OXIDATION OF 3β,28-DIACETOXY-18-LUPEN-21-ONE WITH PEROXY ACIDS: A WAY TO DES-E-LUPANE DERIVATIVES*

Eva KLINOTOVÁ[a], Martin REJZEK[a], Hana ZŮNOVÁ[a], Jan SEJBAL[a], Jiří KLINOT[a] and Jiří URBAN[b]

[a] *Department of Organic Chemistry, Charles University, 128 40 Prague 2, The Czech Republic*
[b] *The J. Heyrovský Institute of Physical Chemistry, Academy of Sciences of the Czech Republic, 182 23 Prague 8, The Czech Republic*

Received December 16, 1992
Accepted January 2, 1993

Dedicated to Professor Otto Wichterle on the occasion of his 80th birthday.

Oxidation of 3β,28-diacetoxy-18-lupen-21-one (*I*) and its 18β,19β-epoxy derivative *III* with peracetic acid, catalyzed with strong acids, proceeds with cleavage of the bond between C-19 and C-21 under formation of E-seco derivatives with hydroxyl and isobutyryl groups on C-18 (spiro lactones *V* – *VII* and acid *VIII*). Oxidative removal of the isobutyryl fragment in spiro lactone *VI* by treatment with lead tetraacetate leads to the tetranor derivative – keto lactone *XI* which in an alkaline medium loses formaldehyde from C-17 to give des-E acid *XVI*.

In connection with the preparation of highly oxidized E-seco derivatives of pentacyclic triterpenes, we studied the potentialities of the Baeyer–Villiger oxidation of 3β,28-diacetoxy-18-lupen-21-one (*I*) and 18,19-epoxy ketone *III* with peroxy acids.

The starting α,β-unsaturated ketone *I* was prepared by oxidation of 18-lupen-3β,28-diol diacetate with chromium trioxide in acetic acid according to ref.[1]. On treatment with hydrogen peroxide in an alkaline medium, this ketone did not give the desired epoxy ketone *III*, the only reaction being hydrolysis of the acetate groups under formation of diol *II* which for comparison was prepared from compound *I* by alkaline hydrolysis. The double bond in ketone *I* was epoxidized with 3-chloroperbenzoic acid. Although the reaction proceeded slowly, it gave the epoxide *III* as the only product and neither Baeyer–Villiger oxidation products (unsaturated lactones) nor products of their further transformation were found in the reaction mixture. Peracetic acid is less suitable

* Part CI in the series Triterpenes; Part C: Collect. Czech. Chem. Commun. *58*, 1675 (1993).

for the preparation of epoxide *III* because it afforded a complex mixture of products containing, in addition to *III*, many further polar compounds.

The structure of epoxy ketone *III* follows from the infrared, mass and NMR spectra, especially from the ^{13}C NMR spectrum (Table I) which confirmed the presence of two quaternary carbon atoms of the epoxide ring (δ 72.3 and 78.1) and of the keto group (δ 210.2). The configuration of the epoxide group could not be determined from the spectral data; however, because in 18-lupene derivatives peroxy acids attack the 18(19)-double bond exclusively from the β-side[2-5], we suggest the 18β,19β-configuration. This configuration is also supported by the epoxidation of diol *II* in which we can expect[6] a syn-directing effect of the axial 17β-hydroxymethyl group preferring formation of the β-epoxide. The reaction of diol *II* with 3-chloroperbenzoic acid afforded solely an epoxy derivative, identical with derivative *IV*, obtained by alkaline hydrolysis of acetate groups in epoxy ketone *III*; on the other hand, acetylation of derivative *IV* gave the epoxy ketone *III*.

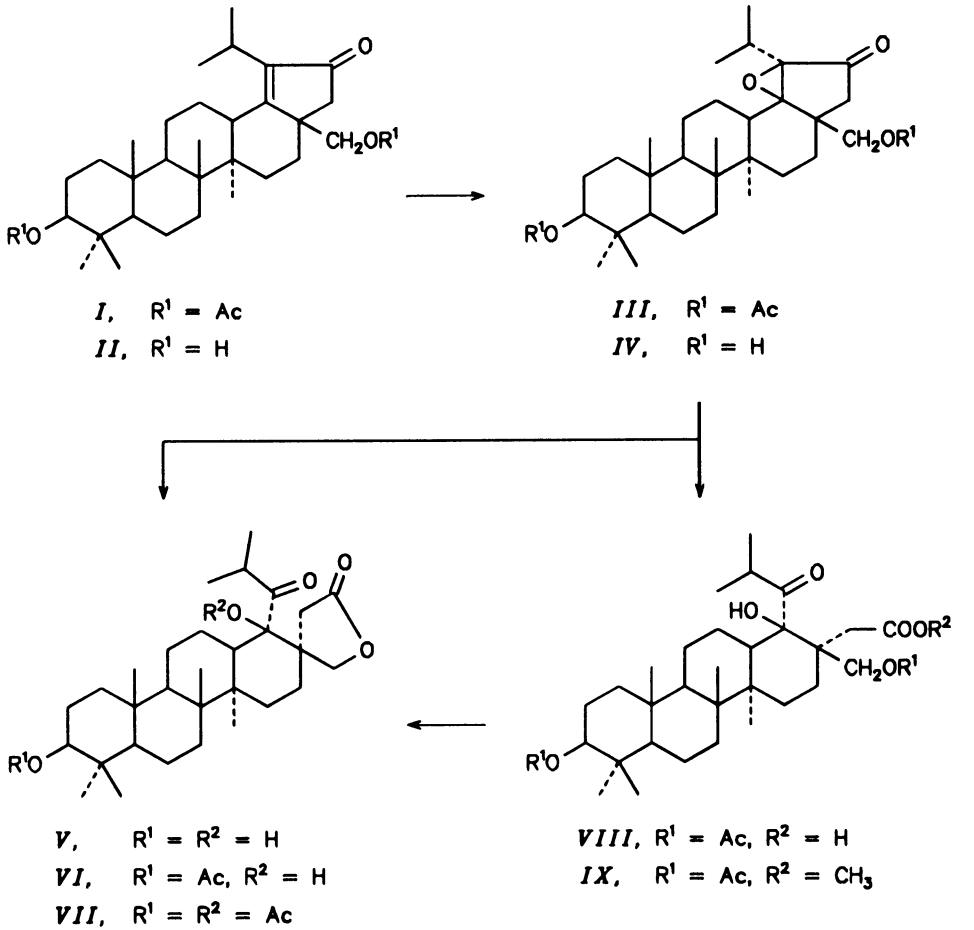
TABLE I

Carbon-13 chemical shifts of compounds *III*, *VI* and *XIII*; measured at 50.31 MHz in $CDCl_3$, for other conditions see Experimental

Carbon	*III*	*VI*	*XIII*	Carbon	*III*	*VI*	*XIII*
1	38.40	38.32	38.43	19	72.31[c]	219.30	–
2	23.46	23.55	23.62	20	26.32	46.09	–
3	80.53	80.68	80.71	21	210.17	177.12	175.67
4	37.64	37.73	37.80	22	41.09[b]	38.91	39.75
5	55.35	55.14	55.43	23	27.81	27.91	27.95
6	17.95	18.09	18.06	24	16.38	16.47	16.49
7	33.96	32.97	32.83	25	16.27	16.04[g]	16.27
8	41.28	41.99[e]	41.10	26	16.62[d]	16.17[g]	15.93[i]
9	51.13	49.57	50.33	27	16.62[d]	14.13	15.31[i]
10	37.03	36.91	37.11	28	64.73	73.40	73.45
11	21.11	21.23	20.59	29	17.33[d]	17.97	–
12	28.81	23.18[f]	25.60[h]	30	18.22[d]	20.82	–
13	38.04	38.13	38.01	OAc:			
14	43.02	42.48[e]	43.99	CH_3	20.75	21.29	20.93
15	[a]	26.36[f]	26.68[h]		21.15	–	21.31
16	[a]	30.32	26.86[h]	C=O	170.73	171.01	170.45
17	44.64[b]	47.90	39.89		170.89	–	170.98
18	78.12[c]	83.62	76.93				

[a] Signal not found. [b-i] Signals with the same symbol may be interchanged.

The ring E in the unsaturated ketone *I* and epoxy ketone *III* is oxidatively cleaved by action of peracetic acid under catalysis with strong acids. Ketone *I* reacted with peracetic acid in the presence of *p*-toluenesulfonic acid to give spiro lactone *VI* as the principal product and small amount of spiro lactone *V* without acetate group at C-3 (Scheme 1). Small amounts of compound *III* were also isolated from the reaction mixture; apparently, this derivative is an intermediate in the formation of spiro lactones *V* and *VI*. Under the same conditions, also the epoxy ketone *III* afforded predominantly the lactone *VI*, again with lactone *V* as the side-product. In addition, we isolated negli-

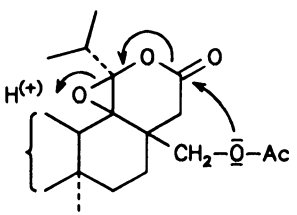


SCHEME 1

gible amounts of 18-acetoxy lactone *VII*. The spiro lactone *VI* was also obtained in high yield (about 80%) from epoxy ketone *III* in the presence of sulfuric acid.

In the presence of the above-mentioned acids as catalysts, the oxidative cleavage with peracetic acid proceeded slowly (1 – 4 weeks at room temperature) and, in addition to the spiro lactones *V* – *VII*, other undesired products were formed which were not studied further. A more suitable method proved to be the oxidation in the presence of trifluoroacetic acid which is faster and gives only E-seco derivatives: beside the spiro lactone *VI*, accompanied by minor amount of compound *VII*, we also isolated the corresponding E-seco acid *VIII* which was characterized as its methyl ester *IX*. The acid *VIII* was converted into spiro lactones *V* and *VI*: after alkaline hydrolysis of the acetate groups the subsequent acidification closed the lactone ring under formation of 3-hydroxy lactone *V*. The compound *V* was obtained in the same manner from lactone *VI*. Acetylation of compound *V* with acetic anhydride afforded 3-acetate *VI*; there was no acetylation of the tertiary hydroxyl in position 18.

The formation of E-seco derivatives *V* – *VIII* can be explained by Baeyer–Villiger oxidation of epoxy ketone *III* to δ-lactone *X* in which the epoxide and lactone rings are opened by the action of acids to give rise to α-hydroxy ketone system. The carbonyl group in position 21 becomes a part of the γ-lactone ring either via the acid *VIII* or directly by intramolecular attack of the 28-oxygen atom under simultaneous loss of acetyl group.

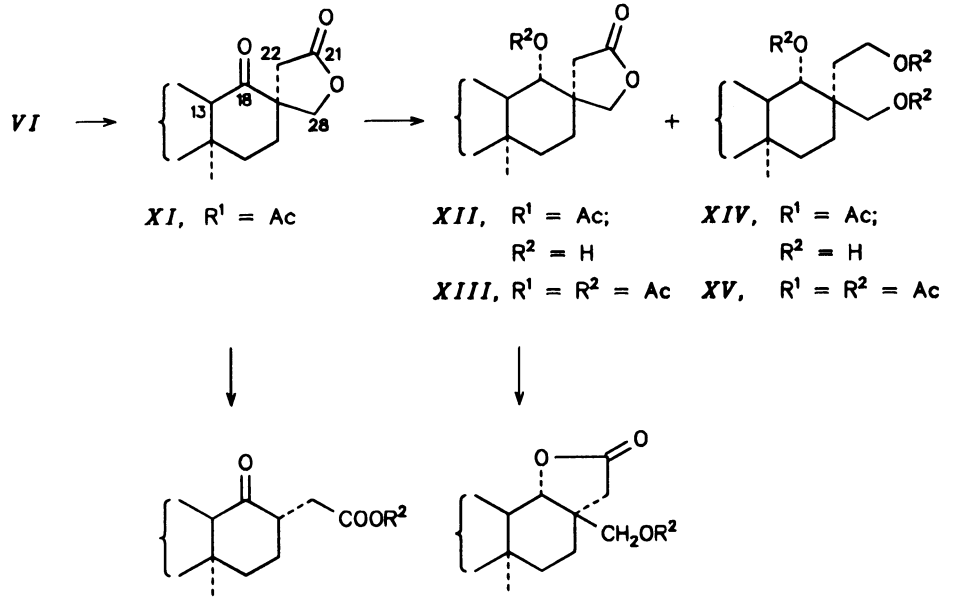


X, R^1 = Ac

The structure of compounds *V* – *IX* follows from their spectral data. The infrared spectra show the presence of a ketonic carbonyl (about 1 710 cm^{-1}) which for compound *VI* was confirmed also by ^{13}C NMR spectrum (δ(C-19) 219.3). The characteristic heptet of H-20 at δ ca 3, together with two doublets of methyl protons in the ^1H NMR spectra of compounds *VI* – *IX*, indicate the presence of an isobutyryl group. In the mass spectra, the loss of isobutyryl residue represents main fragmentation process that gives rise to highly abundant ion *m/z* 71 which in the spectra of compounds *VII* – *IX* is the base peak. In addition, the spectra exhibit ions $[M - 71]^+$ and ions corresponding to combination of loss of 71 mass units and acetic acid or water from the molecular ion (base peaks in the spectra of compounds *V* and *VI*). We have no direct evidence for the configuration at C-18; on the basis of the above-mentioned explanation of the forma-

tion of E-seco derivatives from 18β,19β-epoxy ketone *III* we suggest the α-configuration for the isobutyryl group and β-configuration for the hydroxyl. The presence of a γ-lactone ring in spiro lactones *V* – *VII* is confirmed by infrared spectra (band at about 1 775 cm^{-1}), ^1H NMR spectra (two AB systems of CH_2 groups in the regions δ 2.3 and 4 – 5; $J \approx 19$ and 9 Hz, respectively), and in compound *VI* by the ^{13}C NMR spectrum (δ(C-21) 177.1).

The α-hydroxy ketone grouping on C-18 in compound *VI* was confirmed by oxidative cleavage with lead tetraacetate which led to the tetranor derivative, keto lactone *XI* (Scheme 2). In accord with the presence of keto group in position 18, the doublet of doublets of H-13β is shifted downfield to δ 2.40 and the values of coupling constants *J*(12,13) (11.6 and 3.5 Hz) show that the trans-annelation of rings C and D remained



XI, R^1 = Ac

XII, R^1 = Ac; R^2 = H

XIII, $R^1 = R^2$ = Ac

XIV, R^1 = Ac; R^2 = H

XV, $R^1 = R^2$ = Ac

XVI, $R^1 = R^2$ = H

XVII, R^1 = H; $R^2 = CH_3$

XVIII, R^1 = Ac; R^2 = H

XIX, R^1 = Ac; $R^2 = CH_3$

XX, $R^1 = R^2$ = H

XXI, R^1 = Ac; R^2 = H

XXII, $R^1 = R^2$ = Ac

SCHEME 2

preserved. Reduction of keto lactone *XI* with sodium borohydride afforded 18-hydroxy lactone *XII* as the principal product; in a side-reaction, the lactone group was in part reduced under formation of tetrol monoacetate *XIV*. Compounds *XII* and *XIV* were converted into diacetate *XIII* and tetraacetate *XV*, respectively, on treatment with acetic anhydride in pyridine. The small vicinal coupling constant $J(13,18) \approx 3$ Hz in the ^{1}H NMR spectra of acetates *XIII* and *XV*, together with further splitting of the H-18 signal ($J \approx 1.2$ Hz) in the spectrum of diacetate *XIII* (obviously due to a long-range coupling with the equatorial proton H-16β) show the equatorial position of the proton on C-18. This means that the hydride attacks the molecule from the sterically less hindered β-side and that the hydroxyl group on C-18 has α-configuration. Reaction of compound *XII* with potassium hydroxide, followed by acidification, afforded lactone *XX* in which the γ-lactone ring is closed in position 18. Since an insoluble potassium salt precipitated during the reaction, the 3-acetate group was not completely hydrolyzed and in addition to the lactone *XX* we also isolated its 3-acetate *XXI*. Both derivatives *XX* and *XXI* were acetylated to give the same diacetate *XXII*. All spectral data for compounds *XI* – *XV*, *XXI* and *XXII* agree with the discussed structures. The spiro lactones *XI* and *XIII*, whose C(28)H_2 group is part of the lactone ring, can be easily distinguished from derivatives with the C(28)H_2–OH (and OAc) group (*XIV*, *XV*, *XXI*, *XXII*) on the basis of the geminal coupling constant of protons of this methylene group: in the first case $J(28,28') \approx 9$ Hz (similarly as for spiro lactones *VI* and *VII*), in the second the constant is about 11.5 Hz.

Alkaline hydrolysis of keto lactone *XI* is accompanied by retroaldol elimination of formaldehyde from C-17 under formation of pentanor acid *XVI* which was characterized as its methyl ester *XVII*, acetate *XVIII* and methyl ester acetate *XIX*. The unchanged β-configuration of H-13 is obvious from the coupling constants of derivative *XIX* (11.6 and 3.4 Hz); as concerns the configuration at C-17, we assume that the side-chain is in the more advantageous equatorial position and has thus the α-configuration.

The peracetic acid oxidation of 3β,28-diacetoxy-18-lupen-21-one (*I*), which is easily accessible from betulin[1,7], and its 18β,19β-epoxy derivative *III* represents an advantageous method for conversion of common lupane compounds into 19,21-secolupane derivatives. The structure of the obtained spiro lactones *V* – *VII* with isobutyryl side-chain at C-18 is similar to that of radermasinin, a cytotoxic substance recently isolated from natural material[8]. The cleavage of the bond between C-19 and C-21 in lupane compounds is considered to be one of possible paths of radermasinin biosynthesis[9]. Oxidative degradation of spiro lactone *VI*, followed by elimination of formaldehyde, enables the preparation of des-E compounds (*XVI* – *XIX*) of skeleton similar to that of natural sesterterpenoids derived from scalarane[10,11].

EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. Optical rotations were measured in chloroform (*c* 0.3 – 0.8) on an automatic polarimeter ETL-NPL (Bendix–Ericsson), accuracy ±2°. Infrared spectra were recorded in chloroform on a PE 684 (Perkin–Elmer) spectrometer, wavenumbers are given in cm^{-1}. NMR spectra were measured on FT-NMR spectrometers Tesla BS 587 A (^1H at 80 MHz) and Varian XL-200 (^1H at 200 MHz, ^{13}C at 50.31 MHz) in deuteriochloroform. Tetramethylsilane was used as the internal standard for proton chemical shifts. The values of proton chemical shifts (ppm, δ-scale) and interproton coupling constants (in Hz) were obtained by the first order analysis; the two-spin systems of protons at C-22 and C-28 were analyzed as AB systems. Carbon-13 chemical shifts were referenced to the signal of solvent and recalculated to tetramethylsilane using the relation $\delta(CDCl_3)$ = 77.00 ppm. The structural assignment of carbon chemical shifts in Table I is tentative and is based on proton decoupled "attached proton test" (APT) spectra and on the assignment in ketone *I* (ref.[1]). Mass spectra were measured on an INCOS 50 (Finnigan MAT) spectrometer, ionizing electrons energy 70 eV, ion source temperature 150 °C. The samples were introduced from direct exposure probe at heating rate 10 mA/s. Relative abundance is related to the most abundant ion in the region of $m/z > 50$.

The identity of substances prepared by different procedures was checked by thin-layer chromatography, melting points and IR spectra. Thin-layer chromatography (TLC) was carried out on silica gel G (Merck), detection by spraying with 10% sulfuric acid and heating, or on Silufol foils (Kavalier, Votice), detection by 5% ethanolic phosphomolybdic acid and heating. Preparative TLC was carried out on silica gel G (Merck), column chromatography on silica gel Silpearl (Kavalier, Votice). Acetates were prepared by treatment with a 1 : 1 mixture of pyridine and acetic anhydride at room temperature for 12 h. The "usual work-up procedure" denotes partition of the reaction mixture between water and ether, washing the ethereal phase with water, dilute hydrochloric acid, sodium bicarbonate solution and water, drying over sodium sulfate and evaporation of the solvents. Commercial 34% peracetic acid Persteril (Chemical Works, Sokolov) was used in the peracetic acid oxidations. Analytical samples were dried over phosphorus pentoxide at 100 °C under reduced pressure.

3β,28-Dihydroxy-18-lupen-21-one (*II*)

Ketone *I* (440 mg, 0.81 mmol) (ref.[1]) was refluxed with 2.5% solution of potassium hydroxide in a 1 : 1 benzene–ethanol mixture (10 ml) for 3 h. The mixture was poured in water and worked up in the usual manner. Crystallization from chloroform–methanol afforded dihydroxy derivative *II* (295 mg, 80%), m.p. 268 – 274 °C, $[\alpha]_D$ −91°. IR spectrum: 3 625, 1 690, 1 600. For $C_{30}H_{48}O_3$ (456.7) calculated: 78.89% C, 10.59% H; found: 78.56% C, 10.73% H.

The same product *II* (70 mg, 83%) was also obtained by four days' standing of a solution of ketone *I* (100 mg, 0.18 mmol), sodium hydroxide (0.3 g, 5.3 mmol) and 30% aqueous hydrogen peroxide (5 ml, 1.5 mmol) in a mixture of dioxane (13 ml) and ethanol (2 ml) at room temperature.

3β,28-Diacetoxy-18β,19β-epoxylupan-21-one (*III*)

A) A solution of ketone *I* (2.0 g, 3.7 mmol) and 70% 3-chloroperbenzoic acid (1.6 g, 6.5 mmol) in chloroform (20 ml) was set aside at room temperature for 11 days. The reaction mixture was diluted with chloroform and washed successively with 5% sodium iodide solution, saturated solution of sodium sulfite, saturated solution of sodium hydrogen carbonate and water. After drying and evaporation of the solvent, the residue was crystallized from chloroform–methanol. The obtained epoxy ketone *III* (1.43 g, 69%) had m.p. 254 – 256 °C, $[\alpha]_D$ +28°. IR spectrum: 1 735, 1 720 sh, 1 256. ^1H NMR spectrum (200 MHz): 0.85 s, 6 H, 0.90 s, 3 H, 1.08 s, 3 H, 1.13 s, 3 H, 1.20 d, 3 H (J = 6.8)

and 1.28 d, 3 H (J = 6.8) (7 × CH_3); 2.04 s, 3 H and 2.06 s, 3 H (2 × OAc); 1.71 d, 1 H and 2.36 d, 1 H (2 × H-22, J = 18.6); 2.55 dd, 1 H (H-13β, J = 4, J = 13); 4.47 m, 1 H (H-3α, $\Sigma J \approx 16$); 4.11 d, 1 H and 4.55 d, 1 H (2 × H-28, J = 10.8). For ^{13}C NMR spectrum see Table I. Mass spectrum, *m/z* (%): 556 (M^+, 11), 496 (4), 483 (3), 383 (8), 293 (100), 189 (15), 71 (25), 69 (26). For $C_{34}H_{52}O_6$ (556.7) calculated: 73.34% C, 9.41% H; found: 73.02% C, 9.34% H.

B) Compound *IV* (80 mg, 0.17 mmol) was acetylated in the usual manner to give the epoxy ketone *III* (70 mg, 74%), m.p. 251 – 256 °C (chloroform–methanol), $[\alpha]_D$ +25°, which was identical with a sample prepared by procedure *A*.

18β,19β-Epoxy-3β,28-dihydroxylupan-21-one (*IV*)

A) A mixture of compound *III* (200 mg, 0.36 mmol) and 2.5% solution of potassium hydroxide in a 1 : 1 benzene–ethanol mixture (4 ml) was refluxed for 2 h. After pouring into water, the mixture was worked up as usual to give derivative *IV* (120 mg, 71%), m.p. 182 – 183 °C (chloroform–methanol), $[\alpha]_D$ +28°. IR spectrum: 3 623, 1 737. For $C_{30}H_{48}O_4$ (472.7) calculated: 76.22% C, 10.24% H; found: 75.84% C, 10.22% H.

B) A mixture of compound *II* (220 mg, 0.48 mmol), 70% 3-chloroperbenzoic acid (300 mg, 1.2 mmol) and chloroform (3 ml) was allowed to stand at room temperature for 10 days. The mixture was diluted with chloroform and washed successively with 5% solution of sodium iodide, saturated solution of sodium sulfite, saturated solution of sodium hydrogen carbonate, and water. After drying over sodium sulfate, the solvent was distilled off and the product was crystallized from chloroform–methanol to afford derivative *IV* (210 mg, 92%), m.p. 180 – 183 °C, $[\alpha]_D$ +32°, identical with a sample prepared according to procedure *A*.

Reaction of *III* with Peracetic Acid

A) Peracetic acid (34%, 20 ml, 89 mmol) and trifluoroacetic acid (2 ml, 17 mmol) were added to a solution of compound *III* (1.1 g, 1.98 mmol) in chloroform (8 ml). The two-phase system was made homogeneous by addition of acetic anhydride (3 ml) and acetic acid (3 ml). After standing at room temperature for 5 days, the mixture was poured in water and the product was taken up in ether. The ethereal layer was successively washed with saturated solution of sodium hydrogen carbonate, 5% solution of sodium iodide, solution of thiosulfate, and water. After drying over sodium sulfate, the ether was distilled off and the residue was chromatographed on a column of silica gel (benzene, benzene–ether 2 : 1) to give lactone *VII* (10 mg, 1%), acid *VIII* (120 mg, 10%) and lactone *VI* (660 mg, 63%).

Lactone VI: m.p. 342 – 346 °C (chloroform–methanol), $[\alpha]_D$ +16°. IR spectrum: 3 615, 1 773, 1 718, 1 710, 1 255. ^1H NMR spectrum (200 MHz): 0.85 s, 12 H, 0.97 s, 3 H, 1.03 d, 3 H (J = 6.5) and 1.06 d, 3 H (J = 6.5) (7 × CH_3); 2.04 s, 3 H (OAc); 2.29 d, 1 H and 2.34 d, 1 H (2 × H-22, J = 19); 2.41 s, 1 H (OH); 2.70 m, 1 H; 3.08 heptet, 1 H (H-20, J = 6.5); 4.12 d, 1 H and 4.49 d, 1 H (2 × H-28, J = 8.9); 4.46 m, 1 H (H-3α, $\Sigma J \approx 16$). For ^{13}C NMR spectrum see Table I. Mass spectrum, *m/z* (%): 530 (M^+; 3), 459 (46), 417 (10), 399 (100), 381 (32), 357 (8), 201 (16), 189 (42), 71 (17). For $C_{32}H_{50}O_6$ (530.8) calculated: 72.42% C, 9.50% H; found: 72.13% C, 9.62% H.

Lactone VII: m.p. 285 – 287 °C (chloroform–methanol), $[\alpha]_D$ +30°. IR spectrum: 1 777, 1 718, 1 370, 1 254. ^1H NMR spectrum (80 MHz): 0.84 s, 9 H, 0.86 s, 3 H, 1.04 s, 3 H, 1.09 d, 3 H (J = 6.8) and 1.09 d, 3 H (J = 6.8) (7 × CH_3); 2.03 s, 3 H and 2.10 s, 3 H (2 × OAc); 2.38 bs, 2 H (2 × H-22); 2.91 m, 1 H; 2.95 heptet, 1 H (H-20, J = 6.8); 3.99 d, 1 H and 4.91 d, 1 H (2 × H-28, J = 9.7); 4.47 m, 1 H (H-3α, $\Sigma J \approx 16$). Mass spectrum, *m/z* (%): 572 (M^+; 32), 512 (9), 459 (5), 452 (6), 441 (7), 399 (9), 381 (12), 357 (7), 189 (75), 71 (100).

Acid VIII: m.p. 243 – 246 °C (chloroform–methanol), $[\alpha]_D$ +32.5°. IR spectrum: 3 400 – 3 000, 1 723, 1 254. ^{1}H NMR spectrum (80 MHz): 0.84 s, 12 H, 1.04 s, 3 H, 0.93 d, 3 H (J = 6.6) and 1.00 d, H (J = 6.6) (7 × CH_3); 2.02 s, 3 H and 2.05 s, 3 H (2 × OAc); 2.47 d, 1 H and 2.79 d, 1 H (2 × H-22, J = 18.5); 3.35 heptet, 1 H (H-20, J = 6.6); 4.45 bs, 2 H (2 × H-28); 4.46 m, 1 H (H-3α, $\Sigma J \approx 16$); 6.0 bs, 2 H (OH, COOH, $W_{1/2} \approx 50$ Hz). Mass spectrum, *m/z* (%): 572 (M^+ – 18; 0.5), 519 (5), 512 (2), 459 (8), 399 (4), 381 (8), 371 (5), 293 (8), 189 (40), 71 (100). For $C_{34}H_{54}O_8$ (590.8) calculated: 69.12% C, 9.21% H; found: 68.96% C, 9.01% H.

Methyl ester IX. Prepared by reaction of acid *VIII* with ethereal diazomethane; m.p. 248 – 252 °C (chloroform–methanol), $[\alpha]_D$ +37°. IR spectrum: 3 383, 1 726, 1 712, 1 254. ^{1}H NMR spectrum (80 MHz): 0.84 s, 12 H, 1.05 s, 3 H, 0.93 d, 3 H (J = 6.6) and 1.01 d, 3 H (J = 6.6) (7 × CH_3); 2.02 s, 3 H and 2.05 s, 3 H (2 × OAc); 2.42 d, 1 H and 2.75 d, 1 H (2 × H-22, J = 18.3); 3.38 heptet, 1 H (H-20, J = 6.6); 3.63 s, 3 H (OCH_3); 4.42 bs, 2 H (2 × H-28); 4.47 m, 1 H (H-3α, $\Sigma J \approx 16$); 5.78 bs, 1 H (OH). Mass spectrum, *m/z* (%): 533 (M^+ – 71; 11), 473 (3), 459 (7), 441 (15), 381 (12), 269 (7), 191 (46), 189 (45), 71 (100). For $C_{35}H_{56}O_8$ (604.8) calculated: 69.51% C, 9.33% H; found: 69.19% C, 9.35% H.

B) A mixture of epoxy ketone *III* (300 mg, 0.54 mmol), 34% peracetic acid (5 ml, 22 mmol), chloroform (2 ml), acetic acid (5 ml) and concentrated sulfuric acid (0.3 ml, 6 mmol) was allowed to stand for 10 days at room temperature. The mixture was worked up as described for procedure *A* and the crude product was crystallized from chloroform–methanol to give 180 mg (63%) of spiro lactone *VI*, identical with the product obtained under *A*. Thin-layer chromatography of the mother liquors on silica gel in light petroleum–acetone (4 : 1) afforded a further amount of *VI* (50 mg, 17%) and 13 mg of an unidentified compound, m.p. 278 – 282 °C (chloroform–methanol).

C) A mixture of epoxy ketone *III* (600 mg, 1.08 mmol), 34% peracetic acid (10 ml, 45 mmol), chloroform (4 ml), acetic acid (12 ml) and *p*-toluenesulfonic acid monohydrate (100 mg, 0.53 mmol) was set aside at room temperature for 20 days and then worked up as described under *A*. Chromatography of the crude product on a column of silica gel (elution with benzene and benzene–ether 10 : 1 to 4 : 1) afforded a mixture of 4 nonpolar compounds (110 mg) which was subjected to repeated thin-layer chromatography on silica gel (elution alternately with light petroleum–acetone 4 : 1 and benzene–ether 2 : 1). This procedure gave the starting epoxy ketone *III* (19 mg, 3%) and spiro lactone *VII* (6 mg, 1%), identical with authentic samples, and further unidentified compounds. Further elution of the column afforded spiro lactone *VI* (340 mg, 59%) and spiro lactone *V* (30 mg, 6%), identical with the sample described below. The usual acetylation procedure converted lactone *V* into lactone *VI*.

Reaction of Ketone *I* with Peracetic Acid

A mixture of ketone *I* (200 mg, 0.37 mmol), *p*-toluenesulfonic acid monohydrate (40 mg, 0.21 mmol), 34% peracetic acid (5 ml, 22 mmol), chloroform (1 ml), acetic acid (2 ml) and acetic anhydride (2 ml) was set aside for 26 days at room temperature and then worked up as in the preceding experiment. The mixture of products was separated by column chromatography on silica gel (elution with benzene and benzene–ether mixture (10 : 1 to 4 : 1) to give ketone *III* (25 mg, 12%), lactones *VI* (100 mg, 51%) and *V* (30 mg, 17%), identical with authentic samples, and further, unidentified compounds.

Spiro Lactone *V*

A) Spiro lactone *VI* (300 mg, 0.57 mmol) was refluxed with 2.5% ethanolic potassium hydroxide (20 ml) for 90 min. The reaction mixture was poured in dilute hydrochloric acid, the precipitate was filtered, washed with water, air-dried and crystallized from chloroform–methanol and then from chlo-

roform. The obtained lactone *V* (150 mg, 54%) melted at 356 – 361 °C (decomp.), $[\alpha]_D$ +20°. IR spectrum: 3 620, 1 773, 1 711. Mass spectrum, *m/z* (%): 488 (M^+; 8), 417 (78), 399 (100), 381 (37), 375 (30), 207 (23), 189 (50), 71 (38). For $C_{30}H_{48}O_5$ (488.7) calculated: 73.73% C, 9.90% H; found: 73.50% C, 9.83% H.

B) Acid *VIII* (200 mg, 0.34 mmol) was treated as described under *A* to give lactone *V* (110 mg, 66%), m.p. 345 – 351 °C (decomp.), identical with the product obtained under *A*.

Keto Lactone *XI*

A mixture of lactone *VI* (500 mg, 0.94 mmol), lead tetraacetate (2.0 g, 4.5 mmol), acetic acid (25 ml) and acetic anhydride (5 ml) was refluxed for 3.5 h. The usual work-up procedure, followed by crystallization from chloroform–methanol, afforded lactone *XI* (400 mg, 93%), m.p. 275 – 278 °C, $[\alpha]_D$ −11.5°. IR spectrum: 1 775, 1 715, 1 255. ^{1}H NMR spectrum (200 MHz): 0.85 s, 6 H, 0.86 s, 3 H, 0.91 s, 3 H and 1.11 s, 3 H (5 × CH_3); 2.05 s, 3 H (OAc); 2.06 d, 1 H and 3.42 d, 1 H (2 × H-22, J = 17.8); 2.46 dd, 1 H (H-13β, J = 11.6, J' = 3.5); 4.11 dd, 1 H (J = 9.0, J' = 0.8) and 4.36 d (J = 9.0) (2 × H-28): 4.48 m, 1 H (H-3α, $\Sigma J \approx 16$). Mass spectrum, *m/z* (%): 458 (M^+, 0.5), 398 (50), 383 (17), 355 (21), 189 (100). For $C_{28}H_{42}O_5$ (458.6) calculated: 73.33% C, 9.23% H; found: 73.41% C, 9.30% H.

Reduction of Keto Lactone *XI* with Sodium Borohydride

A solution of sodium borohydride (200 mg, 5.3 mmol) in methanol (3 ml) was added at 0 °C to a solution of keto lactone *XI* (110 mg, 0.24 mmol) in benzene (5 ml). After 15 min the mixture was processed as usual. Crystallization from chloroform–methanol furnished hydroxy derivative *XII* (56 mg, 51%), m.p. 334 – 341 °C (decomp.), $[\alpha]_D$ +19°. IR spectrum: 3 620, 1 774, 1 720, 1 255. For $C_{28}H_{44}O_5$ (460.7) calculated: 73.01% C, 9.63% H; found: 72.88% C, 9.56% H.

Acetate XIII. Prepared from hydroxy derivative *XII* by usual acetylation procedure; m.p. 342 – 346 °C (methanol), $[\alpha]_D$ +56°. IR spectrum: 1 780, 1 736, 1 724, 1 246. ^{1}H NMR spectrum (200 MHz): 0.84 s, 3 H, 0.86 s, 6 H, 0.96 s, 3 H and 1.13 s, 3 H (5 × CH_3); 2.05 s, 3 H and 2.10 s, 3 H (2 × OAc); 2.11 d, 1 H and 2.46 d, 1 H (2 × H-22, J = 17.5); 4.01 dd, 1 H (J = 9.3, J' = 1.2) and 4.24 d, 1 H (J = 9.3) (2 × H-28); 4.99 dd, 1 H (H-18β, J = 3.0, J' = 1.2); 4.48 m, 1 H (H-3α, $\Sigma J \approx 16$). For ^{13}C NMR spectrum see Table I. Mass spectrum, *m/z* (%): 502 (M^+; 0.5), 442 (23), 427 (6), 399 (14), 382 (11), 367 (6), 339 (6), 203 (22), 189 (100). For $C_{30}H_{46}O_6$ (502.7) calculated: 71.68% C, 9.22% H: found: 71.42% C, 9.14% H.

Tetrol monoacetate XIV. Mother liquors from the crystallization of hydroxy derivative *XII* on thin-layer chromatography (benzene–ether 1 : 1) afforded another portion of hydroxy derivative *XII* (12 mg, 11%) and tetrol monoacetate *XIV* (18 mg, 18%), m.p. 230 – 240 °C (chloroform–heptane), $[\alpha]_D$ +23°. IR spectrum: 3 620, 3 450 broad, 1 725, 1 255. ^{1}H NMR spectrum (200 MHz): 0.84 s, 3 H, 0.85 s, 3 H, 0.87 s, 3 H, 0.99 s, 3 H and 1.15 s, 3 H (5 × CH_3); 2.05 s, 3 H (OAc); 3.60 bs, 1 H (H-18β); 3.59 d, 1 H and 3.61 d, 1 H (2 × H-28, J = 11.5); 3.84 t, 2 H (2 × H-21, J = 5.3); 4.48 m, 1 H (H-3α, $\Sigma J \approx 16$). Mas spectrum, *m/z* (%): 446 (M^+ – 18, 8), 428 (8), 402 (10), 386 (7), 343 (14), 203 (15), 189 (62), 181 (100).

Tetraacetate XV. Prepared from compound *XIV* by usual acetylation procedure; m.p. 158 – 162 °C (chloroform–methanol), $[\alpha]_D$ +18°. IR spectrum: 1 725, 1 255. ^{1}H NMR spectrum (200 MHz, $CDCl_3$): 0.84 s, 3 H, 0.85 s, 3 H, 0.86 s, 3 H, 0.99 s, 3 H and 1.09 s, 3 H (5 × CH_3); 2.00 s, 3 H, 2.04 s, 3 H, 2.10 s, 3 H and 2.11 s, 3 H (4 × OAc); 4.07 d, 1 H and 4.14 d, 1 H (2 × H-28, J = 11.5); 4.08 t, 2 H (2 × H-21, J = 7.4); 4.47 m, 1 H (H-3α, $\Sigma J \approx 16.1$); 4.96 d, 1 H (H-18β, J = 3.0). ^{1}H NMR spectrum (200 MHz, C_6D_6): 0.62 s, 3 H; 0.76 s, 3 H; 0.82 s, 6 H and 1.01 s, 3 H (5 × CH_3); 1.63 s, 3 H, 1.65 s, 3 H, 1.69 s, 3 H and 1.76 s, 3 H (4 × OAc); 3.99 d, 1 H and 4.09 d, 1 H

(2 × H-28, J = 11.9); 4.17 m, 2 H (2 × H-21, AB part of ABXY system); 4.62 m, 1 H (H-3α, $\Sigma J \approx 16$); 5.09 d, 1 H (H-18β, J = 2.1). Mass spectrum, m/z (%): 530 (M^+ – 60, 27), 515 (8), 487 (5), 470 (15), 410 (9), 337 (5), 262 (12), 203 (21), 189 (100). For $C_{34}H_{54}O_8$ (590.7) calculated: 69.12% C, 9.21% H; found: 69.01% C, 9.07% H.

Reaction of Compound *XII* with Potassium Hydroxide

A mixture of compound *XII* (70 mg, 0.15 mmol), potassium hydroxide (300 mg, 5.4 mmol), benzene (10 ml) and ethanol (10 ml) was refluxed for 6 h. The hydrolysis products began to separate from the reaction mixture before the starting compound completely dissolved. The mixture was acidified with dilute hydrochloric acid and worked up in the usual manner. Preparative thin-layer chromatography in benzene–ether (1 : 1) afforded the starting lactone *XII* (8 mg, 11%), the isomeric lactone *XXI* (15 mg, 21%) and lactone *XX* (35 mg, 55%).

Lactone XXI: m.p. 295 – 300 °C (decomp.) (chloroform–heptane), $[\alpha]_D$ –22°. IR spectrum: 3 629, 3 500 broad, 1 769, 1 721, 1 254. ^{1}H NMR spectrum (80 MHz): 0.85 s, 9 H, 0.97 s, 3 H and 1.06 s, 3 H (5 × CH_3); 2.04 s, 3 H (OAc); 2.11 d, 1 H and 2.79 d, 1 H (2 × H-22, J = 17.6); 3.64 d, 1 H and 3.66 d, 1 H (2 × H-28, J = 11.5); 4.28 bs, 1 H (H-18β); 4.48 m, 1 H (H-3α, $\Sigma J \approx 16$). For $C_{28}H_{44}O_5$ (460.7) calculated: 73.01% C, 9.63% H; found: 72.79% C, 9.46% H.

Lactone XX. Obtained by the above-mentioned thin-layer chromatography; m.p. 328 – 330 °C (methanol–ether).

Diacetate XXII. The usual acetylation procedure converted the derivatives *XXI* and *XX* into diacetate *XXII*, m.p. 355 – 360 °C (decomp.) (chloroform–heptane), $[\alpha]_D$ +56°. IR spectrum: 1 771, 1 742 sh, 1 728, 1 253. ^{1}H NMR spectrum (80 MHz): 0.85 s, 6 H, 0.87 s, 3 H, 0.98 s, 3 H and 1.07 s, 3 H (5 × CH_3); 2.04 s, 3 H and 2.10 s, 3 H (2 × OAc); 2.21 d, 1 H and 2.63 d, 1 H (2 × H-22, J = 17.0); 4.06 d, 1 H and 4.20 d, 1 H (2 × H-28, $J \approx 12.4$); 4.10 bs, 1 H (H-18β); 4.48 m, 1 H (H-3α, $\Sigma J \approx 16$). Mass spectrum, m/z (%): 442 (M^+ – 60, 29), 427 (10), 399 (12), 360 (9), 203 (19), 189 (100). For $C_{30}H_{46}O_6$ (502.7) calculated: 71.68% C, 9.22% H; found: 71.46% C, 9.08% H.

Reaction of Keto Lactone *XI* with Potassium Hydroxide

A mixture of keto lactone *XI* (230 mg, 0.5 mmol), potassium hydroxide (250 mg, 4.5 mmol) and ethanol (10 ml) was refluxed for 3 h. The starting compound gradually dissolved during the boil. The reaction mixture was then poured into dilute hydrochloric acid and extracted with ether. The ethereal layer was washed with water and passed through a layer of silica gel. The solvent was evaporated and the residue was repeatedly crystallized from ether–heptane and from ether.

Acid XVI (105 mg, 52%), m.p. 157 – 159 °C; after crystallization m.p. 210 – 214 °C, $[\alpha]_D$ +6°.

Methyl ester XVII. Prepared from acid *XVI* by reaction with ethereal diazomethane; m.p. 154 – 156 °C (chloroform–heptane), $[\alpha]_D$ +4°. IR spectrum: 3 610, 1 731, 1 710. ^{1}H NMR spectrum (80 MHz): 0.77 s, 3 H, 0.80 s, 3 H, 0.88 s, 3 H, 0.97 s, 3 H and 1.12 s, 3 H (5 × CH_3); 2.4 – 2.7 m, 3 H; 3.20 m, 1 H (H-3α, $\Sigma J \approx 16$); 3.67 s, 3 H (OCH_3). For $C_{26}H_{42}O_4$ (418.6) calculated: 74.60 %C, 10.11 %H; found: 74.42 %C, 9.98 %H.

Acetate XVIII. Prepared from acid *XVI* by standard acetylation procedure. Chromatographic purification on silica gel (elution with benzene), followed by crystallization from chloroform–heptane afforded the product, m.p. 198 – 204 °C, $[\alpha]_D$ +14°. IR spectrum: 3 510, 3 000 broad, 1 716, 1 255. ^{1}H NMR spectrum (80 MHz): 0.80 s, 3 H, 0.85 s, 6 H, 0.91 s, 3 H and 1.12 s, 3 H (5 × CH_3); 2.03 s, 3 H (OAc); 2.4 – 2.8 bm, 2 H; 4.48 m, 1 H (H-3α, $\Sigma J \approx 16$).

Methyl ester acetate XIX. Prepared from methyl ester *XVII* (80 mg, 0.19 mmol) by acetylation in the usual manner. The product *XIX* (75 mg, 86%) melted at 199 – 201 °C (chloroform–methanol), $[\alpha]_D$ +4°. IR spectrum: 1 727, 1 255. ^{1}H NMR spectrum (200 MHz): 0.79 s, 3 H, 0.85 s, 6 H,

0.90 s, 3 H and 1.11 s, 3 H (5 × CH_3); 2.04 s, 3 H (OAc); 2.56 dd, 1 H (H-13β, J = 11.6, J' = 3.4); 2.66 – 2.87 bm, 2 H; 3.67 s, 3 H (OCH_3); 4.48 m, 1 H (H-3α, $\Sigma J \approx 16$). Mass spectrum, *m/z* (%): 460 (M^+, 24), 429 (4), 400 (43), 385 (16), 357 (21), 197 (100), 189 (82), 183 (62). For $C_{28}H_{44}O_5$ (460.7) calculated: 73.01% C, 9.63% H; found: 72.93% C, 9.71% H. The same acetate *XIX* was also obtained by reaction of acetate *XVII* with ethereal diazomethane.

The authors are indebted to Dr S. Hilgard for recording the infrared spectra, Dr B. Máca for measurement of the mass spectra, and Mrs Čečrdlová for performing the elemental analyses.

REFERENCES

1. Sejbal J., Klinot J., Budějínský M., Protiva J.: Collect. Czech. Chem. Commun. *56*, 2936 (1991).
2. Suokas E., Hase T.: Acta Chem. Scand., B *31*, 231 (1977).
3. Suokas E., Hase T.: Acta Chem. Scand., B *31*, 633 (1977).
4. Klinotová E., Protiva J., Klinot J., Vystrčil A.: Collect. Czech. Chem. Commun. *49*, 141 (1984).
5. Klinotová E., Klinot J., Protiva J., Urban J., Vystrčil A.: Collect. Czech. Chem. Commun. *48*, 3444 (1983).
6. Berti G.: Top. Stereochem. *7*, 93 (1973).
7. Suokas E., Hase T.: Acta Chem. Scand., B *29*, 139 (1975).
8. Rice G. K., Yokoi T., Hayashi T., Suzuki H., Lee K.-H., McPhail A. T.: J. Chem. Soc., Chem. Commun. *1986*, 1397.
9. Connolly J. D., Hill R. A.: Nat. Prod. Rep. *6*, 475 (1989).
10. Alvi K. A., Crews P.: J. Nat. Prod. *55*, 859 (1992).
11. Hanson J. R.: Nat. Prod. Rep. *9*, 481 (1992).

Translated by M. Tichý.