The Structures of Five New Didehydropeptides related to Neoechinulin, isolated from *Aspergillus amstelodami*

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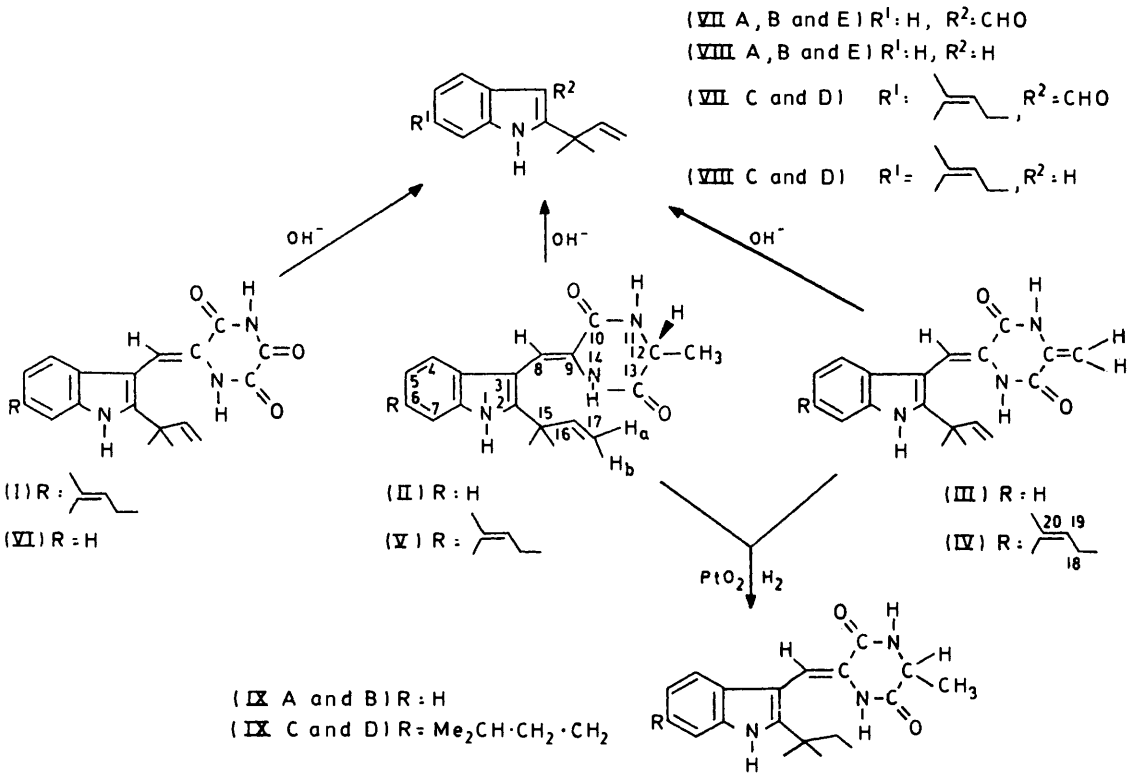
Five new neoechinulin-type metabolites have been isolated from the mycelium of *Aspergillus amstelodami*, grown on molasses beet cultures. They are all characterised by a didehydrotryptophyl structure with a reversed isoprenic chain at the 2-position of the indole nucleus, and have been named neoechinulins A—E. On the basis of chemical and spectral evidence they have been shown to be, respectively, *cyclo*-alanyl-2-(1,1-dimethylprop-2-enyl)-didehydrotryptophyl (II), *cyclo*-didehydroalanyl-2-(1,1-dimethylprop-2-enyl)didehydrotryptophyl (III), *cyclo*-didehydroalanyl-2-(1,1-dimethylprop-2-enyl)-6-(3-methylbut-2-enyl)didehydrotryptophyl (IV), *cyclo*-alanyl-2-(1,1-dimethylprop-2-enyl)-6-(3-methylbut-2-enyl)didehydrotryptophyl (V), and *cyclo*-oxamyl-2-(1,1-dimethylprop-2-enyl)didehydrotryptophyl (VI).

In the course of investigations on the biosynthesis of neoechinulin [1] (I) from *Aspergillus amstelodami*, we have isolated several co-occurring structurally related metabolites, which may either be biogenetic intermediates or be derived from them on ' shunt ' pathways. They are all characterised by a didehydrotryptophyl structure with a reversed isoprenic chain at the 2-position of the indole nucleus. Their isolation allows speculation on natural processes such as prenylation of tryptophan systems and oxidation of amino-acid residues. Recently, the presence of amino-acid residues at higher oxidation level in microbial peptides has been connected with the epimerization of amino-acids occurring in the biosynthesis of antibiotics [2] and with their biological activity.[3]

Preliminary accounts of the identification of neoechinulins A,[4] B,[4] C,[4,5] and D [6] have been published, and neoechinulins A and D have been isolated also from the mycelium of *Aspergillus ruber*.[7,8] Their common biosynthetic origin from *cyclo*-L-alanyl-L-tryptophyl has been established.[9] We now report the full structural



Scheme

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[6] A. Dossena, R. Marchelli, and A. Pochini, *Experientia*, 1975, **31**, 1249.
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characterization of neoechinulins A—E [(II)—(VI); see Scheme].

The fungal mat of *Aspergillus amstelodami*, grown on molasses beet cultures, was extracted continuously with light petroleum (b.p. 60—80 °C), diethyl ether, and chloroform. Both the latter two extracts were shown to contain, beside a large amount of echinulin [10] and other didehydrotryptophyl compounds,[11] minor amounts of neoechinulins A—E and neoechinulin. Separation was achieved by column chromatography (silica gel) with hexane–ethyl acetate as eluant. All metabolites were further purified by preparative t.l.c., and recrystallised.

Their u.v. spectra enabled them to be classified as indoles (see Table 1). In particular, the additional

TABLE 1

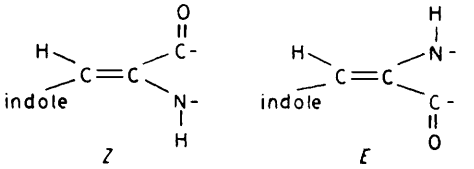
U.v. absorption maxima for neoechinulins and related compounds

Metabolite	$\lambda_{max.}$ (95% EtOH)/nm (log ε)			
Neoechinulin (I)	231 (4.51)	287 (4.12)	420 (3.99)	
Neoechinulin A (II)	229 (4.35)	286 (4.07)	292 (4.02)	338 (3.98)
Neoechinulin B (III)	228 (4.45)	273 (4.28)	284 (4.26)	374 (4.02)
Neoechinulin C (IV)	231 (4.54)	275 (4.35)	290 (4.27)	380 (4.08)
Neoechinulin D (V)	231 (4.54)	265 (4.06)	296 (4.02)	345 (4.04)
Neoechinulin E (VI)	228 (4.57)	281 (3.96)	410 (3.91)	
Telomycin	222 (4.80)	277 (4.14)	290 (4.07)	339 (4.34)

band at higher wavelength, present in the spectra of all the metabolites, suggested a didehydrotryptophanic structure, in agreement with the chromophore present in telomycin.[12]

Neoechinulin A (II), $C_{19}H_{21}N_3O_2$ showed i.r. bands at 3 360 and 2 980 (NH) and 1 670 cm^{-1} (C:O). A band at 1 630 cm^{-1} was attributed to C=C stretching, as in benzylidene-substituted 2,5-diones.[13] The ^1H n.m.r. spectrum was consistent with the presence of an unsubstituted aromatic ring [δ 7.0—7.5 (4 H, m)] and a 1,1-dimethylprop-2-enyl substituent [δ 1.50 (6 H, s, 2 CH_3), 5.04 and 5.06 (2 H, 2 dd), and 6.12 (1 H, dd), analysed as A, B, and X of an ABX system with J_{cis} 10.6, J_{trans} 17.4, and J_{gem} 1.5 Hz]. The presence of an alanyl unit was confirmed by signals at δ 4.15 (1 H, qd) and 1.42 (3 H, d). The stereochemistry at C-12 was assigned on the basis of $^3J_{NH-CH}$, which is a function of the torsion angle between the H–N–C(12) and N–C(12)–H planes.[14] The observed value (1.5 Hz) is consistent with an angle θ of 105°,[15] and with a pseudoequatorial orientation of the CH_3 group (as in the Scheme).

The *Z*- (*cis*-) stereochemistry about the 8,9-double bond was deduced from the chemical shift * of the olefinic proton (H-8). The deshielding effect of the carbonyl group on β-vinyl protons is well known,[16] and has been used for configurational assignments for a variety of substances, including 3-arylmethylene-6-methylpiperazine-2,5-diones.[17] In the *E*-isomer the *trans*-position with respect to the carbonyl would be expected to result in a shift of the H-8 signal towards higher field, as found in analogous systems.[18] The



Z *E*

aromatic solvent-induced shift δ ($CDCl_3$) − δ (C_6D_6) = 0.07 p.p.m. is also specific for a *cis*-position of the H-8 with respect to the carbonyl.[19]

Neoechinulin B (III), $C_{19}H_{19}N_3O_2$ was shown to be the didehydroalanine analogue of neoechinulin A. The presence of the ·C=CH_2 group was demonstrated by two ^1H n.m.r. singlets at δ 5.02 and 5.34, overlapping with the ·CH=CH_2 signals. These two systems were analysed independently by the standard procedure and the parameters obtained were refined by use of the iterative LAOCOON program; the calculated spectra are in good agreement with the experimental values (see Figure).

By alkaline hydrolysis (NaOH–EtOH) neoechinulin A and B were each converted into two main products, (VII) (A and B) and (VIII) (A and B). Compounds (VIIA and B) were identical [R_F 0.32 in hexane–ethyl acetate (8 : 2); positive reaction with 2,4-dinitrophenylhydrazine, and u.v. absorption characteristic of 3-formylindoles], and were identified as 2-(1,1-dimethylprop-2-enyl)indole-3-carbaldehyde by comparison with the spectral data of an authentic specimen.[7] Compounds (VIIIA and B) were also identical, showing the same retention times on five different g.l.c. columns, R_F 0.78, a very intense purple colour with the Ehrlich reagent, and identical spectra (typical indole absorption and molecular ion at *m/e* 185). In the n.m.r. spectrum (CCl_4) a singlet at δ 6.15 (indole 3-proton) and signals due to the isoprenic substituent allowed identification of the compound as 2-(1,1-dimethylprop-2-enyl)indole.

* In $(CD_3)_2SO$ the chemical shifts are strongly concentration dependent. However, the very low chemical shifts of H-8 in $CDCl_3$ (*e.g.* neoechinulin at δ 7.61; neoechinulin A at 7.5) are probably due to their position closer to the plane of the carbonyl bond, where anisotropic deshielding is at a maximum. This situation is probably not completely allowed in $(CD_3)_2SO$, because of extensive interactions of the solvent molecules with the N–H groups.

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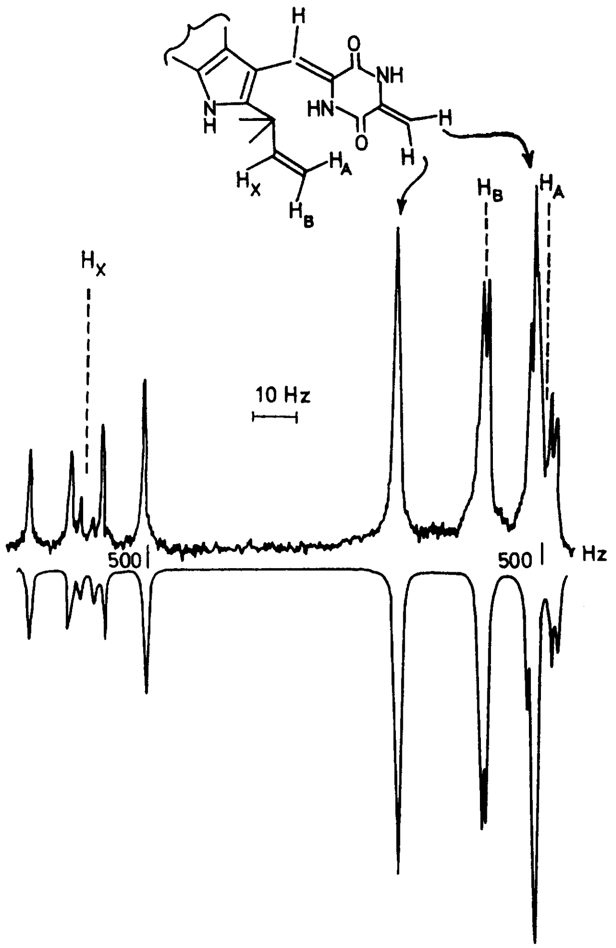
[19] P. Lazlo, *Progr. NMR Spectroscopy*. 1967, **3**, 349.

The aldehyde (VII) may be formed by a retro-aldol condensation,[1] and by alkaline deformylation[20] may produce the indole (VIII). The ratio between the products (VII) and (VIII) depended on the temperature and reaction time, lower temperatures and shorter times giving more of the aldehyde.



^{1}H N.m.r. spectrum of neoechinulin B in the olefinic proton region: top, experimental; bottom, calculated spectrum

Upon hydrogenation over platinum oxide at 100 atm in glacial acetic acid, both neoechinulins A and B produced a compound [(IXA) = (IXB)] identified as *cyclo*-alanyl-2-(1,1-dimethylpropyl)didehydrotryptophyl (M^+ 325), as confirmed by an intense $(M - 71)^+$ peak in the mass spectrum. The unchanged u.v. pattern indicated that the 8,9-double bond was not hydrogenated.

Neoechinulin C (IV), $C_{24}H_{27}N_3O_2$, was shown to be an analogue of neoechinulin B with an additional isoprenic chain on the aromatic nucleus from its ^{1}H n.m.r. spectrum (δ 1.76, 3.40, and 5.35). The mass spectrum showed prominent peaks at $(M - C_5H_9)^+$ and $(M - 2C_5H_9)^+$. Conclusive evidence for structure (IV) was provided by chemical degradation. Alkaline hydrolysis of (IV) afforded 2-(1,1-dimethylprop-2-enyl)-6-(3-methylbut-2-enyl)indole-3-carbaldehyde (VIIC) and the corresponding deformylated indole (VIIIC), identified by comparison with authentic samples obtained from hydrolysis of neoechinulin (mixed m.p.s, t.l.c., and i.r., u.v., and mass spectra).

Neoechinulin D (V), $C_{24}H_{29}N_3O_2$, was shown to be the 6-(3-methylbut-2-enyl) analogue of neoechinulin A [n.m.r. signals at δ 1.72, 3.40, and 5.3; mass spectral base peak at $(M - C_5H_9)^+$]. On alkaline hydrolysis neoechinulin D afforded compounds (VIID) and (VIIID) identified by comparison with authentic samples obtained from neoechinulin C and neoechinulin.

On catalytic hydrogenation over platinum oxide, neoechinulins C and D afforded the same compound [(IXC) = (IXD)] with both the allylic chains hydrogenated (intense $M - 71$ peaks in the mass spectrum), but the 8,9-double bond remaining, as observed for neoechinulins A and B.

Indirect evidence for the aromatic substitution pattern of neoechinulins C and D was provided by the retention of tritium during incorporation experiments with *cyclo*-L-[U-C^{14}]alanyl-L-[5,7-$^3H_2$]tryptophyl.[3]

Neoechinulin E (VI), $C_{18}H_{17}N_3O_3$, appeared to have a trioxopiperazine ring ($\nu_{max.}$ 1 740 and 1 690 cm^{-1}). Its ^{1}H n.m.r. spectrum was almost identical with that of neoechinulin, except for the absence of signals due to the 3-methylbut-2-enyl substituent. By alkaline hydrolysis it produced compounds (VIIE) and (VIIIE) whose physical and chemical data were consistent with those of the products from neoechinulins A and B.

EXPERIMENTAL

M.p.s were taken with a Büchi apparatus. I.r. spectra (KBr) were recorded with a Perkin-Elmer 457 spectrophotometer; u.v. spectra for solutions in 95% ethanol were determined with a Beckman DK-2 instrument; ^{1}H n.m.r. spectra were obtained with a 100 MHz Varian instrument for solutions in $(CD_3)_2SO$ containing tetramethylsilane as internal standard; accurate masses were measured with a Hitachi–Perkin-Elmer RMV 6D mass spectrometer. T.l.c. was performed on silica gel HF_{254} (Merck). Preparative t.l.c. was carried out on 1 mm thick layers. Merck silica gel (70—230 mesh) was used for column chromatography. G.l.c. was performed with a Varian Aerograph 1400 instrument, with a flame ionization detector.

Isolation of Neoechinulins A—E [(II)—(VI)].—*Aspergillus amstelodami* (Mang) Thom and Church (1968) was grown in static liquid sugar molasses cultures for 23 days at 28 °C. The mycelium, washed with warm water and dried at 50 °C (14.36 g l^{-1}), was ground and continuously extracted (Soxhlet) with light petroleum (b.p. 60—80 °C), diethyl ether, and chloroform for 24 h. The latter two extracts gave a brown resin which was fractionated by column chromatography. By varying the hexane–ethyl acetate ratio from 9 : 1 to 1 : 1 (v/v), six metabolites were collected in the following order: C, B, neoechinulin, E, D, A (for t.l.c. see Table 2). They were further purified by preparative t.l.c. in various solvent systems (Table 2).

Neoechinulin A (II) (11 mg l^{-1}) formed ivory crystals, m.p. 264—265° (from methanol) (Found: M^+, 323.1634. $C_{19}H_{21}N_3O_2$ requires M, 323.1633); $\nu_{max.}$ (KBr) 3 360,

[20] J. Powers, *Tetrahedron Letters*, 1955, 655.

2 980, 1 670, and 1 630 cm^{-1}; *m/e* 323 (60%), 280 (10), 254 (100), 182 (50), 168 (20), 167 (25), 99 (10), and 69 (10); δ 1.42 (3 H, d, *J* 6.5 Hz, CH_3-12), 1.50 (6 H, s, CH_3-15), 4.15 (1 H, dd, *J* 6.5 and 1.5 Hz, H-12), 5.04 (1 H, A part of ABX, *J* 1.5 and 18 Hz, H_a-17), 5.06 (1 H, B part of ABX, *J* 10.8 and 1.5 Hz, H_b-17), 6.12 (1 H, X part of ABX, *J* 18 and 10.8 Hz, H-16), 6.93 (1 H, s, H-8), 7.0—7.50 (4 H, m, aromatic), 8.30 (2 H, s, H-1 and -11), and 10.96 (1 H, s, H-14).

TABLE 2

T.l.c. R_F values

Metabolites	Eluants * S_1	S_2	S_3
Neoechinulin C	0.80	0.53	0.39
Neoechinulin	0.75	0.50	0.30
Neoechinulin B	0.77	0.46	0.26
Neoechinulin E	0.51	0.36	0.17
Neoechinulin D	0.37	0.23	0.12
Neoechinulin A	0.26	0.17	0.09
Echinulin	0.14	0.12	0.05

* S_1 Ethyl acetate–hexane (2 : 1); S_2 benzene–ethyl acetate (1 : 1); S_3 benzene–ethyl acetate (2 : 1).

Neoechinulin B (III) afforded yellow crystals (from methanol) (29 mg l^{-1}), m.p. 234—236° (Found: M^+, 321.1477. $C_{19}H_{19}N_3O_2$ requires *M*, 321.1477); $\nu_{max.}$ 3 360, 2 980, 2 925, 1 680, and 1 645 cm^{-1}; *m/e* 321 (90%), 306 (10), 278 (20), 252 (100), 196 (30), 182 (90), 168 (30), 167 (20), 97 (20), 69 (10), and 44 (40); δ 1.50 (6 H, s, CH_3-15), 5.07 (1 H, A part of ABX, *J* 1.05 and 17.9 Hz, H_a-17), 5.09 (1 H, B part, *J* 11.1 and 1.05 Hz, H_b-17), 6.07 (1 H, X part, *J* 17.9 and 11.1 Hz, H-16), 5.34 and 5.08 (each 1 H, d, *J* 0.5 Hz, $=CH_2$-12), 6.85 (1 H, s, H-8), 6.7—7.50 (4 H, m, aromatic), 8.36 (1 H, s, H-1), 10.61 (1 H, s, H-11), and 10.86 (1 H, s, H-14).

Neoechinulin C (IV) gave yellow crystals (from light petroleum–benzene, 1 : 1) (88 mg l^{-1}), m.p. 205—207° (Found: M^+, 389.2102. $C_{24}H_{27}N_3O_2$ requires *M*, 389.2103); $\nu_{max.}$ 3 350, 2 970, 2 925, 1 680, and 1 640 cm^{-1}; *m/e* 389 (70%), 346 (10), 320 (60), 251 (20), 186 (10), 182 (20), 149 (30), 138 (30), 130 (40), and 79 (100); δ 1.48 (6 H, s, CH_3-15), 1.76 (6 H, s, CH_3-20), 3.40 (2 H, s, H_2-18), 5.06 (1 H, A part of ABX, *J* 1.1 and 17.8 Hz, H_a-17), 5.08 (1 H, B part, *J* 11.2 and 11 Hz, H_b-17), 6.07 (1 H, X part, *J* 11.2 and 17.8 Hz, H-16), 4.96 and 5.27 (each 1 H, d, *J* 0.5 Hz, $=CH_2$-12), 5.35 (1 H, s, H-19), 7.01 (1 H, s, H-8), 6.8—7.4 (3 H, m, aromatic), 8.66 (1 H, s, H-1), 10.79 (1 H, s, H-11), and 10.96 (1 H, s, H-14).

Neoechinulin D (V) formed ivory crystals (from methanol) (10 mg l^{-1}), m.p. 223—225° (Found: M^+, 391.2259. $C_{24}H_{29}N_3O_2$ requires *M*, 391.2259); $\nu_{max.}$ 3 340, 2 940, 1 670, and 1 630 cm^{-1}; *m/e* 391 (80%), 376 (10), 348 (10), 322 (100), 251 (20), 250 (20), 182 (20), 154 (20), 149 (50), 69 (100), 60 (30), and 43 (60); δ 1.48 (6 H, s, CH_3-15), 1.53 (3 H, d, *J* 6.5, CH_3-12), 1.72 (6 H, s, CH_3-20), 3.40 (2 H, s, H_2-18), 4.25 (1 H, dd, *J* 6.5 and 1.5 Hz, H-12), 5.06 (1 H, A part of ABX, *J* 1.0 and 17.5 Hz, H_a-17), 5.08 (1 H, B part, *J* 1.0 and 10.6 Hz, H_b-17), 6.11 (1 H, X part, *J* 10.6 and 17.5 Hz, H-16), 5.33 (1 H, s, H-19), 7.01 (1 H, s, H-8), 6.95—7.40 (3 H, m, aromatic), 8.32 (1 H, s, H-1), 10.38 (1 H, s, H-11), and 10.62 (1 H, s, H-14).

Neoechinulin E (VI) afforded orange-red crystals (from methanol) (17 mg l^{-1}), m.p. 275° (Found: M^+, 323.1266. $C_{18}H_{17}N_3O_3$ requires *M*, 323.1267); $\nu_{max.}$ 3 330, 3 150, 3 020, 2 840, 1 740, 1 690, and 1 600 cm^{-1}; *m/e* 323 (60%), 308 (8), 280 (7), 254 (49), 209 (30), 182 (63), 181 (100), 170 (26), 167 (45), 155 (14), 90 (21), 69 (10), 44 (8), and 41 (17); δ 1.54 (6 H, s, CH_3-15), 5.06 (1 H, A part of ABX, *J* 1.10 and 12.8 Hz, H_a-17), 5.20 (1 H, B part, *J* 10.5 and 1.0 Hz, H_b-17), 6.06 (1 H, X part, *J* 10.5 and 17.8 Hz, H-16), 7.40 (1 H, s, H-8), 6.9—7.55 (4 H, m, aromatic), 8.64 (1 H, s, H-1), 10.58br (2 H, s, H-11 and H-14).

Hydrolysis of Neoechinulins A—E.—On heating with 0.1N-sodium hydroxide–ethanol (1 : 1; 100 ml) under nitrogen at 75 °C for 24 h, neoechinulins A, B, and E (250 mg) each gave two main products [(VII) and (VIII) (A, B, and E)], which were separated by preparative t.l.c. (hexane–ethyl acetate, 8 : 2). The products (VII) had identical spectra, R_F 0.34, and gave an orange hydrazone with 2,4-dinitrophenylhydrazine. They were identified as 2-(1,1-*dimethylprop*-2-*enyl*)*indole*-3-*carbaldehyde*; $\nu_{max.}$ (KBr) 3 360, 2 870, 1 720, and 1 620 cm^{-1}; $\lambda_{max.}$ (EtOH) 215 (log ε 4.50), 247 (4.25), 270 (4.00), and 312 nm (3.94); (Found: M^+, 213.1154. $C_{14}H_{15}NO$ requires *M*, 213.1153). The products (VIIIA, B, and E) were identical, showing the same retention time on five different columns (isothermal): XE-60 (5%) (150 °C), Carbowax 20 M (5%) (170 °C), OV-17 (3%) (150 °C), SE-30 (5%) (150 °C), and DEGS (5%) (140 °C), R_F 0.80, purple colour with Ehrlich reagent. They were identified as 2-(1,1-*dimethylprop*-2-*enyl*)*indole*, $\nu_{max.}$ (film) 3 360 and 2 960 cm^{-1}; $\lambda_{max.}$ (hexane) 224.5 (log ε 4.47), 270 (3.83), 283 (3.70), 288 (3.68), and 295 nm (3.70) (Found: M^+, 185.1210. $C_{13}H_{15}N$ requires *M*, 185.1204).

Similarly, neoechinulins C and D, under the same conditions, gave two products each [(VII) (C and D) and (VIII) (C and D)]; which were identical with the corresponding products from neoechinulin. Compound (VII) (C and D), R_F 0.45, gave an orange-red hydrazone with 2,4-dinitrophenylhydrazine, and was shown to be 2-(1,1-dimethylprop-2-enyl)-6-(3-methylbut-2-enyl)indole-3-carbaldehyde, $\nu_{max.}$ (Nujol) 3 150 and 1 630 cm^{-1}; $\lambda_{max.}$ (EtOH) 217 (log ε 4.51), 249.5 (4.26), 273 (4.05), and 312 nm (4.02); *m/e* 281, 266, 253, 252, 226, 225, 69, and 41; δ ($CDCl_3$) 10.44 (1 H, s, CHO), 8.72 (1 H, s, NH). Compounds (VIII) (C and D) were also identical, showing the same retention time on five different g.l.c. columns (isothermal): Carbowax 20 M (5%) (210 °C), XE-60 (5%) (190 °C), SE-30 (5%) (190 °C), DEGS (5%) (190 °C), and Carbowax (5%)–Bentone (5%) (200 °C), R_F 0.85, purple with Ehrlich reagent. They were identified as 2-(1,1-dimethylprop-2-enyl)-6-(3-methylbut-2-enyl)indole, $\nu_{max.}$ (film) 3 400, 2 960, 1 450, and 920 cm^{-1}; $\lambda_{max.}$ (hexane) 224.5 (log ε 4.47), 270 (3.83), 283 (3.70), 288 (3.68), and 295.5 nm (3.70); *m/e* 253, 238, 226, 184, 169, 69, and 41; δ (CCl_4) 1.59 (6 H, s, CH_3-15), 1.90 (6 H, s, CH_3-20), 3.33 (2 H, d, H-18), 4.8—5.5 (3 H, m, H-17 and -19), 6.0 (1 H, dd, H-16), 6.1 (1 H, s, H-3), 6.72 (1 H, dd, H-5) (J_{ortho} 8, J_{meta} 1.5 Hz), and 7.52 (1 H, s, NH).

Hydrogenation of Neoechinulins A—D.—Each metabolite (50 mg) was hydrogenated in glacial acetic acid (50 ml) at room temperature, at 100 atm over platinum oxide (50 mg) for 24 h. The solvent was removed and the residue purified by preparative t.l.c. (EtOAc–hexane, 2 : 1). Neoechinulins A and B gave the same product (IX) (A and B), which was identified as cyclo-*alanyl*-2-(1,1-*dimethylpropyl*)*didehydrotryptophyl* (Found: M^+, 325.1795. $C_{19}H_{23}N_3O_2$ requires *M*, 325.1790), *m/e* 325 and 254 (*M* —

71)$^+$; $\nu_{max.}$ 3 360, 2 980, 1 670, and 1 630 cm^{-1}; $\lambda_{max.}$ 228 (log ε 4.35), 286 (4.07), 292 (4.02), and 338 nm (3.98). Similarly, neoechinulins C and D afforded the same compound (IX) (C and D), identified as cyclo-*alanyl*-2-(1,1-*dimethylpropyl*)-6-(3-*methylbutyl*)*didehydrotryptophyl* (Found: M^+, 395.2570. $C_{24}H_{33}N_3O_2$ requires M, 395.2572), *m/e* 395, 366, and 324; $\nu_{max.}$ 3 340, 2 940, 1 670, and 1 630 cm^{-1}; $\lambda_{max.}$ 230 (log ε 4.55), 265 (4.06), 290 (4.00), and 345 nm (4.04).

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