ORAL CONTRACEPTIVES—PART V. ANTHELMINTICS AS ANTIFERTILITY AGENTS[1]

By

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In the previous communications the potent antifertility effect of *Mallotus philippinensis* was traced by Gujral *et al.* (1960a and b) to the presence of the chromenochalkone rottlerin which appeared to act by counteracting the effect of chorionic gonadotropin (Varma *et al.*,1959 b). In view of this encouraging result it was decided to pursue these studies further to search for newer compounds based on the nature of the pharmacophore responsible for this effect.

Although rottlerin and pelletierine, the main constituent of *Punica granatum* (which also was shown by Gujral *et al.*, 1960a, to possess some antifertility effect), differ widely in their chemical structures, their common anthelmintic and antifertility effects pointed to a possible parallelism in these two bioresponses. The precise mode of action of anthelmintics is still obscure. It is, however, believed that most of these drugs, by virtue of their redox or other activated systems, are effective inhibitors of essential enzymatic processes in the helminths. Rottlerin, for instance, has been reported to be an anti-oxidant for protecting vitamin A, especially in the presence of other redox systems like hydroquinone, maleic acid or oleic acid (Ramaswamy and Banerjee,1948). It is conceivable that a similar action may be involved in blocking essential metabolic pathways leading to interference at some stage in mammalian reproduction. On the basis of this assumption it was thought advisable to test whether other anthelmintics also share the antifertility effect. Attention was to be directed particularly to those, which structurally resembled rottlerin or some of its active centres because of its better response as compared to that of *Punica granatum* which had produced only pseudo-pregnancies. The present communication deals with the antifertility effect of ten anthelmintics selected on the following basis.

Basis of selection of anthelmintics.—Kousso and aspidium were selected as representatives of other naturally occurring anthelmintics possessing chemical structures either similar to that of rottlerin or based on some of its structural fragments. Kousso, an ether extract of the dried female flowers of

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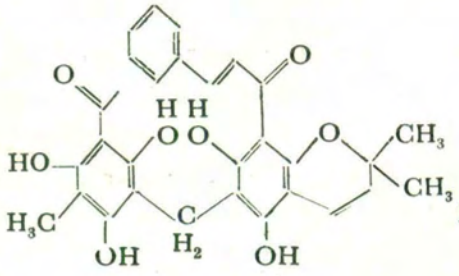
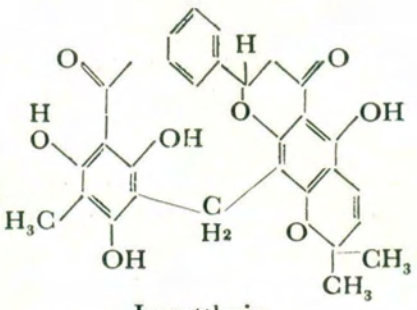
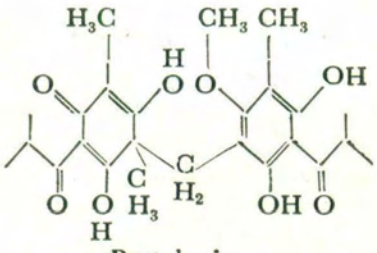
Hagenia abyssinica (also known as *Brayera anthelmintica*, family Rosaceae), has recently been shown by Birch and Todd (1952) to be a mixture of protokosin[1], ∝-kosin and β-kosin, which, like rottlerin, are alkyl-acyl-phloroglucinol moieties joined by methylene bridges.



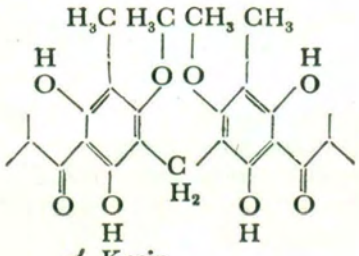
Rottlerin



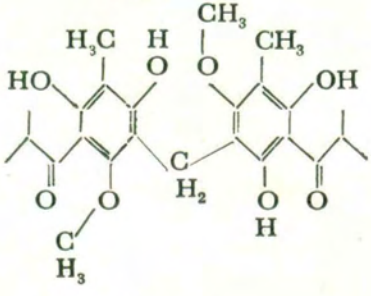
Isorottlerin



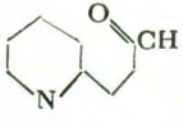
Protokosin



∝-Kosin



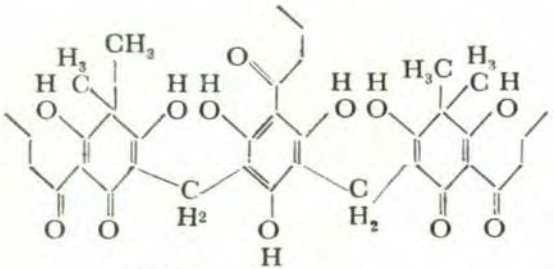
β-Kosin



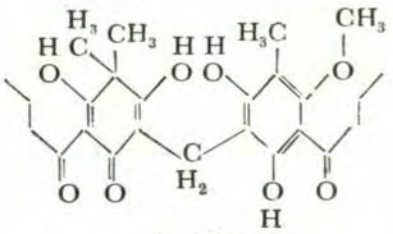
Pelletierine

Aspidium or *Filix Mas* is an ethereal extract of male fern containing a number of closely related compounds. Its major bioactive constituents are filixic acid, aspidin, albaspidin, flavaspidic acid, filicinic acid and aspidinol (Watkins, 1958 ; Hassal, 1958 ; Bally, 1959).

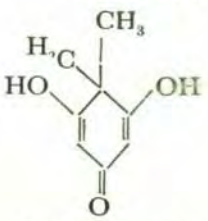
1 According to Riedl (1956) the number of structural units and the position of the methoxy groups are slightly different.



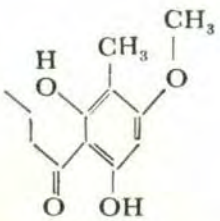
Filixic acid



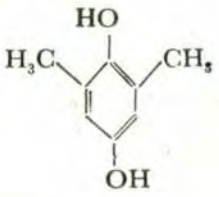
Aspidin



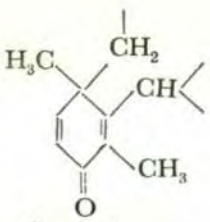
Filicinic acid



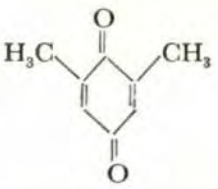
Aspidinol



m-Xylohydroquinone



Santonin
(Ring A)



(Oxidized form of
m-xylohydroquinone)

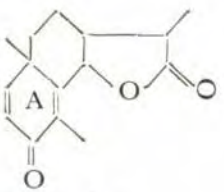
These or their degradation products contain structural moieties similar to that of rottlerin as mono- or poly-units conjugated through formaldehyde residues (methylene bridges). The total extract of the male fern was to be used in the screening programme as all of its constituents have been considered to have anthelmintic activity.

The next compound chosen in this series was santonin, the active principle of *Artemisia maritima*. It is a sesquiterpenoidal lactone containing an α-β-unsaturated ketone and an angular methyl group. Its potent anthelmintic activity has been attributed to a combination of these structural features (Baldwin, 1948; Nakabayashi, 1954). Further, its ring A closely resembled filicinic acid in chemical structure and it also possessed, like kousso and aspidium, a specific action against nematodes.

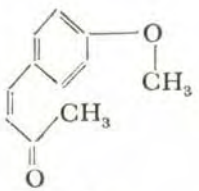
A careful analysis of the structures of rottlerin, kosotoxins, filicinic acid and santonin reveals the existence of a structural similarity, in the form of an activated carbonyl (or semiquinone) either as such or in their active

metabolite forms. In this manner these can also be correlated to the oxidised from of Sanyal's m-xylohydroquinone and its analogues (Sanyal, 1952, 1957).

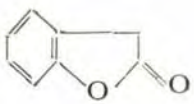
Three synthetic analogues of santonin, S_1 (p-methoxybenzylidene acetone), S_2 (β-γ-phenylene-butyrolactone, 2-coumaranone) and S_3 (α-β-phenylene-butyrolactone, 1-isocoumaranone or phthalide) were also selected on the basis of their known anthelmintic activity (Baldwin, 1948 ; Nakabayashi, 1954). S_1 retained the α-β- unsaturated carbonyl- and S_2 and S_3 the lactonic-grouping of santonin.



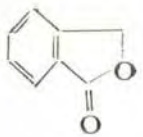
Santonin



S_1



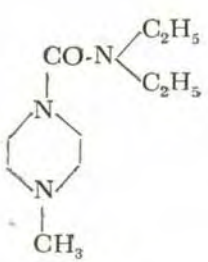
S_2



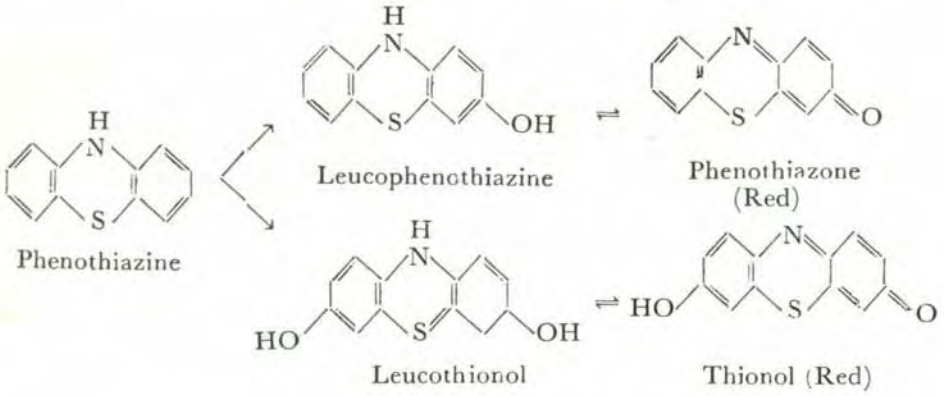
S_3

Besides, three other synthetic anthelmintics, piperazine, hetrazan and phenothiazine, were also selected for screening their antifertility effect. Piperazine and hetrazan are not only effective against nematodes but are also powerful filaricides. Phenothiazine is valuable in nematode infections of sheep and cattle. Its redox system could conceivably interfere with essential enzymatic processes in the worm (Collier *et al.*, 1942, 1952 and 1953 ; Davey and Innes, 1942). It probably brings about paralysis of the muscular system of helminths and causes failure in their reproductive system (Watkins, 1958). It has also been shown to reduce the conception rate in sheep (Blackwell and Allen, 1955). As N-substitution, which blocks its redox system, is known to decrease its anthelmintic activity, other phenothiazines like chlorpromazine or its analogues were not included although chlorpromazine has been reported to inhibit ovulation in the rat (Barraclough and Sawyer, 1957).

Piperazine



Hetrazan



Pappaya (seeds) was tested as a representative of an entirely different class of anthelmintics. It may probably belong to the "proteolytic enzyme" group. It is known that gastro-intestinal helminths are able to withstand the action of digestive enzymes. Ascarids, for instance, produce an antienzyme, ascarase, which has an anti-trypsin and anti-pepsin activity, thus protecting the helminth cuticle from damage (Watkins, 1958). However, a few proteolytic enzymes from plant products such as ficin (Berrio, 1911), raigan (McOwen *et al.*, 1953), bromelin (Watkins, 1958) and papain (Hassler, 1928) are known to be effective in digesting the parasites.

METHODS

Effect of the drugs on the breeding behaviour of mice —Albino mice, of either sex and weighing 18-25 g, maintained in our laboratory at 23° throughout the year and on a standard diet reported earlier (Gujral *et al.*, 1960a), were used in all experiments. The animals were selected, divided into batches and the drug was administered as already described (Gujral *et al.*, 1960b). In the preliminary experiments 10 females constituted a group and the female-male ratio of 5 : 1 was maintained. However, in some experiments 6 females were used with a female-male ratio of 3 : 1. Drugs showing a significant antifertility effect were repeated using 20 females in a group and a female-male ratio of 2 : 1. In the latter experiments, the doses used were increased or decreased depending upon the toxicity.

Each drug was suspended in gum acacia and was administered by mouth using a blunt, wide-bore injection needle as the feeding cannula. The drugs were administered once daily, a few hours after the usual quota of food was served in order to avoid interference with food intake. The dose (calculated from its usual therapeutic dose) and duration of treatment of each drug are shown in Tables I and II. The per cent infertility in one group was statistically compared with that in the other by using the formula:

TABLE I

Effect of some anthelmintics on the fertility of mice

Drug	Dose (mg/100 g)	Treated females						Normal females					
		Treated males			Normal males			Treated males			Normal males (control)		
		No. of females	No. of infertile females	% Infertile females	No. of females	No. of Infertile females	% Infertile females	No. of females	No. of infertile females	% Infertile females	No. of females	No. of infertile females	% Infertile females
Kousso	50	—	—	—	6 (2)	3	75	—	—	—	6 (1)	0	0
Aspidium	40	—	—	—	6 (2)	2	50	—	—	—	6 (1)	0	0
Santonin	3	—	—	—	6 (1)	3	60	—	—	—	6 (1)	0	0
Do	4	10 (2)	8	100	10 (2)	7	87	10 (1)		22	10	1	10
Do	6	20 (2)	18	100	20	20	100	20	4	20	20	4	20
S_1	4	10	3	30	10 (1)	2	22	10 (2)	1	12	10	2	20
S_2	4	10	3	30	10 (3)	3	43	10	2	20	10	2	20
S_2	4	10	7	70	10	8	80	10	5	50	10	2	20
S_3	6	20	8	40	20	7	35	20	5	25	20	2	10

Infertile females were those which had sterile mating, abortion, resorption, still-births or pseudo-pregnancies. The figures in brackets represent the number of females that died before mating.

S E of the difference between the two percentages $= \sqrt{\frac{p_1 \times q_1}{n_1} + \frac{p_2 \times q_2}{n_2}}$

(Robson and Keele, 1950).

Effect of the drugs on the oestrus cycle of mice was determined using the method reported by Gujral *et al.* (1960a) and that on mating by noting the interval between the day when cohabitation was started and the day of the first mating. The approximate lethal dose was determined by the method of Smith (1950).

RESULTS AND DISCUSSION

Table I gives a summary of the effect of kousso, aspidium, santonin and its three analogues on the breeding behaviour (fertility) of mice. Kousso and aspidium, which closely resembled rottlerin in their chemical structure, produced significant antifertility effect. As these were toxic they did not warrant further attention. Isorottlerin, which also closely resembled rottlerin both in chemical structure and anthelmintic properties was, however, found to possess only a slight antifertility effect (Gujral *et al.*, 1960b). Three doses of santonin were tried. The lowest dose, 3 mg/100 g daily for 10 days, produced infertility in 60 per cent of animals. A dose of 4 mg/100 g daily for 15 days before and 15 days during mating rendered 87 per cent of the mice infertile, and a dose of 6 mg/100 g daily for the same period produced sterility in 60 per cent and infertility in 100 per cent of the animals. With the dosage of 4 mg/100 g daily, there was only one pregnancy when only females were treated resulting in the delivery of 9 normal young ones. However, the sample was too small to draw any conclusion as to whether santonin had any effect on the foetus. It did not produce abortions. Among the three synthetic analogues of santonin, S_1 and S_2, at a dosage of 4 mg/100 g daily by mouth for 15 days before and 15 days during cohabitation, were devoid of antifertility effect. S_3 at the same dosage, on the other hand, produced infertility in 87 per cent of animals with 5 pseudo-pregnancies (not shown in the Table) when only females were treated and infertility in 50 per cent of animals with 2 pseudo-pregnancies when only males were treated, compared to infertility in 20 per cent and 2 pseudo-pregnancies in control animals. However, a test of significance showed that the infertility due to treatment of males may be a chance occurrence. A dosage of 6 mg/100 g for 30 days, although produced statistically significant antifertility effect, was rather weaker than that with the lower dose. It is well known that pseudo-pregnancy can occur not only due to mating with a sterile male, but also in conditions where matings or males are not involved, i.e. it commonly occurs if a few females are grouped together in one cage or even when placed one to a cage but subjected to routine physical examination such as administration of the drugs or the taking of vaginal smears (Dewar, 1959). In the case of S_3 the pseudo-pregnancies

may be ascribed to the latter unavoidable factors rather than to a decreased fertility of male mice since the occurrence of pseudo-pregnancy was more frequent in the group in which females were treated compared to the group in which males were treated. More experiments are needed to uncover the dose-response relationship of the drug and explain this paradoxical finding. The drug did not produce abortions and had no effect on the litter size, size of the young ones or their viability. The oral approximate lethal dose of this compound in mice was found to be 10 g/kg which is quite high. This apparent non-toxicity may be explained by the slow rate of absorption from the gut as indicated by the long (12 hrs in contrast to santonin where dose was 300 mg/kg with mortality within 2 hrs) time-interval between drug administration and death. Nevertheless, the dose can be safely increased to improve its potency as an antifertility agent. The compound, therefore, deserves further investigation. Until more data are available, it is not possible to class it among antifertility agents.

Table II shows the effect of other anthelmintics. Hetrazan 20 mg/100 g for 10 days produced infertility in 50 per cent of mice while piperazine 100 mg/100 g for 10 days produced infertility in only 16 per cent. Although phenothiazine has been reported to reduce conception rate in sheep (Blackwell and Allen, 1955), it produced infertility in only 16 per cent of mice which was not statistically significant. Pappaya seeds at a dose of 400 mg/100 g were found to be lethal to a large number of mice (about 67 per cent) and the apparent antifertility effect may only be due to the toxicity of the substance.

TABLE II

Effect of anthelmintics on the fertility of mice (treatment was given for 5 days before and 5 days during cohabitation)

Drug	Dose (mg/100 g)	Treated female x normal male			Normal female x normal male		
		No. of females	No. of infertile females	% Infertile females	No. of females	No. of infertile females	% Infertile females
Piperazine	100	6	1	16	6 (1)	0	0
Hetrazan	20	6	3	50	6 (1)	0	0
Phenothiazine	75	6	1	16	6 (1)	0	0
Pappaya seeds	400	6 (4)	2	100	6 (1)	0	0
	150	6 (1)	3	60	6 (1)	0	0

The figures in brackets represent the number of female mice that died before mating.

TABLE III

Effects of santonin, S_1, S_2 and S_3 on the oestrus cycle of mice

Drug	Dose (mg/100 g)	No. of animals	Control period of 15 days			Treated period of 15 days			t	p
			Total No. of cycles	Mean duration of each cycle		Total No. of cycles	Mean duration of each cycle			
				Days	S E		Days	S E		
Santonin	4.0	18	41.0	5.3	±0.30	26.0	8.4	±0 67	4 25	<0.001
S_1	5.0	20	52.5	5.6	±0.25	35.0	7.2	±0.493	2.018	<0.05
S_2	5.0	17	41.0	5.6	±0.31	28.0	6.3	±0.34	1.32	<0.05
S_3	5.0	19	44.0	5.6	±0.405	35.5	6.7	±0.396	1.93	<0.05

In studies on the oestrus cycle of mice, it was observed that santonin significantly prolonged the duration of the oestrus cycle to 8.4 ± 0.67 days ($P < 0.001$) in comparison with 5.3±0.30 days of the control animals (Table III). The duration of the dioestrus phase was also prolonged. Since oestrus is usually followed by ovulation in a few hours, one is tempted to assume that santonin reduced fertility in mice by preventing or delaying ovulation and keeping the animal in hormonal quiescence (dioestrus) for longer than normal. None of its synthetic analogues tested was found to possess this action.

During experiments on the effect of drugs on the breeding behaviour of mice it was observed that santonin delayed the occurrence of matings. The average interval between cohabitation and mating was found to be 7·1 days in contrast to 5.1 days in the control group. Its analogues had no such effect (Table IV). Since mating does not occur unless the female animal is in oestrus, the effect on mating confirms the observations on the oestrus cycle. The inability of S_3 to affect the oestrus cycle or mating may be correlated to its weak antifertility action which, in turn, may be due to its slow and probably irregular rate of absorption from the gut.

TABLE IV

Effect of some anthelmintics on the mating behaviour of mice when females were treated

Drug	Dose (mg/100 g)	Number of females	Number of matings	Days between cohabitation and the first mating	
				Mean	Range
Control	—	20 (1)	21	5.1	1-9
Santonin	6	20 (12)	8	7.1	2-12
S_1	4	10	14	6.0	1-14
S_2	4	8	11	4.9	1-6
S_3	4	10 (2)	10	5.8	3-7

The figures in brackets represent the number of females which did not mate during the cohabitation period of 15 days.

SUMMARY

The antifertility effect of ten anthelmintics in albino mice was studied. Santonin was found to be a potent antifertility agent and its effects, which were mainly on the female, seemed to be due to a delay in oestrus, ovulation and the onset of matings.

Amongst the synthetic analogues of santonin S_1 (p-methoxybenzylidene-acetone) and S_2 (β, γ'-phenylene-butyrolactone, 2-coumaranone) were found to be devoid of antifertility effect but S_3 (α-β-phenylene-butyrolactone, 1-isocoumaranone or phthalide) had a weak but statistically significant antifertility effect on the female mice. However, it had no effect on the matings or oestrus cycle. The low potency was probably due to a slow and irregular absorption from the gut which also explains its very high ALD in mice.

Kousso, aspidium and pappaya seeds also decreased fertility but these were highly toxic. Phenothiazine and piperazine were devoid of antifertility effect but hetrazan produced a significant degree of infertility in female mice.

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