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ESTROGEN ACTIVITY OF TERPENOIDS FROM PLANTS OF THE GENUS *Ferula*

S. S. Nazrullaev, A. I. Saidkhodzhaev, Kh. S. Akhmedkhodzhaeva, V. N. Syrov, B. F. Rasulev, and Z. A. Khushbaktova*

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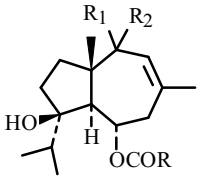
The relationship between chemical structure and estrogen activity of a series of terpenoid esters with aromatic and aliphatic acids was studied. The studied compounds were isolated from the underground and aerial organs of Ferula tenuisecta, F. akitschkensis, F. lapidosa, F. juniperina, F. ceratophylla, F. pallida, F. tschimganica, *and* F. prangifolia. *The estrogen activity was determined* in vivo *tests at a dose of 1 mg/kg.*

Key words: terpene acid esters, carotane, germacrane, humulane, camphane, estrogen activity.

We established previously that plants of the genus *Ferula* L. contain terpenoid esters of aromatic and aliphatic acids, many of which exhibit pronounced estrogen activity [1], in addition to terpenoid coumarins and sesquiterpene lactones. This was confirmed by other investigations [2]. In continuation of that work, we studied the estrogen activity of esters of terpenoid acids isolated from plants of the genus *Ferula*.



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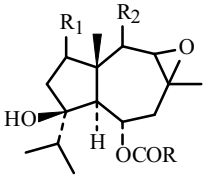

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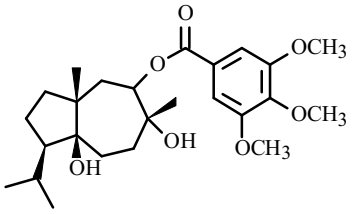

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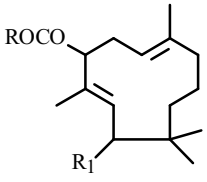

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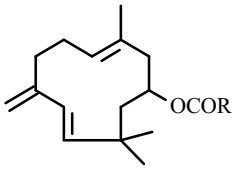

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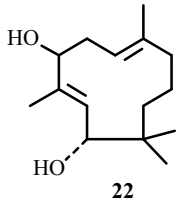

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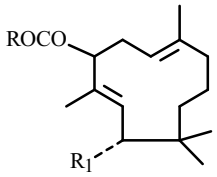

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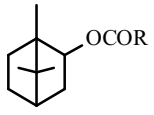

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1: R_1R_2 = O, R = CH_3-CH=C(CH_3)-CH_3; **2:** R_1 = H, R_2 = OAc, R = CH_3-CH=C(CH_3)-CH_3; **3:** R_1 = R_2 = OAc, R = CH_3-CH=C(CH_3)-CH_3
4: R_1 = R_2 = OAc, R = 3,4-dimethoxyphenyl; **5:** R_1 = OAc, R_2 = H, R = 3,4-dimethoxyphenyl
6: R_1R_2 = O, R = 3,4,5-trimethoxyphenyl; **7:** R_1R_2 = O, R = 2-methoxy-3,4-methylenedioxyphenyl
8: R_1 = R_2 = H, R = 3,4,5-trimethoxyphenyl; **10:** R_1 = R_2 = H, R = *p*-hydroxyphenyl
11: R_1 = R_2 = H, R = 3,4-dimethoxyphenyl; **12:** R_1 = R_2 = H, R = *p*-methoxyphenyl
13: R_1 = R_2 = H, R = 3-hydroxy-4-methoxyphenyl; **14:** R_1 = R_2 = H, R = 3-methoxy-4-hydroxyphenyl
15: R_1 = R_2 = H, R = *p*-OAc-phenyl; **16:** R_1 = R_2 = H, R = phenyl; **17:** R_1 = R_2 = H, R = *p*-hydroxyphenyl
18: R = 3-methoxy-4-hydroxyphenyl, R_1 = OH; **19:** R = *p*-hydroxyphenyl, R_1 = OAc
20: R = *p*-hydroxyphenyl; **21:** R = 3-methoxy-4-hydroxyphenyl; **23:** R = *p*-hydroxyphenyl, R_1 = OH
24: R = *p*-hydroxyphenyl, R_1 = OAc; **25:** R = *p*-hydroxyphenyl; **26:** R = 4-hydroxy-3-methoxyphenyl

S. Yu. Yunusov Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (99871) 120 64 75, e-mail: zainab@icps.org.uz. Translated from Khimiya Prirodnykh Soedinenii, No. 5, pp. 463-467, September-October, 2008. Original article submitted June 20, 2002.

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TABLE 1. Effect of Terpenoids on Growth of Uterus and Ovaries of Immature Rats (Dose 1 mg/kg) (M ± m; n = 6-10)

Compound	Formula	Source, ref.	Uterus growth, % of control	P (>or<)	Ovaries growth, % of control	P (>or<)
Lapidine (**1**)	$C_{20}H_{30}O_4$	*Ferula lapidosa* [3]	-39.5±9.05	>0.01	-42.5±7.3	>0.5
Lapiferine (**2**)	$C_{22}H_{34}O_6$	*F. lapidosa* [4]	-41.2±6.7	<0.05	-39.2±2.3	<0.001
Lapidoline (**3**)	$C_{24}H_{36}O_8$	*F. lapidosa* [5]	-38.4±2.7	<0.001	-49.14±2.04	<0.001
Lapidolinine (**4**)	$C_{28}H_{38}O_{10}$	*F. lapidosa* [5]	-28.4±12.4	<0.001	-27.6±4.6	<0.001
Lapiferinine (**5**)	$C_{26}H_{36}O_8$	*F. lapidosa* [6]	-19.9±2.7	>0.5	-41.68±3.3	<0.001
Palliferine (**6**)	$C_{25}H_{32}O_7$	*F. pallida* [7]	-28.9±11.2	≥0.05	-11.04±4.4	<0
Palliferinine (**7**)	$C_{24}H_{30}O_7$	*F. pallida* [7]	5.12±5.1	>0.5	12.6±3.5	>0.5
Palliferidine (**8**)	$C_{25}H_{36}O_7$	*F. pallida* [8]	-15.06±4.3	<0.05	-5.2±2.6	>0.5
Pallidine (**9**)	$C_{25}H_{38}O_7$	*F. pallida* [9]	22.4±5.5	<0.05	1.0±2.0	>0.5
Tenuferidin (**10**)	$C_{22}H_{30}O_5$	*F. tenuisecta* [10]	141.5±9.9	<0.001	13.2±3.0	<0.05
			308.3±36.3	<0.001		
Akiferine (**11**)	$C_{24}H_{34}O_5$	*F. akitschkensis* [11]	157.3±10.5	<0.001	29.1	<0.01
			227.3±25	<0.001		
Ferutidine (**12**)	$C_{23}H_{32}O_4$	*F. kuhistanica* [11]	198.4±13.8	<0.001	5.8±1.6	<0.5
			319.4±15.3	<0.001		
Ferutin (**13**)	$C_{23}H_{32}O_5$	*F. tenuisecta* [12]	268.0±12.4	<0.001	31.2±4.6	<0.002
			386.3±31.3	<0.001		
Teferin (**14**)	$C_{23}H_{32}O_5$	*F. tenuisecta* [13]	268.6±19.1	<0.001	46.4±4.2	<0.001
			556.5±52.8	<0.001		
Ferutinine monoacetate (**15**)	$C_{24}H_{32}O_5$	Partial synthesis	273.6±24.2	<0.001	24.6±7.4	<0.002
			445±39.5	<0.001		
Teferidine (**16**)	$C_{22}H_{30}O_3$	*F. tenuisecta* [14]	328.0±16.3	<0.001	42.2±4.7	<0.001
			415.3±28.1	<0.001		
Ferutinine (**17**)	$C_{22}H_{30}O_4$	*F. tenuisecta* [15]	359.0±29.1	<0.001	44.6±7.4	<0.001
			619±49.5	<0.001		
Juniferine (**18**)	$C_{23}H_{32}O_5$	*F. juniperina* [16]	27.47±5.7	0.05	7.4±3.8	<0.5
Juniferinine (**19**)	$C_{24}H_{32}O_5$	*F. juniperina* [16]	20.49±4.9	<0.25	12.7±2.9	<0.5
Ferocin (**20**)	$C_{22}H_{28}O_3$	*F. ceratophylla* [17]	16.8±4.8	>0.05	6.9±3.1	<0.5
Ferocinin (**21**)	$C_{23}H_{30}O_4$	*F. ceratophylla* [17]	13.25±2.0	>0.5	12.5±2.8	>0.5
Juniferol (**22**)	$C_{15}H_{26}O_2$	*F. juniperina* [16]	-44.7±7.1	<0.001	-14.56±6.3	>0.1
Juniferdine (**23**)	$C_{22}H_{30}O_4$	*F. juniperina* [18]	32.45±1.46	<0.001	7.1±78	>0.05
			80.6±2.1	<0.001		
Juniferidine (**24**)	$C_{22}H_{30}O_4$	*F. juniperina* [19]	108.2±2.1	<0.001	2.1±1.78	>0.5
			148.6±11.6	<0.001		
Tschimgine (**25**)	$C_{17}H_{22}O_3$	*F. tschimganica* [20]	120.5±14.3	<0.001	-10.34±3.0	<0.25
Tschimganine (**26**)	$C_{18}H_{24}O_4$	*F. tschimganica* [20]	107.2±13	<0.001	24.34±8.5	<0.05
			175.0±18.8	<0.001		
Tschimganidine (**27**)	$C_{23}H_{32}O_5$	*F. tschimganica* [21]	219±18.5	<0.001	16.55±4.07	<0.05
			324.1±23.4	<0.001		
Epoxytschimganidine (**28**)	$C_{23}H_{32}O_5$	Partial synthesis	4.9±3.6	<0.001	7.3±4.3	>0.05
Diethylstilbestrol (**29**)	$C_{23}H_{32}O_5$	Reference preparation	364.6±18.7	<0.001	6.2±0.9	>0.05
			703.4±32.2			

Uterus mass without liquid in the numerator; with liquid, in the denominator.

The terpenoid esters with aromatic and aliphatic acids that were studied in the present work were carotanes, humulanes, germacranes, and camphanes. We found among the studied compounds some that inhibited and some that stimulated growth of murine uterus and its hydration. Table 1 shows compounds of the carotane class (**1**-**17**) had the most varied degree of effect on the estrogen activity according to the change of uterus mass and hydration. These compounds could be divided into two subgroups, esters of aliphatic (**1**-**3**) and aromatic (**4**-**17**) acids. Esters such as lapidine (**1**), lapiferine (**2**), and lapidoline (**3**) inhibited growth of uterus, causing it to decrease by 28.4-41.2%.

Terpenoid esters with aromatic acids having two (**4** and **5**) or three methoxyls (**6**) as substituents were less inhibiting (by 19.9-28.8%) of estrogen activity and decreased growth of ovaries by 42.5-49.1%. Replacing two methoxyls in **6** by methylenedioxy in palliferinin (**7**) increased insignificantly such activity. This may have been due to opening in the organism of the methylenedioxy group of **7**. Then, like for tenuferidin (**10**), the presence of *p*-hydroxybenzoic acid would lead to the appearance of rather pronounced estrogen activity.

The estrogen activity of the sesquiterpene alcohol ferutinol and *p*-hydroxybenzoic acid were determined in order to establish the significance of the aromatic substituent to the activity of the terpenoid esters. It was found that they did not separately affect the growth of uterus and its hydration. The studied ferutinol esters exhibited different degrees of estrogen activity besides **8**. Ferutinin (**17**) had the highest estrogen activity. Its estrogen activity was exhibited over a rather broad dose range (from 0.01 to 10 mg/kg) not only for immature mice and rats but also for animals after ovarectomy (Table 2). Administering **17** at a dose of 0.01 mg/kg *per os* to ovarectomized animals that showed no signs of estrus for three weeks after the operation caused estrus in 100% of the test rats. The estrogen activity of **17** was also established on mature females, administration of **17** to which changed the functional state of the ovaries by lengthening estrus to 190%.

However, the presence of an epoxy group in tenuferidin (**10**) almost halved the growth of uterus (both with liquid and without it) and decreased growth of ovaries by eight times.

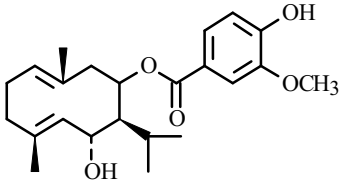
The investigations showed that the presence of a hydroxyl in the *p*-position of the aromatic ring (as in **17**) apparently played a defining role in the manifestation of activity. The estrogen activity of the benzoate ferutinol (teferidin, **16**) in which it is absent was significantly decreased. Growth of uterus with liquid upon administration of **16** was 415.3% whereas it was 645.0% upon administration of **17**. The decrease of effectiveness of **16** in this instance was 229.7% compared with animals that received **17**. Compound **15**, which had an acetyl in the *p*-position, also was less active than **17**. Use of *O*-methylferutinin, which had a hydroxyl in the *p*-position and a methoxyl in the third position (teferin, **14**), reduced estrogen activity compared with **17**. The growth of uterus without liquid decreased by 105.3% whereas the uterus with liquid decreased by only 88.5% compared with **17**. This was evidently explained by its lower influence on cAMP [22, 23]. The presence in the *p*- and *m*-positions of methoxyls (akiferin, **11**) decreased the estrogen activity compared with **17** by more than two times. The activity also decreased by 105.9-258.7% upon changing the *p*-hydroxyl in **17** by a methoxyl with a hydroxyl in the *m*-position (ferutin, **13**).

Replacing the *p*-hydroxyl (in **17**) by methoxyl (ferutidin, **12**) caused the appearance of activity intermediate between that of **11** and **13**. The activity of **12** was more pronounced (by 26-40%) compared with **11** and less (by 25.9-17.2%) than that of **13** and almost half that of **17**. The three methoxyls in pallidin (**9**) caused a complete loss of estrogen activity. The dependence of estrogen activity of the carotanes on the type of fusion (*cis*- or *trans*-) of the five- and seven-membered rings could not be established. This was clearly seen by comparing the data for **8** and **9** (Table 1).

The precursors of the bicyclic sesquiterpenes, monocyclic humulane sesquiterpenes, exhibited less estrogen activity. In this series, juniferol (**22**) decreased distinctly the growth of uterus and ovaries whereas compounds with an aromatic ring on C-10 (ferocin, **20**; ferocinin, **21**) and on C-5 (juniferin, **18**; juniferinin, **19**) enhanced growth of uterus without liquid by 13.25-27.47% despite the hydroxyl in the aromatic ring of the last two. Juniferdin (**23**) and juniferidin (**24**) had more pronounced estrogen activity, increasing growth of uterus with and without liquid.

Esters of the monoterpene alcohol borneol, tschimgin (**25**) and tschimganin (**26**), had twice the estrogen activity. They increased the uterus and had practically no effect or decreased the ovaries of rats. In contrast with **26**, **25** did not enhance grwoth of uterus with liquid (Table 1).

The germacrane representative, tschimganidin (**27**), exhibited rather distinct estrogen activity compared with **12** and **13** although it contained the ten-membered germacrane macrocycle [21]. However, its epoxy derivative **28** had practically no activity. It is possible that not **27** itself but its metabolite, the guaian derivative microferinin, which was also prepared chemically from **27** [24], had estrogen activity.



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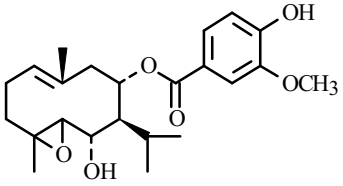

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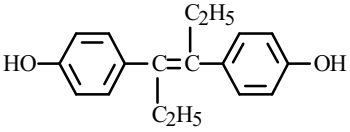

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TABLE 2. Estrogen Activity of Ferutinine Compared with Diethylstilbestrol in Immature and Ovarectomized Mice and Rats (M ± m; n = 8-10)

Experimental conditions	Dose, mg/kg	Uterus mass, mg	P (<or>)	Ovaries mass, mg	P (<or>)	Percent ovarectomized rats with estrus
			Mice			
Control	-	199.6±11.3		51.3±1.7		-
Ferutinine	0.01	233.4±11.6*	<0.001			
		125.4±8.5*	<0.001	53.2±1.7	>0.05	10
	0.1	170+13.2*	<0.001			
		289.7+11.6*	<0.001	56.3±1.1	>0.02	50
	1	246.8+10.4*	<0.001			
		404.5+22.5*	<0.001	55.8+3.3	>0.02	100
	5	299.0+6.2*	<0.001			
		581.9+32.6	<0.001			100
			Rats			
Control	-	64.6±2.3	-	38.9±0.6	-	-
Ferutinine	0.01	102.8±3.7*	<0.001	42.8±3.2	<0.5	60
		125.4±8.5*	<0.001			
	0.1	170+13.2*	<0.001	42.6+3.2	>0.25	100
		289.7+11.6*	<0.001			
	1	296.8+10.4*	<0.001	56.4+2.5*	<0.05	100
		464.6+22.5*	<0.001			
	5	299.0+6.2*	<0.001	52.1+1.6*	<0.05	100
		581.9+11.6*	<0.001			
Control	-	74.9±1.7	-	52.5±1.4		-
Diethylstilbestrol	0.01	249.9±10.5*	<0.001			100
		452.0±49.7*	<0.001	56.4±3.0	<0.1	
	0.1	294.9+10.5*	<0.001			100
		561.3+13.5*	<0.001	55.8+6.3	<0.5	
	1	348.0+22.0*	<0.001		<0.5	100
		601.8+27.0*	<0.001	59.5+3.0*		

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Uterus mass without liquid in the numerator; with liquid, in the denominator.

Considering the importance to estrogen activity of the distance between oxygens of the alcohol hydroxyl and the functional group in the *p*-position of the benzene ring (hydroxyl or methoxyl), we attempted to analyze it based on literature data and our own results. It was found earlier that azomethine compounds in which the distance between the alcohol and phenyl hydroxyls was 14.5 Å exhibited estrogen activity [25]. A determination of this distance in ferutinol esters, which exhibited estrogen activity, showed that the distance between the oxygens was of the order of 10 Å for almost all compounds with estrogen activity. Compound **10** exhibited the lowest activity among compounds **10** and **12-16**. This distance for it was the shortest at 9.85 Å. Compound **17** had the highest estrogen activity. The distance between the oxygens in it was 10.21 Å whereas for estradiol it was 10.85 Å. Compound **17** exhibited an estrogen effect equal to diethylstilbestrol at a dose almost two times higher. This was seen by comparing their ED_{50} (dose causing estrus in 50% of ovarectomized rats). The ED_{50} of **17** (determined for tefestrol consisting mainly of **17**) was 0.0075 mg/kg whereas it was 0.0042 mg/kg for diethylstilbestrol. The results again confirmed that the distance between these functional groups was very important in addition to the other parameters described above.

The results for the activity of terpenoid esters with aromatic and aliphatic acids confirmed the literature data that the presence of an aromatic substituent in the estrogen molecule is one of the factors required for appearance of estrogen activity [25-28]. Terpenoids containing an aliphatic group or carbonyl group in the terpenoid part of the molecule in addition to a benzene ring did not exhibit estrogen activity. The absence of a phenyl hydroxyl decreased or erased estrogen activity. Humulane and germacrane compounds, which were based on 10- or 11-membered macrocycles, exhibited weak estrogen activity.

Our studies again confirmed that terpenoids with a relatively rigid terpenoid conformation that contained phenol and alcohol hydroxyls and an aromatic ring were the most active.

EXPERIMENTAL

The studied compounds were isolated from the underground and aerial organs of *F. tenuisecta*, *F. akitschkensis*, *F. lapidosa*, *F. juniperina*, *F. ceratophylla*, *F. pallida*, *F. tschimganica*, and *F. prangifolia*.

Alcohol extraction of raw material followed by separation into acidic, phenolic, and neutral fractions and column chromatography of the resulting fractions using various solvents were used to separate the terpenoids. The structures and stereochemistries of the terpenoids were proved using chemical and spectral methods [3-21]. The synthesis method and physicochemical properties of **15** and **28**, which were prepared by partial synthesis, will be published separately.

Estrogen activity was determined using immature female rats (30-40 g) and female mice (10-12 g) by the Evans method [29], for which the indicator of activity is the mass increase of uterus and ovaries. The studied compounds were administered *per os* once per day at a dose of 1 mg/kg for three days. Estrogen activity in tests with ovarectomized female rats (170-200 g) was determined by the literature method [30]. The tested preparations were administered over three days. The onset times of estrogen activity and the duration of action of the preparations after their withdrawal were observed in the animals.

The effect of the studied preparations on the estrus cycle was tested in mature intact female rats (150-170 g) with normal cycles. Preparations were administered over 30 d. The estrus cycle was observed (using vaginal smears) during administration of the preparations and for a month after they were no longer administered. Vaginal smears were taken daily, colored by the Gims—Romanovskii method, and examined under a microscope to determine the phase of the estrus cycle.

The reliability of the differences between the test and control groups was determined using the Student *t*-criterion.

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