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FIRST ISOLATION OF JACARANONE FROM AN ALGA, *DELESSERIA SANGUINEA*. A METAMORPHOSIS INDUCER OF *PECTEN* LARVAE

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It has long been known that some marine invertebrate larvae are stimulated to settle and metamorphose by specific chemicals found on living substrata (1). For example, the brown marine alga *Sargassum tortile* induces the settling of swimming larvae of the hydrozoan *Coryne uchidai*; the active compounds are δ-tocotrienol [2H-1-benzopyran-6-ol, 3,4-dihydro-2,8 dimethyl-2-(4′,8′,12′ trimethyl-3′,7′,11′ tridecatrienyl)] and its 11′-12′ epoxide (2). Abalone larvae settle and metamorphose rapidly upon exposure to crustose red algae, γ-aminobutyric acid (GABA) and its analogues being responsible (3). GABA and its analogues display similar activities towards some other marine gastropods. In the black chiton *Katharina tunicata* Wood, GABA induces crawling behavior but does not induce developmental metamorphosis in the absence of the crustose coralline alga *Lithothamnium* sp. (4).

We have found that the metamorphosis of *Pecten maximus* L. larvae occurs more easily in the presence of some living red algae or in the presence of algal extracts. We want to report here the isolation and characterization of an active compound, jacaranone (**1**), isolated from one of these Rhodophyceae, *Delesseria sanguinea* Lamouroux (Delesseriaceae). The biological experiments will be described in detail elsewhere (5). Jacaranone has been encountered previously in *Jacaranda caucana* Pittier (Bignoniaceae) (6,7) and in Compositae (8-11).

RESULTS AND DISCUSSION

Aqueous EtOH extracts of the freeze-dried alga, *D. sanguinea*, are able to promote metamorphosis of *Pecten* larvae. After removing most of the EtOH and partitioning the aqueous residue with Et_2O, we obtained an Et_2O soluble material that retained most of the alga's activity. From this mixture, the active compound was purified by a multistep chromatographic procedure monitored by biological assays. This procedure included chromatography on Sephadex LH-20 and silica gel. Purification was achieved by hplc on Lichrosorb RP 18.

The active compound was isolated as needles, and its structure was established as 2,5-cyclohexadiene-1-acetic acid-1-hydroxy-4-oxo methylester (jacaranone) (**1**) from the molecular composition ($C_9H_{10}O_4$ by hrms), ^1H- and ^{13}C-nmr data (see Experimental section). Definitive identification was established by comparing physical and spectral properties of **1** with data reported for jacaranone (6-8) and by direct comparison (ir, tlc) with an authentic sample isolated from *J. caucana*. Ir spectra of both jacaranones obtained from *D. sanguinea* and *J. caucana* were strictly identical (see Experimental section). Jacaranone has been also prepared synthetically by a route used for synthesizing its ethyl homologue **2** (12).

In hatchery experiments, jacaranone, obtained by extraction or synthesis, induces larval settlement and metamorphosis of *P. maximus* at a concentration of 0.5 mg/liter (3.10^{-6}M). Among the

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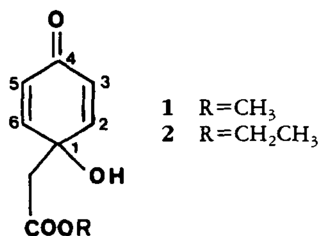

compounds isolated from *D. sanguinea* (13) only **1** displays this property. Jacaranone resembles neither of the known types of algal morphogenes, GABA or δ-tocotrienol.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—The ^{1}H- and ^{13}C-nmr spectra were obtained in $CDCl_3$ on a JEOL FX 100 spectrometer with TMS as internal standard. High resolution mass spectra were recorded on a VG Micromass 70-70 F mass spectrometer. The ir and uv spectra were obtained respectively on a Pye-Unicam SP 2000 and a Beckman Acta III spectrophotometers. Sephadex LH 20 came from Pharmacia, silica-gel from Merck (Kieselgel 60, 70-230 mesh). Hplc separation was carried out using a 6000 A pump, an U6K injector, a M-450 uv detector monitoring at 225 nm (all from Waters) and a Lichrosorb RP-18 250×10 mm column packed with 7μ particles (from Merck). Tlc analysis and Plc separations were carried out on Merck Si 60 F_{254} silica gel plates developed in cyclohexane-EtOAc (1:1, v/v).

PLANT MATERIAL.—*D. sanguinea* was collected by scuba diving in the vicinity of Roscoff, France, in June 1978, and in the Bay of Brest in May 1979. Plant material has been identified by Dr. J. Cabioch (Station Biologique de Roscoff, Roscoff, France) or by Dr. J.Y. Floc'h (Université de Bretagne Occidentale, Brest, France). Because *D. sanguinea* is a very well-known alga in Brittany, it was felt that there was no need to deposit a specimen.

EXTRACTION AND PURIFICATION.—The freeze-dried alga (563 g) was extracted with 70% aqueous EtOH, and the extract was evaporated under reduced pressure. The resulting aqueous residue (2 liters) was extracted three times with Et_2O (1 liter). The Et_2O soluble fraction (3.38 g) was chromatographed on a Sephadex LH 20 column (60×6 cm) using CH_2Cl_2-MeOH (1:1, v/v) as the mobile phase. The first brown or green-brown fraction was discarded. The following yellow or green-yellow fraction was concentrated to give a green gummy residue (1.42 g) which was transferred to a 30-g silica gel column. Elution by cyclohexane-EtOAc (3:2, v/v) gave a multicomponent fraction containing **2**. Purification of **2** was achieved by semi-preparative reverse phase hplc using H_2O-CH_3CN-MeOH (75:20:5, v/v/v) as an eluent at a flow rate of 4 ml/min. Yield: 54 mg.

PHYSICAL DATA OF **1**.—Compound **1** recrystallized as needles from MeOH mp 77° [lit. 76-77° (7)]; Rf 0.2; hrms *m/z* 182.0572 ($M^{+\cdot}$; calcd for $C_9H_{10}O_4$, 182.0579); uv λ max (EtOH) 227 (12500); ir ν max (KBr) cm^{-1} 3390 (s), 2975 (w), 2950 (w), 1720 (vs), 1670 (vs), 1625 (s), 1605 (m), 1445 (sh), 1440 (s), 1430 (m), 1400 (m), 1390 (m), 1365 (s), 1345 (m), 1285 (s), 1255 (s), 1245 (s), 1190 (w), 1175 (sh), 1165 (vs), 1080 (m), 1060 (s), 1040 (s), 1000 (w), 990 (m), 930 (w), 915 (w), 880 (w), 865 (vs), 800 (w), 720 (m), 635 (m), 610 (m), 520 (m), 460 (w), 435 (w), 420 (w), 355 (m), 330 (m); ^{1}H nmr (100 MHz) δ 2.75 (2H, s, CH_2) 3.75 (3H, s, OMe) 6.15 (2H, d, J=10 Hz·H_3 and H_5) 6.98 (2H, d, J=10 Hz·H_2 and H_6; ^{13}C nmr (25.2 MHz) δ 190 (s, C_4), 174 (s, COO), 154 (d, C_2 and C_6), 130 (d, C_3 and C_5), 70 (s, C_1), 55 (q, CH_3), 47 (t, CH_2).

SYNTHESIS OF **1**.—A 150-ml, three-necked flask was fitted with a reflux condenser and a pressure-equalized dropping funnel. In the flask were placed an excess of activated (14) Zn (3.5 g), enough anhydrous Et_2O for covering the Zn, and some crystals of iodine. In the dropping funnel was placed 4.25 g of pure methylbromoacetate. A few drops of pure bromoacetate were added and then some drops of methyl magnesium iodide to initiate the reaction. When the reaction was started, the methylbromoacetate was diluted with 50 ml anhydrous Et_2O, and the flask was heated to reflux in the water bath of an ultrasound laboratory cleaner to accelerate the reaction (15). The methylbromoacetate ethereal solution was added in 1 h. After the addition, the reaction was allowed to reflux for 2 h more. As observed by Siegel and Keckeis (12), the solution turned yellow, and a gummy precipitate appeared, but we obtained only a poor yield of the Reformatsky reagent (16). The yellow solution was decanted and transferred to another three-necked flask. An ethereal solution of benzoquinone (1.275 g) was added slowly to this solution. A precipitate appeared which was filtered, washed with with anhydrous Et_2O, and hydrolyzed by small portions in hot H_2O. The aqueous solution was reextracted by Et_2O, the organic layer was dried with anhydrous Na_2SO_4 and evaporated. After evaporation, the oily residue (125 mg) was purified by plc. Compound **1** (95 mg) as a colorless residue was obtained (yield=4.4%).

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