# Triterpene Saponins, Quaternary Ammonium Compounds, Phosphatidyl Cholines, and Amino Acids in the Pronotal and Elytral Secretions of *Platyphora opima* and *Desmogramma subtropica*

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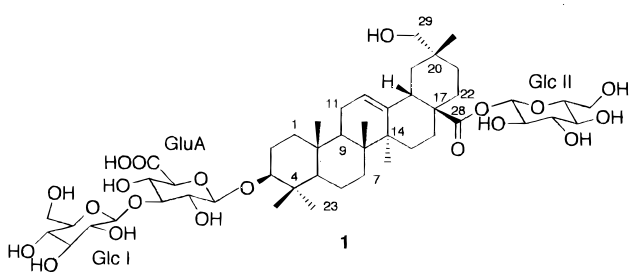
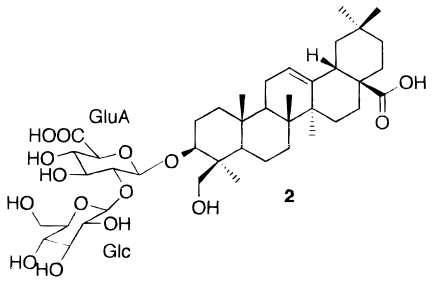
Secretions of the pronotal and elytral glands of adults of the chrysomelid beetle *Platyphora opima* from Panama have been shown to contain two oleanane triterpene saponins: the known 3-*O*-*β*-D-glucopyranosyl-(1→3)-*β*-D-glucuronopyranosyl-oleanolic acid-28-*O*-*β*-D-glucopyranoside and compound **1**, whose structure was established as 3-*O*-*β*-D-glucopyranosyl-(1→3)-*β*-D-glucuronopyranosyl-29-hydroxyoleanolic acid-28-*O*-*β*-D-glucopyranoside by a combination of 1D and 2D NMR methods (COSY, HMQC, HMBC, and TOCSY) and FABMS. The secretions also contained *N*,*N*,*N*-trimethylcadaverine and its 1,2-dehydro derivative **3**, as well as the nicotinamide derivative **4**. Secretions of *Desmogramma subtropica*, also from Panama, contained as sole triterpene derivative 3-*O*-*β*-D-glucopyranosyl-(1→2)-*β*-D-glucuronopyranosyl-24-hydroxyoleanolic acid (**2**), together with glutamic acid, glutamine, pyroglutamic acid, and arginine. A mixture of phosphatidylcholines was also present in the secretions of both species.

In the course of our ongoing studies on the chemical defense mechanisms of insects, we have undertaken the chemical examination of the secretions of pronotal and elytral glands of adult chrysomelid beetles from Panama. We recently reported the isolation and structure determination of two oleanane triterpene saponins together with chlorogenic acid from the glands of *Platyphora ligata*.[1] We report now on the isolation and identification of three other triterpene saponins, including the new compounds **1** from *Platyphora opima* Stål and **2** from *Desmogramma subtropica* Bechyné, two species of leaf-beetles collected in Panama, and belonging to the subtribe Doryphorina (Chrysomelidae). In addition, the secretions of *P. opima* contained *N*,*N*,*N*-trimethylcadaverine, its 1,2-dehydro derivative **3**, as well as the nicotinamide derivative **4**, while those of *D. subtropica* contained glutamic acid, glutamine, pyroglutamic acid, and arginine. The secretions of both species also contained mixtures of phosphatidylcholines.




**1**




**2**

## Results and Discussion

The secretions of *P. opima* were obtained as described in the Experimental Section[2] and stored in MeOH. TLC analysis (*n*-BuOH−HOAc−$H_2O$, 8:2:2) showed the presence of five spots. The corresponding compounds were separated using a combination of Sephadex LH-20 and reversed-phase chromatographies. This procedure led to the isolation of the new compounds **1**, **3**, and **4**, together with the known 3-*O*-*β*-D-glucopyranosyl-(1→3)-*β*-D-glucuronopyranosyl-oleanolic acid-28-*O*-*β*-D-glucopyranoside,[3] of *N*,*N*,*N*-trimethylcadaverine (ascophylline),[4] and of a mixture of phosphatidylcholines.[5] The known compounds were identified by comparing their $^1$H and $^{13}$C NMR spectra and FABMS with those reported in the literature. Compound **1** had the molecular formula $C_{48}H_{76}O_{20}$, determined from its negative- and positive-ion FABMS (quasi-molecular ions at *m*/*z* 971 $[M - H]^-$ and 995 $[M + Na]^+$, respectively). Its $^1$H and $^{13}$C NMR spectra indicated the presence of a *β*-D-glucopyranosyl-(1→3)-*β*-D-glucuronopyranoside moiety linked to C-3 of the aglycon. The most striking difference between the $^1$H NMR spectra of **1** and that of 3-*O*-*β*-D-glucopyranosyl-(1→3)-*β*-D-glucuronopyranosyl-oleanolic acid-28-*O*-*β*-D-glucopyranoside was the lack of the C-29 methyl signal in the $^1$H NMR spectrum of the former, which was replaced by a $CH_2OH$ group ($\delta_H$ 3.17, m, 2H). Thus, the aglycon of **1** was 29-hydroxyoleanolic acid (mesembryanthemoidigenic acid), which was confirmed by comparison with literature data.[6] It follows that **1** is 3-*O*-*β*-D-glucopyranosyl-(1→3)-*β*-D-glucuronopyranosyl-29-hydroxyoleanolic acid-28-*O*-*β*-D-glucopyranoside.

*N*,*N*,*N*-Trimethylcadaverine[4] and compound **3** were isolated as a 3:1 mixture (by $^1$H NMR) that showed, in positive-ion FABMS, two molecular ions at *m*/*z* 145.172 (calcd for $C_8H_{21}N_2$, 145.171) and 143.155 (calcd for $C_8H_{19}N_2$,

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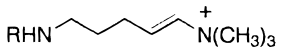
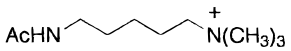
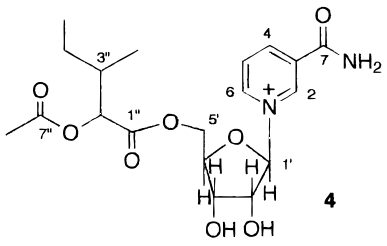
143.155), respectively, in a ratio 100:43. The $^1$H and $^{13}$C NMR spectra (see Experimental Section) indicated that the two compounds were quaternary ammonium salts [$-N^+(CH_3)_3$ at $\delta_H$ 3.14, s, and 3.31, s, for *N,N,N*-trimethylcadaverine and **3**, respectively]. This was confirmed by acetylation of the mixture with $Ac_2O$/pyridine, yielding the monoacetyl derivatives **5** and **6** ($M^+$ at *m/z* 187 and 185, respectively). The presence of a $NHCOCH_3$ group in **5** and **6** was proved by obtaining an $^1$H NMR spectrum in DMSO-$d_6$ ($NHCOCH_3$ at $\delta_H$ 7.87). Compound **3** was identified as the 1,2-dehydro derivative of *N,N,N*-trimethylcadaverine by its NMR data [e.g., H-1 at $\delta_H$ 6.36, br d, $J$ = 13.8 Hz and H-2 at $\delta_H$ 6.32, m; HMBC cross-peaks between the $N^+(CH_3)_3$ protons at $\delta_H$ 3.31 and both the C-1 at $\delta_C$ 136.9 and the C-2 at $\delta_C$ 127.2]. This hypothesis was confirmed by submitting the mixture of **5** and **6** to a catalytic hydrogenation, to afford **5** as a single compound. Compound **3** (*N,N,N*-trimethyl-1,2-dehydrocadaverine) appears to be a new natural product.




**3**: R = H
**6**: R = Ac




**5**: R = Ac




**4**

Compound **4** was strongly $UV_{254}$-positive in TLC and had the molecular formula $C_{19}H_{27}N_2O_8$ as demonstrated by its $M^+$ at *m/z* 411.177 in positive ion HRFABMS. Comparison of its $^1$H and $^{13}$C NMR spectra with literature data[7] showed that it contained a nicotinamide-$\beta$-ribofuranoside moiety, esterified at C-5′ of the ribose. This identification was strengthened by the observation of HMBC cross-peaks between H-1′ ($\delta_H$ 6.19) of the ribose moiety and C-2 ($\delta_C$ 142.0) and C-6 ($\delta_C$ 143.8) of the nicotinamide moiety, as well as between H-2 ($\delta_H$ 9.52) and H-4 ($\delta_H$ 9.05) and the C-7 amide carbonyl ($\delta_C$ 165.2). The ester moiety located at C-5′ of the ribose was identified as 2-acetoxy-3-methylpentanoate, again by NMR methods. Particularly telling were the HMBC cross-peaks between $H_2$-5′ ($\delta_H$ 4.44 and 4.78), H-2″ ($\delta_H$ 4.90), and the C-1″ carbonyl ($\delta_C$ 171.7) and between H-2″ and $H_3$-8″ ($\delta_H$ 2.04) and the acetate carbonyl ($\delta_C$ 173.0). Structure **4** was further supported by diagnostic fragment ions at *m/z* 289 ($M^+$ − nicotinamide), 157 ($C_8H_{13}O_3^+$), and 123 (nicotinamide$^+$) in its positive-ion FABMS.

As was the case for *P. ligata*[1] and *P. opima*, the secretions of *D. subtropica*, collected in Panama, showed the presence of several polar spots in TLC. The corresponding compounds were separated by a combination of Sephadex LH-20 and reversed-phase chromatographies, leading to the isolation of a mixture of phosphatidylcholines,[1,5] a triterpene glycoside (**2**), and several amino acids. Compound **2** displayed quasi-molecular ions at *m/z* 809 [M − H]$^-$, 849 [M + K]$^+$, and 833 [M + Na]$^+$, in negative- and positive-ion FABMS, respectively, and thus had the molecular formula $C_{42}H_{66}O_{15}$. Its negative-ion FABMS displayed prominent fragment ions at *m/z* 647 (M − H − Glc]$^-$) and 471 ([A − H]$^-$, $C_{30}H_{47}O_4$), thus showing that **2** possessed two sugars and that the aglycon contained 30 carbon and four oxygen atoms. The aglycon was identified as 4-epihederagenin by comparison of its NMR data with those of the literature.[8] The diglycoside portion was identified as $\beta$-D-glucopyranosyl-(1→2)-$\beta$-D-glucuronopyranoside and was attached to C-3 of the aglycon on the basis of 2D NMR data as previously discussed. This sugar moiety is identical to that already evidenced in the two triterpene glycosides of *P. ligata*.[1] Thus, compound **2** is 3-*O*-$\beta$-D-glucopyranosyl-(1→2)-$\beta$-D-glucuronopyranosyl-24-hydroxyoleanolic acid. Finally, the major free amino acids present in the secretion of *D. subtropica* were identified by NMR ($^1$H, COSY, and 1D TOCSY) as glutamic acid, glutamine, pyroglutamic acid, and arginine in a 3:2:3:2 ratio. This identification was confirmed by derivatization of the amino acid mixture using the method of Mabbott,[9] followed by GC−MS analysis. The ninhydrin-positive fraction was also submitted to acid hydrolysis by treatment with 6 N HCl under reflux for 12 h. After derivatization,[9] GC−MS analysis showed the presence of glutamic acid (major peak, corresponding to Glu, Gln, and pyroGlu), accompanied by small amounts of proline, aspartic acid (or asparagine, as the two are identical after derivatization), ornithine, and lysine. Under these conditions, arginine was not detected.[9]

We have now analyzed the secretions of three species of chrysomelid beetles from Panama belonging to the subtribe Doryphorina. Oleanane glycosides and phosphatidylcholines have been found in the three species, accompanied in *P. ligata* by chlorogenic acid,[1] in *P. opima* by the quaternary ammonium compounds *N,N,N*-trimethylcadaverine and **3** and by the nicotinamide derivative **4**, and in *D. subtropica* by large quantities of free amino acids (mostly glutamic acid and derivatives). Previous studies of Doryphorina secretions demonstrated the presence of either amino-acid derivatives or cardenolides,[10,11] although one species, *Zygogramma suturalis* Fabricius was found to contain both.[11] In this context, the discovery of oleanane triterpene saponins in the secretions of several Doryphorina from Panama is particularly interesting, especially taking into account that these beetles feed on host plants that, according to the literature, are devoid of oleanane triterpenes.[12] Thus, the origin of these compounds should be traced, and we plan to address this problem soon.

## Experimental Section

**General Experimental Procedures.** UV spectra were taken on a Philips PU 8700 UV-vis spectrophotometer in $CH_3OH$. IR spectra were recorded on a Bruker IFS 25 instrument as a film on a NaCl disk. EIMS, FABMS, and HRFABMS measurements were performed on a Fisons VG Autospec. The FABMS were obtained from a glycerol matrix, unless otherwise stated. The NMR spectra were recorded in $CD_3OD$ at 25 °C on a Varian UNITY 600 spectrometer ($^{13}$C nominal frequency of 150.87 MHz). The chemical shifts ($\delta$) are reported in parts per million (ppm) from the solvent, and the coupling constants are given in hertz. The optical rotations were measured on a Perkin-Elmer 141 polarimeter (Na-vapor lamp) in a 10-cm cell at room temperature. Thin-layer chromatography analyses (TLC) were performed on Polygram SilG/$UV_{254}$ precoated plates (0.25 mm). The compounds were visualized under $UV_{254}$ light, and/or by spraying with Dragendorff's reagent, a 0.2% ethanolic solution of ninhydrin or a 5% ethanolic solution of phosphomolybdic acid followed by a 3% ceric sulfate solution in 2 N $H_2SO_4$. In the two latter cases, spraying was followed by heating at 120 °C for 5 min.

**Biological Material.** *P. opima* was reared for several generations in Brussels on *Marsdenia maculata* Hook f. (Asclepiadaceae). The rearing started with five beetles and two larvae collected in Panama City, Park Metropolitan, on *M. maculata* in August 1997. The host-plant originating from Gamboa (Panama) was grown in a greenhouse (Brussels). *D. subtropica* was reared in Brussels on *Mikania micrantha* H. B. K. (Astereaceae) grown in a greenhouse, starting with a single pair collected 3 km north of Santa Fe de Veraguas (Panama) in early May 1997, on *Mikania* sp. The food plant originated from Gamboa. Identification of *P. opima*, *D. subtropica*, and host-plants was made by comparison with specimens in the Smithsonian Tropical Research Institute (STRI) insect collections and herbarium. The identification of *D. subtropica* was confirmed by M. Daccordi. Vouchers of plants and beetles are stored in the STRI collections. The beetles were stimulated with forceps; the secretion oozing from pores along the elytra and pronotum were collected on bits of filter paper and stored in MeOH. Beetles were remilked at intervals of about 15 days. These beetles are long-lived, especially *P. opima*, and one *P. opima* individual was milked no fewer than 17 times.

**Extraction and Isolation.** For *P. opima*, the secretions of 592 individuals collected on bits of filter paper were stored in MeOH. After filtration, the papers were exhaustively extracted with $CH_2Cl_2$/MeOH (1:1) mixture . Evaporation of the pooled extracts under reduced pressure afforded 55.2 mg of a yellowish oil, the TLC of which (eluent: *n*-BuOH−HOAc−$H_2O$, 8:2:2) showed the presence of one Dragendorff's reagent-positive spot ($R_f$ 0.0) and four major molybdophosphoric acid-positive spots ($R_f$ 0.25, 0.33, 0.65 and 0.70). The crude extract was submitted to a chromatography on Sephadex LH-20, using MeOH as eluent. This furnished three fractions: A ($R_f$ 0.25, 3.5 mg), B ($R_f$ 0.0, 0.65 and 0.70, 26.2 mg), and C ($R_f$ 0.33, 11.6 mg). The $^1$H NMR and FABMS analyses of fraction A showed that it contained a mixture of phosphatidylcholines, differing from each other by the nature of the acyl residues. No attempt was made to fully characterize this fraction. Fraction B contained two types of compounds, which were separated by reversed-phase chromatography (RP $C_{18}$) using a gradient of $H_2O$ + 0.5% TFA and increasing percentages of MeOH + 0.5% TFA as eluent. This furnished two new fractions: F1 (two compounds, $R_f$ 0.65 and 0.70, 10.5 mg) and F2 ($R_f$ 0.0, 13.9 mg). Further reversed- phase (RP $C_{18}$) purification of fraction F1, using a gradient of $H_2O$ + 0.5% TFA and increasing percentages of MeOH + 0.5% TFA as eluent, afforded compounds **1** (3.4 mg, $R_f$ 0.65) and **2** (5.8 mg, $R_f$ 0.70).

**Compound 1**: $[\alpha]^{25}_D$ +29.8° (*c* 0.57, MeOH); $^1$H NMR ($CD_3$-OD, 600 MHz) aglycon, $\delta$ 5.26 (1H, br s, H-12), 3.18 (1H, m, H-3), 3.17 (2H, m, H-29), 2.88 (1H, dd, $J$ = 13.2, 3.6 Hz, H-18), 1.16 (3H, s, H-27), 1.03 (3H, s, H-23), 0.94 (3H, s, H-25), 0.91 (3H, s, H-30), 0.82 (3H, s, H-24), 0.79 (3H, s, H-26); C-3 sugars, GluA $\delta$ 4.37 (1H, d, $J$ = 7.8 Hz, H-1′), 3.41 (1H, m, H-2′), 3.58 (2H, m, H-3′, H-4′), 3.69 (1H, m, H-5′); Glc I $\delta$ 4.62 (1H, d, $J$ = 7.8 Hz, H-1″), 3.28 (1H, m, H-2″), 3.35 (1H, m, H-3″), 3.30 (1H, t, $J$ = 9.6 Hz, H-4″), 3.28 (1H, m, H-5″), 3.63 (1H, dd, $J$ = 12.0, 3.6 Hz, H-6″a), 3.84 (1H, br d, $J$ = 12.0 Hz, H-6″b); C-28 sugar, Glc II $\delta$ 5.37 (1H, d, $J$ = 7.8 Hz, H-1‴), 3.30 (1H, m, H-2‴), 3.40 (1H, m, H-3‴), 3.35 (2H, m, H-4‴, H-5‴), 3.68 (1H, dd, $J$ = 10.8, 4.2 Hz, H-6‴a), 3.80 (1H, d, $J$ = 10.8 Hz, H-6‴b); $^{13}$C NMR ($CD_3OD$, 150.87 MHz) aglycon, $\delta$ 179.0 (C-28), 145.0 (C-13), 124.2 (C-12), 91.2 (C-3), 74.8 (C-29), 57.0 (C-5), 49.2 (C-9), 43.2 (C-14), 41.5 (C-19), 40.9 (C-8), 40.3 (C-4), 40.0 (C-1), 38.1 (C-10), 37.0 (C-20), 33.7 (C-7), 29.8 (C-21), 29.0 (C-15), 28.5 (C-23), 26.3 (C-27), 24.6 (C-11), 24.0 (C-16), 19.5 (C-30), 17.9 (C-26), 17.0 (C-24), 16.1 (C-25); C-3 sugars, GluA $\delta$ 106.8 (C-1′), 75.1 (C-2′), 86.6 (C-3′), 73.0 (C-4′); Glc I $\delta$ 105.1 (C-1″), 75.4 (C-2″), 77.9 (C-3″), 71.5 (C-4″), 78.3 (C-5″), 62.8 (C-6″); C-28 sugar, Glc II $\delta$ 96.0 (C-1‴), 74.6 (C-2‴), 78.2 (C-3‴), 71.4 (C-4‴), 79.0 (C-5‴), 62.5 (C-6‴); FABMS (negative mode) *m*/*z* 971 (17, $[M - H]^-$), 809 (7, $[M - H - Glc]^-$), 763 (6, $[M - H - Glc - HCO_2H]^-$); FABMS (positive mode) *m*/*z* 995 (0.6, $[M + Na]^+$).

Fraction F2 was made up of two compounds, *N,N,N*-trimethylcadaverine and **3**, in a 3:1 ratio. The spectroscopic properties of **3** could be assigned separately. **Compound 3**: white solid; UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 204 (3.52) nm; IR (NaCl, film) $\nu_{max}$ 3376, 2958, 1690, 1203, 1136 cm$^{-1}$; $^1$H NMR ($D_2O$, 600 MHz) 6.36 (H-1, br d, $J$ = 13.8), 6.32 (H-2, m), 3.31, (3 × $H_3$-6, s), 3.05 ($H_2$-5, t, $J$ = 7.8), 2.29 ($H_2$-3, dt, $J$ = 7.8, 6.6), 1.86 ($H_2$-4, qu, $J$ = 7.8); $^{13}$C NMR ($D_2O$, 150.87 MHz) 136.9 (C-1), 127.2 (C-2), 55.1 (C-6), 39.1 (C-5), 25.9 (C-4), 25.6 (C-3); FABMS (positive mode) *m*/*z* 143.155 ($[M]^+$, calcd for $C_8H_{19}N_2$, 143.155); Electrospray MS *m*/*z* 143 $[M]^+$.

**Acetylation of *N,N,N*-trimethylcadaverine and 3.** A mixture of *N,N,N*-trimethylcadaverine and **3** (5.0 mg) in $Ac_2O$/pyridine (1:1, 1 mL) was allowed to stand at room temperature (25 °C) overnight. After addition of water and evaporation under reduced pressure, a reversed-phase chromatography (RP $C_{18}$) of the solid residue using a gradient of $H_2O$ + 0.5% TFA and increasing percentages of MeOH + 0.5% TFA as eluent afforded compounds **5** and **6**.

**Compound 5**: IR (NaCl, film) $\nu_{max}$ 3478−3293, 1688, 1203, 1128 cm$^{-1}$; $^1$H NMR ($D_2O$, 600 MHz) 3.32 ($H_2$-1, m), 3.20 ($H_2$-5, t, $J$ = 7.2), 3.12 (3 × $H_3$-6, s), 1.99 ($H_3$-8, s), 1.82 ($H_2$-2, qu, $J$ = 7.8), 1.58 ($H_2$-4, qu, $J$ = 7.8), 1.39 ($H_2$-3, qu, $J$ = 7.8); $^{13}$C NMR ($D_2O$, 150.87 MHz) 174.4 (C-7), 66.9 (C-1), 53.2 (C-6), 39.3 (C-5), 28.1 (C-4), 23.2 (C-3), 22.3 (C-2 and C-8); FABMS (positive mode) *m*/*z* 187 $[M]^+$.

**Compound 6**: UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 205 (3.44) nm; IR (NaCl, film) $\nu_{max}$ 3418−3293, 1688, 1203, 1128 cm$^{-1}$; $^1$H NMR ($D_2O$, 600 MHz) 6.27 (H-1 and H-2, m), 3.29 (3 × $H_3$-6, s), 3.20 ($H_2$-5, t, $J$ = 7.2), 2.20 ($H_2$-3, m), 1.99 ($H_3$-8, s), 1.67 ($H_2$-4, qu, $J$ = 7.2); $^{13}$C NMR ($D_2O$, 150.87 MHz) 174.4 (C-7), 136.5 (C-1), 128.1 (C-2), 55.2 (C-6), 38.7 (C-5), 27.3 (C-4), 25.9 (C-3), 22.3 (C-8); FABMS (positive mode) *m*/*z* 185 $[M]^+$.

**Catalytic Hydrogenation of 5 and 6.** A solution of **5** and **6** (1 mg) in an EtOH/$H_2SO_4$ 97:3 solution (0.5 mL) was hydrogenated over Pd/C (10%, 0.15 mg) for 18 h. The resulting solution was filtered through Celite, eluted through Amberlite IRA 400AG, and evaporated under reduced pressure. The residue was identified as pure **5**, on the basis of its spectral properties ($^1$H NMR and FABMS). Fraction C contained a major compound, **4**.

**Compound 4**: $[\alpha]^{25}_D$ −1.6° (*c* 0.19, MeOH); UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 210 (3.8), 260 (3.44) nm; IR (NaCl, film) $\nu_{max}$ 3300, 2905, 1743, 1693, 1617, 1379, 1237 cm$^{-1}$; $^1$H NMR ($CD_3OD$, 600 MHz) 9.52 (H-2, br s), 9.19 (H-6, d, $J$ = 6.0), 9.05 (H-4, d, $J$ = 8.4), 8.30 (H-5, dd, $J$ = 7.8, 6.6), 6.19 (H-1′, d, $J$ = 4.8), 4.90 (H-2″, d, $J$ = 4.2), 4.78 (H-5′, dd, $J$ = 12.6, 4.8), 4.59 (H-4′, m), 4.44 (H-5′, dd, $J$ = 12.6, 3.0), 4.31 (H-2′, t, $J$ = 4.8), 4.16 (H-3′, dd, $J$ = 4.8, 3.6), 2.04 ($H_3$-8″, s), 1.95 (H-3″, m), 1.45 (H-4″, m), 1.30 (H-4″, m), 0.97 ($H_3$-6″, d, $J$ = 6.6), 0.92 ($H_3$-5″, t, $J$ = 7.2); $^{13}$C NMR ($CD_3OD$, 150.87 MHz) 173.0 (C-7″), 171.7 (C-1″), 165.2 (C-7), 146.7 (C-4), 143.8 (C-6), 142.0 (C-2), 136.3 (C-3), 129.6 (C-5), 101.7 (C-1′), 87.8 (C-4′), 79.5 (C-2′), 77.0 (C-2″), 72.1 (C-3′), 65.0 (C-5′), 37.9 (C-3″), 27.1 (C-4″), 20.7 (C-8″), 14.9 (C-6″), 12.2 (C-5″); FABMS (positive mode) *m*/*z* 411 (72, $[M]^+$), 289 (8, $[M - \text{nicotinamide}]^+$), 157 (8), 150 (85), 133 (35), 123 (100), 115 (17); HRFABMS (positive mode, nitrobenzyl alcohol) *m*/*z* 411.177 ($M^+$, calcd for $C_{19}H_{27}N_2O_8$, 411.177).

**Desmogramma subtropica**. The same isolation procedure as for *P. opima* was applied. Extraction of 2128 sretions gave 39.3 mg of crude extract. A chromatography on Sephadex LH-20, using MeOH as eluent followed by a reversed-phase chromatography (RP $C_{18}$), using a gradient of $H_2O$ + 0.5% TFA and increasing percentages of MeOH + 0.5% TFA as eluent, furnished three fractions: A ($R_f$ 0.25, 3.3 mg), B ($R_f$ 0.09 to 0.67, 10.0 mg), and C ($R_f$ 0.66, 11.2 mg). The $^1$H NMR and FABMS analysis of fraction A showed that it was again a mixture of phosphatidylcholines. Fraction B contained a mixture of ninhydrin-positive compounds, the major ones of which were identified as glutamic acid, glutamine, pyroglutamic acid, and arginine by NMR ($^1$H, COSY, and 1D TOCSY). Identifications were confirmed by derivatization using the method of Mabbott[9] followed by GC/MS analysis using an OV1701 capillary column. The column temperature

was programmed from 30 to 100 °C at a rate of 15°/min and then 100 to 220° at 6°/min, with an injection port temperature of 220 °C. Fraction C contained only compound **2**.

**Compound 2**: $[\alpha]^{25}_{D}$ +30.9° (*c* 0.3, MeOH); $^{1}$H NMR ($CD_3$-OD, 600 MHz) aglycon, $\delta$ 5.22 (1H, br s, H-12), 4.09 (1H, d, $J$ = 11.4, H-24), 3.35 (1H, dd, $J$ = 12.0, 4.8 Hz, H-3), 3.20 (1H, m, H-24), 2.83 (1H, br dd, $J$ = 13.2, 3.0 Hz, H-18), 1.19 (3H, s, H-23), 1.14 (3H, s, H-27), 0.93 (3H, s, H-30), 0.89 (3H, s, H-29), 0.84 (3H, s, H-25), 0.78 (3H, s, H-26); C-3 sugars, GluA $\delta$ 4.51 (1H, d, $J$ = 7.8 Hz, H-1′), 3.53 (1H, t, $J$ = 9.0 Hz, H-2′), 3.61 (1H, t, $J$ = 8.4 Hz, H-3′), 3.53 (1H, t, $J$ = 9.0 Hz, H-4′), 3.81 (1H, d, $J$ = 9.5 Hz, H-5′); Glc $\delta$ 4.76 (1H, d, $J$ = 7.8 Hz, H-1″), 3.18 (1H, m, H-2″), 3.33 (1H, m, H-3″), 3.41 (1H, t, $J$ = 9.6 Hz, H-4″), 3.19 (1H, m, H-5″), 3.70 (1H, dd, $J$ = 12.0, 3.6 Hz, H-6″a), 3.79 (1H, br d, $J$ = 12.0 Hz, H-6″b); $^{13}$C NMR ($CD_3$-OD, 150.87 MHz) aglycon $\delta$ 182.0 (C-28), 145.3 (C-13), 123.9 (C-12), 92.7 (C-3), 64.3 (C-24), 57.5 (C-5), 48.9 (C-9), 47.7 (C-17), 47.1 (C-19), 44.5 (C-4), 42.8 (C-18), 42.8 (C-14), 40.5 (C-8), 39.6 (C-1), 37.5 (C-10), 34.9 (C-21), 34.0 (C-7), 33.8 (C-22), 33.5 (C-29), 31.5 (C-20), 28.9 (C-15), 27.0 (C-2), 26.3 (C-27), 24.7 (C-11), 24.0 (C-16), 23.9 (C-30), 22.9 (C-23), 19.5 (C-6), 17.5 (C-26), 16.0 (C-25); C-3 sugars, GluA $\delta$ 105.1 (C-1′), 81.0 (C-2′), 78.3 (C-3′), 72.9 (C-4′), 76.7 (C-5′), 173.2 (C-6′); Glc $\delta$ 104.7 (C-1″), 75.7 (C-2″), 78.2 (C-3″), 70.4 (C-4″), 78.3 (C-5″), 62.0 (C-6″); FABMS (negative mode) *m*/*z* 809 (100, $[M - H]^-$), 647 (35, $[M - H - Glc]^-$), 471 (33, $[M - H - Glc - GluA]^-$); FABMS (positive mode) *m*/*z* 849 (10, $[M + K]^+$), 833 (10, $[M + Na]^+$).

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