SYNTHESIS OF A TRITIUM LABELLED PYRROLIZIDINE ALKALOID-DISENECIOYL RETRONECINE

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SUMMARY

Retronecine (7β-hydroxy-1-hydoxymethyl-1,2-dehydro-8α-pyrrolizidine) (II) which is prepared from the hydrolysis of monocrotaline (I) was oxidized with manganese dioxide in the presence of potassium cyanide to methyl 1,2-dehydro-7β-hydroxy-8α-pyrrolizidine-1-carboxylate (III). Retronecine (II') was ^{3}H labelled at the 1 methyl proton by the reduction of III with $LiAl^{3}H_{4}$. The semi-synthetic pyrrolizidine alkaloid disenecioyl retronecine (Di-3,3-dimethylacrylic acid ester of retronecine), having a high specific activity, was prepared by the acylation of ^{3}H retronecine (II') with senecioyl chloride (3,3-dimethylacryloyl chloride).

INTRODUCTION

The pyrrolizidine alkaloids (PAs) (1,2) are a class of naturally occurring compounds capable of causing carcinogenic changes in the tissues of experimental animals. In addition, it has been shown that they are also potent mitotic inhibitors. In order for these compounds to be active in the body they must be metabolized. By the use of radioactive PAs, the clarification of the mode of action of these compounds will be markedly simplified. However, the radioactive PAs available to date have been isolated from plants grown in a radioactive environment (3). The specific activity of the labeled PAs prepared in this way is low, with the label being randomly distributed (4). Since it has been shown that the retronecine moiety (5) of the PA or its optical isomer, heliotridine, is responsible for biological activity (6), labelling on this part is

most appropriate for evaluation of biological effects of the PAs. Retronecine, labelled at the 1 methyl proton with ^3H, was prepared by the reduction of III with $LiAl^3H_4$ (Figure 1). By means of acylation of this labelled retronecine, a semi-synthetic PA with high specific activity (7) was obtained.

RESULTS AND DISCUSSION

The ^3H labelled disenecioyl retronecine that was prepared (Figure 1) has a specific activity of about 40 μCi/mg. A higher specific activity can be obtained by increasing either the specific activity of the $LiAl^3H_4$ or by increasing the ratio of $LiAl^3H_4$ to $LiAlH_4$. After recrystallizing three times, the synthesized compound is radiochemically pure as shown in Figure 2. This compound has an LD_{50} of about 95 mg/kg i.p. and is a potent mitotic inhibitor. Heliotridine (7α-hydroxy-1-hydroxymethyl-1,2-dehydro-8α-pyrrolizidine), the optical isomer of retronecine, may also be prepared similarly by reduction of methyl 1,2-dehydro-7α-hydroxy-8α-pyrrolizidine-1-carboxylate (8) with $LiAl^3H_4$. Furthermore, there are other PAs, i.e. heliotrine, supine (9), fulvine, crispatine, and dichrotaline (1), which can be prepared by esterification of retronecine or heliotridine with

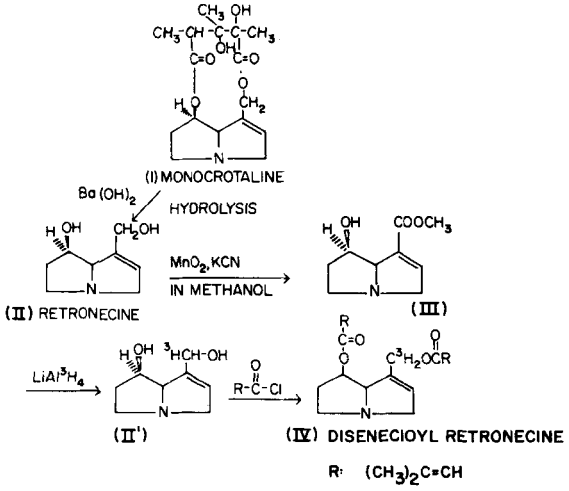

Figure 1. Scheme for the synthesis of ^3H labelled disenecioyl retronecine.

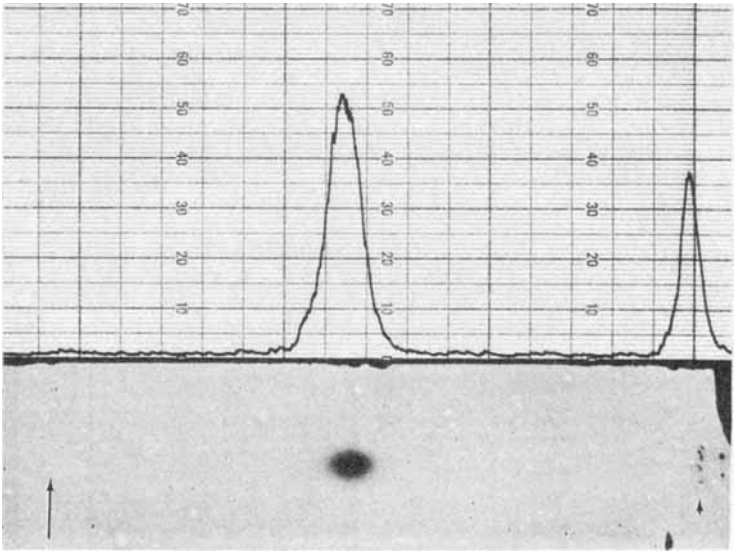

Figure 2. Radiochromatogram of TLC of ^{3}H labelled disenecioyl retronecine. About 3 µg of labelled compound was spotted on the TLC (→) and developed. The plate was scanned with a radiochromatogram scanner (Packard Model 7201, linear range 3 x 10^{3}, collimeter 2.5 mm, time constant at 10 sec. chart speed, 1 cm/min.) and then placed in an iodine tank (see text). A small amount of this compound was also applied above the solvent front as a marker (←).

appropriate organic acids. Therefore, those alkaloids labelled with ^{3}H at the 1 methyl position can be prepared by a procedure similar to that described.

EXPERIMENTAL PROCEDURES

Microanalyses were performed by Spang Microanalytical Laboratories (Ann Arboer, Michigan, U.S.A.). Melting points were measured on a med-temp capillary m.p. apparatus. NMR spectra were run on a Varian A60 NMR spectrometer using D_2O or $CDCl_3$ as solvents. Mass spectra were measured on a MS 902 mass spectrometer and IR spectra on a Hitachi spectrometer (Model 247). TLC was carried out on 8" x 2" silica gel plates developed in the solvent system chloroform:methanol:ammonia (85:14.5:0.5). Radio-

chemical purity was determined by scanning the TLC plates with a Packard Tri-Carb chromatogram scanner. Radioactivity was measured in a Packard Tri-Carb spectrometer, using Aquasol (New England Nuclear, Boston, Massachusetts, U.S.A.) as the scintillation solution.

Methyl 1,2-dehydro-7β-hydroxy-8α-pyrrolizidine-1-carboxylate (III)

Two grams (12 mmole) of retronecine (II) (10) which was prepared from the hydrolysis of monocrotaline (I) (S.B. Penick and Co., New York, New York, U.S.A.) in methanol (20 ml) was added to a mixture of acetic acid (5 ml), potassium cyanide (8 gm), and manganese dioxide (88 gm) in methanol (180 ml) (11). After stirring for 38 hours the reaction mixture was evaporated on a rotary evaporator. The oily residue was dissolved in hydrochloric acid (30 ml), washed with chloroform, basified with ammonia and extracted with chloroform (3 x 25 ml). The yield was about 600 mg of oily residue after evaporation of the chloroform. The oily residue was further purified by elution on a silica gel column with chloroform:methanol:ammonia (85:14.5:0.5) yielding 200 mg (1.1 mmole, 10%) of methyl 1,2-dehydro-7β-hydroxy-8α-pyrrolizidine-1-carboxylate. The solvent was evaporated and the compound crystallized from acetone-hexane as cubic crystals [m.p. 122°; IR. (KBr) 1705 (c=o), 1635 cm^{-1}(c=c); NMR ($CDCl_3$), singlet δ3.77 (O-Me); mass spec. m/e, 183(M^+)].

^{3}H-Retronecine (II')

A solution of III (200 mg in approximately 5 ml tetrahydrofuran) was added drop by drop to a solution of $LiAl^3H_4$ (50 mg $LiAlH_4$ + 10 mg $LiAl^3H_4$ (25 mci), New England Nuclear, Boston, Massachusetts, U.S.A.) in 10 ml tetrahydrofuran. When the addition was completed the solution was refluxed for 5-6 hours, until the starting material disappeared as monitored by TLC. The excess reagent was decomposed by the addition of 10 ml of ethyl acetate and 20 ml water. The mixture was filtered and the filtrate evaporated to dryness with the residue being purified by preparative TLC. The compound on the TLC plate having an R_f value similar to that of retronecine was eluted from the silica gel with methanol. After removing the solvent, 140 mg

(0.9 mmole, 81%) of oily residue was obtained. The sample had a mass spectrum identical to authentic retronecine.

Disenecioyl retronecine (IV)

Disenecioyl retronecine (IV) was synthesized from 140 mg of ^{3}H-retronecine (II') heated at about 90°C with excessive amounts of senecioyl chloride (1-2 ml) on a heating mantal for 1-2 hours until the retronecine had practically disappeared as monitored by TLC. The excess senecioyl chloride was decomposed with 5 ml of 3N sodium hydroxide, and the ester was extracted with ether (2 x 6 ml). After removal of the ether, the residue was dissolved in a minimum amount of ethanol and added to a picric acid solution (100 mg in 10 ml ethanol). Yellow crystalline needles of picrate were obtained (m.p. 112-114°C). The diester was recovered from the picrate by shaking with aqueous ammonia and extracting with ether. The ether solution was added to 0.3 ml of 2N hydrochloric acid and evaporated to dryness. The diester of retronecine hydrochloride formed crystalline colorless needles from acetone-ether. After three additional recrystallizations in acetone-ether, the compound (63 mg, 0.2 mmoles, 22%, m.p. 123-125) was free from impurity as evaluated by TLC and scanning with a chromatogram scanner. The mass spectrum (m/e 319 M^{+}) and the IR. spectrum [1700 cm^{-1} (-c=o), 1730 cm^{-1} (-c=c-)] were identical to the compound prepared by acylation of authentic retronecine. Anal. calcd. for $C_{18}H_{26}NOCl$: C, 60.7; H, 7.3; N, 3.9. Found: C, 60.46; H, 7.17; N, 3.93.

Acknowledgements

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