A ROUTE TO 24-epiBRASSINOLIDE FROM ERGOSTEROL AVOIDING THE USE OF OSMIUM TETROXIDE*

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Received March 1, 1990
Accepted April 27, 1990

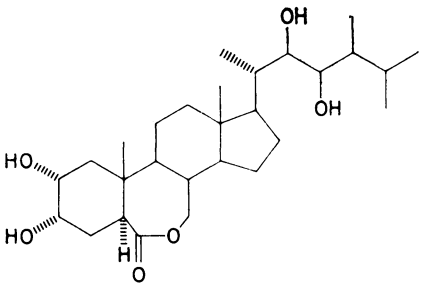
A synthesis of (22*R*,23*R*)-2α,3α,22,23-tetrahydroxy-5α-ergostan-6-one (*V*) from the dienone *IV* via the diepoxide *XXII* is described. The tetrol *V* is a key intermediate in the synthesis of 24-epibrassinolide. The reactivity of the side chain was studied on 5α-ergost-22-en-6-one (*XIV*) as a model compound. ^{1}H and ^{13}C NMR spectra of 24-epibrassinolide derivatives are discussed.

Brassinolide (*I*), the highly active plant growth promotor[1], is not readily accessible either by isolation from natural material or by synthesis. It is therefore conceivable that the efforts of many laboratories focussed on the preparation of more readily accessible analogs. One such compound that can be more easily prepared and possesses high activity is 24-epibrassinolide (*II*). In its synthesis from ergosterol (*III*) (ref.[2]), the key intermediate is (22*E*)-5α-ergosta-2,22-dien-6-one (*IV*) which is converted by treatment with osmium tetroxide to a mixture of 2α,3α,22,23-tetrols (*V* and *VI*) stereoisomeric at positions 22 and 23. The isomer *V* can be easily converted to 24-epibrassinolide (*II*). Since OsO_4 is an expensive and highly toxic reagent, we proposed[3] a procedure avoiding the use of OsO_4 and providing 2α,3α-diols in about the same yield as the osmylation method. This procedure can also be applied to 6-ketones. Conversion of the iodo derivative *X* to *XIa* is performed by the action of a suitable peroxyacid. Alternatively, the halohydrin acetate *X* can be converted to the diol *XII* by treatment with sodium or cupric acetate in acetic acid followed by hydrolysis. As will be mentioned, this transformation occurs by way of the acetyl derivatives *XIa*, *XIb* and *XIc*.

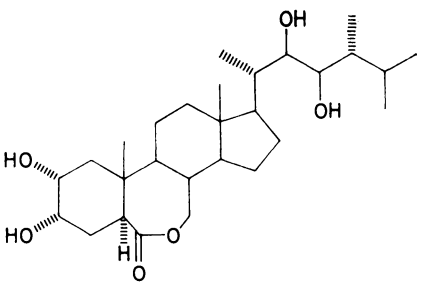
It thus appeared obvious to apply this epoxide method to a preparation of the tetrol *V*, i.e. to a synthesis of 24-epibrassinolide. However, application of the method to the dienone *IV* under conditions optimal for 2,3-olefins was unsuccessful. It could be conceived that the conditions suitable for the 2,3-double bond are not applicable for the transformation of the side chain double bond. To obtain the necessary infor-

* Part CCCLIV in the series On Steroids; Part CCCLIII Collect. Czech. Chem. Commun. *55*, 2510 (1990).

mation on its reactivity, we investigated the dihydroxylation of the side chain on a model exluding the interfering reactions in the ring A. (22*E*)-5α-Ergost-22-en-6-one (*XIV*) was chosen as such a model. This compound is readily accessible by reduction of (22*E*)-3α,5-cyclo-5α-ergosta-7,22-diene-6-one (*XIII*) with sodium or lithium in liquid ammonia. Epoxidation of the olefin *XIV* gives rise to two epoxides (*XV* and *XVI*) differing by the configuration of the oxirane ring. Their cleavage with hydro-



I



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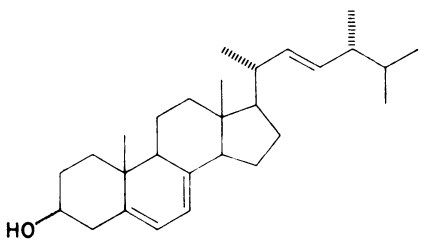
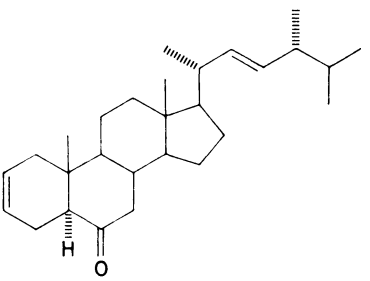
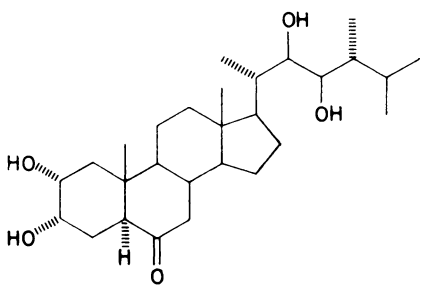


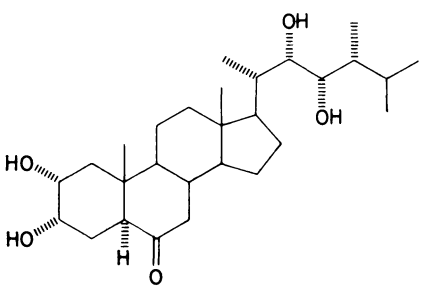
III



IV

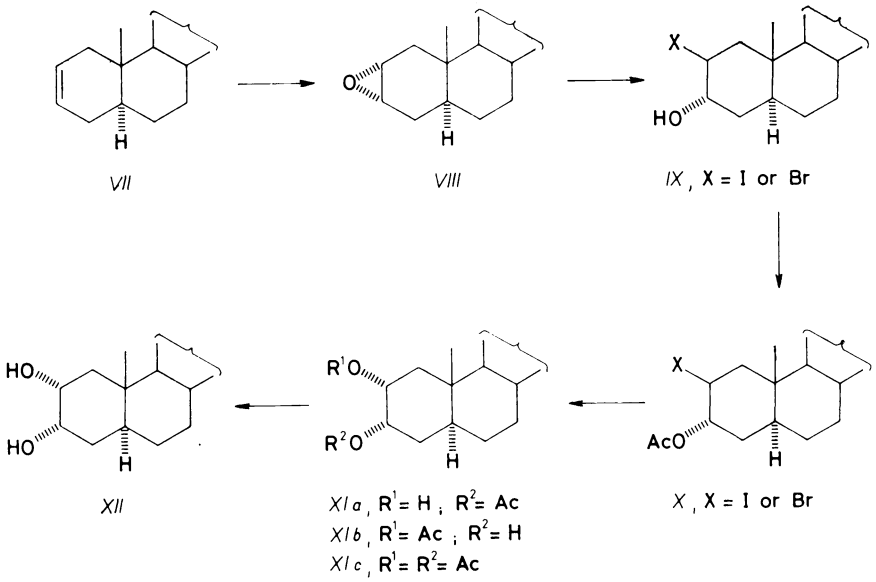


V

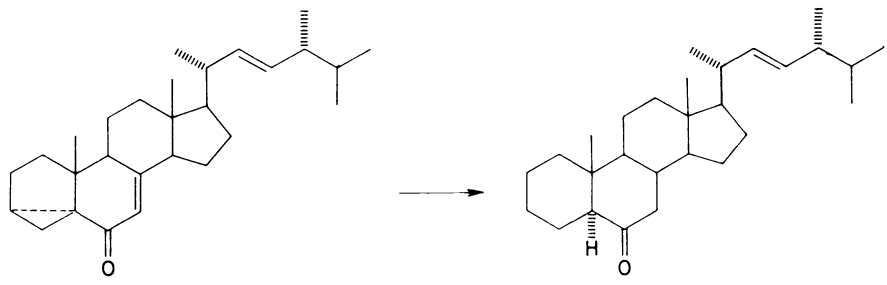


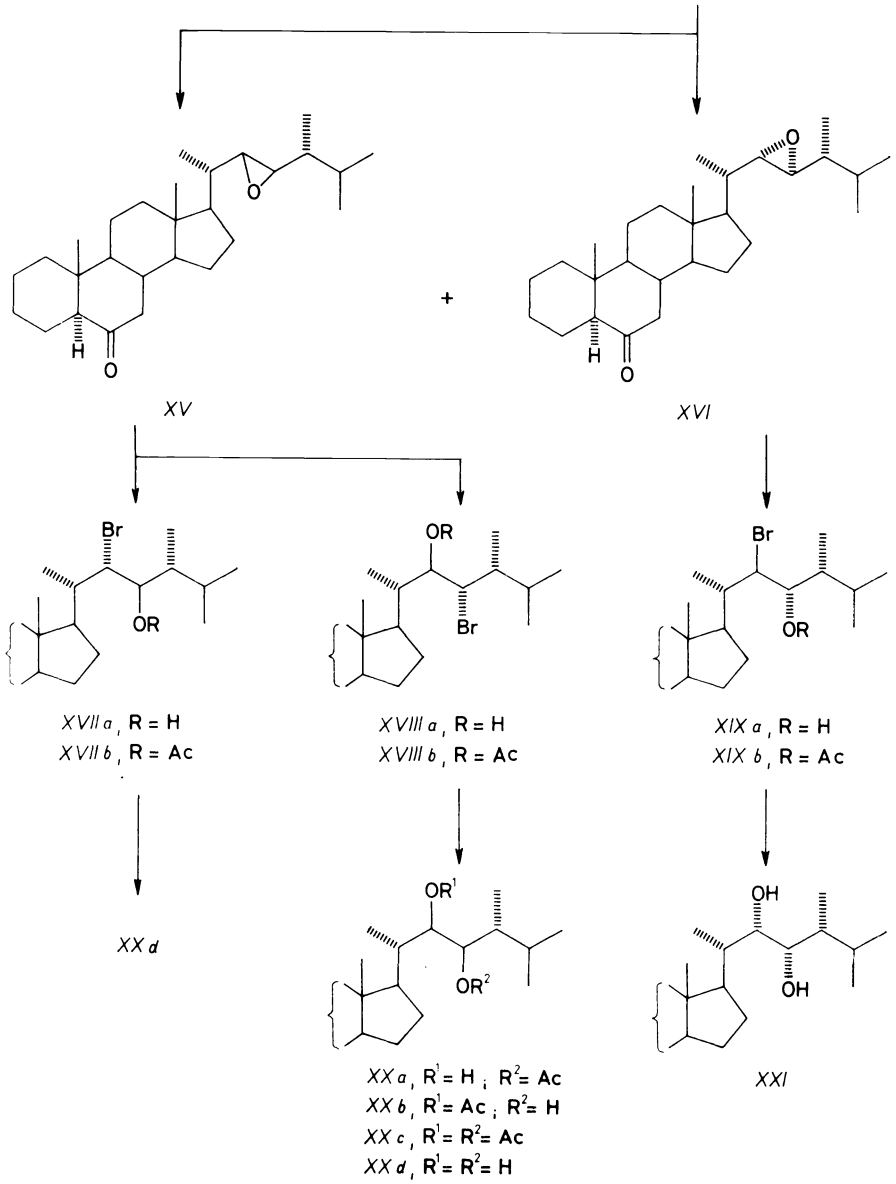
VI

bromic acid is much more difficult than that of 2α,3α-epoxides. Whereas 2α,3α--epoxides are converted to the respective bromohydrine on shaking their chloroform solution with aqueous 48% hydrobromic acid, the same conditions applied to epoxides *XV* and *XVI* result in an extremely slow reaction. However, the conversion can be achieved by treatment with aqueous 48% hydrobromic acid in an acetone solution.



The behavior of the epoxides *XV* and *XVI* is not quite analogous: whereas the epoxide *XV* yields two bromohydrins (*XVIIa* and *XVIIIa*), the epoxide *XVI* gives practically only *XIXa*. The mass spectrum of the bromohydrin *XVIIa* shows the presence of the fragments $C_{22}H_{35}O^{79}Br$ and $C_{22}H_{35}O^{81}Br$ in agreement with the presence of Br at C-22 and with the formula *XVIIa*. By exclusion, we formulate





the second isomer as *XVIIIa*. Acetylation of *XVIIIa* proceeds easier (15 h) than that of *XVIIa* (72 h). The subsequent transformation of the acetylated bromohydrins by the action of refluxing acetic acid in the presence of sodium or cupric acetate requires a longer reaction time compared with the analogous procedure

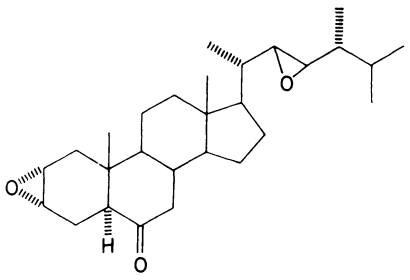
applied to the ring A. On monitoring the reaction by TLC the same picture is observed with all three compounds (*XVIIb*, *XVIIIb* and *XIXb*): along with a small amount of the least polar minor product, two more polar major products are present in about the same proportion. The IR spectrum of these major products demonstrates the presence of both hydroxyl and acetoxy groups in each compound, i.e. the structures of diol monoacetates (e.g. *XXa* and *XXb*). Both bromohydrin acetates (*XVIIb* and *XVIIIb*) yield identical products. The formation of these monoacetates is due to the intramolecular attack of the acetoxy group on the neighboring bromine-bearing carbon to form an intermediary acetoxonium ion then undergoing a symmetric hydrolysis to both regioisomeric monoacetates. The minor product with a polarity between the starting bromohydrin acetate and the monoacetates must be formulated as the corresponding diacetate arising from the bromohydrin monoacetate by the external attack of AcO^-. Alkaline hydrolysis converts the acetates (*XXa*, *XXb* and *XXc*) to homogeneous diol *XXd* identical with the diol obtained analogously from the compound *XVIIb*.

The structure of the bromohydrin *XIXa* could not be derived from the mass spectrum which shows preferential splitting off of HBr and hence the ensuing fragmentation is not informative. Proof of the bromine atom position at C-22 and of the hydroxy group at C-23 was presented by means of a ^1H–^1H homocorrelated 2D-NMR experiment with the corresponding acetate *XIXb* (see NMR discussion) prepared from the bromohydrin *XIXa*. Reactions of *XIXa* proceed analogously as with *XVIIa* and *XVIIIa* under formation of the diol *XXI*.

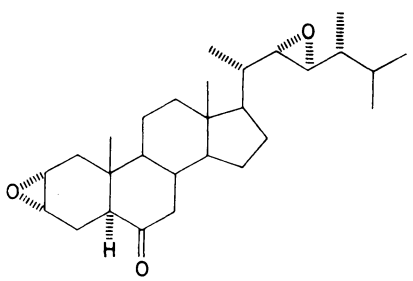
The model experiments on the epoxides *XV* and *XVI* led to the important conclusion that the conversion of the 22,23-oxirane ring to the respective diol requires more energetic conditions than the analogus reaction sequence at the position 2, 3; knowledge of these conditions made it possible to verify the applicability of this procedure to the intended purpose.

Consequently, the reaction sequence *VIII*–*XII* (using HBr for cleavage of the oxirane rings) was applied both to the epoxides *XXII* and *XXIII*. The epoxides *XXII* and *XXIII* were thus converted to the known[2] tetrols *V* and *VI*, respectively. This correlation presents proof of the configuration at C-22 and C-23 in *XXII* and *XXIII*. Conversion of *XXII* to *VI* is also a formal synthesis of 24-epibrassinolide (*II*) since synthesis of *II* from *V* was reported earlier[2]. The transformation of *IV* to *V* can be performed without separation of the isomeric epoxides *XXII* and *XXIII* to give the required tetrol *V* in 32% yield of the pure product; the tetrol *VI* was obtained in 25% yield from *IV*. This result is more favorable than the conversion of the dienone *IV* into *V* by means of catalytic osmylation (26% in our hands).

The knowledge of the configuration at C-22 and C-23 in *XXII* and *XXIII* offered the possibility of establishing the configuration at these chiral centers in the monoepoxides *XV* and *XVI* based on analogous physical properties. ^1H and ^{13}C NMR spectra unequivocally demonstrated identical configuration at C-22 and C-23 in the



XXII



XXIII

pairs *XV*, *XXII* and *XVI*, *XXIII*, respectively (cf. NMR discussion). Also the lower negative value of optical rotation is in agreement with the 22*R*,23*R* configuration for the compound *XV*.

NMR *Discussion*

^{1}H and ^{13}C NMR spectra of the compounds *XV*–*XXIII* (Tables I and II), as yet unreported in the literature, were measured with the aim of achieving maximal possible structural assignment of the signals and utilizing them for structure elucidation. ^{1}H NMR spectra of compounds *XV*–*XXIII* could not be analysed fully. The presence of the 6-keto group allowed easy identification of the H-5 hydrogen signal, which in all substances under investigation appears at δ 2·29–2·30 (dd, $J \approx 13$ and 4·5 Hz), with the exception of diepoxides *XXII* and *XXIII* where a slight downfield shift is observed (δ 2·33–2·34) due to the presence of 2,3-epoxy group. Easily identifiable are also signals of both angular methyls where the position of the 19-Me is again constant at δ 0·73 in the whole series except for a small upfield shift for diepoxides *XXII* and *XXIII* (δ 0·71). Due to closer proximity to the structural modifications in the side chain, the chemical shift of the 18-methyl shows somewhat greater variability (δ 0·64–0·72). The structural assignment of the side chain signals associated with four secondary methyl groups and methine protons at the positions 22 and 23 presents a substantially more difficult problem. In the epoxy derivatives *XV*, *XVI*, *XXII* and *XXIII* the second order effects did not permit unequivocal identification of the four secondary methyls even in 200 MHz spectra and could be safely identified only on 500 MHz frequency. In the above mentioned group of four epoxides the similarity of side chain hydrogen signals permitted a clear correlation of pairs with identical absolute configuration at C-22 and C-23 as shown in Table III. The coupling constants between the epoxide protons prove a *cis*-configuration of the epoxide at 2,3-position ($J(2, 3) = 4{\cdot}0$ Hz) in the compounds *XXII* a *XXIII* and *trans*-configuration of the epoxide at 22,23-position ($J(22, 23) \approx 2{\cdot}3$ Hz) in the

TABLE I
Proton NMR data of compounds *XV*—*XXIII*

Parameter	*XV*	*XVI*	*XVIIa*	*XVIIIa*	*XIXa*[a]	*XVIIb*[b]
δ(H-5)	2·29	2·30	2·29	2·30	2·29	2·29
J(5, 4α)	4·5	4·5	4·2	4·2	4·5	4·1
J(5, 4β)	13·1	13·1	12·5	12·5	13·0	12·5
δ(18-Me)	0·651	0·656	0·670	0·713	0·719	0·640
δ(19-Me)	0·731	0·729	0·726	0·726	0·729	0·725
δ(21-Me)	1·089	1·000	1·192	0·975	1·028	1·102
J(21, 20)	6·4	6·4	6·8	6·3	6·4	7·0
δ(H-22)	2·63	2·57	4·32	3·85	4·07	4·45
J(22, 20)	8·3	6·8	1·8	1·5	1·9	2·0
J(22, 23)	2·2	2·4	10·2	10·2	9·6	10·7
δ(H-23)	2·37	2·45	3·82	4·20	3·97	5·25
J(23, 24)	8·2	7·9	2·9	1·6	2·2	2·8
δ(26-Me)	0·963	0·976	0·934	0·963	0·966	0·937
J(26, 25)	6·9	6·8	6·7	6·1	6·7	6·8
δ(27-Me)	0·919	0·929	0·916	0·950	0·948	0·860
J(27, 25)	6·8	6·8	6·7	6·2	6·7	7·0
δ(24[1]-Me)	0·906	0·950	0·893	0·903	0·840	0·808
J(24[1], 24)	7·0	6·5	6·8	6·7	6·9	7·2

Additional assigned signals: [a] OH: 1·44 d (*J* = 6·2 Hz); [b] OAc: 2·066 s; [c] OAc: 2·803 s; [d] OAc: 2·055 s; [e] NH: 8·49 s and 8·43 s; [f] NH: 8·60 s and 8·54 s; [g] H-2: 3·27; H-3: 3·12; *J*(2, 3) = 4·0;

whole group of four epoxides in agreement with 22*R*,23*R* or 22*S*,23*S* configurations following from chemical consideration. The problem of structural assignment of the side chain signals arises mainly from the symmetry of the fragment CH_3—C(20)H—C(22)HX—C(23)HY—C(24)H—CH_3 leading to identical multiplicity of the H-22 and H-23 signals. Consequently, it means that for compounds *XVIIa* to *XIXb* even correct assignment of the signals CHBr and CHOR (based on coupling ^3J(CH, OH) or on different substitution effects of Br and OAc) does not permit to assign the positions of Br and OR substituents. Whereas for bromohydrins *XVIIa*, *XVIIIa* and their bromoacetates *XVIIb*, *XVIIIb* this information was obtained from mass spectrometric measurement, we utilized ^{1}H–^{1}H homocorrelated 2D-NMR spectra for bromohydrin *XIXa* and bromoacetate *XIXb*. The procedure is illustrated on bromoacetate *XIXb* in Fig. 1. The key point for the assignment was identification of hydrogen H-25 at δ 1·40 that alone shows vicinal coupling with two secondary methyls (26- and 27-Me at δ 0·888 and 0·969) and is further coupled with hydrogen

TABLE I
(*Continued*)

XVIIIb[c]	*XIXb*[d]	*XXd*	*XXd* +TAI[e]	*XXI*	*XXI* +TAI[f]	*XXII*[g]	*XXIII*[h]
2·29	2·29	2·29	2·26	2·29	2·30	2·33	2·34
4·0	4·5	4·2	4·1	4·3	4·3	4·2	4·2
12·3	13·0	12·7	12·5	12·5	12·8	13·1	13·1
0·702	0·668	0·674	0·694	0·691	0·685	0·644	0·649
0·727	0·728	0·731	0·728	0·730	0·725	0·710[i]	0·707[i]
0·935	0·988	0·918	1·070	1·023	1·056	1·088	0·999
6·1	6·1	6·9	6·8	6·8	6·9	6·4	6·4
5·24	4·14	3·70	5·36	3·72	5·38	2·65	2·57
1·0	1·6	4·5	1·0	4·4	5·2	8·3	6·8
10·5	10·0	4·5	8·3	3·2	4·5	2·2	2·3
4·29	5·38	3·41	5·19	3·59	5·21	2·37	2·45
1·8	2·0	5·0	4·1	3·1	3·4	8·1	7·9
0·942	0·969	0·977	0·982	0·970	0·983	0·961	0·973
6·7	6·6	6·4	6·7	6·7	6·4	6·8	6·6
0·920	0·888	0·869	0·912	0·884	0·910	0·918	0·927
6·4	6·7	6·8	6·7	6·8	6·4	6·8	6·8
0·937	0·862	0·848	0·952	0·905	0·918	0·903	0·949
6·9	6·9	7·1	7·0	6·9	6·6	6·7	6·9

[h] H-2: 3·26; H-3: 3·12; $J(2, 3) = 4·0$; [i] doublet with $J(19, 1\alpha) = 0·9$ Hz.

H-24 at δ 1·95. Through hydrogen H-24 can be assigned the 24[1]-Me group (δ 0·862) and hydrogen H-23 at δ 5·38 where the chemical shift shows that it bears an acetoxy group. From hydrogen H-23 it is possible to continue to the assignment of hydrogen H-22 at δ 4·14 which bears a bromine atom and, subsequently, to hydrogen H-20 at δ 1·45 and to the last secondary methyl 21-Me at δ 0·988. The use of NMR spectra for the determination of relative configurations at carbon atoms C-22 and C-23 in the substances *XVIIa–XIXb* is very difficult in view of unknown conformation of the side chain. Moreover, application of conformational analysis to all combinations of configurations (22*R*,23*R*; 22*R*,23*S*; 22*S*,23*R*; 22*S*,23*S*) leads to energetically most favored conformations characterized by antiperiplanar arrangement of H-22 and H-23 hydrogens. Indeed the values of $J(22,23)$ for the compounds *XVIIa–XIXb* are very similar ($\approx$10 Hz) and are in a good agreement with preferred conformations having antiperiplanar hydrogens. Since the absolute configuration at 22 and 23 can be derived from the genesis of these compounds (see above) it is possible to describe

TABLE II

Carbon-13 NMR chemical shifts of compounds *XV*—*XXIII*

Carbon	*XV*	*XVI*	*XVIIa*	*XVIIIa*	*XIXa*	*XVIIb*[a]	*XVIIIb*[b]	*XIXb*[c]	*XXd*	*XXI*	*XXII*	*XXIII*
C-1	38·12	38·14	38·14	38·16	38·13	38·12	38·18	38·10	38·14	38·14	38·36	38·35
C-2	21·38	21·40	21·39	21·42	21·39	21·40	21·40	21·38	21·42	21·39	49·86	49·85
C-3	25·15	25·16	25·16	25·18	25·15	25·19	25·15	25·14	25·17	25·16	50·06	50·09
C-4	20·42	20·44	20·42	20·44	20·42	20·45	20·42	20·42	20·45	20·43	20·99	21·00
C-5	58·83	58·82	58·84	58·86	58·83	58·88	58·80	58·82	58·84	58·86	52·29	52·33
C-6	212·45	212·47	212·62	212·66	212·67	212·42	212·54	212·59	212·79	212·70	211·15	211·14
C-7	46·75	46·74	46·73	46·76	46·72	46·70	46·70	46·70	46·77	46·73	46·82	46·82
C-8	37·87	37·88	37·94	37·97	37·95	37·87	37·89	37·95	38·02	37·91	37·38	37·40
C-9	56·42	56·40	56·26	56·86	56·62	56·71	56·80	56·55	56·69	56·45	56·10	56·08
C-10	41·72	41·71	41·72.	41·77	41·76	41·71	41·71	41·75	41·80	41·75	37·83	37·86
C-11	21·07	21·10	21·14	21·15	21·14	21·08	21·10	21·09	21·15	21·10	20·99	21·00
C-12	39·36	39·43	39·44	39·61	39·50	39·74	39·55	39·35	39·54	39·52	39·16	39·22
C-13	43·26	43·27	43·59	42·99	42·85	43·49	42·93	42·74	42·82	43·44	43·04	43·06
C-14	54·31	54·38	54·29	54·32	54·24	54·24	54·28	54·19	54·25	54·26	53·05	53·13
C-15	24·22	24·12	24·00	23·81	23·70	23·90	23·86	23·68	23·87	24·14	24·20	24·11
C-16	27·75	26·82	27·83	27·32	27·02	27·60	27·77	27·17	27·75	27·86	27·69	26·74
C-17	53·58	55·91	54·98	52·78	54·24	55·61	52·72	52·93	52·66	52·57	53·49	55·84
C-18	12·05	12·10	11·91	12·01	12·65	11·70	11·74	12·38	11·85	11·91	11·92	11·99
C-19	13·01	13·01	13·03	13·06	13·27	13·05	13·04	13·03	13·06	13·03	14·97	14·98
C-20	39·44	38·60	40·27	37·41	36·23	40·70	38·18	36·49	40·21	41·93	39·45	38·57
C-21	16·88	16·04	15·37	11·27	14·91	14·40	12·42	14·77	12·43	13·98	16·89	16·05
C-22	63·66	64·09	61·94	73·32	65·10	57·67	74·95	61·57	76·37	73·23	63·68	64·02
C-23	62·95	60·28	77·66	61·83	72·56	76·81	59·22	73·62	72·67	70·24	62·90	60·30
C-24	42·44	42·25	43·33	40·29	40·85	44·12	40·68	41·13	41·43	43·86	42·42	42·33
C-25	30·97	31·09	25·75	31·94	30·86	26·21	31·89	30·31	27·00	29·80	30·97	31·09
C-26	20·20	20·40	22·88	21·03	20·76	21·50	20·86	20·87	22·12	21·47	20·19	20·41
C-27	18·54	19·47	17·96	20·55	20·35	17·69	20·68	20·16	17·30	18·88	18·54	19·47
C-24[1]	12·51	13·64	11·43	12·70	9·32	10·89	13·23	10·34	10·84	9·92	12·52	13·63

The signals of acetate group: [a] 169·43 and 22·58; [b] 169·84 and 21·00; [c] 169·82 and 21·12.

Collect. Czech. Chem. Commun. (Vol. 55) (1990)

TABLE III

The comparison of side-chain proton and carbon chemical shifts of epoxy derivatives *XV*, *XXII*, *XVI* and *XXIII*

Compound	Configuration	H(22)	H(23)	21-Me	26-Me	27-Me	24^1-Me
XV	22*R*, 23*R*	2·63	2·37	1·089	0·963	0·919	0·906
XXII		2·65	2·37	1·088	0·961	0·918	0·903
XVI	22*S*, 23*S*	2·57	2·45	1·000	0·976	0·929	0·950
XXIII		2·57	2·45	0·999	0·973	0·927	0·949

		C-20	C-21	C-22	C-23	C-24	C-25	C-26	C-27	C-24^1
XV	22*R*, 23*R*	39·44	16·88	63·66	62·95	42·44	30·97	20·20	18·54	12·51
XXII		39·45	16·89	63·68	62·90	42·42	30·97	20·19	18·54	12·52
XVI	22*S*, 23*S*	38·60	16·04	64·09	60·28	42·25	31·09	20·40	19·47	13·64
XXIII		38·57	16·05	64·02	60·30	42·33	31·09	20·41	19·47	13·63

the preferred rotamers around C(22)—C(23) bond for the substances *XVIIa*–*XIXb* by the formulae *A* and *B*. Their common feature is the antiperiplanar arrangement

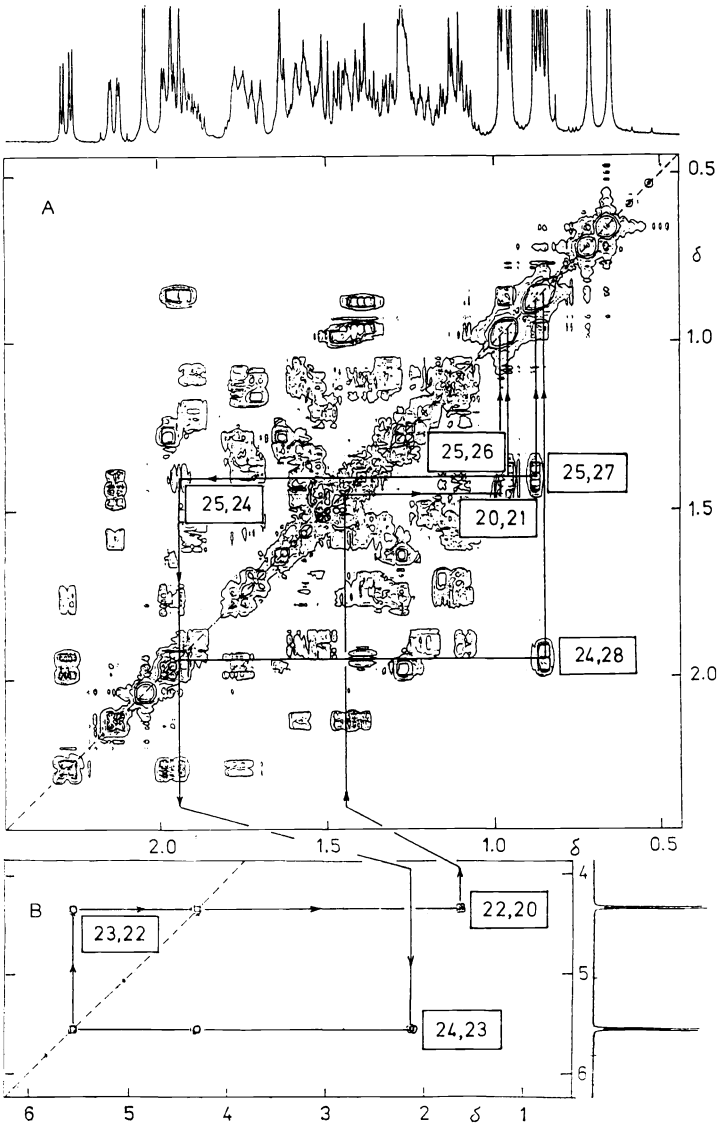
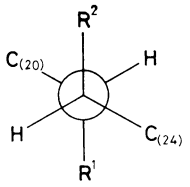


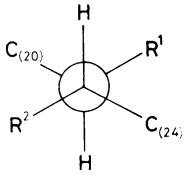
FIG. 1

Proton homocorrelated 2D COSY NMR spectrum (at 500 MHz) of bromoacetate *XIXb* (divided into **A** and **B** parts of different scale; the assignment of side-chain protons is indicated with full lines; the cross peaks are marked by numbers of corresponding protons)

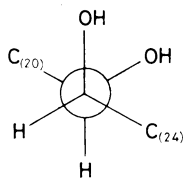
of Br and OR substituents and of carbon atoms C-20 and C-24 leading to an extended form of the side chain. The whole group of six compounds *XVIIa–XIXb* is also characterized by very small values of coupling constants $J(20, 22)$ and $J(23,24)$ (1·0 to 2·8 Hz), pointing to a pronounced preference of rotamers with gauche arrangements of hydrogens around the bond C(20)—C(22) and C(23)—C(24).



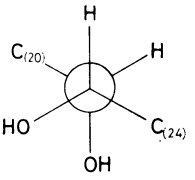
A
XVIIa, *XVIIb*



B
XVIIIa, *XVIIIb*
XIXa, *XIXb*



C
XXd



D
XXI

The presence of two hydroxyl groups in diols *XXd* and *XXI* was proved by in situ TAI acylation in an NMR tube. Formation of di-OTAC derivatives was demonstrated by two signals of NH protons of carbamoyl groups and by characteristic acylation shifts of H-22 and H-23 hydrogens (cf. Table I). However, the diols *XXd* and *XXI* show different conformational behavior compared to the discussed bromohydrins and bromohydrin acetates. The values of $J(22,23) \approx 4{\cdot}5$ Hz indicate a marked preference of rotamers with gauche arrangements of hydrogens around the C(22)—C(23) bond. Evidently, of the two possible rotamers the one which permits formation of a hydrogen bridge most probably stabilizing the given conformation preponderates. With known absolute configurations at C-22 and C-23, the preferred rotamers for *XXd* and *XXI* can be formulated as *C* and *D*, again leading to an extended form of the side chain.

^{13}C NMR spectra of compounds *XV–XXIII* (Table II) are all in general agreement with the proposed structures for these substances. The structural assignment of the signals was based on experimentally obtained information on the number

of directly bonded hydrogens ("attached proton test" method) and on reported[4] chemical shift data of the parent hydrocarbon ergostane together with the known[5] effect of the 6-oxo group on chemical shifts of carbons in the steroid skeleton. It was thus possible to assign reliably the signals of C-1 to C-17 carbons. Their chemical shifts show only very small differences in the whole series except the ring A substituted diepoxides *XXII* and *XXIII*. In these compounds, the epoxide carbons C-2 and C-3 are characteristically shifted downfield ($\delta \approx 50$) and the carbons C-5 and C-10 show marked upfield shifts caused by a γ-effect. The configuration at C-22 and C-23 in the monoepoxides *XV* and *XVI* can be unequivocally correlated with the diepoxides *XXII* and *XXIII* by analogous ^{13}C chemical shifts of the side chain carbons (cf. Table III). Concerning the side chain carbons, unequivocal assignment can be done for signals CHOR and CHBr on the basis of distinct shifts induced by these substituents[6]. The signals of secondary methyl groups and of methin carbons C-20, C-24 and C-25 were assigned tentatively on the basis of chemical shift calculations using empirical parameters[7] and from expected values of acetylation effects in the corresponding pairs of bromohydrins and bromohydrin acetates.

EXPERIMENTAL

Melting points were determined on a Kofler block, Optical rotations were measured in chloroform. The infrared spectra were recorded on a Zeiss UR-20 spectrometer (wavenumbers in cm^{-1}). Mass spectra were measured on a ZAB-EQ instrument (VG Analytical). Column chromatography was performed on silica gel according to Pitra, 60–120 μm, thin layer chromatography on silica gel G Woelm DC. Before evaporation, solutions in organic solvents were dried over anhydrous magnesium sulfate. ^1H and ^{13}C NMR spectra of compounds *XV*–*XXIII* were measured on a FT NMR spectrometer Varian XL-200 (^1H, 200 MHz; ^{13}C, 50·3 MHz) in $CDCl_3$. The chemical shifts and coupling constants of hydrogens were obtained by first order analysis from expanded spectra using tetramethylsilane (TMS) as internal reference. ^1H NMR spectra of compounds *XV*, *XVI*, *XIXa*, *XIXb*, *XXII* and *XXIII* were also measured on a FT NMR spectrometer Bruker AM-500 (at 500 MHz); in the case of compound *XIXb* the homocorrelated 2D COSY spectrum was also measured. The diols *XXd* and *XXI* were acylated in situ with trichloroacetylisocyanate (TAI) in NMR tube[8] to give di-OTAC derivatives characterized by ^1H NMR spectra. Proton NMR data of compounds *XV*–*XXIII* are listed in Table I. ^{13}C NMR spectra were measured by application of APT technique[9] allowing to determine the number of hydrogens directly bonded on the corresponding carbons. Chemical shifts of ^{13}C carbons were referenced to the solvent signal, recalculated to TMS ($\delta(CDCl_3) = 77{\cdot}0$ ppm) and their values for the substances *XV*–*XXIII* are given in Table II.

(22*E*)-5α-Ergost-22-en-6-one (*XIV*)

Small pieces of lithium (350 mg, 50 mmol) were dissolved in liquid ammonia (140 ml) and a solution of the compound *XIII* (3·2 g, 0·81 mmol) in ether (110 ml) was added with vigorous stirring. Stirring was continued for 2 min, the solution was decolorized with ammonium chloride, the mixture poured on ice and the product extracted with ether. After washing with water, the solution was dried and the solvent removed under reduced pressure. The residue was purified

by chromatography on silica gel (100 g) to yield the product *XIV* (3 g). Crystallization from ethanol gave (2·8 g, 86%), m.p. 120–122°C, $[\alpha]_D$ −33° (*c* 1·6). IR spectrum (CCl_4): 1 712 (C=O); 1 672 (C=C). For $C_{28}H_{46}O$ (398·6) calculated: 84·35% C, 11·63% H; found: 84·20% C, 11·57% H.

(22*R*,23*R*)-22,23-Epoxy-5α-ergostan-6-one (*XV*) and
(22*S*,23*S*)-22,23-Epoxy-5α-ergostan-6-one (*XVI*)

Olefin *XIV* (2·0 g, 5·0 mmol) was treated with 3-chloroperbenzoic acid (82%, 2·5 g, 11·9 mmol) in benzene (120 ml) solution at room temperature for 1 h. The solution was washed several times with water, 1% aqueous sodium hydroxide solution and water and the solvent removed under reduced pressure. The residue was chromatographed on a column of silica gel (50 g) in benzene giving the epoxide *XV* (780 mg) and *XVI* (500 mg). The compound *XV* was crystallized from ethanol (with a trace of pyridine) to give the pure product (670 mg, 32%), m.p. 138–140°C, $[\alpha]_D$ −10° (*c* 1·3). For $C_{28}H_{46}O_2$ (414·7) calculated: 81·10% C, 11·18% H; found: 81·05% C, 11·12% H. The compound *XVI* was crystallized in the same way to furnish the pure product (450 mg, 22%), m.p. 170–171°C, $[\alpha]_D$ −28° (*c* 1·4). For $C_{28}H_{46}O_2$ (414·7) calculated: 81·10% C, 11·18% H; found: 81·21% C, 11·12% H.

(22*S*,23*R*)-22-Bromo-23-hydroxy-5α-ergostan-6-one (*XVIIa*) and
(22*R*,23*S*)-23-Bromo-22-hydroxy-5α-ergostan-6-one (*XVIIIa*)

The epoxy derivative *XV* (200 mg, 0·48 mmol) was dissolved in acetone (15 ml) and aqueous HBr (48%, 0·60 ml, 3·56 mmol) was added. After 3 min the reaction was completed (TLC), the mixture was poured in water, extracted in ether, the solution washed with water and hydrogen sodium carbonate. After drying with magnesium sulfate, the solvent was removed under reduced pressure to leave a residue (245 mg) which was chromatographed on silica gel (30 g). Petroleum ether–ether (9 : 1) eluted the first compound *XVIIa* (145 mg, 60%), m.p. 173–175°C, raised to 178–179°C by crystallization from ether, $[\alpha]_D$ +6° (*c* 1·5). IR spectrum ($CHCl_3$): 3 625 (OH); 1 709 (C=O). Mass spectrum (high resolution), *m/z*: 394·1832 ($C_{22}H_{35}{}^{79}BrO$), 396·1846 ($C_{22}H_{35}{}^{81}BrO$). For $C_{28}H_{47}BrO_2$ (495·6) calculated: 67·85% C, 9·56% H, 16·13% Br; found: 68·11% C, 9·58% H, 16·60% Br. Continued elution with petroleum ether–ether (4 : 1) gave the second compound *XVIIIa* (105 mg, 40%), m.p. 215–216°C; crystallization from chloroform–petroleum ether raised the m.p. to 216·5–217·5°C, $[\alpha]_D$ −28° (*c* 1·6). IR spectrum ($CHCl_3$): 3 625, 3 605 (OH); 1 708 (C=O). For $C_{28}H_{47}BrO_2$ (495·6) calculated: 67·85% C, 9·56% H, 16·13% Br; found: 67·83% C, 9·81% H, 16·66% Br.

(22*S*,23*R*)-22-Bromo-6-oxo-5α-ergostan-23-yl Acetate (*XVIIb*)

The bromohydrin *XVIIa* (285 mg, 0·575 mmol) was acetylated with acetic anhydride (2 ml) in pyridine (6 ml) at room temperature for 72 h. The mixture was poured on ice, extracted with ether, washed with water, 5% hydrochloric acid, water, sodium hydrogen carbonate, and dried. After evaporation of solvent the residue (290 mg) melted at 193–194°C. Crystallization from chloroform–petroleum ether gave the pure product (150 mg, 49%), m.p. 194–195°C, $[\alpha]_D$ 0° (*c* 1·4). IR spectrum (CCl_4): 1 745, 1 233 (OAc); 1 712 (C=O). For $C_{30}H_{49}BrO_3$ (537·6) calculated: 67·02% C, 9·19% H, 14·78% Br; found: 67·06% C, 9·25% H, 14·82% Br.

(22*R*,23*S*)-23-Bromo-6-oxo-5α-ergostan-22-yl Acetate (*XVIIIb*)

The bromohydrin *XVIIIa* (395 mg, 0·797 mmol) was acetylated with acetic anhydride (3 ml)

in pyridine (10 ml) at room temperature overnight. After the workup as in the preceding experiment the residue (390 mg, m.p. 225–227°C) was crystallized from chloroform–petroleum ether to give pure *XVIIIb* (280 mg, 65%), m.p. 230–232°C, $[\alpha]_D$ −19° (*c* 1·4). IR spectrum (CCl_4): 1 745, 1 237 (OAc); 1 714 (C=O). For $C_{30}H_{49}BrO_3$ (537·6) calculated: 67·02% C, 9·19% H, 14·87% Br; found: 67·09% C, 9·23% H, 15·09% Br.

(22*R*,23*R*)-22,23-Dihydroxy-5α-ergostan-6-one (*XXd*)

a) The bromo derivative *XVIIb* (180 mg, 0·335 mmol) was refluxed with sodium acetate (250 mg, 3·0 mmol) in acetic acid (18 ml) for 7 h. The mixture was poured onto ice, neutralized with sodium hydroxide and extracted into ether. After washing the solution with water and drying the solvent was removed and the residue (198 mg, oil, two products) was separated from a trace of less polar impurity by chromatography on silica gel using benzene as eluant. This two-compound mixture was refluxed with potassium hydroxide (dissolved in a minimal amount of water) in methanol (15 ml) for 90 min. After this time the TLC showed complete transformation of both components into a single product. The mixture was concentrated to a small volume, poured in water neutralized with hydrochloric acid and extracted several times with ether. The solution was washed with water, sodium hydrogen carbonate and water. Drying and removal of the solvent left a residue that was crystallized from ether–petroleum ether to give the pure diol (75 mg, 52%),m.p. 164–165°C, $[\alpha]_D$ −16° (*c* 1·3). IR spectrum (CCl_4): 3 637, 3 630 (OH, free), 3 585, 3 555 (OH bonded). For $C_{28}H_{48}O_3$ (432·7) calculated: 77·72% C, 11·18% H; found: 77·85% C, 11·35% H.

b) A mixture of the bromo derivative *XVIIIb* (200 mg, 0·372 mmol) and cupric acetate monohydrate (250 mg, 1·25 mmol) was refluxed in acetic acid for 4 h. The mixture was filtered, the inorganic material washed with acetic acid, the combined filtrates poured onto ice and most of the acetic acid neutralized with sodium hydroxide. The product was taken up in ether, the solution washed with water, sodium hydrogen carbonate and water and the solvent evaporated. The product consisted of two more polar components identical by TLC in several systems with the products of acetolysis of *XVIIb* (under *a*). The mixture was separated by chromatography on silica gel in benzene solution first giving some unreacted starting compound (50 mg, 25%). The more polar component (30 mg) (IR (CCl_4) characteristics: 3 625, 3 610 (OH); 1 742, 1 259, 1 026 (OAc); 1 712 (C=O)) was hydrolyzed by refluxing with potassium hydroxide (200 mg, dissolved in a small volume of water) in methanol (15 ml) for 1 h. After concentration under reduced pressure the mixture was diluted with water, acidified with 5% hydrochloric acid and extracted with ether. After washing the solution with water, sodium hydrogen carbonate, drying and removing the solvent, the residue (19 mg, 12%, glass), $[\alpha]_D$ −19° ($CHCl_3$, *c* 1·6) was identical by IR spectrum ($CHCl_3$): 3 625 (OH); 1 707 (C=O) and its R_F in various systems with the diol *XXd* obtained under *a*) and *c*).

c) The most polar component of the acetolysis obtained under *b*) (27 mg) (IR (CCl_4): 3 635, 3 610 (OH); 1 742, 1 251, 1 025 (OAc); 1 714 (C=O)) was hydrolyzed in the same manner as given for the less polar component under *b*). The glassy crude material was filtered through a layer of silica gel in benzene–ether (19 : 1) to remove a trace of polar impurity. The material (20 mg, 12%), $[\alpha]_D$ −13° (*c* 1·9) was identical by its IR spectrum ($CHCl_3$) and R_F in a variety of solvent systems with the diol *XXd* obtained under *a*) and *b*).

(22*R*,23*S*)-22-Bromo-23-hydroxy-5α-ergostan-6-one (*XIXa*)

The epoxide *XVI* (730 mg, 1·76 mmol) was dissolved in acetone (75 ml) and hydrobromic acid (48%, 2·4 ml, 14·3 mmol) was added. After 15 min ice water was added, the product collected

and washed with water. Crystallization from chloroform–petroleum ether gave pure *XIXa*. (500 mg, 57%), m.p. 234–236°C, $[\alpha]_D$ −10° (*c* 1·5). IR spectrum ($CHCl_3$): 3 620 (OH); 1 707 (C=O). For $C_{28}H_{47}BrO_2$ (495·6) calculated: 67·85% C, 9·56% H, 16·13% Br; found: 67·76% C, 9·45% H, 16·61% Br.

(22*R*,23*S*)-22-Bromo-6-oxo-5α-ergostan-23-yl Acetate (*XIXb*)

The bromohydrin *XIXa* (500 mg, 1·01 mmol) was dissoved in pyridine (6 ml), acetic anhydride (4 ml) was added and the mixture left at room temperature for 70 h. After addition of ice, the product was collected with suction, washed with water and crystallized from chloroform–petroleum ether to give the pure product (370 mg, 68%), m.p. 212–213°C, $[\alpha]_D$ −11° (*c* 1·4). IR spectrum (CCl_4): 1 746, 1 235, 1 023 (OAc); 1 713 (C=O). For $C_{30}H_{49}BrO_3$ (537·6) calculated: 67·01% C, 9·19% H, 14·86% Br; found: 67·30% C, 9·15% H, 15·00% Br.

(22*S*,23*S*)-22,23-Dihydroxy-5α-ergostan-6-one (*XXI*)

A mixture of the acetyl derivative *XIXb* (415 mg, 0·77 mmol), acetic acid (35 ml) and sodium acetate (600 mg) was refluxed for 8 h, poured into ice, neutralized with sodium hydroxide and taken up in ether. After removing the solvent under reduced pressure, the residue (360 mg) was dissolved in methanol (50 ml), a concentrated aqueous solution of potassium hydroxide (1·0 g) was added and the mixture was refluxed for 1·5 h, concentrated to a small volume, poured in water, acidified with hydrochloric acid, filtered with suction, taken up in ether, dried and the solvent removed under reduced pressure. The residue was dissolved in benzene–ether (10%) and filtered through a small layer of silica gel. Evaporation gave a residue which was crystallized from chloroform–petroleum ether to give the diol *XXI* (188 mg, 56%), m.p. 172–174°C, $[\alpha]_D$ −17° (*c* 1·5). IR spectrum ($CHCl_3$); 3 639, 3 626 (OH free); 3 583, 3 562 (OH bonded); 1 707 (C=O). For $C_{28}H_{48}O_3$ (432·7) calculated: 77·72% C, 11·18% H; found: 77·70% C, 11·10% H.

(22*R*,23*R*)-2α,3α;22,23-Diepoxy-5α-ergostan-6-one (*XXII*) and
(22*S*,23*S*)-2α,3α;22,23-Diepoxy-5α-ergostan-6-one (*XXIII*)

The dienone *IV* (1·0 g, 2·52 mmol) was dissolved in benzene (55 ml) and 3-chloroperbenzoic acid (82%, 2·2 g, 10·4 mmol) was added and the solution was left standing at room temperature. After 4 h the reaction was complete (TLC) and the solution was washed with water, sodium hydroxide, sodium sulfite solution and water, dried and the solvent was removed under reduced pressure. The residue was chromatographed on a column of silica gel (100 g, kept 1 h under NH_3 atmosphere) in benzene–ether (1%) to give *XXII* (542 mg, m.p. 126–130°C). Crystallization from aqueous acetone yielded pure *XXII* (402 mg, 27·5%), m.p. 130–132°C, $[\alpha]_D$ +5° (*c* 1·5). IR spectrum (CCl_4): 1 712 (C=O); 913, 907. For $C_{28}H_{44}O_3$ (428·6) calculated: 78·45% C, 10·35% H; found: 78·84% C, 9·98% H.

Continued elution yielded the isomeric epoxide (392 mg, m.p. 180–185°C) which after crystallization from ether gave pure *XXIII* (278 mg, 26%), m.p. 183–185°C, $[\alpha]_D$ −21° (*c* 1·3). For $C_{28}H_{44}O_3$ (428·6) calculated 78·45% C, 10·35% H; found: 78·75% C, 10·10% H. IR spectrum (CCl_4): 1 712 (C=O); 910.

(22*R*,23*R*)-2α,3α,22,23-Tetrahydroxy-5α-ergostan-6-one (*V*)

The diepoxide *XXII* (440 mg, 0·103 mmol) was dissolved in acetone (60 ml) and aqueous hydrobromic acid (48%, 1·5 ml, 8·9 mmol) was added and left standing 7 min, the mixture poured in

water, the product taken up in ether, the solution washed with water, sodium hydrogen carbonate, dried and the solvent removed under reduced pressure ($t < 35°C$). The residue was acetylated with pyridine (15 ml) and acetic anhydride (10 ml) for 70 h, the mixture poured on ice, extracted with ether, the solution washed with water, hydrochloric acid (5%), sodium hydrogen carbonate, dried and the solvent removed under reduced pressure ($t < 35°C$). The residue was dissolved in acetic acid (90 ml), sodium acetate (450 mg, 5·5 mmol) and cupric acetate monohydrate (450 mg, 2·5 mmol) were added and the mixture refluxed for 11 h, poured on ice, most acidity neutralized with sodium hydroxide and the product taken up in ether. After washing with sodium hydroxide and water the solvent was removed under reduced pressure and the residue dissolved in methanol (100 ml), potassium hydroxide was added (900 mg, dissolved in 2 ml of water) and the mixture refluxed for 1·5 h. Most methanol was then removed in vacuo, the mixture diluted with water and the product taken up in ether, the solution washed with water, hydrochloric acid, water, sodium hydrogen carbonate and water, dried and the solvent was removed under reduced pressure. The residue (430 mg) was purified by chromatography on silica gel (20 g) using chloroform–methanol (2%). The pure fraction (150 mg, 31%, m.p. 240–250°C) was crystallized from aqueous ethanol to give the pure product, m.p. 245–247°C, $[\alpha]_D$ −8° (*c* 1·4); literature[2] reports 241–242°C, $[\alpha]_D$ 0°. The compound is identical with an authentic sample[2] (IR spectrum, mixture m.p., TLC).

(22*S*,23*S*)-2α,3α,22,23-Tetrahydroxy-5α-ergostan-6-one (*VI*)

The diepoxide *XXIII* (330 mg, 0·77 mmol) was dissolved in acetone (45 ml), aqueous hydrobromic acid (48H, 1 ml, 5·9 mmol) was added and left standing 10 min, the mixture poured in water, the product taken up in ether, the solution washed with water, sodium hydrogen carbonate, dried and the solvent removed under reduced pressure ($t < 35°C$). The residue was acetylated with acetic anhydride (10 ml) in pyridine (15 ml) for 70 h, the mixture poured on ice, extracted with ether, the solution washed with water, hydrochloric acid, sodium hydrogen carbonate and with water, dried and the solvent removed under reduced pressure. The residue was dissolved in acetic acid (60 ml), sodium acetate (350 mg, 4·3 mmol) and cupric acetate monohydrate (350 mg, 1·9 mmol) were added and the mixture refluxed for 11 h, poured on ice, most acidity neutralized with sodium hydroxide, taken up in ether, the extract washed with water, sodium hydroxide and water, dried and the solvent removed under reduced pressure. The residue was dissolved in methanol (50 ml), potassium hydroxide was added (500 mg, 8·9 mmol, dissolved in 1·5 ml of water) and the mixture refluxed for 1·5 h. Most methanol was then removed in vacuo, the mixture diluted with water, acidified with hydrochloric acid and taken up in ether. The solution was washed with water, sodium hydrogen carbonate and water, dried and the solvent removed in vacuo. The residue (312 mg) was chromatographed on silica gel (15 g) in chloroform–methanol (1%) and the main fraction (187 mg, 52%) was crystallized from aqueous ethanol and from ethyl acetate to give the tetrol *VI* (66 mg, 18%), m.p. 189–190°C, $[\alpha]_D$ −8° ($CHCl_3$ + 5% ethanol), identical (TLC, IR) with an authentic sample[2]. Literature[2] reports m.p. 182–183°C, $[\alpha]_D$ −2°.

(22*R*,23*R*)-2α,3α,22,23-Tetrahydroxy-5α-ergostan-6-one (*V*) and
(22*S*,23*S*)-2α,3α,22,23-Tetrahydroxy-5α-ergostan-6-one (*VI*)

The dienone *IV* (1·76 g, 4·43 mmol) was dissolved in benzene (100 ml), 3-chloroperbenzoic acid (82%, 4·0 g, 18·9 mmol) was added and the solution left standing at room temperature. After 1·5 h the reaction was complete (TLC) and the solution was washed with water, sodium hydroxide, sodium sulfite solution and water, dried and evaporated under reduced pressure. The residue

was dissolved in acetone (200 ml) and aqueous hydrobromic acid (48%, 3·5 ml) was added. After 3 min (TLC), the reaction mixture was poured in water, extracted with ether, the extract washed with water, sodium hydrogen carbonate, water, dried and the solvent was removed under reduced pressure at $t < 35°C$. The residue was dried azeotropically with benzene (reduced pressure, $t < 35°C$) and acetylated in pyridine (45 ml) with acetic acid anhydride (25 ml) at room temperature. After 70 h the mixture was poured on ice, extracted with ether, the solution washed with water, hydrochloric acid (5%), water, sodium hydrogen carbonate, water. After removal of the solvent under reduced pressure ($t < 35°C$) acetic acid (250 ml) and anhydrous potassium acetate (8 g) were added and the mixture refluxed for 9 h. The mixture was poured on ice, the acid partially neutralized with sodium hydroxide, the product extracted with ether and washed with water, sodium hydrogen carbonate, water. After removal of the solvent under reduced pressure, the residue was hydrolyzed with potassium hydroxide (5 g, dissolved in a minimum of water) in methanol (200 ml). After refluxing for 2 h, the reaction mixture was concentrated to ca 30 ml, poured into water, taken up in ether, the solution washed with water, hydrochloric acid (5%) and sodium hydrogen carbonate. After drying the solvent was evaporated under reduced pressure, the residue (2·2 g) chromatographed on a column of silica gel (90 g) in chloroform–methanol (1%) to yield the less polar *VI* (673 mg), giving the pure product (500 mg, 25%), m.p. 182–183°C, $[\alpha]_D$ −8° (*c* 1·4) after crystallization from ethyl acetate; the literature[2] gives m.p. 182–183°C, $[\alpha]_D$ −2°. The more polar fraction (910 mg) was crystallized from ethyl acetate to give pure *V* (645 mg, 32%), m.p. 245–247°C, $[\alpha]_D$ −1° ($CHCl_3$ + 1% ethanol, *c* 1·6). Literature[2] gives m.p. 241–242°C, $[\alpha]_D$ 0°. Both *V* and *VI* were identical in R_F. IR spectrum and mixture m.p. with authetic sample[2].

The analyses were carried out in the analytical laboratory of this Institute under the direction of Dr V. Pechanec. Our thanks are due to Dr J. Smolíková for the interpretation of the IR *spectra and to Dr J. Kohoutová for the interpretation of the mass spectrometric measurements. We also thank Mrs J. Neumannová for her technical assistance.*

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Translated by the author (M.B.).