UTEROTONIC EFFECT OF *EVODIA RUTAECARPA* ALKALOIDS

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ABSTRACT.—Rutaecarpine (**1**) and dehydroevodiamine (**2**) were isolated from the unripe fruit of *Evodia rutaecarpa*. *In vitro* uterotonic assay on rat uterus showed both alkaloids to be active. Dehydroevodiamine was further shown to be active in the *in vivo* uterotonic assay using rats as the animal model. Reference evodiamine hydrochloride (**3**) was also evaluated and found to be devoid of uterontonic activity at the doses administered.

The unripe fruits of *Evodia rutaecarpa* (Juss.) Benth. (Rutaceae), have been used in Chinese medicine for two millenia in the treatment of headache, abdominal pain, dysentery, postpartum hemorrhage and amenorrhea (1, 2). Aqueous extracts of this plant have been reported to decrease mouse litter size (3) and to have *in vitro* a stimulatory effect on hamster uterus (4).

Phytochemical studies on the fruits of this plant have shown the presence of numerous compounds, including the alkaloids evodiamine (5–11), rutaecarpine (5–12), wuchuyine (5, 8), hydroxyevodiamine (rhetsinine) (6, 11–13), evocarpine (6), 1-methyl-2-pentadecyl-4(1H)-quinolone (9), 1-methyl-2-tridecyl-4(1H)-quinolone (dihydroevocarpine) (9), 1-methyl-2-undecyl-4(1H)-quinolone (9), dihydrorutaecarpine (9), 14-formyldihydrorutaecarpine (9) and 7-carbomethoxy-8,13,13b,14-tetrahydro-14-methylindolo(2',3':3, 4) pyrido (2, 1–B) quinazolin-5 (7H)-one (10). The alkaloids, dehydroevodiamine (14) and hydroxyevodiamine (rhetsinine) (14) have been found in the leaves of this plant.

The non-alkaloid constituents of the fruits include rutaevin (15, 17), limonin (evodin) (5, 8, 11, 12, 16, 17), evodol (17, 18), evodinone (12), evogin (12), gushuyic and other fatty acids (19, 20).

In view of the reported uterine stimulant activity, which was confirmed in a series of preliminary experiments in this laboratory, the present study was initiated to isolate the active principle(s) responsible for the observed biological effect.

EXPERIMENTAL[3]

PLANT MATERIAL.—The unripe fruits of *Evodia rutaecarpa* were purchased from Kwun Wo Drug Company, Hong Kong. According to the dealer, these fruits originated from Szech'uan province in China. Two batches, 10.5 kg and 5.24 kg, of these fruits were used in this work. A sample was saved for future reference.[4]

ISOLATION OF RUTAECARPINE **1**.—Fruits (5.24 kg) were ground and defatted with petroleum ether (60–80°), followed by exhaustive extraction with methanol. The methanolic extract was concentrated to a thick syrup *in vacuo*. Hydrochloric acid (5%) was added and the methanol was subsequently removed *in vacuo*. The aqueous solution was made alkaline with 28% ammonia and extracted with chloroform. The chloroform solution was dehydrated with an-

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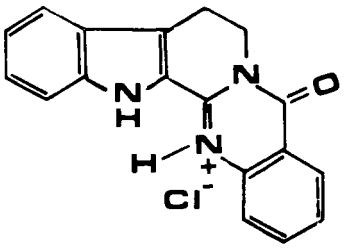
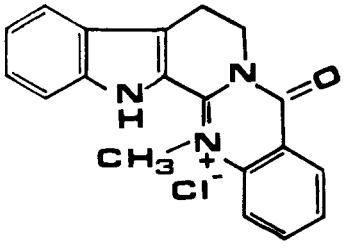
[3]Melting points were determined with a Kofler hot plate and are uncorrected. The uv spectra were obtained with a Beckman model 25 doulble beam spectrophotometer-recorder. The ir spectra were determined with a Beckman IR 10 spectrophotometer with polystyrene calibration at 1601 cm^{-1}. Absorption bands are recorded in wave numbers (cm^{-1}). Nmr spectra were recorded in $CDCl_3$ or CD_3OD solutions with Jeol C-60HL and Jeol PS-100 nmr spectrometers. Tetramethysilane was used as an internal standard and chemical shifts are reported in δ(ppm) units.

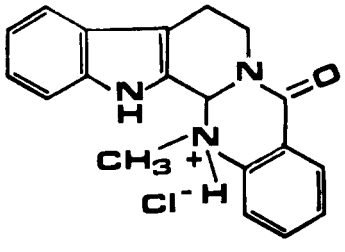
[4]Voucher specimen No. 137-1001.



Rutaecarpine · HCl **1**



Dehydroevodiamine Chloride **2**



Evodiamine · HCl **3**

hydrous sodium sulfate and taken to dryness *in vacuo*. The chloroform extract (28.3 g) obtained was subjected to column chromatography on silica gel 60 (70–230 mesh). The column was developed with benzene and 16 ml fractions were collected. The eluant was changed to chloroform at fraction 246. Fractions 431–820 afforded a crystalline substance (13.9 mg), mp 262–264°. The physical data (uv, ir, tlc, nmr) were in agreement with a reference sample of rutaecarpine.[5] Both the isolate and rutaecarpine showed the same ir characteristics and nmr spectra: ir ν max (KBr) cm^{-1}: 3345, 1650, 1601, 1540, 1385, 1350, 1310, 1210, 1130, 750, 720. Nmr (CD_3OD): δ3.2 (2H,t,J=7Hz), 4.5 (2H,t,J=7Hz), 7.2–7.6 (8H,m) and 9.3 (1H,m) (21).

[5]Reference sample of rutaecarpine was generously provided by Professor U. Sankawa, Faculty of Pharmaceutical Sciences, University of Tokyo, Tokyo, Japan.

ISOLATION OF DEHYDROEVODIAMINE (DHE) **2**.—A second sample of *Evodia rutaecarpa* fruits (10.5 kg) was processed for alkaloids as described above. The non-quaternary alkaloids were converted to their corresponding hydrochloride salts by treatment with 1 N HCl. Repeated crystallization of the total alkaloid fraction (14.2 g) from ethanol yielded 3.5 g of a yellow crystalline compound, mp 208–209°. It gave the following data: nmr (CD_3OD): δ3.38 (2H,t, J=7Hz), 4.44 (3H,s), 4.56 (2H,t,J=7Hz), 7.2–7.8 (7H,m), 8.32 (1H,dd,J=8 Hz and J=1.5Hz); ir ν max (KBr) cm^{-1}: 3400, 1700, 1608, 1544, 1497, 1425, 1333, 1210, 1101, 756. Anal: Calcd. for $C_{19}H_{16}N_3OCl \cdot 1/4\ H_2O$: C, 66.67; H, 4.85; N, 12.27, Found: C, 66.61; H, 4.75; N, 11.97. The nmr spectrum of the isolate was very similar to that of evodiamine and indicated the presence of eight aromatic protons, two methylene groups in adjacent positions, and an *N*-methyl group. A direct comparison of the ir and nmr spectra (CD_3OD and CF_3COOD) of the isolate and a reference sample of dehydroevodiamine chloride[6] showed them to be identical. As further evidence for its identification, the authentic sample of DHE chloride was prepared from a reference sample of rhetsinine[6] by the method of Naksato *et al.* (14). The ir spectra of the isolate and the conversion product were identical, and an unchanged mixture mp of the two compounds unequivocally established the identity of the isolate.

DETERMINATION OF *in vitro* UTEROTONIC ACTIVITY OF RUTAECARPINE, DEHYDROEVODIAMINE AND EVODIAMINE.—The alkaloids, dehydroevodiamine (DHE) and rutaecarpine, isolated during the course of this investigation, and a reference sample of evodiamine (3)[6], in the form of hydrochloride salts, were evaluated for *in vitro* uterotonic activity using isolated rat uteri. Proestrous (determined by vaginal smear) Sprague-Dawley rats were pretreated with 100 μg of estradiol (intramuscular injection in peanut oil) 24 hours prior to use. The middle one-third segment of the isolated uterine horn was mounted in a 10 ml organ bath containing Van Dyke and Hasting solution (22) at 32° and aerated with 95% O_2:5% CO_2. Isometric contraction was recorded on a Beckman R 511A dynograph through a Statham (model UC 3) tension transducer. The uterus was allowed to stand for half an hour and was tested with acetylcholine chloride at 3 x 10^{-6}M prior to the administration of each of the alkaloids in the form of their water soluble hydrochlorides. The results of these experiments are summarized in table 1,

TABLE 1. Uterotonic effects of *Evodia rutaecarpa* alkaloids.

	Alkaloid	Effective dose range	Maximum response
In vitro	Dehydroevodiamine·Chloride	0.6–6.6 μg/ml	2.15±0.33(12)[c]
	Rutaecarpine·HCl	<1.0 μg/ml[a]	0.72±0.40(8)
	Evodiamine·HCl	N.A.[b]	N.D.[d]
In vivo.....	Dehydroevadiamine·Chloride	80–2,400 g/kg	25.3±3.8(3)[e]

[a]Since rutaecarpine·HCl was not completely soluble in water, the exact concentration was not determined.

[b]Not active up to 20 μg/ml.

[c]Maximum response presented as mean ± S.E.M. of maximum peak tension in each preparation with (n) denoting the number of preparations and expressed as unit of g-force.

[d]Not determined.

[e]Maximum peak contraction was measured and expressed in mm Hg. The response was measured at the dose of 2,400 μg/kg body wt.

and the dose response curve determined for dehydroevodiamine hydrochloride is presented in figure 1. It was observed during the course of these experiments that the activity of DHE can be totally suppressed by methysergide at 3 x 10^{-9}M. It was also found that evodiamine hydrochloride (**3**) was not active at doses up to 20 μg/ml.

DETERMINATION OF *in vivo* UTEROTONIC ACTIVITY OF DEHYDROEVODIAMINE.—The observed *in vitro* uterotonic activity of dehydroevodiamine (Cl^-) was confirmed by means of the intrauterine pressure measurement by the sponge-tipped catheter method (23) adapted for rats in the *in vivo* situation. In this study, estrous Sprague-Dawley rats as determined by vaginal smears were anesthetized with sodium pentobarbital (40 mg/kg, i.p.). The lower mid section of abdomen was opened, and the lower portion of the right uterine horn was lifted up with a hemostat. A small incision (3–5 mm) was made at a position 0.8–1.0 cm from the cervix. A catheter with a sponge tip filled with physiological saline solution was inserted into the uterine cavity. The other end of the catheter was attached to a Statham P23 BB transducer coupled

[6]Reference samples of dehydroevodiamine (Cl^-), rhetsinine and evodiamine (HCl) were generously provided by Dr. M. Badawi, Department of Pharmacognosy and Pharmacology, University of Illinois, Chicago, Ill., U.S.A.

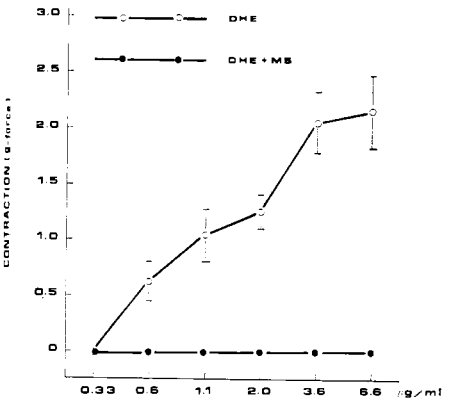


FIG. 1. Log dose response curve of *in vitro* uterotonic effect of DHE. The maximum peak tension within 20 minutes after drug addition was measured as the response. Its effect was totally suppressed by 3×10^{-9}M methysergide (MS) which was added 5 minutes before the addition of DHE. The data were represented as mean ± S.E.M. with n=12 at every point.

to a Beckman 9872 strain gauge coupler in a Beckman 511A dynograph. Dehydroevodiamine (Cl^-) was dissolved in water and administered by i.v. injections. The results are presented in table 1 and figures 2 and 3.

DISCUSSION

There is no previous report of uterotonic compounds isolated from the fruits of *Evodia rutaecarpa*. A degradation product of rutaecarpine, rutamine, of unknown structure was reported to be uterotonic, but detailed information was not available (24).

In the present study, the known alkaloids, dehydroevodiamine and rutaecarpine were isolated from the unripe fruits of *Evodia rutaecarpa* and found to be two of the active principles responsible for the uterotonic effect of the aqueous extracts reported in the literature and confirmed in our laboratory.

In the *in vitro* situation, it was found that the effect of dehydroevodiamine (Cl^-) on isolated rat uteri was not blocked by atropine at a concentration of 3×10^{-8} M, but was blocked by methysergide at a concentration of 3×10^{-9} M. These results suggest that DHE chloride may be a serotoninergic agonist. At doses equal to or greater than 3.6 µg/ml, it was very difficult and sometimes impossible to wash away the uterotonic effect of DHE chloride. The occurrence of this phenomenon may be due to the irreversible binding of DHE chloride to the cell membrane or to the fact that DHE had penetrated into the cell and the observed uterotonic effect was caused by a post-membraneous event.

Since rutaecarpine (HCl) was only sparingly soluble in water, it was not possible to determine the final concentration of this alkaloid in the organ bath. The effective *in vitro* uterotonic dose was thus estimated to be less than 1 µg/ml. The contraction pattern of rutaecarpine is the same as that induced by DHE chloride.

Although evodiamine was not isolated during the course of this investigation, a reference sample of this known constituent was obtained for biological evaluation. It was found to be devoid of *in vitro* uterotonic activity at the doses tested.

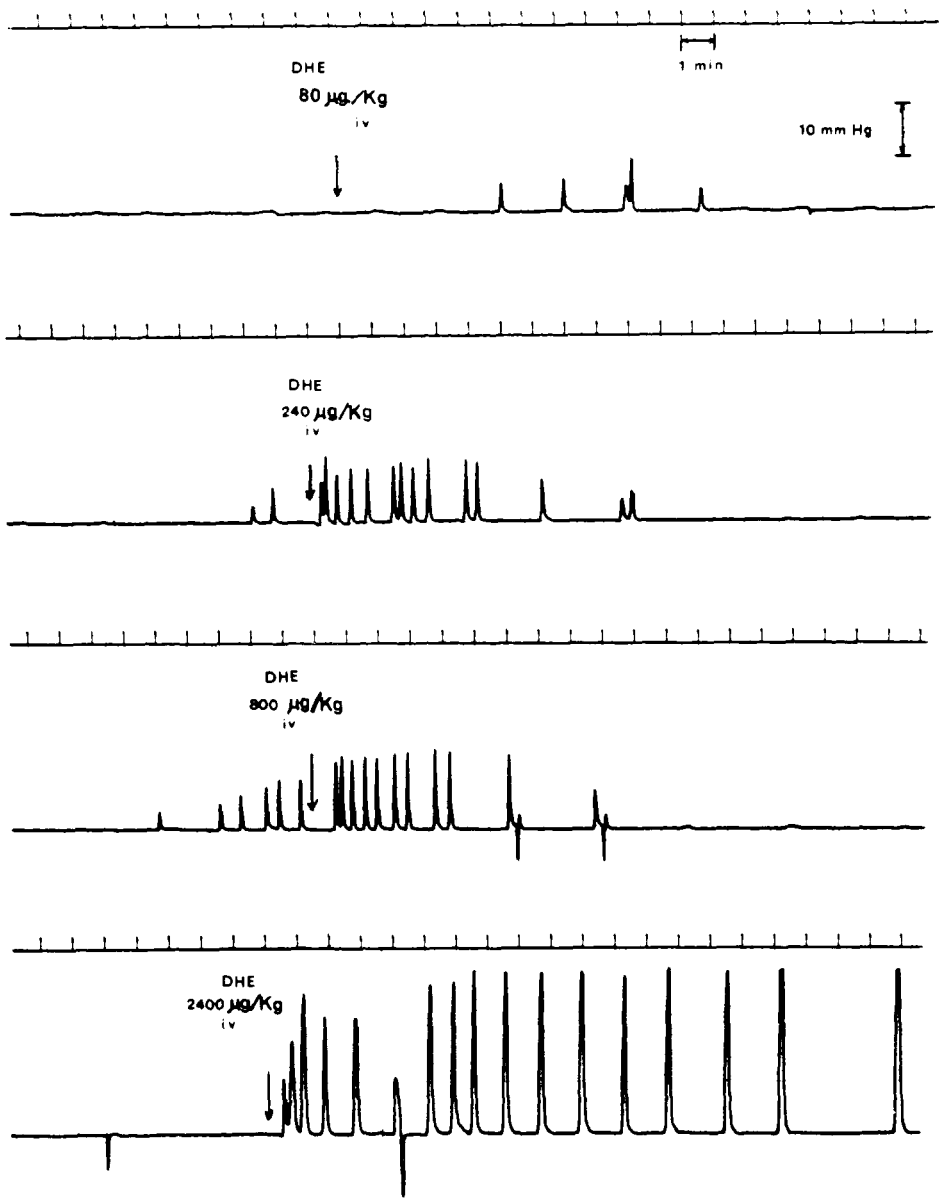


FIG. 2. A typical example of effect of DHE on rat uterus in intact rat (*in vivo*). Arrow indicates the administration of DHE. All dosages are subsequent doses given to the same rat in this experiment.

In the *in vivo* uterotonic assay, where DHE chloride acts through the systemic route, it elicited an initial positive response at a dose of 80 µg/kg body weight. Figure 2 shows that DHE induces clonic contractions with regular frequency and strength, with the intensity of the contractions being dose related. A linear dose response relationship is seen in figure 3. The maximum response dose has not been tested because of the limited solubility of DHE chloride.

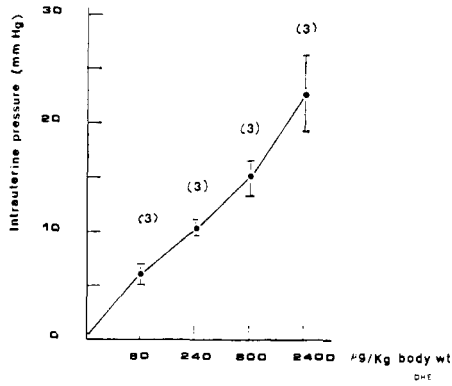


FIG. 3. Log dose response curve of *in vitro* uterotonic effect of DHE. Maximum peak contractions were measured as response. Three rats were used in this experiment.

If the biological data (on rats) from these experiments can be extrapolated into the human situation, the presence of the uterotonic alkaloids in the unripe fruit of *Evodia rutaecarpa* can form the basis for the rational use of this drug in traditional Chinese medicine for the treatment of "female reproductive disorders".

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