267. Stereochemical Structure of the Dihydrothiazines. I. ^{1}H-NMR. Determination of the Stereochemistry of two Reduction Products of Benzo[*b*]-5α-17β-hydroxy-androst-2-eno[2,3-*e*][1,4]thiazine

by **Oronzo Sciacovelli**

Istituto di Chimica fisica della Facoltà di Scienze, Università di Bari, Via Amendola 173, Bari (Italia)

and **Paolo Marchini, Gaetano Liso** and **Antonia Reho**

Istituto di Chimica Organica della Facoltà di Farmacia, Università di Bari, Via Amendola 173, Bari (Italia)

(3.VI.77)

Summary

The stereochemical structure of two reduction products of benzo[*b*]-5α-17β-hydroxy-androst-2-eno[2,3-*e*] [1,4]thiazine has been determined by ^{1}H-NMR. spectrometry using the INDOR technique.

In a series of preceding papers we have described a new synthesis of 1,4-thiazines from 2,2'-dithiodianiline and ketones. A mechanism has also been suggested for the reaction, which has proved to be a general method for preparing 1,4-thiazines [1].

Later, the results were reported of a pharmacological investigation performed on some derivatives of 5a,6,7,8,9,10,10a,11-octahydrobenzo[*b*]cyclohepta[*e*][1,4]-thiazine [2], which can be obtained by sodium borohydride reduction of salts of 1,4-thiazines.

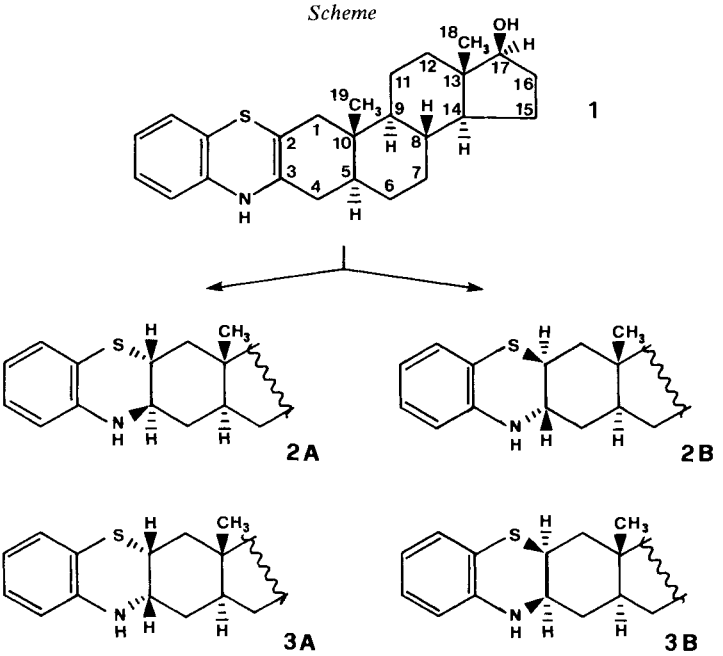
In order to investigate the relationships that exist between structure and pharmacological activity we have undertaken a NMR. study on the stereochemistry of the dihydro-1,4-thiazines. In the present paper we report on the reduction products of benzo[*b*]-5α-17β-hydroxy-androst-2-eno[2,3-*e*] [1,4]thiazine (**1**).

The reduction of the 1,4-thiazine **1** hydrochloride with sodium borohydride afforded two compounds with very similar Rf values. Resolution of the mixture by preparative chromatography yielded two products: the first higher-melting (h.m.) in a yield of 60% (m.p. 228–230°), and the second lower-melting (l.m.) with a smaller Rf, in a yield of 40% (m.p. 204–206°).

The results of elemental analysis and the IR. spectra showing the presence of NH and OH bands and the lack of bands attributable to imine or enamine double bonds, together with the chemical genesis, enable us to attribute the structure of dihydro-1,4-thiazine to both of these products.

In order to determine which of the four diastereoisomeric structures theoretically predictable (see *Scheme*) corresponds to those of the two compounds isolated, we performed a ^{1}H-NMR. study.

Scheme



In the ^{1}H-NMR. spectrum of the l.m. compound the four aromatic protons absorb between 6.3 and 7.0 ppm. At higher field *(Fig. 1a)*, two absorption regions can be distinguished 0.4–2.3 ppm and 2.3–3.9 ppm. The sharp singlet at 0.7_0 ppm could be assigned to the C(18) methyl in agreement with the general observation that in steroids, substituents on ring A have no influence on the chemical shift of the C(18) methyl [3]. Moreover the positive NOE (see below) observed in the integral of H–C(2) by irradiation at 0.8_6 ppm allowed the assignment of this resonance to the C(19) methyl, confirming the location of the methyl group resonances. Integration of the signal areas shows that the region from 2.3–3.9 ppm contains five protons. The broad signal at 2.9 ppm which disappeared on addition of D_2O has been assigned to the NH and OH protons. Decoupling experiments *(Fig. 1b)* showed that the protons at 3.1_0 and 3.8_4 ppm are coupled to each other and can therefore be attributed to H–C(2) and H–C(3). Consequently the resonance at 3.5_4 ppm (two $J \simeq 8.0$ Hz) can be attributed to H–C(17). Irradiation at 3.1_0 ppm simplified the signal at 3.8_4 ppm to an apparent triplet, not well resolved, with two coupling constants of about 3 Hz. At this stage, to complete the assignment of the multiplets at 3.1_0 and 3.8_4 ppm to H–C(2) and H–C(3), and to obtain further information on the vicinal coupling constants between the protons of ring A of the androstane, it was necessary to perform some INDOR experiments [4]. The frequencies of the INDOR signals obtained by monitoring the lines of the proton at 3.1_0 ppm decoupled[1]) from the proton at 3.8_4 ppm *(Fig. 1c)* are given in

[1]) The INDOR spectra were recorded by monitoring the lines of the decoupled proton to obtain a better signal-to-noise ratio. Because of the poor solubility of the compound the signal of the coupled proton are of too low intensity to be used as monitor lines.

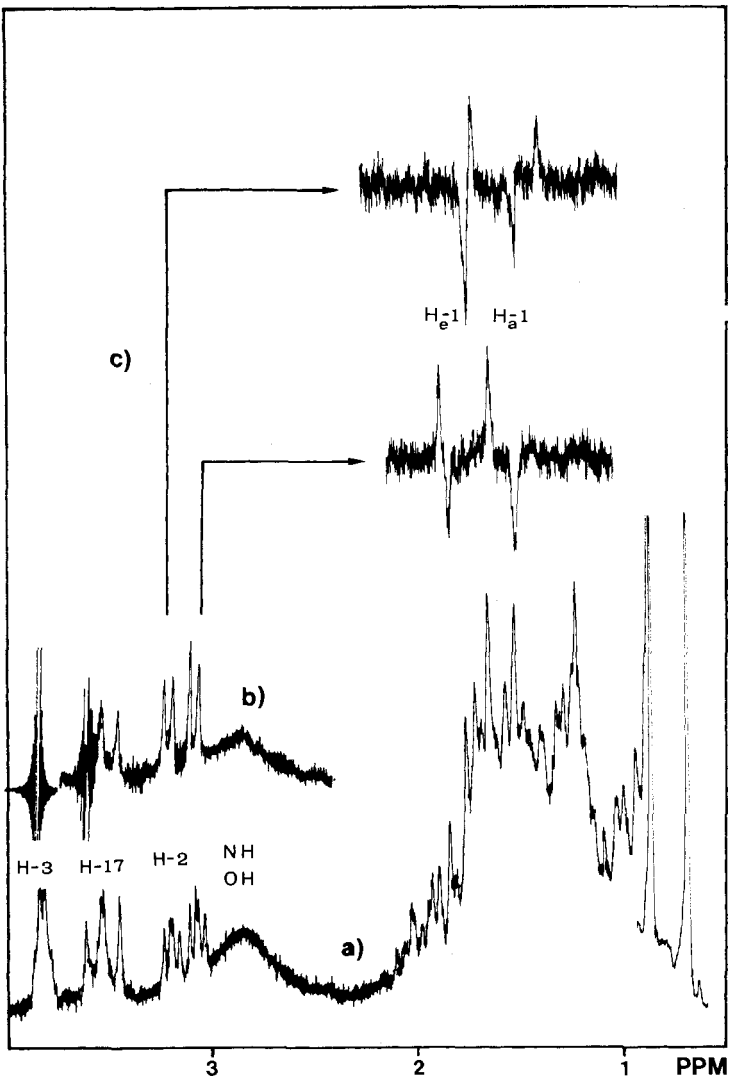


Fig. 1. (a) *High field part of the* ^{1}*H-NMR. spectrum* (100 MHz) *of the l.m. compound in* $CDCl_3$; (b) *part of the spectrum decoupled from the proton at* 3.8_4 *ppm;* (c) *INDOR spectra of the outer lines of the proton at* 3.1_0 *ppm by simultaneous decoupling at* 3.8_4 *ppm*

Table 1. These experiments allowed to locate two hidden protons at ~1.5 and ~1.7 ppm. Starting from the frequencies of all lines of the three protons the set of the ^{1}H-NMR. parameters was computed *(Table 4).*

The results of the INDOR experiments and the values of $J_{1a,2}$, $J_{1e,2}$ and $J_{1a,1e}$ clearly indicated that the resonances at 1.4_9 and 1.7_4 ppm originated from two geminal protons which showed vicinal coupling constants only with the proton at 3.1_0 ppm. It follows that the protons at 1.4_9, 1.7_4 and 3.1_0 ppm correspond,

Table 1. *Frequencies* (in Hz at 100 MHz) *of the INDOR signals*[a]) *obtained by monitoring the lines of H–C(2)*[b]) *decoupled from H–C(3) of the l.m. compound*

Monitored lines of H–C(2)	Observed INDOR signals							
	H_a–C(1)				H_e–C(1)			
301.5			↓147.5	↑160.3			↓179.8	↑184.3
306.0	↓134.5	↑146.6					↑180.1	↓184.4
314.2			↑147.6	↓159.8	↓167.2	↑171.2		
318.6	↑134.3	↓146.5			↑167.2	↓171.0		
Averaged frequencies	134.4	146.6	147.6	160.1	167.2	171.1	180.0	184.4

[a]) The arrows indicate positive (↑) or negative (↓) INDOR signals (6); the reproducibility of the INDOR frequencies in this case is ±0.2 Hz.

[b]) For sake of clarity the correct assignments of the protons have been used.

Table 2. *Frequencies* (in Hz at 100 MHz) *of the INDOR signals*[a]) *obtained by monitoring the lines H_e–C(1) of the h.m. compound*

Monitored lines of H_e–C(1)	Observed INDOR signals								
	H_a–C(1)				H–C(2)				
184.5[b])	↓87.5		↑100.5				↓317.0	↑320.0	↓325.0	↑329.0
188.0[b])		↓99.5		↑113.0			↑317.0	↓320.0	↑325.0	↓329.0
197.5[c])	↑87.5		↓100.5		↓301[d])	↓308[d]) br.	↑313 br.			
200.5[c])		↑99.0		↓112.0		↓312				

[a]) The reproducibility of the INDOR frequencies is ±0.5 Hz. Only frequencies of well developed INDOR signals have been reported.

[b]) Signals obtained from INDOR spectra on the transitions at 317.0 and 320.0 Hz (see *Fig. 3b*).

[c]) Signals localized by decoupling of H–C(2).

[d]) Low intensity INDOR signals. br. = broad.

respectively, to H_a–C(1), H_e–C(1) and H–C(2). Hence the multiplet at 3.8_4 ppm could be assigned to H–C(3) and the value of $J_{23} = 3.2$ Hz accounted for a gauche dihedral angle between these protons. Furthermore the two small coupling constants ($J \simeq 3$ Hz) observed on H–C(3) decoupled from H–C(2) indicate also a gauche dihedral angle between H–C(3) and both H_a–C(4) and H_e–C(4) protons. Moreover, from a comparison between the values of the ^1H, ^1H coupling constants in ring A of the androstane and the corresponding data in the chair conformation of cyclohexane [5] *(Table 4)*, for the thiazine junction the configuration shown in *Figure 2* can be formulated, which corresponds to structure 3A of the *Scheme*. NOE experiments supported this assignment. In fact, for the product treated with D_2O to eliminate the interfering effects from the protons of the NH and OH groups on the integral of the signals at 3.1_0 and 3.8_4 ppm, irradiation at 0.8_6 ppm singlet afforded a positive NOE (15%) only on the integral of H–C(3) (3.1_0 ppm). Irradiation at 0.7_0 ppm did not affect the integrals of H–C(2) and H–C(3).

In the ^{1}H-NMR. spectrum of the h.m. compound the four aromatic protons absorb between 6.3 and 7.0 ppm. Five protons resonate in the region from 2.7–3.7 ppm. The remaining protons of the steroid show signals between 0.5 and

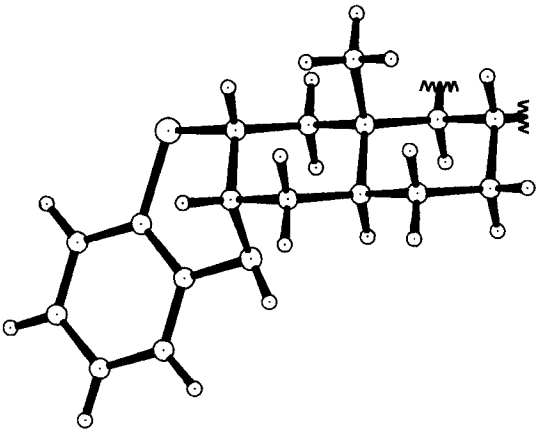


Fig. 2. *Partial stereodiagram of the l.m. compound corresponding to structure 3A in the* Scheme

2.2 ppm *(Fig. 3a)*. The assignment of the two singlets at 0.7_0 and 0.8_5 ppm to the C(18) and C(19) methyl groups, respectively, was made on the basis of the considerations already illustrated for the l.m. compound and were confirmed by the NOE experiment described below. The apparent triplet at 3.5_4 ppm (1 H) could be attributed to H–C(17) on the basis of the coincidence of the chemical shift and of the coupling constants ($J = 7.5$ and $J = 8.5$ Hz) with the corresponding parameters of H–C(17) in the l.m. isomer. The broad signal at 2.9 ppm (2 H) disappears on addition of D_2O and has been assigned to the protons of the NH and OH groups. The signals between 2.9_5 and 3.3_5 ppm (2 H) originated from H–C(2) and H–C(3). In this range the absence of well-resolved lines prevents an extended application of the INDOR technique. Thus, it has not been possible to locate a number of hidden resonances sufficient to define the structure of the fragment $-C(1)H_2-C(2)H-C(3)H-C(4)H_2-$. Nevertheless, the stereochemistry of the thiazine linkage has been defined. By monitoring the maxima at 317.0 and 320.0 Hz *(Fig. 3a)* it has been possible to locate some INDOR signals *(Fig. 3b)* originated from two protons which resonate at ~1 and ~2 ppm. Decoupling experiments, performed by irradiation at ~3.1 ppm, allowed to find in the ^{1}H-NMR. spectrum two other resonance lines, at 197.5 and 200.5 Hz, which could be assigned to the proton at 2 ppm. The four frequencies of the proton at 2 ppm have in turn been used for further INDOR experiments.

The analysis of the INDOR experiments showed that the resonances at 1.0_0 and 1.9_3 ppm are due to the protons of a methylene group flanked by a quaternary C atom and should therefore be assigned to H_a–C(1) and H_e–C(1). In the consecutive INDOR experiments on the signals at 184.5 and 188.0 Hz four resonance lines of H–C(2) could be located *(Fig. 3c)*. It is interesting to note that the two lower field signals originate from transitions of very small intensity which are difficult to detect in the single resonance spectra. The approximate value of $J_{2,3} \sim 9$ Hz, obtained as the difference of two INDOR signals of the same type – progressive or regressive – indicates a antiperiplanar arrangement for the protons of the thiazine linkage. Irradiation of the methyl group at 0.7_0 ppm has no effect on the integral of the signals between 2.9_5 and 3.3_5 ppm (2 H) whereas irradiation at 0.8_5 ppm afforded an enhancement of 6%, therefore the NOE relative to a single proton

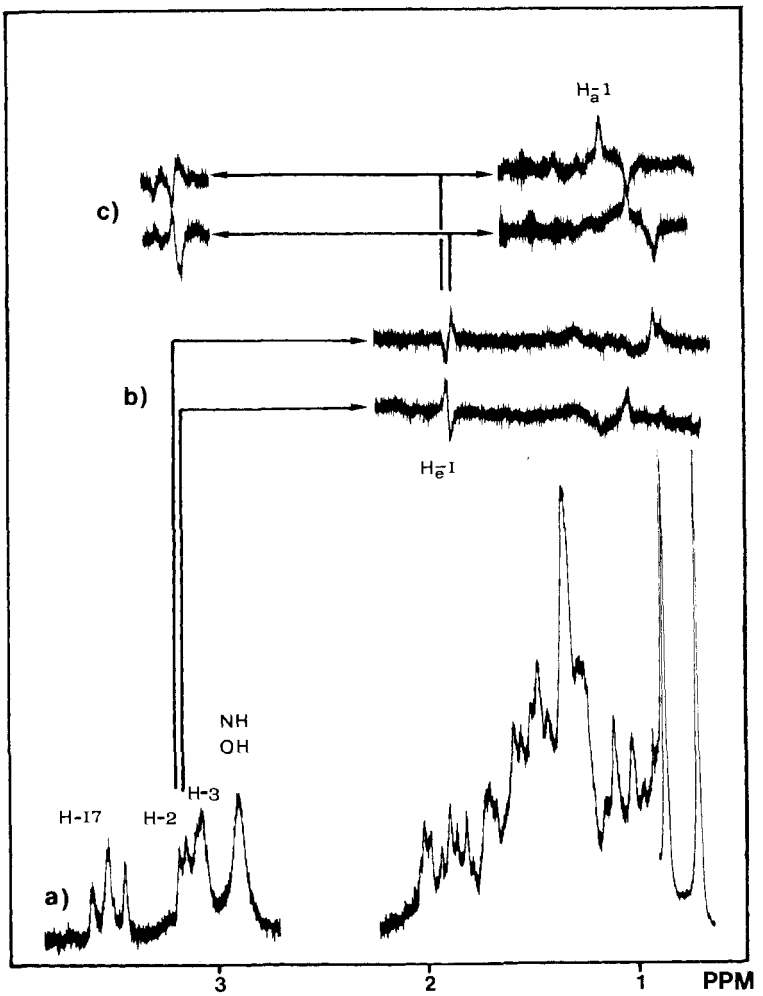


Fig. 3. (a) *High field part of the* ^{1}*H-NMR. spectrum* (100 MHz) *of the h.m. compound in* $CDCl_3$; (b) *and* (c) *primary and consecutive INDOR experiments*

corresponds to 12%. To avoid interferences from the NH and OH resonance (2.9 ppm) the sample for NOE experiments was previously exchanged with D_2O. Because of the overlapping resonances the NOE could not be attributed to H–C(2) or H–C(3). This circumstance and the values of $J_{1a,2}$, $J_{1e,2}$ and $J_{2,3}$ do not permit to distinguish between the two structures 2A or 2B *(Figs. 4* and *5)* which requires H–C(2) or H–C(3) to be adjacent to the C(19) methyl group alternatively. We tried to obtain more information about the stereochemistry of the h.m. compound from its diacetyl derivative **4** which shows separated resonances for H–C(2) and H–C(3) *(Fig. 6a)*. Acetylation of the NH group and esterification of the OH group caused downfield shifts of the H–C(3) (4.2 ppm) and of H–C(17) (4.6_5 ppm, $J = 7.5$ Hz and $J = 8.5$ Hz) resonances. The multiplet at 2.7_5 ppm could be attributed to H–C(2). The structure of this multiplet indicates the presence of two large

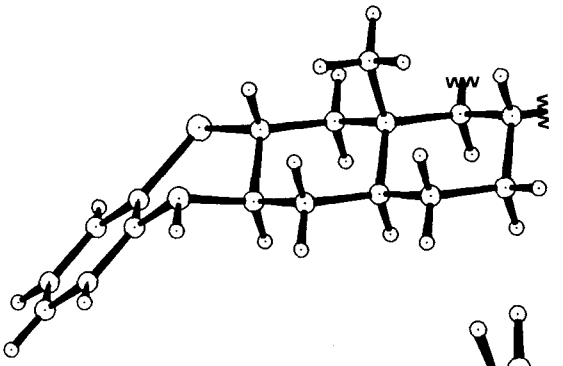


Fig. 4. *Partial stereodiagram of the h.m. compound corresponding to structure 2A on the* Scheme

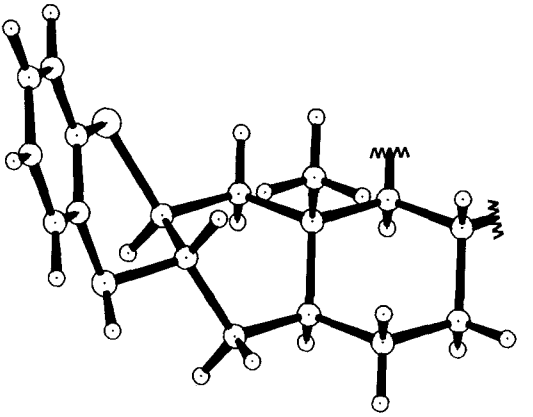


Fig. 5. *Partial stereodiagram of the structure 2B on the* Scheme

(~ 12 Hz) and one small (~ 3 Hz) coupling constants. The protons of the two methyl groups of the steroid skeleton resonate at 0.7_6 and 0.6_3 ppm, while those of the two CH_3–CO– groups have practically the same resonance frequency: 1.9_1 ppm. Irradiation at 0.6_3 or 0.7_6 ppm did not give intensity enhancement in the integral of H–C(2) or H–C(3); probably the introduction of the two acetyl groups and, particularly, the one on the nitrogen atom, gives rise to relaxation phenomena which annihilate the NOE. By INDOR experiments performed on the lines of H–C(2) *(Fig. 6b)* the four lines of H_a–C(1) and the four lines of H_e–C(1) could be located *(Table 3)*, while H–C(3) gives broad INDOR responses. Monitoring of two lines of H_e–C(1), 203.6 and 206.6 Hz, *(Fig. 6c)* confirms the geminal coupling of this proton with H_a–C(1) and allows to obtain $J_{2,3}$ from the two progressive or regressive INDOR signals of H–C(2). Because of the broad resonance of H–C(3) no values can be determined for the coupling constants between H–C(3) and the protons of the methylene group at C(4). The ^{1}H-NMR. parameters of H_a–C(1), H_e–C(1), H–C(2) and H–C(3) obtained from a first-order analysis of the spectra are given in the third column of *Table 4.* In conclusion, in the h.m. isomer and its diacetyl derivative the value of $J_{2,3}$ indicates that H–C(2) and H–C(3) have the antiperiplanar arrangement, while the NOE experiments do not provide indications of the spatial proximity of the C(19) methyl group and H–C(2) or H–C(3).

The structure corresponding to the h.m. compound can be deduced by comparing its spectral data with those of the l.m. compound. Inspection of *Dreiding* models shows that the anisotropic effect of the benzo ring on the chemical shifts

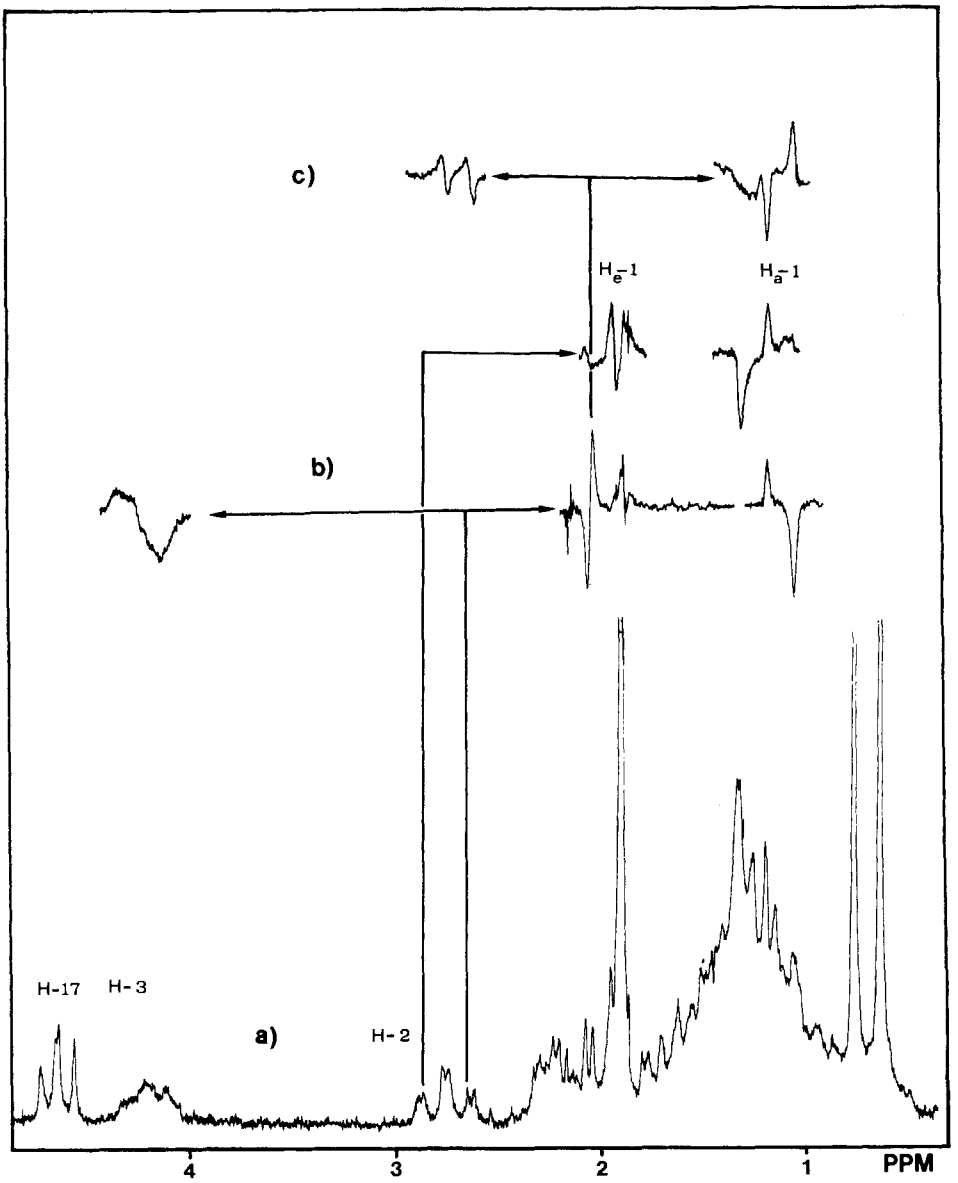


Fig. 6. (a) *High field part of the* ^{1}*H-NMR. spectrum* (100 MHz) *of* **4** *in* $CDCl_3$; (b) *and* (c) *primary and consecutive INDOR experiments*

of H–C(2), H–C(3) and C(19) methyl protons is of the same order in 2A and 3A. Therefore, the remarkable constancy of the H–C(2) and C(19) methyl proton resonances within the l.m. and h.m. compounds, and the chemical shift difference, $(\nu_3)_{\text{l.m.}} - (\nu_3)_{\text{h.m.}} = 0.7_4$ ppm, which agrees fairly well with that recorded for H–C(9) in *cis* and *trans*-decahydroquinolines in twin-chair conformations, $(\nu_9)_{cis} - (\nu_9)_{trans} = 0.7_5$ ppm [7], can be taken as evidence for an identical conformation of ring A

Table 3. *Frequencies* (in Hz at 100 MHz) *of the INDOR signals*[a]) *obtained by monitoring some lines of H–C(2) of compound* **4**

Monitored lines of H–C(2)	Observed INDOR signals							
	H_a–C(1)				H_e–C(1)			
261.6		↓117.8		↑130.6			↓203.5	↑206.7
264.8	↓105.4		↑118.0				↑203.6	↓206.5
286.1		↑117.5		↓130.0	↓191.4	↑194.5		
289.1	↑105.6		↓118.0		↑191.2	↓194.2		
Averaged frequencies	105.5	117.7	118.0	130.3	191.3	194.4	203.6	206.6

[a]) The reproducibility of the INDOR frequencies in this case is ±0.2 Hz.

Table 4. *^{1}H-NMR. parameters*[a])

^{1}H-NMR. parameters	Compound			
	l.m.	h.m.	**4**	cyclohexane[b])
ν_{1a}	1.4_9	0.9_9	1.1_8	
ν_{1e}	1.7_4	1.9_2	1.9_9	
ν_2	3.1_0	3.2	2.7_5	
ν_3	3.8_4	3.1	4.2	
$J_{1a,1e}$	−13.2	−13.0	−12.3	-13.0_5 –
$J_{1a,2}$	+12.9	+12.4	+12.6	$+13.1_2$
$J_{1e,2}$	+ 3.9	+ 3.5	+ 3.0	$+ 3.6_5$
$J_{2,3}$	+ 3.2	+ 8.5	+12.5	
$J_{3,4a}$	3–4			
$J_{3,4e}$	3–4			

[a]) Chemical shifts are referred to TMS. Coupling constants are expressed in Hz. The sign combination has been derived from the connection of the monitor- and INDOR-transitions in the energy level diagram assuming a positive value for the vicinal coupling constants.

[b]) Low-temperature values for the chair conformation (5).

in the two isomers. It must be emphasized that the effects on the chemical shifts due to a different orientation of the nitrogen lone-pair in the two isomers have been ignored. However, when the evidence is taken in conjunction with the remark that $J_{1a,2}$ in the h.m. isomer and its diacetyl derivative **4** has the same value as in the l.m. isomer indicating an antiperiplanar coupling, structure 2A *(Fig. 4)* appears to be the most probable.

The authors would like to thank Dr. *S. Cerrini,* Laboratorio di Strutturistica Chimica 'G. Giacomello', Monterotondo (Roma), for the program of the stereodiagram. The work was financially supported by the Consiglio Nazionale delle Ricerche (Roma).

Experimental Part

All melting points were determined with a *Kofer* apparatus and are uncorrected. IR. spectra were recorded in nujol mulls on a *Perkin-Elmer* 257 grating spectrophotometer. The ^{1}H-NMR. spectra were recorded at 100 MHz on a *Varian* HA 100 spectrometer using an expansion scale of 1 Hz mm^{-1} and a sweep rate of 0.5 Hz s^{-1}. All compounds were dissolved in $CDCl_3$. Because of the low solubility of the compounds the solutions are close to saturation.

A small amount of $CHCl_3$ was added to generate both the lock and internal reference signal. All samples were carefully degassed by the freeze-pump-thaw technique, and the NMR. tubes were sealed under vacuum (10^{-5} Torr).

For the INDOR experiments a sweep rate of 0.2 Hz s^{-1} was used. The intensity of the saturation frequency was low enough to avoid tickling effects on the irradiated transitions. It was necessary to use $CHCl_3$ as the lock signal because the methylene proton frequency was close to the tetramethylsilane frequency, and attempts to saturate the methylene proton region resulted in unlocking the spectrometer. All frequencies are referred to TMS ($\delta_{CHCl_3} = 7.17$).

The audio-modulation frequencies for INDOR, double and triple resonance experiments were obtained from two *Hewlett-Packard* 4204-A digital oscillators.

The position of the INDOR peaks was obtained by stopping the pen of the recorder in correspondence with the maximum of the negative or positive signals. The calibration was made by a *Hewlett-Packard* 5521A frequency counter.

The NOE increments are the mean values of ten integrals. For simulation and iteration of the spectra a modified LAOCOON III program was used.

Reduction of benzo[b]-*5α-17β-hydroxy-androst-2-eno*[*2,3*-e][*1,4*]*thiazine with* $NaBH_4$. To 30 ml of EtOH saturated with HCl 1.6 g of **1** was added under nitrogen. The solution was evaporated under vacuum and the solid thus obtained was dissolved in CH_3OH (70 ml). To this solution 2 g of $NaBH_4$ were added in portions, with stirring and cooling in ice. After 20 min the solvent was evaporated and the residue was dissolved in ether. The organic layer was washed with water and dried over Na_2SO_4. After evaporation of the solvent a solid was obtained (1.5 g), which on TLC. appeared to be a mixture of two products. On preparative PLC. (*Merck* P.F. 254 silica, cyclohexane/EtOAc 8:2) it was possible to isolate two products:

1) 0.7 g, m.p. 228–230° (from EtOH), showing in the IR. spectrum two bands at 3300–3360 cm^{-1} (OH and NH).

$C_{25}H_{35}NOS$ Calc. C 75.51 H 8.87 N 3.52 S 8.06% Found C 75.66 H 8.95 N 3.52 S 8.00%

2) 0.55 g, m.p. 204–206° (from EtOH), showing in the IR. spectrum two bands at 3300–3390 cm^{-1} (OH and NH).

$C_{25}H_{35}NOS$ Calc. C 75.51 H 8.87 N 3.52 S 8.06% Found C 75.70 H 8.85 N 3.53 S 8.09%

Preparation of the diacetyl derivative of the low-melting compound: a solution of 0.12 g of the l.m. product (204–206°) dissolved in 5 ml of $(CH_3CO)_2O$ was boiled under reflux for 1 h. After evaporation of the solvent the residue was crystallized from EtOAc and petroleum ether (m.p. 268–270°).

$C_{29}H_{39}NO_3S$ Calc. C 72.31 H 8.16 N 2.91 S 6.65% Found C 71.80 H 8.05 N 2.89 S 6.65%

REFERENCES

[1] *V. Carelli, P. Marchini, M. Cardellini, F. Micheletti Moracci, G. Liso & M.G. Lucarelli,* Tetrahedron Letters *1969,* 4619; *V. Carelli, P. Marchini, M. Cardellini, F. Micheletti Moracci, G. Liso & M.G. Lucarelli,* Ann. Chimica Roma *59,* 1050 (1969).

[2] *F. Micheletti Moracci, F. Libratore, P. Marchini, G. Liso & M. Cardellini,* J. med. Chemistry *17,* 463 (1974).

[3] *R.F. Zuercher,* Helv. *46,* 2054 (1963); *L.H. Knox, E. Velarde, S. Berger, D. Cuadriello & A.D. Cross,* J. org. Chemistry *29,* 2187 (1963); *N.S. Bhacca & D.M. Williams,* Application of NMR. Spectroscopy in Organic Chemistry. Illustrations from the Steroid Field. Prentice Hall, San Francisco 1964.

[4] *E.B. Baker,* J. chem. Physics *37,* 911 (1962); *E.B. Baker & L.W. Burd,* Rev. Sci. Instruments *34,* 238 (1963); *E.B. Baker, L.W. Burd & C.V. Root,* Rev. Sci. Instruments *34,* 243 (1963); *R. Kaiser,* J. chem. Physics *39,* 2435 (1963).

[5] *E.W. Garbisch & M.S. Griffith,* J. Am. chem. Soc. *90,* 6543 (1968).

[6] *O. Sciacovelli, W. von Philipsborn, C. Amith & D. Ginsburg,* Tetrahedron *26,* 4589 (1970); *W. von Philipsborn,* Pure appl. Chemistry *40,* 159 (1974).

[7] *H. Booth & A.H. Rostock,* J. chem. Soc. Perkin II *1972,* 615.