to previous studies of MnBK-induced neuropathy which described axonal pathology with secondary demyelination (see review in Spencer and Schaumberg[13]) possibly because of the low qualities of MnBK used in this study. Low doses of a toxicant such as lead or colchicine can cause a segmental demyelination and high doses cause axonal degeneration or both[15-17]. Extensive segmental demyelination with some Wallerian degeneration has been reported in animals intoxicated with *Karwinskia humboldtiana* and in tellurium neuropathy[18,19].

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The principal toxin of *Delphinium brownii* Rydb., and its mode of action

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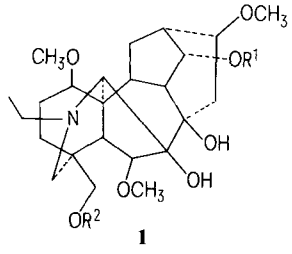
Summary. Examination of *D. brownii*, a stock-poison of Western Canada, revealed that the principal toxin was methyllycaconitine: a potent neuromuscular blocking agent which appears to act competitively at nicotinic receptors.

In North America, *Delphinium* are held responsible for more cattle deaths than any other kind of poisonous plant[2]. Although the problem is an old one, and the toxicity of the plants has long been attributed to the alkaloids which they contain, systematic studies of the toxins and their modes of action are lacking: a matter of substance in determining the best treatment for poisoned animals. We report here the results of a study of the toxins of *D. brownii* Rydb., an important stock-poison of the ranges of Western Canada.

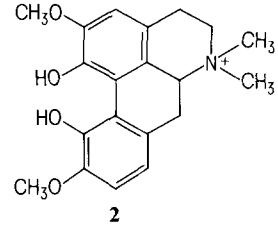
The leafy stems of *D. brownii* were collected in early (crown) stages of growth, and a methanolic extract of the plants was separated by conventional procedures[3] into non-alkaloidal, and alkaloidal fractions; the latter being subdivided into water-insoluble bases, and water-soluble quaternary ammonium salts. Further fractionation[3] of the bases yielded as the major constituents the tertiary diterpenoid alkaloids methyllycaconitine (MLA, **1a**), browniine **(1b)**, and browniine acetate **(1c)**. The principal component of the quaternary ammonium salts was the aporphine magnoflorine **(2)**, although this compound was only present in the plants in very minor amount.

Prominent among the symptoms of *D. brownii* poisoning is paralysis. Together with the published results of work in the Soviet Union on the pharmacology of some diterpenoid alkaloids[4-8], this suggested that a primary site of action of the toxins was the neuromuscular junction. Accordingly, in addition to preliminary acute toxicity tests on mice, the extracts were screened using 2 in vitro assays. Thus the depressant effects of the extracts were examined on the responses of the electrically-induced muscle twitch of the isolated rat phrenic nerve-diaphragm preparation[9], and an electrically stimulated longitudinal muscle strip of guinea-pig ileum[10]. Aconitine hydrochloride, and (+)-tubocurarine chloride hydrochloride were used as reference standards. Alkaloids were tested as water-soluble hydrochloride preparations, while water-insoluble extracts were added to organ baths as suspensions in dilute Cremophor EL (polyoxylated castor oil, BASF (Canada)) produced by sonication.

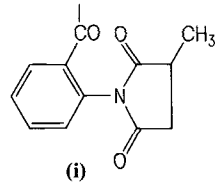
The toxicity of the plant extracts clearly resided in the tertiary base fraction. At the individual alkaloid level none of the compounds **1a–c**, or **2**, exhibited much muscarinic activity (as tested on the ileum preparation). However the crude tertiary, alkaloid fractions showed significant nicotinic blocking action (in the phrenic nerve-diaphragm system). Alkaloids **1b–c**, and **2** were of relatively low activity in this test, but **1a** was much more effective (see table), and as a



1



2



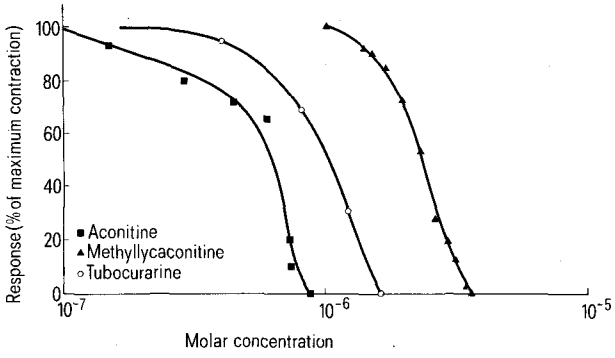
(i)

1a $R^1 = OCH_3$ $R^2 = (i)$
1b $R^1 = OH$ $R^2 = OCH_3$
1c $R^1 = OCOCH_3$ $R^2 = OCH_3$
1d $R^1 = OCH_3$ $R^2 = OH$

Concentrations of alkaloids of *D. brownii* producing 50% (ED_{50}) and 100% (ED_{100}) depression of the response of the rat phrenic nerve-diaphragm preparation

Fraction	Concentration* ED_{50} M/l	g/ml	ED_{100} M/l	g/ml
Aconitine hydrochloride	5.7×10^{-7}	3.9×10^{-7}	8.8×10^{-7}	6.0×10^{-7}
Tubocurarine chloride hydrochloride	1.0×10^{-6}	6.8×10^{-6}	1.6×10^{-6}	1.1×10^{-6}
Methyllycaconitine hydrochloride	2.3×10^{-6}	1.6×10^{-6}	3.6×10^{-6}	2.5×10^{-6}
Extract A**		3.0×10^{-4}		$\sim 6.0\times10^{-4}$
Extract B**		4.0×10^{-5}		$\sim 6.0\times10^{-5}$

* Final bath concentrations (regression values). ** Total methanol extract (after defatting with petroleum ether) (A), and mixture of water-insoluble tertiary bases (B). These fractions were suspended in Cremophor EL.



Dose-response for rat phrenic nerve-diaphragm.

good approximation the activity of the tertiary base fraction could be accounted for in terms of its MLA content. The toxicity of *D. brownii* thus appears to be very largely due to this alkaloid.

The dose-response curve of MLA seemed to parallel those of the reference compounds (figure), although regression analysis revealed a closer similarity to aconitine. The blockade by MLA was at least partially reversed by eserine, and the alkaloid could slowly be washed out on equilibration of the paralysed tissue with fresh physiological solution.

A number of preliminary electrophysiological and mechanical assays have also been performed using the sciatic nerve-sartorius muscle preparation of the frog, *Rana pipiens*. During nerve stimulation, ca. 10^{-7}M MLA produced a 50% inhibition of post-synaptically recorded action potentials within 20 sec. Complete muscle paralysis was achieved after 5 min. With ca. 10^{-8}M MLA the same level of inhibition was achieved after 50 sec, though total inhibition was not achieved at this concentration. The alkaloid could be washed out completely in both cases, but its action was only partially antagonised by 10^{-5}M eserine. Electrical conduction along the sciatic nerve, or over directly stimulated sartorius muscle was not affected by MLA at 10^{-7} M. Direct stimulation of the paralysed muscle elicited a normal contraction.

We conclude that a primary mode of action of MLA is by competitive blockade at nicotinic receptors, in substantial agreement with Dozortseva's findings[7], and the sometime clinical use of the hydroiodide salt of the alkaloid as a curare substitute[6], in the USSR. However, like aconitine, it may also affect sodium channels[11,12]. It is interesting that the neuro-muscular activity of MLA is essentially destroyed if the aromatic ester function is removed, for we found the parent alkaloid, lycoctonine **(1d)** to be essentially devoid of activity.

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A prostaglandin-like activity in small intestine and postirradiation gastrointestinal syndrome[1]

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Summary. A correlation between the postirradiation increase of the small intestine motility and the prostaglandin-like activity in this organ during gastrointestinal syndrome was observed. Indomethacin decreased the elevated motility of intestine and reduced the prostaglandin-like activity in this syndrome.

It was found recently that the increase of prostaglandin content in gastrointestinal tract which appears in such pathological conditions as colitis ulcerosa[2-4], thyroid medullar carcinoma[5] or cholera[6,7], induces diarrhea probably by stimulating the intestinal motility and reducing the epithelial water transport[8,9]. Also the therapeutic effect of diphenolic laxatives, like bisacodyl and phenophtalein, seems to be exerted by stimulation of prostaglandin synthesis[10].

The gastrointestinal syndrome which is manifested by