This work was supported by grant *SF* 11,091 of the U.S. National Heart Institute.

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Isolation and Identification of the Toxic Principle of *Gastrolobium grandiflorum*

SERIOUS losses in livestock in Queensland have been attributed[1,2] to *Gastrolobium grandiflorum* F. Muell, known locally as 'heart leaf poison bush'. Other common names are 'wall-flower poison bush' and 'desert poison bush'.

The plant is reported to occur mainly in inland districts of northern Queensland, the Northern Territory and the northern parts of Western Australia[3,4]. It is the only toxic species of *Gastrolobium* within Australia which extends beyond the boundaries of Western Australia and is the only species found within the tropics[5].

Bailey[6] refers to *G. grandiflorum* as "The most poisonous to stock of any in the Queensland flora". Many instances of sheep being killed with as little as 200 g or less of the leaf have been recorded[7]. Annual losses on some Central Queensland properties have been estimated at 150–300 bullocks on one property and at 500 bullocks on another; the loss of 2,000 sheep at one time on another property in the affected area has also been reported[7].

The isolation and identification of the toxic principle of *G. grandiflorum* as monofluoroacetic acid is now reported.

The isolation of the toxic principle involved continuous extraction, for 96 h, of milled air-dried leaf with 95 per cent ethanol containing excess sodium bicarbonate using an all-glass Soxhlet apparatus of approximately 800 g capacity. The combined extracts from 4 kg of leaf were used. Following transfer to an aqueous medium, and acidification, this extract was submitted to continuous extraction with sulphuric ether for 24 h. After drying and concentrating, the mixed acids were separated by microfractional distillation followed by gas chromatographic separation on a column of 'Tween 80' (2·5 g) dispersed on acid washed 'Embacel' kieselguhr (7·5 g) at 130° C using dry nitrogen as a carrier gas, in a Perkin–Elmer model 154*D* vapour fractometer.

Identification as monofluoroacetic acid was by infra-red spectroscopy, gas chromatography and micro-analysis, comparison being made with an authentic sample of monofluoroacetic acid and its butyl- and 2-chloroethanol-esters. Quantitative analysis[8] showed a level of 120 mg monofluoroacetic acid/kg in a sample of air-dried leaf collected from the Clermont district in central Queensland.

Toxicity testing with plant extracts and with equivalent amounts of monofluoroacetic acid produced characteristic symptoms in rats and sheep and resulted in comparable increases in the levels of citric acid in blood and organs.

The first identification of monofluoroacetic acid as the toxic principle in a plant responsible for serious stock losses was in *Dichapetalum cymosum* in South Africa[9]. Later in Australia it was identified as the toxin in the leaves and pods of *Acacia georginae* in Queensland[10], and afterwards in the Northern Territory[11]. The levels present in *G. grandiflorum* are intermediate between the very high levels present in *D. cymosum* and the relatively low concentrations found in *A. georginae*.

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Photochemical Reactions of Tetrachlorosalicylanilide

PHOTODERMATITIS caused by the use of soap containing the fluorescent germicide 3,5,3′,4′-tetrachlorosalicylanilide (T_4CS) has been described by Calnan[1], Wilkinson[2] and von Frenk[3].

Burckhardt[4,5] proposed that the action of light on a drug which causes photoallergy converts it into a new compound with allergenic properties and that this compound can produce its subsequent biological effect in the absence of light.

Pathak *et al.*[6] have suggested that photosensitization by psoralens involves their excitation to triplet states the energy of which is sufficient to produce free radicals in neighbouring molecules. The free radicals cause the biological changes in the irradiated system.

We have found that light interacts with T_4CS in the following ways:

(1) When T_4CS (0·7 per cent in 0·056 N aqueous alcoholic sodium hydroxide; *p*H 13·1) is irradiated by ultra-violet or sunlight it decomposes to 5,3′,4′-trichlorosalicylanilide (T_3CS), which can be identified by its mixed melting point and infra-red spectrum. A quantitative yield of T_3CS was obtained from T_4CS irradiated in aqueous alcoholic phosphate buffer solution (*p*H 7·3). No apparent decomposition occurred when T_4CS was irradiated in aqueous alcohol alone (*p*H 3·3) or in aqueous alcoholic hydrogen chloride (*p*H 2·2). As the *pKa* value of T_4CS is near 5·6, these results show that it is the ionized form which loses a chlorine atom to give T_3CS on irradiation.

(2) Irradiated T_4CS reacts with protein.

A mixture of T_4CS (1 ml. saturated solution in 0·9 per cent sodium chloride) and γ-globulin (10 mg) (*p*H 6·6) was exposed to daylight for 5 h. When eluted with 0·9 per cent aqueous sodium chloride from a 'Sephadex *G*–25' column, the γ-globulin fluoresced strongly. When exposure to daylight was omitted the eluted γ-globulin did not fluoresce strongly. T_4CS itself cannot be eluted from the column with 0·9 per cent aqueous sodium chloride or by γ-globulin in 0·9 per cent aqueous sodium chloride.

A mixture of T_4CS (0·2 ml. saturated aqueous solution) and rat serum (0·2 ml.) of *p*H 7·1, previously irradiated by ultra-violet light for 30 min, was examined electrophoretically. The strong fluorescence associated with T_4CS travelled with the serum proteins which were located by staining with bromophenol blue. Taken separately rat serum did not fluoresce strongly and T_4CS did not move from the base line under identical conditions.