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THE ISOLATION AND STRUCTURE OF MODIFIED BIOACTIVE NUCLEOSIDES FROM *JASPIS JOHNSTONI*

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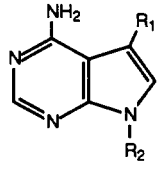
ABSTRACT.—Two cytotoxic pyrrolo[2,3-*d*]pyrimidine nucleosides, toyocamycin [**2**] and 5-(methoxycarbonyl)tubercidin [**1**], have been isolated from the Fijian sponge *Jaspis johnstoni*. The structures were solved primarily by ^{13}C nmr and mass spectral methods and confirmed by comparison to reported values in the literature. Small amounts of the corresponding aglycones were also isolated.

The first nucleosides of marine origin were reported in the 1950s by Bergmann and co-workers (1–3) and eventually led to the development of the analogue compound cytarabine, a drug with proven antiviral and antitumor properties (4). Since then, various marine sources have yielded novel nucleosides, including several with a substituted pyrrolo[2,3-*d*]pyrimidine base. These include tubercidin (5), 5-iodo-5′-deoxytubercidin (6), 5-bromotubercidin aglycone (6), and the mycalisines (7). We now wish to report the first isolation from a marine source of two known, bioactive, pyrrolo[2,3-*d*]pyrimidines along with the corresponding aglycones. The nucleoside 5-(methoxycarbonyl)tubercidin [**1**] has been previously reported (8,9) as an intermediate in syntheses of other pyrrolo[2,3-*d*]pyrimidines, but this is the first report of it as a natural product. Toyocamycin [**2**] was originally reported in 1961 by Ohkuma (10) to be produced by an unidentified strain of *Streptomyces*; however, this is the first isolation of toyocamycin from a marine source.

The MeOH extract of 300 g of freeze-dried *Jaspis johnstoni* Schmidt (Jaspidae) sponges, collected at Suva Harbor, Fiji, was subjected to solvent partition and the resulting fractions assayed for activity against *Candida albicans* and the L1210 murine leukemia cell line. The CCl_4- (0.67 g) and $CHCl_3$- (1.23 g) soluble material exhibited activity in both assays. Tlc showed that the CCl_4 fraction contained only jaspamide, an anticandidal, insecticidal, and cytotoxic metabolite previously isolated from this sponge (11), and that the $CHCl_3$-soluble material contained several highly polar, uv active, and nitrogenous compounds in addition to jaspamide. A crude preparation of these polar metabolites was also found to be active against *C. albicans* and the L1210 cell line. Subsequent separation of these compounds yielded 43.0 mg of **1**, 24.7 mg of **2**, 8.6 mg of **3** and 3.5 mg of **4**, all as white solids.



1 R_1=CO_2Me, R_2=ribose
2 R_1=CN, R_2=ribose
3 R_1=CO_2Me, R_2=H
4 R_1=CN, R_2=H

The nucleoside nature of 5-(methoxycarbonyl)tubercidin [**1**] was first evident from the ^1H-nmr spectrum (Table 1), which showed two aromatic singlets, several signals indicative of a ribose moiety, and an *O*-methyl. Eims gave a molecular ion at *m/z* 324, which was further substantiated by liquid chromatography mass spectrometry (lcms), which produced an $[MH]^+$ ion at *m/z* 325. Characteristic fragment ions in the

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TABLE 1. ^{1}H nmr Data for *Jaspis* Nucleosides.[a]

Proton	Compound	
	1	2
H-2[b]	8.62 (s, 1H)	8.71 (s, 1H)
H-6[b]	8.60 (s, 1H)	8.60 (s, 1H)
H-1′	6.94 (d, 5.3, 1H)	6.81 (d, 4.8, 1H)
H-2′	5.31 (dd, 5.3, 5.1, 1H)	5.18 (dd, 4.8, 4.8, 1H)
H-3′	5.28 (dd, 6.2, 5.1, 1H)	5.02 (dd, 4.8, 4.4, 1H)
H-4′	4.74 (m, 1H)	4.72 (m, 1H)
H-5′	4.30 (dd, 12.2, 2.6, 1H)	4.31 (dd, 12.1, 2.4, 1H)
H-5′	4.18 (dd, 12.2, 2.9, 1H)	4.20 (bd, 12.1, 1H)
OMe	3.66 (s, 3H)	

[a]200 MHz, pyridine-d_5, multiplicity and coupling constants in parentheses, referenced to center signal of residual pyridine-D_4H (7.55 ppm).

[b]May be interchanged. Assignments are made by analogy to other pyrrolo[2,3-*d*]pyrimidines which show H-2 is downfield of H-6.

ei mass spectrum of *m/z* 192 (base + 1, 100%), 193 (base + 2), 205 (base + 14), 221 (base + 30), and 235 (base + 44) confirmed that **1** was a ribosyl nucleoside and also served to place the *O*-methyl group on the base portion of the molecule rather than the sugar (12). The ^{13}C-nmr spectrum of **1** (Table 2) contained a signal at 165.4 ppm which, along with the 1701 and 1239 cm^{-1} absorption maxima in the ir spectrum and the *O*-methyl singlet in the ^{1}H nmr, indicated the presence of an aromatic methyl ester. The 13 signals in the ^{13}C-nmr spectrum together with the mass spectral data permitted a molecular formula of $C_{13}H_{16}N_4O_6$. The ^{13}C spectrum also provided the first indication that **1** did not contain a normal purine base. The upfield signals at 106.2 and 100.7 ppm corresponded more closely to a pyrrolo[2,3-*d*]-pyrimidine ring system than to a purine (13). The final structure was determined to be **1** by identical comparison to reported spectroscopic data (9,13).

TABLE 2. ^{13}C-nmr Data for *Jaspis* Nucleosides.[a]

Carbon	Compound	
	1	2
C-2	153.2	153.6
C-4	157.7	157.0
C-4a	100.7	101.3
C-5	106.2	83.0
C-6	130.0	132.4
C-7a	151.2	150.2
C-1′	87.6	87.8
C-2′	74.2	74.3
C-3′	70.4	70.2
C-4′	85.5	85.5
C-5′	61.3	61.2
CN		115.4
CO	165.4	
OMe	52.0	

[a]50 MHz, DMSO-d_6, referenced to internal DMSO-d_6 (39.5 ppm).

Toyocamycin [**2**] had uv maxima and prominent ir bands all in good agreement with reported values (10). Eims and lcms established a mol wt of 291 and a molecular formula of $C_{12}H_{13}N_5O_4$. The eims again showed the characteristic nucleoside fragmentation pattern of base plus 1, 2, 14, 30, and 44 mass units (12). The ^{1}H nmr is consistent with the structure (Table 1). The ^{13}C-nmr spectrum of **2**, like that of **1**, was again the key piece of data in solving the structure of the modified purine base. The extremely shielded C-5 signal (83.0 ppm) required that it be substituted with the nitrile that was evident from the ir band at 2230 cm^{-1} and the ^{13}C signal at 115.4 ppm. The structure of **2** was substantiated by comparison of ^{13}C-nmr spectral data to published values (13).

The aglycones **3** and **4** of 5-(methoxycarbonyl)tubercidin and toyocamycin, respectively, were also isolated in small quantities, and their structures were based on comparison of nmr and eims data with that of the parent compounds. Isolation conditions were never harsh enough to hydrolyze the nucleosides; therefore, these are naturally occurring compounds.

The anticandidal activity of the crude polar metabolites could be attributed entirely to toyocamycin (1.25 mg/6.4 mm disk gave a 13-mm zone of inhibition). The L1210 cytotoxicity IC_{50} for **2** was 0.0026 μg/ml and that of **1** was 0.027 μg/ml. 5-(Methoxycarbonyl)tubercidin had previously been reported in the literature to extend the lives of mice afflicted with L1210 leukemia by as much as 39% (8). Compounds **3** and **4** were not submitted for biological evaluation.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—All nmr spectra were recorded on an IBM AF 200 spectrometer operating at 200 MHz for ^1H and 50 MHz for ^{13}C. ^1H spectra are referenced to the center signal of residual pyridine-D_4H (7.55 ppm) and ^{13}C spectra are referenced to internal DMSO-d_6 (39.5 ppm). Ir spectra were recorded on a Beckman FT-2100 spectrophotometer calibrated with polystyrene at 1601 cm^{-1} using zinc selenide cells. Uv spectra were obtained on a Beckman DU-8 spectrophotometer. Eims data were obtained on a Varian MAT-112 spectrometer, and lcms data were collected on a noncommercial quadrupole mass spectrometer using thermospray ionization. Hplc separations were performed on a Waters/Millipore system employing a model 501 pump, U6K injector, and R401 differential refractometer detector.

SPONGE COLLECTION.—The bright orange sponge *J. johnstoni* (1.4 kg) was collected using SCUBA at depths of 3 to 10 m in Suva Harbor, the Fiji Islands during November 1986. The animals were immediately frozen at −20° and remained so until lyophilization. The sponge was identified by Dr. Avril Ayling, Sea Research, Daintree, Queensland 4873, Australia (14). A voucher specimen, CI-84-4-13, is kept at Sea Research.

EXTRACTION, SEPARATION, AND PURIFICATION OF COMPOUNDS **1–4**.—The lyophilized sponges (300 g) were covered with MeOH (1 liter) for 14 h, decanted, and soaked in MeOH (1 liter) again for 10 h. Following removal of the solvent the animals were homogenized and soaked in MeOH (750 ml) for an additional 13 h. The MeOH-soluble material was then pooled and concentrated in vacuo to a total volume of 750 ml, made 10% aqueous and extracted with hexanes (3 × 500 ml), made 20% aqueous and extracted with CCl_4 (3 × 500 ml), and finally made 40% aqueous and extracted with $CHCl_3$ (3 × 500 ml). The $CHCl_3$-soluble material (1.23 g) was passed over a C_{18} reversed-phase scrub column [Whatman P-40 ODS-3, 1.5 × 15 cm, MeOH-H_2O (80:20)] followed by hplc [Whatman Partisil 10 ODS-3, 9.2 mm × 50 cm; H_2O-MeOH (60:40), 3 ml/min] which produced four fractions (24.7, 3.5, 43.0, and 8.6 mg).

5-(METHOXYCARBONYL)TUBERCIDIN [**1**].—The third compound to elute from the hplc was 5-(methoxycarbonyl)tubercidin: uv (MeOH) λ max 281, 232(sh), 211 nm; ir (neat film) ν max 3300, 1701, 1239, 1095 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2. Mass spectral data are described in the text.

TOYOCAMYCIN [**2**].—The first compound to elute from the hplc was identified as toyocamycin: uv (MeOH) λ max 279, 231(sh), 206 nm; ir (neat film) ν max 3430, 3300, 2230, 1670 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2. Mass spectral data are described in the text.

5-(METHOXYCARBONYL)TUBERCIDIN AGLYCONE [**3**].—The fourth peak to elute from the hplc was identified as the aglycone of **1**. Compound **3**: eims *m/z* $[M]^+$ 192; ^1H nmr (DMSO-d_6/D_2O) δ 8.13 (s, 1H), 7.88 (s, 1H), 3.90 (s, 3H); ^{13}C nmr (DMSO-d_6) δ 165.7 (s), 157.5 (s), 152.9 (d), 131.1 (d), 105.2 (s), 100.5 (s), 51.5 (q).

TOYOCAMYCIN AGLYCONE [**4**].—The second compound to elute from the hplc was found to be the aglycone of **2**. Compound **4**: eims *m/z* $[M]^+$ 159; ^1H nmr (DMSO-d_6/D_2O) δ 8.14 (s, 1H), 8.05 (s, 1H); ^{13}C nmr (DMSO-d_6) δ 156.8 (s), 153.3 (d), 151.5 (s), 133.2 (d), 116.3 (s), 101.2 (s), 81.6 (s).

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LITERATURE CITED

1. W. Bergmann and R.J. Feeney, *J. Am. Chem. Soc.*, **72**, 2809 (1950).
2. W. Bergmann and R.J. Feeney, *J. Org. Chem.*, **16**, 981 (1951).
3. W. Bergmann and D.C. Burke, *J. Org. Chem.*, **20**, 1501 (1955).
4. T.W. North and S.S. Cohen, *Pharmacol. Ther.*, **4**, 81 (1979).
5. J.J. Barchi Jr., T.R. Norton, E. Furusawa, G.M.L. Patterson, and R.E. Moore, *Phytochemistry*, **22**, 2851 (1983).
6. R. Kazlauskas, P.T. Murphy, R.J. Wells, J.A. Baird-Lambert, and D.D. Jamieson, *Aust. J. Chem.*, **36**, 165 (1983).
7. Y. Kato, N. Fusetani, S. Matsunaga, and K. Hashimoto, *Tetrahedron Lett.*, **26**, 3483 (1985).
8. K.V. Rao, *J. Med. Chem.*, **11**, 939 (1968).
9. D.E. Bergstrom, A.J. Brattesani, M.K. Ogawa, and M.J. Schweikert, *J. Org. Chem.*, **46**, 1423 (1981).
10. K. Ohkuma, *J. Antibiot., Ser. A*, **14**, 343 (1961).
11. T.M. Zabriskie, J.A. Klocke, C.M. Ireland, A.H. Markus, T.F. Molinski, D.J. Faulkner, C. Xu, and J. Clardy, *J. Am. Chem. Soc.*, **108**, 3123 (1986).
12. J.A. McCloskey, in: "Basic Principles in Nucleic Acid Chemistry." Ed. by P.O.P. Ts'o, Academic Press, New York, 1974, Vol. 1, pp. 209–309.
13. M.T. Chenon, R.J. Pugmire, D.M. Grant, R.P. Panzica, and L.B. Townsend, *J. Am. Chem. Soc.*, **97**, 4627 (1975).
14. J.C. Braekman, D. Daloze, B. Moussiaux, and R. Riccio, *J. Nat. Prod.*, **50**, 994 (1987).

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