BIOSYNTHETIC RELATIONSHIPS AMONG THE SECALONIC ACIDS

ISOLATION OF EMODIN, ENDOCROCIN AND SECALONIC ACIDS FROM *PYRENOCHAETA TERRESTRIS* AND *ASPERGILLUS ACULEATUS**

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Cynodontin, emodin, endocrocin and secalonic acids A, E and G have been isolated from five strains of *Pyrenochaeta terrestris*. *Aspergillus aculeatus* produces emodin, endocrocin and secalonic acids B, D and F. No cynodontin was detected. Isolation of emodin in small amounts supports previous evidence that it is an intermediate in secalonic acid biosynthesis. Isolation of cynodontin and endocrocin, which are co-produced with secalonic acids in other organisms, suggests that these compounds are formed by a common branching pathway. A natural isolate of *P. terrestris* contained variant strains which produced different relative amounts of secalonic acids A, E and G. From the combinations of secalonic acids produced in organisms so far examined it is concluded that precursor tetrahydroxanthone units are formed in pairs differing in stereo-chemistry only at C-5 or at the *trans*-invariant C-6 : C-10a positions. A possible biosynthetic pathway is discussed.

Secalonic acids are members of the ergochrome group of secondary metabolites, reviewed by FRANCK and FLASCH[1]. Seven different kinds have been isolated from fungi and lichens (Fig. 1). Secalonic acids A (**1**), B (**2**) and C (**3**) were obtained from *Claviceps purpurea*[2,3], secalonic acids A, E (**4**) and G (**5**) from *Pyrenochaeta terrestris*[4,5], and secalonic acids D (**6**) and F (**7**) from *Aspergillus aculeatus*[6]. Secalonic acids A and C have also been obtained from *Cetraria ornata*[7], secalonic acid A was found in *Aspergillus ochraceus*[8] and *Parmelia entotheiochroa*[9], and secalonic acid D was isolated from *Penicillium oxalicum*[10]. We report here, in addition, the isolation of secalonic acid B from *A. aculeatus* strain MIT-M29.

In *C. purpurea* and *P. oxalicum* secalonic acids are reported to incorporate label from [^{14}C]emodin and [^{3}H]emodin but not from [^{14}C]endocrocin[1,11]. Lack of incorporation from the latter is not unexpected since STEGLICH *et al.*[12] have shown that, in *Dermocybe sanguinea*, emodin is formed independently and not by decarboxylation of endocrocin. That emodin is a precursor is more surprising, since this compound possesses a C-3 hydroxyl that is not present in the secalonic acids. Both emodin (**8**) and endocrocin (**9**) have been found in *D. sanguinea* and *D. semisanguinea*[13], *Penicillium islandicum*[14] and *Penicillium tardum*[15]. Endocrocin has been isolated along with secalonic acids from *C. purpurea*[16] and *Cetraria ornata*[7]. However, endocrocin has not been identified in other secalonic acid-producers and there is no evidence that emodin is a normal metabolite of *C. purpurea* and *P. oxalicum*. We

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report here the isolation of both emodin and endocrocin from five strains of *P. terrestris* and one strain of *A. aculeatus*.

Cynodontin (**10**) was initially isolated from *Helminthosporium cynodontis* and *H. euchlaenae*[17]; it has since been found in some other *Helminthosporium* species[18~20] and in *Phoma* species[21~23]. We have now found this compound in *P. terrestris*.

From the observation that *P. terrestris* and *A. aculeatus* each produce three different secalonic acids, some biosynthetic relationships can be deduced.

Fig. 1. Biosynthetic relationships among the secalonic acids*.

Organism	Monomeric precursor	Secalonic acid (2,2'-dimer)
Claviceps purpurea	A →	A (A-A) **1**
Cetraria ornata	A, B →	C (A-B) **3**
	B →	B (B-B) **2**
Pyrenochaeta terrestris	A →	A (A-A) **1**
	A, E →	G (A-E) **5**
	E →	E (E-E) **4**
Aspergillus aculeatus	B →	B (B-B) **2**
	B, D →	F (B-D) **7**
	D →	D (D-D) **6**

* Secalonic acid A has been isolated from *Aspergillus ochraceus* and *Parmelia entotheiochroa*; secalonic acid D has been isolated from *Penicillium oxalicum*.

Materials and Methods

Cultures

Pyrenochaeta terrestris strain C 44-1 was obtained from Dr. W. M. HESS of Brigham Young University, Provo, Utah. *Pyrenochaeta terrestris* strain T-66 and T-67 were obtained from Dr. D. C. FOLEY of Iowa State University, Ames, Iowa. *Aspergillus aculeatus* strain MIT-M29 was obtained from Dr. A. L. DEMAIN of the Massachusetts Institute of Technology, Cambridge, Massachusetts.

Media

The inoculum medium for *P. terrestris* consisted of (per liter): D-glucose, 10 g; Neopeptone, 5 g; and corn steep liquor, 5 ml. Production medium for this fungus contained (per liter): soluble starch, 50 g; and Neopeptone, 10 g. The inoculum medium for *A. aculeatus* was similar to that used by DEMAIN and co-workers[24] but with corn flour replacing yeast extract for improved sporulation. It consisted of (per liter): sucrose, 30 g; corn flour, 5 g; Casamino acids, 5 g; $NaNO_3$, 3 g; K_2HPO_4, 1 g; $MgSO_4 \cdot 7H_2O$, 0.5 g; KCl, 0.5 g; $FeSO_4 \cdot 7H_2O$, 0.01 g; and agar, 15 g. Two production media were used for this fungus. The first, consisting of corn flour (60 g per liter) in water, has been used previously[6]. The second contained non-glutinous rice flour (120 g/liter) in water and supported a higher yield of secalonic acids than did the corn flour suspension.

Isolation of Substrains

Portions of a soil stock culture of *P. terrestris* strain C 44-1 were suspended in sterile distilled water and plated on potato-dextrose agar. Differently colored colonies were transferred to fresh potato-dextrose-agar plates to form single giant colonies. Spores from such colonies were replated in the same manner and the procedure repeated until colonies of uniform and constant morphology and pigmentation were obtained. Substrain C 44-1 A formed grayish-green colonies and was easily isolated after one transfer; isolation of the yellow C 44-1 B and pink C 44-1 C substrains required at least three transfers.

Fermentation

All cultures grown in Erlenmeyer flasks were incubated at 25°C on a platform rotating at 220 rpm.

Spores and mycelium of *P. terrestris* grown on potato-dextrose-agar slants for two weeks were transferred to 50 ml of inoculum medium in a 250-ml Erlenmeyer flask and incubated for 4 days. The mycelium was homogenized and 2.5-ml portions were used to inoculate 50-ml portions of production

medium in 250-ml Erlenmeyer flasks. The cultures were incubated for 12 days.

Spores of *A. aculeatus*, grown for 2 weeks at 25° on a thin-layer of inoculum medium in 120-ml milk-dilution bottles, were collected by suspending in 10 ml of 0.01% (w/v) sodium lauryl sulfate solution; 2.5-ml portions were used to inoculate 50-ml portions of production medium in 250-ml Erlenmeyer flasks. The cultures were incubated for 12 days.

Isolation and Purification

Mycelium collected by filtration was leached with acetone and the residue obtained on removing solvent *in vacuo* was partitioned at pH 3 between water and ethyl acetate. The residue from evapo-

Table 1. Analytical and spectral properties of the metabolites.

Compound	M.p. (°C)	$[\alpha]_D^{20}$ (*c*) in pyridine	*m/e*	λ^*_{max}	ν^{KBr}_{max} (cm^{-1})
Secalonic acid A	208~209	−200.1° (*c* 0.275)	638, 620, 602 588, 579	239 (4.24) 267 (4.19) 338 (4.51)	3450 m (H-bonded OH) 1735 m (aliphatic C=O) 1605 s (H-bonded C=O)
Secalonic acid B	251~253	+201.2° (*c* 0.115)	638, 620, 602 588, 579	240 (4.31) 265 (4.26) 338 (4.53)	3600 m (free OH) 3180 m (H-bonded OH) 1735 m (aliphatic C=O) 1600 s (H-bonded C=O)
Secalonic acid D	207~208	+198.4° (*c* 0.256)	638, 620, 602 588, 579	239 (4.26) 267 (4.21) 338 (4.53)	3450 m (H-bonded OH) 1735 m (aliphatic C=O) 1605 s (H-bonded C=O)
Secalonic acid E	255~256	−202.4° (*c* 0.561)	638, 620, 602 588, 579	240 (4.29) 265 (4.24) 338 (4.51)	3600 m (free OH) 3180 m (H-bonded OH) 1735 m (aliphatic C=O) 1600 s (H-bonded C=O)
Secalonic acid F	242~243	+201.1° (*c* 0.306)	638, 620, 602 588, 579	240 (4.23) 265 (4.23) 338 (4.56)	3600 m (free OH) 3450 m (H-bonded OH) 3180 w (H-bonded OH) 1735 m (aliphatic C=O) 1600 s (H-bonded C=O)
Secalonic acid G	206~207	−201.6° (*c* 1.105)	638, 620, 602 588, 579	240 (4.32) 265 (4.26) 338 (4.57)	3600 m (free OH) 3450 m (H-bonded OH) 3180 w (H-bonded OH) 1735 m (aliphatic C=O) 1600 s (H-bonded C=O)
Emodin	257~259		270, 255, 253 242, 241, 225 214	217 (4.48) 254 (4.31) 292 (4.22) 303 (4.18) 448 (3.92) 504 (3.65)	3400 m (H-bonded OH) 1620 s (H-bonded C=O)
Endocrocin	289~314 (decomposes)		314, 296, 270 255, 253, 242 241, 225, 214	227 (4.39) 256 (4.20) 274 (4.25) 287 (4.16) 312 (4.04) 441 (3.95)	3390 m (H-bonded OH) 1712 m (aliphatic C=O) 1610 s (H-bonded C=O)
Cynodontin	261~262		286, 269, 257 239	238 (4.11) 296 (3.47) 474 (3.50) 483 (3.56) 505 (3.69) 516 (3.74) 542 (3.71) 554 (3.74)	3420 m (H-bonded OH) 1575 s (H-bonded C=O)

* λ_{max} for secalonic acids were measured in dioxane and for other compounds in ethanol.

rating the ethyl acetate extract was fractionated by chromatography on a column of silicic acid using benzene-ethyl acetate mixtures for elution. Products were recovered at the following elutriant ratios: cynodontin, 19: 1; emodin 9: 1; secalonic acids B or E, 6: 1; endocrocin and secalonic acids F or G, 4: 1; and secalonic acids A or D, 2: 1. Endocrocin was separated from secalonic acid F or G by partitioning the mixture between ethyl acetate solution and 1% (w/v) aqueous $NaHCO_3$. No further fractionation was required since the enantiomeric secalonic acids that eluted at the same benzene-ethyl acetate ratios did not occur together in cultures. Secalonic acid A and secalonic acid D each crystallized as orange needles from ethyl acetate - ethanol (2: 1), secalonic acid B and secalonic acid E as pale-yellow prisms from chloroform, secalonic acid F and secalonic acid G as yellow needles from ethanol, cynodontin as reddish-orange plates from pyridine, emodin as orange plates from chloroform, and endocrocin as reddish-orange plates from pyridine. Some physical properties of these compounds are listed in Table 1.

Acetylation of Cynodontin

Cynodontin (31 mg) was acetylated with acetic anhydride (1 ml) in pyridine (1 ml) for 90 minutes at room temperature. After crystallization from methylene chloride and ethanol (3: 1), cynodontin tetraacetate (**11**) was obtained as yellow plates (48 mg): m.p. 202~203°C; *m/e* at 454, 412, 384, 370, 354, 328, 312, 286; $\lambda_{max}^{ethanol}$ (log ϵ) 254 (3.94), 345 (4.35) nm; ν_{max} (KBr) 1755 cm^{-1} (C=O).

Results and Discussion

NMR Assignments

^1H nmr assignments for **8**, **9** and **11** were straightforward (Table 2), based on the effect of substituents on chemical shifts[25] and multiple irradiation experiments. Chemical shift information on **8**[26~28] and more complete data on its trimethylsilyl derivative[29,30] are available in the literature but no ^1H nmr studies on **9**, **10** and **11** have been reported previously.

Assignment of resonances in the ^1H broadband-decoupled ^{13}C spectrum of **9** (Table 3) is based on the correlation of ^1H and ^{13}C spectra by off-resonance decoupling (ORD)[31] multiplicities in the high resolution (HR) spectrum and on substituent effects observed for a variety of anthraquinones[32,33]

Table 2. ^1H Nmr data for emodin (**8**), endocrocin (**9**) and cynodontin tetraacetate (**11**)*.

	H-2	H-4	H-5	H-7	H-11	1-OH	8-OH	3-OH
8	6.54 d $J_{2,4}$ 2.4	7.05 d	7.40 bdq $J_{5,7}$ 1.6 $J_{5,11}<0.4$	7.08 bdq $J_{7,11}$ 0.7	2.38 bdd	11.94 s	12.02 s	11.31 bs
	H-4		H-5	H-7	H-11	1,6,8,12-OH		
9	7.37 bs		6.99 d $J_{5,7}$ 2.4	6.48 d	2.37 bs	7.40~9.30 bs		
	H-3		H-6	H-7	H-11	1-$COCH_3$	4,5,8-$COCH_3$	
11	7.27 q $J_{3,11}$ 0.7		7.37 s	7.37 s	2.29 d	2.42 s	2.38 s	

* Spectrometer, Varian HA-100; frequency 100.1 MHz; compounds **8** and **9** in dimethylsulphoxide-d_6 and **11** in methylene chloride-d_2; chemical shifts in ppm relative to TMS; coupling constants ($J_{p,q}$) in Hz.

Table 3. ^{13}C Nmr data for endocrocin (**9**)*.

C-1	C-2	C-3	C-4	C-4a	C-5	C-6	C-7
157.90	130.26	143.58	120.44	134.65†	108.94	165.68	107.83
s	dq	dq	dq	d	dd	dd	dd
	^3J_d 6.7	^2J_d 1.4	^1J 166.6	^2J 1.0	^1J 166.3	^2J 1.8	^1J 162.9
	^3J_q 4.9	^2J_q 6.1	^3J 5.2		^3J 5.4	^2J 3.2	^3J 4.5
C-1	C-8a	C-9	C-9a	C-10	C-10a	C-11	C-12
164.47	108.75	189.12	113.54	180.53	132.17†	19.69	167.04
d	dd	d	d	dd	s	dq	bs
^2J 3.3	^3J 5.3	^4J 0.9	^3J 6.2	^3J 4.3		^1J 128.3	
	^3J 6.5			^3J 4.4		^3J 4.5	

* Spectrometer, Varian XL-100/15 FOURIER transform; frequency 25.16 MHz; acquisition time 1.6 s (^1H broadband decoupled) and 3.2 s (high-resolution), data accuracy ± 0.3 and ± 0.15 Hz; spectral width 5120 Hz; flip angles 40 and 48°; internal pulse lock to ^2H in DMSO-d_6 (solvent); temperature 30°C, concentration 90.1 mg in 0.5 ml, 5 mm tube; internal reference TMS. For off-resonance decoupling, protons at δ_H 6.79 ppm ($\gamma Hz/2\pi \sim 400$ Hz) and 7.79 ppm (1600 Hz) were irradiated. Broadband ^1H decoupling was by phase modulation of decoupling field from 0 to 180° with $\gamma Hz/2\pi$ 3800 Hz.

† Assignments may be interchanged.

and naphthalenes[34,35]. Thus C-4, C-5, C-7 and C-11 were identified by the ORD experiments and the characteristic low-field signals for the anthraquinone-type carboxyl carbons[32,33] could be readily distinguished because C-10 was coupled to H-4 and H-5, whereas C-9 was coupled to only one hydrogen, probably H-7. The poorly relaxed carboxyl carbon (C-12) also resonated in a typical region of the spectrum. Other low field resonances arising from carbons bonded to hydroxyl groups were also readily identified because C-6 was coupled to H-5 and H-7 and C-8 to H-7, whereas C-1, as expected, was not similarly coupled. Only two of the quaternary aromatic carbons (C-4a and C-10a) could not be assigned unequivocally, the remainder being easily recognized from their chemical shifts and characteristic multiplicities in the HR spectrum. Thus, C-2 was coupled to H-4 and H-11, C-3 to H-4 and H-11, C-8a to H-5 and H-7 and C-9a to H-4.

Yields of Metabolites

Yields of purified metabolites recovered from typical production cultures are recorded in Table 4. Emodin and endocrocin are produced by both *P. terrestris* and *A. aculeatus*, but cynodontin is produced only by *P. terrestris*. Strains C 44-1 A and C produced cynodontin in quite high yield. No other aromatic metabolites were present in substantial amounts and, in particular, no accumulation of xanthone precursors of secalonic acid was found.

Emodin, endocrocin and the secalonic acids are yellow; cynodontin has a very strong pink color. Since emodin and endocrocin are produced in only small amounts and the proportion of secalonic acid G is also quite low, these substances make little contribution to the color of a culture. The color of *P. terrestris* colonies is accounted for by the amounts of secalonic acids A and E relative to cynodontin. Strains C 44-1 B, T-66 and T-67, which produce secalonic acids A and E predominantly, are yellow, whereas strain C 44-1 C, which produces a higher proportion of cynodontin, is pink. Strain C 44-1 A produces large amounts of both secalonic acids and cynodontin, giving it a brownish green color. Because *A. aculeatus* produces secalonic acids D and F and no cynodontin the fermentation broth is yellow.

Table 4. Production (mg/liter) of pigments by *Pyrenochaeta terrestris* and *Aspergillus aculeatus**.

		Secalonic acid			Emodin	Endocrocin	Cynodontin
		A	E	G			
P. terrestris	C 44-1 A	969	787	143	2.5	11.9	47.6
	C 44-1 B	590	427	67.1	1.3	6.0	18.8
	C 44-1 C	63.2	370	38.4	1.7	3.4	50.7
	T-66	331	525	55.3	0.9	15.2	1.8
	T-67	422	372	70.1	1.6	10.6	12.8
		B	D	F			
A. aculeatus	Corn flour medium	3.7	116	90.4	6.0	13.6	Nil
	Rice flour medium	9.7	409	347	1.3	5.6	Nil

* Each strain was grown in 4 litres of medium; production is recorded as the yield of product obtained after one crystallization.

Biosynthetic Relationships

Secalonic acids, A, B and C, have been isolated from *C. purpurea*[2,3]. We isolated three secalonic acids, A, E and G, from *P. terrestris*[5] and we have now found three different secalonic acids, B, D and F, to be produced by *A. aculeatus*. Secalonic acid C is a "hybrid dimer" containing the monomeric units of both secalonic acids A and B[2]; secalonic acid G similarly contains a monomeric unit from each of A and E[5], while secalonic acid F is a hybrid of B and D[6]. The simplest interpretation of these observations is that each fungus produces two different monomeric precursor units which are then coupled to give three different secalonic acids (Fig. 1). The exact nature of these precursor units has not been established, but we have shown that dimerization occurs after formation of the xanthone ring system[3,6]. While it is not known whether insertion of the oxygen function at C-5 and reduction of the xanthone structure occurs before or after 2,2′-coupling, the absence of dimeric products in which the two halves of the molecule are at different oxidation levels argues in favour of the first possibility. Thus, although alternative sequences are not excluded, the present information on the biosynthesis of secalonic acids can be accommodated by the biosynthetic pathway shown in Fig. 2.

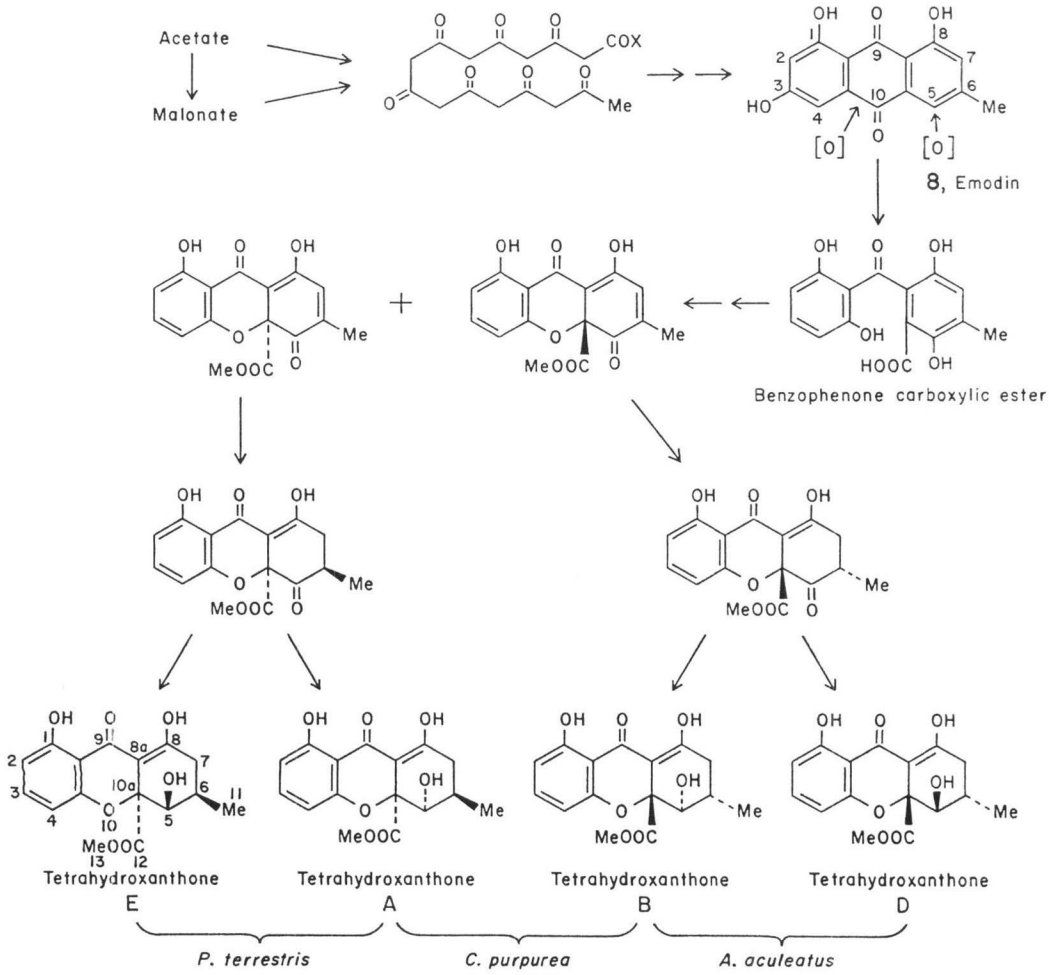
Taking into account the contributions from both "pure" and "hybrid" secalonic acids we have calculated the amounts of each postulated tetrahydroxanthone monomer that must be made by the fungi (Table 5). Since the metabolites are excreted from the mycelium and collect as an insoluble residue in the culture medium, degradation, if it occurs, is probably slow. The values obtained are, therefore, assumed to represent reasonably well the total biosynthetic commitment.

Table 5. Production (mg/liter) of postulated tetrahydroxanthone intermediates A, B, D and E*.

		Tetrahydroxanthone monomer		Ratio
		A	E	A/E
P. terrestris	C 44-1 A	1,041	859	1.21
	C 44-1 B	624	461	1.35
	C 44-1 C	82.4	389	0.21
	T-66	359	553	0.65
	T-67	457	407	1.12
		B	D	B/D
A. aculeatus	Corn flour medium	48.9	161	0.30
	Rice flour medium	183	583	0.31

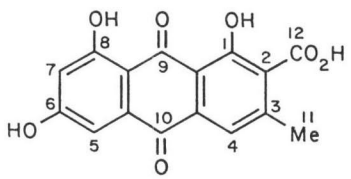
* Calculated from the data of Table 4.

Fig. 2. A possible route for the formation of stereoisomeric tetrahydroxanthone precursors of secalonic acids.

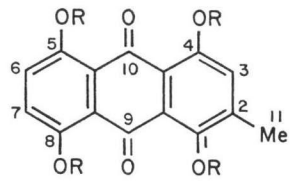


Synthesis of the tetrahydroxanthone A and E monomers in *P. terrestris* is reflected closely in the yields of secalonic acids A and E because of the low yield of secalonic acid G. The ratio of A: E monomers differs for the five strains of *P. terrestris*. It is high for strains C 44-1 A, C 44-1 B and T-67, and low for strains C 44-1 C and T-66.

In the secalonic acids produced by *A. aculeatus* the amount of tetrahydroxanthone B component is quite substantial. Nevertheless, the ratio of B: D monomers favours the latter so that strain MIT-M29 is predominantly a type-D producer. It is possible that *Penicillium oxalicum*[10] is a more extreme example of the same type since only secalonic acid D was isolated from this fungus. Previous studies[6] on secalonic acid production by *A. aculeatus* failed to detect secalonic acid B and the yield was very low in our experiments. The tetrahydroxanthone B monomer is mainly sequestered in the hybrid secalonic acid F. A parallel situation appears to exist in the lichen *Cetraria ornata* from which only secalonic acids A and C have been isolated[7]. Here the tetrahydroxanthone B monomer is predominantly, if not entirely, combined with the A monomer to form secalonic acid C. *C. ornata* can thus be classified in the same group as *C. purpurea*. Other possible members of this group are the fungus *Aspergillus*



9 Endocrocin



10 R = H, Cynodontin

11 R = COMe, Cynodontin tetraacetate

ochraceus[8] and several lichens of the genus *Parmelia*[9], from which only secalonic acid A has been isolated.

Clearly it is not possible to postulate a biosynthetic pathway in which secalonic acids are formed by dimerization of completed tetrahydroxanthone units without also accounting for a substantial measure of specificity in the coupling reaction. In the species where linking of two tetrahydroxanthone B units is not favoured, reaction between one such unit and a tetrahydroxanthone A or tetrahydroxanthone D unit appears to compete strongly with A-A or D-D dimerization. One possible explanation is that the enzyme responsible for coupling the precursors in these organisms has marked specificity for the initial monomeric substrate bound, but is relatively non-specific in binding the second, recognizing primarily the benzenoid ring. Thus while the B monomer is virtually excluded from initial binding and so cannot readily form "pure" dimers it can compete with the A or D monomers to form a hybrid product. The proportion of pure non-B and hybrid dimers formed would then reflect precursor availability, although a close correspondence in the ratios would not be expected because of sequestration of the non-B monomer at the initial binding site.

The foregoing model can be adapted to explain the results in *P. terrestris* where much less secalonic acid G than either A-A or E-E dimers is formed. Here the enzyme responsible for coupling is assumed to be less specific than that in other species, so that the initial binding site can be occupied by either of the tetrahydroxanthone A or E monomers. The almost exclusive formation of "pure" dimers is most readily explained by spatially separate synthesis of the two types of monomeric precursors. Since no accumulation of xanthone intermediates was detected, dimerization of the completed monomer probably occurs close to the site of formation at an enzyme surface with high substrate affinity. As a result there would be a strong probability that the second substrate bound would be of the same type as the first, even though the enzyme has little selectivity.

Although four isomeric tetrahydroxanthones can be formed during cyclization and reduction of the benzophenone carboxylic acid ester intermediate, each organism appears to make only two. The pairs differ in configuration either at the single location, C-5, or at the *trans*-substituted, C-6 and C-10a positions. Cyclization can generate two products, differing in configuration at C-10a. The tetrahydroxanthone components of the secalonic acids produced by *P. terrestris* have the same stereochemistry at this position, indicating that the cyclization is catalysed by a specific enzyme giving a single product. This is true also of *A. aculeatus*, except that the products have the opposite configuration at C-10a. On the other hand, the tetrahydroxanthone components of *C. purpurea* and *C. ornata* show both configurations, implying that in these organisms the cyclization is not stereospecific.

In view of the uniformly *trans* orientation of the C-6 and C-10a substituents it is likely that the

C-10a configuration determined during cyclization directs the stereochemistry of $\Delta^{6,7}$ reduction. For this reason the $\Delta^{6,7}$ reduction step is shown as the penultimate reaction in Fig. 2 and is followed by reduction of the C-5 carbonyl. In *P. terrestris* and *A. aculeatus* this latter reduction lacks stereospecificity whereas *C. purpurea* and *C. ornata* produce secalonic acids in which the tetrahydroxanthone components have a uniform configuration. It is of interest that the D: E combination of tetrahydroxanthone pairs, differing from A and B only in having the alternative configuration at C-5, has not so far been encountered among secalonic acid producers. The literature likewise contains no report of an organism producing secalonic acids formed by the coupling of A-D or B-E precursors which would be expected if both the cyclization and C-5 reduction lacked stereospecificity.

Emodin has been found in numerous fungi[37] and is frequently converted to more highly substituted and complex metabolites[38]. If it is an intermediate in the biosynthetic pathway to secalonic acids, as proposed for *P. oxalicum* and *C. purpurea*, it should be formed in all fungi and lichens that produce these compounds. The previous absence of any report of its co-occurrence with the secalonic acids raised a question about its biosynthetic function in unsupplemented cultures. Our isolation of emodin supports the radiotracer evidence in showing that the compound is a normal metabolite in at least two secalonic acid producers. Moreover, the fact that we obtained it from *P. terrestris* and *A. aculeatus* in very low yield suggests that it is converted rapidly to later intermediates. The secalonic acid pathway thus appears to have a common feature with that in *Penicillium* species where emodin (or more efficiently, emodinanthrone) is metabolized to islandicin and other anthraquinonoid products with loss of the C-3 hydroxyl group[39]. Endocrocin is believed to have the same octaketide intermediate in its biosynthetic pathway as the secalonic acids but to represent a branch product[12]. Cynodontin is probably also in this category; the relatively high yield of these pigments compared to that of emodin suggests that, like the secalonic acids, they are terminal metabolites.

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