Synthetic Modifications of Withanolides with an α-Orientated Side-Chain

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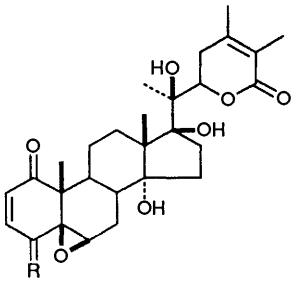
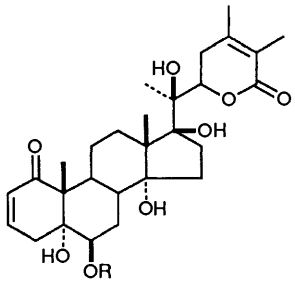
In view of the antitumour activity of withanolide E [(20*S*,22*R*)-5β,6β-epoxy-14α,17β,20-trihydroxy-1-oxowitha-2,24-dienolide] and 4β-hydroxywithanolide E, synthetically modified analogues were prepared. In these compounds, the α-orientation of the side-chain was retained. Its degree of bending with respect to the carbocyclic skeleton depends on the presence or absence of the 14α-OH group. Elimination of this group leads to the formation of the 14,20-oxido-bridged and Δ^{14}-compounds. Epoxidation of the latter afforded 14α,15α-epoxides. With the exception of several 5,6-chlorohydrins, most compounds possess an epoxide ring, allylic or homoallylic with respect to the ring A enone.

The withanolides are a group of highly oxygenated natural steroids built on an ergostane-type skeleton, many of them with an unsaturated δ-lactone in the side-chain. Several compounds of this group were found to have antitumour activity, promising results being obtained with withanolide E[1] **1a** and 4β-hydroxywithanolide E[2] **1b**. The tests were done by the National Cancer Institute (USA) on P388 leukaemia cells and on B16 melanoma tumour systems.

Screening of a large number of withanolides led to the conclusion that, within this group of compounds, two structural features are mandatory for antitumour activity: the epoxide in ring B and the α-orientation of the side-chain. Opening of the epoxide ring in withanolide E **1a** leads to the formation of the 5α,6β-diol **2a** (withanolide S[1]) which is devoid of such activity. The 17-epimer of withanolide E (isowithanolide E[3]), in which the side-chain has the regular β-orientation, is also inactive.



1a R = H_2
1b R = β-OH, α-H
1c R = β-OAc, α-H



2a R = H
2b R = mesyl

Based on these findings, we undertook an investigation of synthetic modifications of withanolide E, in the hope of obtaining compounds active enough to be useful in cancer chemotherapy. In the design of the modified compounds we were guided by the two features outlined above:

(a) The molecule should possess an epoxide in a homoallylic (5,6) or allylic (4,5) position with respect to the ring A enone.

(b) The side-chain should be α-orientated, its degree of bending with respect to the carbocyclic skeleton being manipulated by elimination of the 14-OH group: formation of a 14,15-double bond, epoxidation of the latter, or formation of an additional ring by closure of a 14,20-oxide bridge.

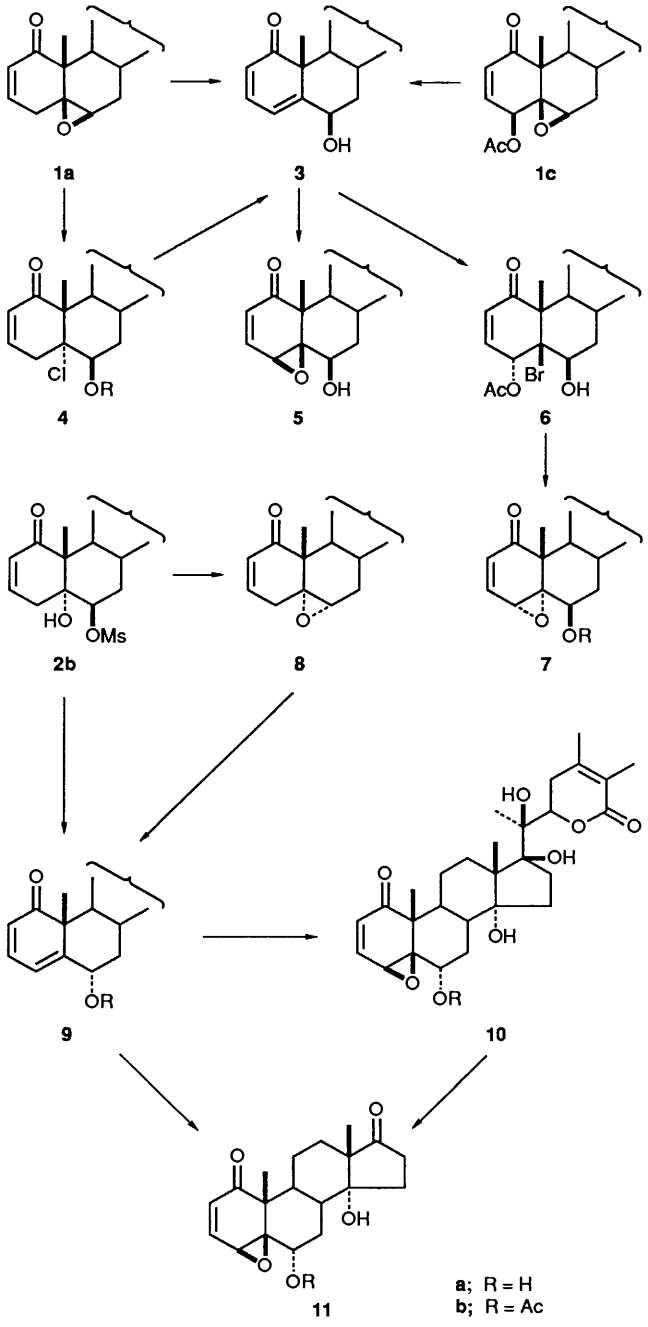
None of these compounds was sufficiently active to justify clinical investigation. The chemical aspects of this work are described in the present paper.

The first group of withanolide E analogues were modified only in rings AB. A key intermediate was the 6β-hydroxy-2,4-dien-1-one **3**, which was prepared initially[4] by treatment of 4β-acetoxywithanolide E **1c** with tetrakis(triphenylphosphine)-palladium. Although this reaction affords compound **3** in good yield, we preferred to follow an alternative route by using withanolide E **1a** as starting material. Whereas 4β-hydroxy-withanolide E **1b** was obtained by a rather tedious chromatographic fractionation of an extract of *Physalis peruviana*,[2a] withanolide E was obtained in significantly higher yield from *Withania somnifera* chemotype III.[5]

Withanolide E was quantitatively isomerised to the 6β-hydroxy dienone **3** by treatment with triethylamine in methanol solution. Alternatively, compound **3** was obtained in a two-step process by treatment of withanolide E with hydrochloric acid in tetrahydrofuran (THF) to give the corresponding diaxial chlorohydrin **4a**, which was dehydrochlorinated with aqueous sodium hydroxide in pyridine solution. Obviously, this two-step process has no advantage over the direct triethylamine isomerisation of withanolide E when the preparation of compound **3** is contemplated. The chlorohydrin **4a** is a useful intermediate in the preparation of the dienone moiety in ring D-modified compounds, such as the 4β,5β-epoxides **23** and **26** (see Scheme 3, below).

Direct epoxidation of compound **3** with *m*-chloroperbenzoic acid (MCPBA) afforded the 4β,5β-epoxy derivative **5**. The stereoisomeric 4α,5α-epoxy derivative **7a** was obtained, albeit in poor yield, by treatment of compound **3** with *N*-bromoacetamide (NBA) in the presence of lithium acetate,[6] leading to the 5β-bromo-4α-acetate **6**, which was transformed under basic conditions (aq. sodium hydroxide in pyridine solution) into the desired 4α,5α-epoxy derivative **7a**. The 5α,6α-epoxy stereoisomer of withanolide E was prepared by conversion of the epoxide ring of **1a** into the corresponding 5α,6β-diaxial diol **2a** (withanolide S[1]) by treatment with a trace of perchloric acid in acetone solution at −10 °C. The corresponding mesyl ester **2b** was transformed into the 5α,6α-epoxy-2-en-1-one **8** on being heated in the presence of silica gel. Alternatively, when the mesyl derivative was treated with a solution of sodium hydroxide in methanol instead of being adsorbed onto silica gel, the isomeric 6α-hydroxy-2,4-dien-1-one **9a** was obtained directly. The same compound was obtained when the epoxide **8** was treated with sodium hydroxide or with triethylamine in methanol. Direct epoxidation of the allylic alcohol **9a** with MCPBA gave only the 4β,5β-epoxy 6α-alcohol **10a**. The steric course of this epoxidation is the same as with cholesta-2,4-dien-1-one[7] and

§ Deceased 5th January, 1988.

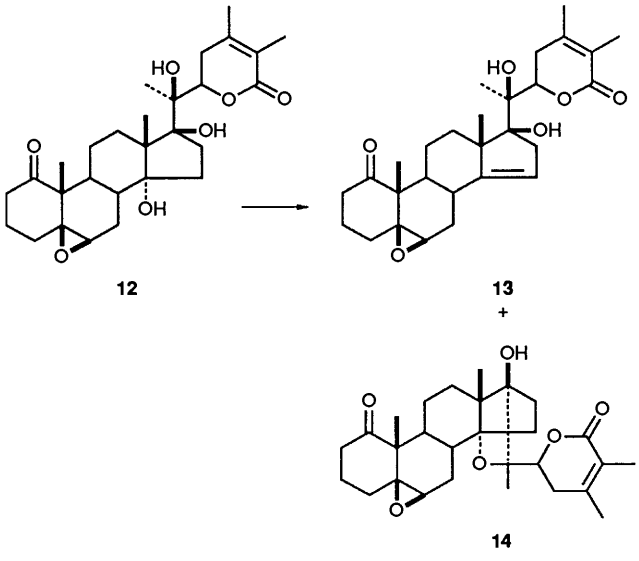


Scheme 1 Ms = mesyl

with 6β-hydroxycholesta-2,4-dien-1-one. Although 4α,5α-epoxy-6α-hydroxycholesta-2,4-dien-1-one was prepared[6] from the corresponding allylic alcohol (Δ^4-6α-ol) by the NBA/lithium acetate procedure, attempted application of this reaction to the withanolide-type allylic alcohol **9a** failed to yield the corresponding 4α,5α-epoxide.

Much as we could have been expected to obtain a 4α,5α-epoxy-6α-hydroxywithanolide by treatment of the allylic alcohol **9a** with Sharpless reagent [$Bu^tO_2H/VO(acac)_2$], the epoxide obtained under these conditions was β-orientated, the same as in the MCPBA epoxidation; hence, in both epoxidations, steric factors prevailed on the directional effect of the allylic 6α-hydroxy group. Additional to the epoxidation of the Δ^4 compound, Sharpless reagent induced cleavage of the side-chain[7] (*vide infra*), thus leading to 4β,5β-epoxy-6α,14α-dihydroxyandrost-2-ene-1,17-dione **11a**. Both compounds, the withanolide **10a** and the androstane **11a**, had similar positive Cotton effects at 330 and 332 nm, respectively, thus pointing to the same configuration of the 4,5-epoxide. The measurements were done on the corresponding acetates **10b** and **11b**. The final proof was achieved by cleavage of the side-chain in compound **10b**, thus transforming it into the androstane derivative **11b** (Scheme 1).

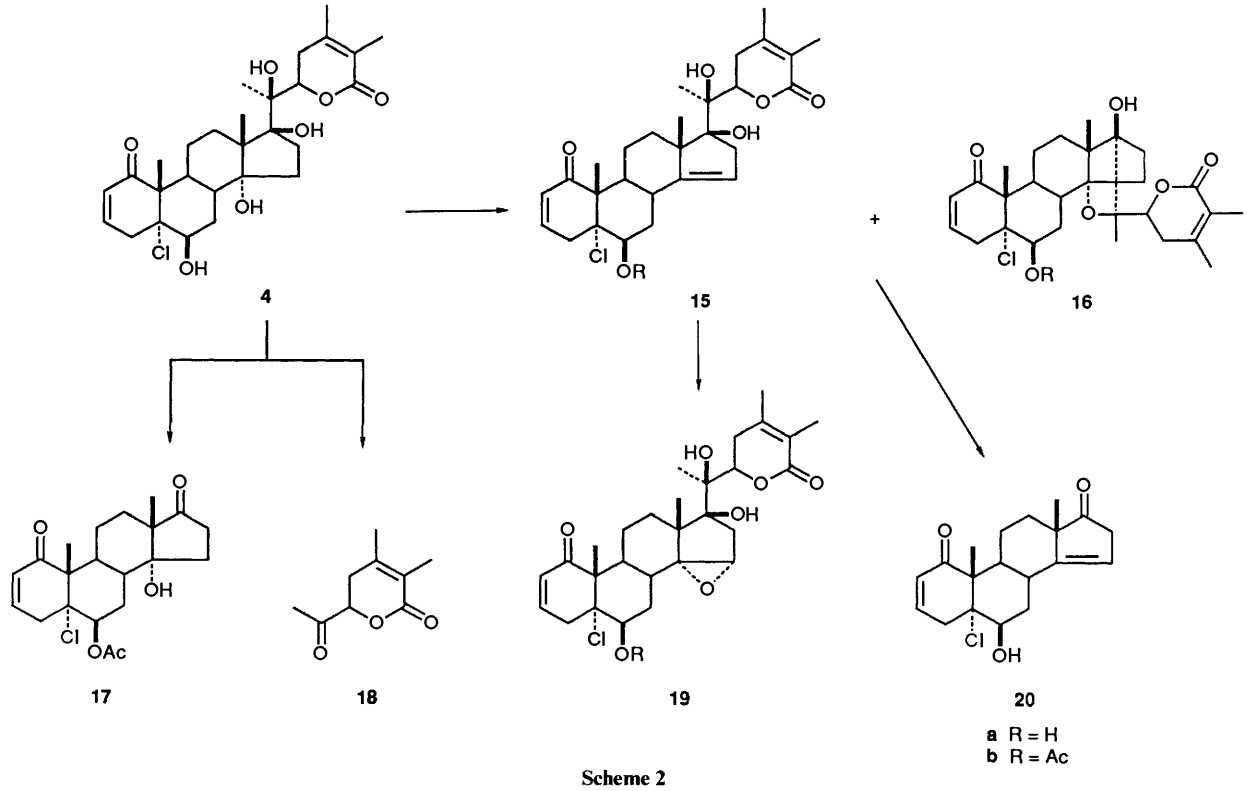
In a previous investigation[1] performed on 2,3-dihydrowithanolide E **12** we succeeded in inducing an acid-catalysed elimination of the 14α-hydroxy group, leaving untouched the 5,6-epoxide ring. The reaction resulted in one major elimination product (Δ^{14}), compound **13**, accompanied by trace amounts of another component, erroneously considered as the $\Delta^{8(14)}$-isomer of the former. Both compounds gave the molecular ion, M^+ 470. Subsequently, it was shown[8] that the latter, as well as other related compounds, in fact possesses the 14,20-oxide structure **14**.



Much as we were interested in compounds of type **13** and **14** in the withanolide E series, we could not reproduce this reaction in order to prepare $\Delta^{2,14}$- and Δ^2-14,20-oxide-compounds without opening of the 5,6-epoxide ring. At best, the only result was the hydrolytic opening of the epoxide ring, as in the preparation of withanolide S **2a**. In the presence of hydrochloric or hydrobromic acid the corresponding halogenohydrins were obtained (*e.g.*, the chlorohydrin **4a**). When this chlorohydrin **4a** was treated with base (aq. NaOH in methanol or in pyridine solution), elimination occurred, leading to the 6β-hydroxy-2,4-dien-1-one **3** in 80% yield. Eventually, when compound **4a** in chloroform–methanol solution was adsorbed on silica gel and heated for 10 h at 50 °C under reduced pressure, the epoxide ring was reclosed leading to the original withanolide E **1a** in *ca.* 50% yield.

The work was continued with the chlorohydrin **4a** under acidic conditions. Treatment of compound **4a** with sulphuric acid in acetone solution afforded a mixture of Δ^{14}-5,6-chlorohydrin **15a** and 14,20-oxidochlorohydrin **16a** which could easily be separated by chromatography. Milder conditions favoured a large proportion of Δ^{14}-chlorohydrin. These two compounds (**15a** and **16a**) were used for further modifications of the withanolide system.

A few years ago, we discovered that ditertiary glycols are smoothly cleaved[9] by vanadyl bis(acetoacetonate) $VO(acac)_2$. The reaction was performed with a catalytic amount of this reagent, in the presence of t-butyl hydroperoxide (Sharpless reagent), or in the absence of hydroperoxide, but with a stoichiometric amount of reagent. Under these conditions the 17–20 bond in withanolide E **1a** was smoothly cleaved at room temperature, whereas the same bond in 17-isowithanolide E was



Scheme 2

cleaved only after prolonged heating at 70 °C. The different behaviour of the ditertiary glycol systems in these two compounds was attributed to conformational factors.

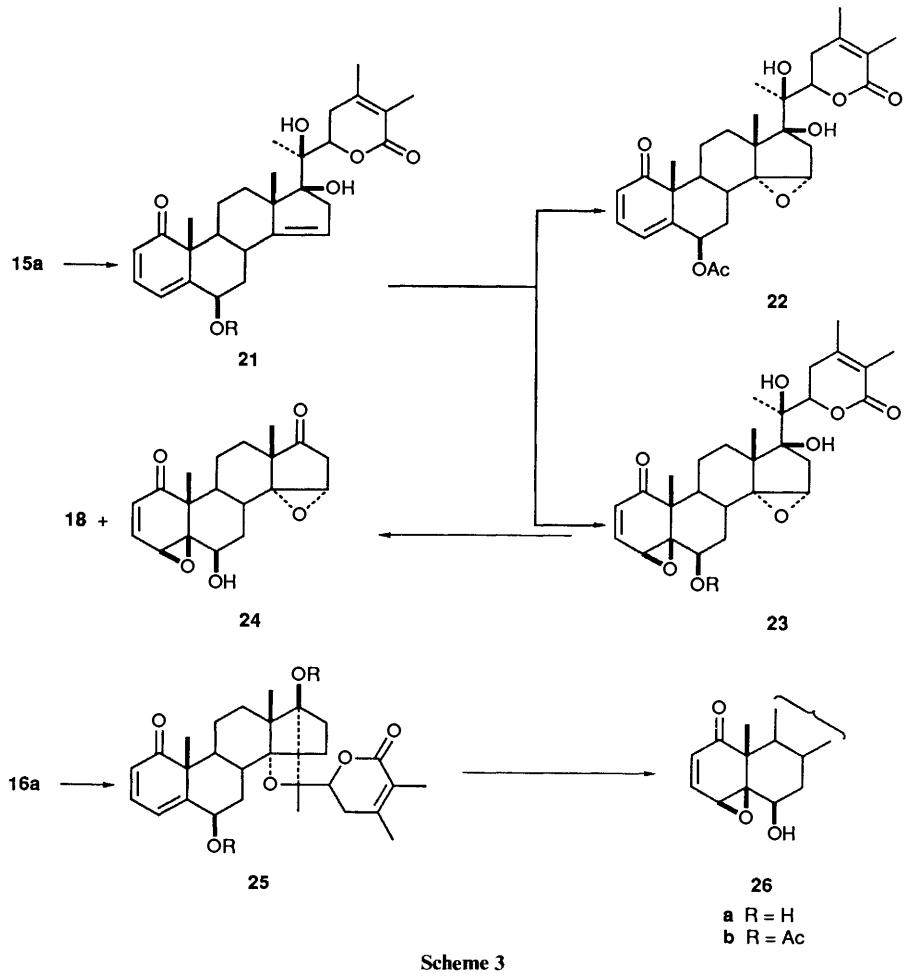
The chlorohydrin acetate **4b** behaves in the same manner as withanolide E either in the presence of Sharpless reagent or in the presence of $VO(acac)_2$ only; after cleavage of the 17–20 bond, the androstane derivative **17** and the keto lactone **18** were obtained. The latter was identified by comparison with an authentic sample.[10] The Δ^{14}-chlorohydrin **15a** behaved in a different manner when submitted to these reactions. In the presence of stoichiometric amount of $VO(acac)_2$, the 17–20 bond was completely cleaved after 2 h at room temperature, leading to the androstane derivative **20** (Scheme 2). However, when the reaction was performed with a catalytic amount of $VO(acac)_2$ in the presence of t-butyl hydroperoxide, only epoxidation of the 14,15-double bond took place, leading to the 14,15-epoxychlorohydrin **19a**. Apparently, in a substrate like **15a** which possesses an epoxidable double bond and a glycol function prone to cleavage, both sites can react with $Bu^tO_2H/VO(acac)_2$.

It can be assumed that, in the case of chlorohydrin **15a**, the rate of epoxidation of the double bond is considerably faster than the rate of glycol cleavage and, therefore, the 14,15-epoxide withanolide-type compound **19a** is obtained. Once this epoxide is formed, the cleavage of the 17,20-glycol becomes more difficult, presumably due to a conformational change involving the relative positions of the 17- and 20-hydroxy groups. The same 14,15-epoxide **19a** was obtained by treatment of compound **15a** with MCPBA.

Whereas the stereochemistry of the peracid epoxidation of the Δ^4-moiety in steroidal 2,4-dien-1-ones is well documented,[6,7] the stereochemical course of the peracid epoxidation of the Δ^{14}-isomer in steroids with an α-orientated side-chain has been less well investigated. In steroids with a β-orientated side-chain, peracid epoxidation of the Δ^{14}-compound affords a 14α,15α-epoxide.[5,11] The stereoisomeric 14β,15β-epoxide was obtained indirectly (treatment with NBA followed by base). To our knowledge, the only example of peracid epoxidation of Δ^{14}-species in a withanolide with a 17α-orientated side-chain (5β,6β-epoxy-17β,20-dihydroxy-1-oxowitha-14,24-dienolide) is in a previous work from our laboratory.[1] We assumed at the time that the 14,15-epoxide thus obtained was α-orientated, in view of the pattern of the 15-H NMR signal, which allows a differentiation between 15β-H (14α,15α-epoxide) and 15α-H (14β,15β-epoxide) to be made.[12] In the above compound there are two hydroxy groups able to influence the direction of epoxidation of the 14,15-double bond: the homoallylic 17β-OH from the top side, and the bis-homoallylic 20-OH which, in view of the α-orientation of the side-chain, can direct the epoxidising agent from the rear side of the molecule. In the present investigation the substrate was the Δ^{14}-chlorohydrin **15a.** Neither the 17- nor the 20-hydroxy group is close enough to the double bond to have an unequivocal directing effect on the action of a peracid. The situation is quite different in epoxidations with Sharpless reagent: a homoallylic, as well as a properly situated bis-homoallylic, hydroxy group can be responsible for the steric outcome of the epoxidation. Both reagents afforded the same 14,15-epoxide compound **19a**. The pattern of the 15-H signal in this compound suggests the α-orientation for the epoxide. The difference between the chemical shift of the 13-Me group in the Δ^{14}-compound **15a** and that in the 14,15-epoxide **19a** (a shift of 0.02 ppm towards higher field) is in agreement with such a configuration. This conclusion is supported by an investigation[12] in the cardenolide series: in comparison with the 13-Me signal in a Δ^{14}-cardenolide, introduction of a 14α,15α-epoxide induces an upfield shift (0.05 ppm), whereas introduction of a 14β,15β-epoxide shifts this signal downfield (0.11 ppm).

One may conclude that in Δ^{14}-derivatives of withanolide E, the directing influence of the bis-homoallylic hydroxy group (20-OH) is significantly stronger than that of the homoallylic 17β-OH group. This difference is attributed to the degree of bending of the side-chain to the rear of the carbocyclic system, thus bringing the 20-OH group into near proximity with the 14,15-double bond.

Dehydrochlorination of compound **15a** afforded 6β-hydroxy-



Scheme 3

1-oxowitha-2,4,14,24-tetraenolide **21a**, characterised by its NMR spectrum, as well as by that of the corresponding acetate **21b**. Treatment of the latter with MCPBA afforded a mixture of 14α,15α-monoepoxide **22** and 4β,5β:14α,15α-diepoxide **23b**. Treatment of the allylic alcohol **21a** with Sharpless reagent afforded the diepoxide **23a** whose acetylation product was identical with compound **23b**. The 17,20-glycol moiety in compound **23b** could be cleaved with Sharpless reagent only after prolonged heating (*ca.* 20 h at 70 °C) to yield a mixture of the keto lactone **18** and the androstane derivative **24** (Scheme 3).

The 14,20-oxido chlorohydrin **16a** was dehydrochlorinated under the same mild conditions as other chlorohydrins in this series, to give the corresponding dienone **25a**. Acetylation of the latter (acetic anhydride–pyridine) at 90 °C afforded the 6,17-diacetate **25b**, known from previous work.[8] Epoxidation of the dienone **25a** with either MCPBA or $Bu^tO_2H/VO(acac)_2$ afforded the corresponding 4β,5β-epoxide **26** (Scheme 3).

Experimental

M.p.s were taken with a Fisher-Johns apparatus and are uncorrected. Optical rotations were measured with an automatic Perkin-Elmer 141 polarimeter. ^1H NMR spectra were determined at 80 MHz on a Varian FT-80A instrument. Mass spectra were obtained by Mrs M. Chernyak by direct probe inlet in a Finnigan 4600 quadrupole instrument, in the electron-impact mode at 70 eV, unless otherwise stated. CD spectra were determined with a Cary 60 instrument. We are grateful to Professor A. Gedanken, Bar Ilan University, for these measurements. Column chromatography was done on silica gel 60, 70–230 mesh. TLC was carried out on plates of silica gel 60 F_{254}; preparative chromatoplates (1 mm thick) were prepared with silica gel PF_{254}.

(20S,22R)-6β,14α,17β,20-*Tetrahydroxy*-1-*oxowitha*-2,4,24-*trienolide* **3**.—(a) *From withanolide E* **1a**. To a solution of withanolide E **1a** in methanol (10 cm^3) was added triethylamine[13] (dried over NaOH pellets) (0.2 cm^3). The solution was heated to reflux until total disappearance of the starting material (TLC). The solvent was removed under reduced pressure and the residue was dissolved in chloroform, the solution was dried (Na_2SO_4), and the solvent was removed again. The product was pure (TLC, NMR) and was identical with compound **3** prepared according to ref. 4, m.p. 240–241 °C (from MeOH) (yield after crystallisation, 90%)

(b) *From chlorohydrin* **4a**. To a stirred solution of chlorohydrin **4a** (50 mg) in pyridine (2 cm^3) was added aq. 10% NaOH (0.2 cm^3). The reaction was complete after 1 h at room temperature. Glacial acetic acid was added (~2 cm^3), followed by ethyl acetate (50 cm^3). The solution was washed with cold, aq. NaCl until neutral and the crude product after evaporation of the solvent, was chromatographed. The fractions eluted with ethyl acetate and ethyl acetate + 5% methanol were combined to give title compound **3** (38 mg, 81%), homogeneous on TLC.

(20S,22R)-5α-*Chloro*-6β,14α,17β,20-*tetrahydroxy*-1-*oxowitha*-2,24-*dienolide* **4a**.—To a stirred solution of withanolide E **1a** (500 mg) in THF (25 cm^3), at 0 °C was added conc. hydrochloric acid (3 dm^3) dropwise. The reaction (TLC) was complete after 1 h at that temperature. After neutralisation (10% aq. $NaHCO_3$), the solvent was partly removed under reduced pressure, water was added, and the precipitate was dried (490

Table 1 ^{1}H NMR data[a]

Compd.	2-H	3-H	4-H	6-H	15-H	22-H	Me groups 19-H_3	18-H_3	21-H_3	Other (OAc)
3	5.97d (10)	6.88dd (10;6)	6.08d (6)	4.6m ($w_{\frac{1}{2}}$ 8)		4.81t (8)	1.48	1.17	1.40	
4a	5.87dd (10;2.5)	6.64ddd (10;5;2)		4.06m ($w_{\frac{1}{2}}$ 8)		4.89t (8)	1.26	1.13	1.42	
5	5.95dd (10;1)	6.99dd (10;4)	3.25m ($w_{\frac{1}{2}}$ 7.5)	3.62m ($w_{\frac{1}{2}}$ 6)		4.82t (8)	1.53	1.12	1.38	
6	6.00d (10)	6.67dd (10;5)	6.11d (5)	4.05m ($w_{\frac{1}{2}}$ 7.5)		4.81t (8.5)	1.62	1.08	1.42	2.19
7b	5.93dd (10;1)	6.88dd (10;4)	3.61dd (4;1)	4.57m ($w_{\frac{1}{2}}$ 6)		4.91t (6)	1.27	1.20	1.43	2.08
8	5.90dd (10;2)	6.69ddd (10;5;2)		3.05d (4)		4.87t (8)	1.31	1.04	1.36	
9a	5.99d (10)	6.99dd (10;6)	6.30d (6)	4.45br m		4.74t (8)	1.23	1.12	1.36	
9b	5.96d (10)	6.99dd (10;6)	6.15d (6)	5.5br m		4.81t (8)	1.29	1.12	1.37	2.11
10b	5.99dd (10;1)	7.05dd (10;4)	3.63dd (4;1)	5.36dd (12;5)		4.79t (8)	1.34	1.07	1.37	1.99
11a	6.04d (10)	7.08dd (10;4)	3.76d (4)	4.25dd (12;5)			1.31	0.98		
11b	6.03dd (10;1)	7.07dd (10;4)	3.66dd (4;1)	5.42dd (12;5)			1.39	1.01		2.03
15a	5.88dd (10;2.5)	6.64ddd (10;5;2)		4.1m ($w_{\frac{1}{2}}$ 8)	5.19m ($w_{\frac{1}{2}}$ 5)	4.67dd (10;6)	1.31	1.22	1.40	
16b	5.91dd (10.2.5)	6.60ddd (10;5;2)		5.21m ($w_{\frac{1}{2}}$ 5)		4.43dd (12.5;4)	1.28	1.04	1.23	2.10
17	5.94dd (10;2.5)	6.61ddd (10;5;2)		5.27t (2.5)			1.36	1.04		2.14
19a	5.91dd (10;2.5)	6.66ddd (10;5;2)		4.05m ($w_{\frac{1}{2}}$ 5)	3.62m ($w_{\frac{1}{2}}$ 4)	4.55dd (11.5;5)	1.28	1.20	1.42	
20	5.93dd (10;2.5)	6.67ddd (10;5;2)		4.15m ($w_{\frac{1}{2}}$ 8)	5.55m ($w_{\frac{1}{2}}$ 5)		1.42	1.14		
21a	5.97d (10)	6.90dd (10;6)	6.13d (6)	4.6 *b*	5.1m ($w_{\frac{1}{2}}$ 5)	4.6 *b*	1.51	1.23	1.26	
21b	5.99d (10)	6.91dd (10;6)	6.30d (6)	5.58t (2.5)	5.11m ($w_{\frac{1}{2}}$ 5)	4.64t (8)	1.41	1.23	1.26	2.06
22	6.00d (10)	6.91dd (10;6)	6.29d (6)	5.5m ($w_{\frac{1}{2}}$ 7.5)	3.46	4.53dd (11.5;5)	1.36	1.23	1.23	2.03
23a	5.96dd (10;1)	7.00dd (10;4)	3.22dd (4;1)	3.55m *b*	3.50 *b*	4.47dd (11.5;5)	1.21	1.15	1.44	
25a	5.99d (10)	6.89dd (10;6.5)	6.12d (6.5)	4.58m ($w_{\frac{1}{2}}$ 5)		4.5m *b*	1.41	1.09	1.23	
26	5.98dd (10;1)	6.98dd (10;4)	3.25dd (4;1)	3.6m ($w_{\frac{1}{2}}$ 6)		4.64dd (11.5;5)	1.45	1.04	1.26	

[a] Recorded at 80 MHz; solvent $CDCl_3$; δ-values; coupling constants (Hz) in parentheses. [b] Obscured.

mg) and purified by crystallisation, m.p. 155–157 °C (decomp.) (from MeOH–$CHCl_3$) (yield after crystallisation, 78%). Acetylation with acetic anhydride and pyridine overnight at room temperature afforded the 6-*acetate* **4b**, m.p. 230–232 °C (decomp.) (from MeOH–EtOAc), $[\alpha]_D$ +97° (*c* 0.5, pyridine) (Found: C, 63.5; H, 7.25. $C_{30}H_{41}ClO_8$ requires C, 63.8: H, 7.3%); *m/z* (30 eV) 529 (M^+ − Cl, 2%), 510 (M^+ − HCl − H_2O, 37), 450 (M^+ − HCl − H_2O − AcOH, 32) and 414 (M^+ − HCl − $3H_2O$ − AcOH, 100).

Reclosure of the 5β,6β-*Epoxide in Compound* **4a**.—To a solution of chlorohydrin **4a** (20 mg) in chloroform (5 cm^3) was added silica gel (3 g) and the mixture was heated under reduced pressure (Rotavapor) for 10 h. The mixture was introduced onto a short chromatographic column and the product was eluted with ethyl acetate. In the crude product (12 mg), the TLC spot of the chlorohydrin **4a** practically disappeared; the major spot was that of withanolide E **1a**. The NMR spectrum confirmed the TLC evidence.

(20S,22R)-4β,5β-*Epoxy*-6β,14α,17β,20-*tetrahydroxy*-1-*oxo-witha*-2,24-*dienolide* **5**.—To a stirred solution of dienol **3** (48 mg) in dry chloroform (20 cm^3) at 0 °C was added commercial MCPBA (~85%) (30 mg) and the mixture was stirred overnight at room temperature. The obtained solution was washed successively with 5% aq. ammonia (3 × 5 cm^3) and water (2 × 10 cm^3). Evaporation of the solvent from the dried (Na_2SO_4) solution afforded *compound* **5** (42 mg, 84%), homogeneous on TLC, m.p. 195–198 °C (from acetone–hexane) (Found: C, 67.1; H, 7.5. $C_{28}H_{38}O_8$ requires C, 66.9; H, 7.6%).

(20S,22R)-4α,5α-*Epoxy*-6β,14α,17β,20-*tetrahydroxy*-1-*oxo-witha*-2,24-*dienolide* **7a**.—(a) *Formation of the bromohydrin acetate*[6] **6**. To a stirred solution of compound **3** (48 mg) in glacial acetic acid (2 cm^3) were added NBA (18 mg) and lithium acetate (160 mg). After 1 h at room temperature, water was added and the precipitate was filtered off. The product was purified on a preparative chromatoplate; it was identified as 4α-acetoxy-5β-bromo-6β,14α,17β,20-tetrahydroxy-1-oxowitha-2,24-dienolide **6** by its NMR spectrum.

(b) *Transformation of compound* **6** *into the epoxide* **7a**. To a stirred solution of bromohydrin acetate **6** (30 mg) in pyridine

(2 cm^3) was added 10% aq. sodium hydroxide (0.1 cm^3); the mixture was stirred for 1 h at room temperature. Water was added, and the product was extracted with chloroform; the extract was washed successively with aq. $NaHCO_3$ and with saturated aq. sodium chloride until neutral. The crude product **7a** was purified on a preparative chromatoplate (overall yield ~20%). Acetylation with acetic anhydride and pyridine, overnight, afforded the acetate **7b**, m.p. 170–172 °C (from MeOH); $[\alpha]_D$ +103° (*c* 0.3, pyridine) (Found: C, 66.0; H, 7.5. $C_{30}H_{40}O_9$ requires C, 66.2; H, 7.4%); *m/z* (30 eV) 543 (M^+ − 1, 10%), 526 (M^+ − H_2O, 100) and 401 (M^+ − H_2O − 125, 80).

(20S,22R)-5α,6α-*Epoxy*-14α,17β,20-*trihydroxy*-1-*oxowitha*-2,24-*dienolide* **8**.—(a) *Formation of the mesyl derivative* **2b** *from withanolide S* **2a**. To a solution of withanolide S **2a** (1 g) in pyridine (7 cm^3) at 0 °C was slowly added freshly distilled mesyl chloride (1.5 cm^3). The mixture was kept overnight at the same temperature, then poured onto crushed ice; the precipitate was filtered off, washed with saturated NaCl solution, and used as such for the next step.

(b) *Transformation of mesyl derivative* **2b** *into the epoxide* **8**. The crude mesyl derivative was dissolved in chloroform and the turbid solution was dried (Na_2SO_4) and filtered. To this solution was added silica gel (20 g) and the mixture was heated under reduced pressure (Rotavapor) for 6 h at ~90 °C (bath temperature). The dry mixture was introduced onto a short chromatographic column and the product was eluted with ethyl acetate. The obtained *compound* **8** (750 mg), was homogeneous on TLC, m.p. 176–179 °C (from MeOH) (Found: C, 69.0; H, 7.8. $C_{28}H_{38}O_7$ requires C, 69.1; H, 7.9%).

(20S,22R)-6α,14α,17β,20-*Tetrahydroxy*-1-*oxowitha*-2,4-24-*trienolide* **9a**.—(a) *From epoxide* **8**. The reaction was performed in the presence of triethylamine, as described for the preparation of compound **3**. The obtained product **9** was homogeneous on TLC and was characterised by NMR spectroscopy; *m/z* 468 (M^+ − H_2O, 9%); 450 (M^+ − $2H_2O$, 75), 361 (M^+ − 125, 52) and 343 (M^+ − 125 − H_2O, 90).

(b) *From mesyl derivative* **2b**. To a solution of mesyl derivative **2b** (500 mg) in methanol (10 cm^3) was added a 5% solution of sodium hydroxide in methanol (10 cm^3) and the obtained solution was heated to reflux for 4 h. Water was then added, the solution was neutralised with 1 mol dm^{-3} HCl, the product was extracted with chloroform, the extract was washed with brine until neutral, and dried (Na_2SO_4), and the solvent was removed. The residue (300 mg) had the same R_f-value and the same NMR spectrum as the product obtained from epoxide **8**. It could not be crystallised.

(20S,22R)-4β,5β-*Epoxy*-6α,14α,17β,20-*tetrahydroxy*-1-*oxo*-*witha*-2,24-*dienolide* **10a**.—The epoxidation of compound **9a** was carried out with MCPBA as described for the preparation of compound **5**. The obtained product **10a** was homogeneous on TLC. Acetylation with acetic anhydride and pyridine afforded the acetate **10b**, characterised by its NMR spectrum; CD (MeOH) Δε +4.6.

(20S,22R)-4β,5β-*Epoxy*-6α,14α-*dihydroxyandrost*-2-*ene*-1,17-*dione* 6-*Monoacetate* **11b**.—(a) *By treatment of compound* **10b** *with Sharpless reagent*. To a solution of compound **10b** (0.03 mmol) in dry methylene dichloride (5 cm^3) under nitrogen was added a solution of $VO(acac)_2$ (7.5×10^{-4} mmol) in the same solvent (0.2 cm^3) and 2.7 mol dm^{-3} t-butyl hydroperoxide in the same solvent (0.6 cm^3). The reaction was stopped after 2 h at 40 °C. The solution was filtered through Florisil (2 g) and elution was completed with ethyl acetate. The crude product (140 mg) showed two spots on TLC, with the same R_f-values as those of keto lactone **18** (upper spot) and compound **11b** (lower spot), identical with a sample prepared by procedure (b).

(b) *By treatment of compound* **9a** *with Sharpless reagent*. The reaction was performed with substrate **9a** (49 mg) under the same experimental conditions as above, but for 24 h at 40 °C. The crude product (36 mg) was acetylated with acetic anhydride and pyridine and was subsequently purified on a preparative chromatoplate. The *acetate* **11b** had m.p. 280 °C (from ethyl acetate–hexane); $[\alpha]_D$ +26.4° (*c* 0.3, pyridine); CD (MeOH) Δε +3.6 (Found: C, 65.2; H, 7.4. $C_{19}H_{26}O_6$ requires C, 65.1; H, 7.5%); *m/z* 374 (M^+, 2%), 314 (M^+ − AcOH, 22) and 296 (M^+ − AcOH − H_2O, 44).

(20S,22R)-5α-*Chloro*-6β,17β,20-*trihydroxy*-1-*oxowitha*-2,14,24-*trienolide* **15a** *and* (22R)-5α-*Chloro*-14α,20ξ-*epoxy*-6β,17β-*dihydroxy*-1-*oxowitha*-2,24-*dienolide* **16a**.—To a solution of chlorohydrin **4a** (500 mg) in pure acetone (250 cm^3) at −10 °C under nitrogen was slowly added a solution of 98% sulphuric acid (1 cm^3) in acetone (25 cm^3) during 1 h. After the mixture had been stirred for 2 h at the same temperature, the ice–salt-bath was removed and the mixture was stirred overnight at room temperature before being filtered through a column packed with a mixture of $KHCO_3$–K_2CO_3 (9:1) (30 g). The acetone was evaporated off under reduced pressure at room temperature and the crude product was chromatographed over silica gel (15 g). Elution with chloroform–ethyl acetate (4:1) afforded compound **15a** (100 mg); further elution with chloroform–ethyl acetate (1:1) afforded compound **16a** (190 mg). *Compound* **15a** had m.p. 185–186 °C (first crystallised from EtOAc, then from MeOH), $[\alpha]_D$ +218° (*c* 0.3, pyridine) (Found: C, 66.4; H, 7.2. $C_{28}H_{37}ClO_6$ requires C, 66.6, H, 7.4%); *m/z* (15 eV) 468 (M^+ − HCl, 15%), 450 (M^+ − HCl − H_2O, 10), 432 (M^+ − HCl − $2H_2O$, 25) and 414 (M^+ − HCl − $3H_2O$, 100). *Compound* **16a** had m.p. 201–202 °C (from MeOH, ×2); $[\alpha]_D$ +18.3° (*c* 0.4, pyridine) (Found: C, 66.5; H, 7.3. $C_{28}H_{37}ClO_6$ requires C, 66.6; H, 7.4%); *m/z* 468 (M^+ − HCl, 9%), 414 (M^+ − HCl − $3H_2O$, 35) and 125 (lactone, 100). Acetylation afforded the 6-acetate **16b**, which was used for the NMR spectrum (Table 1).

5α-*Chloro*-6β,14α-*dihydroxyandrost*-2-*ene*-1,17-*dione* 6-*Monoacetate* **17** *and* (Z,5R)-5-*Hydroxy*-2,3-*dimethyl*-6-*oxohept*-2-*enoic Acid Lactone* **18**.—The reaction was performed on the chlorohydrin acetate **4b** (56 mg) with Sharpless reagent as described for the preparation of compound **11b**, but at room temperature (TLC monitoring); it was stopped after 90 min. The crude product was separated on a preparative chromatoplate. *Compound* **17** (36 mg) (the lower spot) had m.p. 224–225 °C (from benzene); $[\alpha]_D$ +48° (*c* 0.3, pyridine) (Found: C, 61.6; H, 7.25. $C_{19}H_{27}ClO_5$ requires C, 61.5; H, 7.3%); *m/z* 334 (M^+ − AcOH, 100%); 306 (M^+ − 28, 67); 298 (M^+ − AcOH − HCl, 55) and 280 (M^+ − AcOH − HCl − H_2O, 90).

Compound **18** (the upper spot) was identified by comparison of its NMR spectrum with that of an authentic sample.

(20S,22R)-5α-*Chloro*-14α,15α-*epoxy*-6β,17β,20-*trihydroxy*-1-*oxowitha*-2,24-*dienolide* **19a**.—(*a*) *By epoxidation of compound* **15a**. Compound **15a** (30 mg) with MCPBA as described for the preparation of compound **5**, gave the *title compound* **19a**, m.p. 176–178 °C (from CH_2Cl_2); $[\alpha]_D$ +106° (*c* 0.4, pyridine) (Found: C, 64.7; H, 7.0. $C_{28}H_{37}ClO_7$ requires C, 64.5; H, 7.2%).

(b) *By epoxidation with Sharpless reagent*. Compound **15a** was epoxidised with Sharpless reagent as described for the preparation of compound **11b**. The product was purified on a preparative chromatoplate, and was identical with that prepared by procedure (a); *m/z* (30 eV) 484 (M^+ − HCl, 5%) and 412 (M^+ − HCl − $4H_2O$, 100).

5α-Chloro-6β-hydroxyandrosta-2,14-diene-1,17-dione **20**.—To a solution of compound **15a** (10 mg) in dry methylene dichloride (3 cm^3) under nitrogen was added a solution of $VO(acac)_2$ (5.4 mg) in the same solvent (3 cm^3). The reaction was stopped after 90 min at room temperature. Work-up was carried out as described for Sharpless epoxidation. The reaction mixture showed (TLC) a spot identical with that of the keto lactone **18** and a lower spot with the same R_f-value as that of the starting material; however, according to its NMR spectrum, this product was the expected androstane derivative **20**.

(20S,22R)-*6β,17β,20-Trihydroxy-1-oxowitha-2,4,14,24-tetraenolide* **21a**.—Dehydrochlorination of compound **15a** (50 mg) was carried out as described for that of chlorohydrin **4a**, to give *compound* **21a** (30 mg), m.p. 261–262 °C (from EtOAc); $[\alpha]_D$ +174° (*c* 0.3, pyridine) (Found: C, 71.7; H, 7.8. $C_{28}H_{36}O_6$ requires C, 71.8; H, 7.7%); *m/z* 450 ($M^+ - H_2O$, 52%), 325 ($M^+ - H_2O$ − 125, 30), 299 (M^+ − 169, 30) and 281 (M^+ − 169 − H_2O, 90).

Acetylation with acetic anhydride and pyridine afforded the 6-acetate **21b**, characterised by its NMR spectrum.

(20S,22R)-*14α,15α-Epoxy-6β,17β,20-trihydroxy-1-oxowitha-2,4,24-trienolide 6-Monoacetate* **22** *and* (20S,22R)-*4β,5β:14α,15α-Diepoxy-6β,17β,20-trihydroxy-1-oxowitha-2,24-dienolide* **23a**.—(a) *By treatment of compound* **21a** *with Sharpless reagent*. The reaction was carried out with compound **21a** (50 mg) as described for the preparation of compound **10b**, at 40 °C overnight. The crude product (41 mg) was purified on a chromatoplate. It could not be crystallised and was characterised as the diepoxide **23a** according to its NMR spectrum.

(b) *By treatment of compound* **21b** (30 mg) *with MCPBA*. The reaction was carried out as described for the oxidation of compound **3**, and the crude product was separated on a chromatoplate into two components: the monoepoxide 6-acetate **22** and the diepoxide 6-acetate **23b**. The latter was identical with the acetylation product of triol **23a** obtained according to procedure (a). Both compounds **22** and **23b** were characterised by their NMR spectra.

Compound **23a** remained unchanged upon treatment with $VO(acac)_2$ for 2 h at room temperature, according to the procedure described for cleavage of the C-17–C-20 bond in compound **15a**. The reaction was repeated at 70 °C for 24 h: this time the crude product was a mixture of keto lactone **18** and 4β,5β:14α,15α-diepoxy-6β-hydroxyandrost-2-ene-1,17-dione **24**.

(22R)-*14α,20ξ-Epoxy-6β,17β-dihydroxy-1-oxowitha-2,4,24-trienolide* **25a**.—The dehydrochlorination of compound **16a** (100 mg) was performed as described for that of chlorohydrin **4a**. The crude product was chromatographed over silica gel; elution with ethyl acetate afforded *title compound* **25a** (65 mg; homogeneous on TLC), m.p. 284–285 °C (from MeOH); $[\alpha]_D$ −156.5° (*c* 0.3, pyridine) (Found: C, 71.9; H, 7.7. $C_{28}H_{36}O_6$ requires C, 71.8; H, 7.7%); *m/z* 469 ($M^+ - 1$, 2%), 450 ($M^+ - H_2O$, 6), 343 ($M^+ - 125 - H_2O$, 14), 299 (M^+ − 169, 14), 169 (whole side-chain, 32) and 125 (lactone, 40). Acetylation with acetic anhydride and pyridine, overnight at 95 °C, afforded the 6,17-diacetate **25b**, identical with the same compound from previous work.*

(22R)-*4β,5β:14α,20ξ-Diepoxy-6β,17β-dihydroxy-1-oxowitha-2,24-dienolide* **26**.—Epoxidation of compound **25a** (50 mg) was carried out with MCPBA as described for the oxidation of compound **3**. The crude product was purified on a chromatoplate to give the *diepoxide* **26**, m.p. 247–248 °C (from MeOH); $[\alpha]_D$ −23° (*c* 0.3, pyridine). Epoxidation with Sharpless reagent afforded the same product (Found: C, 69.2; H, 7.4. $C_{28}H_{36}O_7$ requires C, 69.4; H, 7.5%); *m/z* 359 (M^+ − 125, 90%) and 341 ($M^+ - 125 - H_2O$, 75).

* Compound **6** of ref. 8.

References

1 E. Glotter, A. Abraham, G. Günzberg and I. Kirson, *J. Chem. Soc., Perkin Trans. 1*, 1977, 341 and references cited therein.
2 (*a*) I. Kirson, A. Abraham, P. D. Sethi, S. S. Subramanian and E. Glotter, *Phytochemistry*, 1976, **15**, 340; (*b*) K. Sakurai, H. Ishii, S. Kobayashi and T. Iwao, *Chem. Pharm. Bull.*, 1976, **24**, 1403.
3 I. Kirson and H. E. Gottlieb, *J. Chem. Res.*, 1980, (*S*) 338; (*M*) 1178.
4 E. Keinan, M. Sahai and I. Kirson, *J. Org. Chem.*, 1983, **48**, 2550.
5 E. Glotter, I. Kirson, A. Abraham and D. Lavie, *Tetrahedron*, 1973, **29**, 1353.
6 M. Ishiguro, A. Kajikawa, T. Haruyama, Y. Ogura, M. Okubayashi, M. Morisaki and N. Ikekawa, *J. Chem. Soc., Perkin Trans. 1*, 1975, 2295.
7 M. Weissenberg, E. Glotter and D. Lavie, *Tetrahedron Lett.*, 1974, 3063; *J. Chem. Soc., Perkin Trans. 1*, 1977, 796.
8 I. Kirson, G. Günzberg, H. E. Gottlieb and E. Glotter, *J. Chem. Soc., Perkin Trans. 1*, 1980, 531.
9 M. Zviely, A. Goldman, I. Kirson and E. Glotter, *J. Chem. Soc., Perkin Trans. 1*, 1986, 229.
10 F. Frolow, A. B. Ray, M. Sahai, E. Glotter, H. E. Gottlieb and I. Kirson, *J. Chem. Soc., Perkin Trans. 1*, 1981, 1029.
11 P. Hofer, H. Linde and K. Meyer, *Helv. Chem. Acta*, 1962, **45**, 1041.
12 K. Tori and K. Aono, *Annu. Rep. Shionogi Res. Lab.*, 1965, **15**, 130.
13 D. H. R. Barton and Y. Houminer, *J. Chem. Soc., Perkin Trans. 1*, 1972, 919.

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