FUNCTIONALIZATION OF LUPANE-3β,28-DIYL DIACETATE WITH CHROMIUM(VI) OXIDE*. PART 2**

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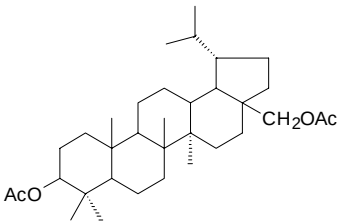
Oxidation of lupane-3β,28-diyl diacetate (**1**) with chromium trioxide was reported already earlier to give small amounts of products functionalized in position 19β and 11 (19β-hydroxylupane-3β,28-diyl diacetate (**3**) and 11-oxolupane-3β,28-diyl diacetate (**6**)), together with products of subsequent oxidation, 21-oxolup-18-ene-3β,28-diyl diacetate (**4**) and anhydride **5**). Now we identified further products, arising by functionalization in positions 12, 16 and 21 (12-hydroxy-11-oxolup-12-ene-3β,28-diyl diacetate (**8**), 12-oxolupane-3β,28-diyl diacetate (**9**), 11-oxolup-12-ene-3β,28-diyl diacetate (**17**), 21-oxolupane-3β,28-diyl diacetate (**20**) and 16-oxolupane-3β,28-diyl diacetate (**22**)), either as such or after conversion into other derivatives. The obtained compounds were further transformed and structure of the prepared derivatives was confirmed by ^{1}H NMR, ^{13}C NMR and mass spectroscopy.
Key words: Triterpenes; Triterpenoids; Lupane derivatives; Oxidation; Functionalization; Chromium (VI) oxide.

Some positions in natural triterpenoids bear substituents very often, some only rarely. Lupane derivatives are triterpenes in which functional groups are attached mostly in positions 3 and 28 and in the isopropyl group (*i.e.* in positions 20, 29 and 30) whereas in other positions substitution occurs only very rarely. During the past years, functionalization of lupane derivatives in less common positions has been attempted; (see *e.g.* refs[2–9] and references therein). For lupane and lupan-3β,28-diyl diacetate (**1**) reactions with selective radical reagents were studied and high selectivity of the position 19 toward the radical attack has been found[7–9]. In our paper[3], we used chromium(VI) oxide in a less selective oxidation of lupane and obtained in low yields products of functionalization in ring A and further in positions 12, 16, and 19.

* Part CVIII in the series Triterpenes; Part CVII: see ref.[1].
**Part 1: see ref.[2].

In order to prepare also derivatives of lupane-3β,28-diyl diacetate (**1**) substituted in positions other than 19, we used in our previous paper[2] chromium(VI) oxide in boiling acetic acid for the functionalization. From the reaction mixture after oxidation we isolated products both from the chromatographically homogeneous fractions that upon crystallization afforded a single compound, and from fractions which on reduction with lithium aluminium hydride gave one major product. The compounds were products of functionalization in positions 11 and 19 (derivatives **6** and **3**), products of subsequent reactions (**4** and **5**) and the 3-oxo derivative **7** as product of modification of the functional group at C-3. The present communication extends the mentioned publication[2] and describes chromatographic separation and purification of at that time unseparated compounds obtained after conversions of fractions *A*, *B* and *C*.



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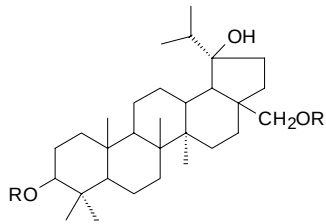
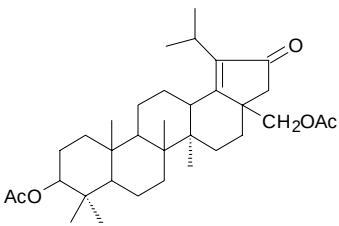


2, R = H
3, R = Ac



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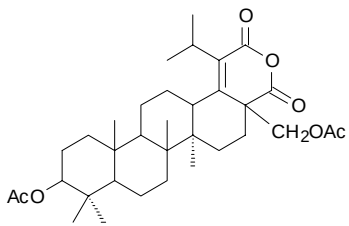

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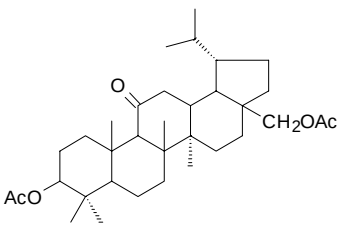

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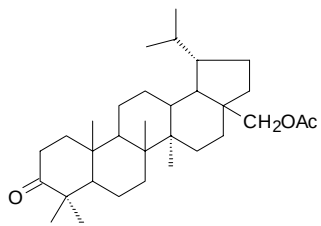

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Fraction *A* was separated by repeated chromatography which gave the enol form of diketone **8** whose NMR spectra are very similar to those of the unsaturated ketone **17** (*vide infra*). In the ^{13}C NMR spectrum (Table I), more significant differences between

TABLE I
Carbon-13 chemical shifts of lupane derivatives, for conditions see Experimental

Atom	**8**	**11**	**15**	**17**	**18**	**20**	**21**
1	37.04	38.29	37.84	36.75	38.63	38.32	38.87
2	23.49	23.62	23.40	23.55	24.26	23.60	27.35
3	80.42	80.80	80.80	80.61	80.52	80.74	78.82
4	37.99	38.80	37.84	37.99	37.89	37.74	38.87
5	54.96	55.58	54.87	55.06	51.43	55.29	55.33
6	17.45	18.12	17.62	17.46	18.11	18.05	18.15
7	34.72	36.70	32.73	34.66	32.92	33.87	33.98
8	44.16	42.96	40.31	45.18	42.45[a]	40.98	40.59
9	58.92	44.85	54.70	60.83	153.97	49.90	51.33[a]
10	38.84	34.32	36.36	38.82	37.05	36.99	37.21
11	195.52	30.13	128.00	199.30	115.58[b]	20.68	21.60[b]
12	119.58	69.34	130.18	124.33	116.73[b]	26.78	27.01
13	142.48	40.92	87.74	163.13	142.17	36.43	39.82
14	43.31	41.34	44.90	43.79	42.36[a]	42.67	46.50
15	30.97	28.84	24.55	30.59	26.65	26.98	44.58
16	29.70	29.19	31.96	28.05	31.08	30.19	216.69
17	48.32	46.98	54.02	48.42	47.07	41.54	50.60[a]
18	50.61	45.27	52.57	51.06	50.63	46.79	53.78[a]
19	42.08	44.47	42.81	41.58	42.53	54.05	49.05
20	29.43	28.77	30.56	28.05	28.98	29.80	32.49
21	21.42	21.46	23.33	21.83	22.15	218.47	28.37[c]
22	34.07	34.38	32.57	33.89	34.36	52.17	31.51[c]
23	28.35	27.94	27.58	28.30	28.12	27.88	27.97
24	16.60	16.54[a]	16.49	16.61	16.74	16.26	15.40
25	19.81	15.71	17.90	18.43	17.77	16.00	16.09
26	28.04	16.56[a]	19.49	21.25	21.51	16.06	16.41
27	16.75	19.20	18.58	16.74	25.56	14.67	14.57
28	62.89	62.58	73.06	62.30	63.06	64.30	–
29	22.30	23.09	22.59	22.35	22.65	22.18	21.09[b]
30	15.09	14.89	16.09	15.56	15.51	16.45	18.97
OAc	171.25	171.64	170.97	171.03	171.40	170.93	–
	170.99	170.96	–	170.92	171.01	170.93	–
	21.31	21.31	21.32	21.16	21.31	21.25	–
	20.98	21.04	–	20.85	21.01	20.81	–

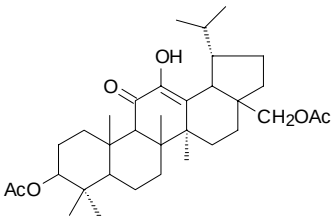
[a–c] Signals designated by the same letters may be interchanged.

the compounds **8** and **17** occur only in the case of signals of C-11, C-12, C-13 and C-26 (both the olefinic carbon atoms, ketone carbon atom and carbon atom of the 8β-methyl group). Similarly, in the ^{1}H NMR spectra of both compounds a significant difference was observed between the shift of singlet at δ 5.55 ppm for compound **17** and at 6.36 ppm for compound **8**. There is also difference in position of the H-19 signal which in derivative **8** is shifted to δ 2.86 whereas in derivative **17** remains in the envelope of skeletal proton signals. The signal at δ 6.36 in the spectrum of **8** disappeared on addition of tetradeuterioacetic acid. The mass spectrum showed that compound **8** contains one oxygen atom more than the compound **17**. On the basis of these facts compound **8** is the enol form of the 11,12-dioxo derivative with 12(13)-double bond.

Assuming that fractions *B* and *C* may contain various 3β,28-diacetoxylupanones, we reduced them. Since the reduction with sodium borohydride led to complex mixtures we tried the reduction with lithium aluminium hydride. Reduction of fraction *B*, followed by acidification of the reaction mixture, afforded three chromatographically separable products **2**, **10** and **14**. The most polar of them proved to be identical with lupane-3β,19β,28-triol (**2**), obtained already in ref.[2]. The second reduction product was assigned the structure of lupane-3β,12α,28-triol (**10**) on the basis of the following data. Upon acetylation with acetic anhydride in pyridine, compound **10** afforded acetate **11** whose 12-(trichloroacetylcarbamoyl) derivative **12** exhibits a considerable shift of both signals of isopropyl methyl groups (**11**: δ 0.86 and 0.88, **12**: δ 0.74 and 0.84). Diacetate **11** reacted with phosphorus oxychloride to give the known[6] 12-unsaturated derivative **13**. The ^{1}H NMR methyl signals of 12α-hydroxy derivative **11** are shifted relative to those of diacetate **1** by increments characteristic[10] of 12α-hydroxylupane derivatives.

The third reduction product, compound **14**, was acetylated to give acetate **15** and oxidized with sodium dichromate to afford ketone **16**. Proton NMR spectrum of acetate **15** exhibited an ABX system of two olefinic protons interacting with another proton, and further signals of protons in positions 3α and 28, indicating the presence of oxygen substituents at the C-3 and C-28 carbon atoms. The geminal coupling constant of the proton pair in position 28 (J = 8 Hz) shows that the atom C-28 is incorporated in a five-membered ether ring. ^{13}C NMR spectrum of acetate **15** indicates the presence of two olefinic carbon atoms, each bearing one proton (δ 128.0 and 130.2), and one oxygen-bearing quaternary carbon atom (δ 87.7). All the three compounds **14–16** have the same system of olefinic proton signals and protons in position 28, which indicates that the acetylation and oxidation took place only in position 3.

The above-mentioned data show that the compounds **14–16** are derivatives of 13β,28-epoxylup-11-ene. According to TLC, neither of these derivatives **14–16** was present in the chromatographic fraction *B* before reduction. However, the ^{1}H NMR spectrum of this fraction exhibits a signal at δ 5.55 which is close to the value 5.54 for 11-oxolup-12-en-28-yl acetate, described in the literature[11] (for 11-oxolup-12-en-3β-yl acetate, devoid of the spatially close 28-acetoxy group, ref.[12] reports a somewhat different value



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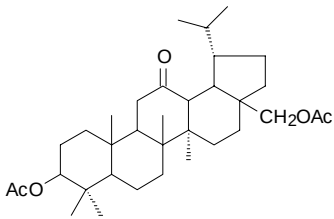

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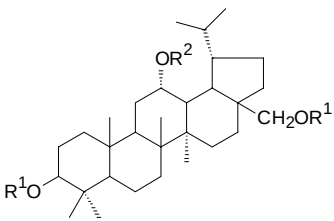
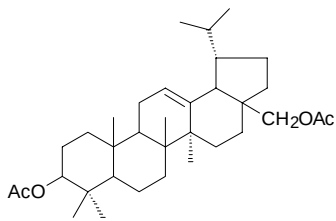
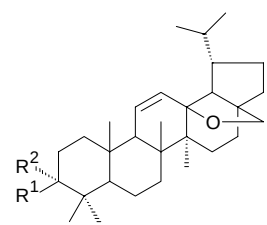
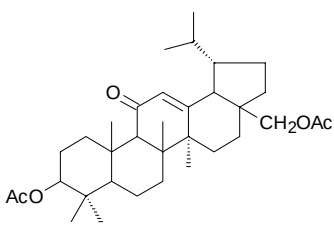




13

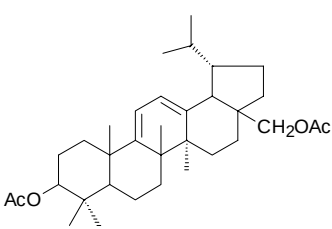
10, $R^1 = R^2 = H$
11, $R^1 = Ac$; $R^2 = H$
12, $R^1 = Ac$; $R^2 = CONHCOCCl_3$





17

14, $R^1 = OH$; $R^2 = H$
15, $R^1 = OAc$; $R^2 = H$
16, $R^1 + R^2 = O$



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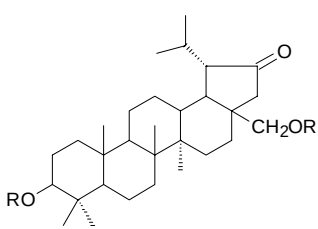


19, R = H
20, R = Ac

δ 5.38). Therefore, we prepared ketone **17** and studied its behaviour under conditions applied to fraction *B*.

Allylic oxidation of lup-12-ene derivative **13** with chromyl chloride under conditions analogous to those reported in ref.[12] afforded the unsaturated ketone **17** in 87% yield. Its structure follows from its preparation, from the NMR spectra and further from the mass spectra exhibiting ions *m/z* 331, 290 and 271 whose formation is described in ref.[11] for a similar 12-unsaturated ketone. Reduction of the unsaturated ketone **17** with lithium aluminium hydride and subsequent acidification of the reaction mixture (*i.e.* the same conditions as in the case of reduction of fraction *B*) gave epoxylupene **14** as the only product isolated. Thus, the formation of the 13β,28-epoxylupene derivative in the reduction of fraction *B* can be explained in the following way. Fraction *B* contains ketone **17** which is reduced to lup-12-ene-3β,11β,28-triol. In an acidic medium, this triol undergoes allylic rearrangement with forming of ether bridge between positions 13β and 28. Similar rearrangements were described for oleanane derivatives[13,14]. The unsaturated triol could not be detected even when the reaction mixture was worked up in a neutral medium.

Reaction of derivative **15** with acetic anhydride under catalysis with 4-toluenesulfonic acid afforded 9(11),12-diene **18** as the sole product. This fact is interesting because for similar reactions of oleanane derivatives formation of a mixture of 9(11),12- and 11,13(18)-dienes was reported[14,15]. The structure of the diene **18** follows from its UV spectrum (band at 277 nm, ε 11 200, characteristic of homoannular dienes), as well as from the ^{1}H NMR spectrum exhibiting an AB system of two olefinic protons with coupling constant 6.0 Hz. In the ^{13}C NMR spectrum of diene **18**, it has been shown by APT experiment that of the four olefinic carbon atoms (115.6, 116.7, 142.2 and 154.0) the first pair belongs to tertiary and the second pair to quaternary atoms. This again confirms the presence of a C=CH–CH=C grouping and the structure **18** for the diene obtained.

A part of the chromatographically homogeneous fraction *C* was reduced with lithium aluminium hydride; however, the thus-obtained mixture again could be separated neither chromatographically nor by crystallization. On the other hand, from the original fraction *C*, we obtained 21-oxo derivative **20** after precipitation with light petroleum, chromatography and crystallization. The structure of the derivative **20** was established on the basis of spectral data of **20** and the corresponding diol **19**. The mass spectra confirm the saturated ketone structure of **20** and **19** (M^+ *m/z* 542 and 458) and fragmentation leading to the ion *m/z* 189 indicates that the rings A and B are unchanged. The band at 1 730 cm^{-1} in the infrared spectrum of diol **19** is in accord with the presence of a five-membered ring; the same can be derived from the geminal coupling constant (16 Hz) of the $-CR_2-CH_2-CO-$ system in diacetate **20**. Isolation of the methylene group from other spin systems indicates the 21-oxo derivative structure. Decisive for the structural assigment proved to be the ^{13}C NMR spectrum in which, based on known effects of the

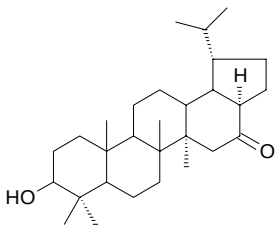
keto group on chemical shifts of the individual atoms in cyclic ketones, we were able to assign all the signals with only negligible differences. Comparison of the measured spectrum with the spectra estimated for keto groups in positions 6, 7, 12, 15, 16 and 22 shows, at least for two or three signals, differences greater than 8 ppm. The suggested structure is also supported by the presence of a cross peak in the ^{1}H,^{1}H-COSY spectrum of ketone **20** between one branch of the AB system of protons on C-28 and one proton in the methylene group adjacent to the keto group. This interaction is possible only in the 15-oxo and 21-oxo derivatives.

Alkaline hydrolysis of the mother liquors after crystallization of ketone **20** afforded a mixture from which we obtained, in addition to the dihydroxy ketone, a compound whose polarity corresponded to a monohydroxy ketone. We assigned it the structure 3β-hydroxy-28-nor-17α-lupan-16-one (**21**) on the basis of the following data. The infrared frequency ν(C=O) is 1 689 cm^{-1} which corresponds to the value found[6] for 28-nor-17α-lupan-16-one (**24**). Mass spectrum of compound **21** exhibits all ions (with the corresponding intensities) that can be expected in fragmentation of 28-norlupan-16-one derivatives according to the fragmentation schemes given in ref.[11]. In the ^{1}H NMR spectrum, there is an AB system of protons on C-15 with long-range coupling J(15α,17) = 1.5 Hz; on the other hand, an AB system of protons on C-28 is absent. The formation of the hydroxy ketone **21** in the hydrolysis can be explained by a retro-aldol reaction in which formaldehyde is eliminated from the carbon atom α to the 16-carbonyl group. Alkaline isomerization in position 17 leads to the product with *cis*-annelation of rings D and E. An analogous isomerization was observed also in the case of a similar 16-oxo derivative[6] in an acidic medium. The polarity of fractions, from which the hydroxy ketone **21** was obtained, corresponds to a ketone diacetate and thus the original mixture probably contained ketone **22**.

The configuration in position 17 in compound **21** was determined in the following way. The coupling constants of proton in position 17 (12.5, 6.9 and 6.8 Hz) indicate the configuration 17α-H with *cis*-annelation of rings D and E. However, ^{1}H,^{1}H-COSY spectrum shows that the 18α-proton and one of the protons in position 22 have almost the same chemical shift. Thus the coupling constant values may be false. Vystrcil and Protiva[6] describe a similar compound, 28-nor-16-lupanone (**24**) which differs only by the absence of acetoxy group in position 3β. The compound was assigned *cis*-annelation of rings D and E (17α-derivative), mainly on the basis of its CD spectrum. Our 16-oxolupane derivative **21** shows completely identical shifts of protons of the methyl groups C-26 and C-27, and in the side chain. We therefore assume that the annelation of rings D and E in the compound **24** and in our derivative **21** is the same, 17α. Recently, another compound with *cis*-annelation of rings D and E, 17α-lup-20(29)-en-3β-yl acetate (**25**), has been described[16]. On the basis of molecular modelling two energy minima have been found for this compound, both with a boat form of ring D. If the ring D in 17α-lupane derivatives exists indeed in a boat form, this means that the configura-

tion derived[6] from the CD spectrum is not reliable and cannot be used for assignment of configuration of our compound **21**.

We therefore carried out a conformational analysis for the pair of isomers differing in configuration at C-17, 28-norlupan-16-one (**23**) and 28-nor-17α-lupan-16-one (**24**). For each isomer we found several energy minima corresponding to different conformations of the ring D and of the side chain. Energies of the most advantageous chair and boat conformations of the ring D in both isomers **23** and **24** are given in Table II. All the three force fields used (Dreiding II, Amber B and MM2) show that the *cis*-fusion of the rings D and E (17α-H isomer **23**) is substantially more stable than the *trans*-annelation (17β-H isomer **24**). At the same time, the computations show that in both isomers **23** and **24** the chair form of the ring D is preferred.



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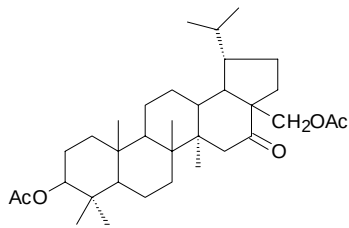

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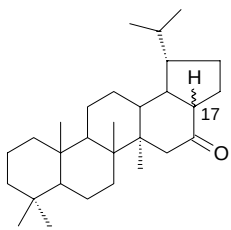
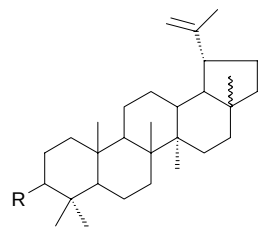


23, 17β-H (*trans*)
24, 17α-H (*cis*)



25, 17α-CH_3 (*cis*) R = OAc
26, 17β-CH_3 (*trans*) R = H
27, 17α-CH_3 (*cis*) R = H

In order to compare and verify the results in ref.[16], we performed energy minima calculations for isomeric 20(29)-lupenes with different configuration in position 17 (compounds **26** and **27**). Compound **27** is an analogue of the derivative modelled in ref.[16]. The results are again summarized in Table II and as concerns the conformation of the ring D and stability of both epimers they are similar to those found for the isomeric pair **23** and **24**. Its is likely that the authors found only local minima instead of the global ones. Further details about the molecular modelling results will be published elsewhere.

The results of the modelling support the assumption that compound **21** has 17α-H configuration. All the lupane derivatives studied prefer the chair conformation of ring D which is in accord with the CD-spectral results published in ref.[6]. Moreover, the marked energy difference between *cis*- and *trans*-annelated rings D and E indicates that in the case of isomerization in this position (α to the keto group), the mixture at thermodynamic equilibrium will contain almost exclusively the 17α-derivative.

In conclusion, we can summarize the results of this and the previous[2,5] papers. In the oxidation of lupane-3β,28-diyl diacetate (**1**) with selective radical reagents, the 19β position is attacked exclusively. The use of a little selective functionalization reagent, chromium(VI) oxide, leads again to predominant attack in position 19β under formation of 19β-hydroxy derivative **3** and its dehydration and subsequent oxidation products, ketone **4** and anhydride **5**. In addition, there is some oxidation in position 3 (formation of ketone **7**) and attack in positions 11, 12, 16 and 21 in the rings C, D and E leading to products **6**, **8**, **9**, **17**, **20** and **22** which were isolated from the reaction mixture either directly or after conversion into other compounds.

EXPERIMENTAL

Melting points were determined on a Kofler block and are uncorrected. Optical rotations were measured in chloroform on an automatic polarimeter ETL-NPL (Bendix–Ericsson) with accuracy ±2° (*c* 0.4–0.8). Infrared spectra were recorded in chloroform on a PE 684 (Perkin–Elmer) instrument.

TABLE II
The lowest energy conformers of compounds **23**, **24**, **26** and **27** with ring D in chair and boat conformation

Compound	D-ring conformation	Energy, kcal mol^{-1}[a] force field		
		Dreiding II	Amber B	MM2+
23	Chair	143.28	42.60	124.45
	Boat	[b]	50.29	[b]
24	Chair	138.32	37.94	121.85
	Boat	146.09	43.41	127.98
26	Chair	151.85	44.01	130.92
	Boat	[b]	[b]	138.97
27	Chair	150.04	42.10	127.58
	Boat	152.14	43.61	129.12

[a] 1 kcal = 0.239 kJ. [b] Energy minimum not found.

Wavenumbers are given in cm^{-1}. ^1H NMR spectra were measured on a Tesla BS 487A instrument (80 MHz, FT mode) in deuteriochloroform with hexamethyldisiloxane as internal standard (chemical shifts were referenced to tetramethylsilane using the value δ(HMDS) 0.063 ppm and rounded to two decimal places) or on Varian XL-200 (at 200 MHz, FT mode) and Varian Unity-500 (at 500 MHz, FT mode) instruments in deuteriochloroform with tetramethylsilane as internal standard. COSY spectra were measured on a Varian-Unity 500 (500 MHz) spectrometer, using standard pulse sequence and absolute mode. Chemical shifts (δ-scale, ppm) and coupling constants (Hz) were obtained by first order analysis. ^{13}C NMR spectra were measured on a Varian XL-200 (50.31 MHz, "attached proton test" technique) in deuteriochloroform. Chemical shifts were referenced to the solvent signal and calculated from the value δ($CDCl_3$) 77.00 ppm. Mass spectra were measured on Varian MAT 311 and AEI MS 902 instruments, ionizing electron energy 70 eV, direct inlet temperatures ranged between 150 and 180 °C; data are given in the *m/z* (%) formate. Ultraviolet spectrum was measured on a Unicam SP-700 instrument in cyclohexane. Calculation of the most stable conformers of 28-nor-lupan-16-one (**23**), 28-nor-17α-lupan-16-one (**24**), lup-20(29)-ene (**26**) and 17α-lup-20(29)-ene (**27**) was performed using the Biograf program (version 3.2.1, Molecular Simulations) on a Sparc 4 computer. For each structure 300 different starting conformations were generated, differing in the orientation of the side chain and torsion angles in rings D and E. The structures were minimized using the Dreiding II, Amber B and MM2+ force fields. The conjugate gradient method for minimizations was used to convergence (less than 0.05 kcal mol^{-1} RMS force). Further details of the method used will be published elsewhere.

The identity of samples prepared by different methods was verified by thin-layer chromatography and IR and ^1H NMR spectra. Thin-layer chromatography was performed on plates of silica gel G (Merck) with detection by spraying with 10% sulfuric acid and subsequent heating or on Silufol foils (Kavalier, Votice), detection by spraying with 5% ethanolic phosphomolybdic acid followed by heating. Preparative thin-layer chromatography was carried out on Kieselgel 60G (Merck), column chromatography was run on silica gel Silpearl (Kavalier, Votice). "The usual workup" means pouring the reaction mixture into water, extraction of the product with ether and washing the ethereal layer successively with water, dilute hydrochloric acid (1 : 4), water, saturated solution of sodium hydrogen carbonate and again water, drying over sodium sulfate and evaporation of the solvent under diminished pressure. Analytical samples were dried *in vacuo* over phosphorus pentoxide at room temperature.

Yields of the products obtained from fractions *A*, *B* and *C*, either directly or after reduction, are related to the amount of the starting diacetate **1** (40 g, see ref.[2]).

12-Hydroxy-11-oxolup-12-en-3β,28-diyl Diacetate (**8**)

Fraction *A* (0.12 g), obtained by separation of oxidation products from diacetate **1** (40 g, see ref.[2]), was rechromatographed on a plate of silica gel (10 g; four developments in light petroleum–ether 10 : 1). The main zone upon crystallization from ethanol afforded diosphenol **8** (43 mg, 0.1%) m.p. 233–235 °C, $[\alpha]_D$ +19°. IR spectrum: 3 400 (OH); 1 728, 1 255 (OAc); 1 672, 1 634 (diosphenol); 1 031 (C–O–C). ^1H NMR spectrum (200 MHz): 0.75 d, 3 H, J = 6.6; 0.86 d, 3 H, J = 6.8, 0.88 s, 3 H, 0.89 s, 3 H, 1.18 s, 3 H, 1.20 s, 3 H and 1.31 s, 3 H (7 × CH_3); 2.05 s, 3 H and 2.06 s, 3 H (2 × OAc); 2.20 bd, 1 H, J(18,19) = 11.4 (H-18); 2.37 bs, 1 H (H-9); 2.69 dt, 1 H, J(gem) = 13.4, J(1,2) = 3.6 and 3.6 (H-1β); 2.86 tdd, 1 H, J = 11.1, 11.1, 5.0 and 3.5 (H-19); 3.90 bd, 1 H and 3.98 dd, 1 H, J(gem) = 11.6, J(long-range) = 2.0 (2 × H-28); 4.52 m, 1 H, ΣJ = 16 (H-3α); 6.36 s, 1 H (OH-12, disappeared on addition of CD_3CO_2D). Mass spectrum: 556 (M^+, 18), 541 (8), 496 (39), 481 (24), 468 (16), 453 (22), 277 (16), 217 (32), 203 (28), 191 (52), 189 (100).

13β,28-Epoxylup-11-en-3β-ol (**14**) and Lupane-3β,12α,28-triol (**10**)

A part (600 mg) of fraction *B* (2.5 g; obtained by separation of the oxidation products of diacetate **1**, see ref.[2]) was dissolved in anhydrous ether (50 ml). To this solution was added lithium aluminium hydride (100 mg) and the mixture was refluxed for 20 min. Ethyl acetate was added dropwise and the reaction mixture was poured into a mixture of ice and hydrochloric acid (5 : 1). The products were taken up in ether and the ethereal layer was worked up in the usual manner. The residue (480 mg) was chromatographed on a column of silica gel (50 g) in light petroleum–ether (10 : 1). The chromatography afforded successively the following compounds.

13β,28-Epoxylup-11-en-3β-ol (**14**), yield 160 mg (2.0%), m.p. 205–207 °C (ethanol), $[\alpha]_D$ +37°. IR spectrum: 3 613 (OH); 1 017 (C–O–C). ^{1}H NMR spectrum (80 MHz): 0.78 s, 3 H, 0.81 d, 3 H, J = 6, 0.88 d, 3 H, J = 6, 0.91 s, 3 H, 0.95 s, 3 H, 0.98 s, 3 H and 1.09 s, 3 H (7 × CH_3); 3.20 m, 1 H, ΣJ = 16 (H-3α); 3.49 bs, 2 H (2 × H-28); 5.64 d, 1 H, J = 11; 5.76 dd, 1 H, J = 11 and 1.2 (H-11 and H-12). Mass spectrum: 440 (M^+, 100), 425 (18), 407 (20), 397 (21), 357 (21), 189 (41). For $C_{30}H_{48}O_2$ (440.7) calculated: 81.76% C, 10.98% H; found: 81.52% C, 10.75% H. When the reduction and the above-described work-up was performed with the unsaturated ketone **17** (60 mg), the reaction afforded epoxylupene **14** (83 % yield after crystallization from chloroform–ethanol) which was identical with the sample obtained above.

Acetate **15** was prepared by acetylation of compound **14** with acetic anhydride in pyridine at room temperature for 24 h and was crystallized from methanol; m.p. 192–194 °C, $[\alpha]_D$ +49°. IR spectrum: 1 721 and 1 259 (OAc); 1 017 (C–O–C). ^{1}H NMR spectrum (200 MHz): 0.81 d, 3 H, J = 6.6, 0.86 s, 3 H, 0.86 s, 3 H, 0.88 d, 3 H, J = 6.9, 0.92 s, 3 H, 0.94 s, 3 H and 1.09 s, 3 H (7 × CH_3); 1.82 m, 1 H (H-9); 1.97 d, 1 H, J = 9.5 (H-18); 2.05 s, 3 H (OAc); 3.48 dd, 1 H and 3.50 d, 1 H, J(gem) = 8.0, J(long-range) = 1.1 (2 × H-28); 4.49 m, 1 H, ΣJ = 16 (H-3α); 5.65 dd, 1 H, J = 10.6 and 2.8 and 5.76 dd, 1 H, J = 10.6 and 1.3 (H-11 and H-12). Mass spectrum: 482 (M^+, 100), 467 (8), 439 (23), 422 (52), 407 (15), 399 (21), 332 (27), 302 (15), 299 (30), 281 (14), 257 (21), 255 (17), 229 (27). For $C_{32}H_{50}O_3$ (482.7) calculated: 79.62% C, 10.44% H; found: 79.93% C, 10.31% H.

Lupane-3β,12α,28-triol (**10**), yield 210 mg (2.5%), m.p. 290–293 °C (chloroform–acetone), $[\alpha]_D$ –13°. IR spectrum: 3 605 (OH), 1 018. Mass spectrum: 460 (M^+, 2.5), 442 (13), 411 (20), 393 (13), 207 (59), 204 (51), 203 (51), 191 (55), 189 (100), 177 (74). For $C_{30}H_{52}O_3$ (460.7) calculated: 78.20% C, 11.38% H; found: 78.43% C, 11.50% H.

3,28-Diacetate **11** was prepared by acetylation of triol **10** with acetic anhydride in pyridine at room temperature for 24 h and crystallization from methanol: m.p. 276–278 °C, $[\alpha]_D$ –1°. IR spectrum: 3 618 (OH); 1 721 and 1 256 (OAc); 1 030 (C–O–C). ^{1}H NMR spectrum (200 MHz): 0.85 s, 3 H, 0.86 s, 3 H, 0.86 d, 3 H, J = 6.6, 0.88 d, 3 H, J = 6.6, 0.91 s, 3 H, 1.05 s, 3 H and 1.21 s, 3 H (7 × CH_3); 2.04 s, 3 H and 2.06 s, 3 H (2 × OAc); 3.99 m, 1 H, ΣJ = 12 (H-12β); 3.84 d, 1 H and 4.19 dd, 1 H, J(gem) = 11.2, J(long-range) = 1.5 (2 × H-28); 4.49 m, 1 H, ΣJ = 16 (H-3α). ^{1}H NMR spectrum of trichloroacetyl carbamate **12**, prepared by reaction with trichloroacetyl isocyanate in NMR cell (200 MHz): 0.74 d, 3 H, J = 6.8, 0.84 d, 3 H, J = 6.8 , 0.85 s, 3 H, 0.86 s, 3 H, 0.89 s, 3 H, 1.08 s, 3 H and 1.28 s, 3 H (7 × CH_3); 2.04 s, 3 H and 2.07 s, 3 H (2 × OAc); 3.84 d, 1 H and 4.20 d, 1 H, J(gem) = 11.2 (2 × H-28); 4.49 m, 1 H, Σ J = 16 (H-3α); 5.03 m, 1 H, Σ J = 7 (H-12β); 8.20 s, 1 H (NH). Mass spectrum of diacetate **11**: 544 (M^+, 1.5), 526 (5), 484 (8), 466 (8), 441 (7), 393 (7), 391 (6), 381 (6), 363 (5), 294 (8), 264 (22), 216 (16), 204 (80), 203 (44), 189 (100). For $C_{34}H_{56}O_5$ (544.8) calculated: 74.95% C, 10.36% H; found: 75.08% C, 10.48% H.

Lupane-3β,19β,28-triol (**2**), yield 10 mg (0.03%), m.p. 290 °C, identical with the compound obtained in ref.[2].

Lup-12-ene-3β,28-diyl Diacetate (**13**)

To a solution of diacetate **11** (60 mg, 0.11 mmol) in pyridine (3 ml) was added phosphorus oxychloride (0.3 ml, 3.3 mmol). The mixture was refluxed for 10 min, cooled, poured on a mixture of ice (30 g) and hydrochloric acid (5 ml) and worked up in the usual manner. Crystallization of the product from ethyl acetate afforded **13** (40 mg, 70%), m.p. 203–204 °C, identical with an authentic sample described in ref.[6] .

11-Oxolup-12-ene-3β,28-diyl Diacetate (**17**)

A solution of diacetate **13** (100 mg, 0.19 mmol) in acetone (25 ml) was cooled to –70 °C and freshly prepared chromyl chloride (250 mg, 1.61 mmol) was added at this temperature. The mixture was stirred for 1 h, warmed to room temperature and stirred for another 1 h. Methanol (10 ml) was added and the reaction mixture was worked up in the usual manner to give unsaturated ketone **17** (89 mg, 87%), m.p. 223–224 °C (ether–methanol), $[\alpha]_D$ +4.5°. IR spectrum: 1 724 and 1 252 (OAc); 1 668 (C=O); 1 635 (C=C). ^{1}H NMR spectrum (200 MHz): 0.75 d, 3 H, J = 6.6, 0.88 d, 3 H, J = 6.8, 0.88 s, 3 H, 0.89 s, 3 H, 1.17 s, 3 H, 1.23 s, 3 H and 1.34 s, 3 H (7 × CH_3); 2.04 s, 3 H and 2.05 s, 3 H (2 × OAc); 2.23 bdd, 1 H, J(18,19) = 10.8, J(12,18) = 1.7 (H-18); 2.30 bs, 1 H (H-9); 2.69 dt, 1 H, J(gem) = 13.4, J(1,2) = 3.5 and 3.5 (H-1β); 3.74 bd, 1 H and 3.88 dd, 1 H, J(gem) = 11.2, J(long-range) = 2.0 (2 × H-28); 4.54 m, 1 H, ΣJ = 16 (H-3α); 5.55 d, 1 H, J(12,18) = 1.7 (H-12). UV spectrum: λ_{max} 237 nm, ε 9 400. Mass spectrum: 540 (M^+, 4), 525 (1), 480 (7), 465 (7), 437 (2), 331 (100), 290 (12), 271 (9). For $C_{34}H_{52}O_5$ (540.8) calculated: 75.51% C, 9.69% H; found: 75.33% C, 9.81% H.

13β,28-Epoxy-11-lupen-3-one (**16**)

A solution of hydroxy derivative **14** (30 mg, 0.07 mmol), sodium dichromate (30 mg, 0.10 mmol) and sodium acetate (100 mg, 1.22 mmol) in acetic acid (10 ml) was allowed to stand at room temperature for 24 h and then diluted with water. The products were taken up in ether and the ethereal layer was worked up in the usual manner. The residue was crystallized from chloroform–methanol to give ketone **16** (19 mg, 63%), m.p. 186–189 °C, $[\alpha]_D$ +30°. IR spectrum: 1 703 (C=O), 1 017 (C–O–C). ^{1}H NMR spectrum (200 MHz): 0.81 d, 3 H, J = 6.6, 0.89 d, 3 H, J = 6.8, 0.96 s, 3 H, 1.03 s, 3 H, 1.04 s, 3 H, 1.09 s, 3 H and 1.13 s, 3 H (7 × CH_3); 1.98 d, 1 H, J(18,19) = 9.5 (H-18); 2.07 ddd, 1 H, J = 12.9, 7.2 and 3.9; 2.12 m, 1 H (H-19); 2.3–2.7 m, 2 H (2 × H-2); 3.49 dd, 1 H and 3.51 bd, 1 H, J(gem) = 7.8, J(long-range) = 1.3 (2 × H-28); 5.68 dd, 1 H, J = 10.6 and 2.6 and 5.77 dd, 1 H, J = 10.6 and 1.3 (11-H and 12-H). Mass spectrum: 438 (M^+, 100), 395 (40), 355 (32), 341 (38), 288 (35), 273 (38), 243 (38), 229 (34), 203 (31), 189 (48). For $C_{30}H_{46}O_2$ (438.7) calculated: 82.13% C, 10.57% H; found: 82.5% C, 10.39% H.

Lupa-9(11),12-diene-3β,28-diyl Diacetate (**18**)

A mixture of acetate **15** (150 mg, 0.31 mmol), 4-toluenesulfonic acid monohydrate (20 mg, 0.11 mmol) and acetic anhydride (2 ml, 18.0 mmol) was heated at 100 °C for 2 h, then poured on ice, the products were taken up in ether and the ethereal layer was worked up in the usual manner. The residue was purified by chromatography on a column of silica gel (10 g) in light petroleum–ether (20 : 1). Crystallization from ethyl acetate afforded diene **18** (120 mg, 75%), m.p. 198–199 °C, $[\alpha]_D$ +23°. IR spectrum: 1 732, 1 255 (OAc). ^{1}H NMR spectrum (200 MHz): 0.76 d, 3 H, J = 6.6, 0.88 s, 3 H, 0.89 s, 3 H, 0.91 s, 3 H, 0.91 d, 3 H, J = 6.6, 1.16 s, 3 H and 1.25 s, 3 H (7 × CH_3); 2.05 s, 3 H and 2.06 s, 3 H (2 × OAc); 3.83 d, 1 H and 3.97 dd, 1 H, J(gem) = 11.6, J(long-range) = 1.9 (2 × H-28); 4.52 m, 1 H, ΣJ = 16 (H-3α); 5.49 dd, 1 H, J(11,12) = 6.0, J(12,13) = 2.4 (H-12); 5.62 bd, 1 H, J(11,12) = 6.0 (H-11). UV spectrum: λ_{max} 277 nm, ε 11 200. Mass spectrum: 524 (M^+, 100), 509 (3), 464 (7), 449

(39), 389 (21), 365 (17), 339 (25), 327 (9), 313 (43), 253 (58), 251 (20). For $C_{34}H_{52}O_4$ (524.8) calculated: 77.82% C, 9.99% H; found: 77.67% C, 10.10% H.

21-Oxolupane-3β,28-diyl Diacetate (**20**) and 3β-Hydroxy-28-nor-17α-lupan-16-one (**21**)

A part (2 g) of fraction *C* (3.8 g) obtained by separation of the oxidation product od diacetate **1** (40 g, see ref.[2]) was dissolved in chloroform (2 ml), and the solution was mixed with light petroleum (20 ml). The precipitate was removed and the solution was chromatographed on a column of silica gel (150 g) in light petroleum–ether (10 : 1). After elution with 2 l of the solvent mixture, the products were eluted in 8 fractions à 40 ml. The last three fractions were combined and repeatedly crystallized from chloroform–ethanol to give 21-oxo derivative **20** (230 mg, 1.1%), m.p. 250–254 °C, $[\alpha]_D$ +46°. IR spectrum: 1 730 and 1 251 (C=O); 1 033, 979. ^{1}H NMR spectrum (500 MHz): 0.82 d, 3 H, J = 7.0, 0.85 s, 3 H, 0.86 s, 3 H, 0.88 s, 3 H, 1.05 s, 3 H, 1.08 s, 3 H and 1.15 d, 3 H, J = 7.1 (7 × CH_3); 2.02 s, 3 H and 2.05 s, 3 H (2 × OAc); 1.80 bd, 1 H and 2.37 d, 1 H, J(gem) = 16.4 (2 × H-22); 3.73 d, 1 H and 4.38 dd, 1 H, J(gem) = 11.5, J(long-range) = 1.5 (2 × H-28); 4.48 m, 1 H, ΣJ = 16 (H-3α). Mass spectrum: 542 (M^+, 4), 499 (3), 482 (38), 467 (18), 439 (23), 407 (4), 400 (8), 397 (2), 379 (5), 303 (5), 293 (7), 277 (12), 249 (5), 217 (11), 203 (23), 189 (100).

The mother liquors and chromatographic fractions, from which no diacetate **20** was obtained, were combined (0.7 g) and hydrolyzed by refluxing with potassium hydroxide (500 mg, 8.9 mmol) in ethanol (10 ml) for 1 h. After the usual work-up, the product mixture was chromatographed on a silica gel column (100 g) in light petroleum–ether (2 : 1). The first isolated compound was 3β-hydroxy-28-nor-17α-lupan-16-one (**21**), yield 60 mg (0.4%), m.p. 209–211 °C (chloroform–methanol), $[\alpha]_D$ –30°. IR spectrum: 3 614 (OH); 1 689 (C=O). ^{1}H NMR spectrum (500 MHz): 0.77 s, 3 H, 0.82 d, 3 H, J = 6.5, 0.86 s, 3 H, 0.86 d, 3 H, J = 7.5, 0.87 d, 3 H, J = 1.3, 0.98 s, 3 H and 1.02 s, 3 H (7 × CH_3); 1.51 octet, 1 H, J = 6.5 (H-19); 1.93 dd, 1 H, J(gem) = 13.7, J(15α,17) = 1.5 (H-15α); 2.43 dddd, 1 H, J(17,22β) = 12.5, J(17,18) = 6.8, J(17,22α) = 6.9, J(15α,17) = 1.5 (H-17); 2.50 dq, 1 H, J(gem) = 13.7, J(15β,27) = 1.3 (H-15β); 3.20 dd, 1 H, J = 11.6 and J = 4.8 (H-3α). Mass spectrum: 428 (M^+, 74), 410 (80), 395 (45), 367 (28), 345 (13), 328 (9), 301 (8), 283 (7), 231 (6), 218 (13), 207 (69), 189 (100).

Further elution with the same solvent mixture afforded 20 mg of an unidentified compound and then 3β,28-dihydroxylupan-21-one (**19**), 95 mg (0.5%), m.p. 300–302 °C (chloroform–methanol), $[\alpha]_D$ +62°. IR spectrum: 3 621 (OH); 1 727 (C=O); 1 027, 982. ^{1}H NMR spectrum (80 MHz): 0.77 s, 3 H, 0.81 d, 3 H, J = 7, 0.85 s, 3 H, 0.98 s, 3 H, 1.05 s, 3 H, 1.07 s, 3 H and 1.15 d, 3 H, J = 7 (7 × CH_3); 2.45 d, 1 H, J = 16.5 (H-22); 3.21 m, 1 H, ΣJ = 16 (H-3α); 3.43 d, 1 H and 3.82 d, 1 H, J = 11 (2 × H-28). Mass spectrum: 458 (M^+, 2), 443 (25), 440 (3), 425 (2), 415 (26), 400 (12), 397 (7), 370 (12), 342 (21), 340 (25), 312 (100), 297 (42), 281 (30), 207 (21), 191 (21), 189 (26), 187 (22).

Hydrolysis of diacetate **20** (100 mg, 0.18 mmol) by boiling with ethanolic potassium hydroxide (50 mg, 0.89 mmol) afforded diol **19** (60 mg, 71%), identical with the compound prepared above.

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