REVISION OF THE STRUCTURE OF 3-METHOXY-14α-HYDROXY-D--HOMO-1,3,5(10)-ESTRATRIEN-17a-ONE. A SIMPLE ^{1}H NMR METHOD FOR THE DETERMINATION OF CONFIGURATION OF HYDROXY GROUP IN POSITION 5 AND/OR 14 OF THE D-HOMO-STEROID SKELETON*

Miloš BUDĚŠÍNSKÝ, Alexander KASAL, Želimír PROCHÁZKA, Huynh Kim THOA, Soňa VAŠÍČKOVÁ and Pavel KOČOVSKÝ

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

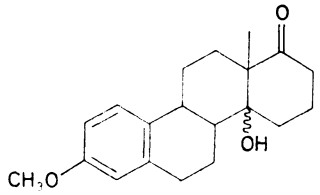
Received August 10, 1990
Accepted September 20, 1990

Eignerová and Procházka found in 1974 the Cotton effect value for 3-methoxy-14α-hydroxy-D--homo-1,3,5(10)-estratrien-17a-one (*Ia*) to be $\Delta\varepsilon$ −2·76. Calculation of the $\Delta\varepsilon$ value for this compound led, however, to a substantially lower value, which suggested the hypothesis that the compound was in fact rather an epimer with the hydroxy group in position 14β. This hypothesis was studied by means of ^{1}H NMR spectra of synthetic models, using the changes of the chemical shifts of angular methyls, induced by in situ acylation of the angular hydroxyl with an α- or β-configuration with trichloroacetyl isocyanate (TAI). The observed TAI-acylation shifts on model compounds indicated the structure *Ib* with a 14β-configuration of the hydroxyl group. Independent proof has been given by the synthesis of both 14-hydroxy epimers, *Ia* and *Ib*. A simple ^{1}H NMR method is proposed for the determination of configuration of the hydroxyl in position 5 or 14 of D-homo-steroid skeleton.

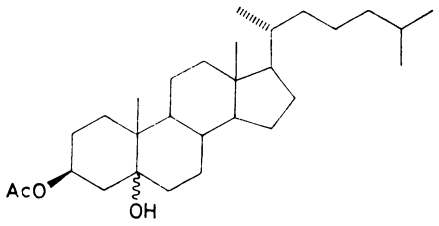
In 1974 Eignerová and Procházka[1] described the resolution of racemic 3-methoxy--14α-hydroxy-D-homo-1,3,5(10)-estratrien-17a-one (*Ia*), prepared by Torgov and co-workers[2]. Using the knowledge that ketones can be reduced with *Saccharomyces cerevisiae* or *Rhizopus nigricans* to alcohols of the (*S*) series (configuration), they correctly assigned the natural configuration to one of the enantiomers, but they overlooked a printer's error in their paper[1] concerning the sign of the Cotton effect of both enantiomers (they assigned the optically active derivative *Ia* the value $\Delta\varepsilon$ −2·76). This paper aroused the interest of the late Prof. W. Klyne, who came to the conclusion, on the basis of his calculations (carried out using the method of Kirk and Klyne[3]), that the calculated value of $\Delta\varepsilon$ for the published structure *Ia* is considerably different from that published, and, hence, that the relative configuration

* Part CCCLVIII in the series On Steroids; Part CCCLVII Collect. Czech. Chem. Commun. *56*, 1340 (1991).

of the above mentioned synthetic derivative might be incorrect[4]. The calculation was based on the following considerations: *1*) D-homo-5α,14α-estran-17a-one has a very small negative Cotton effect ($\Delta\varepsilon$ −0·2 in hexane or dioxan); *2*) the aromatic ring A has no great influence on the Cotton effect of the D-ring carbonyl; *3*) the axial hydroxyl group (in β-position to the carbonyl) has only a small effect on the $\Delta\varepsilon$ value (for example the 5α-hydroxyl in 5α-cholestan-3-one has an $\Delta\varepsilon$-contribution of only +0·5). From this it followed that the $\Delta\varepsilon$ value given in the mentioned paper[1] is evidently excessively high and that it would correspond better to the structure *Ib*. In view of the fact that the 14α-configuration of the hydroxyl group in the racemic *Ia* was proposed[2] merely on the basis of a mass spectrum[5], we considered it useful to check it by other methods as well. Since this synthetic derivative was not available, we endeavoured to solve the question of configuration by means of ^{1}H NMR spectroscopy.



Ia, 14α-OH
Ib, 14β-OH



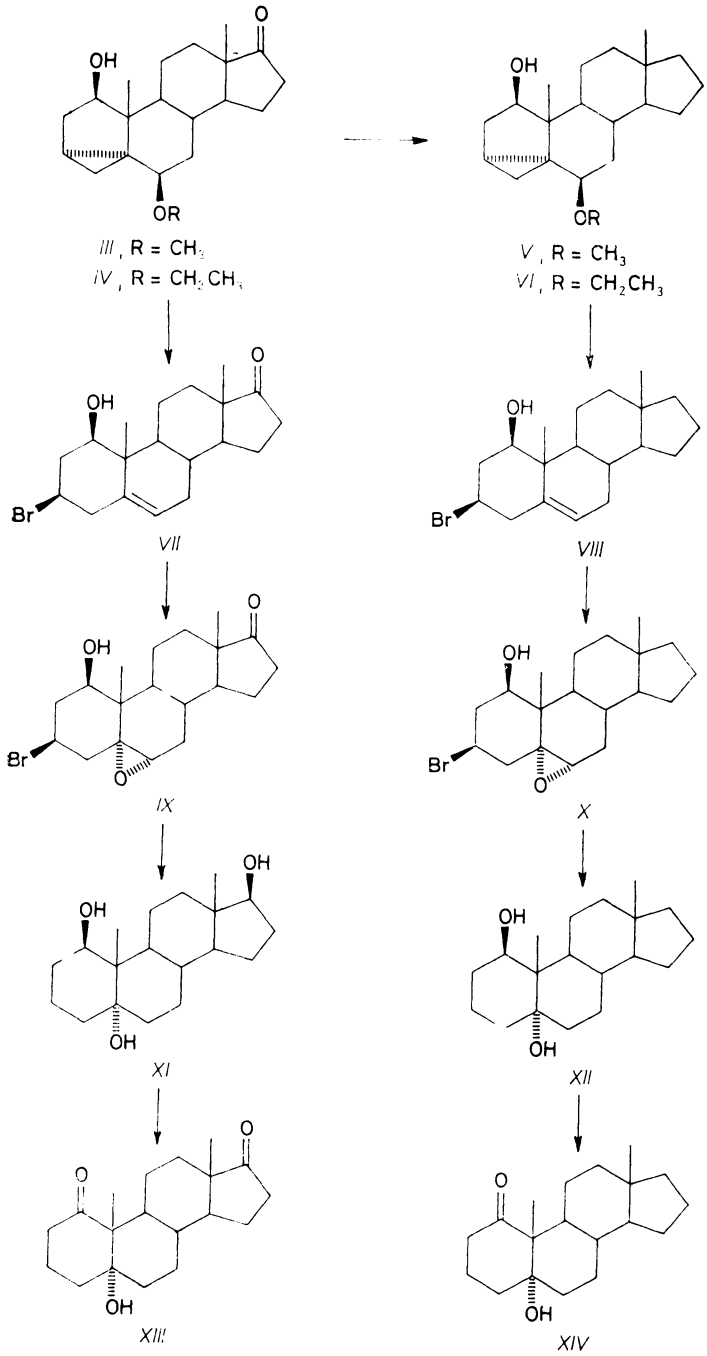
IIa, 5α-OH
IIb, 5β-OH

For the proof of the relative configuration of the methyl group and the hydroxyl on the vicinal tertiary carbon atoms C-13 and C-14 the coupling constant or the chemical shift value of the methyl itself cannot be used, or the described shielding contributions of the 14α- or 14β-hydroxyl either, owing to the presence of further magnetically anisotropic groups and the structural difference of the whole skeleton, as compared to the classical steroidal skeleton. Therefore we tried to use the acylation effects[6,7] observed for the in situ acylations of hydroxy derivatives, carried out with trichloroacetyl isocyanate (TAI) in an NMR tube. The hydroxy groups of tertiary alcohols are also acylated under formation of trichloroacetylcarbamoyl derivatives (TAC), which is accompanied by more or less characteristic shifts of the ^{1}H NMR signals of hydrogens close to the reaction centre[7]. In our case it may be expected that the methyl signal Me-18 will be more strongly affected by the TAI acylation of the closer *cis*-oriented 14β-hydroxy group than of the remoter *trans*-oriented 14α-hydroxy group. However, since the values of the acylation shifts ($\Delta\delta$ = $= \delta$(R-OTAC) − δ(R-OH)) cannot be satisfactorily predicted for the given system, we decided to use models, which would best simulate our case. As the best available we considered the known 3β-acetoxy-5α- and 3β-acetoxy-5β-cholestanols[8,9] (*IIa*, *IIb*),

which we prepared as the first pair of models. The TAI-acylation shifts of their methyl hydrogens H-19 are 0·075 or 0·14 ppm, when the higher value belongs, as expected, to the 5β-hydroxy derivative *IIb*. Thus, a comparison with the value 0·12 ppm found for the investigated homoestrone indicated again rather the 14β--hydroxy group and thence the structure *Ib*.

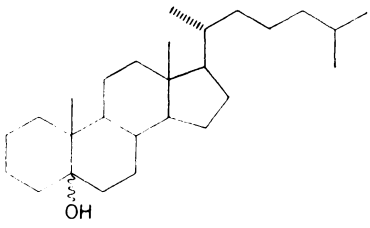
We decided to check the possibility of using the TAI-acylation shifts for the determination of configuration of the angular hydroxyl on further models, especially such as would be structurally close to the case studied. We made use of the fact that we had at our disposal 1β-hydroxy-6β-methoxy-3α,5-cyclo-5α-androstan-17--one[10] (*III*) as well as its homologue[11] (*IV*) and we converted them to 5-hydroxy-5α--androstan-1,17-dione (*XIII*) and 5-hydroxy-5α-androstan-1-one (*XIV*) by a reaction sequence shown in Scheme 1. The TAI-acylation shifts of the Me-19 signal in both models with the 5α-hydroxy group were equally low (0·03 ppm). We then extended the series of the model compounds by further substances (*XV*–*XIX*) prepared in our laboratory[16–19]. In the pairs of epimeric 5ξ-cholestan-5-ols *XVa*, *XVb*, we found acylation shifts with $\Delta\delta$ 0·06 or 0·14, respectively, that is, again a distinctly higher value for the A/B *cis* deriative *XVb* (with synclinal arrangement of the C(19)—C(10)—C(5)—OH fragment). For A/B *trans* derivatives *XVI*–*XIX* with an antiperiplanar arrangement we obtained the values $\Delta\delta$ 0·06 to 0·10 ppm. The final proof of the structure of the investigated methyl ether of 14-hydroxy-D-homo-estrone was gained by the preparation of both 14α- and 14β-OH epimers *Ia* and *Ib* by homologization[12] of corresponding methyl ethers of epimeric 14ξ-hydroxy-estrones *XXa* and *XXb* (ref.[13]). Thus, it was confirmed that the previously described[1] 14-hydroxyhomoestrone methyl ether is identical with the synthetized 14β-OH epimer and its structure has, therefore, to be revised in the sense of formula *Ib*, which is in accordance with the conclusions made from the CD spectra measurements and the TAI-acylation shifts in the ^{1}H NMR spectra.

The chemical shifts of the angular methyls (Me-19 or Me-18 in D-homo steroids) and their $\Delta\delta$ values induced by TAI acylation of the tertiary hydroxyl groups are surveyed in Table I. From the table it also follows that – within the frame of the available models – the $\Delta\delta$ values lower than 0·10 ppm are characteristic of the *trans*--arrangement of the CH_3 and OH groups, while the $\Delta\delta$ values higher than 0·10 ppm indicated a *cis*-arrangement of these groups. Thus the in situ TAI-acylation in the NMR tube represents a very simple method for the determination of configuration of the tertiary hydroxyl in position 5 or in position 14 of D-homo-steroidal skeleton, which does not require the possession of both corresponding epimers or further model compounds and which is realisable with a minimum quantity of sample (approx. 1 mg). However, the method is limited to cases of monohydroxy derivatives or such polyhydroxy compounds where no further hydroxy group is present in the proximity of the angular methyl investigated, the TAI-acylation of which would distinctly contribute to the resulting induced shift of the methyl.

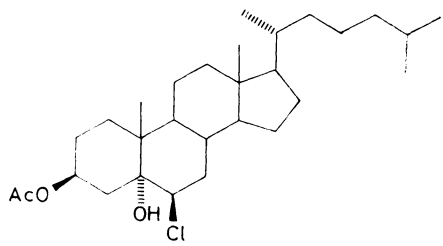

III, R = CH3
IV, R = CH2CH3
V, R = CH3
VI, R = CH2CH3
VII
VIII
IX
X
XI
XII
XIII
XIV

SCHEME 1

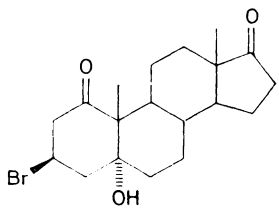
The $\Delta\delta$ values obtained for epimeric 14-hydroxyestrones *XXa*, *XXb* (0·10 and 0·07 ppm, resp.) show that the mentioned empiric rule cannot be simply extended to structural fragments with the annellation of six- and five-membered cycles, i.e. for Me-13 in the normal steroid series. This may be due to the flexibility of the five-membered ring. Checking the possibility of using the TAI-acylation shifts for these cases too would require the study of a larger series of epimeric 14-hydroxy steroids.



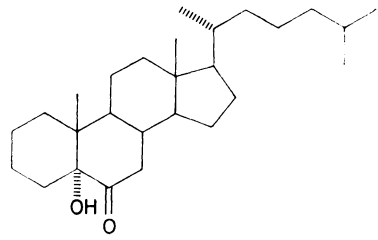
XV a, 5α - OH
XV b, 5β - OH



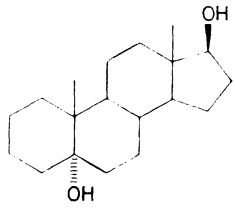
XVI



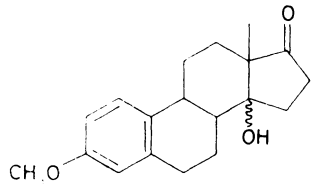
XVII



XVIII



XIX



XX a, 14α - OH
XX b, 14β - OH

The ^{13}C NMR data of compounds *Ia*, *Ib*, *XVa*, *XVb*, *XXa* and *XXb* are surveyed in Table II. For the assignment of the signals in compounds *Ia*, *Ib* and *XXa*, *XXb* we used in addition to the experimental data on the number of directly bound hydro-

gen atoms also the data for 3-methoxy-1,3,5(10)-estratrien-17-one (ref.[14]). In both pairs of the 14-hydroxy epimers – *Ia*, *Ib* and *XXa*, *XXb* – the signal of the methyl carbon C-18 appears at a distinctly lower field in the 14α-hydroxy epimer with the *trans*-annellation of the C/D rings (a difference of about 6 ppm). In contrast to this it is known from literature (refs[14,15]) that the downfield position of the methyl carbon C-19 in the pairs of 5αH- and 5βH-epimers, similarly to that of the hydrogen C-18 in the pairs 14αH- and 14βH-epimers indicates a *cis*-annellation of the A/B or C/D ring, respectively, in consequence of the decrease of the number of γ-gauche interactions in *cis*-isomers. The reason for the reversed order of the methyl signals of 14ξOH and 14ξH derivatives evidently consists in the shielding effect of the 14-hydroxy group present. This was confirmed by our data for the pair of epimeric 5-hydroxycholestanes *XVa* and *XVb* (Table II) and the literature values for the corresponding 5α and 5β cholestane (ref.[14]) which showed substitution effects of +3·9 ppm for 5α-OH and −7·1 ppm for 5β-OH on carbon C-19.

EXPERIMENTAL

The melting points were determined on a Kofler block and they are not corrected. The circular dichroism curves were measured on a Jobin Yvon Mark V instrument in methanol. The ^{1}H NMR spectra of compounds *I*–*XX* were measured on a Tesla BS-467 (60 MHz) and Varian HA-100 (100 MHz) instrument in CW-mode or on a FT NMR spectrometer Varian XL-200 (200 MHz) in deuteriochloroform with tetramethylsilane as internal reference. The TAI-acylation shifts were obtained by repeated measurements of the spectrum after addition of a mild excess of

TABLE I

Chemical shifts of angular methyl protons (Me-18 and/or Me-19) and the acylation shifts Δδ induced by in situ TAI-acylation of tertiary hydroxy groups in positions 5 ("normal steroid") and/or 14 (D-homo-steroid) at two relative configurations (CH_3, OH)

Compound	*Trans*		Compound	*Cis*	
	$\delta(CH_3)$	$\Delta\delta(CH_3)$		$\delta(CH_3)$	$\Delta\delta(CH_3)$
Ia	1·244 (Me-18)	0·06	*Ib*	1·196 (Me-18)	0·12
IIa	0·995 (Me-19)	0·075	*IIb*	0·970 (Me-19)	0·14
XVa	0·962 (Me-19)	0·06	*XVb*	0·893 (Me-19)	0·14
XIII	1·295 (Me-19)	0·03			
XIV	1·280 (Me-19)	0·03			
XVI	1·273 (Me-19)	0·095			
XVII	1·325 (Me-19)	0·06			
XVIII	0·775 (Me-19)	0·08			
XIX	1·002 (Me-19)	0·10			

TAI. In the case of compounds *Ia*, *Ib*, *XVa*, *XVb*, *XXa* and *XXb* the ^{13}C NMR spectra were also measured on a Varian XL-200 instrument (50·3 MHz) in $CDCl_3$, referenced to the solvent signal and the chemical shifts were calculated using the relation $\delta(CDCl_3) = 77{\cdot}00$. The infrared spectra were measured on a PE 684 Perkin–Elmer spectrometer; the frequencies are given in cm^{-1}. The purity of the compounds was checked by thin-layer chromatography on Silufol plates. Before evaporation the solutions were dried over anhydrous sodium sulfate.

14α-Hydroxy-3-methoxy-17a-homoestra-1,3,5(10)-trien-17a-one (*Ia*)

Potassium cyanide (30 mg) was added to a solution of 17-ketone[13] *XXa* (100 mg, 0·33 mmol) n ethanol (1·5 ml) and hydrogen cyanide (1 ml), and the mixture was stirred at room tempera-

TABLE II

Carbon-13 chemical shifts of epimeric pairs of steroid alcohols *Ia*, *Ib*, *XVa*, *XVb*, *XXa*, and *XXb*

Carbon	*Ia*	*Ib*	*XXa*	*XXb*	*XVa*	*XVb*
C-1	126·67	127·01	126·70	126·70	31·64	28·69
C-2	111·27	112·08	111·72	111·94	20·94	20·42
C-3	157·56	157·67	157·58	157·72	20·70	21·83
C-4	113·39	113·71	113·78	113·79	34·42	31·43
C-5	137·38	137·69	137·30	137·57	73·24	73·65
C-6	30·20	30·97	30·12	30·31	34·63	32·54
C-7	25·30	26·84	22·14	22·03	26·32	26·57
C-8	36·59	39·50	41·06	44·96	34·85	35·01
C-9	42·38	46·15	37·13	39·97	46·19	43·21
C-10	132·63	131·83	132·49	131·27	39·32	36·38
C-11	26·99	26·67	25·01	25·67	20·94	21·16
C-12	28·52	34·48	25·24	26·74	40·10	40·05
C-13	52·12	54·56	52·93	53·44	42·70	42·51
C-14	79·12	78·82	81·09	81·82	56·31	56·72
C-15	22·27	22·59	29·78	32·14	24·10	24·26
C-16	20·23	20·47	33·16	33·32	28·28	28·26
C-17	35·78	37·12	218·32	220·92	56·26	56·24
C-17a	213·91	215·30	—		—	—
C-18	20·54	14·25	18·33	12·95	12·15	11·99
C-19	—	—	—	—	16·02	17·16
C-20	—	—	—	—	35·85	35·78
C-21	—	—	—	—	18·66	18·66
C-22	—	—	—	—	36·19	36·18
C-23	—	—	—	—	23·89	23·84
C-24	—	—	—	—	39·53	39·52
C-25	—	—	—	—	28·02	28·02
C-27	—	—	—	—	22·83	22·81
C-28	—	—	—	—	22·57	22·57
OCH_3	55·22	55·22	55·21	55·21	—	—

ture for 48 h. The major part of the volatile components was eliminated in vacuo on a rotatory evaporator and the concentrated solution was dried by filtration over a small column of sodium sulfate. The product was chromatographed on thin layers of silica gel (2 plates 20 × 20 × 0·1 cm in benzene–ether 1 : 1). The required cyanohydrin (37 mg, 34%) was the lipophilic component of the mixture.

The cyanohydrin was acetylated with acetic anhydride (0·3 ml) in pyridine (0·5 ml) at room temperature for 18 h. The excess of the acylation reagent was decomposed by standing with methanol (3 ml) and the volatile components were evaporated in a vacuum.

The raw cyanohydrin acetate was dissolved in benzene (10 ml) and the azeotropic mixture was distilled off (6 ml). The solution was then added dropwise to a stirred solution of lithium aluminium hydride (200 mg) in diethyl ether (15 ml) at 0°C. The reduction was terminated by two hours' boiling under argon. The excess of the reducing agent was decomposed by addition of several drops of water, the mixture was alkalized with a sodium hydroxide solution (10%, 5 ml) and the precipitate of the aminosteroid (0·3 g) was filtered off and washed with water until neutral. The crude 17ξ-aminomethyl-3-methoxy-estra-1,3,5(10)-triene-14,17-diol was extracted with acetone in a Soxhlet extractor for 8 h, the extract was concentrated in a vacuum to dryness and the residue was dissolved in aqueous acetic acid (5%, 5 ml). Neutral substances were eliminated by extraction with ether. The aqueous phase was then alkalized with potassium hydroxide (10%) and the liberated amine was extracted with ether. Deamination of the amine was carried out in solution in aqueous acetic acid (33%, 5 ml) with sodium nitrite (60 mg) dissolved in water (2 ml) at 0°C. The mixture was allowed to stand at 0°C for 3 h and then 20 h at room temperature. The product formed was filtered off under suction and washed with water (11 mg, 11% from ketone *XXa*). After crystallization from ethanol D-homoketone *Ia* was obtained, m.p. 195 to 197°C, $[\alpha]_D$ +15° (chloroform, *c* 0·9). IR spectrum (tetrachloromethane): 3 620 (OH), 1 610, 1 510, 1 257 ($ArOCH_3$), 1 718 and 1 744 sh (CO). Mass spectrum, *m/z* (%): 314 (M^+, 21), 296 (100), 281 (44), 256 (17), 225 (38); high resolution: 314·1838. CD spectrum: $\Delta\varepsilon_{288}$ −0·63 (*c* 1·63, ethanol). ^{1}H NMR spectrum: 1·244 s (3 H, Me-18); 3·78 s (3 H, OMe); 6·63 d (1 H, H-4, *J*(4, 2) = 2·8); 6·74 dd (1 H, H-2, *J*(2, 1) = 8·5; *J*(2, 4) = 2·8); 7·24 d (1 H, H-1, *J*(1, 2) = 8·5). ^{1}H NMR spectrum after addition of TAI: 1·306 s (3 H, Me-18); 3·77 s (3 H, OMe); 6·63 d (1 H, *J*(4, 2) = 2·7); 6·74 dd (1 H, H-2, *J*(2, 1) = 8·6; *J*(2, 4) = 2·7); 7·24 d (1 H, H-1, *J*(1, 2) = = 8·6); 8·17 s (1 H, NH).

14β-Hydroxy-3-methoxy-17a-homoestra-1,3,5(10)-trien-17a-one (*Ib*)

Ketone *XXb* (100 mg, 0·33 mmol) was dissolved in a mixture of ethanol and toluene (2·5 ml, 3 : 2) and converted to the cyanohydrin intermediate (70 mg) with hydrogen cyanide (1 ml) under catalysis of potassium cyanide (30 mg). Further steps were identical as above. The yield of *Ib* was 39 mg (36·5%), m.p. 195–197°C (ethanol), $[\alpha]_D$ +151° (chloroform, *c* 1·1). IR spectrum (chloroform): 3 615 (OH), 1 709 (CO), 1 612, 1 507, 1 262 ($ArOCH_3$). Mass spectrum, *m/z* (%): 314 (M^+, 82), 296 (91), 281 (40), 256 (30), 242 (25), 27 (23), 213 (18), 202 (19), 186 (100); high resolution: 314·1836. CD spectrum: $\Delta\varepsilon$ +3·24 (*c* 1·03, ethanol). ^{1}H NMR spectrum: 1·196 (3 H, Me-18); 3·78 s (3 H, OMe); 6·64 d (1 H, H-4, *J*(4, 2) = 2·8); 6·74 dd (1 H, H-2, *J*(2·1) = = 8·7; *J*(2, 4) = 2·8); 7·21 d (1 H, H-1, *J*(1, 2) = 8·7). ^{1}H NMR spectrum after addition of TAI: 1·314 s (3 H, Me-18); 3·79 s (3 H, OMe); 6·65 d (1 H, H-4, *J*(4, 2) = 2·8); 6·74 dd (1 H, H-2, *J*(2, 1) = 8·7; *J*(2, 4) = 2·8); 7·19 d (1 H, H-1, *J*(1, 2) = 8·7); 8·16 s (1 H, NH). For $C_{20}H_{26}O_3$ (314·4) calculated: 76·40% C, 8·34% H; found: 76·27% C, 8·39% H.

5-Hydroxy-5α-cholestan-3β-yl Acetate[8,9] (*IIa*)

^{1}H NMR spectrum: 0·653 s (3 H, Me-18); 0·862 d (6 H, Me-26 and Me-27, *J* = 6·5); 0·900 d

(3 H, Me-21, $J = 6{\cdot}5$); 0·955 s (3 H, Me-19); 2·00 s (3 H, OAc); 5·15 m (1 H, H-3). ^{1}H NMR spectrum after addition of TAI: 0·668 s (3 H, Me-18); 0·861 d (6 H, Me-26 and Me-27, $J = 6{\cdot}5$); 0·905 d (3 H, Me-21, $J = 6{\cdot}5$); 1·070 s (3 H, Me-19); 2·00 s (3 H, OAc); 4·82 m (1 H, H-3); 8·32 s (1 H, NH).

5-Hydroxy-5β-cholestan-3-yl Acetate[8,9] (*IIb*)

^{1}H NMR spectrum: 0·755 s (3 H, Me-18); 0·864 d (6 H, Me-26 and Me-27, $J = 6{\cdot}5$); 0·903 d (3 H, Me-21, $J = 6{\cdot}5$); 0·970 s (3 H, Me-19); 2·07 s (3 H, OAc); 5·23 m (1 H, H-3). ^{1}H NMR spectrum after addition of TAI: 0·674 s (3 H, Me-18); 0·867 d (6 H, Me-26 and Me-27, $J = 6{\cdot}5$); 0·910 d (3 H, Me-21, $J = 6{\cdot}5$); 1·108 s (3 H, Me-19); 1·95 s (3 H, OAc); 5·17 m (1 H, H-3); 8·27 s (I H, NH).

1β-Hydroxy-6β-methoxy-3α,5-cyclo-5α-androstan-17-one[10] (*III*)

^{1}H NMR spectrum: 0·937 s (3 H, Me-18); 1·133 s (3 H, Me-19); 2·78 t (1 H, H-6, $J(6, 7\alpha) \approx \approx J(6, 7\beta) \approx 3$); 3·36 s (3 H, OMe); 3·92 bd (1 H, H-1, $J(1, 2) = 6$ and $\leqq 1$). ^{1}H NMR spectrum after addition of TAI: 0·927 s (3 H, Me-18); 1·100 s (3 H, Me-19); 2·80 t (1 H, H-6, $J(6, 7\alpha) \approx \approx J(6, 7\beta) \approx 3$); 3·35 s (3 H, OMe); 5·16 bd (1 H, H-1, $J(1, 2) = 6$ and $\leqq 1$); 8·33 s (1 H, NH).

6β-Methoxy-3α,5-cyclo-5α-androstan-1β-ol (*V*)

This compound was prepared from compound *III* analogously as the homologue *VI* described below. M.p. 106–109°C, $[\alpha]_D$ +30° (*c* 2·26, chloroform). ^{1}H NMR spectrum: 0·775 s (3 H, Me-18); 1·108 s (3 H, Me-19); 2·71 t (1 H, H-6, $J(6, 7\alpha) \approx J(6, 7\beta) \approx 3$); 3·32 s (3 H,OMe); 3·93 bd (1 H, H-1, $J(1, 2) = 6{\cdot}5$ and $\leqq 1$). ^{1}H NMR spectrum after addition of TAI: 0·762 s (3 H, Me-18); 1·083 s (3 H, Me-19); 2·74 t (1 H, H-6, $J(6, 7\alpha) \approx J(6, 7\beta) \approx 3$); 3·32 s (3 H, OMe); 5·19 bd (1 H, H-1, $J(1, 2) = 6{\cdot}5$ and $\leqq 1$); 8·30 s (1 H, NH). For $C_{20}H_{32}O_2$ (304·5) calculated: 78·89% C, 10·60% H; found: 78·72% C, 10·35% H.

6β-Ethoxy-3α,5-cyclo-5α-androstan-1β-ol (*VI*)

Compound[18] *IV* (582 mg, 1·75 mmol) was mixed with potassium hydroxide (3·16 g, 56·4 mmol), hydrazine hydrate (98%, 3·13 ml, 63 mmol) and ethylene glycol (20 ml) and the volatile components were distilled off until the temperature of the mixture was 190°C. The mixture was then refluxed for 32 h. After cooling the mixture was diluted with water, the product extracted with ether, the extract washed with water, dried and evaporated in a vacuum. The residue was submitted to chromatography on an alumina column (20 g), using chloroform as eluent. The fractions containing the required product *VI* were combined, concentrated and crystallized from 50% ethanol. M.p. 115–118°C, $[\alpha]_D$ +25·6° (*c* 1·64, chloroform). ^{1}H NMR spectrum: 0·777 s (3 H, Me-18); 1·103 s (3 H, Me-19); 1·14 t (3 H, CH_3CH_2O, $J = 7$); 2·81 t (1 H, $J(6, 7\alpha) \approx \approx J(6, 7\beta) \approx 3$); 3·49 q (2 H, CH_3CH_2O, $J = 7$); 3·92 bd (1 H, H-1, $J(1, 2) = 6{\cdot}5$ and $\leqq 1$). ^{1}H NMR spectrum after addition of TAI: 0·783 s (3 H, Me-18); 1·083 s (3 H, Me-19); 1·14 t (3 H, CH_3CH_2O, $J = 7$); 2·85 t (1 H, H-6, $J(6, 7\alpha) \approx J(6, 7\beta) \approx 3$ Hz); 3·49 q (2 H, CH_3CH_2O, $J = 7$); 5·20 bd (1 H, H-1, $J(1, 2) = 6{\cdot}5$ and $\leqq 1$); 8·40 s (1 H, NH). For $C_{21}H_{34}O_2$ (318·5) calculated: 79·19% C, 10·76% H; found: 78·56% C, 10·53% H.

3β-Bromo-1β-hydroxyandrost-5-en-17-one (*VII*)

Compound *III* (300 mg, 0·94 mmol) was dissolved in acetone (16·5 ml), hydrobromic acid (46%,

0·66 ml) was added to it and the mixture allowed to stand at room temperature for 2 h. After evaporation in a vacuum the residue was diluted with ether (50 ml) and the ethereal layer washed with a saturated hydrogen carbonate solution and water. After drying and evaporation the residue (350 mg) was crystallized from ether (1 ml). Yield, 329 mg (95%), m.p. 285–289°C. For $C_{19}H_{27}$. .BrO_2 (367·3) calculated: 62·12% C, 7·41% H; found: 62·67% C, 7·33% H.

3β-Bromoandrost-5-en-1β-ol (*VIII*)

Compound *VI* (568 mg, 1·78 mmol) was dissolved in acetone (30 ml), hydrobromic acid (46%, 3 ml) was added and the mixture was allowed to stand at 20°C for 3 h. Acetone was evaporated under reduced pressure and the residue diluted with water (30 ml). After extraction with ether (3 × 50 ml) the extract was washed with a sodium hydrogen carbonate solution and water, dried, filtered and evaporated to dryness. The residue (628 mg) was repeatedly crystallized from heptane, m.p. 132–134°C, $[\alpha]_D$ −39·5° (*c* 2·03, chloroform). ^{1}H NMR spectrum: 0·72 s (3 H, Me-18); 1·06 s (3 H, Me-19); 3·40 dd (1 H, H-1, $J(1, 2) = 11·2$ and 4·9); 3·76 m (1 H, H-3); 5·58 bd (1 H, H-6, $J(6, 7) = 5$ and $\leqq 1$). ^{1}H NMR spectrum after addition of TAI: 0·70 s (3 H, Me-18); 1·21 s (3 H, Me-19); 3·85 m (1 H, H-3); 4·70 dd (1 H, H-1, $J(1, 2) = 11·2$ and 4·9); 5·66 bd (1 H, H-6, $J(6, 7) = 5$ and $\leqq 1$); 8·28 s (1 H, NH). For $C_{19}H_{29}BrO$ (353·3) calculated: 64·53% C, 8·27% H, 22·62% Br; found: 65·42% C, 8·34% H, 21·92% Br. Compound *VIII* was also prepared quite analogously from compound *V*.

3β-Bromo-1β-hydroxy-5,6α-oxido-5α-androstan-17-one (*IX*)

Compound *VII* (320 mg, 0·87 mmol) was dissolved in a mixture of ether and benzene 1 : 1 (16 ml) and a solution of perphthalic acid (655 mg in 12 ml of ether) was added to it. The reaction mixture was allowed to stand in a refrigerator for 22 h and it was then diluted with an ether–benzene 1 : 1 mixture (80 ml). The solution was washed with a saturated hydrogen carbonate solution and water, dried and evaporated to dryness. The residue (405 mg) was crystallized from methanol (0·8 ml). Yield, 323 mg of crystalline product. Chromatography of the mother liquors on thin layers of silica gel (2 plates, 200 × 200 × 0·7 mm) afforded another 25 mg of product. Yield, 77%, m.p. 185–189°C. For $C_{19}H_{27}BrO_3$ (383·4) calculated: 59·53% C, 7·10% H; found: 59·13%C, 7·49% H.

3β-Bromo-5,6α-oxido-5α-androstan-1β-ol (*X*)

Compound *VIII* (350 mg, 0·99 mmol) was dissolved in chloroform (10 ml), a solution of *m*-chloroperbenzoic acid (85%, 350 mg, 1·72 mmol) in chloroform (10 ml) was added to it and the mixture was allowed to stand at room temperature (24°C) for 24 h. The reaction mixture was diluted with chloroform (40 ml) and the solution washed with a sodium hydrogen carbonate solution. After drying of the chloroform solution and evaporation of the solvent the residue was chromatographed on a column of alumina (1 g) with benzene–light petroleum 1 : 1 (30 ml) and then with pure benzene. The fractions containing the required product according to TLC were combined and concentrated. The residue (320 mg) was crystallized from heptane. Yield, 311 mg (85%), m.p. 114–116°C, $[\alpha]_D$ −70° (*c* 1·67, chloroform). ^{1}H NMR spectrum: 0·65 s (3 H, Me-18); 1·11 s (3 H, Me-19); 2·89 d (1 H, H-6, $J(6, 7) = 4·2$ and ≈ 0); 3·86 dd (1 H, H-1, $J(1, 2) = 10·7$ and 5·2); 4·18 m (1 H, H-3). ^{1}H NMR spectrum after addition of TAI: 0·64 s (3 H, Me-18); 1·29 s (3 H, Me-19); 2·95 d (1 H, H-6, $J(6, 7) = 4·0$ and ≈ 0); 4·19 m (1 H, H-3); 4·99 dd (1 H, H-1, $J(1, 2) = 10·9$ and 5·3); 8·27 s (1 H, NH). For $C_{19}H_{29}BrO_2$ (369·3) calculated: 61·78% C, 7·92% H; found: 62·35% C, 8·10% H.

5α-Androstane-1β,5,17β-triol (*XI*)

Lithium aluminium hydride (800 mg, 21 mmol) was added to a solution of compound *IX* (250 mg, 0·65 mmol) in ether (50 ml) and the mixture was refluxed for 96 h. The excess of the hydride was decomposed with methanol and acidified with 10% sulfuric acid. The product was extracted with ether and the extract washed with a sodium hydrogen carbonate solution and water. After drying the extract and filtration the ether was evaporated to dryness. The residue (225 mg) was crystallized from ether and ethyl acetate. Yield, 48 mg. The mother liquors were chromatographed on 3 plates with 200 × 200 × 0·7 mm silica gel thin layers, affording a further crop (18·6 mg) of product. Total yield of *XII* was 25%, m.p. 189–193°C, $[\alpha]_D$ −14° (*c* 1·28, ethanol). For $C_{19}H_{32}O_3$ (308·5) calculated: 73·98% C, 10·46% H; found: 73·28% C, 9·87% H.

5α-Androstane-1β,5-diol (*XII*)

Compound *X* (250 mg, 0·68 mmol) was dissolved in ether (50 ml), lithium aluminium hydride (912 mg, 24 mmol) was added to it and the mixture refluxed for 100 h. After decomposition with methanol (5 ml) and sulfuric acid (10%, 60 ml) and dilution with ether (100 ml) the ethereal extract was washed with aqueous sodium hydrogen carbonate and water, dried, filtered and evaporated to dryness. The residue (168 mg) was crystallized repeatedly from methanol, m.p. 205–208°C, $[\alpha]_D$ −23° (*c* 1·36, ethanol). ^{1}H NMR spectrum ($CDCl_3$ and CD_3SOCD_3 mixture 4 : 1): 0·68 s (3 H, Me-18); 0·91 s (3 H, Me-19); 2·49 s (1 H, C(5)—OH); 2·70 d (1 H, C(1)—OH, J(OH, 1) = 6·5); 3·90 m (1 H, H-1, $\sum J$ = 21). For $C_{19}H_{32}O_2$ (292·5) calculated: 78·03% C, 11·03% H; found: 78·08% C, 10·28% H.

5-Hydroxy-5α-androstane-1,17-dione (*XIII*)

Triol *XI* (44 mg, 0·14 mmol) was dissolved in acetone (2 ml) and the solution cooled with ice. Jones's reagent (5 drops) was then added dropwise to it and the mixture was allowed to react for 10 min. The solvent was evaporated in a vacuum and the reaction mixture extracted with ether (3 × 20 ml). The extract was washed with a 10% aqueous sodium hydrogen carbonate solution and water, then dried, filtered and evaporated to dryness. The residue (42·5 mg) was crystallized from an ether–benzene 5 : 2 mixture (0·7 ml), affording 21 mg (48%) of a pure product of m.p. 186–190°C, $\Delta\varepsilon_{290}$ +3·83. ^{1}H NMR spectrum: 0·862 s (3 H, Me-18); 1·295 s (3 H, Me-19). ^{1}H NMR spectrum after addition of TAI: 0·876 s (3 H, Me-18); 1·329 s (3 H, Me-19); 8·33 s (1 H, NH). For $C_{19}H_{28}O_3$ (304·4) calculated: 74·96% C, 9·27% H; found: 75·24% C, 9·07% H.

5-Hydroxy-5α-androstan-1-one (*XIV*)

Diol *XII* (77 mg, 0·26 mmol) was dissolved in a mixture of acetone (12 ml) and ether (3 ml), the solution was cooled with ice and water and Jones's reagent (about 0·2 ml) was added to it. The excess of the reagent was eliminated by addition of isopropyl alcohol. The solvents were evaporated almost to dryness, the residue was diluted with ether (40 ml) and washed with water, aqueous sodium hydrogen carbonate solution and water, dried and concentrated. The residue (69·5 mg) was crystallized from methanol. Yield, 37 mg (48%) of compound *XIV*, m.p. 206 to 210°C, $[\alpha]_D$ +112° (*c* 1·7, chloroform). ^{1}H NMR spectrum: 0·695 s (3 H, Me-18); 1·280 s (3 H, Me-19). ^{1}H NMR spectrum after addition of TAI: 0·710 s (3 H, Me-18); 1·310 s (3 H, Me-19); 8·27 s (1 H, NH). For $C_{19}H_{30}O_2$ (290·4) calculated: 78·57% C, 10·41% H; found: 78·47% C, 10·57% H.

5α-Cholestan-5-ol[16] (*XVa*)

^{1}H NMR spectrum: 0·649 s (3 H, Me-18); 0·859 d and 0·864 d (6 H, Me-26 and Me-27, J = 6·6); 0·898 d (3 H, Me-21, J = 6·6); 0·962 s (3 H, Me-19). ^{1}H NMR spectrum after addition of TAI: 0·663 s (3 H, Me-18); 0·857 d and 0·862 d (6 H, Me-26 and Me-27, J = 6·6); 0·903 d (3 H, Me-21, J = 6·4); 1·019 s (3 H, Me-19); 8·22 s (1 H, NH).

5β-Cholestan-5-ol[16] (*XVb*)

^{1}H NMR spectrum: 0·643 s (3 H, Me-18); 0·861 d and 0·866 d (6 H, Me-26 and Me-27, J = 6·6); 0·893 s (3 H, Me-19); 0·902 d (3 H, Me-21, J = 6·6). ^{1}H NMR spectrum after addition of TAI: 0·659 s (3 H, Me-18); 0·863 d and 0·867 d (6 H, Me-26 and Me-27, J = 6·6); 0·906 d (3 H, Me-21, J = 6·6); 1·035 s (3 H, Me-19); 8·16 s (1 H, NH).

6β-Chloro-5-hydroxy-5α-cholestan-3β-yl Acetate[17] (*XVI*)

^{1}H NMR spectrum: 0·699 s (3 H, Me-18); 0·861 d and 0·865 d (6 H, Me-26 and Me-27, J = 6·6); 0·906 d (3 H, Me-21, J = 6·6); 1·273 s (3 H, Me-19); 2·03 s (3 H, OAc); 2·31 dd (1 H, H-4β, J(4, 3) = 11·0; J(4, 4) = 13·1); 3·83 dd (1 H, H-6, J(6, 7) = 3·8 and 2·0); 5·10 m (1 H, H-3). ^{1}H NMR after addition of TAI: 0·716 s (3 H, Me-18); 0·859 d and 0·863 d (6 H, Me-26 and Me-27, J = 6·6); 0·912 d (3 H, Me-21, J = 6·4); 1·368 s (3 H, Me-19); 2·02 s (3 H, OAc); 2·34 dd (1 H, H-4β, J(4, 4) = 14·1; J(4, 3) = 11·7); 2·91 dd (1 H, H-4α, J(4, 4) = 14·1; J(4, 3) = 4·9); 4·75 m (1 H, H-3); 5·09 dd (1 H, H-6, J(6, 7) ≈ 4 and 2); 8·35 s (1 H, NH).

3β-Bromo-5-hydroxy-5α-androstane-1,17-dione[18] (*XVII*)

^{1}H NMR spectrum: 0·865 s (3 H, Me-18); 1·325 s (3 H, Me-19); 2·15 dd (1 H, H-4α, J(4, 4) = 14; J(4, 3) = 6); 2·57 dd (1 H, H-4β, J(4, 4) = 14; J(4, 3) = 12); 2·79 dd (1 H, H-2α, J(2, 2) = 13; J(2, 3) = 6); 4·52 m (1 H, H-3). ^{1}H NMR after addition of TAI: 0·88 s (3 H, Me-18); 1·38 s (3 H, Me-19); 2·55 dd (1 H, H-4β, J(4, 4) = 15; J(4, 3) = 12); 2·87 dd (1 H, H-2α, J(2, 2) = 13; J(2, 3) = 6); 3·20 t (1 H, H-2β, J(2, 2) = 13; J(2, 3) = 12); 3·37 dd (1 H, H-4α, J(4, 4) = 15; J(4, 3) = 6); 4·12 m (1 H, H-3); 8·41 s (1 H, NH).

5-Hydroxy-5α-cholestan-6-one[11] (*XVIII*)

^{1}H NMR spectrum: 0·644 s (3 H, Me-18); 0·775 s (3 H, Me-19); 0·863 d (6 H, Me-26 and Me-27, J = 6·5); 0·908 d (3 H, Me-21, J = 6·5). ^{1}H NMR after addition of TAI: 0·647 s (3 H, Me-18); 0·855 s (3 H, Me-19); 0·860 d (6 H, Me-26 and Me-27, J = 6·5); 0·908 d (3 H, Me-21, J = 6·5); 8·75 s (1 H, NH).

5α-Androstane-5,17β-diol[18] (*XIX*)

^{1}H NMR spectrum: 0·739 s (3 H, Me-18); 1·002 s (3 H, Me-19); 3·60 m (1 H, H-17). ^{1}H NMR after addition of TAI: 0·874 s (3 H, Me-18); 1·101 s (3 H, Me-19); 4·24 m (1 H, H-17); 8·35 s and 8·37 s (2 H, 2 × NH).

14-Hydroxy-3-methoxyestra-1,3,5(10)-trien-17-one[13] (*XXa*)

^{1}H NMR spectrum: 1·033 s (3 H, Me-18); 3·78 s (3 H, OMe); 6·65 d (1 H, H-4, J(4, 2) = 2·8); 6·73 dd (1 H, H-2, J(2, 1) = 8·6; J(2, 4) = 2·8); 7·22 d (1 H, H-1, J(1, 2) = 8·6). ^{1}H NMR spectrum after addition of TAI: 1·136 s (3 H, Me-18); 3·78 s (3 H, OMe); 6·63 d (1 H, H-4, J(4, 2) = 2·8); 6·74 dd (1 H, H-2, J(2, 1) = 8·6; J(2, 4) = 2·8); 7·24 d (1 H, H-1, J(1, 2) = 8·6); 8·26 s (1 H, NH).

14-Hydroxy-3-methoxy-14β-estra-1,3,5(10)-trien-17-one[13] (*XXb*)

^{1}H NMR spectrum: 1·084 s (3 H, Me-18); 3·78 s (3 H, OMe); 8·65 d (1 H, H-4, $J(4, 2) = 2·7$); 6·73 dd (1 H, H-2, $J(2, 1) = 8·6$; $J(2, 4) = 2·7$); 7·20 d (1 H, H-1, $J(1, 2) = 8·6$). ^{1}H NMR spectrum after addition of TAI: 1·158 s (3 H, Me-18); 3·79 s (3 H, OMe); 6·66 d (1 H, H-4, $J(4, 2) = 2·8$); 6·75 dd (1 H, H-2, $J(2, 1) = 8·6$; $J(2, 4) = 2·8$); 7·20 d (1 H, H-1, $J(1, 2) = 8·6$); 8·28 s (1 H, NH).

We thank the departments of analytical chemistry (Dr J. Horáček, head) and molecular spectroscopy (Dr J. Smolíková, head) for elemental analyses and the IR *and* CD *spectra and Miss J. Sklenářová for technical assistance. Our thanks are also due to Prof. G. Schubert of the Zentralinstitut for Mikrobiologie und experimentelle Therapie in Jena and Prof. K. Ponsold of the same Institute for the kind donation of the samples of compounds* XXa *and* XXb.

REFERENCES

1. Eignerová L., Procházka Ž.: Collect. Czech. Chem. Commun. *39*, 2828 (1974).
2. Zakharychev A. V., Kasymov S. K., Ananchenko S. N., Torgov I. V.: Izv. Akad. Nauk SSSR, Ser. Khim. *8*, 1790 (1969).
3. Kirk D. N., Klyne W.: J. Chem. Soc., Perkin Trans. 1 *1974*, 1076.
4. Klyne W.: Private communication.
5. Wulfson N. S., Zaretskii V. I., Sedovskaya V. L., Zakharychev A. V., Ananchenko S. N., Torgov I. V.: Tetrahedron *23*, 3667 (1967).
6. Goodlett V. W.: Anal. Chem. *37*, 431 (1965).
7. Samek Z., Buděšínský M.: Collect. Czech. Chem. Commun. *44*, 558 (1979).
8. Plattner P. A., Petrzilka T., Lang W.: Helv. Chim. Acta *27*, 513 (1944).
9. Plattner P. A., Heusser H., Feurer M.: Helv. Chim. Acta *32*, 587 (1949).
10. Huynh Kim Thoa, Procházka Ž., Buděšínský M., Kočovský P.: Collect. Czech. Chem. Commun. *43*, 2305 (1978).
11. Huynh Kim Thoa, Procházka Ž.: Unpublished results.
12. Tieffeneau M., Weill P., Tchoubar B.: C. R. Acad. Sci. *205*, 54 (1937); Birch A. J., Harrisson R. J.: Aust. J. Chem. *8*, 519 (1955).
13. Schubert G., Ponsold K.: Pharmazie *34*, 323 (1979).
14. Blunt J. W., Stothers J. B.: Org. Magn. Reson. *9*, 439 (1977).
15. Smith W. B.: Annu. Rep. NMR Spectrosc. *8*, 221 (1978).
16. Shoppee C. W., Howden M. E. H., Killick R. W., Summers G. H. R.: J. Chem. Soc. *1959*, 630.
17. Růžička L., Bosshard W.: Helv. Chim. Acta *20*, 244 (1937).
18. Kočovský P.: Unpublished results.
19. Reich H., Walker F. E., Collins R. W.: J. Org. Chem. *16*, 1753 (1951).

Translated by the author (Ž.P.).