PREPARATION OF TRITIUM-LABELLED α-DIHYDROGRAYANOTOXIN II.

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α-Dihydrograyanotoxin II has been labelled with tritium at the C-10 and C-19 positions by catalytic hydrogenation of grayanotoxin II. Pd-catalyzed reduction in tetrahydrofuran produced the α-form exclusively. The compound was obtained with specific activity 1.21 Ci/mmole and with 99% radio-chemical purity.

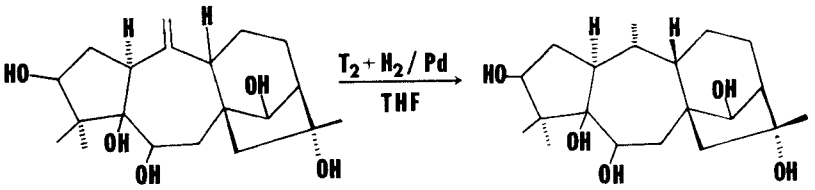
INTRODUCTION

Grayanotoxins I, II, and III are toxic principles which are contained in the leaves of Ericacae species. The chemistry (1, 2, 3), toxicology (4), and pharmacology (5) of grayanotoxins have been studied by many researchers. Seyama and Narahashi (6) reported recently that α-dihydrograyanotoxin II (α-2HG-II), a derivative of grayanotoxin II (G-II), increased the resting sodium permeability of the squid axon membranes, whereas β-2HG-II had little or no effect.

In order to study the binding activity with nerve preparations, the tritium-labelled α-2HG-II was needed with high specific activity.

DISCUSSION

For non-radioactive synthesis, Iwasa et al. (7) reported that the catalytic hydrogenation of G-II proceeded easily over platinum oxide (Adams) in acetic acid,



to form exclusively α-2HG-II. To minimize exchange of tritium with the solvent as reported by Eidenoff et al. (8) and Nunez (9), the conditions were changed. When dioxane was used as a solvent over palladium chloride, the absorption of hydrogen was slow. Better results were obtained by using tetrahydrofuran over palladium chloride.

The optimum amount of the catalyst was determined by measuring the reaction time to get saturation of hydrogenation. These were 70, 10, and 10 minutes for 40, 60, and 100 mg of 5% palladium on charcoal, respectively against 100 mg of G-II. Therefore, 50 mg of the catalyst was used in hot runs with 100 mg of G-II.

α-2HG-II was purified by recrystallization from methanol:ethyl acetate (1:1). G-II and β-2HG-II, which were potential contaminants, were not precipitated from this solvent system. Identification of α-2HG-II was accomplished, by comparing the melting point (of the unlabelled compound only), Rf-value and stains on silica gel thin-layer chromatogram with an authentic standard.

The melting points of standard chemicals were 198°C, 260-261°C, and 217°C for G-II, α-2HG-II, and β-2HG-II. The Rf values on the tlc plates developed with ethyl acetate:methanol (95:5) did not show clear separation of G-II (Rf = 0.25), α-2HG-II (Rf = 0.33) and β-2HG-II (Rf = 0.29). This solvent system was the best among numerous combinations of n-heptane, ethyl acetate, methanol and acetic acid. However, the three compounds could be distinguished because the stains visualized with 10% sulfuric acid or vanillin - sulfuric acid were different for each compound as shown in Table I.

Table I

Color after spraying tlc plate and heating for 3 minutes at 80 - 90°C.

	Time after heating	G-II	α-2HG-II	β-2HG-II
10% H_2SO_4	zero	tan	pink	light purple
	one day	pink	gray	gray
Vanillin $-H_2SO_4$*	zero	green	blue	purple
	one day	green	dark green	blue

*Vanillin 3% and H_2SO_4 0.5% in ethanol.

EXPERIMENTAL

The hydrogenation was carried out in a simple apparatus enabling a more convenient handling of small volumes of gases.

Chemicals

G-II and authentic chemicals, α- and β-2HG-II, were kindly donated by Dr. Junkichi Iwasa of Okayama Unviersity, Japan. Tritium gas, carrier-free, was purchased from New England Nuclear, Boston, Massachusetts.

Procedures

A solution of 100 mg (0.284 mmoles) of G-II in 4 ml of tetrahydrofuran with 50 mg of 5% palladium chloride on charcoal as a catalyst was stirred by means of a magnetic stirrer under hydrogen containing 2 Ci of tritium at one atom and room temperature. Gas uptake began immediately and was estimated graphically to be complete after 25 minutes, when about 7 ml (theoretical amount) of the gas was consumed. The reaction was then discontinued. The catalyst was removed by filtration, the solvent was evaporated and the residue was treated four times with 10 ml of methanol to remove labile tritium.

The residue containing some impurities (Rf. 0.17) was recrystallized from methanol-ethyl acetate (0.7 ml + 0.7 ml). Combining the first, second and third

crystal crops, 80.4 mg and 256 mCi (12.8% for 2 Ci.) of tritium-labelled α-2HG-II was obtained. The specific activity was 1.21 Ci/mmole. Silica gel thin-layer chromatographs were developed with ethyl acetate-methanol as described above. After making a readiochromatogram and autoradiogram (both showed a single spot), 99% of radiochemical purity was established by liquid scintillation counting of its radioactivity of the silica gel. All activity measurements were carried out on a Tri-Carb Model 3375 (Packard) scintillation counter.

Activity balance of the experiments:

Tritium used: 2 Ci

Activity in CuO trap = 0.07 mCi, in the reaction mixture = 519 mCi, in the distillate = 212 mCi, in pure α-2HG-II = 256 mCi, and in mother liquid and $PdCl_2$= 51 mCi.

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