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STRUCTURE AND TOTAL SYNTHESIS OF CHRYSCANDIN, A NEW ANTIFUNGAL ANTIBIOTIC

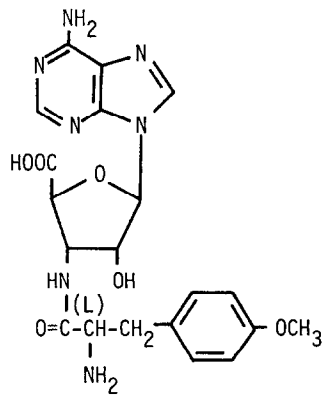
Michio Yamashita, Yoshio Kawai, Itsuo Uchida, Tadaaki Komori
Masanobu Kohsaka, Hiroshi Imanaka, Kazuo Sakane[1)],
Hiroyuki Setoi and Tsutomu Teraji[1)]

Exploratory Research Laboratories, Fujisawa Pharmaceutical Co.,Ltd.
1-6, 2-Chome, Kashima, Yodogawa-ku, Osaka 532, Japan

Summary : The structure elucidation and total synthesis of chryscandin (1), a new antifungal antibiotic produced by Chrysosporium pannorum No.4629, is reported.

A number of nucleoside antibiotics have been isolated as antibacterial, antifungal and antitumor agents. In the course of our screening program for new antifungal antibiotics, a strain of fungi, Chrysosporium pannorum No.4629, was found to produce a peptidyl nucleoside antibiotic, designated as chryscandin (1).[2)] In this report, we describe the structure and total synthesis of this antibiotic.

Chryscandin was purified by chromatography of the cultured broth using Diaion HP-20 (40 % aq. MeOH) and CM sephadex C-25 (H^+ type, 0.1N HCl), followed by crystallization from 0.1N HCl : colorless needles ; mp. 215-233°C (dec.) ; $[\alpha]_D^{22}$ + 34° (c 1.0, 1N HCl) ; $C_{20}H_{23}N_7O_6$ (FD mass : m/z 458 (M^+ + 1) and elemental analysis [3)]). The UV absorption at 260 nm (ε 32,500) and the two proton signals at δ 8.18 (s) and 8.46 (s) in the ^{1}H-NMR spectrum suggested the presence of the adenine nucleus in the chryscandin molecule. This was proved by acid hydrolysis of 1 (6N HCl, 110°C, 12 h), thus yielding adenine and O-methyl-L-tyrosine. The signals attributed to O-methyl-L-tyrosine in the ^{1}H-NMR spectrum of 1 (DMSO-d_6) were observed at δ 2.95 (1H, dd, J=7 and 14 Hz), 3.10 (1H, dd, J=6 and 14 Hz), 3.73 (3H, s), 4.10 (1H, dd, J=7 and 6 Hz), 6.90 (2H, d, J=8.5 Hz) and 7.23 (2H, d, J=8.5 Hz). In addition, the ^{1}H-NMR spectrum of 1 showed an anomeric proton signal (δ 6.12, d, J=2 Hz) and an additional three proton signals (δ 4.37, 1H, d, J=7 Hz ; δ 4.70, 1H, m ; δ 4.78, 1H, m), implying the presence of a sugar amino acid moiety in 1.



Chryscandin (1)

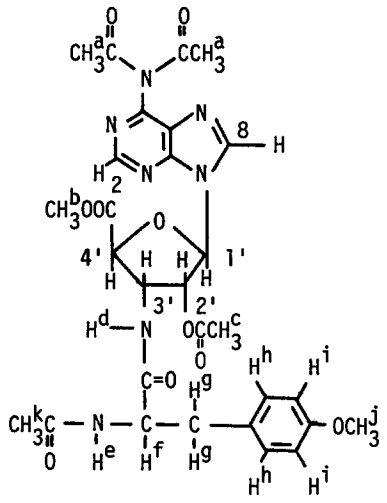


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Table

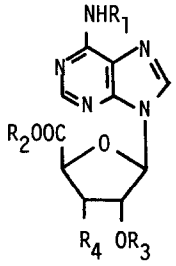
Proton	δ (multiplicity, J Hz) 100 M Hz in $CDCl_3$
2 - H	8.75 (s)
8 - H	8.92 (s)
1'- H	6.30 (d, 2)
2'- H	5.76 (dd, 2, 5)
3'- H	5.09 (m)
4'- H	4.41 (d, 6)
H^a (6H)	2.35 (s)
H^b (3H)	3.72 (s)
H^c (3H)	2.13 (s)
H^d	7.25 (d, 7.5)
H^e	6.50 (d, 7.5)
H^f	4.65 (t, d, 7.5, 7.5)
H^g (2H)	2.95 (d, 7.5)
H^h (2H)	7.15 (d, 8)
H^i (2H)	6.83 (d, 8)
H^j (3H)	3.76 (s)
H^k (3H)	1.96 (s)

The presence of both carboxylic acid and amide functions in the chryscandin molecule was indicated from the IR (ν_{max}^{KBr} : 1720 (sh), 1695, 1665 cm^{-1}) and ^{13}C-NMR (δ 168.3 (s) and δ 171.2 (s)) spectra. Further corroboration on the structure of 1 was obtained as follows.

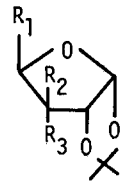
Acetylation of 1 with Ac_2O in pyridine (r.t.,overnight), followed by treatment with MeOH gave 2. Analysis of the ^{1}H-NMR spectrum of 2 using a double resonance technique (Table) revealed the structure including relative stereochemistry of the sugar moiety as shown in the structure 2 ($J_{1',2'}$ = 2, $J_{2',3'}$ = 5, $J_{3',4'}$ = 6 Hz).[4] Alkaline hydrolysis of 1 (1N methanolic NaOH, reflux, 20 h) caused the peptidyl bond cleavage to give 3 (yield 66 %) : ν_{max}^{Nujol} : 3600-2100, 1650 cm^{-1} ; FD mass : m/z 281 (M^+ + 1) ; $[\alpha]_D^{20}$ -28° (c 0.25, 1N HCl) ; δ (D_2O-DCl) 5.13 (1H, dd, J=2 and 6 Hz), 6.33 (1H, d, J=2 Hz), 8.34 (1H, s), 8.43 (1H, s), together with O-methyl-L-tyrosine (57 %). In order to confirm the deduced structure and to determine the absolute configurations, total synthesis of chryscandin was carried out as follows.

The diol (4)[5] transformed from D-xylose was selectively benzoylated (1.3 eq. C_6H_5COCl, pyridine, 78 %) and subsequently treated with $(CF_3SO_2)_2O$ in pyridine at -10°C to give the triflate (5, 73 %). Heating 5 with NaN_3 in EtOH for 40 h resulted in a S_N2 reaction to afford the 3α-azide (6)[9] (47 %) with a concomitantly formed 3,4-elimination byproduct (7, 49 %)[6] which was separated from 6 by column chromatography. After alkaline hydrolysis (1N NaOH-MeOH, r.t.,1 h) of 6, the resulting alcohol was oxidized with $KMnO_4$ (KOH, r.t.,

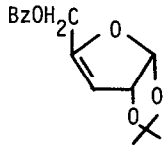
1 h) to provide the carboxylic acid (83 %), which was methylated (CH_2N_2) to give 8.[9] Cleavage of the 1,2-isopropylidene group of 8 with 75 % HCOOH (50°C, 2 h), followed by acetylation (Ac_2O, pyridine) led to the diacetate (9)[7] in 84 % yield. Glycosidation of adenine with 9 was achieved by the procedure modified by Lichtenthaler et al.[8] Thus, the silylated benzoyl adenine and 9 were stirred together with $SnCl_4$ in dichloroethane at 60-70°C for 4 h to afford the β-glycosidation product (10)[9] in the yield of 57 %. The ester groups of 10 were cleaved with 0.5N NaOH (r.t., 30 min) and the resulting azide (11) was hydrogenized (Pd-black, 3 atm) to give the 3'-amino glycoside (12)[9] (10 → 12, 73 %). Debenzoylation of 12 with n-butylamine (MeOH, reflux, 1 h) afforded 3-amino-1-(6-amino-9H-purin-9-yl)-1,3-dideoxy-β-D-ribofuranuronic acid (80 %) which was identical with the compound (3) derived from chryscandin by alkaline hydrolysis in all respects (IR, NMR, FD mass, $[\alpha]_D$). Coupling of the key intermediate (12) with O-methyl-L-tyrosine was carried out by the conventional manner (Z-O-methyl-L-tyrosine-OSu, Et_3N, THF-H_2O, 1 day) to yield (13) in 56 % yield. Finally, deprotection of 13 with n-butylamine (78 %), followed by catalytic hydrogenation (Pd-black, 3 atm, dil.HCl) afforded 1-(6-amino-9H-purin-9-yl)-1,3-dideoxy-3-(O-methyl-L-tyrosylamino)-β-D-ribofuranuronic acid,



3 R_1=H, R_2=H, R_3=H, R_4=NH_2
10 R_1=Bz, R_2=Me, R_3=Ac, R_4=N_3
11 R_1=Bz, R_2=H, R_3=H, R_4=N_3
12 R_1=Bz, R_2=H, R_3=H, R_4=NH_2



4 R_1=CH_2OH, R_2=OH, R_3=H
5 R_1=CH_2OBz, R_2=OTf, R_3=H
6 R_1=CH_2OBz, R_2=H, R_3=N_3
8 R_1=COOMe, R_2=H, R_3=N_3



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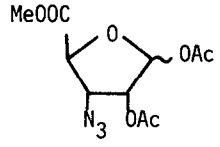

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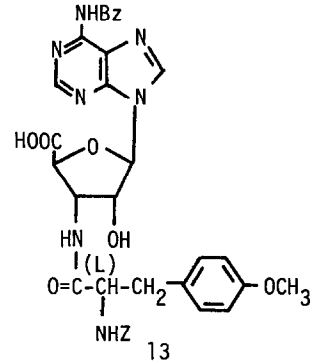

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which was crystallized from 0.1N HCl as the 2HCl salt. All the spectral data of this synthetic compound was completely in accord with the natural chryscandin·2HCl salt (1) : synthetic, $[\alpha]_D^{20}$ + 33° (c 1, 1N HCl) ; natural, $[\alpha]_D^{22}$ + 34° (c 1, 1N HCl). Consequently, the absolute structure of chryscandin was established to be as shown in formula 1. To our knowledge, chryscandin is the first naturally occurring glycoside possessing 3-aminoribofuranuronic acid moiety in the molecule.

References and Notes

1. Present address ; Research Laboratories, Fujisawa Pharmaceutical Co., Ltd. Osaka, Japan
2. Chryscandin has an activity against Candida albicans.
3. 1 : Anal, Found : C, 43.49 ; H, 4.82 ; N, 18.25 ; Cl, 13.04 %
   Calcd. for $C_{20}H_{23}N_7O_6 \cdot 2HCl \cdot H_2O$: C, 43.80 ; H, 4.96 ; N, 17.88 ; Cl, 12.93%
4. O. Jardetzky, J. Am. Chem. Soc. 85, 1823 (1963)
5. R. B. Baker and R. E. Schaub, J. Am. Chem. Soc. 77, 5900 (1955)
6. A. M. Ozols, A. V. Azhayev, N. B. Dyatkina and A. A. Krayevsky, Synthesis, 557 (1980)
7. NMR analysis of 9 showed that the β-anomer is exclussively predominant.
8. F. W. Lichtenthaler, P. Voss and A. Heerd, Tetrahedron Lett. 2141 (1974)
9. Spectroscopic data of typical compounds are shown below ;

   6 : $\nu_{max}^{CHCl_3}$: 2130, 1725 cm^{-1} ; δ ($CDCl_3$) 1.36 (3H, s), 1.61 (3H, s), 3.43 (1H, dd, J=4 and 8 Hz), 4.26-4.66 (3H, m), 4.79 (1H, t, J=4 Hz), 5.85 (1H, d, J=4 Hz), 7.26-7.62 (3H, m), 7.99-8.14 (2H, m)

   8 : $\nu_{max}^{CHCl_3}$: 2990, 2130, 1750 cm^{-1} ; δ ($CDCl_3$) 1.37 (3H, s), 1.57 (3H, s), 3.70 (1H, dd, J=3.5 and 9 Hz), 3.83 (3H, s), 4.55 (1H, d, J=9 Hz), 4.73 (1H, t, J=3.5 Hz), 5.88 (1H, d, J=3.5 Hz)

   10 : $\nu_{max}^{CHCl_3}$: 3120, 3000, 2130, 1750, 1640 cm^{-1} ; δ ($CDCl_3$) 2.13 (3H, s), 3.80 (3H, s), 4.60 (1H, d, J=5 Hz), 4.87 (1H, t, J=5 Hz), 5.84 (1H, t, J=5 Hz), 6.29 (1H, d, J=5 Hz), 7.20-7.61 (3H, m), 7.70-8.12 (2H, m), 8.42 (1H, s), 8.71 (1H, s), 8.97 (1H, s)

   12 : ν_{max}^{Nujol} : 3600-2200 (br.), 1685, 1640, 1620 cm^{-1} ; δ (D_2O-DCl) 5.23 (1H, dd, J=2 and 6 Hz), 6.57 (1H, d, J=2 Hz), 7.30-7.80 (3H, m), 7.80-8.20 (2H, m), 8.97 (1H, s), 9.05 (1H, s)

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