NOTES

The Microbial Modification of A58365A, an Angiotensin Converting Enzyme Inhibitor

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We have previously reported the discovery[1,2], isolation[3], and structure elucidation[4], of A58365A (**1**) and A58365B (**2**), a pair of angiotensin converting enzyme (ACE) inhibitors produced by fermentation of *Streptomyces chromofuscus* NRRL 15098. We now report the structure of a microbial modification product (**3**) produced from the dimethyl ester of A58365A (**4**). A screen of forty micro-organisms for their ability to bioconvert dimethyl A58365A (**4**) yielded an organism, *Streptomyces rimosus*, NRRL 2234, capable of converting **4** to a more polar derivative in high yield. A scale-up was performed to provide sufficient **3** for structural elucidation. Seventy-five milligrams of **4** was incubated with 150 ml of *Streptomyces rimosus* at 30°C in shaken flasks for 48 hours. The culture was filtered to remove the mycelium and the filtrate loaded onto a column of Diaion HP-20 adsorbent. The conversion product **3** was eluted with acetonitrile - water (1 : 1). Final purification was afforded by reversed phase HPLC on Dupont Zorbax-ODS using a mobile phase of acetonitrile - water - formic acid (8.0 : 91.8 : 0.2) to give 47 mg of pure **3**; $[\alpha]_{589}^{25}$ +154.5° (*c* 6.5, water). The structure of **3** was derived by examining results from ^{1}H NMR, ^{13}C NMR, and UV spectroscopies and from field desorption mass spectrometry, and by comparing these results to similar data for compounds **1** and **4**.

The UV spectra of **1**, **3**, and **4** in methanol solution are summarized in Table 1. The conversion product retains the pyridone chromophore of A58365A (**1**) but the chromophore no longer contains a titratable group, suggesting that the modification of **4** to produce **3** has involved a change at R_3. The field desorption mass spectrum of **3** exhibits major peaks at *m/z* 454 ($M+H^+$), 453 (M^+), and 295 ($[M-158]^+$). The molecular weight of dimethyl A58365A (**4**) is 295, suggesting that modification of **4** to form **3** involved the addition of a single moiety which is readily cleaved in the mass spectrometer.

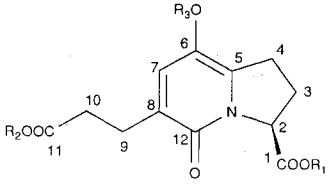
The ^{1}H NMR spectrum of **3** was examined in D_2O solution, using the solvent resonance as a chemical shift reference (residual HDO 4.80 ppm); the spectrum is very similar to that of **4**, with two exceptions: the aromatic singlet at 7.33 ppm in **4** (proton at position 7) is shifted to 7.61 ppm in **3**, and there are four new single-proton resonances. The proton spectra of **3** and **4** are compared in Table 2. Decoupling experiments indicated the following connectivity for the four new resonances of **3**: –CH (6.07 ppm)–CH (4.23 ppm)–CH (4.13 ppm)–CH (5.54 ppm)–. The 5.54 ppm resonance and the peak at 7.61 ppm each exhibit small NOEs when the other is irradiated, indicating that they are in close spatial proximity. The 6.07 ppm doublet sharpens slightly when the 4.13 ppm triplet is irradiated, removing a small four-bond coupling.

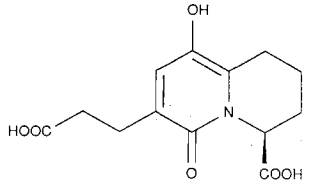
Table 1. Ultraviolet spectroscopy of **1**, **3**, and **4***.

Compound	Neutral solution	After addition of base
1	λ=325 nm (ε=7,600)	λ=353 nm (ε=7,400)
	λ=232 nm (ε=6,000)	λ=243 nm (ε=7,200)
3	λ=317 nm (ε=6,850)	No change
	λ=233 nm (ε=10,700)	
4	λ=333 nm	λ=362 nm
	λ=235 nm	λ=246 nm

* UV spectra recorded on a Cary 118 spectrometer; solvent = methanol.

Fig. 1. Structures of A58365 ACE inhibitors and modification products.





1 $R_1 = R_2 = R_3 = H$

2

4 $R_1 = R_2 = CH_3$, $R_3 = H$

3 $R_1 = R_2 = CH_3$, $R_3 = X$

5 $R_1 = R_2 = H$, $R_3 = X$

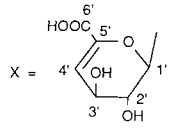


Table 2. Proton NMR comparison of **3** and **4**; chemical shifts (δ) and coupling constants (J)*.

Position	**4** δ (ppm), multiplicity	**3** δ (ppm), multiplicity, J (Hz)
2	5.13 (1H, dd)	5.22 (1H, dd; J=10, 4)
3	2.50 (1H, m),	2.62 (1H, m),
	2.30 (1H, m)	2.36 (1H, m)
4	3.11 (2H, t)	3.19 (2H, t; J=8)
7	7.33 (1H, s)	7.61 (1H, s)
9	2.80 (2H, m)	2.81 (2H, t; J=7)
10	2.63 (2H, t)	2.68 (2H, t; J=7)
(R_1, R_2)	3.75 (3H, s),	3.81 (3H, s),
	3.63 (3H, s)	3.67 (3H, s)
		6.07 (1H, d; J=4)
		5.54 (1H, d; J=3.5)
		4.23 (1H, t; J~3.5)
		4.13 (1H, t; J~3.5)

* ^{1}H NMR spectrum of **4** recorded in $CDCl_3$; spectrum of **3** recorded in D_2O. Spectra recorded using a Bruker WH360 spectrometer.

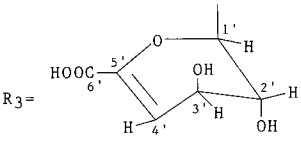
Table 3. Carbon NMR parameters for **4** and **3***.

4 δ (ppm)	**3** δ (ppm), multiplicity, coupling	Assignment
173.45	176.68 s	11
170.55	173.20 s	1
	166.13 s	6′
158.86	161.26 s	12
	141.90 s	5′
135.25	141.53 s	6
133.83	136.50 d; ^{1}H=7.61	7
132.16	136.13 s	5
128.54	129.29 s	8
	112.90 d; ^{1}H=6.07; $^1J_{C-H}$=171 Hz	4′
	101.57 d; ^{1}H=5.54; $^1J_{C-H}$=173 Hz	1′
	70.09 d; ^{1}H=4.13	2′
	66.17 d; ^{1}H=4.23	3′
62.10	63.93 d	2
52.52	54.18 q	(R_1,
51.34	52.99 q	R_2)
32.54	33.20 t	10
27.11	28.38 t	4
26.67	26.68 t	3
25.84	25.86 t	9

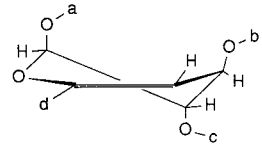
* ^{13}C NMR spectrum of **4** recorded in $CDCl_3$; spectra of **3** recorded in D_2O. Spectra recorded using a Bruker WM270 spectrometer.

The ^{13}C NMR spectrum of **3** was examined in D_2O solution using internal dioxane as the chemical shift reference (dioxane 67.4 ppm). In addition to a broad-band decoupled carbon spectrum, several spectra were recorded using single-frequency proton decoupling, and a fully coupled spectrum was also collected. The results of these experiments are summarized in Table 3.

Both the lack of a titratable group (by UV spectroscopy) and the NOEs between the resonances at 7.61 ppm and 5.54 ppm (^{1}H NMR) suggest that the new moiety in **3** is attached to the rest of the molecule through the hydroxyl group at position 6; the ^{13}C NMR results presented in Table 3 reveal that the group R_3 contains six carbon atoms, two of which are non-protonated (166.13 ppm and 141.90 ppm). The 166.13 ppm resonance is suggestive of a conjugated carboxyl group, while the 141.90 ppm resonance should arise from an sp^2 carbon attached to a heteroatom (oxygen in this case). The connectivities of the four protonated carbons are known (from the proton homonuclear and heteronuclear decoupling); both the ^{1}H and ^{13}C results indicate that the carbons at 101.57, 70.09, and 66.17 ppm are attached to oxygen (two oxygens for 101.57 ppm). These data suggest that R_3 has the elemental composition $C_6O_5H_7$, which is confirmed by the ready loss of a 158 fragment in the FD-MS ($C_6O_5H_7$=159). The proposed structure for R_3 is that of an unsaturated sugar acid, 4-deoxy-α-L-*threo*-hex-4-enopyranuronate:†

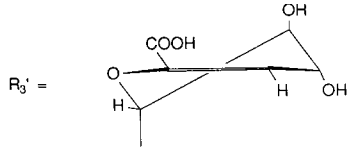


The four protons in positions 1′~4′ must all be *gauche* to their neighbors ($^3J_{H-H}$<4 Hz), and the large $^1J_{C-H}$ at position 1′ (173 Hz) indicates that the anomeric proton is equatorially oriented[5]. The small coupling of the H-2′ and H-4′ resonances is consistent with a W orientation of the four bonds connecting the protons[6]. The addition of sodium borate to a D_2O solution of **3** (pH meter reading=9.0) caused only minor changes in the ^{13}C NMR spectrum of the compound, indicating that the hydroxyl groups at 2′ and 3′ are not oriented *cis* to each other[7]. The accumulated NMR results indicate that R_3 adopts a half-chair conformation similar to that reported for the tri-acetyl methyl ester of the parent sugar[8]:



3: a=A58365A dimethyl ester, b=c=H, d=COOH; Methyl triacetyl-4-deoxy-α-L-*threo*-hex-4-enopyranuronate: a=b=c=$COCH_3$, d=$COOCH_3$.

† Note that the enantiomeric structure R_3' is equally probable on the basis of the observed NMR parameters:



Since the dimethyl ester of A58365A is inactive in the *in vitro* ACE inhibition assay[1], **3** was hydrolyzed to the free acid with 0.5 N NaOH prior to assay. The free acid (**5**) gave an IC_{50} of 1×10^{-6} M, two orders of magnitude less active than A58365A.

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