# AN ACTIVE GLYCOSIDE FROM *ALBIZIA* SPECIES AND ITS ACTION ON ISOLATED UTERUS AND ILEUM

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An active oxytocic principle was extracted from *Albizia gummifera* (Lipton, 1959) by continuous percolation of the dry bark with methanol. Further purification was by dialysis, fractional extraction and precipitation with organic solvents, and by chromatography on alumina. The concentration of activity achieved was 200 times that of the dried bark, measured *in vitro* on strips of uterus. The active principle is a saponin, giving on hydrolysis an unsaturated triterpene acid (probably $C_{30}H_{48}O_5$), and four sugars, glucose, rhamnose, xylose and arabinose. The principle has a powerful action on isolated uterine tissues from different species, resistant to atropine, antihistamines and brief boiling in aqueous or alcoholic solution, but it fails to excite guinea-pig ileum even in high concentrations. The material, for which the name 'albitocin' is proposed, is thought to be potentially valuable in the study of smooth muscle, particularly uterine muscle. The use of these plants by African native doctors, and their possible implication in the high incidence of uterine rupture in Uganda is discussed.

EXTRACTS of members of the plant genus *Albizia* have been used to accelerate labour and procure abortion in East African women. (Lipton, 1959). These women use native medicines, even when in hospital, in an attempt to accelerate birth at or near term, and the excessively high incidence of uterine rupture in Uganda has been attributed in part to powerful uterine spasmogens in these medicines (Rendle-Short, 1960). Cold aqueous extracts of the bark of *Albizia gummifera* (Gmel.) C. A. Smith var. *gummifera* were previously reported to produce powerful contractions in strips from the gravid uteri of mice, rats, guinea-pigs, sheep, cows and man (Lipton, 1959). The extraction and partial characterisation of the active material, and its actions on smooth muscle preparations is now described.

## EXPERIMENTAL METHODS

### *Isolation of Active Principle*

Ground bark (60–80 mesh) (11·36 kg.) was defatted with light petroleum (b.p. 40–60°), then extracted with methanol in a soxhlet-type apparatus until exhausted. The extract, concentrated to 1·5 litres, was then completely precipitated by the addition of acetone. Filtered and dried, this crude solid weighed 661 g. It was dialysed in water (5 litres) for 60 hr. and the residual aqueous liquors treated with charcoal (50 g.). Concentration to 500 ml. *in vacuo* below 35° and finally freeze-drying gave 205 g. of light tan coloured amorphous material. That portion soluble in methanol was again reprecipitated with acetone and the resulting solid in 50 per cent aqueous ethanol passed through a column of deactivated alumina, elution

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being carried out with the same solvent. Freeze-drying yielded a colourless amorphous Fraction A, melting point 220–225° (softening at 205–210° $[\alpha]_{5461}^{22}$ −23·8° [*c*, 1 in MeOH]). This fraction has pharmacological activity about 200 times that of the original dry bark, and contained more than 80 per cent of all the activity. It was found that concentrations of 4–5 μg./ml. in an isolated organ bath gave approximately equivalent responses to 1·0 milliunit/ml. of Pitocin (Parke, Davis & Co.) with the gravid guinea-pig uterus preparation.

The Fraction A, which has so far not been crystallised, was highly soluble in water, giving a copious stable froth. It was insoluble in ether, acetone and benzene, very sparingly soluble in cold ethanol but readily soluble in methanol. It was precipitated from aqueous solution with basic lead acetate but not by neutral lead acetate and from methanol solution by ethanolic potassium hydroxide or baryta. Precipitates were not obtained with the usual alkaloid reagents and elementary analysis showed the absence of nitrogen and sulphur. Reactions with Fehling's, Barfoed's and Benedict's solutions were negative but a positive Molisch test was given. The Liebermann-Burchardt reaction gave a purple-blue colour, and stannic chloride in thionyl chloride, a blue colour. These tests suggested a saponin, probably triterpenoid in nature. Further confirmation of the presence of saponin was found in that an emulsion of olive-oil in water was stabilised indefinitely by solutions of concentrations greater than 50 μg./ml., while fresh rabbit's blood diluted 1 : 100 with isotonic saline was completely haemolysed *in vitro* but not *in vivo*, by concentrations greater than 10 μg./ml. (Lipton, 1960).

Saponins have been found previously in *Albizia* spp. and a number have been isolated and characterised (Tschirch, 1925; Peyer and Liebisch, 1928; Watt and Breyer-Brandwijk, 1929; Sannil, Lapin and Varshney, 1957; Tschesche and Fortsmann, 1957; Barua and Raman, 1958; Farooq, Varshney and Hasan, 1959).

We also found oxytocic activity in aqueous extracts of three other species of *Albizia*, *A. grandibracteata* (Taub.), *A. chinensis* (Osbeck) Merrill, and *A. isenbergiana* (A. Rich) Fourn., but aqueous extracts of *A. coriaria* Welw. ex. Oliv., *A. schimperiana* (Oliv.) var. *tephrocalyx* (Bren.), *A. ferruginea* (Benth.) and *A. zygia* (DC.) Macbr. gave no responses in doses up to 100 times those required for uterine contractions with *A. gummifera* extracts.

Some preliminary work has been done on the nature of the saponin in Fraction A. Brief boiling in neutral, acid or alkaline solution did not measurably affect the action on the uterus, but prolonged boiling, particularly in acid solution, resulted in a steady decline of activity.

Hydrolysis of Fraction A with dilute aqueous acid gave an insoluble prosapogenin and a mixture of sugars which were characterised by paper chromatography (Whatman No. 1 paper; isopropanol: water, 6:4) as arabinose, rhamnose, xylose and a trace of glucose. Further hydrolysis of the prosapogenin with aqueous ethanolic sulphuric acid gave a crude sapogenin and glucose. The saponin was hydrolysed directly to the sapogenin and the four sugars with aqueous ethanolic sulphuric acid.

The sapogenin was purified by conversion to its insoluble potassium salt, which was converted to the free acid and treated with diazomethane and then acetic anhydride in pyridine. Chromatography on alumina gave as the main fraction a crystalline solid, melting point 170–171°$[\alpha]_D^{20} = +76{\cdot}6°$ (*c*, 0·1 in $CHCl_3$) the analysis of which corresponds to the methyl ester triacetate of the triterpene acid $C_{30}H_{48}O_5$. In spite of repeated attempts constant acetyl values could not be obtained. (Found: C, 70·7; H, 9·3 per cent. $C_{37}H_{56}O_8$ requires C, 70·7; H, 8·9 per cent). Prolonged alkaline hydrolysis gave the free acid m.p. 304–305° (decomp.) $[\alpha]_D^{20} = +79{\cdot}4°$ (*c*, 0·2 in MeOH). (Found: C, 74·3; H, 9·7 per cent. $C_{30}H_{48}O_5$ requires C, 73·7; H, 9·9 per cent).

*Assay Methods*

Isolated gravid rodent uteri were 5–10 times more sensitive than non-gravid preparations. It was found essential to increase the potassium and decrease the magnesium content of Dale's solution (Dale and Laidlaw, 1912) to ensure low spontaneous activity, good sensitivity to Fraction A and good survival of the gravid guinea-pig uterus. The composition of the 'G.U.' solution is given in Table I together with those of other media used.

TABLE I

THE BATH MEDIA USED

(Salts in g./litre distilled water.)

| | G.U. solution | 1953 B.P. solution | De Jalon's solution | Rat tyrode | Dale's solution |
|---|---|---|---|---|---|
| Sodium chloride .. .. | 9·0 | 9·0 | 9·0 | 9·0 | 9·0 |
| Potassium chloride .. .. | 0·25 | 0·42 | 0·42 | 0·42 | 0·20 |
| Calcium chloride .. .. | 0·20 | 0·24 | 0·06 | 0·06 | 0·20 |
| Magnesium chloride .. | 0·05 | 0·0025 | — | 0·0025 | 0·10 |
| Sodium bicarbonate .. .. | 1·00 | 0·50 | 0·50 | 0·50 | 1·00 |
| Sodium acid phosphate .. | 0·05 | — | — | — | 0·05 |
| Dextrose .. .. .. | 0·50 | 0·50 | 0·50 | 0·25 | 1·00 |

An automatic apparatus for testing preparations *in vitro* was used, based on earlier designs, which ensured absolute uniformity in dose volume, bath volume, time intervals between doses, and time of exposure to doses (Schild, 1954; Lock, 1961). Recording was by Schild's method (1946).

The required bath solution was freshly prepared each day from stock solutions of the constituent salts (Table I), except for the dextrose and bicarbonate which were kept dry in sealed test tubes and added just before use.

Drugs were dissolved in the appropriate bath medium and checked for neutrality with universal indicator. Doses were warmed to bath temperature before delivery. Washing was by the overflow method to avoid tissue stimulation by stretching and cooling.

*Rodent uteri.* These were prepared by the standard methods (Dale and Laidlaw, 1912; Holton, 1948; British Pharmacopoeia, 1953; De Jalon, Bayo and de Jalon, 1954) (see Table I). The mouse uteri were suspended in Dale's solution or Rat Tyrode (Table I) with a lever load of about 0·3 g. Strips were obtained from 17 non-gravid guinea-pig uteri, and from

43 gravid guinea-pig uteri in which foetuses varied from recent implantations up to a crown to rump length of 5 cm., and were used for quantitative examination of the various plant extracts. A further 16 gravid uterine strips were obtained from uteri in which the foetuses were of more than 5 cm. crown-rump length, but because of excessive spontaneous activity, or because the slope of the dose-response curve was too steep, only 7 of these were completed as quantitatively successful assays (Lipton, 1960). Nineteen dioestrus rat uteri and 10 gravid rat uterine strips were used, but the latter always showed vigorous spontaneous activity and were of value only for qualitative comparison. Four non-gravid and two gravid mouse uteri were used.

*Ungulate uteri.* These were obtained from freshly slaughtered animals; thin strips from the myometrium were immersed in aerated Dale's or G.U. Solution (Table I) at 4°. By replacing the solution about every half hr. the strips could be preserved for several hr. without serious deterioration.

*Human uteri.* These were obtained as described by Robson (1933), and stored at 4° in aerated Dale's solution or G.U. solution. These preparations were at first relatively insensitive, but after remaining in the bath for 30–60 min. with frequent changes of solution and vigorous aeration to remove volatile anaesthetic maximal activity was restored. Robson (1933) and Scott Russell (1943) also observed that the human uterine strips they used did not immediately exhibit maximal activity.

*Guinea-pig ileum.* Seven of the non-gravid females (weighing 400–600 g.) used for uterine preparations also had short lengths removed from the ileum at least 10 cm. from the ileo-caecal junction, for tests in Tyrode's solution with Fraction A, acetylcholine and histamine.

## RESULTS

All the rodent uterus preparations responded vigorously to Fraction A or extracts containing it. Amounts of 0·2 $\mu$g./ml. or more for gravid guinea pig uteri, and 1–5 $\mu$g./ml. for non-gravid guinea-pig uteri, up to 10$\mu$g./ml. for dioestrus virgin rat uteri, and up to 60 $\mu$g./ml. for gravid and non-gravid mouse uteri, were needed for assay. The gravid rat uteri with one exception, showed increased rate and force of spontaneous contractions, with doses of more than 2 $\mu$g./ml. Fraction A. (Fig. 1 a, c, d).

Although there was no significant difference in the sensitivity of six oestrus and eleven dioestrus guinea-pig uteri, the gravid guinea-pig uteri were always several times more sensitive than the non-gravid uteri; the sensitivity and the log dose: response slope of the gravid preparations increasing as gestation advanced (Lipton, 1960) (Fig. 3).

Three cow uteri, all gravid, (foetuses from 9–20 cm. in crown to rump length), three sheep uteri (two gravid and one virgin), and 2 gravid goat uteri all gave vigorous responses to doses of 20–50 $\mu$g./ml. of Fraction A, the sheep uterus showing an increase in force and frequency of spontaneous contractions with 10 $\mu$g./ml. or more Fraction A, while a quiescent strip from the lower segment of a gravid goat uterus required 50 $\mu$g./ml. or more for powerful responses (Fig. 1b).

Strips from 6 full-term gravid human uteri, four obtained at Caesarian

section and two subsequent to uterine rupture showed increased activity in response to doses as low as 0·2 μg./ml. of Fraction A, and strips from 3 non-gravid human uteri showed increased activity in response to doses from 1–4 μg./ml. (Fig. 2).

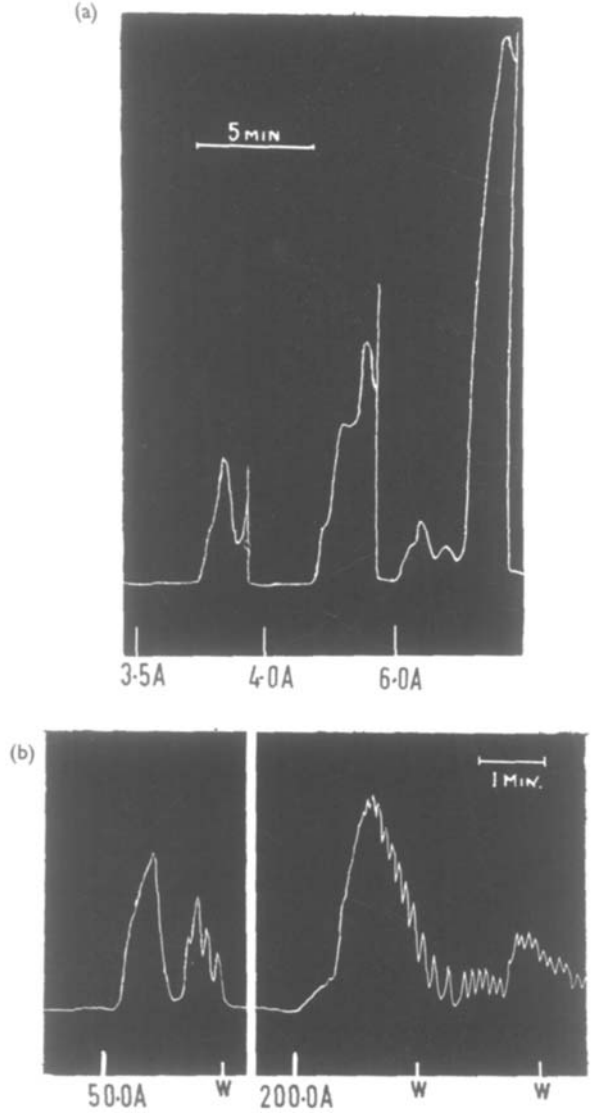


FIG. 1. Responses of various mammalian preparations *in vitro* to extracts of plants of *Albizia* genus. A = μg./ml. albitocin. P = μg./ml. pitocin. w = bath change.
(a) Guinea-pig. Gravid uterus, non-gravid horn, ovarian end, 2 × 2·8 cm. foetuses. Load 1·5 g. G.U. solution* 33° C.
(b) Goat, Gravid uterus, lower segment, 22 cm. foetus, G.U. solution* 36°.
* See Table I for bath media.

All the uterine preparations continued to respond to Fraction A in the presence of sufficient antihistamine (pyranisamine maleate or promethazine hydrochloride) to eliminate the responses to previously adequate doses of

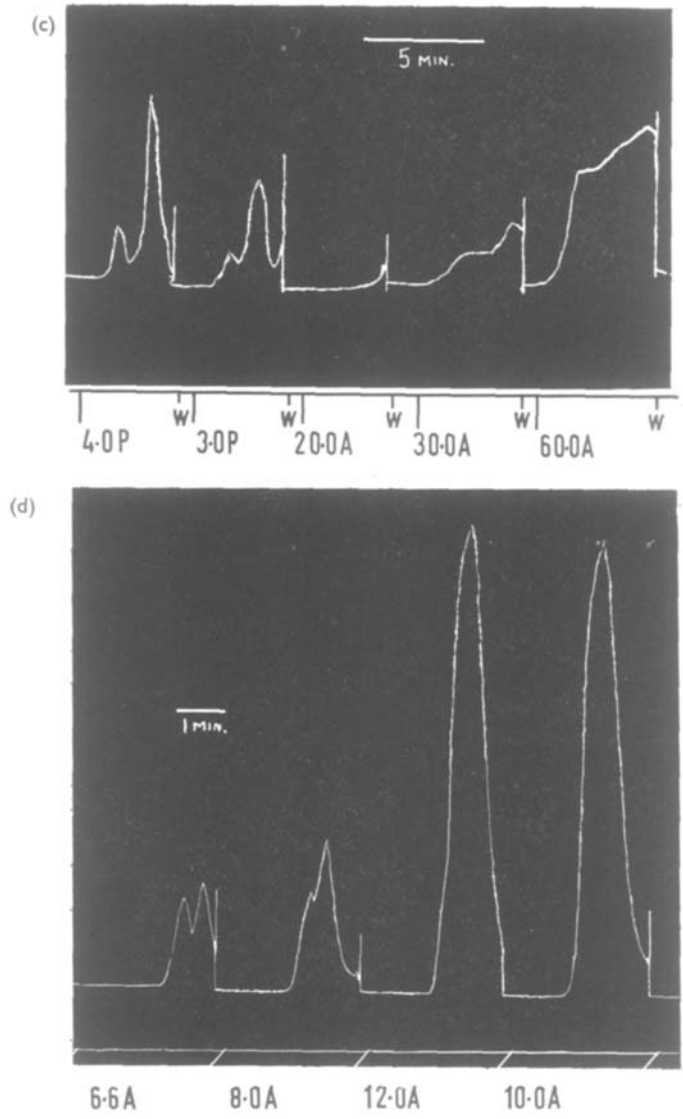


FIG. 1. *continued.*

(*c*) Mouse. Uterus post-partum. Load 0·4 g. Rat tyrode* 32°.
(*d*) Rat. Uterus dioestrus virgin. Load 0·75 g. Rat tyrode* 32°.

histamine, and the rodent uteri continued to respond in the presence of sufficient atropine to prevent responses to acetylcholine.

When chemical studies showed the total absence of nitrogen from Fraction A, similar eliminations of other known alkaloid and polypeptide spasmogenic substances were deemed unnecessary.

*The guinea pig ileum preparation.* All seven preparations, while exhibiting normal responses to histamine (up to 0·2 μg./ml.) and acetylcholine (up to 0·4 μg./ml.) showed no response to doses of Fraction A of up to 500 μg./ml.; that is 100 to 500 times the dose found effective with uterine preparations from the same non-gravid animals (Fig. 4).

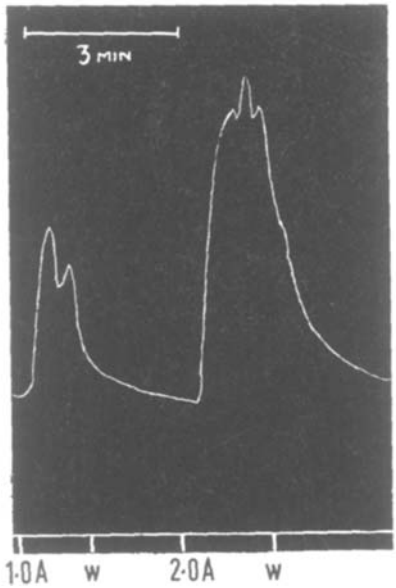


FIG. 2. Human non-gravid uterus response to extracts of plants of *Albizia* genus. Section at hysterectomy for fibroids. Multiparous. Dale's solution* 36·5°. See Fig. 1 for key.

## DISCUSSION

The evidence presented shows that the active principle elicits powerful responses in isolated strips of uterine muscle from different mammalian types, acting to induce contractions when the tissue is quiescent and to increase the frequency and force of contractions when spontaneous activity is present.

The exceptionally high incidence of uterine rupture in Bantu women in Uganda (Rendle-Short, 1960) may be partly explained by the known use of this drug and others with similar properties.

There is a difference in sensitivity to the drug in different species, and also between individuals of the same species, depending on whether the uterus from which the strip was obtained was gravid or not, and also on the stage of gestation. The effect of the stage of gestation on the response of the guinea-pig uterus to this drug (Fig. 3) is reported in detail elsewhere (Lipton, 1960). This evidence is of potential value in the design of experiments aimed at greater comprehension of the changes occurring in

the uterine musculature during gestation. Since it also specifically acts on uterine and not on intestinal smooth muscle this would appear to enhance its value in the study of the important differences which undoubtedly exist between these two kinds of muscle.

Work by Csapo and others (Csapo, 1956) and recent work by the present author supports the idea that oxytocic substances in general act on the initiation of contraction, probably by a change at the membrane of the smooth muscle cell, and since other glycosides, e.g. digitalis glycosides, have been shown to have this type of action on isolated heart muscle (Csapo, 1956), this may also be the mode of action of Fraction A. The fact that it does not affect intestinal smooth muscle activity suggests an important difference in the contraction initiation process between this type of smooth muscle and that of the uterus.

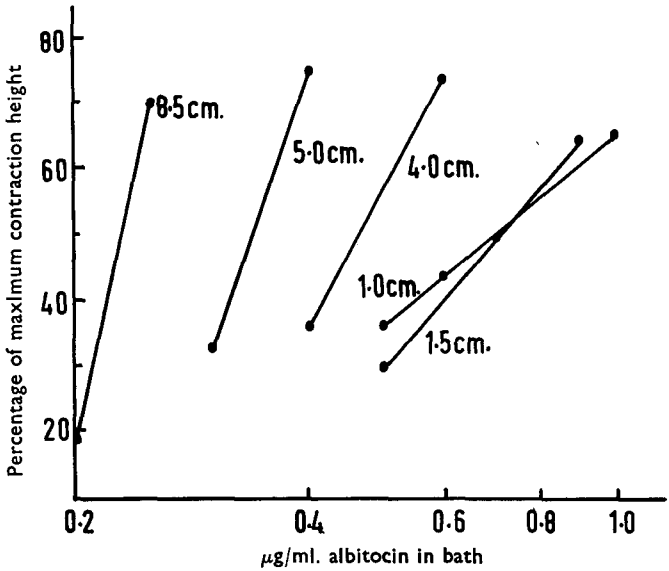


FIG. 3. Log. dose/response relationships of gravid guinea-pig uteri to albitocin *in vitro*, at different stages of gestation. G.U. solution in all cases. Strips from ovarian end, non-placental sites. The response slopes are labelled with the mean crown-rump lengths of the foetuses in the uteri from which the strips were obtained. Each point is the mean of six or more responses.

For convenience in subsequent descriptions of activity we propose the name "albitocin" for the active oxytocic principle in extracts of *A. gummifera*, and our present hypothesis is that it is identical with the Fraction A obtained. This is the first triterpenoid glycoside shown to have this kind of action, to our knowledge, and it would appear to be worth while to examine other compounds of this type for similar activity.

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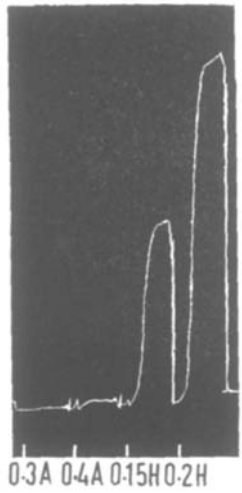


FIG. 4. Response of isolated guinea-pig ileum to albitocin and histamine. The doses of albitocin are about 100 times greater than those required for maximal responses with an *in vitro* uterus preparation from the same animal.
Dale's solution 36°. A = Albitocin in mg./ml. H = Histamine in $\mu$g./ml.

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