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MACROMOLECULAR CHEMISTRY AND POLYMERIC MATERIALS

Polymeric Derivatives of Dipterocarpol, a Dammarane Triterpenoid

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Abstract—Polymeric derivatives of dammarane triterpenoids with both labile and strong (covalent) polymer–triterpenoid bonds were prepared from *N*-vinylpyrrolidone copolymers.

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Treatment of virus diseases requires development of effective nontoxic antiviral preparations. Synthesis of polymeric derivatives of low-molecular-weight biologically active substances (BASs) opens prospects for development of improved medicinals of reduced toxicity, enhanced efficiency, and prolonged effect.

Polymers based on *N*-vinylpyrrolidone (VP) are among the most widely used polymeric carriers [1, 2]. VP copolymers containing amino groups show promise as BAS carriers. Alkylation with various alkyl halides of VP copolymers with primary and tertiary amines allows preparation of polymers containing, along with the ionic ammonium group, alkyl substituents providing hydrophobic interaction in aqueous solutions [3]. Preparation of water-soluble derivatives (salts) of polyene antibiotics Levorin and Nystatin on the basis of such copolymers was reported in [4].

Previous studies revealed more pronounced antiviral properties of polymeric derivatives of certain triterpene acids compared to the nonmodified acids [5].

The goal of this study was to develop procedures for preparing from VP copolymers polymeric derivatives of triterpenoids of the dammarane series differing in the type and lability of the polymer–triterpenoid bond.

Among dammarane triterpenoids, the highest antiviral activity is exhibited by alnincanone, 24-methyl-20S,24R-epoxydammaran-3-one [6]; however, its content in plant sources is insufficient for more comprehensive biological studies. As a model compound we chose 20(S)-hydroxydammar-24-en-3-one (dipterocarpol) **I**. It can be prepared in amounts required for the studies from *Dipterocarpus alatus* latex [7].

We prepared dammarane derivatives containing at C-17 an open chain (VII), a γ -lactone ring (III), and a tetrahydrofuran ring (IV). Diol VII was prepared by reduction of dipterocarpol with sodium borohydride in ethanol. To prepare compounds III and IV, dipterocarpol was oxidized with chromic anhydride in acetic acid to lactone II. Reduction of II with sodium borohydride in ethanol yielded hydroxy lactone III, and reduction with sodium borohydride in the presence of $BF_3 \cdot OEt_2$ in THF yielded epoxydammaranol IV. From compounds III, IV, and VII (Scheme 1) containing the 3β-hydroxy group, we prepared by refluxing with phthalic anhydride phthalic acid monoesters V, VI, and VIII, respectively (Scheme 1). Through the free carboxy group, these compounds were bonded to an amino group of the polymer.

To prepare polymeric derivatives of triterpenoids of the dammarane series with a labile BAS–polymer bond (BAPs), we used a copolymer of VP with dimethylaminoethyl methacrylate (DMAEM) and with its derivative quaternized with dodecyl iodide (DMAEM $\cdot C_{12}H_{25}I$) [8]. Polymeric salts of phthalic acid monoesters were prepared by Scheme 2.

As solvent we used methanol in which all the reactants (triterpenoid hydrogen phthalates and the polymer) and products (polymeric salts) are soluble. After the synthesis completion, methanol was gradually replaced by water [4]. As a result, we obtained polymeric derivatives which, in contrast to the starting



triterpenoid hydrogen phthalates, are soluble in water. The UV spectra of all the derivatives contain absorption bands of the corresponding triterpenoid hydrogen phthalates **V**, **VI**, and **VIII** ($\lambda_{max} = 274$ nm), which allowed determination of the BAS content in the polymeric products. The results are given in Table 1.

It is known that polyvinylpyrrolidone (PVP) forms complexes with many substances [1, 2]. We attempted to prepare PVP derivatives of triterpenoid hydrogen phthalates by the procedure similar to that used for preparing salts of VP–DMAEM–DMAEM · $C_{12}H_{25}I$ copolymer. We failed to obtain water-soluble products. Addition of water to a solution of the reactants in methanol resulted in precipitation of triterpenoid hydrogen phthalates. This fact confirms that, to obtain a water-soluble product, the starting copolymer should contain ionic groups. The products were separated by gel permeation chromatography (GPC) on a column packed with Sephadex LH-20 gel (eluent ethanol). The absorption of the fractions taken was measured at 274 nm (the starting polymer is virtually transparent in this range). The chromatograms of all the polymeric salts obtained

Table 1. Polymeric salts of hydrogen phthalates ofdipterocarpol derivatives*

Run no.	Hydrogen phthalate	$^{\mathrm{e}}$, l mol ⁻¹ cm ⁻¹	[R–]	
			wt %	mol %
$\begin{array}{c}1\\2\\3\end{array}$	VIII V VI	1600 1600 1300	10.2 9.1 11.6	2.3 2.2 2.9

^{*} The salts are water-soluble.

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exhibit peak I in the same region as for the unmodified BAS (Fig. 1). There is also a weak peak II in the region of small retention volumes corresponding to polymer-containing fractions (for comparison, we show a chromatogram of a copolymer of VP with *N*-hydroxyphthalimide ester of acrylic acid). The UV spectra of fractions I and II are similar to the spectra of the low-molecular-weight BAS and starting polymer, respectively. To prepare polymeric derivatives of hydrogen phthalates **V**, **VI**, and **VIII** in which the BAS is linked to the polymer by a strong covalent bond, we used a copolymer of VP with allylamine (AlAm) [9].

The reactions of triterpene hydrogen phthalates with this copolymer were performed in CH_2Cl_2 in the presence of *N*, *N*'-dicyclohexylcarbodiimide (DCC), as shown in Scheme 3.

The reaction should yield derivatives in which BAS is linked to the polymeric carrier by an amide bond. The characteristics of the reaction products are given in Table 2. Their composition, similarly to the polymeric salts, was determined by UV spectroscopy, as the absorption band at $\lambda_{max} = 274$ nm was observed in the spectra of all the products. As seen from Table 2, in all the cases, as in the case of the polymeric salts, the derivatives containing ≤ 12 wt % triterpenoid hydrogen phthalate are water-soluble (run nos. 5–7). At a high BAS content, as in the case of betulinic and betulonic acid derivatives [5], the solubility in water is lost (run no. 4).

The chromatograms of covalent derivatives of triterpenoid hydrogen phthalates (Fig. 2) differ from those of polymeric salts (Fig. 1). As seen from Fig. 2, for the covalent derivatives, with the same gel (LH-20) and eluent (ethanol), peak I is weak, and peak II, on the contrary, strong. The UV spectra of the fractions corresponding to peak II, as those of the initial BASs, contain a band with $\lambda_{max} = 274$ nm. Similar chromatograms are obtained with Sephadex G-75 gel and water as eluent. This fact confirms that, in contrast to the derivatives obtained in run nos. 1–3, in the products from run nos. 4–7 the BAS is strongly bound to the polymeric carrier, because its chromatographic behavior is similar to that of the carrier.

EXPERIMENTAL

20(*S*)-Hydroxydammar-24-en-3-one I. Dipterocarpol I was eluted with hexane–ethyl acetate (15 : 1) in the course of chromatographic separation (silica gel, 5/40 μ m) of an ethyl acetate solution of *Dipterocarpus alatus* latex; mp 133–135°C [7]. IR spectrum, cm⁻¹: 3480, 1690. Yield 67%.

25,26,27-Trinordammaran-3-on-20(*S*),**24-olide II.** Dipterocarpol **I** (10 g) was dissolved in 50 ml of benzene and 30 ml of acetic acid; 4 g of CrO_3 and 3 ml of H₂O were added, The mixture was cooled with ice and allowed to stand for 12 h at room temperature, after which it was diluted with water. The precipitate was filtered off and washed with water to neutral reaction. The dry precipitate was chromatographed on a column packed with silica gel.

Fractions containing dipterocarpol lactone **II** were combined, and the solvent was evaporated. The residue was crystallized from ethyl acetate. Yield of dipterocarpol lactone **II** (25,26,27-trinordammaran-3-on-20(S),24-olide) 7.9 g, mp 180–182°C (published

Table 2. Polymeric derivatives of hydrogen phthalates of dammarane triterpenoids with an amide bond BAS–polymer

Run no.	Hydrogen phthalate	[R–]		Degree of	Solubility
		wt %	mol %	sion, %	in water
4	VIII	19.6	4.1	75	Insoluble
5	VIII	8.6	1.7	62	Soluble
6	VI	11.6	2.6	91	Soluble
7	V	10.2	2.2	80	Soluble

data [7]: mp 184–186°C). IR spectrum, cm⁻¹: 1765 (C=O in γ -lactone), 1695 (C=O).

25,26,27-Trinordammaran-3 β **-ol-20**(*S*),**24-olide III.** Dipterocarpol lactone **II** (2 g) was refluxed with 250 mg of NaBH₄ in 85 ml of isopropyl alcohol for 24 h, after which the solution was diluted with water and acidified to pH < 2. The precipitate was filtered off, washed with water, dried, and recrystallized from ethyl acetate. Yield of reduced lactone **III** 1.5 g



Fig. 1. Chromatograms (LH-20, eluent ethanol): (1) dammar-24-ene- 3β ,20(*S*)-diol hydrogen phthalate **VIII**, (2) copolymer of VP with *N*-hydroxyphthalimide ester of acrylic acid, and (3) polymeric salt of dammar-24-ene- 3β ,20(*S*)-diol hydrogen phthalate **VIII** and VP–DMAEM–DMAEM · C₁₂H₂₅I copolymer (run no. 1).



Fig. 2. Chromatogram (LH-20, eluent ethanol) of the derivative of dammar-24-ene-20(S), 3β -diol hydrogen phthalate **VIII** and VP-AlAm copolymer (run no. 5).

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(75%), mp 199–201°C (published data [7]: mp 203–204°C). IR spectrum, cm⁻¹: 3450 (OH), 1765 (C=O in γ -lactone).

Dammar-24-ene-20(*S*),3 β -diol VII. Dipterocarpol I [20(*S*)-hydroxydammar-24-en-3-one] was reduced with NaBH₄ under the same conditions as compound II. Diol VII was obtained in 40% yield; mp 132–134°C (published data [10]: mp 131–133°C). ¹³C NMR spectrum, δ , ppm: 78.4 (C-3 β –OH).

25,26,27-Trinor-20(S),24-epoxydammaran-3β-ol **IV.** A solution of 5 g of dipterocarpol lactone **II** in 100 ml of THF and 10 ml of BF₃ · OEt₂ were added over a period of 3 h at -15°C to 1.28 g of sodium borohydride in 50 ml of THF. The mixture was allowed to stand for 2 h at -15°C, after which it was refluxed on a water bath for 5 h. Then the mixture was cooled, diluted with a fivefold volume of water, and acidified with 2 N HCl to pH < 2. The precipitate was filtered off, washed with water, and dried. The residue (4.7 g) was chromatographed on a column packed with 150 g of silica gel (70/230 μ m). Elution with hexane-5% ethyl acetate gave 2.9 g (65%) of IV, mp 182-183°C (published data [7]: mp 180-182°C). IR spectrum, cm⁻¹: 3450 (OH stretching vibrations) [7]. ¹H NMR spectrum, δ , ppm: CH₃ (0.79 s, 0.89 s, 1.05 s, 0.9 s, 0.69 s, 0.79 s); 3.09 t; 3.6 m.

Hydrogen phthalates of compounds **III**, **IV**, and **VII**. Hydroxy derivative **III**, **IV**, or **VII** (0.01 mol) and phthalic anhydride (0.02 mol) were refluxed in pyridine for 48 h, after which the mixture was diluted with a fourfold volume of water and acidified with 10% HCl to pH < 2. The white precipitate was filtered off, washed with water to neutral reaction, and dried; the residue was recrystallized from petroleum ether–ethyl acetate.

Dammar-24-ene-20(*S*),3 β -diol hydrogen phthalate VIII: mp 208–210°C. ¹H NMR spectrum, δ , ppm: 4.8 (α -H at C-3) and 7.5–7.9 (4H).

25,26,27-Trinor-20(*S*),**24-epoxydammaran-3**β-ol hydrogen phthalate VI: mp 219–221°C. ¹H NMR spectrum, δ , ppm: 4.75 (α-H at C-3) and 7.6–7.9 (4H).

25,26,27-Trinordammaran-3 β **-ol-20**(*S*),**24-olide V:** mp 258–260°C. ¹H NMR spectrum, δ , ppm: 4.75 (α -H at C-3) and 7.6–7.9 (4H).

Synthesis of polymeric derivatives of triterpenoids. Polymeric salts of triterpenoids were prepared from the VP (87.8 mol %)–DMAEM (9.2 mol %)–DMAEM $\cdot C_{12}H_{25}I$ (3.0 mol %) copolymer, [η] at

25°C in 0.1 M CH₃COONa 0.13 dl g⁻¹, molecular weight $M_{\eta} = 21000$, by the following procedures.

Example 1. VP–DMAEM–DMAEM · $C_{12}H_{25}I$ copolymer (0.35 g) was dissolved in 30 ml of methanol, and 0.044 g of dammar-24-ene-3 β ,20(*S*)-diol hydrogen phthalate **VIII** was added. The mixture was stirred for 24 h at room temperature. The polymeric salt was isolated by gradual replacement of methanol as solvent by water (stepwise addition of water with distillation of methanol) [4]. The polymer was isolated from the resulting aqueous solution by freeze drying. Yield 0.38 g (96%) (run no. 1).

Under similar conditions we prepared the polymeric salts of hydrogen phthalates V [yield 61% (0.24 g) (run no. 2)] and VI [yield 94% (0.37 g) (run no. 3)].

Polymeric derivatives of triterpenoids with a polymer–BAS covalent bond were prepared from the VP (94.5 mol %)–AlAm (5.5 mol %) copolymer, [η] at 25°C in 0.1 M CH₃COONa 0.12 dl g⁻¹, M_{η} = 18000.

Example 2. A solution of 0.23 g of VP–AlAm copolymer, 0.075 g of VIII, and 0.025 g of DCC in 4.5 ml of CH₂Cl₂ was kept for 2 h at -18° C and then for 72 h at -5° C. The molar ratio of the reactants was 1 : 1 : 1. Then the polymer was precipitated into diethyl ether, and the precipitate was separated on a glass frit and vacuum-dried to constant weight. The low-molecular-weight impurities were separated by gel permeation chromatography on a column packed with Sephadex LH-20 gel (h = 44 cm, d = 1.5 cm), eluent ethanol. The high-molecular-weight fraction was collected, and ethanol was distilled off on a rotary evaporator.

Run no. 5: polymeric derivative of **VIII**, yield 89% (0.31 g); run no. 6: polymeric derivative of **VI**, yield 69% (0.24 g); run no. 7: polymeric derivative of **V**, yield 64% (0.22 g).

CONCLUSIONS

(1) Previously unknown 25,26,27-trinordammaran-3 β -ol-20(*S*),24-olide hydrogen phthalate **V**, 25,26,27trinor-20(*S*), 24-epoxydammaran-3 β -ol hydrogen phthalate **VI**, and dammar-24-ene-20(*S*),3 β -diol hydrogen phthalate **VIII** were prepared.

(2) Procedures were developed for preparing polymeric water-soluble derivatives of \mathbf{V} , \mathbf{VI} , and \mathbf{VIII} with *N*-vinylpyrrolidone copolymers, with both labile and strong covalent bonding of the polymer with the triterpenoid.

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