SYNTHESIS OF 3,4-, 4,5- AND 5,6-UNSATURATED 19-SUBSTITUTED CHOLESTANE DERIVATIVES AND RELATED EPOXIDES*

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Starting from the 5,6-unsaturated compound *X*, 19-hydroxy-, 19-methoxy- and 19-acetoxy derivatives with the double bond in positions 5,6- (*XIV—XVI*), 4,5- (*XXI—XXIII*) and 3,4- (*XXX, XXXIII, XXXIV*) were prepared by stepwise transposition of the 5,6-double bond. The route to 4,5-unsaturated steroids involves hypobromous acid addition (*XVI→XVIII*) followed by reductive removal of bromine and dehydration (*XIX→XXI*). Transposition of the 4,5-double bond to the 3,4-position is based on the conversion of the 4,5-olefin into 4β-alcohol (*XXIII→ XXVII*) and pyrolysis of its benzoate (*XXVIII→XXX*).

For our studies on some aspects of neighboring group participation in electrophilic additions to double bonds in the steroid skeleton[1-3], we needed derivatives of the olefins *I*, *II* and *III* bearing hydroxyl, methoxyl or acetoxyl in position 19, and the corresponding epoxides.

An obvious route to these compounds seemed to be the addition of hypobromous acid to olefins *I–III* presumed to provide the corresponding bromohydrins *IV–VI*; their conversion to the bromo epoxides *VII–IX* by lead tetraacetate procedure followed by zinc reduction was expected to give rise to the desired unsaturated 19-hydroxy derivatives. However, only a complex mixture of products was obtained from the olefins *I* and *II* on reaction with hypobromous acid. On the other hand, the 3,4-olefin *III* gave the required bromohydrin[4] *VI* (though accompanied by its 3α-hydroxy-4β-bromo isomer) but this product reacted with lead tetraacetate to give a complex mixture containing no bromo epoxide *IX*. Therefore, a more complicated procedure for the preparation the 19-substituted olefins is described in the present paper.

The synthesis started from 19-benzoyloxy derivative *X* which is comparatively easily accessible[5] from cholesterol. This alternate approach is based on the removal of the substituent from the position 3 followed by stepwise shift of the double bond into 4,5- and then 3,4-position. The diester *X* was selectively saponified to provide the monoester *XI* the mesylate *XII* of which was reduced with zinc and sodium

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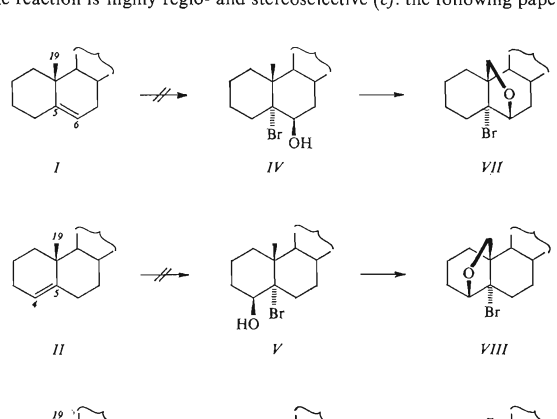
iodide[6,7] to give the 3-deoxy derivative *XIII*. The latter was converted to all three desired derivatives, *i.e.* the alcohol *XIV*, methyl ether *XV* and acetate *XVI*.

The shift of the double bond from 5,6- to 4,5-position was conducted in the following manner. Addition of hypobromous acid to the 5,6-double bond of the 19-acetate *XVI* gave rise to the diequatorial bromohydrin *XVIII* in practically quantitative yield. The reaction is highly regio- and stereoselective (*cf.* the following papers[8-11])

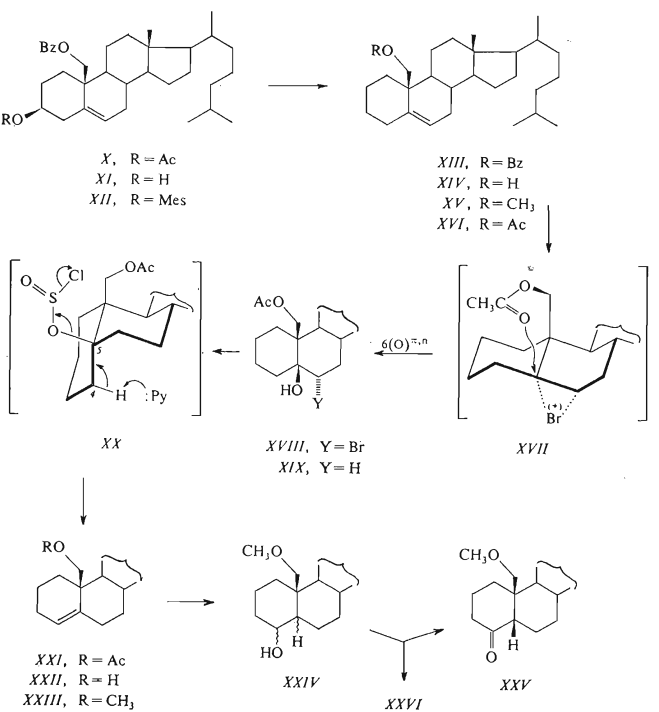


and involves a $6(O)^{n,n}$ participation (for notation cf. ref.[1]). Reduction of *XVIII* with Raney-nickel[12,13] yielded the alcohol *XIX* that was subjected to dehydration with thionyl chloride. In the intermediate *XX* of this reaction, only 4α-hydrogen assumes an antiperiplanar orientation with respect to the leaving group. This fact accounts for the high regioselectivity and high yield of the 4,5-olefin *XXI* in which the maximal content of the isomeric 5,6-olefin *XVI* amounts to 5% (TLC evidence). The 4,5-unsaturated 19-acetoxy derivative *XXI* was readily converted to the alcohol *XXII* and methyl ether *XXIII*.

In the following preparation of derivatives of the 3,4-unsaturated series from 4,5-unsaturated compounds, we utilized the 19-methoxy derivative *XXIII* to achieve effective protection of the 19-oxygen moiety[13,14]. The synthetic sequence planned for this double bond shift involved the preparation of a 4β-alcohol and its conversion

to a suitable derivative capable of *syn*-elimination. Since only 3β-H is accessible for a *syn*-elimination a 3,4-olefin should be formed regioselectively.

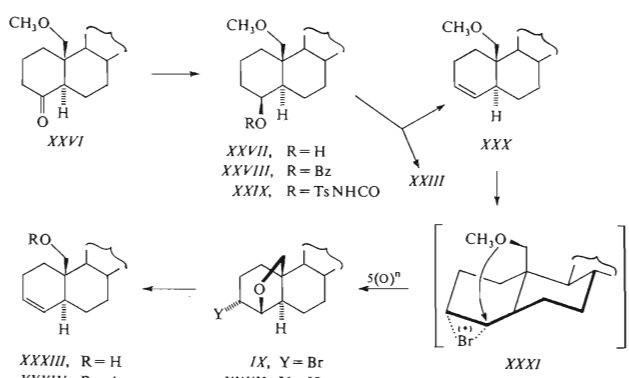
Hydroboration of the 4,5-double bond of the methyl ether *XXIII* gave a mixture of alcohols *XXIV* which was oxidized without separation to yield 5-epimeric 4-ketones *XXV* and *XXVI* in *c.* 2 : 1 proportion. Equilibration in a basic medium (*cf.* the 19-unsubstituted series[15]) improved this ratio to 1 : 3. The ketone *XXVI* was reduced with lithium aluminum hydride to give stereoselectively the 4β-alcohol *XXVII*. We first converted this alcohol to the benzoate *XXVIII* and subjected it to pyrolysis under the conditions applied in the 19-unsubstituted series[16]. The result was disappointing since the reaction was not smooth and the major product of the



reaction mixture was 4,5-olefin *XXIII*. We therefore focused our attention on the urethane *XXIX* which was easily generated from *XXVII* and *p*-toluenesulfonyl isocyanate[17]. However, the urethane *XXIX* was resistant to refluxing in benzene and prolonged boiling in xylene yielded essentially the 4,5-olefin *XXIII* containing, as shown by ^{1}H-NMR evidence, only about 10% of the 3,4-olefine *XXX*. This result indicates isomerization of the initially formed 3,4-olefin to the more stable 4,5-isomer in the reaction medium. On the other hand, when pyrolysis of the benzoate *XXVIII* was repeated on smaller scale under controlled conditions, the desired 3,4-olefin *XXX* was obtained in 72% yield and its 4,5-unsaturated isomer *XXIII* (15%) could be separated by chromatography.

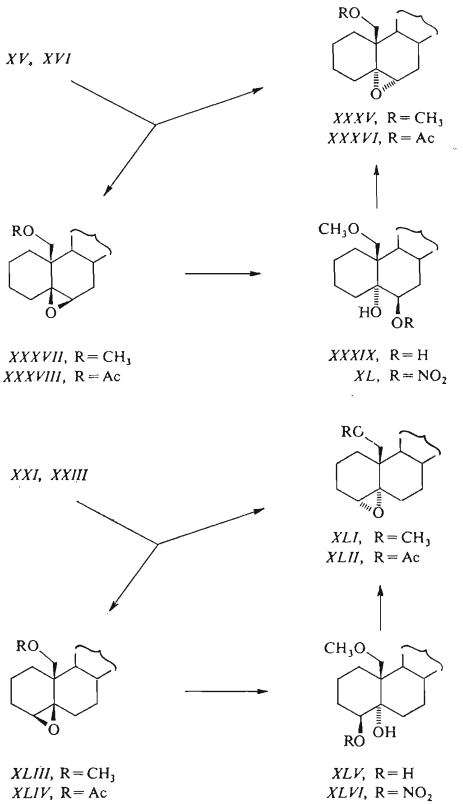
The next task was to convert the 3,4-unsaturated methyl ether *XXX* to the 19-hydroxy derivative and its acetate. Deprotection[13,14] of the 19-hydroxyl was conducted using the following two-step procedure: Addition of hypobromous acid to the double bond proceeds *via* the bromonium ion *XXXI* with 5(O)^n participation to provide the bromo epoxide *IX* in practically quantitative yield[9]. Its structure was proved by reduction with Raney-nickel to the known[18] cyclic ether *XXXII*. This conversion constitutes also structural proof of all the related compounds involved in the preceding synthetic steps. Reduction of the bromo epoxide *IX* with zinc in acetic acid yielded the alcohol *XXXIII*. The acetoxy derivative *XXXIV* was prepared from *XXXIII* in the conventional manner.

The remaining group of compounds to be synthesized were the corresponding α-epoxy derivatives. They were prepared by epoxidation of the olefins with *m*-chloroperoxybenzoic acid but were always accompanied by β-epimers. In the case of 5,6-unsaturated 19-methoxy derivative *XV* this epoxidation led to an inseparable

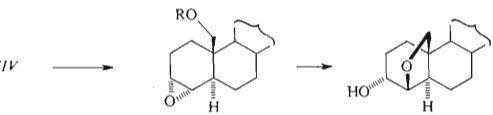


XXVI *XXVII*, R = H; *XXVIII*, R = Bz; *XXIX*, R = TsNHCO *XXIII* *XXX* *XXXI* *IX*, Y = Br; *XXXII*, Y = H *XXXIII*, R = H; *XXXIV*, R = Ac

mixture of epoxides in which the undesired 5β,6β-epoxide *XXXVII* highly predominated. For converting the mixture to pure 5α,6α-epoxide *XXXV*, we utilized a simple procedure that we published earlier[19]. Acid catalyzed hydrolysis of the mixture gave the single diol *XXXIX* convertible to the nitrate *XL* which cyclized easily in a basic medium to give the 5α,6α-epoxide *XXXV*. Epoxidation of the 5,6-unsaturated 19-acetate *XVI* provided a separable mixture of epimeric epoxides



XXXVI (49%) and *XXXVIII* (41%). Epoxidation of the 4,5-unsaturated 19-methoxy derivative *XXIII* gave a mixture of epimeric epoxides *XLI* (19%) and *XLIII* (75%). Both epoxy derivatives were separated chromatographically and the 4β,5β-epoxide *XLIII* was converted to the 4α,5α-epoxide *XLI via* the diol *XLV* and nitrate *XLVI*. Epoxidation of the 3,4-unsaturated 19-methoxy derivative *XXX* gave a mixture of epoxides. The ^{1}H-NMR spectrum of this mixture shows that the major component



XLVII, R = CH_3
XLVIII, R = Ac

IL

(>80%) is the desired 3α,4α-epoxide *XLVII* since the chemical shifts and splitting patterns of 3-H and 4-H fully correspond to these protons in the spectrum of 19-unsubstituted 3α,4α-epoxide[4,20]. Attempts to separate this epoxide from the mixture were unsuccessful due to the instability of the α-epoxide *XLVII*. After chromatography on silica gel, we isolated a new compound proved to be[11] the transannular epoxide *IL*. Thus, the instability of the 3α,4α-epoxide *XLVII* is due to extremely easy 5(O)^n participation of the 19-methoxyl (*cf.* the following paper[11]). On the other hand, the 19-acetate *XXXIV* yielded the 3α,4α-epoxide *XLVIII* stereoselectively. This compound is comparatively stable and can be easily isolated.

Neighboring group participation in hypobromous acid addition to the olefins[8,9] and in cleavage of the epoxides[10,11] is reported in subsequent communications.

EXPERIMENTAL

Melting points were determined on a Kofler block. Analytical samples were dried at 50°C/26 Pa (0·2 Torr). Optical 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 elemental compositions of ions were determined by accurate mass measurements. 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*.

3α-Bromo-4β,19-epoxy-5α-cholestane (*IX*)

The olefin *XXX* (1·2 g) was dissolved in dioxane (60 ml), a solution of 70% perchloric acid (2·5 ml) in water (6 ml) was added and the mixture was treated with N-bromoacetamide (0·5 g) at room temperature for 3 min. The mixture was treated with ether and water, the ethereal layer was washed with water, a 5% aqueous potassium hydrogen carbonate solution and water, dried and evaporated. The residue was crystallized from a mixture of chloroform and acetone to yield the bromo epoxide *IX* (0·98 g), m.p. 125—126°C, $[\alpha]_D^{20}$ +43° (*c* 1·6). ^{1}H-NMR spectrum: 0·65 (3 H, s, 19-H), 3·15 (1 H, m, W = 10 Hz, 4α-H), 4·24 (1 H, m, W = 20 Hz, 3β-H), 3·66 (1 H, d, J = 9 Hz, 19-H), 3·18 (1 H, d, J = 9 Hz, 19-H). For $C_{27}H_{45}BrO$ (465·6) calculated: 69·66% C, 9·74% H, 17·16% Br; found: 69·47% C, 9·81% H, 17·33% Br.

5-Cholesten-3β,19-diol 19-Monobenzoate (*XI*)

The diester *X* (50 g) was dissolved in chloroform (200 ml) and methanol (1 l) and treated with conc. hydrochloric acid (30 ml) at 40°C for 6 h. The mixture was concentrated *in vacuo*, the residue was dissolved in ether and the ethereal solution was washed with water, a 5% aqueous potassium hydrogen carbonate, water, then dried and evaporated. The residue was crystallized from a mixture of acetone, methanol and water to yield the monoester *XI* (41 g), m.p. 144—145°C $[\alpha]_D^{20}$ —55° (*c* 2·4). ^{1}H-NMR spectrum: 0·60 (3 H, s, 19-H), 3·50 (1 H, m, W = 30 Hz, 3α-H), 4·25 (1 H, d, J = 12 Hz, 19-H), 4·55 (1 H, d, J = 12 Hz, 19-H). For $C_{35}H_{50}O_3$ (506·7) calculated: 80·58% C, 9·95% H; found: 80·36% C, 9·78% H.

5-Cholesten-19-ol 19-Benzoate (*XIII*)

The alcohol *XI* (40 g) was dissolved in pyridine (250 ml) and treated with methanesulfonyl chloride (25 ml) at 0°C for 1 h. The mixture was decomposed with ice and water, the product was taken up into ether and the ethereal layer was worked up as usual to afford the crude mesylate *XII* (*c* 43 g). The mesylate *XII* was dissolved in 1,2-dimethoxyethane (100 ml), dioxane (400 ml) and water (20 ml), sodium iodide (30 g) and zinc (30 g) were added and the mixture was stirred at 80°C for 5 h. The inorganic material was filtered off, the solution was evaporated *in vacuo*, the residue was treated with ether and water, the ethereal layer was washed with water, 5% aqueous hydrochloric acid, water, a 5% aqueous potassium hydrogen carbonate solution, 5% aqueous sodium thiosulfate solution, water, dried and evaporated. The residue was dissolved in light petroleum and the solution was filtered through a column of aluminum oxide (150 g). The filtrate was evaporated to yield the oily olefin *XIII* (32 g), $[\alpha]_D^{20}$ —63° (*c* 2·0). For $C_{34}H_{50}O_2$ (490·8) calculated: 83·21% C, 10·27% H; found: 83·21% C, 10·31% H.

5-Cholesten-19-ol (*XIV*)

The benzoate *XIII* (30 g) was dissolved in ether (500 ml) and treated with lithium aluminum hydride (3 g) at room temperature overnight. The mixture was decomposed with water and 5% aqueous hydrochloric acid and the solution was worked up as usual. The residue was crystallized from aqueous acetone to afford the alcohol *XIV* (24 g), m.p. 86—87°C, $[\alpha]_D^{20}$ —37° (*c* 3·1). ^{1}H-NMR spectrum: 0·70 (3 H, s, 18-H), 3·52 (1 H, d, J = 12 Hz, 19-H), 3·82 (1 H, d, J = 11 Hz, 19-H), 4·62 (1 H, s, OH), 5·65 (1 H, m, W = 13 Hz, 6-H). For $C_{27}H_{46}O$ (402·7) calculated: 80·54% C, 11·51% H; found: 80·45% C, 11·36% H.

19-Methoxy-5-cholestene (*XV*)

A solution of the alcohol *XIV* (5 g) in 1,2-dimethoxyethane (30 ml) was treated with sodium hydride (500 mg) and methyl iodide (5 ml). The mixture was stirred at 60°C for 2 h, then 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 a mixture of chloroform and methanol to yield the methoxy derivative *XV* (4·6 g), m.p. 87—88°C, $[\alpha]_D^{20}$ —66° (*c* 2·1). ^{1}H-NMR spectrum: 0·67 (3 H, s, 18-H), 3·27 (3 H, s, CH_3O), 3·25 (1 H, d, $J = 10$ Hz, 19-H), 3·61 (1 H, d, 19-H), 5·53 (1 H, m, $W = 14$ Hz, 6-H). For $C_{28}H_{48}O$ (400·7) calculated: 83·93% C, 12·07% H; found: 83·71% C, 11·94% H.

5-Cholesten-19-ol 19-Acetate (*XVI*)

The alcohol *XIV* (25 g) was dissolved in pyridine (150 ml) and treated with acetic anhydride (30 ml) at room temperature overnight. The mixture was decomposed with ice and water, the product was extracted with ether and the ethereal layer was worked up as usual to yield the oily acetate *XVI* (23 g) $[\alpha]_D^{20}$ —58° (*c* 2·7). ^{1}H-NMR spectrum: 0·67 (3 H, s, 18-H), 2·00 (3 H, s, CH_3CO_2), 3·94 (1 H, d, $J = 12$ Hz), 4·46 (1 H, d, $J = 12$ Hz, 19-H), 5·50 (1 H, m, $W = 12$ Hz, 6-H). For $C_{29}H_{48}O_2$ (428·7) calculated: 81·25% C, 11·29% H; found: 81·33% C, 11·08% H.

6α-Bromo-5β-cholestane-5,19-diol 19-Monoacetate (*XVIII*)

The olefin *XV* (20 g) was dissolved in dioxane (400 ml), a solution of 70% perchloric acid (10 ml) in water (50 ml) was added and the mixture was treated with N-bromoacetamide (9 g) at room temperature for 1 h. The mixture was treated with ether and water, the ethereal layer was washed with water, a 5% aqueous potassium hydrogen carbonate solution, 5% aqueous sodium thiosulfate solution, water, dried and evaporated to yield the oily bromohydrin *XVIII* (19 g), $[\alpha]_D^{20}$ +17° (*c* 2·3). ^{1}H-NMR spectrum: 0·60 (3 H, s, 18-H), 2·05 (3 H, s, CH_3CO_2), 4·28 (2 H, s, 19-H), 4·75 (1 H, m, $W = 25$ Hz, 6β-H). For $C_{29}H_{49}BrO_3$ (525·6) calculated: 66·27% C, 9·40% H, 15·20% Br; found: 65·93% C, 9·27% H, 15·43% Br.

5β-Cholestane-5,19-diol 19-Monobenzoate (*XIX*)

The bromohydrin *XVIII* (18 g) was dissolved in ethanol (300 ml), Raney-nickel (*c.* 30 g) was added and the mixture was stirred at 80°C for 12 h. The inorganic material was filtered off, the filtrate evaporated, the residue dissolved in ether and the ethereal solution was worked up as usual. The residue was chromatographed on a column of silica gel (600 g) using a mixture of light petroleum and ether (93 : 7 and 90 : 10) which eluted impurities; a mixture of the same solvents (85 : 5) eluted the product. Crystallization from aqueous acetone yielded the monoacetate *XIX* (11 g), m.p. 69—71°C, $[\alpha]_D^{20}$ +25° (*c* 1·8). ^{1}H-NMR spectrum: 0·65 (3 H, s, 18-H), 2·05 (3 H, s, CH_3CO_2), 4·35 (2 H, brd s, 19-H). For $C_{29}H_{50}O_3$ (446·7) calculated: 77·97% C, 11·28% H; found: 77·92%, 11·37% H.

4-Cholesten-19-ol 19-Acetate (*XXI*)

The alcohol *XIX* (8 g) was dissolved in pyridine (50 ml) and treated with thionyl chloride (5 ml) at 0°C for 15 min. The mixture was decomposed with ice and water, the product was extracted

with ether and the ethereal layer was worked up as usual. The residue was crystallized from a mixture of acetone, methanol and water to yield the olefin *XXI* (6·5 g), m.p. 58—60°C, $[\alpha]_D^{20}$ +80° (*c* 5·8). ^{1}H-NMR spectrum: 0·65 (3 H, s, 18-H), 2·01 (3 H, s, CH_3CO_2), 4·07 (1 H, d, J = 11 Hz, 19-H), 4·45 (1 H, d, J = 11 Hz, 19-H), 5·50 (1 H, m, W = 15 Hz, 4-H). For $C_{29}H_{48}O_2$ (428·7) calculated: 81·25% C, 11·29% H; found: 81·36% C, 11·22% H.

4-Cholesten-19-ol (*XXII*)

The acetate *XXI* (5 g) was treated with lithium aluminum hydride (500 mg) in ether (100 ml) at room temperature overnight. The mixture was decomposed with water, diluted with ether and 5% aqueous hydrochloric acid and the ethereal layer was worked up as usual. The residue was crystallized from aqueous acetone to yield the alcohol *XXII* (3·7 g), m.p. 85—86°C, $[\alpha]_D^{20}$ +55° (*c* 1·9). For $C_{27}H_{46}O$ (402·7) calculated: 80·54% C, 11·51% H; found: 80·71% C, 11·49% H.

19-Methoxy-4-cholestene (*XXIII*)

A solution of the alcohol *XXII* (4 g) in 1,2-dimethoxyethane (30 ml) was treated with sodium hydride (400 mg) and methyl iodide (4 ml). The mixture was stirred at 60°C for 2 h, then decomposed with water and 5% aqueous hydrochloric acid, diluted with ether and the ethereal layer was worked up as usual, to afford *XXIII* (3·9 g), m.p. 47—49°C, $[\alpha]_D^{20}$ +93° (*c* 1·9). ^{1}H-NMR spectrum: 0·67 (3 H, s, 18-H), 3·31 (3 H, s, CH_3O), 3·36 (1 H, d, J = 9 Hz, 19-H), 3·65 (1 H, d, J = 9 Hz, 19-H), 5·50 (1 H, m, W = 13 Hz, 4-H). For $C_{28}H_{48}O$ (400·7) calculated: 83·93% C, 12·07% H; found: 83·77% C, 11·95% H.

19-Methoxy-5β-cholestan-4-one (*XXV*)

The olefin *XXIII* (4 g) was dissolved in ether (120 ml), boron trifluoride etherate (9 ml) was added at —10°C while stirring and the mixture was treated with a solution of lithium aluminum hydride (2 g) in ether (120 ml) at —15°C while stirring for 1 h. The mixture was decomposed with a saturated aqueous solution of sodium sulfate, the inorganic material was filtered off, washed with ether and the filtrate was evaporated at 20°C. The residue was dissolved in tetrahydrofuran (40 ml) and dioxane (60 ml) and then treated with aqueous 30% hydrogen peroxide (30 ml) and a solution of potassium hydroxide (10 g) in water (100 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 to yield the crude mixture of isomeric alcohols *XXIV* (*c.* 4 g). The crude product was dissolved in acetone (60 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 solution was washed with water, a 5% aqueous hydrogen carbonate solution, water, dried and evaporated to afford the crude mixture of epimeric ketones *XXV* and *XXVI* in about 2 : 1 ratio as indicated by TLC. The mixture was dissolved in dioxane (20 ml) and methanol (100 ml) and refluxed with potassium hydroxide (2 g) for 4 h. The mixture was concentrated *in vacuo*, treated with ether and water and the ethereal solution was worked up as usual. The residue was chromatographed on a column, of silica gel (140 g) using a mixture of light petroleum and benzene (40 : 20) which eluted impurities. A mixture of light petroleum and ether (95 : 5) eluted the ketone *XXV* (1·04 g), m.p. 61—63°C, $[\alpha]_D^{20}$ +45° (*c* 1·9). ^{1}H-NMR spectrum: 0·63 (3 H, s, 18-H), 3·21 (1 H, d, J = 9 Hz, 19-H), 3·65 (1 H, d, J = 9 Hz, 19-H), 3·35 (3 H, s, CH_3O). For $C_{28}H_{48}O_2$ (416·7) calculated: 80·71% C, 11·61% H; found: 80·57% C, 11·54% H.

19-Methoxy-5α-cholestan-4-one (*XXVI*)

Continued elution using the same mixture of solvents gave polar fractions. These fractions were collected and evaporated to yield the ketone *XXVI* (2·57 g) which on crystallization from a mixture of acetone, methanol and water afforded the *XXVI* (1·8 g), m.p. 92—93°C, $[\alpha]_D^{20}$ +50° (*c* 1·9). ^{1}H-NMR spectrum: 0·65 (3 H, s, 18-H), 3·17 (3 H, s, CH_3O), 3·36 (2 H, brd s, 19-H). For $C_{28}H_{48}O_2$ (416·7) calculated: 80·71% C, 11·61% H; found: 80·53% C, 11·59% H.

19-Methoxy-5α-cholestan-4β-ol (*XXVII*)

The ketone *XXVI* (1·1 g) was dissolved in ether (40 ml) and treated with lithium aluminum hydride (200 mg) at room temperature for 2 h. 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 a mixture of acetone, methanol and water to yield the alcohol *XXVII* (1·0 g), m.p. 120—121°C, $[\alpha]_D^{20}$ +31° (*c* 1·8). ^{1}H-NMR spectrum: 0·63 (3 H, s, 18-H), 3·35 (3 H, s, CH_3O), 3·36 (1 H, d, J = 10 Hz, 19-H), 3·80 (1 H, d, J = 10 Hz, 19-H) 3·56 (1 H, m, W = 13 Hz, 4α-H). For $C_{28}H_{50}O_2$ (418·7) calculated: 80·32% C, 12·04% H; found: 80·18%C, 11·93% H.

19-Methoxy-5α-cholestan-4β-ol 4-Benzoate (*XXVIII*)

The alcohol *XXVII* (1 g) was dissolved in pyridine (10 ml) and treated with benzoyl chloride (1·5 ml) at room temperature overnight. The mixture was decomposed with ice, the product was taken up in ether and the ethereal solution was worked up as usual. The residue was crystallized from a mixture of acetone, methanol and water to afford the benzoate *XXVIII* (790 mg), m.p. 85—87°C, $[\alpha]_D^{20}$ +29° (*c* 2·2). ^{1}H-NMR spectrum: 0·66 (3 H, s, 18-H). 3·35 (3 H, s, CH_3O), 3·75 (1 H, d, J = 10 Hz, 19-H), 4·10 (1 H, d, J = 10 Hz, 19-H), 5·22 (1 H, m, W = 13 Hz, 4α-H). For $C_{35}H_{54}O_3$ (522·8) calculated: 80·41% C, 10·41% H; found: 80·37% C, 10·32% H.

19-Methoxy-5α-cholest-3-ene (*XXX*)

The benzoate *XXVIII* (350 mg) was pyrolyzed at 345°C for 10 min *in vacuo*. The residues of ten runs were collected by dissolving in ether, the solution was washed with water, 5% aqueous potassium hydrogen carbonate, water and evaporated. The residue was chromatographed on a column of silica gel (200 g) using light petroleum as eluent. The lipophilic fractions were collected and evaporated to yield the oily olefin *XXX* (1·93 g) which on crystallization from a mixture of chloroform and methanol gave *XXX* (1·67 g), m.p. 87—88°C, $[\alpha]_D^{20}$ —66° (*c* 2·1). ^{1}H-NMR spectrum: 0·69 (3 H, s, 18-H), 3·29 (3 H, s, CH_3O), 3·35 (1 H, d, J = 9 Hz, 19-H), 3·53 (1 H, d, J = 9 Hz, 19-H), 5·25 (1 H, m, W = 20 Hz) and 5·65 (1 H, m, W = 30 Hz) 3-H and 4-H. For $C_{28}H_{48}O$ (400·7) calculated: 83·93% C, 12·07% H; found: 83·68% C, 11·99% H. Collection and evaporation of polar fractions afforded the isomeric olefin *XXIII* (0·80 g), m.p. 46—48°C.

4β,19-Epoxy-5α-cholestane (*XXXII*)

The bromo epoxide *IX* (25 mg) in ethanol (3 ml) was refluxed while stirring with Raney-nickel (100 mg) for 8 h. The inorganic material was removed by filtration, the filtrate was evaporated, dissolved in ether, and the ethereal solution was worked up as usual. The residue was chromatographed on a preparative plate of silica gel (5 × 20 cm) using a mixture of light petroleum, ether and acetone (90 : 5 : 5) as eluent. Corresponding zones were eluted with ether and the filtrate

was evaporated. The residue was crystallized from a mixture of acetone, methanol and water to afford the epoxide *XXXII* (12 mg), m.p. 117—119°C, identical with an authentic sample[18].

5α-Cholest-3-en-19-ol (*XXXIII*)

The bromo epoxide *IX* (500 mg) was dissolved in a hot mixture of dioxane (5 ml) and acetic acid (15 ml) and treated with powdered zinc (2 g) at 100°C for 5 min. The inorganic material was filtered off, the filtrate diluted with water, extracted with ether, the ethereal layer was washed with water, 5% aqueous potassium hydrogen carbonate, water, dried and evaporated. The residue was disolved in a mixture of light petroleum and benzene (3 : 1) and filtered through a column of aluminum oxide (10 g). The filtrate was evaporated and the residue crystallized at —78° from a mixture of acetone and methanol to yield the alcohol *XXXIII* (360 mg), m.p. 95—96°C, $[\alpha]_D^{20}$ +44° (*c* 3·6). ^{1}H-NMR spectrum: 0·70 (3 H, s, 18-H), 3·72 (1 H, d, J = 10 Hz, 19-H), 3·91 (1 H, d, J = 10 Hz, 19-H), 5·30 (1 H, m, W = 20 Hz) and 5·65 (1 H, m, W = 30 Hz), 3-H and 4-H. For $C_{27}H_{46}O$ (386·7) calculated: 83·87% C, 11·99% H; found: 83·63% C, 12·06% H.

5α-Cholest-3-en-19-ol 19-Acetate (*XXXIV*)

The alcohol *XXXIII* (250 mg) was dissolved in pyridine (5 ml) acetic anhydride (1 ml) was added and the solution was refluxed for 10 min. The mixture was decomposed with ice and water, the product was extracted with ether and the ethereal solution was worked up as usual to afford the oily acetate *XXXIV* (238 mg), $[\alpha]_D^{20}$ +62° (*c* 2·6). ^{1}H-NMR spectrum: 0·63 (3 H, s. 18-H), 2·01 (3 H, s, CH_3CO_2), 4·03 (1 H, d, J = 12 Hz, 19-H), 4·38 (1 H, d, J = 12 Hz, 19-H), 5·30 (1 H, m, W = 18 Hz) and 5·60 (1 H, m, W = 28 Hz), 3-H and 4-H; For $C_{29}H_{48}O_2$ (428·7) calculated: 81·25% C, 11·29% H; found: 81·19% C, 11·35% H.

5,6α-Epoxy-19-methoxy-5α-cholestane (*XXXV*)

The nitrate *XL* (400 mg) was dissolved in a mixture of dioxane (6 ml) and methanol (10 ml) and refluxed with a 5% aqueous solution of potassium hydrogen carbonate (2 ml) for 5 min. The mixture was treated with ether and water, the ethereal layer was washed with water, dried and evaporated. The residue was crystallized from aqueous acetone to yield the epoxide *XXX* (290 mg), m.p. 90—91°C, $[\alpha]_D^{20}$ —13° (*c* 1·9). ^{1}H-NMR spectrum: 0·63 (3 H, s, 18-H), 2·95 (1 H, d, J = 3 Hz, 6β-H), 3·29 (3 H, s, CH_3O), 3·45 (1 H, d, J = 10 Hz, 19-H), 3·64 (1 H, d, J = 10 Hz, 19-H). For $C_{28}H_{48}O_2$ (416·7) calculated: 80·71% C, 11·61% H; found: 80·56% C, 11·69% H.

5,6α-Epoxy-5α-cholestan-19-ol 19-Acetate (*XXXVI*)

The olefin *XVI* (400 mg) was dissolved in chloroform (10 ml) and treated with *m*-chloroperoxybenzoic acid (200 mg) in the presence of potassium acetate (100 mg) at room temperature for 3 h. The mixture was diluted with ether and water, the ethereal layer was washed with water, 5% aqueous potassium hydrogen carbonate, water, then dried and evaporated, The residue was chromatographed on a column of silica gel (40 g) using a mixture of light petroleum and ether (95 : 5) as eluent to yield the oily 5α,6α-epoxide (*XXXVI*) (203 mg), $[\alpha]_D^{20}$ —77° (*c* 1·9). ^{1}H-NMR spectrum: 0·60 (3 H, s, 18-H), 2·03 (3 H, s. CH_3CO_2), 2·93 (1 H, d, J = 3 Hz, 6β-H), 4·22 (1 H, d, J = 13 Hz, 19-H), 4·46 (1 H, d, J = 13 Hz, 19-H). For $C_{29}H_{48}O_3$ (444·7) calculated: 70·33% C, 10·88% H; found: 70·25% C, 10·97% H.

5,β-Epoxy-5β-cholestan-19-ol 19-Acetate (*XXXVIII*)

Prolonged elution with a mixture of light petroleum, ether and acetone (93 : 5 : 2) after isolation of the 5α,6α-epoxide *XXXVI* gave a more polar fraction of the oily 5β,6β-epoxide *XXXVIII* (171 mg), $[\alpha]_D^{20}$ —22° (*c* 1·7). ^{1}H-NMR spectrum: 0·64 (3 H, s, 18-H), 2·05 (3 H, s, CH_3CO_2), 2·88 (1 H, d, J = 2 Hz, 6α-H), 4·00 (1 H, d, J = 12 Hz, 19-H), 4·49 (1 H, d, J = 12 Hz, 19-H). For $C_{29}H_{48}O_3$ (444·7) calculated: 70·33% C, 10·88% H; found: 70·19% C, 10·89% H.

19-Methoxy-5α-cholestan-5,6β-diol (*XXXIX*)

The olefin *XV* (500 mg) was dissolved in chloroform (10 ml) and treated with *m*-chloroperoxybenzoic acid (300 mg) at room temperature for 2 h. The mixture was diluted with ether, water, the ethereal solution was washed with water, an aqueous 5% potassium hydrogen carbonate solution, water, dried and evaporated to yield the nonseparable mixture of 5α,6α- and 5β,6β--epoxides *XXXV* and *XXXVII*. This mixture was dissolved in a mixture of dioxane (7 ml) and water (0·5 ml) and treated with 10% aqueous perchloric acid (1·2 ml) at room temperature for 2 h. The mixture was diluted with ether and water, the ethereal layer was washed with water, a 5% aqueous potassium hydrogen carbonate solution, water, dried and evaporated. The residue was chromatographed on a column of silica gel (20 g) with a mixture of light petroleum, ether and acetone (90 : 9 : 1) which eluted impurities and then with a mixture of the same solvents in a ratio of 88 : 10 : 2 which eluted the diol *XXXIX* (410 mg), m.p. 130—131°C, $[\alpha]_D^{20}$ +6° (*c* 1·9). ^{1}H-NMR spectrum: 0·66 (3 H, s, 18-H), 3·42 (1 H, d, J = 10 Hz, 19-H), 3·93 (1 H, d, J = 10 Hz, 19-H), ~3·40 (1 H, m, 6α-H, overlapped by 19-H signal). IR spectrum: 2820, 3416, 3610, 3634 cm^{-1}. For $C_{28}H_{50}O_3$ (434·7) calculated: 77·36% C, 11·59% H; found: 77·29% C, 11·63% H.

19-Methoxy-5α-cholestane-5,6β-diol 6-Nitrate (*XL*)

A solution of the diol *XXXIX* (400 mg) in chloroform (12 ml) was introduced at —40°C into a reagent prepared from acetic anhydride (3·5 ml) and 65% nitric acid (0·8 ml) at —40°C. The mixture was stirred at —40 to —20°C for 8 h, poured onto ice and neutralized with a 5% aqueous potassium hydrogen carbonate solution and solid sodium carbonate. The product was taken up in ether, the etheral solution was washed with water, a 5% aqueous potasium hydrogen carbonate solution, water, dried and evaporated to give the oily nitrate *XL* (410 mg) $[\alpha]_D^{20}$ —20° (*c* 1·8). IR spectrum: 1280, 1638, 2810, 3620 cm^{-1}. For $C_{28}H_{49}NO_5$ (479·7) calculated: 70·11% C, 10·30% H; found: 70·08% C, 10·32% H.

4α,5-Epoxy-19-methoxy-5α-cholestane (*XLI*)

a) *From* 19-*methoxycholest*-4-*ene* (XXIII): The olefin *XXIII* (500 mg) was dissolved in chloroform (7 ml) and treated with *m*-chloroperoxybenzoic acid (300 mg) in the presence of potassium acetate (100 mg) at room temperature for 1 h. The mixture was worked up as given for *XXXVI* to yield a mixture of the epoxides *XLI* and *XLIII*. This mixture was chromatographed on a column of silica gel (25 g) using a mixture of light petroleum and ether (95 : 5) as eluent. The lipophilic fractions were collected and evaporated to yield the 4α,5α-epoxide *XLI* (101 mg) which on crystallization from a mixture of acetone, methanol and water gave the pure *XLI* (73 mg), m.p. 78—79°C, $[\alpha]_D^{20}$ +84° (*c* 1·7). ^{1}H-NMR spectrum: 0·67 (3 H, s, 18-H), 2·92 (1 H, m, W = 10 Hz, 4β-H), 3·29 (3 H, s, CH_3O) 3·53 (2 H, brd s, 19-H). For $C_{28}H_{48}O_2$ (416·7) calculated: 80·71% C, 11·61% H; found: 80·65% C, 11·77% H.

b) *From* 19-*methoxy*-5α-*cholestane*-4β,5-*diol* (XLVI): The nitrate *XLVI* (350 mg) was dissolved in a mixture of dioxane (6 ml) and methanol (10 ml) and refluxed with a 5% aqueous potassium hydrogen carbonate solution (2 ml) for 5 min. The mixture was diluted with ether and water, the ethereal layer was washed with water, dried and evaporated. The residue was crystallized from a mixture of acetone, methanol and water to yield the epoxide *XLI* (245 mg), m.p. 78—79°C.

4α,5-Epoxy-5α-cholestan-19-ol 19-acetate (*XLII*)

The olefin *XXI* (800 mg) in chloroform (10 ml) was treated with *m*-chloroperoxybenzoic acid (500 mg) in the presence of potassium acetate (100 mg) at 0°C for 3 h. The mixture was worked up as given for *XXXVI* and the residue was chromatographed on a column of silica gel (50 g) using a mixture of light petroleum and ether (95 : 5) as eluent. The corresponding lipophilic fractions were collected and evaporated to yield the oily 4α,5α-epoxide *XLII* (400 mg), $[\alpha]_D^{20}$ +78° (*c* 1·9). ^{1}H-NMR spectrum: 0·67 (3 H, s, 18-H), 2·01 (3 H, s, CH_3CO_2), 2·92 (1 H, m, W = 11 Hz, 4β-H), 4·30 (2 H, brd s, 19-H). For $C_{29}H_{48}O_3$ (444·7) calculated: 78·33% C, 10·88% H; found: 78·14% C, 10·97% H.

4β,5-Epoxy-19-methoxy-5β-cholestane (*XLIII*)

Prolonged elution with the same mixture of solvents after isolation of the 4α,5α-epoxide *XLI* gave the polar fractions. These fractions were collected and evaporated to afford the oily 4β,5β-epoxide *XLIII* (389 mg) which on crystallization from a mixture of acetone, methanol and water gave the pure *XLIII* (310 mg), m.p. 61—63°C, $[\alpha]_D^{20}$ +24° (*c* 2·2). ^{1}H-NMR spectrum: 0·68 (3 H, s, 18-H), 2·78 (1 H, m, W = 9 Hz, 4α-H), 3·28 (3 H, s, CH_3O), 3·22 (1 H, d, J = 10 Hz, 19-H), 3·56 (1 H, d, J = 10 Hz, 19-H). For $C_{28}H_{48}O_2$ (416·7) calculated: 80·71% C, 11·61% H; found: 80·67% C, 11·73% H.

4β,5-Epoxy-5β-cholestan-19-ol 19-Acetate (*XLIV*)

Prolonged elution with the same mixture of solvents after isolation of the 4α,5α-epoxide *XLII* gave the polar fraction. Evaporation yielded the oily epoxide *XLIV* (320 mg), $[\alpha]_D^{20}$ +22° (*c* 4·1). ^{1}H-NMR spectrum: 0·65 (3 H, s, 19-H), 2·05 (3 H, s, CH_3CO_2), 2·79 (1 H, m, W = 12 Hz, 4α-H), 4·10 (1 H, d, J = 11 Hz, 19-H), 4·36 (1 H, d, J = 11 Hz, 19-H). For $C_{29}H_{48}O_3$ (444·7) calculated: 78·33% C, 10·68% H; found: 78·25% C, 10·93% H.

19-Methoxy-5α-cholestan-4β,5-diol (*XLV*)

The epoxide *XLIII* (1 g) was dissolved in a mixture of dioxane (12 ml) and water (0·5 ml) and treated with 10% aqueous perchloric acid (1·2 ml) at room temperature for 2 h. The solution was diluted with ether and water, the ethereal layer was washed with water, a 5% aqueous potassium hydrogen carbonate solution, water, dried and evaporated. The residue was chromatographed on a column of silica gel using a mixture of light petroleum and ether (90 : 10), which eluted the lipophilic impurities, and then applying a mixture of light petroleum, ether and acetone (88 : 10 : 2) to give the polar fraction. Evaporation afforded the diol *XLV* (720 mg) which on crystallization from a mixture of ethyl acetate and n-heptane gave the pure *XLV* (490 mg), m.p. 141—143°C, $[\alpha]_D^{20}$ +21° (*c* 2·0). ^{1}H-NMR spectrum: 0·63 (3 H, s, 18-H), 3·34 (3 H, s, CH_3O), 3·40 (1 H, m, 4α-H), 3·52 (1 H, d, J = 10 Hz, 19-H), 3·96 (1 H, d, J = 10 Hz, 19-H). For $C_{28}H_{50}O_3$ (434·7) calculated: 77·36% C, 11·59% H; found: 77·25% C, 11·68% H.

19-Methoxy-5α-cholestane-4β,5-diol 4-Nitrate (*XLVI*)

A solution of the diol *XLV* (400 mg) in chloroform (10 ml) was added at —40°C to a reagent prepared from acetic anhydride (3·5 ml) and 65% nitric acid (0·8 ml) at —40°C. The mixture was stirred at —40 to —20°C for 8 h, poured onto ice and neutralized with a 5% aqueous potassium hydrogen carbonate solution and solid sodium carbonate. The product was taken up in ether, the ethereal solution was washed with water, a 5% aqueous potassium hydrogen carbonate solution, water, dried and evaporated. The residue was crystallized from a mixture of acetone, methanol and water to give the nitrate *XLVI* (330 mg), m.p. 105—106°C dec., $[\alpha]_D^{20}$ +35° (*c* 1·4). IR spectrum: 1280, 1638, 2810, 3620 cm^{-1}. For $C_{28}H_{49}NO_5$ (479·7) calculated: 70·11% C, 10·30% H; found: 70·01% C, 10·37% H.

3α,4α-Epoxy-19-methoxy-5α-cholestane (*XLVII*)

The olefin *XXX* (250 mg) was dissolved in chloroform (5 ml) and stirred with *m*-chloroperoxybenzoic acid (180 mg) in the presence of potassium acetate (100 mg) at 0°C for 5 h. The mixture was diluted with ether, washed with water, a 5% aqueous potassium hydrogen carbonate solution, 10% aqueous potassium thiosulfate solution, water, dried and evaporated to yield the crude mixture of three compounds (TLC indication). The most lipophilic compound (*c* 5%), probably the 3β,4β-epoxide, and the 3α,4α-epoxide *XLVII* (*c* 80%), ^{1}H-NMR spectrum: 0·63 (3 H, s, 18-H), 2·71 (1 H, d, J = 4 Hz, 4β-H), 3·20 (1 H, m, W = 10 Hz, 3β-H), 3·28 (3 H, s, CH_3O), 3·41 (1 H, d, J = 9 Hz, 19-H), 3·61 (1 H, d, J = 9 Hz, 19-H). Third component is identical with the cyclic ether *IL* (*c* 10—20%). By preparative thin layer chromatography practically all the epoxide *XLVII* was converted to this ether, and all attempts at purification of the epoxide *XLVII* failed.

3α,4α-Epoxy-5α-cholestan-19-ol 19-Acetate (*XLVIII*)

The olefin *XXXIV* (400 mg) was dissolved in chloroform (10 ml) and stirred with *m*-chloroperoxybenzoic acid (320 mg) in the presence of potassium acetate (200 mg) at 0°C for 5 h. The mixture was worked up as given in the previous experiment to yield a practically pure product, which on crystallization from a mixture of acetone, methanol and water (with a drop of pyridine) gave *XLVIII* (278 mg), m.p. 94—97°C. $[\alpha]_D^{20}$ +12° (*c* 2·5). ^{1}H-NMR spectrum: 0·62 (3 H, s, 18-H), 2·03 (3 H, s, CH_3CO_2), 2·73 (1 H, d, J = 5 Hz, 4β-H), 3·19 (1 H, m, W = 11 Hz, 3β-H), 3·96 (1 H, d, J = 12 Hz, 19-H), 4·34 (1 H, d, J = 12 Hz, 19-H). For $C_{29}H_{48}O_3$ (444·7) calculated: 78·33% C, 10·88% H; found: 78·25% C, 11·03% H.

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REFERENCES

1. Kočovský P., Černý V., Synáčková M.: This Journal *44*, 1483 (1979).
2. Kočovský P., Černý V.: This Journal *44*, 1496 (1979).
3. Kočovský P., Kohout L., Černý V.: This Journal *45*, 559 (1980).
4. Fürst A., Scotoni R. jr: Helv. Chim. Acta *36*, 1332 (1953).
5. Fajkoš J., Joska J.: This Journal *43*, 1142 (1978).

6. Fujimoto Y., Tatsumoto T.: Tetrahedron Lett. *1976*, 3325.
7. Kočovský P., Černý V.: This Journal *44*, 246 (1979).
8. Kočovský P., Černý V.: This Journal *45*, 3023 (1980).
9. Kočovský P., Černý V.: This Journal *45*, 3030 (1980).
10. Kočovský P., Černý V.: This Journal *45*, 3190 (1980).
11. Kočovský P., Černý V.: This Journal *45*, 3199 (1980).
12. Kočovský P.: This Journal *44*, 2156 (1979).
13. Kočovský P.: Tetrahedron Lett. *21*, 555 (1980).
14. Kočovský P.: This Journal *45*, 2998 (1980).
15. Bull J. R., Jones E. R. H., Meakins G. D.: J. Chem. Soc. *1965*, 2601.
16. Barton D. H. R., Rosenfelder W. J.: J. Chem. Soc. London *1951*, 1048.
17. Valenta Z.: Private communication.
18. Tureček F., Kočovský P.: This Journal *45*, 274 (1980).
19. Kočovský P., Černý V., Tureček F.: This Journal *44*, 234 (1979).
20. Kasal A.: This Journal *41*, 140 (1976).

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