THE DISCOVERY OF FUNGAL SEX HORMONES: I. SIRENIN

MICHAEL J. CARLILE

Department of Biology, Imperial College, Silwood Park, Ascot, Berkshire, SL5 7PY

Sirenin was the first fungal sex hormone to have its structure determined. It is produced by female gametangia and gametes of the chytridiomycete genus *Allomyces* and attracts male gametes of the genus. It was discovered in 1958 by Leonard Machlis and, with the help of organic chemists, was purified and had its structure determined by 1968. Machlis's success is attributable to his association at Berkeley with the world authority on the genus, Ralph Emerson, to his meticulous physiological work on the genus in the 1950s, to his skill in devising bioassays and to his organising ability and drive. Subsequent work on sirenin has been carried out almost wholly by a single worker, Jeffrey Pommerville, and has for the time being come to an end.

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Hormones are substances produced by one type of cell or organ and having highly specific effects on another type of cell or organ. Those involved in the sexual process are known as sex hormones. By 1960 there was abundant evidence for the existence of fungal sex hormones, but in no instance was their chemical nature known. Ten years later the structures of three, sirenin from *Allomyces*, antheridiol from *Achlya* and trisporic acid from *Mucor,* had been elucidated. The nature of these hormones, the way in which they were discovered, and the personalities involved,were very different. The hormone here considered, sirenin, was detected (Machlis, 1958a), produced on a large scale and purified (Machlis *et al.*, 1966), and had its structure determined (Machlis *et al.*, 1968) in an efficient campaign under a single commander, Leonard Machlis (Jones *et al.*, 1978).

In 1948 Machlis (1915-1976) was the plant physiologist and Ralph Emerson (1912-1979) the mycologist in the Department of Botany at the Berkeley campus of the University of California. Emerson was the world authority on the chytridiomycete genus *Allomyces*, and in that year his graduate student, John Ingraham, started work on devising a defined medium for its culture. Machlis provided advice on physiological problems, and in so doing became converted to the study of the fungus, and during the period 1953-57 published meticulous studies of a kind now unfashionable on its physiology. Working with shaken liquid cultures, he proceeded from Ingraham's defined medium to a minimal medium in which there were no unnecessary components, and then determined optimal concentrations of those that were needed. A range of strains were studied and the requirements for the growth of the haploid (gamete-producing) and diploid (zoospore-producing) phases shown to be identical. Conditions for the efficient production and germination of meiosporangia (diploid sporangia in which meiosis occurs) were determined, enabling the haploid phase to be produced readily. As a result of these studies Machlis had, by the late 1950s, an unprecedented ability to grow *Allomyces* efficiently and throughout its life-cycle under precisely defined and readily repeatable conditions.

Machlis then became fascinated by the sight, in haploid cultures, of male gametes swarming around maturing female gametangia, ready to fertilize female gametes as they emerged (Fig 1). He found, with *Allomyces macrogynus*, that the male gametes were still attracted when female gametangia were embedded in agar, and deduced that an attractant, a sex hormone, was diffusing through the agar. He then undertook the task of isolating the hormone in order to determine its structure. To make progress he needed to produce the hormone in large amounts and to devise an objective assay for the hormone which

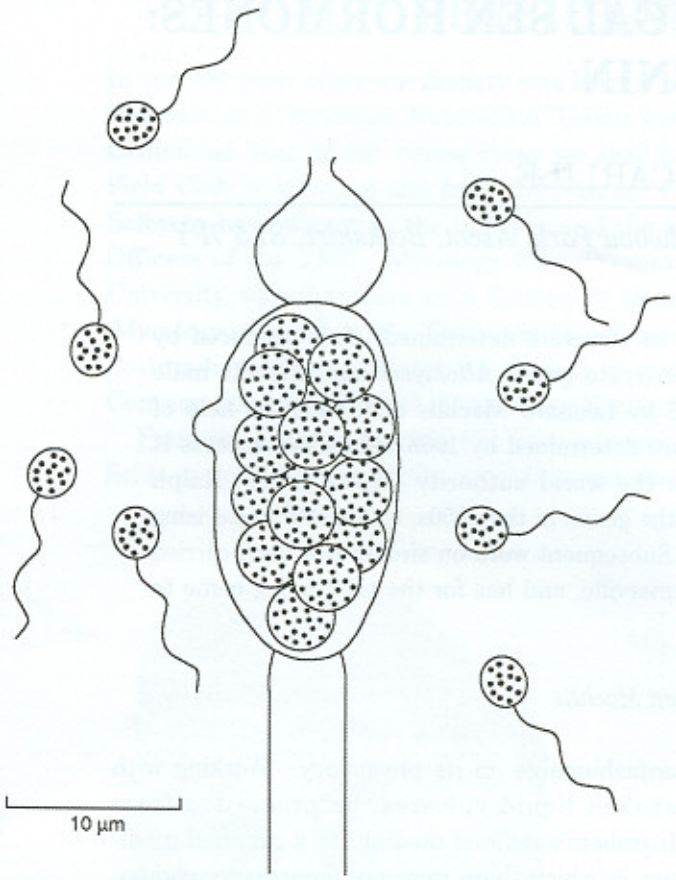

Fig 1 Male gametes have been discharged from a terminal male gametangium, now empty, of *Allomyces macrogynus*. They are swimming around the sub-terminal female gametangium, being attracted by sirenin emitted by the nearly mature female gametes within the gametangium. The male gametes are thus well placed to fuse with female gametes when the latter swim out of the gametangium through a ruptured papilla. In *Allomyces arbusculus* the female gametangium is terminal and the male gametangium sub-terminal. It is hence not surprising that in hybrids between the two species sexual development is disturbed with some strains producing almost exclusively male gametangia and others female. Freshour & Barstow (1987) provide photomicrographs illustrating gametangium development and discharge, and Fuller & Pommerville (1976) light and electron micrographs of gametes and fertilization.

would indicate if attempts at concentration were proving successful. Unfortunately mating will soon occur in gamete-producing cultures of *A. macrogynus*, with the haploid phase being succeeded by the diploid and hormone production coming to an end. The solution to this problem, and to the attainment of an effective bioassay, was the development of unisexual *Allomyces* strains. Emerson had already shown that hybrids between *Allomyces arbusculus* and *A. macrogynus* often had numbers of gametangia that differed markedly from the normal 1:1 male to female ratio. Machlis extended this hybridization work, and succeeded in obtaining some strains that produced almost exclusively female gametangia and some almost exclusively male. Both types therefore remained much longer in the haploid state than did wild strains. The female strains yielded the hormone, and the male strains gave gametes that could be used in bioassay. In this assay, solutions containing the hormone were placed on one side of a transparent permeable membrane, and a suspension of male gametes on the other. The number of gametes settling on the membrane indicated the potency of the hormone preparation. Having devised efficient methods for the production and assay of the hormone, he was able to publish conclusive evidence for its existence (Machlis, 1958a) and to concentrate it (Machlis, 1958b,c). His preparations were so potent, being active at 10 µg 1^{-1} (1 part per 100 million), that he believed them to be nearly pure. In fact they were only *ca* 0.2% pure, and hence the empirical formula that he published at that time was erroneous. He was persuaded to name the hormone *sirenin* (Machlis, 1958b), after the Sirens of classical mythology who possessed a fatal attraction for sailors in the Mediterranean, although he told me that he had intended to call it *lorelin*, after the Lorelei, a comparable navigational hazard on the Rhine.

Machlis then realised the need for expert assistance in the purification of sirenin, and obtained the help of the Berkeley organic chemist, Henry Rapoport. By late 1963 it had been purified and its approximate molecular weight was known. At that point I arrived at Berkeley for a year's work with Machlis, and had the good fortune to be the first fungal physiologist to have available pure sirenin. Soon after my arrival I carried out an experiment in which male gametes were exposed to 10^{-6} M sirenin and, as expected, observed attraction. The next day I obtained attraction with 10^{-7} M sirenin, and the day after with 10^{-8} M. Attraction was still perceptible when 10^{-10} M was reached (Carlile & Machlis, 1965), showing that sirenin was active at 10^{-8} g 1^{-1} (1 part per 50 000 million), and thus remarkably potent. I then tested concentrations of sirenin higher than 10^{-6} M, and found that at 10^{-4} M attraction was weak; clearly there was so much sirenin everywhere that the gametes did not know which way to turn! I also found that male gametes destroyed sirenin. This probably prevents their sirenin receptors from becoming completely saturated

and permits sensitivity over an enormous concentration range, 10^{-10} to 10^{-5} M or a hundred thousand fold. It also provides another reason why female rather than hermaphrodite wild strains are best for sirenin production, there then being few male gametes to destroy the sirenin.

Machlis saw himself as being in a race for the first fully characterized fungal sex hormone, with the *Achlya* and *Mucor* workers as his competitors. By 1966 2.5 g of sirenin had been accumulated, and the methods for the production and isolation of sirenin were published, along with the correct molecular formula and some information on its chemical nature (Machlis *et al.*, 1966). The full chemical structure (Fig 2) was reported in a preliminary note (Machlis *et al.*, 1968), a little before that of the *Achlya* sex hormone, antheridiol (Arsenault *et al.*, 1968). Although the structure of the *Mucor* sex hormone, trisporic acid C, had been established earlier (Caglioti *et al.*, 1967), it was as an inducer of carotenoid synthesis in *Blakeslea trispora*, and it was only later that it became clear that it was the sex hormone being sought in *Mucor*. So Machlis could well regard himself as having won the race for the first fungal sex hormone.

A fuller account of the structure of sirenin was subsequently published from Berkeley (Nutting *et al.*, 1968). The structure of sirenin, a bicyclic sesquiterpenediol, was of such interest that its synthesis was reported not only from Berkeley (Plattner *et al.*, 1969) but also independently from Harvard (Corey *et al.*, 1969), New York (Grieco, 1969) and Tokyo (Mori & Matsui, 1969). All these syntheses were of racemic mixtures of *d*- and *l*-sirenin. Subsequently Plattner & Rapoport (1971) synthesised *d*- and *l*-sirenin separately, and Machlis showed that only *l*-sirenin attracted male gametes; its mirror image or optical isomer, *d*-sirenin, was without activity as were various synthetic analogues of sirenin (Machlis, 1973b). This paper, which also considered the kinetics of sirenin uptake by male gametes was, along with one on refinements to the sirenin bioassay (Machlis, 1973a), the last publication on sirenin by Machlis, who died three years later. Earlier he had shown that male gametes of wild strains of *Allomyces macrogynus* and *A. arbusculus*, as well as the hybrid strain used in most of his studies, responded to sirenin (Machlis, 1968).

Machlis was a competent mechanic and metal

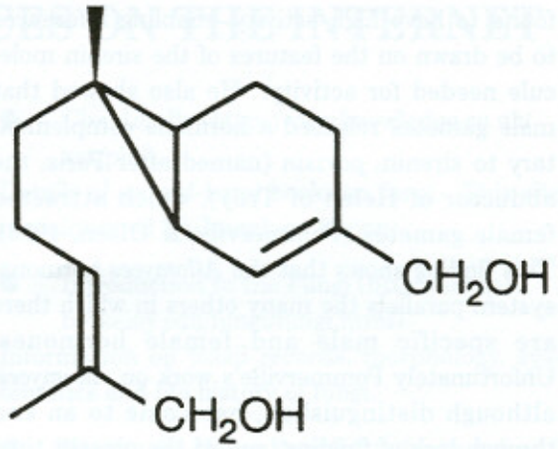

Fig 2 Sirenin, a bicyclic sesquiterpene ($C_{15}H_{24}O_2$: MW 236) released by female gametangia and gametes of *Allomyces* and attracting male gametes. Only one of the many analogues synthesised has activity, and differs from sirenin only in lacking the hydroxy-methyl group on the cyclohexane ring. All other features appear to be needed for activity.

worker, and the prototypes of his assay kits were made by himself, prior to being copied in adequate numbers in the departmental workshop. He was relatively uninterested in theory, his only book being a laboratory manual (Machlis & Torrey, 1956). Instead, he was fascinated by experimental detail, and made repeated improvements (especially Machlis, 1969, 1973a) to the sirenin assay. In parallel with his sirenin work he, with his co-worker Mascarenhas (Mascarenhas & Machlis, 1962a,b, 1964), identified for the first time a chemotropic factor that guided the growth of pollen tubes into a plant stigma, and with other co-workers demonstrated the occurrence of the sperm attractant in the alga *Oedogonium cardiacum* (Machlis *et al.*, 1974). These studies also involved devising bioassay apparatus and procedures. The success of Machlis in the first isolation of a fungal sex hormone in part resulted from the presence at Berkeley of a great mycologist, Ralph Emerson, and was in part due to his own industry, organising ability and meticulous attention to experimental detail.

Since Machlis died, substantial studies on sirenin have been carried out by only one worker, Jeffrey Pommerville, and his associates. He analysed the path taken by male gametes in their response to sirenin (Pommerville, 1977, 1978) and examined the effect of further analogues of sirenin on male gametes (Harding *et al.*, 1988; Pommerville *et al.*, 1988); just one was

found to have high activity, enabling inferences to be drawn on the features of the sirenin molecule needed for activity. He also showed that male gametes released a hormone complementary to sirenin, *parisin* (named after Paris, the abductor of Helen of Troy), which attracted female gametes (Pommerville & Olsen, 1988). This finding shows that the *Allomyces* hormonal system parallels the many others in which there are specific male and female hormones. Unfortunately Pommerville's work on *Allomyces*, although distinguished, has come to an end through lack of funding, and at the present time there seems to be no work being done on sirenin.

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