Notes

SYNTHESIS OF CAIROMYCIN A

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Recently SHIMI and coworkers have isolated three peptide antibiotics from the culture broth of *Streptomyces* sp. strain AS-C-19 collected from the Cairo area and designated them as cairomycins A, B and C. Based primarily on analytical physico-chemical and spectral data these workers reported the structures[1,2] of cairomycin B and cairomycin A. Cairomycin A was shown to contain L-valine and L-aspartic acid and has been tentatively assigned as 6-isopropyl-2,5-diketopiperazine-3-acetic acid (**5**)[2]. In this paper we report the synthesis of this compound (**5**), cairomycin A.

t-Butyloxycarbonyl-L-valine (**1**) and L-aspartic acid dibenzyl ester-*p*-toluene sulfonate (**2**) were prepared from L-valine and L-aspartic acid by standard methods[3,4]. Dicyclohexylcarbodiimide and *N*-hydroxysuccinimide mediated coupling of these protected derivatives (**1** and **2**) gave the protected dipeptide (**3**) in 80% yield (Scheme 1). Compound **3** was deblocked at the *N*-terminus with anhydrous trifluoroacetic acid in quantitative yield and the trifluoroacetate salt was refluxed with an equivalent amount of triethylamine in dichloromethane to give the protected 2,5-diketopiperazine (**4**) in 56% yield. Catalytic hydrogenation of **4** over palladised charcoal in 5% acetic acid in methanol gave the deprotected diketopiperazine (**5**) in 94% yield.

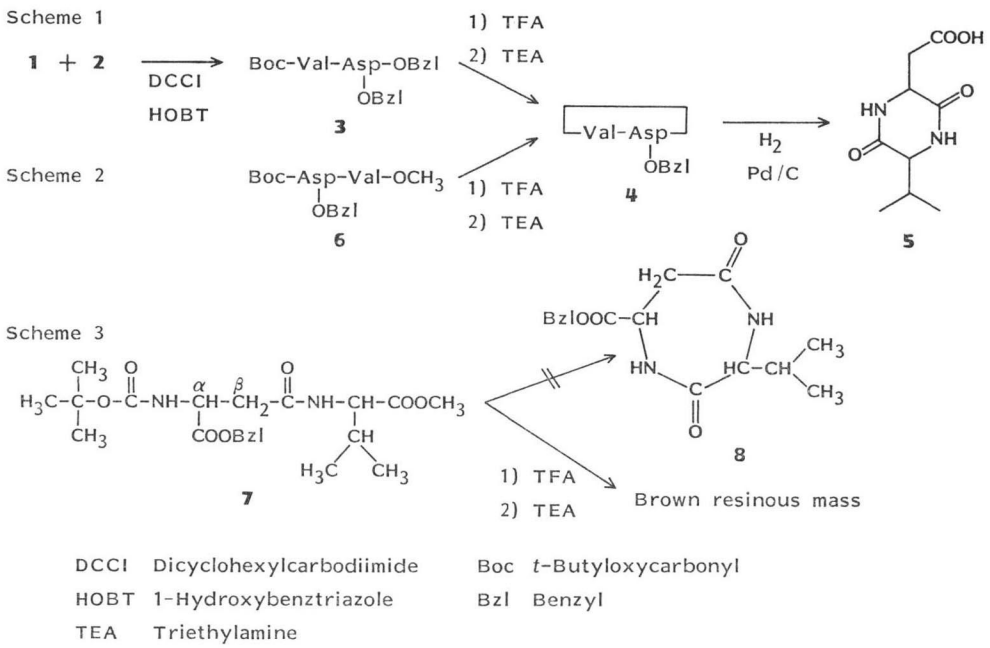
Although diketopiperazine ring formation from dipeptide esters is known to be a facile process[5,6], formation of a seven membered ring compound (**8**) from internal cyclization of **7** could be seen as a possibility and such a compound, if formed, would show physico-chemical properties similar to those of cairomycin A (**5**). A dipeptide (**7**) was prepared from *t*-butyloxycarbonyl-L-aspartic acid-α-benzyl ester and L-valine methyl ester hydrochloride in THF by dicyclohexylcarbodiimide and 1-hydroxybenztriazole mediate coupling in 80% yield. However, under similar conditions as employed in the preparation of **5** (Scheme 3), a dark brown resinous mass was obtained which even on extensive chromatographic separation did not yield homogeneous products. To exclude the possibility of formation of seven membered ring compound (**8**), a dipeptide (**6**) was prepared from *t*-butyloxycarbonyl-L-aspartic-β-benzyl ester and L-valine methyl ester hydrochloride by dicyclohexylcarbodiimide - 1-hydroxybenztriazole mediated coupling in 95% yield. Deprotection at the *N*-terminal followed by cyclization (Scheme 2) as above gave a compound identical to **4**. On a catalytic hydrogenation the target compound (**5**) was obtained in 52% overall yield. All the intermediates and the target compound (**5**) were obtained chromatographically homogeneous and were fully characterized†.

Physico-chemical properties particularly elemental analysis, mass, ^{1}H NMR and ^{13}C NMR spectra indicate the synthetic compound (**5**) to be 6-isopropyl-, 2,5-diketopiperazine-3-acetic acid which is also the structure of cairomycin A as proposed by SHIMI *et al.* Discrepancies in melting point and physical appearance suggest that perhaps cairomycin A isolated from natural

† Satisfactory NMR, IR and elemental analysis data were obtained on all the compounds. TLC (silica gel) purity was checked in at least two solvent systems. System (A): $CHCl_3$ - MeOH (9:1), system (B): BuOH - CH_3COOH - H_2O (4:1:1). **3** Rf (A) 0.78, Rf (B) 0.70; mp 77~78°C; **4** Rf (A) 0.69, Rf (B) 0.57; mp 205~207°C, $[\alpha]_D^{22}$ −26.8° (*c* 0.33, acetic acid); **6** Rf (A) 0.90, Rf (B) 0.82; mp 105~106°C; **7** Rf (A) 0.69, Rf (B) 0.73; mp 93~95°C.

Selected data: **5** Rf (A) 0.54, Rf (B) 0.72; $[\alpha]_D^{22}$ −56.7° (*c* 0.84, acetic acid); ninhydrin negative; acid hydrolysate of **5** (6 N, HCl, 110°C) showed only aspartic acid and valine; MS *m/z* 214 (M^+); ^{1}H NMR (90 MHz; $CDCl_3$+a drop of TFA) 1.0 and 1.12 (6H, two separate doublets, $CH(CH_3)_2$, J=7 Hz), 2.55~2.60 (1H, multiplet, $CH(CH_3)_2$), 2.95~3.2 (2H, two distorted doublets, CH_2COOH), 4.12 (1H, triplet, α-CH), 4.6 (1H, multiplet, α-CH); ^{13}C NMR (200 MHz, $CDCl_3$ + a drop of TFA) 175.68, 169.78, 169.12, 60.69, 51.32, 38.31, 31.68, 18.43, 16.18.

Fig. 1.



DCCI Dicyclohexylcarbodiimide
HOBT 1-Hydroxybenztriazole
TEA Triethylamine
Boc *t*-Butyloxycarbonyl
Bzl Benzyl

Table 1. Physico-chemical properties of natural and synthetic cairomycin A.

	Cairomycin A[2)]	Synthetic compound
Appearance	Yellowish brown powder	Colorless crystalline compound
MP	110~112°C	238~240°C
UV Spectrum	No characteristic absorption	No characteristic absorption
IR (cm^{-1}) Strong bands at	1735, 1625 and 1030	3200, 1740, 1455, 1370, 1260 and 830
^1H NMR (selected data)	($CDCl_3$) 1.02 (6H, doublet, $CH(CH_3)_2$), 1.56 (1H, heptet, $CH(CH_3)_2$, 3.04 (2H, doublet, CH_2COOH)	1.0 and 1.12 (6H, two separate doublets, J=7 Hz, $CH(CH_3)_2$), 2.25~2.6 (1H, multiplet, $CH(CH_3)_2$), 2.95~3.2 (2H, two distorted doublets, CH_2COOH)
MS (M^+)	214	214
Anal Found:	C 50.50, H 6.51, N 12.93	C 50.62, H 6.59, N 12.89
Calcd ($C_9H_{14}N_2O_4$):	C 50.47, H 6.54, N 13.08	

source could have been further purified. Diketopiperazines from natural amino acids are usually crystalline compounds with high melting points[6)]. Synthetic cairomycin A was tested for antimicrobial activity against *Staphylococcus aureus* and *E. coli* and found to have minimum inhibitory concentration (25 μg/ml) as similar to the value reported for natural cairomycin A.

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