THE SYNTHESIS AND MASS SPECTRA OF SOME OXYGEN-BRIDGED 5α-CHOLESTANE AND B-HOMO-5α-CHOLESTANE DERIVATIVES*

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The synthesis and mass spectrometric behavior of a series of oxygen-bridged 5α-cholestanes and B-homo-5α-cholestanes *I*—*X* are described. The differences in fragmentation patterns of these compounds are discussed in dependence on the position of the oxygen bridge, position and configuration of additional substituents and conformation of the A and B rings of the steroid skeleton.

In steroid chemistry, compounds bearing substituents in position 19 have found use in the synthesis of 19-norderivatives[1-3]. Substitution at $C_{(19)}$ may also offer an excellent tool for controlling the stereochemistry of electrophilic additions to the double bonds located in A and B rings[4-12]. Some naturally occurring 19-substituted steroids, *e.g.* strophanthidin, ouabain and others are important cardiotonics. Hence, mass spectrometry of $C_{(19)}$-modified steroids became of interest and an extensive series of model compounds was investigated[13-18]. The presence of an oxygen function at $C_{(19)}$ results in a ready loss of the whole $C_{(19)}$-moiety under electron impact, the elimination being accompanied by a hydrogen transfer in dependence both on the nature of the substituents at position 19 and on the skeleton.

In the present paper we report the mass spectra of a group of $C_{(19)}$-modified 5α-cholestanes and B-homo-5α-cholestanes *I*—*X* in which the 10β-methyl is linked by the oxygen bridge to several positions in the A and B rings. In this way the ether group is introduced to 2β, 4β, 6β, 7β and 7aβ positions at which the axial arrangement of the ether function is imposed by bridging so that axial-to-equatorial flipping is avoided. Thus, substances *I*—*X* represent a useful set for studying electron-impact-induced skeletal fragmentations of compounds with the ether group fixed in axial conformation. As an example of cyclic ether with equatorial arrangement of the ether moiety we used the derivative *XI*.

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SYNTHESIS OF THE COMPOUNDS

The compounds *Ib*, *IIIa*, *IIIb*, *Vc*, *VIc*, *VIIa*, *VIIb*, *VIIIa* and *XI* are known[4,12,19–22]. The synthesis of the remaining compounds *Ia*, *IIa*, *IV*, *Vb*, *VIa*, *VIb*, *VId*, *IX* and *X* is described in the present paper.

The transannular epoxides *Ia* and *VIa* were prepared from the corresponding bromo epoxides *Ib* and/or *VIb* by reduction with Raney-nickel in boiling ethanol in practically quantitative yields. Despite the smooth course of the reduction of the compounds containing the bromine atom at the secondary carbon atom, the reaction completely failed when applied to the bromo epoxide *IIIb* with the bromine at the tertiary carbon atom: Under the same conditions the bromo epoxide *IIIb* gave a complicated mixture of at least five products which was not further worked up. The 4β,19-epoxide *IIa* was prepared from the known[23] 4β-alcohol *XII* by cyclization with lead tetraacetate in the presence of iodine under conventional conditions. For the preparation of the deuterated 6β,19-epoxide *IV* we modified the known methods[24]: The 5-cholestene[25] (*XIII*) was deuteroborated to afford mainly the (5α-^{2}H)-6α-alcohol *XV*. As a minor component we isolated the product of the reagent approach from the β-side, namely alcohol *XIV*. In order to invert the configuration of the hydroxy group in alcohol *XV* we oxidized the latter compound and reduced the ketone *XVI* thus obtained to 6β-alcohol *XVII*. Again, treatment of this compound with lead tetraacetate smoothly furnished the 6β,19-epoxide *IV*.

The bromo epoxide *Vb* was prepared in good yield from olefin *XVIII* by the addition of hypobromous acid. The addition commences by formation of the intermediary 6α,7α-bromonium ion *XIX* which is predominantly opened by an attack of the methoxyl oxygen as an internal nucleophile to afford the bromo epoxide *Vb*. Such behavior was discussed in detail in our previous papers[4–12] and may be characterized as a typical example of 5(O)^n neighboring group participation (for notation ref.[7]). Similarly, both the B-homo-6,7-unsaturated derivatives *XX* and *XXI* with different substituents at $C_{(19)}$ gave the bromo epoxide *VIb* quantitatively in the same reaction.

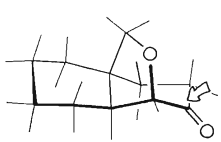
The ketones *IX* and *X* were prepared without difficulty from the known[12] alcohols *Vc* and *VIc* using Jones' reagent. The hydride reduction of ketone *IX* gave stereospecifically the axial 7α-alcohol *Vc*. No trace of the epimeric 7β-alcohol was detected by TLC. On the other hand, under the same conditions the B-homo-ketone *X* afforded a mixture of epimeric alcohols *VIc* and *VId* in which the latter compound slightly prevailed (44 : 56). Inspection of Dreiding models explained the diversity in the course of the reaction in both closely analogous ketones: In the case of the six-membered ring ketone *IX*, approach of the hydride reagent from the β-side (yielding the α-alcohol *Vc*) is relatively unhindered, while approach from the α-side is greatly hindered by axial 5α, 9α and 14α-hydrogen atoms (Fig. 1). On the other hand, the ketone *X* with seven-membered B-ring may exist *a priori* in three con-

formations. In the $C_{(5)}$-chair conformation the β-side is well accessible and a high yield or exclusive formation of the α-epimer *VIc* is predictable. A different situation arises when the $C_{(7)}$-twist chair or $C_{(9)}$-chair conformations are considered: Approach of the reagent from the β-side is hindered by the oxygen bridge and by 8β-H, whereas the α-side remains relatively free. Preferential formation of the β-epimer *VId* may therefore be expected for these conformations. Formation of the mixture of both epimers *VIc* and *VId* in which the β-alcohol *VId* slightly prevails indicates the conformational nonhomogeneity of the B-ring with slight domination of the $C_{(7)}$-twist--chair of $C_{(9)}$-chair conformations in the reduction process.

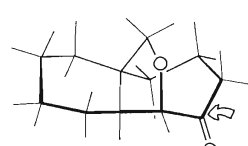
MASS SPECTRA

The mass spectra of the series of our model compounds are summarized in Tables I–V. For the sake of brevity, only important ion species of the selected representatives (*Ia*, *IIa*, *IIIa*, *VIIa*, *VIIIa*, *IX*, *X* and *XI*) are given. If necessary, full spectra of all compounds can be acquired from the authors on request.

The mass spectrum of the cyclic ether *Ia* (Table I) exhibits the expected pattern: Ion species arising from the molecular ion by the loss of CH_2O, C_8H_{17} and by the cleavage of the D ring are the most abundant in this case. In addition to these ubiquitous fragmentations, the molecular ions of *Ia* and *Ib* decompose via skeletal clea-

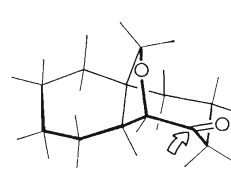


IX



X, $C_{(5)}$-Chair

X, $C_{(7)}$-Twist-Chair



X, $C_{(9)}$- Chair

FIG. 1
Conformations of the Ketones *IX* and *X*

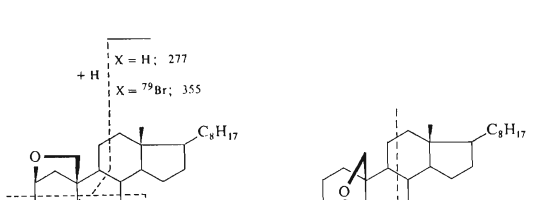
vage leading to ions $(M-C_7H_9O)^+$ (m/z 277 and m/z 357–355) for *Ia* and *Ib*, respectively, and to ions m/z 315 $(M-C_5H_{11})^+$ and $(M-C_5H_{10}Br)^+$ for *Ia* and *Ib*, respectively. As shown by the bromine label, $C_{(2)}$ is retained in the $(M-C_7H_9O)^+$ ion, so that the formation of the latter can be reasonably explained by cleavage of the $C_{(2)}$—$C_{(3)}$, $C_{(5)}$—$C_{(10)}$, $C_{(8)}$—$C_{(9)}$ and $C_{(12)}$—$C_{(13)}$ bonds which must be accompanied by the transfer of one hydrogen atom to the neutral fragment. In a similar manner, the loss of $C_5H_{10}Br$ from the molecular ion of *Ib* indicates a cleavage of $C_{(2)}$—$C_{(3)}$, $C_{(5)}$—$C_{(10)}$ and $C_{(7)}$—$C_{(8)}$ bonds accompanied by a transfer of two hydrogens onto the neutral fragment. The hydrogens transferred may originate from

TABLE I
Mass Spectra of *Ia* and *IIa*

m/z	Relative intensity[a]		Composition	Parent ion(s) (m/z)[b]
	Ia	*IIa*		
386	55·6	20·8	$C_{27}H_{46}O$	$M^{+\cdot}$
371	2·9	—	—	386
356	20·9	100	$C_{26}H_{44}$	386
353	—	12·5	—	386
341	2·1	38·9	$C_{25}H_{41}$	356
327	—	1·5	—	356
315	5·8	—	$C_{22}H_{35}O$	386
314	—	1·2	—	356
301	7·6	—	$C_{21}H_{33}O$	386
299	—	1·4	—	
277	10·0	—	$C_{20}H_{37}$	386
273	9·6	—	$C_{19}H_{29}O$	386
271	—	4·6	—	356
260	—	3·6	$C_{18}H_{28}O$	386
246	14·2	—	$C_{17}H_{26}O$	386
243	—	58·7	$C_{18}H_{27}$	356
232	37·3	—	$C_{16}H_{24}O$	386
231	44·7	—	$C_{16}H_{23}O$	386
230	—	12·2	$C_{17}H_{26}$	356
217	—	40·7	$C_{16}H_{25}$	386, 356
201	100	68·5	$C_{15}H_{21}$	356 (*Ia*, *II*) 386, 231 (*Ia*)
124	39·7	—	$C_8H_{12}O$	386
121	—	69·2	C_9H_{13}	386

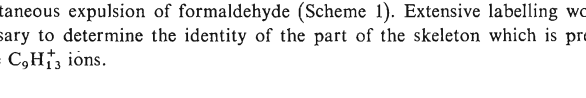
[a] Related to the most abundant ion reported in the table; [b] determined from observed metastable transitions in the 1st field-free region.

$C_{(1)}$ and $C_{(9)}$ but no attempt at deuterium labelling has been made in this case. In general, the 2β,19-ether group induces no low-activation-energy fragmentation of the steroid skeleton, so that the aforementioned ions are of modest abundance only.



SCHEME 1

The fragmentation pattern of the cyclic ether *IIa* (Table I) shows prominent ions arising by the loss of formaldehyde and by the cleavage of the D ring. The 4β,19-ether group has a negligible effect on the cleavage of the A ring (*cf.* ions *m/z* 314). The formation of the abundant $C_9H_{13}^+$ ion from the molecular ion of *IIa* can be tentatively accounted for by fission of the $C_{(11)}$—$C_{(12)}$, $C_{(9)}$—$C_{(10)}$ and $C_{(6)}$—$C_{(7)}$ bonds with the simultaneous expulsion of formaldehyde (Scheme 1). Extensive labelling would be necessary to determine the identity of the part of the skeleton which is preserved in the $C_9H_{13}^+$ ions.



SCHEME 2

The mass spectrum of the 6β,19-epoxide *IIIa* (Table II) differs considerably from those of the isomeric epoxides *Ia* and *IIa*. The cleavage of the B-ring (Scheme 2) leads to ions *m/z* 181 which consequently eliminate acetic acid producing ions *m/z* 121, the base peak of the spectrum. The alternative reaction path, *i.e.* $M^{+\cdot} \rightarrow (M - CH_3.COOH)^{+\cdot} \rightarrow m/z$ 121, which also appears probable, could not be proved experimentally due to the insufficient range of the accelerating voltage scan. The origin of the hydrogen transferred was determined by using the labelled derivative *IV*. Although

there are abundant ions m/z 124 (superposition of $C_8H_{10}{}^2HO$, $C_8H_{12}O$ and $C_7{}^{13}CH_{11}O$ species) in the spectrum of *IV*, the regio-specificity of the 5α-hydrogen transfer can be estimated to be about 70%. The cleavage of the B-ring was observed earlier[18] with various $C_{(6)}$-substituted steroids. However, the closely related 6β-methoxycholestane was reported[16] to give corresponding ions (m/z 139) of only modest abundance. While not sensitive to the configuration at $C_{(6)}$, the cleavage of the B ring is crucially dependent on the configuration at the site from which the hydrogen is abstracted. Thus the 5α-^{2}H alcohol *XV* exhibits abundant ions $C_8H_{13}O^+$ in its mass spectrum, while in the spectrum of the 5β-isomer *XIV* these ions are virtually absent. Recently, Grube and Spiteller[17] described analogous stereochemical effects for 3,6,20-pregnanetriols. The occurrence of this type of cleavage is not restricted to steroids, for analogous effects of annulation have been found with tricyclododecanols[26]. Introducing a substituent at $C_{(7)}$ (compounds *Vb*, *Vc*, *IX*) further promotes cleavage of the B ring, thus markedly increasing the abundances of the $C_8H_9O^+$ and $C_{10}H_{13}O_3^+$ ions in the total ion current. Substitution of 5α-hydrogen for the 5α-bromine (compound *IIIb*) has a dramatic effect on the fragmentation pattern. The ions corresponding to the B ring cleavage are absent in the spectrum of the bromoepoxide *IIIb*. It should be noted, however, that the tertiary bromine at $C_{(5)}$ is readily lost under electron impact thus producing even-electron ions which are known to behave differently than the odd-electron ones. As mentioned above, the configuration of the oxygen function at $C_{(6)}$ has only a small effect on the cleavage of the B-ring.

The mass spectrum of the cyclic ether *XI* (Table III), in which the 6α-oxygen is kept equatorial by bridging to the 4α-methyl, exhibits abundant $C_{10}H_{15}O^+$ ions. Another cleavage of the B ring in both the molecular ion and the $(M-CH_2O)^{+\cdot}$ ion of *XI* also produces $C_{19}H_{32}^{+\cdot}$ ions whose formation involves a transfer of two hydrogens onto the C_{10}-neutral fragment.

A striking feature of the mass spectra of *Ia*, *Ib*, *IIa*, *IIIa*, *IV* and *XI* is the loss of formaldehyde or CH_2OH radical from the molecular ions. Tables I–III show that only the 6β,19-epoxy derivatives *IIIa* and *IV* eliminate CH_2OH under electron impact, while in the remaining compounds with 2β,19 and 4β,19 as well as 4α-$CH_2$6α bridging (*Ia*, *Ib*, *IIa*, *XI*), a simple elimination of formaldehyde takes place. This effect can be rationalized on stereochemical grounds. Inspection of Dreiding models shows that in 6β,19-epoxy derivatives the secondary 4β and the tertiary 8β hydrogens are suitably oriented with respect to the 6β-oxygen to enable CH_2OH elimination (Fig. 2). While the secondary 4β-H can be bent away from the 6β-oxygen by conformational flipping of the A-ring, the tertiary 8β-hydrogen is fixed at a distance allowing a transfer (0·22 nm; Fig. 2). Although appropriate deuterium labelling would provide a keener insight as to which hydrogen is lost in the CH_2OH fragment, there is an analogy[27] showing that a γ-tertiary hydrogen is transferred preferentially onto the oxygen function provided both groups are *cis*-oriented. It should also be noted that the rigid arrangement of the 6β-oxygen and the 8β-hydrogen favors the

TABLE II
Mass Spectrum of *IIIa*

m/z	Relative intensity	Composition	Parent ion(s) *m/z*
444	9·1	$C_{29}H_{48}O_3$	$M^{+\cdot}$
429	1·9	—	444
413	2·5	$C_{28}H_{45}O_2$	444
384	75·6	$C_{27}H_{44}O$	444
369	17·4	$C_{26}H_{41}O$	384
366	17·4	$C_{27}H_{42}$	384
356	15·6	$C_{25}H_{40}O$	384
353	43·4	$C_{26}H_{41}$	384
341	4·0	—	444
331	3·5	—	—
311	3·5	—	366

TABLE III
The Mass Spectrum of *XI*

m/z	Relative intensity	Composition	Parent ion(s) *m/z*
414	6·4	$C_{29}H_{50}O$	$M^{+\cdot}$
399	5·2	—	414
384	100	$C_{28}H_{48}$	414
369	58·1	—	399, 384
355	3·0	—	384
341	1·3	—	—
329	1·5	—	—
328	1·5	—	—
314	5·8	—	384
313	5·2	—	—

transfer of the latter to the lone oxygen electron pairs (Fig. 2). In 4β,19-epoxide *IIa* the distances between the 4β-oxygen and the secondary 2β-H and 6β-H appear to be too large to allow the transfer of the secondary hydrogens[28]. Moreover, both the A and B rings in *IIa* preserve some conformational flexibility so that both poten-

TABLE II
(*Continued*)

m/z	Relative intensity	Composition	Parent ion(s) *m/z*
300	10·0	$C_{22}H_{36}$	384
271	33·1	$C_{19}H_{27}O$	384
263	15·6	$C_{19}H_{35}$	384
253	14·9	—	366
247	14·4	—	—
244	16·9	—	384
229	25·0	—	384, 369
181	18·7	$C_{10}H_{13}O_3$	444
122	42·5	—	—
121	100	C_8H_9O	181

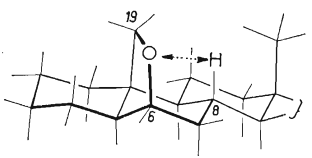
TABLE III
(*Continued*)

m/z	Relative intensity	Composition	Parent ion(s) *m/z*
301	5·8	—	414
287	3·4	—	—
274	6·4	—	—
271	17·4	—	384
260	13·4	$C_{19}H_{32}$	414, 384
247	10·5	—	414, 384
245	7·0	—	—
244	7·0	—	—
229	20·3	—	384
151	51·2	$C_{10}H_{15}O$	414

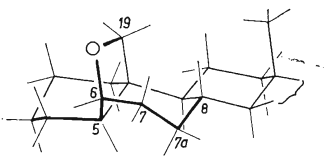
tially accessible axial hydrogens can be removed from the oxygen during the conformational changes of the skeleton. The same would be valid for the 2β,19-epoxide *Ia* in which the secondary axial hydrogen at $C_{(4)}$ can be made unaccessible to the ether group by conformational flipping of the A-ring. The necessity of available hydrogen

TABLE IV
The Mass Spectrum of *VIa*

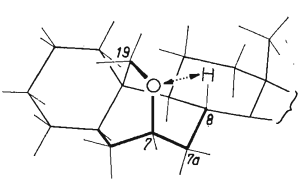
	m/z	Relative intensity	Composition	Parent ion(s) *m/z*
	430	7·0	$C_{29}H_{50}O_2$	$M^{+\cdot}$
	412	2·6	—	430
	398	19·0	$C_{28}H_{46}O$	430
	383	3·0	—	—
	380	3·4	—	—
	370	4·0	$C_{26}H_{42}O$	398
	354	2·1	—	—
	341	2·9	—	—
	315	3·3	—	—



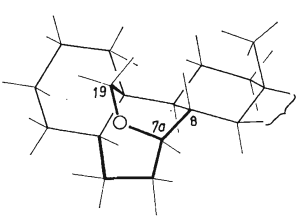
IIIa



VIa, $C_{(9)}$-Chair



VIIa, $C_{(6)}$-Chair



VIIIa, $C_{(8)}$-Twist-Chair

FIG. 2
Conformation of the Cyclic Ethers *IIIa*, *VIa*, *VIIa* and *VIIIa*

TABLE IV
(*Continued*)

m/z	Relative intensity	Composition	Parent ion(s) *m/z*
300	2·7	$C_{22}H_{36}$	398
285	11·3	$C_{20}H_{29}O$	398
275	2·8	$C_{20}H_{35}$	430
267	4·6	—	285
257	3·4	$C_{18}H_{25}O$ $C_{19}H_{29}$	398
247	8·5	$C_{18}H_{31}$	430
153	100	$C_9H_{13}O_2$	430
121	68·7	C_8H_9O	153

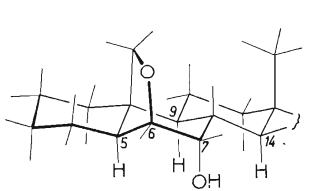
TABLE V
The Mass Spectra of *VIIa* and *VIIIa*

m/z	Relative intensity		Composition	Parent ion(s) *m/z*
	VIIa	*VIIIa*		
458	1·4	37·1	$C_{30}H_{50}O_3$	$M^{+\cdot}$
427	1·1	—	$C_{29}H_{47}O_2$	458
398	33·3	100	$C_{28}H_{46}O$	458
383	5·1	13·6	—	398
380	6·4	10·6	$C_{28}H_{44}$	398
370	—	16·8	$C_{26}H_{42}O$	398
367	6·7	—	—	427, 398
357	8·2	—	$C_{25}H_{41}O$	398
339	5·6	—	—	357
305	17·6	—	$C_{21}H_{37}O$	398
285	15·7	62·6	$C_{20}H_{29}O$	398
267	12·8	22·9	—	285
258	—	20·9	$C_{18}H_{26}O$	398
247	16·5	—	$C_{18}H_{31}$	398, 357
243	9·9	25·3	$C_{17}H_{23}O$	398
135	100	96·3	$C_9H_{11}O$(*VIIa*) $C_{10}H_{15}$ (*VIII*)	398 (*VIIa*)

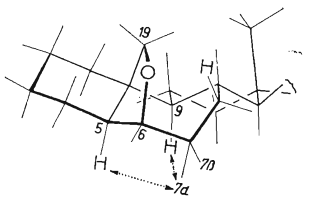
for the elimination of CH_2OH to occur is clearly demonstrated by the spectrum of the cyclic ether *XI* in which only the $(M-CH_2O)^{+\cdot}$ ions are present. In the compound *XI* there are no hydrogens accessible to the ether group. The loss of CH_2O and CH_2OH from *XI* and *IIIa*, respectively, parallels the elimination of methanol from 6α and 6β-methoxycholestanes[16].

The mass spectrum of the B-homo-6β,19-epoxide *VIa* (Table IV) exhibits abundant $C_9H_{13}O_2^+$ and $C_8H_9O^+$ ions. The former ion arises from $M^{+\cdot}$ by fission of the B ring in a way similar to that described for *IIIa*. Skeletal fragmentation with a reversed hydrogen transfer, *i.e.* from the hydrocarbon to the oxygen-containing fragment, produces $C_{20}H_{35}^+$ ions, *m/z* 275.

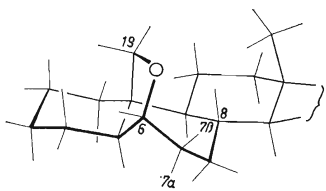
The presence of the substituent at $C_{(7)}$ (compounds *VIb*–*VId*) greatly facilitates the cleavage of the B ring increasing the relative abundance of the $C_8H_9O^+$ and $C_{10}H_{13}O_3^+$ ions. The spectra of the epimeric alcohols *VIc* and *VId* contain abundant $(M-H_2O)^{+\cdot}$ ions in contrast to *Vc* which does not. The abundance ratios $(M-H_2O)^{+\cdot}/M^{+\cdot}$ (91·7, 8·6 and 0·19 for *VId*, *VIc* and *Vc*, respectively) merit a comment. Due to the presence of the 6β,19-oxygen bridge, the conformation of the six-membered B ring in *Vc* is frozen as may be seen by inspection of the Dreiding models (Fig. 3). The axial 7α-hydroxyl is rather remote from the potentially accessible 5α, 9α and 14α-hydrogens which cannot be approached unless an additional strain is involved.



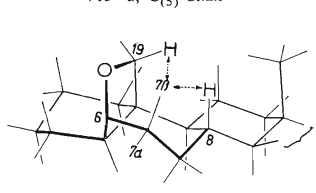
Vc



VIc—d, $C_{(5)}$-Chair



VIc—d, $C_{(7)}$-Twist-Chair



VIc—d, $C_{(9)}$-Chair

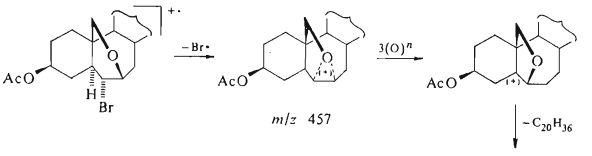
FIG. 3

Conformations of the Hydroxy Epoxides *Vc* and *VIc—d*

On the other hand, the bridged seven-membered B ring in *VIc* and *VId* preserves some conformational flexibility (Fig. 3). Dreiding models show that the 7α-hydroxyl in *VIc* can easily approach the 5α- and 9α-hydrogens in $C_{(5)}$-chair conformation (the distances being 0·21 and 0·25 nm respectively), while in *VId* only the 8β-hydrogen is sufficiently close to the 7β-hydroxyl (0·24 nm) in the $C_{(9)}$-conformation and no hydrogen atoms are accessible in the $C_{(7)}$-twist-chair form. Easier water elimination from the 7α-epimer *VIc* may therefore be expected. However, the ratio of $(M-H_2O)^{+\cdot}/M^{+\cdot}$ is much higher in the case of 7β-epimer *VId*. This behavior could be tentatively explained by loss of 19-H of the 7β-alcohol *VId* in the $C_{(9)}$-chair conformation (the distance between OH and 19-H makes 0·20 nm), but no attempt at deuterium labelling has been made in this case.

The base peak in the mass spectrum of the 7β,19-epoxy derivative *VIIa* corresponds to the $C_9H_{11}O^+$ ions, *m/z* 135 (Table V). Formation of these ions can be rationalized in a similar manner as described for the $C_8H_9O^+$ ions of *IIIa* and *VIa*. The origin of the hydrogen transferred to the neutral fragment was not checked by labelling in this case.

It is noteworthy that the prerequisite axial hydrogen at the site adjacent to the oxygen function is present in *VIIa*. The absence of abundant ions *m/z* 195 in the spectrum of *VIIa* and the observed metastable transition *m/z* 398 → *m/z* 135 indicate that fission of the B ring in *VIIa* is slower than that of *IIIa* and *VIa*. Both the molecular and $(M-CH_3COOH)^{+\cdot}$ ions in *VIIa* eliminate the CH_2OH radical (Table V). Surprisingly, the bromo epoxide *VIIb* shows abundant $C_8H_9O^+$ and $C_{10}H_{13}O_3^+$ ions in its mass spectrum. The latter ion originates from $(M-Br)^+$ ions, so that its formation requires the oxygen bridge to migrate from $C_{(7)}$ to $C_{(6)}$ in a process of $3(O)^n$ participation (Scheme 3). Similar migrations of a hydroxyl group to the adjacent cationic site in even-electron ions have been proved earlier by collision-activation studies[29,30].

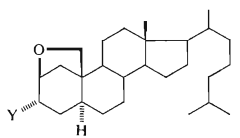


SCHEME 3

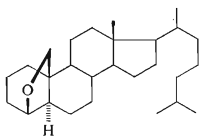
The mass spectrum of the epoxide *VIIIa* shows no abundant ions arising by the B ring cleavage (Table V). The high mass region contains ions due to the combined losses of acetic acid, water, cholestane side chain and cleavage of the D ring.

The spectra of the B-homocholestane derivatives *VIa*, *VIIa* and *VIIIa* differ markedly in the relative abundance of $(M-CH_2OH)^+$ ions. In *VIIa* the facile elimination

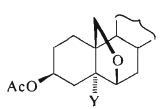
of CH_2OH can be related to the close proximity of the bridge oxygen to the 8β-hydrogen 0·24 nm (Fig. 2) as discussed in detail with *IIIa*. The epoxide *VIIIa* has no tertiary hydrogen suitably oriented with respect to the ether group. Accordingly, no elimination of CH_2OH from the molecular ion of *VIIIa* takes place. In *VIa* the seven-membered B-ring can assume three different chair conformations (Fig. 3). In the $C_{(5)}$-chair and $C_{(7)}$-twist-chair conformation the 8β-hydrogen is close to the ether group (0·25 and 0·21 nm respectively, while in the $C_{(9)}$-chair conformation both groups are remote (0·36 nm). The absence of the $(M-CH_2OH)^+$ ions in the spectrum of *VIa* indicates that the latter conformation is probably favored. This



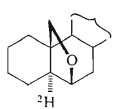
Ia, Y = H
Ib, Y = Br



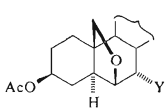
IIa



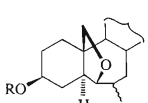
IIIa, Y = H
IIIb, Y = Br



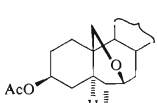
IV



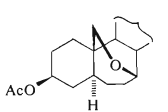
Vb, Y = Br
Vc, Y = OH



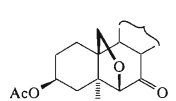
VIa, R = CH_3, Y = α-H
VIb, R = CH_3, Y = α-Br
VIc, R = Ac, Y = α-OH
VId, R = Ac, Y = β-OH



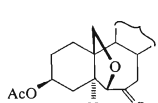
VIIa, Y = H
VIIb, Y = Br



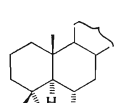
VIIIa



IX



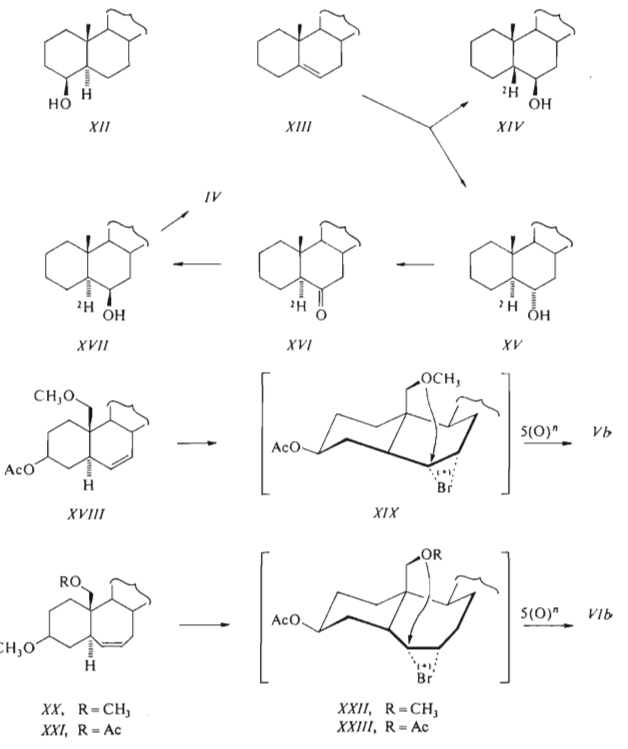
X



XI

would also account for the rather peculiar loss of water from the epimeric alcohols *VIc* and *VId* as discussed above.

It can be concluded that in the compounds under investigation the position of the oxygen bridge may be reliably located by means of mass spectrometry only for 6β,19- 6α,4α-methyl- and 7β,19-epoxy derivatives (*V*, *VI*, *VII*, *XI*), which exhibit abundant ions due to the cleavage of the B-ring. The stereochemistry of electron impact-induced fragmentations of compounds containing cycloheptane rings appears to differ considerably from that of cyclohexane analogs.



EXPERIMENTAL

Melting points were determined on a Kofler block. Analytical samples were dried at 50°C/26 Pa. Optical activity measurements were carried out in chloroform with an error of ±3°. The infrared spectra were recorded on a Zeiss UR 20 spectrometer in tetrachloromethane unless otherwise stated. The ^{1}H-NMR spectra were recorded on a Tesla BS 476 instrument (60 MHz) in deuteriochloroform at 30°C with tetramethylsilane as internal reference. Chemical shifts are given in ppm. Apparent coupling constants were obtained from the first order analysis. The CD spectra were recorded on a Dichrographe II (Jouan–Roussel) in dioxane. The mass spectra were recorded on a Jeol JMS D-100 spectrometer operating at 14—75 eV. The samples were introduced using a direct inlet at lowest temperature enabling evaporation. The ion source temperature was maintained at 140°C. The elemental compositions of all ions in Tables I—V were determined by accurate mass measurements. The decompositions of metastable ions in the 1st field-free region were monitored by using the accelerating voltage scan method. The identity of the samples prepared by different routes was checked by mixture melting point determination, by thin-layer chromatography (TLC) and by infrared and ^{1}H-NMR spectra. Usual work up of an ethereal solution means washing the solution with 5% aqueous hydrochloric acid, water, a 5% aqueous potassium hydrogen carbonate solution, water, drying with sodium sulfate and evaporation of the solvent *in vacuo*.

2β,19-Epoxy-5α-cholestane (*Ia*)

Raney-nickel (100 mg) was added to a solution of the bromo epoxide[4,7] *Ib* (30 mg) in ethanol (3 ml) and the mixture was refluxed and stirred for 6 h. The inorganic material was removed by filtration and washed with a mixture of ethanol and acetone, the filtrate was evaporated, the residue was dissolved in ether, and the ethereal solution was washed with water, dried and evaporated. The residue was crystallized from aqueous ethanol to yield the epoxide *Ia* (12 mg), m.p. 97—98°C, $[\alpha]_D^{20}$ +42° (*c* 1·2) in accordance with the literature[3,1]. ^{1}H-NMR spectrum: 0·60 (3 H, s, 18-H), 3·60 (1 H, d, J = 8 Hz, 19-H), 3·81 (1 H, d, J = 8 Hz, 19-H), 4·27 (1 H, m, W = 20 Hz, 2α-H).

2β,19-Epoxy-3α-bromo-5α-cholestane (*Ib*)

The compound was prepared earlier[6]. Mass spectrum: *m/z* (rel. intensity): 466—464 (89·8), 385 (35·6), 357—355 (21·2), 355 (59·3), 353—351 (21·2), 326—324 (14·4), 315 (30·5), 311—309 (100), 301 (32·2), 281—279 (50·8), 231 (28·8).

4β,19-Epoxy-5α-cholestane (*IIa*)

A mixture of lead tetraacetate (50 mg) and calcium carbonate (30 mg) in benzene (5 ml) was refluxed while stirring for 30 min. A solution of the alcohol[2,3] *XII* (60 mg) in benzene (5 ml) and iodine (10 mg) were added and the mixture was refluxed while stirring for 2 h. The inorganic material was filtered off, washed with ether and the filtrate was washed with water, 5% aqueous hydrochloric acid, water, a 5% aqueous potassium hydrogen carbonate solution, an aqueous sodium thiosulfate solution, water, and then dried and evaporated. The residue was chromatographed on a plate of silica gel (10 × 20 cm) using a mixture of light petroleum, ether and acetone (90 : 5 : 5) as eluent. Corresponding zones were collected, washed with ether and the filtrate was evaporated. The residue was crystallized from aqueous acetone to yield the epoxide *IIa* (9 mg), m.p. 116—118°C. For $C_{27}H_{46}O$ (386·7) calculated: 83·87% C, 11·99% H; found: 83·66% C, 12·07% H.

6β,19-Epoxy-5-bromo-5α-cholestan-3β-ol 3-Acetate (*IIIb*)

The compound was prepared earlier[20]. Mass spectrum: *m/z* (rel. intensity): 524—522 (0·4), 464—462 (0·6), 443 (8·5), 383 (100), 365 (16·9), 353 (18·3), 289 (8·0), 243 (14·9), 229 (30·3).

[5-^{2}H]-6β,19-Epoxy-5α-cholestane (*IV*)

The alcohol *XVII* (300 mg) was treated with lead tetraacetate (300 mg), calcium carbonate (100 mg) and iodine (20 mg) in benzene (20 ml) as in the previous experiment and worked up. The residue was crystallized from aqueous acetone to yield the epoxide *IV* (240 mg, m.p. 89 to 90°C, $[\alpha]_D^{20}$ +21° (*c* 2·1). ^{1}H-NMR spectrum: 0·69 (3 H, s, 18-H), 3·69 (2 H, s, 19-H), 3·87 (1 H, d, J = 2 Hz, 6α-H). IR spectrum: 1 029, 2 160 cm^{-1}. Mass spectrum; *m/z* (rel. intensity): 387 (46·9), 372 (12·6), 369 (13·1), 356 (45·2), 274 (35·6), 264 (36·8), 256 (16·8), 247 (38·3), 202 (29·7), 124 (100), 123 (96·5).

6β,19-Epoxy-7α-bromo-5α-cholestan-3β-ol 3-Acetate (*Vb*)

The olefin *XVIII* (150 mg) was dissolved in dioxane (5 ml) and water (0·5 ml) and treated with 10% aqueous perchloric acid (0·3 ml) and N-bromoacetamide (60 mg) for 30 min. The mixture was then diluted with ether and water, the ethereal layer was washed with water, 5% aqueous potassium hydrogen carbonate, an aqueous sodium thiosulfate solution, water, and then dried and evaporated. The residue was chromatographed on two preparative plates of silica gel using a mixture of light petroleum, ether and acetone (90 : 5 : 5) as eluent. The lipophilic zone was collected and eluted with ether, the filtrate was evaporated to yield the crude *Vb* (130 mg), which on crystallization from a mixture of acetone, methanol and water gave *Vb* (105 mg), m.p. 130 to 131°C, $[\alpha]_D^{20}$ —63° (*c* 2·6), identical with the known compound[12]. Mass spectrum; *m/z* (rel. intensity): 524-522 (0·1), 464—462 (1·4), 443 (12·1), 383 (74·7), 365 (23·8), 355 (6·8), 353 (10·7), 289 (8·7), 243 (12·1), 229 (20·9), 181 (35·4), 121 (100).

6β,19-Epoxy-5α-cholestane-3β,7α-diol 3-Monoacetate (*Vc*)

The ketone *IX* (20 mg) was dissolved in tetrahydrofurane (1 ml) and treated with lithium tris-(tert-butoxy)hydridoaluminate (20 mg) at room temperature for 3 h. The mixture was diluted with ether, the excess of reagent was decomposed with water, and the ethereal solution was worked up as usual to yield the crude product. The TLC analysis showed contamination by about 5% of another more polar substance. The crude mixture was crystallized from aqueous methanol to yield the pure 7α-alcohol *Vc* (12 mg), m.p. 157—158°C identical with an authentic sample[12]. Mass spectrum; *m/z* (rel. intensity): 460 (1·9), 400 (15·3), 382 (3·1), 369 (1·9), 351 (2·0), 278 (2·2), 269 (2·0). 245 (3·3), 181 (23·3), 121 (100).

3β-Methoxy-6β,19-epoxy-B-homo-5α-cholestane (*VIa*)

The bromo epoxide *VIb* (45 mg) in ethanol (3 ml) was treated with Raney-nickel (100 mg) as given for *Ia* to yield the oily epoxide *VIa* (31 mg), $[\alpha]_D^{20}$ +18° (*c* 1·3). ^{1}H-NMR spectrum: 0·67 (3 H, s, 18-H), 3·00 (1 H, m, W = 30 Hz, 3α-H), 3·30 (3 H, s, CH_3O), 3·65 (2 H, s, 19-H), 4·00 (1 H, m, W = 10 Hz, 6α-H). For $C_{30}H_{50}O_2$ (442·7) calculated: 81·39% C, 11·38% H; found: 81·16% C, 11·45% H.

3β-Methoxy-6β,19-epoxy-7α-bromo-B-homo-5α-cholestane (*VIb*)

The olefin[1,2] *XX* (90 mg) was dissolved in dioxane (5 ml) and water (0·5 ml) and treated with 10% aqueous perchloric acid (0·2 ml) and N-bromoacetamide (40 mg) for 15 min. The mixture was diluted with ether and water, the ethereal layer was washed with water, 5% aqueous potassium hydrogen carbonate, aqueous sodium thiosulfate solution, water, dried and evaporated to yield the oily epoxide *VIb* (88 mg), $[\alpha]_D^{20}$ −21° (*c* 2·0). ^{1}H-NMR spectrum: 0·65 (3 H, s, 18-H), 3·05 (1 H, m, W = 30 Hz, 3α-H), 3·34 (3 H, s, CH_3O), 3·72 (2 H, brd s, 19-H), 4·00 (1 H, m, W = = 16 Hz, 7β-H), 4·18 (1 H, m, W = 7 Hz, 6α-H). For $C_{30}H_{49}BrO_2$ (521·6) calculated: 69·08% C, 9·47% H, 15·32% Br; found: 68·87% C, 9·31% H 15·60% Br.

6β,19-Epoxy-B-homo-5α-cholestan-3β,7α-diol 3-Monoacetate (*VIc*)

The compound was prepared earlier[1,2]. Mass spectrum; *m/z* (rel. intensity): 474 (0·3), 456 (2·2), 414 (8·1), 396 (2·6), 283 (1·8), 247 (2·3), 181 (24·2), 121 (100).

6β-19-Epoxy-B-homo-5α-cholestan-3β,7β-diol 3-Monoacetate (*VId*)

The ketone *X* (90 mg) in tetrahydrofurane (3 ml) was treated with lithium tris(tert-butoxy)-hydridoaluminate (100 mg) at room temperature for 1 h. The mixture was decomposed with water and 5% aqueous hydrochloric acid, ether was added and the solution was worked up as usual. The residue was chromatographed on preparative silica gel plate (20 × 20 cm) using a mixture of light petroleum, ether and acetone (76 : 12 : 12) as eluent. The polar zone was collected and washed with ether to yield the 7α-alcohol *VIc* (38 mg), m.p. 186—187°C, identical with an authentic sample[1,2]. In the same manner the lipophilic zone afforded the 7β-alcohol *VId* (48 mg), m.p. 125—126°C, $[\alpha]_D^{20}$ +6° (*c* 3·8). IR spectrum: 1 243, 1 738, 3 564 cm^{-1}. ^{1}H-NMR spectrum: 0·68 (3 H, s, 18-H), 2·01 (3 H, s, CH_3CO_2), 3·74 (2 H, s, 19-H), 3·80 (2 H, m, W = = 35 Hz, 6α-H and 7α-H), 4·55 (1 H, m, W = 30 Hz, 3α-H). Mass spectrum; *m/z* (rel. intensity): 474 (0·1), 456 (9·6), 414 (6·0), 396 (5·7), 181 (27·0), 121 (100). For $C_{30}H_{50}O_4$ (474·7) calculated: 75·90% C, 10·62% H; found: 75·81% C, 10·53% H.

7β,19-Epoxy-6α-bromo-B-homo-5α-cholestan-3β-ol 3-Acetate (*VIIb*)

The compound was prepared earlier[1,2]. Mass spectrum; *m/z* (rel. intensity): 538—536 (0·3), 478—476 (11·6), 457 (5·5), 415 (4·5), 397 (81·8), 379 (43·2), 369 (7·2), 303 (7·0), 181 (30·1), 121 (100).

3β-Acetoxy-6β,19-epoxy-5α-cholestan-7-one (*IX*)

The alcohol[1,2] *Vc* (25 mg) was dissolved in acetone (3 ml) and treated with Jones' reagent at room temperature for 5 min. The excess of reagent was decomposed with methanol, the mixture was treated with ether and water, the ethereal layer was washed with water, 5% aqueous potassium hydrogen carbonate, water, and then dried and evaporated. The residue was crystallized from aqueous methanol to yield the ketone *IX* (21 mg), m.p. 192—194°C. IR spectrum (KBr): 1 262, 1 710, 1 724 cm^{-1}. CD spectrum: $\Delta\varepsilon$ = +0·18, 316 nm. Mass spectrum; *m/z* (rel. intensity): 458 (2·3), 398 (4·7), 370 (1·1), 181 (32·6), 121 (100). For $C_{29}H_{46}O_4$ (458·7) calculated: 75·94% C, 10·11% H; found: 75·82% C, 10·04% H.

3β-Acetoxy-6β,19-epoxy-B-homo-5α-cholestan-7-one (*X*)

The alcohol[12] *VIc* (100 mg) was dissolved in acetone (8 ml) and treated with Jones' reagent at room temperature for 5 min. The excess of reagent was decomposed with methanol, diluted with ether and water, the ethereal solution was washed with water, 5% aqueous potassium hydrogen carbonate solution, water, dried and the solvent evaporated. The residue was chromatographed on one silica gel plate (20 × 20 cm) using a mixture of light petroleum, ether and acetone (80 : 10 : 10) as eluent. The corresponding zone was collected, washed with ether and the solvent evaporated to yield the oily ketone *X* (93 mg), $[\alpha]_D^{20}$ —51° (*c* 2·2). IR spectrum: 1243, 1711, 1737 cm^{-1}. CD spectrum: $\Delta\varepsilon$ = —1·97, 315 nm. Mass spectrum; *m/z* (rel. intensity): 472 (0·9), 412 (4·6), 384 (0·9), 181 (28·2), 121 (100). For $C_{30}H_{48}O_4$ (472·7) calculated: 76·23% C, 10·24% H; found: 79·97% C, 10·33% H.

[5-^{2}H]-5β-Cholestan-6β-ol (*XIV*)

The olefin[25] *XIII* (1·5 g) was dissolved in ether (30 ml). boron trifluoride etherate (0·8 ml) was added at —10°C while stirring and the mixture was treated with a solution of lithium aluminum deuteride (200 mg) in ether (60 ml) at —10°C while stirring for 1 h. The mixture was decomposed with a saturated aqueous sodium sulfate solution, the inorganic material was filtered off, washed with ether and the filtrate was evaporated at 20°C. The residue was dissolved in tetrahydrofurane (30 ml) and treated with aqueous 30% hydrogen peroxide (30 ml) and a solution of potassium hydroxide (5 g) in water (50 ml) at 0°C while stirring for 1 h. The mixture was treated with ether and water, the ethereal solution was washed with water, dried and evaporated. The residue was chromatographed on a column of silica gel (100 g) with a mixture of light petroleum and benzene (70 : 30), then with light petroleum and ether (95 : 5) which eluted impurities. Elution with a mixture of light petroleum and ether (93 : 7) afforded the fractions which after collection and evaporation yielded the oily 6β-alcohol *XIV* (160 mg), $[\alpha]_D^{20}$ +23° (*c* 4·8). Mass spectrum; *m/z* (rel. intensity): 389 (9·1), 374 (8·7), 371 (91·2), 356 (25·0), 274 (6·2), 258 (15·9), 231 (27·8), 216 (69·5), 134 (100). The content of deuterium in ionized molecules: 92·6% d_1, 7·4% d_0.

[5-^{2}H]-5α-Cholestan-6α-ol (*XV*)

Prolonged elution with the same mixture of solvents after isolation of the 6β-alcohol *XIV* gave the polar fractions. These fractions were collected and evaporated to yield the crude 6α-alcohol *XV* (750 mg). The sample was crystallized from aqueous acetone to yield *XV*, m.p. 130—131°C. The unlabelled compound[32] has m.p. 128—130°C. IR spectrum: 2100, 3375, 3630 cm^{-1}. ^{1}H-NMR spectrum: 0·66 (3 H, s, 18-H), 0·78 (3 H, s, 19-H), 3·35 (1 H, m, *W* = 23 Hz, 6β-H). Mass spectrum; *m/z* (rel. intensity): 389 (45·6), 374 (14·4), 371 (75·0), 356 (48·2), 301 (3·3), 291 (7·5), 258 (13·8), 249 (15·0), 234 (85·0), 216 (100), 125 (51·9). The content of deuterium in ionized molecules: 1·4% d_2. 93·9% d_1, 4·7% d_0.

[5-^{2}H]-5α-Cholestan-6-one (*XVI*)

The alcohol *XV* (700 mg) was dissolved in a mixture of acetone (20 ml) and benzene (5 ml), deuterium oxide (2 ml) was added and the solution was treated with Jones' reagent at room temperature for 10 min. The excess of reagent was decomposed with methanol, the mixture was diluted with ether and water, the ethereal solution was washed with water, a 5% aqueous

potassium hydrogen carbonate solution, water, dried and the solvent was evaporated. The residue was crystallized from a mixture of chloroform and methanol to yield the ketone *XVI* (570 mg), m.p. 99—100°C, $[\alpha]_D^{20}$ —6° (*c* 2·0). Literature[33] gives for the unlabelled compound m.p. 98°C, $[\alpha]_D^{20}$ —7°. IR spectrum: 1 711, 2115 cm^{-1}.

[5-^{2}H]-5α-Cholestan-6β-ol (*XVII*)

The ketone *XVI* (500 mg) was reduced with lithium aluminum hydride (100 mg) in ether (20 ml) at room temperature for 30 min. The mixture was decomposed with water and 5% aqueous hydrochloric acid, diluted with ether and water and the ethereal layer was worked up as usual. The residue was crystallized from aqueous acetone to yield the 6β-alcohol *XVII* (360 mg), m.p. 79—81°C, $[\alpha]_D^{20}$ +5° (*c* 2·0). Literature[34] gives for the unlabelled compound m.p. 80 to 82°C, $[\alpha]_D^{20}$ +8°.

The analyses were carried out in the Analytical Laboratory of this Institute (under the direction of Dr J. Horáček). The IR *spectra were recorded by Mrs K. Matoušková and Mr P. Formánek and interpreted by Dr S. Vašíčková. The* CD *spectra were recorded and interpreted by Dr S. Vašíčková.* ^{1}H-NMR *spectra were recorded by Mrs J. Jelínková.*

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