Table I. Purification of Bovine Parotid DNase

| Fraction | Total protein (g) | Total activity (units) | Specific activity (units/mg) | Yield of DNase (%) |
|---|---|---|---|---|
| Crude extract from the gland | 800 | $416\times10^3$ | 0.52 | 100 |
| Extract of acetone-dried powder | 53 | $106\times10^3$ | 2.0 | 25 |
| Precipitate with ammonium sulfate (0.5—1.0 saturation) | 8 | $92\times10^3$ | 11.5 | 20 |
| DEAE-cellulose | 0.62 | $36\times10^3$ | 58 | 9 |
| Sephadex G-100 | 0.21 | $22\times10^3$ | 105 | 5 |

and had requirement for magnesium ion, as shown in Fig. 4. A result of analysis of a complete digest of the DNA with purified DNase according to the procedure described earlier[10] showed that the digest was composed of oligonucleotides from mononucleotide to pentanucleotide and the mononucleotide fraction contained 5′-dAMP, 5′-dCMP, 5′-dTMP and 5′-dGMP, which were assigned by their chromatographic behaviour on AG $1\times2$ columns. The approximate molecular weight of the parotid DNase was estimated to be 38000 by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis using the method of Dunker and Rueckert[11] and the molecular weight of pancreatic DNase (Miles Laboratories, Grade I) was also estimated to be 38000. The sugar in purified parotid DNase was determined by the phenol–$H_2SO_4$ method[12] and the content was 3.0% as calculated from a calibration curve based on glucose and this result is coincident with that of pancreatic DNase.[13] Rundblad, *et al.* reported that DNases from pancreas and parotid glands were immunologically different. However we could not obtain the biochemical results which showed differences between pancreatic DNase and parotid DNase. It was concluded that parotid DNase was very similar to pancreatic DNase.

10) S. Takemura, T. Mizutani, and M. Miyazaki, *J. Biochem.*, **64**, 827 (1968).
11) A.K. Dunker and R.R. Rueckert, *J. Biol. Chem*, **244**, 5074 (1969).
12) M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Reber, and F. Smith, *Anal. Chem.*, **28**, 350 (1956).
13) J. Salnikow, S. Moore, and W.H. Stein, *J. Biol. Chem.*, **245**, 5685 (1970).

[Chem. Pharm. Bull. 22(11)2725—2728(1974)]

UDC 547.264.1'233.04

## A Simple Synthesis of Amino-containing Bunte Salts by the Reaction of Aminothiols with Chlorosulfonic Acid

Tomohide Tanaka, Hiroshi Nakamura, and Zenzo Tamura

*Faculty of Pharmaceutical Sciences, University of Tokyo*[1]

(Received April 1, 1974)

Organic thiosulfates, so-called "Bunte salts," were first prepared by Bunte in 1874.[2] Bunte salts are remarked as surfactants,[3] intermediates in organic syntheses,[4] protectors

1) Location: *Hongo-7-3-1, Bunkyo-ku, Tokyo, 113, Japan.*
2) H. Bunte, *Chem. Ber.*, **7**, 646 (1874).
3) E. Schirm, *Chem. Abstracts*, **29**, 6670 (1935).
4) H.B. Footner and S. Smiles, *J. Chem. Soc.*, **127**, 2887 (1928).

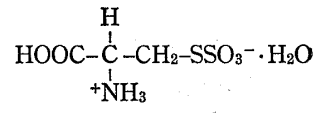
from radiation,[5] and biologically active substances.[6,7] There have been some methods for preparation of the salts, using reactions between thiosulfate and alkyl halides,[2] sulfite and disulfides,[8] or sulfur trioxide and thiols[9,10] and so on.[11] Dörr introduced a synthesis of Bunte salts by sulfation of alkyl mercaptans ($C_8$—$C_{18}$) with chlorosulfonic acid ($ClSO_3H$).[12] We succeeded in extending the method to synthesize amino-containing Bunte salts of biological significance by changing the reaction conditions. The greatest difference is the choice of acetic acid instead of ethyl ether as a solvent.

## Experimental

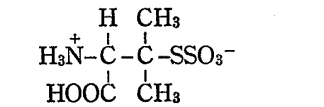
All melting points were measured on a Yanagimoto Melting Point Apparatus and were uncorrected.

**Reagents and Materials**——Methanol, ethanol, *n*-butanol, formic acid, acetic acid, $ClSO_3H$, and ethyl ether were extra pure reagents of Kanto Chemical Co. Ltd. L-Cysteine, cysteamine, dimethylaminoethanethiol hydrochloride, penicillamine, *p*-aminothiophenol, and benzyl mercaptan were Tokyo Kasei's guaranteed reagents. Amberlite CG-120, and Amberlite XAD-2 were supplied by Organo Co. Ltd. Paper chromatography and electrophoresis were performed with Toyo Filter Paper No. 514.

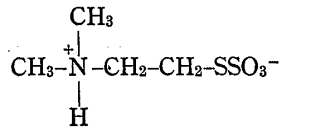
**Cysteine-S-sulfate Monohydrate (I)**——Two mmoles (0.13 ml) of $ClSO_3H$ were added to the suspension of L-cysteine (121 mg, 1 mmole) in 5 ml of acetic acid under stirring. The mixture was soon cleared and stirred for 30 min at room temperature (15°). White precipitates formed were filtered and washed with 10 ml of ethyl ether, dissolved in 1 ml of water and applied on a column of Amberlite CG-120 (H, 1×5 cm) to adsorb contaminous cystine, and washed with 20 ml of distilled water. Effluent was evaporated to dryness below 30° under reduced pressure. The residue was recrystallized from ethanol–ethyl ether. Needles, yield 83% (180 mg). mp 204—205° (decomp.) (Lit.[13] 184—185°). *Anal.* Calcd. for $C_3H_7O_5NS_2 \cdot H_2O$ (mw. 219.32): C, 16.43; H, 4.13; N, 6.39; S, 29.18. Found: C, 16.62; H, 4.04; N, 6.54; S, 29.52.



**Cysteamine-S-sulfate (II)**——Two mmoles of $ClSO_3H$ were added dropwisely within 1 min to the solution of cysteamine (77 mg, 1 mmole) in 5 ml of acetic acid. The solution was stirred for 20 min at room temperature. Twenty ml of ethyl ether was poured into the reaction mixture. Resulting oily precipitates were separated by decantation and dissolved in 1 ml of methanol and added with 5 ml of ethyl ether. The reprecipitated oily product was dissolved in 1 ml of water, applied on a column of Amberlite CG-120 (H, 1×5 cm), and washed with 10 ml of water. Effluent was evaporated to dryness below 30° under reduced pressure. The product was recrystallized from ethanol–ethyl ether. Needles, yield 77% (120 mg). mp 195—196° (decomp.) (Lit.[14] 195—196° decomp.). *Anal.* Calcd. for $C_2H_7O_3NS_2$ (mw. 157.22): C, 15.28; H, 4.49; N, 8.91; S, 40.71. Found: C, 15.33; H, 4.49; N, 9.19; S, 40.87.



**Penicillamine-S-sulfate (III)**——Two mmoles of $ClSO_3H$ were added dropwisely to the suspension of penicillamine (149 mg, 1 mmole) in 5 ml of acetic acid. Clarified solution was stirred for 30 min at room temperature. White precipitates formed were filtered and recrystallized from ethanol–ethyl ether. Block crystals, yield 72% (165 mg). mp 202—203° (decomp.). *Anal.* Calcd. for $C_5H_{11}O_5NS_2$ (mw. 229.28): C, 26.20; H, 4.84; N, 6.10; S, 27.91. Found: C, 26.43; H, 4.99; N, 6.09; S, 27.95.

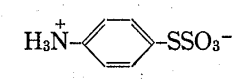


**Dimethylaminoethanethiol-S-sulfate (IV)**——Two mmoles of $ClSO_3H$ were added to the suspension of dimethylaminoethanethiol hydrochloride (142 mg, 1 mmole) in 5 ml of acetic acid. Clarified solution was stirred for 5 min. Then 20 ml of ethyl ether was poured into the reaction mixture. Resulting oily precipitates were separated and dissolved in 1 ml of methanol and added with 5 ml of ethyl ether to form precipitates The precipitates were dissolved in 1 ml of water, applied on a column of Amberlite CG-120 (H, 1×5 cm), and washed with water. Portions of 5 ml were collected. Fractions 7—10

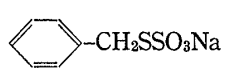
5) L. Eldjarm and A. Phil, *J. Biol. Chem.*, **223**, 341 (1956).
6) M. Yoshioka and Z. Tamura, *Chem. Pharm. Bull.* (Tokyo), **19**, 178 (1971).
7) M. Yoshioka and Z. Tamura, *Chem. Pharm. Bull.* (Tokyo), **19**, 186 (1971).
8) I.H. Segel and M.J. Johnson, *Anal. Biochem.*, **5**, 330 (1963).
9) M. Schmidt and G. Talsky, *Chem. Ber.*, **94**, 1352 (1961).
10) P. Baumgarten, *Chem. Ber.*, **63**, 1330 (1930).
11) H. Distler, *Angew. Chem.*, **79**, 520 (1967).
12) E.L. Dörr, U.S. Patent 2921952 (1960) [*Angew. Chem.*, **79**, 520 (1967)].
13) F. Michell and H. Emde, *Z. Physiol. Chem.*, **265**, 266 (1940).
14) H.Z. Lecher and E.M. Hardy, *J. Org. Chem.*, **20**, 475 (1955).

were pooled and evaporated to dryness below 30° under reduced pressure. The residuewas recrystallized from ethanol–ethyl ether. Block crystals, yield 69% (128 mg). mp 191—192° (decomp.) (Lit.[15] 189—193° decomp.). *Anal.* Calcd. for $C_4H_{11}O_3NS_2$ (mw. 185.27): C, 25.93; H, 6.00; N, 7.60; S, 34.54. Found: C, 25.77; H, 6.11; N, 7.61; S, 34.41.

**p-Aminothiophenol-S-sulfate (V)**——To the solution of *p*-aminothiophenol (125 mg, 1 mmole) in 5 ml of acetic acid, 2 mmoles of $ClSO_3H$ were added. The mixture was stirred for 30 sec at room temperature. White needles formed were filtered and recrystallized from water–ethanol. Needles, yield 98% (201 mg). mp 254—255° (decomp.). *Anal.* Calcd. for $C_6H_7O_3NS_2$ (mw. 205.26): C, 35.10; H, 3.43; N, 6.82; S, 31.18. Found: C, 35.14; H, 3.42; N, 6.95; S, 31.40.



**Benzyl Mercaptan-S-sulfate Sodium Salt (VI)**——To the solution of benzyl mercaptan (124 mg, 1 mmole) in ethyl ether (5 ml), the solution of $ClSO_3H$ (0.13 ml) in ethyl ether (5 ml) was added dropwisely within a min under cooling in the ice bath (3°). After stirring for 15 min appropriate amount of sodium ethoxide (or sodium hydroxide in methanol) was added. Resulting precipitates were filtered and dissolved in 5 ml of water. The solution was applied on a column of Amberlite XAD-2 (2×20 cm), washed with 100 ml of water and eluted with 200 ml of methanol. The eluate was evaporated to dryness under reduced pressure at 30°. The residue was washed with 10 ml of water. The washing was evaporated to dryness under reduced pressure at 30°. The residue was recrystallized from ethanol–ethyl ether. Needles, yield 55% (124 mg). mp not definite (*ca.* 200° decomp.). *Anal.* Calcd. for $C_7H_7O_3S_2Na$ (mw. 226.26): C, 37.16; H, 3.12. Found: C, 36.97; H, 3.10.



## Result and Discussion

Our products have been stable for more than a month in a desiccator containing silica gel under reduced pressure (15 mmHg) or even in aqueous solutions at room temperature. We found that cysteine methyl ester-S-sulfate, homocysteine-S-sulfate and glutathione-S-sulfate were prepared in a similar fashion.

The properties of the products are as follows. Ninhydrin test[16] (I—IV) or Ehrlich test[17] (V) was positive. Nitroprusside test[18] was negative. After the treatment with 5% KCN, $SO_3^{2-}$ was detected with $10^{-4}$M malachite green.[19] Under the conditions employed $ClSO_3H$ reacted only with -SH groups and not with $-NH_2$ groups, though $ClSO_3H$ is well known to react with both $-NH_2$ groups[20] and aromatic hydrocarbons.[21] This is probably because $ClSO_3H$ is more reactive to -SH groups than protonated $-NH_2$ groups in acetic acid.

*Rf* values and electrophoretic mobilities of the products are shown in Table I.

Table I. Paper Chromatography and Paper Electrophoresis of the Products

| Compd. | *Rf*[a] | *Rf*[b] | Distance (cm)[c] | Color reaction |
|---|---|---|---|---|
| I | 0.54 | 0.28 | +7.5 | brown[d] |
| II | 0.52 | 0.26 | 0.0 | red-violet[d] |
| III | 0.58 | 0.34 | +6.8 | dark yellow[d] |
| IV | 0.62 | 0.31 | 0.0 | brown[d] |
| V | 0.61 | 0.38 | +1.2 | yellow[e] |

*a*) *n*-butanol: acetic acid: water=5:2:3 (v/v)
*b*) *n*-butanol: acetic acid: water=8:1:2 (v/v)
*c*) 0.1N formic acid, pH 2.3, 15 v/cm, 2 hr
*d*) with ninhydrin
*e*) with Ehrlich reagent

15) A. Kaluszyner, P. Czerniak, and E.D. Bergmann, *Radiation Research*, **14**, 23 (1961).
16) S. Ruhemann, *J. Chem. Soc.*, **1911**, 792, 1486.
17) P. Ehrlich and C.A. Herter, *Z. Physiol. Chem.*, **41**, 379 (1904).
18) I.W. Grote, *J. Biol. Chem.*, **93**, 25 (1931).
19) E. Votocek, *Chem. Ber.*, **40**, 414 (1907).
20) R.N. Johnson and S. Smiles, *J. Chem. Soc.*, **123**, 2384 (1923).
21) B. Yu. Tasnitskii, *Zhur. Obshchei Khim.*, **23**, 107 (1953).

As mentioned above, our methods are much superior to the conventional ones,[8,13–15] in reaction time, simplicity, purity and yield.

We could increase the reaction size up to 100 mmoles of thiols without any decrease of yields, although variation of the ratio of $ClSO_3H$ or acetic acid to thiol led to reduce yields.

**Acknowledgements** We would like to thank Analytical Laboratory of this faculty for analysis of carbon, hydrogen and nitrogen, and also Chugai Co. Ltd. for analysis of sulfur. We are grateful to Mr. Yazawa for his assistance in our work.

---

[Chem. Pharm. Bull. 22(11)2728—2731(1974)]

*UDC 547.751.04 : 546.15.04*

# Reaction of Skatole with Iodine in the Presence of Thiourea

TOHRU HINO, MAMORU ENDO, and MASAKO NAKAGAWA

*Faculty of Pharmaceutical Sciences, Chiba University*[1]

(Received April 30, 1974)

The reaction of 3-carboethoxy-2,4-dimethylpyrrole (**1**) and other pyrrole derivatives with $I_2$-KI in the presence of thiourea has been investigated to give S-($\alpha$ or $\beta$-pyrryl)-pseudothiourea hydroiodides such as **2**.[2] The reaction was successfully applied to indole (**3**) and 3-mercaptoindole (**5**) was prepared *via* 3-indolylpseudothiourea hydroiodide (**4**) in excellent yield. The intermediacy of sulfenyl iodide (**6**), which presumably be formed by the reaction of thiourea with iodine, has been postulated to react with pyrrole or indole to form pseudothiourea derivatives.

This results led us to investigate the similar reaction of 3-alkylindoles in the hope that a new synthetic route to 2-indolinethiones[3] *via* **7** might result. We describe here the reaction of skatole with iodine in the presence of thiourea.

When a solution of skatole (**8**) and thiourea in aqueous ethanol was treated with one mole of iodine solution following the Haris procedure, a number of products were obtained unexpectedly. Separation of the products by column chromatography and fractional recrystallizations yielded the expected pseudothiourea (**9**, 12%), mp 204—205°, 3-oxindolylpseudothiourea (**10**, 23%), mp 134—135.5°, a dimeric product (**11**, 13%), 2-indolyl sulfide (**12**, 2%), oxindole (**13**, 3%), and the dioxindole (**14**, 6%). The structure of **9** was elucidated from correct elemental analysis and the following spectral data. UV $\lambda_{max}^{EtOH}$ nm ($\varepsilon$): 219 (46700), 284 (12500). IR (KBr) $cm^{-1}$: 3430—3100 (multiplets, NH), 1654 (C=N). NMR ($D_2O$): $\delta$ 2.33 (s, 3H, $CH_3$). Further confirmation of structure **9** was obtained by the comparison with an authentic specimen prepared by the reaction of 2-bromoskatole (**15**) with thiourea in the presence of an acid.[4]

The compound (**10**) showed $\lambda_{max}^{EtOH}$ at 214 (37600) and 295 (1400) nm, similar to those of 3-bromo-3-methyloxindole.[5] The infrared (IR) spectrum of **10** was consistent with the suggested structure and in particular, the new band at 1700 $cm^{-1}$ and the bands at 1654, 3400—3100 $cm^{-1}$ in its spectrum supported the presence of a carbonyl and a pseudothiourea, res-

---

1) Location: *Yayoi-cho, Chiba-shi, 280, Japan.*
2) R.L.N. Haris, *Tetrahedron Letters*, **1969**, 4465; *idem*, *Austr. J. Chem.*, **23**, 1199 (1970).
3) T. Hino, K. Tsuneoka, M. Nakagawa, and S. Akaboshi, *Chem. Pharm. Bull.* (Tokyo), **17**, 550 (1969); T. Hino, T. Suzuki, S. Takeda, N. Kano, Y. Ishii, A. Sasaki, and M. Nakagawa, *Chem. Pharm. Bull.* (Tokyo), **21**, 2739 (1973).
4) T. Hino, M. Tonozuka, and M. Nakagawa, *Tetrahedron*, **30**, 2123 (1974).
5) R.L. Hinman and C.P. Bauman, *J. Org. Chem.*, **29**, 2431 (1964).