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Synthesis of Compounds with Potential Antitumor Activity: IV.* Modification of Lupinine and Menthol by the Taxol Amino Acid Moiety

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Abstract—The synthesis and the results of biological testing of novel *N*-benzoylphenylisoserine-modified L–lupinine and L-menthol are reported.

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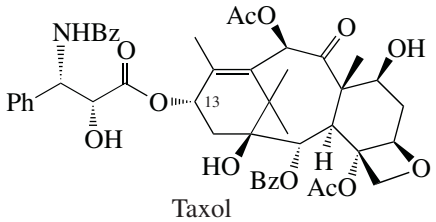
Taxol, isolated from extracts of the *Taxus brevifolia* bark, is currently the most efficient antitumor drug. Its action is based on its ability to bind to tubulin and disturb the dynamics of the microtubule cytoskeleton (taxol stabilizes microtubules and thereby prevents a cell from dividing). It is worth noting that the clinical use of taxol is limited by the fact that it should be obtained by a semisynthetic method from natural sources. This necessity is caused by its intricate molecular structure. Therefore, development of taxol analogues with a simpler structure is an important task.



Taxol

In the framework of a program of synthesis of analogues in which the complicated taxol skeleton is replaced by the simpler adamantane or bicyclo[3.3.1]nonane moieties [1–9], we showed that tubulin aggregation is induced only by cage compounds containing *N*-benzoylphenylisoseryl as the only substituent (the substituent at the C-13 atom in taxol). This fact has stimulated our study of modification of different mono- and polycyclic structures with *N*-benzoylphenylisoserine. As such structures, we used the natural compounds lupinine (**1**), used in the form of N-oxide, and menthol (**2**). Esterification was performed as described in [1] by protected amino acid **3** with subsequent opening of the oxazolidine ring (Scheme 1). The structures of the resulting new optically active intermediates (**4**, **5**) and end compounds (**6**, **7**) were determined by means elemental analysis, IR spectroscopy, and NMR. The overall yields of *N*-benzoylphenylisoseryl derivatives **6** and **7** were 51 and 64%, respectively.

Our biological activity tests for the ability of the resulting compounds to stimulate tubulin polymerization in vitro and to stabilize microtubules in vivo in cells showed that neither the lupinine (**6**) nor the menthol (**7**) analogue has an effect on microtubules (at the same time, compound **7** has a strong effect on cell adhesion). It should be emphasized that the known *N*-benzoylphenylisoseryl derivatives of galactopyranose, gibberellic acid, and guanosine are unable to promote tubulin polymerization or to stabilize microtubules in cells [10, 11] (for the guanosine analogue, only a weak cytotoxic effect was reported [11]). The results of the present study confirms our previous conclusion that definite cage moieties in the structure of "simplified" taxol analogues play an important role in tubulin-aggregating activity.

EXPERIMENTAL

The ^{1}H and ^{13}C NMR spectra were recorded on a Varian Avans-400 spectrometer (400 MHz) in $CDCl_3$ with trimethylsilane as the internal reference. The reaction course was monitored by thin-layer chromatography (TLC) on Silufol plates. Chromatographic separation was carried out on columns packed with Merck-60 silica gel (220–440 mesh ASTM).

**3-*tert*-Butyl 5-[((1*S*,9a*S*)-5-oxidooctahydro-2*H*-quinolizin-1-yl)methyl] (4*S*,5*R*)-2,2-dimethyl-4-phe-

* Parts I–III, see [1–3].

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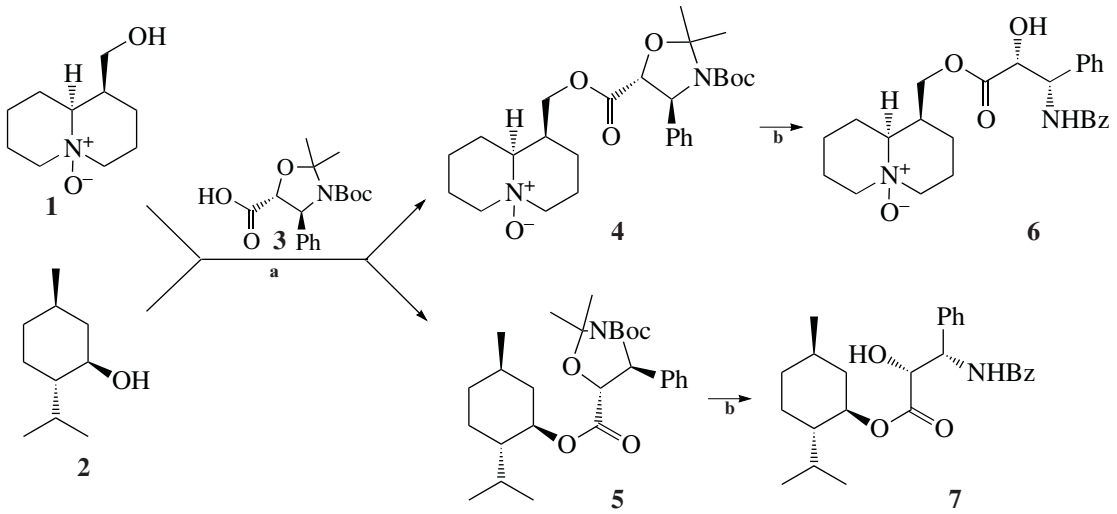

Scheme 1.

nyl-1,3-oxazolidine-3,5-dicarboxylate (4) was synthesized as described in [1] from 0.060 g (0.32 mmol) of lupinine N-oxide (**1**) and 0.050 g (0.16 mmol) of protected amino acid **3** in absolute CH_2Cl_2. The product was purified chromatographically (elution with ethyl acetate–petroleum ether (1 : 10) at 40–70°C). The yield was 0.053 g (68%) of compound **4** as a colorless liquid. ^{1}H NMR ($CDCl_3$/TMS), δ, ppm: 1.18–1.39 (m, 19H, backbone + *t*-Bu); 1.61–1.96, including 1.67 and 1.77 (m, 12H, backbone + Me_2C); 3.65 and 4.07 (both dd, 2H, CH_2O); 4.64 (d, 1H, OCHCHN); 5.44 (s, 1H, OCHCHN); 7.27–7.36 (m, 5H, Ph).

3-*tert*-Butyl 5-[(1*R*,2*S*, 5*R*)-2-isopropyl-5-methylcyclohexyl] (4*S*,5*R*)-2.2-dimethyl-4-phenyl-1,3-oxazolidine-3,5-dicarboxylate (5) was synthesized as described in [1] from 0.070 g (0.45 mmol) of (–)-menthol **2** and 0.068 g (0.21 mmol) of protected amino acid **3** in absolute CH_2Cl_2. The product was chromatographically purified (elution with ethyl acetate–petrol ether (1 : 7), 40–70°C). The yield was 0.070 g (72%) of compound **5** as a colorless liquid. ^{1}H NMR ($CDCl_3$/TMS), δ, ppm: 0.75 (d, 3H, MeCH), 0.86–0.92 (m, 6H, Me_2CH), 1.00–1.51 + 1.17 (s, 16H, backbone + *t*-Bu), 1.67–2.05 + 1.71 + 1.79 (m, 8H, backbone + Me_2C), 4.46 (d, 1H, OCHCHN), 4.81 (m, 1H, CHO_2C), 5.12 (s, 1H, OCHCHN), 7.26–7.37 (m, 5H, Ph).

(1*S*,9a*S*)-5-Oxidooctahydro-2*H*-quinolizin-1-yl)methyl (2*R*,3*S*)-3-(benzoylamino)-2-hydroxy-3-phenylpropanoate (6) was synthesized as described in [1] from 0.053 g (0.11 mmol) of **4** in 5 mL of 85% formic acid. The intermediate product was benzoylated with 0.02 mL (0.17 mmol) of benzoyl chloride. For end product **6**, ^{1}H NMR ($CDCl_3$/TMS), δ, ppm: 1.34–2.18 (m, 17H, backbone + OH), 3.66–4.13 (m, 2H, CH_2O_2C), 5.08 (d, 1H, CHOH), 5.92 (dd, 1H, CHN), 7.16 (br s, 1H, NH), 7.29–7.81 (10H, arom.). ^{13}C NMR ($CDCl_3$/HMDS), δ, ppm: 24.71, 24.91, 25.48, 25.58, 28.42, 28.43, 33.22, 48.71, 52.35, 52.97 (CHN), 77.70 (CHOH), 126.856–134.42 (arom.), 166.55 (NHOCBz), 172.02 (COO). IR (KBr), cm^{-1}: 1530, 1630, 1670, 1730, 3250 br, 3380.

(1*R*,2*S*,5*R*)-2-Isopropyl-5-methylcyclohexyl (2*R*,3*S*)-3-(benzoylamino)-2-hydroxy-3-phenylpropanoate (7) was synthesized as described in [1] from 0.070 g (0.15 mmol) of cyclic ester **5** in 5 mL of 85% formic acid. The intermediate hydroxyamino acid ester was benzoylated with 0.02 mL (0.17 mmol) of benzoyl chloride. The end product was purified chromatographically (elution with ethyl acetate–petroleum ether 1 : 9, then 1 : 3). The yield was 0.048 g (76%) of compound **7** as colorless crystals with mp 127–128°C, $[\alpha]_D^{26}$ – 44.04 (c = 0.005, CH_2Cl_2).

For $C_{26}H_{33}NO_4$ anal. calcd. (%): C, 73.73; H, 7.85; N, 3.31.

Found (%): C, 73.55; H, 7.90; N, 3.15.

^{1}H NMR ($CDCl_3$/TMS), δ, ppm: 0.55 (d, 3H, MeCH); 0.79 (d, 3H, MeC); 0.88–1.25, including 0.93 (d) (m, 6H, backbone + MeC); 1,48 (m, 2H); 1.68–1.71 (m, 2H); 1.83 (m, 1H); 1.99 (m, 1H); 3.44 (1H, OH); 4.60 (1H, CHOH); 4.87 (m, 1H, CHO_2C); 5.73 (dd (1H, CHN); 7.14 (br s, 1H, NH); 7.28–7.81 (10H, arom.). ^{13}C NMR ($CDCl_3$/TMS), δ, ppm: 15.46, 20.84, 21.95, 22.71, 25.60, 31.48, 34.00, 40.70, 46.78, 54.78 (CHYN), 73.78 (CHOH), 77.35 (CHO_2C), 126.86–138.97 (arom.), 166.37 (NHOCBz), 172.51 (COO). IR (KBr), cm^{-1}: 1520, 1580, 1650, 1720, 3420 br.

For biological tests of esters **6** and **7**, 5 mM initial solutions of these compounds and taxol in DMSO were prepared. The tests were carried out in vivo and in vitro in a concentration range of 10–100 μmol/L of **6** and **7**. A 25 μM taxol solution was used as a positive control. The ability of the tested compounds to stimulate in vitro assembly of microtubules (MTs) from protein

tubulin (1–2 mg/mL) isolated from bovine brain [12]. The formation of MTs was monitored by light video-enhanced contrast microscopy (AVEC DIC microscopy [13]) and sedimentation analysis [14]. The ability of compounds **6** and **7** to inhibit in vivo cell division was studied by fluorescence microscopy of *Vero* fibroblasts and PtK_2 epithelial cells stably transfected and expressing fluorescent labeled YFP tubulin.

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