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SYNTHESIS OF ASPERPHENAMATE, A NOVEL FUNGAL METABOLITE

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Key Word Index—*Aspergillus flavipes*; asperphenamate; fungal metabolite; synthesis.

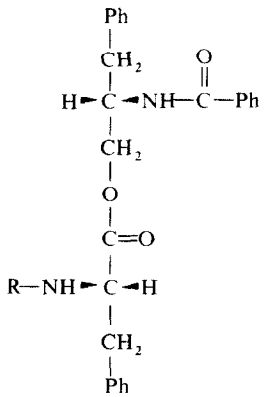
Abstract—The proposed structure of asperphenamate (**1**), a novel fungal metabolite, was confirmed by synthesis. Esterification of *N*-benzoyl-L-phenylalaninol with *N*-carbobenzoxy-L-phenylalanine, followed by deprotection of the carbobenzoxy group and benzoylation yielded a product which was identical to the natural fungal metabolite.

INTRODUCTION

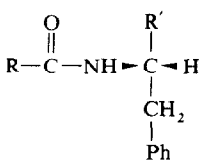
Asperphenamate (**1**), a novel fungal metabolite of *Aspergillus flavipes* (ATCC 11013), has been characterized as (*S*)-*N*-benzoylphenylalanine-(*S*)-2-benzamido-3-phenyl propyl ester. This proposed structure was based upon the isolation of *N*-benzoyl-L-phenylalaninol (**2**) and *N*-benzoyl-L-phenylalanine (**3**) upon basic hydrolysis. The spectroscopic data was also in accord with the proposed structure [1]. A synthesis of asperphenamate is now reported which confirms the proposed structure.

RESULTS AND DISCUSSION

Theorectically, it should be possible to synthesize asperphenamate by the esterification of *N*-benzoyl-L-phenylalanine (**3**) with *N*-benzoyl-L-phenylalaninol (**2**). However, it is known that *N*-acylamino acids of this type, when subjected to dehydration conditions, undergo racemization via azlactone formation [2]. Therefore, it was necessary to use *N*-carbobenzoxy-L-phenylalanine (**4**) since it does not undergo cyclization to the azlactone. The anhydride of *N*-carbobenzoxy-L-phenylalanine, prepared from *N*-carbobenzoxy-L-phenylalanine and dicyclohexylcarbodiimide, was reacted with *N*-benzoyl-L-phenylalaninol (**2**) and gave the ester **5**. The ester (**5**) had ^{1}H and ^{13}C NMR spectra very similar to that reported for **1** [1] with an additional signal at δ 4.99 (2H, *s*) in the ^{1}H MNR spectrum and δ 65.4 (*t*) in the ^{13}C NMR spectrum. The carbobenzoxy protecting group was removed by hydrogenolysis using 5% $Pd/BaSO_4$ and benzoylation of the deprotected ester yielded the final product, which was identical in all respects to asperphenamate (**1**).



1. R = COPh
5: R = $COOCH_2Ph$



2: R = Ph
R′ = $-CH_2OH$
3: R = Ph
R′ = $-CO_2H$
4: R = —O—CH_2—Ph
R′ = $—CO_2H$

Two related phenylalanine-derived compounds, both having amide linkages, have also been reported. Asperglaucide (*O*-acetyl-*N*-(*N*′-benzoyl-L-phenylalanyl)-L-phenylalaninol) was isolated from the fungus *Aspergillus glaucus* [3] and the plant *Euphorbia fisheriana* [4]. *N*-Acetyl-L-phenylalanyl-L-phenylalaninol was isolated as a metabolite of the fungus *Emericellopsis salmo*-synnemata [5].

EXPERIMENTAL

Mps are uncorr. ^{1}H NMR spectra were recorded using TMS as int. stand.; chemical shift values are reported in δ (ppm) units. The ^{13}C NMR spectra were recorded at 15.03 MHz using the deuterium signal of $CDCl_3$ as the lock signal and TMS as int. stand. Circular dichroic spectra were obtained on a Jasco J-40 recording spectropolarimeter.

N-Carbobenzoxy-L-phenylalanine anhydride. To a soln of 4.99 g of *N*-carbobenzoxy-L-phenylalanine (1.63×10^{-2} M) in dry Et_2O (50 ml) was added 1.92 g dicyclohexylcarbodiimide (9.30×10^{-3} M) A white ppt. formed immediately. The reaction was allowed to stand at room temp for 1 hr. The suspension was then cooled to 4° and filtered. The ppt. was air dried for 15 min. then extracted with 100 ml cold dry EtOAc. The EtOAc suspension was filtered to remove dicyclohexyl urea. The filtrate was diluted with petrol until the soln became cloudy, then placed in the refrigerator for crystallization. Crystallization by this method (×3) yielded a total of 3.065 g anhydride as needles, mp 148–150°, $[\alpha]_D^{25}$ −16.49° (*c* 0.94, Py); IR (KBr) ν_{max} 3332 (N—H), 1850 and 1770 (OC—O—CO), 1700 (O—CO—N), and 1632 (N—CO) cm^{-1}

Carbobenzoxy-L-phenylalanyl-N-benzoyl-L-phenylalaninol (**5**). To a soln of 500 mg *N*-benzoyl-L-phenylalaninol (**2**) [1] (1.96×10^{-3}M) in 50 ml anhydrous tetrahydrofuran was added 0.34 ml dry Py followed by 3.0 g *N*-carbobenzoxy-L-phenylalanine anhydride (5.16×10^{-3}M). The soln was allowed to stand at room temp. for 30 hr. A chip of ice was added to the reaction mixture to destroy the excess anhydride. After standing at room temp. for 1 hr, the soln was evapd *in vacuo*. *Ca* 50 ml C_6H_6 was added to the residue and the C_6H_6 was evapd *in vacuo*. This procedure was repeated ×2. The residue was then suspended in 150 ml dry EtOAc and washed successively with 100 ml cold 2 N citric acid, 100 ml cold satd. aq. $NaHCO_3$, and finally 100 ml H_2O. The EtOAc layer was dried and

evapd *in vacuo* to leave a solid residue (994 mg). The coupled product (**5**) was crystallized from EtOH as needles, mp 185°, $[\alpha]_D^{25°}$ −39.7° (*c* 0.99, Py); IR (KBr) ν_{max} 3340 (N—H), 1740 (O—CO), 1702 (O—CO—N), 1645 (N—CO) cm^{-1}; UV λ_{max}^{MeOH} 236 nm (*sh*, ε 8.54 × 10^3) and 211 nm (ε 1.61 × 10^4); CD (MeOH) $[\theta]_{230}$ −11530; ^{1}H NMR ($CDCl_3$) δ 2.70–3.16 (*m*, 4H), 3.90–4.76 (*m*, 4H), 4.99 (*s*, 2H), 6.5 (*br s*, 2H), 7.06–7.83 (*m*, 20 Ar-H); ^{13}C NMR ($CDCl_3$) δ 37.3 (*t*), 37.9 (*t*), 50.2 (*d*), 55.4 (*d*), 65.4 (*t*), 67.2 (*t*), four signals at 127.1, 128.6, 128.7 and 129.2 representing 18 Ar-C, 131.5 (*s*), 134.3 (*s*), 135.6 (*s*), 136 (*s*), 137 (*s*), 155.9 (*s*), 167.2 (*s*), 172.1 (*s*). Analysis: found C, 73.85%; H, 6.09%; N, 5.03%; calculated for $C_{33}H_{32}N_2O_5$: C, 73.86%; H, 6.01%; N, 5.22%.

(S)-N-*Benzoylphenylalanine*-(S)-*2-benzamido-3-phenyl propyl ester* (*asperphenamate*) (**1**). To a suspension of 20 mg carbobenzoxy-L-phenylalanyl-*N*-benzoyl-L-phenyl alaninol (**5**) in 10 ml of 80% HOAc was added 20 mg of 5% Pd/$BaSO_4$. The suspension was shaken with hydrogen in a Parr hydrogenator under 1 kg/cm^2 pressure for 24 hr. The catalyst was removed by filtration through Celite 545 and the reddish brown filtrate was evapd *in vacuo* to leave 45 mg dark brown residue. Purification by PLC (10% MeOH–$CHCl_3$) yielded 14 mg of the deprotected ester [IR ν_{max}^{KBr} 3320 (N—H), 1740 (O—CO), 1640 (N—CO) cm^{-1}]. A soln of 14 mg of the deprotected product in $CHCl_3$ (3 ml) was treated at room temp. with 0.01 ml PhCOCl followed by 0.5 ml NEt_3. After 30 min it was evapd *in vacuo* and the residue was purified by PLC (Brinkmann Sil G-50, 0.5 mm thickness, 4% MeOH–$CHCl_3$). Crystallization from EtOH yielded asperphenamate (**1**) (2 mg) as needles, mp 201–204°. The synthetic product was identical in all respects with the natural product (mp, mmp, TLC, co-TLC, IR, CD) obtained from the fungus.

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(+)-α(S)-BUTYRAMIDO-γ-BUTYROLACTONE FROM *LYNGBYA MAJUSCULA*

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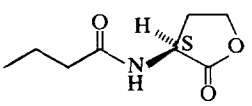
Key Word Index—*Lyngbya majuscula*; Oscillatoriaceae; (+)-α(S)-butyramido-γ-butyrolactone.

Abstract—(+)-α(S)-Butyramido-γ-butyrolactone is a minor constituent of the toxic marine blue–green alga *Lyngbya majuscula*.

Lyngbya majuscula is a toxic marine cyanophyte that is responsible for occasional outbreaks of a contact dermatitis known as 'swimmers' itch'. The toxin associated with the dermatitis-producing strain is debromoaplysiatoxin [1], but whether this compound actually causes the dermatitis is not known for certain. The toxin associated with the non-dermatitis-producing variety is a different substance [2]. In an investigation of this latter toxin, we have isolated a minor constituent of the alga and have identified it as (+)-α(*S*)-butyramido-γ-butyrolactone (**1**).



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The lactone was found in the gel filtration fraction that was eluted immediately after the toxin. Its structure was determined in a straightforward manner from spectral data (see Experimental) and confirmed by by acid hydrolysis to *n*-butyric acid and α-amino-γ-butyrolactone and synthesis from (−)-α(*S*)-amino-γ-butyrolactone and butyryl chloride.

EXPERIMENTAL

PMR and C^{13}NMR spectra were obtained using a 100 MHz spectrometer equipped with a Fourier transform system. Single frequency off-resonance decoupled C^{13}NMR spectra were determined with the proton decoupler at δ 14. Chemical shifts are reported in δ units (ppm) relative to TMS (δ = 0) as an int. stand All chromatographic separations were continuously assayed for UV absorption at 254 and 280 nm and all fractions were tested for toxicity in mice by intraperitoneal injection.

Isolation. Lyngbya majuscula was collected at Kahala Beach, Oahu in July 1976. The freeze-dried alga (680 g) was extracted twice with $CHCl_3$ and twice with MeOH to give 37 g of a dark brown oil. The extract (34 g) was chromatographed on a column of Si gel (550 g) using a hexane–$CHCl_3$–Me_2CO–