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SHORT COMMUNICATION

ECDYSONES FROM GAMETOPHYTIC TISSUES OF A FERN

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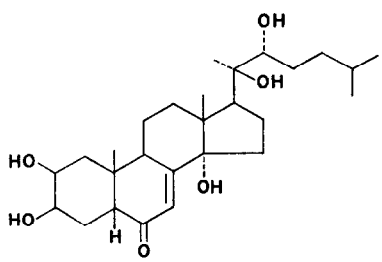
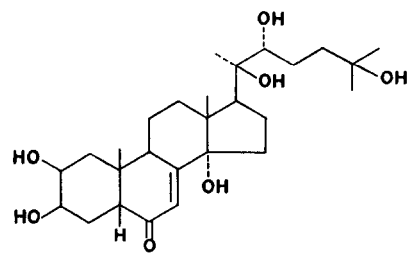
Abstract—Ponasterone A and crustecdysone have been isolated from culture filtrates of the fern gametophyte *Pteridium aquilinum* (L.) Kuhn.

INTRODUCTION

During studies directed towards isolating and chemically identifying antheridogen-A, the male reproductive organ inducing-substance[1] from cultures of bracken fern gametophytes, we found ponasterone A and crustecdysone (ecdysterone, β-ecdysone), steroids not previously reported from the haploid generation of plants. Sporophytic tissues of various dicotyledonous plants, *Achyranthes*, for example, have been reported to yield (β)-ecdysone,[2] inokosterone[2] and rubrosterone.[3] Sporophytic tissues of the gymnosperm *Podocarpus* have been a source of (β)-ecdysone,[2] ponasterone-A,[4] ponasterones B and C[5,6] and makisterones A-D.[7] Within the ferns, pterosterone has been identified in the sporophytes of *Lastrea* and *Onoclea*,[8] polypodine B in *Polypodium*,[9] lemmasterone in *Lemmaphyllum*[10] and (α)- and (β)-ecdysone in *Pteridium*[11] and *Cheilanthes*;[12] *Cheilanthes* also contains cheilanthones A and B.[12] Despite the identification of ecdysones in the *sporophytes* of these several ferns, we are unaware of any previous report of their production by the *gametophytes* of ferns or any other plants. Thus, it is of interest to find them represented in the haploid part of the life cycle of bracken (*Pteridium aquilinum* (L.) Kuhn).



Ponasterone A



Crustecdysone

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RESULTS

Culture filtrates of gametophytes of *P. aquilinum* were concentrated, (see Experimental) then extracted with ethyl acetate which removed practically all the active material from the aqueous phase. The ethyl acetate was distilled off under reduced pressure and the residue chromatographed on silica gel. Biological assay of the fractions from chromatography indicated two broad peaks of activity. The active fractions gave partly crystalline material which on further chromatography was found to consist mainly of one compound. This had spectral properties indicative of an ecdysone. It was identified as ponasterone A by comparison with an authentic sample. More polar fractions from the chromatography gave smaller quantities of a second crystalline steroid identified as crustecdysone. Mass spectral evidence suggested the presence of still other ecdysones in the culture filtrates of the fern. The amount of ponasterone A obtained from a batch of 260 l. of culture filtrate was approximately 100 mg.

Because of the presence of ecdysones in the active fractions from chromatography, we examined the possibility that these compounds might be involved in antheridium-inducing activity. It has been suggested that the ecdysones, which are well known as molting hormones of insects and crustaceans, may play a role in physiological processes in the plant.[11] Samples of authentic ecdysones were therefore tested, but none was found to exhibit any biological activity in antheridium induction.

The substances responsible for this activity are acidic and can be separated from the ponasterone A by extraction with sodium bicarbonate solution. We hope to report on the nature of these antheridogens in due course.

EXPERIMENTAL

Axenic cultures of bracken fern gametophytes (*Pteridium aquilinum* (L.) Kuhn) were grown from spores which had been surface sterilized;[1,13] with 20% commercial hypochlorite bleach (Chlorox). The spores were aseptically inoculated into Blake bottles containing 1 l. of an inorganic salts medium.[1] Cultures were maintained in continuous light at an intensity of approximately 60 lx and constant temperature of 18°. Culture filtrates were harvested at 10 weeks. The filtrates were concentrated *in vacuo* to 5% their initial volume in a circulating evaporator at temperatures not exceeding 45°.

A batch of 13 l. of concentrate was extracted twice with EtOAc (8 l.). Less than 5 per cent of the original activity remained in the aqueous phase. The EtOAc was distilled off under reduced pressure leaving a brown semi-solid residue (~1 g). This was chromatographed on a column of silica gel (0·05–0·2 mm, 200 g) with $CHCl_3$–MeOH (9:1). Fractions of 30 ml were collected and aliquots of each were used for biological assay. Peaks of activity occurred at about fractions 20 and 34. Fractions 26–32 were crystalline and consisted mainly of one compound (TLC). Further purification by preparative TLC gave white crystals, 80 mg, m.p. 258–260 (dec.). The UV (λ_{max} 244 nm, 12,000), IR (in KBr·ν_{max} 3400, 1645 cm^{-1}) and mass spectral properties were the same as those reported for ponasterone A.[4] Comparison with authentic ponasterone A confirmed the identity. Later fractions showed a second fluorescent spot more polar than the spot for ponasterone A. Separation by preparative TLC gave crystalline material (5 mg) whose IR spectrum was identical to that of crustecdysone. The mass spectra were also identical.

Ponasterone A and ponasterone C exhibited no biological activity in the antheridium induction bioassays with red light grown *Pteridium*[14] or white light grown *Onoclea*[15] or *Anemia*.[16] Concentrations tested were 10^{-4}, 5×10^{-5}, 10^{-5} and 10^{-6} g/ml. In similar, assays, ecdysone and crustecdysone at the following concentrations were ineffective in antheridium induction: 2×10^{-2}, 2×10^{-3} and 2×10^{-4} g/ml.

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