TABLE I

METHIONINE YIELDS FROM PENICILLIUM CELLS

Expt. no.	Wt. of cells extracted, mg.	Vol. of H_2O used, ml.	Wt. of cold methionine added, mg.	Wt. of solids[c] extracted from cells, mg.	Activity of extract, μc.	% of cell activity in extract	Sp. act. of methionine, d.p.m./mg.	% of starting activity in methionine
1	100	5	100	..	..	..	426,000	19
2	400	25	100	137	198[b]	71	1,500,000	17.5
3	1000	25	250	376	404	58	2,160,000	24.5[d]
4	727	25	None	251	168	33	.....	...
5	946	50[a]	153	305	305	45	1,625,000	8.3

[a] 80% Ethanol–water (v./v.) was used for this extraction. [b] Two additional water extractions removed 12.5 and 5.8 μc. in that order. [c] These solids contain about 6 mg. of methionine and traces of other ninhydrin reacting substances; most of the weight is accounted for by salts and undetermined organic constituents. [d] Further extraction of cells gave another 100 μc. of activity which raises the potential radioactive yield of methionine to 30%.

will release methionine in amounts which represent a 30% conversion from the precursor sulfur-35. Since the conversion of sulfate-sulfur to penicillin itself is of the order of 20–30%, it can be seen that the role played by methionine in sulfate metabolism must be a large one.

As cited earlier, cystine has been shown to be incorporated, probably as a single unit, into penicillin.[5] Conversion of methionine to cysteine by way of cystathionine has been demonstrated by Rachele, *et al.*,[9] in the rat. Horowitz[10] has found the reverse reaction in *Neurospora*, and Lampen, *et al.*, have found evidence for methionine–cysteine conversions in both directions using *E. coli* mutants.[11] Hence, the conversion of inorganic sulfur to penicillin and methionine in approximately equal amounts, may suggest a role for methionine in penicillin biosynthesis.

Williams and Dawson,[12] and Wood and Mills[13] have prepared radioactive methionine by the hydrolysis of yeast protein, followed by a number of other manipulations. In our preparation, aqueous extraction of the mycelium followed by a single crystallization with added carrier gives methionine with a radiopurity of >99% in yields, based on starting radiosulfate, of up to 30%. These yields represent a four-fold to thirty-fold increase over those obtained from yeast biosynthesis.[12,13]

Experimental

The data from several experiments are summarized in Table I.

It may be seen from Table I that omitting carrier methionine during the mycelium extraction step reduces the amount of radioactivity removed from the cells (column 7). Hence, the methionine is probably reversibly bound to the mycelium and can be displaced by added methionine. Aqueous ethanol (exp. 5, Table I) is an unsatisfactory solvent for this step. Most of the radioactivity in the aqueous extract is in the form of methionine, the balance being present mainly as inorganic sulfate. Only traces of cysteine have been detected in these extracts.

The methionine was initially observed on autoradiographs of two-dimensional paper chromatograms[14] of mycelial extracts. Addition of carrier methionine to the extracts resulted in exact correspondence of the position and shape of the ninhydrin spots on paper due to carrier and the autoradiographic spots on X-ray film. As additional evidence, the methionine sulfoxide artifact always found on paper chromatograms[15] of methionine likewise duplicated its counterpart on film. Crystallization of the radioactive methionine plus carrier from 80% ethanol (v./v.) gave a product whose specific activity did not change on repeated crystallization. Rechromatographing this crystalline material likewise failed to show any separation of radioactivity and methionine ninhydrin color. Conversion of the crystalline methionine to the crystalline benzoate and acetate gave no change in the molar specific activity. Oxidation of the methionine to the sulfone with H_2O_2 by the method of Dent[15] gave a product whose chromatogram and autoradiograph showed exact coincidence of methionine sulfone spots. Thus, methionine, its sulfoxide, and the sulfone have been demonstrated to be radioactive in these experiments.

Anal. Calcd. for methionine, $C_5H_{11}O_2NS$: S, 21.4. Found: S, 21.3.

Preliminary experiments have indicated that carrier-free methionine of very high specific activity could be isolated from cell extracts by a combination of charcoal[16] and ion-exchange resin chromatography.[17]

(9) J. R. Rachele, *et al.*, *J. Biol. Chem.*, **185**, 817 (1950).

(10) N. H. Horowitz, *ibid.*, **171**, 255 (1947).

(11) J. O. Lampen, R. R. Roepke and M. J. Jones, *Arch. Biochem.*, **13**, 55 (1947).

(12) R. B. Williams and R. M. C. Dawson, *Biochem. J.*, **52**, 314 (1953).

(13) J. L. Wood and G. C. Mills, THIS JOURNAL, **74**, 2445 (1952).

(14) A. A. Benson, J. A. Bassham, M. Calvin, T. C. Goodale, V. A. Haas and W. Stepka, *ibid.*, **72**, 1710 (1950).

(15) C. E. Dent, *Biochem. J.*, **43**, 169 (1948).

(16) H. G. Cassidy and J. Wachtel, *Science*, **95**, 233 (1942).

(17) S. Moore and W. H. Stein, *J. Biol. Chem.*, **192**, 663 (1951).

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16-Substituted Steroids. X. Androstan-17β-ol-3,16-dione

BY MAX N. HUFFMAN AND MARY HARRIET LOTT

RECEIVED MARCH 5, 1954

Lieberman, Praetz, Humphries and Dobriner[1] have recently established that oxygenation at C_{16} is a general pattern in the metabolism of steroids.

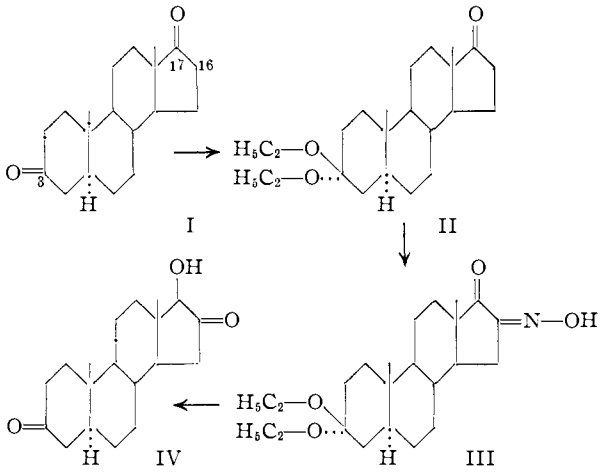
In our syntheses of 16-oxygenated steroids we have had occasion to prepare the compound androstan-17β-ol-3,16-dione, which is of considerable interest as a possible catabolite of male sex hormone in the human organism.[2]

In the present synthesis it was preferred to use as starting material an androstan-17-one with a preformed carbonyl at C_3, for the 16-keto-17-hydroxy-steroid is quite labile to oxidizing agents. Thus, androstane-3,17-dione was protected at C_3 by formation of the 3-diethyl ketal, and this derivative was nitrosated and then reduced following our established procedures. During the last step the 3-diethyl ketal is simultaneously hydrolyzed, yielding as the end-product the free androstan-17β-ol-3,16-dione.

(1) S. Lieberman, B. Praetz, P. Humphries and K. Dobriner, *J. Biol. Chem.*, **204**, 491 (1953).

(2) Also of interest in this connection are our compounds androstane-3α,17β-diol-16-one and androstane-3β,17β-diol-16-one, previously described (M. N. Huffman and M. H. Lott, *J. Biol. Chem.*, **207**, 431 (1954)).

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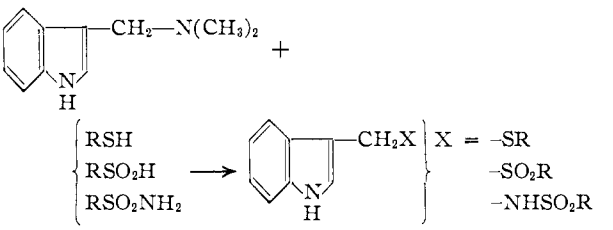
(3) M. N. Huffman and M. H. Lott, *J. Biol. Chem.*, **172**, 325 (1948).
(4) L. Ruzicka and M. W. Goldberg, *Helv. Chim. Acta*, **19**, 99 (1936).
(5) M. N. Huffman and M. H. Lott, THIS JOURNAL, **75**, 4327 (1953).
(6) All melting points listed and cited are uncorrected.
(7) A. Serini and H. Köster,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