiff base–thiol adducts in cold benzene solution has been reported: A. Martani, *Ann. chim.* (*Rome*), **43**, 282 (1953); *C. A.*, **48**, 12737 (1954).

(15) R. W. Bost, J. O. Turner and R. D. Norton, THIS JOURNAL, **54**, 1985 (1932).

The benzene phase was concentrated in volume, N-benzylideneaniline (III) crystallizing from the residue. After this was washed with cold ether, reasonably pure III was obtained; yield 1.00 g. (62%), m.p. 47–50°.

Behavior of the Adduct VI with Dilute Alkali.—A solution of 2.00 g. (0.006 mole) of VI in 30 ml. of *p*-xylene was extracted with several portions of 10% sodium hydroxide (total, 200 ml.). The *p*-xylene phase was washed with water and dried over anhydrous sodium sulfate. After removal of solvent and recrystallization of the resulting residue from ethanol, N-benzylideneaniline (III) was obtained; yield 0.600 g. (50%), m.p. 48–50°. After the alkaline extract had been acidified with cooling, *p*-toluenethiol precipitated; yield 0.475 g. (58%).

Cleavage of Benzylideneaminobenzoic Acids by Thiols. (a) ***m*-Benzylideneaminobenzoic Acid (X).**—A solution of 5.63 g. (0.025 mole) of X and 12.42 g. (0.10 mole) of *p*-toluenethiol in 50 ml. of *p*-xylene was heated under reflux for 24 hours. As the mixture cooled, a crystalline precipitate was formed, was removed by filtration, and was washed with petroleum ether (b.p. 35–60°); this proved to be *m*-aminobenzoic acid; yield 1.87 g. (55%), m.p. 165–173°. The identity was confirmed by a mixed melting point determination with an authentic sample.

The *p*-xylene filtrate from the above was extracted with several portions of 5% sodium hydroxide solution and then was dried over Drierite. The *p*-xylene was removed, and a solid residue was obtained which weighed 5.34 g., m.p. 69–79°. Upon recrystallization from ethanol, this proved to be the mercaptal XI; yield 4.24 g. (51%), m.p. 78–79°. A mixture of this with an authentic sample[16] of XI (m.p. 79.5–80.5°) showed no depression in melting point (78–79.5°).

α,α-Bis-(*p*-tolylsulfonyl)-toluene was obtained by the following procedure since the reported method[16] proved to be unsatisfactory in our hands. To a solution of 0.336 g. (0.001 mole) of XI in 25 ml. of glacial acetic acid was added dropwise with swirling 10 ml. of an 8% solution of potassium permanganate. The mixture was chilled in an ice-bath, and the excess permanganate and manganese dioxide were eliminated by the dropwise addition of 3% hydrogen peroxide. The resulting suspension was poured into 75 ml. of ice-cold water, and the solid product was collected by filtration; yield 0.296 g. (74%), m.p. 182.5–183.5°. Recrystallization from glacial acetic acid afforded 0.226 g. (56%) of colorless crystals, m.p. 181–182°. A sample of the same substance was prepared from the authentic material.[16] Since the melting point of our product varied considerably from that reported by Fromm and Raiziss (m.p. 163°[16]), a sample was submitted for analysis.

Anal. Calcd. for $C_{21}H_{20}O_4S_2$: C, 62.97; H, 5.03; S, 16.01. Found: C, 62.95; H, 5.14; S, 16.07.

(b) **N-Benzylideneanthranilic Acid (I).**—In this case and in the following experiments involving reduction, the quantities of reactants and period of heating were identical to those of experiment a. The main difference between this and (a) was that water deliberately was added to the reaction mixture by way of *p*-xylene, which previously had been saturated with water at room temperature.

After the reaction mixture had been cooled in an ice-bath, crude anthranilic acid precipitated and was removed by filtration; yield 1.53 g. (45%), m.p. 130–138°. To remove acidic materials, the *p*-xylene filtrate was washed with several portions of 5% sodium hydroxide solution. The *p*-xylene then was dried and distilled leaving a residue which was dissolved in 15 ml. of hot ethanol. The mercaptal XI crystallized at room temperature to yield 4.70 g. m.p. 77–80°; this was recrystallized from ethanol to give 4.13 g. (49%), m.p. 80–81°.

Reduction of Benzylideneaminobenzoic Acids by Thiols. (a) ***m*-Benzylideneaminobenzoic Acid (X).**—The conditions for reduction of X differed from the cleavage described above in (a) only in that considerable care was taken to eliminate moisture. The solvent and all reactants were dried rigorously by appropriate means as was the apparatus in which the reaction was carried out, and the reaction mixture was protected from atmospheric moisture throughout the 24-hour heating period.

When, in contrast to (a) above, relatively little precipitate was obtained on cooling, the solvent was removed by distillation (A). The residue was taken up in ether (B), and by the addition of petroleum ether (b.p. 35–60°) in small portions (B), several crystalline and oily fractions separated. Crystalline material was obtained from the oily fractions by treating with small portions of ether (C). Recrystallization of the combined crystalline materials from *p*-xylene gave 0.430 g. of a substance melting at 170–171°; by a mixed melting point determination, this proved to be *m*-aminobenzoic acid.

The *p*-xylene removed by distillation (A) and the combined filtrates (B) were extracted with several portions of 5% sodium hydroxide solution to remove acidic substances and excess *p*-toluenethiol. The solvent from the organic layer so extracted was removed by distillation; no mercaptal XI was isolated from the oily residue.

The ether filtrates (C) from above were extracted with 5% sodium hydroxide solution; when the extracts were acidified with dilute hydrochloric acid, a gum formed. After ether was added to the mixture, some of the gum dissolved leaving crystalline material (D) in the aqueous phase. The ether phase was dried, and then a stream of anhydrous hydrogen chloride was introduced to give more crystalline material (E). The combined crystalline materials (D, E) in addition to other small fractions of similar material that were isolated from this reaction mixture appeared to be the hydrochloride of *m*-benzylaminobenzoic acid, the anticipated reduction product; total yield 2.13 g. (32%). A sample of *m*-benzylaminobenzoic acid was obtained from some relatively pure hydrochloride. The hydrochloride was dissolved in dilute sodium hydroxide solution, and the *p*H adjusted to 5.5 with dilute hydrochloric acid. The resulting solution was then extracted with four portions of ether and, after the combined extracts had been processed in the usual manner, a product melting at 108–111° was obtained. This was recrystallized once from a mixture of 4 ml. of benzene and 3 ml. of petroleum ether and then twice from 50% ethanol, m.p. 114–115°.[17]

Because of difficulties in isolating the amino acid in a pure condition, a sample of hydrochloride was converted to **N-acetyl-*m*-benzylaminobenzoic acid.** By warming the mixture on a steam-bath, a 0.200-g. sample of hydrochloride was dissolved in 30 ml. of 10% sodium acetate solution. The mixture was cooled in an ice-bath, and 2 ml. of acetic anhydride was added with agitation. Almost immediately the solution became turbid, and an oil formed. The cold mixture was made strongly acidic with hydrochloric acid, and then it was extracted with four portions of ether. The combined extracts were processed in the usual manner, and the residue was dissolved in toluene and treated with Norite; yield 0.093 g., m.p. 130–131°. The extent of reduction based on this pure acetyl derivative was 15%. An analytical sample was recrystallized twice from toluene; m.p. 132.5–133°.

Anal. Calcd. for $C_{16}H_{15}NO_3$: C, 71.35; H, 5.61; N, 5.20. Found: C, 71.52; H, 5.32; N, 5.38.

(b) **N-Benzylideneanthranilic Acid (I).** Benzenethiol was employed in this experiment rather than *p*-toluenethiol. After the *p*-xylene had been removed by distillation, the residue was taken up in 200 ml. of ether, which was extracted with four portions of 10% sodium bicarbonate solution. The combined extracts then were acidified with dilute hydrochloric acid, and the resulting precipitate was separated to give the crude reduction product, N-benzylanthranilic acid; yield 0.62 g., m.p. 151–167°. Recrystallization from chloroform afforded a pure product, 0.42 g., m.p. 174.5–175°. The ether phase from the above separation was then extracted with several portions of 10% sodium hydroxide solution. A crystalline material, which was discovered in the interphase during this separation, proved to be the sodium salt of some additional reduction product, which upon acidification gave 0.34 g., m.p. 172–173°. The extent of reduction was 13% as based on the total amount of recrystallized product, 0.75 g., m.p. 174–175°.[18]

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(16) Prepared in 90% yield by passing anhydrous hydrogen chloride through an ether solution of *p*-toluenethiol and benzaldehyde; E. Fromm and G. Raiziss, *Ann.*, **374**, 90 (1910).

(17) G. Lockemann and H. Rein, *Chem. Ber.*, **80**, 485 (1947), reported m.p.'s 115°, 119°.

(18) The reported melting point for N-benzylanthranilic acid is 175° (ref. 17).

which a part of this work was carried out. This investigation also was supported in part by a grant from the National Science Foundation.

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[CONTRIBUTION FROM DEPARTMENT OF BIOCHEMISTRY, UNIVERSITY OF WASHINGTON]

Studies on Thiols. I. Oxidation of Thiol Groups by 2,6-Dichlorophenol Indophenol[1]

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Using a spectrophotometric method, the oxidation of a variety of thiols by the dye, 2,6-dichlorophenol indophenol, has been investigated. Depending upon the structure of an individual thiol, the reaction rate is either relatively fast and approaches a final stoichiometry of oxidant : reductant reacting of approximately 1:1 or slow, whereupon the stoichiometry approaches approximately 1:2. The reaction rate is directly proportional to the concentration of both oxidant and reductant and hydrogen ion concentration. Metal ions, such as Cu^{++}, Ni^{++} and Fe^{++} depress the rate and shift the stoichiometry toward a 1:2 reaction; Versene reverses this effect. In light of these kinetic studies the mechanism of reaction is discussed, and a quantitative assay for purified thiols has been developed. Acyl thiols, disulfides and certain other sulfur-containing compounds do not react in this system. "Potential" thiols, such as thiazolidines, thiazolines and thiazoles, react with indophenol, but with a rate several orders of magnitude slower than "open" thiols.

The importance of thiol[3] groups in biological systems[4] is due to the widespread occurrence of this functional group in many proteins and in simple molecules such as glutathione, cysteine, Coenzyme A[5] and lipoic acid. In addition to the well-known reversible oxidation–reduction to the disulfide stage, thiol groups have recently taken on additional significance as carriers of acyl groups in enzymatic reactions.[6]

The chemistry of thiol groups, however, still contains areas of uncertainty, especially with regard to oxidation processes.[7] This difficulty arises in part from the fact that thiols can undergo oxidation to a series of higher states.[8] Oxidation of thiols is also affected by factors[4] such as: (a) steric and inductive effects of other groups in the molecule, which may shield the thiol group, cause hydrogen bonding or cause ring formation; (b) the presence of metal ions and (c) the nature of the oxidant.

The present investigation was undertaken to gain further information pertinent to the above problems. The dye, 2,6 dichlorophenol indophenol, was chosen as the oxidant, since it permits the reaction to be followed spectrophotometrically. Although the oxidation of thiols by this dye may be complicated by non-oxidative side reactions (*e.g.*, addition of the thiol to the quinoid form of the dye[4]), the rapidity of the oxidation–reduction reaction in dilute solutions minimizes this difficulty, and makes it possible to obtain data on the kinetics and stoichiometry of a typical oxidative reaction involving thiols.

Experimental

Chemicals.—All chemicals were purchased from commercial sources unless otherwise specified.

Pantethine was kindly supplied by Dr. Bird of Parke, Davis Co. and alatheine (β-alanylcysteine) from Drs. Cheldelin and King. Thioglycolic acid, a redistilled Merck product, and Versene from the Bersworth Chemical Co., were generously supplied by Dr. P. E. Wilcox.

Thiazolidine-4-carboxylic acid was prepared by the method of Ratner and Clarke[9] (m.p., with decomposition, 197–200°). L-Cystine disulfoxide was synthesized by the method of Toennies and Lavine[10] and converted to cysteinesulfinic acid by the method of Lavine[11]; the purity of both compounds was determined iodometrically. Perbenzoic acid (used in the preparation of cystine disulfoxide) was prepared by the method of Braun.[12] 2-Methyl-Δ^2-thiazoline was obtained from L. Light and Co., Ltd., or synthesized by the method of Gabriel and Hirsch.[13] Both products had physical constants (b.p. and m.p. of the picrate) slightly lower than those listed in Beilstein[14,15] and may have contained the 5-methyl isomer as an impurity.

Colorimetric Assay of Thiols.—The following system was used in all assays of thiols except where noted.

10^{-3} *M* Indophenol	0.15 ml.
10^{-1} *M* Phosphate buffer, *p*H 7	1.00 ml.
Water	1.85 ml.
1.25×10^{-2} *M* Thiol	0.01 ml.

The indophenol, buffer and water are mixed in a 1-cm. cuvette, and the log I_0/I value read in the Beckman spectrophotometer, model DU, at 600 mμ against a blank composed of buffer and water. The log I_0/I (or E_{600}) remains constant with time as long as no reducing substance is added. At zero time, 0.01 ml. of thiol is added to both experimental and blank cuvettes and the decrease in E_{600} followed at 15- or 30-seconds intervals. Initial velocity is defined

(1) This material is taken from the Dissertation of Robert E. Basford offered in partial fulfillment of the requirements for the degree of Doctor of Philosophy. The work was supported in part by grants from Eli Lilly and Co. and by Initiative 171, State of Washington. A preliminary report of this investigation has been given at the 37th Meeting of the Federated Societies of American Biologists at Chicago in April 1953 (F. M. Huennekens and R. E. Basford, *Federation Proc.*, **12**, 221 (1953)).

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(3) The term "thiol" will be used in preference to "sulfhydryl groups" or "mercaptans."

(4) For excellent reviews of thiol compounds and their role in biological processes, see E. S. G. Barron, *Adv. Enzymol.*, **11**, 201 (1951); C. Fromageot, *ibid.*, **7**, 369 (1947).

(5) Abbreviations used in this and the subsequent paper include: CoA, Coenzyme A; ATP, adenosine triphosphate; DPN, diphosphopyridine nucleotide; indophenol, 2,6-dichlorophenolindophenol; CSH, CSSC, reduced and oxidized cysteine; GSH, CSSG, reduced and oxidized glutathione; RSH, thiol.

(6) F. Lynen and E. Reichert, *Angew. Chem.*, **63**, 47 (1951).

(7) For example, Freedman and Corwin (*J. Biol. Chem.*, **181**, 601 (1949)) have reviewed recently the widely divergent values which have been reported in the literature for the oxidation potential of the cysteine–cystine couple.

(8) For cysteine (RSH), the higher oxidation states are cystine (RSSR), cysteinesulfenic acid (RSOH), cysteinesulfinic acid (RSO_2H) and cysteic acid (RSO_3H).

(9) S. Ratner and H. Clarke, THIS JOURNAL, **59**, 200 (1937).

(10) G. Toennies and T. F. Lavine, *J. Biol. Chem.*, **113**, 571 (1936).

(11) T. F. Lavine, *ibid.*, **113**, 583 (1936).

(12) G. Braun in "Organic Syntheses," Coll. Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1941, p. 431.

(13) S. Gabriel and C. F. von Hirsch, *Ber.*, **29**, 2609 (1896).

(14) "Beilstein," Fourth Ed., Vol. 27, p. 13 (1937).

(15) Ref. 14, Fourth Ed. Suppl., p. 206 (1938).