HERBICIDAL NUCLEOSIDES FROM MICROBIAL SOURCES

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The structures of five naturally-occurring herbicidal nucleosides have been determined by spectral analysis. Three (5'-deoxyguanosine, coaristeromycin and 5'-deoxytoyocamycin) are novel natural products while the remaining two (coformycin and adenine 9-β-D-arabinofuranoside) are known natural products which have not previously been reported to be herbicidal.

Description

Metabolites of microorganisms have the potential to provide agricultural researchers with novel structures which can serve as models in synthetic programs aimed at providing commercial herbicides. Several reports of naturally-occuring nucleosides having interesting herbicidal properties have appeared††. In the course of screening microbial fermentation broths for herbicidal activity, we have encountered a number of phytotoxic nucleosides. This report††† presents the isolation and structure elucidation of three bioactive compounds which were previously unknown from natural sources and two known natural substances not previously reported to have herbicidal activity.

The cell-free filtrate from a 500-ml fermentation of *Thermoactinomycete* sp. A6019 was found to contain a water soluble herbicidal metabolite. Following concentration to dryness and trituration with methanol, the methanol-soluble bioactive material was purified by C_{18} RP-flash chromatography (FC) and C_{18} RP-HPLC (water-methanol gradient) to provide *ca.* 1 mg of bioactive material. Analysis of the UV, ^{1}H NMR and thermospray HPLC-MS data suggested that the active component was a guanosine nucleoside containing a reduced pentose moiety. Comparison of the HPLC Rt and ^{1}H NMR spectral data with that obtained with synthetically derived material[5] confirmed the presence of the novel natural product 5'-deoxyguanosine (**I**). 5'-Deoxyguanosine was found to be very active against *Lemna minor* at 100 μg/ml.

Coaristeromycin (**II**), also known synthetically[6], but not from natural sources, was found to be produced as a phytotoxic metabolite of *Streptomyces* sp. A6308. An 850 ml sample of the cell-free fermentation filtrate was purified by sequential fractionation on Amberlite XAD-2, C_{18} RP-FC and C_{18} RP-HPLC (water-methanol gradient) to yield 10 mg of **II**. Analysis of the UV, ^{1}H NMR and ^{13}C NMR data suggested that **II** was structurally related to aristeromycin[7] (**III**) which was also isolated from this fermentation. Thermospray HPLC-MS analysis provided a molecular ion at *m/z* 267 ($(M+H)^+$) indicating a MW one mass unit less than aristeromycin. On the basis of the coproduction of **II** and **III**

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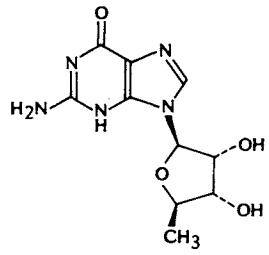
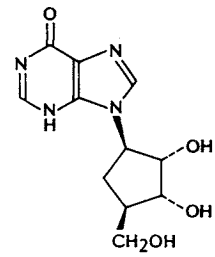
†† Among these are formycin A[1], herbicidins A, B, E, F, and G[2], and gougerotin[3].

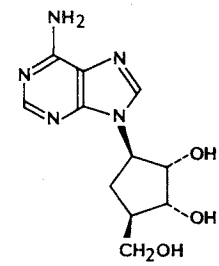
††† Compounds **I**, **II**, **V**, and **VI** were presented at a Society of Chemical Industry Pesticides Group Symposium entitled "Natural Products as a Source for New Agricultural Chemicals"[4].



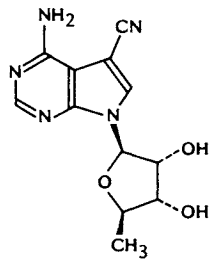
5′-Deoxyguanosine (**I**)



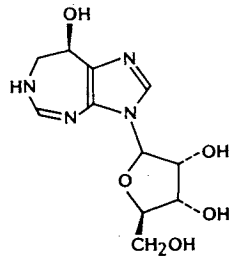
Coaristeromycin (**II**)



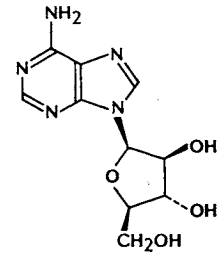
Aristeromycin (**III**)



5′-Deoxytoyocamycin (**IV**)



Coformycin (**V**)



Ara-A (**VI**)

and their spectroscopic similarity, **II** was assigned the structure shown. Confirmation of this assignment was provided by spectroscopic (^{1}H and ^{13}C NMR) comparison with authentic material obtained by the deamination of aristeromycin[6]. Coaristeromycin was herbicidally active against yellow nutsedge, johnsongrass, barnyard grass and Indian mustard at an application rate of 6 kg/ha.

A third nucleoside not previously isolated from natural sources but known synthetically[8] has been characterized as 5′-deoxytoyocamycin (**IV**). Compound **IV** (5.2 mg) was isolated from 20 ml of the filtered fermentation broth of *Streptomyces* sp. A14345 by extraction with ethyl acetate followed by C_{18} RP-FC. The identification of **IV** was based upon analysis of its UV, ^{13}C NMR and HRFAB mass spectra as well as comparison of its ^{1}H NMR spectrum with that reported by WANG *et al.*[8]. 5′-Deoxytoyocamycin caused bleaching of *L. minor* at 10 μg/ml.

In addition to compounds **I**, **II** and **IV**, two nucleosides known to occur naturally but for which herbicidal activities have yet to be reported, have also been isolated. Crude coformycin[9] (**V**) was obtained from the fermentation broth of an unclassified actinomycete A990 by successive Amberlite XAD-2 and C_{18} RP-FC. Methanol trituration of the combined active fractions followed by fractionation of the methanol insoluble fraction on Sephadex LH-20 (1 : 1 methanol-water) provided 2.7 mg of pure **V**. The ^{1}H, ^{13}C NMR and MS data for compound **V** were identical to those reported previously[9]. Coformycin was found to be active against seedling johnsongrass, barnyard grass, morning glory, crabgrass and Indian mustard at an application rate of 6 kg/ha.

Adenine 9-β-D-arabinofuranoside (Ara-A, **VI**) was isolated from 32 ml of the cell-free fermentation broth of *Actinoplane* sp. A9222 by concentration and fractionation by C_{18} RP-FC. The active fractions were combined and further purified by C_{18} RP-HPLC to give 1.4 mg of Ara-A[10]. Compound **VI** and commercially available Ara-A were indistinguishable by HPLC-MS, ^{1}H NMR and UV analysis. Ara-A was found to inhibit the germination of *Arabidopsis thaliana* at 25 μg/ml.

In conclusion, three new naturally occurring herbicidal nucleosides and two known nucleosides not

previously reported to be herbicidal have been isolated from five different actinomycetes. The isolation of these five compounds provides further evidence that microorganisms represent an important source of interesting herbicidally active compounds.

Experimental

Analytical Procedures

HPLC purifications were performed on either a Vydac Semi-prep 201HS1010 (25 cm × 10 mm) or a Waters μBondapak (25 cm × 9.4 mm) C_{18} column. ^1H and ^{13}C NMR spectra were obtained on either a Varian XL-300 or VXR-300 spectrometer. UV data was obtained on a Hewlett-Packard 8450A. Thermospray mass spectral analysis was performed on a Finnigan MAT Model 4535 with a Vestec thermospray interface and FAB analysis was obtained on a VG-ZAB instrument.

Fermentation and Physical Data

5′-Deoxyguanosine (**I**): A6019 was isolated from a soil sample taken from Northwood, Rhode Island, U.S.A. A6019 was fermented for 3 days at 55°C while shaking at 250 rpm in seed medium which consisted of glucose 0.25 g, malt extract 0.25 g, KNO_3 0.125 g, baker's yeast 0.125 g, $CaCO_3$ 0.075 g, K_2HPO_4 0.025 g and $MgSO_4 \cdot 7H_2O$ 0.025 g in 50 ml of water. The seed culture was then added to a medium composed of glucose 0.25 g, Maltrin M-100 5 g, baker's yeast 1.25 g, K_2HPO_4 0.125 g, $MgSO_4 \cdot 7H_2O$ 0.125 g and NaCl 0.5 g in 500 ml of water, adjusted to pH 7.5 prior to sterilization, and fermented for 7 days at 55°C in a 2-liter baffled Erlenmeyer flask shaken at 250 rpm. Following the isolation sequence described above, *ca.* 1 mg of 5′-deoxyguanosine was obtained. UV $\lambda_{max}^{H_2O}$ nm 251, 273; ^1H NMR (300 MHz, DMSO-d_6) δ 7.86 (1H, s), 6.50 (2H, br s), 5.63 (1H, d, J=5 Hz), 4.44 (1H, br d), 3.88 (2H, m), 1.26 (3H, d, J=6 Hz); positive ion thermospray HPLC-MS m/z 268 $(M+H)^+$.

For comparison with the natural product, 5′-deoxyguanosine was prepared synthetically according to the method of McGee and Martin[5]: ^1H NMR (300 MHz, DMSO-d_6) δ 10.59 (1H, br s), 7.85 (1H, s), 6.44 (2H, br s), 5.63 (1H, d, J=5 Hz), 5.36 (1H, d, J=6 Hz), 5.05 (1H, d, J=5 Hz), 4.44 (1H, q, J=5 Hz), 3.87 (2H, m), 1.26 (3H, d, J=6 Hz).

Coaristeromycin (**II**): A6308 was isolated from a soil sample obtained in Northwood, Rhode Island, U.S.A. A seed culture of A6308 was grown for 3 days at 30°C while shaking at 250 rpm in a medium which contained Tryptone 0.25 g and yeast extract 0.15 g in 50 ml of water. The seed culture was then added to, and fermented for 5 days in, medium containing dextrin 10 g, glucose 1 g, soybean flour 5 g, yeast extract 1.5 g, $CaCO_3$ 1.5 g and Amberlite XAD-7 20 g in 1 liter of water adjusted to pH 6.5 prior to sterilization. The culture was fermented in two 500 ml portions in 2-liter Erlenmeyer flasks at 30°C with shaking at 250 rpm. Following purification as outlined above 10 mg of coaristeromycin was obtained. UV $\lambda_{max}^{H_2O}$ nm 250; ^1H NMR (300 MHz, D_2O) δ 8.27 (1H, s), 8.19 (1H, s), 4.89 (1H, dt, J=11 and 9 Hz), 4.51 (1H, dd, J=6 and 9 Hz), 4.12 (1H, dd, J=3 and 6 Hz), 3.78 (1H, dd, J=11 and 7 Hz), 3.73 (1H, dd, J=11 and 7 Hz), 2.54 (1H, dt, J=13 and 8 Hz), 2.32 (1H, m), 1.86 (1H, ddd, J=9, 11 and 13 Hz); ^{13}C NMR (75 MHz, D_2O) δ 160.1, 146.9, 142.0, 125.2, 76.6, 73.3, 64.5, 61.0, 46.2, 30.3.

Aristeromycin was converted to coaristeromycin by the method of Marumoto *et al.*[6]. ^1H NMR (300 MHz, D_2O) δ 8.27 (1H, s), 8.20 (1H, s), 4.89 (1H, dt, J=11 and 9 Hz), 4.52 (1H, dd, J=6 and 9 Hz), 4.12 (1H, dd, J=3 and 6 Hz), 3.78 (1H, ddd, J=2, 6 and 11 Hz), 3.73 (1H, ddd, J=2, 6 and 11 Hz), 2.54 (1H, dt, J=13 and 8 Hz), 2.32 (1H, m), 1.86 (1H, ddd, J=9, 11 and 13 Hz); ^{13}C NMR (75 MHz, D_2O) δ 160.1, 146.9, 142.0, 125.2, 76.8, 73.2, 64.5, 61.0, 46.2, 30.3.

5′-Deoxytoyocamycin: A14235 was isolated from a soil sample taken from Kepong, Malaysia. A14345 was grown in a 250-ml Erlenmeyer flask at 30°C with shaking at 250 rpm in media containing casein 0.5 g, glucose 0.25 g, malt extract 1.25 g, tomato paste 1.0 g, $MgSO_4 \cdot 7H_2O$ 0.05 g, K_2HPO_4 0.05 g, $CaCO_3$ 0.25 g, $MgHPO_4 \cdot 3H_2O$ 0.25 g, iron EDTA 0.009 g, and 5 mg each of $FeSO_4 \cdot 7H_2O$, $MnCl_2 \cdot 4H_2O$, $ZnSO_4 \cdot 7H_2O$, $CoSO_4 \cdot 5H_2O$, and $CuSO_4 \cdot 6H_2O$ in 50 ml of water. Isolation of the active component as detailed in the discussion section provided 5.2 mg of 5′-deoxytoyocamycin. UV λ_{max}^{MeOH} nm 207, 231, 274 (sh), 279, 289 (sh); ^1H NMR (300 MHz, pyridine-d_5) δ 8.63 (1H, s), 8.32 (1H, s), 7.81 (2H, br s), 6.83 (1H, d, J=3.9 Hz), 4.98 (1H, t, J=4.8 Hz), 4.57 (1H, dq, J=6 and 6.3 Hz), 4.44 (1H, t, J=5.4 Hz), 1.59 (1H, d, J=6.6 Hz);

^{13}C NMR (75 MHz, CD_3OD) δ 158.6, 154.6, 151.5, 132.7, 116.0, 90.6, 85.5, 81.5, 76.3, 75.9, 19.1; positive ion FABHR-MS m/z 276.1120 (Calcd for $C_{12}H_{14}N_5O_3$: 276.1098 $(M+H)^+$).

Coformycin: A990 was isolated from a soil sample taken from Winterville, Georgia, U.S.A. A seed culture of A990 was prepared by fermentation in a medium containing Tryptone 0.25 g and yeast extract 0.15 g in 50 ml of water which was maintained at 30°C for 3 days while shaking at 250 rpm. Actinomycete A990 was fermented at 30°C for 5 days with shaking at 250 rpm after the addition of the seed culture to a medium which contained dextrin 10 g, glucose 1 g, soybean flour 5 g, yeast extract 1.5 g and $CaCO_3$ 1.5 g in 1-liter of water which was adjusted to pH 6.5 prior to sterilization. The fermentation was conducted in two 2-liter Erlenmeyer flasks which contained 500 ml of broth each. Following the purification scheme outlined above, 2.7 mg of coformycin was obtained. ^1H NMR (300 MHz, D_2O) δ 7.49 (1H, s), 6.99 (1H, s), 5.65 (1H, d, $J=6.6$ Hz), 4.96 (1H, d, $J=3.9$ Hz), 4.46 (1H, t, $J=5.4$ Hz), 4.13 (1H, m), 4.02 (1H, m), 3.60 (2H, m), 3.32 (1H, dd, $J=13.5$ and 3.9 Hz), 3.18 (1H, d, $J=13.5$ Hz); ^{13}C NMR (75 MHz, D_2O) δ 152.7, 138.1, 134.9, 131.4, 89.9, 87.9, 76.0, 73.3, 69.5, 64.2, 49.2; positive ion thermospray HPLC-MS m/z 285 $(M+H)^+$.

Ara-A: A9222 was isolated from a soil sample taken in the Ulmstead State Park, North Carolina, U.S.A. The fermentation of A9222 was conducted in media which contained glucose 0.025 g, dextrin 0.125 g, cotton-seed oil 0.25 ml, yeast extract 0.025 g, Proflo 0.125 g, soybean flour 0.25 g and $MgHPO_4 \cdot 3H_2O$ 0.025 g in 50 ml of water. The fermentation was maintained at 30°C in a 250 ml Erlenmeyer flask for 5 days while shaking at 250 rpm. UV $\lambda_{max}^{H_2O}$ nm 258; ^1H NMR (300 MHz, D_2O) δ 8.25 (1H, s), 8.13 (1H, s), 6.31 (1H, d, $J=5.7$ Hz), 4.46 (1H, t, $J=5.4$ Hz), 4.26 (1H, t, $J=6.3$ Hz), 3.96 (1H, m), 3.85 (1H, dd, $J=12.9$ and 3 Hz), 3.77 (1H, dd, $J=12.9$ and 5.1 Hz); negative ion thermospray HPLC-MS m/z 266 $(M-H)^-$; positive ion thermospray HPLC-MS m/z 268 $(M+H)^+$.

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References

1) HORI, M.; E. ITŌ, T. TAKITA, G. KOYAMA, T. TAKEUCHI & H. UMEZAWA: A new antibiotic, formycin. J. Antibiotics, Ser. A 17: 96~99, 1964
2) TERAHARA, A.; T. HANEISHI, M. ARAI, T. HATA, H. KUWANO & C. TAMURA: The revised structure of herbicidins. J. Antibiotics 35: 1711~1714, 1982
3) MURAO, S. & H. HAYASHI: Gougerotin, as a plant growth inhibitor, from *Streptomyces* sp. No. 179. Agric. Biol. Chem. 47: 1135~1136, 1983
4) AYER, S. W.; B. G. ISAAC, D. M. KRUPA, K. E. CROSBY, L. J. LETENDRE & R. J. STONARD: Herbicidal compounds from micro-organisms. Pestic. Sci. 27: 221~223, 1989
5) MCGEE, D. P. & J. C. MARTIN: Acyclic nucleoside analogs: methods for the preparation of 2',3'-secoguanosine, 5'-deoxy-2',3'-secoguanosine, and (*R,S*)-9-[1-(2-hydroxyethoxy)-2-hydroxyethyl]guanine. Can. J. Chem. 64: 1885~1889, 1986
6) MARUMOTO, R.; Y. YOSHIOKA, Y. FURUKAWA & M. HONJO: Synthesis of aristeromycin analogs. Chem. Pharm. Bull. 24: 2624~2628, 1976
7) KISHI, T.; M. MUROI, T. KUSAKA, M. NISHIKAWA, K. KAMIYA & K. MIZUNO: The structure of aristeromycin. Chem. Pharm. Bull. 20: 940~946, 1972
8) WANG, Y.; H. P. C. HOGENKAMP, R. A. LONG, G. R. REVANKAR & R. K. ROBINS: A convenient synthesis of 5'-deoxynucleosides. Carbohydr. Res. 59: 449~457, 1977
9) NAKAMURA, H.; G. KOYAMA, Y. IITAKA, M. OHNO, N. YAGISAWA, S. KONDO, K. MAEDA & H. UMEZAWA: Structure of coformycin, an unusual nucleoside of microbial origin. J. Am. Chem. Soc. 96: 4327~4328, 1974
10) LEE, W. W.; A. BENITEZ, L. GOODMAN & B. R. BAKER: Potential anticancer agents. XL. Synthesis of the β-anomer of 9-(D-arabinofuranosyl)-adenine. J. Am. Chem. Soc. 82: 2648~2649, 1960