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INHIBITORS OF HUMAN ADRENAL C_{17-20} LYASE AND C_{19}-5-ene, 3β-HYDROXYSTEROID DEHYDROGENASE

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SUMMARY

The effect of several synthetic substrate derivatives on the activity of 3β-hydroxysteroid-oxidoreductase, 5 → 4-ene,3-ketosteroid isomerase, and C_{17-20} lyase in human adrenal microsomes has been determined *in vitro*. These steroids inhibit all three enzymes to a very strong degree and one of them appears to be a selective C_{17-20} lyase inhibitor, namely 3β-hydroxy-5-androstene 17α-nitrile,3β-acetate. Thus, several potent compounds inhibit these enzymes *in vitro* and may be candidates for potential use as inhibitors *in vivo*.

INTRODUCTION

3β-Hydroxysteroid oxidoreductase and 5 → 4-ene-3-ketosteroidisomerase are microsomal enzymes involved in the early steps of steroid biosynthesis essential for the production of all biologically active steroid hormones [1]. Steroid C_{17-20} lyase is a microsomal enzyme which cleaves C_{21}-steroids to the corresponding C_{19}-androgens which are involved in the synthesis of active androgens and oestrogens. Two substrate analogues (2α-cyano-4,4-17α-trimethyl-17β-hydroxy-5-androsten-3-one and 17β-hydroxy-4,4-17α-trimethyl-5-androsten-{2,3d}-isoxazole) are both stoichiometric inhibitors of the oxidoreductase and isomerase in rat adrenals and gonads [1–4]. These analogues stoichiometrically bind to the enzymes' active sites, titrate activity of the enzymes, cannot be removed from the active sites by dilution. However, there have been very few similar reports of inhibitors of the lyase and, therefore, we have performed screening experiments using human adrenal microsomes to determine whether the conversion of labelled dehydroepiandrosterone or labelled 17-hydroxyprogesterone to androstenedione can be inhibited by some inhibitors effective on the corresponding enzymes in rat adrenal tissues [5].

MATERIALS AND METHODS

Chemical

[4-^{14}C]-17-Hydroxyprogesterone (61 μCi/μmol), [4-^{14}C]-dehydroepiandrosterone (52 μCi/μmol) and [7α-^{3}H]-androstenedione (3·1 mCi/μmol) were purchased from the Radiochemical Centre, Amersham, Bucks., England, and were purified by paper chromatography before use. The maker's specific activities were not checked. Nicotinamide-adenine dinucleotide (NAD^+) and reduced nicotinamide-adenine dinucleotide phosphate (NADPH) were purchased from British Drug Houses Ltd., Poole, Dorset, England. The organic solvents used were also supplied by B.D.H. Ltd.; toluene and ethyl acetate were redistilled before use. 1,4-Bis-{2-(4-methyl-5-phenyloxazolyl)}-benzene (dimethyl POPOP) and 2,5-diphenyloxazole (PPO) were obtained from the Packard Instrument Company, Inc.

Clinical

Human adrenal glands were obtained from patients with metastatic cancer of the breast who were undergoing bilateral adrenalectomy. Immediately after removal the glands were frozen on Cardice and remained in that state until used. The adrenals were grossly examined for the presence of tumour secondaries and those glands which were found to contain malignant deposits were discarded.

Preparation and incubation of adrenal microsomes

The method of Allfrey *et al.*, 1964[6] as modified by Jensen *et al.*, 1972[7] was used in the preparation of adrenal microsomes.

The procedures for incubation and isolation of androstenedione are described in detail elsewhere [8].

Inhibitors

The synthetic inhibitors are given numbers as shown in the tables.

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Table 1. C_{19} and C_{18} inhibitors of human adrenal C_{17-20} lyase and C_{19}-3β-hydroxysteroid oxidoreductase: 5 → 4-ene,3-ketosteroid isomerase

Inhibitor No.	Substituents on position 1	2	3	4	5	16	17	19	C_{17-20} Lyase % Inhibition A	% Inhibition B	I_{50}	Oxidoreductase: Isomerase % Inhibition A	% Inhibition B	I_{50}
5-Androstene derivatives														
1	—	—	βOH		Δ	Δ	C=N		59·5	99·5	1·6	32·7	71·1	2·9
2	—	—	βOH		Δ		βOH,αC=N		63·5	100·0	1·4	72·6	43·6	3·6
3	—	—	βCH_3OCO	—	Δ	Δ	C=N		64·8	72·6	1·8	0·9	23·3	8·4
4	—	—	βCH_3OCO	—	Δ	—	βOH,αC=N	—	24·1	19·3	14·2	13·9	37·3	5·1
5	—	—	βCH_3OCO		Δ	—	βH,αC=N		48·6	53·1	3·3	23·7	3·1	/
4-Androstene derivatives														
6	Δ	—	keto	Δ			αH,β-NHCONH$_2$		50·3	94·5	1·7	13·2	53·1	4·0
7	Δ		keto	Δ	—	—	*	—	31·0	80·5	2·3	36·2		2·9
5α,β-Androstane derivatives														
8	—	O,	αC=N		αH	—	$\beta OCOCH_3$		34·2	59·2	2·9	24·7	56·4	3·6
9	αC=N	—	keto	—	αH		$\beta OCOCH_3$	—	29·6	48·6	4·2	40·8	49·0	4·1
10	αC=N	Δ	—		αH	—	$\beta OCOCH_3$		49·4	90·0	1·7	17·0	50·0	3·6
11	—	—	keto	—	βC=N		$\beta OCOCH_2CH_3$		12·1	46·7	3·9	23·9		3·6
19 Nor-Androstane derivatives														
12		—	keto	—	βC=N	—	βOH,αC = CH	Nor	65·1	63·7	6·1	14·4	23·6	9·7
Estratriene derivatives														
13	—	—	CH_3O	—	—		$\beta N(CH_3)_2$,αCH_3	Nor	26·3	53·9	3·2	33·2	60·8	3·0
14	—	—	CH_3O	—		—	βOH,αC=N	Nor	31·1	58·5	3·5	36·4	55·7	3·5

A: Inhibitor concentration 0·19 μg/ml: B: Inhibitor concentration 1·35 μg/ml; I_{50} = Inhibitor concentration (μM) required for 50% inhibition; % Inhibition:

$$\frac{\text{1-nG products formed with inhibitor}}{\text{nG products formed without inhibitor}} \times 100\%.$$

* 2,3′α-tetrahydrofuran-2′-spiro-17.

Compounds 1–5, 15–20 were purchased from Steraloids (Pawling, New York); 6 and 7 were the kind gifts of Dr. Glen Arth, Merck, Sharpe and Dohme, Raway, New Jersey; 8–10 those of Dr. Friedmund Neumann, Schering A/G. Berlin, Germany; 11 and 12 those of Dr. W. H. Hunt, Searle Co., Chicago, Illinois, and 13–14 and 21 and 22 those of Dr. John Babcock, Upjohn Co., Kalamazoo, Michigan.

RESULTS

5-Androstene derivatives. The effect of various substituted inhibitors on the conversion of 17-hydroxyprogesterone and dehydroepiandrosterone to androstenedione by the human adrenal microsomes are shown in Tables 1 and 2. The uppermost panel of Table 1 shows that two synthetic derivatives of dehydroepiandrosterone, namely 16-ene-17-cyano "1" and the 17β-hydroxy, 17α-cyano "2" derivatives are potent inhibitors of both the oxidoreductase-isomerase system and of the C_{17-20} lyase. When a 3β-acetate derivative of "1" is formed "3", the degree of inhibition of the oxidoreductase-isomerase is about one-third but the inhibition of the lyase remains about equally potent. Conversion of "2" to the 3β-acetate "4" however, markedly diminishes inhibitory capacity with each enzyme. A 3β-acetate of dehydroepiandrosterone having a 17α-cyano and a 17β-hydrogenase substitution is quite potent inhibitor of C_{17-20} lyase, however, it does not affect the oxidoreductase-isomerase system.

4-Androstene derivatives. The 1-en-17β-ureido derivative of testosterone is a potent inhibitor of both

Table 2. 4-ene and 5-ene-C_{21} inhibitors of human adrenal C_{17-20} lyase and C_{19}-3β-hydroxysteroid oxidoreductase: 5 → 4-ene,3-ketosteroid isomerase

Inhibitor No.	Substituents on Position 1	2	3	4	5	6	16	17	20	21	C_{17-20} Lyase % Inhibition A	% Inhibition B	I_{50}	Oxidoreductase: Isomerase % Inhibition A	% Inhibition B	I_{50}
4-Pregnene derivates																
15	—	—	keto	Δ	—	—	αCH_3	—	keto	—	35·7	65·8	2·8	27·4	13·7	46·8
16	—	—	keto	Δ	—	—	βCH_3	—	keto	—	60·0	83·9	1·7	0	32·9	6·2
17	—	—	βOH	Δ	—	—	—	—	βC=N	—	51·9	73·5	2·2	59·5		3·6
5-Pregnene derivatives																
18	—	—	βOH		Δ	—	αBr		keto		63·7	94·2	1·6	27·2	64·6	2·4
19	—	—	βOH		Δ	—	αC=N	—	keto		30·6	56·6	3·2	39·3	27·9	9·6
20	—	—	βOH	—	Δ	—	αC=N	αOH	keto		38·6	33·9	6·3		5·7	31·6
21			βOH, βOH 3COO-4′H	—	—	—			keto	—	30·5	30·6	7·6	43·5		4·1
22			3…5α	—	—	$\beta OCOCH_3$	αC=N	—	keto	—	24·2	36·7	5·8	7·7	21·8	10·3

Abbreviations: See Table 1.
3COO-4′H: 4′H,4′H-pregnen-3′carboxylic acid.
3…5α: 3α,5α-cyclopregnane.

enzymes as is the 17–20 spiroderivative of testosterone (Table 1).

5α,β-Androstane derivatives. Three 5α-reduced androstane derivatives, a 17β-acetate and 3α-cyano, 2,3-epoxy derivative "8", a 3-keto, 1α-cyano, 17β-acetate derivative "9", and 1-ene-1α-cyano, 17β-acetate substitution are all equally potent inhibitors of the 3 enzymes (Table 1). A 5β-cyano 3-keto, 17β-propionate substituted derivative "11" is also a good inhibitor of all three enzymes.

19-Nor-androstane derivative. A 19-nor-androstane derivative substituted with 5β-cyano, 3-keto, 17β-hydroxy and 17α-ethinyl groups "12" is a somewhat less potent inhibitor of the three enzymes (Table 1).

Estratriene derivatives. Two estratriene derivatives having a 3-methyl ether and 17β-diethyl, 17α-methyl substitutions "13" or a 17β-hydroxy-17α-cyano "14" substitution are also potent inhibitors of the three enzymes.

4-Pregnene derivatives. 16α-Methyl-progesterone "15" is a potent inhibitor of the lyase but is considerably less active on the oxidoreductase-isomerase system (Table 2). When 16β-methyl-progesterone "16" is tested, it is more potent on the oxidoreductase-isomerase system. 20β-cyano-3β-hydroxyprogesterone "17" is also a potent inhibitor of all three enzymes.

5-Pregnene derivatives. Several pregnenolone derivatives are effective inhibitors of all three enzymes, with 16α-bromopregnenolone "18" being the most effective (Table 2). It is interesting to note that a 17α-hydroxy substitution "20" of 16α-cyanopregnenolone "19" causes considerably greater loss of inhibition of the oxidoreductase system than that of the lyase system.

DISCUSSION

The present report has delineated several potent inhibitors of the human adrenal microsomal 3β-hydroxysteroid-oxidoreductase-5 → 4-ene, 3-keto-steroid isomerase and C_{17-20} lyase. We have previously reported that compounds 1–5, 7 and 19 inhibit the 3β-hydroxy-5-ene-steroid oxidoreductase in human placenta with dehydroepiandrosterone as substrate [9]. However, we have reported that compounds 6, 8, 10, 12–17, 18, 20–22, are without effect on the human placental C_{19} oxidoreductase [9]. These observations suggest that these inhibitors are capable of demonstrating tissue-dependent isozymic active site differences, which have the capacity to be inhibited by these synthetic steroids in one tissue but not in that of another. In the present case all the compounds inhibit the human adrenal C_{19} oxidoreductase *in vitro* whereas only compounds 1–5, 7 and 19 inhibit the corresponding isozyme in the human placenta [9].

We have made preliminary reports of the inhibition of testicular steroidogenesis in the rat, selectively at the level of gonodal oxidoreductase system 17α-hydroxylase and C_{17-20} lyase by compounds 1–4 [5]. The 17β-ureido derivative "6" of testosterone also selectively inhibits rat gonodal 17α-hydroxylase and C_{17-20} lyase and the steroidal excretion patterns of rats treated with these inhibitors *in vivo* are completely consistent with the proposed mode of action of these inhibitors *in vitro* [5]. Stoichiometric inhibitors have been used to identify differences in enzymes in one tissue from those of another, where other studies have not been able to detect such distinctions. By antisera cross-reaction studies [10, 11], and by aminoacid analysis [11], it has been shown that lactic acid dehydrogenases from heart and skeletal muscle in the same animal are quite different, but the heart lactic acid dehydrogenase from different species are more similar. Thus, in the present report compounds 6, 8–10, 12–17, 18, 20–22, give selective inhibition of tte adrenal 3β-hydroxysteroid oxidoreductase system but do not affect the human placental enzyme. Attempts are now being made to study in detail the nature of these inhibitions.

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