7-Keto-Δ5-Steroids: Key-Molecules Owning Particular Biological and Chemical Interest

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Abstract: 7-keto-Δ5-steroids have been suggested for the treatment of several diseases. Their significant biological profile resulted in the development of a great number of methods and reagents for the allylic oxidation of Δ5-steroids. These methods and the biological evaluation of the main oxidized Δ5-steroids are summarized.

Key words: 7-keto-Δ5-steroids, allylic oxidation, 7-keto-cholesterol, 7-keto-DHEA, anticancer activity, aromatase inhibitors, apoptosis.

INTRODUCTION

Several oxidized products of Δ5-steroids have been found in animal tissues and nutritional additives [1]. Among them, the most frequently encountered products are those with a ketone function at C-7 (7-keto-Δ5-steroids). Over the last decades, a significant number of studies regarding the biological profile of these compounds have been reported in the literature. These reports resulted in the establishment of these ketones as compounds of high and stable biological and medicinal interest and they mainly concern the biological activity of oxysterols (among them is 7-keto-cholesterol) and of oxidized androstenic derivatives (as 7-keto-dehydroepiandrosterone or 7-keto-DHEA). In parallel, 7-keto-Δ5-steroids are known as inhibitors of mammalian steroidal hormones biosynthesis [2,3].

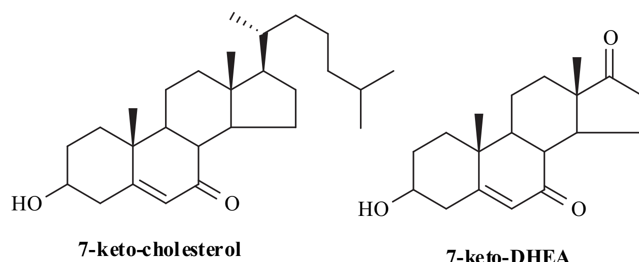

Fig. (1). Chemical structures of the most significant 7-keto-Δ5-steroids.

Although the biological profile of these compounds has not been fully disambiguated yet, a large number of information reported suggests them as molecules of potent application against a variety of diseases. The observation that some of the oxidized Δ5-steroids are more toxic toward cancerous than non-cancerous cells [4], as well as their ability to inhibit cell replication [2] is probably the most important reason indicating them as potent agents for cancer treatment.

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From the point of view of their chemical behavior, 7-keto-Δ5-steroids constitute a class of compounds of high synthetic interest. A large number of reagents and methods have been reported for the allylic oxidation of Δ5-steroidal substrates to the corresponding α, β-unsaturated enones. All of these studies provide useful synthetic "tools" and alternatives for the researchers in order to apply or modify these procedures accordingly to the synthetic problems faced. Moreover, these allylic steroidal ketones can be used as intermediates for the preparation of other steroidal derivatives such as 7-amino-cholestenic analogues which are fungicidal [5], 7-[O-(carboxymethyl)oxime] derivatives which have been devised as immunoassay components [6], steroidal guanidines which are proposed as peptidomimetics [7], as well as 7,7-gem-difluorosteroids of androstane and pregnane series which exhibit a high degree of antinflammatory activity [8]. They can also be used for the synthesis of new steroidal skeletons derived from the functionalization or conversion of the keto-group to other moieties as happened in steroidal B-lactams [9] and $\Delta^{5,7}$-steroids [10], or for the synthesis of new drug-hormone conjugates as steroidal polyamines of squalamine, a new class of naturally occurring antibiotics [11], or the steroidal esters of alkylating agents which exhibit a significantly improved antileukemic activity with reduced toxicity in relation to the alkylating agents themselves [12].

All the above mentioned rather vindicate the researchers' interest for this field, as well as the large number of methods referred in the literature for the preparation of allylic oxidized steroidal derivatives. These methods are summarized below, while the biological activity of 7-keto-Δ5-steroids is also presented.

ALLYLIC OXIDATION OF Δ5-STEROIDS TO THE CORRESPONDING 7-KETO-Δ5- ANALOGUES

Since the first reference for the synthesis of a 7-keto-Δ5-steroid cited in the literature in 1937 [13], an unfailingly increased number of reports on this field has been observed. These reports dilate the development and/or the improvement of methods for the allylic oxidation of several steroidal alkenes, resulting in a kaleidoscope of oxidizing

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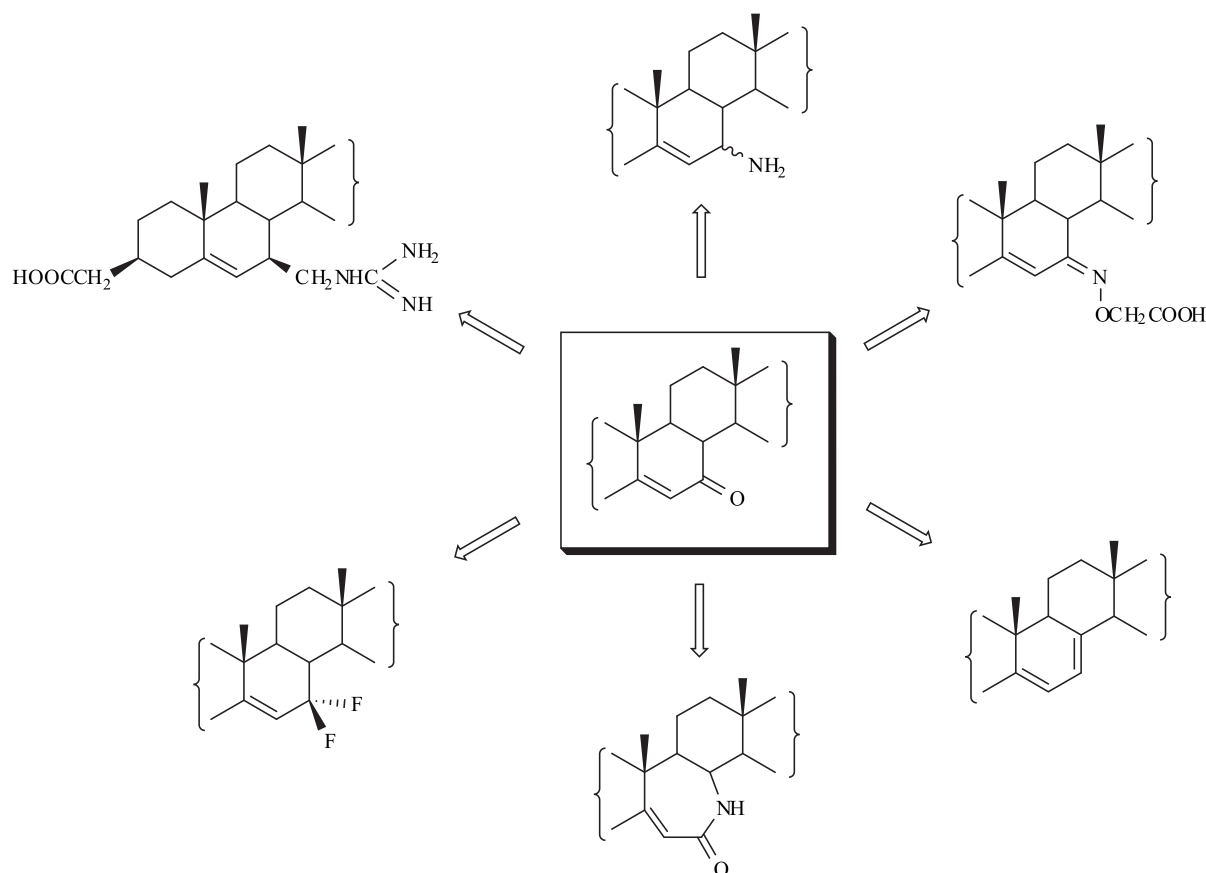

Fig. (2). Structural modifications on steroidal moiety using 7-keto-Δ^5-steroids as intermediate molecule

reagents, catalysts and experimental conditions. The initial approaches for the introduction of a keto-group at the 7-position of the Δ^5-steroids embraced more than one synthetic steps, which led to the synthesis of the desired product in poor yield and/or with simultaneous production of undesired by-products [14-16].

Chromium (VI) Oxide-Mediated Oxidation

The classical and probably more well-known variation of the allylic oxidation is the chromium (VI) oxide-mediated oxidation by the application of several chromium reagents as oxidizing factors, which were quite popular and several of

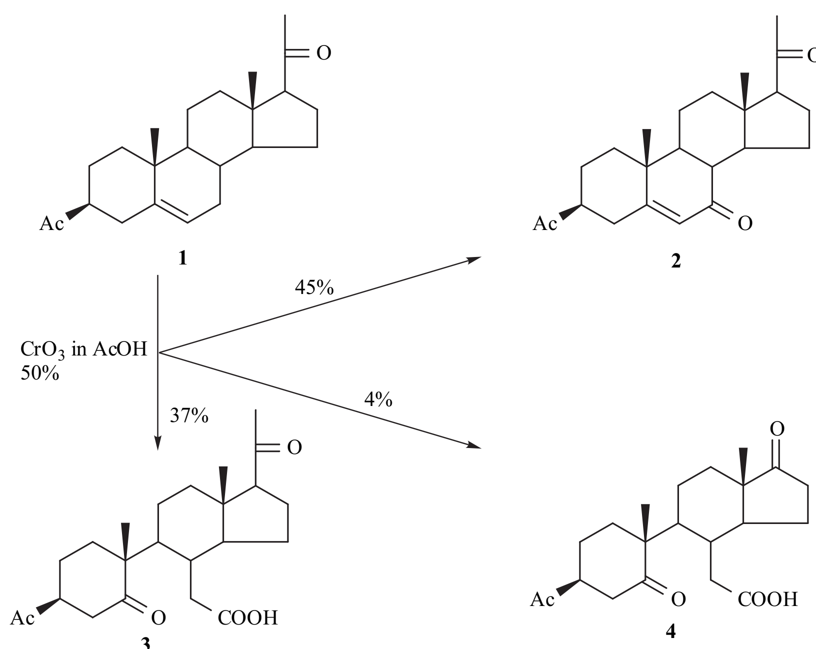

Fig. (3). Allylic oxidation of 3β-acetate-5-pregnen-20-one using CrO_3 in acetic acid.

them are in use with some success up to date. CrO_3 [17-19], or sodium chromate [20,21] and t-butyl chromate [22,23,24] in acetic acid have been used for the allylic oxidation with only limited success. For the CrO_3 method the yield of the allylic ketone was less than 50% and a significant percentage of fusion products were reported [19] (Figure (**3**)). The author suggested that such analogues containing an additional oxygen atom in the B ring are more soluble in body fluids and thus potentially more useful for the treatment of pain, anxiety and sleep disorders.

Some complexes of CrO_3, developed over recent years as oxidizing agents became very popular, as they proved more successful in performing this transformation. The chromium trioxide-pyridine complex ($CrO_3.2C_5H_5N$) was initially reported in 1948 [25], but used for the allylic oxidation of Δ^5-steroids to Δ^5-7-ketones in 1969 [26]. This and relative reports described the synthesis of 7-keto-Δ^5-steroids in yields of 52-81% [27,28]. A modification of the original method by Ratcliffe and Rodehorst resulted in an improved method [29], which was applied in several studies [10, 30-33]. Despite some disadvantages concerning that procedure [24, 26, 28, 32], researchers have applied it in order to prepare 7-keto-Δ^5-steroids as key step for the synthesis of 7α-hydroxylated oxysterols which are of considerable interest because of their possible involvement in regulation of cholesterol metabolism [33].

In 1973, Corey and Fleet [35] developed the chromium trioxide-3,5-dimethylpyrazolium complex ($DMP.CrO_3$), which was applied successfully in the allylic oxidation of Δ^5-steroids to 7-keto-analogues [11, 24, 36-38]. From the data cited in these reports, it can be estimated that the process of this method is dependent on the nature and the structure of the substrate used. The most recent studies concerned the preparation of 7-keto-cholesterol as starting material for the synthesis of heptafluoro-derivatives which were believed to block major metabolism of 7-keto-cholesterol resulting in an enhancement of its potential *in vivo* effects on serum cholesterol levels and other parameters [38], and of an androgenic derivative relevant to the body's immune system regulation [36]. Both studies referred a yield of 64% for this oxidation method.

The chromium trioxide-benzotriazole complex method [34] is more convenient, but the usefulness of this method is difficult to be appreciated as this was applied for only one steroidal substrate and in a small synthetic scale, while only one report can be located in the literature.

Pyridinium chlorochromate (PCC) is a long-stable chromium trioxide complex, which can easily be prepared [39] and has been used with pyridine, aromatic amines, pyrazolium and dimethylpyrazolium for the oxidation of allylic alcohols to aldehydes and ketones [40-42]. It is also effective for the allylic oxidation of Δ^5-steroids, although the first attempt was not successful [43]. In a later report [44], the authors described the oxidative procedure obtained with another chromium trioxide complex. It was the pyridinium dichromate (PDC), which was prepared by Corey and Schmidt in 1979 [45]. PDC has also been used in combination with *tert*-butyl hydroperoxide (TBHP), resulting in a mild method with good yield and smaller reaction-time [46]. This method was applied by other researchers too, with small modifications in the molar equivalents used for the reactants and gave sufficient yields [3]. PDC and PCC are used in allylic oxidation of steroidal alkenes until nowadays [47,48]. Pyridinium fluorochromate (PFC) is a newer reagent and it has been reported to be a selective and mild oxidizing complex for the performance of allylic oxidation [51]. It has been successfully used for the preparation of 7-keto-Δ^5-steroids, giving a 87-88% yield in product but the reaction-time of the oxidizing procedure was relatively long [52] (Figure (**4**)).

In 2001, Marwah and Lardy [49], in order to prepare a series of steroid glucuronosides, ethers and alkylcarbonates with thermogenetic activity, synthesized 7-keto-steroidal alkenes in 87% yield, using the combination of N-hydroxyphthalimide (NHPI) with PDC (Figure (**5**)). N-hydroxyphthalimide has also been used in the presence of sodium dichromate and chromium perchlorate in acetone affording allylic oxidation steroidal products in 74% yield [50].

The newest of the chromium trioxide complexes used in the allylic oxidation of Δ^5-steroids is 3,5-dimethyl-

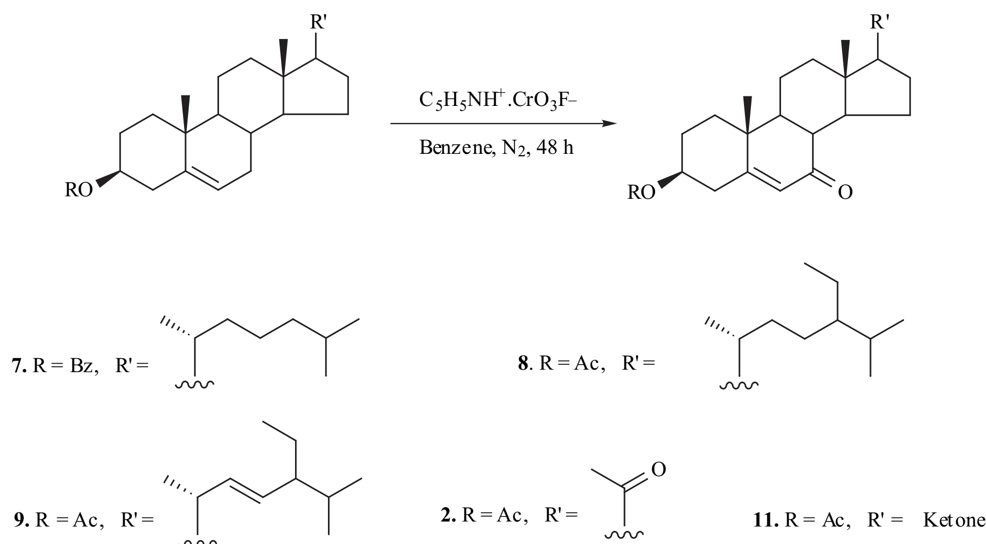

Fig. (4). Allylic oxidation of Δ^5-steroids with pyridinium fluorochromate (PFC).

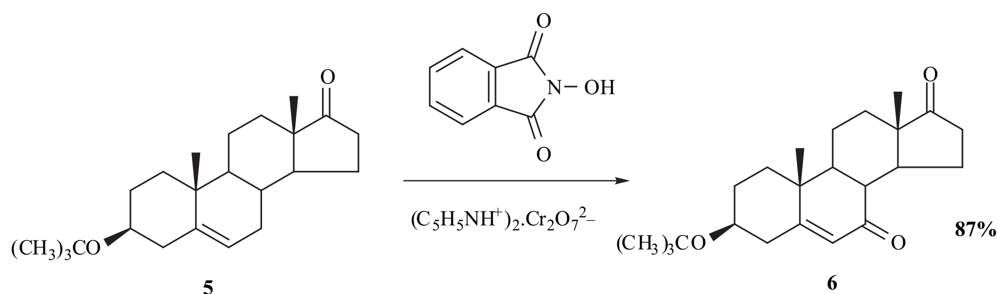

Fig. (5). Allylic oxidation of 3β-*tert*-butyl ether of DHEA using pyridinium dichromate (PDC) and N-hydroxypthalimide (NHPI).

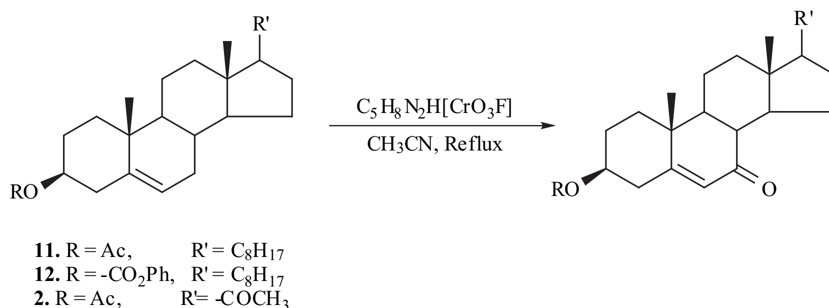

Fig. (6). Synthesis of 7-keto-Δ^5-steroids using the 3,5-dimethylpyrazolium fluorochromate (VI) method.

pyrazolium fluorochromate (VI) (DmpzHFC) (Figure (**6**)). Its synthesis was published in the literature recently and it was found to be a quite effective oxidizing agent in several substrates [53]. This reagent was described as less acidic than those mentioned above and it was developed in order to overlook the limitations resulted from their acidity and limited solubility.

Almost all of the chromium-based reagents, which have been developed so far, have some limitations that cannot be overlooked. These include the inherent problems of acidity of the reagents and their limited solubility in organic solvents, which make them unsuitable for oxidations in several occasions. Moreover, a large excess of solvents and reagents are required and in some cases the reaction conditions are stringent and the reaction times are long. The above mentioned data and the requirement for the preparation of some of these reagents before each reaction, along with a difficult and complicated work-up of the environmentally hazardous chromium residues led to the parallel development of other methods of allylic oxidation.

tert-Butyl Hydroperoxide-Mediated Oxidation

Of great synthetic interest is the use of hydroperoxides, as *tert*-butyl hydroperoxide (TBHP) with different types of catalysts to perform allylic oxidations. In 1984, the first application of the CrO_3 as catalyst gave steroidal Δ^5-7-ketones in yields fluctuant from 32% to 52% [54].

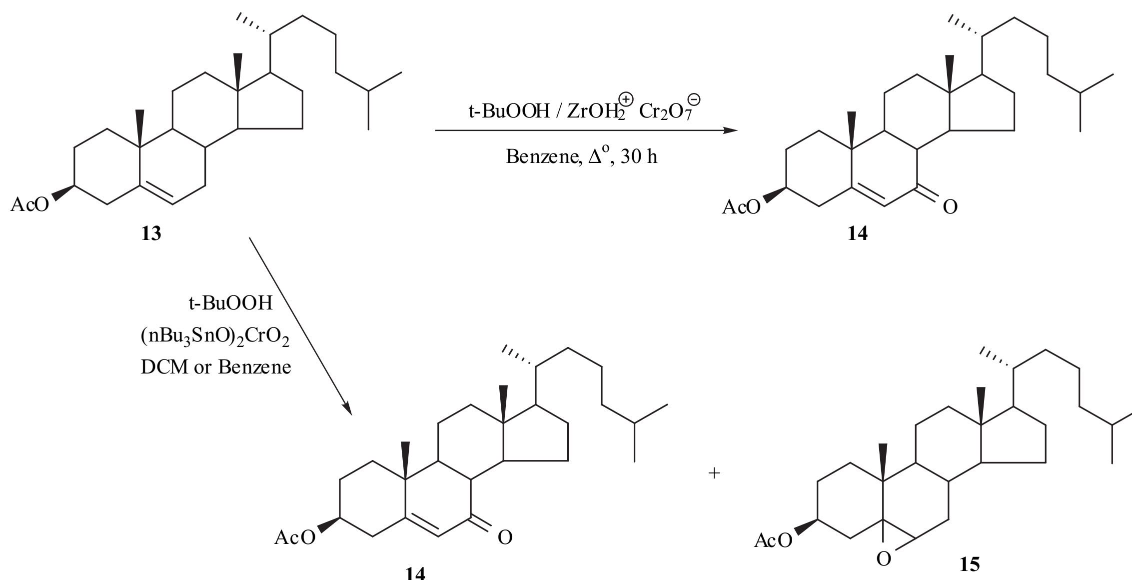

Fig. (7). Allylic oxidation of 3β-acetyl-5-cholestene with t-BuOOH and chromium (VI) absorbed on SiO_2/ZrO_2 or bis-(tributyltinoxide) dioxochromium as catalyst.

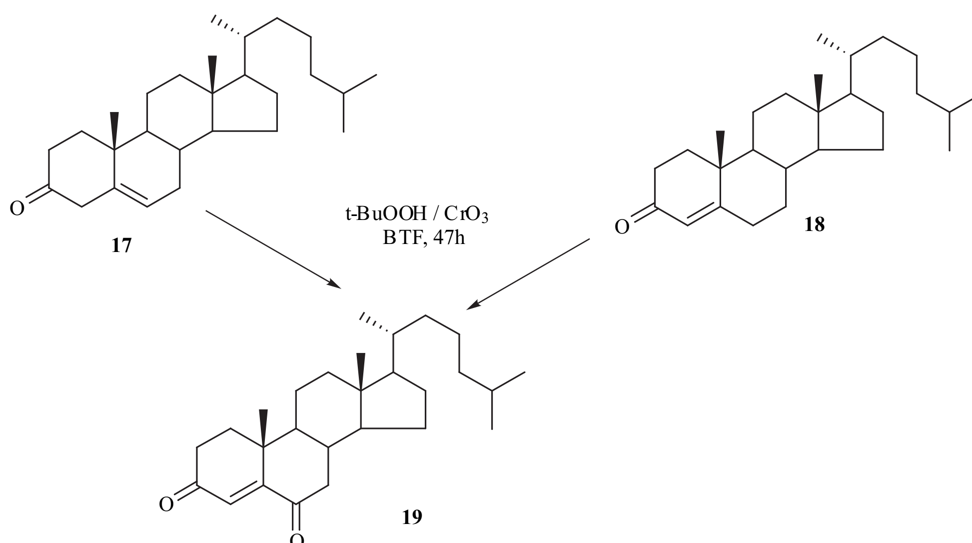

Fig. (8). Allylic oxidation of Δ^5 and Δ^4- steroid substrates with t-BuOOH and CrO_3 in benzotrifluoride.

Disadvantages such as the epoxidation of the double bond led to modifications of this method, concerning the use of chromium (VI) absorbed on SiO_2/ZrO_2, which gave similar yields without epoxidation but with a much longer reaction-time [55], and the replacement of the initial used DCM by benzotrifluoride resulting in a significant variation of the yields (40-70%) while the product isolated was surprisingly the Δ^4-6-keto-analogue [56].

Better yields in relation to the later method were obtained by the use of bis-(tributyltinoxide) dioxochromium as catalyst [57], although epoxidation of the 5,6 double bond was also occurred. High yields of allylic oxidized Δ^5-steroids are reported by performing the methods where the TBHP's oxidizing activity is catalysed by hexacarbonyl chromium [58, 59] or ruthenium trichloride [60]. No by-products were reported for these methods, they can be successfully applied on substrates carrying a free secondary hydroxyl group and moreover they are efficient in scale-up synthesis. However, the high toxicity of hexacarbonyl chromium and the high cost of the ruthenium catalyst, along with the high risk of the strongly exothermic reaction, which can be occurred from the accumulation of TBHP in the presence of ruthenium trichloride in larger-scale reactions [61], led other researchers to find new methods for this type of oxidation. One of the resulting methods is the use of TBHP with a copper catalyst, either Cu(II) and Cu(I) salts or Cu metal [62]. This method proved efficient for the allylic oxidation of Δ^5-steroids. Furthermore, the copper-catalysed oxidation by TBHP under phase transfer conditions [63,64] was applied for this type of allylic oxidation, giving impressive results (85-89 % yield in short reaction time) although this method had never been used before for the synthesis of α,β-unsaturated steroidal ketones. (recent data from our laboratory; accepted for publication to *Steroids*).

TBHP has also been used for the synthesis of 7-keto-Δ^5-steroids in the presence of a catalytic amount of $KMnO_4$ in acetic acid [65] and in the presence of sodium periodate in a cosolvent system of water and organic solvents [66] or sodium hypochloride in ethyl acetate giving the allylic oxidation products in 67-68 % yield [67]. The most recent application of TBHP concerns a procedure found to be selective for the allylic oxidation of unsaturated steroids [68,69]. Cobalt acetate and supported cobalt acetate ($Co(OAc)_2/SiO_2$) were used as catalysts affording a 84% yield of oxidized product. This method was referred to be selective for this kind of allylic oxidation, while the catalyst can be easily recovered and reused.

Oxygen or Air-Mediated Oxidation

In 1991, the more environmental friendly oxidation by molecular oxygen or air and N-hydroxyphthalimide as a catalyst [70] was reported to be suitable for the allylic oxidation of steroids as it gives good yields of products, is technically readily applicable and cheap, the catalyst can be recovered practically completely and avoids the use of metal compounds and consequently a contamination of the product by metal compounds. The application of this method by other researchers gave satisfactory results for the allylic oxidation of other steroidal substrates in larger scale-

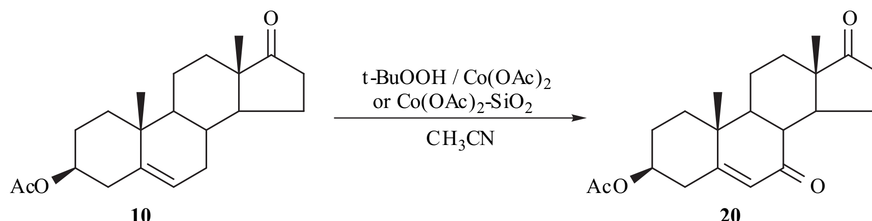

Fig. (9). Synthesis of 3β-acetyl-7-keto-DHEA using t-BuOOH in MeCN in presence of cobalt acetate or supported cobalt acetate as catalyst.

Table 1. Reagents for the Allylic Oxidation of Δ^5-Steroidal Substrates in Chronological Order

Chromium (VI) oxide in acetic acid	CrO_3-AcOH	1937[13]
Chromium (VI) oxide-pyridine complex or *Collins reagent*	$CrO_3 \cdot 2C_5H_5N$	1948[25]
Sodium chromate in acetic acid	$Na_2Cr_2O_7$ –ACOH	1950[20]
tert-Butyl chromate		1957[22]
Chromium (VI) oxide-3,5-dimethylpyrazolium complex	DMP·CrO_3	1978[24]
tert-Butyl hydroperoxide-hexacarbonyl chromium	TBHP-$Cr(CO)_6$	1984[58]
tert-Butyl hydroperoxide-chromium (VI) oxide	TBHP-CrO_3	1984[54]
Chromium (VI) oxide-benzotriazolium		1985[34]
Pyridinium chlorochromate	PCC	1986[43]
Pyridinium dichromate	PDC	1987[44]
tert-Butyl hydroperoxide-bis-(tributyltinoxide)diochromium	TBHP-$[(n.Bu_3SnO)_2CrO_2$	1989[57]
Oxygen-N-hydroxyphthalimide	O_2-NHPI	1991[70]
tert-Butyl hydroperoxide-Potassium permanganate	TBHP-$KMnO_4$	1991[65]
Pyridinium fluorochromate	PFC	1996[52]
tert-Butyl hydroperoxide-Ruthenium trichloride	TBHP-$RuCl_3$	1996[60]
tert-Butyl hydroperoxide-Copper or copper salts	TBHP-Cu	1997[62]
tert-Butyl hydroperoxide- supported chromium (VI) oxide	TBHP-$CrO_3/SiO_2/ZrO_2$	1999[55]
N-hydroxyphthalimide-Sodium dichromate or chromium perchlorate		1999[50]
tert-Butyl hydroperoxide-Sodium periodate	TBHP-Na_5IO_6	2000[66]
Pyridinium dichromate-N-hydroxyphtlalimide	PDC-NHPI	2001[49]
tert-Butyl hydroperoxide-Sodium hypochloride	TBHP-NaOCl	2001[67]
tert-Butyl hydroperoxide-Cobalt acetate or supported cobalt acetate	TBHP-$Co(OAc)_2/SiO_2$	2001[68]
3,5-dimethylpyrazolium fluorochromate complex	DmpzHFC	2001[53]

reactions [49,71]. Despite these, its application to the laboratory scale has refereed to be restricted due to the technical and safety problems connected with a reaction under an oxygen atmosphere [61].

BIOLOGICAL PROFILE OF THE MAIN 7-KETO-Δ^5-STEROIDS.

Over the recent decades a large number of studies concerning the multilevel and complicated expression of biological effects of oxidized Δ^5-steroids have been carried out. The majority of these studies focused on the biological actions of oxysterols, such as 7-keto-cholesterol, 7-keto-DHEA, ect. Of great interest appears to be their antiproliferative and/or cytotoxic properties, which render them as molecules of potent value for the cancer treatment, while these properties may provide sanative effects for other types of diseases.

7-keto-Cholesterols

7-keto-cholesterol is produced from the autoxidation of cholesterol by the air in room temperature [1], while it was found that several microorganisms in the human intestine (Clostridia, Eubacterium, E. Coli, B. Fragilis, C. Absonum and C. Sporosphaeroides) oxidize bile acids to 7-keto- and 7-hydroxy-derivatives [72]. The observation that oxidized products of cholesterol inhibit the mammalian sterol biosynthesis [2] (7-keto-cholesterol is one of the most potent inhibitors) was of great interest for further studying and application of these molecules for specific inhibition of several cellular functions.

More specifically, oxysterols inhibit the enzyme of 3-hydroxy-3-methylglutaryl-CoA reductase (HMGR) – the regulatory enzyme of the cholesterol biosynthesis - *in vitro* and *in vivo* [73,74]. This action is achieved either by the inhibition of the formation of the enzyme or by a mediated inactivation of the enzyme, although the *in vivo* activity of 7-keto-cholesterol is weaker due to their metabolism and the induction of other types of enzymes, which can cause their inactivation [75]. It has also been reported that 7-keto-cholesterol acts on HMGR and not on the pathway downstream of HMGR [76]. Other data indicated that HMGR is not the sole target enzyme of sterol biosynthesis inhibition of oxysterols, but lanosterol demethylation and/or the subsequent transformation ultimately leading to cholesterol are also affected by these compounds [77].

Moreover, oxidized sterols were reported to bind to the nuclear proteins LXR and PXR, which belong to the orphan nuclear receptors. The activation of LXRα induces the transcription of several genes as well as the increased activity of Cyp7α, which is the regulatory enzyme of the biosynthesis rate of bile acids [78, 79].

Cholesterol plays an important structural role in the lipid core of biological membranes. The high concentrations of cholesterol found in many membranes have also an influence on membrane functions such as passive transport, carrier-mediated transport and enzymatic activity of membrane-bound enzymes, which mean that cholesterol has a dynamic regulatory function in biological membranes [80]. As a result of oxysterols cytotoxicity is the structural and functional changes they cause to cellular membrane [81], which is essential for cell's viability. Oxysterols have been reported to alter membrane fluidity [82], permeability for cations [83], glucose [84] and membrane-bound kinase C activity [85]. Results of this cytotoxicity are perhaps the effect on human polymorphonuclear leukocyte chemotaxis, echinocyte formation of red blood cells and platelet aggregation in plasma. It has been suggested that cholesterol oxides act and undirectly upon cell membrane repressing LDL-receptor functions by down-regulating LDL-receptor gene expression [86]. It has been also reported that cellular membranes influence the decision of the cell to divide. Addition of 7-keto-cholesterol in a serum-free medium culture of L-cells resulted in depression of HMGR and reduction of sterol biosynthesis for hours, while afterwards a reduction of the DNA synthesis was also observed [87-89]. The growth of the culture stopped 24-36 hours after the addition of 7-keto-cholesterol and 48 hours later the cells became rounded and detached from the surface of the flask. The cells died unless an appropriate sterol was supplied in the medium. It was concluded that the inhibition of sterol biosynthesis led to the development of a reversible accumulation of cells in the G1 phase of the cell cycle [90]. The inhibition of the growth of L-cell cultures by oxysterols correlated well with their capacity to inhibit sterol biosynthesis and to depress levels of HMGR activity, affirming that the growth arrest resulted from this inhibition [91]. A causal relationship was observed between the supply of lipid and restricted passage of cells in the S phase of the cell cycle. This implied a coordination between lipid metabolism and the onset of DNA synthesis. Chen and his colleagues [92] suggested that cholesterol synthesis is a prerequisite for DNA synthesis in PHA-stimulated lymphocytes and DNA synthesis is inhibited when cholesterol synthesis is blocked in L-cells. A rationale for the existence of coupling between membrane lipid and DNA synthesis was based on the suggestion that the duplication of DNA, which is a prerequisite for cell division, must be coupled with the necessary doubling of membrane mass if a state of balanced growth is to be maintained [90]. This necessity of cholesterol biosynthesis was depicted in studies carried out on normal lymphocytes and leukemic cells [93,94], where the DNA synthesis was also abolished after the depression of HMGR by 7-keto-cholesterol. A relationship between lymphocyte proliferation and cholesterol synthesis was also indicated by the fact that leukemic cells of mice [95,96], guinea pigs [97] and man [98,99] all showed high rates of sterol biosynthesis. Moreover, in human lymphocytes, *de novo* sterol synthesis was a prerequisite for DNA synthesis, even though cells were cultured in cholesterol-medium [100]. It has also been reported that cells normally synthesize sterol needed for cell division even though cholesterol may be present in extracellular fluids (dietary cholesterol or blood levels of the sterol). Proliferating tissues and cells with high rates of sterol synthesis include intestinal, mucosa, epidermis, spontaneous-or well differentiated-transplantable tumors and developing brain. *De novo* synthesis is a requirement for division of these cells under physiological conditions, as it is in lymphocyte cultures *in vitro* [2]. All the above mentioned data support rather well the suggestion that oxysterols can inhibit cell replication and these effects on cell division might be useful in the control of abnormal rates of cell division, such as those occurring in cancer.

Moreover, the oxygenated cholesterol derivatives were widely described as lethal to highly proliferating or tumor cells, both *in vitro* and *in vivo*. Smith and Johnson described 28 oxysterols as toxic [101]. The anticancer activity of 7-keto-cholesterol has been reported over the recent years by other authors too [102]. Several studies concerning the anticancer activity of oxysterols against some types of brain cancer have been carried out and the 7-keto-cholesterol was not found to be cytotoxic for normal astrocyte cells [76], but it is active against reactive astrocyte (with increased cAMP levels), C6 glioma cell lines and neuroblastoma cells [103-107]. Local administration of 7-keto-cholesterol-3-oleate in rat brain C6 glioblastoma cells decreased the tumor volume by 50%, as well as it affected the extent of reactive gliosis. Exposure of human neuroblastoma cells to 7-keto-cholesterol caused 50% of neuronal cells to die within 30 hours. It was considered to be potentially useful for chemotherapy since it would expect to cross the blood-brain and/or brain-hematotumoral barriers. 7-keto-cholesterol's cytotoxicity in central nervous system tumors has been attributed to the alteration of catalytic efficiency of HMGR, to effects of oxysterols on the growth and activity of MAP kinase, as well as to the provocation of necrotic processes. It has been also reported that 7-keto-cholesterol inhibited endogenous cholesterol biosynthesis in injured brain by 32% causing inhibition of astrogliosis and intracranial glioblastoma growth [108].

Several studies have shown that 7-keto-cholesterol increases the induction of apoptosis in a series of cell lines as smooth muscle cells [109,110], endothelial cells[111-114], monocytic cells [115,116] and human promonocytic leukemia cells [117]. The precise mechanism of apoptosis is unclear. Interesting reports made by several authors suggest that:

a. These molecules caused a progressive disruption of actin microfilaments and loss of vinculin, preceding cell detachment and cell death by apoptosis as revealed by the subsequent leakage of cytosolic enzymes and nuclear fragmentation [112].

b. Oxysterols-induced apoptosis involves some death-regulating genes (*ced*), which encode either for proteins, which promote or protect cells from death. These molecules led to a dramatic decrease of bcl-2 protein (homolog of *ced*-9 gene product, which protects cell from death in multiple contexts)

[110,117] and activated CPP32 protease (a caspase homolog of *ced*-3 gene product, which promotes apoptosis by cleaving and inactivating the poly-(ADP ribose) polymerase) [117].

c. 7-keto-cholesterol induced nuclear condensation and/or fragmentation and internucleosomal DNA fragmentation (which is a typical feature of apoptosis) [112,117], induced apoptosis in a dose-depended manner [109,114] and this apoptosis was not inhibited by exogenous cholesterol [110] or by PKC activation [109].

Cytotoxicity and apoptosis are also induced by oxidized low-density lipoproteins (ox-LDL), whose major component is 7-keto-cholesterol [109, 118-124]. They led cells to apoptosis causing uncontrolled calcium rise, increased DNA fragmentation, and alterations in cytoskeletal network.

Besides the above mentioned, oxysterols possess angiotoxic properties [77] and they are suspected to be atherogenic [117,125], although they have been suggested to be useful chemotherapeutic agents in individuals predisposed to atherosclerosis with homozygous and even heterozygous familiar hypercholesterolemia [126]. When 7-keto-cholesterol was fed to mice with the diet, growth was suppressed and mature mice lost weight [127]. 7-keto-cholesterol had also immunosuppressive activity [128], while natural antibodies of it have been found in human serum [129].

7-keto-Dehydroepiandrosterone (7-keto-DHEA) Derivatives

It was known since 1962 that dehydroepiandrosterone (DHEA) is converted *in vitro* to 7-hydroxy and 7-keto-DHEA [130,131]. Some of DHEA's biological effects ascribed to those metabolites [132]. These were the anti-obesity effects [133,134], the decreasing of blood cholesterol concentrations in several species [135], the normalization of blood sugar in diabetic mice [136-138], the enhancement of immune system [139], the suppression of tumor development [140], as well as the improvement of the memory in aged mice [141]. 7-keto-DHEA is not convertible to compounds with androgenic or estrogenic activity and they are therefore potentially useful medications for women whereas DHEA is not, because it causes masculinization [132]. It had no toxicity to rats and monkeys and was well tolerated by men who received it up to 200 mg /day per 4 weeks [142].

A significant number of studies about 7-keto-DHEA and other oxidized derivatives of DHEA concern their ability to inhibit the enzyme of aromatase. Aromatase is enzyme complex, which catalyzes the conversion of C19 steroids to estrogens and is expressed in a variety of normal tissues, as well as in endometrial and breast cancer tissues [143]. As the role of estrogens, in the development and promotion of hormone-depended breast tumors is already accepted [144], inhibitors of aromatase could be potential treating agents against hormone-depended tumors. It has been found that C19 androstanes of Δ^5-7-keto structure do inhibit aromatase competitively, even though there is no oxygen atom at the C3 of the molecule. Among them, only 7-keto-5-en-steroids inactivate the enzyme in a mechanism-based (suicide) manner [145]. It has also been reported that 7-keto-group is not essential for strong binding of the steroids to the active site of the enzyme but is essential for the irreversible inactivation of aromatase [146]. Also, the lipophilicity of C7 substituent is an important factor for the inhibitory activity against placental aromatase [147].

7-keto-DHEA has also been found to be more efficient than DHEA as an inducer of liver mitochondrial sn-glycerol-3-phosphate dehydrogenase and cytosolic malic enzyme and so is an enhancer of thermogenesis [148]. That observation led other researchers to studies, which indicated that 7-keto-DHEA indeed confers to the anti-obesity action of its parental molecule. 7-keto-DHEA combined with moderate exercise and a reduced-calorie diet significantly reduced body weight and body fat. In addition it significantly elevated T_3 levels, but did not affect TSH or T_4 levels indicating that it does not adversely affect thyroid function in the short term [149]. It has also been suggested as dietary supplement for promoting healthy hormonal balance in adult subjects and especially in elderly subjects, as it stimulated the human growth hormone by pituitary [150].

The suggestion of 7-keto-DHEA's contribution to the enhancement of cellular immunity by DHEA led to some interesting results. This oxidized metabolite has been suggested to have prophylactic, modulatory and ameliorative activity against HIV-wasting syndrome [151], against inflammatory Bowel disease [152], and in the treatment of lupus erythematosus [153] and of arthritis [154]. It is also helpful in treating primakes infected with Simian Immunodeficiency Virus (SIV), as it improved their physical state, weight, behavior and clinical condition [155]. Of great interest is the suggestion of an effective treatment of Alzheimer's disease and other immune deficiency disorders by the 7-keto-DHEA-modulation of immune system [156]. The later suggestion may be also relevant to the results of some biological and electrophysiological experiments indicating that DHEA is a potent allosteric inhibitor of γ-aminobutyric acid subtype A ($GABA_A$) receptors [157, 158]. The antagonizing activity of 7-keto-DHEA-acetate for the $GABA_A$ receptor was the base of a study resulted in that this molecule could overcome the cholinergic dysfunction produced by scopolamine, which induces amnesia in young mice. It was also reported to have a potential beneficial effect for memory retention in old mice [159].

Besides the above reported, 7-keto-androstenic derivatives have been suggested for moisturization of human skin [160], for treating skin ageing symptoms alone [161], or in combination with a NO-synthase inhibitor [162], a carotenoid [163], an anti-glycation agent [164], a depigmentation agent [165] and a vitamin and/or an enzymatic cofactor [166]. 7-keto-DHEA has also been suggested for the treating of disorders related to excessive 5α-reductase activity as acne, seborrhea, hirsutism and androgenic alopecia [167], while in combination with an antimicrobial agent it can be used against skin disorders such as greasy skin with acne, acne scalp dandruff and bad odors [168].

From the data reported in this mini-review, it is obvious that the allylic oxidation of Δ^5-steroids constitutes a significant chapter of steroids chemistry. A large number of oxidizing methods and reagents have been developed for the

synthesis of 7-keto-Δ^5-steroids, and a vast amount of research has occurred to the study of their biological activities. Potential therapeutic targets for these molecules are cancer, immune deficiency disorders, diabetes, Alzheimer's disease. The continuous development and study of oxidized Δ^5-steroids will greatly assist in determining their utility and future, so for the synthesis of new steroidal derivatives as for the treatment of several pathological disorders.

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ABBREVIATIONS

DCM	=	Dichloromethane
DHEA	=	Dehydroepiandrosterone
DMP	=	3,5-Dimethylpyrazole
DmpzHFC	=	3,5-Dimethylpyrazolium fluorochromate
DMSO	=	Dimethylsulfoxide
$GABA_A$	=	γ-aminobutyric acid subtype A receptor
HMGR	=	3-hydroxy-3-methylglutaryl-CoA reductase
7-keto-DHEA	=	7-Keto-dehydroepiandrosterone
NHPI	=	N-Hydroxyphthalimide
ox-LDL	=	Oxidized low-density lipoproteins
PCC	=	Pyridinium chlorochromate
PDC	=	Pyridinium dichromate
PFC	=	Pyridinium fluorochromate
SIV	=	Simian immunodeficiency virus
TBHP	=	*t*-Butyl hydroperoxide

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