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Structure and Biosynthesis of Cetoniacytone A, a Cytotoxic Aminocarba Sugar Produced by an Endosymbiontic *Actinomyces*†

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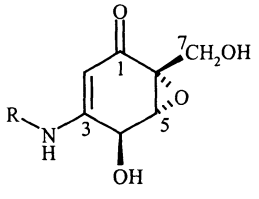
Cetoniacytone A (**1**) and some related minor components (**2**, **6**, **7**) were produced by *Actinomyces* sp. (strain Lu 9419), which was isolated from the intestines of a rose chafer (*Cetonia aureata*). The structures of the novel metabolites were established by detailed spectroscopic analysis. The absolute configuration of **1** was determined by X-ray analysis and derivatisation with chiral acids. **1** exhibits a significant cytotoxicity against selected tumor cell lines. The biosynthesis of **1** was studied by feeding ^{13}C labelled precursors. The results suggest that the characteristic *p*-C_7N skeleton of the aminocarba sugar is formed *via* the pentose phosphate pathway by cyclisation of a heptulose phosphate intermediate.

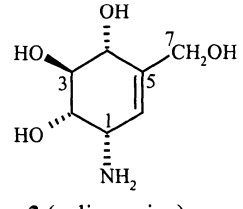
In the course of our screening program for new secondary metabolites[2] we investigated endosymbionts, which were isolated from several members of Crustacea (wood-lice), Myriapoda (millipedes) and Hexapoda (insects)[3]. In the culture broth of *Actinomyces* sp. (strain Lu 9419), which was isolated from the intestines of a rose chafer (*Cetonia aureata*) we identified two novel aminocarba sugars, which were named cetoniacytone A (**1**) and B (**2**). Carba sugars and aminocarba sugars are widespread metabolites produced especially by actinomycetes. Many of their natural derivatives are biologically active, well known examples are validamycin A[4] and acarbose[5], both containing valienamine (**3**). Structurally related to the cetoniacytones are epoxyquinomicin C (**4**) and D (**5**), which were isolated from the culture broth of an *Amycolatopsis* sp.[6]. They possess anti-arthritic effects on type II collagen-induced arthritis in mice[7] and inhibited the histidine decarboxylase in rat embryos[8].

Different biosynthetic pathways leading to carbocyclic

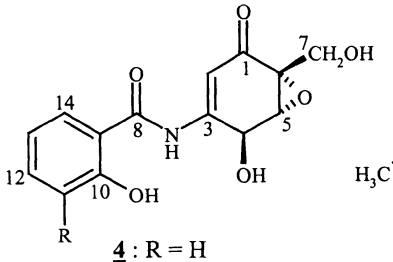
Fig. 1. Structural formulae of cetoniacytones and related compounds.



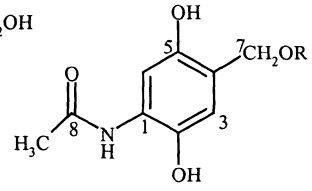
1 : R = $COCH_3$
2 : R = H



3 (valienamine)



4 : R = H
5 : R = Cl



6 : R = H
7 : R = CH_3

† Art. No. 43 on secondary metabolites by chemical screening. Art No. 42: See ref. 1.

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C_7 and C_7N units are known. Valienamine (**3**) is biosynthesized involving a C_7 sugar, which is formed from one C_3 and two C_2 units *via* the pentose phosphate pathway[9]. **3** is also an aliphatic analogue of *m*-C_7N units that are part of many antibiotics, *e.g.* the mitomycines, pactamycin, geldanamycin or the rifamycines, but their *m*-C_7N unit is synthesized *via* a branch of the shikimate pathway[10]. Similar fungal metabolites like epoxydon, enaminomycin C and terremutin represent carba sugars formed by a polyketide assembly[11]. In this paper we describe the fermentation of strain Lu 9419 as well as the isolation, characterisation, structural elucidation and biogenesis of cetoniacytone A (**1**) as main compound. Additionally we report the structures of three novel minor components, cetoniacytone B (**2**) and two aromatic analogues (**6**, **7**).

Fermentation and Isolation

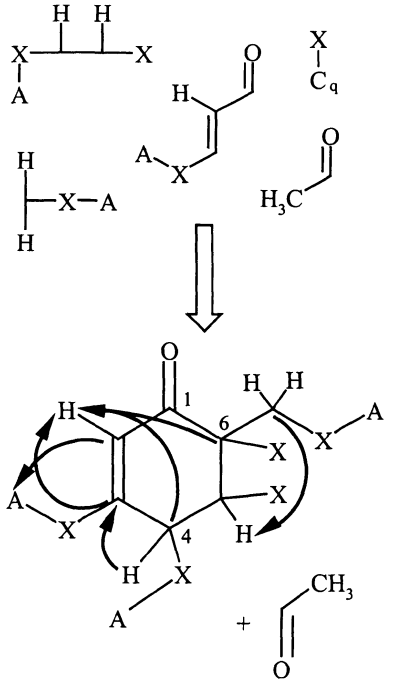
Actinomyces sp. (strain Lu 9419) was cultivated in shaking flasks, using oatmeal medium with sodium acetate (1 g/liter) as supplement for 96 hours at 28°C. The described metabolites were only found in the culture filtrate, which was separated from the mycelium by centrifugation. The filtrate was passed through Amberlite® XAD-2, from which the metabolites were eluated with methanol. The evaporation residue was separated by successive column chromatography on silica gel and Sephadex LH-20 leading to 10~15 mg/liter of cetoniacytone A (**1**). Besides 4.8 mg/liter of **1** a cultivation in absence of sodium acetate yielded 5.6 mg/liter of cetoniacytone B (**2**). Addition of glucose (1 g/liter) after 48 hours, when the production of **1** has just started, increased the yield of **1** to 20~25 mg/liter. However, addition of glucose at the beginning of the fermentation had no influence on the yield of **1**. A scale-up using a 50-liter stirring fermenter yielded 85 mg of **1** and allowed the isolation of minor components: 34 mg of 2,5-dihydroxy-4-hydroxymethylacetanilide (**6**), 4.1 mg of 2,5-dihydroxy-4-methoxymethylacetanilide (**7**) and 9.4 mg of 2-acetamido-phenol. All compounds are detectable on silica gel TLC-plates with UV-light at 254 nm and when treated with anisaldehyde-H_2SO_4 show clearly recognizable colour reactions after heating.

Structure Elucidation

The molecular formula of **1** was determined by HREI-MS to be $C_9H_{11}NO_5$ (M^+: m/z=213). A characteristic fragment ion at m/z=171 [M^+−42] in the EI-MS spectrum was attributed to the loss of an acetyl group. The IR spectrum displays characteristic absorption bands of an α, β unsaturated ketone (1698 cm^{-1}) and an amide carbonyl group (1648 cm^{-1}).

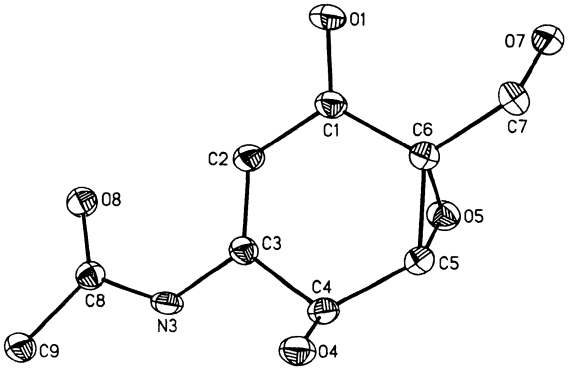
The ^1H and ^{13}C NMR spectra of **1** show the presence of eleven protons and nine carbon atoms, respectively. The carbon atoms in **1** are classified into one methyl, one methylene, two aliphatic and one olefinic methines, as well as four quaternary carbons including two carbonyl carbons by their chemical shifts and the APT spectrum. The connectivities of proton and carbon atoms were confirmed by a HMQC spectrum and indicate the presence of three interchangeable protons with signals at δ_H=9.87, 5.92~6.08 and 4.73. The connectivities between the proton-bearing groups were revealed by a ^1H-^1H COSY experiment and the elaborated fragments were connected due to a HMBC spectrum (Fig. 2).

Fig. 2. Partial structure fragments of **1** derived from ^1H-^1H COSY NMR spectra and their connection due to selected HMBC correlations (X=heteroatom, A=exchangeable protons).



The presence of five double-bond equivalents suggested a bicyclic structure for **1**. Due to their characteristic chemical shifts, the carbon signals for C-5 (δ=57.1) and C-6 (δ=58.1) could be assigned to an epoxy moiety. The

Fig. 3. Perspective view of cetoniacytone A (**1**).



relative configuration of **1** was derived from the coupling constants and was proven by an X-ray analysis of crystallized **1**. The ORTEP-type plot of **1** is shown in Fig. 3. The absolute configuration of **1** was assigned by applying the Helmchen method[12]. The esterification of **1** with 2-(*S*)- and 2-(*R*)-phenylbutyric acid and ^{1}H NMR analysis of the isolated diastereomeric diesters **1a** and **1b** (Fig. 4) revealed the *S*-configuration of the center of chirality at C-4. Therefore cetoniacytone A (**1**) shows (4*S*,5*R*,6*R*) configuration, which is the same as assigned for epoxyquinomicin D (**5**)[13].

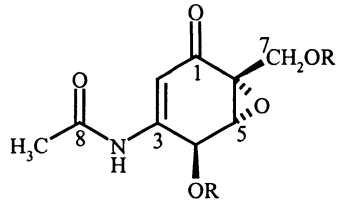
The molecular formula of cetoniacytone B (**2**) was determined by HREI-MS to be $C_7H_9NO_4$. **2** was revealed to be the *N*-deacetyl derivative of **1** by comparison of the ^{1}H and ^{13}C NMR spectral data.

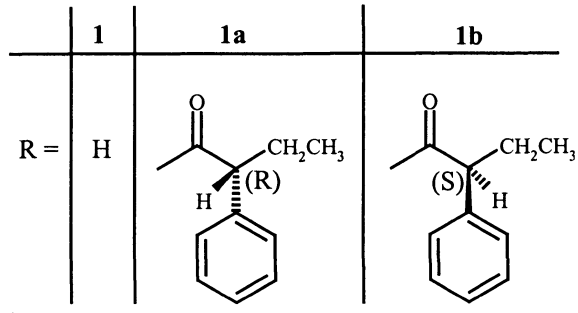
The HREI-MS of 2,5-dihydroxy-4-hydroxymethyl-acetanilide (**6**) (m/z=197 [M^+]) leads to the empirical formula $C_9H_{11}NO_4$, and a characteristic fragment ion at m/z=155 [M^+-42] points out to an acetyl group. The ^{1}H and ^{13}C NMR spectra indicate the presence of a 1,2,4,5-substituted benzene with two phenolic hydroxy groups, an acetamido group and a hydroxymethyl group as substituents. The substitution pattern of **6** was determined by a HMBC experiment.

The molecular formula of 2,5-dihydroxy-4-methoxymethyl-acetanilide (**7**) was established to be $C_{10}H_{13}NO_4$ by HREI-MS (m/z=211 [M^+]). The molecular weight difference of 14 compared to **6**, as well as very similar ^{1}H and ^{13}C NMR spectra, quickly showed **7** to be a methyl derivative of **6**. The methylation of the hydroxymethyl group derives from the fact, that the ^{13}C signal of C-7 is shifted 10 ppm downfield compared to that of **6** while the other signals remain nearly unchanged. 2-Acetamido-phenol was identified by comparison with the data reported in the literature[14].

Fig. 4. Comparison of the ^{1}H-NMR data of **1** and their derivatives measured in DMSO-d_6 (δ_H values).

	1	1a	1b	$\Delta\delta_H$ 1a-1b
2-H	6.65	6.67	6.68	- 0.01
3-NH	9.87	10.03	10.09	- 0.06
4-H	4.49	5.81	5.77	+ 0.04
5-H	3.72	3.69	3.62	+ 0.07
7-H_a	3.75	4.39	4.28	+ 0.11
7-H_b	3.87	4.54	4.38	+ 0.16
9-H_3	2.05	1.94	1.98	- 0.04





Biological Activities

In agar plate diffusion assays cetoniacytone A (**1**) showed no antimicrobial activity against Gram-positive and Gram-negative bacteria at concentrations up to 1 mg/ml. **1** was also tested against three human cancer cell lines HMO2 (stomach adenocarcinoma), HEP G2 (hepatocellular carcinoma) and MCF 7 (breast adenocarcinoma) according to the NCI guidelines[15]. In this test **1** showed a significant growth inhibition against HEP G2 (GI_{50}=3.2 μmol/liter) and MCF 7 (GI_{50}=4.4 μmol/liter).

Feeding Experiments

The increased production of cetoniacytone A (**1**) after

addition of glucose to the culture medium indicates, that the formation of **1** proceeds *via* the shikimate or the pentose phosphate pathway, because glucose stimulates both pathways. Feeding experiments with [1-^{13}C]glucose however, showed no significant incorporation of ^{13}C. This result can be best explained by a loss of the label during the pentose phosphate pathway. In this case the cetoniacytones are build *via* sedoheptulose-7-phosphate (S-7-P), which originates from ribose-5-phosphate (R-5-P) by transfer of a C_2 fragment from xylulose-5-phosphate (X-5-P). Both, R-5-P and X-5-P derive from ribulose-5-phosphate by isomerisation and epimerisation. Ribulose-5-phosphate itself is formed by decarboxylation of glucose.

To prove this hypothesis [U-$^{13}C_3$]glycerol was fed to cultures of the producing strain. If **1** is build *via* the pentose phosphate pathway glucose as well as glycerol will be incorporated during the biosynthesis. Indeed labelling of all C-atoms was observed, although the production of **1** decreased to 1 mg/liter. All ^{13}C-signals showed ^{13}C-^{13}C couplings, resulting from the intact incorporation of two C_2 and one C_3 segments of the precursor. This was further proved by feeding [U-$^{13}C_6$]glucose, which yielded **1** in an amount of 23.4 mg/liter and resulted in the same labelling pattern (Table 1).

Finally, feeding of sodium [1-^{13}C]acetate led to the labelling of the carbonyl atom of the acetyl group only. This corresponds with the proposed pathway, in which the introduction of the acetyl side chain takes place during the late biosynthesis.

Table 1. ^{13}C-^{13}C Couplings of **1** after feeding of [U-$^{13}C_3$]glycerol and [U-$^{13}C_6$]glucose.

C-atom	δ_C [ppm]	^{13}C-^{13}C coupling
1	194.5	d (J = 60 Hz)
2	106.6	d (J = 60 Hz)
3	151.7	d (J = 48 Hz)
4	63.4	dd (J = 48 Hz)
5	57.0	d (J = 48 Hz)
6	58.0	d (J = 50 Hz)
7	56.2	d (J = 50 Hz)
8	170.8	d (J = 53 Hz)
9	24.3	d (J = 53 Hz)

Discussion

The cetoniacytones A (**1**) and B (**2**) are novel aminocarba sugars produced by an endosymbiontic *Actinomyces*. **1** resembles to epoxyquinomicin C (**4**) and D (**5**) and inhibits the growth of two tumor cell lines. In contrast **4** and **5** show no cytotoxic activity, but display some other interesting biological properties[5,6].

The biosynthesis of **1** was established to proceed *via* the pentose phosphate pathway with sedoheptulose-7-phosphate as the key intermediate. These findings correspond with results from biosynthetic studies on valienamine[16] and the gabosines[17]. We assume that sedoheptulose-7-phosphate (S-7-P) cyclises by an aldol reaction between C-2 and C-7 to yield 2-*epi*-5-*epi*-valiolone (**9**) according to recent results (Fig. 5)[18]. Compound **9** seems to be the key intermediate in the formation of carba and aminocarba sugars in actinomycetes. To form the cetoniacytones a considerable number of steps such as transamination, dehydration, epoxidation and acetylation are necessary. A lot of questions remain open concerning the sequence of these steps. As a result of our investigations the amino group is introduced in *para* position to the hydroxymethyl group and the center of chirality at C-4 of **1** remains unchanged compared to S-7-P. The epoxidation needs an unsaturated precursor and proceeds *trans* to 4-OH.

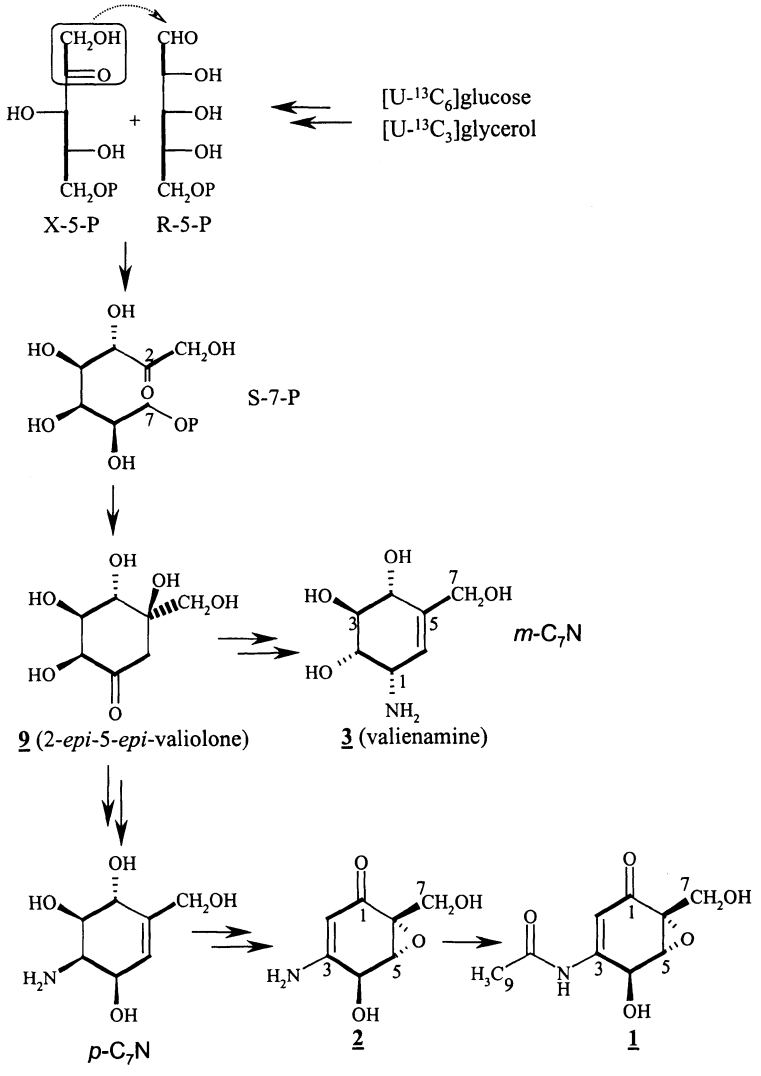
The novel aromatic minor compounds **6** and **7** seem to be shunt products. Their biosynthesis probably branches from an intermediate before the epoxidation has taken place.

Experimental

General

MP's were determined on a Reichert hot stage microscope and are not corrected. ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 and CD_3OD with Varian Unity 300 (300 MHz) and Varian Inova 500 (500 MHz) instruments. Chemical shifts are expressed in δ values (ppm) with solvents as internal standards. The mass spectra were taken by Finnigan MAT 95 (EI-MS: 70 eV, high resolution with perfluorkerosine as internal standard) and by Finnigan LQC. IR spectra were recorded on a Perkin Elmer FT IR-1600 spectrometer as KBr pellets. UV spectra were recorded on a Kontron Uvikon 860 spectrophotometer. Optical rotation values were recorded with a Perkin Elmer 241 polarimeter. TLC was carried out on silica gel 60 F_{254} plates (Merck, 0.25 mm) and column chromatography on silica gel (Macherey & Nagel, <0.08 mm) or Sephadex LH-20 (Pharmacia). Rf values were determined on 20×20

Fig. 5. Proposed biosynthetic pathway to cetoniacytone A (**1**) starting from sedoheptulose-7-phosphate (S-7-P).



Bold bonds mark the labelling pattern after feeding of [U-$^{13}C_3$]glycerol and [U-$^{13}C_6$]glucose.

cm plates, the evaluation length was 10 cm. Compounds were detected under UV lamp at 254 nm and sprayed with anisaldehyde-H_2SO_4 followed by heating.

Fermentation and Isolation

Actinomyces sp. (strain Lu 9419) was maintained as a stock culture on agar slants consisting of malt extract (1%), yeast extract (0.4%), glucose (0.4%), $CaCO_3$ (0.03%), agar (2%), pH=7.0 prior to sterilization. Fermentations were carried out in 300 ml Erlenmeyer flasks with three indentations and in a 50-liter fermenter (Biostat U, Braun). Each flask was filled with 100 ml of oatmeal medium with sodium acetate (1 g/liter) as supplement and sterilized 30 minutes at 121°C. 1 liter oatmeal medium consists of 20 g oatmeal and 2.5 ml trace element solution (1 liter contains 3 g $CaCl_2 \times 2\ H_2O$, 1 g Fe(III)-citrate, 0.2 g $MnSO_4$, 0.1 g $ZnCl_2$, 25 mg $CuSO_4 \times 5\ H_2O$, 20 mg $Na_2B_4O_7 \times 10\ H_2O$, 4 mg $CoCl_2$, 10 mg $Na_2MoO_4 \times 2\ H_2O$). The pre-cultures were inoculated at room temperature with a 1 cm^2 piece of agar from 7 day old cultures and incubated for 48 hours at 28°C on a rotary shaker (180 rpm). The main-cultures were inoculated with 5 ml of these pre-cultures and incubated for 96 hours at 28°C.

The fermenter was filled with 46 liters of oatmeal medium with sodium acetate (1 g/liter) as supplement and inoculated with 3.5 liters of a 48 hours old pre-culture. The

fermentation was carried out at 28°C with an aeration of 1.8 vvm and 200 rpm for 96 hours.

The harvested culture broths were separated from the mycelia by centrifugation (2500 rpm, 25 minutes) and the culture filtrates were passed through Amberlite® XAD-2. The more lipophilic constituents were eluated with methanol, which was evaporated to dryness under reduced pressure. The crude residue was purified by column chromatography on silica gel (CH_2Cl_2/methanol, 9 : 1) and Sephadex LH-20 (methanol).

Labelled Compounds

^{13}C labelled compounds were of 99% ^{13}C atom purity. 12.0 mmol/liter [1-^{13}C]acetate (Deutero GmbH), 10.4 mmol/liter [U-$^{13}C_3$]glycerol (CIL Inc.), 5.5 mmol/liter [1-^{13}C]glucose (CIL Inc.) and [U-$^{13}C_6$]glucose (CIL Inc.).

Feeding Experiments

Feeding experiments were carried out in 300 ml Erlenmeyer flasks under conditions as described before. Labelled precursors were dissolved in sterile water and adjusted to pH 7. The labelled precursors were added in five equal aliquots following the pulse feeding method 48, 54, 60, 66 and 72 hours after incubation. The cultures were harvested after 96 hours and worked up as described before.

Cetoniacytone A (**1**)

Colourless solid; Rf=0.11 ($CHCl_3$/MeOH=9 : 1); colour reaction with anisaldehyde-H_2SO_4: brown; MP 163°C; UV (MeOH) λ_{max} nm (ε) 284 (10500); IR ν_{max} (KBr) cm^{-1} 3460, 3350, 3302, 1698, 1648, 1537, 1265, 1031; ^1H NMR (300 MHz, DMSO-d_6) δ 2.05 (s, 3H, 9-H_3), 3.72 (d, J=1.5 Hz, 1H, 5-H), 3.75 (dd, J=13.0, 5.0 Hz, 1H, 7-H_a), 3.87 (dd, J=13.0, 5.0 Hz, 1H, 7-H_b), 4.49 (s, 1H, 4-H), 4.73 (br t, J=5 Hz, 1H, 7-OH), 6.00 (br s, 1H, 4-OH), 6.65 (s, 1H, 2-H), 9.87 (s, 1H, 3-NH); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ 24.4 (q, C-9), 56.4 (t, C-7), 57.1 (d, C-5), 58.1 (s, C-6), 63.6 (d, C-4), 106.8 (d, C-2), 151.6 (s, C-3), 170.9 (s, C-8), 194.6 (s, C-1); EI-MS *m/z* (%) 213 (4) [M^+, calcd. for $C_9H_{11}NO_5$ and found], 171 (32), 153 (30), 142 (40), 122 (30), 112 (76), 43 (100) $[CH_3CO]^+$.

X-Ray Crystallography of Cetoniacytone A (**1**)

A single colorless crystal of dimensions 0.5×0.2×0.2 mm^3 was obtained from a saturated solution in methanol and mounted inside a nylon cryo-loop (Hampton Research) using perfluorated polyether oil[19]. Crystal data: $C_9H_{11}NO_5$, M=213.19 g/mol, monoclinic, space group $P2_1$, a= 7.2218(14) Å, b=7.3290(15) Å, c=8.9174(18) Å, β= 96.76(3) Å, V=468.71(16) $Å^3$, Z=2, D_{calc}=1.511 Mgm^{-3}, F(000)=224, μ(Mo-K_α)=0.12 mm^{-1}. All measurements were made using a four-circle diffractometer equipped with a Stoe fine-focus sealed tube X-Ray generator (graphite monochromated Mo-Kα radiation), Siemens CCD area detector, Huber goniometer and low-temperature device. The diffractometer was controlled using the SMART program[20]. Intensities were measured by means of ϕ- and ω-scans with a step width of 0.5°. Frame integration was carried out using the SAINT program[21]. Of the 11564 reflections measured, 960 were used to determine the cell parameters. Within the θ range of 2.30~27.48° ($-9\leq h\leq 9$, $-9\leq k\leq 9$, $0\leq l\leq 11$), 2136 independent reflections were observed, representing 99.7% of the unique data. The structure was solved by direct methods and refined by full-matrix least-squares against F^2 using the programs SHELXS97 and SHELXL97, respectively[22,23]. Anisotropic displacement parameters were refined for all non-hydrogen atoms. Carbon-bound hydrogen atoms were placed at geometrically calculated positions and refined by a riding model, whereas the coordinates of those attached to heteroatoms were refined with distance restraints. All hydrogen atoms were refined isotropically with displacement parameters constrained to multiple U_{eq}-values of the attached atoms. In total, 146 structure parameters were refined using 4 restraints, leading to a final R1 of 0.0328 (reflections with $|F_o|>4\sigma(F_o)$), wR2 of 0.0855 (all data) and a restrained goodness of fit of 1.103. The residual electron density after the final difference Fourier synthesis was observed between −0.182 and 0.294 $eÅ^{-3}$ with a root mean square deviation of 0.041 $eÅ^{-3}$. Average estimated standard deviations are 0.002 Å for the C–C bonds and 0.1° for the C–C–C angles. Crystallographic data have been deposited at the Cambridge Crystallographic Data Centre.

Preparation of **1a** and **1b**

Dicyclohexylcarbodiimide (12.8 mg; 0.062 mmol), 4-dimethylaminopyridine (0.6 mg; 0.005 mmol) and (*R*)-2-phenylbutyric acid (10.2 mg; 0.062 mmol) were added at room temperature to a solution of **1** (6.0 mg; 0.028 mmol) in 2 ml absolute CH_2Cl_2. The mixture was stirred for 2 hours and the reaction was finished by adding 0.5 ml of water. The solvent was evaporated *in vacuo* and the residue was purified by chromatography on Sephadex LH-20 (CH_2Cl_2) to yield 8.1 mg (57%) of **1a**. **1b** was obtained by using (*S*)-2-phenylbutyric acid (10.2 mg; 0.062 mmol) instead of (*R*)-2-phenylbutyric acid under the same conditions. The analogous purification yielded 6.9 mg (49%) of **1b**. For partial ^1H NMR assignments of **1a** and **1b** see Fig. 4. ESI-MS for **1a** and **1b**: *m/z* (%) 528 (100) $[M+Na]^+$.

Cetoniacytone B (**2**)

Colourless solid; Rf=0.29 ($CHCl_3$/MeOH=4 : 1); colour reaction with anisaldehyde-H_2SO_4: yellow; UV (MeOH) λ_{max} nm (ε) 296 (7507); IR ν_{max} (KBr) cm^{-1} 3423, 1705, 1606, 1384, 1252; ^1H NMR (500 MHz, CD_3OD) δ 3.69 (d, J=1.5 Hz, 1H, 5-H), 3.91 (d, J=13.0 Hz, 1H, 7-H_a), 3.98 (d, J=13.0 Hz, 1H, 7-H_b), 4.48 (s, 1H, 4-H), 5.06 (s, 1H, 2-H); ^{13}C NMR (125.7 MHz, CD_3OD) δ 58.6 (s, C-6), 59.1 (d, C-5), 59.4 (t, C-7), 65.6 (d, C-4), 95.2 (d, C-2), 167.1 (s, C-3), 193.4 (s, C-1); EI-MS *m/z* (%) 171 (50) [M^+, calcd. for $C_7H_9NO_4$ and found], 112 (100).

2,5-Dihydroxy-4-hydroxymethylacetanilide (**6**)

Colourless solid; Rf=0.10 ($CHCl_3$/MeOH=9 : 1); colour reaction with anisaldehyde-H_2SO_4: orange; MP 176°C; UV (MeOH) λ_{max} nm (ε) 302 (3400), 245 (4843), 209 (12300); IR ν_{max} (KBr) cm^{-1} 3400, 1652, 1624, 1542, 1522, 1433, 1378; ^1H NMR (300 MHz, DMSO-d_6) δ 2.06 (s, 3H, 9-H_3), 3.16 (s, 1H, 7-OH), 4.37 (s, 2H, 7-H_2), 6.79 (s, 1H, 3-H), 7.20 (s, 1H, 6-H), 8.65 (br s, 1H, 2-OH or 4-OH), 8.87 (br s, 1H, 2-OH or 4-OH), 9.20 (s, 1H, 1-NH); ^{13}C NMR (75.5 MHz, DMSO-d_6) δ 23.6 (q, C-9), 58.0 (t, C-7), 108.6 (d, C-6), 115.1 (d, C-3), 124.7 (s, C-1 or C-4), 124.8 (s, C-1 or C-4), 140.0 (s, C-2), 146.2 (s, C-5), 168.8 (s, C-8); EI-MS *m/z* (%) 197 (60) [M^+, calcd. for $C_9H_{11}NO_4$ and found], 178 (16), 155 (50), 137 (100), 109 (30), 43 (34) [$CH_3CO]^+$.

2,5-Dihydroxy-4-methoxymethylacetanilide (**7**)

Colourless solid; Rf=0.35 ($CHCl_3$/MeOH=9 : 1); colour reaction with anisaldehyde-H_2SO_4: red; ^1H NMR (500 MHz, CD_3OD) δ 2.15 (s, 3H, 9-H_3), 3.35 (s, 3-H, OCH_3), 4.40 (s, 2H, 7-H_2), 6.75 (s, 1H, 3-H), 7.26 (s, 1H, 6-H); ^{13}C NMR (125.7 MHz, CD_3OD) δ 23.6 (q, C-9), 58.2 (q, 7-OCH_3), 70.6 (t, C-7), 110.4 (d, C-6), 117.9 (d, C-3), 122.4 (s, C-4), 127.3 (s, C-1), 141.9 (s, C-2), 149.4 (s, C-5), 172.0 (s, C-8). EI-MS *m/z* (%) 211 (38) [M^+, calcd. for $C_{10}H_{13}NO_4$ and found], 179 (28) [M–$CH_3OH]^+$, 168 (22), 137 (100), 109 (24), 43 (24) [$CH_3CO]^+$.

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References

1) BODE, H. B.; M. WALKER & A. ZEECK: Cladospirones B to I from *Sphaeropsidales* sp. F-24'707 by variation of culture conditions. Eur. J. Org. Chem.: 3185~3193, 2000
2) GRABLEY, S.; R. THIERICKE & A. ZEECK: The chemical screening approach. *In* Drug Discovery from Nature. *Eds.*, S. GRABLEY & R. THIERICKE, pp. 124~148, Springer, Berlin-Heidelberg, 1999
3) GEBHARDT, K.; J. SCHIMANA, J. MÜLLER, H. P. FIEDLER, H. G. KALLENBORN, M. HOLZENKÄMPFER, P. KRASTEL, A. ZEECK, J. VATER, A. HÖLTZEL, J. RHEINHEIMER & K. DETTNER: Screening for biologically active metabolites with endosymbiotic bacilli isolated from arthropods. FEMS Microbiol. Lett.: in preparation
4) IWAZA, T.; E. HIGASHID, H. YAMAMOTO & M. SHIBATA: Studies on validamycins, new antibiotics. II. Production and biological properties of validamycins A and B. J. Antibiotics 24: 107~113, 1971
5) SCHMIDT, D. D.; W. FROMMER, B. JUNGE, L. MÜLLER, W. WINGENDER, E. TRUSCHEIT & D. SCHAFTER: Alpha-glucosidase inhibitors—new complex oligosaccharides of microbial origin. Naturwissenschaften 64: 535~537, 1977
6) MATSUMOTO, N.; T. TSUCHIDA, M. UMEKITA, N. KINOSHITA, H. IINUMA, T. SAWA, M. HAMADA & T. TAKEUCHI: Epoxyquinomicins A, B, C and D, new antibiotics from *Amycolatopsis*. I. Taxonomy, fermentation, isolation and antimicrobial activities. J. Antibiotics 50: 900~905, 1997
7) MATSUMOTO, N.; H. IINUMA, T. SAWA, T. TAKEUCHI, S. HIRANO, T. YOSHIOKA & M. ISHIZUKA: Epoxyquinomicins A, B, C and D, new antibiotics from *Amycolatopsis*. II. Effect on type II collagen-induced arthritis in mice. J. Antibiotics 50: 906~911, 1997
8) MATSUMOTO, N.; N. AGATA, H. KUBOKI, H. IINUMA, T. SAWA, T. TAKEUCHI & K. UMEZAWA: Inhibition of rat embryo histidine decarboxylase by epoxyquinomicins. J. Antibiotics 53: 637~639, 2000
9) MAHMUD, T.; J. TORNUS, E. EGELKROUT, E. WOLF, C. UY, H. G. FLOSS & S. LEE: Biosynthetic studies on the α-glucosidase inhibitor acarbose in *Actinoplanes* sp.: 2-epi-5-epi-valiolone is the direct precursor of the valienamine moiety. J. Am. Chem. Soc. 121: 6973~6983, 1999
10) FLOSS, H. G. & J. M. BEALE: Untersuchungen zur Biosynthese von Antibiotika. Angew. Chem. 101: 147~179, 1989
11) TURNER W. B. & D. C. ALDRIDGE: Fungal metabolites II. pp. 60~69, Academic Press, London, 1983
12) HELMCHEN, G.: Über eine neue Methode zur Bestimmung der absoluten Konfiguration an sekundären Alkoholen und Aminen: NMR-Spektroskopie von Diastereomeren Estern und Amiden der α-Phenylbutter- und Hydratropansäure. Tetrahedron Letters 16: 1527~1530, 1974
13) MATSUMOTO, N.; T. TSUCHIDA, R. SAWA, H. IINUMA, H. NAKAMURA, H. NAGANAWA, T. SAWA & T. TAKEUCHI: Epoxyquinomicins A, B, C and D, new antibiotics from *Amycolatopsis*. III. Physico-chemical properties and structure determination. J. Antibiotics 50: 912~915, 1997
14) PUSECKER, K.; H. LAATSCH, E. HELMKE & H. WEYLAND, Dihydrophenocomycin methyl ester, a new phenazine derivative from a marine streptomycete. J. Antibiotics 50: 479~483, 1997

15) GREVER, M. R.; S. A. SCHEPARTZ & B. A. CHABNER: The National Cancer Institute: Cancer drug discovery and development program. Semin. Oncol. 19: 622~638, 1992
16) DEGWERT, U.; R. VAN HÜLST, H. PAPE, R. E. HEROLD, J. M. BEAL, P. J. KELLER, J. P. LEE & H. G. FLOSS: Studies on the biosynthesis of the α-glucosidase inhibitor acarbose: Valienamine, a *m*-C_7N unit not derived from the shikimate pathway. J. Antibiotics 40: 855~861, 1987
17) HÖFS, R.; S. SCHOPPE, R. THIERICKE & A. ZEECK: Biosynthesis of gabosines A, B and C, carba sugars from *Streptomyces cellulosae*. Eur. J. Org. Chem.: 1883~1887, 2000
18) HAIJUN, D., M. TAIFO, I. TORNUS, S. LEE & H. G. FLOSS: Biosynthesis of the validamycins: Identification of intermediates in the biosynthesis of validamycin A by *Streptomyces hygroscopicus* var. *limoneus*. J. Am. Chem. Soc. 123: 2733~2742, 2001
19) KOTTKE, T. & D. STALKE: Crystal handling at low temperatures. J. Appl. Crystallogr. 26: 615, 1993
20) SIEMENS: *SMART*. Version 4.202. Siemens Analytical X-ray Instruments Inc., Madison, Wisconsin, 1996
21) BRUKER: *SAINT*. Version 5.0. Bruker AXS Inc., Madison, Wisconsin, 1997
22) G. M. SHELDRICK: Phase annealing in SHELX-90: direct methods for larger structures. Acta Cryst. A46: 467~473, 1990
23) G. M. SHELDRICK: *SHELXS97* and *SHELXL97*. University of Göttingen, 1997