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Secalonic Acids D and F Are Toxic Metabolites of *Aspergillus aculeatus*

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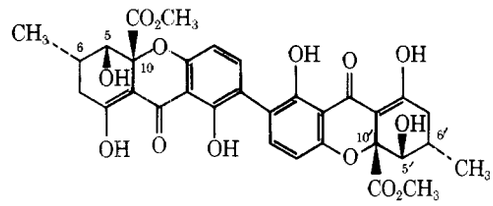
Continuing our studies of toxic substances produced by food spoilage fungi, we have investigated the metabolites of *Aspergillus aculeatus* Iizuka grown on white corn. Purification of the crude methylene chloride extracts by petroleum ether precipitation and subsequent preparative thin layer chromatography on silica gel yielded two toxic metabolites, secalonic acid D and F. Secalonic acid D has previously been isolated from *Penicillium oxalicum*.[3] Other secalonic acids are known metabolites of *Aspergillus ochraceus* (secalonic acid A),[4] *Claviceps purpurea* (secalonic acids, A, B, C)[5] and *Phoma terrestris* (secalonic acids, A, E).[6] Secalonic acid F is a new member of this group. In the following, we report the chemical identification, preparation and biological activity of the two toxins of *A. aculeatus* Iizuka. The more polar compound, obtained as yellow needles, was identical with secalonic acid A[7] as judged by comparison of infrared, ultraviolet, mass, and proton magnetic resonance spectra. Its optical rotation, $[\alpha]^{20}D$ +64° (*c* 0.14, $CHCl_3$), however, was opposite in sign from that of secalonic acid A and hence the toxin



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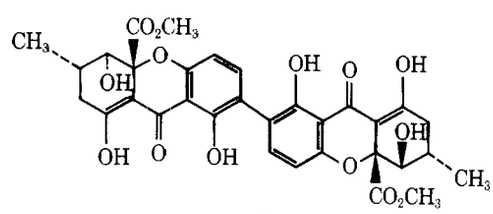

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is secalonic acid D (**1**)[3] (ergochrome EE), the enantiomer of secalonic acid A.

The second toxin crystallized from benzene–cyclohexane in the form of yellow needles, $[\alpha]^{20}D$ +190° (*c* 0.131, pyridine), *m/e* 638.15987 (calcd for $C_{32}H_{30}O_{14}$, 638.16356).[8] It was soluble in aqueous potassium carbonate, gave a positive ferric chloride test, and exhibited ultraviolet and infrared absorptions typical for secalonic acids.[5,9] The aromatic region of the proton magnetic resonance spectrum displayed four one-proton doublets at δ 6.51, 6.55, 7.30, and 7.33 each with a coupling constant of 8 Hz indicating that the new substance was an unsymmetrical dimer.[10] This hypothesis received further support from the appearance of the carbinol proton signals at C-5 and C-5′. A broad singlet at δ 4.08 and a doublet at δ 3.88, J = 11 Hz, suggest that hydroxyl and methyl groups are cis and trans oriented, respectively, in the two structural moieties. The circular dichroism spectrum was found to exhibit a large positive Cotton effect at 333 nm and comparison with values obtained for other secalonic acids[11] left no doubt that both C-10 and C-10′ have the *R* configuration. If it is assumed, in analogy to all known secalonic acids and ergochromes, that the C-6 and C-6′ methyl groups are trans to the C-10 and C-10′ carbomethoxy groups, respectively,[11-13] the new toxin, which we have named secalonic acid F (ergochrome BE),[14] should have structure **2**. This was confirmed as follows. When submitted to oxidation with potassium permanganate secalonic acid F (**2**) gave (*S*)-(−)-methylsuccinic acid[11] identical with a sample obtained by analogous oxidation of secalonic acid D (**1**). The two metabolites showed antimicrobial activity against *Bacillus megaterium*,[15] secalonic acid F being somewhat less active than secalonic acid D. Secalonic acid A had been reported to inhibit *Bacillus subtilis* and *Piricularia oryzae* but not other tested microorganisms.[4] Toxicity data will be presented in a forthcoming paper by Professor Gerald N. Wogan, Department of Nutrition and Food Science, M.I.T.

Experimental Section

Melting points were measured on a Kofler hot stage or a Büchi SMP20 oil bath apparatus and are corrected. Optical rotations were measured on a Perkin-Elmer 141 polarimeter. The following spectrometers were used: IR, Perkin-Elmer 567; ultraviolet, Cary 14; ^1H NMR, Hitachi Perkin-Elmer R22 90 MHz; CD, Cary 60; mass spectra, Hitachi Perkin-Elmer RMU-6L and CEC 110B (Du Pont Industries).

Aspergillus aculeatus Iizuka was screened on a variety of grains in 2.8-l. Fernbach flasks on the shaker for 10 days at 30 °C.[15] The best were white corn and minute rice. The more traditional procedure of unagitated fermentation on glutinous rice yielded no toxin. After growth on white corn, the cultures were homogenized in a blender with methylene chloride, the homogenate was filtered, and the methylene chloride filtrate was concentrated in vacuo. Precipitation with petroleum ether gave ~400 mg of toxic petroleum ether insolubles (PEI)/kg of substrate corn. The PEI (814 mg) was dissolved in 40 ml of hot methylene chloride and filtered to remove 50 mg of nontoxic precipitate. The filtrate was concentrated in vacuo and chromatographed on silica gel GF254 plates containing 6% tartatic acid (solvent 2-pentanone–chloroform, 2:8). Two yellow bands (R_f 0.17 and 0.29), which both gave red-brown ferric chloride tests, contained toxic substances.

Secalonic Acid D (1). The slow-moving band (R_f 0.17) yielded 94 mg of a yellow glass which was crystallized first from carbon tetrachloride and then from chloroform to give 74 mg (9.1% of PEI) of light yellow needles (mp 281–283 °C in evacuated capillary, 255–259 °C on hot stage). High-resolution mass spectrum M^+ 638.16088 (calcd for $C_{32}H_{30}O_{14}$, 638.16353); $[\alpha]^{25}D$ +64° (*c* 0.14, chloroform); UV max (ethanol) 236, 265, and 338 nm (ε 17 800, 15 100, and 37 800); ^1H NMR (Me_2SO-d_6–1% Me_4Si) δ 1.05 (d, 6 H, J = 4 Hz), 2.0–3.0 (m, 6 H), 3.63 (s, 6 H), 3.80 (d of d, 2 H, J = 6 and 10 Hz), 6.02 (d, 2 H, J = 6 Hz, exchanges), 6.64 (d, 2 H, J = 8 Hz), 7.47 (d, 2 H, J = 8 Hz), 11.70 (s, 2 H, exchanges), 13.72 (bs, 2 H, exchanges); CD (*c* 2.9 × 10^{-2} mg/ml dioxane) λ 400 (Δε 0), 332 (+13.5), 290 (0), 270 (−4), 260 (−1.5), 225 (−48), 215 (−23). Further, the compound was found to have TLC behavior (silica gel GF254 containing 6% tartaric acid, solvent 2-pentanone–chloroform, 2:8) and an IR spectrum [(KBr) 3505, 1735, 1610, 1585, 1432, 1232, and 1061 cm^{-1}] identical with those of an authentic sample of secalonic acid A.

Oxidation of Secalonic Acid D (1). Secalonic acid D (54 mg) was dissolved in 5 ml of 2 N sodium hydroxide. The solution was cooled to 0 °C and added to 5 ml of saturated potassium permanganate solution at 0 °C. The reaction mixture was maintained at 0 °C for 48 h and then clarified at 0 °C with sulfur dioxide. Extraction with ethyl acetate (3 × 50 ml) gave 40 mg of crude reaction products which were chromatographed on a 1-mm Avicel F plate (solvent ammonia–1-propanol, 3:7, developed twice). The desired band (R_f 0.37) was eluted

from the cellulose with 40 ml of methanol–water (9:1). Removal of the methanol in vacuo followed by partitioning of the aqueous residue between 1 N hydrochloric acid (10 ml) and ethyl acetate (2 × 25 ml) gave 2.6 mg of crystalline, ethyl acetate soluble residue. Crystallization (benzene–cyclohexane) gave 1.9 mg of (*S*)-(−)-methylsuccinic acid, mp 109–110 °C, $[\alpha]^{25}D$ −12° (*c* 0.09, ethanol). IR ($CHCl_3$) and mass spectra were identical with spectra of authentic, racemic methylsuccinic acid.

Secalonic Acid F (2). The fast-moving band (R_f 0.29) yielded 53 mg of a yellow glass which upon crystallization (benzene–cyclohexane) gave 26 mg (3.2% of PEI) of yellow needles, mp 218–221 °C (hot stage), 253–256 °C (evacuated capillary). A high-resolution mass spectrum indicated M^+ 638.15987 (calcd for $C_{32}H_{30}O_{14}$, 638.16356). Secalonic acid F showed $[\alpha]^{20}D$ +202°, $[\alpha]^{20}_{578}$ +214° (*c* 0.13, pyridine); UV max (ethanol) 236, 263, and 388 nm (ε 19 250, 17 300, 37 000); IR (KBr) 3520, 1748, 1610, 1590, 1442, 1238, 1068, and 1045 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.14 (d, 6 H, *J* = 7 Hz), 2.0–3.0 (m, 6 H), 2.67 (b, 1 H, exchanges), 2.86 (b, 1 H, exchanges), 3.67 (s, 6 H), 3.87 (d, 1 H, *J* = 10 Hz), 4.09 (b, 1 H), 6.52 (d, 1 H, *J* = 9 Hz), 6.58 (d, 1 H, *J* = 9 Hz), 7.35 (d, 1 H, *J* = 9 Hz), 7.39 (d, 1 H, *J* = 9 Hz), 11.65 (s, 1 H, exchanges), 11.80 (s, 1 H, exchanges), 13.70 (s, 1 H, exchanges), 13.88 (s, 1 H, exchanges); mass spectrum (70 eV) *m/e* (rel intensity) M^+ 638 (20), 579 (100), 561 (20), and 501 (20); CD (*c* 4.8 × 10^{-2} mg/ml dioxane) λ 400 nm (Δε 0), 332 (+17), 275–260 (0), 223 (−43), and 215 (−25).

Oxidation of Secalonic Acid F (2). Secalonic acid F (57 mg) was oxidized with potassium permangate as described for secalonic acid D to give 2.6 mg of (*S*)-(−)-methylsuccinic acid, mp 109–111 °C, $[\alpha]^{25}D$ −13° (*c* 0.12, ethanol). IR ($CDCl_3$) and mass spectra were identical with spectra of authentic racemic methylsuccinic acid.

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Registry No.—1, 35287-69-5; 2, 60687-07-2; (*S*)-(−)-methylsuccinic acid, 2174-58-5.

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A Synthesis of (±)-Methyl *n*-Tetradeca-*trans*-2,4,5-trienoate, an Allenic Ester Produced by the Male Dried Bean Beetle *Acanthoscelides obtectus* (Say)[1]

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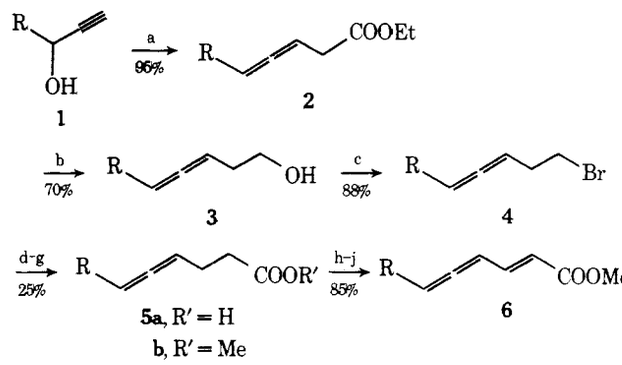
In 1970, Horler[2] isolated a novel optically active allenic ester from the hexane extracts of male dried bean beetles [*Acanthoscelides obtectus* (Say)] for which structure **6** was suggested based on spectrometric and chemical evidence. Subsequent total synthesis has corroborated the assigned structure.[3–6] As a putative sex pheromone, there are two aspects of **6** which are unusual: first, the compound is present in rather large amounts (ca. 0.5% of the body weight) compared with other insect sex pheromones, and second, the ester **6** is unstable ($t_{1/2}$ = 10 h at room temperature) and polymerizes readily. We report below a synthesis of racemic **6** starting with undec-1-yn-3-ol (**1**).

The synthetic plan outlined in Scheme I (R = *n*-octyl) was conceived with two strategic strictures in mind: (1) an intermediate (e.g., carboxylic acid **5a**) was desired which could be resolved and later used to ascertain the absolute configuration

Scheme I



a, $CH_3C(OEt)_3$, EtCOOH, 135 °C: b, $(i\text{-Bu})_2AlH/C_6H_6$–hexane, 0 °C; c, CBr_4–Ph_3P/CH_2Cl_2, 0 °C; d, Mg/THF; e, CO_2; f, H_3O^+; g, MeOH, *p*-TsOH; h, $(i\text{-Pr})_2NLi$/THF, −78 °C; i, PhSeSePh; j, $NaIO_4$/THF–H_2O, 25 °C, 10 h

(as yet unknown) of the allenic moiety, and (2) the instability inherent in the conjugated allenenic ester suggested that the introduction of the completely conjugated chromophore be relegated to the last step of the synthesis. A key intermediate **5** which fulfills these requirements was prepared in essentially four steps from the acetylenic alcohol **1**[7] (Scheme I).

A modified Claisen rearrangement[8,9] converted **1** to the β-allenic ester **2** in 95% yield. Reduction of **2** to the corresponding alcohol **3** with lithium aluminum hydride gave poor yields of **3**; rather, the predominant reaction was proton abstraction from the highly activated position α to the ester function (as evideced by copious gas evolution) whereupon subsequent aqueous workup returned an isomeric mixture of methyl trideca-2,4-dienoates as the major product.[10] The desired reduction was successfully achieved in 70% yield with diisobutylaluminum hydride.

The reaction of the homoallenic alcohol **3** with PBr_3 under a variety of conditions gave poor yields of the desired substitution product **4**.[11] However, the bromide **4** was conveniently prepared in 76% yield from the alcohol **3** using CBr_4–Ph_3P in CH_2Cl_2 at ice bath temperatures. Although the analogous chlorination with CCl_4–PPh_3 has been amply documented,[12] the corresponding bromination has received only sporadic attention[13] despite the often preferable reaction properties of the bromides. Since we could find no systematic evaluation of the scope and limitation of this mild bromination, we have examined a number of additional cases. Invariably, primary alcohols gave good yields of the bromide (see Table I). With the exception of 2-octanol, which gave a 90% yield of 2-bromooctane, secondary alcohols such as 3-pentanol and cyclohexanol gave consistently poor yields of the bromide. Thus the synthetic utility of the reaction appears to be restricted to primary bromides, in which case we found that best yields were obtained when the reactions were run in CH_2Cl_2 at 0–25 °C in the presence of 1.25 equiv of CBr_4 and 1.5 equiv of Ph_3P.