Agricultural and Biological Chemistry
Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/tbbb19

New Anthracycline Antibiotic CG12 Obtained by Microbial Glycosidation of α-Citromycinone

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Published online: 09 Sep 2014.

To cite this article: Osamu Johdo, Akihiro Yoshimoto, Tomoyuki Ishikura, Hiroshi Naganawa, Tomio Takeuchi & Hamao Umezawa (1986) New Anthracycline Antibiotic CG12 Obtained by Microbial Glycosidation of α-Citromycinone, Agricultural and Biological Chemistry, 50:6, 1657-1659

To link to this article: http://dx.doi.org/10.1080/00021369.1986.10867629

Note

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Received January 13, 1986

Aiming at obtaining new antitumor anthracycline antibiotics, we have studied the production of hybrid anthracyclines by biosynthetic glycosidation of natural and semi-synthetic anthracyclinones using antibiotic-negative mutants of anthracycline-producing microorganisms.[1～3] We have prepared 16 hybrid anthracyclines,[4～7] among which betaclamycins,[7] trisarubicinol,[5] and 2-hydroxyaclacinomycin A[4] had excellent antitumor effects on leukemic L1210 cells in mice.

In this paper we describe the production of an additional new hybrid anthracycline antibiotic, CG12, by microbial glycosidation of α-citromycinone using the aclacinomycin-negative mutant strain KE303 of *Streptomyces galilaeus* MA144-MI.[2] CG12 was identified as 10-*O*-(cinerulosyl-2-deoxyfucosyl-rhodosaminyl)-α-citromycinone (Fig. 1).

The aglycone α-citromycinone used as the glycosidation substrate was obtained by acid hydrolysis of an anthracycline mixture extracted from the culture broth of a blocked mutant, strain SU2-270, which was derived from rhodomycin-producing *Streptomyces violaceus* A262 (unpublished data).

Microbial conversion using strain KE303 was done by flask culture in the manner previously described.[1] Thus, 320 mg of α-citromycinone was glycosylated with a total of 32 liters of conversion culture. The glycosylated products were extracted from the mycelial cake with a total of 10 liters of acetone. The solvent extract was evaporated *in vacuo* and extracted twice with 3 liters of chloroform. After evaporation of the chloroform layer, the pigmented residue was dissolved in about 50 ml of the chloroform–methanol mixture (1 : 2), and put on the top of a Sephadex LH-20 column (ϕ3.2 × 50 cm), which was eluted with the same chloroform–methane mixture. Fractions were monitored by thin-layer chromatography (TLC) on a silica gel plate 60 F_{254} (E. Merck) using a solvent of chloroform–methanol (15 : 1) and those containing the glycosylated products were pooled and evaporated to dryness. The resulting residue was then chromatographed on a silica gel C-200 (Wako Junyaku) column (ϕ3.0 × 15 cm), which was eluted with chloroform–methanol (100 : 5). The main fractions containing CG12 were evaporated to dryness followed by further chromatographic purification on preparative silica gel 60 PF_{254} (E. Merck) using chloroform–methanol–aqueous ammonia (120 : 10 : 0.3) as the solvent. The main pigment band corresponding to CG12 was scraped off and eluted with a chloroform–methanol (7 : 1) mixture. After evaporation, the pigment residue was dissolved in 30 ml of 0.1 M acetate buffer (pH 3.2). This solution was washed with 20 ml of toluene, neutralized with sodium bicarbonate, and extracted with chloroform. The chloroform layer was dried over sodium sulfate and evaporated to a small volume. An excess of *n*-hexane was added to it to precipitate CG12. Thus, CG12 was obtained as a pure yellow powder in a yield of 49 mg.

Physico-chemical properties of CG12 are as follows: mp 154～157°C; IR (KBr) cm^{-1}: 3420, 2930, 1730, 1630, 1610, 1460, 1380, 1330, 1270, 1120, 1020; $\lambda_{max}^{90\%\ MeOH}$ nm ($E_{1\ cm}^{1\%}$): 209 (sh, 274), 233 (494), 257 (312), 290 (sh, 106), 435 (150); FD-MS: *m/z*: 770 $(M+H)^+$.

Acid hydrolysis of CG12 in 0.1 N HCl at 85°C for 30 min gave the aglycone which was identified as α-citromycinone by comparing its ^{1}H-NMR, UV, and IR spectra with those of an authentic sample. The sugar mixtures were composed of L-rhodosamine, 2-deoxy-L-fucose, and L-cinerulose by chromatographic accordance with those detected in the acid hydrolysate of aclacinomycin A when the sugars were analyzed by silica gel TLC.[8]

Partial methanolysis of CG12 in 0.01 N methanolic hydrogen chloride at room temperature for 45 min gave a methyl glycoside and rhodosaminyl-α-citromycinone. The sugar was identical with authentic methyl L-cinerulosyl-2-deoxy-L-fucoside obtained by methanolysis of aclacinomycin A.[9]

The chemical shifts and assignments of ^{1}H-NMR and ^{13}C-NMR spectra of CG12 are shown in Tables I and II, respectively, in comparison with those of aclacinomycin A and α-citromycinone. In both spectra, the peaks corresponding to the three sugar components were superimposable between CG12 and aclacinomycin A. To find

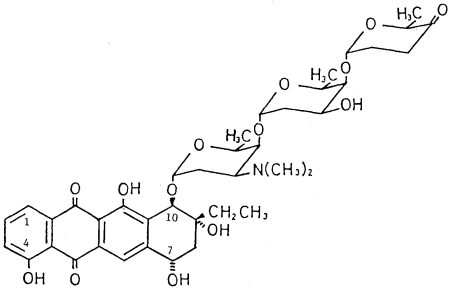

FIG. 1. Structure of CG12.

TABLE I. CHEMICAL SHIFT ASSIGNMENTS OF ^{1}H-NMR SPECTRA OF CG12 AND ACLACINOMYCIN A

Assignment	CG12 δ ppm	Aclacinomycin A* δ ppm	Remarks
1-H	7.74 d	7.79 dd	Aglycone
2-H	7.63 t	7.69	moiety
3-H	7.25 d	7.22	
6-H	7.95 s	—	
7-H	5.04 t	5.28 b	
8-CH_2	2.4 m	2.4 m	
10-H	5.01 s	4.90 bs	
13-CH_2	1.75	1.7 m	
14-CH_3	1.09 t	1.09 t	
16-OCH_3	—	3.70 s	
1′-H	5.38 bd	5.25 b	Rhodosamine
2′-CH_2	1.9 m	1.9 m	moiety
3′-H	2.4 m	2.4 m	
3′-$N(CH_3)_2$	2.15 s	2.17 s	
4′-H	3.69 s	3.78 bs	
5′-H	3.87 q	4.02 q	
6′-CH_3	1.26 d	1.29 d	
1″-H	4.85 bd	5.03 b	2-Deoxyfucose
2″-CH_2	1.8 m	1.90 m	moiety
3″-H	4.05 b	4.1 m	
4″-H	3.62 s	3.7 bs	
5″-H	4.49 q	4.56 q	
6″-H	1.13 d	1.16 d	
1‴-H	5.04 t	5.07 t	Cinerulose
2‴-CH_2	2.1	2.0 m	moiety
3‴-CH_2	2.4 m	2.4 m	
5‴-H	4.47 q	4.50 q	
6‴-H	1.29 d	1.33 d	

The spectra were measured in $CDCl_3$ using TMS as an internal reference with a 400 MHz spectrometer.

* Data cited from ref. 8 measured with a 100 MHz spectrometer.

TABLE II. CHEMICAL SHIFT ASSIGNMENTS OF ^{13}C-NMR SPECTRA OF CG12 AND α-CITROMYCINONE

Carbon	CG12 ppm	α-Citromycinone ppm	Carbon	CG12 ppm
5	187.4	188.0	1′	100.1
12	187.4	187.9	2′	29.7
4	162.7	162.7	3′	61.4
11	161.9	162.3	4′	74.5
6a	148.9	147.0	5′	68.5
2	136.7	137.0	6′	18.1
12a	133.1	133.5	$N(CH_3)_2$	43.3
5a	132.4	133.2	1″	99.4
10a	131.4	132.4	2″	34.3
3	124.9	125.0	3″	65.3
1	120.8	121.3	4″	82.9
6	119.4	120.0	5″	66.7
4a	116.0	116.4	6″	16.9
11a	114.7	115.0	1‴	96.6
7	67.1	67.5	2‴	27.7
9	73.4	73.6	3‴	33.5
10	69.1	66.0	4‴	210.0
8	36.3	34.5	5‴	71.8
13	31.1	30.7	6‴	14.8
14	6.4	6.3		

The spectra were measured in $CDCl_3$ using TMS as an internal reference.

whether the trisaccharide moiety linked to C-7 or C-10, CG12 was acetylated with acetic anhydride to give 4,7, 11,3″-tetra-*O*-acetyl CG12: FD-MS: *m/z* 938 $(M+H)^+$; $C_{48}H_{59}O_{18}N$ (M_w 937.99). The ^{1}H-NMR and ^{13}C-NMR spectra revealed that the sugar-linkage site was the hydroxyl group at C-10 since the chemical shifts assigned to H-7 and C-7 of the acetylated CG12 shifted down from δ5.0 to δ6.1 (m) ppm and from δ67.1 to δ71.7 ppm, respectively, compared with those for CG12. Whereas, the chemical assignments of H-10 and C-10 were not different between the compounds: δ5.01 and δ69.1 ppm for CG12 and δ5.17 and δ69.1 ppm for the acetylated CG12, respectively. The CD spectrum of CG12 was similar to that of β-rhodomycinone[10] indicating that CG12 has a stereochemical configuration of 7(*S*), 9(*R*) and 10(*R*).

CG12 was weakly active against cultured L1210 cell leukemia. The concentration required to inhibit the growth and the biosynthesis of DNA and RNA by 50% (IC_{50} value) were about 0.1, 10 and 10 μg/ml, respectively. CG12 was about 20 fold less inhibitory to L1210 cell growth than betaclamycin A.[7] We also showed that a new hybrid anthracycline CG11 (6-deoxy CG10) recently isolated was far less active than CG10.[6] These findings suggest that a hydroxyl group at C-6 is important in the bioactivity of the anthracycline antibiotics.

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