J. steroid Biochem. Vol. 35, Nos 3/4, pp. 465–471, 1990
Printed in Great Britain.

0022-4731/90 $3.00 + 0.00

STRUCTURE–ACTIVITY RELATIONSHIPS IN THE *IN VITRO* MODULATION OF RAT HEPATIC MICROSOMAL ANDROST-4-ENE-3,17-DIONE HYDROXYLASE ACTIVITIES BY DERIVATIVES OF 5α- AND 5β-ANDROSTANE

MICHAEL MURRAY* and ISHITA MEHTA
Liver Research Unit, Department of Medicine, University of Sydney, Westmead Hospital, Westmead, N.S.W. 2145, Australia

(*Received* 21 *June* 1989)

Summary—The relationships between structure and inhibitory potency toward microsomal cytochrome *P*-450 (*P*-450)-mediated androst-4-ene-3,17-dione hydroxylase activities were investigated in rat liver with a series of 5α- and 5β-androstane derivatives. 5β-Reduced steroids (containing a *cis*-A/B ring junction) were more potent inhibitors than the 5α-reduced epimers (containing a *trans*-A/B ring junction) except in the case of the 17β-hydroxy-substituted derivatives. The most effective inhibitor was 5β-androstane-3β-ol which exhibited I_{50} values of 7 and 27 μM against androstenedione 16α- and 6β-hydroxylase activities, which are catalysed by *P*-450 IIC11 and IIIA2, respectively. In general, these two pathways of steroid hydroxylation were more susceptible to inhibition than the 7α- and 16β-hydroxylase pathways. The 7α-hydroxylase enzyme (*P*-450 IIA1) was only inhibited by 5β-reduced steroids that contained an oxygenated function at C17. All of the test compounds elicited type I spectral binding interactions with *P*-450 in oxidised microsomes. The most effective steroid inhibitors generally exhibited the greatest capacity to interact with *P*-450. Additional studies with one of the more potent compounds, 5β-androstane-3β-ol-17-one, revealed that the inhibition kinetics were competitive and that preincubation of the inhibitor with NADPH-supplemented microsomes prior to substrate (androstenedione) addition decreased the extent of inhibition observed. These findings are consistent with the assertion that the inhibition of hepatic steroid hydroxylases by 5β-androstanes involves an effective competitive interaction with the steroid substrate at the *P*-450 active site. Since the relative overproduction of 5β-reduced metabolites of certain androgens has been reported in clinical conditions, such as androgen insensitivity, it now appears important to investigate the hepatic drug oxidation capacity of patients with hormonal abnormalities.

INTRODUCTION

Many hydrophobic substances are converted to more polar metabolites by the hepatic microsomal cytochrome *P*-450 (*P*-450)-linked mixed-function oxidase (MFO) system. A number of closely related, but distinct, isoforms of *P*-450 are present in liver and this multiplicity appears to be responsible for the broad substrate specificity of the MFO system. Although a large number of substrates of the MFO system have been identified, recent studies have demonstrated that several steroids are subject to *P*-450 isoform-specific hydroxylation [1, 2]. Thus, the 2α- and 16α-hydroxylations of C-19 and C-21 steroids are catalysed by the male-specific *P*-450 IIC11 [3, 4], steroid 6β-hydroxylation is catalysed by *P*-450 IIIA2 [3, 4] and immunochemically similar forms, and 7α- and 16β-hydroxylations are catalysed by the *P*-450s IIA1 and IIB1, respectively [3, 4]. Hepatic microsomes contain enzymes, other than the *P*-450s, that catalyse the 5α-reduction of 4-ene-steroids (4-ene-3-oxosteroid 5α-oxidoreductase) [5] and the reduction of 17-oxosteroids (17β-hydroxysteroid oxidoreductase) [6]. In contrast, the hepatic cytosol contains a 4-ene-3-oxosteroid 5β-oxidoreductase [5, 7] and a 3α-hydroxysteroid oxidoreductase [8]. These enzymes function in the deactivation of many steroid hormones and probably have an important regulatory role in the duration of steroid action.

Steroids are also inhibitors of foreign compound metabolism mediated by the MFO system [9–11]. Of considerable interest is the observation that certain 5β-steroids appeared to be more potent inhibitors of drug metabolism than the 5α-isomers [9]. Since one site of steroid metabolism is the liver, it appears that hepatic enzymes are capable of catalysing the conversion of androgens to steroids that are themselves inhibitory toward *P*-450s involved in steroid and drug metabolism. The present study was undertaken

*To whom correspondence should be addressed.

to investigate the effects of several structural variations in a series of hydroxy- and ketoandrostanes as inhibitors of *P*-450-mediated microsomal androst-4-ene-3,17-dione (androstenedione) hydroxylation *in vitro*. Of particular interest was the importance of the A/B ring junction stereochemistry and the nature of the oxy-functions at C-3 and C-17. The effect of conformation on binding of the steroids to ferric microsomal *P*-450 was also investigated in this study.

MATERIALS AND METHODS

Animals

In these experiments untreated male Wistar rats (approx. 350 g) were used for the preparation of hepatic microsomes as described previously [12].

Chemicals

[4-^{14}C]Androstenedione (sp. act. 59 mCi/mmol) and [4-^{14}C]testosterone (sp. act. 59 mCi/mmol) were obtained from Amersham Australia, Sydney, N.S.W. Unlabelled steroids were purchased from either Sigma Chemical Co., St Louis, Mo. or the MRC Steroid Reference Collection, Queen Mary's College, London, U.K.

Biochemicals were also obtained from Sigma. Solvents and other analytical reagents were from Ajax Chemicals, Sydney, N.S.W.

Steroid hydroxylase assays

Microsomal androstenedione hydroxylase activity was estimated essentially as described previously [13, 14]. The substrate and protein [15] concentrations were 175 μM and 0.75 mg/ml, respectively, and incubations were conducted for 10 min. After extraction with chloroform, metabolites were separated by thin-layer chromatography (F_{254} silica gel 60; Merck, Darmstadt, F.R.G.) using the solvent system chloroform:ethyl acetate (1:2, w/v) [2]. Zones on TLC plates that comigrated with metabolite standards were visualised under u.v. light, scraped into vials and quantified by liquid scintillation counting (ACS II, Amersham, Sydney, Australia).

Testosterone hydroxylase activity was estimated in hepatic microsomal fractions as described elsewhere [14]. Metabolites of testosterone were separated by TLC as described for androstenedione metabolites except that the first solvent system was dichloromethane:acetone (4:1, v/v) and the second system was chloroform:ethyl acetate:ethanol (4:1:0.7, v/v/v) [2]. Rates of metabolite formation were again quantified by liquid scintillation spectrometry.

I_{50} values (concentrations of inhibitors that elicit a 50% decrease in the rate of steroid metabolite formation) were determined at least in duplicate from plots of log inhibitor concentration vs percent control activity using at least four inhibitor concentrations; inhibitors were added in ethanol (final concentration 2.5%). Additional experiments assessed the inhibition kinetics associated with one of the inhibitors (5β-androstan-3β-ol-17-one). The range of substrate (androstenedione) concentrations employed in these experiments was 32–380 μM and the range of inhibitor concentrations was 0–50 μM. Incubation time was 5 min. Kinetic parameters were determined from plots of reciprocal substrate concentration vs reciprocal product formation (Lineweaver–Burk analysis) and inhibitor concentration vs reciprocal product formation (Dixon analysis). Further experiments assessed the effect of preincubation of 5β-androstan-3β-ol-17-one with hepatic microsomes and an NADPH-generating system for varying times prior to the addition of androstenedione. Incubations were continued for 10 min after substrate addition after which time metabolites were extracted, separated by TLC and quantified as described above.

Spectral binding studies

Optical difference spectra were determined in an Aminco-Chance DW-2a spectrophotometer operating in the split beam mode. Microsomes were resuspended in 0.1 M potassium phosphate buffer (pH 7.4) to a concentration of 1 mg protein/ml, divided equally between two cuvettes and a baseline of zero light absorbance established at 37°C. Test compounds were added to the sample cuvette in microlitre quantities of ethanol and solvent was added to the reference cuvette. Total solvent addition did not exceed 1%. Plots of reciprocal ligand concentration vs reciprocal spectral change (peak to trough) were constructed. Spectral dissociation constants (K_s) and maximal absorbance change (ΔA_{max}) parameters were determined from the x-axis and y-axis intercepts, respectively, of the double reciprocal plots. Cytochrome *P*-450 was estimated by the standard procedure of Omura and Sato [16].

Cytochrome P-450 nomenclature

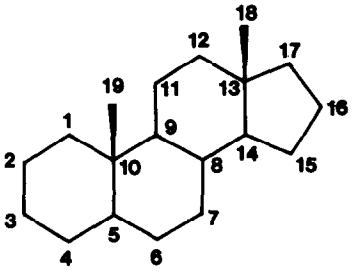
The recommended nomenclature of Nebert *et al.*[17], which is based upon gene designation, is used in this paper. Thus, *P*-450 IIC11 is the male-specific steroid 16α-hydroxylase, also termed *P*-450 *UT-A* [3], *P*-450 2c [3], *P*-450$_{16\alpha}$ [18] or *P*-450 h [4]. *P*-450 IIA1 is the steroid 7α-hydroxylase, also termed *P*-450 *UT-F* [3], *P*-450 3 [3] or *P*-450 a [1]. *P*-450 IIIA2 is the male-specific steroid 6β-hydroxylase that belongs to the same subfamily as the pregnenolone 16α-carbonitrile-inducible forms of the cytochrome such as *P*-450 *PCN-E* [3]. *P*-450 IIB1 is the phenobarbital-inducible steroid 16β-hydroxylase otherwise termed *P*-450 *PB-B* [3], *P*-450 PB-4 [3] and *P*-450 b [1].

RESULTS AND DISCUSSION

Inhibition of microsomal androstenedione hydroxylation in rat liver by hydroxy- and ketoandrostanes

The data in Table 1 demonstrate that, of the four principal pathways of androstenedione hydroxylation

Table 1. Inhibition of rat hepatic microsomal androst-4-ene-3,17-dione hydroxylases by derivatives of 5α- and 5β-androstane



General structure of androstane derivatives.

No.	Compound 5-H	3	17	Androst-4-ene-3,17-dione hydroxylase I_{50} (μM)[a] 16α	16β	6β	7α
1	β	β-ol	—	7	—[b]	27	—
2	α	β-ol	—	71	—	110	—
3	β	α-ol	one	95	—	—	—
4	β	β-ol	one	51	87	47	60
5	α	α-ol	one	—	—	—	—
6	α	β-ol	one	—	140	120	—
7	β	—	β-ol	65	—	110	24
8	α	—	β-ol	26	47	46	—
9	α	one	one	—	—	—	—
10	β	one	one	—	—	—	84

[a] I_{50} values (concentrations of 5α- and 5β-androstane derivatives that elicit 50% inhibition of androst-4-ene-3,17-dione positional hydroxylation) were determined in duplicate as described in Materials and Methods using at least four different concentrations of inhibitor. [b] Less than 50% inhibition observed below 150 μM.

in male rat hepatic microsomes, formation of the 6β- and 16α-alcohols was usually more susceptible to inhibition by the androstane steroids than formation of the 7α- and 16β-alcohols. The most effective inhibitor of the series was 5β-androstane-3β-ol (compound **1**), which exhibited an I_{50} of 7 μM against *P*-450 IIC11-mediated steroid 16α-hydroxylation and an I_{50} of 27 μM against androstenedione 6β-hydroxylase activity catalysed by *P*-450 IIIA2. In contrast, the 5α-epimer (compound **2**) was 10- and 4-fold less potent, respectively, against 16α- and 6β-hydroxylation. Similar findings, with the 5β-isomers being more effective than corresponding 5α-isomers, were noted in the 5-androstane-3α/β-ol-17-one series (compounds **3**–**6**, Table 1). Testosterone hydroxylation pathways were inhibited in a similar fashion to those of androstenedione (data not shown). Thus, testosterone 2α- and 16α-hydroxylation (I_{50}s of 63 and 71 μM, respectively) and androstenedione 16α-hydroxylation (I_{50} = 51 μM) were similarly susceptible to inhibition by compound 4, whereas the 3α-epimer (compound **3**) was distinctly less effective against these activities. These findings are not surprising as the same enzyme (*P*-450 IIC11) is active in all of these pathways of steroid oxidation [2]. Analogous results were obtained for the inhibition of the 6β-, 7α- and 16β-hydroxylations of both steroids by compounds **3** and **4**.

The presence of a 17β-hydroxyl substituent also led to compounds that were relatively effective inhibitors of *P*-450 activity although, in the case of compounds **7** and **8**, it was noted that the 5α-reduced isomer was more potent than its 5β-epimer (except toward steroid 7α-hydroxylation). The isomeric 5-androstane-3,17-diones (compounds **9** and **10**, Table 1) were both essentially ineffective inhibitors of microsomal androstenedione hydroxylation. It was apparent, however, that steroid 7α-hydroxylation was relatively susceptible to inhibition by the 5β-isomer.

The present series of compounds also provided an opportunity to assess the importance of the stereochemistry of the 3-hydroxyl group to *P*-450 inhibition. When the hydroxyl substituent was in the β-(equatorial) configuration, as in compounds **4** and **6**, *P*-450 activity was inhibited to a greater extent than when the hydroxyl was in the α-(axial) configuration. As indicated above, replacement of the 3-hydroxyl group with a ketone function resulted in androstane derivatives that were ineffective inhibitors of *P*-450 activity. When considered along with the information obtained from the 3β- and 17β-alcohols of 5α- and 5β-androstane, it appears likely that the hydroxyl function in these C19 steroids could be involved in a hydrogen-bonding interaction with a keto-containing amino acid residue in the active sites of the 16α- and 6β-hydroxylases. This possibility has been suggested previously by Swinney *et al.* to account for the observed ratios of 2α/16α-hydroxy products of different steroid substrates produced by *P*-450 IIC11 [4].

The only three androstanes that inhibited androstenedione 7α-hydroxylation were compounds **4**, **7** and **10** (Table 1). The structural features that these derivatives share are the *cis*-A/B ring junction and an oxygenated substituent (either hydroxyl or keto) at C17. It is perhaps not surprising that 5β-androstane

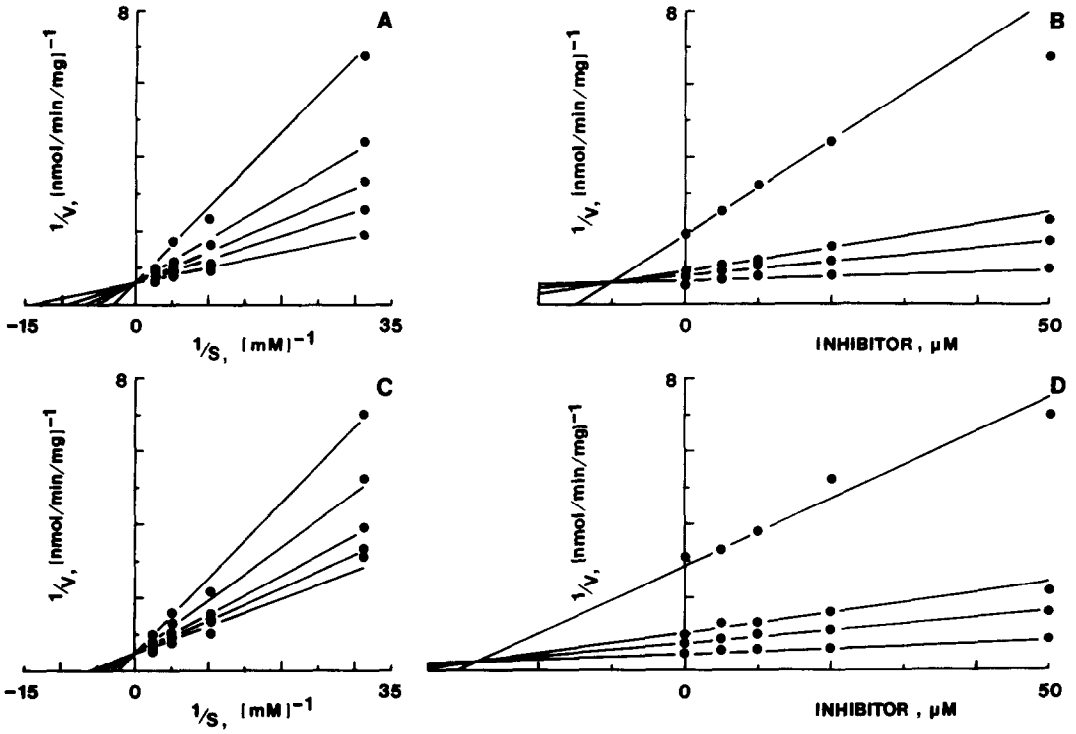


Fig. 1. Inhibition of rat hepatic microsomal androst-4-ene-3,17-dione 6β- and 16α-hydroxylase activities by 5β-androstane-3β-ol-17-one. Panels (A) and (B) show the Lineweaver–Burk and Dixon plots for competitive inhibition of the 6β-hydroxylase by the steroid. Panels (C) and (D) show the analogous plots for the inhibition of 16α-hydroxylase activity.

derivatives were effective inhibitors of the 7α-hydroxylase as the C7 position is sterically hindered due to the A/B-ring *cis* stereochemistry and the C19-methyl group. Further studies are required to delineate fully the features of the steroid 7α-hydroxylase (*P*-450 IIA1) active site that appear to be important for substrate and inhibitor binding. Nevertheless, from the present data set it appears possible that the three 5β-steroids may preclude metabolism by *P*-450 IIA1 so that these derivatives continue to compete effectively with androstenedione at the catalytic centre of the enzyme.

One of the more potent inhibitory steroids, 5β-androstane-3β-ol-17-one (compound **4**), was investigated further. Kinetic analysis of the inhibition of androstenedione 6β- and 16α-hydroxylation revealed that compound **4** was a competitive inhibitor of these activities. From the Dixon plots of the inhibition of androstenedione 6β- and 16α-hydroxylation, dissociation constants of the enzyme-inhibitor complexes (K_is) were determined to be 12 and 34 μM, respectively (Fig. 1 (B and D)). Further experiments were conducted in which 5β-androstane-3β-ol-17-one was preincubated with microsomes and an NADPH-generating system for varying periods of time prior to substrate (androstenedione) addition. Without preincubation, 6β- and 16α-hydroxylation were inhibited by 43 and 41% percent, respectively, in the presence of 50 μM of compound **4**. Preincubation decreased the extent of inhibition until, after 30 min, steroid 6β- and 16α-hydroxylations were inhibited by 23 and 20%, respectively (Fig. 2). Thus the extent of inhibition was decreased under conditions that support oxidation, a finding that is consistent with the slow conversion of the steroid to less inhibitory metabolites.

Spectral binding interactions of androstane derivatives with oxidised hepatic microsomes from male rats

The hydroxy- and keto-substituted androstanes in the present study all interacted in type I fashion

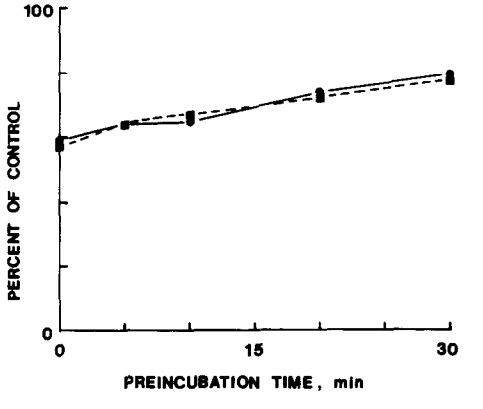


Fig. 2. Effect of preincubation of hepatic microsomes with an NADPH-generating system for varying periods prior to substrate (androst-4-ene-3,17-dione) addition on the inhibition of 6β (■)- and 16α (●)-hydroxylase activities by 5β-androstane-3β-ol-17-one (50 μM).

Table 2. Spectral binding parameters of the interaction of 5α- and 5β-androstane steroids with ferricytochrome *P*-450 in male rat hepatic microsomes

Compound	K_s (μM)	ΔA_{max} (abs. units/nmol *P*-450)	$\Delta A_{max}/K_s$ (abs. units/nmol *P*-450/M)
1	2.0	0.048	24400
2	4.2	0.045	10800
3	3.1	0.014	4500
4	1.7	0.046	27000
5	3.4	0.005	1500
6	1.9	0.026	14000
7	0.8	0.022	27500
8	2.2	0.023	10500
9	5.0	0.013	2600
10	3.5	0.025	7100

($\lambda_{max} \sim 386$ nm, $\lambda_{min} \sim 420$ nm) with oxidised hepatic microsomal *P*-450 (not shown). As shown in Table 2, all of the test steroids elicited binding interactions of relatively high affinity (K_s range 0.8–5.0 μM). Certain derivatives were found to produce only small maximal absorbance changes (ΔA_{max}), which reflect the proportion of total *P*-450 undergoing the binding reaction, and these derivatives were usually non-potent inhibitors (e.g. compounds **5** and **9**). A further parameter ($\Delta A_{max}/K_s$) was used as an indicator of the relative efficiency of ligand binding to the cytochromes *P*-450 present in microsomes. The range of values for this parameter was 1500 abs. units/nmol *P*-450/M (Table 2; for compound **5**, which was an ineffective inhibitor) to 27,500 (for compound **7**, which was an effective inhibitor). The significance of this parameter in binding studies is analogous to that of V_{max}/K_m in catalytic reactions. It is clear, however, that it is not possible to equate binding parameters directly with the capacity of the steroids to inhibit androstenedione hydroxylase activities. Indeed, it should be recalled that the type I interaction merely reflects the capacity of a particular compound to interact with *P*-450 prior to the initiation of the catalytic cycle [19]. That is, it is quite possible for a particular steroid to have relatively high affinity for the oxidised form of the cytochrome, but to have a greatly decreased binding capacity for the reduced form of the enzyme. Furthermore, these spectral binding parameters pertain only to the binding of ligands to the substrate-free cytochrome. In the presence of substrate the capacity of the androstane steroids to interact with *P*-450 may be impaired. Indeed, we have estimated K_s values of 3.4 and 2.1 μM, respectively, for the type I interactions of androstenedione and testosterone with oxidised microsomal *P*-450 (data not shown). Thus, it is likely that some degree of competition between steroids occurs at the active site of oxidised *P*-450. The present observations, however, confirm that most androstane derivatives interact strongly with ferric *P*-450 and that, in general, the more potent inhibitors of androstenedione hydroxylation were effective type I ligands for *P*-450.

General discussion

It has been reported that steroids have the capacity to modulate hepatic microsomal drug oxidation [8–11]. Although not a surprising finding, in view of the well-established role of the *P*-450 enzymes in the biotransformation of steroids as well as drugs, it is now clear that certain structural features enhance the potency of *P*-450 inhibitory steroids. The most striking finding is that 5β-reduced steroids appeared to be more potent than the 5α-epimers as inhibitors of specific *P*-450s (the 6β- and 16α-hydroxylases of rat liver were generally more susceptible to inhibition in the present study). 5β-Androstane-5β-ol-17-one was found to be a competitive inhibitor of these hydroxylation pathways in rat hepatic microsomes. Preincubation of this steroid with microsomes and an NADPH-generating system before substrate addition resulted in decreased inhibition. It was found that long preincubation times were necessary for significant reversal of inhibition suggesting that the 5β-conformation (*cis* A/B ring junction) is not the preferred conformation for *P*-450 steroid substrates. Despite this point it is clear that the 5β-reduced steroids fulfil the requirements for binding to ferric *P*-450.

In vitro experiments have demonstrated that the 4-ene-3-oxosteroid 5α-reductase is a microsomal enzyme while the 5β-reductase is located in the hepatic cytosol [5]. Interestingly, however, Forchielli and Dorfman showed that perfusion of livers with 4-ene-3-oxosteroids yielded only 5α-reduced products [5]. Thus it was inferred that, in the normal situation, the functioning of the 5β-oxidoreductase enzyme is suppressed (presumably in the intact animal). Nevertheless, steroids possessing a 5β-reduced conformation have been detected in urine of humans treated with testosterone and conjugates [20, 21], and in animals treated with testosterone glucuronide [22]. Indeed, human males with psuedohermaphroditism have been shown to excrete a greater proportion of 5β-reduced steroids (the urinary etiocholanolone:androsterone ratio is in the range 1.0–4.0 whereas it is normally about 0.5–1.0). Studies by Gustafsson and co-workers investigated the regulation of rat hepatic cytosolic 5β-reductase activity, as well as a number of other steroid metabolising enzymes [23, 24]. They noted that the rate of 5β-reduction was greater in males than in females and that neonatal castration of male rats prevented the developmental expression of 5β-reductase activity in adult life. Postnatal castration

(at 7 days and older) actually increased apparent 5β-reductase activity. These findings are consistent with induction of 5β-reductase activity at birth and irreversible suppression during the adult period. Considered together these findings indicate that hepatic 5β-reductase activity is normally suppressed but may assume greater importance in hormonal disturbances, such as those associated with 5α-reductase deficiency, androgen insensitivity and Acute Intermittent Porphyria [25]. Thus, from animal studies, it appears that disease states associated with hormonal imbalances, or treatments that produce hormonal imbalances, may themselves produce alterations in the relative production of 5β-reduced products.

The 5β-reduced steroids also constitute a useful series of probes for investigation of the active sites of different *P*-450s. The differences in inhibitory potency between epimeric pairs (5α/5β) are not attributable to hydrophobic effects, which have been shown in several studies to influence the extent of *P*-450 inhibition [26–28]. In fact, the 5α-reduced steroids are likely to possess slightly greater hydrophobic character than their 5β-epimers since steroids with *trans*-A/B ring junctions adopt conformations that expose a greater surface area to the aqueous environment. Electronic factors are also unlikely to contribute to inhibition so that steric factors appear to be the primary determinant of androstenedione hydroxylase inhibition by androstane derivatives. Indeed, steric factors have been shown to be important in the inhibition of other MFO activities by imidazoles and benzimidazoles [27, 29, 30]. It is feasible that the *cis*-A/B ring conformation of the 5β-reduced steroids facilitates binding to the hydrophobic substrate site on the *P*-450 apoprotein and that inhibition is enhanced due to minimal rates of inhibitor metabolism. In contrast, the 5α-reduced steroids are readily oxidised by *P*-450s [23] and it may be partly for this reason that less potent MFO inhibition was observed.

The results in the present study emphasise the importance of conformational factors in the inhibition of microsomal androstenedione hydroxylase activities by a series of androstane steroids. The finding that 5β-reduced androstanes were relatively more potent inhibitors of MFO activity has potential significance as hormonal disturbances in certain clinical situations (e.g. male pseudohermaphroditism and Acute Intermittent Porphyria) are documented to result in the overproduction of 5β-reduced steroids relative to 5α-reduced steroids. It is yet to be determined whether oxidative steroid and drug metabolism is impaired in such patients.

Acknowledgement—This work was supported by the Australian National Health and Medical Research Council (NH&MRC). M.M. is an NH&MRC Senior Research Fellow.

REFERENCES

1. Wood A. W., Ryan D. E., Thomas P. E. and Levin W.: Regio- and stereoselective metabolism of two C_{19} steroids by five highly purified and reconstituted rat hepatic cytochrome *P*-450 isozymes. *J. Biol. Chem.* **258** (1983) 8839–8847.
2. Waxman D. J., Ko A. and Walsh C.: Regioselectivity and stereoselectivity of androgen hydroxylations catalyzed by cytochrome P-450 isozymes purified from phenobarbital-induced rat liver. *J. Biol. Chem.* **258** (1983) 11937–11947.
3. Waxman D. J., Dannan G. A. and Guengerich F. P.: Regulation of rat hepatic cytochrome P-450: age-dependent expression, hormonal imprinting, and xenobiotic inducibility of sex-specific isozymes. *Biochemistry* **24** (1985) 4409–4417.
4. Swinney D. C., Ryan D. E., Thomas P. E. and Levin W.: Regioselective progesterone hydroxylation catalyzed by eleven rat hepatic cytochrome P-450 isozymes. *Biochemistry* **26** (1987) 7073–7083.
5. Forchielli E. and Dorfman R. I.: Separation of Δ^4-5α- and Δ^4-5β-hydrogenases from rat liver homogenates. *J. biol. Chem.* **223** (1956) 443–448.
6. Milewich L., Garcia R. L. and Gerrity L. W.: 17β-Hydroxysteroid oxidoreductase: A ubiquitous enzyme. Interconversion of estrone and estradiol-17β in BALB/c mouse tissues. *Metabolism* **34** (1985) 938–944.
7. Einarsson K., Gustafsson J.-A. and Stenberg A.: Neonatal imprinting of liver microsomal hydroxylation and reduction of steroids. *J. Biol. Chem.* **248** (1973) 4987–4997.
8. Lau P. C. K., Layne D. S. and Williamson D. G.: Comparison of the multiple forms of the soluble 3(17)α-hydroxysteroid dehydrogenases of female rabbit kidney and liver. *J. Biol. Chem.* **257** (1982) 9450–9456.
9. Tephly T. R. and Mannering G. J.: Inhibition of drug metabolism: V. Inhibition of drug metabolism by steroids. *Molec. Pharmac.* **4** (1968) 10–14.
10. Soyka L. F. and Long R. J.: *In vitro* inhibition of drug metabolism by metabolites of progesterone. *J. Pharmac. Exp. Ther.* **182** (1972) 320–327.
11. Soyka L. F. and Deckert F. W.: Further studies on the inhibition of drug metabolism by pregnanolone and related steroids. *Biochem. Pharmac.* **23** (1974) 1629–1639.
12. Murray M., Zaluzny L. and Farrell G. C.: Drug metabolism in cirrhosis: selective changes in cytochrome P-450 isozymes in the choline-deficient rat model. *Biochem. Pharmac.* **35** (1986) 1817–1824.
13. Gustafsson, J.-A. and Ingelman-Sundberg M.: Regulation and substrate specificity of a steroid sulfate-specific hydroxylase system in female rat liver microsomes. *J. Biol. Chem.* **250** (1975) 3451–3458.
14. Murray M., Zaluzny L. and Farrell G. C.: Impaired androgen 16α-hydroxylation in hepatic microsomes from carbon tetrachloride-cirrhotic male rats. *Gastroenterology* **93** (1987) 141–147.
15. Lowry O. H., Rosebrough N. J., Farr A. L. and Randall R. J.: Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193** (1951) 265–275.
16. Omura T. and Sato R.: The carbon monoxide binding pigment of liver microsomes. I. Evidence for its hemoprotein nature. *J. Biol. Chem.* **239** (1964) 2370–2378.
17. Nebert D. W., Nelson D. R., Adesnik M., Coon M. J., Estabrook R. W., Gonzalez F. J., Guengerich F. P., Gunsalus I. C., Johnson E. F., Kemper B., Levin W., Phillips I. R., Sato R. and Waterman M. R.: The P-450 Superfamily: updated listing of all genes and recommended nomenclature for the chromosomal loci. *DNA* **8** (1989) 1–13.
18. Morgan E. T., MacGeoch C. and Gustafsson J.-A.: Sexual differentiation of cytochrome P-450 in rat liver:

Evidence for a constitutive isozyme as the male-specific 16α-hydroxylase. *Molec. Pharmac.* **27** (1985) 471–479.
19. Schenkman J. B., Remmer H. and Estabrook R. W.: Spectral studies of drug interaction with hepatic microsomal cytochrome P-450. *Molec. Pharmac.* **3** (1967) 113–123.
20. Shinohara Y., Baba S. and Kasuya Y.: Absorption, metabolism, and excretion of oral testosterone in humans by mass fragmentography. *J. Clin. Endocr. Metab.* **51** (1980) 1459–1462.
21. Fukushima D. K., Matsui M., Bradlow H. L. and Hellman L.: Metabolism of testosterone *N*-acetylglucosaminide. *Steroids* **19** (1972) 385–410.
22. Ghraf R., Hoff H.-G., Lax E. R. and Schriefers H.: The 5β-metabolites of testosterone: mode and sex-specificity of their formation in the rat liver. *Acta Endocr.* **73** (1973) 577–584.
23. Gustafsson J.-A. and Stenberg A.: Irreversible androgenic programming at birth of microsomal and soluble rat liver enzymes active on androstene-3,17-dione and 5α-androstane 3α,17β-diol. *J. Biol. Chem.* **249** (1974) 711–718.
24. Gustafsson J.-A., Ingelman-Sundberg M. and Stenberg A.: Neonatal androgenic programming of hepatic steroid metabolism in rats. *J. Steroid Biochem.* **6** (1975) 643–649.
25. Kappas A., Bradlow H. L., Gillette P. N. and Gallagher T. F.: Abnormal steroid metabolism in the genetic liver disease acute intermittent porphyria. *Ann. N.Y. Acad. Sci.* **179** (1971) 611–624.
26. Wilkinson C. F., Hetnarski K., Cantwell G. P. and Di Carlo F. J.: Structure-activity relationships in the effects of 1-alkylimidazoles on microsomal oxidation *in vitro* and *in vivo*. *Biochem. Pharmac.* **23** (1974) 2377–2386.
27. Murray M., Ryan A. J. and Little P. J.: Inhibition of rat hepatic microsomal aminopyrine *N*-demethylase activity by benzimidazole derivatives. Quantitative structure–activity relationships. *J. Med. Chem.* **25** (1982) 887–892.
28. Murray M., Hetnarski K. and Wilkinson C. F.: Selective inhibitory interactions of alkoxymethylenedioxybenzenes towards monooxygenase activity in rat hepatic microsomes. *Xenobiotica* **15** (1985) 369–379.
29. Rogerson T. D., Wilkinson C. F. and Hetnarski K.: Steric factors in the inhibitory interaction of imidazoles with microsomal enzymes. *Biochem. Pharmac.* **26** (1977) 1039–1042.
30. Murray M.: New heterocyclic modifiers of oxidative drug metabolism. II. Steric factors in the interaction of isomeric 2-(naphthyl)methylbenzimidazoles with rat hepatic microsomal cytochrome P-450 and monooxygenase activities. *Biochem. Pharmac.* **36** (1987) 463–468.