J. Pharm. Pharmac., 1967, **19**, 87–94 Received August 25, 1966

Chemical and pharmacological observations on some *Hebe* species

(MRS.) JUNE GRADY, the late J. J. LEWIS, M. MARTIN-SMITH, T. C. MUIR AND G. SUBRAMANIAN

Examination of oven-dried leaves and twigs of four species of *Hebe* indigenous to New Zealand—*H. stricta*, *H. odora*, *H. bollonsii* and *H. corriganii*—has revealed D-mannitol and condensed tannins to be major constituents. Identification of some of the minor constituents, namely alkanes, fatty acids and aliphatic alcohols was achieved by means of gas-liquid chromatography. Pharmacological studies with the condensed tannin fractions of *H. stricta* and *H. odora* have indicated that the reputation of *H. stricta* as a constipatory agent is attributable to tannins; this agrees with earlier assumptions.

SOME of the larger-leaved shrubs of the genus *Hebe* (Family Scrophulariaceae)* have long enjoyed a medicinal reputation in New Zealand, the leaves and tender shoots being employed by the Maoris in the treatment of certain skin diseases (Goldie, 1905) and by both Maoris and Europeans for the alleviation of diarrhoea (e.g. Newman, 1879; Kesteven, 1880; Baber, 1886; Bell, 1890; Martindale & Westcott, 1898; Best, 1905; Beattie, 1920; Gardner, 1923; Wall & Cranwell, 1943; Brooker & Cooper, 1961). In the absence of any conclusive pharmacological studies, it has been generally assumed that the ability of these plants to arrest loose bowel movements could be attributed to the astringent action of tannins, although this view has not gone unchallenged (see Gardner, 1924). The total aqueous extracts from several *Hebe* species are without action against certain micro-organisms producing amoebic and bacillary dysentery (Professor L. H. Briggs, personal communication).

We have made a chemical and pharmacological examination of two *Hebe* species—viz. *H. stricta* (Benth.) L. B. Moore (which is one of several species included by earlier writers under the broad name *H. salicifolia* or *Veronica salicifolia*) and *H. odora* (Hook f.) Ckn. (syn. *H. buxifolia*)—in an attempt to isolate and characterize any active principles present. In addition a chemical investigation was made of two other species, *H. bollonsii* and *H. corriganii* (specimens of which were kindly supplied by Miss Lucy B. Moore) and the leaf alkane distribution patterns determined by gas-liquid chromatography as an extension of earlier work (Eglinton, Hamilton & Martin-Smith, 1962a) directed towards a possible chemotaxonomic differentiation of individual members of the genus, which is characterized by extreme ease of hybridization (Cockayne & Allan, 1934).

From the Division of Experimental Pharmacology, Institute of Physiology, The University, Glasgow, W.2, and the Department of Pharmacy, University of Strathclyde, Glasgow, C.1.

* Botanical names as in H. N. Allan, *Flora of New Zealand*, Vol. 1, 1961, Wellington, Government Printer.

Materials and methods

ISOLATION PROCEDURE

The same method was employed for the isolation of the chemical constituents of the four species, *Hebe bollonsii*, *H. corriganii*, *H. odora* and *H. stricta*. Finely ground oven-dried twigs and leaves (80 g) were exhaustively extracted with 95% ethanol (200 ml) in a Soxhlet apparatus and the resulting solution evaporated to dryness under reduced pressure. Standard tests (Paech & Tracey, 1955) showed the absence of alkaloids in the solid residue in each case. Thorough extraction of each residue with redistilled light petroleum (b.p. 40–60°) gave material exhibiting –OH, C=O and $[CH_2]_4$ absorption in the infrared region.

Each residue, which was insoluble in light petroleum, was fractionally crystallized from 95% ethanol giving, as the least soluble fraction, crystalline material of m.p. 163–165°, which was undepressed on admixture with authentic D-mannitol. Infrared spectra (KCl discs) of the natural and authentic specimens were identical. Yields of D-mannitol (based on dry weight of plant material) were *H. stricta*, 0·25%; *H. odora*, 1·0%; *H. corriganii*, 3·7%; *H. bollonsii*, 2·9%. The bitter-tasting glassy residue remaining after removal of the D-mannitol and ethanol showed reactions characteristic of catechin-type condensed tannins. Each residue developed a pink tinge on prolonged exposure to air and was very hygroscopic (cf. Haworth, 1961), and all gave a deep green colouration with ferric chloride and afforded precipitates with gelatin solution, phenazone lead acetate and bromine water. On boiling with dilute hydrochloric acid phlobaphenes were formed, confirming the materials to be condensed tannins. Paper chromatography on Whatman No. 1 sheet and thin-layer chromatography on silica employing butanol-acetone-water showed the presence of several components, but individual components could not be obtained in crystalline form after column chromatography over paper rolls, silica gel, charcoal-kieselguhr or alumina pretreated with acetic acid. Application of standard colour tests (Campbell, 1959) indicated the absence of compounds of the chromone type and application of the Gibbs' test (1927) indicated the absence from the tannin fraction of phenols possessing a free *para* position.

ISOLATION OF ALKANES

In all instances the total alkane fraction, uncontaminated with compounds of other chemical groups, was isolated by the procedure described previously by Eglinton & others (1962a), and then subjected to gas-liquid chromatographic analysis on a 0·5% Apiezon L column. Identification of alkanes in the natural mixture was achieved through appropriate intensification experiments with added n-nonacosane and n-untriacontane followed by a plot of log retention time against carbon atom number. The resulting straight line plot (cf. James, 1960) then permitted identification of the remaining n-alkanes present. Percentages of individual components were obtained through integration of the areas under each peak

on the gas-liquid chromatographic traces of the natural mixtures. Repetition experiments indicated an accuracy of $\pm 2\%$.

ANALYSIS OF FATTY ACIDS AND n-ALKANOLS

In the cases of *H. odora*, *H. corriganii* and *H. bollonsii*, analyses were made of the total fatty acids and n-alkanols present without distinction between those occurring free and those occurring combined as esters. Glycerol was not detected in the products of saponification.

Total light petroleum solubles (1 g) from the particular *Hebe* species under investigation were refluxed for 2 hr in aqueous ethanol (1 : 2, 20 ml) containing sodium hydroxide (3 g). The solution was then evaporated to dryness under reduced pressure and the residue thoroughly extracted with dry ether. The combined ethereal solutions were taken to dryness under reduced pressure and the residue refluxed in acetic anhydride (5 ml) for 4 hr to convert the mixed alcohols into the corresponding acetates which were obtained free from coloured impurities by filtration in ethanol through neutral alumina and then subjected to gas-liquid chromatographic analysis on a 10% polyethyleneglycol adipate column.

Intensification experiments with added n-octyl acetate and n-decyl acetate and plots of log retention time against carbon atom number permitted identification of the individual components.

The ether-insoluble residue resulting from the aqueous ethanolic saponification of the light petroleum extractives from each plant was taken up in water (30 ml), and the solution acidified with dilute hydrochloric acid to liberate the free carboxylic acids from their sodium salts before being exhaustively extracted with ether. After removal of the solvent from the ethereal solution, the residue of mixed carboxylic acids was dissolved in methanol and treated with an excess of an ethereal solution of diazomethane. Removal of solvents under reduced pressure then afforded the methyl esters which were taken up in ethanol, filtered through a column of neutral alumina and subjected to gas-liquid chromatographic analysis on a 10% polyethyleneglycol adipate column.

Intensification experiments with added methyl laurate, methyl palmitate and methyl stearate and plots of log retention time against carbon atom number permitted identification of individual components.

GAS-LIQUID CHROMATOGRAPHY

The instrument employed was a standard Pye Panchromatograph, giving preheating of the argon carrier gas and fitted with standard glass tubes, containing the column packing, of 5 feet in length and internal diameter about $\frac{3}{16}$ inch. The detector was the standard Lovelock argon ionization type, fitted with a ^{90}Sr source and the current from the detector was fed into a Honeywell Brown (Newhouse, Lanarkshire, Scotland) pen recorder with sensitivity 0–10 mV.

Column packings were prepared on the silane-treated support Gas-Chrom Z in the manner described by Bryce, Martin-Smith, Osske, Schreiber & Subramanian (1966).

Pharmacology

In view of the difficulty in securing individual components of the tannin fractions in pure form, the pharmacological examination of *H. odora* and *H. stricta* necessarily had to be made on the crude fractions. Hence, although nitrogenous compounds were established as being absent, tests were included for local anaesthetic action, ability to mimic or antagonize acetylcholine on various preparations, ability to mimic or antagonize adrenaline and ability to antagonize histamine, barium chloride and dimethylphenylpiperazine iodide to ascertain that compounds other than tannins were not contributing to the activity—comparison of the overall results with the known pharmacological properties of tannins (e.g. Ware, 1926) making definite conclusion possible.

CONSTIPATORY EFFECT

The number of stools passed by healthy adult rats fed solutions (2 ml) of the tannin fraction (50 mg/ml) by stomach tube was compared statistically, using Student's *t*-test, with that produced by control animals given equal volumes of water,

GANGLION BLOCKING ACTIVITY

Sympathetic. The effect of previously (30 sec) administered aqueous solution of tannin fraction (10 mg/kg) via the external jugular vein on electrically-induced (12 V, 2 msec, 800/sec) contractions of the cat nictitating membrane elicited by supramaximal stimulation of the preganglionic sympathetic chain was compared with that of hexamethonium bromide (0·25 mg/kg).

Parasympathetic. The effect of adding a solution (0·16 mg/ml) of the tannin fraction 30 sec before the next stimulus to inhibit the peristaltic reflex in the guinea-pig ileum (Trendelenburg's experiment, Feldberg & Lin, 1949) was compared with that of hexamethonium bromide (0·05 mg/ml).

EFFECTS ON NON-VASCULAR INTESTINAL SMOOTH MUSCLE

Guinea-pig ileum. Reproducible submaximal contractions to the agonists acetylcholine chloride (0·1 μg/ml), histamine acid phosphate (0·5 μg/ml), barium chloride (100 μg/ml), and dimethylphenylpiperazine iodide (1·4 μg/ml), each acting for 30 sec were recorded. Solutions of total tannin fraction (0·5–5 mg/ml) were added 30 sec before the next addition of agonist.

Rat uterus. Reproducible submaximal contractions to acetylcholine chloride (0·1 μg/ml) acting for 30 sec were obtained. Solutions of tannin fraction (0·5 and 1·0 mg/ml) were added 30 sec before the next dose of agonist.

Rabbit duodenum. The effect of addition of tannin (0·3 mg/ml) solution on the spontaneous activity of the rabbit duodenum suspended in Locke's solution was observed and compared with that of papaverine hydrochloride (5 μg/ml).

VASCULAR SMOOTH MUSCLE

The isolated rat hindquarters preparation was perfused using the method of Burn (1952). Adrenaline hydrogen tartrate (2·5 μg) was injected into the abdominal aorta until a reproducible response was obtained. A solution of the tannin fraction (10 mg) was injected before the next dose of adrenaline hydrogen tartrate and the effect on the outflow recorded.

THE BLOOD PRESSURE OF THE ANAESTHETIZED CAT

The blood pressure of cats anaesthetized by intraperitoneal injection of sodium pentobarbitone (50–60 mg/kg) was recorded from a common carotid artery. The effect of a solution of tannin fraction (25 mg/kg) injected via the external jugular vein was recorded.

ISOLATED PERFUSED RABBIT HEART (Langendorff's method, 1895)

The effects of a solution of tannin fraction (8 mg) injected into the aorta, on heart rate, coronary flow and amplitude of contraction were noted.

FROG GASTROCNEMIUS MUSCLE-SCIATIC NERVE PREPARATION

The effect on contractions of the gastrocnemius muscle of the frog, elicited by indirect supramaximal electrical stimulation (4 V, 1–2 msec, 12/min) via the sciatic nerve, obtained by addition of a solution of the tannin fraction (2 ml of 50 mg/ml solution) to the bath was noted and compared with the inhibition of twitch height achieved by the addition of procaine hydrochloride (10 mg/ml) under identical conditions.

Results and discussion

The results of the alkane analyses of *H. bollonsii* and *H. corriganii* are summarized in Table 1 and in Fig. 1 which also shows earlier (Eglinton & others, 1962a) results obtained with the twigs and leaves of *H. odora*, *H. parviflora*, *H. diosmifolia* and *H. stricta* for comparison. It is to be noted that none of the species contain more than traces of branched alkanes (none being detected in *H. odora*, *H. corriganii* or *H. bollonsii*). It is of interest that *H. corriganii* and *H. bollonsii* possess very similar alkane distribution patterns, which might imply that the utility of plant alkane analysis as a method of "fingerprinting" individual species for chemotaxonomic and pharmacognostical purposes may prove to be more limited than had been originally hoped (Eglinton & others, 1962a, b, c).

TABLE 1. n-ALKANES FROM DIFFERENT *Hebe* SPECIES*

Plant	Total alkane fraction present (based on dry weight of leaves), %	Number of carbon atoms in alkane, %								
		C_{25}	C_{26}	C_{27}	C_{28}	C_{29}	C_{30}	C_{31}	C_{32}	C_{33}
Hebe corriganii ..	0·054	2	1	14	2	34	2	41	2	2
Hebe bollonsii ..	0·074	3	2	15	2	32	5	32	3	6

* The quantities of the individual alkanes are expressed as a percentage of the total alkanes isolated.

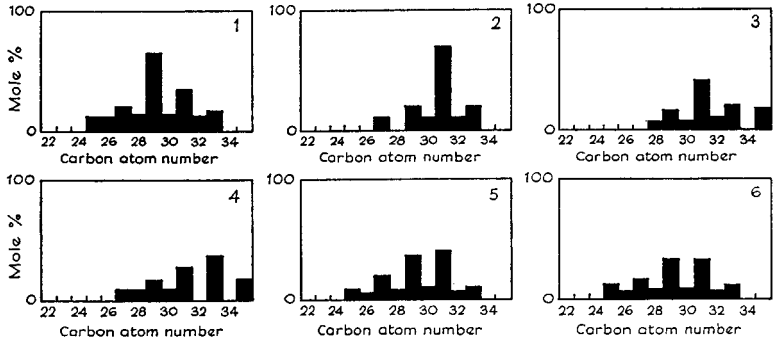

FIG. 1. Distribution in mole % of n-alkanes in six *Hebe* species. Twigs and leaves: 1. *H. odora*, 2. *H. parviflora*, 3. *H. diosmifolia*, 4. *H. stricta*. Leaves: 5. *H. corriganii*, 6. *H. bollonsii*.

The results of the fatty acid and n-alkanol analyses as shown in Tables 2 and 3 reveal little potential application for such analyses in chemotaxonomic differentiation of the genus *Hebe*. The total number of representatives in each series in no case exceeds five, with decan-1-ol the predominant alcohol in all cases and lauric acid the major acid except in *H. odora*.

TABLE 2. FATTY ACIDS FROM DIFFERENT *Hebe* SPECIES*

Plant	Total acid fraction present (based on dry weight of leaves), %	Capric (decanoic), %	Lauric (dodecanoic), %	Myristic (tetradecanoic), %	Palmitic (hexadecanoic), %	Stearic (octadecanoic), %
Hebe odora ..	—	32	28	22	12	8
Hebe corriganii	0·93	26	42	31	11	2
Hebe bollonsii ..	1·12	1	49	29	21	

* The quantities of the individual acids are expressed as a percentage of the total acids isolated.

TABLE 3. ALCOHOLS FROM DIFFERENT *Hebe* SPECIES*

Plant	Total alcohol fraction present (based on dry weight of leaves), %	Octan-1-ol %	Decan-1-ol %	Dodecan-1-ol %	Hexadecan-1-ol %
Hebe odora ..	—	24	46	19	14
Hebe corriganii	0·68	29	43	17	12
Hebe bollonsii ..	0·64	30	48	22	

* The quantities of the individual alcohols are expressed as a percentage of the total alcohols isolated.

Consistent results were obtained on repeat analyses to within $\pm 2\%$. No unsaturated acids could be detected. All peaks on the gas-liquid chromatographic traces obtained with the mixed methyl esters fell on the one straight line when log retention time was plotted against carbon atom

number indicating that all resulted from the one (saturated) homologous series (James, 1960).

The short chain lengths of the acids (C_{10}–C_{18}) and alcohols (C_8–C_{16}) as compared to the chain lengths of the alkanes (C_{25}–C_{33}) are as to be expected in terms of current biogenetic theory in which it is considered (Eglinton & Hamilton, 1963) that at least one route leading to the formation of n-alkanes involves the coupling of two molecules of fatty acid before decarboxylation and reduction to the alkane, much as in the biogenesis of corynomycolic acid (Gastambide-Odier & Lederer, 1959).

In accord with previous observations (Chibnall, Piper, Pollard, Williams & Sahai, 1934; Waldron, Gowers, Chibnall & Piper, 1961; Eglinton & others, 1962b) in all the *Hebe* species, n-alkanes with an odd number of carbon atoms form the major components whilst the acids and alcohol appear restricted only to those with an even number of carbon atoms.

In keeping with the established astringent properties of tannins (Ware, 1926) the crude total tannin fractions from *H. odora* and *H. stricta* were without appreciable activity against acetylcholine or histamine on the guinea-pig ileum, but at a concentration of 0·625 mg/ml solutions of the crude fractions completely inhibited the effect of barium chloride (0·125 mg/ml) thus exhibiting a direct effect upon the intestinal musculature. This was also seen on the rabbit duodenum where an aqueous solution of the tannins (3 mg/ml) produced a slow strong relaxation without inhibiting spontaneous activity—an effect equivalent to that produced by 50 μg of papaverine.

The total crude tannins showed neither sympathetic nor parasympathetic ganglion blocking properties and had no adrenaline-like action on the isolated rabbit heart. Again these results are as to be expected for tannins. The apparent antagonism of the effect of acetylcholine on the rat uterus can also be ascribed to the expected direct action of tannins. Absence of inhibition of the contraction in the frog rectus abdominis muscle induced by acetylcholine and failure to block contraction in the frog gastrocnemius muscle sciatic nerve-preparation indicated the absence of any neuromuscular-blocking activity or local anaesthetic activity in the tannin fractions.

No statistically valid difference between the number of stools passed by healthy rats fed with solutions of the condensed tannins by stomach tube and the number of stools passed by control rats fed equal volumes of water could be discerned, but this test is not necessarily comparable to the loose bowel conditions for which extracts of *H. stricta* are efficacious. A further complication here was introduced by the necessity to oven-dry the plant material for purposes of preservation and transport and this heat process, coupled with the later hot solvent extraction of the ground dried plant, may have induced polymerization of simpler tannins (cf. Haslam, 1966). Indeed there have been conflicting reports over the efficacy of various extracts from *H. stricta* prepared by different methods (Baber, 1886; Bell, 1890; Gardner, 1923) and these would certainly be explicable in terms of the polymerization of the tannins present.

In view of the total pharmacological results, especially the absence of

any effect against nervously controlled intestinal movements and the marked potency against muscle spasm induced by barium chloride solution, the constipatory properties of *H. stricta* and *H. odora* can be explained in terms of their tannin content.

Acknowledgments. One of the authors (M.M-S.) cordially thanks The Royal Society and the Pharmaceutical Society of Great Britain for generous financial assistance towards the collection of plant material. He also thanks Dr. A. T. Johns, Plant Chemistry, Grasslands Division, D.S.I.R., Palmerston North, New Zealand, for the use of drying facilities, The kind co-operation of Miss Lucy B. Moore is gratefully acknowledged.

References

Baber, J. (1886). *Trans. Proc. N.Z. Inst.*, **19**, 319–322.
Beattie, H. (1920). *Ibid.*, **52**, 53–77.
Bell, T. W. (1890). *N.Z. med. J.*, **3**, 129–145.
Best, E. (1905). *J. Polynes. Soc.*, **14**, 1–23.
Brooker, S. G. & Cooper, R. C. (1961). *New Zealand Medicinal Plants*, Handbook of the Auckland War Memorial Museum, pp. 34–35.
Bryce, T. A., Martin-Smith, M., Osske, G., Schreiber, K. & Subramanian, G. (1966). *Tetrahedron*, in the press.
Burn, J. H. (1952). *Practical Pharmacology*, pp. 65–69. Oxford: Blackwell.
Campbell, N. (1959). In *Chemistry of Carbon Compounds*, ed. Rodd, E. H., IVB, pp. 904–906, London: Elsevier.
Chibnall, A. C., Piper, S. H., Pollard, A., Williams, E. F. & Sahai, P. N. (1934). *Biochem. J.*, **28**, 2189–2208.
Cockayne, L. & Allan, H. H. (1934). *Ann. Bot.*, **48**, 1–55.
Eglinton, G. & Hamilton, R. J. (1963). In *Chemical Plant Taxonomy*, ed. Swain, T., pp. 197–217, New York: Academic Press.
Eglinton, G., Hamilton, R. J. & Martin-Smith, M. (1962a). *Phytochemistry*, **1**, 137–145.
Eglinton, G., Gonzalez, A. G., Hamilton, R. J. & Raphael, R. A. (1962b). *Ibid.*, **1**, 89–102.
Eglinton, G., Hamilton, R. J., Raphael, R. A. & Gonzales, A. G. (1962c). *Nature, Lond.*, **193**, 739–742.
Feldberg, W. & Lin, R. C. Y. (1949). *Br. J. Pharmac. Chemother.*, **4**, 33–34.
Gardner, R. (1923). *N.Z. J. Sci. Tech.*, **6**, 147–151.
Gardner, R. (1924). *Ibid.*, **7**, 220–221.
Gastambide-Odier, M. & Lederer, E. (1959). *Nature, Lond.*, **184**, 1563–1564.
Gibbs, H. D. (1927). *J. biol. Chem.*, **72**, 649–664.
Goldie, W. H. (1905). *Trans. Proc. N.Z. Inst.*, **37**, 1–120.
Haslam, E. (1966). *Chemistry of the Vegetable Tannins*, New York: Academic Press.
Haworth, R. D. (1961). *Proc. chem. Soc.*, 401–410.
James, A. T. (1960). In *Methods of Biochemical Analysis*, ed. Glick, D., **8**, pp. 1–59, New York: Interscience.
Kesteven, L. (1880). *Lancet*, **2**, 457.
Langendorff, O. (1895). *Pflügers Arch. ges. Physiol.*, **61**, 292–339.
Martindale, W. & Westcott, W. W. (1898). *Extra Pharmacopoeia*, **9**, p. 463.
Newman, A. K. (1879). *Trans. Proc. N.Z. Inst.*, **12**, 433–438.
Paech, K. & Tracey, M. V. (1955). *Modern Methods of Plant Analysis*, **4**, pp. 373–374, Berlin: Springer-Verlag.
Waldron, J. D., Gowers, D. S., Chibnall, A. C. & Piper, S. H. (1961). *Biochem. J.*, **78**, 435–442.
Wall, A. & Cranwell, L. M. (1943). *The Botany of Auckland*, 2nd ed., p. 35, Auckland: Wilson & Horton.
Ware, A. H. (1926). *Pharm. J.*, 162–167.