Heterocyclic Steroids. VI. Steroidal Pyridazones and Pyridazinones[1]

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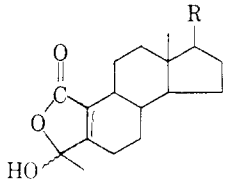
Received April 16, 1964

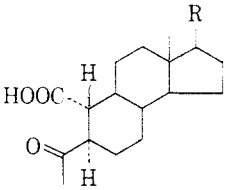
The preparation of 2,3-diaza-4-methyl-1-one steroids from γ-keto acids is described. Some of the diaza steroids and their intermediate γ-keto acids were assayed for their biological activity.

The γ-keto acids I and II obtained from the oxidation[3] of 1-hydroxy-4-methyl-1,3,5(10)-triene steroids presented themselves as likely intermediates for the preparation of 2,3-diaza steroids, pyridazones, and pyridazinones,[4] which could be used for biological evaluation.

Starting with the appropriate hydroxy-2,3-bisnor-1,4-secoketo acids (I and II), preparation of the pyridazones and pyridazinones was easily accomplished by condensation with hydrazine hydrate in ethanol. The 17-keto- and 20-ketodiaza steroids were obtained by chromium trioxide–pyridine oxidation of the corresponding hydroxy compound. The compounds thus prepared and their physical constants are listed in Table I.



Ia, R = OH
b, R = CHOH(β)CH$_3$
c, R = COCH$_3$

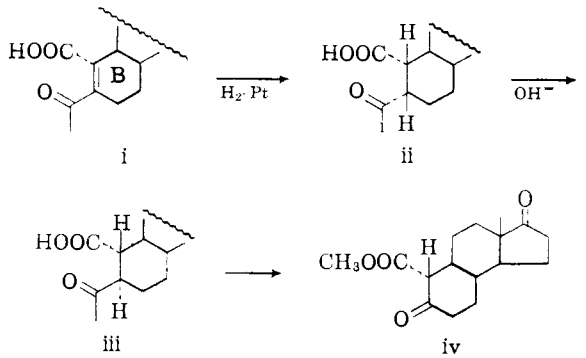


IIa, R = OH
b, R = CHOH(β)CH$_3$
c, R = COCH$_3$

(1) (a) The work was supported by Grants A5326, CA-02193, and CA-07137-01 from the U. S. Public Health Service. (b) Part V: D. M. Piatak and E. Caspi, *Steroids*, **3**, 631 (1964).

(2) (a) Summer student supported by Grant GE 2363 from NSF Undergraduate Science Education Program. (b) Recipient of Public Health Service Research Career Program Award, CA-K3-16614, from the National Cancer Institute.

(3) (a) E. Caspi, P. K. Grover, and D. M. Piatak, *Chem. Ind.* (London), 1495 (1963); (b) E. Caspi, P. K. Grover, D. M. Piatak, and Y. Shimizu, *J. Chem. Soc.*, in press. The conformation of the acids was established by the following sequence.

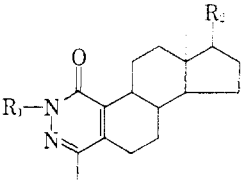


i ii iii iv

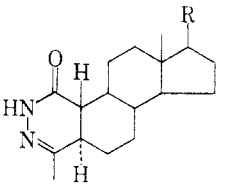
Compound iv was prepared from iii by methylation, Baeyer-Villiger oxidation, saponification, remethylation, and Jones' oxidation. Compound iv was shown to be identical with a known compound [F. Sondheimer, R. Mechoulam, and M. Sprecher, *Tetrahedron Letters*, **22**, 38 (1960)].

(4) (a) For a review of this heterocyclic system, see T. L. Jacobs in "Heterocyclic Compounds," Vol. VI, R. C. Elderfield, Ed., John Wiley and Sons, Inc., New York, N. Y., 1957, Chapter 4. (b) See F. L. Weisenborn, D. C. Remy, and T. L. Jacobs, *J. Am. Chem. Soc.*, **76**, 552 (1959), for the preparation of a steroidal 3,4-pyridazinone. (c) W. G. Overend, L. M. Turton, and L. F. Wiggins, *J. Chem. Soc.*, 3500 (1950).

The proof of structure rests upon elemental analysis and spectroscopic data. The pyridazones IIIa, c, and



IIIa, R$_1$ = H; R$_2$ = OH
b, R$_1$ = CH$_3$; R$_2$ = OH
c, R$_1$ = H; R$_2$ = CHOH(β)CH$_3$
d, R$_1$ = H; R$_2$ = COCH$_3$
e, R$_1$ = CH$_3$; R$_2$ = CHOH(β)CH$_3$
f, R$_1$ = CH$_3$; R$_2$ = COCH$_3$

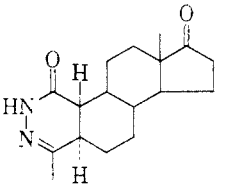


IVa, R = OH
b, R = CHOH(β)CH$_3$
c, R = COCH$_3$

d exhibited the expected[4a] ultraviolet maximum at 286 mμ with a shoulder at 230 mμ. In addition infrared spectra showed characteristic peaks at 3130 (NH), 1635 (C=O), 1580, and 1530 cm.$^{-1}$ (double bonds). An n.m.r. spectrum of IIIa had peaks at τ 7.78 and 9.21 for the methyls at C-4 and -13, respectively.

Methylation of pyridazones IIIa and IIIc with dimethyl sulfate[4c] yielded the 2-methyl steroidal pyridazones IIIb and IIIe. The fact that N-methylation rather than O-methylation occurred was determined by infrared spectroscopy, which showed *no* NH absorption in the 3100–3200 cm.$^{-1}$ region and strong absorption at 1620, 1580, and 1525 cm.$^{-1}$ for the carbonyl and double bonds. Ultraviolet spectra of IIIb and IIIf had the maximum at 290 mμ and a shoulder at 230 mμ.

In the case of the pyridazinones IV and V the proof



V

of structure was again based on elemental analysis and spectroscopy. Infrared spectra revealed split absorption at 3230 and 3100 cm.$^{-1}$ for the NH group and bands at 1665 and 1625 cm.$^{-1}$ for the carbonyl and the double bond. The ultraviolet maximum was at 245 mμ, characteristic of the >C=N– group.[4a,b]

The stereochemical assignment of 10β and 5α to pyridazones IV and V can be rationalized from the diequatorial configuration[3b] of the parent γ-keto acids (II). It is probable that pyridazinone formation first proceeds by attack on the C-4 ketone, followed by ring closure *via* amide formation. Both of these reactions entail nucleophilic attack of nitrogen on the carbonyls and do not involve carbon–carbon enolization. Hence,

TABLE I
PHYSICAL CONSTANTS AND ANALYSES OF COMPOUNDS[a]

Compd.	M.p., °C.	$[\alpha]_D$[b]	Ultraviolet λ_{max}, mμ	ε	Infrared, cm.$^{-1}$	Calcd., % C	Calcd., % H	Calcd., % N	Found, % C	Found, % H	Found, % H
IIIa	>280[c]	181	sh 230	3100	3440, 3130, 1635,	70.80	8.39	9.71	70.35	8.39	9.55
			286	3800	1585, 1535				70.56	8.43	
IIIb	187–188[d]	363	sh 230	2800	3410, 1620, 1580,	71.49	8.67		71.56	8.45	
			290	4100	1530						
IIIc	>300[c]	301	sh 230	3700	3440, 3130, 1630,	72.11	8.92		71.94	8.36	
			286	4100	1585, 1525						
IIId	>300[c]	416	sh 230	2900	3130, 1705, 1630,	72.58	8.34	8.91	72.50	8.31	9.05
			286	3600	1580, 1530						
IIIf[f]	182–184[d]	429	sh 230	2700	1690, 1625, 1580	73.13	8.59	8.53	72.88	8.35	8.37
			290	3800							
IVa	206–210[g]	352	244	5200	3460, 3240, 3100,	70.31	9.02	9.65	70.24	8.62	10.11
					1675, 1630						
IVb	183–189[g]	351	245	5500	3640, 3230, 3100,	71.66	9.50	8.80	71.48	9.45	9.06
					1650, 1625						
IVc	197–199[e]	410	245	5500	3230, 3100, 1690,	72.11	8.92	8.85	71.50	8.99	8.91
					1665, 1625						
V	>270[d]	453	244	6000	3260, 3100, 1715,	70.80	8.39	9.71	70.87	8.39	10.05
					1665, 1620						

[a] Compounds I and II are fully reported in ref. 3. [b] Rotations taken in chloroform at 20° except for IIIc and IVa which were taken in methanol. [c] Recrystallized from chloroform–ethanol. [d] From ethyl acetate. [e] From methylene chloride–methanol. [f] The corresponding 20β-hydroxy compound was isolated and the crude product oxidized directly to IIIf. [g] From isopropyl ether.

it seems reasonable to assume that the original stereochemistry of the acid was retained. The assumption is valid since it is unlikely that the acid II would change from its stabilized diequatorial conformation. Inspection of models also reveals that the 10β,5α-conformation of the pyridazinones is the most stable.

TABLE II
BIOLOGICAL ACTIVITIES OF CERTAIN γ-KETO ACIDS, PYRIDAZONES, AND PYRIDAZINONES

Bioassay (route)	Compd.	Total no. of animals used	Relative potency (std. = 100)	Standard
Estrogenic activity (s.c.)	Ia	19	<0.05	Estrone
	IIIa	20	<0.05	Estrone
	IIId	20	<0.05	Estrone
	IVa	19	<0.005	Estrone
	IVb	19	<0.05	Estrone
Antiestrogenic activity (s.c.)	Ia	20	50	Progesterone
	Ic	30	<20	Progesterone
	IIa	28	100	Progesterone
	IIc	30	35	Progesterone
	IIIa	20	<50	Progesterone
	IIId	20	<50	Progesterone
	IVa	19	50	Progesterone
	IVb	19	<50	Progesterone
	IVc	47	50	Progesterone
Chick androgen (inunction)	Ia	19	<0.5	Testosterone
	IIIa	21	<0.5	Testosterone
	IIId	23	<0.5	Testosterone
	IVb	21	<0.5	Testosterone
	IVc	26	<0.5	Testosterone
Androgen–anabolic assay (s.c.)	IVa	7	<5	Testosterone
Mouse antiandrogenic assay (s.c.)	Ic	15	70	Progesterone
	IIa	12	50	Progesterone
	IIc	8	100	Progesterone
	IIIa	16	<30	Progesterone
	IIId	16	<30	Progesterone
	IVa	9	70	Progesterone
	IVb	7	70	Progesterone
	IVc	9	<20	Progesterone
Antitumor activity, rat mammary fibroadenoma (s.c.)	Ia	8 (12 tumors)	<50	2α-Methyl-17β-hydroxy-5α-androstan-3-one
	IIa	7 (11 tumors)	<100	2α-Methyl-17β-hydroxy-5α-androstan-3-one
	IIc	11 (17 tumors)	<50	2α-Methyl-17β-hydroxy-5α-androstan-3-one
	IIIa	8 (15 tumors)	<50	2α-Methyl-17β-hydroxy-5α-androstan-3-one
	IIId	8 (14 tumors)	<50	2α-Methyl-17β-hydroxy-5α-androstan-3-one
	IVa	10 (18 tumors)	<50	2α-Methyl-17β-hydroxy-5α-androstan-3-one

Experimental[5]

Hydroxypyridazones and -pyridazinones.—In a typical experiment a solution of Ia (100 mg.) and hydrazine hydrate (0.1 ml.) in ethanol (2.0 ml.) was heated at reflux for 4 hr. The reaction was diluted with water, and the solid was collected by filtration. When the product did not crystallize on dilution, it was recovered by extraction with ether.

2-Methylpyridazones.—The preparation is exemplified by the following experiment. To a solution of pyridazone IIIa (300 mg.) in potassium hydroxide (30%, 1.0 ml.) and methanol (5.0 ml.) was added dimethyl sulfate (0.5 ml.). The mixture was heated at reflux for 2 hr., then diluted with water. The steroid was taken up in ether, washed with water, then dried. Evaporation of the solvent gave a residue, which crystallized on trituration with solvent.

Oxidation of 17β- or 20β-Hydroxy Steroids.—To a suspension of chromium trioxide (100 mg.) in pyridine (5.0 ml.) was added a solution of a hydroxy steroidal pyridazone or pyridazinone (100 mg.) in pyridine (2.0 ml.). After 3.5 hr., ethyl acetate was added and the solids were removed by filtration with Celite. The filtrate was washed with sodium bicarbonate and water, then dried. Evaporation of the solvents left a residue containing traces of pyridine which were removed by repeated evaporations with ethyl acetate. The product was crystallized directly or purified by thin layer chromatography.

Biological Activity.—Representative new compounds were studied for mouse estrogenic,[6] mouse antiestrogenic,[7] chick androgenic,[8] mouse antiandrogenic,[9] and rat antitumorogenic[10] activities. The results are listed in Tables II and III. Antiestrogenic activity was detected for five compounds and the tricyclic compound IIa showed an activity by injection equal to that of the reference standard, progesterone. The other four antiestrogens appear to be less active than progesterone.

TABLE III

RELATIVE ANTIESTROGENIC AND ANTIANDROGENIC POTENCY OF THE 7-KETO ACIDS AND PYRIDAZINONES

Compd.	Antiestrogenic activity[a] A	Antiandrogenic activity[a] B	Ratio A/B
Ic	<20	70	<0.3
IIa	100	50	2
IIc	35	100	0.3
IVa	50	70	0.7
IVb	<50	70	<0.7
IVc	50	<20	>2.5

[a] Progesterone = 100.

Five compounds, including two tricyclic compounds, were antiandrogenic in the mouse assay. Compound IIa was judged to be about one-half as active as the standard, progesterone; IIc was about as active as progesterone; and Ic, IVa, and IVb were rated as 70% as active as the standard.

Six compounds were studied in both the antiestrogenic and antiandrogenic assays and the results indicate that the two activities were not correlated (Table III).

(5) Melting points are corrected. Ultraviolet spectra were taken in methanol and infrared spectra in potassium bromide wafers. N.m.r. spectra were determined for chloroform-*d* solutions containing tetramethylsilane as the internal standard on a Varian V4300 B spectrometer. The values are expressed in τ-units.

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Studies on the Aromatization of 10β-Hydroxy-3-keto Steroids[1]

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Received February 27, 1964

The rate of aromatization of the A-ring of 17α-ethynylestr-4-ene-10β,17β-diol-3-one (17α-ethynyl-10β-hydroxy-19-nortestosterone) by different concentrations of hydrochloric acid has been studied both in methanol and in water. In methanol the reaction was relatively rapid, and at concentrations of HCl of 0.010 *M* and 0.003 *M*, respectively, the half-life of 17α-ethynyl-10β-hydroxy-19-nortestosterone was 5.0 and 11.0 min. The reaction rate in aqueous solution was very much slower and the acid concentration had to be 3 *M* before measurements could be obtained. The half-life of 17α-ethynyl-10β-hydroxy-19-nortestosterone under these conditions was 55.5 min. It is concluded that the aromatization of this compound under aqueous acid conditions such as those in the human stomach would be slow, although the production of physiologically active amounts of phenolic estrogen during a period of several hours is possible.

The synthetic steroid 17α-ethynylestr-5(10)-en-17β-ol-3-one (norethynodrel) has received considerable use as an inhibitor of ovulation and has been reported[3] to possess estrogenic activity. In a study of the metabolism of this compound after its oral administration to humans and animals we have shown that it is partially converted by gastric juice to 17α-ethynylestr-4-ene-10β,17β-diol-3-one (17α-ethynyl-10β-hydroxy-19-nortestosterone).[4] Since the acid-catalyzed dehydration of 10β-hydroxy and 10β-acetoxy steroids to phenols and their derivatives has been established by several workers[5-9] we have been interested in the extent to which such steroids might be aromatized *in vivo*, in particular in the stomach. The present paper reports a study of the rate of aromatization of estr-4-ene-10β,17β-diol-3-one (10β-hydroxy-19-nortestosterone) and 17α-ethynyl-10β-hydroxy-19-nortestosterone by solutions of HCl at 37°. Also presented is a countercurrent distri-

(1) Supported in part by Grant No. 00087 from the National Institute of Child Health and Human Development.

(2) Holder of Public Health Service research career program award No. Am-K3-18, 319 from the National Institute of Arthritis and Metabolic Diseases.

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