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SYNTHESIS FROM POLYPRENOLS AND BIOLOGICAL ACTIVITY OF POLYPRENYLACETIC ESTERS

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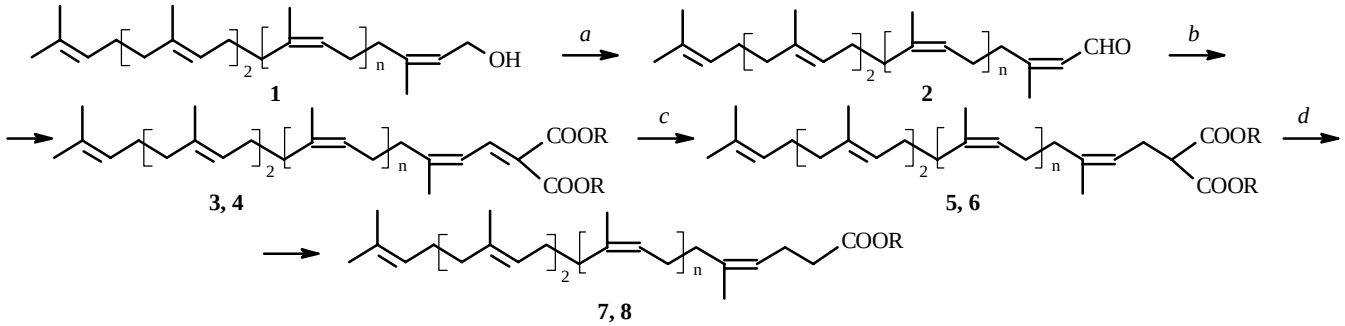
Narrow fractions of polyprenols isolated from birch (C_{35}-C_{45}) and fir (C_{70}-C_{85}) greenery were used to prepare esters of polyprenylacetic acids that exhibited high anti-ulcer activity comparable and in several instances exceeding that of the known anti-ulcer preparation omeprazole (omez).

Key words: polyprenylacetic acid esters, polyprenols, synthesis.

Esters of prenylacetic acids possess distinct anti-ulcer activity with practically no harmful side effects [1]. Among the known highly active representatives, solanesylacetic acid and its closest (n+1)-homolog decaprenylacetic acid are highly recommended [2]. The synthesis of pure long-chain oligoisoprenoids is a labor-intensive task. On the other hand, methods have been developed to isolate narrow fractions of polyprenols from greenery and wood of broad-leaf and coniferous trees [3] that can be used as raw material to synthesize homologs of polyprenylacetic acids and their esters. Such a method may be promising for creating anti-ulcer preparations because they have anti-ulcer activity.

An array of polyprenylacetic acids containing 7-9 isoprene units in the hydrocarbon chain was obtained from the total isoprenols isolated from birch greenery.

The physiological activity of the esters of the aforementioned acids depends on the radical in the carbalkoxy group [4]. Therefore, we prepared their methyl and ethyl esters in order to compare the biological activity. The polyprenol (**1**) OH group was oxidized to the aldehyde. The resulting formyl derivatives (**2**) underwent Knoevenagel condensation with diethyl- or dimethylmalonates. The synthesis of the esters (**7** and **8**) ended with reduction of the Δ-2 double bond and subsequent decarboxylation of diesters (**5**) or (**6**) by heating with LiI in DMF. Polyprenols **1** were oxidized into total aldehydes **2** using MnO_2. The double bond in the α-position could be selectively reduced to the carbalkoxy using $NaBH_4$ owing to its activation by the two electron-accepting substituents [5].



n = 3 - 5

R = Me (**3**, **5**, **7**), Et (**4**, **6**, **8**)

a. MnO_2; *b*. $CH_2(COOR)_2$/Py; *c*. $NaBH_4$; *d*. LiI/DMF

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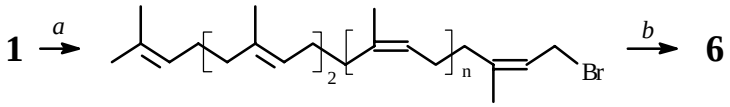
The triplet near 3.35 ppm that belongs to the H in the α-position relative to the two esters confirms that the reaction proceeded as planned.

Alternative approaches based on alkylation of the malonoic ester by prenylhalides, prenylhydroxymesylates, or prenylhydroxytosylates were developed because the yield from condensation of aldehydes **2** with the dimethyl- or diethylmalonates was 40-45%.

The sequence polyprenols—prenylbromides—prenyl derivatives of malonic ester did not increase the yield, which remained low both for the substitution of hydroxy by bromine (~65%) and the alkylation (~40%).

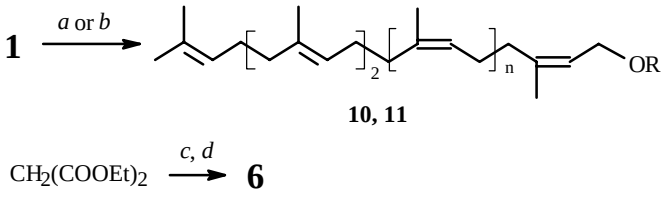


9

n = 3 - 5

a. CBr_4/PPh_3; *b*. $CH_2(COOEt)_2/NaH$

The prenylhydroxytosylates (**10**) and prenylhydroxymesylates (**11**), in contrast with the bromides, were readily prepared in high yield by treatment of polyprenols **1** with tosyl- or mesylchlorides. Subsequent alkylation of malonic ester **10** or **11** in yields of about 70 and 80%, respectively, formed polyprenyl-substituted esters of malonic acid.



n = 3 - 5

R = Ts (**10**); Ms (**11**)

a. $TsCl/Et_3N\text{-}Me_3N \cdot HCl\text{-}CH_2Cl_2$; *b*. $MsCl/Et_3N\text{-}Me_3N{\cdot}HCl\text{-}CH_2Cl_2$;

c. NaH; *d*. **10** or **11**

The anti-ulcer activity of the methyl (**7**) and ethyl (**8**) esters of polyprenylacetic acids and their precursor diesters **5** and **6** were studied using experimental models of stomach ulcers caused by administration of NPVS and ethanol [6, 7]. The reference preparations were omeprazol (omez) [8] and the starting polyprenols, which can also act as anti-ulcer agents.

Pharmacotherapeutic investigations on white mice showed that the prenylacetic esters obtained from birch-greenery polyprenols produce a statistically significant decrease the destruction of the stomach mucous membrane using indomethacin ulcer as a model. The maximal protective effect was found for the mixture of diethylesters of polyprenylacetic acids **6**. These compounds at a dose of 10 mg/kg are statistically significant (by 3.2 times) relative to the control and more effectively than the reference (by 1.5 times) decrease stomach ulceration. Therefore, they possess ulcer-healing activity. The mixture of methyl esters of polyprenylacetic acids **7** exhibited similar activity. The activity of the remaining compounds was similar to the anti-ulcer activity of the starting polyprenols **1** (Table 1).

The studied compounds exhibit effective gastro-protective activity upon administration per os of a C_2H_5OH solution (60%) in HCl solution (150 mM) by preventing destruction of the stomach mucous membrane.

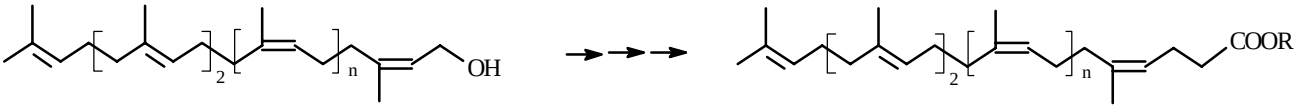
Esters **8** increase by 1.8 times compared with the control both bile secretion by liver hepatocytes (mg/min per 100 g) and cholagogic activity.

The length of the hydrocarbon chain of polyprenylacetic acid derivatives is known to influence the pharmacological activity. Therefore, chemical transformations of polyprenols from fir greenery (14-17 isoprene units) and the analogous sequences **1**-**11**-**5**-**7** were carried out. The anti-ulcer activity of these compounds was studied.

TABLE 1. Effect of Polyprenylacetic Acids Derivatives on Experimental Stomach Ulcers

Compound No.	Number of ulcers destroyed by	
	indomethacin	ethanol
1	14.0±1.1*	6.0±0.5*
6	8.7±3.0*	6.6±0.2*
7	9.5±1.9*	7.6±0.5*
Omez	13.2±1.9*	6.0±0.5*
Control	28.0±1.6	13.0±0.5

*Confidence relative to control, $p < 0.05$. Number of animals in groups, 6; dose of polyprenylacetic acids derivatives, 10 mg/kg; in control, 0.



12 **13, 14**

n = 10 - 13

R = Me (**13**), Et (**14**)

Biological testing of the longer chain esters **13** and **14** that were prepared by transformation of fir polyprenols showed that these compounds also exhibit gastro-protective activity although it is less evident than for esters **6-8**. However, it is noticeably significant with respect to the control. It should be noted that they are most effective for models of acute ethanol ulcers.

EXPERIMENTAL

IR spectra were recorded on a UR-20 instrument in thin layers or nujol. PMR spectra were obtained on a Bruker spectrometer (300 MHz) at working frequency 75 MHz, $CDCl_3$ solvent, and TMS internal standard. TLC was performed on Silufol plates (Chemapol, Czech Rep.) using the solvent system petroleum ether:ethylacetate (4:1). Compounds were developed by anisaldehyde:EtOH:H_2SO_4 (1:18:1) followed by heating at 100-120°C for 1-2 min.

Mixture of Polyprenals 2. Total prenols (**1**, 1.0 g) and freshly prepared MnO_2 (2.82 g) in dry CCl_4 (5 mL) were stirred at room temperature for 30 min. The reaction mixture was filtered. The filtrate was evaporated. The solid was chromatographed (SiO_2, petroleum ether:ethylacetate, 3:1). Yield 0.94 g of the mixture of compounds **2**. IR spectrum (KBr, ν, cm^{-1}): 860 w, 1640 m (C=C), 1680 m (CHO).

PMR spectrum (δ, ppm): 1.66, 1.70, 1.90 (all s, CH_3), 2.05 (m, CH_2), 5.14 (br.s, C=CH), 6.04 (s, H-2), 9.60 (s, CHO).

Polyprenylbromides 9. A solution of polyprenols (**1**, 1.0 g) and Ph_3P (0.27 g) in CH_2Cl_2 (10 mL) was stirred and treated in one portion with CBr_4 (0.38 g). Stirring was continued for 2 h. The reaction mixture was filtered through a thin layer of silica gel and purified by chromatography (SiO_2, petroleum ether). Yield 0.75 g of bromides **9**, identical to those described in the literature [4].

***p*-Tolylsulfonyloxypolyprenols 10.** A mixture of polyprenols (**1**, 1.3 g), Et_3N (0.2 g), and $Me_3N{\cdot}HCl$ (0.18 g) in CH_2Cl_2 (7 mL) at -7 to -5°C was treated with *p*-TsCl (0.25 g) and stirred at this temperature for 1 h and then at room temperature for 4 h. The reaction mixture was poured into icewater (15 mL). The product was extracted with CH_2Cl_2. The organic layer was washed successively with NaOH solution (10%), HCl (3%), and saturated NaCl solution; dried over $MgSO_4$; filtered; and evaporated. Yield 1.2 g of **10**, which was used without further purification to synthesize esters **6**.

Methylsulfonyloxypolyprenols 11. These were prepared by treatment of polyprenols (**1**, 1.0 g), Et_3N (0.18 g), and $Me_3N{\cdot}HCl$ (0.5 g) in CH_2Cl_2 (5 mL) with MsCl (0.21 g) under the conditions described for compounds **10**. Yield 0.95 g of **11**.

Synthesis of Diesters 3, 4. A mixture of aldehydes (**2**, 1.0 g), dimethyl- or diethylmalonate (0.4 mL), Py (0.27 mL), and piperdine (0.1 mL) was stirred for 3 h at 120°C, cooled to 20°C after the reaction was complete, diluted with diethylether, washed with HCl (1 N) and saturated solutions of $NaHCO_3$ and NaCl, dried over $MgSO_4$, and filtered. The solid was chromatographed (SiO_2, petroleum ether:ethylacetate, 9:1). Yield 0.52 g of **3** or 0.64 g of **4**.

For Dicarbomethoxy Derivatives 3. IR spectrum (KBr, ν, cm^{-1}): 870 m, 1180 s, 1250 m, 1640 m (C=C), 1710 m (COOMe).

PMR spectrum (δ, ppm, J/Hz): 1.61, 1.68 (all s, CH_3), 1.88 (s, CH_3–C-5), 1.96 (m, CH_2–C=C), 3.69 (s, OCH_3), 5.03 (m, HC=C), 6.28 (d, J = 12.1, H-4), 7.65 (d, J = 12.1, H-3).

For Dicarboethoxy Derivatives 4. IR spectrum was identical to that given above.

PMR spectrum (300 MHz, $CDCl_3$, ppm, J/Hz): 1.19 and 1.24 (two t, J = 7.5, $OCH_2\underline{CH_3}$), 1.61, 1.68 (all s, CH_3), 1.88 (s, CH_3–C-5), 1.98 (m, CH_2C=C), 4.15, 4.20 (two q, J = 7.5, CH_2O), 5.00 (m, HC=C), 6.28 (d, J = 12.0, H-4), 7.64 (d, J = 12.0, H-3).

α-Carbalkoxypolyprenylacetic Esters 5, 6. a. A solution of esters (**3**, 0.52 g or **4**, 0.70 g) in abs. MeOH (2.0 or 2.5 mL) at 5-10°C was stirred, treated in portions with $NaBH_4$ (0.04 or 0.052 g), and stirred until the starting materials disappeared (TLC monitoring). After the reaction was complete, the temperature was adjusted to room temperature and HCl (8.3 mL, 8%) was added. The product was extracted with diethylether. The organic layer was washed with $NaHCO_3$ solution (5%) and water, dried over $MgSO_4$, filtered, and evaporated. The solid was chromatographed (SiO_2, petroleum ether:ethylaceate, 7:3). Yield 0.5 g of **5** or 0.63 g of **6**.

b. A mixture of NaH (0.04 g) in THF (4 mL) at 0°C was treated dropwise with diethylmalonate (0.26 mL) in THF (2 mL), stirred for 30 min, and treated dropwise with bromides (**9**, 1.16 g) in THF (2 mL). The temperature was increased to room temperature. Stirring was continued for 12 h. The reaction mixture was poured into NH_4Cl solution. The product was extracted with diethylether. The combined organic layer was washed successively with water and NaCl solution, dried over $MgSO_4$, filtered, and evaporated. The solid was chromatographed (SiO_2, petroleum ether:ethylacetate, 7:3). Yield 0.77 g of esters **6**.

c. A mixture of NaH (0.05 g) in THF (6 mL) at 0°C was treated dropwise with diethylmalonate (0.32 mL) in THF (5 mL), stirred for 30 min, and treated dropwise with mesylates (**11**, 1.5 g) or tosylates (**10**, 1.4 g) in THF (5 mL). The temperature was raised to room temperature. The mixture was stirred for 12 h and poured into NH_4Cl solution. The product was extracted with diethylether and isolated analogously to that described in part a. Yield 1.26 g or 1.18 g of esters **6**.

For Esters 5. IR spectrum (KBr, ν, cm^{-1}): 860 w, 1180 m, 1250 m, 1640 m (C=C), 1735 m (COOMe).

PMR spectrum (δ, ppm, J/Hz): 1.60, 1.69 (all s, CH_3), 1.96 (m, CH_2–C=C), 2.61 (t, J = 7.5, H-3), 3.40 (t, J = 7.5, H-2), 3.68 (s, OCH_3), 5.03 (m, HC=C).

For Esters 6. The IR spectrum was identical to that given above. PMR spectrum (δ, ppm, J/Hz): 1.27 (t, J = 7.5, $OCH_2\underline{CH_3}$), 1.61, 1.68 (all s, CH_3), 1.96 (m, CH_2–C=C), 2.63 (t, J = 7.5, H-3), 3.35 (t, J = 7.5, H-2), 4.18 (q, J = 7.5, CH_2O), 5.02 (m, HC=C).

Prenylacetic Esters 7, 8. Diesters (**5**, 0.5 g or **6**, 0.63 g) were dissolved in DMF (2-3 mL), treated with LiI (0.31 or 0.44 g), stirred for 3 h at 150°C, cooled, and treated with water (6-8 mL). The product was extracted with diethylether. The combined organic extracts were washed with water, dried over $MgSO_4$, filtered, and evaporated. The products were purified by chromatography (SiO_2, petroleum ether:ethylacetate, 15:1). Yield 0.17 g of **7** or 0.19 g of **8**.

For Esters 7. IR spectrum (KBr, ν, cm^{-1}): 860 w, 1175 m, 1245 m, 1640 m (C=C), 1730 m (COOMe).

PMR spectrum (δ, ppm): 1.62, 1.69 (all s, CH_3), 1.99 (m, CH_2C=C), 2.31 (m, H-2, H-3), 3.65 (s, OCH_3), 5.02 (m, HC=C).

For Esters 8. The IR spectrum was identical to that given above.

PMR spectrum (δ, ppm, J/Hz): 1.28 (t, J = 7.0, $OCH_2\underline{CH_3}$), 1.63, 1.69 (all s, CH_3), 2.01 (m, CH_2–C=C), 2.38 (m, H-2, H-3), 4.18 (q, J = 7.0, OCH_2), 5.06 (m, HC=C).

Polyprenolacetic Esters 13, 14. These were prepared from total polyprenols (**12**, 4.0 g) analogously using the sequence of transformations **1-11-5-7** in ~75% yield.

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