RADIOLABELLING OF THE HISTAMINE LIBERATING SESQUITERPENE LACTONE, THAPSIGARGIN

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SUMMARY

Thapsigargin *(1)* selectively labelled in the butyrate moiety of the molecule was isolated after hydridocarbonyltris(triphenylphosphine)rhodium(I) catalyzed reduction of the product *(5)* obtained by reacting debutanoylthapsigargin *(2)* with 3-butenoic anhydride. The use of tritium gas as a reducing agent afforded labelled thapsigargin with a specific activity of 78 Ci/mmol.

Key Words: Thapsigargin, Histamine release, Deuterium, Tritium, Hydridocarbonyltris(triphenylphosphine)rhodium(I).

INTRODUCTION

Although the release of histamine from mast cells and leucocytes plays a major role in the development of acute allergic reactions and generalized inflammatory responses only a fragmentary knowledge of the events involved in the secretion is available.[1-5] Peritoneal rat mast cells incubated with histamine secratogogues as concanavalin A, compound 48/80, or ionophore A 23187 are often used as test systems for the investigation of histamine secretion.[2-4] Thapsigargin *(1)* a sesquiterpene lactone isolated from the umbelliferous plant, *Thapsia garganica*,[6-7] has been recognized as a potent non-cytotoxic histamine liberator in this test system.[8] A number of investigations have revealed that *(1)* is capable of inducing histamine release

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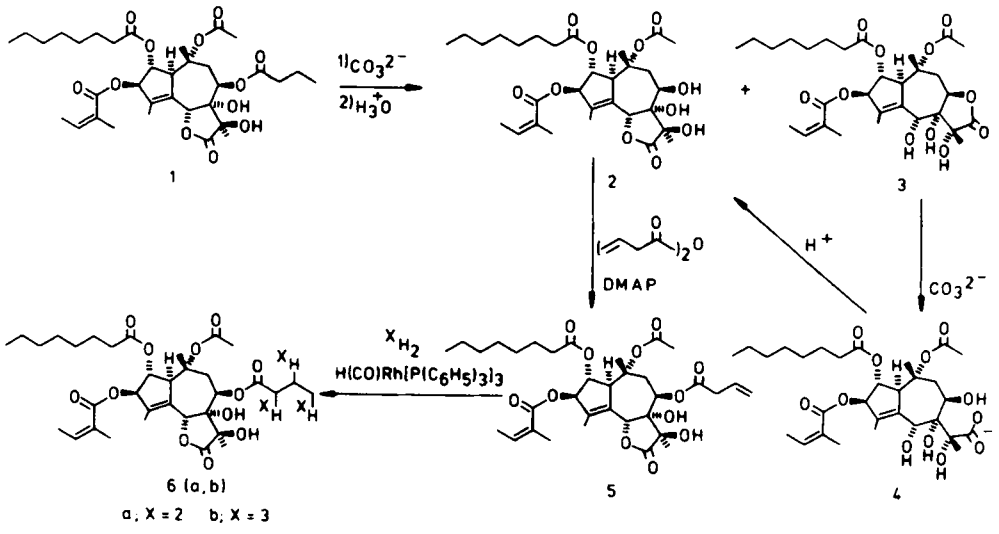
by a mechanism different from those of other non-cytotoxic secretagogues.[9,10] The procedure for tritiumlabelling of *(1)* described belove has been developed in preparation for studies on the mechanism for histamine release, with special emphasis on characterization of a potential receptor for the action of thapsigargin.

RESULTS AND DISCUSSION

The strategy for labelling of *(1)* was to introduce a double bond, which could be saturated by treatment with tritium or deuterium gas. The molecule contains in advance a smoothly hydrogenated trisubstituted double bond and a tetrasubstituted double bond. The ability of hydridocarbonyltris(triphenylphosphine)rhodium(I) to selectively catalyze reduction of terminal double bonds[11] and the formation of *(2)*[6] by hydrolysis of *(1)* made the approach outlined in Scheme 1 attractive.

Sodium carbonate catalyzed saponification of thapsigargin gave a mixture of *(1)*, *(2)*, and *(3)*. Conversion of *(3)* into a mixture of *(2)* and *(3)*, presumably *via* the carboxylate *(4)*, by dissolving *(3)* in 0.25 M sodium carbonate and acidification of the obtained solution, increased the overall yield of *(2)*. Compound *(2)* contains secondary and tertiary hydroxy groups. Although both types of alcohols react with anhydrides in the presence of 4-dimethylaminopyridine (DMAP)[12] the greater reactivity of the secondary hydroxy group enabled a selective acylation. Hydridocarbonyltris(triphenylphosphine)rhodium(I) catalyzed reduction of *(5)* using deuterium gas as reagent gave *(6a)*. Investigation of the reaction



Scheme 1

mixture by HPLC did not reveal the presence of *(1)* reduced at the trisubstituted double bond. Mass spectrometric and ^1H NMR spectrometric investigations of *(6a)* evidenced that all deuterium atoms were attached to the butyrate moiety. The successful incorporation of deuterium encouraged to a tritiation of *(5)* using tritium gas and the same catalyst. The specific activity of the obtained *(6b)* was determined to 78 Ci/mmol.

EXPERIMENTAL

HPLC was performed over Lichrosorb RP 18 in one of three systems: A: a Knauer Prepacked column, dimensions 8 x 250 mm, particle size 7 µm, eluent: MeOH-0.5% aqueous AcOH (5:1), flow 2.5 ml/min; B: same column as in A, eluent: MeOH-0.5% aqueous AcOH (9:1), flow 2.5 ml/min; C: a Knauer Prepacked column, dimensions 16 x 250 mm, particle size 10 µm, same eluent as in A, flow 9.9 ml/min. In all cases a combined

UV/RI detector (Knauer Dualdetektor model 61.00) was used. TLC was performed on Kiselgel 60 F 254 (Merck 5549) 5 x 7.5 cm, eluent: tolueneethyl acetate (7:3). Radioactive spots were visualized using AGFA-gevaert osray M3-DW film. Mass spectra were obtained using the chemical ionisation technique on a VG 7070 instrument equipped with a dual EI/CI ion source. Reagent gas isobutane, ion source temperature 220^{0}C. The NMR spectra were obtained on a Bruker HX-270 S spectrometer. The radioactivity was determined in a Packard liquid scintillation spectrometer, model 2405, using Instagel, Packard as a scintillation fluid. The counting efficiency was determined to 30% by counting a tritium labelled toluene standard.

Debutanoylthapsigargin *(2)* from thapsigargin. Thapsigargin *(1)*[6] (120 mg; 0.18 mmol) was dissolved in a 0.25 M solution of Na_2CO_3 (2 ml) in MeOH-H_2O (1:1), and the solution was stirred at room temperature for 15 min. The mixture was made acidic with 4 M hydrochloric acid, added H_2O (10 ml), and extracted with ether (3 x 10 ml). The combined organic phases were dried ($MgSO_4$) and evaporated *in vacuo* to give a gum (115 mg). HPLC (system C) gave pure *(3)*[6] (19 mg; 18%; retention time 8.6 min.), *(2)*[6] (23 mg; 22%; retention time 10 min.), and *(1)*[6] (50 mg; 42%; retention time 14 min.).

Debutanoylthapsigargin *(2)* from *(3)*. Compound *(3)* (46 mg) was dissolved in 0.25 M solution of Na_2CO_3 (2.5 ml) in MeOH-H_2O (1:1). As soon as a clear solution was obtained 4 M hydrochloric acid (1 ml) and H_2O (9 ml) was added and the mixture extracted with ether (2 x 20 ml). The combined organic phases were dried ($MgSO_4$) and evaporated *in vacuo* to give a gum from which *(3)* (19 mg; 41%) and *(2)* (22 mg; 48%) was isolated by HPLC (system C).

Acylation of *(2)*. A solution of *(2)* (29 mg; 50 µmol), 3-butenoic anhydride (100 µl, 1 mmol)[13], and 4-dimethylaminopyridine (20 mg; 0.18 mmol) in CH_2Cl_2 (2 ml) was left for 1 h. The mixture was washed with 0.4 N hydrochloric acid, dried ($MgSO_4$) and evaporated to give an oil, from which *(5)* (24 mg; 80%; retention time 17.8 min.) was isolated by HPLC (system A).

In the ^1H NMR spectrum (C^2HCl_3) the signals originating in the 3-butenoate moiety were found at δ 3.05 (complex d, 2H), 5.1-5.3 (m, 2H), 5.8-6.2 (m, 1H). The part of the spectrum due to the other protons of the molecule was superimposable with this due to the analogous protons in *(1)*. Mass spectrum [m/z values (intensity)]: 649 (1), 589 (3), 549 (5), 445 (6), 503 (40), 489 (8), 463 (100), 357 (3), 359 (55), 345 (2), 403 (80), 271 (9), 259 (13), 171 (3).

Deuteration of *(5)* to give *(6a)*. A solution of *(5)* (6 mg; 9 µmol) and hydridocarbonyltris(triphenylphosphine)rhodium(I) (5 mg) in toluene (5 ml) was stirred in a deuterium atmosphere for 1.5 h. The solution was evaporated *in vacuo* and the residue purified by HPLC (system A) to give *(6a)* (3 mg; 50%) having the same retention time as *(1)* (20.6 min.). The ^1H NMR was superimposable with the spectrum of *(1)*[6] except for the signals originating in the butyrate moiety, which are overlapped by the signals of the octanoate moiety. An estimate based on the integration curve indicates an average of 1 deuterium attached to each of the three tetrahedral carbons. Mass spectrometry [m/z values (intensity)]: 656 (0.3), 655 (1.0), 654 (1.2), 595 (1.2), 594 (2.1), 593 (2.2), 503 (42), 463 (100), 403 (83), 359 (75); indicating an average of 1.9 deuterium/molecule.

The very low intensity of these peaks compared to the intensity of the corresponding peaks in *(1)* indicates that some unexpected secondary isotope effects take place. This could explain the different incorporation of deuterium according to the NMR and mass spectra.

Tritiation of *(5)* to give *(6b)*. A solution of *(5)* (10 mg; 17 μmol) and hydridocarbonyltris(triphenylphosphine)rhodium(I) (10 mg) in toluene (2 ml) was stirred for 2 h in an atmosphere of tritium gas. An uptake of 0.5 ml (21 μmol) was noted. Labile tritium was removed *in vacuo* using MeOH-0.5% aqueous AcOH (5:1). (Because of missing access to equipment suitable for handling radioactive gases the step involving tritium gas was performed by New England Nuclear, Massachusetts, USA. The working up of the crude reaction mixture and determination of specific activity and radiochemical purity all was done by the author). From the reaction mixture *(6b)* (6 mg; 60%) was isolated by HPLC (system B). The product has the same retention time (9.0 min.) as *(1)* and a specific activity of 78 Ci/mmol. The radiochemical purity of a diluted methanolic solution, which had been stored for a month at -20^{0}C, was by TLC verified to be greater than 96%.

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