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ESTROGENIC ACTIVITY AS A FUNCTION OF CHEMICAL STRUCTURE IN *Haplophyllum* QUINOLINE ALKALOIDS

S. S. Nazrullaev, I. A. Bessonova, and Kh. S. Akhmedkhodzhaeva

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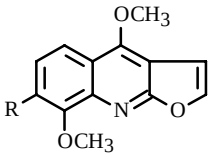
The estrogenic activity of 15 quinoline alkaloids isolated from plants of the Haplophyllum *genus and two derivatives was studied. These compounds exhibit estrogenic activity that depends on the type of heterocyclic skeleton, N atom basicity, and substituent.*

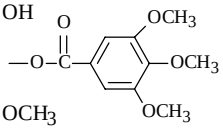
Key words: *Haplophyllum*, Rutaceae, quinoline alkaloids, estrogenic activity.

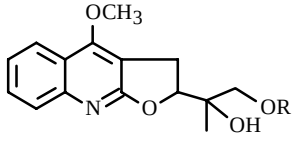
Plants of the *Haplophyllum* A. Juss. (Rutaceae) genus have long been known for their medicinal properties [1]. The extracts of certain plants are widely used in folk medicine as analgesics, antispamodics, diuretics, and sedatives and as topical agents for skin diseases, etc. [2-4]. Ruta is also known to affect reproductive organs, induce abortion, and act as a contraceptive [5].

One third of the more than 200 quinoline alkaloids isolated from Rutaceae plants occur in the *Haplophyllum* genus, being a unique source of various quinoline alkaloids [6, 7].



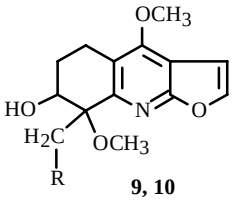
1 - 6

1: R = H
2: R = OH
3: R = 
4: R = OCH_3
5: R = O-α-L-Rha*p*
6: R = $OCH_2CH(OH)C(OH)(CH_3)_2$



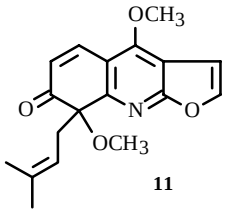
7, 8

7: R = H
8: R = Ac



9, 10

9: R = $CH_2C(OH)(CH_3)_2$
10: R = $CH{=}C(CH_3)_2$



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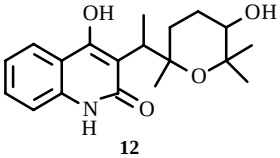

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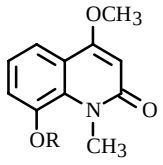
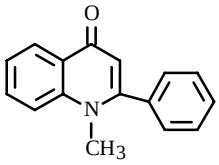


13 - 15

13: R = H
14: R = Ac
15: R = $CH_2CH(OH)C(OH)(CH_3)_2$



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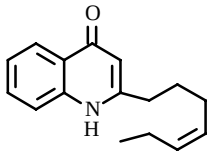

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S. Yu. Yunusov Institute of the Chemistry of Plant Substances, Academy of Sciences of the Republic of Uzbekistan, Tashkent, fax (99871) 120 64 75. Translated from Khimiya Prirodnykh Soedinenii, No. 6, pp. 472-475, November-December, 2001. Original article submitted October 22, 2001.

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Pharmacological research showed that *Haplophyllum* alkaloids possess a wide spectrum of pharmacological activity with little toxicity. The majority of them have a distinct depressive effect on the CNS, exerting a sedative, soporific, antispasmodic, and other effects. Certain alkaloids exhibit stimulant and antiarrhythmic activity and affect the uterine and intestinal muscle tone [8-15].

The present article contains results of research on 15 quinoline alkaloids and two derivatives for estrogenic activity in animals (estrogenic activity, effect on the menstrual cycle, and embryogenesis). This research reveals the dependence between the chemical structure and estrogenic activity of quinoline alkaloids belonging to the furanoquinoline (**1**-**6**), dihydrofuranoquinoline (**7**, **8**), modified furanoquinoline (**9**-**11**), 4-hydroxyquinolin-2-one (**12**-**15**), and 2-phenyl(alkyl)-quinolin-4-one (**16**, **17**) systems.

It was found that almost all these compounds possess estrogenic activity to some degree or another. Compounds **1**-**7**, **9**-**12**, **15**, and **17** exhibit this effect at a dose of 10 mg/kg (Table 1). Administration of γ-fagarine (**1**), glycoperine (**5**), perforine (**9**), and foliosidine (**15**) increased the uterine mass in immature rats by 122.1-193.9% without liquid. Certain alkaloids also cause the uterus to hydrate. This is characteristic of estrogenic compounds. The greatest increase of uterine mass with liquid was observed for administration of γ-fagarine (**1**), 283.6%.

All compounds had a dose-dependent effect. Quinoline alkaloids had the greatest estrogenic activity at doses of 50-100 mg/kg (1/20-1/10 of LD_{50}). Thus, γ-fagarine (**1**), a derivative of **3**, glycoperine (**5**), evoxine (**6**), and acutine (**17**) at a dose of 50 mg/kg increase the uterine mass by 251.7-403.9% without liquid and by 318.0-612.3% with liquid.

The alkaloids γ-fagarine (**1**) and perforine (**9**) a dose of 10 mg/kg reliably increased the uterine mass and also that of ovaries by 21.5 and 29.1%. This indicates that they are gonadotropic or follicule-stimulating. A mass increase of ovaries up to 42.5 and 26.7% was noted upon administration of **8** and **13**, respectively, which exhibit weak estrogenic activity (Table 1).

The estrogenic activity of haplopine (**2**), its derivative **3**, dubinidine (**7**), perfamine (**11**), bucharidine (**12**), and acutine (**17**) is significantly increased at doses of 50 and 100 mg/kg whereas these alkaloids have no effect on mass of ovaries.

These alkaloids at doses of 30, 50, and 100 mg/kg in experiments with ovariectomized rats caused proliferative changes in the vaginal lining upon estrus in 20, 50, and 100% of the rats, respectively. The most active alkaloids were the furanoquinolines **1**, **4**, and **5** and modified furanoquinolines **9** and **10**.

Experiments with mature intact rats demonstrated that skimmianine, dubinidine, and perforine at doses of 50 and 100 mg/kg reliably change the menstrual cycle by lengthening the estrus phase. Thus, whereas the average duration of a single menstruation is one day, these alkaloids extend it to 1.4, 1.3, and 1.4 days, respectively.

These alkaloids at doses of 10-20 mg/kg have essentially no effect on implantation and embryogenesis. However, administration at a dose of 100 mg/kg, especially of skimmianine, caused an embryotoxic effect characteristic of synthetic and natural estrogens [16-18].

Thus, *Haplophyllum* quinoline alkaloids were found to possess an estrogenic effect. However, compared with known estrogens, for example, synestrol, ethynylestradiol, etc., they are effective at higher doses.

One of the principal approaches to determining the structure—activity relationship is to compare the effectiveness of structurally similar compounds that belong to different structural categories. An analysis showed that the greatest estrogenic effect occurs for furanoquinoline alkaloids **1**-**6**. A common element of these is the furanoquinoline skeleton and the C-4 and C-8 methoxyls. Adding hydroxyl, methoxyl, and 3,4,5-benzoyloxy to C-7 has little effect on the estrogenic activity whereas furanoquinoline alkaloids with a glycosyl or hydroxylated isoprenoid substituent in this position have increased activity (Table 1).

Th estrogenic activity sharply decreases for **7** and **8**, which are dihydrofuranoquinolines. These are stronger bases than furanoquinolines **1**-**6** [6]. Apparently the basicity of the N atom plays an important role in producing estrogenic activity because the lack of a double bond in the furan ring increases the electron density on the N atom and, therefore, its basicity, i.e., the electronic state of the N atom effects the degree of estrogenic activity. Modified furanoquinolines **10** and **11**, in which the benzene ring is partially hydrogenated (haplophylidine) or modified into a geminally substituted cyclohexadienone ring (perfamine), except for perforine (**9**), exhibit weaker estrogenic activity than furanoquinolines.

It is interesting to note that the estrogenic activity of **9** is several times greater than that of **10** although they differ chemically only by the structure of the isoprenoid substituent, which has a hydroxyl in perforine instead of the double bond in haplophylidine. Therefore, the estrogenic activity of the alkaloids depends on the nature of the substituent.

TABLE 1. Estrogenic Activity of Quinoline Alkaloids on Immature Rats (n = 10)*

Compound	Dose, mg/kg	Uterine mass gain, % of control		Mass gain of ovaries, % of control
		without fluid	with fluid	
γ-Fagarine (**1**)	10	193.9	283.6	29.1
	50	285.0	612.3	39.7
Haplopine (**2**)	10	74.4	-	14.2
	50	268.5	-	10.6
	100	305.5	456.5	4.2
3,4,5-Trimethoxybenzoylhaplopine (**3**)	10	33.3	-	-9.6
	50	251.7	353.9	6.7
Skimmianine (**4**)	10	22.6	80.8	24.9
	50	97.2	158.2	10.4
Glycoperine (**5**)	10	122.1	-	9.5
	50	396.0	551.2	9.9
Evoxine (**6**)	10	108.3	-	-1.4
	50	403.9	597.9	19.7
Dubinidine (**7**)	10	36.4	-	0.9
	100	132.0	-	4.5
Dubinine (**8**)	100	22.5	68.2	42.5
Perforine (**9**)	10	157.6	-	21.5
	100	184.3	350.6	13.7
Haplophylidine (**10**)	10	26.8	-	30.1
	50	39.3	77.0	32.5
Perfamine (**11**)	10	45.8	-	1.2
	50	87.5	121.4	2.8
Bucharidine (**12**)	10	48.5	-	-1.3
	50	177.4	228.5	8.4
Folifidine (**13**)	100	-4.3	-	26.7
Acetylfolifidine (**14**)	100	-15.2	25.9	-35.0
Foliosidine (**15**)	10	176.1	-	10.6
	50	204.1	257.5	7.6
1-Methyl-2-phenylquinolin-4-one (**16**)	100	18.9	-	11.8
Acutine (**17**)	10	21.5	-	3.4
	50	243.0	318.0	7.6

*True uterine mass gain was reliable relative to the control for all compounds except **13** and **16**; gain of ovaries was reliable for **1**, **4**, **5**, **6**, **8**-**10**, **13**, and **14**.

Bucharidine (**12**) and foliosidine (**15**), which contain hydroxylated isoprenoid moieties in the 3- and 8-position, respectively, have the highest estrogenic activity among the 4-hydroxyquinolin-2-one alkaloids **12**-**15**. Folifidine (**13**) and its acetate (**14**), which differ from foliosidine only by the lack of a hydroxylated isoprenoid chain on C-8, have almost no estrogenic activity. This fact indicates also that the substituent plays a role on the degree of estrogenic activity. In particular, it indicates that the presence of hydroxylated isoprenoids in the alkaloids of the furanoquinoline (evoxine), 5,6,7,8-tetrahydrofuranoquinoline (perforine), and 4-hydroxyquinolin-2-one (bucharidine, foliosidine) types have significantly elevated estrogenic activity.

With respect to **16** and **17** of the 2-phenyl(alkyl)-quinolin-4-one type, only **17** has estrogenic activity. Alkaloid **16**, which has a C-2 phenyl, exhibits a weak estrogenic effect.

Thus, the majority of quinoline alkaloids of *Haplophyllum* plants possess estrogenic activity that depends on the heterocyclic skeleton, basicity of the N atom, and nature of the substituent.

EXPERIMENTAL

Alkaloids **1**, **2**, **4-7**, **9-13**, and **15-17** were prepared from *Haplophyllum pedicellatum*, *H. foliosum*, *H. perforatum*, *H. bucharicum*, and *H. acutifolium* [7]; dubinine (**8**) and acetylfolifidine (**14**), by acetylation of dubinidine [19] and folifidine [20]; 3,4,5-trimethoxybenzoylhaplopine (**3**), by reaction of haplopine (**2**) and 3,4,5-trimethoxybenzoyl chloride in pyridine [21].

3,4,5-Trimethoxbenzoylhaplopine (3), mp 203-204°C (acetone).

Mass spectrum (EI, 70 eV), *m*/*z* (I_{rel}, %): 439 $[M]^+$ (69), 244 (7), 195 (100).

PMR spectrum (400 MHz, DMSO, δ, ppm, J/Hz): 3.83 (3H, s, OCH_3), 3.92 (6H, s, 2×OCH_3), 4.08 (3H, s, OCH_3), 4.48 (3H, s, OCH_3), 7.26 (1H, d, J = 9, H-6), 7.40 (1H, d, J = 3, Hβ), 7.42 (2H, s, H-2′, H-6′), 7.92 (1H, d, J = 3, Hα), 8.04 (1H, d, J = 9, H-5).

The estrogenic activity of the alkaloids was determined by the Dorfman [22] and Allen—Doisy [23] methods. Experiments were performed on immature rats of mass 35-45 g and mature ovariectomized rats of mass 170-200 g. The indicators of an effect were uterine mass increase and appearance of estrus, respectively.

The estral cycle was determined in experiments on mature rats with a 4-5-day cycle. Females in estrus were introduced to males (2:1) to determine the effect of the alkaloids on embryogenesis. The first day of pregnancy was considered the day on which spermatozoids were observed. The animals were sacrificed on the 19-20th day of pregnancy. The results were treated statistically [24].

REFERENCES

1. *Handbook of Medical Science* [in Russian], Abu Ali Ibn Sino, Tashkent (1996), Vol. 3, p. 139.
2. Kh. Kh. Khalmatov, *Wild Medicinal Plants of Uzbekistan* [in Russian], Meditsina, Tashkent (1964), Vol. 113, p. 278.
3. B. P. Makhlayuk, *Medicinal Plants in Folk Medicine* [in Russian], Privolzhskoe Knizhnoe Izd., Saratov (1967), p. 560.
4. *Plant Resources of the USSR* [in Russian], Nauka, Leningrad (1988), Vol. 4, p. 360.
5. V. G. Shimanov, *Hormonal Activity of Field Plants and Their Effect on Karakul Sheep Fertility* [in Russian], Fan, Tashkent (1972), p. 17.
6. I. A. Bessonova and S. Yu. Yunusov, *Khim. Prir. Soedin.*, 303 (1977).
7. I. A. Bessonova, in: *Progress in Research on Alkaloid-Bearing Plants* [in Russian], Kh. N. Aripov, ed., Fan, Tashkent (1993), p. 62.
8. M. B. Sultanov, *Pharmacology of Plant Substances* [in Russian], Fan, Tashkent (1976), p. 3.
9. N. P. Polievtsev and I. K. Kamilov, *Pharmacology of Alkaloids* [in Russian], Fan, Tashkent (1962), No. 1, p. 148.
10. N. I. Evdokimova, N. P. Polievtsev, and M. B. Sultanov, *Pharmacology of Alkaloids and Cardiac Glycosides* [in Russian], Fan, Tashkent (1971), pp. 167, 171, and 174.
11. M. A. Margupova, I. K. Kamilov, and N. P. Polievtsev, *Pharmacology of Alkaloids* [in Russian], Nauka, Tashkent (1962), p. 155.
12. V. V. Berezhinskaya and E. A. Trutneva, *Farmakol. Toksikol. (Moscow)*, **6**, 707 (1963).
13. M. M. Azimov, *Pharmacology of Alkaloids and Glycosides* [in Russian], Fan, Tashkent (1967), p. 30.
14. Kh. S. Akhmedkhodzhaeva, I. K. Kamilov, and S. Kh. Nasirov, *Pharmacology of Alkaloids and Glycosides* [in Russian], Fan, Tashkent (1967), p. 14.
15. F. Sadritdinov and M. M. Margupova, *Dokl. Akad. Nauk UzSSR*, No. 11, 32 (1969).
16. S. I. Fomichev, *Probl. Endokrinol.*, No. 2, 83 (1973).
17. S. Kresnadi, M. Harper, and C. Lioid, *Endocrinology*, **20**, No. 3, 834 (1972).
18. R. Mehrotra and V. Kamboy, *Planta Med.*, **33**, No. 4, 349 (1978).

19. I. A. Bessonova, G. P. Sidyakin, and S. Yu. Yunusov, *Zh. Org. Khim.*, **34**, 347 (1964); *Khim. Prir. Soedin.*, 29 (1969).
20. Z. Sh. Faizutdinova, I. A. Bessonova, and S. Yu. Yunusov, *Khim. Prir. Soedin.*, 257, (1967).
21. Z. Sh. Faizutdinova, G. P. Sidyakin, and S. Yu. Yunusov, *Dokl. Akad. Nauk UzSSR*, No. 1, 35 (1966).
22. R. Dorfman and A. Dorfman, *Endocrinology*, **55**, 65 (1954).
23. Ya. M. Kabak, *Practicum in Endocrinology* [in Russian], Moscow St. Univ., Moscow (1968), p. 24.
24. M. L. Belen′kii, *Elements of Quantitative Assessment of Pharmacological Effects* [in Russian], Gos. Izd. Med. Lit., Leningrad (1963), p. 30.