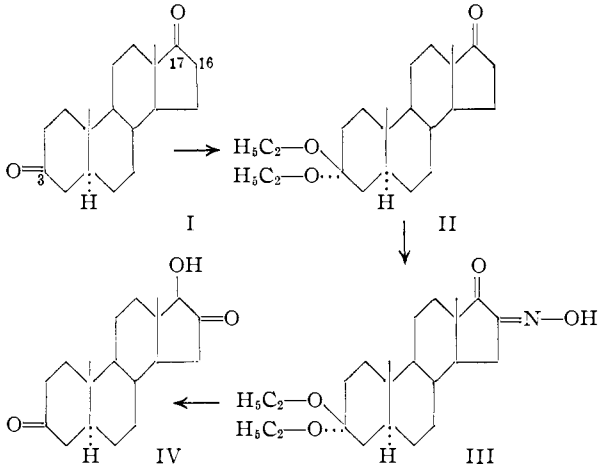
Like all 16-keto-17β-hydroxysteroids which melt as high as 200° androstan-17β-ol-3,16-dione furnishes a yellow-colored melt at its melting point.[3] In keeping with all 16-ketosteroids which we have examined, androstan-17β-ol-3,16-dione shows a strong *levo* shift in optical rotation over that of its analog unsubstituted at $C_{16}$. Thus, the optical rotation of androstan-17β-ol-3-one is +32.4° (in alcohol),[4] whereas that of 16-ketoandrostan-17β-ol-3-one is −146° (in chloroform). Similarly, the optical rotations of estradiol and 16-ketoestradiol are in alcohol +80 and −102°, respectively.[5]




**Experimental**[6]

**3,3-Diethoxyandrostan-17-one (II).**—Androstane-3,17-dione (1.00 g., Ciba) (I) was treated with 0.67 ml. of ethyl orthoformate, 10 ml. of absolute ethanol and 2 drops of 1% sulfuric acid in absolute ethanol. The mixture was refluxed for 30 minutes using adequate moisture protection. After a day at 5° the 3-diethyl ketal was collected on the filter and washed with ice-cold 95% ethanol containing a trace of pyridine. The yield of fine needles was 944 mg. melting at 121.5–122.5° (II). Serini and Köster,[7] who first prepared this compound, gave as its melting point 121–123°.

**Nitrosation of 3,3-Diethoxyandrostan-17-one.**—To 893 mg. of the steroid ketal II, as above, was added 31 ml. of a solution of potassium *t*-butoxide in *t*-butyl alcohol (≈0.76 g. of K). The mixture was stirred mechanically for a sufficient period of time to effect solution and for 5 hours longer, during which latter period 0.6 ml. of isoamyl nitrite was added at 2.5-hour intervals. The reaction mixture was transferred to a separatory funnel containing 100 ml. of aqueous glycine (≈ 15 g. of glycine) with 200 ml. of ice water and 300 ml. of ethyl ether. From the foregoing partition the separated ethereal phase was washed with 300 ml. of 3% sodium bicarbonate, and the nitroso compound then extracted from the ether by two washings with 0.5 *N* potassium hydroxide. Acidification of the combined potassium hydroxide phases (250 ml.) with concentrated hydrochloric acid (10 ml.) precipitated the 16-oximino derivative III. It was allowed to settle overnight, then filtered and washed copiously with water. It is probable that a portion of this product exists with the $C_3$-carbonyl free.

**Androstan-17β-ol-3,16-dione (IV).**—To the 16-oximino derivative III, in the preceding paragraph, was added 70 ml. of 50% acetic acid and 2.8 g. of zinc dust. The mixture was refluxed vigorously for one hour and the hot solution decanted from the zinc. The zinc was rinsed with a total of 20 ml. of acetic acid, the rinsings being combined with the main portion. To this was added 365 ml. of water, and 80% of the acetic acid was gradually neutralized with solid sodium bicarbonate. After a day at room temperature the precipitated steroid was filtered. After six recrystallizations, alternately from acetone–Skellysolve B and from aqueous methanol plus a drop of acetic acid (involving the liberal use of charcoal), there was obtained 108 mg. of leaves of androstan-17β-ol-3,16-dione which melted at 190–191.5° with yellow turning (IV). The compound crystallized as the monohydrate; $[\alpha]^{22}D$ −138° (*c*, 0.937 in chloroform, as the monohydrate), $[\alpha]^{22}D$ −146° (calculated for unhydrated compound).

*Anal.* Calcd. for $C_{19}H_{28}O_3 \cdot H_2O$: C, 70.77; H, 9.38. Found: C, 70.93; H, 9.30. (Calcd. for $C_{19}H_{28}O_3$: C, 74.96; H, 9.27).

The microanalytical data, optical rotation value, and ultraviolet and infrared absorption spectra for this compound were determined by Dr. Seymour Bernstein of the Lederle Laboratories through the courtesy of Dr. C. D. Kochakian.

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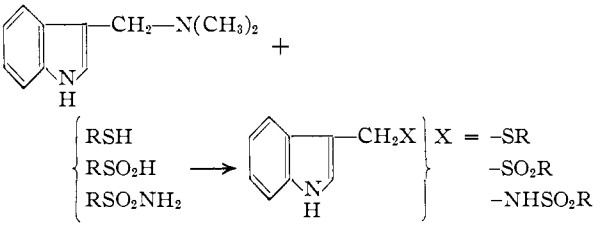
(3) M. N. Huffman and M. H. Lott, *J. Biol. Chem.*, **172**, 325 (1948).
(4) L. Ruzicka and M. W. Goldberg, *Helv. Chim. Acta*, **19**, 99 (1936).
(5) M. N. Huffman and M. H. Lott, THIS JOURNAL, **75**, 4327 (1953).
(6) All melting points listed and cited are uncorrected.
(7) A. Serini and H. Köster, *Ber.*, **71**, 1766 (1938).

## Reactions of Gramine with Sulfur Compounds

BY J. J. LICARI AND GREGG DOUGHERTY

RECEIVED MARCH 16, 1954

The ready substitution of the dimethylamino group of gramine has been utilized recently in the preparation of 3-indolemethyl derivatives which are difficult or thus far impossible to synthesize in other ways. Most of the syntheses thus far effected have been of the nature of C–C alkylations. We have now extended this reaction to mercaptans and sulfinic acids to give sulfides and sulfones, respectively. We have also found that the H of benzenesulfonamide can easily be replaced to give N-(3-indolemethyl)-benzenesulfonamide. To our knowledge this is the first time that an N-substituted sulfonamide has been made in this way. We believe that this is a general reaction and are now in the process of showing this.




The compounds prepared by us and not previously recorded in the literature are given in Table I.

Yields for the sulfides ranged from 70 to 92% using 2:1 mole ratio of mercaptan to gramine, and refluxing only 15 min. 3-Indolemethylsodium sulfonate previously prepared from gramine and sodium sulfite at high temperature and pressure[1] was obtained in 83% yield by simply refluxing the components 15 min. at atm. pressure. In view of the similarity of some of the compounds prepared to sulfanilamide they may possess bacteriostatic activity. The thioglycolic derivative in view of its similarity to the plant growth factor indoleacetic acid is at present being tested for any activity.

### Experimental

Gramine was prepared according to the instructions of Kühn and Stein.[2] All the sulfinic acids were prepared by

(1) H. Erdtman and T. Pettersson, *Acta Chim. Scand.*, **3**, 904 (1950).
(2) H. Kühn and O. Stein, *Ber.*, **70**, 567 (1937).

TABLE I

PROPERTIES OF THE 3-INDOLEMETHYLSULFUR COMPOUNDS

| No. | Compound | Formula | M.p., °C. | Carbon, % Calcd. | Carbon, % Found | Hydrogen, % Calcd. | Hydrogen, % Found |
|---|---|---|---|---|---|---|---|
| 1 | 3-Indolemethyl methyl sulfide | $C_{10}H_{11}NS$ | 87–88 | 67.74 | 67.38 | 6.25 | 5.90 |
| 2 | 3-Indolemethyl ethyl sulfide | $C_{11}H_{13}NS$ | 48–49 | 69.07 | 69.20 | 6.85 | 6.52 |
| 3 | 3-Indolemethyl *n*-propyl sulfide | $C_{12}H_{15}NS$ | 47 | 70.19 | 69.9 | 7.36 | 7.52 |
| 4 | 3-Indolemethyl *n*-butyl sulfide | $C_{13}H_{17}NS$ | 43–44 | 71.50 | 71.48 | 7.81 | 7.81 |
| 5 | 3-Indolemethyl *n*-amyl sulfide | $C_{14}H_{19}NS$ | 47–48 | 72.10 | 72.11 | 8.21 | 8.30 |
| 6 | 3,3′-Diindolemethyl sulfide | $C_{18}H_{16}N_2S$ | 140–141 | 73.94 | 73.49 | 5.52 | 5.63 |
| 7 | S-(3-Indolemethyl)-thioglycolic acid | $C_{11}H_{11}NSO_2$ | 110–111 | 59.71 | 60.00 | 5.01 | 4.72 |
| 8 | 3-Indolemethyl methyl sulfone | $C_{10}H_{11}NO_2S$ | 154–155 | 57.41 | 57.55 | 5.30 | 5.01 |
| 9 | 3-Indolemethyl ethyl sulfone | $C_{11}H_{13}NO_2S$ | 142–144 | 59.17 | 59.74 | 5.87 | 5.87 |
| 10 | 3-Indolemethyl phenyl sulfone | $C_{15}H_{13}NO_2S$ | 160–161 dec. | 66.40 | 66.72 | 4.83 | 4.98 |
| 11 | 3-Indolemethyl *p*-toluenesulfone | $C_{16}H_{15}NO_2S$ | 162 dec. | 67.35 | 67.37 | 5.30 | 5.13 |
| 12 | N-(3-Indolemethyl)-benzenesulfonamide | $C_{15}H_{14}N_2SO_2$ | 160–163 | 62.91 | 63.22 | 4.91 | 4.84 |

reduction of the sulfonyl chloride in ether by Zn or $Na_2SO_3$[3] and used either as the free acid or the Zn or Na salts. The following preparations are submitted as representative.

**3-Indolemethyl *n*-Amyl Sulfide.**—Two grams of gramine (0.0114 mole), 0.5 g. of NaOH and 1.2 g. (0.0114 mole) of *n*-amyl mercaptan were refluxed for 15 min. in 25 cc. of $H_2O$, cooled, acidified with dilute acetic acid to remove unreacted gramine. The oil which at first forms solidifies on cooling and scratching. The solid after slurrying in $H_2O$ is recrystallized from pet. ether (30–50° fraction).

**S-(3-Indolemethyl)-thioglycolic Acid.**—3.4 g. of gramine, 2.0 cc. of thioglycolic acid, 25 cc. of 1 *N* NaOH and 25 cc. of $H_2O$ were refluxed for 30 min. At the end of 15 min. all solid went into soln. Upon cooling and acidifying with dilute acetic acid a solid separated which was washed with $H_2O$ and recrystallized from benzene.

**3-Indolemethyl Methyl Sulfone.**—Two grams (0.0114 mole) of gramine and 2 g. of zinc methyl sulfinate (0.009 mole) in 40 cc. of 95% $C_2H_5OH$ were refluxed for 1 hr. The contents became milky white. The vapor gave a basic test to litmus and the strong odor of dimethylamine was detected. The contents was filtered after cooling to remove small amounts of inorganic zinc salt. The alcohol solution was evaporated almost to dryness, water added, and the whole extracted with ether. The ether extract upon evaporation yielded a solid which was slurried in dilute acetic acid and then $H_2O$; recrystallized from EtOH.

**N-(3-Indolemethyl)-benzenesulfonamide.**—Gramine (1 equiv.) and benzenesulfonamide (2 equiv.) were refluxed in water for 20 min. at which time an oil formed which solidified on cooling. The solid was washed with dil. HOAc, $H_2O$, EtOH, and ether, m.p. 160–163° (from benzene).

**3,3′-Diindolemethyl Sulfide.** (A).—One gram of gramine (0.0057 mole) and 0.72 g. of $Na_2S\cdot 7H_2O$ (0.003 mole) in 20 cc. of $H_2O$ were refluxed for 30 min. and filtered hot. The residue was slurried twice with hot $H_2O$ and recrystallized from $CH_3OH$ to give white crystals, m.p. 140–141°.

(B).—Reaction with $NaSH\cdot 3H_2O$ gave a white solid, m.p. 142°, mixed melting point with A was 142°.

(3) F. Ullmann, *ibid.*, **34**, 1153 (1901).

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## Lack of Inhibition of Desoxyribonuclease by Heat Depolymerized Desoxyribonucleic Acid[1]

BY N. B. KURNICK[2]

RECEIVED MARCH 9, 1954

In a recent report,[3] we noted that desoxyribonuclease (DNase) depolymerized highly polymerized desoxyribonucleic acid (DNA) at a much greater rate than it did heat depolymerized DNA. In order to determine whether the slower depolymerization of heat treated DNA in our experiments was due to inhibition of the enzyme by the products of heat treatment or only to lesser affinity of the DNase for the heated DNA, the following simple experiment was performed.

**Method.**—A sample of the substrate solution (*p*H 7.5) for DNase determination by our methyl green method[4,5] was heated for 30 minutes in a boiling water-bath. After cooling, the rates of depolymerization of heated DNA, untreated substrate, and equal parts of the heated and unheated substrates were determined.[5] The enzymes used were crystalline bovine pancreatic DNase and rabbit serum.

**Results.**—The results are presented in Tables I and II. Typical experiments are shown in Fig. 1. Comparison of the slopes of curves 1 (unheated DNA) and 2 (heated DNA) confirms our earlier finding that heated DNA is depolymerized at a much lower rate than is the unheated substrate. Since the slopes of curves 1 and 3 (mixture of equal parts heated and unheated DNA) are nearly equal, it follows that there is no inhibition by heated DNA. In none of the 9 experiments with crystalline DNase

TABLE I

VELOCITIES OF DEPOLYMERIZATION OF HEATED AND UNHEATED DNA BY CRYSTALLINE DNASE

| Date | DNase, μg./19 ml. substrate–enzyme mixture | Slopes[a] Unheated (P) $v_1$ | Heated (H) $v_2$ | Mixture (P) + (H) $v_3$ | Probability (P) that $v_1 = v_3$ |
|---|---|---|---|---|---|
| 11/19 | 0.53 | 0.52 ± 0.05 | 0.26 | 0.50 ± 0.03 | >0.7 |
| 10/12 | 0.67 | .94 ± .15 | .34 | 0.85 ± .07 | > .6 |
| 11/19 | 1.0 | .95 ± .09 | | 0.62 ± .11 | > .05 |
| 11/24 | 1.0 | 1.17 ± .12 | .72 | 1.19 ± .17 | > .8 |
| 9/30 | 1.22 | 1.19 ± .04 | .25 ± 0.01 | 1.10 ± .09 | > .3 |
| 10/9 | 1.0 | 1.20 ± .15 | .42 ± .06 | 1.11 ± .09 | > .6 |
| 11/13 | 4.0 | 3.6 ± .27 | 1.0 | 3.0 ± .16 | > .2 |
| 11/16 | 4.0 | 4.3 ± .4 | 1.94 | 4.5 ± .3 | > .7 |
| 9/23 | 5.0 | 5.1 ± 1.3 | 1.1 | 3.5 ± 1.4 | > .3 |

[a] The slope is expressed as a positive function ±S.E. The equation for the curves is, therefore, $y = a - bx$, where $b$ is the slope, $y$ is the optical density, and $x$ is expressed in $10^3$ minutes.

(1) This work was supported in part by grants from the National Heart Institute, U. S. Public Health Service (H-714(C3)); the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council; and the Life Insurance Medical Research Fund.

(2) Department of Medicine, University of California Medical School, Los Angeles, California.

(3) N. B. Kurnick, THIS JOURNAL, **76**, 417 (1954).

(4) N. B. Kurnick, *Arch. Biochem.*, **29**, 41 (1950).

(5) N. B. Kurnick, *Arch. Biochem. and Biophys.*, **43**, 97 (1953).