# Neocomplanines A and B, a Complanine Family Isolated from the Marine Fireworm[⊥]

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Two new members of the complanine family, neocomplanines A (**2**) and B (**3**), were isolated as minor components of the methanolic extract of the "marine fireworm" *Eurythoe complanata*. The structures of the neocomplanines were revealed spectroscopically to be a trimethylammonium unit with a saturated carbon chain. The planar structures of neocomplanines A and B were confirmed successfully through total syntheses that used epichlorohydrin as a starting material. The neocomplanines show inflammatory activity and also enhanced PKC activity in combination with TPA in the presence of $Ca^{2+}$ in vitro; both are similar to the effects of complanine (**1**). The molecular mechanism of the effects of complanine-related compounds is discussed.

The marine animals commonly known as "fireworms" are dangerous to humans since careless handling with the bare hands can result in serious dermatitis.[1] We recently isolated a novel amphipathic substance, named complanine (**1**), from the amphinomid polycheate *Eurythoe complanata* as a substance that caused inflammation. The molecular mechanism by which **1** induced inflammation was examined, and the results showed that it activated PKC (protein kinase C) in the presence of $Ca^{2+}$ and TPA (12-*O*-tetradecanoylphorbol 13-acetate).[2] This result suggested that complanine (**1**) could bind PKC at the same site as phosphatidylserine. It has previously been shown that phosphorylation through the action of PKC plays an important role in the production of various signal molecules in inflammation;[3,4] thus, the biological activity of **1** may involve controlling this cascade. From a structural point of view, complanine possesses amphipathic properties due to its characteristic unsaturated carbon chain and a $\gamma$-aminobutyric acid (GABA)-derived trimethyl ammonium substructure. The configuration of the hydroxy-substituted carbon has been determined unambiguously by means of a synthetic methodology that incorporates a chiral synthon approach.[5] The *R*-stereochemistry of this molecule showed that it is closely related to other natural products possessing a *vic*-amino alcohol moiety. Furthermore, it is known that these molecules are biosynthesized by the condensation of various fatty acids and amino acids.[6] This fact indicates that this class of molecules is chemically diverse, and related compounds may be biosynthesized by the same animal.

According to this hypothesis, we carefully searched for minor components of the methanolic extract of *E. complanata* and isolated two related compounds, neocomplanines A (**2**) and B (**3**). In this report, we describe the structures and biological activities of these molecules.

The aqueous layer of the methanolic extract of *E. complanata* (whole bodies, wet weight 225 g) was purified by chromatography on TSK-G3000S (Tosoh) polystyrene gel followed by repeated chromatography on silica gel with elution by $CHCl_3$−Me-OH−$H_2O$−AcOH, as previously reported.[2] After complanine (**1**) was isolated, precise fractionation was carried out on neighboring samples, and a final fraction (2.0 mg) with properties similar to those of **1** was obtained as two inseparable compounds. Hence, the structures were determined without further purification.

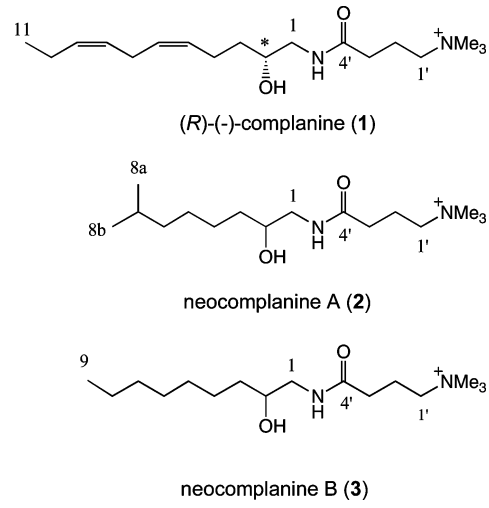


**Figure 1.** Structures of complanine and neocomplanines A and B

The mixture provided a single molecular ion peak at *m*/*z* of 287.2686 by positive HRESIMS, and thus the two molecules were considered to possess the same molecular formula of $C_{16}H_{35}N_2O_2^+$

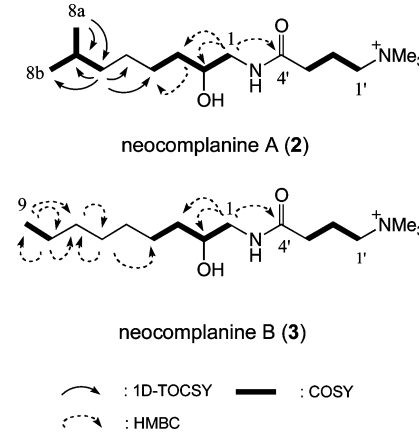


**Figure 2.** HMBC and 1D-TOCSY assignments for compounds **2** and **3**.

[⊥] Dedicated to the late Dr. John W. Daly of NIDDK, NIH, Bethesda, Maryland, and to the late Dr. Richard E. Moore of the University of Hawaii at Manoa for their pioneering work on bioactive natural products.

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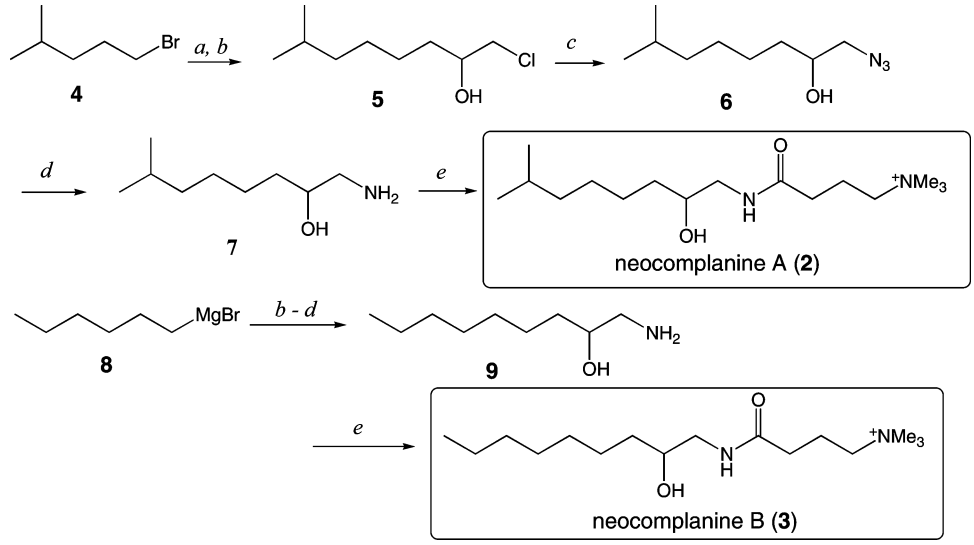
**Scheme 1.** Total Syntheses of Neocomplanines A (**2**) and B (**3**)[a]




[a] Key: (a) Mg−THF; (b) CuI then epichlorohydrin−THF; (c) $NaN_3$−DMF; (d) $H_2$−Pd/C−MeOH; (e) *γ-N,N,N*-trimethylammonium butyroxy succinimide iodide (refs 5 and 8) (2.0 equiv) in MeOH.

**Table 1.** NMR Assignments for Neocomplanines A (**2**) and B (**3**)[a]

| | **2** | | | **3** | | | |
|---|---|---|---|---|---|---|---|
| | $\delta_H$ | $\delta_C$ | HMBC correlations | | $\delta_H$ | $\delta_C$ | HMBC correlations |
| NMe | 3.34 (9H, s) | 52.4 | C-1′ | NMe | 3.34 (9H, s) | 52.4 | C-1′ |
| 1′ | 3.53 (2H, m) | 65.1 | NMe | 1′ | 3.53 (2H, m) | 65.1 | NMe |
| 2′ | 2.25 (2H, quint., $J \approx 7.6$ Hz) | 18.2 | C-1′,C-3′,C-4′ | 2′ | 2.25 (2H, quint., $J \approx 7.6$ Hz) | 18.2 | C-1′,C-3′,C-4′ |
| 3′ | 2.59 (2H, t, $J = 6.4$ Hz) | 31.2 | C-1′,C-2′,C-4′ | 3′ | 2.59 (2H, t, $J = 6.4$ Hz) | 31.2 | C-1′,C-2′,C-4′ |
| 4′ | | 173.1 | | 4′ | | 173.1 | |
| 1 | 3.53 (1H, dd, $J = 8.3,12.8$ Hz), 3.36 (1H, m) | 44.9 | C-4′,C-2,C-3 | 1 | 3.53 (1H, dd, $J = 8.3,12.8$ Hz), 3.36 (1H, m) | 44.9 | C-4′,C-2,C-3 |
| 2 | 3.89 (1H, br m) | 69.4 | | 2 | 3.89 (1H, br.m) | 69.4 | |
| 3 | 1.45, 1.49 (each 1H, m) | 34.0 | C-1,C-2,C-4 | 3 | 1.45, 1.49 (each 1H, m) | 34.0 | C-1,C-2,C-4 |
| 4 | 1.22, 1.38 (each 1H, m) | 24.9 | | 4 | 1.22, 1.38 (each 1H, m) | 24.6 | |
| 5 | 1.05 (2H, m) | 26.4 | | 5 | 1.05 (2H, m) | 28.7 | |
| 6 | 0.88 (2H, q, $J = 7.3$ Hz) | 37.6 | C-8 | 6 | 0.96 (2H, m) | 28.2 | C-4,C-7 |
| 7 | 1.22 (1H, m) | 26.8 | C-8 | 7 | 0.96 (2H, m) | 30.7 | C-6 |
| 8 | 0.66 (6H, d, $J = 6.4$ Hz) | 21.6 (2C) | C-6,C-7 | 8 | 1.03 (2H, m) | 21.6 | C-7,C-9 |
| | | | | 9 | 0.71 (3H, t, $J = 6.4$ Hz) | 13.1 | C-7,C-8 |

[a] Measured with a 600 MHz ($^1$H) and 200 MHz ($^{13}$C) spectrometer. $D_2O$/pyridine-$d_5$ was used as a solvent.

($M^+$), $\Delta = 0.8$ mmu. The compounds were assigned as being cationic, and both compounds were found to possess a *N*-trimethylated GABA moiety similar to that of complanine (by COSY analysis C-1′ to C-4′). Thus, the remaining units were determined to be $C_9H_{20}NO$. For the first compound, named neocomplanine A (**2**), two terminal methyl doublets were observed by $^1$H NMR spectroscopy [$\delta$ 0.66 (6H, d, $J = 4.4$ Hz)]. The connectivities from the C-8a,b methyl to C-7/C-6 and from C-1 to C-4 were analyzed by analysis of the COSY spectrum, and relay coupling was found in the 1D-TOCSY spectrum (irradiated C-6, C-5, and C-4). The second compound, named neocomplanine B (**3**), showed one methyl group of a straight-chain alkyl unit [$\delta$ 0.71 (3H, t, $J = 6.4$ Hz)]. The connectivities from C-9 to C-8 and C-1 to C-4 were analyzed using the COSY spectrum, and the correlations of both units were revealed from the HMBC spectrum (C-8/C-7, C-7/C-6, and C-6/C-4). The molecular ratio of neocomplanine A (**2**) and B (**3**) in this fraction was about 3:4. However, complete NMR assignments were difficult to make because of the overlapping of signals. Therefore, we sought to establish the final structures of neocomplanines by total syntheses.

Synthesis of the proposed structure of neocomplanine A (**2**) was started with a Grignard reagent generated from 1-bromo-4-methylpentane (**4**) and magnesium, which was subjected to copper-catalyzed coupling with epichlorohydrin. The nitrogen function was introduced to this compound with sodium azide, and it was then subjected to catalytic reduction to afford the corresponding amino alcohol[7] (**7**, four steps ca. 20% yield). The reaction between the amino alcohol (**7**) and the activated ester of the GABA unit[2,8] (2.0 equiv) in MeOH gave the desired neocomplanine A in 47% yield. The synthesis of neocomplanine B (**3**) was carried out in a similar manner. The reaction between hexyl magnesium bromide (**8**, commercially available) and epichlorohydrin gave the corresponding *vic*-chlorohydrin (81% yield). This compound was converted into an amino alcohol (**9**) via azide (two steps, 94% yield),[7] and coupling with the activated ester in MeOH gave the desired neocomplanine B (47% yield). The $^1$H and $^{13}$C NMR spectra of these compounds were superimposable with those of the mixture of natural neocomplanines, and thus their planar structures were confirmed to be as proposed.

The biological properties of synthetic neocomplanines were examined. Inflammatory activity was examined by the sc application of a 2% solution to a mouse footpad, and the time-course of footpad swelling was examined over 5 h. Inflammatory activity was evaluated by measuring the thickness of the footpad over time compared to a negative control. The activity (% swelling) was defined as [(thickness of tested footpad − thickness of intact

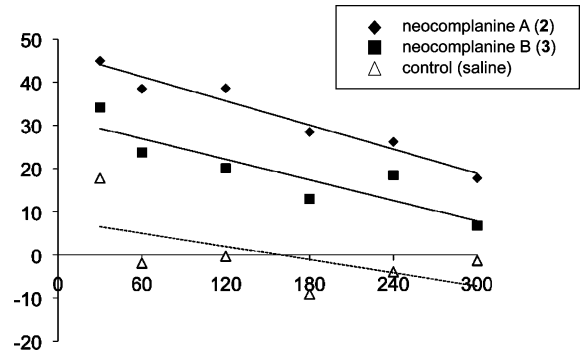



**Figure 3.** Inflammatory activities of neocomplanines. The inflammatory activity (% swelling) is defined as [(thickness of tested footpad − thickness of intact footpad)/(thickness of intact footpad)] × 100 (%). A 1% solution of carrageenan was used as a positive control to confirm reproducibility. Saline was used as a negative control.

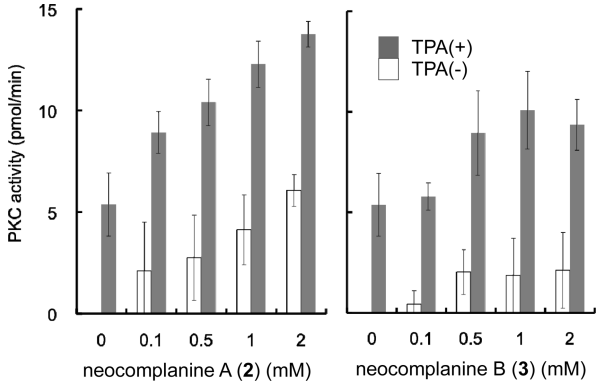



**Figure 4.** PKC activation by neocomplanines A (**2**) and B (**3**). PKC activity was determined after incubation with the indicated concentrations of complanine in the presence (solid columns) or absence (open columns) of 1.2 $\mu$M TPA. Values are the mean ± SD of triplicate experiments.

footpad)/(thickness of intact footpad)] × 100 (%). As shown in Figure 3, these compounds showed activity profiles very similar to that of complanine (**1**). Thus, we concluded that the neocomplanines (**2** and **3**), like complanine (**1**), are also inflammation-inducing substances produced by *E. complanata*.

The molecular mechanism of the inflammation produced by complanine has been shown to involve the enhancement of phosphorylation with PKC in the presence of $Ca^{2+}$ and TPA in a dose-dependent manner.[2] Thus, we examined PKC activation with neocomplanines (**2** and **3**) in the absence and presence of TPA.[9] The results are summarized in Figure 4. The neocomplanines alone showed the weak activation of PKC at concentrations higher than 0.5 mM in the presence of $Ca^{2+}$. However, in the presence of 1.2 $\mu$M TPA, the neocomplanines significantly and dose-dependently enhanced PKC activity. These bioactivity profiles are similar to that of original complanine (**1**), except that neocomplanine A (**2**) showed more potent activity than **1**.[10] We previously hypothesized that complanine (**1**) binds to the phospholipid-binding site of classical PKC, but not to the diacylglycerol/phorbol ester-binding site, since no synergistic activation of PKC is seen with the combination of complanine and L-phosphatidylserine.[2] Under biological conditions, PKC can bind to phosphatidylserine at its C-2 domain for activation, by recognition as a "membrane factor".[11] Indeed, L-phosphatidylserine activated PKC in combination with 1.2 $\mu$M TPA (20.5 ± 1.4 pmol/min, at 8.5 $\mu$g/mL). Thus, complanine and the neocomplanines are considered to bind to the same C-2 domain of PKC, instead of phosphatidylserine. Such amphipathic molecules have been conjectured to form a micellar aggregate in aqueous media, but it is not yet clear whether PKC recognizes these molecules themselves or their membrane-like superstructures in aqueous solution. It has been reported that the binding of phosphatydylserine to the C-1 domain occurs in a nonstereospecific manner, and thus the enantiomer shows almost the same binding activity.[12] In the case of complanine (**1**), the corresponding enantiomer showed almost the same activity for the induction of inflammation and the activation of PKC.[5] The detailed mechanisms that underlie the activation of PKC by complanine and related molecules are now being investigated.

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**Supporting Information Available:** $^1$H NMR data of the neocomplanines A (**2**) and B (**3**) and biological activities of (*R*)- and (*S*)-complanine (**1**). This material is available free of charge via the Internet at http://pubs.acs.org.

## References and Notes

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(7) Spectroscopic data of selected synthetic intermediates **7**: $^1$H NMR (600 MHz, $CD_3OD$) $\delta$ 3.65 (1H, br m), 2.89 (1H, dd, $J$ = 2.8, 12.8 Hz), 2.65 (1H, dd, $J$ = 9.6, 12.8 Hz), 1.49 (1H, m), 1.41 (2H, m), 1.29 (4H, m), 1.15 (2H, m), 0.83 (3 + 3H, d, $J$ = 6.9 Hz); $^{13}$C NMR (150 MHz, $CD_3OD$) $\delta$ 69.6, 46.5, 40.1, 36.1, 29.1, 28.5, 26.7, 23.0 (2C); HRESIMS *m/z* 160.1712 (calcd for $C_9H_{22}NO$ [$(M + H)^+$] 160.1701). **9**: $^1$H NMR (600 MHz, $CD_3OD$) $\delta$ 3.49 (1H, br m) 2.65 (1H, $J$ = 3.5 12.8 Hz), 2.50 (1H, dd, $J$ = 8.0, 12.8 Hz), 1.25−1.50 (12H, br), 0.89 (3H, t, $J$ = 7.1 Hz); $^{13}$C NMR (150 MHz, $CD_3OD$) $\delta$ 73.5, 48.4, 36.0, 33.0, 30.8, 30.4, 26.8, 23.7, 14.4; HRESIMS *m/z* 160.1694 (calcd for $C_9H_{22}NO$ [$(M + H)^+$] 160.1701).
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(9) Purified protein kinase C (PKC), consisting of α, $\beta$, and $\gamma$ isoforms, was purchased from Promega (Madison, WI). 12-*O*-Tetradecanoylphorbol 13-acetate (TPA) was obtained from Sigma Chemical (St. Louis, MO). [$\gamma$ -$^{32}$P]ATP was purchased from Perkin Elmer Bioscience Japan (Tokyo, Japan). PKC activity was measured using the Protein Kinase C enzyme assay system RPN77 from GE Healthcare Bioscience (Tokyo, Japan), with slight modifications. Briefly, the reaction mixture contained 3 mM Ca acetate, 10 mM dithiothreitol, 12 mM [$\gamma$-$^{32}$P]ATP (7.4 kBq), 300 $\mu$M synthetic substrate peptide, and 0.01 unit PKC. The test sample was dissolved in DMSO, and the activation of PKC was determined via incorporation of 32P into the substrate in the presence or absence of TPA or 8.5 $\mu$g/mL phosphatidylserine for 15 min at 37 °C. After stopping solution was added, an aliquot of the mixture was applied to peptide-binding paper discs, and the discs were washed with 75 mM orthophosphoric acid. Radioactivity was determined by a scintillation counter.
(10) PKC activity induced by (*R*)-complanine (**1**) was determined to be 10.9 ± 2.0 pmol/min at 2 mM in combination with TPA (see Supporting Information).
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