# Two New $\boldsymbol{N}$-( $O$-Carbamoylglucopyranosyl)- $\boldsymbol{N}$-dimethylansamitocins from Actinosynnema pretiosum 

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Two new and a known N -( O -carbamoylglucopyranosyl)ansamitocins were isolated from Actinosynnema pretiosum ssp. auranticum ATCC 31565. The known $N$-(4-O-carbamoyl- $\beta$-D-glucopyranosyl)-$N$-demethylansamitocin P 2 ( $=$ ACGP-2;1) was assigned according to 1D- and 2D-NMR data, and the two new compounds were identified as $N$-(6-O-carbamoyl- $\beta$-D-glucopyranosyl)- $N$-demethylansamitocin P 2 (=ACGP-2'; 2) and $N$-(4- $O$-carbamoyl- $\beta$-d-glucopyranosyl)- $N$-demethylansamitocin P 1 ( $=$ ACGP$1 ; \mathbf{3}$ ) on the basis of spectroscopic data interpretation including 2D-NMR and tandem MS analysis.

Introduction. - Maytansinoids are a family of 19-membered macrocyclic lactams having extraordinary cytotoxic and antineoplastic activities [1][2], and are products of a bacterium (Actinosynnema pretiosum) [3], mosses, and three closely related plant families [4], i.e., Celastraceae, Rhamnaceae, and Euphorbiaceae [5]. They are structurally related to ansamycin antibiotics of microbial origin. Floss and co-workers have reported the maytansinoid (ansamitocin) biosynthetic gene cluster of Actinosynnema pretiosum ssp. auranticum ATCC 31565 [6], and recently, our group characterized the glycosyltransferase (asm25) functions in the ansamitocin biosynthesis pathway [7].

To explore the potential of $A$. pretiosum to generate glycosides, this strain was cultivated on solid-state YMG medium in our previous work, and two new $N$-demethyl-$N$-( $\beta$-D-glucopyranosyl)ansamitocins were reported [8][9]. In the present work, two new $N$-( $O$-carbamoyl- $\beta$-D-glucopyranosyl)- $N$-demethylansamitocins were isolated and characterized. Here, we report the isolation and structure elucidation of the new ansamitocins.

Results and Discussion. - In the present work, we applied an improved TLC method. By using preparative TLC as the vital isolation procedure, three $N-(O-$ carbamoyl- $\beta$-D-glucopyranosyl)- $N$-demethylansamitocins, named ACGP $2(\mathbf{1} ; 20 \mathrm{mg})$, ACGP $2^{\prime}(\mathbf{2} ; 3 \mathrm{mg})$, and ACGP $1(\mathbf{3} ; 12 \mathrm{mg})$ were obtained from the AcOEt extract of the strain of $A$. pretiosum ATCC 31565 cultivated on solid-state YMG medium (61) (Fig. 1).

Compound 1 was obtained as straw yellow solid. The positive-ion-mode HR-ESIMS suggested the molecular formula to be $\mathrm{C}_{37} \mathrm{H}_{50} \mathrm{ClN}_{3} \mathrm{O}_{15}\left([M+\mathrm{Na}]^{+}\right.$at $\mathrm{m} / \mathrm{z}$ 834.2833). The structure of compound $\mathbf{1}$ was reported in a patent [10], and its full



Fig. 1. Compounds $\mathbf{1}-\mathbf{3}$, isolated from the strain of A. pretiosum ATCC 31565
NMR data are now provided (Tables 1 and 2). Compound $\mathbf{1}$ was analyzed by tandem MS (Fig. 2). The quasimolecular-ion peak of compound $\mathbf{1}$ at $\mathrm{m} / \mathrm{z} 834\left([M+\mathrm{Na}]^{+}\right)$lost a fragment derived from the 3-ester group to produce the product-ion peak at $\mathrm{m} / \mathrm{z} 760$ $\left([M+\mathrm{Na}]^{+}\right)$in the MS (Fig. 2,a), and in the MS/MS experiment, the parent-ion peak at $m / z 760$ lost a neutral fragment (derived from 4-O-carbamoylglucopyranosyl) to produce the MS/MS product-ion peak at $m / z 555\left([M+\mathrm{Na}]^{+}\right)($Fig. 2,b). Therefore, compound 1 was determined to be $N$-(4- $O$-carbamoyl- $\beta$-D-glucopyranosyl)- $N$-demethylansamitocin P2 and named ACGP 2 (ansamitocin P2 2 2' de(acetylmethylamino)maytansine).

Compound 2 was obtained as straw yellow solid. The positive-ion-mode HR-ESIMS suggested the molecular formula to be $\mathrm{C}_{37} \mathrm{H}_{50} \mathrm{ClN}_{3} \mathrm{O}_{15}\left([M+\mathrm{Na}]^{+}\right.$at $\mathrm{m} / \mathrm{z}$ 834.2846). The ${ }^{13} \mathrm{C}$-NMR spectrum of 2 (Table 1) showed 37 C -atom signals, including $6 \mathrm{Me}, 5 \mathrm{CH}_{2}$, and 15 CH groups and 11 quaternary C-atoms. The NMR data (Tables 1 and 2) of $\mathbf{2}$ was very close to those of $\mathbf{1}$, and the same molecular formula and ${ }^{13} \mathrm{C}-\mathrm{NMR}$ data of $\mathbf{2}$ and $\mathbf{1}$ suggested their isomeric relationship. According to the NMR data, two chemical shifts had obvious dissimilarity: the $\mathrm{H}-\mathrm{C}\left(4^{\prime \prime}\right)$ appeared at $\delta(\mathrm{H}) 3.04-3.10$ $(\delta(\mathrm{C}) 71.5)$ in $\mathbf{2}$ instead of $\delta(\mathrm{H}) 4.24(\delta(\mathrm{C}) 73.0)$ in $\mathbf{1}$, and the chemical shifts of $\mathrm{CH}_{2}\left(6^{\prime \prime}\right)$ at $\delta(\mathrm{H}) 4.38-4.46$ and $4.01-4.20(\delta(\mathrm{C}) 65.4)$ of $\mathbf{2}$ replaced the signals at $\delta(\mathrm{H}) 3.57-$ 3.62 and $3.46-3.50(\delta(\mathrm{C}) 62.9)$ of $\mathbf{1}$. The ${ }^{13} \mathrm{C},{ }^{1} \mathrm{H}$ long-range HMBC data showed that one $\mathrm{H}-\mathrm{C}\left(6^{\prime \prime}\right)$ was correlated with the C -atom at $\delta(\mathrm{C}) 159.3\left(\mathrm{NH}_{2} \mathrm{CO}-\mathrm{C}\left(6^{\prime \prime}\right)\right)$. So, $\mathbf{2}$ was determined to be $N$-(6-O-carbamoyl- $\beta$-d-glucopyranosyl)- $N$-demethylansamitocin P 2 and named ACGP $2^{\prime}$ [8][9][11].

Compound $\mathbf{3}$ was obtained as straw yellow solid. The positive-ion-mode HR-ESIMS suggested the molecular formula to be $\mathrm{C}_{36} \mathrm{H}_{48} \mathrm{ClN}_{3} \mathrm{O}_{15}\left([M+\mathrm{Na}]^{+}\right.$at $\mathrm{m} / \mathrm{z}$ 820.2659). The NMR data of $\mathbf{3}$ (Tables 1 and 2) showed signals for 36 C -atoms including $6 \mathrm{Me}, 4 \mathrm{CH}_{2}$, and 15 CH groups and 11 quaternary C -atoms. According to the relative molecular masses of compounds $\mathbf{3}$ and AGP 1 [8], the presence of a carbamoyl $\left(\mathrm{CONH}_{2}\right)$ group in $\mathbf{3}$ accounted for the increase of its molecular mass by 43 mass units as compared to AGP 1. Compound $\mathbf{3}$ was also analyzed by tandem MS (Fig. 2). The quasimolecular-ion peak of $\mathbf{3}\left(\mathrm{m} / \mathrm{z} 820\left([M+\mathrm{Na}]^{+}\right)\right)$lost a fragment derived from the 3-ester group to produce the product-ion peak at $m / z 760\left([M+\mathrm{Na}]^{+}\right)$in the MS (Fig. 2, c), and in the MS/MS experiment, the parent-ion peak at $m / z 760$ had lost a

Table 1. ${ }^{13} C$-NMR Data of Compounds $\mathbf{1 - 3}$ and $A G P-2$. $\delta$ in ppm.

| C-Atom | 1 | 2 | 3 | AGP 2 |
| :---: | :---: | :---: | :---: | :---: |
| C(1) | 172.9 | 172.8 | 172.9 | 172.9 |
| $\mathrm{CH}_{2}(2)$ | 34.7 | 34.7 | 34.7 | 34.7 |
| H-C(3) | 77.8 | 77.9 | 78.8 | 77.8 |
| C(4) | 62.0 | 62.0 | 62.0 | 62.0 |
| $\mathrm{H}-\mathrm{C}(5)$ | 68.0 | 68.0 | 68.0 | 68.0 |
| $\mathrm{H}-\mathrm{C}(6)$ | 39.2 | 39.2 | 39.1 | 39.2 |
| H-C(7) | 75.9 | 75.9 | 75.9 | 75.9 |
| $\mathrm{CH}_{2}(8)$ | 37.5 | 37.5 | 37.5 | 37.4 |
| C(9) | 81.9 | 81.9 | 82.0 | 81.9 |
| H-C(10) | 89.7 | 89.8 | 89.8 | 89.8 |
| H-C(11) | 129.3 | 129.3 | 129.5 | 129.3 |
| H-C(12) | 134.0 | 134.1 | 134.0 | 134.1 |
| H-C(13) | 125.5 | 125.5 | 125.6 | 125.5 |
| $\mathrm{C}(14)$ | 141.5 | 141.5 | 141.45 | 141.5 |
| $\mathrm{CH}_{2}(15)$ | 47.5 | 47.5 | 47.5 | 47.5 |
| C(16) | 141.4 | 141.2 | 141.49 | 141.4 |
| H-C(17) | 126.3 | 126.3 | 126.2 | 126.3 |
| C(18) | 137.3 | 137.6 | 137.4 | 137.6 |
| C(19) | 123.4 | 123.4 | 123.5 | 123.4 |
| C(20) | 157.1 | 157.1 | 157.2 | 157.1 |
| H-C(21) | 115.2 | 115.2 | 115.3 | 115.1 |
| $\mathrm{C}\left(1^{\prime}\right)$ | 175.3 | 175.2 | 171.8 | 175.3 |
| $\mathrm{CH}_{2}\left(2^{\prime}\right)$ or $\mathrm{Me}\left(2^{\prime}\right)$ | 27.7 | 27.7 | 21.6 | 27.7 |
| $\mathrm{Me}\left(3^{\prime}\right)$ | 8.5 | 8.5 | - | 8.5 |
| $\mathrm{H}-\mathrm{C}\left(1^{\prime \prime}\right)$ | 84.5 | 84.6 | 84.5 | 84.7 |
| $\mathrm{H}-\mathrm{C}\left(2^{\prime \prime}\right)$ | 71.9 | 71.7 | 72.0 | 71.8 |
| $\mathrm{H}-\mathrm{C}\left(3^{\prime \prime}\right)$ | 77.0 | 79.2 | 78.8 | 79.3 |
| $\mathrm{H}-\mathrm{C}\left(4^{\prime \prime}\right)$ | 73.0 | 71.5 | 73.1 | 71.8 |
| $\mathrm{H}-\mathrm{C}\left(5^{\prime \prime}\right)$ | 78.7 | 78.0 | 77.1 | 80.2 |
| $\mathrm{CH}_{2}\left(6^{\prime \prime}\right)$ | 62.9 | 65.4 | 62.9 | 63.3 |
| CONH-C(9) | 155.3 | 155.3 | 155.3 | 155.3 |
| $\mathrm{MeO}-\mathrm{C}(10)$ | 56.9 | 56.9 | 56.9 | 56.9 |
| $\mathrm{MeO}-\mathrm{C}(20)$ | 57.1 | 57.1 | 57.2 | 57.1 |
| $M e-\mathrm{C}(4)$ | 12.1 | 12.1 | 12.1 | 12.1 |
| $M e-\mathrm{C}(6)$ | 14.7 | 14.7 | 14.6 | 14.7 |
| Me-C(14) | 15.8 | 15.8 | 15.8 | 15.8 |
| $\mathrm{NH}_{2} \mathrm{COO}-\mathrm{C}\left(4^{\prime \prime}\right)$ or $-\mathrm{C}\left(6^{\prime \prime}\right)$ | 159.3 | 159.3 | 159.3 | - |

neutral fragment $\left(\mathrm{NH}_{2} \mathrm{COOH}\right)$ to produce an MS/MS product-ion peak at $\mathrm{m} / \mathrm{z} 699$ $\left([M+\mathrm{Na}]^{+}\right)($Fig. 2,d). Comparing the NMR data of 3 and AGP 1 [8], revealed differences in the signals of $\mathrm{H}-\mathrm{C}\left(4^{\prime \prime}\right)$, i.e., $\delta(\mathrm{H}) 4.19-4.23$ and $\delta(\mathrm{C}) 73.1$ for $\mathbf{3}$ and $\delta(\mathrm{C})$ 3.04 and $\delta(\mathrm{C}) 71.9$ for AGP 1 [8], thus establishing the position of the $\mathrm{NH}_{2} \mathrm{COO}$ group at $\mathrm{C}\left(4^{\prime \prime}\right)$ of $\mathbf{3}$. Therefore, $\mathbf{3}$ was determined to be $N$-(4-O-carbamoyl- $\beta$-D-glucopyr-anosyl)- $N$-demethylansamitocin P 1 and named ACGP 1 (ansamitocin P $1=2^{\prime}$-de-(acetylmethylamino)-2'-demethylmaytansine).

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Table 2. ${ }^{l} H-N M R$ and HMBC Data of Compounds 1-3. $\delta$ in ppm, $J$ in Hz.

| H-Atom | 1 |  | 2 |  | $\begin{aligned} & \mathbf{3} \\ & \delta(\mathrm{H}) \end{aligned}$ |
| :---: | :---: | :---: | :---: | :---: | :---: |
|  | $\delta(\mathrm{H})$ | HMBC | $\delta(\mathrm{H})$ | HMBC |  |
| $\mathrm{CH}_{2}(2)$ | 2.47 (dd, $J=13.2,11.9$ ), | $\mathrm{C}(1), \mathrm{C}(3), \mathrm{C}(4)$ | 2.59-2.61 (m), | $\mathrm{C}(3), \mathrm{C}(4)$ | 2.47 (dd, $J=12.1,13.2$ ), |
|  | 2.20 (dd, J=2.8, 13.2) |  | 2.17-2.19 (m) | - | 2.18 (dd, 4.5, 13.7) |
| H-C(3) | 4.76 ( $d d, J=2.9,11.9)$ | $\mathrm{C}\left(1^{\prime}\right)$ | 4.76 ( $d$, $J=11.8$ ) | $\mathrm{C}(4), \mathrm{Me}-\mathrm{C}(4), \mathrm{C}\left(1^{\prime}\right)$ | 4.74 ( $d d, J=3.1,12.0)$ |
| $\mathrm{H}-\mathrm{C}(5)$ | 2.72 ( $d, J=9.7$ ) | $\mathrm{C}(3), \mathrm{C}(6), \mathrm{Me}-\mathrm{C}(4)$ | 2.71-2.73 (m) | $\mathrm{C}(3), \mathrm{C}(4), \mathrm{C}(6), \mathrm{C}(7), M e-\mathrm{C}(4)$ | 2.66-2.70 (m) |
| $\mathrm{H}-\mathrm{C}(6)$ | 1.49-1.58 (m) | - | 1.49-1.52 (m) | - | 1.54-1.58 (m) |
| $\mathrm{H}-\mathrm{C}(7)$ | $4.17(d d, J=3.3,10.6)$ | - | 4.25 ( $t, J=3.0)$ | - | 4.20 (dd, $J=2.8,10.4)$ |
| $\mathrm{CH}_{2}(8)$ | 1.49-1.58 (m) | - | 1.52-1.54 (m) | $\mathrm{C}(7), \mathrm{C}(9)$ | 1.54-1.58 (m) |
| H-C(10) | 3.53-3.58 (m) | $\mathrm{C}(9), \mathrm{C}(12), \mathrm{Me}-\mathrm{C}(10)$ | 3.55-3.59 (m) | $\mathrm{C}(9), \mathrm{C}(11), \mathrm{C}(12), \mathrm{MeO}-\mathrm{C}(10)$ | 3.55-3.63 (m) |
| H-C(11) | 5.53 (dd, $J=9.0,15.4)$ | C(13) | 5.51-5.54 (m) | C(13) | 5.57 (dd, $J=8.8,15.2)$ |
| H-C(12) | 6.60 ( $d d, J=11.1,15.5$ ) | $\mathrm{C}(10), \mathrm{C}(13), \mathrm{C}(14)$ | 6.63 (dd, $J=11.2,15.0)$ | C(10), C(13), C(14) | 6.63 (dd, 10.8, 15.6) |
| H-C(13) | 6.25 ( $d, J=11.1$ ) | $\mathrm{C}(11), \mathrm{C}(12), \mathrm{C}(15), \mathrm{Me}-\mathrm{C}(14)$ | 6.26 ( $d, J=10.8)$ | $\mathrm{C}(11), \mathrm{C}(12), \mathrm{C}(15), \mathrm{Me}-\mathrm{C}(14)$ | 6.26 ( $d, J=11.6)$ |
| $\mathrm{CH}_{2}(15)$ | $\begin{aligned} & 3.53-3.58(\mathrm{~m}), \\ & 3.29-3.31(\mathrm{~m}) \end{aligned}$ | $\mathrm{C}(13), \mathrm{C}(14), \mathrm{C}(21), \mathrm{Me}-\mathrm{C}(14)$ | $\begin{aligned} & 3.55-3.59(\mathrm{~m}), \\ & 3.32-3.35(\mathrm{~m}) \end{aligned}$ | $\mathrm{C}(13), \mathrm{C}(14), \mathrm{C}(17), \mathrm{C}(21), M e-\mathrm{C}(14)$ | $\begin{aligned} & 3.55-3.63(\mathrm{~m}), \\ & 3.28-3.30(\mathrm{~m}) \end{aligned}$ |
| H-C(17) | 7.20 ( $d, J=1.6$ ) | $\mathrm{C}(15), \mathrm{C}(16), \mathrm{C}(18), \mathrm{C}(21)$ | 7.20 (s) | $\mathrm{C}(15), \mathrm{C}(16), \mathrm{C}(18), \mathrm{C}(21)$ | $7.21(d, J=2.0)$ |
| H-C(21) | 7.16 ( $d, J=1.5$ ) | $\begin{aligned} & \mathrm{C}(14), \mathrm{C}(15), \mathrm{C}(16), \\ & \mathrm{C}(20), M e-\mathrm{C}(14) \end{aligned}$ | 7.16 (s) | $\mathrm{C}(15), \mathrm{C}(16), \mathrm{C}(17), \mathrm{C}(20)$ | 7.16 ( $d, J=2.0)$ |
| $\mathrm{CH}_{2}\left(2^{\prime}\right)$ or | 2.72-2.77 (m) | $\mathrm{C}\left(1^{\prime}\right), \mathrm{C}\left(3^{\prime}\right)$ | 2.73-2.75 (m) | $\mathrm{C}\left(1^{\prime}\right), \mathrm{C}\left(3^{\prime}\right)$ | 1.24 (s) |
| $\mathrm{Me}\left(2^{\prime}\right)$ | 2.50-2.56 (m) | - | 2.62-2.64 (m) | - |  |
| $\mathrm{Me}\left(3^{\prime}\right)$ | $1.08(t, J=7.4)$ | $\mathrm{C}\left(1^{\prime}\right), \mathrm{C}\left(2^{\prime}\right)$ | 1.13 ( $t, J=7.3)$ | $\mathrm{C}\left(1^{\prime}\right), \mathrm{C}\left(3^{\prime}\right)$ | - |
| H-C(1") | 5.73 ( $d, J=9.4$ ) | $\mathrm{C}(1), \mathrm{C}(18), \mathrm{C}\left(2^{\prime \prime}\right), \mathrm{C}\left(3^{\prime \prime}\right)$ | $5.71(d, J=9.5)$ | $\mathrm{C}(18), \mathrm{C}(1), \mathrm{C}\left(3^{\prime \prime}\right)$ | 5.74 ( $d, J=9.6$ ) |
| $\mathrm{H}-\mathrm{C}\left(2^{\prime \prime}\right)$ | $3.12(t, J=9.2)$ | $\mathrm{C}\left(1^{\prime \prime}\right)$ | 3.04-3.10 (m) | $\mathrm{C}\left(1^{\prime \prime}\right)$ | 3.08-3.13 (m) |
| $\mathrm{H}-\mathrm{C}\left(3^{\prime \prime}\right)$ | $3.46-3.50$ (m) | $\mathrm{C}\left(5^{\prime \prime}\right)$ | 3.53-3.56 (m) | $\mathrm{C}\left(2^{\prime \prime}\right), \mathrm{C}\left(4^{\prime \prime}\right)$ | 3.43-3.46 (m) |
| $\mathrm{H}-\mathrm{C}\left(4^{\prime \prime}\right)$ | $4.24(t(d d), J=9.6)$ | $\mathrm{C}\left(3^{\prime \prime}\right), \mathrm{C}\left(6^{\prime \prime}\right), \mathrm{NH}_{2} \mathrm{COO}-\mathrm{C}\left(4^{\prime \prime}\right)$ | 3.04-3.10 (m) | $\mathrm{C}\left(3^{\prime \prime}\right), \mathrm{C}\left(6^{\prime \prime}\right)$ | 4.19-4.23 (m) |
| $\mathrm{H}-\mathrm{C}\left(5^{\prime \prime}\right)$ | $3.46-3.50$ (m) | $\mathrm{C}\left(4^{\prime \prime}\right)$ | 3.56-3.59 (m) | - | 3.28-3.30 (m) |
| $\mathrm{CH}_{2}\left(6^{\prime \prime}\right)$ | 3.57-3.62 (m) | - | 4.38-4.46 (m) | $\mathrm{C}\left(5^{\prime \prime}\right), \mathrm{NH}_{2} \mathrm{COO}-\mathrm{C}\left(6^{\prime \prime}\right)$ | 3.55-3.64 (m) |
|  | $3.46-3.50$ (m) | - | 4.01-4.20 (m) | - | 3.43-3.46 (m) |
| $\mathrm{MeO}-\mathrm{C}(10)$ | $3.34(d, J=2.0)$ | C(10) | 3.37 ( $d, J=2.0$ ) | C(10) | 3.35 (s) |
| $\mathrm{MeO}-\mathrm{C}(20)$ | 3.98 (s) | C(20) | 3.98 (s) | C(20) | 3.96 (s) |
| $\mathrm{Me}-\mathrm{C}(4)$ | 0.79 (s) | $\mathrm{C}(4), \mathrm{C}(5)$ | 0.80 (s) | $\mathrm{C}(4), \mathrm{C}(5)$ | 0.80 (s) |
| $\mathrm{Me}-\mathrm{C}(6)$ | $1.21(d, J=6.4)$ | $\mathrm{C}(5), \mathrm{C}(6), \mathrm{C}(7)$ | 1.24 ( $d, J=6.3$ ) | $\mathrm{C}(5), \mathrm{C}(6), \mathrm{C}(7)$ | 1.24 ( $d, J=6.4$ ) |
| $\mathrm{Me}-\mathrm{C}(14)$ | 1.74 (s) | $\mathrm{C}(13), \mathrm{C}(14), \mathrm{C}(15)$ | 1.75 (s) | $\mathrm{C}(13), \mathrm{C}(14), \mathrm{C}(15)$ | 1.74 (s) |



Fig. 2. Tandem MS of a) b) ACGP 2 (1) and c) d) ACGP 1 (3)

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## Experimental Part

General. TLC: Silica gel $G F_{254}$ for separating plates and silica gel $G$ for precoated TLC plates from Qingdao Marine Chemical Factory, Qingdao, P. R. China. Column chromatography (CC): Sephadex LH20 (Amersham Biosciences) reversed-phase $C_{18}$ silica gel (Merck), and silica gel $G$ (200-300 mesh; Qingdao Marine Chemical Factory). Optical rotation: Jasco-DIP-370 digital polarimeter. UV Spectra: Shimadzu UV-2401PC; $\lambda_{\max }(\log \varepsilon)$ in nm. IR Spectra: Paragon-1000pc spectrometer; KBr pellets; in $\mathrm{cm}^{-1}$. NMR-Experiments: Bruker-AM-400 or -DRX-500 spectrometer; chemical shifts $\delta$ in ppm rel. to $\mathrm{Me}_{4} \mathrm{Si}$, coupling constants $J$ in Hz. HR-ESI- and ESI-MS: VG-Auto-Spec-3000 mass spectrometer and Finnigan Trace DSQ; values in $\mathrm{m} / \mathrm{z}$.

Microbial Material. The strain A. pretiosum ssp. aurantium ATCC31565 was obtained from Dr. T.-W. Yu and H. G. Floss of the University of Washington (Seattle, Washington State, U.S.A.), and was conserved in $20 \%$ glycerol at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences (Kunming, Yunnan Province, P. R. China). A. pretiosum was used to inoculate a plate on YMG medium (glucose ( 4.0 g ), malt extract $(10.0 \mathrm{~g})$, and yeast extract $(4.0 \mathrm{~g})$ in 1 liter of $\mathrm{H}_{2} \mathrm{O} ; \mathrm{pH} 7.2$.) at $28^{\circ}$ for $5-8$ days to afford a working seed culture. Solid fermentation was performed with solid YMG medium (61) at $28^{\circ}$ for 7 days.

Extraction and Isolation. The culture agar was chopped, diced, and extracted with AcOEt/MeOH/ AcOH $80: 15: 5$ at r.t. $(4 \times 201$, each three days) to afford the extract $(21 \mathrm{~g})$. The extract $(21 \mathrm{~g})$ was subjected to medium-pressure CC (reversed-phase $C_{18}$ silica gel $(145 \mathrm{~g}), \mathrm{H}_{2} \mathrm{O} / \mathrm{MeOH}$ containing increasing amounts of MeOH ); Fractions 1-4. Fr. 2 ( 864 mg ) was subjected to CC (Sephadex LH-20 $(130 \mathrm{~g}), \mathrm{MeOH})$ and then medium-pressure CC (reversed-phase $C_{18}$ silica gel $(45 \mathrm{~g}), \mathrm{H}_{2} \mathrm{O} / \mathrm{MeOH}$ containing increasing amounts of MeOH ): Frs. 2.1-2.3. Fraction Fr. $2.2(240 \mathrm{mg})$ was dissolved in MeOH and then repeated subjected to prep. TLC (separating plates $\left(G F_{254}\right)$, particular developing agent ( $\mathrm{AcOEt} / \mathrm{MeOH} 5: 1(100 \mathrm{ml})$ and 0.5 ml of $25 \% \mathrm{NH}_{3} / \mathrm{H}_{2} \mathrm{O}$ per plate). The prep. TLCs were run once or twice for best separation; densitometric analyses of the chromatogram were carried out with a ternary wavelength TLC scanner $Z F-I$ at 254 nm . The products were removed from the plates by elution of the scratched zones with the particular developing agent. All the compounds were purified by CC (Sephadex $L H-20(30 \mathrm{~g})$, acetone $)$ : $\operatorname{ACGP} 2(\mathbf{1} ; 20 \mathrm{mg})$, ACGP $2^{\prime}(\mathbf{2} ; 3 \mathrm{mg})$, and ACGP $1(\mathbf{3} ; 6 \mathrm{mg})$.

ACGP 2 ( $=\mathrm{N}$-(4-O-Carbamoyl- $\beta$-D-glucopyranosyl)- N -demethylansamitocin $P 2=22-[4-\mathrm{O}-($ Ami-nocarbonyl)- $\beta$-D-glucopyranosyl]-2'-de(acetylmethylamino)-22-demethylmaytansine; 1): Straw yellow solid. $[\alpha]_{\mathrm{D}}^{15}=-37(c=0.8, \mathrm{MeOH}) . \mathrm{UV}(\mathrm{MeOH}): 201.8$ (4.58), 232.0 (4.37), 253.4 (4.34), 282.8 (3.73) IR (KBr): 3443, 1694, 1659, 1429, 1362, 1161, 1082. NMR: Tables 1 and 2. ESI-MS: $834\left([M+\mathrm{Na}]^{+}\right)$. HR-ESI-MS: $834.2833\left([M+\mathrm{Na}]^{+}, \mathrm{C}_{37} \mathrm{H}_{50} \mathrm{ClN}_{3} \mathrm{NaO}_{15}^{+}\right.$; calc. 834.2828).

ACGP 2' (= N-(6-O-Carbamoyl- $\beta$-D-glucopyranosyl- N -demethylansamitocin $\quad P 2=22-[6-\mathrm{O}-($ Ami-nocarbonyl)- $\beta$-D-glucopyranosyl]-2'-de(acetylmethylamino)-22-demethylmaytansine; 2): Straw yellow solid. $[\alpha]_{\mathrm{D}}^{19}=-52(c=0.56, \mathrm{MeOH})$. UV (MeOH): 202.6 (4.54), 231.0 (4.34), 253.4 (4.28), 282.8 (3.69), 290.6 (3.68). IR (KBr): 3431, 1699, 1634, 1428, 1162, 1082. NMR: Tables 1 and 2. ESI-MS: 834 $\left([M+\mathrm{Na}]^{+}\right)$. HR-ESI-MS: $834.2846\left([M+\mathrm{Na}]^{+}, \mathrm{C}_{37} \mathrm{H}_{50} \mathrm{ClN}_{3} \mathrm{NaO}_{15}^{+}\right.$; calc. 834.2828).

ACGP 1 (= N-(4-O-Carbamoyl- $\beta$-D-glucopyranosyl)-N-demethylansamitocin P $1=22$ - $[4-\mathrm{O}-($ Ami-nocarbonyl)- $\beta$-D-glucopyranosyl]-2'-de(acetylmethylamino)-2',22-didemethylmaytansine; 3): Straw yellow solid. $[\alpha]_{\mathrm{D}}^{19}=-22(c=0.58, \mathrm{MeOH})$. UV (MeOH): 201.8 (4.63), 231.6 (4.43), 253.2 (4.39), 282.4 (3.79). IR (KBr): 3440, 1703, 1657, 1386, 1111, 1042. NMR: Tables 1 and 2. ESI-MS: $820\left([M+\mathrm{Na}]^{+}\right)$. HR-ESI-MS: $820.2659\left([M+\mathrm{Na}]^{+}, \mathrm{C}_{36} \mathrm{H}_{48} \mathrm{ClN}_{3} \mathrm{NaO}_{15}^{+}\right.$; calc. 820.2671).

## REFERENCES

[1] S. M. Kupchan, Y. Komoda, W. A. Court, G. J. Thomas, R. M. Smith, A. Karim, C. J. Gilmore, R. C. Haltiwanger, R. F. Bryan, J. Am. Chem. Soc. 1972, 94, 1354.
[2] S. M. Kupchan, Y. Komoda, A. R. Branfman, A. T. Sneden, W. A. Court, G. J. Thomas, H. P. Hintz, R. M. Smith, A. Karim, G. A. Howie, A. K. Verma, Y. Nagao, R. G. Dailey Jr., V. A. Zimmerly, W. C. Sumner Jr., J. Org. Chem. 1977, 42, 2349.
[3] E. Higashide, M. Asai, K. Ootsu, S. Tanida, Y. Kozai, T. Hasegawa, T. Kishi, Y. Sugino, M. Yoneda, Nature (London, U.K.) 1977, 270, 721.
[4] K. Sakai, T. Ichikawa, K. Yamada, M. Yamashita, M. Tanimoto, A. Hikita, Y. Ijuin, K. Kondo, J. Nat. Prod. 1988, 51, 845
[5] R. G. Powell, C. R. Smith Jr., R. D. Plattner, B. E. Jones, J. Nat. Prod. 1983, 46, 660.
[6] T.-W. Yu, L. Bai, D. Clade, D. Hoffmann, S. Toelzer, K. Q. Trinh, J. Xu, S. J. Moss, E. Leistner, H. G. Floss, Proc. Natl. Acad. Sci. U.S.A. 2002, 99, 7968.
[7] P. Zhao, L. Bai, J. Ma, Y. Zeng, L. Li, Y. Zhang, C. Lu, H. Dai, Z. Wu, Y. Li, X. Wu, G. Chen, X. Hao, Y. Shen, Z. Deng, H. G. Floss, Chem. Biol. 2008, 15, 863.
[8] J. Ma, P.-J. Zhao, Y.-M. Shen, Arch. Pharm. Res. 2007, 30, 670.
[9] C. Lu, L. Bai, Y. Shen, J. Antibiot. 2004, 57, 348.
[10] A. D. Patil, to Millenium Pharmaceuticals, Inc., PCT Int. Appl. 2007, WO 2007067698 A2, 14.06.2007.
[11] K. Hatano, E. Higashide, S.-i. Akiyama, M. Yoneda, Agric. Biol. Chem. 1984, 48, 1721.

