Phytochemistry, Vol. 22, No. 12, pp. 2851–2852, 1983.
Printed in Great Britain.

0031–9422/83 $3.00 + 0.00

SHORT REPORTS

IDENTIFICATION OF A CYTOTOXIN FROM *TOLYPOTHRIX BYSSOIDEA* AS TUBERCIDIN

JOSEPH J. BARCHI, JR.*, TED R. NORTON†, EIICHI FURUSAWA†, GREGORY M. L. PATTERSON* and RICHARD E. MOORE*‡

*Department of Chemistry, University of Hawaii, Honolulu, HI 96822, U.S.A.; †Department of Pharmacology, University of Hawaii, Leahi Hospital, 3675 Kilauea Ave., Honolulu, HI 96816, U.S.A.

(*Received* 9 *May* 1983)

Key Word Index—*Tolypothrix byssoidea*; Scytonemataceae cultured cells; tubercidin; pyrrolo[2,3-d]pyrimidine nucleoside.

Abstract—Tubercidin, a biologically active pyrrolo[2,3-d]pyrimidine nucleoside previously isolated from *Streptomyces tubercidicus*, has been identified as a major metabolite of the cyanophyte *Tolypothrix byssoidea*.

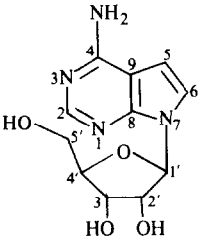
INTRODUCTION

Extracts of a variety of field collected blue–green algae have been found to possess antineoplastic activities [1]. Field collections, however, have frequently provided too little material for isolation and identification of the active agents. We have been able to culture some blue–green algae in the laboratory which display good anticancer activities in their extracts. In one case an ethanolic extract of cultured *Tolypothrix byssoidea* (Berk.) Kirchner was found to show significant inhibitory activity *in vitro* against KB and NIH/3T3 cells and *in vivo* against P-388 lymphocytic leukemia in mice. We report here the bioassay-directed isolation and identification of the active constituent, tubercidin.

RESULTS AND DISCUSSION

Fractionation of the algal extract led to the isolation of **1** as the cytotoxic, antileukemic component. ^{1}H NMR spectral analysis of compound **1** revealed the elements of a furanose carbohydrate glycosidically linked to a heteroaromatic base. ^{1}H and ^{13}C NMR spectral data of compound **1** corresponded precisely with data reported in the literature for tubercidin [2, 3]. Direct comparison of **1** with authentic tubercidin rigorously established its identity. Other supportive evidence was obtained by comparison of UV absorption and CD spectra. Compound **1** and authentic tubercidin (hydrochloride) exhibited UV maxima at 270 and 227–230 nm and showed negative CD peaks at 270 and 230 (shoulder) nm.

Tubercidin has previously been isolated from *Streptomyces tubercidicus* [4, 5]. It is a potent antitumor agent [6] which has found use against some forms of human cancer, such as cutaneous neoplasms [7, 8].



1

Tubercidin is an inhibitor of DNA, RNA and protein synthesis in growing KB cells [9], acting by disruption of nucleic acid structure following incorporation. Synthesis of messenger RNA was found to be particularly susceptible.

EXPERIMENTAL

^{1}H NMR spectra were obtained at 300 MHz in DMSO-d_6 and ^{13}C NMR spectra at 75 MHz in DMSO-d_6. Chemical shifts are reported in δ units (ppm) relative to DMSO-d_6 as internal standard for both ^{1}H (2.49 ppm) and ^{13}C (39.5 ppm). The cytotoxicity assays against KB cells (a human carcinoma cell line) and NIH/3T3 cells (a mouse fibroblast line) were carried out using the method of Furusawa *et al.* [10]. P-388 activity was determined by a literature method [11]. Authentic tubercidin was obtained from Sigma Chemical Company.

Culture conditions. Tolypothrix byssoidea, an epilithic, aerial alga, was collected on the island of Oahu, Hawaii. Clonal cultures were prepared by repeated subculture on solidified media [12]. The alga was cultured in 25 l. bottles containing an inorganic medium [13] modified by replacing citrate buffer with 3 mM 3-(*N*-morpholino) propanesulfonic acid (pH 7) and by supplementing the medium with a comprehensive minor and trace element mixture [14]. Cultures were illuminated continuously at an

‡To whom correspondence should be addressed.

incident intensity of 330 micro Einsteins/m^2/sec from banks of cool-white fluorescent tubes. Cultures were vigorously aerated with 1% CO_2 in air. Incubation temp. was 24 ± 1°. The alga was harvested by filtration; yields typically were of the order of 0.5 g dry wt per liter of culture.

Isolation. The freeze dried alga (10 g) was homogenized with EtOH–H_2O (3:7) in a Waring-type blender. The extract was clarified by centrifugation at 10000 rpm followed by concn *in vacuo* to a vol. of 100 ml. The aq. concentrate was applied directly to an 8 × 45 cm column of Sephadex G-25 and eluted with 30 mM ammonium acetate satd with $CHCl_3$. The cytotoxic agent obtained at V_e/V_0 = 3.4–4.1 was rechromatographed on a 2.5 × 70 cm column of Sephadex G-25 with the same eluant. The purity was *ca* 60% at this point. Final purification was achieved by isocratic HPLC on a 1 × 25 cm column of Ultrasphere 5 micron ODS using 5% CH_3CN in aq. 100 mM triethylamine/120 mM AcOH as the eluant (flow rate 4 ml/min). The major peak (R_t 9.5 min) was collected and lyophilized to give 9.5 mg of tubercidin (**1**, 4-amino-7-(β-D-ribofuranosyl)-pyrrolo-(2,3-d)-pyrimidine) as a white powder: UV $\lambda_{max}^{H_2O}$ 270, 230 nm; CD (H_2O): $[\theta]_{270}$ −4000; ^{1}H NMR (DMSO-d_6): δ 8.024 (1H, *s*, H-2), 7.322 (1H, *d*, *J* = 3.7 Hz, H-6), 7.027 (2H, *br s*, NH_2), 6.567 (1H, *d*, *J* = 3.7 Hz, H-5), 5.970 (1H, *d*, *J* = 6.3 Hz, H-1′), 5.301 (1H, *dd*, *J* = 6.5 and 4.8 Hz, C5′-OH), 5.251 (1H, *d*, *J* = 6.5 Hz, C2′-OH), 5.084 (1H, *d*, *J* = 4.8 Hz, C3′-OH), 4.406 (1H, *q*, *J* = 6.5, 6.3, and 6.0 Hz, H-2′), 4.062 (1H, *m*, *J* = 6.0, 4.8, and 3.0 Hz, H-3′), 3.875 (1H, *q*, *J* = 3.5, 3.0, and 2.9 Hz, H-4′), 3.606 (1H, *ddd*, *J* = −11.9, 4.8, and 3.5 Hz, H-5′), 3.505 (1H, *ddd*, *J* = −11.9, 4.8, and 3.5 Hz, H-5′); ^{13}C NMR (DMSO-d_6): δ 61.82 (C-5′), 70.69 (C-3′), 73.61 (C-2′), 85.02 (C-4′), 87.56 (C-1′), 99.46 (C-5), 103.05 (C-9), 122.24 (C-6), 149.85 (C-8), 151.46 (C-2), 157.48 (C-4).

The tubercidin from *T. byssoidea* and the authentic sample each showed complete kill of both KB and NIH/3T3 cells at 70 ppb.

Acknowledgments—This research has been supported by PHS Grant CA 12623-10, awarded by the National Cancer Institute, Department of Health and Human Services. High field NMR studies at the University of Hawaii were made possible by NSF Grant CHE81-00240.

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