# SYNTHESIS OF TRITIUM LABELLED VERRUCAROL AND VERRUCARIN A

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## SUMMARY

Two tritium labelled trichothecenes, verrucarol (**2**) and verrucarin A (**5**) were synthesized with high specific activity. Oxidation of verrucarol (**1**) to the 15-dehydroverrucarol (**3**) followed by sodium borotritide reduction yielded [15-$^3$H]-verrucarol (**2**). Reduction of 16-mesyloxyverrucarin A (**7**) in a special buffer-organic solvent system with a phase transfer catalyst, produced [16-$^3$H]-verrucarin A (**5**). Final purification of the radioactive products was achieved by preparative thin layer chromatography. Their structures were assigned based on the analytical data of the corresponding nonradiolabelled compounds obtained from the nonradioactive preparations.

Key words: Verrucarol, Verrucarin A, Trichothecenes, Borohydride Reduction, Tritium.

## INTRODUCTION

Trichothecenes are a group of fungal secondary metabolites with unique biological activities.(1-5) These mycotoxins are produced mainly by fungi belonging to the *Fusarium*, *Trichothecium*, *Myrothecium*, and *Stachybotrys*.(4-6) The trichothecenes in general can be divided into two major groups: the simple trichothecenes, e.g., verrucarol, T-2 toxin, and nivalenol, and the macrocyclic trichothecenes, e.g., roridins and verrucarins.(4,5) Naturally occurring trichothecenes are distributed worldwide and are implicated in a diverse variety of toxicoses in man and animals.(4-6,7) Several investigators showed that the toxicity of trichothecenes require the presence of 12,13-epoxide function.(4,5) Examination of various macrocyclic trichothecenes indicates that the macrocyclic moiety, which is formed by the connection of C4 and C15 hydroxyl groups of verrucarol (**1**), has a great influence on biological activity.(4,5,7) Some members of the latter group are among the most

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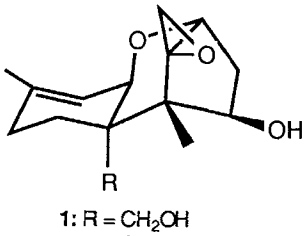
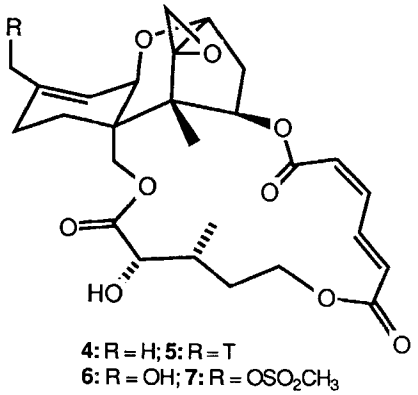
toxic trichothecenes.(7) Recently, it was reported that these toxins may pose serious indoor pollution problems in urban areas.(8) Although there are reasonable amounts of data available on the metabolism(9) and pharmacokinetics(10) of T-2 toxin, there are almost no data reported on the metabolic fate of verrucarol or the macrocyclic trichothecenes. Unknown rearrangements may occur during the *in vivo* detoxification process of verrucarol and macrocyclic trichothecenes. Hydrolysis of the ester bonds connected to carbons 4 and/or 15 of verrucarin A (**4**) will yield verrucarol or monoester, respectively. There is, however, also the possibility of obtaining other presently unknown metabolites. Synthesis of radiolabelled verrucarol and verrucarin A will enable their use in toxicology, metabolism and pharmacokinetics studies. Due to high susceptibility of experimental animals to trichothecenes,(4-6) the labelled toxins with a high radio specific activity are desired for *in vivo* studies. We report here a simple method for the synthesis of tritiated verrucarol (**2**) and verrucarin A (**5**).

## DISCUSSION

In the study of the synthesis of tritium labelled **2** and **5** for the toxicologic studies, special attention was paid to the selective tritium labelling at the positions 15 and 16 of verrucarol (**1**), and verrucarin A (**4**), respectively. These positions were chosen not only because they offer the possibility of introducing the tritium atom, but also because they should not suffer from serious problems due to the loss of labelling protons during the metabolic studies in animals.




**1:** R = $CH_2OH$
**2:** R = CHTOH
**3:** R = CHO




**4:** R = H; **5:** R = T
**6:** R = OH; **7:** R = $OSO_2CH_3$

We considered the oxidation of a primary alcohol of verrucarol to the aldehyde **3**, and subsequent reduction of this aldehyde with $^3$H-sodium borohydride to be the most efficient and rapid

way of achieving a radiolabelled verrucarol. Oxidation of **1** with tris(triphenylphosphine)ruthenium chloride reagent afforded in one step the desired aldehyde **3** despite the presence of the second hydroxyl group in the molecule.(11)

The attempt to synthesize [16-$^3$H]-verrucarin A (**5**) by the reduction of 16-mesyloxyverrucarin A (**7**) with tritium labelled sodium borohydride under phase transfer conditions described in the literature(12) gave unsatisfactory results. The reported conditions(12) were modified in such a way that the rate of 16-mesyloxyverrucarin A reduction would be significantly greater than the decomposition of the sodium borohydride by the phase transfer catalyst. Addition of sodium borohydride to the solvent mixture in the presence of sodium phosphate buffer followed by 16-mesyloxyverrucarin A resulted in the production of the desired tritiated verrucarin A, which was purified by preparative TLC to give radiolabelled compound **5**.

The synthesized [15-$^3$H]-verrucarol (**2**) and [16-$^3$H]-verrucarin A (**5**) were obtained with a very high specific activity and a purity of greater than 95%. Such a significant degree of labelling will enable the tracing of these toxins or their metabolites in animal studies. It also will help in the elucidation of their tissue metabolism and in the process of tracing their metabolites during their isolation from the biological fluids.

## EXPERIMENTAL

Verrucarol (**1**) was prepared by hydrolysis of a crude extract of a culture of Myrothecium verrucaria ATCC 24571 as described previously.(13) Verrucarin A was isolated from a culture of Myrothecium verrucaria as described earlier.(14) 16-Hydroxyverrucarin A (**6**) was synthesized from verrucarin A (**4**) according to the described procedure.(15) Tritiated $NaBH_4$ (11.4 Ci/mol) was purchased from Amersham International. All the following reagents: Tris-(triphenylphosphine)ruthenium(II)chloride, trimethylamine-N-oxide dihydrate, hexadecyltributylphosphonium bromide and methanesulfonyl chloride were purchased from Aldrich Chemical Company, Inc.

Analytical and preparative thin layer chromatography (TLC) were performed on precoated TLC plates of silica gel (Merck-Kiesel gel 60 F254). Visualization was done by spraying the plates with vanillin spray (40 g/l vanillin) in ethanol-sulfuric acid (1:4). Solvent systems employed for analytical TLC were: system I ($CH_3OH$; $CHCl_3$; 5:95) and system II (ethyl acetate: toluene: acetic acid; 6:3:1). The chromatotron (Harrison Research Laboratories) Model 7942 was used for preparatiave TLC. The circular glass plates were coated with silica gel (thickness of 1, 2 or 4 mm) according to the

instructions in the product manual. Filtration chromatography was done with flash grade silica gel (230-240 mesh, E. Merck).

For localization of radioactivity on the plate a Bioscan II radioisotopic scanner, Bioscan Inc., Washington, D.C. 20007 was used. A Mark III, scintillation counter, Searle, Analytic Des Plaines, IL 60018, was used for liquid scintillation measurements. Nuclear magnetic resonance spectra were obtained on an IBM SY-200 spectrometer in deuterated chloroform with tetramethylsilane as an internal standard. Infrared (IR) spectra were determined in chloroform on a Perkin-Elmer Model 183 spectrophotometer.

**15-Dehydroverrucarol (3)**

To a solution of verrucarol (**1**) (266 mg) in 15 ml of dry $CH_2Cl_2$ was added anhydrous trimethylamine-N-oxide (150 mg) and $RuCl_2(Ph_3P)_3$ (959 mg). The mixture was allowed to stir overnight at room temperature. According to the TLC analysis (system I), some unreacted verrucarol remained in the reaction mixture. An additional amount of $RuCl_2(Ph_3P)_3$ (480 mg) was added at once, and the reaction was stirred for 12 hours. The solvent was evaporated, and the crude product was purified by chromatography on a 5 cm x 20 cm flash silica gel column using 1:1 ethyl acetate:hexane as eluent to afford 135 mg of slightly contaminated 15-dehydroverrucarol (**2**). Elution of the column with 3:97 methanol:ethyl acetate afforded 55 mg of recovered verrucarol (**1**). The 15-dehydroverrucarol was further purified by TLC by elution with 2:1 mixture of hexane-ethyl acetate on 2 mm preparative silica gel plate (chromatotron) to afford 102 mg of **3**, m.p. 130°C (crystallized from acetone-hexane) (Lit.[16] 138-140°C). $^1H$ NMR ($CDCl_3$) $\delta$ 0.95 (3 H, s, H-14), 1.65 (3 H, s, H-16), 1.9-2.2 (5 H, m, H-3$\beta$, H-7, H-8), 2.70 (1 H, dd, J=7.4 and 17.6 Hz, H-3$\alpha$), 2.83 and 3.10 (2 H, AB, J=3.8 Hz, H-13), 3.83 (1 H, d, J=4.8 Hz, H-2), 4.25 (1 H, d, J=5.2 Hz, H-11), 4.34 (1 H, dd, J=4.4 and 7.4 Hz, H-4), 5.49 (1 H, d, J=5.2 Hz, H-10), and 9.62 (1 H, s, H-15).

**15-[3H]-Verrucarol (2)**

Tritiated sodium borohydride (200 mCi, specific activity 11.5 Ci/mmol) was added to the solution of the aldehyde **3** (53 mg) in 10 ml of dry tetrahydrofuran. After being stirred for 5 hours at 25° C, non-radioactive sodium borohydride (20 mg) was added and the reaction stirred overnight. Non-radioactive verrucarol (20 mg) was added in order to aid in the recovery of tritiated verrucarol. The reaction mixture was diluted with 25 ml of ethyl acetate and washed twice with 2 x 15 ml of saturated solution of ammonium chloride (10 ml), and the aqueous layer was extracted with additional ethyl acetate (25 ml). The combined organic layers were dried ($MgSO_4$), filtered and evaporated. The crude residue was purified by TLC on two 0.5 mm preparative plates (three elutions with 8:1:1

$CHCl_3$, MeOH, EtOAc). Visualization of verrucarol was done by covering the TLC plate with a clear glass plate and spraying only the edges of the plate with the vanillin spray, followed by heating with hot air. The [$^3$H]-verrucarol band, which was located by cospotting with a verrucarol standard, was removed and verrucarol was eluted with a mixture of acetone: methanol: ethyl acetate (1:1:5). The eluent was concentrated, and the residue rechromtographed on one plate as described above. The 15-[$^3$H]verrucarol (2) was obtained in 32% yield (based on the recovered radioactivity) with a specific activity of 266 mCi/mmol. Radiochromatographic homogenity of 2 was determined by the Bioscan radioisotopic scanner.

**16-Mesyloxyverrucarin A (7)**

To a solution of alcohol 6 (259 mg) and 1 ml of freshly distilled triethylamine in dichloromethane (5 ml) at 0° C, was added 57 mg of mesyl chloride, and the solution stood at 0° C for 4 hours. Ten ml of ethyl acetate was added, and the organic layer was washed with 2 x10 ml of brine, dried ($MgSO_4$), and concentrated *in vacuo*. The residue was purified on the chromatotron (1 mm silica gel plate) using 10-50% of ethyl acetate in hexane as an eluting solvent to obtain 130 mg of 7, mp > 300° C.$^1$H NMR ($CDCl_3$): δ 0.84 (3 H, s, H-14), 1.02 (3 H, d, J=6 Hz, H-12'), 1.64 (3 H, s, H-16), 2.47 (1 H, dd, J=8 and 16 Hz, H-3α), 2.80 and 3.10 (2 H, AB, J=3.8 Hz, H-13), 3.03 (3 H, s, mesyl), 3.62 (1 H, d, J=5 Hz, H-11), 3.86 (1 H, d, J=5 Hz, H-2), 4.23 and 4.72 (2 H, AB, J=12 Hz, H-15), 4.64 (2 H, s, H-16), 5.80 (1 H, dd, J=4 and 8 Hz, H-4), 6.08 (1 H, dd, J=5 Hz, H-10), 6.04 (1 H, d, J=15.5 Hz, H-7'), 6.13 (1 H, d, J=11.4 Hz, H-10'), 6.67 (1 H, dd, J=11.4 and 11.4 Hz, H-9'), and 8.02 (1 H, dd, J=11.4 and 15.5 Hz, H-8). $^{13}$C NMR: δ 7.3 (C-14), 10.1 (C-12'), 19.5 (C-7), 23.2 (C-8), 32.3 (C-4'), 33.2 (C-3'), 34.8 (C-3), 38.0 ($CH_3SO_2$), 44.5 (C-6), 47.7 (C-13), 49.5 (C-5), 61.1 (C-5'), 63.0 (C-15), 64.9 (C-12), 66.1 (C-11), 71.5 (C-16), 74.1 (C-2'), 75.3 (C-4), 79.1 (C-2), 123.5 (C-10), 125.5 (C-11'), 127.6 (C-8'), 137.1 (C-9), 138.7 (C-10'), 139.0 (C-9'), 165.2 (C-6'), 166.1 (C-11'), and 174.7 (C-1'); high resolution mass spectrum (positive CI, methane gas reagent) calcd for $C_{28}H_{36}O_{11}S$+H: 581.6605, found 581.6588.

**Reduction of 16-Mesyloxyverrucarin A with Sodium Borohydride**

To the stirred mixture of 7 (20 mg) in 2 ml of dichloromethane and 0.4 ml of sodium phosphate buffer, (1M, pH 9.5) and 10 mg of hexadecyltributylphosphonium bromide was added 4 mg of sodium borohydride. After 20 minutes, an additional 10 mg of sodium borohydride was added, and the reaction was stirred at room temperature for 12 hours., followed by the addition of 1 ml of

saturated solution of ammonium chloride. The mixture was extracted with 3 x 2 ml of ethyl acetate. The organic layer was washed with brine, dried, filtered and evaporated. The crude product was purified by preparative TLC on a 0.25 mm silica gel plate using system II as eluent. The verrucarin A band, which was located by cospotting with a appropriate standard, was removed, and verrucarin A was eluted with a mixture of acetone: methanol: ethyl acetate 1:1:5. The eluents were concentrated and rechromatographed in a similar way as described above to obtain 4 mg of verrucarin A, which was shown by IR, TLC and $^{1}H$ NMR to be identical to an authentic sample.

### Reduction of 7 with [$^{3}H$]Sodium Borohydride

To the vigorously stirred reaction mixture of ten mg of 16-mesyloxyverrucarin A (7), in a mixture of 2 ml of dichloromethane and 0.4 ml of sodium phosphate buffer (1 M, pH 9.5) and 10 mg of hexadecyltributylphosphonium bromide was added $^{3}H$-sodium borohydride (200 mCi, specific activity 11.4 Ci/mmol) The mixture was stirred for 60 minutes and an additional 20 mg sodium borohydride (cold) was added. After 3 hours, nonradiolabelled verrucarin A (20 mg) was added to the reaction mixture followed by 2 ml of a saturated solution of ammonium chloride. The reaction mixture was extracted with 2 x 25 ml of ethyl acetate. The organic layer was separated and washed with brine, dried ($MgSO_4$), filtered and evaporated. After two thin layer chromatographic purifications, at the conditions described above, the 16-[$^{3}H$]-verrucarin A was obtained in 4% yield (based on the recovered radioactivity. Specific activity of the product **5** was 130 mCi/mmol. Radiochromatographic homogenicity of **5** was determined by the Bioscan radioisotopic scanner.

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