STUDIES ON THE REACTIONS OF 2,3-DIKETOPYRIDO[4,3,2-*de*] QUINOLINES WITH AMINO ACIDS AND AMINO ESTERS

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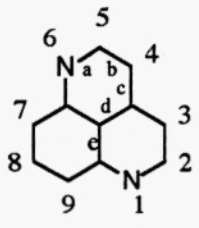
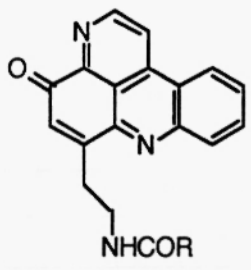
Abstract: A reaction of 2,3-diketopyrido[4,3,2-*de*]quinolines with α, β, γ, δ, ε and ω amino acids and amino esters in the presence of triethylamine is described. The reaction is simple in execution and work-up, occurring under ambient conditions.

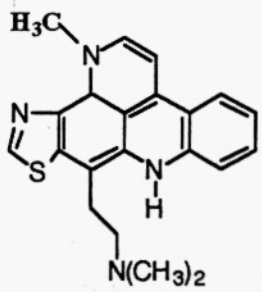
In recent years, many new heterocyclic alkaloids have been isolated from marine sources, characterized and synthesized, and the biological activities have also been reported for some of these compounds[1-5]. The cystodytins are obtained from the Okinawan tunicate, *cystodytes dellechiajei*(Della Valle)[6] and exhibit significant antineoplastic properties. The mixture of cystodytin A and cystodytin B exhibited high cytotoxicity with IC_{50}: 6.7×10^{-7} M toward L1210 murine



pyrido[4,3,2-de]quinoline skeleton



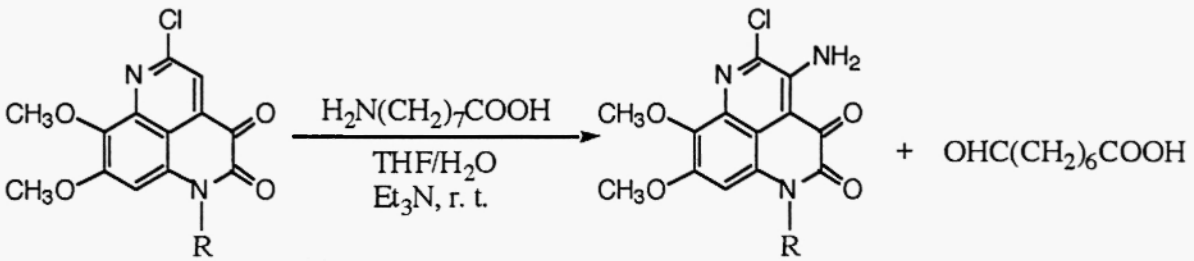
R= $CH=C(CH_3)_2$, Cystoditin A
R = $C(CH_3)=CH(CH_3)$, Cystoditin B



Dercitin

leukemia. Studies of bioactivities for the structurally related dercitin show that the compound has *in vitro* antitumor against P388 (IC_{50} equal to 50 ng/mL) and human tumor cell lines (HCT-8, A-549, T47D, 1.0 (μg/mL) and *in vivo* activity against P388 (T/C 170%, 5mg/kg). Dercitin also exhibits immunosuppressant activity(% murine MLR at 10ng/mL)[7]. These alkaloids contain a

pyrido[4,3,2-*de*]quinoline core structure. Certain pyrrolo[4,3,2-*de*]quinolines and pyrido[4,3,2-*de*]quinolines have been synthesized in our group, and the cytotoxic potency and anticancer activities of these compounds have also been reported[8-13]. The biological evaluation data show that 1-ethoxycarbonylmethyl-2,3-diketopyrido[4,3,2-*de*]quinoline is actively cytotoxic exhibiting a wide spectrum of anticancer activities against 22 cell lines in seven cancer panels with LC_{50} values less than 9 μM, especially in non small cell lung cancer, melanoma cancer, renal cancer, prostate cancer, and breast cancer. In connection with our ongoing interest in the chemistry and the biochemistry of the novel 2,3-diketopyrido[4,3,2-*de*]quinoline alkaloids, an investigation of the unusual and characteristic amination reaction between these compounds and amines, amino acids, peptides and nucleotides was desirable. We have already reported the novel amination and elimination reaction between 2,3-diketopyrido[4,3,2-*de*]quinolines and a limited group of amines[13]. In order to establish the scope of this reaction and to investigate the structural parameters controlling the reaction, we



1. R=H
2. R=$CH_2COOC_2H_5$

3. R=H
4. R=$CH_2COOC_2H_5$

(1)

herein describe the reaction of 2,3-diketopyrido[4,3,2-*de*]quinolines with a wider range of amino acids and amino esters, which includes α, β, γ, δ, ε and ω amino acids and amino esters (Eq. 1, ω amino acids). The reactivity, which in some respects mimics the action of pyridoxal, may be related to their cytotoxicity.

2,3-Diketopyrido[4,3,2-*de*]quinolines reacted with 3 equivalents of amino acids in the presence of triethylamine in tetrahydrofuran and water at room temperature to yield 4-amino-2,3-diketopyrido[4,3,2-*de*]quinolines in yields up to 72% (Table 1). In addition, 2,3-diketo-pyrido[4,3,2-*de*]quinolines can react with amino esters to afford product in 82% yield (Table 1, entry 9). Similar rates of reaction and yields of products were observed for different amino acids (Table 1, entries 1-4, 6 and 13-18). The reaction was also found to be sensitive to electronic effects. The reaction of substrates which contain an α-amino moiety required a longer reaction time and the yield was generally lower. For β- and γ - amino acids, the reaction time is similar to that required for α-amino acids but the yield of products from γ - amino acids was generally higher than for β- amino acids. Within the group of compounds examined, consisting of γ, δ, ε and ω amino acids, they react

Table 1 Amination reaction of 2,3-diketopyrido[4,3,2-*de*]quinolines with various amino acids and esters

Entry [a]	Substrate	Reaction time(h)	Yield (%) [b]
1	$CH_3CH(NH_2)COOH$	72	15
2	$H_2NCH_2CH_2COOH$	52	53
3	$H_2NCH_2CH_2CH_2COOH$	48	69
4	$H_2N(CH_2)_5COOH$	24	71
5	$H_2N(CH_2)_7COOH$	20	72
6	H_2NCH_2COOH	72	28
7	$H_2NCH_2COOCH_2CH_3$	20	78
8	$H_2NCH_2CH_2COOCH_2CH_3$	24	73
9	$H_2NCH_2COOCH_3$	20	82
10	$H_2N(CH_2)_5COOH$	24	11[c]
11	$H_2N(CH_2)_7COOH$	20	9[c]
12	H_2NCH_2COOH	72	6[c]
13	$CH_3CH(NH_2)COOH$	72	8
14	$H_2NCH_2CH_2COOH$	52	42
15	$H_2NCH_2CH_2CH_2COOH$	48	46
16	$H_2N(CH_2)_4COOH$	24	51
17	$H_2N(CH_2)_7COOH$	20	67
18	H_2NCH_2COOH	72	25
19	$H_2NCH_2COOCH_2CH_3$	20	69
20	$H_2NCH_2CH_2COOCH_2CH_3$	24	48
21	$H_2NCH_2COOCH_3$	24	72
22	$H_2N(CH_2)_7COOH$	20	12[c]

a. Entries 1-12: the reaction of compound **1** with amino acids and esters; Entries 13-22: the reaction of compound **2** with amino acids and esters; b. Yield of compound **3** or **4**; c. The elimination products were identified by GC-MS, GC-IR and GC analysis compared with authentic materials.

essentially similarly. Clearly, the position of the amino group is important for the aminative transfer reaction between 2,3-diketopyrido[4,3,2-*de*]quinolines and amino acids.

In order to compare amino acids with esters, the reactions between 2,3-diketopyrido[4,3,2-*de*]quinolines and amino esters were studied (Table 1, entries 7-9 and 19-21). These experiments showed that amino esters generally react more readily than the corresponding amino acids for the amination reaction.

The amino group reactivity and solubility are two important factors affecting the amination reaction. Thus the amination product was isolated in poor yield and the reaction rate was slow when the reaction was performed in water. Upon screening solvent systems, we found that water : tetrahydrofuran (2:1) produced the best combination of amination reactivity and substrate solubility. For substrates which were less soluble in the reaction system, the amination reaction could be enhanced by adding more organic solvent. The aminative transfer does not proceed in the absence of triethylamine. Therefore it is essential for the aminative transfer reaction of 2,3-diketopyrido[4,3,2-*de*]quinolines to maintain basic reaction conditions.

The mechanism for amination and elimination between 2,3-diketopyrido[4,3,2-*de*]quinolines and amino acids and esters resembles that with pyridoxal. The amino group undergoes nucleophilic addition to the double bond between carbon (C3a) and carbon (C4) to form a dihydro intermediate which is then oxidized. Finally, 4-amino-2,3-diketopyrido[4,3,2-*de*]quinolines were formed[13].

In conclusion, we have described the novel amination and elimination reaction that occurs between 2,3-diketopyrido[4,3,2-*de*]quinolines and amino acids which contain α, β, γ, δ, ε and ω amino and ester groups and compared the reactivities of different acids and esters. Studies to extend this reaction to other structural types of amino acids, peptides as well as nucleotides are currently underway and will be reported in due course.

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14. General experimental procedure for the amination reaction summarized in Table 1**:** A solution of 10 mg (0.034 mmol) of 1*H* -2,3-diketopyrido[4,3,2-*de*]quinolines (**1**) was suspended in 8 ml of water and the pH value was adjusted to 10 with triethylamine, then 4 mL of tetrahydrofuran was added, followed by the amino acid 7.5 mg(0.1 mmol, Table 1, entry 6). The resulting mixture was stirred at room temperature for 72 hours. A red product formed in the solution. After removing the solvent under vacuum, the residue was extracted with chloroform (20 mL × 2) and the crude product was purified by flash chromatography on silica gel(Merck, 230-400 mesh) using a solvent mixture of ethyl acetate/hexane(1:1 then 4:1) and then ethyl acetate. The red product was fully characterized spectroscopically.

15. Compound **4**: HRMS: calcd For $C_{17}H_{16}N_3O_6Cl$ m/z 393.0727, found m/z 393.0725 (M^+, 100%); ^1H NMR (400 MHz, DMSO-d_6): δ 9.72 (s, 1H, NH), 8.46 (s, 1H, NH), 7.34 (s, 1H, Ar-H), 5.17 (s, 2H, CH_2), 4.18 (q, 2H, J=7.1 Hz, CH_2), 3.94 (s, 3H, OCH_3), 3.92 (s, 3H, OCH_3), 1.21 (t, 3H, J=7.1Hz, CH_3); Anal. calcd for $C_{17}H_{16}N_3O_6Cl$: C, 51.85; H, 4.06; N, 10.67. Found: C, 51.96; H, 4.07; N, 10.19.

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