PREPARATION AND CONFORMATIONAL STUDY OF B-RING SUBSTITUTED LUPANE DERIVATIVES+

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Dedicated to Professor Antonín Holý on the occasion of his 70th birthday.

New lupane-type triterpenoids with 5(6) double bond were prepared using the method of partial demethylation on carbon C-4. Hydroboration of the double bond led to 6α-hydroxy derivative. By the oxidation and following reduction of 6α-hydroxy derivative the 6-oxo and 6β-hydroxy derivatives were prepared. A new method for selective oxidation of secondary hydroxy group in the presence of primary hydroxy group was performed. The conformation of ring A of new lupane-type 3-oxo derivatives with a substituent on ring B was elucidated on the bases of ^{1}H and ^{13}C NMR spectra and molecular modelling.

Keywords: Triterpenoids; Triterpenes; Hydroboration; Oxidation; Lupane; NMR spectroscopy; Conformation analysis.

Triterpenes are common constituents of higher plants[2]. Recently, interesting anti-HIV and anti-cancer activities have been found for some of them (e.g. refs[3–5]). Most of these compounds perspective for medical use are derived from the lupane skeleton. B-ring substituted lupane derivatives are quite rare in the nature and no synthetic route leading to them has been published yet. Isolations of 41 natural products with the B-ring substituted lupane skeleton have been published so far[6–36]. Some of them show interesting biological activities, for example antibacterial[10,35], hepatoprotective[37]

+ Part CXIV in the series Triterpenes; for part CXIII see ref.[1]

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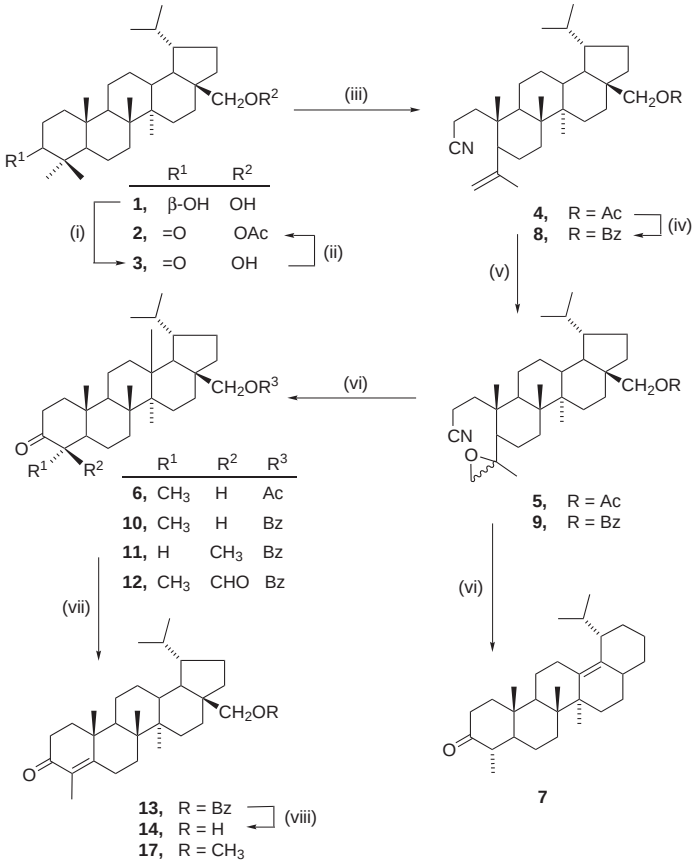
and cytostatic[13,30]. An appropriate starting compound with lupane skeleton is betulin, which is easy to isolate from birch bark.

A method of partial demethylation at carbon C-4 followed by methylation of this carbon with simultaneous introduction of double bond to position 5 was chosen. For the preparation of B-ring substituted lupane derivatives, dihydrobetulin (**1**) was converted in ten reaction steps to unsaturated norketone **14**. 3-Oxolupane-28-yl acetate (**2**) was the key intermediate of this reaction route (Scheme 1). There are two possible routes for prepara-



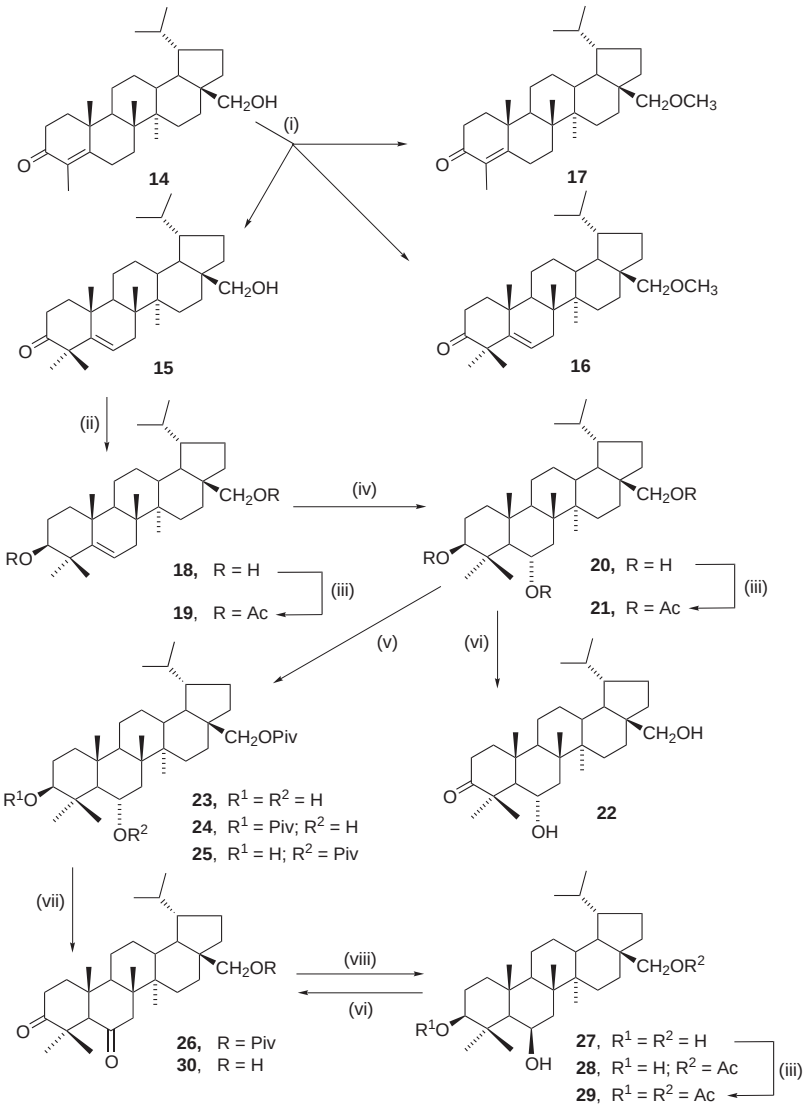
(i) Br_2, Py, –24 °C; (ii) Ac_2O, Py; (iii) see ref.[39]; (iv) 1. NaOH, EtOH 2. benzoyl chloride, Py; (v) 3-chloroperoxybenzoic acid, $CHCl_3$, 0 °C; (vi) 1. BF_3Et_2O, 2. 2 M HCl; (vii) HBr, AcOH, Br_2; (viii) KOH, EtOH

SCHEME 1

tion of this acetoxyketone from dihydrobetulin (**1**). The first consists in selective acetylation of 28-hydroxy group and subsequent oxidation of the 3β-hydroxy group. This partial acetylation is known[38] but the yields are quite poor and the product must be purified by column chromatography. We decided to use the other pathway: selective oxidation of the secondary 3β-hydroxy group and subsequent acetylation of the hydroxy group in position 28. As an oxidation agent we chose aqueous solution of sodium hypochlorite in acetic acid. The selectivity of the oxidation reaction at room temperature was good but the low solubility of diol **1** required huge amounts of acetic acid. When the reaction was carried out at higher temperatures, the solubility of the starting compound was higher but the selectivity was lower. Another oxidation agent was bromine in pyridine. Best results were achieved when the reaction was carried at –24 °C. The selectivity of this reaction was high and the preparative yield of 28-hydroxylupan-3-one (**3**) was higher than 90%. The hydroxyketone **3** was acetylated to acetoxyketone **2**. The conversion of the compound **2** to seconitrile **4** was performed according to ref.[39] The next step, epoxidation of seconitrile **4**, provided a mixture of 4*R* and 4*S* epoxy derivatives **5**. The further reaction was closure of ring A and decarbonylation, with norketone **6** as the expected product. This reaction is known in steroid chemistry[40] and it was successfully used also for preparation of oleanane derivatives[41]. We applied this reaction to the epoxy derivatives **5** but the yields of 3-oxo-24-norlupan-28-yl acetate (**6**) were quite low (15%) since the solvolysis of acetoxy group in position 28 and further rearrangements of ring E led to product **7**. Its structure was confirmed by IR, MS and 2D-NMR spectroscopy. When other Lewis acid ($AlCl_3$, $FeCl_3$, $ZnCl_2$) was used, no traces of norketone **6** were found in the reaction mixture. We decided to use another protective group of the hydroxy group in the position 28. Acetoxyseconitrile **4** gave benzoylseconitrile **8** after alkaline hydrolysis and reaction with benzoyl chloride. Seconitrile **8** reacts with 3-chloroperoxybenzoic acid to give a mixture of epoxides **9**. When a mixture of epoxynitriles **9** was refluxed in toluene with boron trifluoride etherate only small traces of products with rearranged ring E appeared. The reaction mixture contained two main products. The first product was 3-oxo-24-norlupan-28-yl benzoate (**10**), whose structure was confirmed by mass, infrared and NMR spectroscopy (in ^{1}H NMR spectrum a doublet of 4α-methyl group at δ 0.98). The signals of all hydrogens and carbons of compound **10** were assigned using two-dimensional NMR techniques (Tables I and IV). Attempts to isolate the second product failed because it is transformed to norketone **10** during chromatography. ^{1}H NMR data of this product were obtained from the spectrum

of the reaction mixture, suggested structure of the by-product is 4β isomer (**11**) of norketone **10** (doublet of 4β-methyl group at δ 1.11). Steric interaction of the 4β-methyl group with the 10β-methyl group can decrease the thermodynamic stability of compound **11** and explains its easy isomerisation to norketone **10**. A small amount of 3,24-dioxolupan-28-yl benzoate (**12**) was also isolated from the reaction mixture. Its structure was confirmed by mass, infrared and NMR spectroscopy (see Tables I and IV). In ^{1}H NMR spectrum a signal of aldehydic hydrogen (δ 9.71) appears; signals of all hydrogens and carbons of compound **12** were assigned using 2D-NMR techniques and the 4β position of the aldehydic group was confirmed by NOESY spectrum. Norketone **10** was brominated and then dehydrobrominated (without isolation of the bromo derivative) to give unsaturated norketone **13**. The benzoyl ester **13** was hydrolysed to the unsaturated ketone **14**.

It is known[42] that methylation of 3-oxosteroid derivatives with methyl iodide after enolisation with potassium *tert*-butyl alcoholate takes place preferentially at the C-4 and mixtures of 4-monomethyl and 4,4-dimethyl derivatives are usually obtained. This reaction was also applied to an oleanane derivative[1]. It was shown that neither prolongation of the reaction with potassium *tert*-butoxide nor prolongation of the methylation increased the yields of the 4,4-dimethyl derivative. When higher concentrations of potassium *tert*-butoxide and methyl iodide were used, by products with one or two methyl groups in position 2 appeared[1]. Methylation of norketone **14** with methyl iodide (Scheme 2) after refluxing with potassium *tert*-butyl alcoholate gave a mixture of four compounds: starting ketone **14** (38%), 4,4-dimethyl ketone with double bond in position 5 (**15**, 34%) and small amounts of two analogous compounds with methoxy group in position 28 (**16**, 5% and **17**, 5%). Ketone **15** was reduced with sodium borohydride and the resulting dihydroxy derivative **18** was acetylated to diacetoxy derivative **19**. In IR spectra of 3-oxo derivatives **15** and **16** one can find a weak absorption band of C=C valence vibration at 1666 cm^{-1}, hydroxy derivative **18** and acetoxy derivative **19** have this band very weak. Double bond in position 5 gives in ^{13}C NMR spectra of all derivatives two signals – singlet at δ 145–147 (C-5) and doublet at δ 119–121 (C-6). In ^{1}H NMR spectra H-6 appears as doublet of doublets at δ 5.50 with coupling constants J(6,7β) = 5–6 and J(6,7α) = 2–3 Hz, signal of the axial H-7α is easy to identify at δ 2.20 as a very broad doublet with large geminal coupling constant (17–18 Hz). Full assignment of signals of all hydrogens and carbons of compounds **15**, **16** and **18** was made using 2D-NMR techniques (Tables I, II and V). In the mass spectra of ketones **15** and **16** an ion at *m/z*



(i) 1. *t*-BuOK, Δ, Ar, 2. MeI; (ii) $NaBH_4$, C_6H_6, MeOH; (iii) Ac_2O, Py; (iv) 1. BH_3, THF, Δp, 2. NaOH, H_2O; (v) pivaloyl chloride, Py, –10 °C; (vi) Br_2, Py, –24 °C; (vii) CrO_3, Py; (viii) 1. $LiAlH_4$, Et_2O, 2. AcOEt

SCHEME 2

124 appears, this ion was found also in some 4,4-dimethyl steroid[43] and triterpenoid derivatives[1,7] with double bond at position 5.

The double bond in the position 5 can be used for introduction of various substituents into the ring B. In previously published papers the allylic oxidation[44], allylic bromination[1] and epoxidation[1,44] of triterpenoids with double bond in position 5 is described. In this work we studied the hydroboration of olefin **18**, that was prepared by reduction of ketone **15**. The hydroboration did not work under normal conditions. When performed at 80 °C in the pressure vial then after 8 h working up with hydrogen peroxide in alkaline medium provided lupane-3β,6α,28-triol (**20**) as the only product. The structure of this compound follows from full assignment of ^{1}H and ^{13}C NMR chemical shifts using HSQC and HMBC techniques (see Tables II and V). The stereochemistry was confirmed by analysis of *J* couplings and NOESY spectrum. In ^{1}H NMR spectrum proton H(6) appears as a triplet of doublets at δ 4.05 with two large (almost identical) vicinal coupling constants (10.6 Hz) and one smaller (4.0 Hz). The ^{3}J ~ 10.6 Hz indicates *trans*-diaxial coupling of H-6, which must therefore adopt position 6β and the hydroxy group is in position 6α. In addition, the hydrogen H-6 shows NOE contacts (according to NOESY spectrum, see Fig. 1) to the methyl groups 24, 25 and 26, that is possible only in the case H-6 in the position 6β.

Triol **20** containing two secondary and one primary hydroxy group was acetylated to triacetate **21**. It was interesting to examine the reaction of the triol with bromine in pyridine at –24 °C. During this reaction only the 3β hydroxy group is oxidised while the 6β and 28 hydroxy groups do not re-

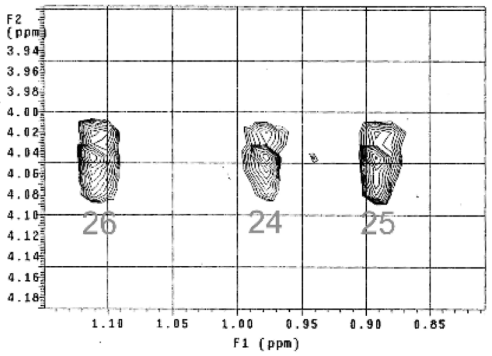
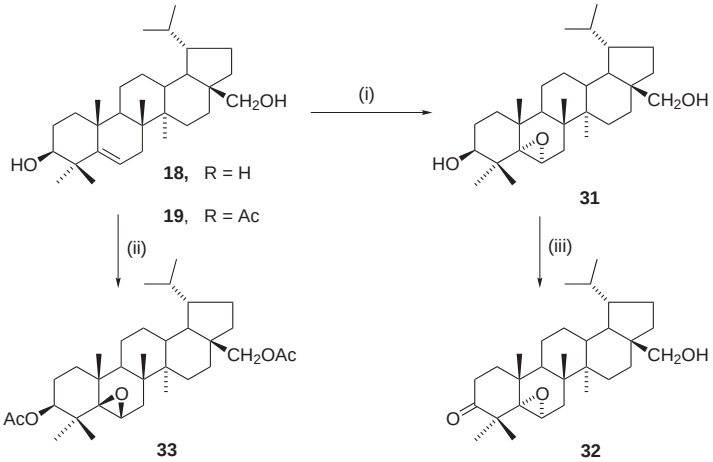


FIG. 1
Part of NOESY spectrum of compound **20**. The NOE cross-peaks of H-6 (at δ 4.05) with methyl protons H-24, H-25 and H-26

act; dihydroxyketone **22** is the only reaction product. A possible reason for distinct reactivity of the 3β and 6β secondary hydroxy group is the steric hindrance of the 6β hydroxy group. To prepare 6-oxo derivatives we had to use a stronger oxidative agent than bromine in pyridine. First, the 28 hydroxy group was protected by transformation to pivaloyloxy derivative. When pivaloyl chloride in pyridine at –10 °C reacted with the triol **20**, protected derivative **23** appeared as a main product but small amounts of dipivalates **24** and **25** were also isolated from the reaction mixture. Dihydroxy derivative **23** was oxidised with chromium trioxide to diketo derivative **26**. The pivalate protective group was removed by reduction with lithium aluminum hydride. During this reaction, the 3 and 6 oxo groups were also reduced and lupane-3β,6β,28-triol (**27**) was the main product. To decompose the residual lithium aluminum hydride ethyl acetate was used and some transesterification took place and 3β,6β-dihydroxylupan-28-yl acetate (**28**) was also isolated from the reaction mixture. The fact that the ester of acetic acid appears only in the position 28 is in agreement with the previous observation[38] that primary hydroxy group in position 28 of lupane derivatives is more reactive in esterifications than secondary hydroxy groups in other positions. The structures of compounds **27** and **28** were confirmed by mass, IR and NMR spectra. Both compounds show in ^{1}H NMR spectra a broad signal at δ 4.53 (H-6). Signals of all hydrogen and carbon atoms of compound **28** were assigned using 2D-NMR techniques (Tables III and VI). Triol **27** was acetylated at room temperature to 6β-hydroxylupane-3β,28-diyl diacetate (**29**), 6β hydroxy group did not react under these conditions. We also studied selective oxidation of triol **27** with bromine in pyridine at –24 °C. Under these conditions both the 3β and 6β hydroxy groups are oxidised, the primary hydroxy group in position 28 does not react and dioxo derivative **30** is the product. Signals of all hydrogen and carbon atoms of compound **30** were assigned using 2D-NMR techniques (see Tables III and VI).

Olefin **18** was epoxidised with 3-chloroperoxybenzoic acid to give 5α,6α-epoxylupan-3β,28-diol (**31**) (Scheme 3). This epoxy derivative was oxidised using bromine in pyridine at – 24 °C to epoxyketone **32**. The structure of epoxyketone **32** follows from full assignment of all ^{1}H and ^{13}C signals using HSQC, HMBC and NOESY spectra. The 5α,6α configuration of the epoxide is in agreement with the previously observed epoxidation of triterpenoid derivatives[1,44]. We also examined the reaction of the diacetoxy derivative **19** with *N*-bromoacetamide. This reaction is used in steroid chemistry to prepare 5α-bromo-6β-hydroxy derivatives[45,46]. In our hands, olefin **19** gave after the reaction with *N*-bromoacetamide 5β,6β-epoxide **33**

as the only product. Its structure was confirmed by full assignment of ^{1}H and ^{13}C NMR signals by HSQC, HMBC and NOESY spectra (Tables III and VI). The different reactivity of olefin **19** can be explained by the steric hindrance of the β side of the B ring of the lupane skeleton. The 6β-hydroxy group (which is probably present in the intermediate) is in *trans*-position to the 5α-bromine, thus intramolecular epoxidation can take place and the resulting 5β,6β-epoxide is less sterically demanding than the 6β-hydroxy derivative intermediate.



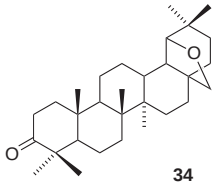
(i) 3-chloroperoxybenzoic acid, $CHCl_3$; (ii) 70% $HClO_4$, *N*-bromoacetamide, CH_2Cl_2, EtOH, H_2O; (iii) Br_2, Py, -24 °C

SCHEME 3

Conformation of Ring A

It is well known (e.g. ref.[47]) that ring A of 3-oxo derivatives of triterpenoids can adopt other than chair conformations (twist-boat, sofa) depending on other substituents. It was shown[1,47] that one can determine the conformation of the ring A on the basis of an analysis of vicinal coupling constants of protons in positions 1 and 2. Three basic conformations of ring A were proposed in ref.[48] They are the chair conformation *C* and two twist-boat conformations T_1 and T_2. Later a sofa conformation was found in crystal structure of a 3-oxolupane-28-nitrile[49] (Fig. 2). The values of characteristic coupling constants of conformations *C*, T_1, T_2 and S are presented in Table VII together with $^{3}J(1,2)$ of 3-oxo derivatives prepared in this work

and allobetulone[47] (**34**). The values for *C*, T_1 and T_2 are taken from ref.[1], the values for *S* are calculated according to a modified Karplus equation[50]. To find out the vicinal constants it was necessary first to assign chemical shifts



34

of hydrogens in positions 1α, 1β, 2α and 2β. For compounds **12**, **15**, **22**, **30** and **32** it was done using NOESY spectra, for compound **26** according to analogy with compound **30** and for compound **16** according to analogy with compound **15**. It is discussed in ref.[47] that ring A of 3-oxo derivatives

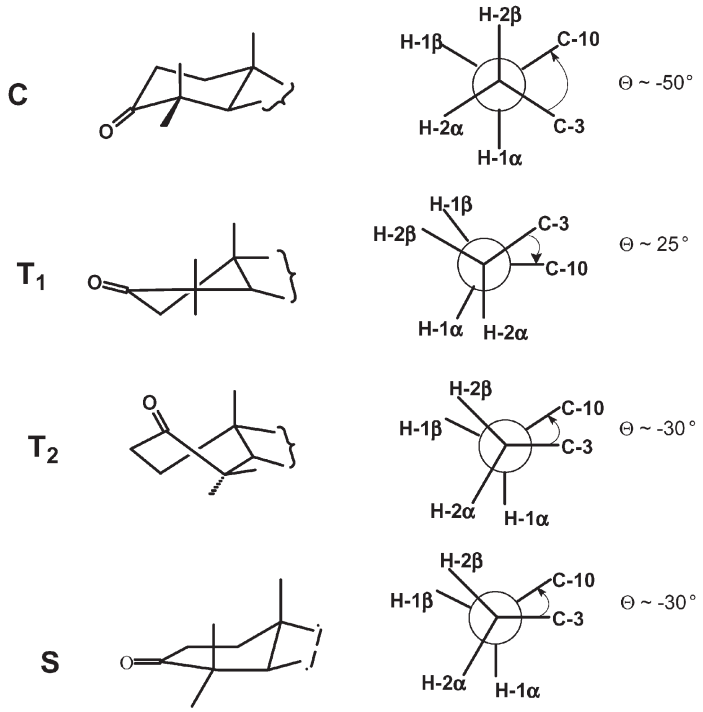


FIG. 2
Possible conformations of ring A of 3-oxo triterpenoids. Θ is the torsion angle C(10)–C(1)–C(2)–C(3)

without other substituents (e.g. allobetulon) is found in an equilibrium of chair and T_1 conformation.

The conformation of ring A of compounds **12**, **15**, **22** and **30** was also studied using molecular modelling. It was done with molecules analogical to these compounds, where isopropyl group in position 19 and hydroxymethyl or benzoyloxymethyl group in position 17 were replaced with methyl groups. These structural changes simplify the calculations and are sufficiently far from ring A to influence its conformation. One hundred of simulated annealings to 1000 K followed by slow cooling to 200 K were performed with every molecule. The calculated structures were then sorted according to the conformation of ring A to several structural types. The results of molecular modelling are presented in Table VIII.

Coupling constants of aldehyde **12** are very close to those of chair conformation and the molecular modelling found also chair conformation as the global energy minima geometry. The replacement of the methyl group in position 4β by the carbaldehyde group thus increases the stability of chair conformation (in comparison with allobetulone (**34**), where equilibrium of *C* and T_1 conformation takes place).

By examination of the coupling constants of derivatives with double bond in the position 5 we can see that they are in the best agreement with the $^3J(1,2)$ values of T_2 conformer. The same conclusion was made for oleanane derivatives in ref.[1] The results of molecular modelling are in good agreement with this conclusion. Figure 3 shows the ring A of the lowest energy conformation of molecule analogous to compound **15**. The energy of other conformers is at least 26 kJ mol^{-1} higher, this means they are almost not presented in the equilibrium.

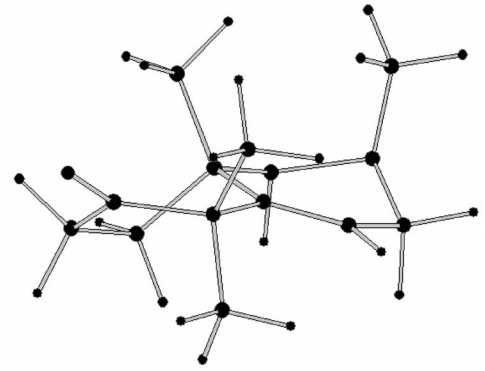


FIG. 3
Rings A and B of lup-5-ene derivatives (results of molecular modelling)

Coupling constants of compound **22** with hydroxy group in position 6α agree best with the values of T_1 conformer. The same result was also obtained by molecular modelling. Figure 4 shows ring A of the lowest energy conformation of a molecule analogous to 6α-hydroxy derivative **22**.

The vicinal coupling constants of compounds **26** and **30** with keto group in position 6 are close to the constants of the chair conformation. The same result was found by molecular modelling. Conformers with other than chair conformation have energy at least 7 kJ mol^{-1} higher.

Additional information on the A ring conformation of 3-oxo triterpenoids could be obtained in general from NOE contacts between sterically close hydrogen atoms. However the inspection of molecular models of four above discussed conformations of ring A showed, that three conformers – *C*, T_2 and *S* – have short interatomic distances (≤3 Å) only between the methyl group in position 25 and hydrogens 1β and 2β and thus the NOESY spectra can not be used to distinguish between these conformations. The corresponding cross peaks were observed in NOESY spectra of compounds **12**, **15**, **30** and **32**. On the other hand, the interatomic distance in the conformation T_1 between the 2β hydrogen and the methyl group in position 25 is larger (close to 4 Å) but the distance between 2α hydrogen and methyl group 23 is shorter (~3 Å). In the NOESY spectrum of 6α-hydroxy derivative **22**, cross-peaks are observed between the methyl group in position 23 and 2α hydrogen and between methyl group 25 and hydrogen 1β but not 2β. This observation is further evidence of the T_1 conformation of the 6α-hydroxy derivative.

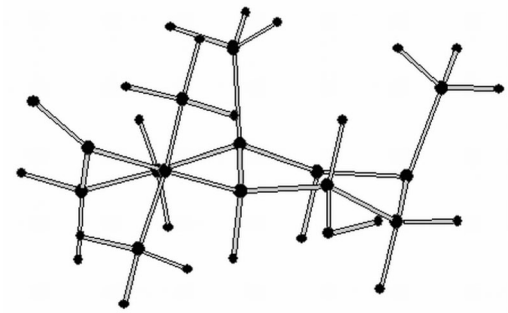


FIG. 4
Rings A and B of 6α-hydroxylupane derivatives (results of molecular modelling)

EXPERIMENTAL

TLC was performed on silica gel plates G (Merck), visualisation by spraying with 10% sulfuric acid and heating. Kieselgel 60 G (Merck) was used for preparative TLC (spraying with 0.3% methanolic solution of morin), column chromatography was carried out on silica gel (Merck). Mixtures of hexane and ether were used as eluents. The usual work-up means dilution of reaction mixture with water, extraction of products with ether and successive washing of ether layer with dilute (1:4) hydrochloric acid (if necessary), water, saturated sodium hydrogencarbonate solution, water, drying over anhydrous sodium sulfate and evaporation of solvents under reduced pressure. Samples for elemental analysis were dried over phosphorus pentoxide under reduced pressure. Starting dihydrobetulin **1** was prepared according to ref.[51]

Melting points were determined on a Kofler block and are uncorrected. Optical rotations were carried out in chloroform (if not specified in experiment), *c* 0.3–0.5, at ambient temperature on an automatic polarimeter ETL-NPL (Bendix–Ericsson) with accuracy ±2 and are given in 10^{-1} deg cm^2 g^{-1}. Infrared spectra were recorded in chloroform solution on a PE 684 (Perkin–Elmer) spectrometer, wavenumbers are given in cm^{-1}. Mass spectra were recorded on Finnigan MAT-Incos 50 instrument, ionising electron energy 70 eV, direct inlet temperatures 150–180 °C. Data are presented in the format *m/z* (%), intensities are scaled to most intensive ion above *m/z* 80. Elemental analyses were carried out on a Perkin–Elmer CHN Analyser 2400, Series II.

NMR spectra were recorded on instrument Varian ^{UNITY}INOVA 400 (^{1}H at 400 MHz, ^{13}C at 100.5 MHz) in deuteriochloroform at 25 °C. For ^{1}H NMR spectra, tetramethylsilane (TMS) was used as internal standard, chemical shifts (δ scale, ppm) and coupling constants (*J*, Hz) were obtained by first order analysis. ^{13}C NMR chemical shifts were obtained from the spectra measured in deuteriochloroform and referenced to solvent signal ($\delta(CDCl_3)$ 77.00). The 2D-H,H-COSY experiments were recorded in absolute value mode using standard two-pulse sequence. 2D-H,H-NOESY experiments were performed in phase sensitive mode with standard three-pulse sequence and mixing time 0.3 s. The 2D-H,C-HSQC and 2D-H,C-HMBC measurements were performed as PFG experiments (HSQC in phase sensitive and HMBC in absolute value mode). All heteronuclear 2D-H,C experiments were recorded with spectral windows 5000 Hz for proton and 25 000 Hz for carbon.

Molecular modelling studies were performed using programme Hyperchem 6 (Hypercube). The conjugate gradient method for energy minimisations was used to convergence (less than 0.2 kJ mol^{-1} RMS force). Force field MM+ was used for all computations. General protocol for obtaining lowest-energy conformers by simulated annealing: optimised starting structure was subjected to dynamic run – 0.2 ps heating from 300 to 1000 °C, 0.4 ps equilibration and 1 ps cooling to 200 °C followed by energy minimisation. Every next run started from the previously minimised structure. A set of 100 structures was so obtained for each compound, from which the lowest-energy conformation was chosen for evaluation.

28-Hydroxylupan-3-one (**3**)

A solution of lupane-3β,28-diol (**1**; 17 g, 38 mmol) in pyridine (340 ml) was cooled to 0 °C and then bromine (12 ml, 240 mmol) was added. The mixture was left standing at –24 °C for three days and then it was diluted with water. The precipitate was filtered off, washed with water and dried at room temperature. Then the precipitate was dissolved in ether and the solution was filtered through silica gel (30 g) and worked up in the usual manner.

Crystallisation from ethanol afforded 28-hydroxylupan-3-one (**3**; 14.5 g, 85%). The physical constants of the compound were in agreement with those published previously[38].

3-Oxolupan-28-yl Acetate (**2**)

To a solution of hydroxyketone **3** (3 g, 6.9 mmol) in pyridine (5 ml), acetic anhydride (5 ml, 55 mmol) was added and the mixture was left standing at room temperature overnight. The mixture was worked up in the usual manner. Crystallisation from chloroform–ethanol gave acetate **2** (2.9 g, 91%). The physical data of the compound were in agreement with those published previously[38].

28-Acetoxy-4,23-epoxy-3,4-secolupane-3-nitrile (**5**)

A solution of 28-acetoxy-3,4-secolup-4(23)-ene-3-nitrile[39] (**4**; 5.1 g, 10 mmol) in chloroform (70 ml) was cooled to 0 °C. Then 3-chloroperoxybenzoic acid (60%, 4 g, 14 mmol) was added and the mixture was left standing at 4 °C for 24 h. Then ether was added and the solution was washed with 5% solution of potassium iodide and then with 5% solution of sodium sulfite to decolourise the solution. This procedure was repeated until the potassium iodide solution changed colour to brown. Then the ether layer was washed with water and worked up in the usual manner. A mixture of epoxy derivatives **5** (5.3 g, 100%) was obtained. The mixture was used without purification in further reaction.

Reaction of Epoxyseconitrile **5** with Boron Trifluoride

A solution of crude mixture of epoxy derivatives **5** (2.4 g, 4.8 mmol) in dry toluene (150 ml) was deoxygenated with low stream of argon and then boron trifluoride diethyl etherate (7.5 ml, 68 mmol) was added. The mixture was refluxed under argon for 10 h. After cooling to room temperature the reaction mixture was worked up in the usual manner. Evaporation residue contained (according to TLC) two components. The mixture was separated by column chromatography on silica gel (250 g). A mixture of ether and hexane (1:10) was used as eluent. Following compounds were isolated (according to increasing polarity).

22(17→28)Abeo-24-norlup-3-one (**7**): 130 mg (7%), m.p. 165–169 °C (chloroform–ethanol), $[\alpha]_D$ –9. IR: 1700 (C=O), 1453, 1207. EI-MS, *m/z* (%): 410 (M^+, 22), 395 (1), 367 (3), 205 (100), 149 (44), 109 (77). ^{1}H NMR: 0.677 d, 3 H (H-29, *J*(29,20) = 6.7); 0.919 d, 3 H (H-30, *J*(30,20) = 6.5); 0.968 s, 3 H (H-26); 0.998 d, 3 H (H-23, *J*(23,4) = 6.5); 1.066 d, 3 H (H-25, *J* = 1.0); 1.115 s, 3 H (H-27); 1.91 dh, 1 H (H-20, J_1 = 10.5, J_2 = 6.5); 2.02 bm, 1 H (H-17, Σ*J* = 25); 2.08 ddd, 1 H (H-1β, *J*(1β,1α) = 13.2, *J*(1β,2α) = 2.4, *J*(1β,2β) = 7.1); 2.28 m, 1 H (H-4β, *J*(4β,5) = 11.8, *J*(4β,23) = 6.6); 2.33 ddd, 1 H (H-2α, *J*(2α,2β = 15.3, *J*(2α,1α) = 5.6, *J*(2α,1β) = 2.4); 2.36 bddd, 1 H (H-19, J_1 = 10.6, J_2 = 4.8, J_3 = 2.6); 2.47 dddd, 1 H (H-2β, *J*(2β,1α) = 13.2, *J*(2β,2α) = 15.3, *J*(2β,1β) = 7.1, *J*(2β,4β) = 1.1); 2.72 bddd, 1 H (H-12β, J_1 = 15.0, J_2 = 5.0, J_3 = 2.4). For ^{13}C NMR, see Table I. For $C_{29}H_{46}O$ (410.7) calculated: 84.81% C, 11.29% H; found: 84.32% C, 11.08% H.

3-Oxo-24-norlupan-28-yl acetate (**6**): 346 mg (15%), m.p. 130–131 °C (chloroform–ethanol), $[\alpha]_D$ 0. IR: 1244 (C–O–C), 1702 (C=O), 1727 (CH_3COO). EI-MS, *m/z* (%): 470 (M^+, 14), 410 (6), 397 (33), 367 (5), 204 (100), 191 (64), 43 (55). ^{1}H NMR: 0.764 d, 3 H (H-29, *J*(29,20) = 6.7); 0.845 d, 3 H (H-30, *J*(30,20) = 6.8); 0.947 d, 3 H (H-27, *J* = 0.7); 0.982 d, 3 H (H-23, *J*(23,4) = 6.6); 1.036 d, 3 H (H-25, *J* = 0.7); 1.104 s, 3 H (H-26); 2.067 s, 3 H (OAc); 2.02 ddd, 1 H (H-1β, *J*(1β,1α) = 13.1, *J*(1β,2β) = 7.0, *J*(1β,2α) = 2.3); 2.27 ddq, 1 H (H-4β, *J*(4β,5) = 12.0,

J(4β,23) = 6.6, *J*(4β,2β) = 1.0); 2.30 ddd, 1 H (H-2α, *J*(2α,2β) = 15.0, *J*(2α,1α) = 5.5, *J*(2α,1β) = 2.3); 2.44 dddd, 1 H (H-2β, *J*(2β,2α) = 15.0, *J*(2β,1α) = 13.4, *J*(2β,1β) = 7.0, *J*(4β,2β) = 1.1); 3.82 dd, 1 H (H-28a, *J*(28a,28b) = 11.0, $J_{l.r.}$ = 1.4); 4.26 dd, 1 H (H-28b, *J*(28b,28a) = 11.0, $J_{l.r.}$ = 1.8). For ^{13}C NMR, see Table I. For $C_{31}H_{50}O_3$ (470.7) calculated: 79.10% C, 10.71% H; found: 79.07% C, 10.94% H.

28-Benzoyloxy-3,4-secolup-4(23)-ene-3-nitrile (**8**)

To the solution of acetate **4** (10 g, 21 mmol) in ethanol (300 ml) a solution of sodium hydroxide (5 g) in ethanol (150 ml) was added. The mixture was refluxed for 1 h then it was cooled to room temperature and diluted with water (1 l). The precipitate was filtered off, washed with water and dried at 100 °C. ^{1}H NMR spectrum of the precipitate was in agreement with NMR data published previously[39] for 28-hydroxy-3,4-secolup-4(23)-ene-3-nitrile. The precipitate was dissolved in pyridine (50 ml) and then benzoyl chloride (20 ml, 110 mmol) was added. The mixture was left standing at room temperature overnight and then it was worked up in the usual manner. Crystallisation from chloroform–ethanol gave 6.5 g (77%) of the title compound **8**, m.p. 162–163 °C, $[\alpha]_D$ –1. IR: 2249 (nitrile), 1714 (C=O), 1454, 1389, 1278, 1120. EI-MS, *m/z* (%): 543 (M^+, 11), 421 (23), 408 (25), 378 (13), 340 (10), 229 (18), 191 (50), 135 (30), 105 (100). For ^{1}H NMR, see Table IV. For ^{13}C NMR, see Table I. For $C_{37}H_{53}NO_2$ (543.8) calculated: 81.72% C, 9.82% H, 2.58% N; found: 81.67% C, 9.91% H, 2.35% N.

28-Benzoyloxy-4,23-epoxy-3,4-secolupane-3-nitrile (**9**)

The solution of compound **8** (10.3 g, 19 mmol) in chloroform (125 ml) was cooled to 0 °C. Then 3-chloroperoxybenzoic acid (60%, 8 g, 28 mmol) was added and the mixture was left standing at 4 °C for 16 h. Then ether was added and the solution was washed with 5% solution of potassium iodide and then with 5% solution of sodium sulfite to decolourise the solution. This procedure was repeated as long as the potassium iodide solution changed colour to brown. Then the ether layer was washed with water and worked up in the usual manner. A mixture of epoxy derivatives (10.6 g, 100%) was obtained. Multiple crystallisation gave one isomer, the title compound **9**, m.p. 179–181 °C, $[\alpha]_D$ –1. IR: 2249 (nitrile), 1712 (C=O), 1454, 1277. EI-MS, *m/z* (%): 559 (M^+, 2), 530 (2), 501 (2), 437 (9), 424 (11), 380 (7), 351 (4), 229 (18), 191 (32), 135 (28), 105 (100). ^{1}H NMR: 0.791 d (*J* = 6.6), 0.866 d (*J* = 6.7); 0.952 s, 0.955 s, 1.109 s, 1.275 s , 6 × 3 H (6 × CH_3); 2.63 d, 1 H (H-23a, *J*(23a,23b) = 4.4); 2.72 d, 1 H (H-23b, *J*(23b,23a) = 4.4); 4.05 d, 1 H (H-28a, *J*(28a,28b) = 11.1); 4.51 dd, 1 H (H-28b, *J*(28b,28a) = 11.1, $J_{l.r.}$ = 1.5); 8.05 m, 2 H, 7.56 m, 1 H and 7.44 m, 2 H (C_6H_5). For ^{13}C NMR, see Table I. For $C_{37}H_{53}NO_3$ (559.8) calculated: 79.38% C, 9.54% H, 2.50% N; found: 78.61% C, 9.63% H, 2.31% N.

3-Oxo-24-norlupan-28-yl Benzoate (**10**)

The solution of crude mixture of isomeric epoxides **9** (5 g, 8.9 mmol) in dry toluene (200 ml) was deoxygenated with low stream of argon and then boron trifluoride etherate (6 ml, 54 mmol) was added. The mixture was refluxed under argon for 2 h, then 2 M hydrochloric acid (30 ml) was added and the mixture was refluxed for another 30 min. After cooling to room temperature the reaction mixture was worked up in the usual manner. The crude product was filtered through a column of silica gel (eluent: chloroform–petroleum ether, 2:1).

TABLE I
^{13}C NMR chemical shifts of compounds **6**–**10** and **12**–**15** in $CDCl_3$

Carbon	**6**	**7**	**8**	**9**	**10**	**12**	**13**	**14**	**15**
1	40.30	40.35	34.36	34.58	40.34	39.83	36.56	36.53	35.28
2	37.44	37.40	11.42	11.63	37.46	36.00	33.31	33.29	33.67
3	213.84	213.94	120.22	119.74	213.9	209.98	198.92	198.96	217.47
4	44.56	44.59	147.00	57.93	44.61	63.59	128.05	128.06	48.41
5	53.25	53.26	50.52	49.91	53.29	57.45	164.42	164.49	146.98
6	22.12	22.14	24.19	20.98	22.16	19.62	24.56	24.58	119.29
7	32.97	33.61	32.59	32.39	33.00	33.95	32.78	32.83	33.12
8	40.56	40.62	40.69	40.55	40.62	40.76	40.52	40.53	38.73
9	47.65	48.26	40.11	40.55	47.69	48.58	49.09	49.10	43.40
10	36.68	36.82	39.44	38.74	36.72	37.22	39.31	39.31	37.12
11	21.40	22.38	21.52	21.20	21.44	21.43	21.54	21.59	22.75
12	26.77[a]	25.14	26.48	26.35	26.80	26.69	26.88	26.87	26.45
13	37.10	132.40	37.20	37.23	37.23	37.22	37.41	36.99	37.00
14	42.93	43.90	43.34	43.29	43.02	43.02	43.22	43.17	42.00
15	26.82[a]	26.12	26.98	26.91	26.94	26.98	26.98	26.91	27.17
16	29.78	25.60	29.94	29.86	30.01	29.95	29.86	29.13	29.09
17	46.40	32.95	46.84	46.79	46.85	46.84	46.93	47.80	47.80
18	48.02	136.00	48.10	48.04	48.15	48.10	47.96	47.95	48.19
19	44.47	45.53	44.55	44.51	44.61	44.57	44.54	44.49	44.63
20	29.41	27.28	29.47	29.45	29.47	29.47	29.44	29.46	29.58
21	21.55	30.37	21.65	21.62	21.65	21.65	21.62	21.70	21.75
22	34.60	22.20	34.81	34.76	34.82	34.81	34.80	33.98	33.97
23	11.55	11.67	113.98	55.41	11.58	201.24	11.02	11.03	27.97
24	–	–	22.78	20.70	–	17.17	–	–	30.01
25	13.46	13.50	19.68	19.76	13.50	15.02	18.14	18.13	17.44
26	15.96	17.77	16.07	15.92	16.03	16.04	15.59	15.52	15.66
27	14.43	20.52	14.52	14.77	14.51	14.54	14.38	14.32	15.23
28	62.77	34.62	63.20	63.12	63.26	63.17	63.19	60.52	60.42
29	14.83	20.97	14.90	14.86	14.89	14.89	14.88	14.88	14.88
30	22.87	20.94	22.89	22.86	22.91	22.90	22.89	22.92	22.93
OAc or OBz									
C=O	171.70	–	166.94	166.91	166.97	166.94	166.95	–	–
1′	21.03	–	130.50	130.44	130.53	130.48	130.47	–	–
2′, 6′		–	129.53	129.53	129.54	129.54	129.53	–	–
3′, 5′		–	128.35	128.83	128.36	128.36	128.36	–	–
4′		–	132.84	132.84	132.84	132.87	132.86	–	–

[a] Signals may be mutually interchanged.

Crystallisation from chloroform–ethanol gave the title compound **10** (2.5 g, 53%), m.p. 196–198 °C, $[\alpha]_D$ +5. IR: 1700 broad (2 × C=O), 1454, 1388, 1316, 1277, 1176, 1118. EI-MS, *m/z* (%): 532 (M^+, 10), 410 (16), 397 (26), 367 (7), 204 (60), 191 (40), 163 (25), 135 (29), 105 (100). For ^{1}H NMR, see Table IV. For ^{13}C NMR, see Table I. For $C_{36}H_{52}O_3$ (532.8) calculated: 81.15% C, 9.84% H; found: 80.80% C, 9.85% H. Preparative TLC of mother liquor gave 25 mg of 3,24-dioxolupan-28-yl benzoate (**12**), m.p. 225–229 °C, $[\alpha]_D$ –18. IR: 2871 (CH=O), 1722, 1709 (2 × C=O), 1278, 1119. EI-MS, *m/z* (%): 532 (M^+ – CO, 55), 425 (5), 410 (3), 397 (4), 229 (15), 191 (20), 135 (27), 105 (100). HRMS, *m/z*: 560.3879 (for $C_{37}H_{52}O_4$ calculated: 560.3866). For ^{1}H NMR, see Table IV. For ^{13}C NMR, see Table I. Before addition of hydrochloric acid, the reaction mixture contained also 3-oxo-23-norlupan-28-yl benzoate (**11**). The attempts to isolate this compound failed because of easy isomerisation to 3-oxo-24-norlupan-28-yl benzoate (**10**). ^{1}H NMR spectrum of 3-oxo-23-norlupan-28-yl benzoate (**11**) was obtained by elimination of signals of compound **10** from a spectrum of the reaction mixture. ^{1}H NMR: 0.791 d, 3 H (H-29, *J*(29,20) = 6.7); 0.864 d, 3 H (H-30, *J*(30,20) = 6.8); 0.988 s, 3 H (H-27); 1.021 s, 3 H (H-25); 1.105 d, 3 H (H-24, *J*(24,4α) = 7.8); 1.133 s, 3 H (H-26); 2.58 ddd, 1 H (H-2β, *J*(2β,2α) = 15.7, *J*(2β,1α) = 12.7, *J*(2β,1β) = 6.9); 4.07 d, 1 H (H-28a, *J*(28a,28b) = 11.0); 4.53 d, 1 H (H-28b, *J*(28b,28a) = 11.0); 8.05 m, 2 H, 7.56 m, 1 H and 7.44 m, 2 H (C_6H_5).

3-Oxo-24-norlup-4-en-28-yl Benzoate (**13**)

Ketone **10** (7.7 g, 14.4 mmol) was dissolved in warm acetic acid (550 ml). The solution was cooled to the room temperature and then 33% solution of hydrogen bromide in acetic acid (0.3 ml) was added. The mixture was continuously stirred and, during 30 min, bromine (2.56 g, 16 mmol) in acetic acid (64 ml) was added dropwise. Then 33% solution of hydrogen bromide in acetic acid (70 ml, 288 mmol) was added and the mixture was left standing at room temperature for two days in the dark. The reaction mixture was then diluted with water, the precipitate was filtered off, washed with saturated solution of sodium hydrogencarbonate and with water and then it was dried on air at room temperature. Precipitate (7.7 g) of the title compound **13** was obtained, pure enough for use for further reaction. A sample for analysis was obtained by preparative TLC (eluent: ether–hexane, 1:2). M.p. 212–217 °C (chloroform–ethanol), $[\alpha]_D$ +47. IR: 1713 (C=O), 1655 (C=O), 1604 (C=C), 1453, 1388, 1315, 1278, 1117. EI-MS, *m/z* (%): 530 (M^+, 87), 365 (8), 351 (5), 229 (25), 191 (27), 147 (18), 138 (36), 105 (100). ^{1}H NMR: 0.793 d, 3 H (H-29, *J*(29,20) = 6.7); 0.873 d, 3 H (H-30, *J*(30,20) = 6.7); 0.953 s, 3 H (H-27); 1.144 s, 3 H (H-25); 1.284 s, 3 H (H-26); 1.791 d, 3 H (H-23, $J_{l.r.}$ = 1.1); 2.01 ddd, 1 H (H-1β, *J*(1β,1α) = 13.1, *J*(1β,2β) = 5.1, *J*(1β,2α) = 2.8); 2.35 ddd, 1 H (H-2α, *J*(2α,2β) = 17.0, *J*(2α,1α) = 4.7, *J*(2α,1β) = 2.7); 2.46 ddd, 1 H (H-2β, *J*(2β,2α) = 17.0, *J*(2β,1α) = 15.0, *J*(2β,1β) = 5.2); 2.64 ddd, 1 H (H-6a, *J*(6a,6b) = 14.8, *J*(6a,7a) = 3.4, *J*(6a,7b) = 3.4); 4.07 dd, 1 H (H-28a, *J*(28a,28b) = 11.1, $J_{l.r.}$ = 1.2); 4.56 dd, 1 H (H-28b, *J*(28b,28a) = 11.1, $J_{l.r.}$ = 1.8); 7.45 m, 2 H, 7.57 m, 1 H and 8.06 m, 2 H (C_6H_5). For ^{13}C NMR, see Table I. For $C_{36}H_{50}O_3$ (530.8) calculated: 81.46% C, 9.49% H; found: 81.25% C, 9.48% H.

28-Hydroxy-24-norlup-4-en-3-one (**14**)

To the solution of benzoate **13** (3.5 g, 6.6 mmol) in ethanol (100 ml) a solution of potassium hydroxide (1.7 g, 30 mmol) in ethanol (50 ml) was added. The mixture was refluxed for 1 h and then it was cooled to room temperature, diluted with water (300 ml) and extracted with ether. The ether layer was worked up in the usual manner. The evaporation res-

idue (2.8 g) was used for another reaction without further purification. A sample for analysis was obtained by column chromatography (see the next experiment) and crystallisation from chloroform–ethanol. M.p. 245–249 °C, $[\alpha]_D$ +124. IR: 3625 (OH), 1656 (C=O), 1607 (C=C), 1458, 1376, 1329, 1018. For ^{1}H NMR, see Table IV. For ^{13}C NMR, see Table I. For $C_{29}H_{46}O_2$ (426.7) calculated: 81.63% C, 10.87% H; found: 81.12% C, 10.45% H.

28-Hydroxylup-5-en-3-one (**15**)

Potassium (220 mg, 5.6 mmol) was dissolved in warm *tert*-butyl alcohol (10 ml). To 7.5 ml of this solution ketone **14** (250 mg, 0.59 mmol) was added. This mixture was refluxed under argon for 1 h. Then methyl iodide (100 µl, 1.6 mmol) was added and the mixture was refluxed another 15 min and then it was left standing at room temperature for 20 min. Then water (30 ml) was added and the mixture was extracted with ether. The ether layer was worked up in the usual manner. The evaporation residue (240 mg) contained (according to TLC) four components. The mixture was separated by column chromatography on silica gel (25 g). Ether–hexane (1:1) was used as eluent. The following compounds were obtained and they were crystallised from chloroform–ethanol (according to increasing polarity).

28-Methoxylup-5-en-3-one (**16**): 15 mg (5.6%), m.p. 185–191 °C, $[\alpha]_D$ +15. IR: 1702 (C=O), 1666 (C=C), 1462, 1384, 1371, 1100, 1024, 966, 816. EI-MS, *m/z* (%): 454 (M^+, 29), 422 (4), 409 (23), 397 (8), 229 (21), 205 (36), 203 (35), 191 (52), 124 (45), 107 (100). For ^{1}H NMR, see Table V. For ^{13}C NMR, see Table II.

28-Methoxy-24-norlup-4-en-3-one (**17**): 14 mg (5.4%), m.p. 218–221 °C, $[\alpha]_D$ +54. IR: 1655 (C=O), 1606 (C=C), 1458, 1376, 1100, 963. EI-MS, *m/z* (%): 440 (M^+, 100), 408 (36), 395 (13), 366 (18), 271 (20), 229 (23), 203 (33), 191 (62), 161 (36), 147 (47), 138 (49), 121 (56). HRMS, *m/z*: 440.3659 (for $C_{30}H_{48}O_2$ calculated: 440.3654). ^{1}H NMR: 0.763 d, 3 H (H-29, J(29,20) = 6.7); 0.854 d, 3 H (H-30, J(30,20) = 6.8); 0.919 s, 3 H (H-27); 1.137 s, 3 H (H-25); 1.253 s, 3 H (H-26); 1.793 d, 3 H (H-23, $J_{l.r.}$= 1.2); 2.00 ddd, 1 H (H-1β, J(1β,1α) = 13.1, J(1β,2β) = 5.2, J(1β,2α) = 2.8); 2.34 ddd, 1 H (H-2α, J(2α,2β) = 17.1, J(2α,1α) = 4.9, J(2α,1β) = 2.6); 2.45 ddd, 1 H (H-2β, J(2β,2α) = 16.9, J(2β,1α) = 15.0, J(2β,1β) = 5.2); 2.64 ddd, 1 H (H-6a, J(6a,6b) = 14.8, J(6a,7a) = 3.5, J(6a,7b) = 3.5); 3.06 dd, 1 H (H-28a, J(28a,28b) = 9.2, $J_{l.r.}$ = 1.1); 3.49 dd, 1 H (H-28b, J(28b,28a) = 9.2, $J_{l.r.}$ = 1.6); 3.35 s, 3 H (OCH_3). For ^{13}C NMR, see Table II.

28-Hydroxylup-5-en-3-one (**15**): 88 mg (34%), m.p. 188–192 °C, $[\alpha]_D$ +22. IR: 3626 (OH), 1701 (C=O), 1666 (C=C), 1462, 1385, 1371, 1111, 1025, 1017. EI-MS, *m/z* (%): 440 (M^+, 72), 422 (8), 409 (15), 247 (15), 217 (18), 203 (21), 191 (22), 177 (20), 152 (38), 133 (28), 124 (100). For ^{1}H NMR, see Table V. For ^{13}C NMR, see Table I. For $C_{30}H_{48}O_2$ (440.7) calculated: 81.76% C, 10.98% H; found: 80.21% C, 11.33% H.

28-Hydroxy-24-norlup-4-en-3-one (**14**): 94 mg (38%), identical with sample obtained by alkaline hydrolysis of benzoate **13** (see previous experiment).

Lup-5-ene-3β,28-diol (**18**)

To a solution of ketone **15** (200 mg, 0.45 mmol) in a mixture of benzene (5 ml) and methanol (7 ml) sodium borohydride (200 mg, 5.3 mmol) was added during 15 min and the mixture was left standing at room temperature. Then 2 M hydrochloric acid (10 ml) was added dropwise and the mixture was extracted with ether, the ether layer was worked up in the usual manner. Crystallisation from chloroform–ethanol gave title compound **18** (162 mg,

TABLE II
^{13}C NMR chemical shifts of compounds **16**–**22** in $CDCl_3$

Carbon	**16**	**17**	**18**	**19**[a]	**20**	**21**[b]	**22**
1	35.27	36.54	38.99	38.66	38.46	37.98	39.56
2	33.68	33.31	27.41	23.83	26.71	23.20	33.02
3	217.50	198.94	78.09	79.91	78.68	80.41	219.96
4	48.42	128.05	42.01	40.81	39.26	39.38	47.15
5	146.96	164.53	146.08	145.35	60.48	58.15	58.56
6	119.36	24.60	119.92	120.51	68.75	70.77	67.82
7	33.13	32.82	34.40	34.40	46.72	41.92	44.48
8	38.73	40.54	38.13	38.12	42.90	42.07	42.89
9	43.41	49.10	46.22	46.16	49.52	49.43	47.93
10	37.13	39.31	37.25	37.19	39.15	37.60	38.19
11	22.80	21.62	22.24	22.15	20.72	20.66	21.71
12	26.47	26.93	26.51	26.46	26.93	26.56	26.80
13	37.22	37.20	37.01	37.30	36.34	36.63	36.51
14	41.97	43.16	42.27	42.28	42.23	42.98	41.70
15	27.36	27.07	27.21	27.20	26.93	26.96	26.91
16	29.85	29.90	29.11	29.65	29.23	29.71	29.14
17	47.19	47.35	47.81	46.35	47.88	46.43	47.88
18	48.34	47.97	48.27	48.31	48.00	48.03	48.43
19	44.81	44.67	44.68	44.60	44.51	44.47	44.48
20	29.60	29.49	29.58	29.52	29.44	29.40	29.48
21	21.94	21.88	21.76	21.62	21.68	21.56	21.71
22	34.72	34.75	33.96	34.57	33.96	34.60	33.97
23	27.97	11.03	27.96	27.86	30.88	30.34	31.90
24	30.01	–	20.28	20.44	15.46	16.74	19.59
25	17.45	18.14	22.80	24.04	17.06	17.05	17.62
26	15.67	15.52	16.63	16.74	17.48	17.27	16.39
27	15.27	14.36	15.48	15.43	14.63	14.60	14.57
28	71.19	71.34	60.47	62.72	60.48	62.70	60.45
29	14.91	14.89	14.89	14.88	14.85	14.86	14.86
30	22.96	22.95	22.94	22.89	22.91	22.87	22.91
OMe	59.66	59.68	–	–	–	–	–

[a] Signals of acetate in the position 3 (δ 170.83 and 21.33), and 28 (δ 171.62 and 21.05); [b] signals of acetate in the position 3 (δ 170.01 and 21.28), 6 (δ 171.05 and 21.96) and 28 (δ 171.58 and 21.00).

81%), m.p. 269–271 °C, $[\alpha]_D$ –22. IR: 3624 (OH), 1462, 1385, 1020. EI-MS, *m/z* (%): 442 (M^+, 36), 424 (20), 359 (89), 203 (26), 187 (100), 175 (42), 161 (30), 147 (38), 133 (68), 119 (72). For ^{1}H NMR, see Table V. For ^{13}C NMR, see Table II. For $C_{30}H_{50}O_2$ (442.7) calculated: 81.39% C, 11.38% H; found: 80.94% C, 11.50% H.

Lup-5-ene-3β,28-diyl Diacetate (**19**)

To a solution of diol **18** (10 mg, 0.023 mmol) in pyridine (0.4 ml), acetic anhydride (0.4 ml, 4.4 mmol) was added and the mixture was left standing at room temperature overnight. The mixture was worked up in the usual manner. Crystallisation from chloroform–ethanol gave diacetate **19** (10 mg, 84%), m.p. 230–235 °C, $[\alpha]_D$ –8. IR: 1724 (C=O), 1460, 1387, 1367, 1252, 1031, 982. EI-MS, *m/z* (%): 526 (M^+, 5), 466 (18), 451 (15), 398 (18), 325 (6), 302 (4), 187 (100), 175 (41). ^{1}H NMR: 0.774 d, 3 H (H-29, *J*(29,20) = 7.2); 0.850 d, 3 H (H-30, *J*(30,20) = 6.8); 0.941 s, 3 H (H-27); 1.054 s, 3 H (H-26); 1.151 s, 3 H (H-24); 1.031 s, 1.103 s, 2 × 3 H (H-23 and H-25); 2.057 s, 2.064 s, 2 × 3 H (2 × OAc); 3.85 dd, 1 H (H-28a, *J*(28a,28b) = 11.1, $J_{l.r.}$ = 1.2); 4.25 dd, 1 H (H-28b, *J*(28b,28a) = 11.1, $J_{l.r.}$ = 1.8); 4.46 m, 1 H (H-3, Σ*J* = 16.5); 5.57 dd, 1 H (H-6, *J*(6,7a) = 3.1, *J*(6,7b) = 5.0). For ^{13}C NMR, see Table II. For $C_{34}H_{54}O_4$ (526.8) calculated: 77.52% C, 10.33% H; found: 76.95% C, 10.46% H.

Lupane-3β,6α,28-triol (**20**)

Olefin **18** (100 mg, 0.23 mmol) was placed into a 3-ml reaction flask with Teflon cap and 1 M solution of BH_3 in tetrahydrofuran (2.5 ml, 2.5 mmol) was added. The flask was covered with metal net and it was warmed to 80 °C in water bath for 8 h and then it was left standing at room temperature overnight. Then a solution of sodium hydroxide (0.4 g, 10 mmol) in ethanol (10 ml) and 30% hydrogen peroxide (6 ml, 58 mmol) was added. The mixture was stirred for 4 h and then it was extracted with ether. The ether layer was washed successively with water, 2 M hydrochloric acid, 5% solution of potassium iodide and with 5% solution of sodium sulfite to decolourise the solution. This procedure was repeated until the potassium iodide solution turned to brown. Then the ether layer was washed with water, dried over anhydrous sodium sulfate and evaporated under reduced pressure. Preparative TLC (eluent: hexane–ether, 1:3) and crystallisation from chloroform–ethanol gave two compounds: starting compound **18** (22 mg, 22%) and triol **20** (58 mg, 56%), m.p. 262–265 °C, $[\alpha]_D$ 0. IR: 3625 (OH), 1463, 1386, 979. EI-MS, *m/z* (%): 460 (M^+, 1), 442 (5,5), 429 (6), 411 (17), 393 (3), 207 (25), 205 (20), 191 (22), 187 (100), 177 (25). HRMS, *m/z*: 460.3926 (for $C_{30}H_{52}O_3$ calculated: 460.3916). For ^{1}H NMR, see Table V. For ^{13}C NMR, see Table II.

Lupane-3β,6α,28-triyl Triacetate (**21**)

To a solution of triol **20** (10 mg, 0.022 mmol) in dry pyridine (0.4 ml) acetic anhydride (0.4 ml, 4.4 mmol) was added and the mixture was left standing at room temperature overnight. Then the mixture was worked up in the usual manner. Crystallisation from methanol gave triacetate **21** (10 mg, 78%), m.p. 175–179 °C. IR: 1726 (C=O), 1460, 1368, 1255, 1030, 980. ^{1}H NMR: 0.766 d, 3 H (H-29, *J*(29,20) = 6.8); 0.839 d, 3 H (H-30, *J*(30,20) = 6.8); 0.904 s, 3 H (H-25); 0.971 s, 0.975 s, 2 × 3 H (H-24 and H-27); 1.028 s, 3 H (H-23); 1.182 s, 3 H (H-26); 2.021 s, 3 H (OAc); 2.052 s, 6 H (2 × OAc); 3.83 d, 1 H (H-28a, *J*(28a,28b) = 11.0); 4.19 dd, 1 H (H-28b, *J*(28b,28a) = 11.0, $J_{l.r.}$ = 1.7); 4.45 m, 1 H (H-3, Σ*J* = 16.5); 5.29 td, 1 H (H-6β, *J*(6β,5α) = 10.9, *J*(6β,7α) = 10.9, *J*(6β,7β) = 4.1). For ^{13}C NMR, see Table II.

6α,28-Dihydroxylupan-3-one (**22**)

To a solution of triol **20** (13 mg, 0.028 mmol) in pyridine (0.5 ml) bromine (20 μl, 0.40 mmol) was added and the mixture was left standing at –24 °C for 3 days. Then the mixture was diluted with water and extracted with ether. The ether layer was washed with water, 2 M hydrochloric acid, 5% solution of sodium sulfite and with saturated solution of sodium hydrogencarbonate and then it was dried over anhydrous sodium sulfate and evaporated under reduced pressure. Ketone **22** (10 mg, 77%) was obtained, amorphous, $[\alpha]_D$ +42. IR: 3620 (OH), 1700 (C=O), 1460, 1384, 1368, 1027. EI-MS, *m/z* (%): 458 (M^+, 4), 440 (5), 427 (29), 409 (40), 203 (100), 191 (55), 177 (40), 149 (91). For ^{1}H NMR, see Table V. For ^{13}C NMR, see Table II. For $C_{30}H_{50}O_3$ (458.7) calculated: 78.55% C, 10.99% H; found: 78.21% C, 11.13% H.

3β,6α-Dihydroxylupan-28-yl Pivalate (**23**)

A solution of triol **20** (70 mg, 0.15 mmol) in dry pyridine (1.8 ml) was cooled to –10 °C and then pivaloyl chloride (250 μl, 2.1 mmol) was added. The mixture was left standing at –10 °C for 2 h, then methanol (20 ml) was added (to remove excess of pivaloyl chloride) and the mixture was left standing at 4 °C overnight. The mixture was extracted with ether, the ether layer was washed with 2 M hydrochloric acid, water and saturated solution of sodium hydrogencarbonate, then it was dried with anhydrous sodium sulfate and evaporated in vacuum. Evaporation residue (83 mg) was according to TLC a mixture of three compounds. The mixture was separated by preparative TLC using ether–hexane (2:1) as eluent. The following compounds were obtained (according to increasing polarity).

6α-Hydroxylupane-3β,28-diyl dipivalate (**24**): 13 mg, 14%, m.p. 295–297 °C (methanol). ^{1}H NMR: 0.770 d, 3 H (H-29, *J*(29,20) = 6.8); 0.840 d, 3 H (H-30, *J*(30,20) = 6.8); 0.981 s, 3 H (H-27); 1.132 s, 3 H (H-26); 0.925 s, 1.081 s, 1.165 s, 3 × 3 H (H-23, H-24 and H-25); 1.206 s, 18 H (2 × pivalate); 3.76 dd, 1 H (H-28a, *J*(28a,28b) = 11.0, $J_{l.r.}$ = 1.0); 4.25 dd, 1 H (H-28b, *J*(28b,28a) = 11.0, $J_{l.r.}$ = 1.8); 4.05 td, 1 H (H-6β, *J*(6β,5α) = 11, *J*(6β,7α) = 11, *J*(6β,7β) = 3.2); 4.40 m, 1 H (H-3, Σ*J* = 16.3). For ^{13}C NMR, see Table III.

3β-Hydroxylupane-6α,28-diyl dipivalate (**25**): 6 mg, 6%, amorphous, $[\alpha]_D$ 0. ^{1}H NMR: 0.765 d, 3 H (H-29, *J*(29,20) = 6.4); 0.839 d, 3 H (H-30, *J*(30,20) = 6.8); 0.824 s, 0.961 s, 0.978 s, 1.187 s, 1.228 s, 5 × 3 H (5 × CH_3); 1.198 s, 1.206 s, 2 × 9 H (2 × pivalate); 3.19 m, 1 H (H-3, Σ*J* = 16.5); 3.80 dd, 1 H (H-28a, *J*(28a,28b) = 11.4, $J_{l.r.}$ = 1.0); 4.17 dd, 1 H (H-28b, *J*(28b,28a) = 11.4, $J_{l.r.}$ = 1.8); 5.32 td, 1 H (H-6β, *J*(6β,5α) = 10.9, *J*(6β,7α) = 10.9, *J*(6β,7β) = 3.9). For ^{13}C NMR, see Table III.

3β,6α-Dihydroxylupan-28-yl pivalate (**23**): 44 mg, 53%, m.p. 125–129 °C (methanol), $[\alpha]_D$ –11. IR: 3608 (OH), 1716 (C=O), 1480, 1462, 1387, 1367, 1174, 1033. EI-MS, *m/z* (%): 544 (M^+, 1), 526 (3), 509 (4), 493 (12), 411 (6), 205 (22), 191 (30), 187 (100). HRMS, *m/z*: 544.4471 (for $C_{35}H_{60}O_4$ calculated: 544.4492). ^{1}H NMR: 0.766 d, 3 H (H-29, *J*(29,20) = 6.8); 0.840 d, 3 H (H-30, *J*(30,20) = 6.8); 0.894 s, 3 H (H-25); 0.979 s, 0.982 s, 2 × 3 H (H-24 and H-27); 1.128 s, 3 H (H-26); 1.314 s, 3 H (H-23); 1.206 s, 9 H (pivalate); 3.18 m, 1 H (H-3, Σ*J* = 16.3); 3.77 dd, 1 H (H-28a, *J*(28a,28b) = 11.1, $J_{l.r.}$ = 1.8); 4.24 dd, 1 H (H-28b, *J*(28b,28a) = 11.1, $J_{l.r.}$ = 1.3); 4.05 td, 1 H (H-6β, *J*(6β,5α) = 10.6, *J*(6β,7α) = 10.6, *J*(6β,7β) = 3.8). For ^{13}C NMR, see Table III.

TABLE III
^{13}C NMR chemical shifts of compounds **23**–**26** and **28**–**33** in $CDCl_3$

Carbon	**23**[a]	**24**[b]	**25**[c]	**26**[d]	**28**[e]	**29**[f]	**30**	**31**	**32**	**33**[g]
1	38.48	38.04	38.45	40.98	40.71	40.37	41.01	34.50	34.85	37.95
2	26.95	23.11	26.84	33.80	27.59	23.89	33.81	27.46	33.53	23.45[h]
3	78.71	80.19	78.73	214.56	79.14	80.88	214.54	75.21	217.5	78.53
4	39.28	39.01	38.57[h]	46.90	39.66	38.65	46.93	38.12	47.77[h]	40.38
5	60.48	60.56	58.08	65.19	55.60	55.61	65.20	68.47	68.69	65.55
6	68.74	68.54	71.58	211.19	68.89	68.75	211.38	54.85	55.36	61.33
7	46.69	46.72	42.05	51.93	42.17	42.22	52.06	32.79	32.37	31.68
8	42.92	42.93	42.98	47.61	40.04	40.01	47.72	36.98	38.26	39.10
9	49.52	49.42	49.57	49.99	50.70	50.59	50.03	41.06	39.94	47.15
10	39.15	39.16	39.76[h]	43.46	36.66	36.63	43.51	41.06	36.20	37.19
11	20.68	20.72	20.64	21.29	20.92	20.90	21.38	20.17	21.51[i]	23.51[h]
12	26.68	26.65	26.60	26.26	26.95	26.98	26.34	26.63	26.40	26.81
13	36.69	36.69	36.64	36.93	36.30	36.23	36.64	36.78	36.82	36.64
14	42.19	42.17	42.10	43.31	43.10	43.06	43.35	43.37	42.69	43.00
15	26.95	26.95	26.93	26.92	26.94	26.87	26.92	26.90	26.90	27.37
16	29.88	29.86	29.68	29.70	29.84	29.79	29.12	29.05	29.02	29.61
17	46.78	46.78	46.76	46.68	46.46	46.44	47.82	47.74	47.66[h]	46.32
18	48.11	48.11	48.08	48.24	48.22	48.19	48.18	48.26	48.29	48.23
19	44.54	44.55	44.51	44.52	44.52	44.59	44.55	44.42	44.54	44.50
20	29.41	29.41	29.40	29.42	29.47	29.42	29.50	29.41	29.52	29.48
21	21.57	21.58	21.55	21.58	21.60	21.57	21.70	21.80	21.77[i]	21.59
22	34.63	34.63	34.57	34.54	34.66	34.62	33.93	33.97	33.94	34.53
23	30.90	30.76	30.82	24.10	27.64	27.57	24.14	21.57	23.03	24.57
24	15.48	16.70	15.49	21.67	16.89	18.13	21.70	19.89	24.32	20.11
25	17.07	17.11	16.96	16.34	17.66	17.66	16.37	20.11[h]	19.55	18.65
26	17.57	17.58	17.18	16.07	16.99	16.96	16.05	20.32[h]	19.15	16.85
27	14.66	14.67	14.64	14.94	14.94	14.89	14.94	14.99	15.33	15.52
28	62.44	62.45	62.34	62.16	62.79	62.75	60.40	60.63	60.49	62.63
29	14.87	14.87	14.87	14.84	14.94	14.86	14.86	14.85	14.85	14.90
30	22.88	22.87	22.86	22.84	22.93	22.91	22.91	22.92	22.92	22.87

[a] 28-OPiv: 178.91, 36.69, 27.28; [b] 3-OPiv: 178.21, 38.32, 27.20, 28-OPiv: 178.92, 36.69, 27.20; [c] 6-OPiv: 178.11, 27.17, 28-OPiv: 178.92, 36.64, 27.27; [d] 28-OPiv: 178.83, 36.93, 27.24; [e] 28-OAc: 171.65, 21.07; [f] 3-OAc: 171.02, 21.33, 28-OAc: 171.60, 21.04; [g] 3-OAc: 170.80, 21.26, 28-OAc: 171.61, 21.05; [h,i] signals with identical symbols may be mutually interchanged.

TABLE IV
^{1}H NMR chemical shifts and coupling constants of compounds **8**, **10**, **12** and **14** in $CDCl_3$

Hydrogen	**8**	**10**	**12**	**14**
H-1α	1.68 m	1.29 m	1.36 m	1.57 m
H-1β	1.68	2.03 ddd (*13.1; 7.0; 2.5*)	2.06 ddd (*13.3; 7.0; 3.0*)	2.00 ddd (*13.1; 5.2; 2.7*)
H-2α	2.20 m[a]	2.33 m	2.43 ddd (*15.5; 5.9; 3.0*)	2.34 ddd (*17.1; 4.9; 2.7*)
H-2β	2.32 m[a]	2.45 td (*13.9; 13.9; 7.2*)	2.64 ddd (*15.5; 12.7; 7.0*)	2.45 ddd (*17.1; 14.8; 5.2*)
H-3α	–	–	–	–
H-4β	–	2.27 m	–	–
H-5α	1.90 m	1.08 m	1.44 m	–
H-6a	1.38 m	1.41 m	1.74 m	2.26 m
H-6b	1.81 m	1.61 m	1.79 m	2.64 dt (*14.8; 3.5; 3.5*)
H-7a	1.38 m	1.41m	1.46 m	1.56 m
H-7b	1.41 m	1.41m	1.51 m	1.56 m
H-9	1.44 m	1.42 m	1.41 m	1.56 m
H-11a	1.53 m	1.40 m	1.38 m	1.29 m
H-11b	1.70 m	1.53 m	1.41 m	1.58 m
H-12a	1.28 m	1.26 m	1.25 m	1.21 m
H-12b	1.66 m	1.67 m	1.67 m	1.66 m
H-13	1.78 m	1.80 m	1.79 m	1.74 m
H-15a	1.06 m	1.06 m	1.06 m	1.08 m
H-15b	1.79 m	1.80 m	1.80 m	1.76 m
H-16a	1.29 m	1.30 m	1.30 m	1.20 m
H-16b	1.95 m	1.98 m	1.96 m	1.93 ddd (*13.3; 4.6; 2.4*)
H-18	1.43 m	1.42 m	1.43 m	1.40 m
H-19	1.84 m	1.85 m	1.85 m	1.75 m
H-20	1.90 m	1.91 m	1.89 m	1.87 m
H-21a	1.38 m	1.53 m	1.52 m	1.51 m
H-21b	1.44 m	1.69 m	1.71 m	1.58 m
H-22a	0.96 m	0.95 m	0.96 m	0.85 m
H-22b	1.91 m	1.92 m	1.91 m	1.84 m
H-23a	4.64 d (*2.1*)	0.98 d (*5.5*)	1.249 s	1.79 d (*1.2*)
H-23b	4.88 m	–	–	–
H-24	1.724 s	–	9.713 s	–
H-25	0.861 s	1.048 d (*0.6*)	0.995 s	1.133 s
H-26	1.126 s	1.141 s	1.137 s	1.235 s
H-27	1.001 s	0.975 s	0.985 s	0.930 s
H-28a	4.06 dd (*11.1; 1.4*)	4.06 dd (*11.1; 1.4*)	4.06 dd (*11.1; 1.2*)	3.34 d (*11.2*)
H-28b	4.52 dd (*11.1; 1.7*)	4.54 dd (*11.1; 1.8*)	4.53 dd (*11.1; 1.7*)	3.80 dd (*11.2; 1.6*)
H-29	0.870 d (*6.8*)	0.791 d (*6.7*)	0.792 d (*6.6*)	0.770 d (*6.9*)
H-30	0.802 d (*6.8*)	0.867 d (*6.9*)	0.868 d (*6.9*)	0.854 d (*6.9*)
OBz:				
H-2′,6′	8.05 m	8.05 m	8.05 m	–
H-3′,5′	7.44 m	7.45 m	7.45 m	–
H-4′	7.56 m	7.56 m	7.56 m	–

[a] Signals may be mutually interchanged.

TABLE V
^{1}H NMR chemical shifts and coupling constants of compounds **15**, **16**, **18**, **20** and **22** in $CDCl_3$

Hydrogen	**15**	**16**	**18**	**20**	**22**
H-1α	1.56 m	1.56 m	0.99 m	0.95 m	1.63 m
H-1β	1.97 m	1.96 m	1.78 m	1.68 m	1.82 m
H-2α	2.54 ddd (*18.9; 9.2; 1.6*)	2.53 ddd (*18.9; 9.3; 1.8*)	1.68 m	1.22 m	2.71 ddd (*15.3; 12.0; 6.6*)
H-2β	2.43 ddd (*18.9; 10.2; 8.7*)	2.43 ddd (*18.9; 10.4; 8.7*)	1.68 m	1.62 m	2.26 ddd (*15.3; 9.8; 3.6*)
H-3α	–	–	3.21 m (Σ*J* = *16.2*)	3.18 m (Σ*J* = *16.4*)	–
H-5α	–	–	–	0.86 m	1.66 m
H-6	5.54 dd (*6.0; 2.4*)	5.55 dd (*5.6; 2.4*)	5.5710 dd (*5.2; 3.2*)	4.05 td (*10.6; 10.6; 4.0*)	3.93 td (*10.9; 10.9; 4.3*)
H-7a	1.73 m	1.72 m	1.68m	1.47 m	1.50 m
H-7b	2.20 bd (*17.2*)	2.20 bd (*16.8*)	2.23 bd (*18.4*)	1.69 m	1.79 m
H-9	1.63 m	1.62 m	1.42 m	1.30 m	1.40 m
H-11a	1.44 m	1.44 m	1.46 m	1.26 m	1.25 m
H-11b	1.54 m	1.56 m	1.54 m	1.50 m	1.48 m
H-12a	1.25 m	1.26 m	1.23 m	1.26 m	1.25 m
H-12b	1.66 m	1.66 m	1.63 m	1.86 m	1.63 m
H-13	1.68 m	1.68 m	1.62 m	1.63 m	1.65 m
H-15a	1.05 m	1.04 m	1.03 m	1.03 m	1.08 m
H-15b	1.83 m	1.71 m	1.77 m	1.76 m	1.76 m
H-16a	1.19 m	1.15 m	1.17 m	1.19 m	1.19 m
H-16b	1.93 m	1.91 m	1.88 m	1.91 m	1.93 ddd (*13.3; 4.6; 2.4*)
H-18	1.40 m	1.34 m	1.37 m	1.35 m	1.47 m
H-19	1.73 m	1.72 m	1.62 m	1.71 m	1.72 m
H-20	1.89 m	1.85 m	1.89 m	1.86 m	1.86 m
H-21a	1.50 m	1.46 m	1.50 m	1.49 m	1.48 m
H-21b	1.62 m	1.60 m	1.63 m	1.62 m	1.62 m
H-22a	0.85 m	0.82 m	0.83 m	0.85 m	0.86 m
H-22b	1.82 m	1.86 m	1.81 m	1.82 m	1.84 m
H-23	1.250 s	1.251 s	1.153 s	1.316 s	1.335 s
H-24	1.229 s	1.228 s	1.081 s	0.981 s	1.319 s
H-25	0.841 s	0.842 s	1.081 s	0.894 s	0.75 d (*0.8*)
H-26	1.070 s	1.080 s	1.048 s	1.112 s	1.088 s
H-27	0.989 s	0.975 s	0.953 s	0.981 s	1.020 s
H-28a	3.33 dd (*11.0; 1.1*)	3.04 dd (*9.2; 0.9*)	3.32 dd (*11.0; 1.2*)	3.31 dd (*10.9; 1.2*)	3.32 d (*10.9; 1.2*)
H-28b	3.80 dd (*11.0; 1.7*)	3.49 dd (*9.2; 1.7*)	3.80 dd (*11.0; 1.8*)	3.76 dd (*10.9; 1.7*)	3.76 dd (*10.9; 1.6*)
H-29	0.784 d (*6.8*)	0.774 d (*6.7*)	0.774 d (*6.8*)	0.767 d (*6.8*)	0.776 d (*6.8*)
H-30	0.861 d (*7.2*)	0.867 d (*6.9*)	0.853 d (*6.8*)	0.843 d (*6.8*)	0.851 d (*6.9*)
OMe	–	3.343 s	–	–	–

TABLE VI
^{1}H NMR chemical shifts and coupling constants of compounds **28**, **30**, **32** and **33** in $CDCl_3$

Proton	**28**	**30**	**32**	**33**
H-1α	0.92 m	1.53 m	1.92 m	1.98 dt (*14.0; 3.5*)
H-1β	1.69 m	2.10 ddd (*13.0; 6.2; 2.7*)	1.80 m	1.39 m
H-2α	1.65 m	2.22 ddd (*14.9; 4.5; 2.6*)	2.77 ddd (*16.2; 11.3; 5.3*)	1.75 m[a]
H-2β	1.65 m	2.77 td (*14.9; 14.9; 6.4*)	2.36 ddd (*16.2; 9.8; 4.4*)	1.80 m[a]
H-3α	3.14 m (Σ*J* = *15.8*)	–	–	4.68 m (Σ*J* = *15.3*)
H-5α	0.70 m	2.44 s	–	–
H-6	4.53 bs	–	3.12 bd (*4.1*)	3.43 t (*2.3*)
H-7a	1.63 m	2.02 d (*12.0*)	1.55m	1.83 m
H-7b	1.67 m	2.4721 d (*12.0*)	2.19 bd (*16.2*)	1.83 m
H-9	1.35 m	1.94 m	1.85 m	1.35 m
H-11a	1.35 m	1.43 m	1.39 m	1.34 m
H-11b	1.52 m	1.50 m	1.60 m	1.42 m
H-12a	1.20 m	1.30 m	1.21 m	1.14 m
H-12b	1.43 m	1.68 m	1.61 m	1.65 m
H-13	1.78 m	1.68 m	1.55 m	1.70 m
H-15a	1.02 m	0.89 m	1.04 m	1.05 m
H-15b	1.77 m	1.73 m	1.74 m	1.75 m
H-16a	1.20 m	1.19 m	1.16 m	1.27 m
H-16b	1.83 m	1.95 m	1.94 m	1.82 m
H-18	1.37 m	1.36 m	1.33 m	1.40 m
H-19	1.78 m	1.73 m	1.68 m	1.78 m
H-20	1.88 m	1.86 m	1.85 m	1.88 m
H-21a	1.46 m	1.50 m	1.45 m	1.45 m
H-21b	1.61 m	1.60 m	1.62 m	1.62 m
H-22a	0.87 m	0.85 m	0.83 m	0.88 m
H-22b	1.73 m	1.84 m	1.80 m	1.74 m
H-23	1.056 s	1.101 s	0.879 s	0.746 s
H-24	1.156 s	1.489 s	1.261 s	1.101 s
H-25	1.210 s	1.136 s	1.014 s	1.101 s
H-26	1.372 s	1.106 s	1.075 s	1.191 s
H-27	0.927 s	1.067 s	0.970 s	0.923 s
H-28a	3.84 dd (*11.1; 1.4*)	3.32 dd (*10.8; 1.0*)	3.31 dd (*11.0; 1.2*)	3.87 bd (*11.1*)
H-28b	4.26 dd (*11.1; 1.9*)	3.73 dd (*10.8; 1.6*)	3.75 dd (*11.0; 1.7*)	4.16 dd (*11.1; 1.8*)
H-29	0.768 d (*6.8*)	0.785 d (*6.8*)	0.769 d (*6.8*)	0.763 d (*6.8*)
H-30	0.846 d (*6.8*)	0.860 d (*6.9*)	0.845 d (*6.8*)	0.839 d (*6.8*)
OAc	2.059 s	–	–	2.057 s 2.058 s

[a] Signals may be mutually interchanged.

TABLE VII
Vicinal coupling constants (in Hz) of ring A protons of 3-oxo derivatives

Compd	Conformation	Substituent	Coupling constants, Hz			
			1α,2α	1α,2β	1β,2α	1β,2β
	C		5.7	13.2	1.4	5.7
	T_1		11.2	3.6	9.1	11.2
	T_2		9.7	10.3	0	9.7
	S		8.6	12.1	0.8	8.6
34[a]			7.9	9.4	4.4	7.8
12		24-oxo	5.9	12.7	3.0	7.0
15		$\Delta^{5,6}$	9.2	10.2	1.6	8.7
16		$\Delta^{5,6}$	9.3	10.4	1.8	8.7
22		6α-OH	12.0	3.6	6.6	9.8
26		6-oxo	4.6	14.8	2.6	6.2
30		6-oxo	4.5	14.9	2.6	6.4
32		5α,6α-epoxy	11.3	4.4	5.3	9.8

[a] Coupling constants (in Hz) taken from ref.[47]

TABLE VIII
Results of molecular modelling

Subst.	Ring A conformation	Number of structures	Energy[a] kJ mol^{-1}	Θ_1, ° (C_{10}–C_1–C_2–C_3)	Θ_2, ° (C_1–C_2–C_3–C_4)
24-oxo	*C*	53	341.7	–49	49
	T_1	21	345.1	21	–57
	sofa	23	346.7	–31	–6
	other	2	377.5		
$\Delta^{5,6}$	T_2	95	290.5	–31	–21
	other	5	316.2		
6α-OH	T_1	88	339.0	24	–60
	sofa	7	345.9	–26	–14
	C	5	347.5	–51	47
6-oxo	*C*	59	339.0	–51	50
	T_1	37	346.3	27	–59
	other	3	352.6		

[a] Lowest energy for a structure of given conformation type.

3,6-Dioxolupan-28-yl Pivalate (**26**)

To a solution of diol **23** (97 mg, 0.18 mmol) in pyridine (5 ml), chromium trioxide (145 mg, 1.45 mmol) was added and the mixture was stirred at room temperature overnight. The mixture was extracted with ether, the ether layer was washed with 2 M hydrochloric acid, 5% solution of potassium disulfite and with saturated solution of sodium hydrogencarbonate and then dried over anhydrous sodium sulfate and evaporated under reduced pressure. The evaporation residue (74 mg, 77%) was pure enough for use in another reaction. A sample for analysis was obtained by multiple crystallisation from methanol. M.p. 235–249 °C. EI-MS, *m/z* (%): 540 (M^+, 3), 524 (19), 509 (8), 425 (8), 409 (6), 217 (12), 203 (18), 191 (15), 57 (100). ^{1}H NMR: 0.784 d, 3 H (H-29, *J*(29,30) = 6.8); 0.856 d, 3 H (H-30, *J*(30,20) = 6.8); 1.062 s, 3 H (H-27); 1.121 s, 3 H (H-26); 1.137 s, 3 H (H-25); 1.098 s, 1.488 s, 2 × 3 H (H-23 and H-24); 1.206 s, 9 H (H pivalate); 2.02 d, 1 H (H-7a, *J*(7a,7b) = 11.9); 2.10 ddd, 1 H (H-1β, *J*(1β,1α) = 13.1, *J*(1β,2β) = 6.0, *J*(1β,2α) = 2.6); 2.21 ddd, 1 H (H-2α, *J*(2α,2β) = 14.9, *J*(2α,1α) = 4.6, *J*(2α,1β) = 2.6); 2.43 s, 1 H (H-5); 2.45 d, 1 H (H-7b, *J*(7b,7a) = 11.9); 2.77 td, 1 H (H-2β, *J*(2β,2α) = 14.8, *J*(2β,1α) = 14.8, *J*(2β,1β) = 6.2); 3.78 dd, 1 H (H-28a, *J*(28a,28b) = 11.2, $J_{l.r.}$ = 1.3); 4.20 dd, 1 H (H-28b, *J*(28b,28a) = 11.2, $J_{l.r.}$ = 1.9). For ^{13}C NMR, see Table III.

Lupane-3β,6β,28-triol (**27**)

To a solution of compound **26** (174 mg, 0.32 mmol) in dry ether (35 ml) lithium aluminium hydride (0.2 g, 5.2 mmol) was added and the mixture was refluxed for 3 h. Then ethyl acetate (25 ml, 315 mmol) was added and the mixture was refluxed for another 30 min. Then the reaction mixture was worked up in the usual manner. The evaporation residue (148 mg) was according to TLC a mixture of two compounds. The mixture was separated by column chromatography on silica gel (15 g) with ether–hexane (3:1) as eluent. Following compounds were obtained and crystallised from methanol (introduced according to their increasing polarity).

3β,6β-Dihydroxylupan-28-yl acetate (**28**): 36 mg, 22%, m.p. 231–235 °C, $[\alpha]_D$ –28. EI-MS, *m/z* (%): 502 (M^+, 4), 484 (12), 451 (10), 429 (35), 411 (11), 205 (60), 191 (55), 187 (100). For ^{1}H NMR, see Table VI. For ^{13}C NMR, see Table III.

Lupane-3β,6β,28-triol (**27**): 60 mg, 41%, m.p. 241–244 °C, $[\alpha]_D$ –28. IR: 3624 (OH), 1460, 1017. EI-MS, *m/z* (%): 460 (M^+, 6), 442 (7), 429 (20), 411 (15), 393 (5), 259 (5), 235 (22), 207 (45), 205 (43), 187 (100), 177 (40), 123 (100). ^{1}H NMR: 0.770 d, 3 H (H-29, *J*(29,20) = 6.8); 0.849 d, 3 H (H-30, *J*(30,20) = 6.8); 0.932 s, 3 H (H-27); 1.060 s, 3 H (H-23); 1.157 s, 3 H (H-24); 1.209 s, 3 H (H-25); 1.354 s, 3 H (H-26); 3.14 m, 1 H (H-3, Σ*J* = 15.7); 3.31 d, 1 H (H-28a, *J*(28a,28b) = 11.0); 3.79 dd, 1 H (H-28b, *J*(28b,28a) = 11.0, $J_{l.r.}$ = 1.4); 4.53 m, 1 H (H-6α, Σ*J* = 8.7). For $C_{30}H_{52}O_3$ (460.7) calculated: 78.21% C, 11.38% H; found: 78.12% C, 11.48% H.

6β-Hydroxylupane-3β,28-diyl Diacetate (**29**)

To the solution of triol **27** (10 mg, 0.022 mmol) in pyridine (0.4 ml) acetic anhydride (0.4 ml, 4.4 mmol) was added and the mixture was left standing at room temperature overnight. The mixture was worked up in the usual manner. Diacetate **29** (10 mg, 85%) was obtained, m.p. 266–270 °C (chloroform–methanol), $[\alpha]_D$ –21. IR: 3620 (OH), 1726 (acetate), 1461, 1387, 1367, 1252, 1031, 981. EI-MS, *m/z* (%): 544 (M^+, 3), 526 (3), 466 (8), 451 (10), 411 (3), 205 (34), 187 (100), 123 (58). ^{1}H NMR: 0.773 d, 3 H (H-29, *J*(29,30) = 6.7); 0.846 d,

3 H (H-30, *J*(30,20) = 6.9); 0.919 s, 3 H (H-27); 0.939 s, 3 H (H-23); 1.232 s, 3 H (H-24); 1.238 s, 3 H (H-25); 1.372 s, 3 H (H-26); 2.059 s, 6 H (2 × OAc); 3.83 dd, 1 H (H-28a, *J*(28a,28b) = 11.0, $J_{l.r.}$ = 1.4); 3.79 dd, 1 H (H-28b, *J*(28b,28a) = 11.0, $J_{l.r.}$ = 1.8); 4.43 m, 1 H (H-3α, Σ*J* = 16.0); 4.52 bs, 1 H (H-6α). For ^{13}C NMR, see Table III.

28-Hydroxylupane-3,6-dione (**30**)

To a solution of triol **27** (13 mg, 0.028 mmol) in pyridine (0.38 ml) bromine (20 μl, 0.40 mmol) was added and the mixture was left standing at –24 °C for 4 days. Then the mixture was diluted with water and extracted with ether. The ether layer was washed with water, 2 M hydrochloric acid, 5% solution of sodium sulfite, with saturated solution of sodium hydrogencarbonate and with water and then it was evaporated under reduced pressure. Dione **30** (10 mg, 78%) was obtained, amorphous, $[\alpha]_D$ –49. IR: 3626 (OH), 1709 (C=O), 1459, 1396, 1386, 1367, 1026. EI-MS, *m/z* (%): 456 (M^+, 25), 425 (100), 235 (15), 203 (15), 191 (40), 177 (25). For ^{1}H NMR, see Table VI. For ^{13}C NMR, see Table III.

5α,6α-Epoxylupane-3β,28-diol (**31**)

To a solution of olefin **18** (30 mg, 0.068 mmol) in chloroform (2 ml) 3-chloroperoxybenzoic acid (60%, 50 mg, 0.22 mmol) was added and the mixture was left standing at room temperature for 2.5 h. Then ether was added and the solution was washed with 5% solution of potassium iodide and then with 5% solution of sodium sulfite to decolourise the solution. This procedure was repeated as long as the potassium iodide solution changed colour to brown. Then the ether layer was washed with water, dried over anhydrous sodium sulfate and evaporated under reduced pressure. Epoxide **31** was obtained (31 mg, 100%), m.p. 279 °C (chloroform–ethanol), $[\alpha]_D$ –27. EI-MS, *m/z* (%): 458 (M^+, 6), 440 (18), 235 (15), 275 (45), 203 (60), 175 (78), 135 (90), 121 (100). HRMS, *m/z*: 458.3742 (for $C_{30}H_{50}O_3$ calculated: 458.3760). IR: 1463, 1388, 1238, 1064, 1021, 978, 952. ^{1}H NMR: 0.761 d, 3 H (H-29, *J*(29,20) = 6.9); 0.835 d, 3 H (H-30, *J*(30,20) = 7.0); 0.873 s, 3 H (CH_3); 0.965 s, 3 H (H-27); 1.089 s, 6 H (2 × CH_3); 1.120 s, 3 H (CH_3); 2.28 dd, 1 H (H-7β, *J*(7β,7α) = 16.6, *J*(7β,6β) = 1.6); 3.15 dd, 1 H (H-6β, *J*(6β,7β) = 1.8, *J*(6β,7α) = 3.1); 3.30 dd, 1 H (H-28a, *J*(28a,28b) = 11.2, $J_{l.r.}$ = 1.2); 3.54 m, 1 H (H-3α, Σ*J* = 16.6); 3.74 dd, 1 H (H-28b, *J*(28b,28a) = 11.0, $J_{l.r.}$ = 1.8). For ^{13}C NMR, see Table III.

5α,6α-Epoxy-28-hydroxylupan-3-one (**32**)

To the solution of diol **31** (18 mg, 0.039 mmol) in pyridine (0.6 ml), bromine (29 μl, 0.60 mmol) was added and the mixture was left standing at –24 °C for 4 days. Then the mixture was diluted with water and extracted with ether. The ether layer was washed with water, 2 M hydrochloric acid, 5% solution of sodium sulfite, with saturated solution of sodium hydrogencarbonate and with water. Then it was dried over anhydrous sodium sulfate and evaporated under reduced pressure. Amorphous title compound **32** (15 mg, 83%) was obtained. EI-MS, *m/z* (%): 456 (M^+, 20), 219 (80), 191 (100), 177 (70), 149 (100), 133 (88). HRMS, *m/z*: 456.3590 (for $C_{30}H_{48}O_3$ calculated: 456.3603). For ^{1}H NMR, see Table VI. For ^{13}C NMR, see Table III.

5β,6β-Epoxylupane-3β,28-diyl Diacetate (**33**)

Olefin **19** (40 mg, 0.076 mmol) was dissolved in a mixture of dichloromethane (0.8 ml), ethanol (1.2 ml) and water (80 μl). The solution was treated with 70% aqueous perchloric acid (60 μl) and *N*-bromoacetamide (40 mg) at room temperature for 105 min. The mixture was diluted with water, the product extracted with ether and the ethereal solution was washed with 5% aqueous sodium hydrogencarbonate solution, 5% aqueous sodium thiosulfate solution and with water. Then it was dried over anhydrous sodium sulfate and evaporated under reduced pressure. The product was purified by preparative TLC, ether–hexane (1:2) was used as eluent. Epoxide **33** was obtained (16 mg, 39%), m.p. 257 °C (chloroform–ethanol), $[\alpha]_D$ –20. IR: 1727, 1461, 1388, 1366, 1252, 1033, 983. EI-MS, *m/z* (%): 542 (M^+, 10), 524 (3), 482 (13), 464 (6), 121 (100). HRMS, *m/z*: 542.3968 (for $C_{34}H_{54}O_5$ calculated: 542.3971). For ^{1}H NMR, see Table VI. For ^{13}C NMR, see Table III.

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REFERENCES

1. Dračínský M., Richtr V., Křeček V., Sejbal J., Klinot J., Buděšínský M.: *Collect. Czech. Chem. Commun.* **2006**, *71*, 387.
2. Connolly J. D., Hill R. A.: *Methods Plant Biochem.* **1991**, *7*, 331.
3. Kim D. S., Pezzuto J. M., Pisha E.: *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1707.
4. Lee S. S., Chen W. C., Huang C. F., Su Y.: *J. Nat. Prod.* **1998**, *61*, 1343.
5. Fujikota T., Kashiwada Y., Kilkuskie R. E., Cosentino L. M., Ballas L. M., Jiang J. B., Chen I.-S., Lee K.-H.: *J. Nat. Prod.* **1994**, *57*, 243.
6. Ali M., Ravinder E., Ramachandram R.: *Pharmazie* **2000**, *55*, 385.
7. Dantanarayana A. P., Kumar N. S., Sultanbawa M. U. S., Balasubramaniam S.: *J. Chem. Soc., Perkin Trans. 1* **1981**, 2717.
8. Narayanan C. R., Naik D. G.: *Indian J. Chem., Sect., Sect. B: Org. Chem. Incl. Med. Chem.* **1981**, *20*, 62.
9. Manzoor-I-Khuda M., Chowdhury S. A., Reza T., Chowdhury A. K.: *J. Bangladesh Acad. Sci.* **1981**, *5*, 55.
10. Mustafa G., Anis E., Ahmed S., Anis I., Ahmed H., Malik A., Shahzad-ul-Hassan S., Choudhary M. I.: *J. Nat. Prod.* **2000**, *63*, 881.
11. Kuroyanaji M., Shiotsu M., Ebihara T., Kawai H., Ueno A., Fukushima L.: *Chem. Pharm. Bull.* **1986**, *34*, 4012.
12. Lunardi I., Peixoto J. L. B., da Silva C. C., Shuqel I. T. A., Basso E. A., Vidotti G. J.: *J. Brazil. Chem. Soc.* **2001**, *12*, 180.
13. Kouzi S. A., Chatterjee P., Pezzuto J. M., Hamann M. T.: *J. Nat. Prod.* **2000**, *63*, 1653.
14. Hess S. C., Delle Monache F.: *J. Brazil. Chem. Soc.* **1999**, *10*, 104.
15. Bilia A. R., Ciampi L., Mendez J., Morelli I.: *Riv. Ital. EPPOS* **1996**, *7*, 17.
16. Bilia A. R., Morelli I., Mendez J.: *J. Nat. Prod.* **1996**, *59*, 297.
17. Hisham A., Kumar G. J., Fujimoto Y., Hara N.: *Phytochemistry* **1996**, *42*, 789.

18. Facundo V. A., Andrade C. H. S., Silveria E. R., Braz-Filho R., Hufford C. D.: *Phytochemistry* **1993**, *32*, 411.
19. Drewes S. E., Mashimbye M. J., Field J. S., Ramesar N.: *Phytochemistry* **1991**, *30*, 3490.
20. Dantanarayana A. P., Kumar N. S., Muthukuda P. M., Wazeer M. I. M.: *Phytochemistry* **1982**, *21*, 2065.
21. Dantanarayana A. P., Kumar N. S., Muthukuda P. M., Balasubramaniam S.: *Phytochemistry* **1983**, *22*, 473.
22. Adnyana I. K., Tezuka Y., Awale S., Banskota A. H., Tran K. Q., Kadota S.: *Chem. Pharm. Bull.* **2000**, *48*, 1114.
23. Adnyana I. K., Tezuka Y., Banskota A. H., Xiong Q., Tran K. Q., Kadota S.: *J. Nat. Prod.* **2000**, *63*, 496.
24. Adnyana I. K., Tezuka Y., Banskota A. H., Tran K. Q., Kadota S.: *J. Nat. Prod.* **2001**, *64*, 360.
25. Jiang Z.-H., Tanaka T., Kouno I.: *Phytochemistry* **1995**, *40*, 1223.
26. Anaya J., Caballero M. C., Grande M., Navarro J. J., Tapia I., Almeida J. F.: *Phytochemistry* **1989**, *28*, 2206.
27. Rasool N., Khan A. Q., Malik A.: *J. Nat. Prod.* **1989**, *52*, 749.
28. Fukunaya T., Nishiya K., Kajikawa I., Watanabe Y., Suzuki N., Takeya K., Itokawa H.: *Chem. Pharm. Bull.* **1988**, *36*, 1180.
29. Chatterjee P., Kouzi S. A., Pezzuto J. M., Hamann M. T.: *Appl. Environ. Microbiol.* **2000**, *66*, 3850.
30. Akihisa T., Kimura Y., Kasahara Y., Kumaki K., Thakur S., Tamura T.: *Phytochemistry* **1997**, *46*, 1261.
31. Huang H.-C., Shen C.-C., Chen C.-F., Wu Y.-C., Kuo Y.-H.: *Chem. Pharm. Bull.* **2000**, *48*, 1079.
32. Atta-ur-Rahman, Khan M. A., Khan N. H.: *Phytochemistry* **1973**, *12*, 3004.
33. Rodriguez-Gamboa T., Fernandes J. B., Rodrigues E., da Silva M. F. D. F., Vieira P. C., Barrios M., Castro-Castillo O., Victor S. R., Pagnocca F. C., Bueno O. C., Hebling M. J. A.: *J. Brazil. Chem. Soc.* **2001**, *12*, 386.
34. Schuhly W., Heilmann J., Calis I., Sticher O.: *Planta Med.* **1999**, *65*, 740.
35. Talapatra S. K., Shrestha K. M., Pal M. K., Basak A., Talapatra B.: *Phytochemistry* **1989**, *28*, 3437.
36. Siddiqui S., Begum S., Hafeez F., Siddiqui B. S.: *Phytochemistry* **1989**, *28*, 1187.
37. Adnyana I. K., Tezuka Y., Banskota A. H., Tran K. Q., Kadota S.: *Biol. Pharm. Bull.* **2000**, *23*, 1328.
38. Endová M., Klinotová E., Sejbal J., Máca B., Klinot J., Protiva J.: *Collect. Czech. Chem. Commun.* **1994**, *59*, 1420.
39. Klinotová E., Křeček V., Klinot J., Endová M., Eisenreichová J., Buděšínský M., Štícha M.: *Collect. Czech. Chem. Commun.* **1997**, *62*, 1776.
40. Cohen K. F., Kazlauskas R., Pinhey J. T.: *J. Chem. Soc., Perkin Trans. 1* **1973**, 2076.
41. Klinot J., Hořejší M., Buděšínský M., Vystrčil A.: *Collect. Czech. Chem. Commun.* **1975**, *40*, 3712.
42. Kirk D. N., Hartshorn M. P.: *Steroid Reaction Mechanisms*, p. 168. Elsevier, Amsterdam 1968.
43. Midgley J. M., Millard B. J., Whalley W. B.: *J. Chem. Soc.* **1971**, 13.
44. King F. E., King T. J.: *J. Chem. Soc.* **1956**, 4469.
45. Pouzar V., Slavíková T., Černý I.: *Collect. Czech. Chem. Commun.* **1997**, *62*, 109.

46. Kočovský P., Černý V.: *Collect. Czech. Chem. Commun.* **1979**, *44*, 2275.
47. Klinot J., Buděšínský M., Klinotová J., Vystrčil A.: *Collect. Czech. Chem. Commun.* **1986**, *51*, 593.
48. Tsuda Y., Kiuchi F.: *Chem. Pharm. Bull.* **1984**, *32*, 4806.
49. Klinot J., Podlaha J., Podlahová J., Malý K., Petříček V.: *Collect. Czech. Chem. Commun.* **1989**, *54*, 725.
50. Haasnoot C. A. G., de Leeuw F. A. A. M., Altona C.: *Tetrahedron* **1980**, 3267.
51. Ruzicka L., Isler O.: *Helv. Chim. Acta* **1936**, *19*, 506.