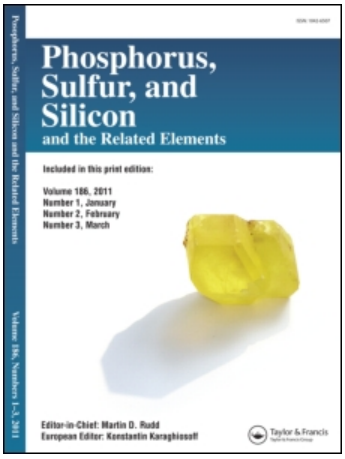

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Studies on Organophosphorus Compounds VII[1,2]: Transformation of Steroidal Ketones with Lawesson's Reagent into Thioxo and Heterofused Steroids. Results of Antimicrobial and Antifungal Activity

Nadia R. Mohamed[a]; Gamal A. Elmegeed[a]; Magdi Younis[b]
[a] National Research Centre, Giza, Egypt [b] South Valley University, Aswan, Egypt

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STUDIES ON ORGANOPHOSPHORUS COMPOUNDS VII[1,2]: TRANSFORMATION OF STEROIDAL KETONES WITH LAWESSON'S REAGENT INTO THIOXO AND HETEROFUSED STEROIDS. RESULTS OF ANTIMICROBIAL AND ANTIFUNGAL ACTIVITY

Nadia R. Mohamed,[a] *Gamal A. Elmegeed,*[a]
and Magdi Younis[b]
National Research Centre, Dokki, Giza, Egypt[a] *and*
South Valley University, Aswan, Egypt[b]

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The reactivity of Lawesson's reagent (LR) toward some steroidal hormones was studied, 4-androsten-3,17-dione ***2*** *reacted with LR to produce the corresponding thioxosteroids* ***3*** *and* ***4****. Epi-androsterone* ***5*** *showed a great activity to LR and produced 3β-mercaptospiro-(androstan-17,4′dithiaphosphetan)thione* ***8*** *and the sulfide derivative* ***9****. Also progesterone* ***10*** *reacted with LR to yield the thiaphospholo[3′, 4′:16,17]androsten-3-one* ***13*** *and the sulfide product* ***16****. The new modified steroidal derivatives thieno[2′,3′:2,3]cholestan* ***18*** *and thieno[2′,3′:2,3]-androstan* ***20*** *were synthesized and examined against LR, the corresponding thiazaphosphorinothieno steroidal derivatives* ***23*** *and* ***24*** *were isolated respectively. The in vitro biological activity of some newly synthesized compounds against bacteria, yeast, and fungi was studied.*

Keywords: 17-Thioxoandrosten-3-one; antibacterial; antifungal; Lawesson's reagent; mercaptooxathiaphospholoandrosten-2-thione; steroids; thiazaphosphorinothieno steroids

The investigation of new modified steroid derivatives condensed with various heterocyclic rings has been given great attention.[3–5] The addition of heterocyclic rings to steroids often leads to a change of their physiological activity and the appearance of new interesting pharmacological and biological properties,[6] especially antiinflammatory,[7] antineoplastic,[8] uterotropic,[9] and antiandrogenic activity.[10] The use of

Address correspondence to Nadia R. Mohamed, Department of Photochemistry, National Research Centre, Dokki, Giza, Egypt. E-mail: nadia_ragab_m@hotmail.com

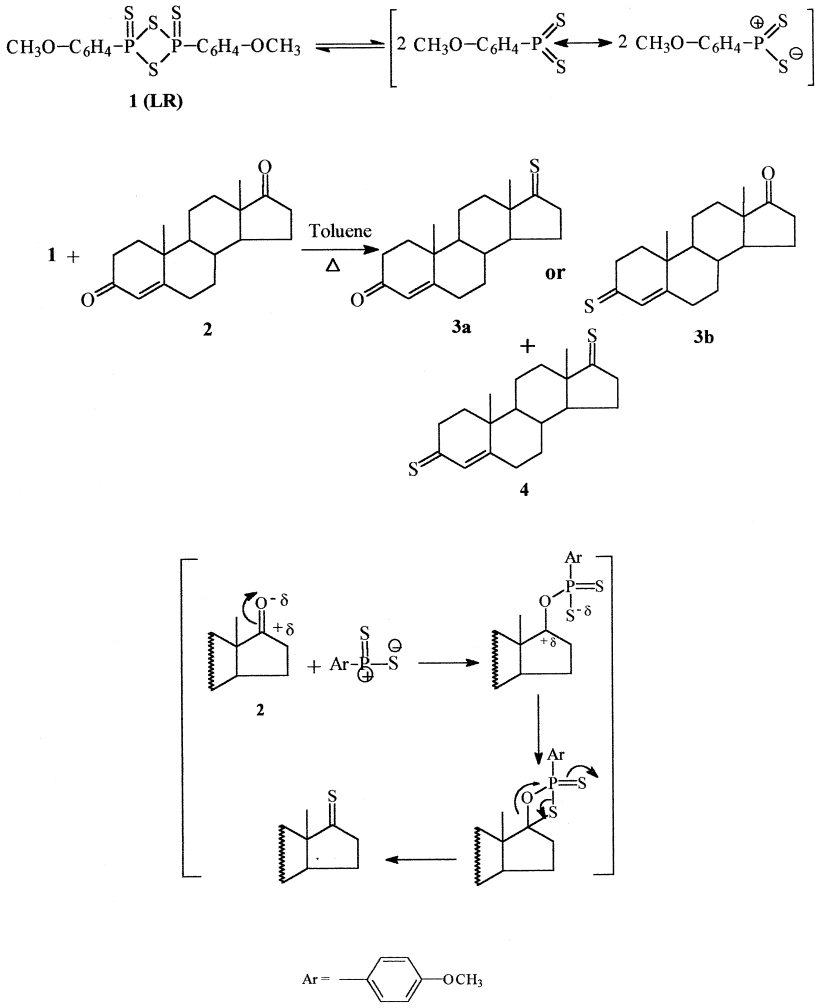
2,4-bis(p-methoxy phenyl)-1,3,2,4-dithiaphosphetane-2,4-disulfide, commonly known as Lawesson's reagent (LR), for the chemical conversion of carbonyls to thiocarbonyl compounds has been well investigated.[11] LR also has been utilized in the synthesis of five- and six-membered phosphorus heterocycles such as oxathiaphospholes,[12] oxathiaphospholidine-2-thiones,[13] oxazaphosphorin-4-thione-2-sulfides,[14] and sulfur containing heterocycles such as thienothiazine-4-thiones[15] and benzothiazole-3-thiones[16]. In continuation to our previous work,[17,18] we felt prompted to study the reactions of LR and steroidal hormones with the aim of synthesize novel biologically active thioxo and hetero-fused steroids. Several steroid derivatives are well authenticated to have antimicrobial activity versus many species of bacteria, yeast, and fungi.[19,20] This article explains the interaction of LR with some androstane, cholestane, and pregnane derivatives and studies the bioactivity of some novel synthesized compounds against a wide spectrum of gram positive, gram negative bacteria, yeast, and fungi.

RESULTS AND DISCUSSION

Chemistry

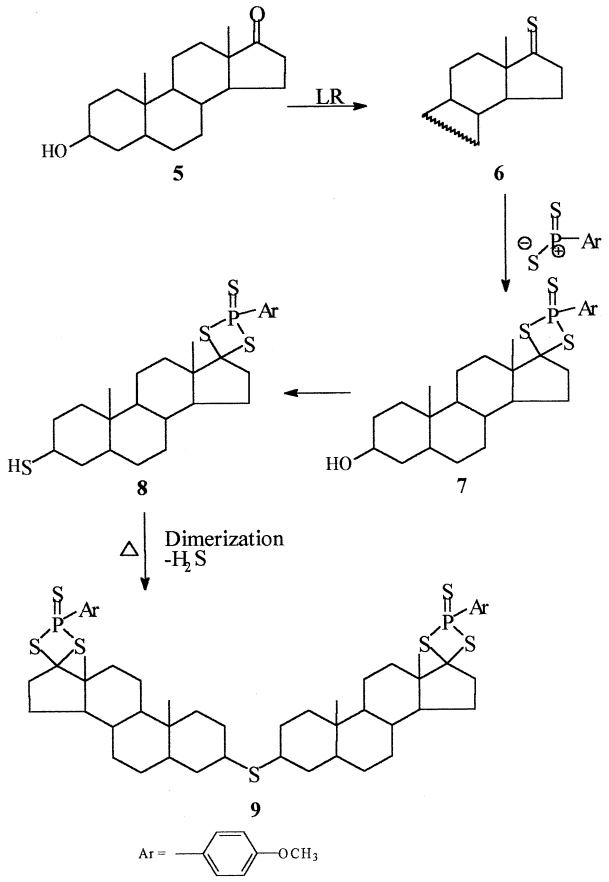
The reaction of LR **1** with an equimolar amount of 4-androsten-3,17-dione **2** in dry toluene under reflux afforded the corresponding 17-thioxoandrosten-3-one **3a** or 3-thioxoandrosten-17-one **3b** and **3,17-**dithioxoandrosten **4**, which were isolated using silica gel column chromatography. It is believed that LR reacted only as thionating agent to form the thioxo derivatives **3** and **4** according to a betain mechanism[21] as explained in (Scheme 1). The IR spectrum of compound **3** revealed absorption band at υ 1698 cm^{-1} for one carbonyl group only beside another absorption band at υ 1124 cm^{-1} for the C=S group. The carbonyl group band at υ 1698 cm^{-1} corresponding to CO group at C-3 as part of a cyclohexenone substructure and not corresponding to the CO group at C-17 as part of a cyclopentanone substructure (about υ 1743 cm^{-1})[22] which excludes structure **3b**. In addition, the literature showed that the enone CO group at C-3 less active than the CO group at C-17.[23] The MS spectrum of each of compounds **3** and **4** showed an ion peak at m/z = 302 and m/z = 318 respectively. The ^{1}H NMR spectra of compounds **3** and **4** showed the characteristic data of the androstane series[24], also the analytical data supported the proposed structures (c.f. experimental section).

The study also extended to the effect of LR on the sex male hormone epi-androsterone **5**. The reaction of equimolar amounts of



SCHEME 1

3β-hydroxy-5α-androstan-17-one **5** with LR by fusion yielded 3β-mercaptospiro(androstan-17,4′-dithiaphosphetan)thione **8** (Scheme 2). It is believed that epi-androsterone **5** transformed at first into the corresponding 17-thione derivative **6**, ring closer with LR occurred to give the 3β-hydroxyspiroandrostanophosphetane **7**. Further thionation of the alcohol **7** with LR produced the final isolated mercapto derivative **8**[25–27] (Scheme 2). On the other hand when the reaction was carried out in dry toluene under reflux for long time the bis[spiro(androstan-17,4′-dithiaphosphetan)thione-3-yl]sulfide **9** was formed (Scheme 2) which

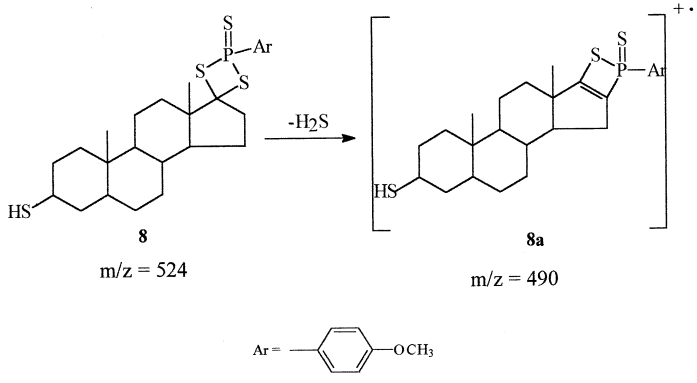


SCHEME 2

could be generated under participation of the mercapto groups of two molecules of **8** by loss of H_2S. Similar spiro ring system and sulfide derivatives were explained before.[12,28]

Structure of compound **8** was supported strongly by the mass spectrum which showed molecular ion peak at m/z = 524 [M^+] and another molecular ion peak at m/z = 490 which corresponded to **8a**. It is believed that this molecular ion was formed by loss of H_2S (Scheme 3).

The ^{31}P NMR shift (VS-H_3PO_4) recoded for compound **8** was δ = 65.5 ppm which supported the structure for the product.[29] Also the ^1H NMR showed a multiplet signal at δ 3.19 ppm for CH-3 proton, singlet signal at 3.81 ppm for SH proton, and singlet signal at 3.85 ppm (3H) corresponding to $-OCH_3$ group beside the other characteristic signals (c.f. Experimental section). The MS spectrum of compound



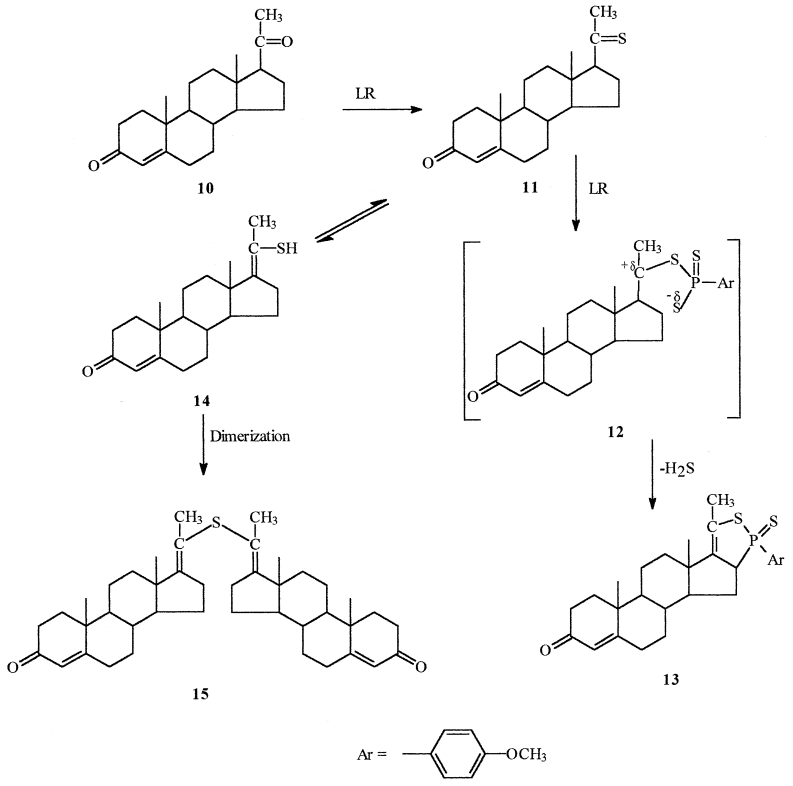
SCHEME 3

9 revealed two ion peaks at m/z = 523 and m/z = 490, which were corresponding to two fragments of the bis product **9**. The ^{1}H NMR of compound **9** showed multiplet signal at 3.65 (2H) for the two C_3—H, singlet signal at δ 3.82 ppm (6H) for the two —OCH_3 groups in addition to multiplet signal at 7.07–7.60 (10H) for aromatic and olefinic protons. All elemental and spectral data of compounds **8** and **9** confirmed the proposed structures (c.f. Experimental section).

The investigation continued with the reaction of LR and the sex female hormone, progesterone **10**, which produced two products **13** and **15** isolated by the use of silica gel column chromatography. The formation of compound **13** occurred via the interaction of the more active C=O group at C-20 of progesterone[23] with LR to from the corresponding thioketone derivative **11** as intermediate. Further interaction of **11** with LR formed compound **12** which is cyclized by loss of H_2S to form the final isolated product substituted thiaphospholo[3′,4′:16,17]androsten–3-one **13** (Scheme 4). On the other hand the substituted bis[oxoprostadien-2-yl]sulfide product **15** was formed from the dimerization of the enol form of 20-mercaptoprogesterone **14** as explained earlier.[12,28]

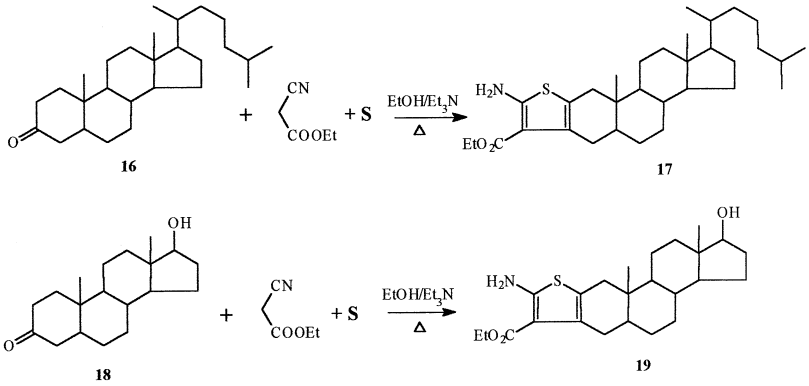
The MS spectrum of compound **13** revealed a molecular ion peak at m/z = 498 (M^+, 51%) which corresponds to the molecular formula $C_{28}H_{35}PO_2S_2$. The ^{1}H NMR of compound **13** showed singlet signal at 3.82 ppm (3H) corresponding to —OCH_3 group, singlet signal at 4.92 ppm (1H) for the C_4—H, and a multiplet signal at 5.17 (1H) for the C_{16}—H. The identity of the latter products **13** and **15** verified by their analytical and spectral data (c.f. Experiment section).

All previous results directed us to synthesize new modified steroidal derivatives to investigate their reactivity toward the reaction with LR.



SCHEME 4

The reactivity of 5α-cholestan-3-one **16** and 17β-hydroxy-5α-androstan-3-one **18** toward the formation of thiophenes studied applying the method of Gewald reaction.[30] Compounds **16** and **18** were reacted with equimolar amounts of ethyl cyanoacetate and elemental sulfur in refluxing ethanolic triethylamine solution to yield the corresponding new modified steroids aminothieno[2′,3′: 2,3]cholestane derivative **17** and aminothieno[2′,3′:2,3]androstane derivative **19** respectively (Scheme 5). Since the initial step of the reaction requires enolization of the ketone followed by reaction with sulfur at the α-position, the reaction products should have structures **17** and **19**, as 3-keto steroids with 5α configuration are known[31] to enolize from C_2 position to give the Δ^2 enol; thus, the thieno[2′,3′:4,3]steroidal isomers were excluded.[32] Identical mass spectra of compounds **17** and **19** indicate that the compounds obtained are free from the angular thieno[2′,3′:4,3]steroidal isomers (c.f. Experimental section). The IR spectrum of each of compounds

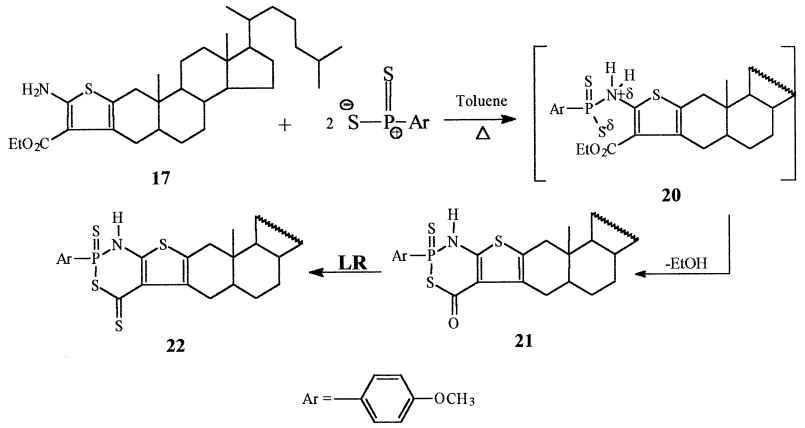


SCHEME 5

17 and **19** revealed the existence of absorption band at υ 1735 cm^{-1} and υ 1730 cm^{-1}, respectively, which corresponds to the ester carbonyl groups and the presence of NH_2 group absorption band at υ 3350 and υ 3335 cm^{-1} respectively. The ^1H NMR spectrum of each compound revealed the presence of a triplet signal at δ 1.35 ppm and δ 1.54 ppm beside a quartet at δ 4.25 ppm and δ 4.20 ppm, respectively, which supported the existence of the ester group. The spectrum also showed D_2O-exchangeable singlet signal corresponding to the NH_2 group at δ 5.65 ppm and δ 5.85 ppm respectively (c.f. Experimental section).

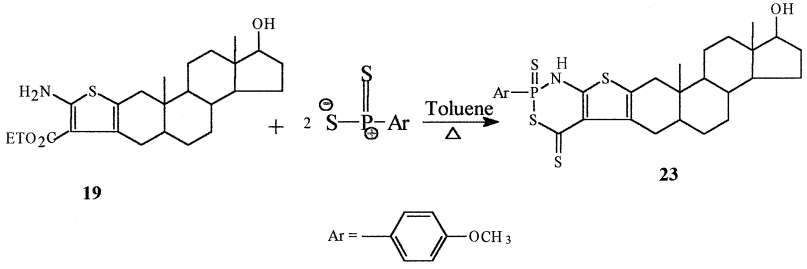
Studies about the activities of both **17** and **19** toward the reaction with LR were carried out. The interaction of **17** with LR in equimolar ratio led to the formation of 1″,3″,2″-thiazaphosphorino [5″,4″:4′,5′]thieno[2′,3′:2,3]cholestan-dithione derivative **22**. The reaction proceeded via a nucleophilic attack of the sulfur atom in LR followed by ring closer and expulsion of ethanol to form compound **21**. Subsequent thionation of **21** with LR produced the dithione derivative **22** (Scheme 6). The IR spectrum of compound **22** revealed the disappearance of the ester carbonyl absorption band and showed a new absorption band at υ 3420–3325 cm^{-1} for the NH group. Also absorption band at υ 1110 cm^{-1} for the C═S group and absorption band at υ 690 cm^{-1} characteristic for the P═S group were recorded respectively. The ^1H NMR spectrum also showed the disappearance of the signals characteristic to the ester group and the appearance of a singlet signal at δ 3.81 ppm for the $-OCH_3$ group in addition to D_2O-exchangeable singlet signal corresponding to the NH group at δ 10.45 ppm.

Similarly the aminothieno[2′,3′:2,3]androstane derivative **19** reacted with LR through the same previous mechanism to form the



SCHEME 6

final product 1″,3″,2″-thiazaphosphorino[5″,4″:4′,5′]thieno[2′,3′: 2,3]androstandithione derivative **23** (Scheme 7) which was confirmed by all spectroscopic and elemental analyses (c.f. Experimental section).



SCHEME 7

Bioactivity

The in vitro antimicrobial activity of the newly synthesized compounds **8**, **9**, **13**, **17**, **22**, and **23** against a wide spectrum of fourteen microbial strains, including six strains of gram positive bacteria, five strains of gram negative bacteria, one strain of yeast, and two strains of fungi was investigated in comparison with ampicillin and nystatin. In general all tested compounds were capable of inhibiting the growth of gram positive bacteria. The four compounds **8**, **9**, **13**, and **17** could be considered as a promising antimicrobial agents. They showed higher activity as antibacterial for gram positive and gram negative bacteria, antiyeast,

TABLE I Antimicrobial Activities of the Tested Compounds

No.	Test organism	Compound							
		8	**9**	**13**	**17**	**22**	**23**	Ampicillin	Nystatin
1	*Bacillus subtilis*	42	24	36	19	10	22	18	—
2	*Micrococcus luteus*	31	26	32	16	12	14	20	—
3	*Bacillus megaterium*	38	30	30	21	10	15	12	—
4	*Staphylococcus aureus*	30	26	34	18	12	12	20	—
5	*Streptomyces sp.*	29	23	22	14	10	13	13	—
6	*Bacillus cereus*	45	36	34	22	12	16	30	—
7	*Serratia Mar.*	32	26	24	16	—	—	20	—
8	*Pseudomonas aeruginosa*	25	19	22	11	—	—	20	—
9	*Escherichia coli*	28	22	20	12	—	—	11	—
10	*Salmonella sp.*	18	11	18	11	—	—	17	—
11	*Pseudomonas sp.*	22	12	14	10	—	—	19	—
12	*Sacharomyces cerevisiae*	24	10	10	10	—	—	—	11
13	*Candida albicans*	25	10	10	10	—	—	—	12
14	*Aspergillus flavus*	18	10	10	10	—	—	—	10

Values show zone of inhibition in mm; conc. of samples 200 μg/ml for the antibacterial study and 100 μg/ml for antifungal and yeast study; Incubation time = 24 h for the antibacterial study and 4 days for antifungal and yeast study; (—): no response.

and antifungal agents. Compounds **22** and **23** were capable of inhibiting the growth of gram positive bacteria but not gram negative, yeast, and fungi. Table I shows the results of the bioassay. The results reported herein are in accordance with previous antimicrobial results reported of plant steroids (phytoecdysteroids), which are analogues of invertebrate steroid hormones.[33] In conclusion, it is possible to report here the importance of these novel compounds as antibacterial and/or antifungal agents. Further studies should be made as to elucidate their mechanism of action and to determine whether their activity is lethal or merely inhibitory to microorganism.

EXPERIMENTAL

Chemical reagents and anhydrous solvents purchased from Aldrich Chemical Co., while starting steroids were from Sigma Company. The appropriate precautions in handling moisture sensitive compounds were undertakes. All melting points are uncorrected. The IR spectra expressed in cm^{-1} and recorded in KBr pellets on a Pa-9721 IR spectrometer. ^1H NMR spectra were obtained on a Varian EM-390 90 MHz spectrometer in DMSO-d_6 and $CDCl_3$ as solvents and TMS as internal reference. Chemical shifts (δ) are expressed in ppm. Mass spectra were recorded on Kartos (75eV) MS equipment. Elemental analyses were

carried out by Microanalytical Data Unit at National Research Centre, Giza, Egypt. All described compounds showed the characteristic spectral data of cyclopentanoperhydrophenanthrene nuclei of each of androstane, pregnane, and cholestane series similar to that which reported in literature.[22,24,34] For the nomenclature of steroid derivatives we used the arbitrary convention described by Fieser and Fieser[35] in addition to the definitive rules for the nomenclature of steroids published by the Commission of the Nomenclature of Biological Chemistry of the International Union of Pure Applied Chemistry.[36] Isolation and identification of bacterial and fungal strains and the antimicrobial study of the new compounds was carried out at the laboratory of Botany Department, Faculty of Science, South Valley University, Aswan, Egypt.

The Reaction of Lawsson's Reagent with 4-Androsen-3,17-dione

General Procedure

To a solution of each of 4-androsten-3,17-dione **2** (0.57 g, 0.002 mmol) in dry toluene (30 ml), LR (0.81 g, 0.002 mmol) was added. The reaction mixture was heated under reflux for 7 h. The solvent was evaporated under vacuum; the remaining residue was applied to a column chromatography prepared by packing slurry of silica gel 60- mesh in n-hexane. Ethyl acetate- n-hexane (1:9 v:v) eluted the thione derivative **3** while ethyl acetate- n-hexane (4:6 v:v) eluted the dithione derivative **4**.

17-Thioxo-4-androsten-3-one (3a). Yellow crystals from n-hexane, yield 0.25 g (42%), m.p. 64°C; IR: $\upsilon = 2942$, 2898 (CH_3, CH_2), 1698 (C═O), 1604 (C═C), 1124 (C═S) cm^{-1}; ^1H NMR (DMSO): $\delta = 0.85$ (s, 3H, 19-CH_3), 1.15 (s, 3H, 18-CH_3), 5.66 (s, 1H, C_4─H); MS (m/z): 302 (M^+, 60%); Found: C, 75.48; H, 8.59; S, 10.59. $C_{19}H_{26}OS$ (302.48). Requires: C, 75.45; H, 8.61; S, 10.60%.

3,17-Dithioxo-4-androstene (4). Brown crystals from methanol, yield 0.22 g (35%), m.p. 95–96°C; IR: $\upsilon = 2933$, 2895 (CH_3, CH_2), 1596 (C─C), 1126, 1052 (2 C─S) cm^{-1}; ^1H NMR (DMSO): $\delta = 0.87$ (s, 3H, 19-CH_3), 1.18 (s, 3H, 18-CH_3), 5.86 (s, 1H, C_4-H); MS (m/z): 318 (M^+, 45%). Found: C, 71.62; H, 8.25; S, 20.16. $C_{19}H_{26}S_2$ (318.55). Requires: C, 71.59; H, 8.22; S, 20.13%.

3β-Mercapto-2′-(4-methoxyphenyl)spiro[5α-androstan-17,4′-dithiaphosphtan]-2′-thione (8). A mixture of equivalent amounts of Epi-androsterone **5** (1.45 g, 0.005 mmol) and LR (2.02 g, 0.005 mmol) was fused in oil bath at 130°C for 30 min. The produced residue upon cooling at room temperature was triturated with methanol the formed solid product was collected by filtration. Compound **8** was formed as brown crystals from ethyl acetate, yield 1.72 g (68%), m.p. 135°C;

IR: $\upsilon = 3063$ (CH-aromatic), 2975, 2860 (CH_3, CH_2), 2590 (SH), 1590 (C=C), 686 (P=S) cm^{-1}; ^1H NMR (DMSO): 0.79 (s, 3H, 19-CH_3), 0.95 (s, 3H, 18-CH_3), 3.19 (m, 1H, C_3-αH), 3.81 (s, 1H, SH), 3.85 (s, 3H, OCH_3), 6.99 (dd, 2H, $^4J_{PH}$ 5Hz, J_{HH} 9Hz), 7.69 (dd, 2H, $^3J_{PH}$ 15Hz, J_{HH} 9Hz); ^{31}P NMR (VS-H_3PO_4) δ 65.5 ppm MS (m/z): 524 (M^+, 28%), 490 (M^+-H_2S, 18%). Found: C, 59.54; H, 6.90; P, 6.03; S, 24.50. $C_{26}H_{37}POS_4$ (524.82). Requires: C, 59.50; H, 7.10; P, 5.90; S, 24.44%.

Bis[2′-(4-methoxyphenyl)spiro(5α-androstan-17,4′-dithiaphosphetan)-2′-thione-3-yl]sulfide (9). To a solution of compound **5** (1.45 g, 0.005 mmol) in dry toluene, an equivalent amount of LR (2.02 g, 0.005 mmol) was added, and the reaction mixture was heated under reflux with stirring for 10 h. The solvent was evaporated after cooling the solid product formed was collected by filtration, dried, and crystallized from methanol to form yellow crystals, yield 2.20 g (45%), m.p. 205°C; IR: $\upsilon = 3075$ (CH-aromatic), 2973, 2862 (CH_3, CH_2), 1602 (C=C), 694, 659 (2P=S) cm^{-1}; ^1H NMR (DMSO): $\delta = 0.82$ (s, 6H, 2 CH_3), 1.03 (s, 6H, 2 CH_3), 3.65 (m, 2H, 2 C_3–αH), 3.82 (s, 6H, 2-OCH_3), 7.07–7.60 (m, 10H, 2-olefinic protons and 8-aromatic protons); MS (m/z): two ion peaks at m/z : 523 (65%), 490 (43%) corresponding to the two fragments of the sulfide product **9**; Found: C, 61.56; H, 7.11; P, 6.13; S, 22.15. $C_{52}H_{72}P_2O_2S_7$ (1015.55). Requires: C, 61.50; H, 7.14; P, 6.09; S, 22.10%.

The reaction of Lawsson's reagent with progesterone (13, 15). To a solution of progesterone **10** (1.57 g, 0.005 mmol) in dry toluene (30 ml), equivalent amount of LR (2.02 g, 0.005 mmol) was added, the reaction mixture was heated under reflux with stirring for 8 h. The solvent was evaporated under vacuum; the remaining residue was applied to a column chromatography prepared by packing slurry of silica gel 60- mesh in n-hexane. Ethyl acetate- n-hexane (2:8 v:v) eluted the product **13**, while ethyl acetate- n-hexane (7:3 v:v)

5′-Methyl-2′-(4-methoxyphenyl)-2′-thioxothiaphospholo[3′,4′:16,17]androst-4-en-3-one (13). Brown crystals from n-hexane, yield 1.19 g (48%), m.p. 212–213°C; IR: $\upsilon = 3053$ (CH-aromatic), 2955, 2835 (CH_3, CH_2), 1695 (C=O), 1605 (C=C), 655 (P=S) cm^{-1}; ^1H NMR ($CDCl_3$): $\delta = 0.73$ (s, 3H, 19-CH_3), 0.98 (s, 3H, 18-CH_3), 1.45 (s, 3H, 21-CH_3), 3.82 (s, 3H, OCH_3), 4.92 (s, 1H, C_4-H), 5.17 (m, 1H, C_{16}–H), 7.02 (dd, 2H, $^4J_{PH}$ 5 Hz, J_{HH} 9 Hz), 7.68 (dd, 2H, $^3J_{PH}$ 15 Hz, J_{HH} 9 Hz); MS (m/z): 498 (M^+, 51%); Found: C, 67.39; H, 7.11; P, 6.32; S, 12.92. $C_{28}H_{35}PO_2S_2$ (498.69). Requires: C, 67.43; H, 7.07; P, 6.21; S, 12.86%.

Bis[3-oxoprosta-4,17-dien-2-yl]sulfide (15). Yellow crystals from methanol, yield 1.10 g (35%), m.p. 220°C; IR: $\upsilon = 2937$, 2925 (CH_3, CH_2), 1675 (2C=O), 1595 (C=C) cm^{-1}; ^1H NMR (DMSO): $\delta = 0.83$ (s, 6H,

2 CH_3), 1.18 (s, 6H, 2 CH_3), 2.02 (s, 6H, 2 CH_3), 5.25 (s, 2H, $2C_4$—H); MS (m/z): 626 (M^+, 48%), 313 (37%), 297 (40%); Found: C, 80.49; H, 9.37; S, 5.07. $C_{42}H_{58}O_2S$ (626.99). Requires: C, 80.45; H, 9.32; S, 5.11%.

Preparation Method of Compounds 17 and 19.

General Procedure

Equimolar amounts of each of 5α-cholestan-3-one **16** (0.77 g, 0.002 mmol), or 17β-hydroxy-5α-androstan-3-one **18** (0.58 g, 0.002 mmol), elemental sulfur (0.064 g, 0.002 mmol) and ethyl cyanoacetate (0.23 g, 0.002 mmol) in ethanol (30 ml) containing a catalytic amount of triethylamine (0.5 ml), were heated under reflux for 3 h then left to cool. The solid product formed after pouring into iced/water containing few drops of hydrochloric acid, in each case, was collected by filtration and crystallized from the proper solvent.

Ethyl 2′-aminothieno[2′,3′:2,3]-5α-cholestan-3′-carboxylate (17). Pale brown crystals from ethanol, yield 0.81 g (79%), m.p. 181°C; IR: υ = 3350 (NH_2), 2965, 2850 (CH_3, CH_2), 1735 (C=O, ester), 1610 (C=C) cm^{-1}; ^{1}H NMR (DMSO): δ = 0.73 (s, 3H, 18-CH_3), 0.95 (s, 3H, 19-CH_3), 1.35 (t, 3H, CH_3, ester), 3.45–3.65 (m, 1H, C_5—αH), 4.25 (q, 2H, CH_2-ester), 5.05 (s, 2H, NH_2, D_2O-exchangeable); MS (m/z): 513 (M^+, 95%), 197 (35%); Found: C, 74.91; H, 9.95; N, 2.80; S, 6.45. $C_{32}H_{51}NO_2S$ (513.83). Requires: C, 74.80; H, 10.00; N, 2.72; S, 6.42%.

Ethyl 2′-amino-17β-hydroxythieno[2′,3′:2,3]-5α-androstan-3′-carboxylate (19). Yellow crystals, from ethanol, yield 0.62 g (74%), m.p. 94–95°C; IR: υ = 3445–3380 (OH) 3335 (NH_2), 2975, 2835 (CH_3, CH_2), 1730 (C=O, ester), 1595 (C=C) cm^{-1}. ^{1}H NMR (DMSO): δ = 0.78 (s, 3H, 18-CH_3), 1.05 (s, 3H, 19-CH_3), 1.54 (t, 3H, CH_3, ester), 3.75–3.82 (m, 1H, C_5—αH), 4.20 (q, 2H, CH_2-ester), 5.85 (s, 1H, NH_2, D_2O-exchangeable), 11.20 (s, 1H, OH). MS (m/z): 417 (M^+, 85%), 197 (55%). Found: C, 68.95; H, 8.40; N, 3.38; S, 7.63. $C_{24}H_{35}NO_3S$ (417.16). Requires: C, 69.02; H, 8.44; N, 3.35; S, 7.67%.

The Reaction of Compounds 17 and 19 with Lawsson's Reagent (22, 23)

General Procedure

To a solution of each of compounds **17** (1.02 g, 0.002 mmol) or **19** (0.83 g, 0.002 mmol) in dry toluene (35 ml), equivalent amount of LR (0.80 g, 0.002 mmol) was added; the reaction mixture was heated under reflux for 12 h. The solvent was evaporated under vacuum. The solid product formed after washing the remaining residue several times with n-hexane was collected by filtration and crystallized.

2″-(4-Methoxyphenyl)1″,3″,2″-thiazaphosphorino[5″,4″:4′,5′]-thieno[2′,3′:2,3]-5α-cholestan-2″,6″-dithione (22). Orange crystals, from benzene, yield 70% (0.96 g), m.p. 110°C; IR: $\upsilon = 3420–3325$ (NH), 3045 (CH-aromatic), 2965, 2870 (CH_3, CH_2), 1598 (C=C), 1110 (C=S), 690 (P=S) cm^{-1}; ^1H NMR (DMSO): $\delta = 0.65$ (s, 3H, 19-CH_3), 0.86 (s, 3H, 18-CH_3), 3.81 (s, 3H, OCH_3), 3.67–3.78 (m, 1H, C_5–αH), 6.95–7.05 (m, 2H, aromatic protons), 7.45–7.60 (m, 2H, aromatic protons), 10.45 (s, 1H, NH, D_2O-exchangeable); MS (m/z): 685 (M^+, 72%); Found: C, 64.73; H, 7.58; N, 2.07; S, 18.73; P, 4.48. $C_{37}H_{52}NPOS_4$ (686.06). Requires: C, 64.77; H, 7.63; N, 2.04; S, 18.69; P, 4.51%.

17β-Hydroxy-2″-(4-methoxyphenyl)-1″,3″,2″-thiazaphosphorino[5″,4″:4′,5′]thieno[2′,3′:2,3]5α-androstan-2″,6″-dithione (23). Brown crystals, from benzene, yield 62% (0.85 g), m.p. 205–206°C; IR: $\upsilon = 3530–3450$ (OH, NH), 3025 (CH-aromatic), 2985, 2875 (CH_3, CH_2),1605 (C=C), 1055 (C=S), 687 (P=S) cm^{-1}; ^1H NMR (DMSO): $\delta = 0.79$ (s, 3H, 19-CH_3), 0.96 (s, 3H, 18-CH_3), 3.81 (s, 3H, OCH_3), 3.85–3.98 (m, 1H, C_5–αH), 6.98–7.15 (m, 2H, aromatic protons), 7.48–7.62 (m, 2H, aromatic protons), 10.65 (s, 1H, NH, D_2O-exchangeable), 11.25 (s, 1H, OH); MS (m/z): 589 (M^+, 67%); Found: C, 59.08; H, 6.11; N, 2.40; S, 21.76; P, 5.28. $C_{29}H_{36}NPO_2S_4$ (589.85). Requires: C, 59.05; H, 6.15; N, 2.37; S, 21.74; P, 5.25%.

Antimicrobial Bioassay

Procedure of Antibacterial Assay

Preparation of Bacterial Suspensions. Suspensions of the microorganisms were prepared by suspending each bacterium in 5 ml sterile nutrient broth media, using a standard loop, then incubating the inoculated nutrient broth at 37°C for 2 h. One ml of each suspension added to the center sensitivity testing plate. A sterile dry cotton wool swap used to spread the inoculums on the media. The inoculants allowed to dry for few minutes.

Preparation of Discs. The compounds were tested as 200 μg/ml (W/V) solutions in sterile DMSO. Discs of 6 mm diameter of filter paper, were placed in Petri dish (each one contains 10 discs) and then sterilized in a hot air oven at 180°C for 1 h. After cooling, 1 ml of the tested compound solution added onto each 10 discs to make 20 μg concentration per one disc. The discs dried in the incubator at 37°C, for 1 h or over phosphorous pentoxide (P_2O_5) in a dissector under vacuum. Then distributed on the inocula by sterile forceps, each disc should be pressed down on the medium and should not be moved once in place. The plates incubated at 37°C overnight. The clear inhibition zones were measured in mm.

Procedure of Antifungal and Antiyeast Assay

The activity of tested compounds against fungi and yeast were carried out by disc-diffusion method,[37] malt-yeast extract agar and Czapek-Dox's agar were used for cultivating the yeast and fungal test organisms respectively. The discs of standard concentration (100 μg/disc) of each one of the tested compounds were appropriately placed on the surface of an agar plate freshly seeded with standard inoculum of young culture (48 h old yeast and 7 days old fungi). The plates kept at 5°C for 1 h to allow diffusion of the compounds through the agar media and then maintained at 30°C for 4 days. At the end of the incubation period, the clear inhibition zones measured in mm.

The preliminary antimicrobial activity[38] was recorded in comparison to standard antibacterial ampicillin (10 mg/ml) and antifungal nystanin (10 mg/ml) in distilled water.

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