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Journal of Molecular Catalysis B: Enzymatic 1 (1996) 103–107

JOURNAL OF MOLECULAR CATALYSIS B: ENZYMATIC

Protease induced novel ring contraction reaction of 1,3-oxazin-4-one derivatives [1]

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Received 7 August 1995; accepted 28 August 1995

Abstract

Protease induced ring contraction reaction of ethyl-4-oxo-3-phenyl-1-oxa-5-azaspiro[5,5]-undec-2-ene-2-carboxylate (**1**) yielded 4-phenyl-3-hydroxy-1H-pyrrole-2,5-dione (**5**). This product and its derivatives have been characterized by comparing their total identity with authentic compounds. Involvement of basic amino acid residues for the initiation of the ring contraction reaction by abstracting the proton at position-3 of the oxazinone ring has been suggested. Chemical evidence for the base catalyzed reaction pathway of compound **1** leading to the formation of compound **5** is presented.

Keywords: Protease; Ring contraction; 1,3-oxazin-4-ones; Maleamides

1. Introduction

The present study is aimed at exploring lead molecules as spermicidal agents. For achieving this objective, the chemical reactivities of a compound in vaginal fluid, cervical mucus, and in seminal plasma have to be studied. Human seminal plasma and sperm contain several proteolytic enzymes [1]. The proteolytic activity of pooled raw samples of human seminal plasma is 1.0–2.9 units/ml [1]. The need for identifying molecular structures capable of interacting with protease thus arose in the present study and it has been found that protease induces a novel ring contraction of the 1,3-oxazin-4-one derivatives and results in the formation of substituted maleamides. The details of this novel observation are presented here.

Ethyl-4-oxo-3-phenyl-1-oxa-5-azaspiro[5,5]-undec-2-ene-2-carboxylate (**1**) on reaction with protease (type IV from *Streptomyces caespitosus*; activity 0.7–1 unit/mg; P0384) in absolute ethanol yielded a mixture of two products as was evident from TLC. Column chromatography of this mixture furnished only one product, 4-phenyl-3-hydroxy-1H-pyrrole-2,5-dione (**5**). During chromatography the other product was either converted to compound **5** or it decomposed on the column. The conclusive evidence of the structure of compound **5** was obtained by establishing total similarity of this compound and its derivatives with the authentic samples prepared by the methods reported in the literature [2]. The molecular ion at m/z 269 of the labile component isolated from TLC suggested

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[1] CDRI Communication No. 5457.

SSDI 1381-1177(95)00018-6

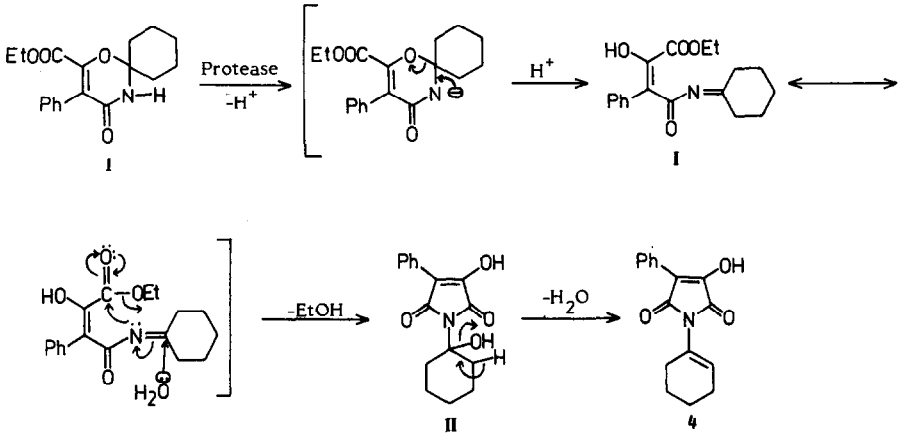
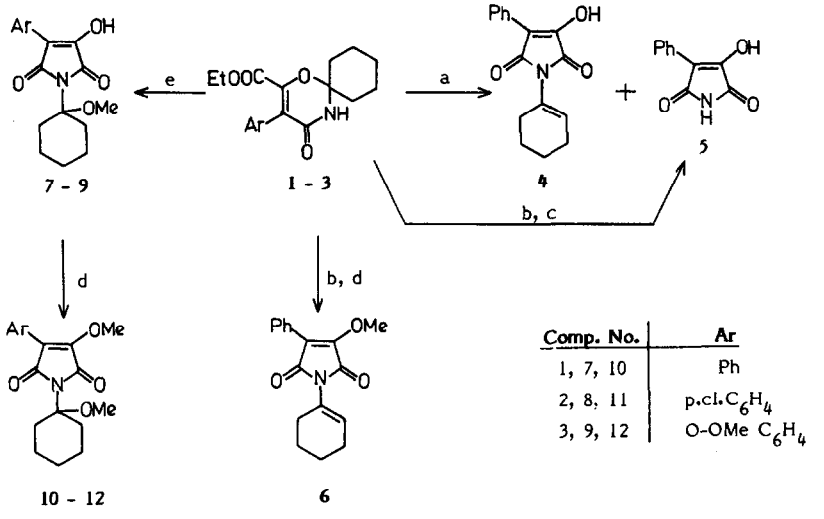


Fig. 1.

it to be compound **4**. Mechanistic considerations suggest that the initiation of the ring contraction reaction possibly occurred by abstracting the proton from position-3 to furnish compounds **4** and **5** (Fig. 1). On the basis of this presumptive pathway and in order to obtain compound **5** without the use of enzyme, compound **1** was reacted with potassium carbonate in dry acetone. This gave compound **4** in good yields which was then hydrolysed with methanolic HCl to furnish compound **5**. Since compound **4** was very labile in nature it was characterized by converting it to its methyl ether **6** by reacting it with methyl iodide in presence of potassium carbonate in dry acetone (Scheme 1).

The presumptive intermediate II (Fig. 1) could not be isolated from the reaction of compound **1** with protease or potassium carbonate because of a faster process of dehydration. In an attempt to trap this intermediate, compound **1** was reacted with sodium methoxide. This led to successful isolation of compound **7** which could be methylated to compound **10**. The synthetic utility of this reaction was obvious from the reaction of compound **2** and **3** with sodium methoxide. The resulting products **8–9** were obtained in good



Comp. No.	Ar
1, 7, 10	Ph
2, 8, 11	p.cl.C_6H_4
3, 9, 12	O-OMe C_6H_4

Scheme 1. a = Protease/Abs. EtOH; b = K_2CO_3/Acetone; c = HCl/MeOH; d = K_2CO_3/MeI/Acetone; e = MeONa/MeOH.

yields which were further converted to their methyl ethers **11–12** (Scheme 1).

On the basis of the chemical reactivity of compound **1**, it was argued that the basic amino acids present in protease was possibly responsible for the ring contraction reaction. This prompted to react compound **1** with arginine or lysine in methanol and the yields of products **4** and **5** lent support to this assumption.

The starting 1,3-oxazin-4-one derivatives (**1–3**), Table 1, were prepared by reacting appropriate β-oxonitriles with cyclohexanone as reported in the literature [3–5]. β-Oxonitriles in turn were prepared by the condensation of phenyl acetonitriles with dimethyloxalate [6–8].

2. Experimental

Melting points were determined on a hot stage apparatus and are uncorrected. IR spectra were measured on a Beckman Acculab 10 spectrophotometer. ^{1}H NMR spectra were recorded on Perkin Elmer R-32 or Bruker 400 FT NMR instrument using $CDCl_3$/DMSO-d_6 as solvents. Mass spectra were determined on a JEOL JMS-D-300 spectrometer. Elemental analyses were carried out on Carlo Erba instrument. Protease enzyme (type IV from *Streptomyces caespitosus*, activity 0.7–1 unit/mg; P0384) was purchased from Sigma Chemical Company.

2.1. Reaction of ethyl-4-oxo-3-phenyl-1-oxa-5-azaspiro-[5,5]-undec-2-ene-2-carboxylate (1) with protease type IV

To a solution of compound 1 (50 mg, 0.16 mmol) in absolute ethanol (20 ml) was added protease (50 mg, type IV from *Streptomyces caespitosus* activity 0.7–1 unit/mg P0384). After stirring for 48 h. at 38°C the insoluble material was filtered off and the filtrate was concentrated in vacuo giving a residue which was subjected to chromatography (silica gel; 99:1 $CHCl_3$/MeOH) to provide compound **5** (24 mg, 77%, m.p. 216°C).

2.2. Preparation of 1-(cyclohex-1-enyl)-3-phenyl-4-hydroxy-1H-pyrrole-2,5-dione (4)

A mixture of compound **1** (300 mg, 1.0 mmol) and freshly baked K_2CO_3 (630 mg, 5 mmol) in dry acetone (30 ml) was stirred at reflux for 12–16 h. The inorganic material was filtered off and the filtrate was concentrated in vacuo to yield crude compound (**4**) (250 mg, 93%). This product without further purification was utilised for the preparation of compounds **5** and **6**.

2.3. Preparation of 1-(cyclohex-1-methoxy)-4-hydroxy-3-substituted phenyl-1H-pyrrole-2,5-diones (7–9) general procedure

To a solution of the appropriate compound (**1–3**, 2 mmol) in dry methanol (30 ml) was added freshly prepared sodium methoxide (4 mmol) and the reaction mixture was stirred at 32°C for 10–12 h. The solvent was removed under reduced pressure and the residue was treated with water and extracted with ethyl acetate (3 × 25 ml). The organic layer was dried (Na_2SO_4) and concentrated in vacuo to give **7–9** (90–95%). These products without further purification were utilised for the preparation of compounds **10–12**.

2.4. Preparation of methyl ethers (6, 10–12) of compounds 4 and 7–9.

2.4.1. General procedure

To a solution of the appropriate compound (**4** or **7–9**, 2 mmol) in dry acetone (50 ml) was added freshly baked K_2CO_3 (5 mmol) and methyl iodide (6 mmol). The resulting reaction mixture was heated at 60–70°C under stirring for 12–16 h. The inorganic material was filtered off and the filtrate was concentrated in vacuo to

Table 1
Physical and spectroscopic data of the compounds synthesized

Compound	Yield (%)	m.p. °C	Mass (m/z)	IR (cm^{-1})	^1H NMR (ppm)	Analysis (%)	Calc.	Found
1	60	150	315	3420 (N–H) 1730 (CO_2) 1670 (CO)	($CDCl_3$ + DMSO-d_6) 1.12 (t, J = 7.8 Hz, 3H, CH_2CH_3), 1.85 (m, 10H, 5×CH_2), 4.0 (q, J = 7.8 Hz, 2H, OCH_2CH_3), 6.74 (s, 1H, NH), 7.28 (m, 5H, Ar-H).	C H N	68.55 6.71 4.44	67.99 6.49 4.61
2	58	159	349	3220 (N–H) 1730 (CO_2) 1670 (CO)	($CDCl_3$ + DMSO-d_6) 1.06 (t, J = 7.6 Hz, 3H, CH_2CH_3), 1.87 (m, 10H, 5×CH_2), 3.94 (q, J = 7.6 Hz, 2H, OCH_2CH_3), 6.68 (s, 1H, NH), 7.18 (m, 4H, Ar-H).	C H N	61.80 5.76 4.00	61.72 5.75 3.99
3	55	171	345	3220 (N–H) 1730 (CO_2) 1680 (CO)	($CDCl_3$ + DMSO-d_6) 0.92 (t, J = 7.6 Hz, 3H, CH_2CH_3), 1.90 (m, 10H, 5×CH_2), 3.70 (s, 3H, $PhOCH_3$), 3.86 (q, J = 7.6 Hz, 2H, OCH_2CH_3), 6.72 (s, 1H, NH), 7.04 (m, 4H, Ar-H).	C H N	66.07 6.71 4.05	65.88 6.46 4.10
5	77	216	189	3281 (N–H) 3263 (O–H)	($CDCl_3$ + DMSO-d_6) 6.4 (brs, 1H, N*H*), 7.20 (m, 3H, Ar-H), 7.94 (m, 2H, Ar-H), 9.76 (brs, 1H, O*H*).	C H N	63.48 3.73 7.40	63.72 3.83 7.57
6	70	oil	283	1710 (CONR)	($CDCl_3$) 1.96 (m, 8H, 4×CH_2), 4.28(s, 3H, C=C–OCH_3), 5.79 (brs, 1H, C=C*H*), 7.30 (m, 3H, Ar-H), 7.84 (m, 2H, Ar-H)	C H N	72.06 6.04 4.94	72.38 6.36 4.92
10	80	oil	315	1710 (CONR)	($CDCl_3$) 1.95 (m, 10H, 5×CH_2), 3.20 (s, 3H, N–C–OCH_3), 4.10 (s, 3H, C=C–OCH_3), 7.28 (m, 3H, Ar-H), 7.69 (m, 2H, Ar-H)	C H N	68.55 6.71 4.44	68.54 6.60 4.67
11	85	oil	349	1690 (CONR)	($CDCl_3$) 1.94 (m, 10H, 5×CH_2), 3.20 (s, 3H, N–C–OCH_3), 4.18 (s, 3H, C=C–OCH_3), 7.25 (d, 2H, J = 8 Hz, Ar-H), 7.70 (d, 2H, J = 8 Hz, Ar-H)	C H N	61.81 5.76 4.00	61.84 5.71 3.92
12	80	oil	345	1700 (CONR)	($CDCl_3$) 1.98 (m, 10H, 5×CH_2), 3.19 (s, 3H, N–C–OCH_3), 3.70 (s, 3H, $PhOCH_3$), 3.74 (s, 3H, C=C–OCH_3), 6.90 (m, 2H, Ar-H), 7.20 (m, 2H, Ar-H)	C H N	66.07 6.71 4.05	65.88 6.75 3.99

obtain an oily residue which was subjected to chromatography (silica gel; 4:1 hexane/chloroform) to give the substituted maleamides **6** or **10–12** as oils in good yields (70–85%).

2.5. Preparation of 4-hydroxy-3-phenyl-1H-pyrrole-2,5-dione (5)

A solution of compound **4** (538 mg, 2 mmol) in methanolic HCl (10 ml, 10%) was refluxed on water bath for 30 min. Excess solvent was removed under reduced pressure to furnish a solid residue which was crystallised from EtOAc–petroleum ether mixture to yield **5** (250 mg, 66%, m.p. 216°C).

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