[CONTRIBUTION FROM THE LEDERLE LABORATORIES DIVISION OF AMERICAN CYANAMID CO.]

# DEHYDROISOANDROSTERYL MERCAPTAN

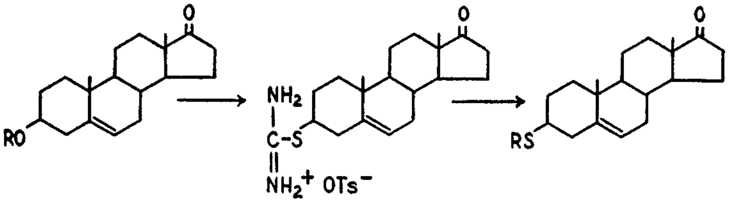
SEYMOUR BERNSTEIN AND KARL J. SAX

*Received November 15, 1950*

Dehydroisoandrosteryl mercaptan (IV) has been prepared to study the effect of the replacement of the hydroxyl by the sulfhydryl group in dehydroisoandrosterone (I) on androgenic activity.[1]

Dehydroisoandrosteryl mercaptan (IV) was prepared from dehydroisoandrosterone (I) by King, Dodson, and Subluskey's procedure (1) for cholesteryl mercaptan. Dehydroisoandrosterone (I) was converted to the known tosylate (II) (2) which, when heated with thiourea in the presence of pyridine and alcohol, gave dehydroisoandrosterylisothiuronium tosylate (III). Hydrolysis of the tosylate (III) with alcoholic sodium hydroxide, and acidification of the reaction mixture gave the desired mercaptan (IV). This product was further characterized by the preparation of the acetate (V) and benzoate (VI).

FLOWSHEET



I. R=H
II. R=Ts

III.

IV. R=H
V. R=Ac
VI. R=Bz

The isothiuronium tosylate (III), mercaptan (IV), acetate (V), and benzoate (VI) have been assigned the $\beta$-configuration at the C-3 carbon atom in accordance with the recent work of Ralls, Dodson, and Riegel (3).

In Figure I the infrared spectra of compounds II, III, IV, and V are presented.

An analysis (4) of the optical rotatory powers of the mercaptan (IV) and its two derivatives according to the Method of Molecular Rotation Differences, is presented in Tables I and II.

In Table I, the data indicated that in the mercaptan series, with the benzoate, a 17-keto-group exerts a slight but definite "vicinal" effect on the C-3 center. This is in contrast to the apparent absence of "vicinal" action in the corresponding oxygen compound (4f). In Table II the influence of the side chain

---

[1] Pertinent to the work presented herein, Malisoff and Malisoff, Abstracts of papers, Division of Organic Chemistry, American Chemical Society Meeting, Pittsburgh, Pa., Sept. 6–10, 1943, have announced the preparation of estrone mercaptan; but no publication on the subject has been forthcoming.

on the molecular rotation difference in the conversion of the $C_3$ $\beta$-OH ($\Delta^{5:6}$) to a $C_3$ $\beta$-SH ($\Delta^{5:6}$) group is examined. The optical anomaly ($\Delta\Delta = 6$) indicates no apparent "vicinal" action by the $C_{17}$-keto-group in this transformation.

In Table III the ultraviolet absorption characteristics ($\lambda_{max}$ and $\epsilon_{max}$) of the acetate, benzoate, thioacetate, and thiobenzoate of both cholesterol and dehydroisoandrosterone are listed. It is apparent that in the C-3 sulfur series there is a bathochromic effect[2] on the principal maximum in both the acetate and benzoate derivatives, *ca.* 30 and 10 m$\mu$, respectively. For the acetate, there would

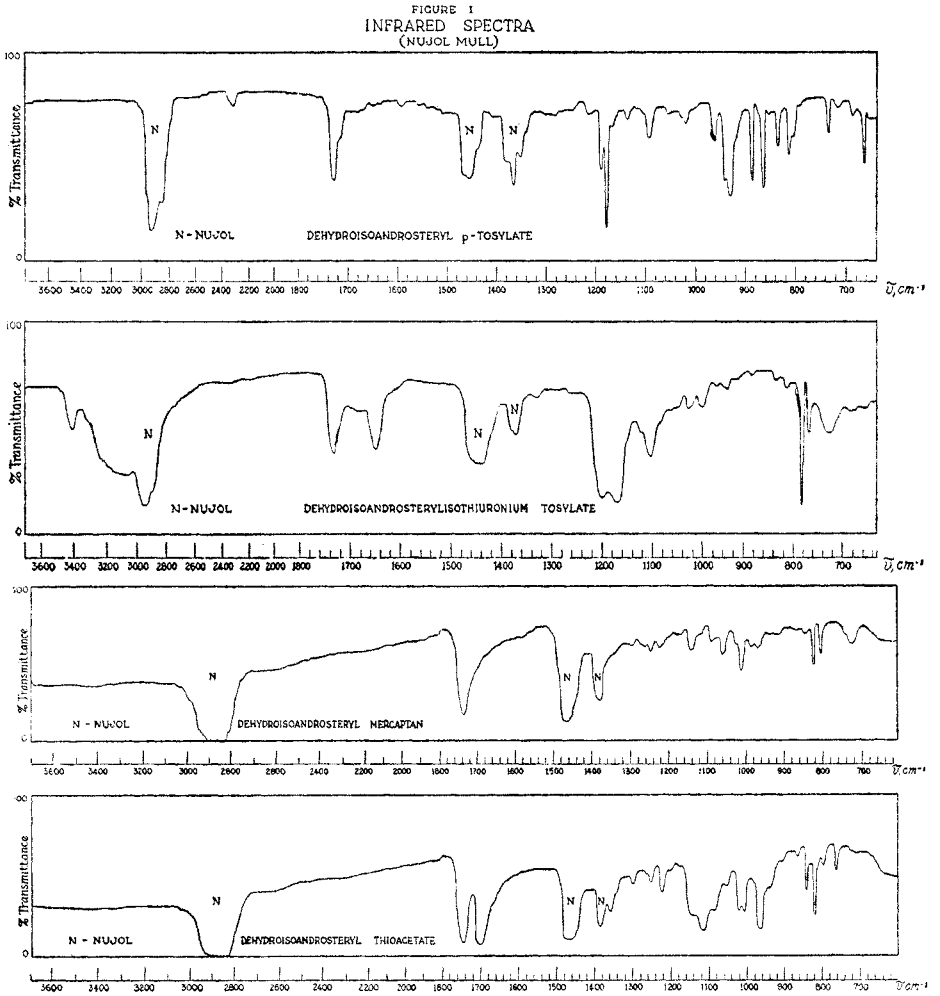


FIG. 1

---

[2] The bathochromic effect of sulfur on the ultraviolet absorption spectrum of steroids has been demonstrated by Ralls, Dodson, and Riegel (3) on 3-substituted $\Delta^{3,5}$-cholestadienes; see also Braude (6).

appear also to be a hyperchromic effect; with the benzoate, a hypochromic effect. It is to be noted that in the oxygen series the benzoate has two minor absorption maxima at *ca.* 273 and 280 mμ; in the sulfur series, there is only one other maximum (*ca.* 270 mμ) which is relatively strong.

The above noted optical rotational anomaly in the thiobenzoate may therefore be explainable to a certain degree by the tentative suggestion of Barton and Cox (4f) of a possible qualitative correlation between anomalies and the ultraviolet absorption spectra of the compounds.

It was interesting to observe that dehydroisoandrosteryl mercaptan (IV) did not give a sparingly soluble digitonide with digitonin under the same qualitative conditions (90% alcohol) that the test for cholesterol was positive. Cholesteryl mercaptan and 7-dehydrocholesteryl mercaptan likewise did not give an insoluble digitonide (5).

TABLE I

ROTATION ANALYSIS: INFLUENCE OF 17-KETO GROUP ON ROTATION OF DERIVATIVES

| SUBSTANCE | $[M]_D$ | | | $\Delta_1$ | $\Delta_2$ | $\Delta\Delta_1$ | $\Delta\Delta_2$ |
|---|---|---|---|---|---|---|---|
| | Mercaptan | Acetate | Benzoate | | | | |
| Cholesteryl mercaptan | $-92^a$ | $-220^a$ | $-138^a$ | −128 | −46 | — | — |
| Dehydroisoandrosteryl mercaptan | +74 | −59 | +54 | −133 | −20 | −5 | +26 |

[a] Ref. (5). Mercaptan, $[\alpha]_D^{29}$ −23° ($CHCl_3$); thioacetate, $[\alpha]_D^{30}$ −49.6° ($CHCl_3$); thiobenzoate, $[\alpha]_D^{29}$ −27.2° ($ChCl_3$).

TABLE II

ROTATION ANALYSIS: INFLUENCE OF 17-KETO GROUP ON $[M]_D$ $C_3$ $\beta$—OH→$[M]_D$ $C_3$ $\beta$—SH

| SUBSTANCE | $[M]_D$ | | $(B \overset{\Delta_1}{-} A)$ | $\Delta\Delta_1$ |
|---|---|---|---|---|
| | A<br>Alcohol | B<br>Mercaptan | | |
| Cholesterol | $-154^a$ | $-92^b$ | +62 | — |
| Dehydroisoandrosterone | $+6^a$ | +74 | +68 | +6 |

[a] Ref. (4f). [b] Ref. (5).

*Bioassay for androgenic activity.*[3] Both dehydroisoandrosterylisothiuronium tosylate (III) and dehydroisoandrosteryl mercaptan (IV) were found to be inactive in the following manner. The test used was a modification of the baby chick test for androgens (7). One week old baby chicks received 50 γ of compound in 0.05 ml. of sesame oil on the comb daily for seven days. A control group received oil only, and the standard reference group, 50 γ of dehydroisoandrosterone. This daily dose of dehydroisoandrosterone doubled the comb

---

[3] We are indebted to Dr. F. I. Dessau of this laboratory for the hormone assays and for supplying us with the pertinent information on the assay method.

weight during the course of the experiment. This relatively weak androgen was chosen as the standard substance for a qualitative screening test for in this way it was felt that potential weak androgens could be recognized more easily.

Experiments are planned to determine whether the mercaptan (IV) may possess any so-called "anti-hormone" properties.

## EXPERIMENTAL

*Melting points.* All melting points were determined with uncalibrated Anschütz thermometers (total immersion).

*Ultraviolet absorption spectra.* All spectra were determined with a Beckman quartz spectrophotometer (Model DU, mfg. by the National Technical Laboratories, Pasadena, California).[4]

*Infrared spectra.* All spectra were determined by Dr. Robert C. Gore, Stamford Research Laboratories, American Cyanamid Company, with a Perkin-Elmer instrument converted to a double-beam spectrophotometer.

TABLE III

ULTRAVIOLET ABSORPTION DATA (220–310 mμ, 1% CA)

| COMPOUND | ABSORPTION MAXIMA MOLECULAR EXTINCTION COEFFICIENTS |
|---|---|
| Cholesteryl acetate | (CO-O-group, $\lambda_{max}^{H_2O}$ 204 mμ, $\epsilon = 40$) (6) |
| Cholesteryl benzoate | $\epsilon_{229.5} = 15{,}300$, $\epsilon_{273} = 970$, $\epsilon_{280} = 760$ (5) |
| Cholesteryl thioacetate | $\epsilon_{232.5-234} = 4{,}630$ (5) |
| Cholesteryl thiobenzoate | $\epsilon_{238-239} = 11{,}500$, $\epsilon_{269-271} = 8{,}350$ (5) |
| Dehydroisoandrosteryl acetate | (see above) |
| Dehydroisoandrosteryl benzoate | $\epsilon_{229} = 14{,}300$, $\epsilon_{272.5} = 990$, $\epsilon_{280} = 790$ (5) |
| Dehydroisoandrosteryl thioacetate | $\epsilon_{233-234} = 4{,}670$ |
| Dehydroisoandrosteryl thiobenzoate | $\epsilon_{239} = 12{,}100$, $\epsilon_{270} = 9{,}150$ |

*Optical rotations.* The sample was dissolved in chloroform to make 2 ml. of solution, (unless otherwise stated), and the rotation was determined in a 1-dcm. semi-micro tube. With one exception, the rotation was determined for two wavelengths, 5893Å (D) and 5461Å (Hg).

*Dehydroisoandrosteryl p-toluenesulfonate (tosylate)* (II). This compound was prepared essentially by the procedure of Butenandt and Grosse (2), m.p. 156.5–160.5°, clear melt with decomposition at 162°; yield 83%.

*Dehydroisoandrosterylisothiuronium tosylate* (III). Dehydroisoandrosteryl tosylate (m.p. 156.5–160.5°, clear melt, d. at 162°) (1.5 g.) (II) 3.1 g. of thiourea, and 1.7 ml. of pyridine in 17 ml. of absolute alcohol were refluxed on the steam-bath for 3 hours. The mixture was diluted with water, and worked with a glass rod. This gave crystals which were cooled in an ice-bath and were washed with 25% alcohol; 1.2 g., m.p. 233–237°d. Three recrystallizations from methanol-acetone gave pure III, m.p. 254°d.,[5] with previous shrinkage at 252–254°. $[\alpha]_D^{30}$ +11.7°, $[\alpha]_{Hg}^{30}$ +18.4° (23.9 mg. in 2 ml. abs. alc., $\alpha_D^{30}$ +0.14°, $\alpha_{Hg}^{30}$ +0.22°); $\alpha_{Hg}/\alpha_D = 1.57$.

---

[4] In UV absorption data, the term $\lambda^{1\%CA}$ refers to a solution in 1 ml. of chloroform diluted to 100 ml. with commercial abs. ethanol.

[5] The m.p. appeared to be dependent on the manner performed (temperature of insertion and rate of heating).

*Anal.* Calc'd for $C_{27}H_{38}N_2O_4S_2$ (518.71): C, 62.51; H, 7.38; N, 5.40; S, 12.36.
Found: C, 62.51; H, 7.63; N, 5.59; S, 12.76.

In another run with 30.2 g. of II, 62 g. of thiourea, 34 ml. of pyridine, and 340 ml. of absolute alcohol (4 hours reflux) there was obtained 16.55 g. of III, m.p. 260–261° d.,[4] and 1.3 g., m.p. 258–260°d.; yield 50%.

*Dehydroisoandrosteryl mercaptan* (IV). Dehydroisoandrosterylisothiuronium tosylate (III) (520 mg.) was refluxed with a solution of 0.12 g. of sodium hydroxide in 15 ml. of alcohol, and gave a slightly turbid solution. Water (1.1 ml.) was added; the turbidity persisted. The mixture was refluxed on the steam-bath for 2 hours, and poured into ice-water. A finely divided precipitate separated after acidification with glacial acetic acid. After standing at room temperature for ½ hour, the crystals were washed with a copious amount of water, wt. 0.28 g., m.p. 172–174° with previous softening at 170–172°. Recrystallization from benzene-petroleum ether (b.p. 64–66°) gave pure IV, wt. 0.23 g., m.p. 173–175°; $\lambda^{1\%\,\mathrm{CA}}_{\mathrm{max}}$ none (220–310 mμ); $[\alpha]^{30}_{\mathrm{D}}$ +24.4°. $[\alpha]^{30}_{\mathrm{Hg}}$ +36.2° (27.05 mg., $\alpha^{30}_{\mathrm{D}}$ +0.33°, $\alpha^{30}_{\mathrm{Hg}}$ +0.49°) $\alpha_{\mathrm{Hg}}/\alpha_{\mathrm{D}} = 1.48$. Yield 75.5%.

*Anal.* Calc'd for $C_{19}H_{28}OS$ (304.47): C, 74.95; H, 9.27; S, 10.53.
Found: C, 74.47; H, 9.33; S, 10.83.

In another experiment with 44.3 g. of III, 1200 ml. of alcohol, 9.6 g. of sodium hydroxide, and 100 ml. of water there was obtained 18.7 g., m.p. 173–175°, and 5.24 g., m.p. 174–176°; yield 92%.

The mercaptan in alcohol gave an immediate yellow precipitate (lead mercaptide) on treatment with lead diacetate in 70% alcohol.

*Dehydroisoandrosteryl thioacetate* (*V*). Dehydroisoandrosteryl mercaptan (1 g.) was refluxed for 40 minutes with 20 ml. of acetic anhydride. The excess anhydride was removed by distillation *in vacuo*. The residue was crystallized from dilute acetone, wt. 0.92 g., m.p. 190–192°. Two recrystallizations from dilute acetone gave 0.60 g.; m.p. 194–195°; $\lambda^{\mathrm{abs.}}_{\mathrm{max.}}$ alc. 232–234 mμ, $\epsilon = 4200$. $\lambda^{1\%\,\mathrm{CA}}_{\mathrm{max}}$ 233–234 mμ, $\epsilon = 4670$. $[\alpha]^{29}_{\mathrm{D}}$ −17.0° (28.3 mg., $\alpha^{29}_{\mathrm{D}}$ −0.24°).

*Anal.* Calc'd for $C_{21}H_{30}O_2S$ (346.51): C, 72.79; H, 8.73; S, 9.25.
Found: C, 73.02; H, 8.82; S, 9.39.

*Dehydroisoandrosteryl thiobenzoate* (VI). The mercaptan (IV) (100 mg.) was dissolved in 3 ml. of pyridine; the solution was cooled in an ice-bath, and treated with 0.1 ml. of benzoyl chloride. The mixture was allowed to stand for 2 days at room temperature. Addition of cold dilute hydrochloric acid gave crystals which were washed successively with cold, dilute hydrochloric acid, water, and methanol, m.p. 212–232°. Recrystallization to constant m.p. from benzene-alcohol gave 80 mg. of VI, m.p. 242–234.5°, $\lambda^{1\%\,\mathrm{CA}}_{\mathrm{max}}$ 239, 270 mμ, $\epsilon_{239} =$ 12,100, $\epsilon_{270} = 9150$, $[\alpha]^{29}_{\mathrm{D}}$ +13.3°, $[\alpha]^{29}_{\mathrm{Hg}}$ +20.0 (18.1 mg. in 2 ml. of chloroform gave $\alpha^{29}_{\mathrm{D}}$ +0.12°, $\alpha^{29}_{\mathrm{Hg}}$ +0.18°, resp., $\alpha_{\mathrm{Hg}}/\alpha_{\mathrm{D}} = 1.52$.

*Anal.* Calc'd for $C_{26}H_{32}O_2S$ (408.58): C, 76.43; H, 7.90; S, 7.85.
Found: C, 76.60; H, 8.07; S, 8.21.

## SUMMARY

1. Dehydroisoandrosteryl mercaptan has been prepared from dehydroisoandrosteryl *p*-toluenesulfonate.

2. The acetate and benzoate of the mercaptan have been prepared and characterized.

3. An analysis of the optical rotatory powers of the mercaptan and its two derivatives according to the Method of Molecular Rotation Differences has been presented.

4. In a modified chick comb test dehydroisoandrosteryl mercaptan showed no androgenic activity at a level of 50 γ daily for seven days.

PEARL RIVER, NEW YORK

## REFERENCES

(1) King, Dodson, and Subluskey, *J. Am. Chem. Soc.*, **70,** 1176 (1948).

(2) Butenandt and Grosse, *Ber.*, **69,** 2776 (1936).

(3) Ralls, Dodson, and Riegel, *J. Am. Chem. Soc.*, **71,** 3320 (1949).

(4) (a) Bernstein, Kauzmann, and Wallis, *J. Org. Chem.*, **6,** 319 (1941); (b) Bernstein, Wilson, and Wallis, *J. Org. Chem.*, **7,** 103 (1942); (c) Barton, *J. Chem. Soc.*, 813 (1945); (d) Barton, *J. Chem. Soc.*, 512 (1946); (e) Barton, *J. Chem. Soc.*, 1116 (1946); (f) Barton and Cox, *J. Chem. Soc.*, 783 (1948), and subsequent publications.

(5) Bernstein and Sax, *J. Org. Chem.*, **16,** following article.

(6) Braude, *Ann. Repts. on Progress Chem.* (*Chem. Soc. London*), **42,** 105 (1946).

(7) Frank and Klempner, *Proc. Soc. Exptl. Biol. Med.*, **36,** 763 (1937); Frank, Klempner, and Hollander, *Proc. Soc. Exptl. Biol. Med.*, **38,** 853 (1938).