Variecolactol: A New Sesterterpene Lactone from the Sclerotia of *Aspergillus auricomus* (Gueguen) Saito

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ABSTRACT

Variecolactol (**1**), a new sesterterpene lactone related to variecolin (**2**), has been isolated from the organic extracts of the sclerotia of *Aspergillus auricomus*. Structure determination of this compound was achieved primarily through HMQC, HMBC, and NOESY experiments. The known compounds dihydropenicillic acid (**3**) and penicillic acid (**4**) were also isolated from *A. auricomus*.

Keywords: Aspergillus auricomus, sclerotia, sesterterpene

INTRODUCTION

Sclerotia are relatively large resting bodies produced by many fungi as a mechanism for their long-term survival and propagation of the species (Wicklow et al., 1988; Gloer et al., 1988). Studies on the chemical constituents of the sclerotia of various *Aspergillus* species have yielded a variety of new natural products with anti-insectan activity (de Guzman et al., 1992; de Guzman et al., 1994; Laakso et al., 1992; Laakso et al., 1994; Whyte et al., 1996; TePaske et al., 1989) and other biological activities (Staub et al., 1992; TePaske et al., 1989; Laakso et al., 1992; de Guzman et al., 1994). We report here the isolation and structure elucidation of variecolactol (**1**), a new sesterterpene lactone related to variecolin (**2**), from the sclerotia of *Aspergillus auricomus* (Gueguen) Saito (NRRL 391). The known compounds dihydropenicillic acid (**3**) and penicillic acid (**4**) were likewise isolated from the semipolar extracts of the sclerotia of *A. ausricomus.*

MATERIALS AND METHODS

General

A culture of *A. auricomus* (NRRL 391) was obtained from the ARS culture collection at the USDA National Center for Agricultural Utilization Research, Peoria, IL. Sclerotia were prepared by solid substrate fermentation on autoclaved corn kernels using general procedures which have been previously described (Wicklow et al., 1988). ^1H and ^{13}C NMR data were obtained in $CDCl_3$. Carbon multiplicities were determined through DEPT experiments. COSY, NOESY, HMQC, and HMBC, experiments were conducted at 600 MHz (^1H dimension) using a Bruker AMX600 spectrometer. HMQC and HMBC data were optimized for J values of 152 and 8 Hz, respectively. All other ^1H and ^{13}C NMR data were obtained using a Bruker AC300 NMR

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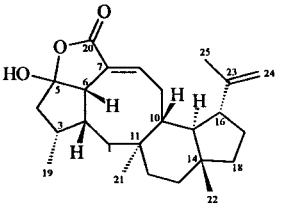
spectrometer. HREIMS data were measured with a VG ZAB-HF mass spectrometer, while the low resolution EI mass spectrum was recorded at 70 eV using a VG Trio 1 quadrupole mass spectrometer. A Beckman ultrasphere 5m 10 mm x 25 cm C_{18}-reversed phase column was used in all HPLC separations. Details of other general experimental procedures have been described elsewhere (Wicklow et al., 1988; Gloer et al., 1988; Gloer et al., 1989).



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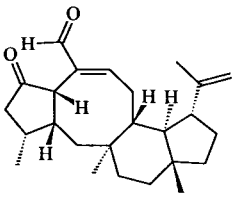

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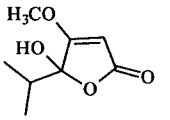

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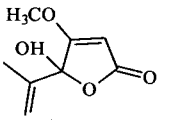

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Extraction and isolation of variecolactol (1)

The sclerotia of *Aspergillus auricomus* (Gueguen) Saïto (NRRL 391) were produced by solid substrate fermentation on autoclaved corn kernels and harvested as described previously (Wicklow et al., 1988). The intact sclerotia (89 g) were extracted at room temperature with hexane (2x500 mL). After removal of the solvent in vacuo, the hexane extract (209 mg) was subjected to flash chromatography using increasing concentrations of EtOAc in hexane as eluent. The fraction that eluted with 8:2 hexane-EtOAc was then subjected to reversed phase HPLC using MeOH to give variecolactol (**1**), (2.1 mg).

A second batch of sclerotia (367.5 g) was ground, then Soxhlet extracted with pentane (1.5 L), and then with CH_2Cl_2 (1.5 L). After removal of the solvent in vacuo, the pentane extract (1.25 g) was likewise subjected to flash chromatography and reversed phase HPLC to give additional variecolactol (4.8 mg).

The CH_2Cl_2 extract, after removal of the solvent, was subjected to flash chromatography using increasing concentrations of EtOAc in hexane, then increasing concentrations of $CHCl_3$ in MeOH. The fraction that eluted with EtOAc was purified by reversed phase HPLC using 1:1 MeOH-H_2O to give dihydropenicillic acid (**3**), (61.4 mg) and penicillic acid (**4**), (7.3 mg).

Table 1. NMR data for variecolactol (1) in $CDCl_3$.

C#	δC[a]	δH[b]	Mult, J_{HH}(H_z)	HMBC[b](C#)	NOESY[b](#H)
1	40.9	1.49α	m	11, 21	1β, 21
		1.08β	dd, 7.3, 0.9	11, 21	1α, 21
2	39.8	2.78β	m	3, 6, 7, 11	3, 6
3	38.0	2.23β	d, 9.7	2, 4, 5	2, 19
4	44.9	2.22β	m	2, 3, 5, 6, 19	4α
		2.09α	t, 9.7	2, 3, 5, 6, 19	4β
5	115.2				
6	51.8	3.57β	dq, 5.2, 1.1	3, 7, 8, 20	2, 10
7	125.2				
8	144.6	6.98	ddd, 2.4, 1.4, 1.0	6, 7, 9, 10, 20	9β
9	29.7	2.75β	m	7, 8, 10, 11, 15, 20	10
		2.13α	m	8, 10, 15	8, 15, 21
10	38.7	2.14β	m	9, 14, 15	6, 22
11	39.0				
12	34.4	1.94β	m	11, 13, 21	12α
		0.97α	m		12β
13	35.1	1.48	m	12, 14, 22	21
14	43.4				
15	48.0	1.48α	m	9, 10	9α,25
16	48.1	2.35β	ddd, 15.4, 8.1, 4.9	10, 15, 23, 24, 25	17β, 22, 24Z
17	30.0	1.97β	m	16	16,17α
		1.30α	m	14, 18, 22	17β
18	39.9	1.44β	m	14, 22	18α, 22
		1.22α	q, 5.2	14	18β
19	15.9	0.68	d, 7	3, 4	3, 4α
20	170.6				
21	21.8	0.89α	s	1, 10, 11, 12	1a, 9a, 13α
22	18.2	0.84β	s	13, 14, 18	10, 16, 18β
23	150.5				
24	110.4	4.94Z	dd, 0.8, 0.3	16, 25	16
		4.61E	dd, 0.8, 1.3	16, 25	25
25	19.3	1.67	s	23,24	15, 24E

[a]75 MHz; [b]600MHz

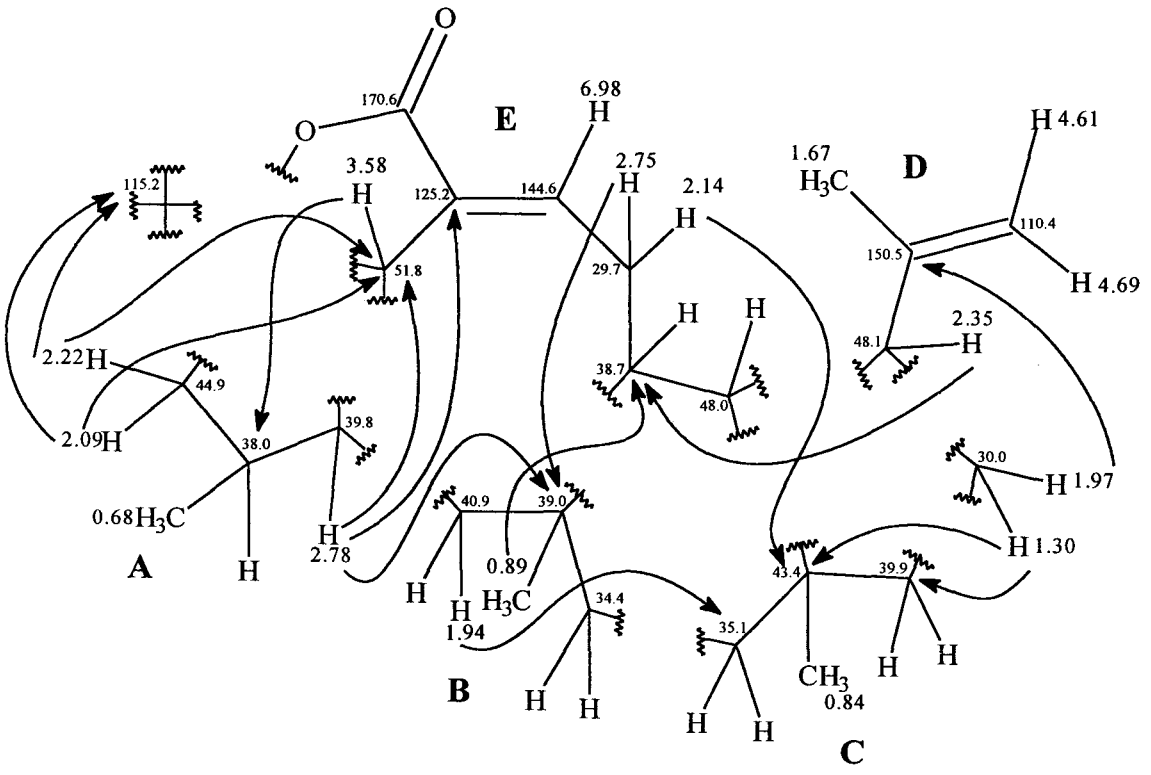

Fig. 1. Partial structures for variecolactol.

Variecolactol (**1**) was isolated as a white solid; HPLC t_R 9.7 min under the conditions above; UV (MeOH) λ_{max} 235 nm (log ε 3.7); EIMS (70eV) *m/z* (rel. int.) 384 (M^+, 2), 366 (5), 274 (17), 256 (25), 167 (22), 149 (88), 69 (100); ^1H and ^{13}C NMR data, Table 1; HREIMS, obsd 384.2656, calcd for $C_{25}H_{36}O_3$, 384.2664.

RESULTS AND DISCUSSION

Variecolactol, isolated from the nonpolar (hexane or pentane) extracts of the sclerotia of *Aspergillus auricomus* had the molecular formula $C_{25}H_{36}O_3$, based on the high resolution EIMS data (M^+ at *m/z* 384.2656, Δ-0.8). Analysis of the ^1H, ^{13}C NMR, HMQC and DEPT data (Table 1) of variecolactol showed the presence of three methyl singlets, one methyl doublet, seven sp^3 methylenes, a terminal alkene, six sp^3 methines, one sp^2 methine, two sp^3, and two sp^2 quaternary carbons, a carbonyl carbon at δ170.6 and a carbon at δ115.2.

Analysis of the HMBC correlations (Table 1) shown by the three methyl singlets at δ1.67, 0.84, and 0.89 and the methyl doublet at δ0.68 gave four partial structures, A-D (Fig. 1). Partial structure E was derived based on the following HMBC results. The vinylic proton at δ6.98 was found to correlate with the carbons at δ170.6, 125.2, 51.8, 29.7, and 38.7. The hydrogen at δ3.58 correlated with the carbons at δ170.6, 125.2, and 144.6 while the hydrogen at δ2.75 was long-range also coupled with the carbons at δ144.6, 125.2, and 38.7, as well as with the carbon at δ48.0. Thus, the carbon bearing the hydrogen at δ3.58 and the carbonyl carbon at δ170.6 must both be attached to the carbon at δ125.2, while the carbon bearing the hydrogen at δ2.75 should be attached to the carbon at δ144.6. The downfield shift of the carbon at δ144.6 can be accounted for by its position beta to the carbonyl.

HMBC correlations were found between the hydrogen at δ2.78 of partial structure A and the carbons at δ51.8 and 125.2 of partial structure E. The proton at δ3.58 showed a correlation with the carbon at δ38.0. Thus, the carbon at δ39.8 of A must be bonded to the carbon at δ51.8 of E. Correlations were also obtained between the protons at δ2.22 and 2.09 and the carbon at δ51.8 and the quaternary carbon at δ115.2. Attaching the carbons at δ44.9 and 51.8 to the carbon at δ115.2 to give a 5-membered ring accounts for all these correlations.

Partial structure B can further be connected to partial structures A and E using HMBC results. The methyl protons at δ0.89 were long-range coupled to the carbon at δ38.7, while the proton at δ2.75 was correlated with the carbon at δ39.0. Thus, carbons at δ39.0 and 38.7 must be bonded to one another. The proton at δ2.78 also showed a correlation with the carbon at δ39.0. Thus, carbons at δ39.8 and 40.9 must also be attached to one another giving rise to an 8-membered ring.

Partial structure C can also be further connected to the AEB substructure as shown by the long-range couplings between the proton at δ2.14 of AEB with the carbon at δ43.4 of partial structure C. Thus, the carbons at δ48.0 and 43.4 must be bonded together. The proton at δ1.94 of AEB was further correlated to the carbon at δ35.1 of partial structure C, inferring the attachment of the carbon at δ34.4 with the carbon at δ35.1 to form a 6-membered ring.

Partial structure D can be attached further to the 3-ring AEBC substructure as shown by the correlations between hydrogen at δ2.35 of D with the carbon of substructure AEBC at δ38.7. Thus, the carbon at δ48.1 must be attached to the carbon at δ48.0. A remaining methylene at δ1.97 and 1.3 showed correlations with the carbons at δ150.5 and 48.1, and also with the carbons at δ43.4 and 39.9. Thus, attachment of the carbons at δ48.1 and 39.9 to the carbon at δ30.0 to form a 5-membered ring could account for these correlations.

Only a hydroxyl group remains to be assigned. The only remaining position is the carbon at δ115.2. Attachment of the ester oxygen and the hydroxyl group to this carbon will account for this chemical shift, giving structure **1** for variecolactol.

The carbon skeleton of this compound is identical to that of variecolin (**2**) (Hensens et al., 1991), compound **1** being the oxidized analog of variecolin with condensation of the carboxylate with the ketone carbonyl group to form a hemiketal. Analysis of the NOESY results (Table 1) showed that the relative stereochemistry of variecolactol is the same as that for variecolin (Hensens et al., 1991). H-2, H-3, H-6, H-10, and H-22 are all syn to one another based on the NOESY correlations shown by H-6 to H-2 and H-10, H-10 to H-22, and H-2 to H-3. H-10 also showed NOESY crosspeaks with H-22, which, in turn, correlated with H-16. These results showed the relative configuration of chiral centers C-2, C-3, C-6, C-10, C-14, and C-16 as that shown in **1**. H-15 correlated with H-9α which, in turn, showed crosspeaks with H-21. These observations would indicate that these protons are syn to one another and suggest the relative stereochemistry shown in **1** for the chiral centers C-11 and C-15. The stereochemistry about C-5 can not be established by the NOESY experiment on variecolactol.

The dichloromethane extract of the sclerotia of *A. auricomus* was found to have potent antibacterial activity against *Bacillus subtilis*. Chemical investigation of this extract yielded dihydropenicillic acid (**3**) (Sassa et al., 1971) as the major component and penicillic acid (**4**) (Wilson, 1976) as the minor component. Penicillic acid was responsible for the antimicrobial activity against *B. subtilis*.

REFERENCES

de Guzman, F. S., J. B. Gloer, D. T. Wicklow, & P. F. Dowd. 1992. New diketopiperazine metabolites from the sclerotia of *Aspergillus ochraceus*. *J. Nat. Prod.* 55(7):931-939.

de Guzman, F. S., D. R. Bruss, J. M. Rippentrop, K. B. Gloer, J. B. Gloer, D. T. Wicklow, & P. F. Dowd. 1994. Ochrindoles A-D: New bis-indolyl benzenoids from the sclerotia of *Aspergillus ochraceus* NRRL 3519. *J. Nat. Prod.* 57(5):634-639.

Gloer, J. B., B. L. Rinderknecht, D. T. Wicklow, & P. F. Dowd. 1989. Nominine: A new insecticidal indole diterpene from the sclerotia of *Aspergillus nomius*. *J. Org. Chem.* 54:2530-2532.

Gloer, J. B., M. R. TePaske, J. S. Sima, D. T. Wicklow, & P. F. Dowd. 1988. Antiinsectan aflavinine derivatives from the sclerotia of *Aspergillus flavus*. *J. Org. Chem.* 53:5457-5460.

Laakso, J. A., J. B. Gloer, D. T. Wicklow, & P. F. Dowd. 1992. Radarins A-D: New antiinsectan and cytotoxic indole diterpenoids from the sclerotia of *Aspergillus sulphureus*. *J. Org. Chem.* 57:138-141.

Laakso, J. A. E. D. Narske, J. B. Gloer, D. T. Wicklow, & P. F. Dowd. 1994. Isokotanins A-C: New bicoumarins from the sclerotia of *Aspergillus alliaceus*. *J. Nat. Prod.* 57(1):128-133.

Hensens, O.D., D. Zink, J. M. Williamson, V. J. Lotti, R. S. L. Chang, & M. A. Goetz. 1991. Variecolin, a sesterterpenoid of novel skeleton from *Aspergillus variecolor* MF138. *J. Org. Chem.* 56:3399-3403.

Sassa, T., S. Hayakari, M. Ikeda, & Y. Miura. 1971. Plant growth inhibitors produced by fungi. Part 1. Isolation and identification of penicillic acid and dihydropenicillic acid. *Agric. Biol. Chem.* 35(13):2130-2131.

Staub, G. M., J. B. Gloer, D. T. Wicklow, & P. F. Dowd. 1992. Aspernomine: A cytotoxic antiinsectan metabolite with a novel ring system from the sclerotia of *Aspergillus nomius*. *J. Am. Chem. Soc.* 114:1015-1017.

TePaske, M. R., J. B. Gloer, D. T. Wicklow, & P. F. Dowd. 1989. Tubingensin A: An antiviral carbazole alkaloid from the sclerotia of *Aspergillus tubingensis*. *J. Org. Chem.* 54: 4743-4746.

Wicklow, D. T., P. F. Dowd, M. R. TePaske, & J. B. Gloer. 1988. Sclerotial metabolites of *Aspergillus flavus* toxic to a detritivorous maize insect (*Carpophilus hemipterus*, Nititulidae). *Trans. Br. Mycol. Soc.* 91(3):433-438.

Wilson, D. M. 1976. Patulin and penicillic acid. *Adv. Chem. Ser.* 149:90-109.

Whyte, A. C., J. B. Gloer, D. T. Wicklow, & P. F. Dowd. 1996. Sclerotiamide: A new member of the paraherquamide class with potent antiinsectan activity from the sclerotia of *Aspergillus sclerotiorum*. *J. Nat. Prod.* 59(11):1093-1095.