[CONTRIBUTION FROM THE CHEMICAL RESEARCH DIVISION OF G. D. SEARLE & CO.]

Addition of Alkanethiolic Acids to $\Delta^{1,4}$ -3-Oxo- and $\Delta^{4,6}$ -3-Oxosteroids¹

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Acylthiosteroids have been prepared by addition of ethanethiolic and propanethiolic acids to $\Delta^{1,4}$ -3-oxo- and $\Delta^{4,6}$ -3-oxosteroids. By analogy to other additions of ethanethiolic acid and to other additions to steroids, by use of molecular rotational data and by consideration of steric interactions, the acylthio groups have been assigned the 1α - and 7α -positions, respectively.

In recent years a number of alterations have been made in the A and B rings of the natural steroid hormones in an effort to enhance certain desirable physiological activities and reduce undesirable side effects.² Since general methods for the preparation of $\Delta^{1,4}$ -3-oxosteroids from the readily available Δ^4 -3-oxosteroids recently have been published,^{3,4} we investigated reactions for the addition of small groups to the 1,2-double bond. Amines,⁵ hydrogen chloride,6 diazomethane7 and ethyl diazoacetate⁸ have been added to Δ^{16} -20-oxosteroids. Attempts to add these reagents to $\Delta^{1,4}$ -3ketones were unsuccessful. While this work was in progress Nussbaum⁹ also found that $\Delta^{1,4}$ -3ketones did not react with diazomethane. It appeared from this evidence that this system was much less reactive toward addition than the Δ^{16} -20-ketones.^{10,11}

Ethanethiolic acid has been added to a number of α,β -unsaturated aldehydes and ketones.¹² These included not only simple compounds such as acrolein, β,β -dimethylacrolein, 2-ethyl-2-hexenal, crotonaldehyde, cinnamaldehyde and mesityl oxide, but also more complicated molecules, *i.e.*, ethyl 6-keto-7-octenoate and 1-p-nitrophenyl-2-dichloro-

(1) Presented before the 133rd National Meeting of the American Chemical Society, San Francisco, Calif., April 6-11, 1958, Abstracts, page 6-M.

(2) (a) W. Klyne, Ann. Reports, 52, 209 (1955); (b) P. Bladon, *ibid.*, 53, 223 (1956); (c) G. B. Spero, J. L. Thompson, F. H. Lincoln, W. P. Schneider and J. A. Hogg, THIS JOURNAL, 79, 1515 (1957); (d) B. J. Magerlein and J. A. Hogg, *ibid.*, 79, 1508 (1957); (e) and other references given in these papers.

(3) J. Fried, R. W. Thoma and A. Klingsberg, *ibid.*, **75**, 5764 (1953).

(4) (a) C. Meystre, H. Frey, W. Voser and A. Wettstein, *Helv. Chim. Acta*, **39**, 734 (1956); (b) S. A. Szpilfogel, T. A. P. Posthumus, M. S. de Winter and D. A. Van Dorp, *Rec. trav. chim.*, **75**, 475 (1956).

(5) D. Gould, E. L. Shapiro, L. E. Finckenor, F. Gruen and E. B. Hershberg, This JOURNAL, 78, 3158 (1956).

(6) R. M. Dodson and P. B. Sollman, U. S. Patent 2,708,201, May 10, 1955.

(7) A. Sandoval, G. Rosenkranz and C. Djerassi, *ibid.*, **73**, 2383 (1951)

(8) G. P. Mueller and B. Riegel, *ibid.*, **76**, 3686 (1954).

(9) A. L. Nussbaum and F. E. Carlon, ibid., 79, 3831 (1957).

(10) D. F. Fukushima and T. F. Gallagher [*ibid.*, **73**, 196 (1951)] have shown that alcohols can be added readily to Δ^{14} -20-oxosteroids in alcoholic alkali, but that no addition occurs with testosterone or with 17-hydroxy-1-androsten-3-one. They attribute the ready addition of alcohol to the Δ^{14} -20-oxosteroids to the relief of strain present in the *trans*-fused cyclopentene system.

(11) After this work had been reported, D. N. Kirk and V. Petrow [J. Chem. Soc., 1334 (1958)] recorded the addition of chlorine to the 1,2-double bond of a $\Delta^{1,4}$ -3-keto system.

(12) (a) W. H. Vinton, U. S. Patent 2,427,582, Sept. 16, 1947; (b)
J. R. Catch, A. H. Cook, A. R. Graham and I. Heilbron, J. Chem. Soc.,
1609 (1947); (c) R. Brown, W. E. Jones and A. R. Pinder, *ibid.*, 2123
(1951); (d) M. W. Bullock, J. A. Brockman, Jr., E. L. Patterson, J. V.
Pierce, M. H. von Saltza, F. Sanders and E. L. R. Stokstad, THIS
JOURNAL, 76, 1828 (1954); (e) J. Farkas and J. Sicher, Chem. Listy, 48,
695 (1954).

acetylamino-2-propenal. In all these cases the sulfur became attached to the carbon atom beta to the carbonyl group. When $\Delta^{1,4}$ -3-oxosteroids were heated with an excess of thiolic acid, the expected addition readily occurred. Because of the presumption that the addition was free radical in nature, the reaction was irradiated initially with ultraviolet light. However, since the same yields were obtained by heating the reaction on a steambath, it appeared that the light was unnecessary.

The acylthic group has been assigned the 1position by analogy to known additions to α,β unsaturated carbonyl systems.¹² This assignment, beta to the ketone, was also supported by the ease of removal of alkanethic acid from the adduct on treatment with base. The possibility that the new group was at the 5-position was eliminated by the failure of cortisone acetate to react under these conditions, and by the ultraviolet spectra of the adducts.

The α -configuration of the 1-acylthio group was based mainly on analogy to the known epoxidations of Δ^{1} -3-oxosteroids, which Tamm and co-workers have proved to give the α -oxide.¹³ Molecular rotatory differences between the 1-acylthiosteroids and their unsubstituted analogs [$MD(RSCOCH_3)$ -MD(RH)] provided further evidence for this assignment of configuration. The molecular rotatory contribution of the acetylthio group in 1 α acetylthio-5 α -androstane-3,17-dione (I) was +179°. For 1 α -acetylthio-4-androstene-3,17-dione (II) the



contribution was $+162^{\circ}$. In the known 1α -acetoxy-4-androstene-3,17-dione (III)¹⁴ the acetoxy group had a molecular rotatory contribution [*M*D-(ROCOCH₃) - *M*D(RH)] of $+116^{\circ}$. While no strictly comparable 1β -acetoxy compounds have been reported, Tamm, *et al.*,¹⁸ have recorded values ranging from -148 to $+27^{\circ}$ for the molecular

(13) (a) P. Striebel and C. Tamm, *Helv. Chim. Acta*, **37**, 1094 (1954);
(b) F. Sallman and C. Tamm, *ibid.*, **39**, 1340 (1956);
(c) W. Schlegel and C. Tamm, *ibid.*, **40**, 160 (1957).

(14) (a) R. M. Dodson, A. H. Goldkamp and R. D. Muir, THIS JOURNAL, **79**, 3921 (1957); (b) W. R. Benn, F. B. Colton and R. Pappo, *ibid.*, **79**, 3920 (1957).



				M.n. (dec.)			Coloulated		Found		Work
R	х	Y	z	°C.	[α]D	ΔM d	Č	H	C	H	upa
CH3	H	=0		197-199	+196.1°	$+162^{\circ}$	69.96	7.83	69.90	7.84	Α
CH3	H	но	н	Glass		• • •	69.57	8.34	69.23	8.20	В
CH3	н	OAc O	Н	171–172	+102	+122	68.28	7.98	68.08	7.72	С
C ₂ H ₅	Η	OĊEt CH₃	н	136.5-138	•••	•••	69.40	8.39	69.61	8.69	С
CH₃	H	ć≔0	н	147.5 - 149	+212.5	+222	71.09	8.30	71.39	8.27	в
CH:	н	CH ₂ OAc	H	149 - 150	+193	+217	67.23	7.67	67.22	7.81	С
CH3	н	ć=0	OH	202-204	+149.5	+239	64.91	7.41	64.76	7.78	в
CH:	0=	CH₂OAc	OH	217-218	+190	+50	63.00	6.77	62.61	7.05	A
CH3	βOH	Ċ=0	OH	212-213	+191	+305	62.74	7.16	62.53	7.27	С
		CH₂OCEt									
C₂H₅	0=	Ċ ≕ O	OH	160-161			64. 26	7.19	64.21	7.32	С
		IVa		217 - 218	+53	+70	66.99	7.50	67.21	7.80	Α
		IVb		191.5 - 192	+47.8	+57	67.66	7.74	67.45	7.68	в
		I		217-218	+137.8	+179	69.58	8.34	69.41	8.34	Α
6 D.	• • • •		.								

^a For meaning of letters see Experimental section.

rotatory contributions of the 1β -acetoxy group in a variety of steroids. Other ΔM D values are given in Table I.

The approach of a thiolic acid molecule to the double bond should occur more readily from the α -side. Approach from the β -side of the molecule to the 1-position would be badly hindered by the axial 10-methyl group. Furthermore, a study of models indicated crowding of an equatorial 1 β -acyl-thio group by the 2α - and 11α -hydrogens and by the 10-methyl group. Steric interference between an 11-carbonyl and the 1β -sulfur atom in a model of 1 β -acetylthiocortisone caused distortion of the model of the molecule.¹⁵ The 1α -isomer avoided this crowded environment; only the 9α -hydrogen was near.

As Connor has reported,¹⁶ alkanethiolic acids are excellent acylating agents. When steroids containing reactive hydroxyl groups were heated for an extended time with thiolic acids, acylation of the hydroxyl group occurred. However, as shown in Chart I, addition of thiolic acids to the 1,2-double bond occurred more rapidly than acylation of a hydroxyl group. With short reaction times, good yields of the hydroxyl compound were obtained.

While the above work was in progress, Agnello and Laubach reported their elegant synthesis of $\Delta^{4,6}$ -3-oxosteroids,¹⁷ thus making these compounds more readily available. Colton¹⁸ had epoxidized the 6,7-double bond in 17 β -hydroxy-4,6-andros-(15) Catalin models.

(16) R. Connor in H. Gilman's "Organic Chemistry," 2nd ed., Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 935.

(17) E. J. Agnello and G. D. Laubach, THIS JOURNAL, 79, 1257 (1957).

(18) F. B. Colton, U. S. Patent 2,738,348, March 13, 1956.

tadiene-3-one and other $\Delta^{4,6}$ -3-oxosteroids, but our attempts (in agreement with Nussbaum's results⁹) to add diazomethane failed. Here, as in the $\Delta^{1,4}$ -3-ketones, the double bond was much less reactive toward addition than the Δ^{16} -20ketones.¹⁰.



As expected from the results with the $\Delta^{1,4}$ -3oxosteroids, alkanethiolic acids added readily to the 6,7-double bond to give a 7-acylthio derivative. The absorption maximum in the ultraviolet spectrum was shifted from the 280 m μ region, characteristic of the starting $\Delta^{4,6}$ -3-oxosteroids, to the 240 m μ region, indicative of Δ^{4} -3-ketones. Most of the compounds had a maximum at 238 to 238.5 m μ with molecular extinction coefficients ranging from 17,700 (7 α -propionylthioprogesterone) to 20,300 (21-acetoxy-7 α -acetylthio - 17 α -hydroxy -4pregnene-3,20-dione). The hypsochromic shift, caused by the 7-acylthio group, was additive to that produced by an 11-ketone, since 7 α -acetylthio-4-pregnene-3,11-20-trione had a maximum at 236

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m μ , ϵ 18,000. A similar shift has been observed with 7-acetoxy compounds.¹⁹ The assignment of the 7-position for the acylthio group also was based on analogy to epoxidation.^{18,20} Colton¹⁸ and Nussbaum, *et al.*,²⁰ have shown that the 6α , 7α epoxide was formed on oxidation of the $\Delta^{4,6}$ -3ketones.

The acetylthic compounds showed characteristic bands in the infrared spectra at about 5.95, 8.8 and 9.1 μ ; although, in a few cases, the 8.8 to 9.1 doublet was merged into a single band. The propionylthic compounds had bands at 5.95 and 10.7 μ .

Pharmacology.—In the androstane–testosterone series the activities of both the 1- and 7-acylthiosteroids were lower than those of the parent compounds (unsubstituted Δ^4 -3-ketones) in an anabolic–androgenic test.²¹ However, 1α -acetylthio-17a-oxa-D-homo-4-androstene-3,17-dione (IVa), had pronounced topical anti-androgenic properties.²²



The 1α -acylthiocorticoids showed outstanding activity in the iritis test.²³ The activities in the other tests (liver glycogen,²⁴ cotton granuloma²⁵ and foot edema²⁶) were not as high. 1α -Acetylthio- 17α ,21-dihydroxy-4-pregnene-3,20-dione 21acetate (V) had anti-inflammatory activity (in the iritis test), as well as liver glycogen activity, in contrast with the parent compound, the acetate of Reichstein's S, and the 7-acetylthio derivative. The above-mentioned compound (V) is the first steroid, lacking an 11-oxygen, reported to show appreciable activity in these tests.

(19) Unpublished data, these laboratories.

(20) A. L. Nussbaum, G. Brabazon, T. L. Popper and E. P. Oliveto, THIS JOURNAL, 80, 2722 (1958).

(21) E. Eisenberg and G. S. Gordan, J. Pharmacol. Exptl. Therap., 99, 38 (1950).

(22) G. Pincus, unpublished test. The comb of a testosterone-fed male chick is anointed with an oil solution of the compound and the comb growth in 7 days is compared with controls.

(23) J. M. Clampit and L. G. Hershberger; unpublished test for local anti-inflammatory activity. The test substance plus the irritant, jequirity powder, is injected into the anterior chamber of a rabbit's eye.

(24) R. E. Olson, F. A. Jacobs, D. Richert, S. A. Thayer, L. J. Kopp and N. J. Wade, *Endocrinol.*, **35**, 430 (1944).

(25) Method of L. G. Hershberger and D. W. Calhoun [*ibid.*, **60**, 153 (1957)], modified for systemic administration of compounds.

(26) Modification of the method of J. J. Selitto and L. O. Randall, Federation Proc., 13, 403 (1954).

In the progesterone series the most active compound was 7α -acetylthio- 17α -propionoxyprogesterone (VI) which was approximately equal to progesterone in potency in the Clauberg test.²⁷

The compounds prepared in this study are listed in Tables I and II.

Experimental²⁸

Starting Materials.—Some of the $\Delta^{1,4}$ -3-ketones used as starting materials were prepared by microbiological fermentations³; others were made from their corresponding Δ^{4} -3-ketones by oxidation with selenium dioxide.⁴ The $\Delta^{4,\delta}$ -3ketones were prepared by the method of Agnello and Laubach.¹⁷ All of the starting materials are known compounds except for the two whose preparations are described.

backing that the internations are described. **4,6-Pregnadiene-3,11,20-trione**.—4-Pregnene-3,11,20trione (10 g.), 10 g. of chloranil and 0.1 g. of *p*-toluenesulfonic acid monohydrate²⁹ were dissolved in 1 liter of xylene. The solution was heated under reflux for 2 hr. and, after having cooled, was chromatographed on 1 kg. of silica gel. The unreacted chloranil was eluted with benzene, and the tetrachlorohydroquinone was eluted with 5% ethyl acetate in benzene. Then the desired product was eluted with 30% ethyl acetate in benzene. The eluents were concentrated, and the solid which formed was crystallized from acetonepetroleum ether, b.p. 60-70°, to yield 2.93 g. of 4,6-pregnadiene-3,11,20-trione, m.p. 152-154°; ultraviolet spectrum: $\lambda_{\text{matchnoil}}^{\text{matchnoil}}$ 281 m μ , ϵ 24,300; $[\alpha]p + 269.5°$.

Anal. Calcd. for $C_{21}H_{26}O_3$: C, 77.27; H, 8.03. Found: C, 76.97; H, 8.24.

An additional 1.18 g. of material, m.p. $144-152^{\circ}$, was obtained from the mother liquors, bringing the total yield to 4.11 g. (41%).

tailed noise include inquise, bringing the total yield to 4.11 g. (41%). The 17 α -Propionoxy-4,6-pregnadiene-3,20-dione.—17 α -Hydroxy-4,6-pregnadiene-3,20-dione, 1.00 g., was mixed with 1.5 ml. of propionic anhydride, 0.2 g. of p-toluenesulfonic acid monohydrate and 25 ml. of benzene, and the solution was warmed on a steam-bath for several hours. After standing 2 days the solution was washed with water and with aqueous sodium bicarbonate, and then concentrated under vacuum. The residue was crystallized from acetone-ether to yield 0.48 g. of 17α -propionoxy-4,6-pregnadiene-3,20-dione, m.p. 168–171°; ultraviolet spectrum: $\lambda_{max}^{methanol}$ 284 ma, ϵ 27,000.

Anal. Calcd. for $C_{24}H_{32}O_4$: C, 74.96; H, 8.39. Found: C, 74.65; H, 8.53.

Addition of Alkanethiolic Acids.—The acylthio compounds were prepared by mixing the unsaturated precursor with an equal weight of alkanethiolic acid. The mixture was heated under reflux for a length of time varying from onehalf to several hours, although, probably, equilibrium was achieved in 15 to 30 minutes. The mixtures were allowed to cool, and the product was separated by one of three methods: A, direct filtration; B, removal of excess alkanethiolic acid under vacuum, followed by addition of ether; or C, chromatography of the reaction product on silica gel using mixtures of ethyl acetate and benzene as eluents.

The letters in the last column of the tables of compounds indicate the method of work-up used for each compound. On chromatography, the acylthio compounds were eluted from the silica gel with a slightly less polar solvent mixture than the unsaturated precursor. A few examples are given below:

Method A, 1α -Acetylthio- 5α -androstane-3,17-dione.— 5α -Androst-1-ene-3,17-dione, 0.99 g., was dissolved in 2.0 ml. of ethanethiolic acid and heated and irradiated with an ultraviolet light for 2 hr. During this time crystals formed. The crystals were separated by filtration and washed with ether to give 0.85 g. (67%) of 1α -acetylthio- 5α -androstane-3,17-dione, m.p. 217-218° dec.; ultraviolet spectrum: $\lambda_{\text{method}}^{\text{method}}$ 232 m μ , e 4350. Method A, 21-Acetoxy- 7α -acetylthio- 17α -hydroxy-4pregnene-3,11,20-trione.—21 - Acetoxy - 17α - hydroxy - 4,6-

Method Å, 21-Acetoxy- 7α -acetylthio- 17α -hydroxy-4pregnene-3,11,20-trione.—21 - Acetoxy - 17α - hydroxy - 4,6pregnadiene-3,11,20-trione, 2.00 g., was dissolved in 2.0 ml. of ethanethiolic acid and irradiated with an ultraviolet light for 2 hr. The solid which formed during this time was sep-

(27) C. W. Emmons, "Hormone Assay," Academic Press, Inc., New York, N. Y., 1950, p. 422.

(28) All melting points were taken on a Fisher-Johns melting point apparatus. The rotations were taken in chloroform at 25° .

(29) Suggested by Dr. J. S. Baran of our laboratories.



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R	x	Y	z	M.p. (dec.), °C.	[<i>α</i>]D	ΔM D	Calcul C	ated — H	C-Fou	nd—— H	Work- up
CH₃	н	O=		Glass	$+87.5^{\circ}$	-228°	69.96	7.83	69.94	8.21	в
C_2H_5	Н	OH O	H	234 - 236	-65.7	- 587	70.20	8.54	70.32	8.52	Α
C_2H_5	Н	OCEt O	Н	Glass	-2.4	-310	69.40	8.39	69.47	8.15	с
CH₃	н	OCC6H5	н	185-186	+37	-466	72.07	7.34	72.08	7.14	в
C_2H_5	Η	CH3	н	134-136	+7.6	-572	71.60	8.51	71.66	8.48	A
C ₂ H ₅	-0	ć=0	н	153 - 155	+95.4	-383	69 .20	7.74	68 .90	7.81	в
CH_3	H	CH ₃	OH O	227-229	-38	-504	68.28	7.97	67.91	7.99	Α
CIT	TT	C=0		000 011	40 1	010	07 70	7 00	07 FF	7.05	
Cri ₃	H		OCEt	209-211	-40.1	-212	67.79	7.88	67.55	7.95	А
C₂H₅	H	CH ₂ OAc	H	92-96	+27.6	-528	67.79	7.88	67.39	8.09	В
CH3	н	Ċ==0	OH	211 - 213	+3	-437	64.91	7.41	64.73	7.52	В
CH_3	-0	CH2OAc	OH	238 - 239	+102.5	-368	63.00	6.77	62.62	6.53	А
CH₃	βOH	Č=0	OH	181-182	+50.5	-367	62.74	7.16	62.34	7.15	Α

arated by filtration and washed with ether. This material was crystallized from methylene chloride-methanol to give

was crystallized from methylene chloride-methanol to give 1.33 g. of 21-acetoxy-7 α -acetylthio-17 α -hydroxy-4-pregnene-3,11,20-trione (56%), m.p. 234-235° dec.; ultraviolet spec-trum: $\lambda_{max}^{methanol}$ 235.5 m μ , ϵ 17,400. Method A, 7 α -Acetylthio-17 α -hydroxyprogesterone. 17 α -Hydroxy-4,6-pregnadiene-3,20-dione, 2.00 g., was mixed with 2.0 ml. of ethanethiolic acid and heated on the steam-bath for 2 hr. When the dark solution was stirred with a spatula, crystals formed. The mixture was allowed to cool and was diluted with ether. The crystals which had to cool, and was diluted with ether. The mixture was allowed formed were separated by filtration to yield 1.60 g. (65%) of 7α -acetylthio-17 α -hydroxyprogesterone, m.p. 227–229° dec.; ultraviolet spectrum: $\lambda_{methanol}^{methanol}$ 238.5 m μ , ϵ 19,200.

Method B, 1a-Accetylthio-17a-oxa-D-homo-4-andros-tene-3,17-dione.—17a-Oxa-D-homo-1,4-androstadiene-3,17dione, 110 g., was mixed with 107 g. of ethanethiolic acid and heated under reflux on a steam-bath for 16 hr. The excess thiolic acid was removed by distillation under vacuum. The solid material was triturated with ether and separated by filtration to give 134 g. of product. This material was synthetic to give 104 g. of product. This internal was crystallized from methylene chloride-methanol to give 107.5 g. (81%) of 1 α -acetylthio-17a-oxa-D-homo-4-andros-tene-3,17-dione, m.p. 218–219° dec.; ultraviolet spectrum: $\lambda_{methanol}^{methanol}$ 239.5 m μ , ϵ 17,900. Anal. Calcd. for $C_{21}H_{28}O_4S$: C, 66.99; H, 7.50; S, 8.51. Found: C, 66.70; H, 7.26; S, 8.64.

Method C, 1a-Acetylthiotestosterone Acetate.-17β-Hydroxy-1,4-androstadiene-3-one, 0.52 g., was dissolved in 1.0 ml. of ethanethiolic acid and irradiated and heated with an ultraviolet light for 24 hr. The reaction mixture was then chromatographed on 25 g. of silica gel. The column was washed with benzene and with a 2% solution of ethyl acetate in benzene. The product was eluted with 10% ethyl acetate in benzene. The residue obtained after concentration of the eluate was diluted with ether, and the crystals which formed were separated by filtration to yield 0.18 g. (22%) of 1 α -acetylthiotestosterone acetate, m.p. 171-172°; ultraviolet spectrum: $\lambda_{methanol}^{methanol}$ 240.5 m μ , ϵ 16,800.

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