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(Journal of the Korean Chemical Society)
Vol. 19, No. 3, 1975
Printed in Republic of Korea

혈압강하제인 4-(β-Guanidinoethyl)-17α-methyl-4-aza-5α-androstan-17β-ol 의 합성 및 평가

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(1975. 2. 5 접수)

The Synthesis and Evaluation of Antihypertensive 4-(β-Guanidinoethyl)-17α-methyl-4-aza-5α-androstan-17β-ol

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(Received Feb. 5, 1975)

요 약. 4-(β-Guanidinoethyl)-17α-methyl-4-aza-5α-androstan-17β-ol(V)을 합성하는데에 필요한 중간화합물인 17α-methyl-4-aza-5α-androstan-17β-ol(IX)은 4 단계을 거쳐 합성하였으며 IX을 chloroacetonitrile과 축합반응을 한후 $LiAlH_4$로서 환원하여 4-(β-aminoethyl)-17α-methyl-4-aza-5α-androstan-17β-ol(XI)을 얻었다. Title compound인 V은 3가지의 시약, 2-methyl-2-thiopseudourea, 3,5-dimethylpyrazole-1-carboxamidine, cyanamide를 각각 XI와 반응시켜 좋은 수득률을 얻었다. 약리작용의 실험결과 V은 classical한 adrenergic neurone blocking agents와 유사하며 혈압강하제로서의 전망은 좋다고 예상된다.

ABSTRACT. The intermediate, 17α-methyl-4-aza-5α-androstan-17β-ol(IX) required for the synthesis of 4-(β-guanidinoethyl)-17α-methyl-4-aza-5α-androstan-17β-ol(V) was obtained through a reaction of 17α-methyl-3,5,-seco-4-norandrostan-17β-ol-5-on-3-oic acid(VI) with ammonium hydroxide followed by two reductions(platinum dioxide with hydrogen and lithium aluminium hydride). Condensation of IX with chloroacetonitrile under anhydrous condition, followed by reduction of the nitrile with lithium aluminium hydride gave 4-(β-aminoethyl)-17α-methyl-4-aza-5α-androstan-17β-ol(XI). The reaction of XI with 2-methyl-2-thiopseudourea or 3,5-dimethylpyrazole-1-carboxamidine, or cyanamide provided the title compound, V. Relaxation of the nictitating membrane, in the absence of mydriasis, is considered to be evidence of adrenergic neurone blockade. Thus the test compound(V) resembles that of the classical adrenergic neurone blocking agents.

INTRODUCTION

Steroids possessing nitrogen in nucleus(azasteroids) have been investigated for a wide variety

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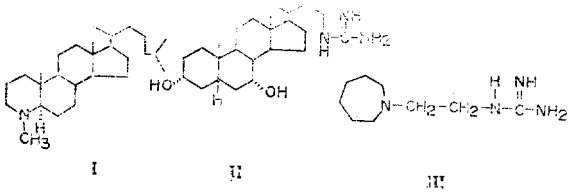
of medicinal properties such as coronary dilatory[1], herbicidal[2], hypotensive[3], antimicrobial(I)[4], and other properties[5]. There are a few reports on the preparation of synthetic guanidino-steroids for the biological activities. For example, 23-guanidino-3α, 7α, 12α-trihydroxy-norcholane(II) was found to inhibit the growth of gram-positive organisms.[6] The discovery of guanethidine(III)[7] has generated considerable interests due to its antihypertensive effects by blockade of the adrenergic neurone. Subsequent structure-activity studies have demonstrated that the azocine ring of guanethidine can be altered without loss of antihypertensive activity[8].



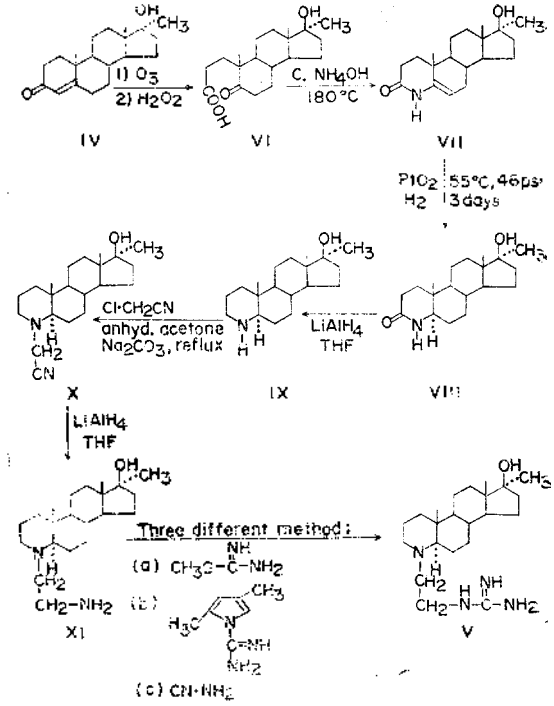
I II III

It is the objective of this investigation to modify the heterocylic ring of guanethidine for better lipophilic steroid moiety, to synthesize azasteroide through the introduction of β-guanidinoethyl function to an azasteroid, namely 17α-methyl-4-aza-5α-androstan-17β-ol, and to evaluate the resulting antihypertensive activity. Guanidine derivative of azasteroids has not been previously reported for the antihypertensive activity.

CHEMISTRY

Synthetic approach to this title compound consisted of preparation of the 4-aza-steroid followed by attachment of the β-guanidinoethyl group to the nitrogen atom. 17α-Methyltestosterone(IV) was used as a starting material for the synhtesis of 4-(β-guanidinoethyl)-17α-methyl-4-aza-5α-androstan-17β-ol(V) (*Scheme* I). 17α-Methyl-3, 5-seco-4-norandrostan-17β-ol-5-on-3-oic acid (VI)[9] was prepared by ozonolysis of 17α-methyltestosterone(IV), followed by oxidative decomposition of the ozonide with aqueous hydrogen

Scheme I.



peroxide. 17α-Methyl-4-aza-5-androstan-17β-ol-3-one(VII) was obtained by heating the keto acid, VI[10] with concentrated ammonium hydroxide in a sealed reaction vessel. An almost quantitative yield was obtained. Wildi[11] described synthesis of the similar kind of lactam.

Catalytic hydrogenation of the 5, 6-double bond of VII was accomplished with platinum dioxide in glacial acetic acid.[12] The course of the reaction was monitored by the disappearance of U. V. absorption at 233 nm, and the reduction product, 17α-methyl-4-aza-5α-androstan-17β-ol-3-one (VIII) showed disappearance of a weak shoulder at 840 cm^{-1} which is characteristic of the carbon-carbon double bond in this lactam. 17α-Methyl-4-aza-5α-androstan-17β-ol (IX) obtained from lithium aluminium hydride reduction of VIII, was condensed with chloroacetonitrile in anhydrous acetone to obtain 4-(β-cyanoethyl)-17α-methyl-4-aza-5α-androstan-17β-ol(X). The condensation

failed to occur when such solvents as anhydrous benzene, DMF and DMSO were used. The nitrile function of X was reduced with lithium aluminium hydride in THF at reflux, and the yellow oily 4-(β-aminoethyl)-17α-methyl-4-aza- 5α-androstan-17β-ol(XI) was obtained. The presence of the free amino group was demonstrated by the appearance of N—H stretching at 3090 and 3220cm^{-1}. For the preparation of the title compound, V, three different methods were employed (see Experimental); XI was reacted with (*a*) 2-methyl-2-pseudothiourea,[13] (*b*) 3,5-dimethylpyrazole-1-carboxamidine,[14] or (*c*) cyanamide.[15] The reaction of XI with cyanamide gave erratic results of maximum 30% yield. However, methods (*a*)and (*b*) were successful to afford good yields of the desired title compound.

Thus, satisfactory synthetic methods were developed for synthesis of the guanidinoazasteroid, 4-(β-guanidinoethyl)-17α-methyl-4-aza-5α-androstan-17β-ol from a readily available methyl testosterone. These methods will be used for synthesis of other guanidinoazasteroids in our future work.

PHARMACOLOGY

Effect on Hypertensive Rats.

Methods. Test compounds (included the intermediates, IX, X, and XI) were tested for antihypertensive activity after intragastric(oral gavage) administration to concious rats. Systolic blood pressure was measured indirectly by means of a pulse transducer applied on the ventral surface of the tail artery distal to an occluding cuff.[16] Recordings were made on an E & M physiograph. Each dose of test compounds was examined in two rats.

Results(*Table* 1). Good antihypertensive activity was obtained with V at 100 mg/kg; guanethidine, however, exhibited a greater po miligram potency. As expected, compounds, IX, X and XI were inactive.

Effect on Cat Nictitating Membrane.

Methods. Each test compounds were examined for their ability to cause relaxation (prolapse) of the nictitating membrane when administered subcutaneously to a separate concious cat (2~4 kg) of either sex in the manner described by McLean, *et al.*[17]

Table 1. Pharmacological activities.

Compd. no.	Effect on hypertensive rat blo od pressure			Effect on cat nictitating membrane	
	Dose mg/kg po	Decrease ofsystolic pressure[a]	Dose mg/kg sc	Deg of relaxation[b]	Duration hr
III	100	++	3	++	30
	30	++	10	+++	>70
V	100	++	10	+	20
	30	0	30	++	47
IX	100	0	10	0	
			30	0	
X	100	0	10	0	
			30	0	
XI	100	0	10	0	
			30	0	

[a]0, inactive; +, 25~39mm; ++, 40~70 mm; +++, >70mm.
[b]0, inactive, +, 25%; ++, 50%; +++, >50%.

Results (*Table* 1). Relaxation of membrane was observed with V(10 and 30 mg/kg) and persisted up to 47 hr with large dose of V. By comparison, a greater degree and more prolonged duration of relaxation was produced by smaller doses of guanethidine III. None of these compounds caused mydriasis. Compounds, IX, X, and XI had no noticeable effect on the nictitating membrane in doses up to 30 mg/kg.

In summary, relaxation of the nictitating membrane, in the absence of mydriasis, is considered to be evidence of adrenergic neurone blockade.[18] Thus, the title compound V resembles that of the classical adrenergic neurone blocking agents.[19]

EXPERIMENTAL

Melting points were taken in open capillaries using a Thomas-Hoover Uni-melt apparatus unless otherwise indicated, and the elemental analyses were obtained from Galbraith Laboratories, Knoxville, Tenn., U.S.A. A Perkin-Elmer 202 UV-visible spectrophotometer was used to record the UV spectra. The R_f[20] values were determined by applying a methanolic solution of the steroid at a rate of 50~100μg of the steroid as a spot on a silica gel TLC plate(Eastman Chromatogram Sheet) and developing in a Erinkmann jar in following solvent systems: solvent A, benzene-methanol-ethyl acetate (85:10:5); solvent B, chloroform-methanol-ammonia (85:14:1). The steroids were detected by iodine vapor.

4-(β-Cyanoethyl)-17α-methyl-4-aza-5α-androstan-17β-ol(X).

To a stirred solution of 5.00g(0.016 mole) of IX and 2.43g(0.032mole) of chloroacetonitrile in 750 m*l* of anhydrous acetone was added 8.00 g of anhydrous sodium carbonate. The mixture was refluxed with vigorous stirring for 17hr and filtered when hot. The filtrate was evaporated *in vacuo* to obtain a light-yellow oily residue. Crystallization from ethanol gave 3.29 g(64%) of a white solid, mp. 183~185°; IR(KBr): 2260 cm^{-1}(C≡N); R_f 0.72 in solvent B.

Anal. Calc. for $C_{21}H_{34}N_2O$: C, 76.31; H, 10.37; N, 8.51. Found: C, 76.27; H, 10.26; N, 8.64.

4-(β-Aminoethyl)-17α-methyl-4-aza-5α-androstan-17β-ol(XI).

To a stirred solution of 1.00g(0.003mole) of X in 150 m*l* of dry tetrahydrofuran was added, in samll portions, 0.57 g(0.015 mole) of lithium aluminium hydride. The mixture was refluxed with stirring for 24 hr. After decomposition of the excess lithium aluminium hydride with methanol and then water, the mixture was filtered and the filtrate was evaporated *in vacuo* to obtain a yellow oily residue. All attempts to obtain crystals were unsuccessful; IR(neat): 3090 and 3210 cm^{-1} (N—H) and disappearance of nitrile peak at 2260 cm^{-1}. The yellow oily residue, XI was made to XI·HCl salt, and crystallized from ethanol-ether to obtain an analytical sample, 0.92 g(77 %) of white solid, mp. 281~284°; IR (KBr): absence of the nitrile peak at 2260 cm^{-1}; R_f 0.74 in solvent B.

Anal. Calc. for $C_{21}H_{40}Cl_2N_2O$: C, 61.90; H, 9.90; N, 6.88. Found: C, 61.59; H, 9.79; N, 6.78.

4-(β-Guanidinoethyl)-17α-methyl-4-aza-5α-androstan-17β-ol(V).

For synthesis of V, three methods were utilized.

Method(*a*)[14]. A solution of 2.50 g(0.007 mole) of XI and 3.45 g(0.007 mole) of 3,5-dimethylpyrazole-1-carboxamidine in 70 m*l* of ethanol was refluxed for 5 hr. On cooling to 0 °C, there deposited 3.00 g(70 %) of cream-colored solid, mp. >300; IR(KBr): 3100 and 3390 cm^{-1} (guanidine absorption) and 1620 and 1660 cm^{-1} (C=N)

Anal. Calc. for $C_{22}H_{40}N_4O$: C, 62.08; H, 9.71; N, 13.13. Found: C, 61.89; H, 9.79;

N, 13.08.

Method(*b*)[13]. One gram(0.0028 mole) of XI and 0.45 g(0.0058 mole) of 2-methyl-2-pseudothiourea were dissolved in 50 m*l* of 50 % aqueous ethanol and heated under reflux with vigorous stirring for 1hr. After chilling, the reaction mixture was filtered to obtain a gray solid. Crystallization from 50 % aqueous acetone gave 2.02 g(82 %) of cream-colored solid, which was identical with the authentic sample from the method(*a*).

Method(*c*)[15]. Equal molar amounts of XI(0.8 g, 0.0024 mole) and cyanamide(0.15 g, 0.0024 mole) were added to 20 m*l* of ethanol and refluxed with vigorous stirring for 3 days. The reaction mixture was evaporated *in vacuo* to obtain an oily residue, which was crystallized as usual, and gave 0.31 g(35 %) of a cream-colored solid; identical with the authentic sample from the method(*a*).

REFERENCES

1. N. J. Doorenbos and M. T. Wu, *J. Pharm. Sci.*, **54**, 1290(1965).
2. K. D. Shvetsova-Shilovskaya, U. S. S. R. 179, 552, Feb. 8, 1966; *Chem. Abstr.*, **65**, 2928c (1966).
3. Neth. Appl. 6,504,900, Oct. 18, 1965; *Chem. Abstr.*, **64**, 15974a(1966).
4. N. J. Doorenbos and J. C. Kim, *J. Pharm. Sci.*, **63**, 620(1974).
5. L. S. Goodman, "The Pharmacological Basis of Therapeutics", The MacMillan Co., New York, 1970.
6. B. Wolters, *Planta Med.*, **14**, 392(1966); *Chem. Abstr.*, **66**, 17283e(1966).
7. R. A. Mull, A. J. Plummer, F. Schneider, H. Povalski and A. I. Daniel, *J. Pharmacol. Exp. Ther.*, **128**, 22(1960).
8. R. P. Mull and R. A. Maxwell, "Antihypertensive Agents", P. 115, E. Schlitter, Ed., Academic Press, New York, 1967.
9. N. J. Doorenbos and W. E. Solomons, *J. Pharm. Sci.*, **62**, 638(1973).
10. N. J. Doorenbos and C. L. Huang, *J. Org. Chem.*, **26**, 4106(1961).
11. R. S. Wildi, *U.S. Patent* 2,897,202, July 28 (1959).
12. N. J. Doorenbos and P. C. Bossle, *J. Pharm. Sci.*, **54**, 1691(1965).
13. B. Rathke, *Chem. Ber.*, **14**, 1774(1881).
14. J. H. Short and T. D. Darby, *J. Med. Chem.*, **10**, 833(1967).
15. J. H. Short, U. Biermacher, D. A. Dunnigan and T. D. Leth, *J. Med. Chem.*, **6**, 275(1964).
16. M. A. Commarato, M. M. Winbury and H. R. Kaplan, *J. Pharmacol. Exp. Ther.*, **187**, 300 (1973).
17. R. A. McLean, R. J. Gens, R. J. Mohrbacker, P. A. Mattis and G. E. Ullyst, *J. Pharmacol. Exp. Ther.*, **129**, 11(1960).
18. A. Grollman, *Proc. Soc. Exp. Bio. Med.*, **57**, 102(1944).
19. F. C. Copp, *Advan. Drug Res.*, **1**, 161(1964).
20. N. J. Doorenbos and J. C. Kim, *J. Chromatogr.*, **21**, 393(1968).