ANTIBACTERIAL ACTIVITY OF PREGNENOLONE DERIVATIVES

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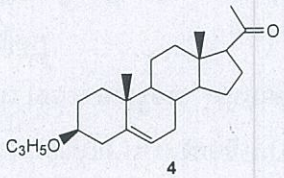
ABSTRACT

Four new synthesized steroids, 3β-alloxy-5-pregnene-20-one **4**, 3β-alloxy-5-pregnene-17-oxime **5**, 3β-stearoate-5-pregnene-20-one **6**, 3β-stearoate-5-pregnene-17-oxime **7**, have been synthesized starting from 3β-hydroxy-5-pregnene-20-one **3**. The structures of these steroids were confirmed by analytical and spectroscopic evidence. All of compounds were examined for antibacterial activity *in vitro* using the disc diffusion method.



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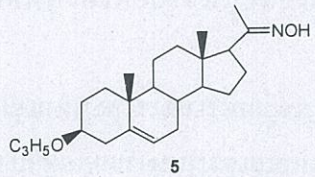

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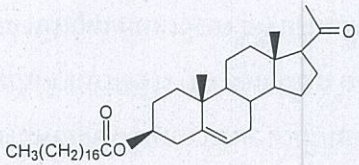

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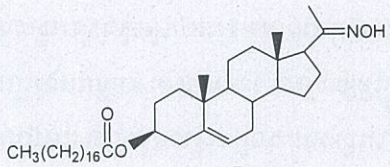

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KEYWORDS: steroid; antibacterial activity; Disc diffusion method; pregnenolone

1. INTRODUCTION

Steroids are an important class of biologically active compounds and a large number are used in therapy. Over the last 20 years the biochemistry of steroids has advanced vary rapidly and such developments have been quite relevent for research of new drugs and led to a renewed interest in steroid [1]. Attention has been devoted in literature to the synthesis of several steroidal derivatives that exhibited marked medicine activity [2]. Some steroids showed potent antineoplastic activity to tomour cell [3]. For example, oximino derivatives, 6-hydroximino-4-en-3-ones **1** and **2** also show a high affinity for human placental, and function as competitive inhibitors of this enzyme [4]. Moreover, several steroids derivatives are well authenticated to have antimicrobial activity versus many species of bacteria and fungi [5].

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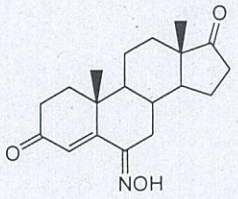

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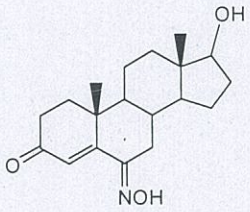

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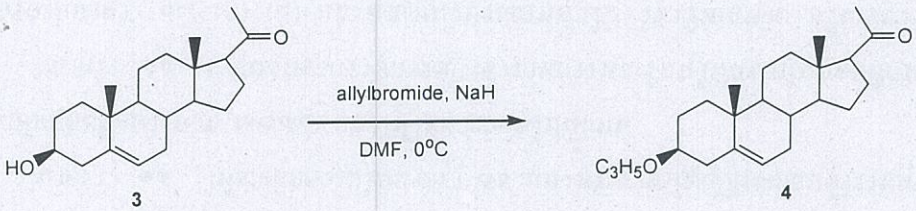
This report describes the synthesis and properties of the pregnenolone derivatives ; 3β-alloxy-5-pregnene-20-one **4**, 3β-alloxy-5-pregnene-17-oxime **5**, 3β-stearoate-5-pregnene-20-one **6**, 3β-stearoate-5-pregnene-17-oxime **7**. The *in vitro* antibacterial activity of the new steroid derivatives was tested against a wide spectrum of bacteria.

2. MATERIALS AND METHODS

Melting points (m.p.) were determined on GALLENKAMP SANYO apparatus. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance DPX 300 spectrometer. Chemical shifts (δ) are give in ppm downfield from TMS as the internal standard. Mass spectra were collected on Agilent 5973 *Network* Mass Selective Detector. IR spectra were obtained with potassium bromide pellets (ν_{max} in cm^{-1}). All solvents were dried and freshly distilled prior to use according to standard procedure. IR spectra were recorded on a Perkin Elmer FT-IR spectrometer. Antibacterial study of synthesized steroids were carried out at laboratory of Department of Stomatology, Faculty of Dentistry, Srinakharinwirot University, Bangkok, Thailand. Commercially available 3β-hydroxy-5-pregnene-20-one **3** was obtained from Fluka and used as starting material for the synthesis of compounds **4** to **7**. Compound **3** : ^{1}H NMR δ ($CDCl_3$) : 1.84;1.08 (2H, H-1), 1.84;1.48 (2H, H-2), 3.54 (1H, H-3), 2.27 (2H, H-4), 5.34 (1H, H-6), 1.97;1.57 (2H, H-7), 1.46 (1H, H-8), 0.98 (1H, H-9), 1.62;1.47 (2H, H-11), 2.04;1.43 (2H, H-12), 1.17 (1H, H-14), 1.68;1.23 (2H, H-15), 2.19;1.66 (2H, H-16), 2.55 (1H, H-17), 0.63 (3H, H-18), 1.00 (3H, H-19), and 2.11 (3H, H-21); ^{13}C NMR δ ($CDCl_3$) : 13.22, 19.39, 21.10, 22.85, 24.50, 31.51, 31.60, 31.79, 31.88, 36.54, 37.29, 38.86, 42.25, 44.01, 50.02, 56.94, 63.72, 71.66, 121.35 [6].

Synthesis of 3β-alloxy-5-pregnene-20-one 4



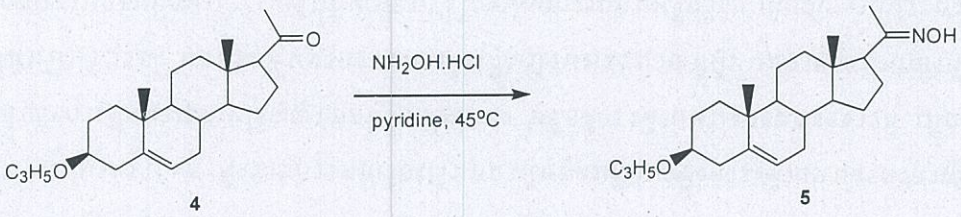
3 4

A solution of 3β-hydroxy-5-pregnene-20-one **3** (300 mg, 0.95 mmol) in dimethylformamide (10 mL) was added to solution of sodium hydride (18 mg, 1.2 mmol) in dimethylformamide (5 mL). The reaction mixture was stirred for 1 hr. at 0 °C under nitrogen atmosphere, then allylbromide (0.06 mL, 1.2 mmol) was added. The reaction mixture was stirred for 2 hrs. at room temperature. The completion of the reaction was determined by TLC. The solvent was evaporated under vacuum, the remaining residue was applied to chromatographic column prepared by packing slurry of silica gel 60, 0.04-0.06 mm for flash chromatography in hexane.

The product was obtained by elution with hexane/ethyl acetate (8:2 v/v) as yellow oil to yield 156.1 mg (69.29 %). IR (KBr) cm^{-1} : 2932 (CH_3), 2830 (CH_2), 1706 (C=O), 1094 (C-O), 1015, 922 (C=C), 1451 (CH_2), 1355 (CH_3); ^{1}H NMR ($CDCl_3$) δ: 0.63 (s, 3H, H-18), 1.09 (s, 3H, H-19), 2.12 (s, 3H, H-21), 2.50 (t, 1H, H-17, J = 8.80 Hz), 3.21 (m, 2H, H-3), 4.03 (d, 2H, H-22, J = 5.51 Hz), 5.13 (dd, 2H, H-24, $^{3}J_{ac}$ cis = 10.26 Hz, $^{3}J_{bc}$ trans = 17.21 Hz, $^{2}J_{ab}$ gem = 1.38 Hz, $^{4}J_{bd}$ allyl = 1.42 Hz), 5.53

(t, 1H, H-6, J = 9.89 Hz), 5.92 (m, 1H, H-23); ^{13}C NMR ($CDCl_3$) δ: 14.56, 20.70, 22.42, 24.18, 25.83, 29.74, 32.85, 33.16, 33.20, 38.24, 38.60, 40.20, 40.44, 45.34, 51.41, 58.29, 65.06, 70.33, 79.77, 117.83, 122.57, 136.80, 142.31, 210.83; EIMS : m/z = 356 (11), 341 (2), 300 (100), 285 (39).

Synthesis of 3β-alloxy-5-pregnene-17-oxime 5

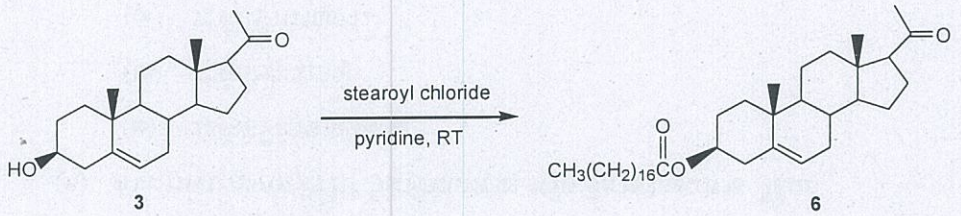


4 5

Hydroxylamine hydrochloride (44.36 mg, 1.2 mmol) was added to a solution of 3β-alloxy-5-pregnene-20-one **4** (190 mg, 0.532 mmol) in dry pyridine (15 mL). The reaction mixture was stirred for 1 hr. at 45 °C. The completion of the reaction was determined by TLC. The solvent was evaporated under vacuum, the remaining residue was applied to chromatographic column prepared by packing slurry of silica gel 60, 0.04-0.06 mm for flash chromatography in hexane.

The product was obtained by elution with hexane/ethyl acetate (9:1 v/v), as white powder to yield 110.9 mg (56.11 %), m.p. 179-181 °C. IR (KBr) cm^{-1} : 3370 (N-OH), 3085 (C=C), 2933 (CH_3), 2851 (CH_2), 1060 (C-O); ^{1}H NMR ($CDCl_3$) δ: 0.63 (s, 3H, H-18), 1.09 (s, 3H, H-19), 1.94 (s, 3H, H-21), 2.44 (t, 1H, H-17, J = 9.20 Hz), 3.24 (m, 2H, H-3), 4.05 (d, 2H, H-22, J = 5.58 Hz), 5.17 (dd, 2H, H-24, $^3J_{ac}$ cis = 9.20 Hz, $^3J_{bc}$ trans = 17.22 Hz, $^2J_{ab}$ gem = 1.51 Hz, $^4J_{bd}$ allyl = 1.44 Hz), 5.53 (t, 1H, H-6, J = 4.88), 6.00 (m, 1H, H-23); ^{13}C NMR ($CDCl_3$) δ: 13.14, 15.15, 19.39, 21.03, 23.19, 24.23, 28.41, 31.81, 32.06, 36.93, 37.27, 38.66, 39.12, 43.89, 50.24, 56.18, 56.72, 69.00, 78.47, 116.50, 121.31, 135.47, 141.02, 158.79; EIMS : m/z = 371 (17), 356 (61), 315 (22).

Synthesis of 3β-stearoate-5-pregnene-20-one 6

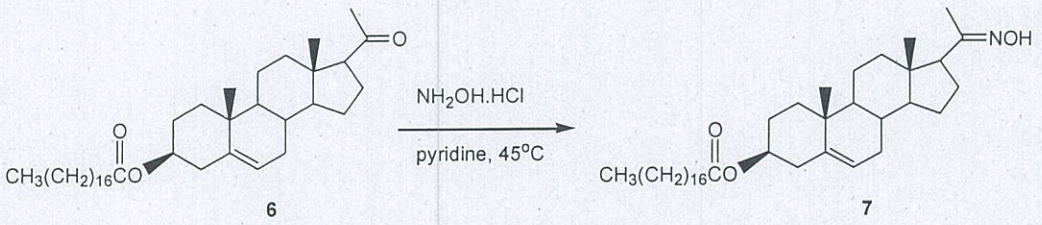


3 6

Stearoyl chloride (0.6 mL, 1.2 mmol) was added to a solution of 3β-hydroxy-5-pregnene-20-one **3** (500.10 mg, 1.58 mmol) in dry pyridine (15 mL). The reaction mixture was stirred for 1 hr. at room temperature. The completion of the reaction was determined by TLC. The solvent was evaporated under vacuum, the remaining residue was applied to chromatographic column prepared by packing slurry of silica gel 60, 0.04-0.06 mm for flash chromatography in hexane.

The product was obtained by elution with hexane/ethyl acetate (9:1 v/v), as white powder to yield 543.1 mg (62.44 %), m.p. 89-91 °C. IR (KBr) cm^{-1} : 1742 (-CO-O), 2915 (CH_3), 2850 (CH_2), 1015 (C-O); ^{1}H NMR ($CDCl_3$) δ: 0.63 (s, 3H, H-18), 1.02 (s, 3H, H-19), 2.12 (s, 3H, H-21), 2.50 (t, 1H, H-17, J = 8.77 Hz), 4.60 (m, 2H, H-3), 5.36 (t, 1H, H-6, J = 4.60 Hz); ^{13}C NMR ($CDCl_3$) δ: 13.62, 19.17, 21.82, 22.48, 24.28, 29.50, 30.07, 31.79, 32.03, 35.10, 37.00, 38.50, 44.36, 50.05, 56.83, 74.06, 140.13, 173.72, 209.89: EIMS : m/z = 584 (23), 569 (40), 269 (24).

Synthesis of 3β-stearoate-5-pregnene-17-oxime 7



6 7

To a solution of 3β-stearoate-5-pregnene-20-one **6** (300 mg, 0.5150 mmol) in dry pyridine (15 mL), hydroxylamine hydrochloride (42.94 mg, 1.2 mmol) was added. The reaction mixture was stirred for 1 hr. at 45 °C. The completion of the reaction was determined by TLC. The solvent was evaporated under vacuum, the remaining residue was applied to chromatographic column prepared by packing slurry of silica gel 60, 0.04-0.06 mm for flash chromatography in hexane.

The product was obtained by elution with hexane/ethyl acetate (9:1 v/v) as white powder to yield 252.3 mg (81.9 %), m.p. 101-103 °C. IR (KBr) cm^{-1} : 3370 (N-OH), 2932 (CH_3), 2830 (CH_2), 1706 (C=O), 1094 (C-O); ^{1}H NMR ($CDCl_3$) δ: 0.63 (s, 3H, H-18), 1.02 (s, 3H, H-19), 1.90 (s, 3H, H-21), 2.24 (t, 1H, H-17), 4.60 (m, 2H, H-3), 5.37 (t, 1H, H-6, J = 3.53 Hz) ; ^{13}C NMR ($CDCl_3$) δ: 13.44, 19.06, 21.76, 22.68, 24.50, 29.68, 31.45, 31.92, 32.20, 36.63, 37.56, 38.12, 43.94, 49.79, 56.45, 69.17, 139.76, 159.30, 173.34; EIMS : *m/z* = 599 (31), 581 (71), 266 (27).

Microoganisms used

Five strains of bacteria used were *Staphylococcus aureus* (MRSA), *Corynebacterium diptheriae*, *Bacillus subtilis* ATCC 26633, *Pseudomonos aeruginosa* ATCC 27853 and *Streptococcus mutans* ATCC 27175. These standard strains were obtained from Department of Stomatology, Faculty of Dentistry, Srinakharinwirot University.

Antibacterial assay

The disc diffusion method [7] was followed for antibacterial susceptibility test. Test compounds were dissolved in dimethyl sulfoxide (DMSO) and two-fold serial dilution was made with DMSO. The bacterial inoculum was prepared by suspending in sterile phosphate buffer saline for colonies from 24 hours culture in Todd-Hewitt broth and Sabouraud liquid medium respectively. The cell density of each inoclum was adjusted to obtain a final concentration of approximately 10^6 cfu (colony forming unit)/ml of bacteria. Sterile filter paper discs (6 mm in diameter) were impregnated with 10 µl of each two-fold serial dilution of the test material and place on the inoculated plates. The plates were then incubated at 37 °C for 16-18 hours for bacterial. DMSO was used as negative control. At the end of the incubation period the diameter of the inhibition zone was measured from the edge of the disc to the inner margin of the surrounding pathogens.

3. RESULTS AND DISCUSSION

3β-Alloxy-5-pregnene-20-one **4** was prepared from pregnenolone **3**. The hydroxyl group of pregnenolone was interaction with a allylbromide to obtain 69.29 % yield. Pregnenolone was acetylated using stearoyl chloride in dry pyridine to give the ester compound **6** in 62.44 % yield. Oxime derivative was obtained from the ketones **4** and **6** by oximation reaction with hydroxylamine hydrochloride in dry pyridine to obtain compound **5** and **7** in 56.11 and 81.9 % yield, respectively.

The *in vitro* antibacterial activity of the synthesized steriods **4**, **5**, **6** and **7** were examined against five types of microorganism tested as illustrated in Table 1. Our results showed that the data of the disc diffusion method for the tested compounds **4** and **5** possessed antibacterial activity. When the compounds **4** and **5** were assayed against the test organism, the mean zones of inhibition obtained were between 3 and 50 mm. The blind control (dimethyl sulfoxide) did not inhibit any of the microorganisms tested.

Table 1 : Antibacterial activity of the tested compounds.

Test organism	Name	Compounds				
		3	4	5	6	7
bacteria	*S aureus* (MRSA)	-	25	-	-	-
	C diptheriae	-	3.125	25	-	-
	B subtilis ATCC 26633	-	50	-	-	-
	P aeruginosa ATCC 27853	-	50	-	-	-
	S mutans ATCC 27175	-	-	12.5	-	-

Values show zone of inhibition in mm ; concentration of sample 50 μg/ml for the antibacterial study of the inhibition zones were : high (x ≥ 14 mm), modulate (11-14 mm), week (8-11 mm), negative (-) (no zone of inhibition).

4. CONCLUSIONS

The results showed that 3β-alloxy **4** and oxime ether derivative **5** have exhibited significant antibacterial activity in contrast to the compounds with 3β-stearoate **6** and oxime ester derivative **7**. Preliminary study, further highlight the importance of ether and oximino functionality to increase potent antibacterial activity.

ACKNOWLEDGEMENTS

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