Phytoecdysteroids and Anabolic-Androgenic Steroids – Structure and Effects on Humans

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Abstract: Phytoecdysteroids are structural analogs of the insect molting hormone ecdysone. Plants comprise rich sources of ecdysteroids in high concentration and with broad structural diversity. Ecdysteroids have a number of proven beneficial effects on mammals but the hormonal effects of ecdysteroids have been proven only in arthropods. Their structures are somewhat similar to those of the vertebrate steroid hormones but there are several structural differences between the two steroid groups. Despite of these essential structural differences, ecdysteroids exert numerous effects in vertebrates that are similar to those of vertebrate hormonal steroids, and they may serve as effective anabolic, hepatoprotective, immunoprotective, antioxidant and hypoglycemic agents.

Ecdysteroids do not bind to the cytosolic steroid receptors, instead, they are likely to influence signal transduction pathways, like the anabolic steroids, possibly *via* membrane bound receptors.

The application of phytoecdysteroids is a promising alternative to the use of anabolic-androgenic steroids because of the apparent lack of adverse effects. The prospective use of phytoecdysteroids may extend to treatments of pathological conditions where anabolic steroids are routinely applied. One of the most cited aspects of phytoecdysteroid application (on the Internet) is the increase of muscle size. However in this field too stringent research is needed as an adequate cytological explanation is not yet available for the anabolic.

This paper reports on the most important structural differences between androgenic hormones, their synthetic analogs and ecdysteroids. The anabolic/hormonal effects and the possible mechanisms of action of these compounds are also discussed as concerns the skeletal muscle.

Keywords: Ecdysteroids, anabolic activity of ecdysteroids, anabolic-androgenic steroids, signal transduction, muscle growth.

INTRODUCTION

The Occurrence of Ecdysteroids and their Effects on Invertebrates

The steroid hormones can be classified according to their biological relevance [1].

The first class is the family of vertebrate steroid hormones: androgens, estrogens, progestogens, corticosteroids and colecalciferols. Brassinolids, the second class, are growth-promoting hormones of plants. Ecdysteroids belong in the third class of steroid hormones, which were discovered in insects, but which are also present in other arthropods, other invertebrate phyla and plants.

In insects, they act as molting hormones, regulating metamorphosis and also several other important life-cycle processes [2]. They may also have roles in the reproduction, embryogenesis and diapause of certain other arthropods (insects, crustaceans, arachnids and myriapods). The hormonal effects of ecdysteroids have been proven only in arthropods. In view of their occurrence in arthropods (1 million species), ecdysteroids are the most widespread steroid hormones.

The theory that insect development is controlled by ecdysteroid hormones was supported by the isolation and structure elucidation of these molecules, as a result of 40 years of intensive research. The milestones in ecdysteroid research were as follows:

It was postulated [3] that the molting of insects must be under hormonal control. Isolation of the first ecdysteroid, ecdysone (**16**), from silkworm pupae [4] proved this assumption. The structure of ecdysone was elucidated 10 years later [5]. In most insect organisms, the main and also the biologically most significant ecdysteroid is 20-hydroxyecdysone (**6**), which was first isolated from crayfish (*Jasus lalandii*) [6]. A huge number of further ecdysteroids have been isolated from various animal sources. It has been suggested that other ecdysteroids may play active roles in insects at different stages of their development [7].

The fortuitous discovery of ecdysteroids from plant sources initiated the systematic and fruitful screening of ecdysteroid-rich plant sources, leading to the isolation of new ecdysteroids. It is generally accepted that ecdysteroids in plants play an important part in the protection against insect predators and soil nematodes, either as a consequence of their antifeedant activity or by inducing the developmental disruption and even the death of non-adapted phytophagous insects or soil nematodes [2]. In the early stages of endocrinology, only vertebrate steroid hormones were known, but the steroid hormone function was not associated with insect evolution.

Phytoecdysteroids were discovered with rapid success in numerous plant species, and later it emerged that ecdysteroids are widely distributed in the plant kingdom [2]. Ecdysteroids occur in unrelated plant species in great structural variety and in exceptionally large amounts. Plant ecdysteroids are often biosynthesized i.e. 2-5 orders of magnitude higher than their concentration in insects. A number of structurally similar phytoecdysteroid molecules exert similar biological activity to that of ecdysteroids on insects.

The relatively large amounts of ecdysteroids isolated from plants permitted the initiation of pharmacological studies.

The Chemical Structure of Ecdysteroids

Ecdysteroids comprise a class of steroids with a polyhydroxylated cyclopentano[α]perhydrophenanthrene ring system as shown in Fig. (**1**). They are generally characterized by a basic skeleton containing 27-29 carbon atoms with a long sterol alkyl side chain on C-17 and the presence of a 7-en-6-one chromophore group in ring B. However, ecdysteroids with 19, 21 or 24 carbon atoms also occur, which may be products of C-27 ecdysteroids formed by cleavage of the sterol side chain. They are mainly steroids of 5β-androstane type. The 14α position is generally hydroxylated, and they contain a β-hydroxy group on C-3. Further hydroxylation is often observed at positions 1, 2, 5, 11, 20, 22, 25 or 26/27. Other

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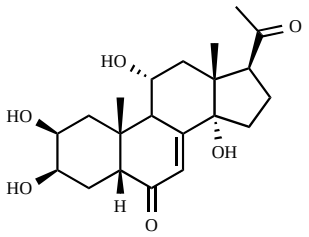
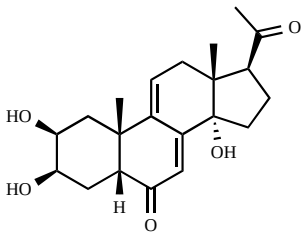
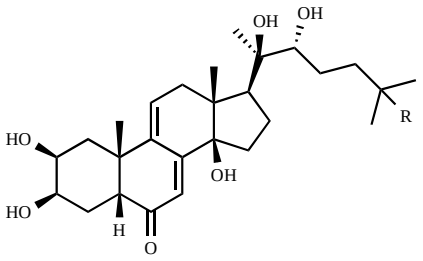


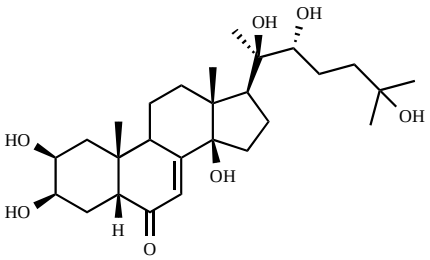
11-α-hydroxypoststerone (**1**)



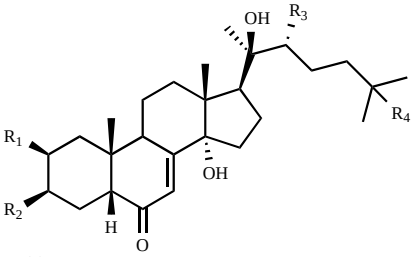
9,11-Didehydropoststerone (**2**)



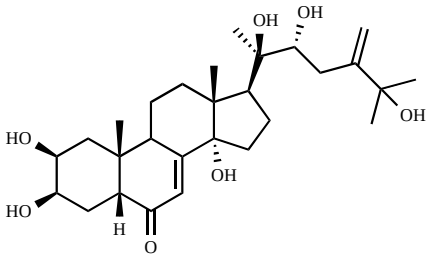
Dacryhainansterone (**3**): R = H
25-Hydroxydacryhainansterone (**4**): R = OH



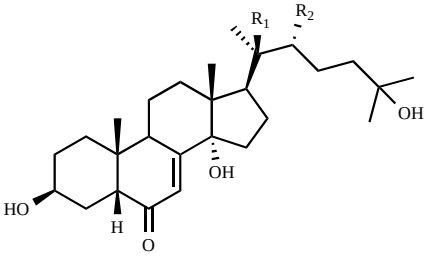
14-Epi-20E (**5**)



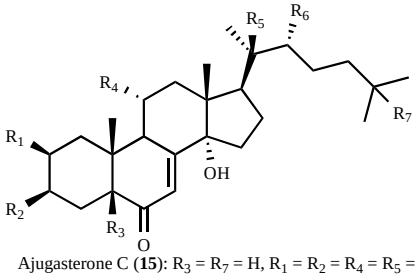
20E (**6**): $R_1 = R_2 = R_3 = R_4 = OH$
20E 2,3,22-triacetate (**7**): $R_1 = R_2 = R_3 = OAc$, $R_4 = OH$
20E 2,3,22,25-tetraacetate (**8**): $R_1 = R_2 = R_3 = R_4 = OAc$
20E 22-acetate (**9**): $R_1 = R_2 = R_4 = OH$, $R_3 = OAc$
20E 22-benzoate (**10**): $R_1 = R_2 = R_4 = OH$, $R_3 = Benzoate$



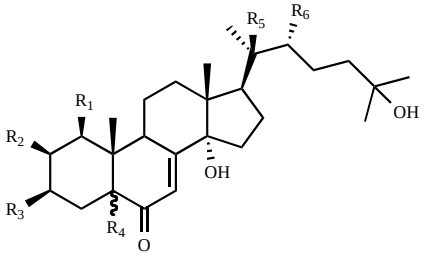
24(28)-Dehydromakisterone A (**11**)



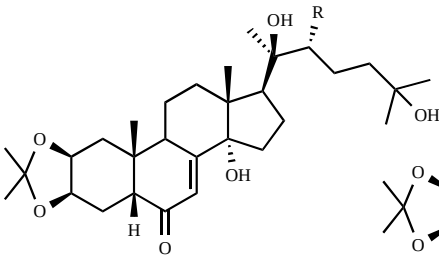
2-Deoxy-20E (**12**): $R_1 = R_2 = OH$
2-Deoxyecdysone (**13**): $R_1 = H$, $R_2 = OH$
2-Deoxyecdysone-22-acetate (**14**): $R_1 = H$, $R_2 = OAc$



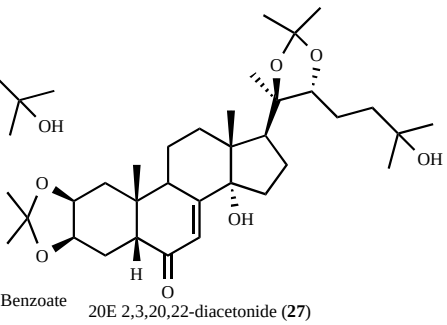
Ajugasterone C (**15**): $R_3 = R_7 = H$, $R_1 = R_2 = R_4 = R_5 = R_6 = OH$
Ecdysone (**16**): $R_3 = R_4 = R_5 = H$, $R_1 = R_2 = R_6 = R_7 = OH$
Polypodine B (**17**): $R_4 = H$, $R_1 = R_2 = R_3 = R_5 = R_6 = R_7 = OH$
Turkesterone (**18**): $R_3 = H$, $R_1 = R_2 = R_4 = R_5 = R_6 = R_7 = OH$
Turkesterone 2,3,11,22-tetraacetate (**19**): $R_3 = H$, $R_5 = R_7 = OH$, $R_1 = R_2 = R_4 = R_6 = OAc$
Viticosterone E (**20**): $R_3 = R_4 = H$, $R_1 = R_2 = R_5 = R_6 = OH$, $R_7 = OAc$



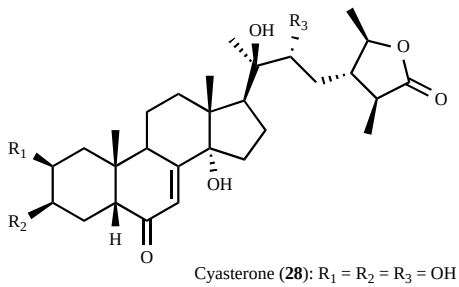
Integristerone A (**21**): $R_4 = ßH$, $R_1 = R_2 = R_3 = R_5 = R_6 = OH$
Sileneoside A (**22**): $R_1 = H$, $R_4 = ßH$, $R_2 = R_3 = R_5 = OH$, $R_6 = O\text{-gal}$
Sileneoside C (**23**): $R_4 = ßH$, $R_1 = R_2 = R_3 = R_5 = OH$, $R_6 = O\text{-gal}$
(5α)-Sileneoside E (**24**): $R_4 = \alpha H$, $R_5 = H$, $R_1 = R_2 = R_6 = OH$, $R_3 = O\text{-glu}$



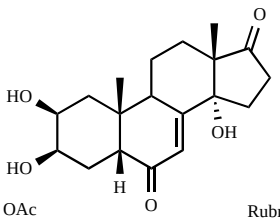
20E 2,3-monoacetonide (**25**): R = OH
20E 2,3-monoacetonide 22-benzoate (**26**): R = Benzoate



20E 2,3,20,22-diacetonide (**27**)



Cyasterone (**28**): $R_1 = R_2 = R_3 = OH$
Cyasterone 2,3,22-triacetate (**29**): $R_1 = R_2 = R_3 = OAc$
Cyasterone 22-acetate (**30**): $R_1 = R_2 = OH$, $R_3 = OAc$



Rubrosterone (**31**)

Fig. (1). The chemical structures of ecdysteroids referred to in this paper. 20E = 20-hydroxyecdysone.

known features include an additional double bound, a second oxo group and they also occur in glucoside, esters or ether form.

PHARMACOLOGICAL EFFECTS OF ECDYSTEROIDS COMPARED TO ANABOLIC-ANDROGENIC STEROIDS ON MAMMALS

The ecdysteroids influence many physiological functions and have a wide array of experimentally proved, beneficial pharmacological effects on mammals, including humans; their low acute toxicity has been repeatedly demonstrated experimentally [8-11], *e.g.* LD_{50} > 6 g/kg and > 9 g/kg for 20-hydroxyecdysone (**6**) administered i.p. or orally to mice, respectively. A dose of 0.1 g/kg of 20-hydroxyecdysone (**6**) administered i.v. to rabbits did not cause a toxic reaction, and the subacute treatment of rats with 2 g/kg/day resulted in no toxic symptoms.

Ecdysteroids affect certain major metabolic pathways in mammals: protein synthesis, lipid and carbohydrate metabolisms and ioncurrents. Ecdysteroids display protective, corrective and preventive effects. They are general tonic and broad-spectrum stimulants, improving the health of mammalian organisms. They may act as effective anabolic, hepatoprotective, immunoprotective, antioxidant and hypoglycemic agents. They are considered adaptogenic, enhancing the physical performance; promoting vitality and increasing the resistance to stress and aging.

Most of the pharmacological experiments have been carried out on the main phytoecdysteroid, 20-hydroxyecdysone (**6**). A 0.1% concentration of 20-hydroxyecdysone (**6**) is not unusual in plants, and several plant species biosynthesize 20-hydroxyecdysone (**6**) in 1-3 % of their dry mass, i.e. a 2-6-fold order of magnitude higher concentration than those of the other ecdysteroid constituents. 20-Hydroxyecdysone (**6**) can be isolated from these plant sources by simple separation procedures [12]. The isolation of minor ecdysteroids requires adequate plant sources, from which these ecdysteroids can be isolated in appropriate quantities by means of sophisticated methods [13]. Such plants are *Ajuga*, *Serratula*, *Silene* species and *Leuzea*. The pharmacological investigation of minor ecdysteroids has recently come into the limelight. The large number of phytoecdysteroids (more than 300) with basically different structures promotes continuous research for the discovery of further pharmacological effects and supervision of the earlier findings.

Until now only a limited number of studies compared the physiological effects of anabolic-androgenic steroids and ecdysteroids in the same experimental system. Most of these works have not been published in international scientific journals and the whole articles are not available in English, therefore it is difficult to evaluate their experimental data. As the investigated problems are still interesting and fundamental, and the main aspects are worth to consider for further enlightenment, these works will be summarized here according to the studied effects.

Effects on Protein Synthesis

The first observed and classical pharmacological activity of ecdysteroids was their protein synthesis-stimulatory effect. Okui *et al*. [14], and Otaka *et al*. [15] reported the stimulatory effect of ecdysteroids on protein synthesis in the mouse liver. The amino acid incorporation was determined after the oral or intraperitoneal administration of ecdysteroids isolated from plants. 4-Chlorotestosterone (**41**), Fig. (**2**), an anabolic-androgen, was used as control. Maximum activity was observed for 20-hydroxyecdysone (**6**) and the anabolic-androgen.

A protein synthesis increase after 20-hydroxyecdysone (**6**) administration was confirmed by Otaka *et al.* [16] in the mouse liver and in the microsomal fraction of the mouse liver. The anabolic activities of 20-hydroxyecdysone (**6**) and cyasterone (**28**) were likewise determined in mice, where enhanced protein synthesis was detected in the liver and kidney [17].

These early studies were extended to examinations of protein synthesis in other tissues and other mammalian species (rats, mice, sheep, pigs, quails) [18-23]. For example improved nitrogen retention and a body weight increase with lowered feed consumption were observed in pigs and Japanese quails (>12% increase) which received ecdysteroid-containing plants and 20-hydroxyecdysone (**6**) in their diets [24].

Comparing the effect of ecdysteroids and anabolic-androgenic steroids it has been reported [25] that an androgen dependent development is a prerequisite before the action of ecdysteroids in rat. 20-Hydroxyecdysone (**6**) in 0.5 mg/100 g dose for 7 days resulted in increased weight gain of the whole body, liver, heart, kidneys and musculus tibialis anterior in rats. The accumulation of protein content was also accelerated. These changes were even more pronounced if the animals were still growing (70-80 g). In sexually immature castrated rats the androgenic action of 20-hydroxyecdysone (**6**) was not demonstrable in contrast to that of methandrostenolone (**36**). Twenty-four years later an extensive comparative study was presented in English using a number of purified ecdysteroids including turkesterone (**18**), 20-hydroxyecdysone (**6**) and methandrostenolone (**36**) [26].

An early report [27] also implied the difference between the mechanisms of actions of ecdysteroids and anabolic steroids. 20-Hydroxyecdysone (**6**), turkesterone (**18**) and 2-deoxyecdysone (**13**) in the same (0.5 mg/100 g) dose were found to stimulate protein synthesis in the liver of laboratory mice. The protein synthesis-increasing ability was associated with polyribosomal activity. The preliminary administration of actinomycin D did not prevent the phytoecdysteroid effect on protein synthesis stimulation. Therefore it has been concluded that the anabolic effect of ecdysteroids is connected with the acceleration of translocation processes instead of the induction of new RNA synthesis. This shows that ecdysteroids are not likely to act as the classical steroids, *via* cytoplasmic receptor and regulation of gene transcriptional activity.

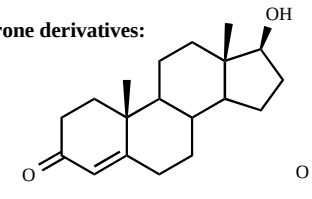
The effect of Nerobol® (**36**) and 20-hydroxyecdysone (**6**) was linked in insulin-dependent processes and in insulin resistance [28]. The insulin resistance was induced by injection of hydrocortisone and the insulin insufficiency by alloxan. The sensitivity of the body to intravenal infusion of insulin and the reaction of isolated fat tissue to the hormone increased after administration of both Nerobol® (**36**) and 20-hydroxyecdysone (**6**). The above effects of the steroid were more dependent on the nonspecific protein synthesis of the cells than on the increase in insulin secretion.

Chermnykh *et al.* [29] compared the anabolic action of ecdysteroids and of methandrostenolone (**36**) on male mice, preconditioned with or without a swimming test.

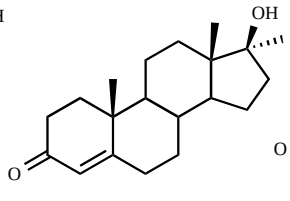
Methandrostenolone (**36**) produced anabolic effects only after constant training, but 20-hydroxyecdysone (**6**) improved the physical ability of the mice both with and without this preconditioning training. Methandrostenolone (**36**) stimulated the biosynthesis of myofibrillar proteins in the musculus soleus, but not in the musculus extensor digitorum longus, while 20-hydroxyecdysone (**6**) increased the amount of myofibrillar proteins in both muscles.

Meanwhile Syrov and coworkers consequently described non androgenic effects of ecdysteroids [25-27], at least one study is at variance with their conclusion. Xu *et al*. [30] have reported that 20-hydroxyecdysone (**6**), the effective compound of the extract from *Antherea pernyi* Pas, was able to increase the weight of prostate-semina and levator ani/muscle-bubocavernosus muscles of castrated mice. The extract was also able to accelerate the growth of younger male mice and enhances the RNA, DNA and protein content in liver. Therefore it has been concluded that 20-hydroxyecdysone (**6**) has androgen-like anabolic action.

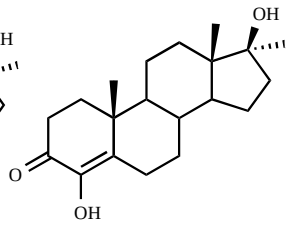
1. Testosterone derivatives:



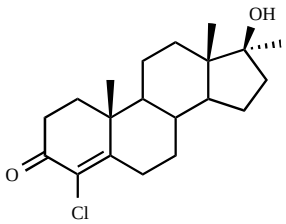
Testosterone (**32**)



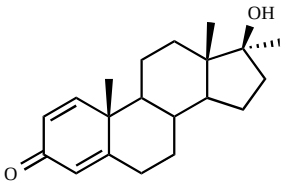
Methyltestosterone (**33**)



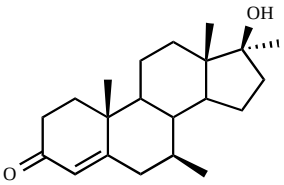
Oxymesterone (**34**)



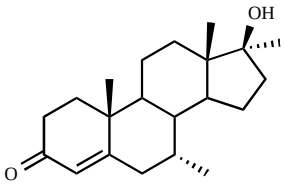
4-Chloro-methyltestosterone (**35**)



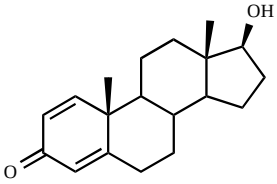
Methandrostenolone / Methandienone / Nerobol® (**36**)



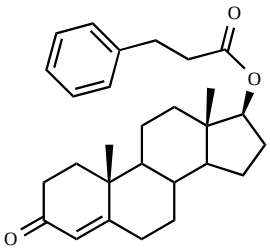
Clausterone (**37**)



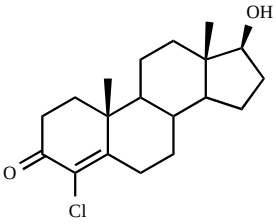
Bolasterone (**38**)



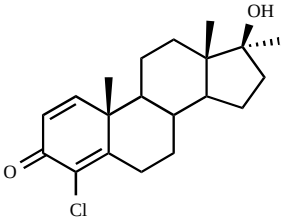
Boldenone (**39**)



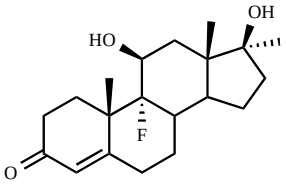
Testosterone phenylpropionate (**40**)



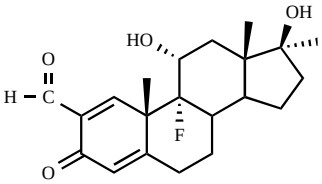
4-Chloro-testosterone (**41**)



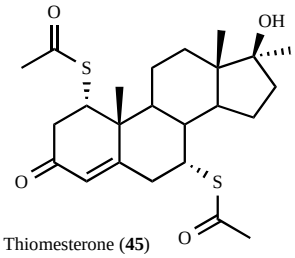
4-Chloro-1,2-didehydro-17α-methyltestosterone (**42**)



Fluoxymesterone (**43**)

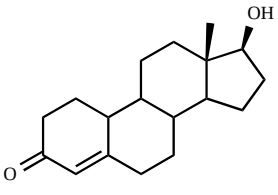


Formebolone (**44**)

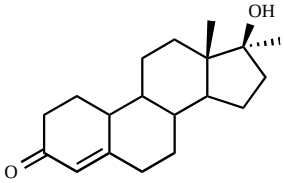


Thiomesterone (**45**)

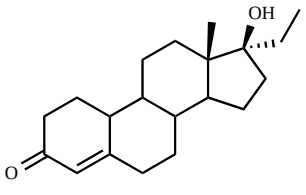
2. 19-nortestosterone derivatives:



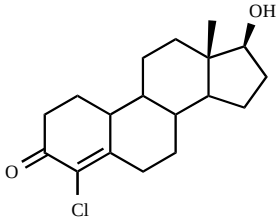
19-Nortestosterone / Nandrolone / Durabolan® (**46**)



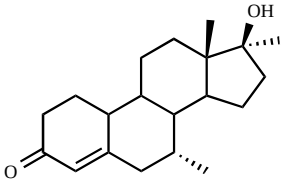
Methylnandrolone (**47**)



Norethandrolone (**48**)

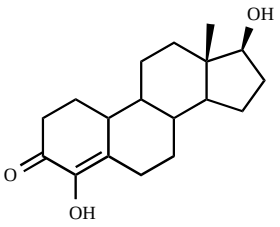


Norclostebol (**49**)

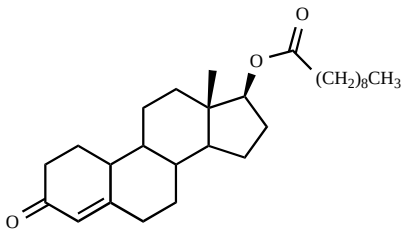


Mibolerone (**50**)

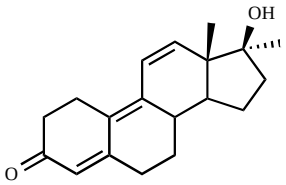
Fig. (2). Contd…..



Oxabolone (**51**)

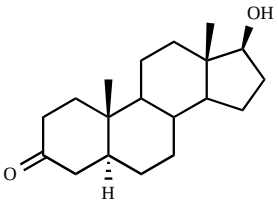


Nandrolone decanoate (**52**)

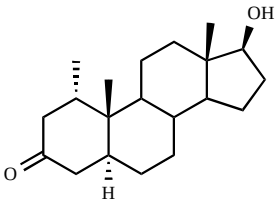


Metribolone (**53**)

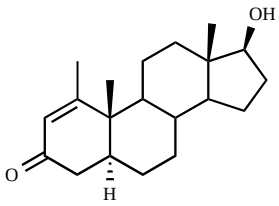
3. Androstane derivatives:



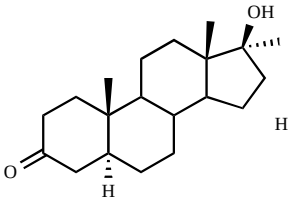
Androstanolone / 5α-DHT (**54**)



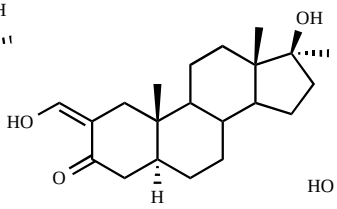
Mesterolone / Proviron® (**55**)



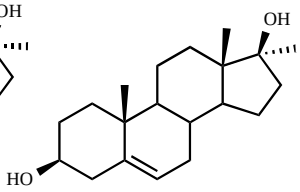
Methenolone / Primobolan® (**56**)



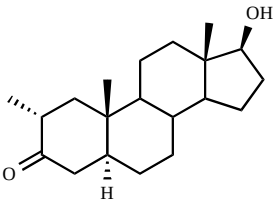
Mestanolone (**57**)



Oxymetholone (**58**)

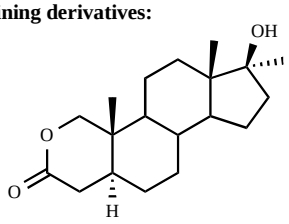


Methylandrostenediol (**59**)

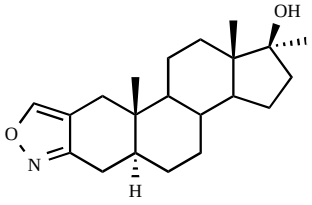


Drostanolone / Masterone® (**60**)

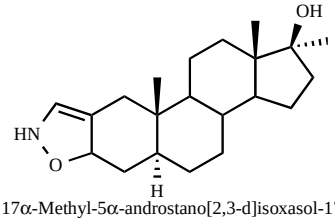
4. Heterocyclic ring containing derivatives:



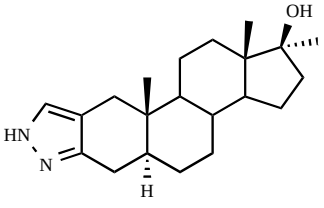
Oxandrolone (**61**)



17α -Methyl-5α-androstano[3,2-c]isoxasol-17β-ol (**62**)



17α-Methyl-5α-androstano[2,3-d]isoxasol-17β-ol (**63**)



Stanozolol (**64**)

Fig. (2). The chemical structures of the most commonly used anabolic-androgenic steroids.

These protein synthesis-stimulating effects of ecdysteroids was determined following the p.o, i.p. or i.v. administration of 0.2-500 mg/kg ecdysteroids from 5 to 150 days.

Hormonal Activity on Mammals

In consequence of their steroidal structure and anabolic action, ecdysteroids have often been suspected of possessing the hormonal effects of vertebrate steroids (estrogens, androgens and corticoids), especially of the androgens. Knowledge of the hormonal nature of various ecdysteroids would be of pharmacological importance because of their possible future use in therapy.

The absence of an androgenic effect of 20-hydroxyecdysone (**6**) and some other ecdysteroids has been widely proven in experimental animals. This conclusion was based on measurement of the in-

crease of the prostate and seminal vesicle mass, where no androgenic effect was observed [31-34].

The estrogenic effect has been assayed only in the case of 20-hydroxyecdysone (**6**). The possibility of estrogenic or antiestrogenic effects of 20-hydroxyecdysone (**6**) was investigated by Prabhu & Nayar [35]. In intravaginal doses of 30- 500 µg, it was compared with 17β-estradiol dipropionate in adult female rats. Neither estrogenic nor antiestrogenic effects of 20-hydroxyecdysone (**6**) were observed.

It has also been reported that the effects of anabolic steroids and ecdysteroids are characteristically different on thymocytes. The systemic administration of testosterone (**32**) and methandrostenolone (**36**) to male rats in doses of 1-2 mg/100 g for 10 days decreased the mass of thymus and reduced the thymic serum factor content. 20-Hydroxyecdysone (**6**) which does not possess androgenic activity failed to influence the thymus mass and the level of the thymic serum factor [36].

An another work [34] presented similar findings: two anabolic steroids, the testosterone (**32**) and methandrostenolone (**36**) decreased mass, the quality of DNA and the incorporation of ^{3}H labeled thymidine in thymocytes after ten-days injection to mice in 5 mg/100 g dose. Ecdysten® (preparation containing various ecdysteroids) did not influence proliferative activity of thymocytes *in vitro* and thymolytic effect *in vivo*. This indicates that the proliferative process may have a functional significance in thymolitical effect of anabolic steroids.

The glucocorticoid effect of ecdysteroids has not been investigated yet.

While these results have not been confirmed by modern radioligand binding assays before, in our laboratory *in vitro* radioligand binding assays using estrogen, glucocorticoid and androgen receptor selective-radioligands were applied to check on the presence or absence of estrogen, glucocorticoid and androgen effects of 11 ecdysteroids (Table **1**). Our unpublished results can be summarized as follows:

The specific binding of all the ligands was higher than 70% at estrogen and glucocorticoid receptors concentration of 10^{-6} M (Table **1**), and therefore the IC_{50} values are higher than 10^{-6} M. On androgen receptor four compounds {20-hydroxyecdysone (**6**), polypodine B (**17**), 20-hydroxyecdysone 22-acetate (**9**), 9,11-didehydropoststerone (**2**)} showed lower specific binding than 60%.

The K_i values of the most active compounds on estrogen receptor {20-hydroxyecdysone (**6**), 20-hydroxyecdysone 22-acetate (**9**), 11α-hydroxypoststerone (**1**)}and on androgen receptor {9,11-didehydropoststerone (**2**)} were measured in competition binding assays. None of the tested ligands had remarkable K_i values on both receptors (Table **2**). Even the most active compound {9,11-didehydropoststerone (**2**)} possessed only a moderate K_i value ($5.15x10^{-7}$ M) on the androgen receptor. The competition binding curves are demonstrated in Fig. (**4**). Various structural changes in the molecule (side chain cleavage, 11-hydroxylation, epimerization at C-14 and esterification) were not associated with significantly different hormonal properties. Rubrosterone (**31**) has certain structural elements common to androst-4-en-3,17-dione. However, the structural similarity of this ecdysteroid with this known anabolic-androgen is not accompanied by a vertebrate steroid hormonal effect.

Based on the radioligand binding assay we can conclude that none of the tested ecdysteroids displayed an estrogenic, glucocorticoid or androgenic effect, which represents that ecdysteroids do not bind to the vertebrate steroid receptors.

Effect on Liver

The beneficial effects of ecdysteroids and anabolic-androgenic steroids were found to be similar in liver. The administration of ecdysteroids {20-hydroxyecdysone (**6**), turkesterone(**18**)} at a dose of 5 mg/kg and the anabolic steroid preparation Nerobol® (**36**) at a 10 mg/kg dose resulted in changes of mitochondrial enzyme activities in experimental hepatitis caused by CCl_4 poisoning in rats. Positive alterations were found in activity of the polyenzymatic systems in membranes of liver mitochondria, simultaneously with an increase in their stability and resistance to the effect of exogenous factors producing the mitochondria degradation (controlled heating, treatment with phospholipase A2 or trypsin). These alterations, which appear to occur due to the development of strong binding forces between phospholipids and proteins of the inner mitochondrial membrane, promoted normalization of the respiratory chain and the outer pathway of electron transport in hepatocytes of rats with hepatitis [37].

In a subsequent work 20-hydroxyecdysone (**6**), turkesterone (**18**) and cyasterone (**28**) were compared to Nerobol® (**36**) in carbon tetrachloride-induced liver lesion. Ecdysteroids were administered in a dose of 0.5mg/100g per os to rats with hepatitis induced by subcutaneous injections of CCl_4. The treatment alleviated the he-

Table 1. Specific Binding of the Estrogen Receptor (ER), Glucocorticoid Receptor (GR) and Androgen Receptor (AR) Selective Radioligands Using 10^{-6} M Competitors (Our Unpublished Results)

Ecdysteroids	Specific binding ± SEM, %		
	ER	GC	AR
20E (**6**)	84.7±2.9	98.7±5.1	50.7±3.7
Polypodine B (**17**)	94.7±4.2	99.2±3.5	54.4±8.3
20E 22-acetate (**9**)	71.7±4.2	98.4±3.1	59.2±6.1
Ajugasterone C (**15**)	96.8±3.5	98.4±3.2	68.8±5.7
Turkesterone (**18**)	96.7±2.4	99.6±5.4	89.0±3.5
14-Epi 20E (**5**)	88.6±6.2	96.4±3.2	100.1±2.9
11α-Hydroxypoststerone (**1**)	78.9±3.9	97.6±4.1	99.0±6.7
Rubrosterone (**31**)	93.2±3.4	96.6±3.2	96.5±9.9
Dachryhainansterone (**3**)	95.3±6.8	98.9±1.5	99.5±5.2
25-Hydroxydacryhainansterone (**4**)	92.8±7.2	97.6±2.5	89.8±7.9
9,11-Didehydropoststerone (**2**)	96.3±3.1	99.6±1.3	40.7±3.4

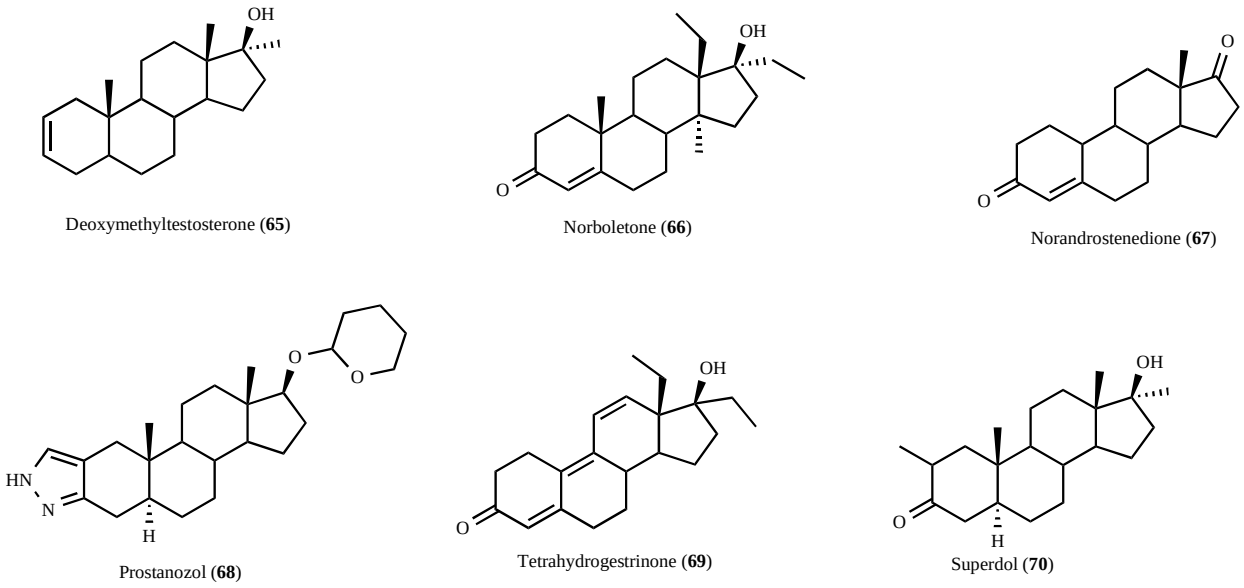


Fig. (3). The chemical structures of some designer steroids.

patic effect of CCl_4 and the normalization of functional and metabolic disorders in the liver. The ecdysteroids and Nerobol® (**36**) noticeably stimulated the recovery of bile secretion, the synthesis of bilirubin and bile acids and cholesterol excretion [38].

Table 2. K_i Values of the Most Active Compounds on Estrogen and Androgen Receptors. The Radioligands were [^{3}H]17β-Estradiol and [^{3}H]Dihydrotestosterone (Our Unpublished Results)

Estrogen receptor	**K_i, nM**
17β-Estradiol	0.39×10^{-10}
20E (**6**)	2.53×10^{-5}
Polypodine B (**17**)	6.95×10^{-5}
20E 22-acetate (**9**)	1.35×10^{-6}
Androgen receptor	
Testosterone (**32**)	1.09×10^{-9}
9,11-Didehydropoststerone (**2**)	5.15×10^{-7}

Effect on Kidney

The nephroprotecting effect of ecdysteroids resembled the action of a steroidal anabolic-androgenic drug Nerobol® (**36**). 20-hydroxyecdysone (**6**) and turkesterone (**18**) isolated from *Ajuga turkestanica* (Rgl.) Brig. decreased the manifestation of uremic intoxication in rats with experimental renal pathology induced by a nephrotoxic mixture (containing uranyl acetate and glycerol). Injected in 0.5 mg/100 g dose, ecdysteroids restored glomerular filtration level, favor the disappearance of the albuminuria and normalized urinary sediments [39].

Anti-Tumor Effect

The influence of 20-hydroxyecdysone (**6**) in combination with the therapeutic and half doses of cisplatin and adriamicin (anticancer drugs) was studied on the development of subcutaneously and intraperitoneally transplanted P388 and L1210 leukemia and metastasizing B16 melanoma in mice. 20-hydroxyecdysone (**6**) significantly stimulated the chemotherapeutic effect of low doses of the cytostatics: inhibition of tumor growth, mice survival rate, their lifespan, and the antimetastatic activity index were comparable or better than those obtained after therapy with high doses of the antitumor drugs. The influence of high and low doses of cisplatin and its low dose in combination with 20-hydroxyecdysone (**6**) on the dynamics of protein and DNA biosynthesis in the liver, pancreas, thymus, spleen, and adrenals of tumor-bearing mice were also studied. Although the therapeutic effect of 4 mg/kg cisplatin by activated protein biosynthesis and DNA repair is comparable or better than that of its low dose (2 mg/kg) in combination with 20-hydroxyecdysone (**6**), the combination with ecdysteroids looks preferable for chemotherapy since the therapeutic dose of cisplatin is toxic for the intact tissues [40].

Cholesterolemic Effect

Ecdysteroids influence the metabolism of cholesterol, the precursor of the biosynthesis of steroid hormones. The most active hypocholesterolemic compound, affecting the absorption of $^3H_1$-cholesterol in small intestine of rats with experimental hypercholesterolemia, proved to be integristerone A (**21**); and the activity was gradually decreased in the following series of compounds: 20-hydroxyecdysone (**6**), steroid sapogenine allyogenone, cyasterone (**28**) and viticosterone E (**20**). Thus, the hypocholesterolemic activity of the preparations enhanced with an increase in the number of hydroxy groups in the molecules. After daily administration of 20-hydroxyecdysone (**6**) at a dose of 2.5 mg/kg to the animals with hypercholesterolemia for 3, 6 and 8 weeks, the cholesterol level of blood plasma was decreased by 7.0%, 16.9% and 29%, respectively. This phenomenon was accompanied by a decrease of the cholesterol content in erythrocyte membranes as well as in microfilaments of erythrocyte border by 26% and 34%, respectively. Amount of phospholipids and the cholesterol/phospholipids ratio were also normalized in the membranes. The data obtained suggest a competition between cholesterol and the hypocholesterolemic componds such as ecdysteroids during the process of binding with the membrane sites depending on their concentrations in blood plasma, intestine lumen and on their content in the membranes [41].

It has not been further enlighted whether the ecdysteroids indeed compete with cholesterol for binding to membrane sites, and the understanding of phytoecdysteroid action in mammals has also not progressed much for tens of years. In the meantime it became accepted that ecdysteroids do not bind to any of the mammalian steroid receptors with suitable affinity [42]. Instead, the ecdysteroid

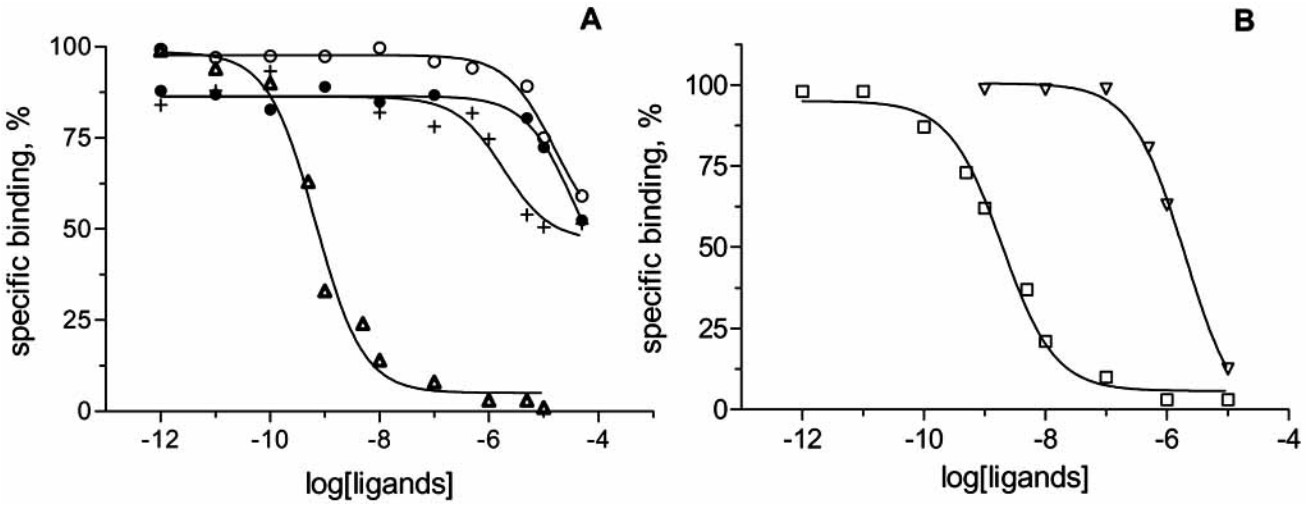


Fig. (4). Competition binding curves of compounds: **A**) 3,17β-Estradiol (Δ) (K_i: 0.39×10^{-10} M), 20E (•) (K_i: 2.53×10^{-5} M), Polypodine B (o) (K_i: 6.95×10^{-5} M) and 20E 22-acetate (+) (K_i: 1.35×10^{-6} M) on rat estrogen receptor. The radioligand was [^{3}H]17β-Estradiol; **B**) Testosterone (□) (K_i: 1.09×10^{-9} M) and 9,11-Didehydropoststerone (∇) (K_i: 5.15×10^{-7} M) on rat prostate cytosol. The radioligand was [^{3}H]Dihydrotestosterone.

receptor in insects is more related evolutionarily to the thyroid and retinoid X receptor [43]. It has been rarely reported even in insects that ecdysteroids acted without binding to a cytosolic receptor, *via* signal transduction systems [44]. This might be appreciated regarding the fact that the action of anabolic-androgenic steroids, an analog to the effect of ecdysteroids in mammals, has still not been clarified until present days.

Ecdysteroid Containing Preparations

The characteristic pharmacological results of ecdysteroid treatment have led to the appearance of numerous ecdysteroid-containing health-improvement products, food supplements and tonics [10,45,46]. Such preparations are widely advertised on the Internet for healthy people, body-builders and sportsmen, and are also available commercially as OTC drugs. Their indications are mainly based on two important effects of the ecdysteroids: the anabolic and adaptogenic effects. The ecdysteroid-containing preparations are used with the aims of increasing physical power and endurance in professional sports and building up the muscle fibers (lean muscle) of body-builders, together with the consumption of protein. These products are made from various plant species, the most important of which are *Leuzea*, certain *Pfaffia*, *Cyanothis*, *Ajuga* and *Polypodium* species. The ecdysteroids affect the metabolic processes of protein synthesis and energy consumption in cells and are responsible for recovery from muscular tiredness during intensive training. They are able to reduce fat tissue. Certain ecdysteroid-containing preparations have been approved officially [10,47]. They have advantages over artificially synthetized products. They appear to be a deserved substitute for popular, but forbidden preparations such as the anabolic-androgenic products: Nerobol® (**36**), Durobolan® (**46** decanoate) or Proviron® (**55**), used in speed and power sports [49]. The administration of these anabolic-androgens is subject to severe limitation in both chemotherapeutic practice and the sports world, where these drugs are classified as prohibited stimulants.

Ecdysteroids have been used by athletes in Asian countries since 1985. Ecdysteroids appear to be harmless to humans, even in high doses (5 mg/kg body weight per day of 20-hydroxyecdysone (**6**) is generally recommended) and are not prohibited [50].

In contrary the therapeutic effects of ecdysteroids have not been adequately tested by means of clinical treatment, in double-blind, placebo-controlled studies. Randomized clinical trials have not been performed to assess their long-term efficacy and safety. Human clinical investigations in the territory of the former Soviet Union are mentioned on the Internet but a full article is not attainable, so the experimental data are therefore incomplete. The results are summarized without further explanation in Table **3**. The growing popularity of the use of ecdysteroid-containing preparations, dietary supplements and pure ecdysteroids demands checks on long-term human treatment prior to further preclinical and clinical applications with numerous patients. Steps must be taken to ensure the quality and the proper use of these preparations.

The protein synthesis-stimulating effect of ecdysteroids in mammals led to their unofficial use in animal breeding. The overall feed consumption can be lowered when the feed contains ecdysteroids. A recently developed preparation (Bioinfusin) with high anabolic activity leads to an increase in the total body weight of about 10-12% following i.m. administration [51]. It has to be noted that further studies are required in the respect of the accepted use of ecdysteroid-containing preparations in agriculture to increase the meat production of domestic animals.

THE MECHANISM OF ACTION OF ANABOLICS

After many years of intense research it has become clear that steroids may exert their action on living cells by at least two ways. One is the well-known genomic pathway, involving hormone binding to intracellular cytosolic (classic) receptors and subsequent modulation of gene transcription followed by protein synthesis. The other alternatives are the operating signal transduction pathways that do not act directly on the genome, therefore indicate nongenomic action. The nongenomic nature of a particularly observed phenomenon is relatively easy to confirm by using transcription or translation inhibitors, but the identification and characterization of these pathways and the ligand binding proteins are more difficult. So far a considerable controversy exists about the identity of receptors that mediate these responses. A number of different approaches have been applied to answer this question, including pharmacology,

Table 3. Human Clinical Trials with Ecdysteroids Revealed by Internet Search

Ecdysteroid containing preparations, Ecdysteroids	Effect	Duration of treatment	Number of subjects	Dosage of 20E	References
Ecdysten® Leveton® Prime Plus® (containing protein)	Elevated muscle mass Anabolic, Increase working capacity (during training)	-	20 athletes (between the age of 17-25)	-	[52]
20E (**6**) + protein	7% increase of lean muscle 10% reduction of fat tissue No hormonal side effects	10 days	78 athletes	5 mg/kg	[53]
-	-	-	117 athletes (between the age of 18-28)	-	[54]
20E (**6**)	Greater performance and speed Improved strength	5 days	112 athletes	-	[55]
Ecdysten® Leveton® Prime Plus® (containing protein)	Reduced fat content under training Increased muscle mass	3 weeks	-	-	[56]
Leveton® Elton®	Increased physical working capacity	20 days	44 athletes	0.4 g/day	[57]

numerous biochemical and molecular biological studies and knock-out animals. Evidence is presented in favor and against the participation of classic receptors, or homologous proteins, as well as for the involvement of poorly understood, novel membrane steroid receptors. In these days the number of clinical implications for a wide array of nongenomic steroid actions is still growing [58]. Since ecdysteroids do not bind to cytoplasmic androgen receptor they are more likely to act analog to the membrane bound androgen receptor mediated pathways or even acting on the membrane bound androgen receptors. These type of binding proteins are of many kinds and presently difficult to asses them. The multiplicity of anabolic steroid binding proteins probably reflects the adhesiveness of the ligands [59]. It has been shown that these receptors are likely act *via* signal transduction pathways [60,61]. Some of the ecdysteroid effects have also been related to induction of PI3K-Akt route [62], a pathway that has also been activated in anabolic steroid action. Skeletal muscle constitutes for a major part of body weight in healthy men and it largely reflects health condition. Anabolic-androgenic steroids have a profound effect on increasing muscle size.

Anabolic Steroid Effect in Muscle

As it is summerised in an excellent review [63], it has been recognized since ancient times that the testes play an important role in the regulation of metabolism and maintaining body composition. However the anabolic effects of androgens on skeletal muscle - the tissue constituting for more than 40% of normal body weight - is still a matter of controversy. An increasing amount of data generated in the last ten years, has now points to the conclusion that androgens indeed have direct anabolic effects on mammalian skeletal muscle. Testosterone (**32**) administration to androgen-deficient men is associated with increased lean body mass and a decreased amount of fat. The effects of testosterone (**32**) on muscle are directly proportional with the administered dose and the prevalent circulating testosterone (**32**) concentrations. Administering supra-physiological doses of testosterone (**32**) to eugonadal men leads to further gains in muscle size and maximal voluntary strength, and a decrease in fat mass. Androgen receptors are also found in muscle, fat, and nerve cells and in satellite cells (mesenchymal pluripotent cells) that reside in the muscle, and likely mediate the effects of androgens on muscle hypertrophy. It is not know whether androgens exert additional effects on these tissues through non-androgen receptor mediated pathways. However data have accumulated in many laboratories that shed light on the mechanisms of anabolic effects of androgens on skeletal muscle (reviewed in [63]).

Remodeling of skeletal muscle has been a subject of intense research in the past twenty years. It seems that Ca-dependent and nondependent signaling pathways play a crucial role in defining muscle type and muscle mass (reviewed in [64]). This is in agreement with the suggestion that the nongenomic anabolic effect of steroids is exerted *via* signal transduction pathways.

The comparison of the effects of anabolic steroids and ecdysteroids on muscle fiber types is summarized in Table **4**. This shows that both anabolic steroids and 20-hydroxyecdysone (**6**) increase muscle fiber size. Only one work is known by us that has reported increase in the number of fast-oxidative-glycolytic type II fibers but no change in the number of slow- oxidative type I fibers in several muscles of the rat [65]. It further complicates the situation that the 20-hydroxyecdysone exerts differential effects on the size of the same fiber types in the different muscles (Table **4**).

The Nongenomic Steroid Effect and the Signal Pathways

The nongenomic effect of androgens takes a shorter period from seconds to minute while the genomic effect is seen later. Testosterone (**32**) and nandrolone (**46**) were found in the seconds to minute period to induce transient intracellular calcium release and to increase the activity of mitogen-activated protein kinases (MAPK/ERK1/2) in cultured myotubes. These multinucleated cells are formed from mononucleated myoblasts and are in an intermediate stage of the muscle differentiation. The fast effect of testosterone (**32**) in this system was the dual phosphorylation of ERK1/2. This was mediated by G protein-coupled receptor activated PLC and subsequently IP3 induced calcium release from the internal stores. The increased intracellular calcium level by an elusive mechanism activated the Ras-MEK-ERK pathway. The phosphorylation of ERK is important in the terminal muscle differentiation involving myoD [76]. The ERK phosphorylation was prevented by dominant negative Ras and MEK, the G-protein and PKC inhibitors and not inhibited by cyproterone, an antagonist of the intracellular androgen receptor. The subcellular location of the androgen receptor was not changed within 5 minutes after the treatment while these changes happened. If the testosterone (**32**) was covalently bound to albumin, therefore was not able to cross the cell membrane, it mimiked the action of a membrane bound androgen recep-

tor and showed the same response as the free hormone. These data strongly support the involvement of a membrane bound androgen receptor in the anabolic action of steroids on muscle [60].

If the muscle cell depletes intracellular Ca stores these compartments regulate plasma membrane channels to let calcium in from the extracellular space. This process is called capacitive calcium entry (CCE). The sarcoplasmic calcium level increased by testosterone (**32**) induces CCE into the myotubes from the extracellular space [61]. As a mechanism it has been suggested that in the sarcolemma the store operated channels (SOCs) [77] interact with the IP3 receptor in case of exhaustion of calcium from the internal store. In case of depletion of the intracellular stores, SOCs are opened and facilitate the extracellular calcium entry into the cell and refill the internal stores.

Role of Calcium in Muscle Remodeling

The intracellular Ca level and Ras are in the center of recently described routes regulating muscle specific gene expression (reviewed in [64]). The calcium and Ras reflect the different patterns of motor nerve activity required for regulating contraction, metabolism and muscle mass in the adaptation process termed muscle plasticity. Calcineurin, a calcium/calmodulin dependent serine phosphatase has been put forward to control gene expression in skeletal and cardiac muscle through dephosphorylating the transcription factor, the nuclear factor of activated T cells (NFAT). The dephosphorylated NFAT is rendered to transfer into the nucleus and activate gene expression together with or without MEF-2, an other transcription factor. Evidence has been shown that meanwhile the function of NFAT in gene transcription is regulated by nerve activity in intact animals; the calcium influx from the transient receptor potential channels (TRPC) is an important factor to determine NFAT activity. TRPC3 is upregulated by neuromuscular activity in cultured skeletal myocytes in a calcineurin-dependent manner. Therefore in case of repeated bouts of contractile activity a positive feedback mechanism is created for escalation of remodeling processes [78]. This mechanism resembles to the capacitive calcium entry and conforms to the suggestion that TRPCs could be candidates for SOCs in CCE [77].

Pathways in Muscle Growth

A number of *in vitro* experiments have enlightened the important role of Akt(PKB)-mTor pathway in the level of protein transla-

Table 4. The Anabolic Effect of Steroids and 20E (6) on Skeletal Muscle Fibers

Compound	Effect on muscle fibers	Duration	Number of subjects	Dosage	References
Testosterone (**32**) Nandrolone decanoate (**46** decanoate) Stanozol (**64**) Methandienone (**36**)	↑ size of IIx, ↓ size of I, ↑ strength of IIA and IIx in VL	2 years	5 body builders	Taking assumed in cycles p.o.	[66]
Testosterone (**32**)	↑ fiber size in m. EDL and m. soleus	10 weeks	9 male mice	1-1.5 mg/g/week for 10 week	[67]
Testosterone (**32**) Nandrolone (**46**) Durobolan® (**46** decanoate) Proviron® (**55**) Primobolan® (**56** acetate) Oxymetholone (**58**) Masteron® (**60** propionate) Stanozolol (**64**)	↑ size of I,IIA, IIAB and IIC, development of all myosin in m. VL	9±3.3 years	9 power lifters	936±527 mg/week p.o.	[68]
Testosterone (**32**)	↑ fiber size of I in m. gastroc.	-	12 male rats	-	[69]
Testosterone (**32**)	↑ size of I, II fibers in m.VL	20 weeks	39 healthy young men	300-600 mg/week, p.o.	[70]
Testosterone (**32**) Nandrolone (**46**) Durobolan® (**46** decanoate) Proviron® (**55**) Primobolan® (**56** acetate) Oxymetholone (**58**) Masteron® (**60** propionate) Stanozolol (**64**)	↑ size of I and IIA in m. trapezius	9±3.3 years	9 body builders	100-500 mg/week, p.o.	[71]
Nandrolone (**46**)	↑ MyHCI / MyHCII ratio in regenerating m. EDL	25 days	8 male rats	2 mg/kg/week, i.p.	[72]
Nandrolone (**46**)	↑ size of IIx/b in diaphragm, IIx/b and I in m. gastroc.	5 weeks	10 female rats	1.5 and 7.5 mg/kg/week i.m.	[73]
Testosterone (**32**)	↑ number of type II fibers	12 weeks	-	"moderate"	[74]
Nandrolone (**46**)	↑ size of average fibers in m. plantaris	6 weeks	7 female rats	0.3 and 0.9 mg/day	[75]
20-Hydroxyecdysone (**6**)	↑ size of I, IIA in m. soleus, ↑ IIx and IIB in m. EDL	1 week	8 male rats	5 mg /kg/day i.p.	Our unpublished results
20-Hydroxyecdysone (**6**)	↑ regeneration of m. soleus	1week	8 male rats	0.5-5 mg/kg/day i.p.	Our unpublished results

↑ increased; ↓ decreased; gastroc.: m. gastrocnemius; EDL: m. extensor digitorum longus; VL: m. vastus lateralis.

tion in muscle cell. An *in vivo* study using transgenic skeletal muscle model [79] have shown that the PI3K–PKB pathway controls nerve activity-dependent muscle growth but not fiber type specification in regenerating skeletal muscle. This model is based on the fact that a few percent of muscle fibers become transfected if the muscle is injected with plasmid expressing a gene. Transfecting regenerating muscle with plasmid expressing constitutively active PKB stimulates muscle fiber growth and this effect was completely prevented by rapamycin; an antibiotic inhibiting mTOR, a protein kinase which regulates ribosomal proteins and protein translation. It was shown [79,80] that activated PKB induces skeletal muscle hypertrophy and prevents denervation atrophy exclusively in transfected fibers in regenerating and adult skeletal muscle. The muscle fibers, overexpressing activated PKB, were hypertrophic and coexisted with normal-size untransfected fibers within the same muscle. This points to a cell-autonomous control of muscle growth by PKB. Therefore PKB appears to act directly in transfected fibers and not through local release of growth factors and autocrine/paracrine loops that could also affect surrounding untransfected fibers. It should be noted that activated PKB is exclusively expressed in muscle fibers and not in mononucleated cells therefore intensively contribute to fiber growth in the regenerating muscle.

The role of calcium is important for the fusion of myoblast with developing muscle fibers. It has been demonstrated [81] that the young developing myofibers called myotubes secrete interleukin-4 (IL-4) in order to stimulate accretion of new myoblasts. The myoblasts express receptor for IL-4, and binding a ligand on this will transform the cell competent to fusion. The IL-4 gene in myofibers is upregulated by an NFAT isoform, therefore this way of regulation is also dependent on the calcium-dependent calcineurin-NFAT pathway. This shows that upregulation of the calcium level might trigger an autocrine/paracrine growth of skeletal muscle.

Satellite Cells and Anabolic Effect

Increase in fiber size during muscle development (or regeneration) and compensatory hypertrophy is accompanied by increased number of myonuclei. The muscle fibers are true multinucleated postmitotic cells with no proliferating internal nuclei. Therefore there is a need of proliferating cells from the outside to support myofiber growth. These are the satellite cells situated between the plasma membrane of myofibers and the lamina basalis. In adapting muscle, satellite cells are activated, proliferate and subsequently fuse with muscle fibers [82]. Androgens regulate satellite cell activity through the androgen receptor but the fastest nongenomic effect of anabolic action has not been shown (reviewed in [83]).

However the ecdysteroid increased muscle fiber size (in the last rows of Table **4**) was accompanied by an adequate increased of myonuclear number. This implies that the satellite cells might also be subject of the anabolic action. One can speculate that the effect of anabolic-androgenic steroids on the signal transduction system induces insulin-like growth factor 1 (IGF-1). There are two types splice variants of the IGF-1 mRNA in skeletal muscle, mechanogrowth factor (MGF) and IGF-1 Ea [84]. The MGF is mechanosensitive and autocrine, while the IGF-1 Ea is similar to the systemic or liver type of IGF-1. The overexpression of MGF induced proliferation and inhibited terminal differentiation in immortalized muscle cell culture. The IGF-1 Ea in the same cells inhibited cell proliferation and helped the fusion of myoblasts into myotubes, therefore stimulated terminal differentiation [85]. Lewis *et al*. [86] has demonstrated that administration of nandrolone (**46**) in rats produced significantly higher level of muscle IGF-1 compared to rats without nandrolone (**46**) treatment. IGF-1 protein expression increased after testosterone (**32**) treatment in humans [87]. The IGF-1 mRNA level was decreased in men after treatment with gonadotropin-releasing hormon (GnRH) analog to make them testosterone (**32**) deficient, meanwhile the circulating IGF-1 remained unchanged [88]. However no distinction has been made which of the IGF-1 isoforms respond to anabolic action.

STRUCTURE-ANABOLIC ACTIVITY RELATIONSHIP

Ecdysteroids

Our knowledge is merely superficial of the structure-activity relationships of ecdysteroids in mammals. Only three publications deal with the protein metabolism, where structure-activity relationship studies are available for 14 [26], 22 [39] and 16 [47] ecdysteroids. Fig. (**1**) depicts the structures of the ecdysteroids used in these experiments. The anabolic effects reflecting amino acid inclusion into the liver proteins of mice, and hypoazotemic effects versus structure relationships of ecdysteroids were also determined.

The experiments concerning the ecdysteroid anabolic activity (14 different ecdysteroids) assessed body weight (Table **5**) and weights of internal organs and skeletal muscles in male rats of various ages and hormonal states (intact and castrated) [26]. The animals in the control group received either methylandrostenediol (**59**) or Nerobol® (**36**). The structure-activity relationship study on the anabolic activity revealed that the presence of a diol group on C-20 and C-22 in the ecdysteroid molecule is important. When the overall stereo-structure is same, the anabolic effect depends on the number and positions of the hydroxy groups. An additional hydroxy group on C-1 decreases the activity, but the presence of an α-hydroxy group on C-11 contributes to an increase in the protein synthesis-stimulating activity of ecdysteroids. The anabolic effects of 20-hydroxyecdysone (**6**) and turkesterone (**18**) are nearly equal to that of Nerobol® (**36**). Slama and Lafont explained the high anabolic activity of turkesterone (**18**) in terms of the presence of the α-hydroxy group on C-11 [9,10]. Le Bizec *et al*. suspected that ajugasterone C (**15**) with an 11-hydroxy group also has high anabolic activity [50]. A hydroxy group on C-20 has a slightly more pronounced effect on protein synthesis than one on C-2. The anabolic activity of 20-hydroxyecdysone (**6**) is 2.5-fold and 2.7-fold higher than those of 2-deoxyecdysone (**13**) and ecdysone (**16**), respectively. The activities of the C-22 glycosides of ecdysteroids are higher than those of the aglycons. The ecdysteroid acetates exhibit lower activity than the free compounds, but the effect depends on the number and the positions of the acetyl groups. The activities of 20-hydroxyecdysone mono-, tri- and tetraacetate display the following sequence: viticosterone E {20-hydroxyecysone 25-acetate (**20**)} > 20-hydroxyecdysone 2,3,22-triacetate (**7**) > 20-hydroxyecdysone 2,3,22,25-tetraacetate (**8**). 24(28)-Dehydromakisterone A (**11**) with a modified side chain at C-24 retains the activity of 20-hydroxyecdysone (**6**).

Rubrosterone (**31**) has been approved as a powerful anabolic in mammals [15]. This ecdysteroid does not contain a long side chain and its structure is close to that of the androgenic androsta-4-en-3,17-dione.

Determination of the anabolic effects of ecdysteroids with structures similar to that of rubrosterone (**31**) should be of interest.

A study of structure-activity versus protein incorporation (Table **5**) demonstrated the importance of 2,3-diol substitution and the hydroxy groups on C-20 and C-11 in the ecdysteroid molecule [39]. This work evaluating 22 compounds, demonstrated that turkesterone (**18**) containing a 2,3-diol and an α-hydroxy on C-11, exerted the most pronounced anabolic effect on the incorporation of radiolabeled amino acids into the liver protein of mice. The anabolic effect of turkesterone (**18**) was comparable to that of Nerobol® (**36**) but without any hormonal consequence. α-Hydroxylation at C-11 resulted in a more active compound {turkesterone (**18**)}, than the C-1-hydroxylated ecdysteroid, and this C-1 hydroxylation caused a greater difference in activity than did hydroxylation at C-5 relative to the activity of turkesterone (**18**). An ecdysteroid with a modified side-chain at C-24 {24(28)-dehydromakisterone A (**11**)} retained the anabolic effects of the parent 20-hydroxyecdysone (**6**), while cyasterone (**28**) with a ring-forming side chain, had a higher activity.

Table 5. The Anabolic Activity of Ecdysteroids. The Anabolic Activity was Determined by the Measurement of the Gain in Body Weight of Male Rats of Various Ages and Hormonal States, the Rate of Incorporation of Radiolabeled ^{14}C-Aminoacid into the Liver Protein of Mice, and Investigation of the Hypoazotemic Effects of Ecdysteroids, with Determination of the Urea and Residual Nitrogen Contents of the Blood [26,39,47]

Name of Ecdysteroids	Change in the structure of 20E	Weight gain			^{14}C Amino acid inclusion into mice liver (%)	Hypoazotemic effect	
		Pubertal rats (%)	Impubertal rats			Change of urea in blood (%)	Change of residual nitrogen in blood (%)
			Intact (%)	Castrated (%)			
20E (**6**)	-	100.00	100.00	100.00	100.00	100.00	100.00
	Hydroxylation						
Integristerone A (**21**)	+βOH at C-1	48.17	45.99	50.00	42.35	50.00	50.00
Polypodine B (**17**)	+βOH at C-5	-	-	-	65.53	95.45	92.31
Turkesterone (**18**)	+αOH at C-11	122.35	147.57	148.21	138.19	136.36	134.62
	Dehydroxylation						
2-Deoxy-20E (**12**)	-βOH at C-2	40.85	42.84	42.86	56.32	63.63	65.38
Ecdysone (**16**)	-βOH at C-20	36.99	33.25	28.57	-	36.36	42.31
2-Deoxyecdysone (**13**)	-βOH at C-2, -βOH at C-20	33.33	28.52	26.79	18.13	27.27	26.92
	Alkyl substitution						
24(28)-Dehydromakisterone A (**11**)	+=CH_2 at C-24	96.34	91.26	89.29	90.04	-	-
	Side chain closure						
Cyasterone (**28**)	+lactone formation	-	-	-	114.26	118.18	115.38
	Acetonide formation						
20E 2,3-monoacetonide (**25**)	+Acetonide group at C-2, C-3	-	-	-	36.11	22.72	23.08
20E 2,3,20,22-diacetonide (**27**)	+Acetonide group at C-2, C-3 +Acetonide group at C-20, C-22	-	-	-	15.16	-	
	Esterification						
Acetylation							
Viticosterone E (**20**)	+Acetyl-group at C-25	77.84	73.79	66.07	78.60	81.81	76.92
20E 2,3,22-triacetate (**7**)	+Acetyl-group at C-2, C-3, C-22	70.36	78.52	60.71	81.13	-	-
20E 2,3,22,25-tetraacetate (**8**)	+Acetyl-group at C-2, C-3, C-25	59.34	68.20	48.21	72.36	-	-
Benzoate formation							
20E 22-benzoate (**10**)	+Benzoyl-group at C-22	-	-	-	56.76	45.45	46.15
	Esterification and other modifications						
Acetylation and hydroxylation/ dehydroxylation							
Turkesterone 2,3,11,22-tetraacetate (**19**)	+αOH at C-11, +Acetyl-group at C-2, C-3 and C-22	114.84	128.52	119.64	128.97	-	-
2-Deoxyecdysone 22-acetate (**14**)	-βOH at C-2, -βOH at C-20, +Acetyl-group at C-22	26.01	22.94	19.64	17.53	18.18	23.08
Acetylation and lactone formation							
Cyasterone 22-acetate (**30**)	+Acetyl-group at C-22	-	-	-	97.33	100.00	96.15
Cyasterone 2,3,22-triacetate (**29**)	+Acetyl-group at C-2, C-3, C-22	-	-	-	88.86	-	-
Benzoate and acetonide formation							

(Table 5). Contd…..

20E 2,3-monoacetonide 22-benzoate (**26**)	+Acetonide group at C-2, C-3 +Benzoyl-group at C-22	-	-	-	28.97	31.81	34.62
	Glycosylation						
Sileneoside A (**22**)	+α-*D*-gal at C-22	107.51	107.04	110.71	108.62	63.63	107.69
	Glycosylation and other modification						
Glycosylation and hydroxylation/ dehydroxylation							
(5α)-Sileneoside E (**24**)	-*β*OH at C-2, +α-*D*-glu at C-3	-	-	-	43.98	-	-
Sileneoside C (**23**)	+OH at C-1, +α-*D*-gal at C-22	40.84	49.15	42.86	51.71	59.09	57.69
	AAS						
Methandrostenolone (**36**)		111.18	137.99	273.21	160.33	-	-
Methylandrostenediol (**59**)		100.00	93.57	187.50	-	-	-

Percentaged values of each ecdysteroid was compared to the control (weight gain for pubertal animals: 151.9% for impubertal intact animals: 182.4% for impubertal castrated animals: 144.8% ^{14}C; amino acid inclusion into mice liver: 167.3%; change in urea blood level: 78%, change in residual nitrogen blood level: 74%) and the results were expressed in relative values where 20E=100%.

The effects of 16 different ecdysteroids on the nitrogen metabolism were studied by investigating their hypoazotemic activity in male rats [47]. The hypoazotemic activity is closely related to the protein metabolism of the ecdysteroids.

The action of ecdysteroids as hypoazotemic agents was proven by their ability to lower the levels of urea and residual nitrogen in the blood. The most pronounced hypoazotemic effect was that of turkesterone (**18**), followed by cyasterone (**28**) (Table **5**).

When the stereochemistry is the same, the hypoazotemic activity depends considerably on the number and positions of the hydroxy groups on the ecdysteroid skeleton as observed in the case of amino acid incorporation in various organs. α-Hydroxylation at C-11 increases, whereas that at C-1 and C-5 decreases this effect. The presence hydroxy group on C-20 is important. Loss of the hydroxy group from C-20 lowers the activity more markedly than loss of that on C-2. Conjugation of ecdysteroids with benzoate, acetate and carbohydrates also lowers their hypoazotemic effect.

Identical structural modifications in the molecules resulted in similar differences in anabolic activity as measured with the three methods.

A clearer understanding of the structure-anabolic activity relationship of ecdysteroids necessitates further pharmacological study, which in turn demands suitable plant sources and isolation techniques for the preparation of these various compounds in adequate amounts.

Anabolic-Androgenic Steroids

The discovery and verification of the anabolic activity of testosterone (**32**) led to the synthesis of a series of anabolic–androgenic steroid derivatives to make orally longer active analogs with high anabolic and low androgenic activity [86]. The structural modifications were mainly carried out in specific positions of ring A, B or C or at position C-17 with appropriate substitutions. Complete separation of the anabolic and androgenic effects was impossible, but there are several preparations with a high anabolic–androgenic ratio among the currently used products. The anabolic and androgenic activities of the steroids mentioned here were quantitatively compared on the basis of experimental data and expressed in anabolic:androgenic dissociation indexes (Table **6**) based on nitrogen retention after oral administration and compared with methyltestosterone (**33**) [86].

Table 6. The Approximate Anabolic-Androgenic Dissociation Indexes Determined by Different Methods in Different Animals (Based on Nitrogen Retention Relative to Methyltestosterone (33)) [87]

Anabolic-androgenic steroid	Anabolic-androgenic ratio
Methyl-testosterone (**33**)	0.67
19-Nortestosterone (**46**)	9.9
Methylnandrolone (**47**)	4.2
Norethandrolone (**48**)	20
Androstanolone (**54**)	0.13
Mestanolone (**57**)	0.8
17α-Methyl-5α-androstano[2,3-c]isoxasol-17β-ol (**62**)	7
17α-Methyl-5α-androstano[2,3-d]isoxasol-17β-ol (**63**)	40
Stanozolol (**64**)	30
Oxymesterone (**34**)	5
4-Chloro-methyltestosterone (**35**)	5.4
Fluoxymesterone (**43**)	2.7
Methandienone (**36**)	3.4
Bolasterone (**38**)	3.2

Most of the natural (endogenous) and synthetically modified anabolic–androgenic steroids contain androstane basic skeleton. These compounds can be classified into four main classes [86-91]:

1. Various substituents on C-17 of the testosterone skeleton

1a. 17α-Alkylated testosterone derivatives

1b. 17β-Hydroxy esterified testosterone derivatives

2. 19-Nortestosterone derivatives

2a. 17α-Alkylated 19-nortestosterone derivatives

2b. 17β-Hydroxy esterified 19-nortestosterone derivatives

3. Heterocyclic ring containing derivatives

4. Further androstane derivatives

An additional group of anabolic-androgenic steroids is the so called "designer steroids", which are designed to be analitically

undetectable in sports doping. Each member of this group can be classified into either of the above chemical classes. Some of these compounds are known to be metabolized to testosterone (**32**) or 19-nortestosterone (**46**) derivatives in the human body *via* enzymatic processes [92,93].

The most commonly used representants of each main class are shown in Fig. (**2**) and Fig. (**3**).

1a. The 17α-alkylated, mainly methylated testosterone derivatives are resistant to metabolism in the liver. The 17α-alkyl group protects the 17β-hydroxy group from oxidation, this hydroxy group is a very important structural element for the anabolic-androgenic effect [87,88]. The 17β-hydroxy group plays a role in the specificity for the androgen receptor. Any substitution of this hydroxy group leads to a decreased activity.

The anabolic:androgenic dissociation index of methyltestosterone (**33**) based on nitrogen retention following oral administration was somewhat more favorable than that of testosterone (**32**).

2. In these compounds, the C-19 methyl group is replaced by hydrogen, and therefore the steric hindrance around the molecule is low. These compounds can readily bind to the receptor [91,92]. Their affinity for the androgen receptor in the skeletal muscle is high, but their specificity is low; they exhibit activity for other steroid receptors (e.g. progesterone) and possess enhanced anabolic activity. Orally 19-nortestosterone (**46**) displays high nitrogen conversing:androgenic index relative to methyltestosterone (**33**). Metabolization of the 19-nor compounds by 5α-reductase results in low acting 5α-dihydronortestosterone derivatives with a decreased affinity for the androgen receptor, in contrast with the highly effective 5α-dihydrotestosterone (**54**) [86-88]. While the presence of 5α-reductase is relatively high in the androgen-dependent organs (such as the prostate or the skin), this metabolic conversion leads to 19-nortestosterone analogs being more myotropic and possessing a markedly diminished androgenic effect as compared to the testosterone derivatives.

2a. Methylnandrolone (**47**), a 17α-methyl derivative of 19-nortestosterone has a lower, the ethyl derivative {norethandrolone (**48**)} has a 2 times higher dissociation index than that of 19-nortestosterone (**46**) (based on nitrogen retention following oral administration).

1b-2b. Derivatives containing an esterified 17β-hydroxyl-group are generally administered parenterally. The 17β-hydroxy group can be esterified with aliphatic (straight-chain), cyclic or aromatic acids, such as acetic, propionic, cypionic, decanoic, undecanoic, enanthic, phenylpropionic acid, etc. The duration of anabolic action depends on the length (carbon number) of the acid used for esterification [87,88]. Esterification with short-chain acids results in short-acting steroids, and esterification with long-chain acids in longer-acting steroids. They can be hydrolyzed to the active parent alcohol and can be aromatized in ring A by aromatase.

3. Besides oxandrolone (**61**) other frequently used synthetic heterocyclic ring containing derivatives of anabolic-androgenic steroids possess a typical androstane skeleton. These compounds have a strongly higher nitrogen conversing:androgenic index than that of methyltestosterone (**33**). The presence of a heterocyclic ring increases the anabolic and decreases the androgenic activity. Table **6** shows the anabolic-androgenic dissociation indexes of 17α-methyl-5α-androstano[2,3-c]isoxasol-17β-ol (**62**), 17α-methyl-5α-androstano[2,3-d]isoxasol-17β-ol (**63**) and stanozolol (**64**). The most markedly anabolic is the (2,3-d)-isoxazole ring-containing derivative of 17α-methyl-5α-androstan-17β-ol (**63**) [86,88,92].

4. Structural modifications at C-10 in ring A make these molecules resistant to the effects of 5α- and 5β-reductase, 3α- and 3β-steroid dehydrogenase, and aromatase [87,88]. These steroids can not be metabolized to the strong androgenic 5α-dihydrotestosterone (**54**) by 5α-reductase, and they can not be aromatized to estrogenic active estradiol derivatives by aromatase.

Androstanolone (**54**) exhibits a less favorable dissociation index relative to methyltestosterone (**33**) (based on nitrogen retention following oral administration) and a decreased dissociation index compared to testosterone (**32**) (based on myotrophic activity following parenteral administration).

4a. Mestanolone (**57**), a methyl derivative of androstanolone (**54**), is a little more anabolic than the parent compound and has a somewhat higher dissociation index (based on its ability to decrease nitrogen retention) [86].

Further structural changes may occur mainly in the classes 1 and 2 of anabolic–androgenic steroids, such as additional hydroxylation, halogenation, extended conjugation or further alkylation.

The additional hydroxy group (besides the 17β-hydroxy) is mainly situated on C-4 or C-11. A higher anabolic activity is observed when it is on C-4 of the methyltestosterone molecule {oxymesterone (**34**)}, with a greater dissociation index relative to methyltestosterone (**33**) (by nitrogen retention following oral administration) [86,88,91,94].

Several Cl or F-containing preparations have been synthesized, with the Cl mainly on C-4 and the F in the 9α position. This yields more anabolic steroids, but in parallel the androgenic activity also increased as observed for 4-chloro-methyltestosterone (**35**) or fluoxymesterone (**43**) relative to methyltestosterone (**33**) (on the basis of nitrogen retention following oral administration) [86,91].

Incorporation of a further double bond into the androstane molecule increases the planarity of the skeleton and the anabolic activity comes into the limelight [91,94]. The extended conjugation is associated with increased anabolic activity and a higher dissociation index (by nitrogen retention orally), as found for methandienone (**36**) relative to methyltestosterone (**33**).

Further alkylation may occur on C-1 or C-7. In methenolone (**56**), where C-1 is alkylated, and clausterone (**37**) and bolasterone (**38**), where C-7 is alkylated, both the androgenic activity and the anabolic activity are more pronounced.

Comparison of the Structures of Phytoecdysteroids and Anabolic-Androgenic Steroids

While the ecdysteroids have the same basic skeleton as the anabolic-androgenic steroids, the ecdysteroids can not bind to the androgenic steroid receptors in mammals. Besides their structural similarities, the ecdysteroids display significant structural differences from anabolic-androgenic steroid hormones, which may explain the different mechanisms of their anabolic action.

The structural similarities of ecdysteroids to anabolic-androgen steroids are as follows and shown in Fig. (**5**):

Both the ecdysteroids and the anabolic-androgens possess *trans* C/D ring junctions in the steroid skeleton.

The ecdysteroids generally, and the anabolic-androgens often contain an oxo group conjugated with a double bond in the steroid ring, but this chromophore group is in ring B in the ecdysteroids and in ring A of the anabolic-androgens.

These two types of steroids exhibit several essential structural differences too. Ecdysteroids bear a long polyhydroxylated alkyl

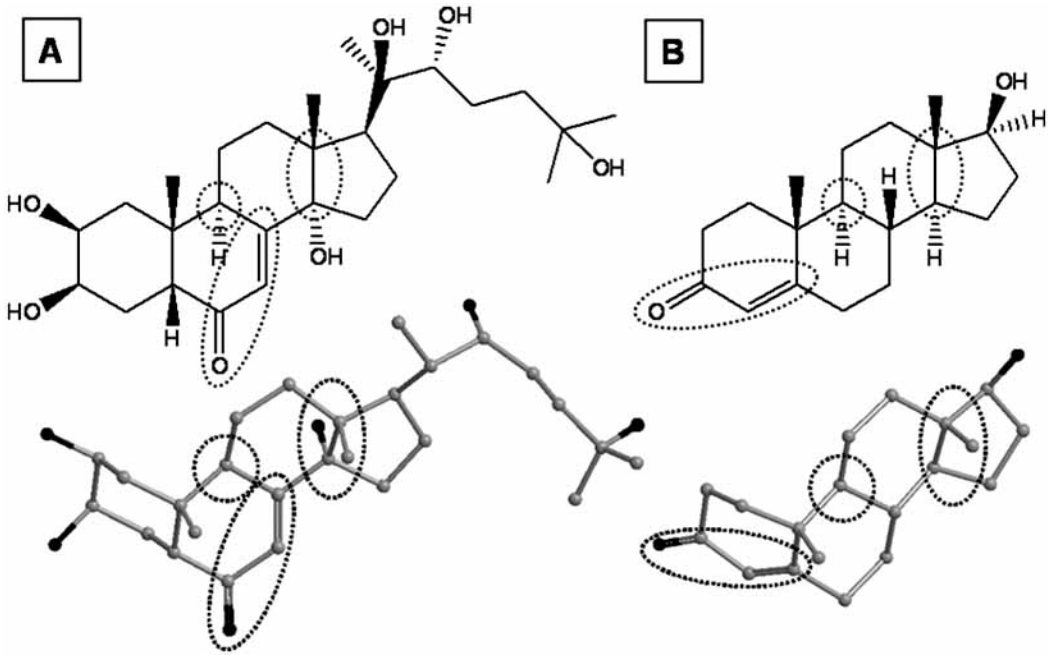


Fig. (5). The structural similarities of ecdysteroids and anabolic-androgenic steroids: **A**) 20-hydroxyecdysone (**6**), **B**) testosterone (**32**).

side chain containing 2-10 carbons attached in the β position at C-17, while in the anabolic-androgenic steroid molecules a methyl or ethyl group is linked in the 17α position (C_{19}-C_{21} steroids). C-7 and C-1 are also methylated sometimes in the anabolic-androgens. The β-hydroxy group on C-17 is characteristic for the latter steroids.

Ecdysteroids with a 19-carbon skeleton also occur, which are metabolites of the common 27-carbon ecdysteroids. These 5β-androstane-type ecdysteroids include compounds containing a β-hydroxy group on C-17. Investigation of the anabolic activity of these ecdysteroids would be of interest.

Ecdysteroids mainly belong in the 5β-androstane series of steroids, where the A/B ring junction is *cis* (5β function). The anabolic-androgenic steroids generally contain a double bound at position 4, or they have a *trans* A/B ring junction (5α steroids). There are some 5α derivatives among the ecdysteroids as well, which function is a further structural similarity to anabolic-androgenic steroids.

Ecdysteroids are highly hydroxylated (2-8 hydroxy groups), and therefore have a more hydrophilic character than the anabolic–androgenic steroid hormones, with almost sugar-like solubility properties. Thus, they are water-soluble, in contrast with the apolar, lipophilic anabolic-androgens, which are monohydroxylated or sometimes diols. In the latter case, the additional hydroxy group is mainly at position 4 or 11 (α or β). The 11-hydroxylation may be important in the manifestation of anabolic activity in ecdysteroids.

A common characteristic with anabolic-androgenic steroids is the extended conjugation, mainly in ring A or throughout the entire skeleton. There are some ecdysteroids with extended conjugation, but the additional double bond is conjugated with the 7-en-6-one group in ring C or D.

FUTURE PERSPECTIVES ACCORDING TO THE HUMAN THERAPY

Presently several anabolic-androgenic steroids are available for the human therapy. These compounds are extensively researched and used in the official medicine for decades. Their main aspects in the view of the mechanism of action, effect and side effect profile just like their structure-activity relationships are well known.

However, considering the risk/benefit ratio of the anabolic-androgenic steroids they may be used relatively safe, it would be a big advance, if the well known side effects (i. e. virilization, influence on the reproductive system of men *etc.*) could be completely avoided.

Ecdysteroids exert a number of beneficial pharmacological effects on mammals including humans. According to the pertinent literature, their protein sysnthesis-increasing effect seems to be the most pronounced one, which is compareable to that of the most widespread anabolic-androgenic steroids. Moreover, ecdysteroids have other relevant benefits, such as:

- They have low toxicity to vertebrates. The harmless consumption of vegetables with a high ecdysteroid content (such as spinach, quinoa or chestnut) and of ecdysteroid-containing preparations is a proof of their safety.
- They do not bind to vertebrate nuclear steroid receptors (estrogenic, glucocorticoid and androgenic), which means they do not exert hormonal side effects characteristic to anabolic-androgenic steroids. The relevant literature data were also supported by our results of radioligand-binding assays.
- The pharmacokinetic parameters of ecdysteroids are advantageous, due to their high polarity and relatively high water-solubility. This property is fundamental in the modern drug-design procedures.

According to the ecdysteroids the following questions are, however, still remained to be determined:

- The mechanism of action is almost completely unknown.
- The number of the known ecdysteroids is near 300 representing high structural variability. Since the profile of activity may highly depend on the structure, much more studies are needed to set on a proper structure-activity relationship both in the view of the anabolic and other possible effects/side effects.
- Only a limited number of human studies are available with small subject numbers and short duration times, in Russian language. In contrast to this, hundreds of ecdysteroid-containing anabolic preparations and products are commercially advertised on the Internet. The questionable origin, legis-

lation and composition of these products does not make them relevant to draw conclusions of efficacy and safety of ecdysteroids in the human therapeutic potency.

In summary, with the clarification of the aspects outlined above, some ecdysteroids may provide promising alternatives to anabolic-androgenic steroids in therapy. Prospective use of ecdysteroids may extend to treatments of pathological conditions where routinely the anabolic steroids are applied, for example to reverse the effect of glucocorticoids in myopathies. Such valuable applications may give further importance and actuality to the ecdysteroid research.

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ABBREVIATIONS

20E	=	20-hydroxyecdysone
5α-DHT	=	5α-dihydrotestosterone
Akt/PKB	=	Protein kinase B
AR	=	Androgen receptor
B16	=	Mouse melanoma cell line
Ca	=	Calcium
CCE	=	Capacitive calcium entry
CCl_4	=	Carbon tetrachloride
DNA	=	Deoxyribonucleic acid
ER	=	Estrogen receptor
ERK	=	Extracellular signal regulated kinase
gal	=	α-D-galactopyranosyl
glu	=	β-D-glucopyranosyl
GR	=	Glucocorticoide receptor
GnRH	=	Gonadotropin releasing hormone
i. m.	=	Intra muscular
i. p.	=	Intraperitoneal
i. v.	=	Intravenous
IGF-1	=	Insulin-like growth factor-1
IL	=	Interleukine
IP3	=	Inositol triphosphate
K_i	=	Binding affinity for receptor
L1210	=	Mouse lymphocytic leukemia cell line
LD_{50}	=	Lethal dose 50%
MAPK/ MEK	=	Mitogen-activated protein kinase
MEF-2	=	Myocyte enhancing factor 2
MGF	=	Mechanogrowth factor
mRNA	=	Messenger ribonucleic acid
mTOR	=	Mammalian target of rapamycin
MyoD	=	Myogenic regulatory factor with a key role in regulating muscle differentiation
m. EDL	=	Musculus extensor digitorum longus
m. gastroc.	=	Musculus gastrochnemius
m. VL	=	Musculus vastus lateralis
NFAT	=	Nuclear factor of activated T cells
OTC	=	Over the counter drug
p. o.	=	Per os (through the mouth)
P388	=	Mouse leukemia cell line
PI3K	=	Phosphoinositide 3-kinase
PKC	=	Protein kinase C
PLC	=	Phospholipase C
Ras	=	Small GTPase
RNA	=	Ribonucleic acid
SOCs	=	Store operated channels
T cell	=	White blood cell, lymphocyte
TÉT	=	Hungarian Science and Technology Foundation
TRPC3	=	Transient receptor potential channel 3

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