

NEW DITERPENE LACTONES FROM Euphorbia pallasii

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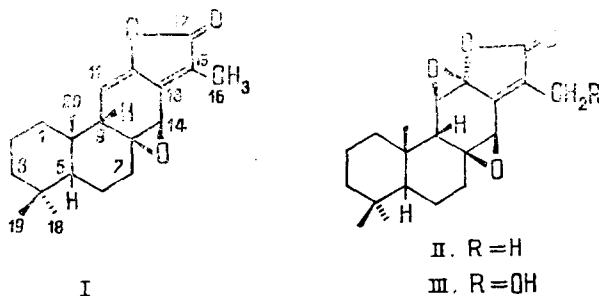
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Three new diterpene lactones have been isolated from a chloroform extract of the roots of Euphorbia pallasii: pseudojolkinolide A pseudojolkinolide B, and 16-hydroxypseudojolkinolide B. Structures have been proposed for them on the basis of chemical transformations and IR, UV,  $^{13}\text{C}$  and  $^1\text{H}$  NMR, and mass spectra.

Euphorbia pallasii Turcz - synonym E. Fisheriana Stendel - (family Euphorbiaceae) is an endemic plant growing in the territory of the USSR in the southeastern regions of Transbaikalia. The roots of the plant enjoy great popularity in Transbaikalian and Siberian folk medicine and are also components of many complex Tibetan preparations.

We have investigated a chloroform extract of the roots of E. pallasii, which exhibits antibacterial action on St. aureus and E. coli. From the acetone-soluble fraction of the extract with the aid of chromatography on silicagel we isolated three new diterpene lactones with the compositions  $\text{C}_{20}\text{H}_{26}\text{O}_3$  (I),  $\text{C}_{20}\text{H}_{26}\text{O}_4$  (II), and  $\text{C}_{20}\text{H}_{26}\text{O}_5$  (III), which we have called pseudojolkinolides A and B and 16-hydroxypseudojolkinolide B, respectively.

The IR spectra of (II) and (III) had strong absorption bands at 1780 and 1680  $\text{cm}^{-1}$  that are characteristic for unsaturated  $\gamma$ -lactones. The UV spectra of (II) and (III) were characterized by absorption maxima at 240 nm. The presence of one more double bond in (I) (1660  $\text{cm}^{-1}$ ) lowered the absorption frequency of the carbonyl group (1760  $\text{cm}^{-1}$ ) and shifted the absorption band in the UV spectrum in the long-wave direction ( $\lambda_{\text{max}}$  288 nm). Consequently, this double bond is conjugated with the first.



The nature of the mass-spectrometric fragmentation of compounds (I-III) confirmed the structure of their carbon skeleton. Breakdown under the action of electron impact took place with cleavage of the ring B (schemes). In the mass spectra of the compounds. In addition to the peaks of the molecular ions, the peaks of fragmentary ions were observed the elementary compositions of which corresponded to the fragments formed as the result of the cleavage of ring B.

In the NMR spectrum of (I) (Table 1), the signals of three methyl groups attached to quaternary atoms at C-4 and C-10 were observed in the strong-field region at 0.94, 0.85, and 0.71 ppm. A signal at 2.05 ppm indicated the presence of another methyl group, attached to a nonterminal double bond at C-15. Two one-proton doublets at 2.62 and 5.45 ppm with  $J = 6$  Hz belonged to the H-9 and H-11 protons, respectively, and a one-proton broadened singlet at 3.71 ppm to H-14. The protons of the other methine and methylene protons of (I) resonated at 1.0-2.0 ppm in the form of a complex multiplet.

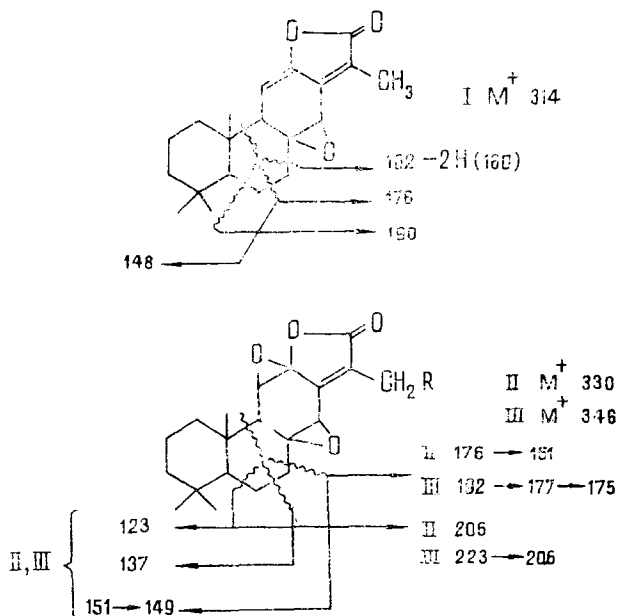
Compounds (II) and (III) had spectral characteristics similar to those of (I). The assignment of the signals of the  $^1\text{H}$  atoms of (II) and (III) is given in Table 1. The signals

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TABLE 1. Spectral Characteristic of the Diterpene Lactones

Compound	mp, °C	[α] <sub>D</sub> deg. CHCl <sub>3</sub>	IR, <sup>ν</sup> <sub>max</sub> , cm <sup>-1</sup>	UV, λ <sub>max</sub> (log ε), CH <sub>3</sub> OH	PMR, δ, ppm, J, Hz, CDCl <sub>3</sub>					CH <sub>3</sub> -group*
					H-9	H-11	H-12	H-14	H-16	
I	237-238		1660, 1680, 1760 (KBr)	206, 288 (4,01; 4,46)	2.62 d (6)	5.45 d (6)		3.71 br.s	2.05 s 3H	0.71 s, 3H, C <sub>18</sub> 0.85 s, 3H, C <sub>19</sub> 0.94 s, 3H, C <sub>20</sub>
IV	220 [1], 220-225 [2], 216-218 [5] 244-244.5	+130, c 0.7 +199, c 1.14	1660, 1670, 1770 (CHCl <sub>3</sub> )	288 (4,29)	2.64 d (6)	5.47 d (6)		3.74 br.s	2.08 s 3H	0.73 s, 3H 0.87 s, 3H 0.97 s, 3H
II			1680, 1780 (KBr)	208, 240 (3,85; 4,10)	2.26 br.s	4.01 br.s		3.66 s	2.06 s 3H	0.79 s, 3H, C <sub>15</sub> 0.82 s, 3H, C <sub>16</sub> 0.91 s, 3H, C <sub>20</sub>
V	215 [1], 212-214 [5]	+220, c 0.4	1690, 1790 (CHCl <sub>3</sub> )	240 (4,22)	2.30 br.s	4.06 d (1,5)		3.70 s	2.10 s 3H	0.83, s, 3H 0.87, s, 3H 0.97, s, 3H
III	199.5-200.5	+140, c 0.36	1680, 1780 (KBr)	240 (4.04)	2.27 br.s	4.04 d (2,6)		4.07 br.s	4.68 s 2H	0.83, s, 3H, C <sub>18,19</sub> 0.92, s, 3H, C <sub>20</sub>
VI	210-212		1680, 1758 3300-3600 (CHCl <sub>3</sub> )		2.04 m	3.57 m	5.57 m	2.69 d (?), 2.51 d (?), J <sub>14-16</sub> = 1.5 J <sub>14-16-18</sub> = 18	1.78 d 3H J <sub>14-16</sub> = 1.5	0.59 s, 3H, C <sub>18</sub> 0.79 s, 3H, C <sub>19</sub> 0.90 s, 3H, C <sub>20</sub>
VII	220-221		1680, 1750 3400-3600 (CHCl <sub>3</sub> )	225 (4,02)		4.98 dd (2,8; 5,6)	5.60 m	2.57 2.72 m 2H	1.80 s 3H	0.67 s, 3H 0.80 s, 3H 0.90 s, 3H
Acetate of (VII)										

\*The assignment of the signals of the protons of the CH<sub>3</sub> groups at C<sub>18</sub>, C<sub>19</sub>, C<sub>20</sub> is not given for (IV), (V), and the acetate of (VII).

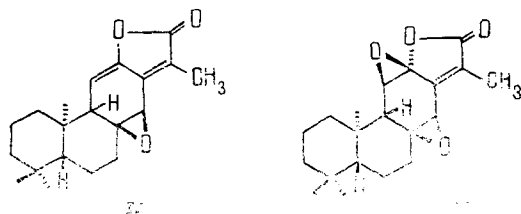


#### Mass-spectrometric fragmentation of pseudojolkinolides

of the protons at C-9 and C-11 appeared in the form of broadened singlets at 2.26 and 4.01 ppm, respectively. Compound (III) was the 16-hydroxy derivative of (II). The presence of a 16-hydroxymethyl group was confirmed by the results of mass spectrometry ( $m/z$  328). In the PMR spectrum it appeared in the form of a broadened two-proton singlet at 4.68 ppm. The broadening of the signal is due to allyl spin-spin coupling with H-14, which also resonated in the form of a broadened singlet, at 4.07 ppm. The signal of the H-11 proton was represented by a doublet with a SSCC of 2.6 Hz. Its spin coupling with H-9 was shown by the double-resonance method.

The main information on the chemical structures of the lactones isolated was obtained by comparing the physicochemical characteristics of compounds (I)-(III) with those of the known jolkinolides [1-5].

As can be seen from Table 1, the spectral characteristics for (I) and (II) coincided with or were very close to those for jolkinolides A (IV) and B (V), isolated previously from *E. jolkini* [1]. The structure and stereochemistry of the latter have been shown by chemical and physicochemical methods, by synthesis, and by x-ray structural analysis [1-5].



In spite of the close values of their spectral characteristics, (I) and (II) differed from (IV) and (V) by their melting points and specific rotations. Since the chemical shifts and nature of the splitting in the weak fields of the PMR spectra of (I) and (II) and those given in the literature (IV) and (V) [1, 2] are practically identical, we assumed that the differences between these compounds related to the stereochemistry of the linkage of rings A and B. Another possibility was that lactones (I) and (II) could be enantiomers of jolkinolides A and B at all the asymmetric centers of ring C. To examine these hypotheses, we used the method of induced paramagnetic shifts in the  $^1\text{H}$  and  $^{13}\text{C}$  PMR spectra. The assignment of the signals of the carbon atoms (Table 2) was made by the off-resonance, INEPT, and shift-reagent techniques and also by mathematical methods and were compared with literature information for related diterpenoids [67]. As the shift reagent we used europium trisdipivaloyl-methanate  $\text{Eu}(\text{dpm})_3$ .

TABLE 2.  $^{13}\text{C}$  Chemical Shifts of the Pseudojolkinolides

Atom No.	I	II	III	VI	Atom No.	I	II	III	VI
1	40,0	39,3	39,3	39,1	11	104,1	61,0	61,6	73,4
2	18,6	18,5	18,5	18,1	12	145,1	85,3	85,5	85,9
3	41,7	41,4	41,4	41,7	13	125,3	130,3	133,2	122,9
4	33,6	33,6	33,6	33,5	14	61,3	55,4	55,3	42,6
5	53,7	53,6	53,6	55,5	15	147,6	148,7	151,0	158,5
6	21,0	21,0	20,9	20,2	16	8,7	8,7	56,6	8,8
7	34,3	39,3	39,2	38,2	17	170,7	169,6	168,2	175,1
8	54,6	66,6	66,8	74,3	18	33,6	33,6	33,6	33,2
9	52,1	48,1	48,0	66,4	19	22,0	22,0	22,0	21,7
10	40,0	35,7	35,7	35,2	20	15,1	15,5	15,7	14,6

In order to form preferential centers for the coordination of the rare-earth ion, compound (II) was subjected to catalytic hydrogenation. As compared with (V), the hydrogenolysis of (II) took place slowly and ambiguously. Chromatographic separation of the reaction mixture yielded a diol  $\text{C}_{20}\text{H}_{30}\text{O}_4$  (VI), analogous to substance (VII) obtained from jolkinolide B. The positions of the hydroxy groups at C-8 and C-11 in (VI) followed from its  $^{13}\text{C}$  NMR spectrum, where the corresponding signals had shifted upfield (74.3 and 73.4 ppm) as compared with (II) (66.1 and 61.0 ppm). The presence of a doublet at 85.9 ppm and of a triplet at 42.6 ppm for C-12 and C-14, respectively, in the off-resonance and INEPT spectra confirmed the assigned positions of the OH groups in (VI). The assignments of the signals in the PMR spectrum of (VI) are given in Table 1.

As follows from the IR spectrum, the diol (VI) contained a strong hydrogen bond ( $3300\text{--}3600\text{ cm}^{-1}$ ). Its intramolecular nature was shown by the dilution method ( $3520, 3605\text{ cm}^{-1}$ ). This serves as an additional proof of the cis arrangement of the OH groups with respect to the plane of ring C.

Then substances (VI) and (III) were used to study the changes in the CSs of the signals of the protons and  $^{13}\text{C}$  nuclei under the influence of  $\text{Eu}(\text{dpm})_3$ . The calculation of the observed isotropic shifts to internuclear distances was made as described in the literature [8], using a simplified form of the McConnell equation

$$\Delta = \frac{3 \cos^2\theta - 1}{R_i^3} .$$

The position of the metal in the complex was determined by the screening method using Dreiding models.

Diol (VI) has two preferred coordination centers. To a first approximation the value of  $\Delta_i$  is inversely proportional to the cube of the distance of the proton from the center of complex-formation. Consequently, the calculations of the paramagnetic shifts were made by summing the contributions of each of the two coordinated europium atoms from the formula  $\Delta_i = 1/R_{i(11)}^3 + 0.9/R_{i(8)}^3$ , where  $R_i$  is the distance from the  $i$ -th atom to Eu at  $C_{11}$  and  $C_8$ . The coefficient 0.9 was calculated from a comparison of the induced shifts of the C-11 and C-8 atoms in the  $^{13}\text{C}$  spectrum of (VI). The basis for this was the assumption that the difference in the shifts was proportional to the field of the rare-earth ion coordinated with the corresponding oxygen atoms. Where the angle  $\theta$  was  $\geq 50^\circ$ , a correction was introduced by using the factor  $3\cos^2\theta - 1$ . If this angle approximated to the critical value for both coordination centers, no calculation was performed.

Treatment of the results obtained led us to the conclusion that the pseudojolkinolides differed from (IV) and (V) by the cis-linkage of rings A/B. In the projection formula of (VIII) and (IX) are shown the stereochemistry and conformation, according to the calculated figures, of the europium complexes of 16-hydroxypseudojolkinolide B (III) and of the diol (VI).

Figure 1 summarizes the results obtained for lactone (III). The facts available for interpretation relate only to the protons of ring C and to the C-20 methyl group. It follows from them that the stereochemistry of ring C coincides with that for the jolkinolides (the protons at C-16, C-11, and C-14 are located on the  $\alpha$ -side of ring C, and those at C-9 on the opposite side). The point for the C-20 methyl group lies accurately on the calculated curve, but the C-19 and C-18 groups ranged almost at the critical angles to the Eu-O bond and it is impossible to calculate  $\Delta_i$  for them with sufficient accuracy.

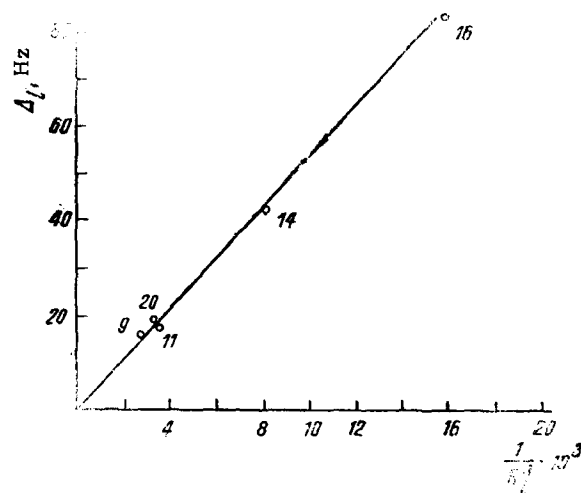
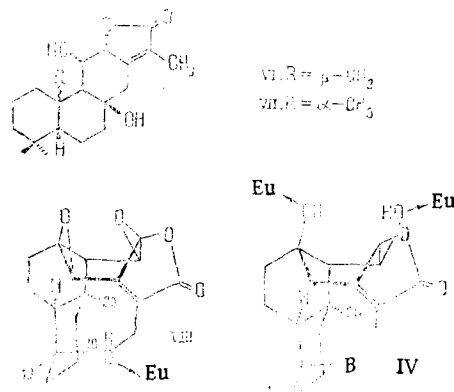


Fig. 1. Dependence of  $\Delta_i$  on the intramolecular distances in the PMR spectrum of (III). Molar ratio of  $\text{Eu}(\text{pdm})_3$  to (III) 1:16.

A larger number of signals is available for analysis in the PMR spectrum of the diol (VI) (IX). As can be seen from Fig. 2, C-18 and C-19 are groups also lying on the calculated curve. Figure 3 gives the results of a correlation of the paramagnetic shifts with the interatomic distances from the  $^{13}\text{C}$  spectrum. It can be seen from this that some atoms of rings A and B also obey the rules. The C-8 and C-11 atoms, located in the immediate vicinity of the europium ion, deviate from it. This figure does not show either the atoms corresponding to the C-atoms present at the critical angles to both the  $\text{Eu}-\text{O}$  bonds or those of which the induced shifts are difficult to determine because of signal overlapping.



Thus, the facts presented permit us to propose with high probability for pseudojolkinolides A and B and 16-hydroxypseudojolkinolide B structures (I), (II), and (III) with the *cis-cis* linkage of rings A/B/C.

In the quantitative respect, pseudojolkinolide B and 16-hydroxypseudojolkinolide B predominated in the extract. Pseudojolkinolide A was detected in minor amounts. The total content of the lactones was ~0.05% on the weight of the dry roots.

16-Hydroxypseudojolkinolide B exhibited a moderate bactericidal action *in vitro* on *St. aureus*. It may be assumed that the pseudojolkinolide form part of the complex of antibacterial substances present in the roots of the plant. We have observed a skin-irritating action of the pseudojolkinolide. One of the authors developed a severe allergy both to an extract of *E. pallasii* and also to the pure substances (I), (II), and (III).

#### EXPERIMENTAL

IR spectra were obtained on a UR-20 instrument in KBr, and UV spectra on a Specord UV-Vis spectrophotometer in methanol. The PMR spectra of (I), (II), and (III) were taken by L. V. Krivdin (A. A. Zhdanov Irkutsk State University) on a Bruker WP-200 sy, 200.13 MHz, spectrometer ( $\delta$  scale, 0 - HMDS) in  $\text{CDCl}_3$ . The PMR spectrum of (VI) and also the spectra

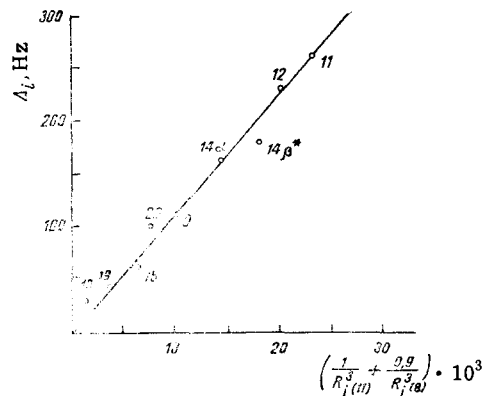


Fig. 2. Dependence of  $\Delta_i$  on intramolecular distances in the PMR spectrum of (VI). Molar ratio of  $\text{Eu}(\text{dpm})_3$  to (VI): 1:5.7. In the calculation of H-14 $\beta$  a correction was made for  $\cos \theta$ .

of (VI) and (III) with additions of Eu were taken on a JEOL FX-90 Q spectrometer at 89.55 MHz in  $\text{CDCl}_3$ .  $^{13}\text{C}$  NMR spectra were obtained on the JEOL FX-90 Q spectrometer at 22.49 MHz in  $\text{CDCl}_3$ . The  $^{13}\text{C}$  chemical shifts were measured relative to internal TMS.

Mass spectra were taken on an MAT-212 chromato-mass spectrometer with the direct introduction of the sample at an ionizing energy of 70 eV. Melting points were measured in sealed capillaries.

Extraction and Isolation of the Pseudojolkinolides. The roots of *E. pallasii* were gathered in August, 1982, in the valley of the R. Talangui near the village of Lozhnikovo, Baleiskii region, Chitinskaya province.

The dried and comminuted roots were extracted three times with chloroform by steeping in large bottles at room temperature. The solvent was distilled off in vacuum. The yield of extract was ~10%. By treatment with hexane, the concentrated extract (200 g) was separated into hexane-soluble and hexane-insoluble fractions (84 g and 116 g, respectively).

The hexane-soluble fraction was chromatographed on  $\text{SiO}_2$ . Elution was performed with hexane-chloroform mixtures with increasing proportions of the latter from 0 to 50% (by volume). Pseudojolkinolide A (I) was isolated from the evaporated eluate eluted with hexane-chloroform (7:3), after crystallization from hexane.

The hexane-insoluble fraction was treated with acetone, and the hexane-soluble fraction was chromatographed on  $\text{SiO}_2$  in two ways:

1) by elution successively with benzene benzene-ethyl acetate (1:1), ethyl acetate, and acetone; and

2) by elution with hexane-acetone with an increase in the proportion of the latter from 0 to 50%.

Elution with benzene and with hexane-acetone (9:1) yielded fractions enriched with pseudojolkinolide B (II); fractions enriched with 16-hydroxypseudojolkinolide B (III) were obtained on elution with benzene-ethyl acetate (1:1) and with hexane-acetone (8:5:1.5). The purification of (II) and (III) was carried out by rechromatography on silica gel in the hexane-acetone (9.7:0.3) system for (II) and (9.5:0.5) for (III), followed by crystallization from hexane-acetone (1:1) or from acetone.

For TLC we used Silufol prepared plates and the following solvent systems: chloroform-methanol (9.8:0.2); benzene-acetone (8:2) and (9:1); The spots were revealed with concentrated sulfuric acid, which colored (I) pink, (II) lemon yellow, and (III) red.

Pseudojolkinolide A (I). Pseudojolkinolide A (I),  $\text{C}_{20}\text{H}_{26}\text{O}_3$ , mp 237-238°C (hexane). UV spectrum ( $\text{CH}_3\text{OH}$ ),  $\lambda_{\text{max}}$ , nm: 206, 288 ( $\log \epsilon$  401, 446). IR spectrum,  $\nu_{\text{max}}^{\text{KBr}}$ ,  $\text{cm}^{-1}$ : 1660, 1680 (C=C), 1760 (C=O of a  $\gamma$ -lactone). Mass spectrum, m/z (%): M+314 (34), 299 (8), 246 (10), 229 (15), 218 (17), 204 (18), 201 (27), 190 (41), 176 (100), 164 (53), 160 (57), 148 (34), 136 (15), 123 (23), 119 (25), 107 (18), 95 (48), 81 (57), 69 (59).

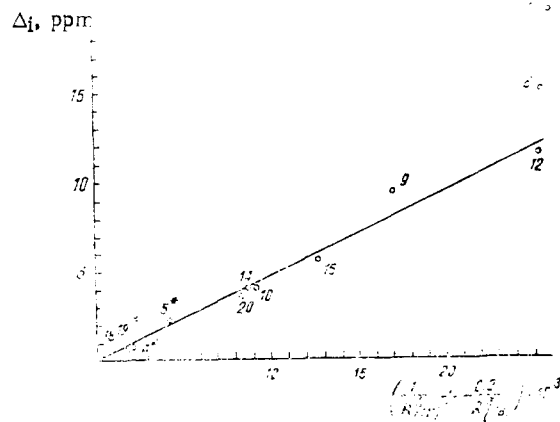


Fig. 3. Dependence of  $\Delta_i$  on intramolecular distances in the  $^{13}\text{C}$  spectrum of the diol (VI). Molar ratio of  $\text{Eu}(\text{dpm})_3$  to (VI): 1:1.8. Corrections for  $\cos \theta$  were introduced into the calculation of the C-atoms marked with asterisks.

Pseudojolkkinolide B (II).  $\text{C}_{20}\text{H}_{26}\text{O}_4$ , mp 244-244.5°C,  $[\alpha] +199^\circ$  (c 1.14; chloroform). UV spectrum:  $\lambda_{\text{max}}$  ( $\text{CH}_3\text{OH}$ ), nm: 208, 240 ( $\log \epsilon$  3.85, 4.10). UV spectrum  $\nu_{\text{max}}^{\text{KBr}}$ ,  $\text{cm}^{-1}$ : 1680 (C=C), 1780 (C=O of a  $\gamma$ -lactone). Mass spectrum, m/z (%): M+ 330 (21), 285 (20), 273 (5), 206 (17), 194 (17), 176 (18), 161 (21), 151 (100), 137 (77), 123 (44), 109 (33), 95 (49), 81 (70).

16-Hydroxypseudojolkkinolide B (III).  $\text{C}_{20}\text{H}_{26}\text{O}_5$ , mp 199.5-200.5°  $[\alpha] +140^\circ$  (c 0.36; chloroform). UV spectrum,  $\lambda_{\text{max}}$  ( $\text{CH}_3\text{OH}$ ), nm: 240 ( $\log \epsilon$  4.04); IR spectrum,  $\nu_{\text{max}}^{\text{KBr}}$ , 1680 (C=C), 1780 (C=O of a  $\gamma$ -lactone), 3510 (OH). Mass spectrum, m/z (%): M+ 346 (11), 328 (9), 299 (40), 285 (10), 223 (14), 192 (24), 177 (40), 175 (48), 163 (26), 149 (100), 137 (89), 123 (56), 109 (35), 95 (24).

6 $\alpha$ ,11 $\alpha$ -Dihydroxy-4,4,8,11 $\beta$ -tetramethyl-2,3,4 $\alpha$ ,5,6,7,10 $\alpha$ , 11 $\beta$ -octahydro-1H-phenanthro-[3,2-b]furan-9-one (VI). The hydrogenation of 310 mg of the diepoxide (II) was carried out in the presence of 30 mg of 10% Pd/C in 6 ml of ethyl acetate at 75°C for 75 h. Then the fresh portion of catalyst (30 mg) was added and hydrogenation was continued at room temperature until the initial (II) had disappeared completely. After the usual working up, the reaction products were dissolved in chloroform and chromatographed on a column of silica gel. Elution with chloroform-methanol (97.5:2.5) gave the diol (VI) (IX)  $\text{C}_{20}\text{H}_{30}\text{O}_4$ , M+ 334, mp 210-212°C; IR spectrum,  $\nu_{\text{max}}^{\text{KBr}}$ ,  $\text{cm}^{-1}$  1680 (C=C), 1750. Yield -15%.

#### SUMMARY

New diterpene lactones have been isolated from the roots of *Euphorbia pallasii* - pseudojolkkinolides A and B and 16-hydroxypseudojolkkinolide B - and structures have been proposed for them on the basis of chemical and spectral characteristics.

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