RESEARCH PAPERS

SOME ACTIONS OF EXTRACTS OF GORSE

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Received August 30, 1954

Introduction

Gorse is used in certain parts of Wales as winter feeding for horses but it is stated by farmers who use it that it is "too hot for cattle," and causes abortion or "slipping of the calf." In Yorkshire an infusion of gorse is a traditional remedy for retained placenta in the cow. In 1943, Smith and Wilson[1] reported that an aqueous extract of gorse (*Ulex gallii*) caused contraction of the isolated uterus of guinea-pig, cat and human pregnant uterus. They also observed that the extract contracted the isolated intestine of the guinea-pig but stated that the response was variable and concluded that the intestine was much less sensitive to the extract than was the guinea-pig uterus.

Correia da Silva[2] showed that an extract prepared in the same way from *Ulex europæus* contracted the uterus and in a later publication reported that this action was also observed with extracts of *Ulex micranthus* and *Ulex nanus* (Correia da Silva[3]). He also demonstrated that the extracts caused a decrease followed by an increase in the amplitude of the spontaneous movements of the isolated rabbit intestine but these effects on the intestine were not constant.

We have investigated the action on the isolated uterus and intestine of a variety of extracts prepared from *Ulex gallii* and have found that the extracts also contract the isolated uterus of the rat, rabbit, goat and pregnant cat. Whilst some of the extracts were shown to contract the isolated intestine of the guinea-pig and rabbit, others caused relaxation with abolition of spontaneous movement. It seemed probable, therefore, that more than one active substance was present in the extracts, and the purpose of this communication is to report some of the attempts which we have made to isolate the active substances in the extracts.

Methods

All the extracts of gorse were prepared from a large stock of dried powdered gorse which was obtained from the green terminal shoots of *Ulex gallii*. Non-pregnant guinea-pig and rabbit uteri were suspended in a 10-ml. bath containing Ringer Solution at 37·5° C. aerated with oxygen. Portions of the upper and lower segments of the rabbit intestine and of the guinea-pig ileum were suspended in a 10-ml. bath of Tyrode Solution at 37·5° C. aerated with oxygen. The extracts were allowed to act for 3 minutes on the uterus and $\frac{1}{2}$ minute on the intestine. Doses of the extracts are expressed in terms of dried gorse. Blood pressure was

recorded in the rat by the method of Landgrebe, Macaulay and Waring[4], modified by Crawford and Outschoorn[5] using urethane as anæsthetic. For the experiments with the perfused rat hind limbs the method described by Burn[6] was used, the fluid output was recorded by a drop recorder incorporating the circuit described by Bernstein and Betts[7].

RESULTS

Our initial experiments were carried out on the concentrated extract "S" prepared according to the method described by Smith and Wilson[1]. Evaporation of this solution produces a brown aromatic gum which is almost completely soluble in methanol and when acetone was added to this, a brown gum separated. When dissolved in water this gum formed a dark brown solution which relaxed the intestine and produced slight contraction of the guinea-pig uterus.

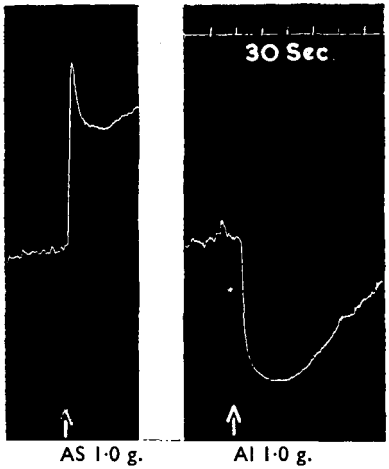

FIG. 1. Isolated guinea-pig intestine, the effect of acetone soluble (AS) and acetone insoluble (AI) fractions of "S." Dose expressed as equivalent of dried gorse.

The methanol acetone solution when evaporated *in vacuo* produced a yellow gum which when dissolved in water contracted the isolated guinea-pig intestine and uterus (Fig. 1). This solution contained about half the uterine contracting activity of "S". These results indicate that extract "S" contains at least two active substances and it was decided to extract dry gorse with organic solvents.

Extraction of gorse with organic solvents

In collaboration with Dr. Jurd of the Department of Organic Chemistry several batches of dried gorse were extracted in turn with light petroleum, diethyl ether, acetone and methanol. Extraction with each solvent was carried out continuously for 21 to 28 days. The results are summarised in Table I. Light petroleum and ether each yielded green extracts which contained on evaporation a large amount of solid material. Aqueous extracts of these residues, however, produced no action on uterus or intestine.

When the acetone extract was concentrated an oily precipitate separated which when dried produced a yellow gum. An aqueous extract of this gum caused relaxation of the intestine but did not contract the uterus. Further concentration of the acetone extract produced another oily precipitate, an aqueous extract of which relaxed the intestine and also contracted the uterus at a concentration five times greater than that of extract "S".

Extraction of gorse with methanol yielded a light yellow gum, an aqueous extract of which contracted the uterus and the intestine in doses equivalent to those of extract "S". Later experiments showed that this gum also contained a substance which relaxed the intestine, for extraction of the gum with butanol produced a butanol-soluble fraction which

TABLE I

EXTRACTION OF GORSE BY ORGANIC SOLVENTS

	Action of extract	
	Uterus	Intestine
Dried gorse extracted with:		
1. Light petroleum ..	Nil	Nil
2. Ether	Nil	Nil
3. Acetone	Nil*	Relaxation
4. Methanol..	Contraction	Contraction

* One fraction of this extract produced slight contraction.

relaxed the intestine but did not contract the uterus. These experiments with organic solvents demonstrate that gorse contains a substance which relaxes the intestine and a fraction which contracts the uterus and the intestine.

Fractionation of aqueous extracts with organic solvents

In view of the separation of the relaxing fraction by acetone and by butanol it was decided to modify the process of extraction originally used by Smith and Wilson[1] by omitting the process of precipitation with basic lead acetate. Several batches of 1 kg. of dried powdered gorse were each boiled with 10 l. of distilled water. After filtration the extract was concentrated *in vacuo* to 500 ml. and to it 1500 ml. of acetone were added. The precipitate which was separated was highly coloured and inactive; the filtrate was evaporated *in vacuo* and yielded a brown gum which was dissolved in methanol and made up to 200 ml. 1800 ml. of acetone was then slowly added to this solution resulting in the precipitation of a brown gum. The filtrate was then evaporated *in vacuo* to dryness and extracted with 200 ml. of water. This aqueous solution was then continuously shaken with an equal volume of butanol for 6 hours. After separation of the butanol layer the aqueous layer was similarly treated on two further occasions with butanol. The combined butanol layers were evaporated *in vacuo* and yielded a brown gum which was soluble in dilute sodium hydroxide from which an aqueous solution was prepared of *p*H 7. This solution is referred to as Bu.S.

The aqueous layer when evaporated *in vacuo* to dryness produced a pale yellow gum which was very soluble in water and in methanol. An aqueous solution of this gum is referred to as BuI and although 20 times more concentrated than extract "S" it was much lighter in colour. Figure 2 shows the activity of these two fractions on the isolated intestine and uterus of the guinea-pig and rabbit. The butanol-soluble fraction (Bu.S) caused relaxation of the longitudinal muscle of the rabbit and guinea-pig intestine but did not contract the isolated rabbit or guinea-pig

uterus. It inhibited contraction of the intestine by acetyl β-methylcholine but not by the butanol-insoluble fraction.

In contrast, the butanol-insoluble fraction (Bu.I) contracted the isolated intestine and uterus of guinea-pig and rabbit. Bu.I is stable in boiling water and does not lose its activity after boiling with dilute hydrochloric acid for one hour. When the butanol-insoluble fraction was dried and

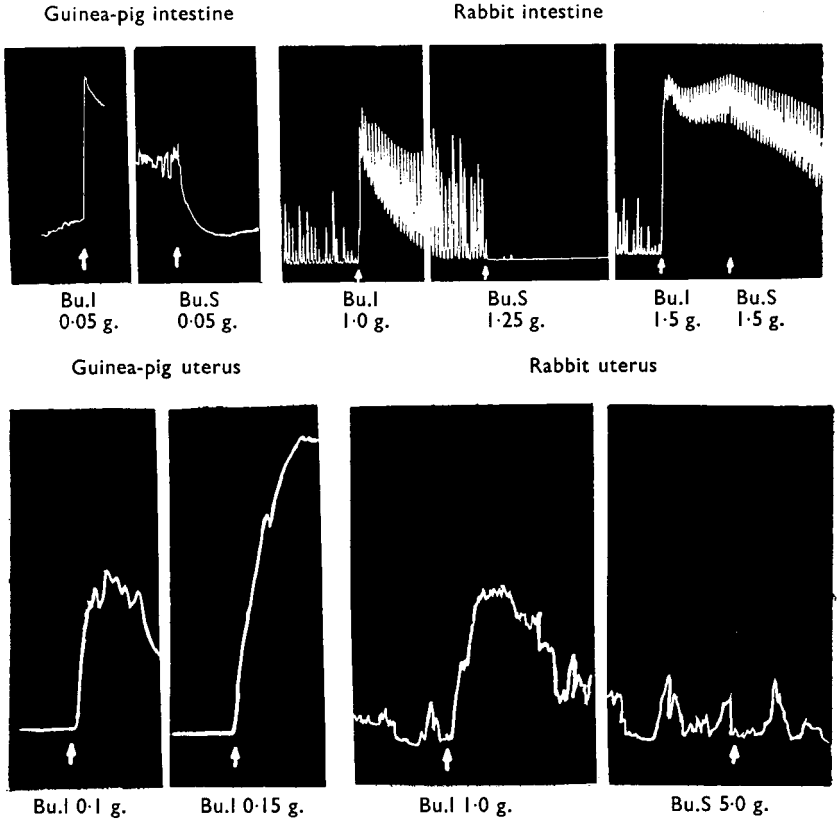

FIG. 2. The effects on the isolated guinea-pig and rabbit intestine and uterus of a butanol insoluble fraction (Bu.I) and a butanol soluble fraction (Bu.S) prepared from an aqueous extract of gorse (*Ulex gallii*). For details of extraction see text.

ignited, a solution of the ash was inactive. Contraction of the intestine by the butanol-insoluble fraction was prevented by atropine (Fig. 3). This effect resembled that of acetylcholine but not that of histamine.

When injected intravenously into rats, the butanol-insoluble fraction caused a fall in blood pressure which was inhibited by atropine (Fig. 4). This fall in blood pressure may be due in part to a peripheral action on blood vessels. Figure 5 shows that addition of the butanol-insoluble fraction to the fluid perfusing the rat hind limbs caused an increase in the outflow, an effect which usually lasted for about 10 minutes.

These results are in substantial agreement with the experiments already described, namely that gorse contains at least two fractions, one of which contracts the intestine and uterus and the other which relaxes the intestine and has no action on the uterus.

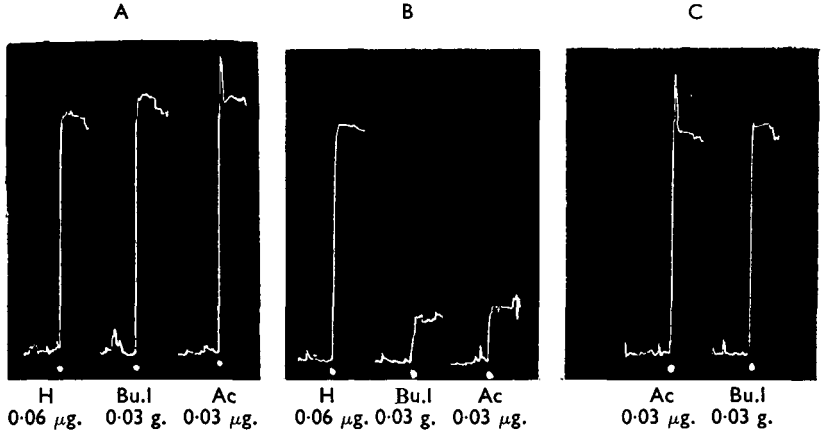

FIG. 3. Isolated guinea-pig intestine, contractions produced by a butanol insoluble fraction of gorse (Bu.I), acetylcholine (Ac) and histamine (H). A and C in the absence of, B in the presence of atropine $\times 10^{-9}$.

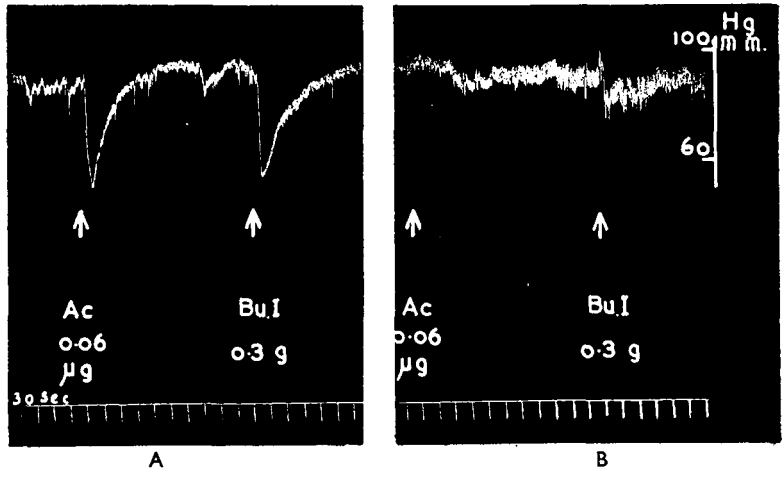

FIG. 4. The effect on the rat arterial blood pressure of a butanol insoluble fraction of gorse (Bu.I) and of acetylcholine (Ac), A before and B after 1 mg. atropine.

Fractionation by chromatography

A number of attempts have been made to separate the two active fractions from gorse extract by means of partition chromatography. Preliminary observations undertaken in conjunction with Dr. Carless of Manchester University showed that extract "S" when examined by paper

chromatography separated into two main zones which in the presence of ultra-violet light were denoted by a green and a blue fluorescence. Attempts were then made to separate these fractions by running extract "S" through a cellulose column containing borate buffer at *p*H 10, using ethyl acetate as developing solvent. In this way it was possible to separate

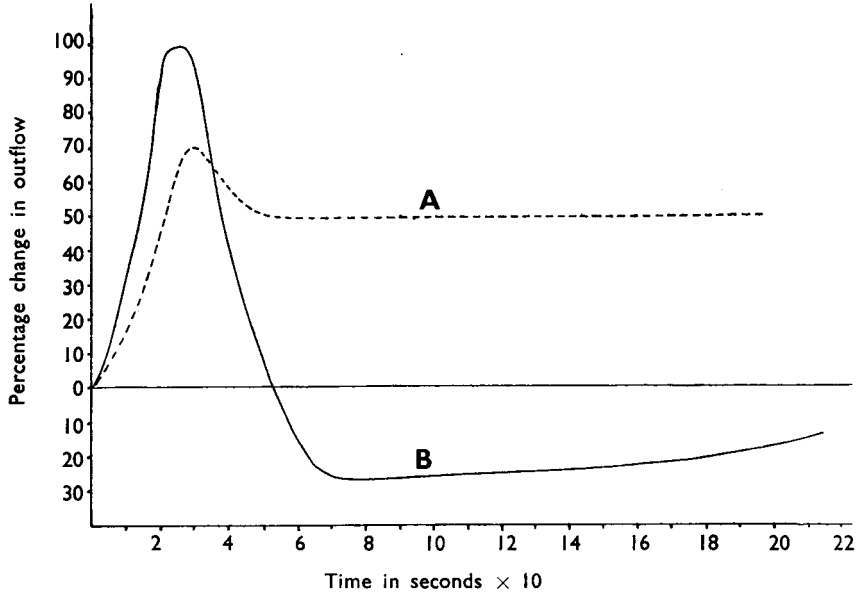

FIG. 5. The effect on the fluid output from the perfused rat hind limbs preparation produced by the addition to the perfusion fluid of: A, 5 g. of butanol-insoluble fraction of gorse (Bu.I); B, 2·5 g. of gorse extract "S."

the green fluorescent material which produced relaxation of intestine but did not contract the uterus.

Separation was also attempted by means of an alumina column using water as the developing solvent. The eluate contained the blue fluorescent material which contracted both the intestine and uterus of the guinea-pig but the green fluorescent fraction was strongly absorbed in the column and was not removed by water. When the oily precipitate obtained from the acetone extraction of gorse was dissolved in methanol and diluted to 100 ml. with acetone and run through an alumina column it was possible to elute all the fraction with relaxing activity by means of methanol but not by acetone. Similar experiments, using methanol extract of gorse resulted in the separation of the uterus and intestine contracting fraction by eluting the alumina column with methanol. In this case only a quarter of the expected activity was separated and after further unsuccessful attempts to increase this yield this process of separation was abandoned. Separation of this fraction by ion exchange resins is at present under investigation.

Discussion

Our results with different extracts of gorse (*Ulex gallii*) have confirmed the observations first reported by Smith and Wilson[1] that gorse contains a substance which contracts the isolated guinea-pig uterus. A substance with a similar type of activity appears also to be present in other species of gorse (Correia da Silva[2,3]). By extracting gorse with a number of organic solvents we have shown that acetone extracts a substance which relaxes the intestine but does not contract the uterus, whilst methanol extracts a substance which contracts both the uterus and the intestine. Separation of these two active fractions has also been obtained by extracting an aqueous extract of gorse with acetone and butanol, whereby the butanol-soluble fraction contains the intestine relaxing substance and the butanol-insoluble fraction contracts the uterus and intestine. It is evident that the aqueous extracts prepared by Smith and Wilson[1] and by Correia da Silva[3] contain a mixture of these two fractions and this probably accounts for the variable effects which they observed on the intestine.

It has not been possible so far to obtain the fractions in a crystalline form. The solutions obtained by the use of organic solvents and by chromatographic separation have yielded gummy residues which though lighter in colour and more active than those obtained by the method used by the previous workers are not sufficiently pure to establish their chemical identity.

A number of reports have been published of the isolation of chemical compounds from gorse, chiefly from *Ulex europæus* but such of these as have been submitted to pharmacological analysis do not appear to possess the actions of the fractions which we have separated. Bridel and Béguin[8] described the isolation from the flowers of *Ulex europæus* of a glycoside ulexoside. This glycoside was also isolated by Paris[9] together with ulexflavone, both of which were reported to be poorly soluble in water and to have little pharmacological activity. No experiments were published on their actions on the isolated uterus or intestine. Two alkaloids have also been separated from *Ulex europæus*, anagyrine by Clemo and Raper[10] and ulexine or cytisine by Gerrard[11,12]. The pharmacology of the former compound has been briefly reported by Gley[13], whilst Dale and Laidlaw[14] have shown that the actions of cytisine closely resemble those of nicotine. We have not attempted to identify the presence of these compounds in our fractions. Further attempts to purify the fractions are in active progress.

Summary

1. Contraction of the isolated guinea-pig uterus by aqueous extracts of gorse (*Ulex gallii*) is confirmed. This effect has also been observed on the isolated uterus of the rat, rabbit, goat and pregnant cat.

2. The fractionation of aqueous extracts of gorse by organic solvents is described and the presence of two active fractions has been demonstrated, one of which contracts the isolated guinea-pig uterus and intestine, the

other relaxes the isolated guinea-pig intestine and does not contract the uterus.

3. The two fractions have also been separated by extraction of gorse with organic solvents, the uterus contracting fraction being extracted by methanol and the intestine-relaxing fraction by acetone. Chromatographic separation of the fractions indicates that the former is associated with a blue fluorescence in ultra-violet light whilst the latter is associated with a green fluorescence.

4. The chemical nature of these fractions has not been identified.

Professor Wilson Smith, University College Hospital Medical School, was actively engaged with one of us (A. W.) in the early stages of this present investigation and it is a pleasure to record his sustained interest in this work. The expenses of the work were in part defrayed by grants from the Medical Research Council.

REFERENCES

1. Smith and Wilson, *Brit. med. J.*, 1943, **2**, 322.
2. Correia da Silva, *Jornal do Medico*, 1950, **16**, 825.
3. Correia da Silva, *An. Faculdade Farm, Porto.*, 1953, **13**, 1.
4. Landgrebe, Macaulay and Waring, *Proc. Roy. Soc. Edin.*, 1946, **62**, 202.
5. Crawford and Outschoorn, *Brit. J. Pharmacol.*, 1951, **6**, 8.
6. Burn, *Practical Pharmacology*, Blackwell Scientific Publications Ltd., Oxford, 1952.
7. Bernstein and Betts, *J. Physiol.*, 1949, **110**, 2P.
8. Bridel and Béguin, *Bull. Soc. Chem. Biol.*, 1926, **8**, 895.
9. Paris, *Ann pharm. franc.*, 1951, **9**, 642.
10. Clemo and Raper, *J. chem. Soc.*, 1935, **1**, 10.
11. Gerrard, *Pharm. J.*, 1886, **17**, 101.
12. Gerrard, *ibid.*, 1886, **17**, 229.
13. Gley, *C. R. Soc. Biol.*, 1892, **40**, 680.
14. Dale and Laidlaw, *J. Pharmacol.*, 1912, **3**, 205.