SYNTHESIS OF 17β-[4-(1,3-THIAZOYL)]ANDROSTANE 3β-HEMISUCCINATE AND GLYCOSIDE*,**

Pavel DRAŠAR, Vladimír POUZAR, Ivan ČERNÝ, Jorga SMOLÍKOVÁ and Miroslav HAVEL

Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, 166 10 Prague 6

Received September 14th, 1983

The protected 20(21)-en-20-ol ether *III* was oxidized with N-methylmorpholine N-oxide monohydrate and osmium tetroxide to give the hydroxy ketone *IV* which was converted into the bromo derivative *VIII via* the mesylate *VI*. Hantzsch reaction of the bromo ketone *VIII* with ethyl thioxamate afforded the thiazole *XI* whose hemisuccinate *XIII* and glycoside *XV* were prepared.

Heterocyclic steroidal derivatives containing sulfur and nitrogen atoms in some of the rings represent a group of compounds important for their biological activity[1]. The present paper is a continuation of syntheses of steroidal thiazoles, carried out in this Institute[2,3]. These thiazole derivatives are structurally related to cardenolides and their study is promising both from the synthetic and biological point of view.

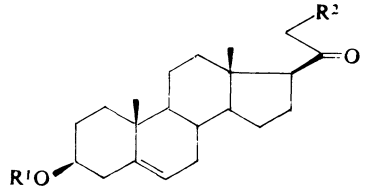
In the synthesis of the thiazole *XI* the key compound was the hydroxy ketone *IV* which was prepared in the following way. Reaction of pregnenolone (*I*) with chloromethyl methyl ether in the presence of N,N-dimethylaniline[4] gave the methoxymethoxy derivative *II* which had been prepared already previously by another method[5].The ketone *II* was transformed by means of lithium diisopropylamide into the enolate with terminal double bond and further by chlorotrimethylsilane and triethylamine into the trimethylsilyl enol ether *III*. Its structure was confirmed by the ^{1}H NMR spectrum, particularly by the signal of the trimethylsilyl group at $\delta = 0{\cdot}18$ and of two olefinic protons at $\delta = 3{\cdot}97$. The hydroxy ketone *IV* was obtained by oxidation of the enol ether *III* with N-methylmorpholine N-oxide monohydrate[6] and osmium tetroxide[7]. Its ^{1}H NMR spectrum displayed the CH_2OH signal at $\delta = 4{\cdot}16$ and IR spectrum exhibited bands due to carbonyl at 1 704 cm^{-1} and hydroxyl at 3 475 cm^{-1}. To prove the structure of the hydroxy ketone *IV*, the methoxymethyl protecting group was removed with *p*-toluenesulfonic acid monohydrate in benzene–methanol[8] to give the known[9] dihydroxy ketone *V*. The hydroxy ketone *IV* was converted[2,3,10] into the mesylate *VI*. The reaction was accompanied by formation of the chloro derivative *VII* which was isolated in 8% yield. The mesylate *VI* showed

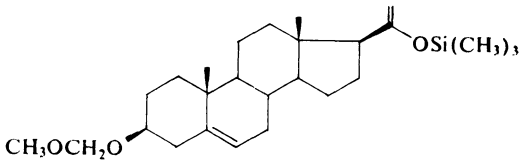
* Part CCCVII in the series On Steroids; Part CCCVI: This Journal *49*, 892 (1984).

** Presented in part at the X. Conference on Isoprenoids, Třeboň, ČSSR 1983.

IR bands at 1 720 cm^{-1} (C=O group) and 1 360 and 1 172 cm^{-1} due to the methanesulfonyl group and by signals of methanesulfonyl protons at $\delta = 3{\cdot}22$ and of—OCH_2.OCH_3 protons at $\delta = 4{\cdot}68$ and $\delta = 3{\cdot}35$ in the ^1H NMR spectrum. The chloro derivative was characterized mainly by a peak at m/z 332 in its mass spectrum, arising by loss of the $C_2H_6O_2$ fragment from the methoxymethyl grouping in the 3β-position. This peak appears as a doublet, characteristic for the presence of a chlorine atom. The presence of chlorine is confirmed also by the fragment at m/z 255, arising by loss of the side chain ($COCH_2Cl$) from the fragment at m/z 332. Reaction of the mesylate *VI* with excess of lithium bromide afforded[2,3] the bromo derivative *VIII*. Its ^1H NMR spectrum resembled that of the chloro derivative *VII*, differing only in shielding of protons at $C_{(21)}$ due to the halogen atom ($\delta = 4{\cdot}06$ and $3{\cdot}90$ for *VII* and *VIII*, respectively). Also the carbonyl bands in the IR spectrum of *VII* are situated at 1 727 and 1 711 cm^{-1}, as compared with those at 1 720 and 1 704 cm^{-1} for *VIII*.

Hantzsch reaction[11] of the bromo ketone *VIII* with ethyl thioxamate in acetonitrile afforded the thiazole *XI*. The reaction affords first the blocked thiazole *X* which, however, is not isolated. The liberated hydrogen bromide catalyses the removal of the methoxymethyl protecting group at $C_{(3)}$ not only in the thiazole *X* but also in the starting bromo ketone *VIII* and therefore in a short time the reaction mixture contains (after disappearance of the starting ether *VIII*) the free thiazole *XI* and the deprotected bromo ketone *IX*, which was indeed isolated. The bromo derivative *IX* has been described already several times[12-14] but its physical constants found by us differ from those given in earlier papers. There is, however, no doubt about the structure of our compound which was fully characterized. The bromo ketone *IX* was also converted under analogous conditions to the thiazole *XI* which exhibits spectral characteristics of a substituted thiazole, as shown in Table I and II.





III

I, $R^1 = H$; $R^2 = H$
II, $R^1 = CH_2OCH_3$; $R^2 = H$
IV, $R^1 = CH_2OCH_3$; $R^2 = OH$
V, $R^1 = H$; $R^2 = OH$
VI, $R^1 = CH_2OCH_3$; $R^2 = OSO_2CH_3$
VII, $R^1 = CH_2OCH_3$; $R^2 = Cl$
VIII, $R^1 = CH_2OCH_3$; $R^2 = Br$
IX, $R^1 = H$; $R^2 = Br$

TABLE I

Characteristic IR bands of steroidal thiazoles *XI*—*XXI*

Compound	Solvent	Wavenumbers, cm^{-1}					
XI	$CHCl_3$	3 110	[a]	1 500	1 303	1 091	1 025
XII	$CHCl_3$	3 110	[a]	1 500	1 304	1 091	1 018
XIII	$CHCl_3$	3 110	1 601	1 500	1 301	1 091	1 016
XIV	$CHCl_3$	3 120	[a]	1 502	1 303	1 096	1 025[b]
XV	KBr	3 105	[a]	1 498	1 302	[a]	[a]
XVI[c]	$CHCl_3$	3 130[d] 3 100[d]	1 635	[a]	1 315	1 148	1 036
XVII[c]	$CHCl_3$	3 120	[a]	1 504	1 305	1 096	1 035
XVIII[e]	$CHCl_3$	3 120	1 603	1 522	1 312	1 040	1 000
XIX[c]	$CHCl_3$	3 120	1 602	1 522	1 311	1 048	1 000
XX[c]	KBr	3 120	1 619	1 508	1 311	1 145	1 009
XXI[f]	$CHCl_3$	3 120	[a]	1 505	1 305	1 095	1 028

[a] Band not discernible; [b] shoulder at the side of a strong acetate band at 1 043 cm^{-1}; [c] taken from ref.[2]; [d] possible overlap with bands of the associated amides; [e] taken from ref.[19]; [f] taken from ref.[3].

TABLE II

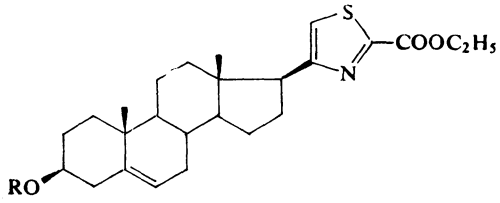
Characteristic signals in the ^1H NMR spectra of steroidal thiazoles in C^2DCl_3. Chemical shifts in ppm, δ scale, multiplet widths (W) in Hz

Compound	$C_{(5')}$—H[a]	$C_{(17)}$—H[b]
XI	7·20	2·83 (W = 18)
XII	7·19	[c]
XIII	7·21	[c]
XIV	7·20	3·00 (W = 20)
XV	7·68[d]	[c]
XVI[c]	6·57[f]	2·80 (W = 25)[f]
	6·80	2·85 (W = 18)
XVII[c]	7·18	2·88 (W = 17)
	6·95[f]	2·63 (W = 16)[f]
XVIII[g]	6·20	—
XIX[g]	6·27	—
XXI[h]	7·06[f]	2·82 (W = 18)[f]

[a] Singlet; [b] multiplet; [c] undeterminable value; [d] in deuteriochloroform — hexadeuteriodimethyl sulfoxide 3 : 1; [e] ref.[2]; [f] in tetrachloromethane; [g] ref.[19]; [h] ref.[3].

Table I shows IR spectral data for 11 steroidal thiazoles which generally exhibit bands at 3 100–3 130, 1 601–1 635, 1 498–1 522, 1 301–1 315, 1 040–1 148 and 1 000–1 036 cm^{-1}, whose position depends on the type of the substituent at $C_{(2')}$ but also to a certain extent on the steroid moiety. The bands were not assigned to the corresponding vibrations, since in some cases the assignment was not unequivocal.

Table II lists the ^1H NMR data for 10 steroidal thiazoles in which the chemical shift of the 5-thiazole proton ranges from $\delta = 6{\cdot}20$ to $\delta = 7{\cdot}21$ depending on the substituent at $C_{(2)}$ of the thiazole, the medium and the steroid moiety. The chemical shift of the $C_{(17)}$ proton signal ($\delta = 2{\cdot}80-2{\cdot}98$) and its shape indicate that in all the compounds the thiazole moiety is bonded to the steroid system in the same stereochemical way.



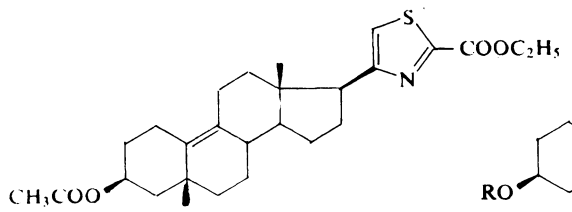
X, R = CH_2OCH_3
XI, R = H
XII, R = $COCH_2CH_2COOCH_2CCl_3$
XIII, R = $COCH_2CH_2COOH$
XVII, R = $COCH_3$

The structure of the thiazole *XI* is confirmed also by its mass spectrum which exhibits fragmentations characteristic for the steroid part of the molecule (*i.e.* loss of water and methyl) as well as the fission of the D-ring. As expected, the substitution on the thiazole ring is characterized by loss of the ethoxycarbonyl fragment. The thiazole moiety bonded to the D-ring gives rise to fragments, containing the whole thiazole grouping together with fragments of the D-ring. The equit mass measurements for the molecular ion $C_{25}H_{35}NO_3S$ and ions $C_{22}H_{30}NOS$, $C_8H_{10}NO_2S$ and $C_7H_9NO_2S$ represent also confirmation of the assumed structure.

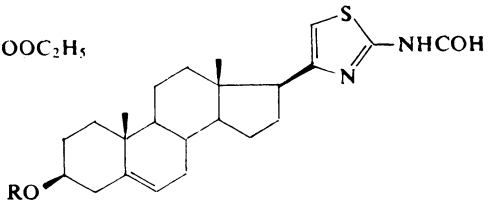
The free thiazole *XI* was converted to the mixed ester *XII* by reaction with 4-(2,2,2-trichloroethoxy)-4-oxobutanoic acid in benzene in the presence of N,N'-dicyclohexylcarbodiimide and 4-dimethylaminopyridine[15,16]. The product *XII* was characterized by IR bands due to the mixed ester structure at 1 752, 1 730 and 1 150 cm^{-1} as well as by the corresponding ^1H NMR signals at $\delta = 2{\cdot}96$ and 4·72. The carboxyl, protected as the trichloroethyl ester, was deblocked with zinc powder at 0°C in a tetrahydrofuran–acetic acid–water mixture[16–18], affording the hemisuccinate *XIII*. The hemisuccinate arrangement was confirmed by IR bands at 3 500 to 2 400, 1 729, 1 715 and 1 171 cm^{-1} and proton signals at $\delta = 2{\cdot}58$ in the ^1H NMR

spectrum. The hemisuccinate *XIII* was prepared as a thiazole derivative with an enhanced affinity towards aqueous medium in which most biological activities are tested.

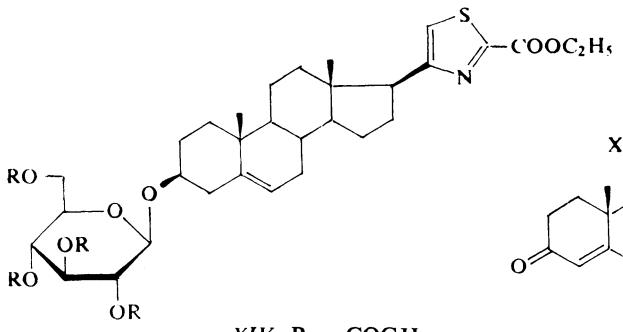
The glycoside *XV* was synthesized as another derivative of the thiazole *XI*. The glycosylation reaction was studied also to investigate its potential use in the reaction with thiazoles of the type *XI*. According to the method developed for synthesis of oligosaccharides[21], the thiazole *XI* was condensed with acetobromoglucose using silver silicate as catalyst[20]. The use of an excess (1·7 equivalent) of the halogenose afforded the acetyl glucoside *XIV* in a 50% yield. The β-configuration of the D-glucopyranose moiety follows from the chemical shift of the proton on $C_{(1'')}$ (δ = 4·60) and the coupling constant ($J_{1'',2''}$ = 8 Hz; *cf.* ref.[20]).



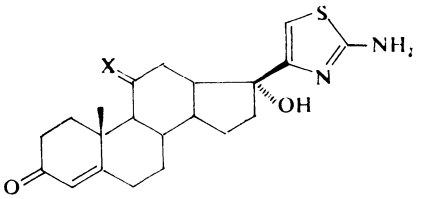
XXI



XVI, R = $COCH_3$
XX R = H



XIV, R = $COCH_3$
XV, R = H



XVIII, X = H_2
XIX, X = O

The hydroxy groups of the sugar component were deblocked under very mild conditions by treatment with sodium ethoxide in ethanol at room temperature for 24 h, to give the glucoside *XV*. The coupling constant $J_{1'',2''}$ = 7·7 Hz, together with the chemical shift of the proton on $C_{(1'')}$ corresponded again to β-configuration of the glycosidic bond[20]. Because of close R_F values of compounds *XI* and *XIV* in thin-layer chromatography, the composition of the reaction mixture was followed by high performance liquid chromatography. As seen from Table III, the relative

TABLE III

Retention times (t_R) and capacity factors (k') in HPLC (Solvent: methanol, flow rate: 2 ml/min, pressure: 4·92 MPa, column packing: Separon Si C_{18} (10 μm), samples were applied in dichloromethane–methanol solution (1 : 1 v/v)

Compound	*XI*	*XIV*	*XV*
t_R, min	2·24	1·88	1·64
k'	0·48	0·24	0·08

retention time and capacity factor decrease with increasing polarity of the compound in the order *XI* > *XIV* > *XV*. Unexpectedly, compounds *XI* and *XIV* were not separated in methanol–water (9 : 1; v/v). Use of systems with a water content higher than 20% resulted in broad tailing peaks due to low solubility of the compounds.*

Neither of the steroidal thiazoles prepared in this study exhibited antimicrobial activity.

EXPERIMENTAL

Melting points were determined on a Boetius melting point microscope (GDR). Optical rotations were measured at 25°C on a Perkin–Elmer 141 MC polarimeter, IR spectra were taken on a Zeiss UR-20 spectrometer and are given in cm^{-1}. ^1H NMR spectra were determined in deuteriochloroform with tetramethylsilane as internal standard on a Tesla B-476 (60 MHz) instrument. Compounds *XIV* and *XV* were measured on a Varian XL-200 (200 MHz) instrument. Chemical shifts are given in ppm (δ-scale), coupling constants (J) and signal widths (W) in Hz. All values were obtained by first order analysis. Mass spectra were taken on an AEI MS 901 spectrometer. Column chromatography was performed on silica gel (according to Pitra; 60—120 μm) or on neutral alumina (Reanal, activity II), thin-layer chromatography was carried out on silica gel G (according to Stahl; Woelm). Prior to evaporation (at about 2 kPa), solutions of the compounds in organic solvents were dried over anhydrous sodium or magnesium sulfate. Analytical samples were dried over phosphorus pentoxide at 40°C and 26 Pa for 12 h. The identity of samples prepared by different routes was checked by comparison of their IR and ^1H NMR spectra, by thin-layer chromatography and mixture melting point determination.

HPLC was performed on an apparatus consisting of a Milton Roy/LDC pump System Support Unit I, stainless steel column (250 × 4 mm), UVM4 UV-detector (Development Workshops, Czechoslovak Academy of Sciences, Prague, Czechoslovakia) and Knauer No 63.00.00 Loop injector. Samples (10 μl) were injected at 25°C. Steroidal thiazoles were detected at 260 nm. Methanol (mobile phase) was *p.a.* grade (Lachema, Brno, Czechoslovakia).

* See also: Drašar P., Pouzar V., Černý I., Havel M.: J. Chromatogr. *283*, 396 (1984).

3β-Methoxymethoxy-5-pregnen-20-one (*II*)

N,N-Dimethylaniline (25·7 g), followed by chloromethyl methyl ether (17 ml), was added to a solution of pregnenolone (*I*; 31·6 g; 0·1 mol) in a mixture of dichloromethane (200 ml) and ether (200 ml). After stirring for 24 h at room temperature, the mixture was diluted with ether (1 litre), and washed with dilute hydrochloric acid, water, potassium hydrogen carbonate solution and water. After the work up, the residue was crystallized from acetone–methanol–water affording 33 g (92%) of the ether *II*; m.p. 104–106°C, $[\alpha]_D$ +18° (*c* 0·2, chloroform). Reported[5] m.p. 102 to 104°C, $[\alpha]_D$ +19°. IR spectrum (tetrachloromethane), 1 705 (C=O), 1 147, 1 105, 1 044 (C—O—C), 3 030, 1 666 (C=C—H). ^{1}H NMR spectrum: 5·36 m (1 H, $C_{(6)}$—H), 4·68 s (2 H, O—CH_2—O), 3·35 s (3 H, OCH_3), 2·11 s (3 H, $C_{(21)}$—H), 1·01 s (3 H, $C_{(19)}$—H), 0·63 s (3 H, $C_{(18)}$—H).

21-Hydroxy-3β-methoxymethoxy-5-pregnen-20-one (*IV*)

To a stirred solution of diisopropylamine (2·8 g; 27·7 mmol) in 1,2-dimethoxyethane (46 ml) a solution of 1-butyllithium in hexane (17 ml; c = 1·6 mol l^{-1}) was added at −78°C under argon. After stirring for 30 min at −78°C, the ketone *II* (5 g; 13·9 mmol) in 1,2-dimethoxyethane (25 ml) was added during 5 min and the mixture was stirred at −78°C for 1 h. Triethylamine (3·22 ml; 23·1 mmol), followed by chlorotrimethylsilane (2·93 ml; 23·1 mmol), was added and the mixture was allowed to attain room temperature during 1 h. The mixture was diluted with benzene (800 ml), washed with potassium hydrogen carbonate solution, dried over anhydrous potassium carbonate and taken down. The residue (5·5 g) consisted of the practically pure enol ether *III*. ^{1}H NMR spectrum (tetrachloromethane, external lock): 5·27 m (1 H, $C_{(6)}$—H), 4·53 s (2 H, O—CH_2—O), 3·97 d (2 H, $C_{(21)}$—H, J = 1·5), 3·25 s (3 H, OCH_3), 0·98 s (3 H, $C_{(19)}$—H), 0·58 s (3 H, $C_{(18)}$—H), 0·18 s (9 H, $OSi(CH_3)_3$). A solution of osmium tetroxide (100 mg) in 2-methyl-2-propanol (10 ml) was added to a solution of N-methylmorpholine N-oxide monohydrate[6] (1·97 g; 14·6 mmol) in a mixture of water (28 ml) and acetone (62·5 ml). The mixture was cooled to −5°C and the crude enol ether *III* (5·5 g) in a mixture of acetone (23 ml) and tetrahydrofuran (46 ml) was added. After stirring for 6 h at 0°C and 6 h at room temperature, potassium hydrogen sulfite (2·5 g), followed by 1M-H_2SO_4, (to an acid reaction) was added and the mixture was taken down *in vacuo*. The residue was partitioned between ethyl acetate and water, the aqueous layer was extracted with ethyl acetate and the combined organic phases were washed with dilute hydrochloric acid, water, potassium hydrogen carbonate and water. After drying and evaporation, the residue was chromatographed on a column of silica gel (150 g). Elution with benzene–light petroleum–ether (45 : 45 : 10) afforded 3·72 g (71%) of the hydroxy ketone *IV*, m.p. 123–124°C (light petroleum–acetone), $[\alpha]_D$ +9° (*c* 0·6, chloroform). IR spectrum (chloroform): 3 475 (OH), 1 704 (C=O), 1 146, 1 101, 1 038 (C—O—C). ^{1}H NMR spectrum: 5·33 m (1 H, $C_{(6)}$—H), 4·67 s (2 H, O—CH_2—O), 4·16 d (2 H, $C_{(21)}$—H, $J_{21,OH}$ = 4), 3·34 s (3 H, OCH_3), 3·24 t (1 H, OH, $J_{21,OH}$ = 4), 0·99 s (3 H, $C_{(19)}$—H), 0·63 s (3 H, $C_{(18)}$—H). For $C_{23}H_{36}O_4$ (376·5) calculated: 73·37% C, 9·64% H; found: 73·11% C, 9·47% H.

3β,21-Dihydroxy-5-pregnen-20-one (*V*)

A mixture of the ether *IV* (100 mg; 0·27 mmol), benzene (3 ml), methanol (7 ml) and *p*-toluenesulfonic acid monohydrate (100 mg) was stirred at 45°C for 10 h. The solvents were evaporated *in vacuo*, the residue partitioned between water and ether and the ethereal layer washed with potassium hydrogen carbonate solution and water. After drying and evaporation, the residue was chromatographed on a silica gel plate (20 × 20 cm) in dichloromethane–acetone (9 : 1), affording 53 mg (60%) of the hydroxy derivative *V*, m.p. 160–163°C (light petroleum–acetone), $[\alpha]_D$ +3°

(*c* 2·0, chloroform). Reported[9] m.p. 160–165°C, $[\alpha]_D$ +7·3°. IR spectrum (chloroform): 3 605, 3 475 (OH), 1 705 (C=O), 1 668 (C=C). ^{1}H NMR spectrum: 5·34 m (1 H, $C_{(6)}$—H), 4·16 d (2 H, $C_{(21)}$—H, $J_{21,OH}$ = 5), 3·24 t (1 H, OH, $J_{21,OH}$ = 5), 1·00 s (3 H, $C_{(19)}$—H), 0·65 s (3 H, $C_{(18)}$—H).

21-Methanesulfonyloxy-3β-methoxymethoxy-5-pregnen-20-one (*VI*)

Methanesulfonyl chloride (1·77 ml; 22·9 mmol) was added at 0°C to a solution of the hydroxyketone *IV* (2·86 g; 7·6 mmol) in pyridine (50 ml). The mixture was stirred at 0°C for 4 h and poured into a mixture of ice (200 g) and water (200 ml). After the ice had melted, the product was taken up into benzene (5 × 150 ml). The combined extracts were washed with 5% hydrochloric acid (3 × 200 ml), water (2 × 200 ml), dried and taken down. Crystallization of the residue from a dichloromethane–ether–light petroleum mixture gave 1·75 g (51%) of the mesylate *VI*; the mother liquors were used for isolation of the chloro derivative *VII*. The product *VI* melted at 148–151°C; $[\alpha]_D^{25}$ −22° (*c* 0·5, chloroform). IR spectrum (tetrachloromethane): 1 720 (C=O), 1 360, 1 172 (SO_2), 3 025, 3 010, 1 680, 1 667 (C=C), 1 103, 1 036 (C—O—C). ^{1}H NMR spectrum: 5·32 bd (1 H, $C_{(6)}$—H, J = 3·5), 4·77 d (2 H, $C_{(21)}$—H, J = 1·5), 4·68 s (2 H, —O—CH_2—O—), 3·35 s (3 H, CH_3—O—), 3·22 s (3.H, CH_3SO_3), 3·22 m (1 H, $C_{(3)}$—H, W = 40), 1·00 s (3 H, $C_{(19)}$—H), 0·67 s (3 H, $C_{(18)}$—H). For $C_{24}H_{38}O_6S$ (454·6) calculated: 63·41% C, 8·42% H, 7·05% S; found: 63·77% C, 8·55% H, 6·71% S.

21-Chloro-3β-methoxymethoxy-5-pregnen-20-one (*VII*)

The mother liquors from crystallization of the mesylate *VI* were taken down and the residue was crystallized from ether–light petroleum and then from methanol–light petroleum. The mother liquors from the second crystallization were taken down and the residue was chromatographed on a column of silica gel (100 ml) in benzene–ether (4 : 1). The obtained product was crystallized from dichloromethane–light petroleum, affording 245 mg (8% based on the starting *IV*) of the chloro derivative *VII*, m.p. 96–98°C, $[\alpha]_D^{25}$ +33° (*c* 0·4; chloroform). IR spectrum (chloroform): 1 727 and 1 711 sh (C=O), 1 150, 1 106 and 1 042 (C—O—C), 1 671 (C=C). ^{1}H NMR spectrum: 5·33 bd (1 H, $C_{(6)}$—H, J = 3·5), 4·67 s (2 H, —OCH_2O—), 4·06 s (2 H, $C_{(21)}$—H), 3·33 s (3 H, CH_3—O—), 3·32 m (1 H, $C_{(3)}$—H, W = 40), 0·99 s (3 H, $C_{(19)}$—H), 0·63 s (3 H, $C_{(18)}$—H). Mass spectrum (*m/z*): 332 (M − $C_2H_6O_2$), 317 (332 − CH_3), 255 (332 − $COCH_2Cl$). For $C_{23}H_{35}ClO_3$ (395·0) calculated: 69·94% C, 8·93% H, 8·98% Cl; found: 70·01% C, 9·36% H, 8·65% Cl.

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

A mixture of the mesylate *VI* (1·55 g; 3·41 mmol), acetone (50 ml) and lithium bromide (3 g) was stirred at 50°C (bath) for 30 min, filtered through a silica gel column (50 g) and washed out with benzene. The filtrate was taken down and the residue crystallized from ether–light petroleum, affording 935 mg (62%) of the bromo derivative *VIII*, melting at 90–91°C, $[\alpha]_D^{25}$ +43° (*c* 0·25; chloroform). IR spectrum (chloroform), 1 720, 1 704 (C=O, rotamers), 1 667 (C=C), 1 146, 1 107, 1 102 (C—O—C). ^{1}H NMR spectrum: 5·33 bd (1 H, $C_{(6)}$—H, J = 3·5), 4·68 s (2 H, —O—CH_2—O—), 3·90 s (2 H, $C_{(21)}$—H), 3·34 s (3 H, CH_3O—), 3·33 m (1 H, $C_{(3)}$—H, W = 33), 0·99 s (3 H, $C_{(19)}$—H), 0·63 s (3 H, $C_{(18)}$—H). For $C_{23}H_{35}BrO_3$ (439·4) calculated: 62·86% C, 8·03% H, 18·18% Br; found: 63·16% C, 7·94% H, 18·08% Br.

17β-[4-(2-Ethoxycarbonyl-1,3-thiazolyl)]-3β-hydroxy-5-androstene (*XI*)

A) A mixture of the bromo ketone *VIII* (500 mg; 1·14 mmol), ethyl thioxamate (155 mg; 1·17 mmol) and acetonitrile (18 ml) was refluxed for 4 h (sodium hydroxide protecting tube), poured into water (150 ml) and extracted with ether (5 × 50 ml). The ethereal extracts were dried, filtered through charcoal and taken down. The residue was chromatographed on a column of silica gel (80 g) in benzene–ether (20 : 1; 500 ml) and benzene–ether (4 : 1; 1000 ml), affording the thiazole *XI* as the more polar of the two chromatographically very similar compounds. Crystallization from ether gave 120 mg (24%) of *XI*, m.p. 158–160°C. The less polar fractions were used in the preparation of the bromo ketone *IX*. The product *XI* had $[\alpha]_D^{25}$ −87° (*c* 0·12; chloroform). IR spectrum (chloroform): 3 600 (OH), 1 729, 1 709, 1 260 (—COO—), 1 665 (C=C, steroid skeleton), 3 115 (C—H, thiazole), 1 090, 1 040 (C—O—C). ^{1}H NMR spectrum: 7·20 s (1 H, $C_{(5')}$—H), 5·33 bd (1 H, $C_{(6)}$—H, $J = 4$), 4·43 q (2 H, CH_3CH_2—, $J = 7·3$), 3·37 m (1 H, $C_{(3)}$—H, $W = 45$), 2·83 m (1 H, $C_{(17)}$—H, $W = 18$), 2·21 bd (2 H, $C_{(7)}$—H, $J = 8·5$, $W = 18$), 1·37 t (3 H, CH_3—CH_2—), 0·94 s (3 H, $C_{(19)}$—H), 0·42 s (3 H, $C_{(18)}$—H). Mass spectrum: 429·2348 ($M^+ = C_{25}H_{35}NO_3S$), 414 (M−CH_3), 411 (M−H_2O), 356 ($C_{22}H_{30}NOS$ = M−$COOC_2H_5$), 184 ($C_8H_{10}NO_2S$), 171 ($C_7H_9NO_2S$). For $C_{25}H_{35}NO_3S$ (429·6) calculated: 69·89% C, 8·21% H, 3·26% N, 7·46% S; found: 69·98% C, 8·54% H, 3·19% N, 7·73% S.

B) A mixture of the bromo ketone *VIII* (1·2 g; 2·73 mmol), acetonitrile (25 ml) and ethyl thioxamate (372 mg; 2·8 mmol) was refluxed for 16 h. During this time further portions of ethyl thioxamate (100 mg; 0·75 mmol) were added in 3 hours intervals. The mixture was mixed with silica gel, taken down and chromatographed on a column of silica gel (100 g) in benzene–ether (20 : 1, 500 ml and 4 : 1, 1 500 ml). The principal fraction on crystallization from ether gave 800 mg (68%) of the thiazole *XI*, identical with the product prepared by the procedure *A*.

C) A mixture of the bromo ketone *IX* (270 mg; 0·68 mmol), acetonitrile (10 ml) and ethyl thioxamate (93 mg; 0·7 mmol) was refluxed for 5 h. Further portion of ethyl thioxamate (20 mg; 0·15 mmol) was added and the mixture was refluxed for further 4 h. After evaporation, the residue was chromatographed on a column of silica gel (50 g) in benzene–ether (4 : 1). The main fraction gave 300 mg of an oil which on crystallization from ether yielded 221 mg (74%) of the thiazole *XI*, identical with that prepared by procedure *A*.

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

The less polar fraction from the chromatography in the preparation of the thiazole *XI* (see method *A*) was taken down and the residue was crystallized from ether, affording 140 mg of *IX* (31% based on the starting ether *VIII*), m.p. 153–155°C, $[\alpha]_D^{25}$ +36° (*c* 0·14; chloroform). IR spectrum (chloroform): 3 600, 1 042 (OH), 1 729, 1 706 (C=O), 1 665 (C=C). ^{1}H NMR spectrum: 5·31 bd (1 H, $C_{(6)}$—H, $J = 4$), 3·87 s (2 H, $C_{(21)}$—H), 3·51 m (1 H, $C_{(3)}$—H, $W = 45$), 2·20 bd (2 H, $C_{(7)}$—H, $J = 8$), 0·97 s (3 H, $C_{(19)}$—H), 0·61 s (3 H, $C_{(18)}$—H). Mass spectrum: 394 (M^+), 376 (M−H_2O), 361 (376−CH_3), 297 (376−Br). Reported m.p. 159–159·5°C (ref.[13]) and 156–158°C (ref.[14]). Reported[14] ^{1}H NMR spectrum: 5·38, 3·92, 3·50, 1·62, 0·67; IR spectrum: 1 719 cm^{-1} and mass spectrum (*m/z*): 394 and 396 (M^+); reference[12] states m.p. 141–141·5°C, IR spectrum: 3 600, 1 715, ^{1}H NMR spectrum: 5·3–5·6, 3·99, 3·3–3·8, 1·02, 0·68.

17β-[4-(2-Ethoxycarbonyl-1,3-thiazolyl)]-3β-hydroxy-5-androstene 3-[4-(2,2,2-Trichloroethoxy)-4-oxobutanoate] (*XII*)

A mixture of the alcohol *XI* (340 mg; 0·81 mmol), 4-(2,2,2-trichloroethoxy)-4-oxobutanoic acid[18] (377 mg; 1·51 mmol), N,N'-dicyclohexylcarbodiimide (185 mg; 0·9 mmol), 4-dimethylaminopyri-

dine (6 mg) and benzene (20 ml) was stirred at room temperature for 2·5 h. The mixture was poured into water and extracted with dichloromethane (3 × 50 ml) and ether (3 × 50 ml). The combined extracts were dried and taken down and the residue was chromatographed on a column of silica gel (80 g) in benzene–ether (10 : 1). The main fraction afforded 380 mg (70%) of the ester *XII*. IR spectrum (chloroform): 3 110, 1 500, 1 304, 1 091, 1 018 (thiazole), 1 730 ($-COOC_2H_5$ on the thiazole ring), 1 670 (C=C), 1 752, 1 730, 1 150 ($-OOCCH_2CH_2COO.CH_2CCl_3$). ^{1}H NMR spectrum: 7·19 s (1 H, $C_{(5')}$—H), 5·36 m (1 H, $C_{(6)}$—H, $W = 14$), 4·72 s (2 H, $—OCH_2CCl_3$), 4·48 m (1 H, $C_{(3)}$—H, $W = 45$), 4·43 q (2 H, $CH_3—CH_2—O—$, $J = 7·2$), 2·69 bs (4 H, $—OCCH_2CH_2CO—$, $W = 12$), 2·27 bd (2 H, $C_{(7)}$—H, $J = 8$), 1·38 t (3 H, $CH_3.CH_2—$, $J = 7·2$), 0·98 s (3 H, $C_{(19)}$—H), 0·45 s (3 H, $C_{(18)}$—H). For $C_{31}H_{40}Cl_3NO_6S$ (661·1) calculated: 56·32% C, 6·10% H, 16·09% Cl, 2·12% N, 4·85% S; found: 56·05% C, 6·06% H, 16·03% Cl, 2·30% N, 4·80% S.

17β-[4-(2-Ethoxycarbonyl-1,3-thiazolyl)]-3β-hydroxy-5-androstene 3-(3-Carboxypropanoate) (*XIII*)

A mixture of the butanoate *XII* (380 mg; 0·57 mmol), tetrahydrofuran (11 ml), glacial acetic acid (11 ml), water (2 ml) and zinc dust (20 mg) was stirred at 0°C (ice bath) for 3 h. During this time further portions (à 20 mg) of zinc dust were added in 30 min intervals. The mixture was filtered and taken down, the residue was dried over sodium hydroxide in an exsiccator under diminished pressure and chromatographed on a silica gel column (50 g) in benzene (100 ml) and then in benzene–ether (5 : 1; 500 ml and 5 : 2; 1 000 ml). The main fraction afforded the hemisuccinate *XIII* (120 mg; 40%), m.p. 95—97·5°C (benzene–ether–light petroleum), $[\alpha]_D^{25}$ −94° (*c* 0·08; chloroform). IR spectrum (chloroform): 3 500—2 400, 1 715 (COOH), 3 010, 1 500, 1 301, 1 091, 1 015 (thiazole), 1 729, 1 171 (—COO—). ^{1}H NMR spectrum: 7·21 s (1 H, $C_{(5')}$—H), 5·35 m (1 H, $C_{(6)}$—H, $W = 14$), 4·50 m (1 H, $C_{(3)}$—H, $W = 40$), 4·42 q (2 H, $CH_3CH_2—$, $J = 7$), 2·58 bs (4 H, $—OCCH_2CH_2CO—$, $W = 16$), 2·25 bd (2 H, $C_{(7)}$—H, $J = 8$), 1·37 t (3 H, $CH_3CH_2—$, $J = 7$), 0·97 s (3 H, $C_{(19)}$—H), 0·44 s (3 H, $C_{(18)}$—H). For $C_{29}H_{39}NO_6S$ (529·7) calculated: 65·76% C, 7·42% H, 2·64% N, 6·05% S; found: 65·46% C, 7·63% H, 2·48%N, 5·92% S.

3β-(2,3,4,6-Tetra-O-acetyl-β-D-glucopyranosyloxy)-17β-[4-(2-ethoxycarbonyl-1,3-thiazolyl)]--5-androstene (*XIV*)

A solution of the thiazole *XI* (90 mg; 0·21 mmol) in 1,2-dichloroethane (1·5 ml) was stirred with a finely powdered molecular sieve (type 4A; 100 mg) and silver silicate[21] (150 mg) for 20 min in an argon atmosphere. A solution of 2,3,4,6-tetra-O-acetyl-α-D-glucopyranosyl bromide (150 mg; 0·36 mmol) in 1,2-dichloroethane (1·5 ml) was added dropwise at room temperature during 30 min and the mixture was stirred in the dark for 48 h at room temperature. The catalyst was filtered through Celite which was then washed with dichloromethane, the filtrate was washed twice with saturated potassium hydrogen carbonate solution and water, dried and taken down. Chromatography on a silica gel column (25 g) in benzene–acetone (50 : 1) gave 80 mg (50%) of the glucoside *XIV* which was crystallized from ethanol; m.p. 177—179°C; $[\alpha]_D^{25}$ −4° (*c* 0·18, chloroform). IR spectrum (chloroform): 3 120, 1 502, 1 303 (thiazole), 1 754, 1 740, 1 255 ($CH_3COO—$), 1 710, 1 740 ($—COOCH_2CH_3$). ^{1}H NMR spectrum (200 MHz, measured on a Varian XL-200 instrument): 7·20 s (1 H, $C_{(5')}$—H, 5·38 bd (1 H, $C_{(6)}$—H, $J_{6,7\alpha} \doteq 4·7$, $J_{6,7\beta} \neq 0$), 5·21 t (1 H, $C_{(3'')}$—H, $J_{3'',2''} = J_{3'',4''} = 9·4$), 5·07 t (1 H, $C_{(4'')}$—H, $J_{4'',3''} = J_{4'',5''} = 9·4$), 4·96 dd (1 H, $C_{(2'')}$—H, $J_{2'',1''} = 8·0$, $J_{2'',3''} = 9·4$), 4·60 d (1 H, $C_{(1'')}$—H, $J_{1'',2''} = 8·0$), 4·46 q (2 H, $—OCH_2CH_3$, $J = 7·0$), 4·27 dd (1 H, $C_{(6'')}$—H_a, $J_{6_a'',6_b''} = 12·3$, $J_{6_a'',5''} = 4·8$), 4·11 dd (1 H,

$C_{(6'')}$—H_b, $J_{6_b'',6_a''} = 12{\cdot}3$, $J_{6_b'',5''} = 2{\cdot}6$), 3·68 ddd (1 H, $C_{(5'')}$—H, $J_{5'',4''} = 9{\cdot}4$, $J_{5'',6_a''} = 4{\cdot}8$, $J_{5'',6_b''} = 2{\cdot}6$), 3·50 m (1 H, $C_{(3)}$—H), 3·00 bt (1 H, $C_{(17)}$—H, $J_{17,16\alpha} \doteq J_{17,16\beta} \doteq 9{\cdot}6$), 2·08, 2·05, 2·02, 2·01 4 s (4 × 3 H, 4 × —$OCOCH_3$), 1·43 t (3 H, —OCH_2CH_3, $J = 7{\cdot}0$), 0·98 s (3 H, $C_{(19)}$—H), 0·49 s (3 H, $C_{(18)}$—H). For $C_{39}H_{53}NO_{12}S$ (759·9) calculated: 61·64% C, 7·03% H, 1·84% N, 4·22% S; found: 61·91% C, 7·23% H, 1·61% N, 4·13% S.

3β-(β-D-Glucopyranosyloxy)-17β-[4-(2-ethoxycarbonyl-1,3-thiazolyl)]-5-androstene (*XV*)

A solution of sodium ethoxide (0·1 ml; $c \sim 0{\cdot}4$ mmol l^{-1}) was added dropwise to a solution o the acetate *XIV* (50 mg; 0·066 mmol) in ethanol (10 ml). After standing at room temperature for 24 h, solid carbon dioxide (about 0·1 g) was added, the solvents were evaporated and the residue dried. Chromatography on a silica gel column (15 g; 30—60 μm) in chloroform–ethanol (10 : 1) afforded the glucoside *XV* which was crystallized from ethanol; m.p. 232—235°C. Yield 20 mg (51%). $[\alpha]_D^{25}$ −9° (*c* 0·2, ethanol); IR spectrum (KBr, taken on Perkin–Elmer 621 instrument): 3 410 (OH), 1 735, 1 710, 1 260 (—$OCOC_2H_5$), 3 105, 1 498, 1 302 (thiazole). ^{1}H NMR spectrum (200 MHz, taken on a Varian XL-200 instrument; deuteriochloroform–hexadeuteriodimethyl sulfoxide (3 : 1)): 7·68 s (1 H, $C_{(5')}$—H), 5·36 m (1 H, $C_{(6)}$—H, $W = 16$), 4·38 q (2 H, —OCH_2CH_3, $J = 7{\cdot}3$), 4·24 d (1 H, $C_{(1'')}$—H, $J = 7{\cdot}7$), 1·37 t (3 H, —OCH_2CH_3, $J = 7{\cdot}3$), 0·98 s (3 H, $C_{(19)}$—H), 0·45 s (3 H, $C_{(18)}$—H). For $C_{31}H_{45}NO_8S$ (591·8) calculated: 62·92% C, 7·66% H, 2·37% N, 5·42% S; found: 62·54% C, 7·94% H, 2·07% N, 5·20% S.

Antibacterial Activity Tests of Thiazole Derivatives *XIII*, *XVI*, *XVII*, *XVIII* and *XIX*

The compound was dissolved in a minimum amount of ethanol, diluted with water to concentrations 1, 10 and 100 mg/l and applied on an *Escherichia coli* culture. Neither of the tested thiazoles *XIII*, *XVI* (ref.[2]), *XVII* (ref.[2]), *XVIII* (ref.[19]) and *XIX* (ref.[19]) exhibited antibacterial activity.

The authors are indebted to Professor J. Škoda for the antibacterial activity assays, to Miss H. Kapičková for taking the IR spectra, to Dr D. Šaman, Mrs J. Jelínková and Dr M. Masojídková for ^{1}H NMR spectral measurements, to Dr M. Buděšínský for measurement and interpretation of the 200 MHz ^{1}H NMR spectra, to Mrs Z. Ledvinová for optical rotation measurements and to Dr A. Trka for mass spectral measurements and their interpretation. The elemental analyses were carried out in the Analytical Laboratory of this Institute (Dr J. Horáček, Head).

REFERENCES

1. Catsoulacos P., Camoutsis C.: J. Heterocycl. Chem. *18*, 1485 (1981).
2. Drašar P., Tureček F., Havel M.: This Journal *46*, 2906 (1981).
3. Kočovský P., Drašar P., Pouzar V., Havel M.: This Journal *47*, 108 (1982).
4. Černý I., Pouzar V., Drašar P., Havel M.: This Journal *48*, 2064 (1983).
5. Fried J. (Olin Mathieson Chemical Corp.) U.S. 3 062 846; Chem. Abstr. *58*, 9193f (1963).
6. VanRheenen V., Kelly R. C., Cha D. Y.: Tetrahedron Lett. *1976*, 1973.
7. McCormick J. P., Tomasik W., Johnson M. W.: Tetrahedron Lett. *22*, 607 (1981).
8. Morris D. S., Williams D. H., Norris A. F.: J. Org. Chem. *46*, 3422 (1981).
9. Sperna Weiland J. H., Arens J. F.: Rec. Trav. Chim. Pays-Bas *79*, 1293 (1960).
10. Tuinman A., Iwasaki S., Schaffner K., Jeger O.: Helv. Chim. Acta *51*, 1778 (1968).
11. Hantzsch A.: Justus Liebigs Ann. Chem. *249*, 1 (1888).
12. Keana J. F. W., Schumaker R. R.: Tetrahedron *26*, 5191 (1970).
13. Reich H., Reichstein T.: Helv. Chim. Acta *22*, 1124 (1939).

14. Numazawa M., Nagaoka M.: J. Chem. Soc., Chem. Commun. *1983*, 127.
15. Drašar P., Pouzar V., Černý I., Havel M.: Czech. Appl. PV 1085-83 (1983).
16. Drašar P., Černý I., Pouzar V., Havel M.: This Journal *49*, 306 (1984).
17. Drašar P., Pouzar V., Černý I., Havel M.: Czech. Appl. PV 860-83 (1983).
18. Okabayashi T., Mihara S., Repke D. B., Moffatt J. G.: Cancer Res. *37*, 619 (1977).
19. Takamura K., Isono C., Takaku S., Nitta Y.: Chem. Pharm. Bull. *11*, 604 (1963).
20. Černý I., Pouzar V., Drašar P., Buděšínský M., Havel M.: This Journal *49*, 881 (1984).
21. Paulsen H.: Angew. Chem. *94*, 184 (1982).

Translated by M. Tichý