Synthesis of [19-^{3}H] Herbimycin A

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SUMMARY

Herbimycin A, an ansamycin antibiotic, is known to reverse the transformed phenotype of cells transfected with tyrosine kinase expressing oncogenes. The synthesis of [19-^{3}H] herbimycin A (**1c**), a potential tool for unraveling the mechanism of this reversal, is described. Reduction of 19-bromoherbimycin A (**4**) employing zinc-copper couple/tritiated water affords hydroquinone (**5c**) which, when treated with manganese dioxide, affords **1c**.

Key Words: herbimycin A, tyrosine kinase, oncogene inhibition, tritium

INTRODUCTION

A number of retroviruses have been identified that encode phosphoproteins which possess protein tyrosine kinase activity (1). These tyrosine kinases have been implicated as playing critical signal transduction roles in the malignant phenotype expressed by cells infected with these retroviruses. The transforming gene of Rous sarcoma virus, *src*, encodes a tyrosine kinase entitled pp60$^{v\text{-}src}$ (2,3). In the course of a screening program Uehara *et al.* found that herbimycin A (**1a**), a benzoquinonoid ansamycin antibiotic (4), converted transformed cell morphology into normal morphology in a cell line infected with the Rous sarcoma virus (5).

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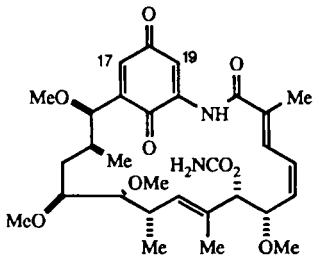
Subsequent studies revealed similar reversals of transformed phenotypes induced by oncogenes expressing other members of the protein tyrosine family (6). Herbimycin A has also recently been shown to inhibit signal transduction (protein tyrosine kinase mediated) in T-cell receptor complexes (7).

Mechanistic studies directed towards identification of the cellular target(s) acted upon by herbimycin A have been reported (8-10). In the case of cells transformed by Rous sarcoma virus, it has been shown that $pp60^{v\text{-}src}$ is down-regulated following treatment with herbimycin A. Though it has been shown that herbimycin A is an irreversible inhibitor of $pp60^{v\text{-}src}$ (9,10), the concentrations necessary to achieve this effect are significantly higher than those required to induce down-regulation and morphological reversion. A radiolabelled derivative of herbimycin A would be a valuable tool for further elucidating the mechanism by which herbimycin A disrupts protein kinase mediated cellular events and in this paper we detail the synthesis of such a compound.



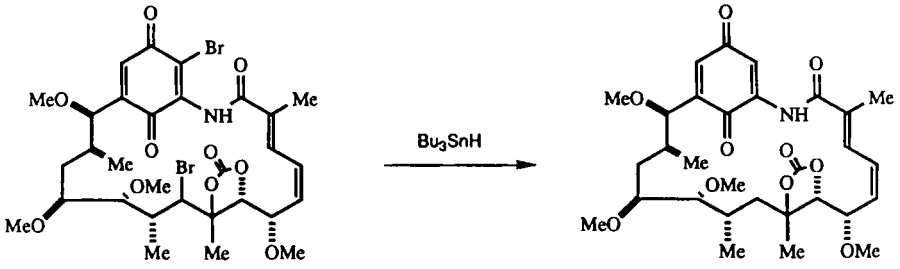
1a

RESULTS AND DISCUSSION

Though there is a wide array of functionality present in the macrocyclic backbone of herbimycin A (1a), there are few options for the specific or nonspecific incorporation of tritium. There are, however, two unsubstituted positions, C_{17} and C_{19}, where chemistry (11,12) has been performed and thus serve as potential sites for the incorporation of tritium. Also, Uehara *et al.* (9) have speculated that herbimycin A irreversibly inhibits pp60*v-src* through a covalent interaction between the benzoquinone group and a sulfhydryl moiety in the

enzyme. Specifically labelling of either the C_{17} or C_{19} positions would aid in sorting out the specifics of this proposed interaction.

During the course of semi-synthetic studies on herbimycin A, it was found that a C_9/C_{19}-dibromide derivative (2) could be efficiently reduced (Scheme 1) with tri-*n*-butyltin hydride to 3 (12). Based on this result, our initial plan was to specifically incorporate tritium at the C_{19}-position of herbimycin A *via* reduction of readily available (12) 19-bromoherbimycin A (4) with tri-*n*-butyltin [^{3}H]-hydride. However, repeated attempts to carry out this transformation, utilizing tri-*n*-butyltin hydride, resulted in complex reaction mixtures, which contained little or no herbimycin A. We are unable to account for the difference in reactivity between 2 and 4; presumably the unconjugated olefin and/or carbamate functionalities present in 4 are responsible for these alternative reaction pathways.

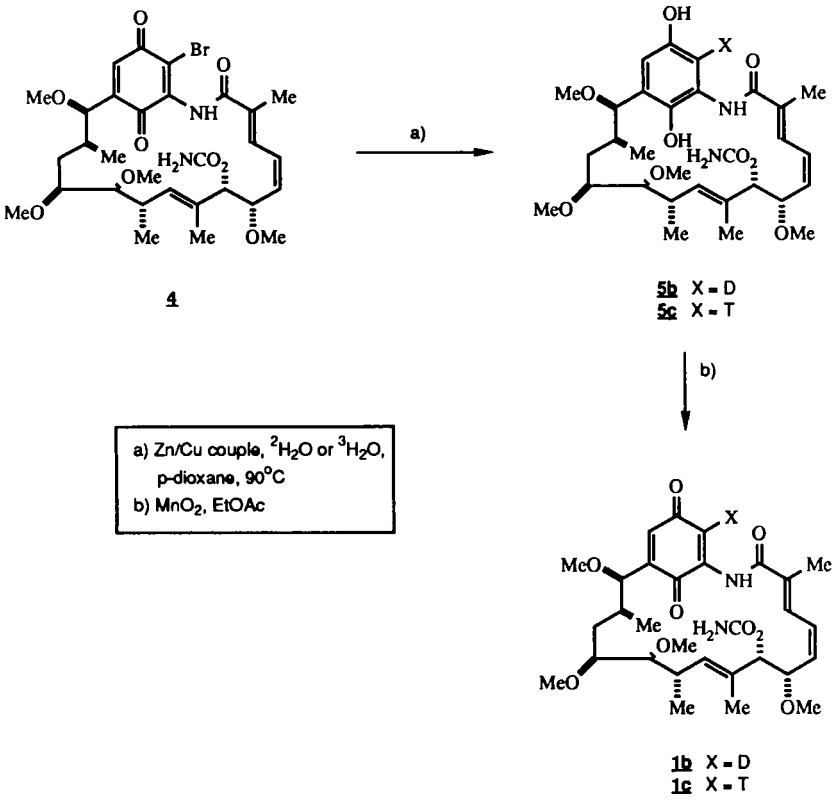


2 3

Scheme 1

Because 4 remained an attractive intermediate for the incorporation of tritium into herbimycin A, we chose to investigate alternative methods for achieving this transformation. The high degree of functionality/sensitivity present in herbimycin A eliminates a number of common reductive conditions one might employ. Stephenson *et al.* (13) have reported a method for the reduction of alkyl, vinyl and aryl halides possessing a range of sensitive functional groups, including carbonyls and conjugated olefins. Specifically, treatment of the halide substrate with zinc-copper couple and deuterium oxide in an ether solvent afforded good to excellent yields of the corresponding deuterated substrate.

When 4 (Scheme 2) was allowed to react with excess zinc-copper couple in the presence of deuterium oxide at 90°C for 3 hours, hydroquinone 5b was produced. Reoxidation of the hydroquinone moiety 5b with manganese dioxide in ethyl acetate provided 19-^{2}H-herbimycin A (1b) of > 95% isotopic purity, as judged by ^{1}H



Scheme 2. Synthesis of [19-^{3}H] Herbimycin A

NMR spectroscopy. The overall yield for this two-step process was 78%. This reaction sequence was repeated utilizing tritiated water to afford 19-^{3}H-herbimycin A (1c) of ≥ 98% radiochemical purity (determined by HPLC) following purification by preparative HPLC. Purified 1c had a specific activity of 4.2 Ci/mmol, as determined by UV spectrophotometry.

EXPERIMENTAL SECTION

Commercial grade reagents were utilized without further purification. Melting points were taken on a Thomas-Hoover melting point apparatus and are

uncorrected. NMR spectra of deuteriochloroform solutions (solvent utilized as an internal standard and deuterium lock) were recorded on a Varian XL-300 spectrometer.

Preparative and analytical high performance liquid chromatography was carried out on a Varian 5040 instrument with a Valco injector (50 μL loop). Scintillation counting was carried out with a Beckman LS 3801 liquid scintillation counter using Fisher Scintiverse LC scintillation cocktail.

[19-^{2}H] Herbimycin A (1b). Deuterated water (99.8 atom %, 0.2 mL) and zinc-copper couple (400 mg, 3.06 mmol) were added to a solution of **4** (100 mg, 0.15 mmol) in anhydrous p-dioxane (1 mL) under a nitrogen atmosphere. The resulting mixture was heated at 90°C for 3 h, whereupon TLC (silica gel, acetone:toluene-40:60), of an aliquot treated for 15 min with an excess of manganese dioxide in ethyl acetate, indicated complete disappearance of **4** (12) (R_f = 0.45). The reaction mixture was taken up in ethyl acetate (100 mL) and filtered through a layer of Celite. The filtrate was washed with water, followed by brine and then dried (Na_2SO_4). Following filtration, the filtrate was concentrated *in vacuo*, redissolved in ethyl acetate (10 mL), treated with manganese dioxide (60 mg, 0.69 mmol) and stirred for 30 min. TLC (silica gel, acetone:toluene-40:60) showed a single component that co-migrated (R_f = 0.59) with authentic herbimycin A (**1a**) indicating that oxidation was complete. The mixture was diluted into ethyl acetate, washed with water, brine, dried (Na_2SO_4) and concentrated *in vacuo* to afford an orange solid. Flash chromatography (silica gel, acetone:toluene-30:70) afforded 69 mg (78%) of **1b** as a yellow solid; m.p. 230°C. ^{1}H NMR analysis revealed ≥95% incorporation of deuterium at C-19. ^{1}H NMR δ 8.74 (br s, 1H, NH), 6.93 (d, 1H, C_3-H), 6.58 (s, 1H, C_{17}-H), 6.45 (dd, 1H, C_4-H), 5.81 (dd, 1H, C_5-H), 5.60 (br s, 1H, C_7-H), 5.45 (br s, 1H, C_9-H), 4.72 (br s, 2H, $CONH_2$), 4.50-4.42 (m, 2H, C_6-H, C_{15}-H), 3.54-3.42 (m, 4H, OMe, C_{11}-H), 3.34-3.24 (m, 10H, 3(OMe), C_{12}-H), 2.60 (br s, 1H, C_{10}-H), 1.98 (s, 3H, C_2-Me), 1.76-1.44 (m, 5H, C_{13}-H, C_{14}-H, C_8-Me), 1.14 (d, 3H, C_{10}-Me), 0.76 (d, 3H, C_{14}-Me). In the ^{1}H NMR of herbimycin A, the C_{19} proton appears at δ 7.34 (d, J = 2.4 Hz).

[19-^{3}H] Herbimycin A (1c). Tritiated water (50 Ci, 2.5 mmol) and zinc-copper couple (100 mg, 0.77 mmol) were added to a solution of **4** (25 mg, 0.038 mmol) in

anhydrous p-dioxane (250 μL) under an argon atmosphere. The resulting mixture was heated at 80°C for 3 h, whereupon TLC (Whatman silica LK6F, acetone:toluene-40:60) of an aliquot, treated for 15 min with an excess of manganese dioxide in ethyl acetate, indicated complete disappearance of starting material (R_f = 0.45). The reaction mixture was taken up in ethyl acetate (10 mL) and filtered through a layer of Celite. The filtrate was washed with water, followed by brine and then dried (Na_2SO_4). Following filtration, the filtrate was treated with manganese dioxide (60 mg, 0.69 mmol) and stirred for 30 min. TLC (silica, acetone:toluene-40:60) showed a single component that co-migrated (R_f = 0.59) with authentic herbimycin A (1a) indicating that oxidation was complete. The mixture was filtered through Celite and solvent was removed *in vacuo* to afford 1140 mCi of unpurified product. A 313 mCi portion of this material was purified by preparative HPLC (Zorbax CN column, 2-propanol:hexane-20:80, 1 mL/min, RT = 10.4 min) to afford 18 mCi of 1c (>98% radiochemical purity) at a specific activity of 4.2 Ci/mmol as determined by UV spectrophotometry (λ max. = 271 nm, ε = 2.46 x 10^4 in 95% ethanol).

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