SYNTHESIS AND in vitro ANTIMETABOLIC EVALUATION OF SOME STEROIDAL THIAZOLES*, **

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Steroidal thiazoles *VI—XII* have been synthesized. The starting bromoketones *XVII* and *XX* were prepared by bromination of pregnan-20-ones in position 21 with copper(II) bromide, and used for synthesis of the thiazole derivatives employing the Hantzsch reaction. Preliminary biological evaluation of thiazoles *I—XII* against the P388 lymphocytic leukemia cell line showed growth inhibition values of ED_{50} 2·9 and 7 μg/ml for thiazoles *II* and *VIII*, respectively.

Preliminary observation that some steroidal thiazoles inhibit significantly (up to 75%) the incorporation of amino acids and nucleic acid components into Ehrlich ascites carcinoma cells prompted us to perform a wider screening study, using the U.S. National Cancer Institute's lymphocytic leukemia P 388 cell line.

The synthetic availability of steroidal thiazoles by the Hantzsch reaction[3-6] enabled us to prepare a wider set of these derivatives. Using this method we prepared compounds *I*, *II* (ref.[3]), *III—V* (ref.[6]) and *VI—XII*, together with some thiazolyl steroids (*XIII—XV*) which were not included in the screening (see Table I). As starting material for the Hantzsch reaction we used 21-bromo-3β-hydroxy-5-pregnen--20-one 3-acetate (*XVII*), obtained from pregnenolone acetate *XVI* by reaction with copper(II) bromide and pyridine in methanol[7]. The Numazawa recation with copper-(II) bromide is very suitable for bromination of a 3-protected pregnenolone, being superior to hitherto used methods of obtaining steroidal 21-bromoketones with respect to yields as well as simplicity (cf. refs[4,6]). Crucial for the reaction is the quality of the employed copper(II) bromide. The best results were obtained when the reagent was prepared by a modified[8] method (see Experimental). Commercial preparations (Fluka, purum: p.a.) can also be used, though with lower yields.

* A part of this communication has been presented in a preliminary form[1].

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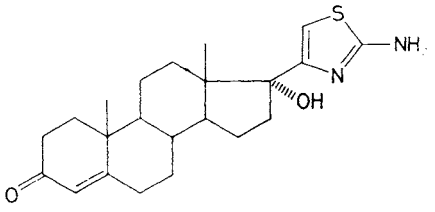

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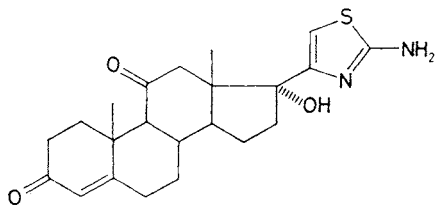

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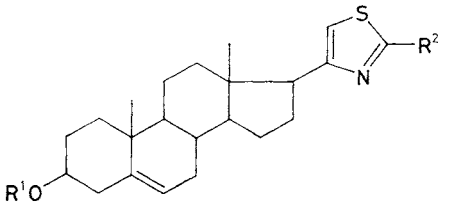


III, R^1 = Ac ; R^2 = $COOC_2H_5$
IV, R^1 = Ac ; R^2 = NHCOH
V, R^1 = H ; R^2 = NHCOH
VI, R^1 = Ac ; R^2 = $N(CH_3)_2$
VII, R^1 = Ac ; R^2 = $NHCH_3$
VIII, R^1 = Ac ; R^2 = NH_2
IX, R^1 = Ac , R^2 = NHC_6H_5
X, R^1 = Ac ; R^2 = C_6H_5
XI, R^1 = Ac ; R^2 = CH_3
XII, R^1 = Ac ; R^2 = CH_2COOCH_3
XIII, R^1 = H ; R^2 = C_6H_5
XIV, R^1 = H ; R^2 = CH_3
XV, R^1 = H ; R^2 = CH_2COOCH_3

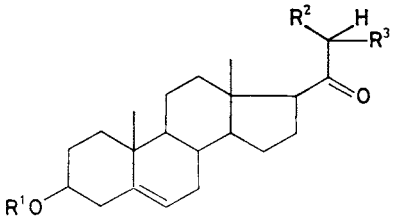
TABLE I

Inhibition of incorporation of nucleic acid and protein precursors into Ehrlich ascites carcinoma cells[a]

Compound	Inhibition of substrate incorporation, %	
	adenine	valine
I	36·4	48·2
II	34·6	34·6
III[b]	74·4	33·5
IV	39·5	56·6

[a] Executed according to ref.[2], concentration 100 μg/ml (corresponds to molar concentration of about $2 \cdot 10^{-4}$ mol l^{-1}), ^{14}C-labeled substrates. [b] Because of low solubility of the compound, the measurement was performed at three times lower concentration.

Bromoketone *XVII* was prepared in 30% yield and characterized by comparison with an authentic sample[6,9]. In a similar yield (34%) we obtained protected bromoketone *XX* (cf. ref.[4]) from the corresponding protected ketone *XIX* (ref.[4]). Bromination of 3-acetate *XVI* gave (in addition to recovered *XVI*) several minor side-products: along with compounds described by Numazawa[7] we also isolated 2·5% of the 21,21-dibromo derivative *XVIII*, characterized by IR bands at 1 725 and 1 255 cm^{-1} (acetate), 1 725 cm^{-1} (ketone) and 1 668 cm^{-1} (double bond), and by ^1H NMR signals at $\delta = 5{\cdot}84$ (H-21) and $\delta = 5{\cdot}37$ (skeletal double bond). The highest peak in the mass spectrum corresponded to a fragment formed by loss of acetic acid from the dibromo molecule.



XVI, R^1= Ac ; R^2= H ; R^3= H
XVII, R^1= Ac ; R^2= Br ; R^3= H
XVIII, R^1= Ac ; R^2= Br ; R^3= Br
XIX, R^1= CH_2OCH_3 ; R^2= H ; R^3= H
XX, R^1= CH_2OCH_3 ; R^2= Br ; R^3= H

Hantzsch reaction with the corresponding thioamides afforded thiazoles in yields ranging from 50% to 85%. In several cases we observed partial deacetylation apparently caused by production of hydrogen bromide. According to the literature, the Hantzsch reaction can be performed in acetonitrile[6,10], N,N-dimethylformamide[6,10], ethanol[10] or acetone[10]. For most experiments, we found ethanol to be the solvent of choice because the work-up was simpler than in the case of N,N-dimethylformamide, the yields and conversion being the same. Reactions in acetonitrile led sometimes to side-reactions which were not followed further; acetone was not evaluated.

The prepared thazoles were characterized by their ^1H NMR spectra. Thus, dimethylaminothiazole *VI* exhibited a signal at $\delta = 3{\cdot}02$ (dimethylamino group), methylaminothiazole *VII* a signal at $\delta = 2{\cdot}90$ (methylamino group), aminothiazole thiazole *VIII* (cf. ref.[6]) a signal at $\delta = 4{\cdot}98$ (amino group). The spectrum of phenylaminothiazole *IX* displayed a multiplet at $\delta = 7{\cdot}10-7{\cdot}50$ due to phenylamine moiety, phenylthiazole *X* a multiplet at $\delta = 7{\cdot}25-8{\cdot}15$ (phenyl), methylthiazole *XI* a methyl signal at $\delta = 2{\cdot}68$ and methoxycarbonylmethyl thiazole *XII* signals at $\delta = 4{\cdot}10$ and 3·78 (methylene group between the thiazole ring and the ester group).

The spectra had all characteristic parameters[4-6,11] for 2'-substituted 1',3'-thiazoles, attached to the steroid moiety by a C(17)—C(4') bond.

As already mentioned, in the preparation of thiazoles *X*–*XII* the isolation procedure led to partial deacetylation to the 3-alcohols *XIII*–*XV* whose physico-chemical parameters were again in accord with the assumed structures[4-6,11]. Since the thiazole *XV* was obtained only in small quantities, it was prepared from the bromoketone *XX*; the reaction was accompanied by loss of the methoxymethyl protecting group[4].

Thiazoles *I*–*XII* were evaluated against the U.S. National Cancer Institute's P388 lymphocytic leukemia cell line (murine)[12]. Substances *II* and *VIII* showed, respectively, ED_{50} 2·9 and 7·0 μg ml ($7{\cdot}5 \cdot 10^{-6}$ and $16{\cdot}9 \cdot 10^{-6}$ mol l^{-1}). All the other thiazoles *I*, *III*–*VII*, *IX*–*XII* were "inactive", with ED_{50} values greater than 10 μg ml. Two steroidal thiazoles *I* and *II* (see ref.[4]), containing a corticoid skeleton and a ketone group in position 3, were included in our set for comparison with data obtained in another Laboratory. The cytostatic activity of thiazoles *II* and *VIII* approximated that of the natural thiazole dolastatin 3 (ref.[13]) but was considerably less than that of dolastatin 10 (ref.[14]).

EXPERIMENTAL

Melting points were determined on a micro melting point apparatus Boetius (G.D.R.). Optical rotations were measured at 25°C on a Perkin–Elmer 141 MC polarimeter. Infrared spectra were recorded on a Zeiss UR-20 spectrometer (wavenumbers in cm^{-1}). ^1H NMR spectra were taken on a Tesla BS-476 instrument (CW model, 60 MHz) at 23°C in deuteriochloroform with tetramethylsilane as internal standard, unless stated otherwise. Chemical shifts are given in ppm (δ-scale), coupling constants (J) and bandwidths (W) in Hz. All values were obtained by the first order analysis. Mass spectra were measured on a Jeol D-100 spectrometer at 75 eV. Colum chromatography was performed on silica gel (according to Pitra, 60—120 μm) or on neutral alumina (Reanal, activity II), thin-layer chromatography on silica gel G according to Stahl (ICN Biochemicals) and on aluminium oxide, neutral grade (Reanal No. 3). Prior to evaporation, solutions in organic solvents were dried over anhydrous magnesium or sodium sulfate. Solvents were evaporated in vacuo (about 2 kPa). Analytical samples were dried over phosphorus pentoxide at 40°C/26 Pa for 12 h. The identity of samples prepared by different routes was checked by comparison of their IR and ^1H NMR spectra, thin-layer chromatography and mixture melting point determination. Commercial copper(II) bromide (quality "purum: p.a.") was purchased from Fluka.

Copper(II) Bromide

Acetyl bromide (45 ml, 0·61 mol) was added dropwise during 10 min to a stirred mixture of copper(II) acetate monohydrate (36·97 g, 0·19 mol) and benzene (140 ml). After stirring for further 30 min, the supernatant was decanted from the solid which was again suspended in benzene (140 ml). Acetyl bromide (45 ml, 0·61 mol) was added dropwise during 10 min to this mixture and the stirring was continued for 30 min. The solid material was separated by decantation and washed with dry benzene (3 × 110 ml). After decanting the last portion of benzene, the solid was dried in vacuo (oil pump) at 40°C until it became loose. Yield 26·5 g (64%) of copper(II) bromide, identical with the compound described in literature[8].

17β-(4-(2-Dimethylamino-1,3-thiazolyl))-5-androsten-3β-ol 3-Acetate (*VI*)

Bromoketone *XVII* (498 mg, 1·14 mmol) and N,N-dimethylthiourea (119 mg, 1·14 mmol) were dried at 10 Pa for 30 min, dissolved in N,N-dimethylformamide (10 ml) and the solution was heated to 140°C (bath) for 1 h. The bright violet reaction mixture was poured into an ice-water mixture (150 ml) and the separated precipitate was collected, washed on the filter with water and dried (together with the filter) in a desiccator at 100 Pa. The cake, together with the filter paper, was cut into small pieces which were applied onto a column of alumina (150 g). Chromatography in dichloromethane (stabilized with 2% of ethanol, 250 ml) afforded the thiazole *VI* (300 g, 60%) as a solid foam. $[\alpha]_D^{25}$ −52° (*c* 0·1, chloroform). IR spectrum (chloroform): 1 725, 1 256 (CH_3COO); 1 556 (N=C thiazole); 1 670 (C=C). ^{1}H NMR spectrum: 0·50 s, 3 H (3 × × H-18); 1·01 s, 3 H (3 × H-19); 2·00 s, 3 H (CH_3COO); 2·28 bd, 2 H (H-7, J = 8); 3·02 s, 6 H ($N(CH_3)_2$); 4·59 m, 1 H (H-3, W = 45); 5·36 bd, 1 H (H-6, J = 4·5); 6·04 s, 1 H (H-5′, thiazole). For $C_{26}H_{38}N_2O_2S$ (442·7) calculated: 70·55% C, 8·65% H, 6·33% N, 7·24% S; found: 70·84% C, 8·52% H, 5·94% N, 6·67% S.

17β-(4-(2-Methylamino-1,3-thiazolyl))-5-androsten-3β-ol 3-Acetate (*VII*)

Bromoketone *XVII* (87 mg, 0·2 mmol) and N-methylthiourea (18 mg, 0·2 mmol) were dried at 10 Pa for 30 min, dissolved in methanol (2 ml) and refluxed for 3·5 h. The reaction mixture was filtered through a column of alumina (50 g) and eluted with a dichloromethane–ethanol mixture (1 : 1). The filtrate was stripped of the solvents and the residue was rechromatographed on a column of alumina (60 g) in dichloromethane (stabilized with 2% of ethanol, 400 ml). The main fraction was collected and the residue on lyophilization from benzene afforded 69 mg (81%) of the solid thiazole *VII*. $[\alpha]_D^{25}$ −70° (*c* 0·2, chloroform). IR spectrum (chloroform): 3 430 (NH); 1 725, 1 255 (CH_3COO); 1 670 (C=C); 1 555 (C=N thiazole). ^{1}H NMR spectrum: 0·52 s, 3 H (3 × H-18); 1·01 s, 3 H (3 × H-19); 2·01 s, 3 H (CH_3COO); 2·31 bd, 2 H (H-7, J = 9); 2·90 m, 3 H ($NHCH_3$, W = 12); 4·59 m, 1 H (H-3, W = 35); 5·36 bd, 1 H (H-6, J = 4·5); 6·08 s, 1 H (H-5′, thiazole). For $C_{25}H_{36}N_2O_2S$ (428·6) calculated: 70·05% C, 8·47% H, 6·54% N, 7·48% S; found: 70·25% C, 8·51% H, 6·53% N, 7·45% S.

17β-(4-(2-Amino-1,3-thiazolyl))-5-androsten-3β-ol 3-Acetate (*VIII*)

Bromoketone *XVII* (174 mg, 0·4 mmol) and thiourea (30 mg, 0·4 mmol) were dried at 10 Pa for 30 min, dissolved in ethanol (3 ml) and refluxed for 5 h. The reaction mixture was filtered through a column of alumina (50 g), the column was washed with dichloromethane–ethanol (1 : 1) and the residue chromatographed on alumina (60 g) in dichloromethane (stabilized with 2% of ethanol, 700 ml). Lyophilization from benzene afforded thiazole *VIII* as a solid foam; yield 130 mg (78%). $[\alpha]_D^{25}$ −62° (*c* 0·2, chloroform), IR spectrum (chloroform): 3 485, 3 395, 1 605 (NH_2); 1 725, 1 255 (CH_3COO); 1 670 (C=C); 1 525 (C=N, thiazole). ^{1}H NMR spectrum: 0·49 s, 3 H (3 × H-18); 0·99 s, 3 H (3 × H-19); 2·00 s, 3 H (CH_3COO); 2·28 bd, 2 H (H-7, J = 8); 3·45 m, 1 H (H-3, W = 35); 4·98 m, 2 H, (NH_2 thiazole, W = 20); 5·34 bd, 1 H (H-6, J = 4·5); 6·06 s, 1 H, (H-5′ thiazole). Mass spectrum, *m/z*: 414 (M^+); this value has already been published[6]. For $C_{24}H_{34}N_2O_2S$ (414·6) calculated: 69·53% C, 8·27% H, 6·76% N, 7·73% S; found: 69·83% C, 8·58% H, 6·66% N, 7·52% S.

17β-(4-(2-Phenylamino-1,3-thiazolyl))-5-androsten-3β-ol 3-Acetate (*IX*)

Bromoketone *XVII* (174 mg, 0·4 mmol) and N-phenylthiourea (61 mg, 0·4 mmol) were dried at 10 Pa for 30 min. After addition of ethanol (3 ml), the mixture was refluxed for 3 3/4 h and

filtered through a column of alumina (50 g). The column was washed with a dichloromethane–ethanol (1 : 1) mixture (60 ml), the filtrates were combined and the solvents evaporated. The residue was chromatographed on a silica gel column (50 g) in dichloromethane (stabilized with 2% of ethanol). The principal fraction afforded 190 mg of crude product which was crystallized from dichloromethane–methanol–ethanol, affording 110 mg (56%) of thiazole *IX*, m.p. 218 to 220°C, $[\alpha]_D^{25}$ −44° (*c* 0·2, chloroform). IR spectrum (chloroform): 3 408 (NH); 1 725, 1 255 (CH_3COO); 1 670 (C=C); 1 499, 1 605 (aromatic nucleus); 1 538 (C=N, thiazole). ^{1}H NMR spectrum: 0·50 s, 3 H (3 × H-18); 1·02 s, 3 H (3 × H-19); 2·01 s, 3 H (CH_3COO); 2·32 bd, 2 H (H-7, J = 8·5); 4·60 m, 1 H (H-3, W = 35); 5·38 bd, 1 H (H-6, J = 4·5); 6·22 s, 1 H (H-5′, thiazole); 7·10–7·50 m, 5 H (phenyl). For $C_{30}H_{38}N_2O_2S$ (490·7) calculated: 73·43% C, 7·81% H, 5·71% N, 6·53% S; found: 73·63% C, 7·89% H, 5·66% N, 6·50% S.

17β-(4-(2-Phenyl-1,3-thiazolyl))-5-androsten-3β-ol 3-Acetate (*X*)

A) Acetyl chloride (0·5 ml) was added to a solution of alcohol *XIII* (40 mg, 0·09 mmol) in pyridine (3 ml). After standing for 2 h, the solvent was removed and the residue was coevaporated with benzene (2 × 5 ml). The dry residue was chromatographed on a preparative plate (200 × × 200 × 0·7 mm) of silica gel in benzene–ether (5 : 1). The principal fraction gave acetate *X* (40 mg, 93%) as a solid foam, $[\alpha]_D^{25}$ −16° (*c* 0·2, chloroform). IR spectrum (chloroform): 1 725, 1 255 (CH_3COO); 1 668 (C=C); 1 510 (thiazole); 1 496 (phenyl). ^{1}H NMR spectrum: 0·51 s, 3 H (3 × H-18); 1·01 s, 3 H (3 × H-19); 2·01 s, 3 H (CH_3COO); 2·32 bd, 2 H (H-7, J = 8); 4·55 m, 1 H (H-3, W = 35); 5·38 bd, 1 H (H-6, J = 4·5); 6·88 s, 1 H (H-5′, thiazole); 7·25 to 8·15 m, 5 H (phenyl). For $C_{30}H_{38}NO_2S$ (476·7) calculated: 75·59% C, 8·03% H, 2·94% N, 6·71% S; found: 75·81% C, 8·33% H, 2·71% N, 6·55% S.

B) The acetate *X* (20 mg, 11%) was also obtained as a minor product (more lipophilic than *XIII*) in the preparation of the hydroxy derivative *XIII* (vide infra); the solid foam was identical with the product prepared ad *A*).

17β-(4-(2-Methyl-1,3-thiazolyl))-5-androsten-3β-ol 3-Acetate (*XI*)

Bromoketone *XVII* (190 mg, 0·43 mmol) and thioacetamide (33 mg, 0·43 mmol) were dried at 10 Pa for 30 min, dissolved in ethanol (5 ml), the solution was refluxed for 3 h and filtered through a column of alumina (50 g), which was then washed with dichloromethane–methanol (1 : 1). The solvents were evaporated and the residue was chromatographed on a column of alumina (140 g) in a 1 : 1 mixture of light petroleum–dichloromethane (stabilized with 2% of ethanol). Of the two principal fractions the less polar one (60 mg) afforded acetylated thiazole *XI* (47 mg; 28%), m.p. 173–176°C (methanol–dichloromethane), $[\alpha]_D^{25}$ −66° (*c* 0·3, chloroform). IR spectrum (chloroform): 1 724, 1 255 (CH_3COO); 1 516 (N=C, thiazole); 1 668 (C=C). ^{1}H NMR spectrum: 0·50 s, 3 H (3 × H-18); 1·02 s, 3 H (3 × H-19); 2·02 s, 3 H (CH_3COO); 2·52 bd, 2 H (H-7, J = 9); 2·68 s, 3 H (CH_3-2′, thiazole); 2·83 m, 1 H (H-17, W = 20); 4·57 m, 1 H (H-3, W = 35); 5·40 bd, 1 H (H-6, J = 4·5); 6·72 s, 1 H (H-5′, thiazole). For $C_{25}H_{36}NO_2S$ (414·6) calculated: 72·42% C, 8·75% H, 3·38% N, 7·53% S; found: 72·63% C, 8·96% H, 3·27% N, 7·34% S.

17β-(4-(2-Methoxycarbonylmethyl-1,3-thiazolyl))-5-androsten-3β-ol 3-Acetate (*XII*)

A mixture of bromoketone *XVII* (300 mg, 0·69 mmol), N,N-dimethylformamide (10 ml) and methyl thiocarbamoylacetate (110 mg, 0·82 mmol) was heated with stirring to 110°C (bath) for 2 h. The reaction mixture was poured into water (100 ml) and the aqueous phase was extracted with

benzene (4 × 50 ml). The combined organic extracts were dried and the solvent removed. The residue was chromatographed on a column of silica gel (80 g) in light petroleum–benzene (1 : 1, 500 ml), light petroleum–benzene–ether (54 : 54 : 2; 4 000 ml) and benzene–ether (4 : 1). The principal fraction on crystallization from ether afforded 312 mg (96%) of thiazole *XII*, m.p. 159–161°C, $[\alpha]_D^{25}$ −61°C (*c* 0·2, chloroform). IR spectrum (chloroform): 3 120, 1 090, 1 020 (thiazole); 1 517 (C=N, thiazole); 1 729, 1 439 ($COOCH_3$); 1 739 sh, 1 258, 1 034 (CH_3COO). ^{1}H NMR spectrum: 0·53 s, 3 H (3 × H-18); 1·06 s, 3 H (3 × H-19); 2·05 s, 3 H (CH_3COO); 2·36 bd, 2 H (H-7, J = 9); 2·83 m, 1 H (H-7, W = 23); 3·78 s, 3 H ($COOCH_3$); 4·10 s, 2 H ($COCH_2$-2′, thiazole); 4·60 m, 1 H (H-3, W = 35); 5·41 bd, 1 H (H-6, J = 4·5); 6·90 s, 1 H (H-5′, thiazole). Mass spectrum, *m/z*: 471 (M^+), 411 (M − CH_3COOH), 396 (411 − CH_3). For $C_{27}H_{37}NO_4S$ (471·6) calculated: 68·76% C, 7·91% H, 2·97% N, 6·80% S; found: 68·79% C, 8·03% H, 2·91% N, 6·62% S.

17β-(4-(2-Phenyl-1,3-thiazolyl))-5-androsten-3β-ol (*XIII*)

Bromoketone *XVII* (174 mg, 0·4 mmol) and thiobenzamide (55 mg, 0·4 mmol) were dried at 10 Pa for 30 min, dissolved in ethanol (5 ml), refluxed for 3 h and filtered through a column of alumina (50 g) which was then washed with a 1 : 1 mixture of dichloromethane–methanol (50 ml). Solvents were removed from the combined filtrates and the residue was chromatographed on a column of alumina in dichloromethane (stabilized with 2% of ethanol). The main fraction, after evaporation and crystallization from dichloromethane–methanol–water, afforded thiazole *XIII* (145 mg, 84%), m.p. 147–149°C; $[\alpha]_D^{25}$ −88° (*c* 0·2, chloroform). IR spectrum (chloroform): 3 610, 3 540 (OH), 1 490, 1 600 (phenyl), 1 668 (C=C), 1 512 (C=N, thiazole). ^{1}H NMR spectrum: 0·53 s, 3 H (3 × H-18); 1·00 s, 3 H (3 × H-19); 2·27 bd, 2 H (H-7, J = 8); 2·83 m, 1 H (H-17, W = 18); 3·51 m, 1 H (H-3, W = 38); 5·40 bd, 1 H (H-6, J = 4·5); 6·88 s, 1 H (H-5′, thiazole); 7·25–8·15 m, 5 H (phenyl). Mass spectrum, *m/z*: 433 (M^+, $C_{28}H_{35}NOS$), base peak; 418 (M − CH_3), 400 (M − OH − CH_3); 188 and 175 (thiazole with fragments of D-ring); peak 175 was the second strongest. For $C_{28}H_{35}NOS$ (433·7) calculated: 77·55% C, 8·13% H, 3·23% N, 7·39% S; found: 77·23% C, 8·32% H, 3·11% N, 7·27% S.

17β-(4-(2-Methyl-1,3-thiazolyl))-5-androsten-3β-ol (*XIV*)

The more polar fraction from the preparation of acetate *XI* afforded 43 mg (27%) of alcohol *XIV*, m.p. 189–191°C (methanol), $[\alpha]_D^{25}$ −66° (*c* 0·4, chloroform). IR spectrum (chloroform): 3 610, 3 520 (OH); 1 666 (C=C); 1 516 (C=N, thiazole). ^{1}H NMR spectrum: 0·50 s, 3 H (3 × H-18); 1·01 s, 3 H (3 × H-19); 2·26 bd, 2 H (H-7, J = 8·5); 2·68 s, 3 H (CH_3-2′, thiazole); 2·83 m, 1 H (H-17, W = 20); 3·48 m, 1 H (H-3, W = 35); 5·35 bd, 1 H (H-6, J = 4·5), 6·71 s, 1 H (H-5′, thiazole). For $C_{23}H_{33}NOS$ (371·6) calculated: 80·81% C, 8·95% H, 3·77% N, 8·63% S; found: 80·95% C, 8·96% H, 3·65% N, 8·34% S.

17β-(4-(2-Methoxycarbonylmethyl-1,3-thiazolyl))-5-androsten-3β-ol (*XV*)

A) A mixture of bromoketone *XX* (500 mg; 1·14 mmol), methyl thiocarbamoylacetate (155 mg, 1·17 mmol) and acetonitrile (18 ml) was refluxed for 4 h. Another portion (50 mg, 0·38 mmol) of methyl thiocarbamoylacetate was added and the reflux was continued for further 20 h. Silica gel (5 g) was added, the solvent was evaporated and the residue chromatographed on a column of silica gel (50 g) in benzene–ether (4 : 1, 2 l). The main fraction was treated with charcoal, filtered and the solvents were evaporated, leaving 700 mg (69%) of thioamide *XV* as a viscous oil; $[\alpha]_D^{25}$ −55° (*c* 0·2, chloroform). IR spectrum (chloroform): 3 120, 1 090, 1 020

(thiazole); 1 517 (C=N, thiazole); 1 739, 1 258, 1 035 (CH_3COO). ^{1}H NMR spectrum: 0·53 s, 3 H (3 × H-18); 1·06 s, 3 H (3 × H-19); 2·37 bd, 2 H (H-7, J = 9); 2·83 m, 1 H (H-17, W = 25); 3·47 m, 1 H (H-3, W = 35); 3·78 s, 3 H (CH_3O); 4·10 s, 2 H ($COCH_2$-2′ of thiazole); 5·41 bd, 1 H (H-6, J = 4·5); 6·90 s, 1 H (H-5′, thiazole). For $C_{25}H_{35}NO_3S$ (429·4) calculated: 69·01% C, 8·11% H, 3·22% N; found: 68·72% C, 8·23% H, 3·45% N.

B) Compound *XV* was also obtained from the minor fraction upon chromatography of compound *XII* (2·7 mg, 0·9%); the product was identical with that prepared ad *A*).

21-Bromo-3β-hydroxy-5-pregnen-20-one 3-Acetate (*XVII*)

Anhydrous copper(*II*) bromide (5 g, 22·4 mmol) was added to a solution of ketone *XVI* (2·68 g, 7·47 mmol) in a mixture of methanol (82 ml) and pyridine (1·81 ml, 22·4 mmol). After refluxing for 12 h, the mixture was filtered, solid material was washed with dichloromethane, the filtrate was mixed with silica gel (20 g) and the solvents were evaporated. The material was applied onto a column of silica gel (70 g) and eluted with dichloromethane–ether (1 : 1, 250 ml). The eluate was again mixed with silica gel (20 g), evaporated and applied onto a column of silica gel (280 g). Chromatography in benzene afforded four fractions which on evaporation left 130 mg, 1·1 g, 30 mg, and 1·7 g of material. The second fraction was crystallized from benzene–acetone to afford bromoketone *XVII* (950 mg, 30%) whose properties were identical with those published[9].

An alternative isolation procedure consisted in replacement of the first chromatography on silica gel column (70 g) by extraction: the reaction mixture was poured into saturated aqueous ammonium chloride and the product was extracted with ether. The combined ethereal extracts were then washed with saturated aqueous ammonium chloride solution, dried, the solvent was evaporated and the product was chromatographed on a silica gel column. This procedure gave the bromoketone *XVII* in the same yield.

21,21-Dibromo-3β-hydroxy-5-pregnen-20-one 3-Acetate (*XVIII*)

The first fraction from chromatography in the preparation of bromoketone *XVII* (130 mg) was crystallized from ether–light petroleum, affording 96 mg (2·5%) of the dibromo derivative *XVIII*, m.p. 109–113°C. $[\alpha]_D^{25}$ +39° (*c* 0·3, chloroform). IR spectrum (chloroform): 1 725, 1 255 (CH_3COO); 1 725 (C=O); 1 668 (C=C). ^{1}H NMR spectrum ($CDCl_3$, Varian XL-200, FT mode (200 MHz)): 0·73 s, 3 H (3 × H-18); 1·02 s, 3 H (3 × H-19); 2·03 s, 3 H (CH_3COO); 2·33 bd, 2 H (H-7, J = 8·5, W = 35); 3·01 t, 1 H (H-17, J = 9); 4·60 m, 1 H (H-3, W = 32); 5·37 bd, 1 H (H-6, J = 4·5); 5·84 s, 1 H (H-21). Mass spectrum, *m/z*: 454 ($M - CH_3COOH$)$^{+\cdot}$ = = $C_{21}H_{28}O^{79}Br_2$; 439; 375 and 374 ($M - CH_3COOH - {}^{79}Br$)$^+$ and ($M - CH_3COOH - H^{79}Br$)$^{+\cdot}$; 255 ($M - CH_3COOH - CH^{79}Br_2$)$^+$ = $C_{19}H_{47}$. For $C_{23}H_{33}Br_2O_3$ (517·3) calculated: 53·40% C, 6·43% H, 30·89% Br; found: 53·36% C, 6·48% H, 30·93% Br.

21-Bromo-3β-methoxymethoxy-5-pregnen-20-one (*XX*)

Anhydrous copper(II) bromide (1·34 g, 3 mmol) was added to a solution of ketone *XIX* (ref.[4], 721 mg, 2 mmol) in a mixture of methanol (22 ml) and pyridine (0·49 ml, 6 mmol). After refluxing for 12·5 h, the mixture was poured into saturated aqueous solution of ammonium chloride and the product was taken up in ether. The ethereal extracts were washed with saturated aqueous ammonium chloride, dried and the solvent was evaporated. Chromatography of the residue on a column of silica gel (70 g) in benzene afforded as the main fraction bromoketone *XX* (300 mg, 34%), m.p. 86–88°C (dichloromethane–light petroleum), identical in all respects with an authentic sample[4].

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