# STUDIES ON *LEONTICE LEONTOPETALUM* LINN.

## PART I. THE ISOLATION OF THE CHEMICAL CONSTITUENTS OF *Leontice leontopetalum* AND SOME PRELIMINARY OBSERVATIONS ON THE PHARMACOLOGICAL ACTION OF LEONTICINE AND PETALINE CHLORIDE

BY J. MCSHEFFERTY, P. F. NELSON, J. L. PATERSON, J. B. STENLAKE AND J. P. TODD

*From the School of Pharmacy, The Royal College of Science and Technology, Glasgow*

Received June 28, 1956

OUR attention was first drawn to *Leontice leontopetalum* Linn. in 1951 by Dr. W. M. Ford-Robertson of the Lebanon Hospital for Mental and Nervous Disorders, Asfuriyeh, Beirut, and the present investigation was undertaken at his request. We are indebted to him for supplying us with material, and with descriptions of the use of the drug as a native remedy for the treatment of epilepsy. The following account of its use is based on these descriptions, and on discussions between one of us (P. F. N.) and Dr. A. S. Manugian, of the Lebanon Hospital.

Dr. Manugian has made a special study of epilepsy in Lebanon, and, in the course of this work, found that a preparation of the tuberous roots of a plant which grows in parts of the Lebanon has a reputation for curing epilepsy. According to the descriptions which we received the stem and leaves of the plant are succulent, and the roots resemble soft watery potatoes. The "juice" prepared from the fresh roots, is bitter and poisonous. It is this juice which is used in the treatment, which we may add, is regarded as "kill or cure". We are informed, however, that several cures of confirmed cases of epilepsy have been observed.

For treatment[1] the tuber is dug up after the aerial parts have died down, cut into small pieces, and pounded in a mortar. The "juice" is given in a dose of about one teaspoonful three times daily. This dosage is continued for three days and during this time the patient is reported to be more or less in *status epilepticus*, having convulsions which recur every two or three minutes. The "juice" is prepared from freshly collected roots for each dose. At the end of this initial treatment the patient is given large quantities of an aqueous extract prepared from the marc left after the preparation of the "juice". The marc is pounded in water, and glassfuls of the extract thus prepared are given frequently every day, usually for a period of several months. The native prescribers say that this extract stops the convulsive action of the drug, but Dr. Manugian believes that this is not an essential part of the treatment.

The present investigation was begun with a small batch of the dried roots supplied by Dr. Ford-Robertson. The plant had been tentatively identified by Mr. R. W. Highwood of the British Council in Beirut as *Leontice leontopetalum* Linn. (Berberidaceae). To confirm this identification and to examine the growing plant in its natural habitat one of us

(P. F. N.) was enabled through the generosity of the Cross Trust, to arrange a short visit to Lebanon early in 1954. Specimens of the entire plant in the flowering and fruiting condition were collected and preserved. On comparison with authentic specimens at the British Museum of Natural History the identity of the material was confirmed as *Leontice leontopetalum* Linn. The part of the plant used as a drug is variously described as a root[2], tuberous root[3–5], corm[2], tuberous stem base[6], or tuberous rhizome[7]. Our observations up to the present indicate that it is an intercalary root tuber, but this awaits confirmation from histological investigations now in progress. A description of the plant is given in Part II.

Plants of *Leontice* species which have been investigated chemically are *Leontice eversmanni* Bge.[8–13] and *Leontice alberti*[11]. They are reported to contain a number of alkaloids, of which leontamine ($C_{14}H_{26}N_2$)[8,9] leontidine ($C_{15}H_{20}ON_2$)[8–11], pachycarpine (sparteine)[10,11] lupanine[10,11] leontine ($C_{15}H_{24}ON_2$)[10,13], isoleontine[11] and taspine ($C_{20}H_{19}O_6N$)[11,12] are found in *L. eversmanii*. *L. alberti* has been less extensively investigated, but has been shown to contain methylcytisine, leontine, leontidine, and an unidentified alkaloid[10]. There has been no report of pharmacological activity either of the plants, extracts, or constituent alkaloids. On the other hand, certain of the Berberis and related alkaloids including capnaurine[14], cryptopine[15], protopine[16], corycavine[17], corycavamine[17], corytuberine[17], glaucine[18], dicentrine[19], domesticine[20], pukateine[21], and boldine[22], have been shown to exhibit convulsant activity in experimental animals. And, with the discovery by us of alkaloids in *L. leontopetalum*, it seemed reasonable to suppose that the activity of the drug resided therein. However, as described in the sequel, *L. leontopetalum* resembles the related species *Caulophyllum thalictroides* (Berberidaceae) in possessing a high content of saponin, and in view of a recent report that caulosaponin, the crystalline glycoside from *C. thalictroides* and the corresponding sapogenin (now identified as hederagenin[23]), exhibit oxytocic activity[24], the work of isolating the alkaloids from *L. leontopetalum* was combined with an investigation of the saponin. We have to report, after careful and repeated examination of both caulosaponin and leontosaponin that we have been unable to find any evidence of oxytoxic activity.

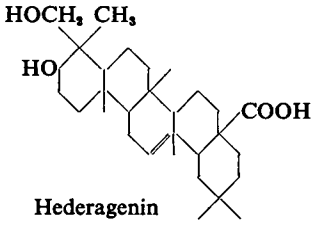
### A. Chemical Examination of L. Leontopetalum

Small quantities of the drug were extracted with various solvents such as water, dilute acid and ethanol, and the resulting solutions examined qualitatively. The aqueous solution was saponaceous, and saponin was shown to be present by (*a*) the voluminous white precipitate with basic lead acetate (glycosides), (*b*) positive Molisch's reaction (carbohydrate), (*c*) reduction of Fehling's solution only after acid hydrolysis. Two alkaloid fractions were shown to be present in the ethanol extract, one extractable from ammoniacal or caustic soda solution with organic solvents. The second non-extractable fraction was freely soluble in water and presumed to be quaternary in type.

## Isolation of Saponins

Several methods of isolating the saponins were examined. Water, although an effective solvent for saponin, was unsuitable, extracting much extraneous material and yielding gummy products. Three saponin fractions were obtained as powderable solids (total yield 17·3 per cent. of the drug), by extracting with absolute ethanol and fractionally precipitating the resulting extract with dry ether. It was essential to use dry solvents, and difficulties were visualised in translating the process to a large scale. Finally a modification of the method used by Power and Salway[25] for the isolation of caulosaponin from the roots of *C. thalictroides* was employed. Three crude saponins were extracted, saponin 1A, IIA, IIIA, which yielded a single pure saponin, leontosaponin. This was obtained as a colourless crystalline solid m.pt. 235–238° C. (decomp.), optically active $[\alpha]_D^{20} + 15{\cdot}1°$, soluble in water to give an almost colourless colloidal solution, slightly acidic in reaction (pH 6·8). Solutions give a copious and persistent froth, and they emulsify oils to form coarse emulsions, which soon break. Solutions readily hæmolyse red blood corpuscles yet appear to be relatively non-toxic *in vivo*; 30 mg. injected into mice (25 g.) showed no apparent effect.

Acetylation of leontosaponin with acetic anhydride and sodium acetate gave leontosaponin acetate. Comparison of the analytical data for leontosaponin and its acetate indicated that the saponin molecule incorporates a sugar chain of about six or more monosaccharide units. Support for this conclusion also comes from the low yield of sapogenin (29 per cent.) which was repeatedly obtained on hydrolysis of leontosaponin with dilute hydrochloric acid (N) in methanol. Hydrolysis proceeded smoothly under these conditions and produced much cleaner products than the more strongly acid conditions recommended by Wall and others[26] for the hydrolysis of steroidal saponins which, however, when adopted led to the isolation of a small quantity of phytosterol. The crystalline



Hederagenin

sapogenin $C_{30}H_{48}O_4$, m.pt. 333–334° C. $[\alpha]_D^{20} + 78°$, was identified as the triperpenoid sapogenin, hederagenin[27–30], by direct comparison with authentic material, and by examination of the diacetate and dibenzoate. The sapogenin gave a yellow colour with tetranitromethane, and showed an ultra-violet absorption maximum (end absorption) at 210 m$\mu$ ($\epsilon$ 2860) as in authentic hederagenin (trisubstituted double bond). Evidence of the carboxyl group was obtained by titration, and by formation of a methyl ester. The proximity of the two hydroxyl groups to each other was demonstrated by the preparation of the ester acetonyl derivative.

The mother liquors from the above hydrolysis, after the isolation of sapogenin and subsequent neutralisation, gave positive reactions for reducing sugars. The presence of glucose was established by specific quantitative tests and the formation of glucosazone and glucose-*NN*-diphenylhydrazone. The solution also gave positive reactions in tests characteristic of pentose sugars. Chromatography of these solutions on paper using the solvent system *n*-butanol-ethanol-water[31] gave three spots[32], one due to traces of leontosaponin, the others attributable to glucose and arabinose, the identity of the latter being further confirmed by the preparation of arabinose-*N*-benzyl-*N*-phenylhydrazone.

## Isolation of Alkaloids

Extraction of the alkaloids was severely handicapped by the high concentration of the saponin also present, and for this reason a large number of alternative extraction procedures were examined. One per cent. hydrochloric acid extracted a large proportion of the alkaloids but yielded only gummy products. Precipitation of the alkaloids with potassium mercuric iodide effectively separated them from solutions, but the complex could not be decomposed.

Maceration of the drug with ammonia or aqueous calcium hydroxide (later shown to be unnecessary), and subsequent extraction in turn with boiling 70, 90 per cent. and absolute ethanol, showed that extraction was not complete with dilute ethanol. The chloroform soluble alkaloid fraction from these extracts was gummy and gave a gummy picrate and hydrochloride. The water soluble (quaternary) alkaloid in the residual liquors was precipitated quantitatively with phosphotungstic acid. The precipitate, however, was slimy, extremely difficult to filter, and recovery of the alkaloid was poor. Mercuric chloride was found to be a more suitable precipitant, the complex being filterable and readily decomposed, although the recovered material was still impure.

The pure quaternary alkaloid was finally obtained after extraction of the chloroform-soluble alkaloids by precipitating as reineckate. The crude reineckate so obtained was readily purified by chromatography from acetone on alumina[33] and obtained as a pale pink amorphous solid, $C_{20}H_{22}O_3N$ $[Cr(NH_3)_2(SCN)_4]$. Decomposition of the pure reineckate[34] gave the pure quaternary alkaloid chloride, designated petaline chloride as bright greenish-yellow scales, m.pt. 140–143° C. (decomp.), $[\alpha]_D^{20} + 11{\cdot}3°$. Petaline chloride, so obtained, has been formulated $C_{20}H_{22}O_3NCl,H_2O$, is extremely hygroscopic, readily soluble in water, methanol and ethanol, but insoluble in ether and chloroform. All attempts to obtain the chloride in a crystalline state failed, largely due to the facility with which it absorbs moisture. The formula of petaline was confirmed by conversion to a picrate, $C_{20}H_{22}O_3N,C_6H_2O_7N_3$; a platinichloride $(C_{20}H_{22}O_3NCl)_2PtCl_4$, and the reineckate already described. An iodide and a perchlorate were also prepared but these proved less suitable for characterisation purposes. Zeisal determination on the chloride and picrate indicated the presence of two methoxyl groups.

In a further attempt to purify the chloroform-soluble alkaloids, the use of ion exchange resins was not successful. Success was achieved with the precipitation of a reineckate, which when chromatographed on paper by the method of Milletti and Adembri[35] indicated the presence of two (possibly three) alkaloids, although there was considerable trailing with some of the spots. Recrystallisation from aqueous acetone, followed by chromatography from acetone on alumina, gave a small yield of a bright-red crystalline reineckate which when chromatographed on paper by the above method gave a single compact spot, $R_F = 0{\cdot}626$. Decomposition of the reineckate, however, yielded a pale brown viscous oil which failed to crystallise.

A crystalline fraction was finally isolated from the chloroform-soluble alkaloids by dissolving in acid, extracting from acid solution with ether and chloroform to remove impurities, basifying with ammonia, and then extracting with ether. A small proportion only of the total alkaloid could be extracted in this way, yielding a clear pale brown viscous oil, which partly crystallised from ethanol, to give a colourless crystalline optically inactive alkaloid, leonticine, m.pt. 118–119° C. The latter readily formed a reineckate, m.pt. 218–221° C. (decomp.), apparently identical with that obtained above. Leonticine has been tentatively formulated as $C_{20}H_{25}O_3N$. The oily residue from the ether extraction was distilled under reduced pressure, giving a colourless oily, strongly basic alkaloid, the properties of which closely resembled those reported for leontamine, the oily base from *L. evermannii*[8]. Insufficient material, however, has so far prevented complete characterisation of this fraction.

The ether insoluble fraction of the total chloroform-soluble alkaloids was precipitated as the reineckate. The latter, however, was not chromatographically homogeneous, and has not yet been resolved into its constituent fractions. Moreover, although decomposition of the reineckate gives a clear solution of the hydrochloride, concentration of the solution and extraction of the alkaloids by normal method leads to considerable decomposition. Work is proceeding on the further examination of this fraction.

## Experimental

Melting points are uncorrected. Rotations were determined in 95 per cent. ethanol, unless otherwise stated, in a 1 dm. tube. Ultra-violet absorption spectra were determined in absolute ethanol on a Hilger Uvispek photoelectric spectrophotometer. We are indebted to Mr. W. McCorkindale and Dr. A. C. Syme for the microanalyses and to Mr. W. Gardiner for technical assistance.

*Material.* This was an authentic sample consisting of the root-tubers of *Leontice leontopetalum* Linn. (Berberidaceae), collected in the Bekaa Plain, Lebanon in the spring of 1953, sliced and dried.

### *Isolation of Leontosaponin*

*Method* 1. The tuber (10 g.) in No. 80 powder was exhausted by boiling with water (3 portions each of 300 ml.). The combined extracts were

evaporated (water bath) to a syrup, filtered, and, whilst still hot, mixed, with stirring, with three times its volume of hot ethanol (60 per cent.), and again filtered. The filtrate poured dropwise into twice its volume of ethanol (90 per cent.) gave a flocculant gummy precipitate (0·68 g. after drying *in vacuo*) of crude leontosaponin. Addition of ether to the filtrate caused further precipitation of crude leontosaponin (0·25 g. after drying *in vacuo*).

*Method* 2. The tuber (400 g.) in No. 80 powder was exhausted by boiling with absolute ethanol (2 l.). The ethanol extract was filtered whilst still hot, and diluted by addition of excess dry ether, when crude leontosaponin was obtained as a yellow flocculent precipitate (saponin I), m.pt. 218–226° C. (decomp.) (45·7 g.). A further yield of 8·5 g. (saponin II) was obtained on concentrating the filtrate, and repeating the precipitation with dry ether. Further concentration and precipitation gave a small yield (3·3 g.) of a dark yellowish-brown saponin (saponin III), m.pt. 188–194° C. (decomp.).

*Method* 3. The drug (933 g.) in No. 80 powder was exhausted by boiling with absolute ethanol. The ethanolic extract was filtered whilst still hot, and on cooling deposited crude leontosaponin (70 g.) as a yellow solid m.pt. 217–221° C. (saponin I A). The filtrate evaporated to dryness gave a dark brown gummy residue, which, after the addition of water and steam distillation, was separated as a dark yellow solid (20 g.), m.pt. 212–222° C. (decomp.) (saponin II A). Repeated extraction of the aqueous filtrate with hot amyl alcohol (10 × 50 ml.), and evaporation of the solvent gave a further yield of dark yellowish brown saponin (62·6 g.), m.pt. 188–220° C. (decomp.) (saponin III A).

*Purification of leontosaponin.* Saponin I A was refluxed with absolute ethanol (1100 ml.), the solution filtered from the insoluble residue, and saponin reprecipitated by the addition of excess dry ether. The product was decolourised by triturating repeatedly with cold absolute ethanol, and finally crystallised from absolute ethanol to yield *leontosaponin* as a colourless crystalline solid, m.pt. 236–238° C. (decomp.) after drying *in vacuo* $[\alpha]_D^{20} + 15{\cdot}1°$. (Found: C, 51·9, 51·5; H, 8·1, 8·1 per cent.) The ethanol-insoluble residue, dissolved in methanol, precipitated with ether, and recrystallised from methanol-ether (charcoal) gave leontosaponin, m.pt. 236–238° C. (decomp.). Recrystallisation of saponins II A and III A, from ethanol similarly gave leontosaponin.

*Leontosaponin acetate.* Leontosaponin (0·76 g.) was heated under reflux (water bath) with acetic anhydride (10 ml.) and sodium acetate (0·56 g.) until solution was complete (30 min.) and thereafter for 2 hours. The reaction mixture was cooled, and poured onto crushed ice (2 g.), and mixture cooled in ice for 30 minutes. The solution was neutralised with NaOH (20 per cent.), and the gummy crystalline precipitate extracted with ether. The ethereal solution, dried ($Na_2SO_4$) and evaporated, yielded a pale cream precipitate (0·54 g.), which, recrystallised from ethanol (70 per cent.), gave *leontosaponin acetate* as an almost colourless crystalline solid, m.pt. 155–156° C. (Found: C, 55·76, 55·69; H, 6·7, 6·8 per cent.)

*Hydrolysis of Leontosaponin*

*Method* 1. Leontosaponin (1·0 g.) was refluxed with 50 per cent. aqueous ethanolic hydrochloric acid (40 ml., 4N) and benzene (16 ml., previously equilibrated by shaking with an equal volume of 50 per cent. aqueous ethanol), for four hours. The sapogenin, precipitated as a pale buff solid was refluxed with benzene (150 ml. including the above fraction) and methanolic potassium hydroxide (50 ml.; 20 per cent.) for one hour. The benzene extract was washed with water, dried ($Na_2SO_4$), and evaporated to yield a yellow solid (20 mg.) m.pt. 112–120° C., giving the typical colour reactions of a phytosterol. Dilution of the methanolic potassium hydroxide solution with water (50 ml.) gave a colourless crystalline precipitate (265 mg.; 26·5 per cent.) of potassium leontosapogenin.

*Method* 2. Crude leontosaponin (35 g.) was heated with a mixture of methanol (900 ml.) and dilute hydrochloric acid (190 ml., 3N) under reflux on a water bath for five hours. After dilution with water (200 ml.), methanol was removed by distillation, and the precipitated sapogenin (8·64 g.; 24·7 per cent.) separated by filtration. The product, recrystallised from 80 per cent. ethanol (charcoal), gave *leontosapogenin*, m.pt. 332–333° C. (slight sintering at 319°), $\epsilon$ max. 2860 at 210 m$\mu$, $[\alpha]_D^{20}$ + 79·6° (*c*, 0·10, $[\alpha]_D^{20}$ + 80·4 (*c*, 1·0 in pyridine). Jacobs[29] gives m.pt. 332–334° C., $[\alpha]_D^{20}$ + 81° (*c*, 2·00 g. in pyridine) for hederagenin. Mixed melting point of leontosapogenin with authentic hederagenin 332–333° C. (Found: C, 76·01, 76·3; H, 10·1; 10·5; equiv. 474·1. Calc. for $C_{30}H_{48}O_4$: C, 76·2; H, 10·2 per cent.; equiv. 472·7.) The following yields of sapogenin were obtained in a series of hydroyses:—26·35; 25·2; 27·4; 28·8; 21·6; and 24·5 per cent.

*Leontosapogenin diacetate.* Leontosapogenin (0·47 g.) refluxed with acetic anhydride (5 ml.) for 1 hour gave leontosapogenin diacetate (from 1:1-aqueous methanol), m.pt. 173–174° C. (sintering at 157–158° C.), $[\alpha]_D^{20}$ + 66·4° (C, 2·08). Jacobs[29] gives m.pt. 172–174° C, $[\alpha]_D$ + 64° (*c*, 1·0) for hederagenin diacetate. Mixed m.pt. with hederagenin diacetate, 172–172° C. (Found: C, 73·3; H, 9·6 Calc. for $C_{34}H_{52}O_6$: C, 73·3; H, 9·4 per cent.)

*Leontosapogenin dibenzoate.* Leontosapogenin (2 g.), refluxed with benzoyl chloride (2 ml.) in pyridine (20 ml.) and the mixture poured into aqueous sodium bicarbonate, gave leontosapogenin dibenzoate, m.pt. 290–291° C., $[\alpha]_D^{20}$ + 114·5° (*c*, 0·1 in $CHCl_3$). Jacobs[29] gives for hederagenin dibenzoate, m.pt. 290–291° C. Mixed m.pt. with hederagenin dibenzoate, 290–291° C. (Found: 3, 77·4; H, 8·3. Calc. for $C_{44}H_{56}O_6$, C, 77·6; H, 8·3 per cent.)

*Leontosapogenin methyl ester.* Leontosapogenin (1 g.) was shaken with excess diazomethane in ether. The ether solution on evaporation yielded a residue which on crystallisation from aqueous methanol gave leontosapogenin methyl ester, m.pt. 236–237° C., $[\alpha]_D^{20}$ + 74·5° (*c*, 1·018). Jacobs[29] gives for hederagenin methyl ester, m.pt. 238–240° C., $[\alpha]_D$ + 75° (*c*, 1·0). Mixed melting point with hederagenin methyl ester (m.pt. 236–

237° C.), 236–237° C. (Found: C, 76·1, H, 10·3. Calc. for $C_{31}H_{50}O_4$: C, 76·5; H, 10·4 per cent.)

*Leontosapogenin methyl ester diacetate.* Leontosapogenin methyl ester (0·177 g.) refluxed with acetic anhydride (4 ml.) for 1 hour yielded leontosapogenin methyl ester diacetate as needles, m.pt. 193–194° C., $[\alpha]_D^{20}$ + 63° (c, 0·564). Van der Haar[27] gives for hederagenin methyl ester diacetate, m.pt. 193° C., $[\alpha]_D^{20}$ + 62° (in absolute ethanol). Mixed m.pt. with hederagenin methyl ester diacetate, 192–193° C. (Found: C, 73·8; H, 10·0. Calc. for $C_{35}H_{54}O_6$: C, 73·6 H, 9·5 per cent.)

*Acetonyl leontosapogenin methyl ester.* A solution of leontosapogenin methyl ester (74 mg.) in acetone (3 ml.) with 3 drops of concentrated hydrochloric acid slowly deposited platelets of acetonyl leontosapogenin methyl ester, m.pt. 250–251° C. (from absolute ethanol) alone or mixed with acetonyl hederagenin methyl ester. (Found: C, 77·5; H, 10·3 Calc. for $C_{34}H_{54}O_4$: C, 77·5; H, 10·3 per cent).

*Isolation and identification of sugars formed by hydrolysis of leontosaponin.* The aqueous filtrate remaining after the separation of leontosapogenin (above) was neutralised with ammonia and decolourised by boiling with activated charcoal. The colourless filtrate gave a positive reaction in Molisch's test for carbohydrates, reduced both Fehling's solution and Barfoed's reagent, gave a blue-green colour in Bial's test (conc. hydrochloric acid and ethanolic orcinol-ferric chloride), and a cherry-red colour in the aniline test (glacial acetic acid and aniline), both the latter reactions being indicative of pentose sugars.

The solution (5 ml.) heated with phenylhydrazine hydrochloride (4 g.) and sodium acetate (0·6 g.) in a boiling water bath for 10 minutes deposited a crystalline osazone from hot solution, m.pt. 201–204° C., and crystalline form characteristic of glucosazone. On further heating the reaction mixture gave a crystalline product, showing a variety of crystalline forms, some characteristic of arabinosazone.

The solution (2 ml.) was treated with a small quantity of lead acetate and boiled. Dilute ammonia solution (5 ml.) was added and the solution boiled again, when a salmon-pink precipitate was obtained, indicative of glucose.

The solution was spotted on Whatman No. 1 filter paper and chromatographed using the solvent system *n*-butanol-ethanol-water, and upward development for 15 hours[31]. After drying, the paper was sprayed with 3 per cent. *p*-anisidine hydrochloride[32], and showed three spots against a pink background, (*a*) yellow $R_F$ = 0·434, (*b*) brown-red $R_F$ 0·259 and (*c*) yellowish-brown $R_F$ 0·214. Control experiments with leontosaponin and known sugars gave the following comparative $R_F$ values, leontosaponin, 0·434 (yellow), arabinose, 0·265 (brown-red), and glucose, 0·213 (yellowish-brown).

The crude sugar residue (3·3 g.) obtained by evaporation of the solution from the hydrolysis, was dissolved in 50 per cent. ethanol (14 ml.). *N*-Benzoyl-*N*-phenylhydrazine (4·09 g.) in absolute ethanol (33 ml.) was added, and the mixed solution left for 24 hours, when a crystalline precipitate was obtained of arabinose-*N*-benzyl-*N*-phenylhydrazone, m.pt. 173·5–

174° C. (from 75 per cent. ethanol), undepressed on admixture with authentic arabinose-*N*-benzyl-*N*-phenylhydrazone. (Found: C, 65·6; H, 6·8; N, 8·5. Calc. for $C_{18}H_{22}O_4N_2$: C, 65·4; H, 6·7; N, 8·5 per cent.) The mother liquors were refluxed with solution of formaldehyde (10 ml.; 40 per cent.) on a water bath for one hour, and the oily formaldehyde -*N*-benzyl-*N*-phenylhydrazone extracted with ethyl acetate. The aqueous solution, which gave reactions for hexoses, but not pentoses, was evaporated to dryness. The residue in water (1 ml.) was refluxed with *NN*-diphenylhydrazine (0·66 g.) in ethanol (17 ml.) to yield glucose-*NN*-diphenylhydrazone, m.pt. 159–160·5° C., undepressed on admixture with authentic glucose-*NN*-diphenylhydrazone. (Found: C, 62·6; H, 6·6; N, 8·1. Calc., for $C_{18}H_{22}O_5N_2$: C, 62·4; H, 6·4; N, 8·1 per cent.)

*Isolation of the Alkaloids*

(*a*) The drug (100 g.) in No. 60 powder was exhausted by percolation with 1 per cent. hydrochloric acid (1830 ml.). A portion of the percolate (610 ml.) was made alkaline with ammonia, and completely extracted with chloroform. The combined chloroform extracts were washed with water, dried ($Na_2SO_4$), and evaporated to a dark brown gummy extract (0·213 g.; 0·64 per cent.). A further portion of the percolate (610 ml.) was treated with solution of potassium mercuri-iodide (30 ml.), when the alkaloid complex was precipitated as a dark-brown amorphous solid (0·614 g.). The latter (0·2 g.) was heated on a water bath with saturated aqueous sodium carbonate (30 ml.) for 30 minutes. After filtering, the solution was neutralised with hydrochloric acid, basified with ammonia and extracted with chloroform. The combined chloroform extracts, washed with water, dried ($Na_2SO_4$) and evaporated, yielded a trace of pale brown alkaloid (15 mg.). Similar treatment with 20 per cent. aqueous sodium hydroxide yielded only traces of alkaloid insufficient for further investigation.

(*b*) The drug (5 kg.) was moistened with dilute solution of ammonia, macerated with ethanol for 24 hours, and percolated to exhaustion with ethanol (10·5 l.). The percolate was concentrated to 3 l., cooled, filtered, acidified with dilute sulphuric acid to pH 4·0, filtered, concentrated to remove ethanol and again filtered. The filtrate was made alkaline with ammonia, and extracted completely with chloroform. The combined chloroform extracts were washed with water, dried ($Na_2SO_4$), and evaporated to a dark gummy solid (22·85 g.; 0·46 per cent).

The crude base (6·43 g.) was dissolved in dilute hydrochloric acid, filtered, the solution poured onto a column of Zeo-Karb 225 (30 g.) and the column washed with water until the eluate was free from acid. Dilute hydrochloric acid failed to displace the alkaloids, but elution with concentrated hydrochloric acid (65 ml.) gave a solution, without resolution of the components, which when basified with ammonia and extracted with chloroform gave a dark-brown gummy residue of alkaloids (4·79 g.).

The crude base (54 mg.) suspended in water (3 ml.) and treated with saturated aqueous ammonium reineckate, gave a pale pink amorphous reineckate m.pt. 180–182° C. (decomp.) (from aqueous acetone). The

reineckate, in acetone was spotted on Whatman No. 1 filter paper, and chromatographed using the solvent system pyridine-water (1:4) and upward development for 16 hours[34]. After drying, the papers were sprayed with a solution of potassium bismuth iodide in dilute acid, and showed evidence of three spots with $R_F$ values about 0·92, 0·62, 0·50, though with considerable tailing.

The ammoniacal solution (1 l.) remaining after the isolation of chloroform-soluble alkaloids was acidified to pH 2·0 with hydrochloric acid and treated with saturated aqueous mercuric chloride (2 l.), when the mercuric chloride complex of the quaternary alkaloid was obtained as an amorphous brown precipitate (39·1 g.). The latter was suspended in ethanol (95 per cent.) and hydrogen sulphide passed into the solution. The solution, filtered from mercuric sulphide, and evaporated gave crude petaline chloride as a dark-brown powderable solid (19·85 g.; 0·4 per cent.).

(*c*) The drug (14·106 kg.) in No. 80 powder was completely extracted by boiling with industrial spirit, and the extract concentrated to give a dark brown soft extract (5·4 kg.). 3·5 kg. of the crude extract was treated with dilute sulphuric acid (5 l.), filtered from precipitated saponin, basified with ammonia, and extracted with chloroform. The combined chloroform extracts were washed with water, dried ($Na_2SO_4$), and evaporated to give a dark-brown crude alkaloid (66·5 g.).

The ammoniacal solution remaining after the isolation of chloroform-soluble alkaloids was acidified with dilute sulphuric acid to Congo red, and treated with saturated aqueous ammonium reineckate solution to precipitate crude petaline reineckate (216 g.). The crude reineckate (137·4 g.) was dissolved in dry acetone (1 l.), filtered from the pale brown insoluble residue (saponin, 50 g.) and the solution chromatographed on a column of alumina (1 kg.) previously washed with dry acetone (500 ml.). Development of the chromatogram with dry acetone, yielded after elution of a small yellow band, a solution of the pure reineckate. Careful evaporation of the acetone eluate below 40° C. gave petaline reineckate m.pt. 179–181·5° C. as a pale pink solid, (49·95 g.). (Found: C, 44·77; H, 5·2 equiv. wt., 678·2; 679·5. $C_{20}H_{22}O_3N$ $[Cr(SCN)_4(NH_3)_2]$ requires C, 44·80; H, 4·4 per cent.; equiv. wt., 642·8.) $R_F$ when chromatographed on paper using pyridine-water (1:4), as described above, 0·68. The reineckate, dissolved in acetone (1 l.) was titrated with silver sulphate solution (0·6 per cent. w/v) until no further precipitate of silver reineckate was formed. Sulphate ions were displaced by quantitative precipitation with barium chloride, the solution filtered, the precipitate repeatedly washed with acetone, and the combined filtrate and washings evaporated (below 60°) to yield *petaline chloride*, m.pt. 140–143° C. (decomp.), $[\alpha]_D^{20} + 11·3°$ as pale grenish-yellow scales (26·6 g.; 100 per cent). (Found, C, 63·35; H, 6·8; N, 3·8; OMe, 16·9. $C_{20}H_{22}O_3NCl·H_2O$ requires C, 63·4; H, 6·65; N, 3·9; OMe, 17·2 per cent.)

*Petaline picrate.*—Petaline chloride (481 mg.) in water, treated with saturated aqueous sodium picrate gave *petaline picrate*, m.pt. 165·5–166° C. (decomp.) (from absolute ethanol). (Found: C, 56·43; H, 4·4; OMe 11·4. $C_{26}H_{24}O_{10}N$ requires C, 56·52; H, 4·4 OMe 11·2 per cent.)

*Petaline chloroplatinate.* Petaline chloride (100 mg.) in water (10 ml.) acidified and treated with 5 per cent. aqueous platinic chloride gave *petaline chloroplatinate*, m.pt. 197–198° C. (decomp.) (from absolute ethanol). (Found: C, 44·34; H, 4·1; Pt, 18·18. $(C_{20}H_{22}O_3NCl)_2PtCl_4$ requires C, 45·42; H, 4·2; Pt, 18·47 per cent.)

*Leonticine reineckate.*—The crude chloroform-soluble alkaloid (1·8 g.) in ethanol (100 ml.) treated with saturated aqueous ammonium reineckate, gave a crystalline reineckate m.pt. 218–221° C. (decomp.) after recrystallisation and chromatography from acetone on alumina. $R_F$ when chromatographed on paper using pyridine-water (4:1) as described above 0·625.

*Leonticine and leontamine.*—The crude chloroform-soluble alkaloid (10 g.) was dissolved in dilute sulphuric acid (300 ml.), extracted first with ether (rejected), and then with chloroform (rejected), basified with ammonia and extracted with ether. The combined ethereal extracts were washed with water, dried ($Na_2SO_4$), and evaporated to yield a pale brown viscid oil, which partly crystallised to yield *leonticine* as a colourless needles, m.pt. 118·5–119·5° C. (from 80 per cent. methanol) $[\alpha]_D^{20} + 0°$. (Found: C, 73·34; H, 7·59; N, 4·44, 4·50. $C_{20}H_{25}O_3N$ requires C, 73, 36; H, 7·7; N, 4·27 per cent.) The oily residue was distilled to yield a colourless oily base, b.pt. 155–160° C. (bath)/4 mm., $n_D^{20}$ 1·5115. (Found: C, 74·73; H, 11·4; N, 12·24. Calc. for $C_{14}H_{26}N_2$ C, 75·61; H, 11·8; N, 12·6 per cent.) Orekhov and Konovalova[8] give for leontamine, b.pt. 118–119° C./4 mm., $n_D$ 1·5113.

## B. Preliminary Pharmacological Investigations

A previous examination by Dr. G. Brownlee of King's College, London, of crude base extracts of *Leontice leontopetalum* indicated the presence of pharmacologically active compounds, and prompted the present preliminary investigation of the quaternary and tertiary bases subsequently isolated.

### *Intravenous Administration of Petaline Chloride in Mouse*

Freshly prepared solutions in normal saline were administered to mice of 25 g. body weight. At doses below 0·05 mg. per mouse recovery from injection was immediate and no functional disturbance was observed. Administration of 0·05 mg. per mouse produced symptoms of sedation and slowed respiration; recovery was complete in approximately 3 minutes and the animals showed no subsequent disorder.

In a group of 10 mice receiving 0·075 mg. per mouse there was one immediate death from respiratory failure, the heart continuing to beat for about 45 seconds after the cessation of respiration; two mice recovered after a period of sedation and reduced respiratory activity lasting 4½ minutes; the remaining 7 mice showed acute respiratory distress with irregular shallow respiratory movements together with varying degrees of stimulation of the central nervous system from slight twitchings of the

extremities to co-ordinated clonic spasms. In the latter, recovery was slow but was complete in all instances in 9–10 minutes after which the animals appeared normal.

Administration of a dose of 0·08 mg. per mouse in a group of 8 mice resulted in death from respiratory failure within 30 seconds of injection in 7 mice, with no convulsive activity; the remaining animal recovered slowly and exhibited mild convulsive activity. In all groups receiving doses of 0·1 mg./mouse and above, death from respiratory failure immediately followed injection.

It is concluded that the Mean Lethal Dose of petaline chloride in mouse is approximately 3·1 mg./kg.

*Intravenous Administration of Petaline Chloride in Rabbit*

From a preliminary investigation it was apparent that the rabbit is much more tolerant of petaline chloride than the mouse. Accordingly a dose of 20 mg. was injected intravenously in a female rabbit of 1·802 kg. body weight. This amount produced no apparent effect in the rabbit and after an interval of 5 minutes a further 10 mg. was injected; the rabbit immediately showed respiratory depression accompanied by mild clonic convulsive movements of the limbs. Within 2 minutes both convulsive and respiratory movements ceased though the heart continued to beat for a further 1 minute. The animal did not recover.

A second female rabbit of 2·725 kg. body weight was injected slowly until symptoms of respiratory failure were produced (after injection of 40 mg.). The animal showed convulsive movements of the limbs as in the previous case and about 1 minute after the termination of the injection respiration ceased, the heart continuing to beat. After a period of apnœa lasting 1 minute respiration recommenced spontaneously and the animal recovered rapidly, resuming its normal activity in 7 minutes. After 48 hours the animal was again injected with 40 mg. petaline chloride; on this occasion only slight respiratory depression was noted and after an interval of 5 minutes, a further 5 mg. was administered. The effects previously observed were repeated and, as spontaneous respiratory movements did not recommence, artificial respiration was attempted; by this means the heart was maintained for a period of 3 minutes after which it gradually slowed and stopped.

It is concluded that the effects of petaline chloride are similar in both mouse and rabbit, and though in the latter the Mean Lethal Dose is much greater, being approximately 15·6 mg./kg.

*The Effect of Petaline Chloride on the Frog Rectus Abdominis muscle Preparation*

The prepared muscle was mounted in an isolated organ bath of 5 ml. capacity and maintained at 15° C. In all cases the spasmogen used was 5·0 $\mu$g. of acetylcholine in oxygenated frog Ringer. Between the recording of contractions the muscle was washed with 3 changes of Ringer's fluid

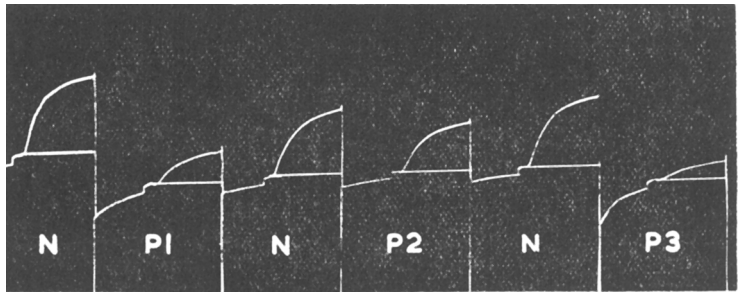
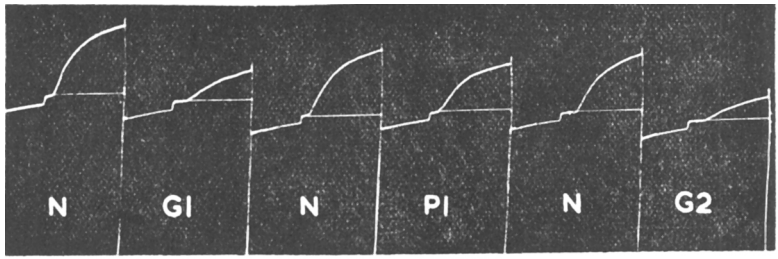



FIG. 1. Response of frog rectus abdominis muscle to acetylcholine 5·0 $\mu$g.
N. Normal contraction
P1. Contraction after application of petaline chloride 100 $\mu$g.
P2. ,, ,, ,, ,, ,, ,, 33 $\mu$g.
P3. ,, ,, ,, ,, ,, ,, 200 $\mu$g.



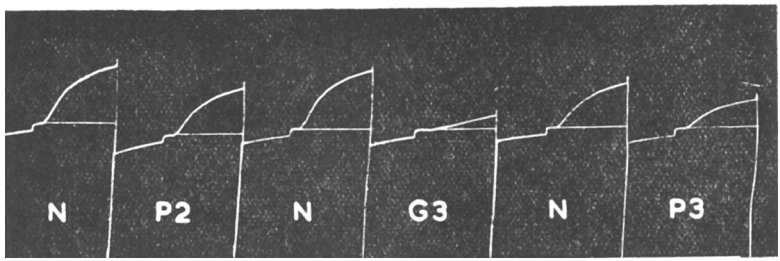




FIG. 2. Response of frog rectus abdominis muscle to acetylcholine 5·0 $\mu$g.
N. Normal contraction.
P1. Contraction after application of petaline chloride 25 $\mu$g.
P2. ,, ,, ,, ,, ,, ,, 33 $\mu$g.
P3. ,, ,, ,, ,, ,, ,, 50 $\mu$g.
G1. Contraction after application of gallamine triethiodide 25 $\mu$g.
G2. ,, ,, ,, ,, ,, 33 $\mu$g.
G3. ,, ,, ,, ,, ,, 50 $\mu$g.

and rested for a period of 4 minutes. The antagonist drugs (petaline chloride and gallamine triethiodide) were added to the bath for a period of 3 minutes before the addition of the acetylcholine. Contraction was recorded for a period of 1 minute.

Typical inhibitory effects of petaline chloride are shown in Figure 1, and compared in Figure 2 with gallamine triethiodide. Mean dose response values to petaline chloride and gallamine triethiodide plotted from a number of observations on the frog rectus muscle are shown in Figure 3. They indicate the relative potency of petaline chloride to gallamine triethiodide to be 0·643 : 1.

Addition of physostigmine (50 $\mu$g.) to the bath antagonised the blocking action of petaline chloride.

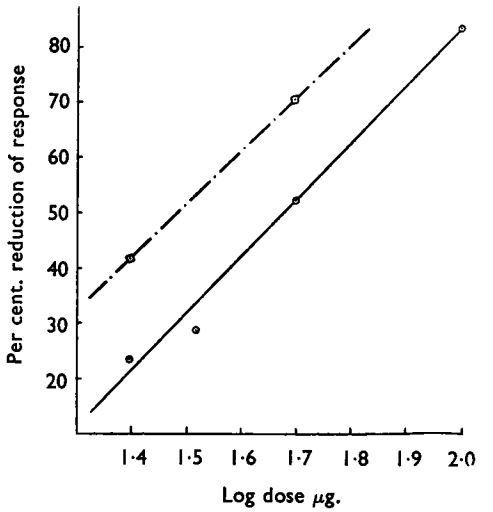



FIG. 3. Inhibition of response of frog rectus abdominis muscle to acetylcholine (5 $\mu$g.).
— · — Gallamine
——— Petaline

*Intravenous Administration of Leonticine in the Mouse*

Solutions of leonticine in 0·05 per cent. aqueous tartaric acid (pH 5·2) were administered to mice of 25 g. body weight.

With doses of 0·5 mg./mouse or less recovery from injection was immediate and no symptoms were observed. Administration of 0·66 mg./mouse produced in all cases evidence of respiratory and cardiac embarrassment; respiration was shallow and slow and the heart rate decreased appreciably. Recovery was protracted, normal activity being resumed after about 15 minutes. In no case was there any convulsive activity though in the majority of individuals there was evidence of increased sensitivity to stimulus, the animal being irritable to touch.

In a group of 8 mice which received 0·7 mg./mouse, 2 instances of complete respiratory failure followed immediately by cardiac failure were recorded; in 4 mice there was slow recovery after an initial period of coma during which the animals were markedly cyanosed. In the remaining 2 mice the period of coma was preceded by relatively powerful clonic spasms of the limbs which lasted for 45 seconds; one of these animals subsequently recovered slowly but in the other the period of coma was terminated by respiratory and cardiac failure.

All groups receiving 0·8 mg./mouse or above showed almost immediate respiratory failure followed in a few seconds by stoppage of the heart. It is concluded that the Mean Lethal Dose of leonticine in mouse is approximately 33·0 mg./kg.

SUMMARY

1. Certain of the chemical constituents of *L. leontopetalum* Linn. have been isolated. The saponin, leontosaponin, yields hederagenin, glucose and arabinose on acid hydrolysis.

2. A small quantity of a crystalline alkaloid, leonticine, and of a colourless oily alkaloid (possibly identical with leontamine) have been obtained from the ether-soluble fraction of the total chloroform-soluble alkaloids.

3. Precipitation of the ammoniacal liquors remaining after the extraction of the chloroform-soluble alkaloids with ammonium reineckate yields a water-soluble alkaloid, petaline chloride, $C_{20}H_{22}O_3NCl,H_2O$.

4. Petaline chloride acts as a central nervous depressant in both mouse and rabbit. It also shows anti-acetylcholine activity on isolated skeletal frog muscle.

5. The effect of leonticine in the mouse appears to be similar to that of petaline chloride, although it is significantly less potent, and bulbar paralysis is preceded by active clonic spasms.

We wish to thank Dr. W. M. Cumming and Mr. D. J. Duff of British Dyewood Co. Ltd. for facilities and help with the large scale extractions. We wish also to thank the Cross Trust and the McCallum Bequest for financial help, and the Pharmaceutical Society of Great Britain for an educational grant to one of us (J. McS.).

REFERENCES

1. Personal communications from Dr. W. M. Ford Robertson and Dr. A. S. Manugian.
2. Post, *Flora of Syria, Palestine and Sinai*, Beirut, p. 28.
3. Loudon, *An Enclyclopædia of Plants*, Longmann, Rees, Orme, Brown and Green, 1829, p. 286.
4. Boissier, *Flora Orientalis*, Vol. I, Georg, Basel and Geneva 1867, p. 99.
5. Bailey, *Standard Cyclopædia of Horticulture*, Vol. 2, MacMillan, New York, 1947, p. 1839.
6. Willis, *Flowering Plants and Ferns*, Cambridge University Press, Cambridge 1919, 378.
7. Bentham and Hooker, *Geneva Plantarum*, Vol. I, Reed and Co., Williams and Norgate, London 1862–1867, pp. 43, 965.
8. Orekhov and Konovalova, *Arch. Pharm., Berl.*, 1932, **270**, 329.
9. Orekhov and Konovalova, *Khim-farm. Prom.*, 1932, **10**, 371.
10. Yunusov and Sorokina, *J. gen. Chem., Moscow*, 1949, **19**, 1955.
11. Platonova, Kuzovkov and Massagetov, *Zhur. Obshchei. Khim.* 1953, **23**, 880.
12. Platonova and Kuzovkov, *ibid.*, 1954, **24**, 2246.
13. Platonova and Kuzovkov, *ibid.*, 1956, **26**, 283.
14. Reynolds and Wand, *Canad. J. Res.*, 1944, **22**, *E*, 64.
15. Heathcote, *J. Pharmacol.*, 1925, **25**, 35.
16. Bohm, *Arch. exp. Path. Pharmak.*, 1940, **195**, 304.
17. Peters, *ibid.*, 1904, **51**, 130.
18. Henry, *The Plant Alkaloids*, 4th Ed., Churchill, London, 1949, p. 313.
19. Iwakawa, *Arch. exp. Path. Pharmak.*, 1911, **64**, 369.
20. Tobitani, *Mitt. med, Ges. Okayama*, 1939, **51**, 1447.
21. Malcolm, quoted by Aston, *J. chem. Soc.*, 1910, **97**, 1381.
22. Raymond-Hamet, *C.R. Soc. Biol., Paris*, 1936, **121**, 431.
23. McShefferty and Stenlake, *J. chem. Soc.*, 1956, 2314.
24. Ferguson and Edwards, *J. Amer. pharm. Ass.*, 1954, **43**, 16.
25. Power and Salway, *J. chem. Soc.*, 1913, 191.
26. Wall, Krider, Rothman and Eddy, *J. biol. Chem.* 1952, **198**, 533.

27. Van der Haar, *Arch. Pharm., Berl.*, 1912, **250**, 424.
28. Van der Haar, *Ber. dtsch. chem. Ges.*, 1921, **54**, 3142.
29. Jacobs, *J. biol. Chem.*, 1925, **64**, 379.
30. Ruzicka and Giaconiello, *Helv. chim. Acta*, 1937, **20**, 299.
31. Bryson and Mitchell, *Nature, Lond.*, 1951, **167**, 864.
32. Hough, Jones and Wadman, *J. chem. Soc.*, 1950, 1702.
33. Karrer and Schmidt, *Helv. chim. Acta*, 1946, **29**, 1853.
34. Dutcher, *J. Amer. chem. Soc.*, 1946, **68**, 419.
35. Milletti and Adembri, *Sperimentale*, 1954, **4** [6], 99.

## DISCUSSION

The paper was presented by MR. J. McSHEFFERTY.

DR. W. MITCHELL (London) said he did not think that alcohol was always the best solvent for drug extraction, because one often extracted too much foreign material. A good general method for alkaloids was to damp the powdered drug with lime and water and then extract with a water-immiscible solvent, such as benzene. He was aware that in the case under discussion petaline would be lost, but other alkaloids might have been extracted in a purer form. Again, the old method of crystallising alkaloids as neutral or acid citrates, tartrates, or oxalates might have been worth while, and fractional extraction of the hydrobromides with chloroform might have produced results. In purifying the saponin he wondered whether the initial purification by alcohol was really necessary, and whether it could have been omitted.

MR. J. J. LEWIS (Glasgow) asked whether the authors had tested the alkaloids for cardiac activity. He also wondered what method they had used for testing for oxytocic activity, and whether they had compared compounds and extracts for anti-convulsive activity against electrically induced convulsions using known anti-convulsive drugs. It would also be interesting to know on what part of the nervous system the drugs were acting. Again, why did the authors use gallamine as a comparison substance and not tubocurarine? When petaline was injected into the rabbit it would be useful to know whether there was any evidence of curareform activity.

MR. N. J. VAN ABBÉ (Loughborough) said it would be interesting to know what sort of cure was claimed for the material.

DR. F. HARTLEY (London) referred to the hydrolysis of leontosaponin and said that the choice of a weaker acid than was often used with steroidal saponins seemed to require a longer time for hydrolysis than four hours in method 1, and five hours in method 2. As the sapogenin yield was in part used as support for the analytical data for the carbohydrate side chain it would be worth checking that eight hours gave no significant addition to the yield.

MR. J. McSHEFFERTY, in reply, said that they had tried moistening the drug with lime and extracting with solvents such as benzene, but this did not extract the alkaloid. Alcohol was the best solvent for the extraction of the alkaloidal fraction. He had endeavoured to separate the tertiary basic fraction by formation of tartrates, but it was not as successful as

the method adopted. The saponin was not very soluble in boiling alcohol, and a large volume had to be used. No test had been made for cardiac activity. The method used for testing oxytocic activity employed the isolated uterus of the guinea-pig. He had no detailed information of the part of the central nervous system on which the alkaloids acted. Both gallamine and tubocurarine were used, and it was found that the former gave the best comparable results with petaline. A number of hydrolyses had been carried out using different conditions and periods of time, and the yields quoted for the particular time were approximately the maximum yields obtainable. The yield was not significantly raised by continuing hydrolysis for another eight hours.

MR. P. F. NELSON, in reply, said that treatment with the drug was regarded as a kill or cure.

DR. J. B. STENLAKE, in reply, said that cardiac activity had not been investigated. Oxytocic activity was measured on the isolated organ and compared with ergometrine.