SYNTHESIS AND PHARMACOLOGICAL EFFECTS OF BUTADIENE AND CYCLOPENTADIENE ADDUCTS OF METHANDROSTENOLONE IN RATS

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ABSTRACT

In this study the reactivity of methandrostenolone or [(17β)-17-hydroxy-17-methylandrosta-1, 4-diene-3-one], as a dienophil in a Diels-Alder type cycloaddition reaction was investigated. The purpose of this approach was to investigate whether the 1-dehydro position of methandrostenolone **1** undergoes a cycloaddition reaction with dienes, such as 1, 3 butadiene or cyclopentadiene, and to investigate the biological behavior of the reaction adducts, i.e, compound **3** {(17β)-17-hydroxy-17-methyl androsta [1α, 2α] cyclohex 3', 4-diene-3-one} and compound **4** {(17β)-17-hydroxy-17-methyl androsta [1α, 2α] cyclohex (2',5' methylene) 3', 4-diene-3-one}, relative to compound **1**. The results indicated that the Diels-Alder reaction did not proceed under the usual circumstances of high pressure and temperature, but could proceed in the presence of a Lewis acid ($AlCl_3$). The structures of compounds **3** and **4** were confirmed by spectroscopic methods. The androgenic behavior of compounds **3** and **4** in comparison to compound **1** in the apomorphine test indicated that both compounds were almost devoid of androgenic activity, but prevented apomorphine mediated penile erection in male rats in a similar manner as cyproterone acetate.

Keywords: Methandrostenolone; 1,3 butadiene; Cyclopentadiene; Diels-Alder adducts; Anti-androgen

INTRODUCTION

For a long period of time the steroidal hormones including androgens, estrogens, and glucocorticoids have found application in medicine both in original and modified forms. The main purpose behind the modifications of the structures of these hormones has been to improve their pharmacodynamic and pharmacokinetic properties. The efforts have been fruitful in all fields, including androgens (1) and Cyproterone acetate (2), Danazol (3), and Deflazacort (4) which are the marketed steroidal medicines with a pentacyclic steroid (PCS) structure. The PCS is obtained by fusion of a carbocyclic ring such as benzene, cyclohexane, or cyclopentane to the steroid nucleus or PCS, or derived by the fusion of a carbocyclic ring to a heterosteroid, exhibit interesting and diverse biological properties (5-8). Moreover, the synthesis of a series of PCS by the fusion of an ethano bridge to positions 4 and 6 of estrone and their anti-fertility properties has been reported (9-11).

Anti-androgens are of interest for the treatment of androgen-dependent diseases such as prostate cancer, acne, seborrhea, and hirsutism. Cyproterone acetate, a PCS, which is derived from hydroxyl-progesterone, is widely used in the diagnosis and treatment of acne and hirsutism in women, has also a progestational activity (12).

The Diels-Alder reaction is the most important cycloaddition reaction from the point of view of synthesis (13), and this reaction has been successfully applied to some cycloalkenones (14, 15). Recently, the synthesis of the butadiene adducts of dexamethasone and prednisolone which are 1-dehyrosteroids have been reported (16). Therefore, it appeared of interest to evaluate the reactivity of another 1-dehydro drug such as **1** as a dienophile in a Diels-Alder reaction and to investigate the androgenic behavior of the new PCS. Fortunately, the carbonyl group on the carbon 3 of compound **1** facilitates this reaction (13). Since the effective receptor binding groups of compound **1**, such as the carbonyl group of carbon 3 and the 17β-hydroxyl group, were left intact in this application, retention of androgen receptor (AR) binding abilities for the new PCS compounds were expected. In this study,

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compounds **3** and **4** were prepared and their potencies were investigated using cyproterone acetate (compound **2**) as a reference anti-androgen in the apomorphine (Apo) test to verify their penile erection (PE) behavior in male rats.

MATERIALS AND METHODS

^{1}H-NMR spectra were run on a Brucker FT-80 spectrometer. Tetramethylsilane was used as an internal standard. Mass spectra were measured with a Finnigan TSQ-70 spectrometer at 70 eV. Butadiene (BD) gas was obtained from a 25 kg cylinder supplied by Arak petrochemical company, Arak, Iran. Cyclopentadiene (CPD) monomer was prepared by distillation of the CPD dimer purchased from Merck Co. Methandrostenolone, cyproterone acetate, and apomorphine were purchased from Sigma Chemical Co.

*Preparation of (17β)-17-hydroxy-17-methyl androsta [1α, 2α] cyclohex 3', 4-diene-3-one **3**.*

To a solution of compound **1** (100 mg, 0.332 mmole) in anhydrous toluene (20 ml) was added a suspension of $AlCl_3$ (50 mg, 0.375 mmole) in anhydrous toluene (10 ml) which was prepared in another flask by stirring with a magnetic bar. Under a stream of N2 gas and at room temperature the mixture was first saturated with BD gas for 2 min and then for 1 min every 30 min during 6 hrs. Then, the mixture was washed with water and dried under vacuum at 30 ^{o} C. The residue was purified by preparative TLC using n-hexane/acetone (7:3) on silica gel HF 60; to give 37.5 mg of compound **3** (32.0 % , mp 159-160 ^{o}C, R_f =0.56).

MS: m/z (rel. int. %): 353 (M^+, 3), 301 (**1**, 9), 282.5 (**1**-H_2O, 15), 212 (100), 54 (BD, 50).

^{1}HNMR ($CDCl_3$) δ: 0.94 (s, 3H of C18), 1.2 (s, 3H of C19), 4.2 (m, 1H of C4'), 4.25 (m, 1H of C3'), 5.72(s, 1H of C4).

*Preparation of (17β)-17-hydroxy-17-methyl androsta [1α, 2α] cyclohex (2', 5' methylene) 3', 4-diene 3-one **4***

From the reaction of compound **1** (100 mg, 0.332 mmole) and CPD (100 mg, 1.47 mmole) under the same experimental conditions described for the synthesis and purification of compound **3** was obtained 31.0 mg of compound **4** (25.3%, mp 157-159°C, R_f = 0.52).

MS: m/z (rel. int. %): 368 (M^+, 6), 313 (3), 279 (6), 236 (18) 149 (100), 121 (32), 66 (CPD, 58).

^{1}HNMR ($CDCl_3$) δ: 0.9 (s, 3H of C18), 1.25 (s, 3H of C19), 4.15 (m, 1H of C3'), 4.25 (m, 1H of C4'), 6.05 (s, 1H of C4).

The compounds **3** and **4** were kept refrigerated in vials under N_2 gas, and their stability were checked by TLC before biological evaluation.

Pharmacology

Animals

Male albino rats (150-250 g) were used in all studies. They were housed in groups of 5 in PVC cages for 1 week at ambient temperature (22-24°C) and with 12 h light and dark cycles and free access to food and water. Six rats were used for each experiment.

Tested compounds

Solutions of compounds **1**, **2**, **3**, and **4** were prepared by dissolving 10 mg of the compounds in 1 ml of 30% ethyl alcohol-water and solution of apomorphine were prepared in de-ionized water. The solutions of tested compounds intraperitoneally and solution of apomorphine were administered subcutaneously.

Apomorphine test.

Various doses of Apo (0.05, 0.1, 0.2 or 0.4 mg kg^{-1}) were injected subcutaneously to male rats. Control rats received normal saline only (17). To evaluate the anti-androgenic effects of compounds **2**, **3**, and **4**, they were injected in different doses intra-peritoneally 1 hr before administration of apo-morphine (0.2 mg kg^{-1}). The number of penile erections in male rats were counted every 5 minutes for 1 hr and registered. The results are shown in Table 1.

Statistical analysis

The results of the experiments were analyzed using analysis of variance (ANOVA) and Newman-Keuls test. $P<0.05$ was considered significant.

RESULTS AND DISCUSSION

Prepatation of PCS compounds **3** and **4** by the Diels-Alder reaction of dehydrosteroid **1** with CPD or BD under various experimental conditions such as high temperature and pressure or the use of organic or aqueous media as solvent were not successful. Although it was expected that compounds **3** and **4** could be prepared by this method based on the hydropholic nature of reactants (18-20). However adducts **3** and **4** could be prepared by carrying the reaction in the presence of $AlCl_3$ as a Lewis acid (21-23).

In each reaction two compounds were formed which were separated by preparative TLC and characterized by spectroscopic methods.

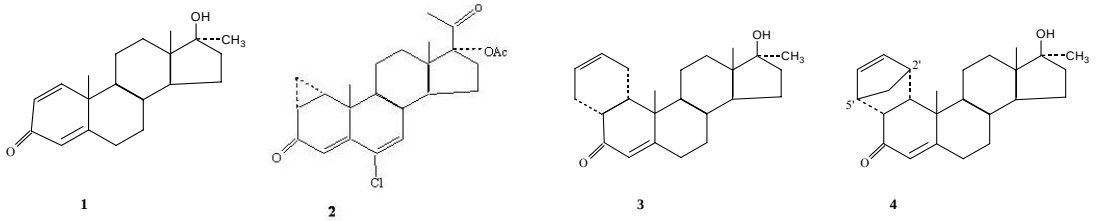

Figure 1. The chemical structures of methandrostonolone (**1**), cyproterone acetate (**2**), butadiene adduct to **1** (**3**) and cyclopentadiene adduct to **1** (**4**).

Table 1. The male rat PE numbers treated with different concentrations of compounds **1-4** (n=6) followed by subcutaneous injection of apomorphine (2 mg kg^{-1}) which were measured during 60 min.

Treatment	Dose (mg kg^{-1})	No. of PE/60 min (mean ± S.E.M.)	PE inhibition (%)
Control[a]	25 µl	3.2 ± 0.06	---
1[b]	2.0	3.3 ± 0.07	-3.0
	4.0	4.2 ± 0.06**	-33.3
2[c]	2.0	1.2 ± 0.01**	62.5
	4.0	0.18 ± 0.002**	94.4
3[c]	0.5	1.0 ± 0.02**	68.7
	2.0	0.2 ± 0.002**	93.7
4[c]	0.1	1.1 ± 0.01**	77.0
	0.5	0.17 ± 0.002**	94.9
	1.0	0.16 ± 0.002**	95.0

*P< 0.05 , **P< 0.01

[a] Control male rats received vehicle (30% hydroalcoholic solution, i.p.)

[b] Injection of **1** (2 mg kg^{-1}) did not induced significant erection, but injection of higher dose (4 mg kg^{-1}) induced significant erections.

[c] Treatment of the rats with different doses of **2**, **3**, or **4** induced significant inhibition of PE induced by Apo.

Only one of these two compounds gave sufficient evidences for the product of Diels-Alder type cycloaddition reaction (Fig. 1).

While the presence of ion peak 54 of butadiene with intensity higher than 50% in the mass spectrum of compound with R_f = 0.56 confirmed the formation of compound **3**, the intensity of this ion peak in the mass spectrum of compound **1** was low and did not exceed 5%.

The ^{1}HNMR of **3** and **4** also confirmed formation of compounds **3** and **4**. The transfer of vinyl protons from the conjugated positions with C3 carbonyl in C1 (δ: C1=6.28, C2=6.15) to non-conjugated positions in the newly formed rings of compounds **3** and **4** led to higher field resonance for C3' and C4' protons (δ: C3'=4.25, C4'=4.2 & C3'=4.25, C4'=4.15 for compounds **3** and **4** respectively). The suggested stereochemistry of **3** and **4** as depicted in Fig 1 is based on a previous study (24), in which, it was found that the addition of 1, 3-butadiene and maleic anhydride to compound 1 led to the formation of a product arising from the endo transition state (1α, 2α) in 85% yield. The steric hindrance of the methyl group 19 (10β-methyl) supports this stereochemistry.

The pharmacological activities of compounds **3** and **4** in comparison with compounds **1** and **2** were determined in terms of their ability to modify the PE behavior exerted by Apo in rats (17) and the results are shown in Table 1. Both compounds **3** and **4** significantly lowered the PE numbers which were measured over 60 min results were comparable to the standard anti-androgen **2**, but with greater potencies. Compounds **2** (4 mg kg^{-1}), **3** (2 mg kg^{-1}) and **4** (1 mg kg^{-1}) exerted almost the same degree of PE inhibition, i.e, 94.4, 93.7 and 95.0%, respectively, and in comparison with compound **2** the compounds **3** and **4** showed 2 and 4 times higher potencies respectively.

From the results of this investigation it may be concluded that introduction of new rings in the structure of dehyrosteroid **1** reduces its androgenic activity and results in formation of an antagonists.

ACKNOWLEDGEMENT

This study was supported by a grant from the Research Council of Tehran University of Medical Sciences and was presented at the 18th International Symposium on Medicinal Chemistry, Copenhagen, Denmark and Malmo, Sweden, 15-19 August 2004.

REFERENCES

1. Wilson JD. Androgens. In: Hardman, JG, Gilman, AG and Limbird, LE, editors. Goodman & Gilman's The Pharmacological Basis of Therapeutics 9th Ed., New York: McGraw-Hill; 1996. p. 1441-1458.
2. Wiechert R, Neuman F. 1, 2α-Methylene–6-dehydeo-17α-hydroxyprogesterone. Ger patent 1965; 1,189,991. Chem Abst 63: p. 1842e.
3. Manson AJ, Stonner FW, Neuman HC, Christiansen RG, Clarke RL, Ackerman JH, Page DF, Dean JW, Philips DK, Potts GO, Arnold A, Beyler AL, Clinton RO. Steroidal Heterocycles.VII Androstano [2, 3-d] isoxazoles and related compounds. J Med Chem 1963; 6: 1-9.
4. Nathansohn G, Winters G, Testa E. Steroids possessing nitrogen atoms. III. Synthesis of new highly active corticoids. [17α, 16α-d] Oxazolino Steroids. J Med Chem 1967; 10: 799-802.
5. Briziarelli G, Castelli PP, Vitali R, Gardi, B. 9α- Fluoro- 11β-hydroxy benzo[d,e] testosterone 17-acetate. Modified steroid highly active on mammary tumors in rats. Experientia 1973; 29: 618-622.
6. Manhas MS, Brown JW, Pandit UK, Houndewind P. Functionalized Enamines-XXII. Annelation of polycyclic α, β-unsaturated ketones; A facile partial synthesis of furo-indolo-and benzsteroids. Total synthesis of 3-oxa-A-norsteroids. Tetrahedron 1975;31: 1325-1330.
7. Redeuil G, Viel C, Leory F, Hospital M. Investigation on the heterosteroids V. Synthesis and configuration of methyl ethers of the benzo-15, 16-and cyclohexano-15, 16-methoxy-2 diazo–8, 13 estrone. J Heterocyclic Chem 1976; 13: 399-403.
8. Coustal S, Fagart J, Davioud E, Marquet A. Synthesis of potential cytochrome P450 11β generated intermediates. Tetrahedron 1995; 51: 3559-3570.
9. Ghosh AC, Hazra BG, Karup-Nelson I. Pentacyclic Steroids 5. Total synthesis of 4, 6β-ethano-3-methoxy-8α-estra-1, 3, 5(10)-trien-17β-ol and 4, 6α-ethano-3-methoxy-8α-estra-1, 3, 5(10), 8, 14-pentaen-17-one. J Org Chem 1979; 44: 683-688.
10. Bull JR, Grundler C, Laurent H, Bohlman R, Mueller- Fahrnow A. Cycloaddition mediated synthesis and rearrangement of 16-functionalized 14α, 17α-ethano-19-norsteroids. Tetrahedron 1994; 50: 6347-6362.
11. Bull JR, Mountford PG, Kirsch G, Neef G, Mueller-Fahrnow A, Wiechert R. Cycloaddition–fragmentation route to 14β-allyl estrone and the derived 14α, 17α-ethano analog of estriol. Ibid 1994; 50: 6363-6376.
12. Zeelen FJ. The Androgens. In: Timmerman H, ed. Medicinal Chemistry of Steroids. The Netherlands: Elsevier; 1990. p. 177-194.
13. Carey FA, Sundberg RJ. Concerted Reactions. 11.3. Cycloaddition Reactions. In: Carey FA and Sundberg RJ, ed. Advanced Organic Chemistry: Structure and mechanisms. New York: Plenum Press; 1990. p. 625-642.
14. Fringuelli F, Pizzo F, Taticchi A, Halls TDJ, Wenkert E. Diels-Alder reactions of cycloalkenones. 1. Preparation and structure of adducts. J Org Chem 1982; 47: 5056-5065.
15. Fringuelli F, Pizzo F, Taticchi A, Wenkert E. Diels-Alder reactions of cycloalkenones. 3. Effects of specific reaction parameters. Ibid 1983; 48: 2802-2808.
16. Sharifzadeh M, Ramazani F, Shamsa F. Anti-inflammatory effects of butadiene Diels-Alder adducts to some 1-dehydroglucocorticoids in rats. Eur J Pharm Sci 2004; 21: 575-579.
17. Sharifzadeh M, Zarrindast R, Samini M. Effects of adenosine analogues on apomorphine-induced penile erection in rats. Gen Pharmacol: The vascular system 1995; 26: 1785-1790.
18. Rideout DC, Breslow R. Hydrophobic acceleration of Diels-Alder reactions. J Am Chem Soc 1980; 102: 7816-7817.
19. Palar H, Bauman R. Diels-Alder reaction of cyclopentadiene with acrylic acid derivatives in heterogenous phases. Angew Chem Int Ed Engl 1981; 20: 1014-1016.
20. Lubineau A, Auge' J, Queneau Y. Water promoted reactions. Synthesis 1994; 8: 741-760.
21. Inukai T, Kasai M. Diels Alder reaction of acrylic acid derivatives catalyzed by aluminium chloride. J Am Chem Soc 1965; 87: 317-321.
22. Houk K, Strozier RW. On Lewis acid catalysis of Diels-Alder reaction. J Am Chem Soc 1973; 95: 4094-4095.
23. Laszlo P, Lucchetti J. Easy formation of Diels-Alder cycloadducts between furans and α, β-unsaturated aldehydes and ketones at normal pressure. Tetrahedron Lett 1984; 25: 4387-4388.
24. Stephenson LM, Smith DE, Current SP. Endo preferences in the Diels-Alder cycloaddition of butadiene and maleic anhydride. J Org Chem 1982; 47: 4170-4171.