ISOLATION OF A NEW POTENTIAL ANTITUMOR COMPOUND FROM *WEDELIA ASPERRIMA*

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Wedelia asperrima Benth. (Compositae) is responsible for serious loss of sheep in northwestern Queensland. The poisoning is acute and the animals die within 24 hr after eating the plant (Mulhearn, (1)). A most marked feature at autopsy is the large quantity of straw colored fluid found in the abdominal cavity and lungs. In the latter case this is so large that the animal often "drowns".

Earlier, Oelrichs and Müller (2) reported the isolation of a toxic principle which they called wedeliatoxin, from the non-dialyzable fraction of an extract of *Wedelia asperrima* which was shown to be highly toxic to laboratory animals and sheep. As no significant structural work was carried out on this compound, we consider it preferable that the name wedeliatoxin no longer be used, to avoid confusion with the present study.

Later investigations of extracts of other plant material revealed a much higher proportion of toxicity in the dialyzable fraction, and a new procedure was developed to isolate the toxic components. In all plant samples examined, there appeared to be one toxic dialyzable compound that was present in larger amounts than others. The following article records the isolation and characterization of this major component and some experiments carried out to determine its effect on tumors produced by aflatoxin B_1 in rats.

EXPERIMENTAL

EXPERIMENTAL ANIMALS.—Toxicity testing was by i.p. injection of female mice (25–30 g) with sterile dilute aqueous solutions of the toxin adjusted to pH 7. Antitumor activity was tested on male and female Wistar rats (200 g), approximately 3 months of age, which had been intraperitoneally injected with a solution of aflatoxin B_1 (0.1%) in aqueous ethanol 50%.

PLANT MATERIAL.—The plants were gathered in northwestern Queensland in an area where sheep loss is common. Samples were identified by the Queensland Government Botanist and specimens were filed by him under the voucher number BRI–082431. The plants were air dried and then hammer-milled to facilitate storage and extraction.

ISOLATION AND PURIFICATION.—The initial steps in the procedure were the same as those outlined in Oelrichs and Müller (2) with the exception that methanol-water (1:1) instead of methanol was used to extract the plant (100 g). Fractions containing the toxin were eluted from the DEAE-cellulose column, combined, acidified with (1:1) sulfuric acid and extracted 3 times with butanol. The butanol extract was washed twice with water, neutralized with ammonia and concentrated to dryness. The residue was dissolved in ammonium formate buffer pH 2.5 and added to a column of B10–RAD AG–50 (x2) 200–400 mesh (100 g) in the NH_4^+ form. After washing, the toxin was displaced with a gradient of ammonium formate buffer at pH 3.4. Fractions containing the toxin were combined and passed through a column of B10–RAD AG–50 (x8) 50–100 mesh in the H^+ form in water. After being concentrated under reduced pressure for removal of the formic acid, the toxin was purified by preparative tlc on Silica-gel "G" with chloroform-methanol-acetic acid-water (65:25:5:5) as the solvent (Rf=0.6). Toxicity levels were monitored at all stages of extraction and purification. The yield of pure toxin (20 mg) gave a single spot when tlc was carried out with two different solvent mixtures, butanol-acetic acid-water (4:1:5) Rf=0.8 and with butanol-acetic acid-

water-pyridine (30:6:24:20) Rf=0.75. All attempts to crystallize the toxin from solution were unsuccessful, but crystals formed when a methanol solution was evaporated to dryness at room temperature.

TOXICITY.—Toxicity testing of the purified compound using mice and rats showed that the smallest LD_{100} dose was approximately 1 mg/kg.

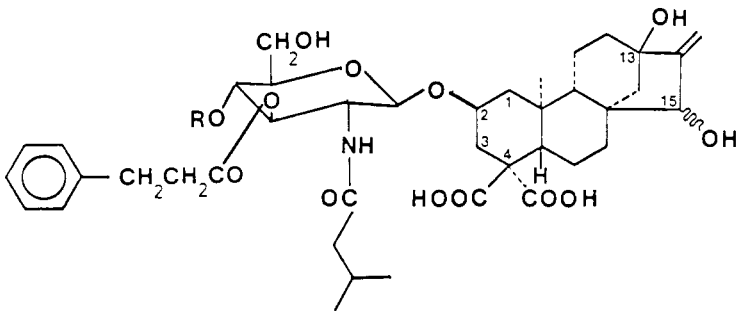
CHEMICAL STRUCTURE.—Due to the small quantity of pure material initially available, the investigations of the structure of the toxin were carried out by mass spectrometry of its permethylated and perdeuteromethylated derivatives (3) and combined GC–MS of its derivatized (permethylated and TMS) hydrolysis products.

When larger quantities of the toxin became available, ^{13}C- and ^{1}H-nmr spectrometry were used for definitive structural assignments by comparison with chemical shift values in synthetic and naturally occurring model compounds. Complete details of the structural elucidation of the toxin which we have named wedeloside (**1**) will be presented in a full paper. As well as the above major toxic compound **1**, another component of the *Wedelia* extract which showed the same level of toxicity was isolated. On examination by mass spectrometry and ^{13}C-nmr spectroscopy, this compound was shown to have structure **2**. It possessed an additional rhamnose moiety attached at the 4-position of the amidoglucoside ring.

All animals were kept till they died from natural causes (2–3 yrs). The 4 controls that received aflatoxin B_1, but no treatment, all showed tumors on autopsy. The 5 animals that received aflatoxin B_1 followed by treatment showed no tumors.

DISCUSSION

The structure of wedeloside **1** is unusual in a number of respects. First, it is one of the very rare examples of an amino-sugar occurring in plants other than in glycoproteins. An earlier reported example of an amino sugar linked to a steroid is halocurtin (4). This is, we believe, the first reported isolation of an amino-sugar glycosidically linked to a diterpene. Second, the presence of a phenyl propionate ester grouping on the glycosidic moiety is extremely rare, if not unique, in plant glycosides. Third, the diterpene aglycone of **1** has an unusual oxygenation pattern on the kaurene ring. In particular, the hydroxyl substituents on both the 2 and the 15 positions have been reported only in atractyligenin (5) and carboxyatractyligenin (6). The latter



1 R=H

2 R=L-rhamnopyranosyl

TESTS FOR ANTITUMOR ACTIVITY.—Tumors were induced in 10 rats (5 female and 5 male) by successive weekly doses over 4–5 weeks of purified aflatoxin B_1 (total dose 1mg/rat). One female animal died 20 weeks after the final injection, and autopsy revealed tumors at a number of sites. Five of the surviving rats (2 male and 3 female) were then dosed weekly for 8 weeks with 0.1 lethal dose (30 μg) of toxin **1**. The remaining 4 animals were left as controls.

compound also possesses the β-dicarboxylic acid grouping at the 18 and 19 positions which are present in wedeloside **1** but lacks the extra hydroxyl substituent at C–13. In all other respects, the aglycones from wedeloside and carboxyatractyloside appear to be identical.

Preliminary experiments with rats suggests that the toxin has an inhibiting effect on tumors produced by aflatoxin B_1 in rats. The method of administration and dose of aflatoxin B_1 has been recorded as producing 100 per cent incidence of liver tumors in rats (7), yet no tumors were present in those animals subsequently treated with the *Wedelia* toxin. Further experiments are also in progress to determine the effect of the toxin at a cellular level and to determine its effect on mitochondria. It is known, for example, that carboxyatractyloside, which is closely related structurally, inhibits oxidative phosphorylation in mitochondria (8) (9).

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