Novel lupane triterpenoids containing allyl substituents in ring A: synthesis and *in vitro* study of antiinflammatory and cytotoxic properties

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Selective methods for the synthesis of *C*(2)-monoallyl and *C*(2)-diallyl derivatives of lupane terpenoids were developed. The methods involve reactions of allyl halides with potassium enolates or potassium enoxy(triethyl)borates generated *in situ* from 3-oxolupanes (betulonic acid, 3-oxo betulin) under the action of $KN(SiMe_3)_2$, KH, or Bu^tOK with subsequent addition of Et_3B. The use of the reagent $KN(SiMe_3)_2—Et_3B$ in 1,2-dimethoxyethane ensured the kinetically controlled generation of enolate anions, which yielded 2β-propenyl lupane terpenoids with high stereoselectivity. Reactions of 3-oxolupanes with excesses of Bu^tOK and allyl halide (2.5 equiv.) gave 2,2-bisallylation products. *In vitro* studies revealed that one of the latter efficiently suppresses the NO production by activated macrophages and has an antitumor effect on Ehrlich carcinoma and P-815 mastocytoma cell lines.

Key words: pentacyclic lupane triterpenoids, ketone enolates, kinetic and thermodynamic control, allylic alkylation, nitrogen monoxide, activated macrophages, antiinflammatory activity, antitumor activity.

Pentacyclic lupane triterpenoids (betulin and betulonic and betulinic acids) constitute a very important class of biologically active compounds with a broad spectrum of biological and pharmacological activity. Particular interest in these compounds is due to their antitumor and antiviral properties.[1,2] Lupane terpenoids show low toxicity against animals even in high concentrations; however, the relatively weak potential of their biological activity presents a serious hindrance to clinical use of these compounds. This was the reason why intensive investigations into the synthesis of derivatives of betulin and betulinic acid have been carried out in the last few decades. Most of them have been aimed at modifying the functional groups at the C(3) and C(28) atoms in betulin and triterpene acids. Some of the derivatives obtained are superior to native terpenoids (betulin and betulinic and betulonic acids) in antitumor and antiviral activity.[3,4] Investigations into the modification of ring A in terpenoids seem to be also promising. There are published data on the synthesis and biological properties of diosphenols, A-seco derivatives of betulinic acid and their anhydrides,[1,5] and compounds with a 1,3-enone fragment in ring A, in which the C(2) atom is bound to sterically small electron-withdrawing groups (CN, Cl, and CHO).[6—8] These C(2)-functionalized derivatives are more cytotoxic than betulinic acid itself and efficiently inhibit the NO production in activated macrophages. Pentacyclic lupane terpenoids with allylic substituents at the C(2) atom that could be further functionalized have not been documented hitherto.

Results and Discussion

The goal of the present study was to develop selective methods for the synthesis of novel 2-allyllupane terpenoids and test them *in vitro* for antiinflammatory and cytotoxic activities. Novel lupane terpenoids were obtained by allyl α-alkylation of potassium enolates or potassium enoxy(triethyl)borates generated from methyl betulonate and 3-oxo betulin.

It is known[9—11] that effective intermediates in α-alkylation of ketones are potassium enoxy(triethyl)borates generated *in situ* by reactions of cyclic or acyclic ketones with the reagents $KH—Et_3B$,[12] $KN(SiMe_3)_2—Et_3B$,[12] and $Bu^tOK—Et_3B$[13] in ethers. In most cases, the use of such enolate anions allows the regio- and stereochemical control of reactions and precludes the formation of *O*-alkylation, dialkylation, and polyalkylation by-products.[12—15]

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We found that a reaction of allyl bromide with enolate anion **A** (generated by treatment of methyl betulonate **1** with $KN(SiMe_3)_2$ in 1,2-dimethoxyethane (DME) followed by addition of Et_3B (the molar ratio **1** : $KN(SiMe_3)_2$: : Et_3B : C_3H_5Br was 1 : 1.3 : 1.3 : 1.3) gives shortly (4 h) methyl 2β-propenyl-3-oxolup-20(29)-en-28-oate (**2**) as the sole reaction product in 65% yield (Scheme 1). Extension of the reaction time to 24 h resulted in the formation of minor 2α-propenyl epimer **3** (2β : 2α = 96 : 4, ^1H and ^{13}C NMR data). Enolization of 3-oxo derivative of betulin TBS ether (**4**) in the system $KN(SiMe_3)_2$—Et_3B—DME followed by alkylation of the resulting enolate anion with allyl bromide was also a kinetically controlled process leading to β-epimer **5** with high stereoselectivity ($\geq$96%). The yield of product **5** in 4 h was 42% because of the incomplete conversion of the reagents. When carrying out α-allylation of enolate (**A**) generated from terpenoid **1** under the action of $KN(SiMe_3)_2$—Et_3B, we found that replacement of DME by THF only slightly changes the selectivity of the reaction over 4 h (2β : 2α = 96 : 4, ^1H and ^{13}C NMR). However, with an increase in the reaction time to 24 h, the kinetically controlled 2β-epimer **2** underwent isomerization, probably into the thermodynamically more stable 2α-epimer **3**. As a result, the reaction yielded a mixture of epimers **2** and **3** (2β : 2α = 60 : 40, ^1H and ^{13}C NMR). The transformation of 2β-epimer **2** into 2α-epimer **3** was confirmed by an additional experiment under thermodynamic equilibrium conditions. When keeping 2β-epimer **2** in THF in the presence of $KN(SiMe_3)_2$—Et_3B for 48 h, we obtained a 60 : 40 mixture of 2β- and 2α-epimers. α-Allylation of enolate **A** generated from methyl betulonate **1** under the action of KH—Et_3B in THF gave a 70 : 30 mixture of epimers **2** and **3** (after 24 h, the conversion was 21%).

In allyl α-alkylation of enolate anion **A** generated from terpenoid **1** in the system Bu^tOK—Et_3B—DME, the composition of products substantially varied with the reaction conditions. A reaction of methyl betulonate **1** with Bu^tOK—Et_3B in DME (**1** : Bu^tOK : Et_3B : C_3H_5Br = = 1 : 1.1 : 1.3 : 1.3) for 12 h gave a mixture of 2β- and 2α-epimers **2** and **3** (2β : 2α = 70 : 30). The total yield of products **2** and **3** did not exceed 20% because of the low conversion of the reagents. When another portion of Bu^tOK (1.1 equiv.) was added to the reaction mixture and the reagents were kept again for 12 h (thermodynamic equilibrium conditions), the reaction product was 2α-epimer **3**.

The high stereoselectivity of the allyl α-alkylation of terpenoids **1** and **4**, as well as the formation of kinetically controlled 2β-epimers **2** and **5**, is probably due to enolization of compounds **1** and **4** under the action of the bulky base $KN(SiMe_3)_2$ in DME. The resulting potassium enoxy(triethyl)borate **A** forms a chelate complex with DME, in which the solvent molecule approaches from the sterically more accessible α-side (because of the presence of the angular methyl group Me-25); so subsequent alkylation of the enolate anion with an allyl electrophile occurs on the β-side. With THF as a solvent, the allylic alkylation of methyl betulonate **1** was much less stereoselective since THF is inferior to DME in chelating properties.

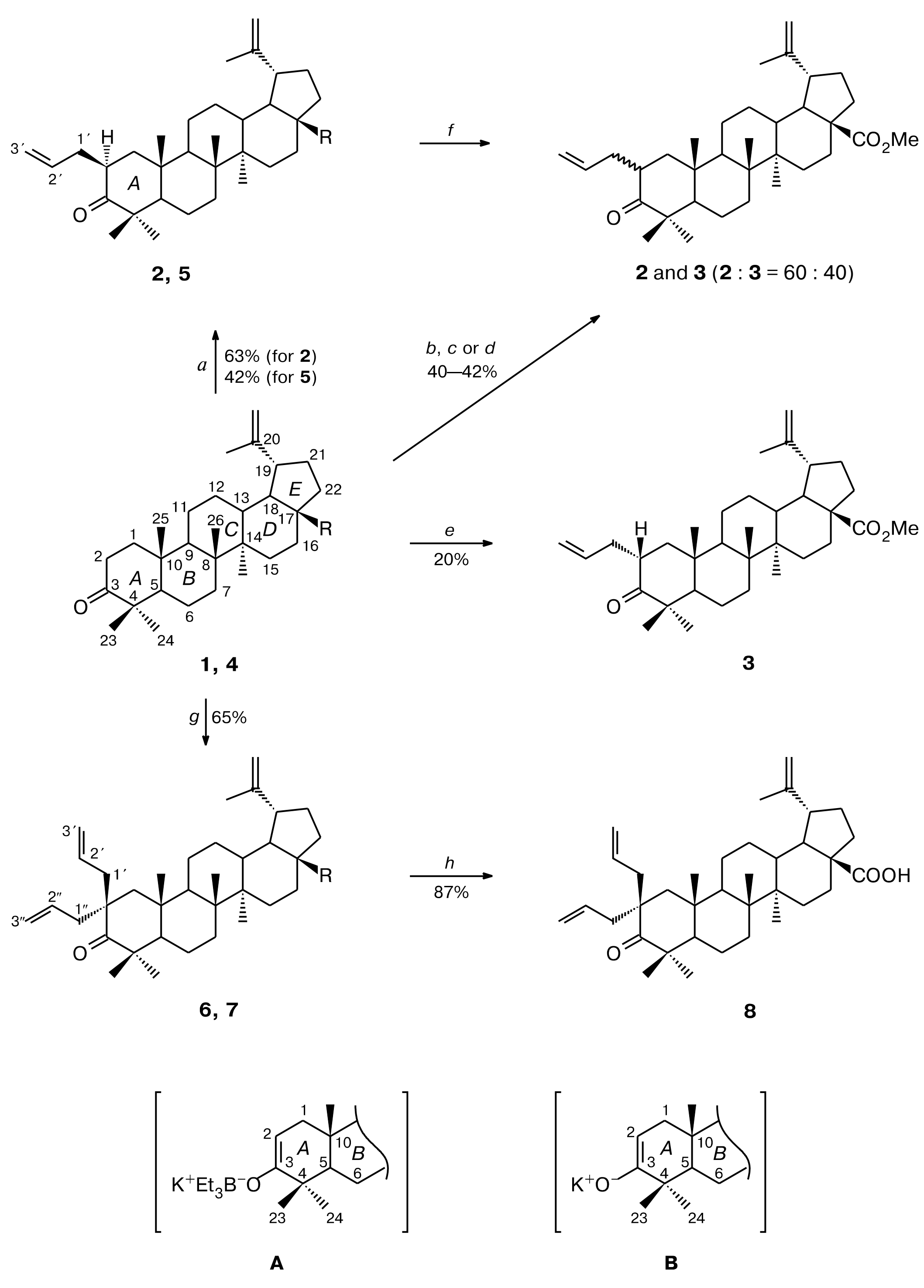
gem-Diallyl derivatives of betulonic acid and 3-oxo betulin are of interest as polyfunctional block synthons for design of novel derivatives of lupane terpenoids. It is known that 1,6-dienes easily undergo radical-induced cyclization**[16,17]** or cyclization catalyzed by transition metal complexes**[18,19]** (including catalysts for olefin metathesis**[20]**). Lupane terpenoids with a 1,6-diene fragment in ring A can be transformed into spiro structures containing cyclopentane and cyclohexane rings at the spiro C(2) atom.

We found that a reaction of allyl bromide with potassium enolate (**B**) generated from terpenoid **1** or **4** under the action of excess Bu^tOK in DME (**1** or **4** : Bu^tOK : C_3H_5Br = = 1 : 2.5 : 2.2) allows highly selective 2,2-bisallylation in 65% yield; the products were terpenoids **6** or **7**, respectively. The carboxyl function in compound **6** was deblocked by halogenolysis with LiBr in DMF to give 2,2-diallyl-3-oxolup-20(29)-en-28-oic acid **8**.

The structures of compounds **2**, **3**, and **5**—**8** were confirmed by 1D (^1H, ^{13}C, APT) and 2D homonuclear (COSY, NOESY) and heteronuclear experiments (HSQC, HMBC). The chemical shifts of the hydrogen atoms of the CH_2 and CH groups of the terpene framework were determined using the 2D heteronuclear correlation technique (HSQC). The ^{13}C NMR spectra of the compounds obtained are given in Table 1.

The MALDI TOF mass spectra of epimers **2** and **3** contain identical molecular ion peaks with *m/z* 531.44 $[M + Na]^+$ and 547.95 $[M + K]^+$. In the ^1H and ^{13}C NMR spectra of these compounds, the positions and multiplicity of the signals for the protons and C atoms of ring A (C(1)—C(5)) and the attached methyl groups (C(23), C(24), C(25)) show substantial changes. The β-orientation of the allyl substituent in compound **2** (2β-epimer) is evident from 1,3-steric couplings of this substituent with the methyl C(23) and C(25) atoms. The resulting shielding accounts for the upfield shifts of the signals for the methyl C atoms (by ~3.9 and ~2.5 ppm, respectively) compared to the signals for the corresponding methyl groups in 2α-epimer **3**. In compound **3**, a 1,3-steric coupling of the α-oriented allyl fragment with the methyl C(24) atom results in an upfield shift of its signal (δ 19.43) (the corresponding signal for 2β-epimer appears at δ 21.72). When determining the configurations of the allyl substituents in the 2α- and 2β-epimers, we also used diagnostic protons in the ^1H NMR spectra: the methine protons at the C(2) atoms and the methylene protons at the C(1) atoms. For instance, the NOESY spectrum of compound **3** shows an intense cross peak between the signal for the methine proton at the C(2) atom (δ 2.77, m) and the signal for the methyl protons at the C(23) atom

Scheme 1



R = CO_2Me (**1**—**3**, **6**), CH_2OTBS (**4**, **5**, **7**)

Reagents and conditions: *a*. 1) $KN(SiMe_3)_2$, BEt_3, 2) C_3H_5Br, DME or THF, 20 °C, 4 h; *b*. 1) $KN(SiMe_3)_2$, BEt_3, 2) C_3H_5Br, THF, 20 °C, 24 h; *c*. 1) Bu^tOK, Et_3B, 2) C_3H_5Br, DME, 20 °C, 12 h; *d*. 1) KH, Et_3B, 2) C_3H_5Br, THF, 24 h; *e*. 1)Bu^tOK, Et_3B, 2) C_3H_5Br, DME, 20 °C, 1 h, 3) Bu^tOK, 12 h; *f*. $KN(SiMe_3)_2$, BEt_3, THF, 20 °C, 48 h; *g*. 1) Bu^tOK, 2) C_3H_5Br, DME, 20 °C, 2 h; *h*. LiBr, DMF, reflux.

Table 1. ^{13}C NMR spectra of compounds **2**, **3**, and **5—8**

No. of the atom	Atom or group	δ					
		2	**3**	**5**	**6**	**7**	**8**
1	CH_2	46.96	48.64	46.82	50.65	50.65	50.67
2	CH_2	41.45	40.84	41.48	46.19	46.21	46.20
3	C	217.10	219.66	217.17	219.78	219.74	219.81
4	C	48.28	46.60	48.35	48.68	48.05	48.70
5	CH	57.28	52.34	57.23	51.58	51.57	51.56
6	CH_2	19.32	20.07	19.31	20.24	20.25	20.22
7	CH_2	34.07	33.00	33.94	32.99	32.85	32.98
8	C	40.74	40.64	40.99	40.49	40.76	40.51
9	CH	50.10	50.11	49.96	49.75	49.62	49.70
10	C	37.37	36.94	37.35	36.93	36.72	36.75
11	CH_2	21.13	22.01	21.11	21.73	21.73	21.69
12	CH_2	25.42	25.69	25.26	25.68	25.43	25.66
13	CH	38.21	38.55	37.35	38.40	37.59	38.56
14	C	42.49	42.42	42.78	42.48	42.78	42.52
15	CH_2	29.65	29.63	29.88	29.60	29.97	29.63
16	CH_2	32.12	32.06	26.99	32.06	26.97	32.10
17	C	56.47	56.54	48.35	56.51	48.33	56.40
18	CH	49.42	49.39	48.30	49.36	48.69	49.16
19	CH	46.86	46.89	48.03	46.91	48.02	46.88
20	C	150.47	150.53	150.89	150.52	150.85	150.38
21	CH_2	36.94	36.78	34.51	30.54	29.91	30.53
22	CH_2	30.54	30.57	29.41	36.74	34.34	36.62
23	CH_3	25.25	29.13	25.17	29.96	29.39	29.96
24	CH_3	21.72	19.43	21.74	23.16	23.18	23.13
25	CH_3	16.08	18.55	16.06	17.36	17.37	17.39
26	CH_3	16.08	15.27	16.06	15.32	15.28	15.40
27	CH_3	14.62	14.63	14.67	14.63	14.70	14.63
28	*	176.57	176.59	60.43	176.60	60.46	182.29
29	CH_2	109.67	109.60	109.44	109.63	109.44	19.36
30	CH_3	19.32	19.43	19.08	19.37	18.31	109.75
1′	CH_2	34.50	34.66	34.32	45.76	45.78	45.78
2′	CH	136.89	136.61	136.93	133.38	133.37	133.36
3′	CH_2	116.17	115.97	116.17	118.37	118.34	118.39
1″	CH_2				42.10	42.12	42.11
2″	CH				134.87	134.92	134.87
3″	CH_2				118.26	118.27	118.30
	OCH_3	51.29	51.29		51.29		
	Me—Si			–5.45		–5.41	
	Bu^t—Si			18.31		19.13	
	Me in Bu^t			25.97		25.97	

* CO for compounds **2**, **3**, **6**, and **8** and CH_2 for compounds **5** and **7**.

(δ 1.06, s). This suggests the spatial vicinity of these protons and hence the β-orientation of the methine H(2) proton. The coupling constant ($^3J_{H(1),H(2)}$ = 10 Hz) of the axial H(1) proton (δ 1.21, t, ^2J = ^3J = 10 Hz, an intense cross peak with the angular methyl group C(25) in NOESY) provides unambiguous evidence for the axial position of the H(2) proton. Therefore, compound **3** contains an α-oriented allyl group in the equatorial position. In epimer **2**, the vicinal coupling constant ($^3J_{H(1),H(2)}$ = 13 Hz) of the axial H(1) proton (δ 2.08, dd, ^2J = 6 Hz, ^3J = 13 Hz) suggests the axial location of the H(2) proton. Therefore, the allyl group in epimer **2** is also in the equatorial location but has the β-orientation. Apparently, the spectroscopic and stereochemical features of methyl 2α- and 2β-propenyl-3-oxolup-20(29)-en-28-oates are associated with the fundamental differences in the conformation of ring A. This assumption is indirectly confirmed by data[21] for 2-bromoallobetulones, according to which ring A in 2α-epimer has a chair conformation, while ring A in 2β-epimer has a planar or boat conformation.

The ^{13}C NMR spectra of bisallylation products **6** and **7** show a new singlet for the quaternary C(2) atom (δ 46.19

and 46.21 for **6** and **7**, respectively), while the signals for the double bonds of the geminal α- and β-oriented allyl substituents differ only slightly: δ 118.26 and 118.37 (CH_2) and δ 133.38 and 134.87 (CH) for compound **6** and δ 118.27 and 118.34 (CH_2) and δ 133.37 and 134.92 (CH) for compound **7**.

Pharmacological tests

Pharmacological *in vitro* tests of compound **8** were carried out in comparison with betulinic acid. The cytotoxic effects of betulinic acid and terpenoid **8** were studied using the MTT assay according to the known method.[22] The MTT assay, which is widely employed for estimation of the cytotoxicity of potential antitumor agents, involves reduction of colorless 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT reagent) to blue crystals of a formazan derivative in DMSO with mitochondrial and cytoplasmic dehydrogenases of metabolically active living cells. The resulting formazan dye is quantified by spectrophotometry. A test compound is said to be cytotoxic if the optical density in MTT assays in the presence of this compound is lower than that in a blank entry.[23] Our experimental data are given in Table 2.

Both betulinic acid and compound **8** proved to be toxic against peritoneal macrophages even in a concentration of 10 μg mL^{-1}, their toxic effects increasing with the dose. For all the concentrations studied (10, 25, and 50 μg mL^{-1}), product **8** was reliably more cytotoxic than betulinic acid.

Using the known procedure,[24] we studied the effects of terpenoid **8** and betulinic acid on the functional state of macrophages by evaluating the NO production by macrophages activated by lipopolysaccharide (LPS).

Our study showed that betulinic acid inhibits the NO production in a concentration of 10 μg mL^{-1} and higher, the inhibitive effect being dependent on the dose (Table 3). Compound **8** was much more superior to betulinic acid in a concentration range from 10 to 50 μg mL^{-1}.

Table 2. Cytotoxic effects of betulinic acid (BA) and terpenoid **8** on peritoneal macrophages ($X \pm m$)[a]

Concentration /μg mL^{-1}	Optical density	
	BA	**8**
—[b]	0.491±0.015	
0.1	0.517±0.018	0.429±0.021
1	0.486±0.032	0.411±0.029
10	0.384±0.009[c]	0.292±0.025[c,d]
25	0.273±0.020[c]	0.153±0.011[c,d]
50	0.209±0.012[c]	0.167±0.014[c,d]

[a] Here and in Tables 3—5, X is the experimental mean and m is the standard error.

[b] Blank entry.

[c] The differences from the blank entry are reliable for $p < 0.05$.

[d] The differences from the entries with BA are reliable for $p < 0.05$.

Table 3. Effects of betulinic acid (BA) and terpenoid **8** on the NO production by peritoneal macrophages ($X \pm m$)[a]

Concentration of the compounds /μg mL^{-1}	Concentration of nitrites/μmol L^{-1} in the presence of	
	BA	**8**
—[b]	77.61±1.40	
0.1	78.57±2.02	76.19±2.31
1	73.97±2.61	74.49±2.03
10	58.02±2.00[c]	22.24±0.62[c,d]
25	28.70±2.65[c]	1.54±0.77[c,d]
50	14.05±1.98[c]	1.82±0.65[c,d]

Note. For the meanings of the superscripts, see Table 2.

The antitumor activities of the compounds obtained were studied with P-815 mastocytoma and Ehrlich carcinoma cell lines. The cell lines were maintained *in vivo* in mice of the lines BALB/c (Ehrlich carcinoma) and DBA/2 (P-815 mastocytoma) using intraperitoneal transplantation. Tumor cells were placed in flat-bottomed 96-well plates ($2.0 \cdot 10^5$ cells per well) and cultured for 48 h (37 °C, 5% CO_2) in the presence of the test compounds at different dilutions. Four hours before the end of the incubation period, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) was added to the wells. After the incubation was completed, the supernatant was removed, the precipitate was dissolved in DMSO, and the absorbances of the solutions at 550 nm were measured on a spectrophotometer. The data obtained are given in Tables 4 and 5.

Betulinic acid showed antitumor activity against P-815 mastocytoma and Ehrlich carcinoma in a concentration of 10 μg mL^{-1}, its activity increasing with the dose. The antitumor activity of compound **8** against both tumor cell lines appeared at a concentration of 10 μg mL^{-1} and was comparable with that of betulinic acid. With an increase in the concentration, compound **8** became

Table 4. Cytotoxic effects of betulinic acid (BA) and terpenoid **8** on P-815 tumor cells ($X \pm m$)[a]

Concentration /μg mL^{-1}	Optical density	
	BA	**8**
—[b]	0.262±0.009	
0.1	0.269±0.009	0.234±0.009
1	0.247±0.013	0.252±0.014
10	0.223±0.006[c]	0.207±0.013[c]
25	0.200±0.010[c]	0.143±0.008[c,d]
50	0.170±0.009[c]	0.116±0.006[c,d]

Note. For the meanings of the superscripts, see Table 2.

Table 5. Cytotoxic effects of betulinic acid (BA) and terpenoid **8** on Ehrlich carcinoma cells ($X \pm m$)[a]

Concentration /μg mL^{-1}	Optical density	
	BA	**8**
—[b]	0.238±0.006	
0.1	0.224±0.011	0.223±0.008
1	0.208±0.016	0.231±0.012
10	0.183±0.007[c]	0.176±0.010[c]
25	0.164±0.006[c]	0.130±0.005[c,d]
50	0.159±0.008[c]	0.113±0.009[c,d]

Note. For the meanings of the superscripts, see Table 2.

more active, being superior to betulinic acid at $c = 25$ and 50 μg mL^{-1}.

To sum up, we were the first to carry out allylic alkylation of potassium enoxy(triethyl)borates of 3-oxolupanes and obtain 2β-propenyl terpenoids in high yields under kinetic control. We developed a simple and efficient method for the synthesis of new C(2)-diallyllupane terpenoids capable of being transformed further. The results of our pharmacological *in vitro* tests suggest that compound **8**, with its cytotoxicity, can have an antitumor effect and substantially inhibit the NO production, acting as an anti-inflammatory agent.

Experimental

IR spectra were recorded on a Specord IR-75 spectrometer (thin films or solutions in $CHCl_3$). ^1H and ^{13}C NMR spectra were recorded on a Bruker AVANCE-400 instrument (400.13 (^1H) and 100.62 MHz (^{13}C)) in $CDCl_3$ with Me_4Si as the internal standard. Mass spectra were measured on a Bruker—Autoflex III spectrometer (MALDI TOF, positive ion mode, 2,5-dihydroxybenzoic and α-cyano-4-hydroxycinnamic acids as the matrices). Optical rotation was determined on a Perkin—Elmer-141 polarimeter. Specific rotation $[\alpha]_D$ was expressed in (deg mL)/(g dm); the concentration of the solution c was expressed in g/(100 mL. Elemental analysis was carried out on a Carlo Erba 1106 analyzer. TLC was carried out on Sorbfil plates (Sorbpolimer, Krasnodar, Russia) in EtOAc—hexane (1 : 10); spots were visualized with anisaldehyde. Silica gel L (KSKG grade, 50—160 μm) was employed for column chromatography. A 25—35% suspension of KH in mineral oil, 1 M BEt_3 in THF, 0.5 M $KN(SiMe_3)_2$ in toluene, Bu^{t}OK, DME, and allyl bromide (Aldrich) were used. Hexane, THF, and DME were refluxed and distilled over metallic sodium. Enolization and α-alkylation of compounds **1** and **4** were carried out under argon. Betulonic acid, its methyl ester **1** (see Refs 25, 26), and betulin derivative **4** (see Ref. 27) were prepared according to known procedures. The carboxyl function in compound **6** was deblocked as described earlier.[24]

Allyl α-alkylation of compounds 1 and 4 with allyl bromide in the system $KN(SiMe_3)_2$—Et_3B (general procedure). A 0.5 M solution of $KN(SiMe_3)_2$ (1.10 mL, 0.55 mmol) in toluene was added under argon at room temperature to a stirred solution of compound **1** or **4** (0.42 mmol) in DME or THF (2.50 mL). After 15 min, 1 M Et_3B (0.55 mL, 0.55 mmol) in THF was added and the mixture was stirred for 1 h. Then a solution of allyl bromide (0.07 g, 0.55 mmol) in DME or THF (1 mL) was added. The reaction mixture was stirred for a specified period of time (4 or 24 h, monitoring by TLC), neutralized with 3 M HCl, and diluted with water (1.5 mL). The product was extracted with EtOAc (4×10 mL). The combined extracts were dried with $MgSO_4$ and concentrated. The residue was chromatographed on SiO_2 with hexane—EtOAc (30 → 1) as an eluent. Depending on the reaction conditions, the products were compound **2** (0.14 g, 65%; DME, 4 h), compound **5** (0.09 g, 42%, $\beta \geq 96\%$; DME, 4 h), a mixture of compounds **2** and **3** (0.09 g, 42%, $2\beta : 2\alpha = 96 : 4$; THF, 4 h), a mixture of compounds **2** and **3** (0.09 g, 42%, $2\beta : 2\alpha = 96 : 4$; DME, 24 h), and a mixture of compounds **2** and **3** (0.08 g, 40%, $2\beta : 2\alpha = 60 : 40$; THF, 24 h).

Methyl 2β-allyl-3-oxolup-20(29)-en-28-oate (2). Colorless crystals, m.p. 128—130 °C (EtOH), $[\alpha]_D^{20}$ −12.8° (c 0.32, $CHCl_3$). Found (%): C, 80.33; H, 10.51. $C_{34}H_{52}O_3$. Calculated (%): C, 80.26; H, 10.30. IR, ν/cm^{-1}: 1725 (C=O). ^1H NMR, δ: 0.92 (m, 1 H, $H^a_{eq}(1)$); 0.95, 0.97, 1.04, 1.05, 1.09 (all s, 3 H each, H(25), H(26), H(27), H(24), H(23)); 1.06 (m, 1 H, $H^a(12)$); 1.12 (m, 1 H, H(5)); 1.14 (m, 1 H, $H^a(15)$); 1.31 (m, 1 H, $H^a(11)$); 1.33 (m, 1 H, H(9)); 1.39 (m, 2 H, H(7); 1 H, $H^a(21)$); 1.40 (m, 2 H, H(6); 1 H, $H^b(15)$; 1 H, $H^a(16)$; 1 H, $H^a(22)$); 1.47 (m, 1 H, $H^b(11)$); 1.58 (t, 1 H, H(18), $^3J = 11$ Hz); 1.68 (s, 3 H, H(30)); 1.73 (m, 1 H, $H^b(12)$); 1.89 (m, 1 H, $H^b(22)$); 1.90 (m, 1 H, $H^b(21)$); 1.95 (dt, 1 H, $H^a(1')$, $^2J = 15$ Hz, $^3J = 7$ Hz); 2.08 (dd, 1 H, $H^b_{ax}(1)$, $^2J = 6$ Hz, $^3J = 13$ Hz); 2.23 (m, 1 H, H(13)); 2.24 (m, 1 H, $H^b(16)$); 2.53 (dt, 1 H, $H^b(1')$, $^2J = 15$ Hz, $^3J = 7$ Hz); 2.70 (dq, 1 H, H(2), $^3J = 13$ Hz, $^3J = 7$ Hz); 3.01 (dt, 1 H, H(19), $^3J = 11$ Hz, $^3J = 4$ Hz); 3.68 (s, 3 H, OMe); 4.60, 4.74 (both br.s, 2 H, H(29)); 4.97—5.03 (m, 2 H, H(3′)); 5.75 (m, 1 H, H(2′)). MS, m/z 531.44 $[M + Na]^+$. $C_{34}H_{52}O_3$.

2β-Allyl-3-oxolup-20(29)-en-28-ol, *tert*-butyl(dimethyl)silyl ether (5). Amorphous solid, $[\alpha]_D^{20}$ −0.89° (c 0.56, CH_2Cl_2). Found (%): C, 78.94; H, 11.02; Si, 4.59. $C_{39}H_{66}O_2Si$. Calculated (%): C, 78.72; H, 11.18; Si, 4.72. IR, ν/cm^{-1}: 1705 (C=O), 1251 ($Si(Me_3)_3$), 1088 (Si—O—C). ^1H NMR, δ: 0.06 (s, 6 H, Me—Si); 0.92 (s, 9 H, Me in Bu^t); 0.94 (m, 1 H, $H^a_{eq}(1)$); 0.97, 1.06, 1.08, 1.09, 1.11 (all s, 3 H each, H(25), H(26), H(27), H(24), H(23)); 1.05—1.90 (m, 21 H, CH_2, CH in the betulin residue); 1.69 (s, 3 H, H(30)); 1.95 (m, 1 H, H(1′)); 2.08 (dd, 1 H, $H^b_{ax}(1)$, $^2J = 6$ Hz, $^3J = 13$ Hz); 2.41 (m, 1 H, H(19)); 2.57 (m, 1 H, H(1′)); 2.71 (m, 1 H, H(2)); 3.28, 3.69 (both d, 1 H each, CH_2—O, $J = 10$ Hz); 4.58, 4.69 (both br.s, 2 H, H(29)); 4.99—5.04 (m, 2 H, H(3′)); 5.76 (m, 1 H, H(2′)). MS, m/z 618.34 $[M + Na]^+$, 633.30 $[M + K]^+$. $C_{39}H_{66}O_2Si$.

Allyl α-alkylation of compound 1 with allyl bromide in the system KH—Et_3B. Hexane (1 mL) was added to a 25—35% suspension of KH (0.06 g) in mineral oil (the actual KH amount was 0.01 g (0.35 mmol)). The suspension was stirred under argon and the hexane was poured out by decantation. The same procedure was repeated twice. Tetrahydrofuran (1 mL) and a solution of compound **1** (0.15 g, 0.32 mmol) in THF (1 mL) were added with stirring to the purified KH. After 15 min, 1 M Et_3B (0.42 mL, 0.42 mmol) in THF was added and the mixture was stirred for 40 min. Then a solution of allyl bromide (0.06 g, 0.48 mmol) in THF (1 mL) was added. The reaction mixture was stirred for an additional 12 h (monitoring by TLC), neutralized with 3 M HCl, and diluted with water (1 mL). The product was extracted with

EtOAc (4×7 mL). The combined extracts were dried with $MgSO_4$ and concentrated. The residue was chromatographed on SiO_2 with hexane—EtOAc (30 → 1) as an eluent. The yield of a mixture of compounds **2** and **3** was 0.03 g (21%, $2\beta : 2\alpha = 70 : 30$).

Allyl α-alkylation of compound 1 with allyl bromide in the system $Bu^tOK—Et_3B$. ***A.*** Potassium *tert*-butoxide (0.06 g, 0.55 mmol) was added under argon at room temperature to a stirred solution of compound **1** (0.24 g, 0.50 mmol) in DME (2.5 mL). After 15 min, 1 *M* Et_3B (0.65 mL, 0.65 mmol) in THF was added and the mixture was stirred for 1 h. Then a solution of allyl bromide (0.08 g, 0.65 mmol) in DME (1 mL) was added and stirring was continued for 1 h. After another portion of Bu^tOK (0.08 g, 0.65 mmol) was added, the reaction mixture was stirred for an additional 12 h (monitoring by TLC), neutralized with 3 *M* HCl, and diluted with water (1.5 mL). The product was extracted with EtOAc (4×10 mL). The combined extracts were dried with $MgSO_4$ and concentrated. The residue was chromatographed on SiO_2 with hexane—EtOAc (30 → 1) as an eluent. The yield of compound **3** was 0.06 g (20%).

Methyl 2α-allyl-3-oxolup-20(29)-en-28-oate (3). Colorless crystals, m.p. 200—201 °C (EtOH), $[\alpha]_D^{20}$ +66.5° (*c* 1.97, $CHCl_3$). Found (%): C, 80.37; H, 10.43. $C_{34}H_{52}O_3$. Calculated (%): C, 80.26; H, 10.30. IR, ν/cm^{-1}: 1720 (C=O). ^1H NMR, δ: 0.67, 0.91, 1.01, 1.03, 1.06 (all s, 3 H each, H(25), H(26), H(27), H(24), H(23)); 1.05 (m, 1 H, $H^a(12)$); 1.07 (m, 1 H, $H^a(11)$); 1.14 (m, 1 H, $H^a(15)$); 1.21 (t, 1 H, $H^a_{ax}(1)$, $^2J = 10.0$ Hz, $^3J = 10.0$ Hz); 1.35 (m, 1 H, $H^b(11)$); 1.36 (m, 1 H, $H^a(21)$); 1.38 (m, 1 H, $H^b(15)$); 1.40 (m, 2 H, H(7); 1 H, $H^a(16)$; 1 H, $H^a(22)$); 1.41 (m, 2 H, H(6)); 1.46 (m, 1 H, H(9)); 1.62 (m, 1 H, H(18)); 1.65 (s, 3 H, H(30)); 1.72 (m, 1 H, $H^b(12)$; 1 H, H(5)); 1.88 (m, 1 H, $H^b_{eq}(1)$); 1.90 (m, 2 H, $H^b(21)$, $H^b(22)$); 1.92 (m, 1 H, $H^a(1')$); 2.25 (m, 2 H, H(13), $H^b(16)$); 2.45 (m, 1 H, $H^b(1')$); 2.77 (m, 1 H, H(2)); 3.01 (m, 1 H, H(19)); 3.67 (s, 3 H, OMe); 4.61, 4.74 (both br.s, 2 H, H(29)); 4.96—5.04 (m, 2 H, H(3′)); 5.76 (m, 1 H, H(2′)). MS, *m/z* 531.97 $[M + Na]^+$, 547.95 $[M + K]^+$. $C_{34}H_{52}O_3$.

B. Potassium *tert*-butoxide (0.04 g, 0.37 mmol) was added under argon at room temperature to a stirred solution of compound **1** (0.16 g, 0.34 mmol) in DME (2 mL). After 15 min, 1 *M* Et_3B (0.44 mL, 0.44 mmol) in THF was added and the mixture was stirred for 1 h. Then a solution of allyl bromide (0.05 g, 0.44 mmol) in DME (1 mL) was added. The reaction mixture was stirred for an additional 12 h (monitoring by TLC), neutralized with 3 *M* HCl, and diluted with water (1 mL). The product was extracted with EtOAc (4×7 mL). The combined extracts were dried with $MgSO_4$ and concentrated. The residue was chromatographed on SiO_2 with hexane—EtOAc (30 → 1) as an eluent. The yield of a mixture of compounds **2** and **3** was 0.03 g (20%, $2\beta : 2\alpha = 70 : 30$).

2β:2α-Epimerization of compound 2. A 0.5 *M* solution of $KN(SiMe_3)_2$ (0.24 mL, 0.12 mmol) in toluene was added under argon at room temperature to a stirred solution of compound **2** (0.06 g, 0.11 mmol) in THF (1 mL). After 15 min, 1 *M* Et_3B (0.14 mL, 0.14 mmol) in THF was added. The mixture was stirred for 48 h (monitoring by TLC), neutralized with 3 *M* HCl, and diluted with water (0.5 mL). The product was extracted with EtOAc (4×4 mL). The combined extracts were dried with $MgSO_4$ and concentrated. The residue was chromatographed on SiO_2 with hexane—EtOAc (30 → 1) as an eluent. The yield of a mixture of compounds **2** and **3** was 0.05 g (89%, $\beta : \alpha = 60 : 40$).

2,2-Bisallylation of compounds 1 and 4 (general procedure). Potassium *tert*-butoxide (0.11 g, 1 mmol) was added at room temperature to a stirred solution of compound **1** or **4** (0.40 mmol) in DME (2.5 mL). After 1 h, a solution of allyl bromide (0.11 g, 0.88 mmol) in DME (1 mL) was added. The reaction mixture was stirred for 2 h (monitoring by TLC), neutralized with 3 *M* HCl, and diluted with water (2 mL). The product was extracted with EtOAc (4×10 mL). The combined extracts were dried with $MgSO_4$ and concentrated. The residue was chromatographed on SiO_2 with hexane—EtOAc (30 → 1) as an eluent to give compound **6** or **7**.

Methyl 2,2-diallyl-3-oxolup-20(29)-en-28-oate (6). Yield 65%, colorless crystals, m.p. 152—154 °C (EtOH), $[\alpha]_D^{20}$ +36.60° (*c* 2.09, $CHCl_3$). Found (%): C, 80.84; H, 10.57. $C_{37}H_{56}O_3$. Calculated (%): C, 80.97; H, 10.28. IR, ν/cm^{-1}: 1720 (C=O), 1750 (COOMe). ^1H NMR, δ: 0.68, 0.93, 1.01, 1.06, 1.14 (all s, 3 H each, H(25), H(26), H(27), H(24), H(23)); 1.06 (m, 1 H, $H^a(12)$); 1.19 (m, 1 H, $H^a(15)$); 1.25 (m, 1 H, $H^a(11)$); 1.37 (m, 1 H, $H^a(21)$); 1.39 (m, 1 H, $H^b(15)$; 1 H, $H^a(16)$; 1 H, H(9)); 1.40 (m, 2 H, H(7)); 1.41 (m, 2 H, H(6)); 1.42 (m, 1 H, $H^a(22)$); 1.48 (m, 1 H, $H^b(10)$); 1.62 (d, 1 H, H(18), $J = 11.0$ Hz); 1.64 (m, 1 H, H(5)); 1.69 (s, 3 H, H(30)); 1.73 (m, 1 H, $H^b(12)$); 1.90 (m, 1 H, $H^b(21)$; 1 H, $H^b(22)$); 2.09 (d, 2 H, H(1), $J = 14.0$ Hz); 2.23 (m, 1 H, H(13)); 2.24 (m, 1 H, $H^b(16)$); 2.25 (m, 2 H, $H^a(1')$, $H^a(1'')$); 2.42 (dd, 2 H, $H^b(1')$, $H^b(1'')$, $J = 15.0$ Hz, $J = 6.0$ Hz); 3.01 (m, 1 H, H(19)); 3.67 (s, 3 H, OMe); 4.60, 4.74 (both br.s, 2 H, H(29)); 4.98—5.07 (m, 4 H, H(3′), H(3″)); 5.55, 5.76 (both m, 1 H each, H(2′), H(2″)). MS, *m/z* 549.82 $[M + H]^+$. $C_{37}H_{56}O_3$.

2,2-Diallyl-3-oxolup-20(29)-en-28-ol, *tert*-butyl(dimethyl)silyl ether (7). Yield 52%, amorphous powder, $[\alpha]_D^{20}$ +34.20° (*c* 0.33, $CHCl_3$). Found (%): C, 79.34; H, 10.98; Si, 4.48. $C_{47}H_{70}O_2Si$. Calculated (%): C, 79.43; H, 11.11; Si, 4.42. IR, ν/cm^{-1}: 1693 (C=O), 1251 ($Si(Me_3)_3$), 1089 (Si—O—C). ^1H NMR, δ: 0.07 (s, 6 H, Me—Si); 0.92 (s, 9 H, Me in Bu^t); 0.91, 1.03, 1.05, 1.08, 1.16 (all s, 3 H each, H(25), H(26), H(27), H(24), H(23)); 1.09—1.95 (m, 18 H, CH_2, CH in the betulin residue); 1.70 (s, 3 H, H(30)); 2.09 (d, 2 H, H(1), $J = 14$ Hz); 2.26 (m, 2 H, H(13), H(16)); 2.26 (m, 2 H, $H^a(1')$, $H^a(1'')$); 2.42 (m, 3 H, $H^b(1')$, $H^b(1'')$, H(19)); 3.29, 3.70 (both d, 1 H each, CH_2—O, $J = 10$ Hz); 4.59, 4.70 (both br.s, 2 H, H(29)); 4.99—5.08 (m, 4 H, H(3′), H(3″)); 5.55, 5.70 (both m, 1 H each, H(2′), H(2″)). MS, *m/z* 633.35 $[M + H]^+$. $C_{47}H_{70}O_2Si$.

2,2-Diallyl-3-oxolup-20(29)-en-28-oic acid (8). Lithium bromide (0.68 g, 7.80 mmol) was added to a stirred solution of compound **6** (0.23 g, 0.42 mmol) in DME (4 mL). The reaction mixture was refluxed for 12 h (monitoring by TLC), diluted with water (2 mL), and neutralized with 5% HCl. The product was extracted with EtOAc (4×10 mL). The combined extracts were dried with $MgSO_4$ and concentrated. The residue was chromatographed on SiO_2 with hexane—EtOAc (30 → 1) as an eluent. The yield of compound **8** was 0.19 g (87%), colorless crystals, m.p. 120—122 °C (EtOH), $[\alpha]_D^{20}$ +4.02° (*c* 0.09, CH_2Cl_2). Found (%): C, 80.91; H, 10.07. $C_{36}H_{54}O_3$. Calculated (%): C, 80.85; H, 10.18. IR, ν/cm^{-1}: 1720 (C=O). ^1H NMR, δ: 0.71, 0.96, 1.03, 1.06, 1.15 (all s, 3 H each, H(25), H(26), H(27), H(24), H(23)); 1.06—1.90 (m, 18 H, CH_2, CH in the betulonic acid residue); 1.71 (s, 3 H, H(30)); 2.09 (d, 2 H, H(1), $J = 14.0$ Hz); 2.23 (m, 1 H, H(13)); 2.25 (m, 3 H, $H^a(1')$, $H^a(1'')$, H(16)); 2.43 (dd, 2 H, $H^b(1')$, $H^b(1'')$, $J = 14.0$ Hz, $J = 6.0$ Hz); 3.04 (m, 1 H, H(19)); 4.63, 4.76 (both br.s, 2 H, H(29)); 5.00—5.08 (m, 4 H, H(3´), H(3″)); 5.57, 5.70 (both m, 1 H each, H(2´), H(2″)); 8.05 (s, 1 H, COOH). MS, *m/z* 557.33 $[M + Na]^+$, 533.29 $[M + H]^+$. $C_{36}H_{54}O_3$.

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