PHOTOTOXIC AND INSECTICIDAL ACTIVITIES OF CHROMENES AND BENZOFURANS FROM *ENCELIA*

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ABSTRACT.—The benzofuran 6-methoxyeuparin and the two chromenes encecalin and 7-demethylencecalin from *Encelia laciniata, E. palmeri* and *E. ventorum* from Baja California, Mexico, were shown to be phototoxic to several bacteria and yeasts in long-wave uv light. Encecalin exhibited also insecticidal activities when applied to petri dishes in which first instar larvae of *Oncopeltus fasciatus* were raised. The observed toxicity may be due to direct contact as well as to volatility of encecalin.

Chromenes and benzofurans are common chemical constituents of certain tribes of the Asteraceae (1). We have recently identified a large number of these natural products in several species of the genus *Encelia* Adans. (tribe Heliantheae, Asteraceae). In the study presented here we have shown that some of these phytochemicals, isolated from *Encelia laciniata, E. palmeri,* and *E. ventorum* (2,3), are phototoxic against microorganisms. This is the first report of the photosensitizing capacity of naturally occurring chromenes and benzofurans. Additionally, we have established that encecalin (**5**), the major chromene of most species of *Encelia*, is insecticidal against the first-instar larvae of the milkweed bug, *Oncopeltus fasciatus*.

EXPERIMENTAL

The plant material, isolation, and identification of compounds **3-7** (figure 1) were described previously (2,3). Precocene I (**1**) and II (**2**) were purchased from Aldrich and 8-methoxypsoralen was purchased from Sigma.

PHOTOTOXICITY TESTS.—The tested organisms were the yeasts *Saccharomyces cerevisiae* and *Candida albicans*, the Gram-positive bacteria *Bacillus subtilis* and *Staphylococcus albus*, and the Gram-negative bacteria *Pseudomonas fluorescens* and *Escherichia coli*. Sabourauds medium was used for culturing the yeasts and nutrient agar plates for culturing the bacteria.

The tested compounds were dissolved at known concentrations in 95% ethanol. Aliquots of 10 μl (containing 100 μg) of each compound were then applied to sterile filter discs and allowed to dry. The dried discs were placed on inoculated agar in petri dishes. The tests were carried out in duplicate, one series being irradiated, the other kept in the dark. Both series were first kept in the dark for 30 min at 37°, then the series to be irradiated was placed under a bank of four black-light-blue uv lamps (max 350 nm, 15 W/m^2) for 2 h. The plates were then transferred to the dark again and were monitored after 24 h. Compounds producing a zone of inhibition in uv and not in the dark were determined to be phototoxic (+). 8-Methoxypsoralen (**8**) was used as a control in all tests (4).

INSECTICIDAL TESTS.—Euparin (**4**), encecalin (**5**), and 7-demethylencecalin (**6**) were dissolved in methanol. Known concentrations of each compound were transferred onto filter paper discs (9 cm diam) and allowed to dry completely. The applied concentrations ranged from 5 mg-10 μg. The impregnated filter discs were placed in petri dishes of the same diameter. Each petri dish contained 25-30 first instar larvae of *Oncopeltus fasciatus*. The insects were raised on milkweed seeds and water. Controls were set up with filter discs that were impregnated only with methanol. All insects were raised until their final moulting to adults. Effects of the applied chemicals were monitored every 24 h.

RESULTS AND DISCUSSION

Figure 1 shows the structures of the seven chromene and benzofuran derivatives that

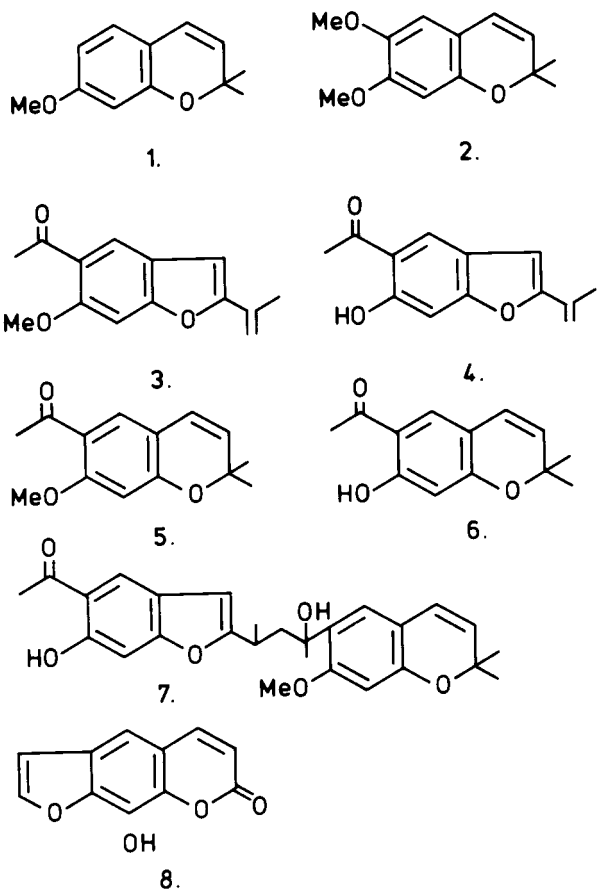

FIGURE 1. Structues of chromene and benzofuran derivatives (**1-7**) that were screened for biological activities. 8-Methoxypsoralen (8) was used as a control in the phototoxicity experiments.

were tested for phototoxic properties against the bacteria and yeasts. 6-Methoxyeuparin (**3**) was moderately active against four of the six microorganisms tested (table 1); encecalin (**5**) and 7-demethylencecalin (**6**) were active against three of the organisms tested (table 1), whereas the remaining compounds showed no activity. Compound **3** was most active, followed by **5** and **6**. None of the seven screened natural products showed any activity in the dark treatments. Most of the tested compounds exhibited absorption maxima between 320 and 350 nm; the uv lamps employed in the phototoxicity tests have a λ max in the region of 350 nm.

Table 1 shows that the Gram-negative bacterium *Escherichia coli* and the Gram-positive bacterium *Staphylococcus albus* were not sensitive to the tested compounds, whereas they were affected by the well-known photosensitizer 8-methoxypsoralen, which was used as the control in all assays. 6-Methoxyeuparin (**3**), however, showed activity against *Pseudomonas fluorescens*, an organism that is unaffected by 8-methoxypsoralen (8) and other known photosensitizers, *e.g.*, furanoquinolines, under these conditions (4).

Preliminary experiments with human erythrocytes on the mode of action of this new class of photosensitizers ruled out an attack on membranes, as reported, for example, for phototoxic polyacetylenes (5,6). It seems more likely rather, that the active compounds **3, 5,** and **6** behave as do the photosensitizing furanocoumarins, by interacting with nucleic acids or intracellular molecules in light (7). Further experiments are planned.

TABLE 1. Phototoxicity of naturally occurring chromenes and benzofurans to various microorganisms.

Compound	Yeasts		Gram-negative Bacteria		Gram-positive Bacteria	
	S. cerevisiae	*C. albicans*	*E. coli*	*P. fluorescens*	*B. subtilis*	*S. albus*
1	−	−	−	−	−	−
2	−	−	−	−	−	−
3	+	+	−	+	+	−
4	−	−	−	−	−	−
5	+	+	−	−	+	−
6	+	+	−	−	+	−
7	−	−	−	−	−	−
8	+	+	+	−	+	+

+ = phototoxic; − = inactive.

It has been shown previously that the two chromenes, precocene I (**1**) and precocene II (**2**) when applied externally, act as antijuvenile hormones against the milkweed bug *Oncopeltus fasciatus* and other insects (8,9). The structural resemblances between precocene I and II and several of the compounds isolated from *Encelia* led us to screen encecalin (**5**) and 7-demethylencecalin (**6**) for antijuvenilizing properties. The benzofuran euparin (**4**) was included in the testing. First instar larvae of *Oncopeltus fasciatus* were treated with different amounts of the three chemicals through their life cycles until they moulted to adults. The amounts of chemicals tested ranged from 5 mg-100 μg/petri dish, with 25-30 individuals per dish. Under similar conditions 248 μg of precocene I and 44 μg of precocene II have been reported to induce precocious metamorphosis in milkweed bug larvae (8). None of the three natural products tested in this study showed antijuvenile hormone activity, but encecalin (**5**) proved to be moderately insecticidal. Although euparin (**4**) had no effect on the health and fitness of the larvae, concentrations of encecalin (**5**) greater than 1.2 mg/petri dish were lethal to the larvae within a period of three to four days. Smaller concentrations of **5** had no effects. 7-Demethylencecalin (**6**) was also toxic to the first instar larvae, requiring however, larger concentrations of approximately 5 mg/petri dish. The toxicity of **5** and **6** to the larvae might be due to the volatility of the compounds as well as to direct contact.

The presence of a methylketone moiety in encecalin (**5**), instead of a methoxy substituent as in (**2**), results in a total loss of antijuvenile hormone activity. The weaker insecticidal activity of **6** compared with **5** could be caused by a more rapid detoxification of phenolic compounds exhibiting free hydroxy groups rather than methoxy groups, as has been proposed in the case of flavonoids (10).

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