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NEW TELEOCIDIN-RELATED METABOLITES, (−)-7-GERANYLINDOLACTAM-V AND BLASTMYCETIN F, FROM *STREPTOVERTICILLIUM BLASTMYCETICUM*

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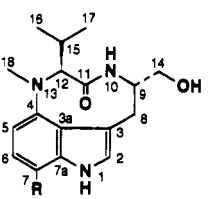
ABSTRACT.—Two new teleocidin-related metabolites, (−)-7-geranylindolactam-V [**2**] and blastmycetin F [**3**], were isolated from fermentation broths of the actinomycete *Streptoverticillium blastmyceticum* NA34-17, and their structures were determined by spectroscopic methods. Compound **2** bound strongly to phorbol ester receptors in a mouse epidermal particulate fraction, suggesting that it is a potent in vivo tumor promoter comparable to teleocidins A-1 [**4**] and B-4 [**5**].

The teleocidins (1–5) are unusual indole alkaloids containing a nine-membered lactam ring and a complex monoterpenoid moiety, which act as potent skin tumor promoters (6). Several teleocidin-producing organisms (actinomycetes and blue-green algae) have been previously reported (7,8). Among these organisms, the actinomycete *Streptoverticillium blastmyceticum* NA34-17 (9,10) is unique in producing (−)-indolactam-V [**1**] (11) in quantity, in addition to minor components. In previous publications we have reported the isolation of several teleocidin-related compounds from this actinomycete (12–15), which have contributed to research on structure-activity relationships (16,17), metabolism in mammals (18), and teleocidin biosynthesis (19,20).

Our continuing research aim to discover new teleocidin-related compounds has



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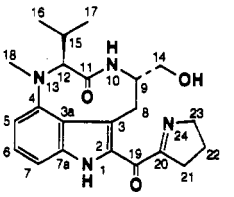

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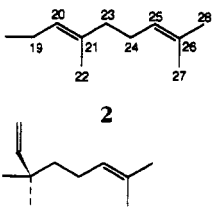

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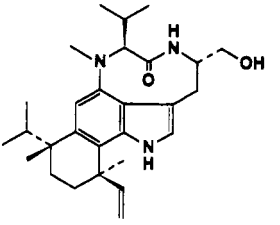

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resulted in the isolation of (−)-7-geranylindolactam-V [**2**] and blastmycetin F [**3**] from *S. blastmyceticum*. This paper describes the isolation, structural determination, and biological activities of these new metabolites.

RESULTS AND DISCUSSION

The purification of the new teleocidin-related metabolites **2** and **3** was guided by Ehrlich's reagent (21), whereby teleocidin-related compounds showed characteristic coloration on tlc (green, blue, or purple). The producing organism, *S. blastmyceticum* NA34-17, was cultured by deep-aerated fermentation for 45 h, and the mycelia (6.5 kg wet weight) were steeped in Me_2CO. Column chromatography of this extract over Si gel, followed by hplc monitored with a photodiode array detector, yielded a new metabolite, **2** (9.9 mg), having the characteristic teleocidin uv chromophore (1). Its molecular formula was determined to be $C_{27}H_{39}N_3O_2$ by high-resolution eims (observed *m/z* 437.3049; calcd *m/z* 437.3042), which was the same as that of teleocidin A-1 [**4**] (5). The ^{1}H-nmr spectrum of **2** in $CDCl_3$ (0.045 M, 27°) revealed that **2** existed as two stable conformers (11) (sofa:twist=1:2.8), clearly indicating the existence of the nine-membered lactam ring present in teleocidins. Three aromatic protons at δ 6.44 (1H, d, J=7.7 Hz), 6.86 (1H, d, J=7.7 Hz), and 6.88 (1H, s) indicated that the indole ring of **2** was substituted at position 5 or 7. Substitution at position 7 was more compatible with the chemical shifts for the two vicinal aromatic protons and with the ratio of the two conformers (22). From these observations, **2** was deduced to be a teleocidin-A type compound (5). The ^{1}H-nmr spectra of **2** furthermore revealed the presence of three methyl groups [δ 1.60 (3H, s), 1.68 (3H, s), and 1.80 (3H, s)] four allyl protons [δ 2.11 (4H, m)], two benzyl protons [δ 3.48 (2H, t, J=7.7 Hz)], and two olefin protons [δ 5.09 (1H, m) and 5.39 (1H, m)] in the substituent at position 7. From these results, **2** was deduced to be 7-geranylindolactam-V. The assignments of all proton signals were established by ^{1}H-^{1}H COSY (Table 1). The configuration at position 20 was confirmed to be trans by the nOe difference spectra of **2** in $CDCl_3$; saturation of the H-20 proton (δ 5.39) caused a remarkable enhancement of the H-23 signal (δ 2.11), while the H-22 signal (δ 1.80) was not affected. To establish the absolute stereochemistry at positions 9 and 12, **2** was synthesized from (−)-indolactam-V [**1**] isolated from *S. blastmyceticum* NA34-17 by the method reported previously (20). Treatment of (−)-14-*O*-acetylindolactam-V (9) with geranyl bromide in HOAc, followed by alkaline hydrolysis, gave (−)-2-, 5-, or 7-geranylindolactam-V at 4.5, 3.0, and 3.8% yield, respectively, along with unreacted starting material (60%). The specific rotation of the synthetic (−)-7-geranylindolactam-V {$[\alpha]^{28}D$ −118° (c=0.33, MeOH)} was very similar to that of **2** {$[\alpha]^{28}D$ −119° (c=0.47, MeOH)}, indicating that the absolute configuration at positions 9 and 12 was *S* and *S*, respectively. On the basis of these data, the structure of **2** was determined to be (−)-7-geranylindolactam-V. Although all monoterpenoid moieties of teleocidins (1–5) have a 1,1-dialkylallyl structure, which is thought to be biosynthesized with the involvement of an aza-Claisen rearrangement from position 1 to position 7 (20), (−)-7-geranylindolactam-V does not have this structure. This is the first example of the isolation of a teleocidin-related compound which does not have a 1,1-dialkylallyl moiety. It is here proposed that (−)-7-geranylindolactam-V [**2**] could be biosynthesized from **1** by a direct attack at position 7 by geranyl diphosphate.

Compound **3** was isolated from filtered *S. blastmyceticum* NA34-17 culture broths (180 liters) by extraction with CH_2Cl_2. The extract was chromatographed over Si gel using toluene and increasing volumes of Me_2CO. An Ehrlich's reagent-positive red compound was found in the 40% Me_2CO eluate. Further chromatography, followed by hplc, gave **3** (4.1 mg) as a red amorphous powder, which was named blastmycetin F. The

TABLE 1. ^{1}H-Nmr Data of (−)-7-Geranylindolactam-V [**3**] in $CDCl_3$ (400 MHz).[a]

Position	^{1}H δ (multiplicity, J in Hz)	
	Twist conformer[a]	Sofa conformer[a]
1	8.02 (1H, br s)	8.30 (1H, br s)
2	6.88 (1H, s)	7.01 (1H, d, J=2.2)
5	6.44 (1H, d, J=7.7)	6.98 (1H, s)
6	6.86 (1H, d, J=7.7)	6.98 (1H, s)
8	3.05 (1H, dd, J=17.2, 3.7)	2.82 (1H, d, J=16.1)
	3.17 (1H, br d, J=17.2)	3.12 (1H, m)
9	4.33 (1H, m)	4.45 (1H, m)
10	7.37 (1H, br s)	4.74 (1H, d, J=11.4)
12	4.35 (1H, d, J=10.3)	2.96 (1H, d, J=10.6)
14	3.56 (1H, m)	ND[b]
	3.74 (1H, m)	ND
15	2.59 (1H, m)	2.38 (1H, m)
16	0.65*[c] (3H, d, J=6.6)	0.93$^+$ (3H, d, J=6.6)
17	0.93* (3H, d, J=6.6)	1.24$^+$ (3H, d, J=7.0)
18	2.90 (3H, s)	2.73 (3H, s)
19	3.48 (2H, t, J=7.7)	ND
20	5.39 (1H, m)	ND
22	1.80 (3H, s)	1.81 (3H, s)
23	2.11 (2H, m)	ND
24	2.11 (2H, m)	ND
25	5.09 (1H, m)	ND
27	1.60# (3H, s)	ND
28	1.68# (3H, s)	ND

[a]Sofa:twist=1:2.8 (0.045 M, 27°).

[b]The signals could not be identified because of their low intensity and because of overlap by the signals of the major conformer.

[c]Assignments bearing the same symbol may be reversed.

molecular formula was determined to be $C_{22}H_{28}N_4O_3$ by high-resolution eims (observed *m/z* 396.2157; calcd *m/z* 396.2161). Its ms fragment pattern [*m/z* 396 (M^+, 78), 378 (6), 353 (21), 300 (100), 266 (38), 170 (56)] suggested the presence of the indolactam core (*m/z* 300). The ^{1}H-nmr spectrum of **3** in $CDCl_3$ (0.02 M, 27°) revealed that **3** existed as two stable conformers (sofa:twist=1:6), clearly showing the existence of the nine-membered lactam ring. Three aromatic protons at δ 6.41 (1H, d, J=7.6 Hz), 6.90 (1H, d, J=8.5 Hz) and 7.19 (1H, t, J=7.9 Hz), and lack of the signal ascribed to the H-2 proton of the indole ring suggested that **3** was substituted at position 2. The ^{1}H-nmr spectrum of **3** also exhibited the presence of three methylenes in the substituent [δ 1.98 (2H, m), 2.99 (2H, m), and 4.29 (2H, m)]. Moreover, the presence of an imino group and a carbonyl group in the substituent was suggested from the ^{13}C-nmr signals in $CDCl_3$ [δ 178.0 and 178.5] and the molecular formula of the substituent (C_5H_6NO). The remarkably low-field chemical shifts of the H_a-8 and H-1 protons (δ 4.38 and 11.84) suggested the attachment of the carbonyl group to position 2 of the indole ring (22). This was also supported by the visible absorption of **3** [λ max (MeOH) nm (ε) 432 (4,800), 344 (11,200), 273 (12,400), 223.5 (27,800)]. These data led us to formulate the structure of blastmycetin F as shown in structure **3**. The assignments of all proton and carbon signals were established by ^{1}H-^{1}H COSY, HSQC (23) and HMBC (24) (Table 2). Compound **3** showed a specific rotation of $[\alpha]^{26}D$ −147° (c=0.21, MeOH) similar to that of **1** {$[\alpha]^{16}D$ −161° (c=0.75, MeOH)} and thus the absolute configuration of **3** was deduced to be 9*S* and 12*S*. In addition, **1** was the common biosynthetic intermediate of

TABLE 2. ^{1}H- and ^{13}C-Nmr Data of Blastmycetin F [**3**] in $CDCl_3$ (500 and 125 MHz).[a]

Position	^{1}H δ (multiplicity, J in Hz)	^{13}C δ[b]	HMBC (coupled ^{1}H)
1	11.84 (1H, br, s)	—	—
2	—	129.2	H-8
3	—	127.2	H-8
3a	—	118.8	H-5,6,8
4	—	149.6	H-6,12,18
5	6.41 (1H, d, J=7.6)	106.0	H-7
6	7.19 (1H, t, J=7.9)	127.8	—
7	6.90 (1H, d, J=8.5)	104.5	H-5
7a	—	140.1	H-6
8	3.07 (1H, m) 4.38 (1H, dd, J=19.2, 3.7)	32.8	—
9	4.29 (1H, m)	54.8	H-8
10	6.89 (1H, br s)	—	—
11	—	173.1	H-12
12	4.29 (1H, m)	70.8	H-16,17
14	3.60 (2H, m)	65.7	H-8
15	2.54 (1H, m)	28.6	H-12,16,17
16	0.54*[c] (3H, d, J=6.7)	19.3[+]	H-17
17	0.89* (3H, d, J=6.4)	21.4[+]	H-12,16
18	2.89 (3H, s)	33.2	H-12
19	—	178.5	—
20	—	178.0	H-22,23
21	2.99 (2H, m)	35.1	H-22
22	1.98 (2H, m)	21.0	—
23	4.29 (2H, m)	62.8	H-22

[a]Chemical shifts only for the twist conformer are expressed: sofa:twist=1:6 (0.02 M, 27°).
[b]Assignments were determined by HSQC and HMBC.
[c]Assignments bearing the same symbol may be reversed.

teleocidin-related compounds in our actinomycete (20). Blastmycetin F is the first teleocidin-related metabolite with a substituent at position 2.

The lack in (−)-7-geranylindolactam-V [**2**] of the 1,1-dialkylallyl moiety common to teleocidins (1–5) prompted us to estimate its possible tumor-promoting activity using two in vitro bioassays related to tumor-promotion: (a) binding affinity to the 12-*O*-tetradecanoylphorbol-13-acetate (TPA) receptor in the mouse epidermal particulate fraction, and (b) ability to enhance incorporation of inorganic phosphate into phospholipids of HeLa cells (Table 3). Binding affinity to the TPA receptor was evaluated by inhibition of the specific binding of [^{3}H]-TPA to the mouse epidermal particulate fraction. This inhibition assay was carried out by the cold Me_2CO filter method (25,26).

TABLE 3. Biological Activities of (−)-7-Geranylindolactam-V [**2**].

Compound	Inhibition of specific [^{3}H]-TPA binding	Incorporation of ^{32}Pi into phospholipids of HeLa cells (relative cpm/mg of protein)			
	IC_{50} (log 1/M)	10^{-10}M	10^{-9}M	10^{-8}M	10^{-7}M
DMSO (control)[a]	—	1.00 (0.16)[b]			
(−)-7-Geranylindolactam-V [**2**]	8.05 (0.05)	0.97 (0.07)	1.41 (0.20)	2.61 (0.29)	6.64 (0.44)
Teleocidin A-1 [**4**]	8.30 (0.06)	1.05 (0.01)	1.57 (0.05)	4.59 (0.44)	8.36 (0.79)
Teleocidin B-4 [**5**]	8.71 (0.23)	1.19 (0.03)	1.82 (0.00)	6.72 (0.17)	7.46 (0.22)

[a]0.2% DMSO.
[b]Standard deviation.

Binding affinity was evaluated by the concentration required to cause 50% inhibition, IC_{50}, which was calculated by a computer program (Statistical Analysis System) with a probit (probability unit) procedure (27). The results are summarized in Table 3. Compound **2** had almost the same binding affinity as teleocidin A-1 [**4**] and teleocidin B-4 [**5**] which were potent tumor promoters in vivo (28). We have also measured the stimulation of radioactive inorganic phosphate ($^{32}P_i$) incorporation into HeLa cell phospholipids because the enhancement of phospholipid metabolism has been reported to play an important role in tumor promotion (29,30). As shown in Table 3, **2** as well as **4** and **5** showed a potent stimulation at 10^{-7} M. These results strongly suggest that **2** is a potent tumor promoter comparable to **4** and **5** in vivo.

Although the activity of blastmycetin F [**3**] was not measured, it is suggested that **3** is not likely to be active because introduction of a large substituent into position 2 of the related (−)-indolactam-V [**1**] resulted in a remarkable decrease in activity (16).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Mps were uncorrected. The following spectroscopic and analytical instruments were used: uv, Shimadzu UV-200; ORD, Jasco Model J-5; ^1H and ^{13}C nmr, JEOL GX400 (400 MHz, 27°) and Alpha-500 (500 MHz, 27°); hplc, Waters Model 600E with Model 484 uv detector: ms, JEOL, JMS-DX300 (70eV, 300 μA). Hplc was carried out on YMC packed A-311 (ODS 6 mm i.d.×100 mm), AQ-323 (ODS, 10 mm i.d.×250 mm), SH-342-5 (ODS, 20 mm i.d.×150 mm), SH-343-10 (ODS, 20 mm i.d.×250 mm), A-023 (Si gel, 10 mm i.d.×250 mm) columns (Yamamura Chemical Laboratory), and a μ-Bondasphere C_{18} (19 mm i.d.×150 mm) column (Waters Associates). Wako C-100 and C-200 gels (Si gel, Wako Pure Chemical Industries) and YMC A60-350/250 gel (ODS, Yamamura Chemical Laboratory) were used for column chromatography.

The reference compounds (−)-indolactam-V [**1**], teleocidin A-1 [**4**], and teleocidin B-4 [**5**] were obtained from the culture broth of *S. blastmyceticum* NA34-17 by the method reported previously (9). Radioisotopes were purchased from NEN Research Products.

ISOLATION OF (−)-7-GERANYLINDOLACTAM-V [**2**] AND BLASTMYCETIN F [**3**].—*Streptoverticillium blastmyceticum* NA34-17 kept on Waksman's medium was transferred to a 500 ml shake flask containing 100 ml of a medium consisting of 1% glucose, 1% polypeptone (Daigo Eiyo Kagaku), 1% meat extract (Wako Pure Chemical Industries) and 0.5% NaCl (pH 7.0). The flask was shaken at 30° for 70 h and this inoculum was transferred to a 30-liter jar fermentor (Marubishi type MSJ-02) containing 20 liters of medium (2% glucose, 1% polypeptone, 1% meat extract, 0.5% NaCl, 0.05% adekanol, pH 7.0). The conditions of the cultivation were as follows: temperature 30°; aeration, 22 liters/min; agitation, 400 rpm; cultivation time, 45 h.

The mycelia (6.5 kg wet weight) were steeped in 20 liters of Me_2CO, and the extract was partitioned between CH_2Cl_2 and H_2O. The CH_2Cl_2 layer was dried over anhydrous Na_2SO_4, and evaporated *in vacuo* to give a brown oily syrup (197 g). The residue was chromatographed on Wako C-100 gel (2.5 kg) using toluene and increasing volumes of Me_2CO to yield a 30% Me_2CO eluate (12.4 g), which was chromatographed on YMC A60-350/250 gel (160 g) using H_2O and increasing volumes of CH_3CN to give an 80% CH_3CN eluate. This was further purified by hplc on YMC SH-343 using 80% CH_3CN, followed on YMC A-023 using 85% hexane, 10% $CHCl_3$ and 5% 2-PrOH, to give (−)-7-geranylindolactam-V [**2**, 9.9 mg] as an amorphous powder; uv λ max (MeOH) nm (ε) 286 (8,900), 229 (30,200); eims *m/z* 437 (M^+, 100), 394 (30), 351 (60), 307 (80), 171 (40). ^1H-Nmr data are shown in Table 1.

The filtered broth (180 liters) was extracted with CH_2Cl_2. The extracts (50 g) were purified by elution through Wako C-100 gel (1.5 kg) with toluene and increasing volumes of Me_2CO. The 40% Me_2CO eluate (20 g) was chromatographed on YMC A60-350/250 gel (160 g) using H_2O and increasing volumes of MeOH to give an 80% MeOH eluate. This was chromatographed on Wako C-200 gel using 85% hexane, 10% $CHCl_3$, and 5% 2-PrOH to give crude blastmycetin F [**3**], which was further purified by hplc on YMC AQ323 using 75% MeOH, followed by YMC A-023 chromatography using 80% hexane, 13% $CHCl_3$, and 7% 2-PrOH to give blastmycetin F [**3**, 4.1 mg] as a red amorphous powder. The ^1H and ^{13}C nmr are presented in Table 2.

INHIBITION OF SPECIFIC [^3H]-TPA BINDING TO THE MOUSE EPIDERMAL PARTICULATE FRACTION.—Inhibition of specific [^3H]-TPA binding was assayed by the cold Me_2CO filter method (25,26) with slight modifications (31). An epidermal particulate fraction was prepared from dorsal epidermis of female ICR mice and subjected to a binding assay as reported previously (31). Binding affinity was evaluated by the

concentration required to cause 50% inhibition, IC_{50}, which was calculated by a computer program (Statistical Analysis System) with a probit procedure (27). Since there existed subtle variations in IC_{50} values, depending on the particulate fraction prepared, all compounds were tested simultaneously using the same particulate fraction.

STIMULATION OF $^{32}P_i$ INCORPORATION INTO HELA CELL PHOSPHOLIPIDS.—Incorporation of $^{32}P_i$ into HeLa cell phospholipids was measured by the method reported previously (32) with slight modifications (17).

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