Metabolism of anabolic androgenic steroids

Wilhelm Schänzer

Anabolic androgenic steroids (AAS) are misused to a high extent in sports by athletes to improve their physical performance. Sports federations consider the use of these drugs in sports as doping. The misuse of AAS is controlled by detection of the parent AAS (when excreted into urine) and (or) their metabolites in urine of athletes. I present a review of the metabolism of AAS. Testosterone is the principal androgenic steroid and its metabolism is compared with that of AAS. The review is divided into two parts: the general metabolism of AAS, which is separated into phase I and phase II metabolism and includes a systematic discussion of metabolic changes in the steroid molecule according to the regions (A–D rings), and the specific metabolism of AAS, which presents the metabolism of 26 AAS in humans.

INDEXING TERMS: sports medicine • drug assays • gas chromatography–mass spectrometry

The high extent of misuse of synthetic anabolic androgenic steroids (AAS) in sports by athletes to improve their physical performance became public in the 1970s.1 The International Olympic Committee (IOC) in 1974 banned the use of synthetic AAS by athletes. This prohibition was also adopted by all national and international sports federations. This ban encouraged drug testing laboratories to develop methods for the detection of misused anabolic steroids. The first methods, based on radioimmunoassay techniques [1, 2], failed to take into account the high extent of metabolism of AAS. For detecting and identifying AAS metabolites, gas chromatography–mass spectrometry (GC-MS) is the current method of choice [3–5]. Because most AAS are extensively metabolized and the parent steroids are detected for only a short period after administration, the detection of AAS metabolites allows one to confirm the misuse of AAS for a longer time. To follow this strategy, investigators must determine the metabolism of anabolic steroids in humans, elucidate the structures of the metabolites, and, if possible, synthesize the metabolites for use as reference materials. This has been done over the past years for the main metabolites of those AAS that are the most frequently misused [6].

Confirmation of AAS misuse is based on comparison of the electron impact (EI) mass spectrum, or selected ion profile, and GC retention time of the trimethylsilyl (TMS) derivatives of the steroid and (or) its metabolite(s) with the corresponding data obtained from synthesized reference substances [6] or from characterized reference substances (e.g., metabolites) isolated from urine in an excretion study.

In 1984 the use of testosterone was also banned by the IOC and by all other sports federations. A method for the detection of administered testosterone was developed by Donike et al. [7] in 1983, in which the ratio of urinary excreted testosterone glucuronide to epitestosterone glucuronide was used as an indicator for testosterone administration.

The following discussion on the metabolism of anabolic steroids is based mainly on published results from various working groups and from my own group’s published and unpublished investigations. All investigations were performed as urinary excretion studies with male subjects. The studies followed the ethical agreement of the University. All studies in our laboratory were based on self-administration of a single oral dose of an anabolic steroid. The identification of metabolites was confirmed by GC-MS. Several metabolites were synthesized to confirm their structures, and in several cases conformation was based on comparison with structurally related steroids. Characteristic fragment ions of AAS as TMS derivatives in their EI mass spectrum were used to confirm the proposed structures. Chemical reactions and enzymatically catalyzed conversions of functional groups were also used as evidence for their structures.

Testosterone and Synthetic AAS

Testosterone (Fig. 1) is the principal androgenic steroid and is produced in males mainly in the testis. In females, smaller amounts of testosterone are produced by the ovary and the adrenal gland. Testosterone was first discovered in 1935 by David et al. [8], who isolated it from the testis of bulls but did not identify its structure. The structure elucidation by synthesis was performed in the same year independently by Butenandt and Hanisch [9] and Ruzicka and Wettstein [10]. For this work Butenandt and Ruzicka were awarded the Nobel prize in 1939.

Interest in testosterone, which possesses anabolic and andro-
The metabolism of anabolic androgenic steroids reviewed

The first synthetic anabolic steroids were methyltestosterone, mestanolone, and metandienol, all synthesized by Ruzicka et al. in 1935 [11]. In all these steroids, a methyl group is introduced at position C-17α, which makes the 17α-methyl steroids orally effective by slowing their metabolism. The 17α-methyl group is not removed and inactivation occurs only after alteration of the A-ring. The importance of the therapeutic use of AAS in treatment of catabolic conditions was recognized in the 1950s, after which an enormous number of steroids were synthesized and tested for potency. For example, metandienone [12, 13] and stanozolol [14, 15], two of the most frequently misused AAS, were synthesized in 1955 and 1959, respectively. Besides 17α-methylation, further modifications were made to reduce the rate of metabolic inactivation. Alteration of the A-ring by introduction of a double bond at C-1,2 yielded metandienone. In stanozolol, a pyrazol ring was condensed to the A-ring, which greatly slowed the rate of metabolic transformation.

The metabolism of testosterone can be discussed as a basic metabolic pathway for all synthetic AAS. The enzymes that convert testosterone to its distinct metabolites are also active towards AAS when similar groups and configurations are present. The metabolism of testosterone has been investigated in various tissues in vivo and in vitro in several animal models and in clinical studies in humans [16–20]. Several of these studies were performed with [14C]testosterone to identify possible testosterone metabolites unambiguously. Overviews on the high number of metabolites have been published [21, 22]. The main excreted testosterone metabolites are hydroxy-5α-androstane-17-one (androstane), 3α-hydroxy-5β-androstane-17-one (etiocholanolone), 3β-hydroxy-5α-androstane-17-one (epiandrosterone), 5α-androstan-3α,17β-diol, 5β-androstan-3α,17β-diol, and 5α-androstan-3β,17β-diol are detected in routine urine samples for drug testing and are part of the so-called steroid profiling. These most abundant metabolites are produced by oxidoreductive reactions at C-3, C-4, C-5, and C-17. Hydroxylated metabolites generated by different isoenzymes of cytochrome P-450 are not discussed here because their excretion into human urine is extremely low.

General Metabolism of AAS

The following overview is a systematic discussion of metabolic changes in the steroid molecule according to the regions (A–D rings; Fig. 1) of the perhydrocyclopentanophenanthrene ring system, the basic structure of all steroids. These changes are generally grouped into two kinds of metabolism, phase I and phase II, the latter also being referred to as conjugation of the steroid.

Phase I Metabolism

Phase I reactions usually convert the steroid by enzymatically catalyzed reactions (e.g., oxidation, reduction, or hydroxylation) into more polar compounds to inactivate the drug and to facilitate its elimination from the body.

A-RING METABOLISM

5α- and 5β-reduction. The initial and rate-limiting step in the metabolism of 3-keto-4-ene steroids, such as testosterone, is the reduction of the C-4,5 double bond. The reduction yields an asymmetric center at C-5, such that two isomers with 5α-(hydrogen at C-5 below the planar molecule) and 5β-configuration (hydrogen at C-5 above the planar molecule) can be formed (Fig. 2). The enzymes catalyzing the reactions, 5α-reductase and 5β-reductase, are located mainly in the liver [23]—5α-reductase primarily in the endoplasmic reticulum and 5β-reductase in the cytoplasm. Both enzymes require NADPH as a cofactor [24]. Once the double bond is reduced, the 3-keto group is immediately transformed, as discussed later.

Hydrogenation of the C-4,5 double bond of metandienone and some structurally related A-ring substituents after reduction of the 3-keto group was discussed by Masse et al. [25].

Table 1 summarizes the proposed and observed reduction to 5α- and 5β-isomers for all discussed 3-keto-4-ene AAS.

The extent of 5α- and 5β-isomer produced depends on the structure of the steroid, as Table 2 summarizes for the metabolism of AAS in one male individual. 3-Keto-androsta-1,4-diene structures, such as metandienone and boldenone, do not produce 5α-isomers [26–28]. Differences in the D-ring structure also strongly influence the activity of both enzymes. As shown in...
Table 1. AAS with a 3-keto-4-en structure, reduction of the C-4,5 double bond in the metabolic pathway.

<table>
<thead>
<tr>
<th>Anabolic androgenic steroid</th>
<th>5α/5β-Reduced metabolites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bolasterone</td>
<td>5β-isomer*</td>
</tr>
<tr>
<td>Boldenone</td>
<td>5β-isomer*</td>
</tr>
<tr>
<td>Calusterone</td>
<td>5α/5β-isomer*</td>
</tr>
<tr>
<td>4-Chloro-1,2-dehydro-17α-methyltestosterone</td>
<td>Detected; only 5β-isomer is proposed</td>
</tr>
<tr>
<td>Clostebol</td>
<td>Detected; both isomers are proposed</td>
</tr>
<tr>
<td>Fluoxymesterone</td>
<td>Detected; 5α/5β-isomer</td>
</tr>
<tr>
<td>Formebolone</td>
<td>Not detected</td>
</tr>
<tr>
<td>Metandienone</td>
<td>5α/5β-isomer*</td>
</tr>
<tr>
<td>Methylenisterone</td>
<td>5α/5β-isomer*</td>
</tr>
<tr>
<td>Mibolerone</td>
<td>Detected; only 5β-isomer is proposed</td>
</tr>
<tr>
<td>Nandrolone</td>
<td>5α/5β-isomer*</td>
</tr>
<tr>
<td>Norclostebol</td>
<td>Detected, both isomers are proposed</td>
</tr>
<tr>
<td>Norethandrolone</td>
<td>Literature, both isomers are reported</td>
</tr>
<tr>
<td>Oxymesterone</td>
<td>Not detected</td>
</tr>
<tr>
<td>Testosterone</td>
<td>5α/5β-isomer*</td>
</tr>
<tr>
<td>Trenbolone</td>
<td>Not detected</td>
</tr>
</tbody>
</table>

* See also Table 2.

Table 2 the metabolism of testosterone to its reduced 5α- and 5β-isomers occurred in a ratio of 1:6, whereas for the 17-keto metabolites (androstenedione and etiocholanolone) the ratio was ∼1:1. For the reduction of 11β-hydroxyandrost-4-ene-3,17-dione, however, which mostly was metabolized to the 5α-isomer, the 5α/5β ratio was ∼15:1.

3α- and 3β-hydroxy- reduction. After reduction of the C-4,5 double bond, which is nonreversible, the 3-keto group in the 5α-isomer is rapidly reduced by either 3α-hydroxysteroid dehydrogenase or 3β-hydroxysteroid dehydrogenase (Fig. 3) [29]. In the metabolism of testosterone after oral administration or intramuscular injection [30], mainly 3α-hydroxy isomers are produced and only small amounts of the 3β-hydroxy-5α metabolite are generated. In the metabolism of AAS having a secondary 17β-hydroxy group, 3β-hydroxy-5α-androstane isomers are formed, e.g., for nandrolone (Schänzer and Donike, unpublished data, 1988–93), drostanolone [31], mesterolone [31, 32], and clostebol (Schänzer, Horning, and Donike, unpublished results, 1993). For clostebol we found that the 3β-sulfate was a long-term excreted metabolite. For no 17β-hydroxy-17α-methyl steroid—whether a 5α-or a 5β-isomer—has a 3β-hydroxy metabolite been reported.

1,2-Hydrogenation of 3-keto-androst-1,4-diene steroids. The hydrogenation of the C-1,2 double bond in 3-keto-androsta-1,4-diene steroids (Fig. 4, left) has been reported for metandienone [26]. An excretion study with orally applied 17α-methyl-5β-androsta-1-ene-3α,17β-diol [26] showed that the C-1,2 double bond was reduced in the presence of the allylic 3-hydroxy group, and both steroids were excreted into urine after conjugation.

1,2-Dehydrogenation of 3-keto-4-en steroids. Endogenous excretion of a 1,2-dehydrogenated steroid (Fig. 4, right) in humans was reported in 1995 [33] for androsta-1,4-diene-3,17-dione (boldenone). Some individuals (at least 3 in 10 000 routine doping control samples) excrete small amounts of the main metabolites of boldenone. The origin of endogenously produced

Table 2. Stereospecific metabolism of 3-keto-4-ene steroids to 5α- and 5β-steroids (in relation to the D-ring structure of the metabolite) for one individual.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Applied amount, mg</th>
<th>17β-Hydroxy</th>
<th>17-Keto</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5α*</td>
<td>5β*</td>
</tr>
<tr>
<td>d5-Testosterone</td>
<td>20</td>
<td>13</td>
<td>87</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>9</td>
<td>91</td>
</tr>
<tr>
<td>d5-11β-Hydroxyandrost-4-en-3,17-dione</td>
<td>20</td>
<td>NE</td>
<td>NE</td>
</tr>
<tr>
<td>Nandrolone</td>
<td>20</td>
<td>15</td>
<td>85</td>
</tr>
<tr>
<td>Methyltestosterone</td>
<td>10</td>
<td>17</td>
<td>83</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>14</td>
<td>86</td>
</tr>
<tr>
<td>Bolasterone</td>
<td>20</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Calusterone</td>
<td>40</td>
<td>22</td>
<td>78</td>
</tr>
<tr>
<td>Boldenone</td>
<td>22</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>80</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>Metandienone</td>
<td>22</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

* Results expressed as % of steroid in 5α or 5β form.

NE, not estimated; d5, deuterated at C-16,16,17; d5, deuterated at C-2,2,3,4,6,6,16,16.

boldenone and its metabolites is unclear. Presumably, unusual bacteria in the gut have 1,2-dehydrogenase activity to convert testosterone, or a precursor, to the 1,2-dehydro steroid. This endogenous production must be considered in drug testing control, with subjects to be followed by endocrinological studies to confirm or rule out the endogenous formation of boldenone and its metabolites. A 1,2-dehydro metabolite is also produced in the metabolism of fluoxymesterone (Schanzer and Horning, unpublished); a 1,2-dehydro metabolite with a 6β-hydroxy structure was identified and its structure elucidated by comparison with a synthesized reference substance. This metabolic reaction occurs to only a small extent (5% of that of the 6β-hydroxylated metabolite). The formation of this 3-ketoandrosta-1,4-diene metabolite is possible because the rate of A-ring metabolism in fluoxymesterone is very slow and a high amount of 6β-hydroxyfluoxymesterone with unchanged A-ring structure is present in the body. The formation of this metabolite in the gut seems possible but could be excluded. 1,2-Dehydrogenation is not seen for other 3-keto-androst-4-ene steroids, in which the A-ring is rapidly metabolized as described above.

Further A-ring metabolism of special A-ring modified anabolic steroids. Depending on the type of modification to the A-ring, further metabolites can be identified, as described in detail in the literature for the metabolism of stanozolol (an A-ring condensed pyrazol derivative) [34], oxymethalone [35, 36], formebolone [37], and metenolone [38]. See also the specific sections on these compounds presented later.

B-RING METABOLISM
6β-Hydroxylation. Metabolism of the B-ring is most pronounced for 17β-hydroxy-17α-methyl steroids where A-ring reduction is hampered by the presence of a C-1,2 double bond, e.g., in metandienone and 4-chlor-1,2-dehydro-17α-methyltestosterone, and by the C-9α fluorine atom in fluoxymesterone. Hydroxylation at position C-6β is the main metabolic route in these anabolic steroids (Fig. 5) [39–41]. Excretion studies have not detected any 6α-hydroxy metabolites.

6,7-Dehydrogenation. 6,7-Dehydrogenation is a minor metabolic pathway, observed only in the metabolism of metandienone [42]. The generation of this metabolite is proposed to originate from an unknown conjugate. In an excretion study with metandienone, the 6,7-dehydro metabolite was obtained after diethyl ether extraction of the alkalinized (pH >12) urine but not at pH 7.0. This conjugate was isolated from urine via fractionation from an Amberlite resin (XAD-2) (Schanzer and Donike, unpublished data, 1988–93; see also Conjugation at the B-ring). When the isolated fractions were alkalinized (pH >12), the 6,7-ene metabolite was obtained. These results can be explained with a basic labile conjugate possibly conjugated at C-6 or C-7, which generates a 6,7-ene when cleaved. A 6,7-dehydro product of testosterone has also been reported in incubation experiments of liver homogenates of rats with testosterone [43].
**C-RING METABOLISM: 12-HYDROXYLATION**

Metabolic changes of anabolic steroids at the C-ring are modest. 12-Hydroxylation was first proposed by Dürbeck et al. [44] for the metabolism of 4-chlor-1,2-dehydro-17α-methyltestosterone. The proposed 12-hydroxylation of metandienone, stanozolol [26, 34], and 4-chlor-1,2-dehydro-17α-methyltestosterone could be confirmed [6]. The TMS derivatives of these metabolites show ion fragments at m/z 143 and 170. The ion m/z 170 was detected in a synthesized 12-hydroxylated 17β-hydroxy-17α-methyl steroid [26]. The ion m/z 143 is the typical D-ring fragment of TMS derivatives of 17β-hydroxy-17α-methyl steroids, whereas m/z 170 is a C-D-ring fragment and occurs after hydroxylation at C-12. The stereochemistry at C-12 (C-12α or C-12β) is not known.

**D-RING METABOLISM**

17-Oxidation of the 17β-hydroxy group. The most well-known metabolic pathway of 17β-hydroxy steroids is enzymatic oxidation by 17β-hydroxysteroid dehydrogenase to form the 17-keto steroid (Fig. 6, top) [45]. 17-Keto metabolites are the main excrated metabolites of testosterone and all AAS having a secondary 17β-hydroxy group, such as boldenone, closebol, drostanolone, mesterolone, methenolone, nandrolone, norclosebol, and stenbolone.

17β-Hydroxylation of 17-keto steroids. The 17-keto group can be converted back to the hydroxy group by the same enzyme, 17β-hydroxysteroid dehydrogenase, to give the 17β-hydroxy configuration. The extent of this equilibrium depends on the rate of subsequent metabolic steps (e.g., 17β-conjugation and A-ring reduction). Oral administration of 20 mg of 16,17-[3H]testosterone to two male volunteers (Schänzer and Donike, unpublished data, 1988–93) was followed by the excretion of 16,17-[3H]testosterone and 16,17-[3H]testosterone in a molar ratio of 2:1. This experiment clearly shows that testosterone is oxidized, and the 17-keto product is, to a considerable extent, reduced back to the 17β-hydroxy steroid.

17α-Hydroxylation of 17-keto steroids. Excretion of a 17α-hydroxy metabolite is observed only in the human metabolism of trenbolone [46]. The 17α-hydroxy group is assumed to be formed via the 17-keto metabolite (Fig. 6, top), which in turn supports the probable existence of a 17α-hydroxysteroid dehydrogenase in humans. This assumption is also supported by the excretion of 17-epitestosterone (17α-testosterone). Its production via the 17-keto metabolite has been discussed by Williams [45], but generation of 17α-testosterone (epitesterone) was not observed after oral administration of testosterone to males [30].

The explanation for this can be the location of the 17α-hydroxysteroid dehydrogenase in testosterone-producing organs (testis, ovary, and kidney). In the organs perhaps epitesterone is formed as a side product in the synthesis of testosterone via androst-4-ene-3,17-dione. An exogenous application of testosterone would be followed by rapid metabolism in the liver to such an extent that unchanged testosterone would scarcely reach the regions where the 17α-hydroxysteroid dehydrogenase enzyme is located.

16α- and 16β-hydroxylation. Hydroxylation at C-16α and C-16β has been reported for several anabolic steroids [6, 26, 34, 44, 47–49]. 16-Hydroxylation is well described in the metabolism of estrogens [50]. Stereospecific hydroxylations are observed at C-16α and C-16β (Fig. 6, bottom), but the extent of formation of both isomers differs for different AAS. There is no general rule, and in some cases only one isomer is excreted. The exact elucidation of the isomers is not always performed. In the metabolism study of stanozolol [34], the possible four isomers with the 16,17-dihydroxy-17-methyl structure were synthesized, and their GC retention indices (determined as per-TMS derivatives) showed the following order: 16β-hydroxy-17-epistanozolol < 16α-hydroxy-17-epistanozolol < 16α-hydroxy-stanozolol < 16β-hydroxy-stanozolol. This elution order was also confirmed for further synthesized 16,17-dihydroxy-17-methyl isomers [26].

16-Oxidation. The excretion of 16-keto metabolites of AAS has not been described before. 16-Oxidation was published for estrogens [50]. 16-Keto steroids are produced by enzymatic oxidation of the corresponding 16α/16β-hydroxy steroids (see Fig. 6). We recently identified 16-keto steroids in the metabolism of 4-chloro-1,2-dehydro-17α-methyltestosterone [51]. This metabolic route of
16-oxidation in humans was also shown in the metabolism of stanozolol in horses.\(^2\)

17-Epimerization. The excretion of 17α-hydroxy-17β-methyl steroids after application of 17β-hydroxy-17α-methyl steroids has been discussed for several years. The formation of the 17-epimers via the sulfoconjugate will be discussed later, in Phase II Metabolism.

Further D-ring metabolism. In the metabolism of norethandroline, a 17β-hydroxy-17α-ethyl AAS, hydroxylation takes place at the β-position of the 17α-ethyl group [52] (see Norethandroline section). A possible hydroxylation at the 17α-methyl group in 17β-hydroxy-17α-methyl steroids has not yet been identified.

18- (19-) HYDROXYLATION
Evidence has been published for 18-hydroxylation of AAS with a secondary 17β-hydroxy group such as mesterolone, methenolone, and stenbolone [53]. Conformation was based on characteristic fragment ions in the EI mass spectrum of the isolated metabolites. The TMS-derivatized primary hydroxy group at C-18 generates a fragment ion m/z 103, which is possible only for a primary hydroxy group. It has been difficult to distinguish whether the location of the hydroxy group is at C-18 or C-19. The structural elucidation of Masse and Goudreault [53] was based on ion fragments of the unchanged A-ring including the unaltered methyl group at C-19. Horning and I also identified a primary hydroxy group in the metabolism of fluoxymesterone, a 17β-hydroxy-17α-methyl steroid (unpublished data). Experiments to localize the position of the hydroxy group were not able to distinguish exactly between the C-18 and C-19 positions.

Phase II Metabolism
Phase II reactions, which are also named conjugation reactions, act to couple the anabolic steroid or its metabolite with glucuronic acid or sulfate. Conjugation helps elimination of the steroid from the body. Both conjugation reactions are enzymatically controlled, glucuronidation involving UDP-glucuronic acid as a substrate and sulfatation 3'-'phosphoadenosine 5'-phosphosulfate.

Not all anabolic steroids and their metabolites are excreted as conjugates. Thus we can distinguish between unconjugated “free” and conjugated metabolites excreted. Unconjugated excreted AAS include oxandrolone, metabolites of oxandrolone, several metabolites of metandienone, fluoxymesterone, 4-chloro-1,2-dehydro-17α-methyltestosterone, formebolone, and two metabolites of stanozolol, in low amounts.

Conjugation of the steroid at the A-ring: sulfatation and glucuronidation of the 3-hydroxy group. In the metabolism of anabolic steroids, reduction of the 3-keto group yields mainly the 3α-

hydroxy-17α-methyl steroids (see Fig. 8, left, with R = CH₃).
It has been proposed that the tertiary hydroxy group is sterically hindered for the enzymatic glucuronidation. Surprisingly, 17β-glucuronidation for 17β-hydroxy-17α steroids could be confirmed and was first presented in 1995 (at the 13th Cologne workshop; Schänzer, unpublished). Metandienone is excreted to a small extent as a glucuronide, a finding confirmed by synthesis of metandienone 17β-glucuronide (Schänzer, Horning, Opfermann, unpublished). Fluoxymesterone and 4-chloro-1,2-dehydro-17α-methyltestosterone are also excreted as 17β-glucuronides, but to a much greater extent than metandienone (ibid.).

3) Sulfation of the secondary 17β-hydroxy group. Sulfation at the 17β-hydroxy group in AAS is possible and is described in the metabolism of testosterone [55] (Fig. 8, right). Sanaullah and Bowers discussed detection of epitestosterone and testosterone sulfates in urine by liquid chromatography/MS in 1995 [56].

4) Sulfation of the tertiary 17β-hydroxy group in 17β-hydroxy-17α-methyl steroids and 17-epimerization. Sulfation at the 17β-hydroxy group in 17β-hydroxy-17α-methyl steroids was first described for metandienone in horses by Edlund et al. [57]. The 17β-sulfate of the tertiary hydroxy group is sterically influenced and decomposes in urine to yield several dehydration products and the corresponding 17-epimeric isomer (17α-hydroxy-17β-hydroxy; Fig. 9). 17-Epimerization has been demonstrated for several 17β-hydroxy-17α-methyl steroids [26, 58-60]. The distribution of reaction products has been similar in several studies of AAS excretion.

Studying fluoxymesterone metabolism, Horning and I were able to isolate the assumed sulfate conjugate of fluoxymesterone and to compare it with synthesized fluoxymesterone 17β-sulfate (unpublished). The urinary compound had the same HPLC retention time and ultraviolet absorbance spectrum as the synthesized fluoxymesterone sulfate. When the isolated metabolite and the synthesized product were dissolved in water, they showed the same route of hydrolysis (t₁/₂ ~ 4 h) and the same distribution of reaction products. From these results we concluded that the precursor of 17β-epimeric steroids is the corresponding 17β-sulfate conjugate.

**Fig. 8. (Left) Glucuronide of secondary and tertiary 17β-hydroxy groups; (right) sulfation of the 17β-hydroxy group.**

**Fig. 9. Degradation of the 17β-sulfate of tertiary 17β-hydroxy groups, with rearrangement to 18-nor-17,17-dimethyl-13-en, 16-ene, 17-ene, 13-hydroxy-17,17-dimethyl and 17-epimeric steroids.**

**Metabolism of Specific Anabolic Steroids**

**BOLASTERONE**

The synthesis of bolasterone (7α,17α-dimethyl-17β-hydroxyandrost-4-en-3-one; Fig. 10) was reported in 1959 by Campbell and Babcock [61]. There being no published data on the metabolism of bolasterone, Donike and I performed two excretion studies to confirm the main metabolites [6]. After oral administration of 20 mg of bolasterone to male volunteers, excretion of the steroids shown in Fig. 10 was confirmed. Bolasterone was detected in the conjugate fraction and it could be hydrolyzed with β-glucuronidase; thus, we assumed that bolasterone was excreted as a 3-enol glucuronide. This assumption is based on the specificity of the highly purified β-glucuronidase. Given the recent identification of 17β-glucuronides of 17β-hydroxy-17α-methyl steroids, a possible 17β-glucuronide of bolasterone must now also be considered. Confirmation of the structure of this glucuronide is in progress.

Two tetrahydro metabolites—7α,17α-dimethyl-5β-androstane-3α,17β-diol (2) and 7α,17β-dimethyl-5β-androstane-3α,17α-diol (4)—were excreted as conjugates (hydrolyzed with β-glucuronidase) and could be detected for a longer time after ingestion than the parent steroid. The structure of both metabolites was confirmed by synthesis of the authentic standards [6, 58]. The generation of the 17-epimer can be explained via a 17β-sulfate (3) (see point 4 in Phase II Metabolism). The tetrahydro metabolites are excreted as bis-conjugates, with glucuronidation at the 3α-hydroxy group and sulfation at the 17β-hydroxy group. The 17β-sulfate undergoes degradation in urine to generate the 17-epimer and the corresponding 18-nor metabolite (5). Both reaction products are still conjugated at the 3α-hydroxy group and can be hydrolyzed with β-glucuronidase.
BOLDENONE

Boldenone was synthesized in 1956 by Meystre et al. [13]. Its metabolism was investigated by Galletti and Gardi [27] in 1971. Donike and I in 1992 published a GC-MS method for screening and identification of boldenone metabolites excreted in human urine [28]. The main metabolic routes in boldenone metabolism are shown in Fig. 11. Boldenone itself is excreted as a 17β-conjugate, presumably as a 17β-glucuronide because of its specific hydrolysis with β-glucuronidase. The reduction of the C-4,5-double bond is stereospecific and yields the 5β-configuration. No 5α-metabolite is detected. The main metabolites of boldenone are 17β-hydroxy-5β-androst-1-en-3-one (2), 5β-androst-1-ene-3α,17β-diol (4), and 3α-hydroxy-5β-androst-1-en-17-one (6). 6β-Hydroxylated metabolites are also excreted but only to a low extent [28]. Excretion of boldenone and its metabolites in low concentrations in urine has been reported without administration of external steroid [33] (see 1,2-Dehydrogenation of 3-keto-4-ene steroids).

CALUSTERONE

Calusterone (7β,17α-dimethyl-17β-hydroxyandrost-4-en-3-one; Fig. 12), first synthesized by Campbell and Babcock in 1959 [61], is the C-7 epimer of bolasterone (see above). Having found no excretion study with calusterone in the literature, Donike and I performed a preliminary study with oral administration of 40 mg of calusterone to a male volunteer; calusterone itself was excreted unchanged but as a conjugate (unpublished data). Given the hydrolysis of the conjugate with β-glucuronidase, we assume glucuronidation at the tertiary 17β-hydroxy group. Compared with bolasterone, the reduction of the C-4,5 double bond yielded not only the 5β-metabolite (7β,17α-dimethyl-5β-androstane-3α,17β-diol; 2), but also a 5α-metabolite (7β,17α-dimethyl-5α-androstane-3α,17β-diol; 3 in Fig. 12). The ratio of
4-CHLOROTESTOSTERONE

The anabolic steroid 4-chloro-1,2-dehydro-17α-methyltestosterone (Fig. 13) was first synthesized in 1960 by Schubert et al. [62]. Marketed by Jenapharm in East Germany, it was the steroid most misused by athletes in East Germany (DDR) through 1990. In metabolic studies of 4-chloro-1,2-dehydro-17α-methyltestosterone in humans, Schubert et al. [48, 49] reported the urinary excretion of the parent steroid, a 6β-hydroxy (2), a 16β-hydroxy, and a 6β,16β-dihydroxy metabolite (3). In 1983 Dürbeck et al. [44] studied the metabolism of 4-chloro-1,2-dehydro-17α-methyltestosterone by GC-MS. They confirmed the formation of the 6β-hydroxy and 6β,16β-dihydroxy metabolite but did not detect the parent drug and the 16β-hydroxy metabolite. Another metabolite present in substantial quantities was detected for which the proposed structure was a 6β,12-dihydroxy metabolite (see above, 12-Hydroxylation). Currently, the misuse of 4-chloro-1,2-dehydro-17α-methyltestosterone is controlled by monitoring the 6β-hydroxy metabolite (2), which has been synthesized and made available as a reference substance [6, 41]. Two additional metabolites identified from excretion studies, the 6β,16β-dihydroxy (3) and 6β,12-dihydroxy [4] forms, are also used to screen for 4-chloro-1,2-dehydro-17α-methyltestosterone in urine samples of athletes [6].

A recent re-investigation of the metabolism of 4-chloro-1,2-dehydro-17α-methyltestosterone has found that several metabolites are excreted for a long time after administration [51]. One long-term metabolite, 4-chloro-3α,6β,17β-trihydroxy-17α-methyl-5β-androst-1-en-16-one (5) (Fig. 13), could be detected for >9 days after oral administration of 40 mg of 4-chloro-1,2-dehydro-17α-methyltestosterone.

CLOSTEBOL (4-CHLOROTESTOSTERONE)

Clostebol (4-chloro-17β-hydroxyandrost-4-en-3-one) was synthesized in 1956 by Camerino et al. [63], and Ringold et al. [64]. The first metabolism studies were published by Starka et al. [65] in 1969 and by Castegnaro and Sala [66] in 1973. The predominant excreted metabolites were oxidized 17-keto products, and the A-ring was reduced to bis and tetrahydro metabolites. The exact configuration of the A-ring-reduced metabolites was not confirmed. After oral administration of 20 mg of clostebol acetate, five main metabolites were detected (Schänzer, Hornig, and Donike, unpublished results, 1993) (Fig. 14). The main metabolite excreted is 4-chloro-3α-hydroxyandrost-4-en-17-one (2), which has been synthesized as a reference material [6]. Two further 17-oxidized and fully A-ring-reduced metabolites (4 and 5) are assumed to be the corresponding C-4-chlorinated analogs of androsterone and etiocholanolone. A further abundant hydroxy metabolite (7), hydroxylated at C-16 and fully A-ring-reduced, was detected and confirmed by GC-MS. The configuration at C-16 and of the A-ring is still unknown.

Besides these metabolites, which are all excreted as glucuronides, a 17-keto tetrahydro metabolite has been isolated as a sulfate conjugate. This metabolite has the most prolonged detection time after administration of clostebol. Because the A-ring is fully reduced and the metabolite is excreted as a sulfate, we propose the configuration 4β-chloro-3β-hydroxy-5α-androstan-17-one (6). The configuration at C-4 (bearing the chloride ion) is still unclear. The exact clarification of the A-ring configuration, including the 4-chloro configuration (α or β), for all tetrahydro products is of interest because it will yield information about the reduction mechanisms of the 5α and 5β reductases.

DROSTANOLONE

Drostanolone (17β-hydroxy-2α-methyl-5α-androstan-3-one; Fig. 15) was first synthesized in 1959 by Ringold et al. [67]. It is taken orally as the 17-propionic acid ester. Its metabolism was investigated by GC-MS and reported by DeBoer et al. in 1992 [31], who confirmed that the parent steroid was excreted as a conjugate that can be hydrolyzed with β-glucuronidase. The main metabolite is the 17-keto-oxidized 3α-hydroxy-reduced product 3α-hydroxy-2α-methyl-5α-androstan-17-one (4), which, in comparison with testosterone metabolism, is the 2α-methyl androsterone analog. This metabolite has been synthesized as a reference substance [6]. Possible reaction pathways for genera-
Metabolism of ethylestrenol

Ethylestrenol (19-nor-17α-preg-4-en-17-ol) was synthesized by De Winter et al. in 1959 [68]. Metabolism of ethylestrenol in humans was reported by Ward et al. in 1977 [69], who found that ethylestrenol metabolism was similar to that of norethandrolone (see below). Metabolism of ethylestrenol proceeds through hydroxylation at C-3 (Fig. 16). The main metabolites 17α-ethyl-5α-estrane-3α,17β-diol (2), 17α-ethyl-5β-estrane-3α,17β-diol (3); synthesis described elsewhere [60], and 17α-ethyl-5α-estrane-3α,17β,21-triol (4) have been confirmed. The 3α-hydroxy configuration of the metabolites is proposed because all metabolites are excreted as conjugates that can be hydrolyzed with β-glucuronidase. Further hydroxy metabolites have been detected, but their structures remain unknown. Recently, Geyer, Donike, and I confirmed the excretion of 3α-hydroxy-5α-estrane-17-one (norandrostenedione) and 3α-hydroxy-5β-estrane-17-one (noretiocoholanolone) in low amounts as metabolites (unpublished), both of which are the main metabolites in the metabolism of nandrolone (19-nortestosterone) (see Nandrolone section).

Fluoxymesterone

Fluoxymesterone (9-fluoro-11β,17β-dihydroxy-17α-methyl-androst-4-en-3-one) was first synthesized in 1956 by Herr et al. [70]. Its metabolism was investigated by Kammerer et al. [71], who excluded the excretion of A-ring-reduced metabolites by comparison with reference substances. Recently, Horning and I prepared an excretion study with 20 and 40 mg of orally applied fluoxymesterone and confirmed the excretion of 20 metabolites after separation by HPLC (unpublished). The main metabolites are shown in Fig. 17. Fluoxymesterone itself is excreted mostly as a 17β-glucuronide and 17β-sulfate. The main metabolites detected are 6β-hydroxy-fluoxymesterone (2), 9-fluoro-17α-methyl-3-androst-4-en-3-one (3), and 9-fluoro-18-nor-17,17-dimethyl-11β-hydroxyandrost-4,13-diene-3-one (5). A little 17-epifluoxymesterone (6) was produced, formed by epimerization of the fluoxymesterone 17β-sulfate.

Compared with other 17-epimerization processes of sulfated 17β-hydroxy-17α-methyl anabolic steroids, where the 18-nor steroid and the epimeric steroid were formed in equal amounts, the amount of 18-nor product produced was ~5 times that of the 17-epimer of fluoxymesterone sulfate. This difference seems
to be due to the presence of the 11\(\beta\)-hydroxy group in fluoxymesterone, which favors the rearrangement process that generates the 18-nor steroid. In contrast to the results of Kammerer et al. [71], we identified fully A-ring-reduced metabolites: two tetrahydro fluoxymesterone metabolites and two 6\(\beta\)-hydroxy tetrahydro metabolites. No evidence of a 11-keto metabolite was found.

**FORMEBOLONE**

Formebolone (2-formyl-11\(\alpha\),17\(\beta\)-dihydroxy-17\(\alpha\)-methylandrosta-1,4-diene-3-one; Fig. 18, left) was synthesized in 1965 by Canonica et al. [72]. GC-MS investigation of formebolone metabolism in humans was published by Masse et al. [37] in 1991, who proposed metabolite structures as related to their EI mass spectra. To confirm the structure of the primary metabolite, Donike and I performed an excretion study with 20 mg of formebolone taken orally [6]. A reduced metabolite, 2-hydroxymethyl-11\(\alpha\),17\(\beta\)-dihydroxy-17\(\alpha\)-methylandrosta-1,4-diene-3-one (2; Fig. 18, left), was found in the unconjugated urine fraction after basic extraction and was identified by comparison with the synthesized compound [6].

**FURAZABOL**

Furazabol (17\(\beta\)-hydroxy-17\(\alpha\)-methyl-5\(\alpha\)-androstano[2,3-c]-furan-3-one; Fig. 18, right) was synthesized in 1965 by Ohta et al. [73]. Metabolic studies in rats were published by Takegoshi et al. [74]. A GC-MS investigation of furazabol metabolism in humans [75] showed that furazabol was excreted as a conjugate that could be hydrolyzed with \(\beta\)-glucuronidase. From this result it is assumed that furazabol is excreted as a 17\(\beta\)-glucuronide. Further work to confirm this assumption is in progress. A 16-hydroxy metabolite was also identified, although its exact configuration (16\(\alpha\)-hydroxy or 16\(\beta\)-hydroxy) is still not determined. 16-Hydroxy metabolites of 17-hydroxy-17-methyl steroids can be easily confirmed by GC-MS from the characteristic EI mass spectra for D-ring fragment ions from the TMS derivative [26, 34].

**MESTANOLONE**

Mestanolone (17\(\beta\)-hydroxy-17\(\alpha\)-methyl-5\(\alpha\)-androst-3-one) was first synthesized in 1935 by Ruzicka et al. [11]. Masse et al. [25] published in 1991 a GC-MS investigation of the mestanolone metabolic pathway (Fig. 19). After oral administration to a male volunteer, 17\(\alpha\)-methyl-5\(\alpha\)-androstane-3\(\alpha\),17\(\beta\)-diol (2)
was excreted as a main conjugate, which can be hydrolyzed with β-glucuronidase. Mestanolone is rapidly reduced by 3α-hydroxy-steroid dehydrogenase. This result is in agreement with the metabolism of methyltestosterone, in which, as an intermediate after 5α-reduction, mestanolone is produced and is further reduced to the corresponding 3α-hydroxy-5α-androstane product. No parent drug nor 3β-hydroxy metabolites were observed.

Recent experiments (Schanzer and Horning, unpublished) have shown that initially a 17-epimer [17β-methyl-5α-androstan-3α,17α-diol (3)] and 18-nor-17,17-dimethyl-5α-androst-13-en-3α-ol (4) are excreted in low amounts (1–2% of the main metabolite), both as conjugates (3α-hydroxy glucuronides). This excretion pattern changes after several days. After 14 days, for example, all metabolites were excreted in comparable low amounts. Even later, after as long as 21 days, the excretion of the epimer exceeds the excretion of the main metabolite. The formation of the epimer follows the epimerization process already described via a 17β-sulfate, and the excretion of the 18-nor-17,17-dimethyl product is in agreement with this mechanism. Because all these metabolites have a 3α-hydroxy structure, they are conjugated with glucuronic acid before excretion into urine. The excreted sulfate is therefore a bis-conjugate and,

**MESTEROLONE**

Mesterolone (17β-hydroxy-1α-methyl-5α-androstane-3-one) was synthesized by Wiegert in 1965 [76]. Human metabolism of mesterolone was reported by DeBoer et al. [31] and Goudreault and Ayotte [32]. Masse and Goudreault reported 18-hydroxylation as a minor metabolic pathway of mesterolone [53]. DeBoer confirmed by GC-MS the urinary excretion of the parent steroid (conjugate, hydrolysis with β-glucuronidase), 1α-methyl-5α-androstane-3α,17β-diol (2) and the androsterone analog (4) as major metabolites (Fig. 20). This metabolite was further elucidated by synthesis [6] as 3α-hydroxy-1α-methyl-5α-androstane-17-one (4).

**METANDIENONE**

Metandienone (17β-hydroxy-17α-methylandrosta-1,4-dien-3-one; Fig. 21) was first synthesized in 1955 by Vischer et al. [12] by microbiological dehydrogenation of methyltestosterone. In 1956 Me்மeर et al. [13] published a dehydrogenation synthesis of methyltestosterone with selenium dioxide. As the main metabolite of this steroid, 6β-hydroxymetandienone (2) was identified in 1963 by Rongone and Segaloff [39]. This metabolite was excreted unconjugated. As discussed above (Conjugation at the B-ring), recent experiments have shown that 6β-hydroxymetandienone is mainly excreted as a labile conjugate, the structure of which is unknown. A further metabolite, 17-epimetandienone (5), was identified and synthesized in 1971 by Macdonald et al. [77]. As reported later [58, 60], the 17-epimeric product results from degradation and rearrangement of an excreted 17β-sulfate. Further publications [42, 44] present results from GC-MS investigations of the unconjugated fraction.

In 1991, we reported identification of the conjugated A-ring-
reduced metabolites [26]. These include (Fig. 21): 17β-methyl-5β-
androst-1-ene-3α,17α-diol (7), 17α-methyl-5β-androst-1-ene-
3α,17β-diol (9), and 17α-methyl-5β-androstan-3α,17β-diol (10).
The 17-epimer, 7, is a long-term excreted metabolite; it can be
detected for a very long time after the administration of metandienone.
Because the formation of this 17-epimer is followed by the
general degradation process to the corresponding 17β-sulfate, it is
also possible to detect the 18-nor product, 18-nor-17,17-dimethyl-
5β-androsta-1,13-dien-3α-ol (8).

METHENOLONE
Methenolone (17β-hydroxy-1-methyl-5α-androstan-3-one;
Fig. 22) was synthesized in 1960 by Wiechert and Kaspar [78].
Methenolone is applied as an acetate or enanthate ester, the
latter being suitable for intramuscular injection. The metabo-
lism of methenolone in humans was investigated by Gerhards et
al. in 1965 [79], who identified 3α-hydroxy-1-methyle]n-5α-
androstane-17-one (4) as a major metabolite. In this metabolic
pathway, the oxidation of the 17β-hydroxy group and reduction of
the 3-keto group are in agreement with the metabolism of
testosterone to androsterone. Interestingly, the C-1,2 double
bond is isomerized to an exocyclic double bond (1-methylene
group) by a mechanism still not clarified. Also, much of the
parent drug is excreted unchanged into urine. Both the parent
drug and 4 are excreted as conjugates that can be hydrolyzed
with β-glucuronidase. In 1990 Goudreault and Masse [38]
published a GC-MS investigation of methenolone metabolism
in humans, describing and characterizing several metabolites by
their El mass spectra.

METHANDRIOL
Methandriol (17α-methylandrostan-5-3β,17β-diol; Fig. 23,
left) was synthesized in 1935 by Ruzicka et al. [11]. To confirm
the main metabolites of methandriol in humans in dope analysis,
we investigated the urinary excretion pattern after oral admin-
istration of 30 mg of methandriol dipropionate and 20 mg of
methandriol [6]. The parent steroid was excreted as a sulfate, possibly as a 3β-sulfate, in low amounts. Hydrolysis of this metabolite was not possible with the β-glucuronidase from *E. coli* but required an arylsulfatase enzyme from *Helix pomatia*. 17α-Methyl-5β-androstan-3α,17β-diol (3; Fig. 23, left) was confirmed as the main metabolite. This metabolite is also a metabolite in the metabolism of metandienone and methyltestosterone; its formation can be explained via an oxidation of the 3β-hydroxy group in methandriol and enzymatic isomerization (steroid-Δ-isomerase) of the C-5,6 double bond to C-4,5. The intermediate so formed (methyltestosterone) is then metabolized mainly to the 5β-isomer. The total amount of excreted metabolites is very low (<5% of the administered steroid); further investigations should be initiated to account for the remaining metabolic products and to elucidate the extent of absorbed substance.

**METHYLTESTOSTERONE**

Methyltestosterone (17β-hydroxy-17α-methylandrost-4-en-3-one; Fig. 24) was synthesized in 1935 by Ruzicka et al. [11]. The metabolism of methyltestosterone in humans was investigated in 1962 by Rongone and Segaloff [80], who identified 17α-methyl-5α-androstan-3α,17β-diol (2) and 17α-methyl-5β-androstan-3α,17β-diol (3) as the main metabolites. These are the classical metabolites as described for the A-ring metabolism of testosterone. Investigations of 17-epimeric metabolites show that the corresponding 17-epimeric steroids are produced but in very low amounts (2–3.5% of the excreted 17β-hydroxy-17α-methyl metabolites). The ratio of the main metabolic isomers 5α/5β was about 0.17 (Table 2) for the individual investigated.

**MIBOLERONE**

Mibolerone (17β-hydroxy-7α,17α-dimethylestr-4-en-3-one) was synthesized in 1963 by Segaloff [81]. The metabolism of mibolerone was investigated by Bowers [82] and after oral application of 20 mg to a male volunteer (Geyer, Schänzer, Donike, unpublished). A major excreted product was the fully A-ring-reduced metabolite, which was excreted as a conjugate. The structure of this metabolite is assumed to be 7α,17α-dimethyl-5β-estrane-3α,17β-diol (2, Fig. 23, right); however, this has not yet been confirmed. The proposed structure is in agreement with the reduction of mibolerone in methanol/potassium hydroxide by hydrogen, with platinum dioxide as a catalyst; the reduction yields only one intermediate with 3-keto structure. The 5β-configuration is based on hydrogenation results for the structurally related steroid bolasterone (Fig. 10), which yields only a 5β-product under the same conditions. The 3α-hydroxy structure is in agreement with the hydrolysis of the conjugate (glucuronide) with β-glucuronidase.

**NANDROLONE**

Nandrolone (17β-hydroxyestr-4-en-3-one; Fig. 25) was synthesized in 1950 by Birch [83] and by Wilds and Nelson [84] in 1953. The metabolism was investigated by Engel et al. in 1958 [85]. The metabolism strongly follows that of testosterone, and the main metabolites have been confirmed as 3α-hydroxy-5α-estr-17-one (3, norandrosterone) and 3α-hydroxy-5β-estr-17-one (2, noretiocholanolone). The structure of both metabolites was elucidated by synthesis in 1960 [86]. Besides these metabolites, a 3β-hydroxy isomer, 5β-hydroxy-5α-estr-17-one (4), is also excreted into urine as a 3β-sulfate in an amount similar to that of the 3α-hydroxy metabolites (Schänzer and Donike, unpublished data, 1988–93).

**NORCLOSTEBOL**

Norclostebol (4-chloro-17β-hydroxyestr-4-en-3-one; Fig. 26) was first synthesized by Camerino et al. [83, 87]. We investigated its metabolism after a single oral dose of 20 mg of norclostebol acetate to a male volunteer (Geyer, Donike, Schänzer, unpublished). Only the glucuronide fraction (hydrolyzable
with β-glucuronidase) has been analyzed. The four metabolites we detected are in agreement with the metabolism of clostebol (see above). All metabolites have a 17-keto group; two (4, 5) are fully A-ring-reduced metabolites (possible androsterone and etiocholanolone analogs); one has a 3-hydroxy-4-ene structure (2); and the fourth is proposed to be C-16 hydroxylated (6). The proposed structures presented in Fig. 26 are based on GC-MS analysis of the per-TMS-derivatized steroids.

**NORETHANDROLONE**

Norethandrolone (17β-hydroxy-17α-ethylestr-4-en-3-one) was synthesized in 1957 by Colton et al. [88]. Brooks et al. [52] investigated in 1971 the metabolism of norethandrolone in humans and reported three metabolites: 17α-ethyl-5α-estrane-3α,17β-diol (2), 17α-ethyl-5β-estrane-3α,17β-diol (3), and a tetrahydro metabolite hydroxylated at the ethyl side chain (4) (Fig. 27). These studies were confirmed in 1977 by Ward et al. [69]. The EI mass spectrum [6] of the TMS derivative of 17α-ethyl-5β-estrane-3α,17β, 21-triol (4) shows abundant D-ring fragment ions at m/z 144 and 157, confirming the hydroxylated position.

**OXANDROLONE**

Oxandrolone (17β-hydroxy-17α-methyl-2-oxa-5α-androstan-3-one; Fig. 28) was synthesized in 1962 by Pappo and Jung [89]. In 1989 Masse et al. [47] published a GC-MS investigation of oxandrolone metabolism in men. Oxandrolone is excreted unchanged and metabolized to its 17-epimer (4), which has already been explained as a urinary rearrangement product of an excreted 17β-sulfate. In this degradation process the 18-nor, 17,17-dimethyl product (5) is also formed. Excretion of further 16-hydroxylated metabolites (2) of oxandrolone have been confirmed, but in low concentrations. All metabolites including the parent steroid are excreted unconjugated.

**OXYMESTERONE**

Oxymesterone (4,17β-dihydroxy-17α-methyandrost-4-en-3-one; Fig. 29, left) was synthesized in 1965 by Camerino [90]. Little metabolized, it is excreted mainly unchanged [58] as a conjugate that can be hydrolyzed with β-glucuronidase. This indicates that oxymesterone is excreted as a 17β-glucuronide.
OXYMETHOLON

Oxymetholone (17β-hydroxy-2-hydroxymethylene-17α-methyl-5α-androstan-3-one) was synthesized in 1959 by Ringold et al. [67]. Its metabolism in humans was discussed by Bi et al. [35, 36], who identified the urinary excretion of acidic metabolites (Fig. 30). They reported a 2-carbonic acidic metabolite (3) and, further, several seco diacetics as metabolites. In the neutral and basic fractions, several hydroxylated and reduced metabolites were detected, but their structural elucidation was not possible. A known metabolite 17α-methyl-5α-androstan-3α,17β-diol (4), which is also a metabolite of methyltestosterone, has been detected. This metabolite originates via oxidation of the 2-hydroxymethylene group to a β-ketocarboxylic acid intermediate (2), which is then decarboxylated to 17β-hydroxy-17α-methyl-5α-androstan-3-one (not shown in Fig. 30). This product is subsequently reduced to the 3α-hydroxy metabolite (4).

QUINBOLONE

Quinbolone [17β-(1-cyclopenten-1-yl)oxy-androsta-1,4-dien-3-one; Fig. 29, right] is the 17β-cyclopentene ether of boldenone and follows the metabolism of boldenone (see above).

STANOZOLOL

Stanozolol (17β-hydroxy-17α-methyl-5α-androst-2-eno[3,2-c]pyrazole; Fig. 31) was synthesized in 1959 by Clinton et al. [14, 15]. Stanozolol is synthesized starting with oxymetholone (see Fig. 30), which is condensed with hydrazine to form a pyrazole ring. In 1989 Masse et al. published a GC-MS method.
for identification of stanozolol metabolites [91] obtained from urine after oral administration of stanozolol.

The metabolism of stanozolol in humans was extensively investigated by Schänzer et al. [34] in 1990. Eleven metabolites were confirmed by GC-MS after separation of the urine extract by HPLC. The main excreted metabolites (Fig. 31) were synthesized: 3′-hydroxystanozolol (2), 3′-hydroxy-17-epistanozolol (3), 16β-hydroxystanozolol (4), and 4β-hydroxystanozolol (5).

Fig. 31. Metabolism of stanozolol (1) to 3′-hydroxystanozolol (2), 3′-hydroxy-17-epistanozolol (3), 16β-hydroxystanozolol (4), and 4β-hydroxystanozolol (5).

16-hydroxy-stenbolone and 17-epitrenbolone are both excreted as conjugates that can be hydrolyzed with β-glucuronidase. From the specific hydrolysis, one can assume that the liberated trenbolone and its 17-epimer are excreted as β-glucuronides. It also seems possible that trenbolone and its 17-epimer are additionally excreted as sulfates. This has not yet been confirmed and will be an interesting subject for future research.

Conformation of the 17-epimer was based on its GC retention time and the EI mass spectra of different derivatives, all of which show an intense cleavage of the 17α-ether (M+ − HO-R) or ester group (M+ − HO-CO-R'). Analysis of trenbolone and its 17-epimer by GC-MS can be problematic when derivatized.

STENBOLONE

Stenbolone (17β-hydroxy 2α-methyl-5α-androst-1-en-3-one) was synthesized in 1960 by Mauli et al. [92] and in 1962 by Counsell et al. [93]. In 1991 Goudreault and Masse [94] published a GC-MS method for identification of urinary excreted metabolites of stenbolone in humans. They identified excretion of unchanged stenbolone, several 17-keto metabolites, and 16-hydroxy metabolites (Fig. 32) and proposed the expected structures.

TRENBOLONE

Trenbolone (17β-hydroxyestra-4,9,11-trien-3-one; Fig. 33), used as a veterinary product, was synthesized in 1963 by Velluz et al. [95]. A GC-MS method for detection of trenbolone and its main metabolite 17-epitrenbolone was first presented in 19893 and was published in 1991 by DeBoer et al. [46]. Trenbolone

Fig. 32. Metabolism of stenbolone (1) to 3α-hydroxy-2-methyl-5α-androst-1-en-17-one (2); 3α-hydroxy-2β-methyl-5α-androstan-17-one (3); 16β-hydroxy-stenbolone (4); 16β-hydroxy-2-methyl-5α-androst-1-ene-3,17-dione (5); and 3α,16β-dihydroxy-2-methyl-5α-androst-1-en-17-one (6).

3 By myself (in June) at the 7th Cologne Workshop in Dope Analysis, and by H. Geyer (in October) at the 4th International Symposium in Doping Control, Moscow.
with MSTFA/TMIS [N-methyl-N-(trimethylsilyl)trifluoroacetamide/trimethylsilylamine]. Enolization of the 3-keto group yields a highly reactive compound that can yield several decomposition products under GC analysis. In this case, different derivatives are used.

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