Anabolic Agents. 2,3-Epithioandrostane Derivatives

P. D. KLIMSTRA,[1] E. F. NUTTING, AND R. E. COUNSELL

Divisions of Chemical and Biological Research, G. D. Searle and Company, Chicago, Illinois 60680

Received March 17, 1966

A number of 2,3-epithioandrostane derivatives were prepared in the hope of obtaining compounds which possessed high anabolic and minimal androgenic activity. A comparison of the biological activity of these episulfide derivatives with the corresponding epoxides is discussed. The chemical pathway to these substances is described in detail.

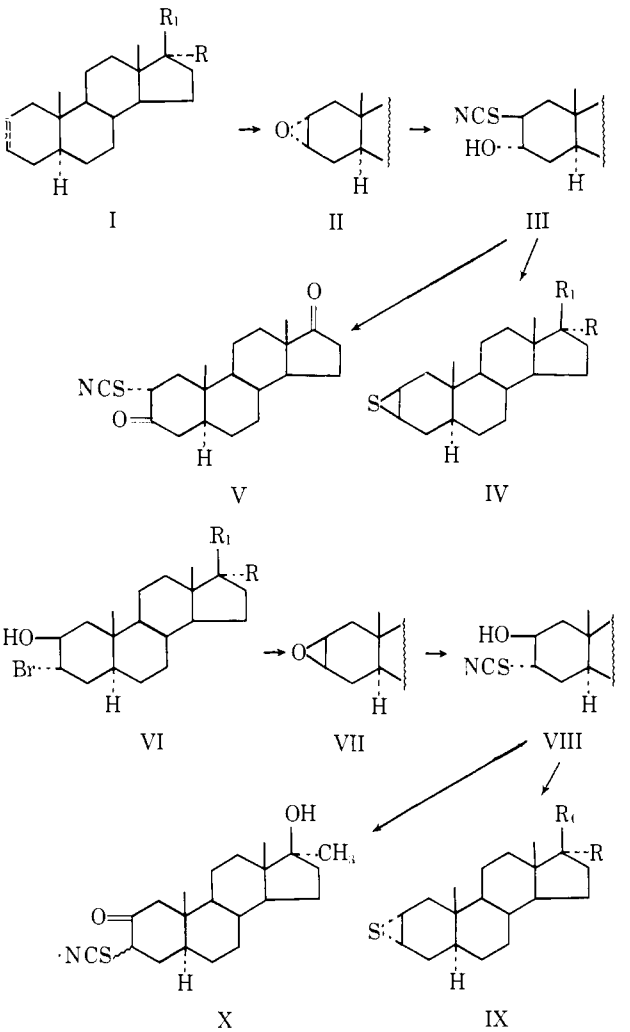
A recent report[2] from the Shionogi laboratories concerning steroidal episulfides has prompted us to describe some of our work in this area. In previous publications[3,4] we recorded attempts made at preparing compounds possessing high anabolic and low androgenic activity. Because of the interesting activity of compounds of the androstane series possessing a 2,3 double bond,[5,6] we felt it of interest to prepare some 2,3-epoxides and episulfides and compare their parenteral and oral anabolic and androgenic activities.

The preparation of steroidal epoxides (Table I) proceeds in good yields either by peracid treatment of an olefin[7,8] or *via* base treatment of the corresponding halohydrins.[4,9] Specifically, the 2,3α-epoxides II were prepared by treating the olefins I with either perbenzoic or *m*-chloroperbenzoic acid. On the other hand, the 2,3β-epoxides VII were derived from the bromohydrins VI upon treatment with base. In most cases, these epoxides were used as intermediates in the preparation of the episulfides as indicated below.

Earlier attempts in these laboratories to prepare the episulfides by direct exchange of the epoxide oxygen with sulfur as reported by Van Tamelen for cyclohexene oxide[10] were unsuccessful affording only unchanged epoxide. However, in 1962, Lightner and Djerassi[11] reported on the conversion of 2,3α-epoxy-5α-cholestane to the 2,3β-episulfide in very low yield by the direct displacement procedure. A more feasible approach to the 2,3-epithio-5α-cholestanes was reported in 1962 by Takeda and Komeno who treated the corresponding epoxide with thiocyanic acid[12] to obtain the thiocyano alcohol which was converted to the episulfide with alcoholic potassium hydroxide.[13]

In our studies, the reaction scheme outlined in Chart I proved to be the most convenient route to the α- and β-episulfides in the androstane series. Briefly, the 2,3α-epoxides II were treated with an ether solution of thiocyanic acid prepared *in situ*[12] to give good yields of

CHART I



a, R, R_1 = O
b, R = H; R_1 = OH
c, R = H; R_1 = OAc
d, R = CH_3; R_1 = OH

the thiocyano alcohols III (Table I). Treatment of III with methanolic potassium hydroxide at room temperature for up to 2 hr afforded the 2,3β-episulfides IV (Table I) in good yield. The 2,3β-epoxides VII were treated similarly to give the 2,3α-episulfides IX.

An alternate procedure (Chart II) which can be utilized for the preparation of the 2,3α-episulfides was shown by the preparation of 2,3α-epithio-5α-androstan-17β-ol (IXb). When purified 2α-bromo-5α-androstane-3,17-dione was treated with potassium thiocyanate in acetone in a manner similar to that described previously

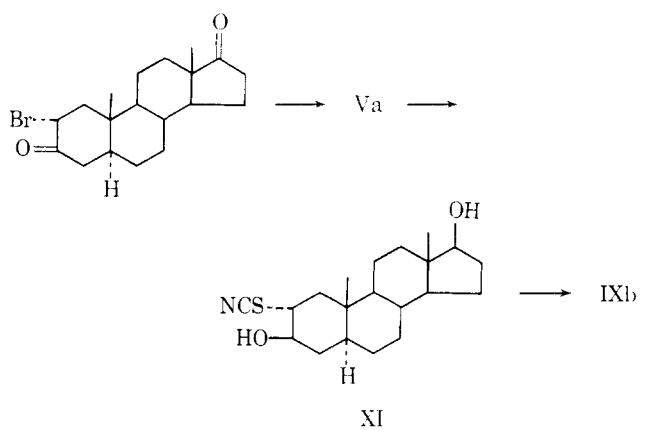
(1) To whom inquiries should be addressed.
(2) K. Takeda, T. Komeno, J. Kawanami, S. Ishihara, H. Kadokawa, H. Tokura, and H. Itani, *Tetrahedron*, **2**, 329 (1965).
(3) R. E. Counsell, P. D. Klimstra, and F. B. Colton, *J. Org. Chem.*, **27**, 248 (1962).
(4) P. D. Klimstra and R. E. Counsell, *J. Med. Chem.*, **8**, 48 (1965).
(5) R. E. Counsell, G. Adelstein, P. D. Klimstra, and B. Smith, submitted for publication.
(6) R. E. Counsell and P. D. Klimstra, U. S. Patent 3,203,966 (1965); J. A. Edwards and A. Bowers, *Chem. Ind.* (London), 1625 (1960).
(7) J. Iriarte, *J. Org. Chem.*, **20**, 543 (1955).
(8) J. Fajkos and F. Sorm, *Collection Czech. Chem. Commun.*, **24**, 3115 (1959).
(9) J. Fried and T. Sabo, *J. Am. Chem. Soc.*, **79**, 1130 (1957).
(10) E. E. Van Tamelen, *ibid.*, **73**, 3444 (1951).
(11) (a) D. A. Lightner and C. Djerassi, *Chem. Ind.* (London), 1236 (1962); (b) *Tetrahedron*, **21**, 583 (1965).
(12) (a) K. Takeda and T. Komeno, *Chem. Pharm. Bull.* (Tokyo), **8**, 468 (1960); (b) *ibid.*, **8**, 672, 680 (1960).
(13) K. Takeda and T. Komeno, *Chem. Ind.* (London), 1793 (1962).

TABLE I

Compd	Recrystn media	Yield, %	Mp, °C	$[\alpha]^{25-28}D$, deg	Formula	Calcd, % C	Calcd, % H	Found, % C	Found, % H
			2,3-Epoxyandrostane Derivatives						
IIa	MeOH	77	125–126[a]	+104	$C_{19}H_{28}O_2$	79.12	9.78	78.94	9.99
b	MeOH–H_2O	87.5	182–184[b]	+25.5	$C_{19}H_{30}O_2$				
d	MeOH–Me_2CO	46	204–205[c]	+0.5	$C_{20}H_{32}O_2$	78.89	10.60	79.04	10.82
VIIa	MeOH–H_2O	97.1	121–123[d]	+132	$C_{19}H_{28}O_2$	79.12	9.79	78.71	9.68
b	MeOH–H_2O	98.6	145–147[e]	+24	$C_{19}H_{30}O_2$	78.57	10.41	78.48	10.49
d	MeOH	72.8	158–161	+25	$C_{20}H_{32}O_2$	78.89	10.59	78.20	10.30
			Thiocyanoandrostane Derivatives						
IIIa	MeOH	51.7	178–180	+78	$C_{20}H_{29}NO_2S$	69.12	8.41	69.56	8.46
b	Me_2CO–hexane	74.5	183–184	+21	$C_{20}H_{31}NO_2S$	68.72	8.94	68.87	8.98
d	Me_2CO	62.5	206.5–210	+35	$C_{21}H_{33}NO_2S$	69.38	9.15	69.19	9.09
VIIIa	MeOH–H_2O	79.6	204–206	+85.5	$C_{20}H_{29}NO_2S$	69.12	8.41	69.13	8.51
b	Me_2CO–hexane	75.6	202–205	+23	$C_{20}H_{31}NO_2S$	68.72	8.94	68.57	9.23
d	Me_2CO–hexane	71.2	183–185	+17.5	$C_{21}H_{33}NO_2S$	69.38	9.15	69.69	8.82
			2,3-Epithioandrostane Derivatives						
IVa	Me_2CO–H_2O	82.2	153–154	+106.5	$C_{19}H_{28}OS$	74.95	9.27	744.41	9.30
b	Me_2CO–H_2O	69.6	124–126, 151–153	+26	$C_{19}H_{30}OS$	74.45	9.87	7.56	9.84
c	Me_2CO–H_2O	95.1	147–149[f]	+17	$C_{21}H_{32}O_2S$	72.36	9.26	72.04	9.57
d	Hexane	85.5	151–153	+12.5	$C_{20}H_{32}OS$	74.95	10.07	74.73	10.07
IXa	Me_2CO–H_2O	82.4	106–108[g]	+98	$C_{19}H_{28}OS$	74.95	9.27	75.07	9.18
b	MeOH–H_2O	97	128–130[h]	+27	$C_{19}H_{30}OS$	74.46	9.86	74.27	9.73
c	Me_2CO	77.5	152–153[i]	+20	$C_{21}H_{32}O_2S$	72.36	9.26	72.42	9.49
d	Me_2CO–H_2O	95	173–174	+4	$C_{20}H_{32}OS$	74.95	10.07	75.27	10.11

[a] Lit.[8] mp 125–126°, $[\alpha]D$ +108°. [b] Lit.[8] mp 183–184°, $[\alpha]D$ +36.3°. [c] Lit.[21] mp 200–203°, $[\alpha]D$ −5°. [d] Lit.[8] mp 121–122°, $[\alpha]D$ +144°. [e] Lit.[8] mp 178–179°, $[\alpha]D$ +50°. [f] Lit.[2] mp 164–165°, $[\alpha]D$ +21.4°. [g] Lit.[2] mp 107–108°, $[\alpha]D$ +107.1°. [h] Lit.[2] mp 127–128°, $[\alpha]D$ +25.4°. [i] Lit.[2] mp 144–145°, $[\alpha]D$ +22°.

CHART II



XI

for the cholestane series,[13] the corresponding thiocyanate Va[14] was obtained. Reduction of Va with lithium tri-*t*-butoxyaluminum hydride afforded 2α-thiocyano-5α-androstane-3β,17β-diol (XI). Treatment of XI with methanolic potassium hydroxide at room temperature gave IXb.

An attempt was made to prepare the 3-keto-2β-thiocyanoandrostanes by mild oxidation of the thiocyano alcohols III. Treatment of IIIa with chromic acid reagent[15] in acetone, however, gave the 2α-thiocyano epimer (V) identical with that obtained above. Apparently, because of the nonbonded 1,3-diaxial interaction with the 19-methyl group, the axial configuration of the 2β-thiocyano substituent is unstable and immediately inverts to the equatorial form. A similar situation was reported by Takeda and Komeno.[2,13]

An Sn2 displacement type reaction of 3α-bromo-17α-methyl-5α-androstan-17β-ol-2-one[4] with potassium thiocyanate in acetone afforded the ketone X possessing a 3β-equatorial thiocyanate group. The spatial configuration of the thiocyanate was confirmed from the nmr spectrum which indicated the presence of a C-3 axial proton at 258 cps (separation between outside peaks of $J_{ax} + J_{bx}$ = 18.5 cps).[16a] On the other hand, the treatment of VIIId in acetone with chromic acid[15] produced a substance which was different from that above. The presence of a peak in the nmr spectrum at 236 cps (width at one-half the peak height $J_{ax} + J_{ex}$ = 7 cps) was indicative of a C-3 equatorial proton.[16b] The above nmr data are consistent for ketone X possessing a 3α-axial thiocyanate group. In this case, apparently, the 5α-axial hydrogen does not play a significant role through 1,3-diaxial interaction to cause spontaneous epimerization of the fairly bulky 3α-thiocyanate group. This situation is somewhat analogous to results reported by Lightner and Djerassi[11b] for the Sn2 displacement of the bromine in 3α-bromo-5α-cholestan-2-one with potassium ethylxanthate to give the 3β-xanthate epimer.

It is interesting to note that while both the 2,3-epoxides and 2,3-episulfides[11,13] are generally unstable to lithium aluminum hydride, they are stable to the reductive action of lithium tri-*t*-butoxyaluminum hydride. This has been demonstrated for 2,3-epoxides

(14) The nmr spectrum of this substance indicated a quartet (J_{ax} + J_{bx} = 20 cps) at 262 cps indicative of an axial proton at C-2. This is consistent with that reported by Takeda and Komeno[13] for 2α-thiocyano-5α-cholestan-3-one.

(15) K. Bowden, I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *J. Chem. Soc.*, 39 (1946).

(16) (a) We wish to thank Dr. R. H. Bible of our laboratories for helpful discussions concerning the nmr spectrum of these compounds. (b) The chemical shift for the C-3 protons in these cases is similar to that observed for α-halo ketones in which the signal for the epimer with an equatorial hydrogen appears at a higher field than that for the epimer containing an axial hydrogen: N. S. Bhacca and D. H. Williams, "Applications of NMR Spectroscopy in Organic Chemistry," Holden-Day, Inc., San Francisco, Calif., 1964, p 73.

by Fajkos[8] and for the 2,3-episulfides by the conversion of the ketone IIIb to the alcohol IVb.

The nmr data obtained for the epoxides (II and VIII) and episulfides (IV and IX) were in agreement with that recently reported by Tori and co-workers[17] for various 2,3-epoxides and episulfides in the cholestane series.

Biological Results.—The androgenic and myotrophic activities were determined by the method of Eisenberg and Gordon[18] as modified by Saunders and Drill.[19] The compounds listed were given to 22-day-old castrated male rats intramuscularly by injection or orally by means of a stomach tube. The relative potencies are given in terms of per cent activity of testosterone propionate (intramuscular) or 17α-methyltestosterone (oral) and were determined from the minimal levels at which significant increases in ventral prostate and seminal vesicle or levator ani muscles weights were obtained. Table II compares the androgenic and anabolic activities for the compound evaluated in this study.

TABLE II

ANDROGENIC–MYOTROPHIC ACTIVITIES[a]

	Intramuscular			Oral[e]		
Compound	Androgenic[b] (SV + VP)/2	Myotrophic LA[c]	M/A[d]	Androgenic (SV + VP/2	Myotrophic LA	M/A
Testosterone propionate	100	100	1.0			
Ib				I	I	
IIb	0.49	0.80	1.6	I	I	
IVb	2.2	13	5.9	I	I	
VIIb	1.3	6.7	5.2	I	I	
IXb	42	308	7.3	25	100	4.0
17α-Methyltestosterone				100	100	1.0
Id						
IId	1.4	4.8	3.4	I	I	
IVd	0.62	2.8	4.5	18	78	4.3
VIId	5.6	36	6.4	I	I	
IXd	27	154	5.7	91	1100	12.1

[a] Potencies are given in terms of per cent of the activity of testosterone propionate and 17α-methyltestosterone. [b] SV = seminal vesicles, VP = ventral prostate; since there is no uniform standard reference for androgenic designation, the values of one-half of the sum of the VP and SV have been used as the criteria of androgenicity. [c] LA = levator ani. [d] Myotrophic to androgenic ratios. [e] The letter I designates inactivity.

Parenterally, the various epoxides (IIb, d and VIIb, d) (Table II) showed very little or no anabolic or androgenic responses when compared to testosterone propionate. The most active of these substances was VIId, the 2,3β-epoxide. Similarly, these same compounds (IIb, d and VIIb, d) demonstrated very low oral activity when compared to 17α-methyltestosterone, failing to stimulate growth of the sex glands. These results, however, should not be too surprising since there are few if any reports of epoxy steroids possessing appreciable endocrine-stimulating properties.[20,21]

Although one would expect the episulfides (IV and IX) to possess biological properties similar to the isosteric epoxides (II and VII), pronounced anabolic–androgenic activity was found for some of the episulfide analogs (Table II). For example, 2,3α-epithio-17α-methyl-5α-androstan-17β-ol (IXd) was found to have approximately equal androgenic and 11 times the anabolic activity of methyltestosterone after oral administration. Even IXb, lacking a 17α-methyl group, showed interesting and significant oral activity. In addition, the activity with respect to structure and configuration appeared to be quite specific for the α-episulfide isomer since the 2,3β-episulfides were much less active.

The 2,3α-episulfides IXb and IXd also displayed significant activity following parenteral administration. For example, IXb was approximately three times as potent as testosterone propionate anabolically with a myotrophic/androgenic ratio of 7.3. Again, there is an association of high anabolic activity with spatial configuration since the 2,3β-episulfide analogs IVb and IVd had very little parenteral anabolic or androgenic activity.

The myotrophic/androgenic ratios of many of the epoxides and episulfides are also shown in Table II. Many of the values may be misleading and possibly of limited value because of the relatively low order of activity of the compounds in both the anabolic and androgenic categories. However, because of the higher order of response observed for compounds IVd, IXb, and IXd, the ratios listed are useful and meaningful. For instance, the 2,3α-episulfide IXd, has one of the best myotrophic/androgenic ratios of those reported so far.[22–25]

It is impossible at this point to rationalize the high activity of the 2,3α-episulfide over the 2,3β isomers. From a chemial standpoint, one could expect the formation of some of the 2-dehydro analogs from the decomposition of the sulfur moiety.[2,13] However, the 17β-hydroxy-2-dehydro analog (Ib) has no oral anabolic activity while the corresponding 2,3α-episulfide does produce a significant oral response. In addition, while both the 2-dehydro and 2,3α-epithio-17α-methyl analogs (Id and IXd) have similar high anabolic activity, the later substance is only about one-half to one-third as androgenic. Similarly, one might expect the much less active 2,3β-epithio-17α-methyl derivative (IVd) to also be transformed into the 2-dehydro analog; however, one might speculate that metabolically this apparently is not the case.

The intermediate thiocyano alcohols III and VIII and the α-thiocyanoketo derivatives Va and Xd were evaluated for anabolic and androgenic activity and were found to be essentially inactive.

While the anabolic–androgenic potencies of the epoxides II and VII are not very pronounced, the results as listed in Table II are of particular interest in considering the hypothesis of Wolff and co-workers[21,26] regarding the mode of adsorption of the steroids on receptors: "The tendency is for the asymmetrical sp^2 system to afford a more active compound when hindrance is greater on the α-face, than when hindrance is greater

(17) K. Tori, T. Komeno, and T. Nakagawa, *J. Org. Chem.*, **29**, 1136 (1964).

(18) E. Eisenberg and G. S. Gordon, *J. Pharmacol. Exptl. Therap.*, **99**, 38 (1950).

(19) F. J. Saunders and V. A. Drill, *Proc. Soc. Exptl. Biol. Med.*, **94**, 646 (1957).

(20) R. E. Counsell and P. D. Klimstra, *J. Med. Pharm. Chem.*, **5**, 477 (1962).

(21) M. E. Wolff, W. Ho, and R. Kwok, *ibid.*, **7**, 577 (1964).

(22) Most of the presently commercially available anabolic agents, and the more potent ones reported in the literature, have ratios of 2–13.

(23) K. Junkman and G. Suchowsky, *Arzneimittel-Forsch.*, **12**, 214 (1962).

(24) F. A. Kincl and R. I. Dorfman, *Steroids*, **1**, 116 (1963).

(25) E. F. Nutting, R. E. Counsell, P. D. Klimstra, and D. Calhoun, submitted for publication.

(26) M. E. Wolff and T. Jen, *J. Med. Chem.*, **6**, 726 (1963).

on the β-face." This hypothesis is set forth to rationalize the increased activity or lack of it of the α over the corresponding β isomers of steroidal A-ring epoxy androgens. In our hands, similar isomeric epoxides appear to have the reverse order of androgenic and myotrophic activity when administered parenterally. For example, the 2,3β-epoxides VIIb and VIId were several times more potent than the corresponding α isomers IIb and IId. This would tend to indicate that the more active epoxides have greater hindrance on the β-rather than on the α-face of the tetracyclic system. These results are somewhat in line with α-face (back side) attack of the androgen molecule by enzymatic surface as proposed by Ringold.[27]

The different results reported as well as the variety of hypotheses expounded seem to further point up the lack of understanding of how and where these substances are bound to receptor sites.

Experimental Section[28]

2,3α-Epoxy-17α-methyl-5α-androstan-17β-ol (IId). General Method.—A solution of Id[29] (10 g) and perbenzoic acid[30] (1.1 *N* in benzene, 75 ml) in benzene (50 ml) was allowed to stand for 20 min at room temperature and then in the refrigerator for 16 hr. The reaction mixture was washed repeatedly with 10% aqueous Na_2CO_3 solution followed by H_2O until free of benzoic acid and dried over anhydrous K_2CO_3 containing Darco. Solvent removal left a solid residue which was recrystallized from acetone–hexane to give IId (5.0 g), mp 198–200°. Further recrystallization from methanol–acetone gave an analytical sample, mp 204–205°.

Thiocyano Alcohols. General Method.—To potassium thiocyanate (40 g) in methanol (20 ml) containing a small amount of ice, was added ether (150 ml). To this mixture was added 85% H_3PO_4 acid (60 g) in small portions with vigorous agitation. The pink ether layer was separated, washed with two 10-ml portions of H_2O, and dried briefly (Na_2SO_4). The ether portion containing thiocyanic acid was combined with IIa (3.7 g) and allowed to stand for 70 hr at room temperature. The reaction mixture was washed with 10% aqueous Na_2CO_3 and with H_2O until neutral and dried (Na_2SO_4). Solvent removal left an oil which solidified. Recrystallization from methanol gave IIIa (1.7 g), mp 178–180°.

2β-Thiocyano-5α-androstane-3α,17β-diol (IIIb). General Method.—To a stirred solution of IIIa (1.0 g) in tetrahydrofuran (THF) (20 ml) cooled in an ice bath and under nitrogen, was added a chilled mixture of lithium tri-*t*-butoxyaluminum hydride (2.5 g) in THF (20 ml). The solution was stirred for 0.75 hr and poured in 10% AcOH and ice. An oily solid formed which gradually solidified. The precipitate was collected, washed with H_2O, and air dried. Recrystallization from acetone–hexane gave IIIb (0.75 g), mp 183–184°.

2,3-Episulfides. General Method.—To a warmed solution of IIIa (5.0 g) in methanol (125 ml) was added a solution of KOH (2.5 g) in methanol (55 ml). The reaction was allowed to stand for 1.75 hr at room temperature. Water (250 ml) was added and the solution cooled. The precipitate was collected and air dried to give the product (3.6 g), mp 146–148°. An analytical sample of IVa was prepared by recrystallization from acetone: mp 153–154°.

2β,3β-Epithio-5α-androstan-17β-ol (IVb).—To a stirred solution of IVa (1.0 g) in THF (20 ml) cooled in an ice bath, was added a mixture of lithium tri-*t*-butoxyaluminum hydride (2.5 g) in THF (20 ml). After 1.5 hr the reaction was poured into an ice and 10% AcOH solution. The precipitate was collected, washed with H_2O, and air dried. Recrystallization from acetone afforded the product IVb (0.7 g), mp 124–126°, 151–153°.

2β,3β-Epithio-5α-androstan-17β-ol Acetate (IVc). General Method.—A solution of IVb (0.25 g) and acetic anhydride (1 ml) in pyridine (2 ml) was allowed to stand at room temperature for 4 hr. The reaction mixture was poured into ice and H_2O and the product was collected, washed with H_2O, and air dried. Recrystallization from acetone afforded IVc (200 mg), mp 135–139°. Further recrystallization from the same solvent system afforded pure IVc, mp 147–149°.

2α-Thiocyano-5α-androstane-3,17-dione (Va). A. *Via* Alcohol.—A stirred solution of IIIa (0.6 g) in acetone (6 ml) was treated with standard chromic acid solution dropwise until the color of the reagent persisted. The excess chromic acid was destroyed with isopropyl alcohol and the inorganic salts were removed by filtration through diatomaceous earth. The filtrate was diluted with H_2O and refrigerated. A crystalline product was collected, washed with H_2O, and air dried. Recrystallization from methanol afforded Va (0.3 g): mp 202–203° dec; $[\alpha]^{25}D$ −44.5°; nmr, 252–257 (2β-H), 69 (C-18 methyl), and 53.5 cps (C-19 methyl).

Anal. Calcd for $C_{20}H_{27}NO_2S$: C, 69.52; H, 7.88. Found: C, 69.44; H, 7.70.

B. *Via* Bromide.—A mixture of 2α-bromo-5α-androstane-3,17-dione (12.0 g) and potassium thiocyanate (8 g) and acetone (400 ml) was refluxed with stirring for 6.5 hr. The reaction gradually became homogeneous and toward the end of the reaction period KBr precipitated. The salt was removed by filtration and the filtrate was concentrated by two-thirds. Water was added and the product which precipitated was collected and washed with H_2O. The precipitate was dissolved in ethyl acetate–benzene (2:1) and dried by azeotroping with the benzene. Solvent removal *in vacuo* afforded a solid (9.6 g) which was recrystallized from ethyl acetate to give Va (8.9 g), mp 212–214° dec. This preparation was shown by mixture melting point and infrared to be identical with that prepared by the above method.

2β,3β-Epoxy-5α-androstan-17-one (VIIa). General Method.—A solution of Na_2CO_3 (0.45 g) in H_2O (10 ml) was added slowly with stirring to a mixture of VIa[31] (2.0 g) in THF (80 ml) and H_2O (30 ml) over 10 min. The reaction was allowed to stand for 41 hr at room temperature and poured into ice and H_2O. The precipitate was collected, washed with H_2O, and air dried. Two recrystallizations from methanol gave pure VIIa (1.1 g), mp 121–123°. The mixture melting point of this sample with the 2α,3α-epoxy isomer was 87–88°.

2β,3β-Epoxy-5α-androstan-17β-ol (VIIb).—To a stirred solution of VIIa[31] (1.0 g) in methanol (20 ml) was added $NaBH_4$ (1.0 g) in methanol (15 ml) and H_2O (1.7 ml). The solution was refluxed with stirring for 5 hr. After cooling, the reaction was poured into ice and H_2O (300 ml) and stirred for 0.5 hr. The precipitate was collected, washed with H_2O, and air dried. Recrystallization from methanol–H_2O gave VIIb (0.65 g), mp 145–147°.

2α,3α-Epithio-5α-androstan-17β-ol (IXb).—A solution of X[1] (1.6 g) in methanol (25 ml) and KOH (0.8 g) in methanol (10 ml) was allowed to stand at room temperature for 2 hr. Water was added to the reaction mixture followed by cooling in the refrigerator. The precipitate was collected and recrystallized from methanol–H_2O to give IXb (1.1 g), mp 128–131°. This preparation was shown by mixture melting point and infrared to be identical with that prepared by ring closure of VIIIa.

17α-Methyl-3β-thiocyano-5α-androstan-17β-ol-2-one (X).—To a solution of 3α-bromo-17α-methyl-5α-androstan-17β-ol-2-one[1] (3.0 g) in acetone (60 ml) was added potassium thiocyanate (2.0 g) in acetone (40 ml). The reaction mixture was refluxed for 2 hr, cooled, and filtered to remove the suspended KBr. The filtrate was poured into H_2O and the precipitate was collected. Recrystallization from acetone–H_2O gave X (3β-thiocyanate, 2.4 g): mp 94–96°; $[\alpha]^{25}D$ −42°; λ_{max} 327 mμ (ε 72); nmr, 248.5–267 (3α-H), 73.5 (C-17 methyl), 51.5 (C-18 methyl), and 48 cps (C-19 methyl).

Anal. Calcd for $C_{21}H_{31}NO_2S$: C, 69.76; H, 8.64. Found: C, 69.51; H, 8.54.

(27) H. J. Ringold in "Mechanism of Action of Steroid Hormones," C. A. Villee and L. L. Engel, Ed., Pergamon Press, New York, N. Y., 1961, pp 200–232.

(28) Optical rotations, spectra, and analytical data were furnished by Mr. E. Zielinski and Mr. J. Damaseus of our analytical department under the supervision of Dr. R. T. Dillon. The optical rotations and infrared spectra were obtained in chloroform and ultraviolet spectra in methanol. The nmr spectra were obtained with a Varian A-60 spectrometer. The melting points were obtained on a Fisher-Johns apparatus and are corrected.

(29) R. E. Counsell and P. D. Klimstra, U. S. Patent 3,203,966 (1965); *Chem. Abstr.*, **63**, 14943 (1965).

(30) *m*-Chloroperbenzoic acid, available from the FMC Corp., could be substituted here to also give good yields of the epoxides.

(31) P. D. Klimstra and R. E. Counsell, U. S. Patent 3,018,298 (1962); *Chem. Abstr.*, **57**, 4733 (1962).

17α-Methyl-3α-thiocyano-5α-androstan-17β-ol-2-one (X).—A solution of VIIId (1.0 g) in acetone (35 ml) was treated dropwise with standard chromic acid solution.[15] The excess reagent was destroyed by a small amount of isopropyl alcohol. The inorganic salts were removed by filtering through diatomaceous earth and the filtrate was concentrated *in vacuo*, the residue was diluted with H_2O, and a crystalline product was collected. Recrystallization from methanol–H_2O afforded the 3α-thiocyanate X (0.7 g), mp 151–153°. An additional recrystallization from methanol gave an analytical sample: mp 153–154°; $[\alpha]^{25}D$ +137.5°; λ_{max} 292 mμ (ε 60); nmr, 232.5–239.5 (3β-H), 73 (C-17 methyl), 50.5 (C-18 methyl), 49 cps (C-19 methyl).

2α-Thiocyano-5α-androstane-3β,17β-diol (XI).—To an ice-cold solution of Va (4.0 g) in THF (100 ml) was added a cold solution of lithium tri-*t*-butoxyaluminum hydride (20 g) in THF (100 ml). The reaction was stirred for 1 hr at about 5° and poured into an ice-cold 5% AcOH solution. The product was extracted with ether and the extracts were washed with H_2O, 5% $NaHCO_3$, and finally H_2O again before drying over anhydrous Na_2SO_4. Solvent removal *in vacuo* left a white solid which was recrystallized from acetone–hexane to give the diol XI (3.6 g), mp 202–205°, $[\alpha]^{25}D$ +23°.

Anal. Calcd for $C_{20}H_{31}NO_2S$: C, 68.72; H, 8.94. Found: C, 68.57; H, 9.23.

Acknowledgment.—We wish to thank Dr. F. B. Colton for his interest and comments during the course of this work.

Pteridinecarboxamide Diuretics. I. Reaction of 4,6-Diamino-5-nitrosopyrimidines with Substituted Malonamides[1]

T. S. OSDENE, ARTHUR A. SANTILLI,[2] LEE E. MCCARDLE, AND MARVIN E. ROSENTHALE

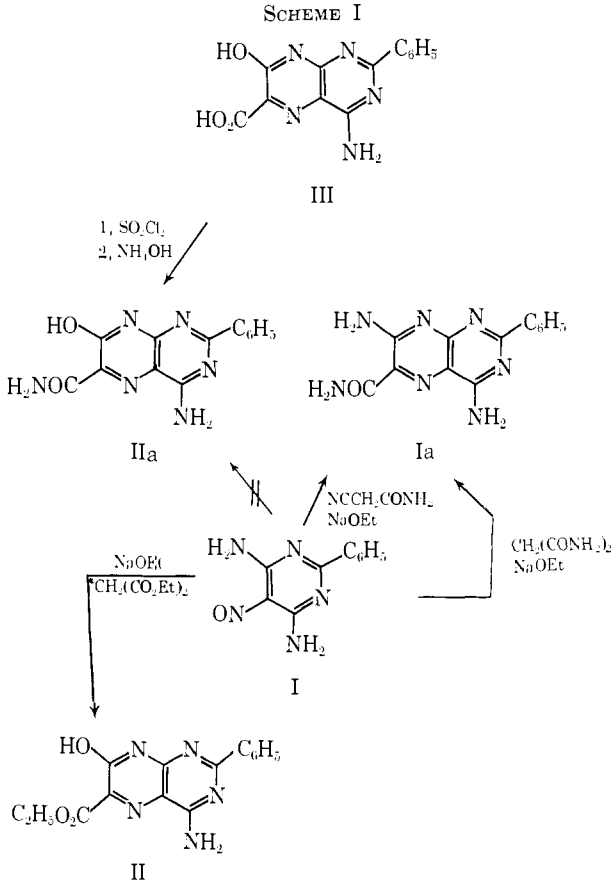
Wyeth Laboratories, Inc., Research and Development Division, Radnor, Pennsylvania

Received March 12, 1966

The reaction of 4,6-diamino-5-nitrosopyrimidines with a number of N,N′-bis-substituted malonamides in the presence of 1 equiv of sodium in ethanol afforded mixtures of 4-amino-7-substituted amino-N-substituted 6-pteridinecarboxamides and 4-amino-7-hydroxy-N-substituted 6-pteridinecarboxamides. The 7-substituted aminopteridinecarboxamides were found to be effective oral diuretics in rats, whereas the 7-hydroxypteridinecarboxamides were inactive at comparable dose levels.

The base-catalyzed reaction of 4,6-diamino-5-nitroso-2-phenylpyrimidine (I) with cyanoacetamide yields 4,7-diamino-2-phenyl-6-pteridinecarboxamide (Ia) (see Scheme I).[3] Variation of substituents on the 2 position of the pyrimidine, as well as substitution on the amide nitrogen of the cyanoacetamide, permits the preparation of many biologically active substituted 6-pteridinecarboxamides. These have been the subject of several recent patents.[4] In each of these reactions, ring closure to the pteridine results from the elimination of 1 equiv of water between the nitroso group of the pyrimidine and the active methylene group of the cyanoacetamide and the concomitant addition of the amino group of the pyrimidine to the nitrile group of the cyanoacetamide.

SCHEME I



In view of what has been reported concerning these types of reactions an attempt was made to prepare 4-amino-7-hydroxy-2-phenyl-6-pteridinecarboxamide (IIa) in a single step by the reaction of I with malonamide in the presence of an equivalent amount of sodium ethoxide. It was expected that water and ammonia would be eliminated in the reaction, thus

(1) A preliminary account of this work was presented before the Division of Medicinal Chemistry, 150th National Meeting of the American Chemical Society, Atlantic City, N. J., Sept 1965, Abstracts, p 16.

(2) To whom inquiries regarding this article should be sent.

(3) T. S. Osdene and E. C. Taylor, U. S. Patent 2,975,180 (1961).

(4) J. Weinstock, U. S. Patent 2,963,478 (1960); T. S. Osdene and A. A. Santilli, U. S. Patents 3,138,595, 3,138,594, 3,138,592, and 3,122,547 (1964).

(5) I. J. Pachter and P. E. Nemeth, *J. Org. Chem.*, **28**, 1187 (1963); I. J. Pachter, P. E. Nemeth, and A. J. Villani, *ibid.*, **28**, 1197 (1963).

(6) T. S. Osdene in "Pteridine Chemistry," W. Pfleiderer and E. C. Taylor, Ed., The Macmillan Co., New York, N. Y., 1964, p 65.