Pergamon

Bioorganic & Medicinal Chemistry Letters 8 (1998) 1969–1972

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

NOVEL ANGUCYCLINE COMPOUND WITH BOTH ANTIGASTRIN- AND GASTRIC MUCOSAL PROTECTIVE- ACTIVITIES

Shinichi Uesato,[a] Takashi Tokunaga[b] and Koji Takeuchi[c]*

[a]*Department of Biotechnology, Faculty of Engineering, Kansai University, Suita, Osaka 564-8680, Japan*
[b]*Japan Tobacco, Inc. Central Pharmaceutical Research Institute, 1-1, Takatsuki City, Osaka 569-1125, Japan*
[c]*Department of Pharmacology & Experimental Therapeutics, Kyoto Pharmaceutical University, Yamashina-ku, Kyoto 607-8414, Japan*

Received 13 May 1998; accepted 18 June 1998

Abstract: An angucycline series compound P371 A1 (**1**) from *Streptomyces* sp. P371 was established to have a novel structure comprising an ureido group at one of four sugar units on the basis of 2D NMR techniques. **1** exhibited an inhibitory activity against the pentagastrin-stimulated acid secretion as well as protective acitvities against HCl/ethanol- and indomethacin-induced gastric lesions.

The advent of H_2-receptor antagonists and proton pump inhibitors revolutionized the medical treatment of ulcer diseases; most of them are cured by both types of medicines instead of surgery operations[1]. However, it has been known that the long-term remedy with H_2-receptor antagonists and proton pump inhibitors causes hyperplasia of enterochromaffin-like (ECL) cells, which may lead to the relapse of ulcer desease and production of gastric cancer[2]. Thus, the anti-gastrin agent has recently been investigated as an alternative anti-ulcer agent[3]. In the course of a screening program to discover new anti-ulcer agents from Actinomyces, we have isolated a novel angucycline series compound P371 A1 (**1**), together with other congeners, former exhibiting not only anti-gastrin activity, but also gastric mucosal protective activity. This paper deals with its structure elucidation and pharmacological activities in rats.

Suspension cultures (25 l)[4] of strain P371 (*Streptomyces* sp.)[5] was extracted with EtOAc after removal of brothes by centrifugation. The EtOAc extract was fractionated through a combination of Silica-gel column chromatography, ODS column chromatography and GPC column chromatography, giving P371A1 (**1**) (83.0mg), P371A2 (155.7 mg), P371 B1 (46.1 mg), P371 B2 (38.1 mg) as orange powders, respectively.

The molecular formula of P371 A1 (**1**) was established to be $C_{48}H_{66}N_2O_{20}$ based on the HRFAB-MS and ^{13}C NMR spectra. The former spectrum showed a parent ion peak at *m/z* 993.4423 ([M+2H+H]$^+$: Calcd. 993.4444) characteristic of quinone structures, whose parent peaks are generally known to appear as reduced forms in the MS spectra[6]. Furthermore, the ^1H and ^{13}C NMR spectra of **1** indicated the presence of a phenolic proton at δ 12.5 (s) and two aromatic protons at δ 7.63 and 7.86 (each d, J= 7.8Hz) as well as two conjugated carbonyl carbons at δ 187.7 and 188.6. These signals reminded us of a naphthoquinone chromophore in the molecule. In addition, three anomeric carbon signals at δ 98.9, 99.4 and 103.1 suggested

0960-894X/98/$ - see front matter
PII: S0960-894X(98)00340-0

that **1** comprises three glycosidic linkages. The stereostructures and connectivities of units X_1, X_2, X_3, X_4, X_5 and X_6 were determined as shown in Fig. 1 on the basis of the ^1H-^1H COSY, ^{13}C-^1H COSY, HMBC and NOESY spectra as well as the

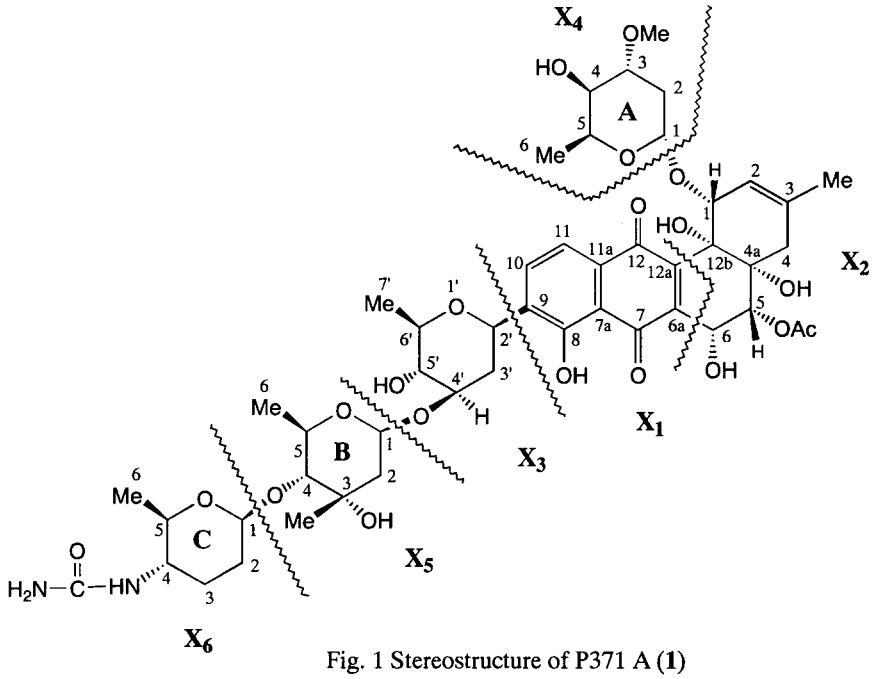
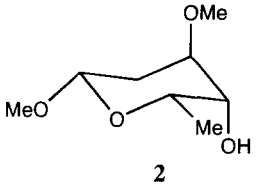


Fig. 1 Stereostructure of P371 A (**1**)

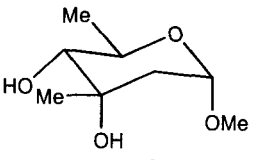
positive FAB-MS spectrum of **1** in the following way. The two- and three-bond HMBC correlations between 6-H at δ 4.94 and 6a-C at δ145.9 and between 6-H and 12a-C at δ140.6 indicated that unit X_1 is joined to unit X_2 through the C-6a−C-6 bond and, hence, through the C-12a−C12b bond. Additionally, the presence of the two-bond HMBC peak between 9-C at δ138.9 and 2'-H at δ 4.84 indicated the connection of units X_1 and X_3 through the C-9−C-2' bond.

The linking positions of the three pyranose moieties (units X_4, X_5 and X_6) were determined from the interpretation of the three-bond HMBC correlation peaks between the following ^1H and ^{13}C: 1_A-H at δ 4.64 / 1-C at δ 80.7, 1_B-H at δ 4.64 / 4'-C at δ 83.1, and 1_C-H at δ 4.47 / 4_B-C at δ 89.5. The positive FAB-MS spectrum showed a parent ion peak at *m/z* 993 $(M+2H+H)^+$, which in turn exhibited fragment peaks at *m/z* 849 $(M+3H+H-145)^+$, 836 $(M+3H-157)^+$, 692 $(M+3H+H-157-145)^+$ and 530 in the MS/MS spectrum. The segments 145 and 157 are in accord with the compositions of units X_4 ($C_7H_{13}O_3$) and X_6 ($C_7H_{13}N_2O_2$), respectively, whereas 530, with the composition ($C_{27}H_{30}O_{11}$) of the reduced form of the naphthoquinone moiety (units X_1, X_2 and X_3). The stereochemistry of three glycosidic moieties in **1** was disclosed in the following way: Methanolysis of **1** with 5% HCl in MeOH afforded three methyl glycosides **2**, **3** and **4** as colourless solids in yields of 58.9, 50.5 and 55.4 %), respectively. By comparison with the data in literatures, **2** was identified with methyl 2,3,6-trideoxy-3-O-methyl-

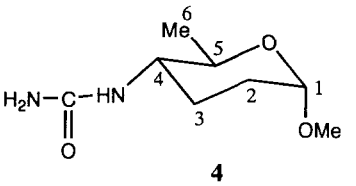
β-L-xylo-hexopyranoside[7] (derivative of unit X_4), whereas **3**, with methyl 2,6-dideoxy-3-C-methyl-α-D-ribo-hexopyranoside[8] (derivative of unit X_5). The remaining methyl glycoside **4** was found to stem form unit X_6 by the IR, FAB-MS [*m/z* 189 $(M+H)^+$], ^1H- and ^{13}C NMR measurements. Especially, the linking position of H_2NCONH- group was deduced to be C-4 in view of the ^{13}C chemical shift (δ 156.3) and IR absorption (1645 cm^{-1}) of its carbonyl group as well as the high field position (δ 66.6) of 4-C relative to those (δ 70 - δ 77) of usual oxymethine carbons. From these findings, **4** was clarified to be methyl 2,3,4,6-tetradeoxy-4-ureido-α-ribo-hexopyranoside. The modes of the glycoside linkages in **1** were regarded as α (X_4), β (X_5) and β (X_6) from the ^1H-^1H coupling constants of the anomeric protons: 4.7 Hz (X_4), 9.2 Hz (X_5), and 9.5 and 1.7 Hz (X_6), together with those of other protons on the pyranose rings. The relative stereochemistry of X_1-X_2 and X_3 was determined based on the NOESY measurement of **1**: In the spectrum, 5-H at δ 5.86 had the cross peaks to 6-H at δ 4.94 and 1-H at δ 4.20,



2



3



4

respectively, thus demonstrating that these three protons should be situated on the same side of the *cis*-decaline ring of X_1-X_2. Furthermore, the NOESY correlation peaks between 2'-H (δ 4.84) / 4'-H (δ 3.71), 2'-H (δ 4.84) / 6'-H (δ 3.70) and 5'-H (δ 3.18) / 7'-Me (δ 1.45) defined the stereochemistry at X_3. The findings mentioned so far, therefore, led us to determine the connectivities and stereostructures of X_1-X_2, X_3, X_4, X_5 and X_6 (including the absolute stereochemistry at X_4 and X_5)[9] for P371 A1 (**1**) as shown in Fig. 1.

P371 A1 (**1**) showed a significant suppression (percentage inhibition: *ca.* 61%) against pentagastrin-stimulated acid secretion, when 10mg/kg was given intraperitoneally to urethane anesthetized rats 30 min prior to the onset of pentagastrin infusion (i.v. 60 μg/kg/hr)[10]. In contrast, **1** did not have any effect on acid secretory response induced by either histamine or carbachol in the experiments carried out under the same condition[10]. Since H_2-receptor antagonists or proton pump inhibitors have been shown to effectively inhibit the acid secretion, irrespective of the stimulus used to activate acid formation[11-13], it is most likely that **1** suppressed the gastric acid secretion by acting as a CCK_B / gastrin receptor antagonist[14] and/or by hindering the gastrin from stimulating a histamine release from ECL cells[15]. Interestingly, intraperitoneal administration of P371 A1 (**1**) to unanesthetized rats 30 min prior to an oral dosage of HCl/ethanol (60% in 150 mM HCl) or a subcutaneous administration of indomethacin (25 mg/kg) prevented significantly and dose-dependently the lesion formation in gastric mucosa. The percentage inhibitions at 1, 3 and 10 mg/kg were 52.6, 81.6 and 83.6%, respectively, in the HCl/ethanol-induced lesion model, and were 57.0, 64.9 and 72.8%, respectively, in the indomethacin-induced lesion model. Further studies are under way to elucidate the detailed mechanisms underlying both gastric antisecretory and mucosal protective actions induced by **1**.

Acknowledgements: We thank Mr. Tsuyoshi Kaieda (Central Pharmaceutical Research Institute, Japan Tobacco, Inc.) for measurements of MS spectra. We are also thankful to Mrs. Masako Akaji (Superconducting NMR Spectrometer Laboratory, Faculty of Pharmaceutical Sciences, Kyoto University) for measurements of 2D NMR spectra.

References and Notes

1. Garner, A., *Scand. J. Gastroenterol.*, **1986**, *21*, 203.
2. Larsson, H.; Carlsson, E.; Ryberg, B.; Fryklund, J.; Wallmark, B., *Am. J. Physiol.*, **1988**, *254*, G33.
3. Makovec, F.; D'Amato, M., *Drug Discovery Today*, **1997**, *2*, 283.
4. A slant culture of strain P371 was inoculated into five flasks (500 ml) which contained a sterilized seed medium (100ml). The flasks were then shaken on the rotary shaker (250 rpm) at 30℃ for 2 days. The resultant seed cultures were inoculated into a 30 l jar fermenter containing the above medium (25 l). The fermentation was carried out at 30℃ under aeration of 20 l / min. and agitation of 200 rpm for 2 days. The medium used for fermentation consists of the following nutrients: starch (2.0 g), glucose (0.1 g), yeast extract (0.5 g), peptone (0.3 g), meat extract (0.3 g), $CaCO_3$ (0.4 g) and a trace metal solution (0.5 ml of 0.5 mg each of $FeSO_4 \cdot 7H_2O$, $MnCl_2 \cdot 4H_2O$, $ZnSO_4 \cdot 7H_2O$, $CuSO_4 \cdot 5H_2O$ and $CoCl_2 \cdot 6H_2O$) in a 100 ml of H_2O solution (pH 7.0).
5. The strain P371 has been deposited at the National Institute of Bioscience and Human Technology, Agency of Industrial Science and Technology, Ibaraki Pref. Japan, as under the accession number FERM BP-4146.
6. Drautz, H.; Zähner, H., *J. Antibiotics*, **1986**, *39*, 1657.
7. Martin, A.; Pais, M.; Monneret, C., *Carbohydrate Research*, **1983**, *113*, 189.
8. Thiem, J.; Elvers, J., *Chem. Ber.*, **1981**, *114*, 1442.
9. The absolute stereochemistry at X_1-X_2, X_3 and X_6 in **1** is under investigation, and will be reported elsewhere.
10. Kato, S.; Kitamura, M.; Korolkiewicz, R. P.; Takeuchi, K., *Bri. J. Pharmacol.*, **1998**, *123*, 839.
11. Daly, M. J.; Humphray, J. M.; Stables, R., *Gut.*, **1980**, *21*, 408.
12. Wallmark, B., *Methods Find. Exp. Clin. Pharmacol.*, **1989**, *11* (suppl), 101.
13. Hirschowitz, B. I.; Keeling, D.; Lewin, M.; Okabe, S.; Parsons, M.; Sewing, K.; Wallmark, B.; Sacks, G., *Dig. Dis. Sci.*, **1995**, *40*, 3S.
14. Nishida, A.; Miyata, K.; Tsutsumi, R.; Yuki, H.; Akuzawa, S.; Kobayashi, A.; Kamato, T.; Ito, H.; Yamano, M.; Katuyama, Y.; Satoh, M.; Ohta, M.; Honda, K., *J. Pharmacol. Exp. Ther.*, **1994**, *269*, 725.
15. Sandvik, A. K.; Waldum, H. L.; Kleveland, P. M.; Schulze, S. B., *Scand. J. Gastroenterol.*, **1987**, *22*, 803.