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Caulerpin, Caulerpicin, *Caulerpa scalpelliformis*: Comparative Acute Toxicity Study

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Abstract

The acute toxicity of caulerpin and caulerpicin isolated from the green algae *Caulerpa* was studied in the mouse. The lack of toxicity of these compounds indicates that they are not responsible for the toxic symptoms observed after ingestion of these algae. On the other hand, comparative studies of various *Caulerpa scalpelliformis* extracts show that the aqueous fraction is poisonous. Chemical and pharmacological characterisation of the toxic substances in this extract provides a new approach to the investigation of the mechanism of *Caulerpa* species toxicity.

Introduction

The genus *Caulerpa* of the family Caulerpaceae is represented by numerous species of algae eaten in the Philippines and various other countries of the Pacific (Doty and Aguilar-Santos 1966).

Certain varieties were first investigated because of their peppery taste though the active metabolite could not be isolated. Several compounds were characterised however, including caulerpin (Aguilar-Santos and Doty 1968, Aguilar-Santos 1970, Aguilar-Santos and Doty 1971, Maiti *et al.* 1978), caulerpicin (Doty and Aguilar-Santos 1966, Aguilar-Santos and Doty 1968, Mahendran *et al.* 1979, Nielsen *et al.* 1982), palmitic acid, β-sitosterol and taraxerol (Aguilar-Santos and Doty 1971), caulerpol (Blackman and Wells 1976), flexilin and trifarin (Blackman and Wells 1978).

A second more important problem concerns the toxic symptoms affecting certain individuals after the ingestion of algae of the *Caulerpa* genus (Doty and Aguilar-Santos 1966, Aguilar-Santos and Doty 1968, 1971, Doty and Aguilar-Santos 1970). The toxic effects described include a mild numbness of the tongue, dizziness, coupled with a cold sensation in the feet and hands, difficulty in breathing and loss of balance.

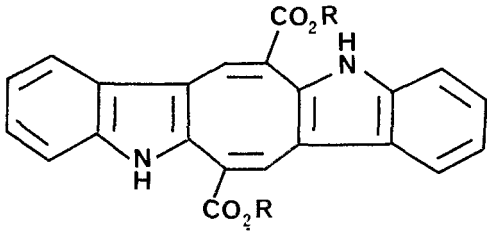
It may be remarked that these symptoms are rather similar to those reported in ciguatera poisoning, which is prevalent in coastal areas of the Pacific where the coral abounds (Bagnis 1973). It is now shown that the causal agent is the dinoflagellate *Gambierdiscus toxicus* and that the toxic complex responsible for ciguatera intoxication consists of two liposoluble substances of different size (ciguatoxin

and saxitoxin) and a water-soluble substance, maitotoxin (Bagnis *et al.* 1980, 1981).

The deterioration of the marine environment stimulates phytoplankton growth and it is not impossible that the algae are contaminated by this dinoflagellate, particularly as the former is considered to be toxic during the rainy season in the Philippines (Aguilar-Santos and Doty 1968).

Ciguatera, however, is unknown in this country (Doty and Aguilar-Santos 1966). According to Doty and Aguilar-Santos (1970), "Caulerpin and caulerpicin are physiologically active and toxic in the mouse and rat" (unpubl. results).

From the structural point of view, only caulerpin has a novel structure *1* (R = CH_3). Caulerpicin has been shown to be composed of a mixture of homologous hydroxy amides: N-acylsphingosines *2* (Mahendran *et al.* 1972) and N-acylsphinganines *3* (Nielsen *et al.* 1982).



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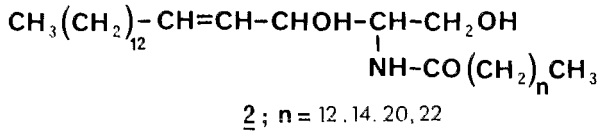
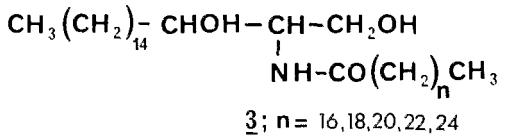


2; n = 12, 14, 20, 22



3; n = 16, 18, 20, 22, 24

We have been able to purify these hydroxy amides ourselves from all the *Caulerpa* species of New Caledonia and from the Caulerpales: *Tydemania expeditionnis* AL 106. The forms *2* and *3* seem to coexist with one of the two forms predominating in each species.

These hydroxy amides are also found in the red algae *Amansia glomerata* and *Laurencia nidifica* (Cardellina *et al.* 1978). Finally it should be noted that sphingosine is the major precursor for all the animal sphingolipids and has not so far been shown to be toxic.

To sum up, no pharmacological studies have been carried out to investigate the toxic metabolites responsible for the symptoms described above which, according to several reviews (Andrews *et al.* 1979, Halstead 1981, Naqui 1980) and books (Southcott 1979, Hoppe *et al.* 1979, 1982, Irwin 1964), are probably of clinical importance. We have therefore decided to present our own findings concerning the acute toxicity of the two metabolites, caulerpin and caulerpicin and of various extracts of *Caulerpa scalpelliformis* AL 122.

Materials and Methods

All the algae used for the study were collected from New Caledonia. *Caulerpa racemosa* var. *clavifera* AL 153 was harvested at a depth of one metre all the year round from Canard Island; *Caulerpa cactoides* (Turner) C. Agardh forma AL 150 at a depth of 12 m in May 1982 from the Cymenia reef; *Caulerpa cupressoides* AL 164 at a depth of 1 metre, all the year round from Canard island and *Caulerpa scalpelliformis* AL 122 (P.B.R.) C. Agardh at a depth of 20 m in May and June 1981 from Maitre island channel.

Extraction and characterisation of caulerpin 1 *(R = CH_3) and caulerpicin* 2 *and* 3

Caulerpin *1* (R = CH_3) was purified from *Caulerpa racemosa* var. *clavifera* AL 153 and *Caulerpa cupressoids* AL 164 by ether extraction in a Soxhlet apparatus followed by recrystallisation from ether and then from acetone, with a yield of approximately 0.2% of the dry weight of the algae. It could not be recovered from *Caulerpa scalpelliformis* AL 122. Its characteristics complied with those given in the literature (Maiti *et al.* 1978): Melting point (acetone) = 317 °C; M^+ 398, H^1NMR; ^{13}C NMR (DMSO-d_6): 165.81 (C = O); 52.10 (OCH_3); 141.58 (*C*H = C) 137.38 (CH = *C*); 137.57 – 132.91 – 126.98 – 125.93 – 122.93 – 122.69 – 117.81 – 111.19 (indole).

Caulerpinic acid *1* (R = H), Melting point = 256 °C dec. M^+ 370 was prepared by hydrolysis of caulerpin *1* (R = CH_3) with ethanolic potassium hydroxide under a reflux condensor (yield = 95%). ^1H NMR (Py d_5): 8H (6.85 m, ArH); 2H (8.1 s, CH = C); 2H (12.5). ^{13}C NMR (CD_3COCD_3): 167.38 (C = O); 143.12 (*C*H = C) 134.19 (CH = *C*) 138.98 – 128.72 – 127.02 – 123.74 – 118.67 – 112.91 and 112.94 (indole).

Caulerpicin (*2* and *3*) was purified by ether extraction in a Soxhlet apparatus followed by recrystallisation from ether and then from methanol. The yield was approximately 0.15% dry weight from *Caulerpa cactoides* AL 150; 0.02% dry weight from *Caulerpa racemosa* var. *clavifera* AL 153, *Caulerpa cupressoides* AL 164 and *Caulerpa scalpelliformis* AL 122.

The previously published spectral data were confirmed (Mahendran *et al.* 1979, Nielsen *et al.* 1982). Caulerpicin is a mixture of N-acylspingosines *2* and N-acylsphinganines *3*. The mixture gives two spots on TLC using a silica chromatoplate and a hexane-ethyl acetate mixture (20/80) as the mobile phase. The mixture methylene chloride-methanol (92/8) gives a single spot. In ^{1}H NMR ($CDCl_3$), the integration of the ethylenic signals at 5.3 is extremely variable. In ^{13}CNMR these signals appear at 129.4 and 129.9.

Acute toxicity

Male mice of the "Swiss" strain were used in this study. They had an average weight of 20 g ± 2 and were maintained in an air-conditioned animal house (21 ± 1 °C). Water alone, without feed, was given for 24 hours before drug administration.

Each dose was administered to groups of 10 animals and the number of groups varied with the number of dosages used. For the behavioural studies each test was carried out in the presence of a control group which received the corresponding dose of the vehicle alone. The experimental conditions for monitoring the animals were those described (Irwin S. 1964).

Toxicity of caulerpin and caulerpicin

The products for oral and intraperitoneal administration were given in a 3% aqueous gum solution.

The oral toxicity study (gastric incubation) was carried out using doses of 0.5 − 1 − 1.5 and 2 g kg^{-1} of caulerpin *1* (R = CH_3) and its sodium salt *1* (R = Na).

Caulerpicin was given at a dose of 1 g kg^{-1}. During the study the body weight was monitored by weighing the animals before drug administration and then on days 5, 10 and 15. The rectal temperature was taken before drug administration and then at times 1, 2, 4 and 6 hrs.

The intraperitoneal study was carried out under the same conditions by the administration of a single dose of 1 g kg^{-1} caulerpicin.

The intravenous study was performed using only the sodium salt *1* (R = Na). As the volume injected was limited to 0.2 ml for a weight of 10 g, the maximum dose administered was 0.2 g kg^{-1}.

Toxicity of the different extracts of the algae Caulerpa scalpelliformis *AL 122*

The dried algae powder (100 g) was extracted successively, at a low temperature, with methylene chloride, methanol and water to give, after decantation, filtration and concentration, crude methylene chloride CS_1 (yield: 2.5%), methanol CS_2 (yield: 30%) and aqueous CS_3 (yield: 19.5%) extacts. The experimental conditions for the study of these extracts have been described already. The animals were given a single dose of 1 g/kg by either the oral or intraperitoneal route.

Results

Acute toxicity study of caulerpin 1 *(R = CH_3)*

Animals were monitored for 15 days after the oral administration of the maximum dose of 2 g kg^{-1} and no mortality was observed. The LD 50 must therefore be higher than this value.

There was no significant difference in the variation in body weight or the mean body temperature between treated and control animals.

Caulerpin was not resorbed after intraperitoneal administration and a soluble caulerpin was therefore prepared by simple hydrolysis of two methyl carboxylate groups. The diacid sodium salt obtained *1* (R = Na) was freely soluble and could be used for further acute toxicity experiments. These are important as the native population chew the Caulerpa leaves and the saliva may stimulate the absorption of caulerpin. The oral toxicity study carried out as described above gave the same results except that one of the mice in the 2 g kg^{-1} group died on the tenth day. During the monitoring period the body weight and temperature were the same as the controls. The toxicity therefore remained very low and the LD 50 was above 2 g kg^{-1}.

No mortality was observed after the intravenous injection of 0.2 g kg^{-1} which was the highest dose that could be administered.

In conclusion, in the mouse the LD 50 of caulerpin and its sodium salt is greater than 2 g kg^{-1} after oral administration and the LD 50 of the sodium salt after IV administration is greater than 0.2 g kg^{-1}.

In view of these results, caulerpin may be classed as a drug with low toxicity. Taking into account the caulerpin content of the edible algae, it alone cannot explain the acute poisoning that may occur in man, although long-term toxicity after repeated administration was not investigated.

Acute toxicity study of caulerpicin 2 and 3

This mixture of hydroxy amides was shown to be non-toxic after oral and intraperitoneal administration of a 1 g/kg dose under the conditions described above. No behavioural modifications were observed either. The LD 50 is therefore greater than 1 g/kg.

Toxicity study of extracts of the algae Caulerpa scalpelliformis *AL 122*

As the two metabolites caulerpin and caulerpicin could not account for the toxicity of *Caulerpa* species, the presence of small quantities of other toxic metabolites in the plant was investigated by screening with three types of extract from the algae *Caulerpa scalpelliformis*: methylene chloride (CS_1), methanol (CS_2) and aqueous (CS_3) extracts, which were prepared in large quantities. As has already been shown, this algae does not contain caulerpin but only small amounts of caulerpicin which can be recovered from the CS_1 extract.

A 1 g kg^{-1} dose of either the CS_1 or the CS_2 extract was not toxic after intraperitoneal or oral administration (no mortality). No behavioural modifications were observed in treated animals.

On the other hand, although à 1 g kg^{-1} dose of the aqueous extract CS_3 was non-toxic after oral administration, there was 100% mortality in the group of animals receiving the same dose by the intraperitoneal route. Thirty minutes after administration motor activity was reduced in 50% of the animals and other tests for muscular and nervous lesions gave positive results in 60% of the animals. Death occurred 24 to 48 hours after administration.

This toxicity was confirmed after intravenous injection of CS_3 in the ethyl-urethane-anesthetised guinea-pig. The simultaneous recording of the respiratory rhythm and the electrocardiogram demonstrated that death occurred as a result of respiratory arrest within an hour of administration of a 80 mg kg^{-1} dose.

Conclusion

The pharmacodynamic tests that we have carried out using the two metabolites present in *Caulerpa* species, caulerpin and caulerpicin, have demonstrated that these two substances are definitely not responsible for the observed toxicity. If one discounts the hypothesis that this algae is contaminated by the dinoflagellate *Gambierdiscus toxicus* which causes ciguatera poisoning, fresh insight into the problem of *Caulerpa* intoxication may follow the characterisation of the toxic metabolites discovered in the aqueous extract of *Caulerpa scalpelliformis*.

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