SYNTHESIS OF TRITIATED SENECIOYL DEHYDRORETRONECINE, A PYRROLIZIDINE ALKALOID PYRROLE

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SUMMARY

Tritiated mono- and disenecioyl dehydroretronecine (3,3-dimethyl-acrylic acid esters of 1-hydroxymethyl-7β-hydroxy-6,7-dihydro-5H-pyrrolizine) (IV and V) were prepared by acylation of 9-H^3-dehydroretronecine (1-hydroxymethyl-7β-hydroxy-6,7-dihydro-5H-pyrrolizine (III)). The synthesis of dehydroretronecine involved a new, higher yield method for preparing retronecinal (1-formyl-7β-hydroxy-6,7-dihydro-5H-pyrrolizine) (II) using pyrolitically prepared activated MnO_2.

Key Words: Pyrrolizidine Alkaloid Pyrrole, Tritium Labeled

INTRODUCTION

Pyrrolizidine alkaloids (PAs) are phytotoxins capable of causing carcinogenic and necrogenic changes in experimental animals (1,2). PAs must be metabolized to be toxic (1,3). The esterified PA pyrroles are the most toxic PA metabolites (4,5,6). Our *in vivo* and *in vitro* studies on the interaction of esterified PA pyrroles with cellular constituents required the development of a synthesis for radiolabeled esterified PA pyrroles since none have been prepared previously.

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RESULTS AND DISCUSSION

The overall yield in the presently reported synthesis (see scheme), from retronecine to senecioyl dehydroretronecine, averages about 13% and compares to an overall yield of disenecioyl dehydroretronecine from retronecine via the previously reported synthesis (7,8) of <0.3%. While the latter (7,8) is a cleaner synthesis with generation of only the diester, our mixture of the mono- and diester pyrroles was adequate for our experimental protocol. Forty-six percent of the label from the tritiated dehydroretronecine (^{3}H-DR) was incorporated into the senecioyl dehydroretronecine (SDR). The specific activity of SDR (2.2 μCi/mg) could be increased either by not diluting the ^{3}H-DR with DR or by increasing the specific activity of the reducing agent, NaB^3H_4. Tritiated dehydroheliotridine (^{3}H-DH), the enantiomer of ^{3}H-DR, may similarly be prepared by oxidizing heliotridine with activated MnO_2, then reducing it with NaB^3H_4. Reacting appropriate acyl chlorides with either ^{3}H-DR or ^{3}H-DH, under conditions similar to those described here, should lead to the synthesis of a variety of esterified ^{3}H-PA pyrroles whose structure-biological activity relationships could then be ascertained.

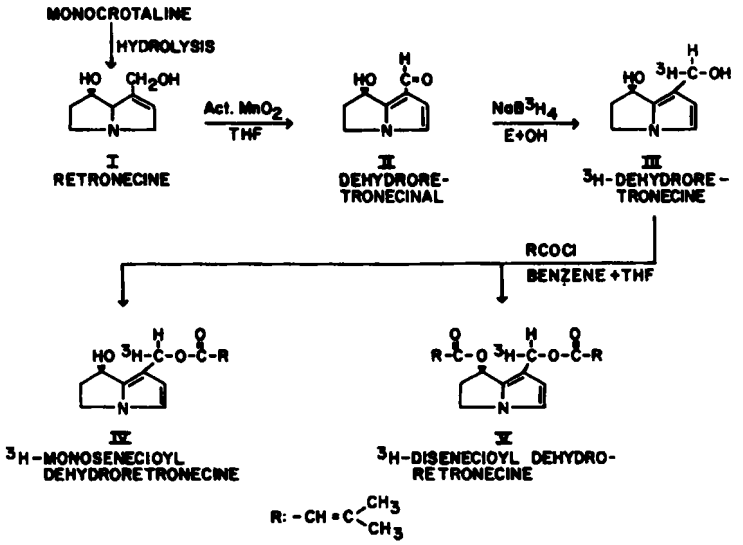

FIG. I. SCHEME FOR THE SYNTHESIS OF TRITIUM LABELED SENECIOYL DEHYDRORETRONECINE

EXPERIMENTAL PROCEDURES

NMR spectra were run on a Varian EM-390 NMR spectrometer using $CDCl_3$ as the solvent. IR spectra were measured on a Perkin-Elmer 247 infrared spectrometer. Mass spectra were taken at 70 eV with a Finnigan 1015 mass spectrometer equipped with a direct insertion probe and a Finnigan 6000 MS data system. Analytical TLC was carried out on pre-coated silica gel 1B plates (J.T. Baker, Phillipsburg, N.J.) developed in 85 $CHCl_3$:14.5 MeOH:0.5 NH_4OH, $CHCl_3$ or acetone. Monosenecioyl dehydroretronecine was separated from disenecioyl dehydroretronecine by preparative TLC on 150 μ silica gel G plates developed in $CHCl_3$. Radiochemical purity was determined by scanning the TLC plates using a Packard Tri-Carb chromatogram scanner. Radioactivity was measured in a Packard Model 3375 Tri-Carb liquid scintillation spectrometer, using Aquasol (New England Nuclear, Boston, Mass.) as the scintillation cocktail.

Dehydroretronecinal (II)

The following reaction was done behind an explosion shield. One gram of retronecine (6.54 mmol) (I), prepared by hydrolysis of monocrotaline (9) was dissolved in 100 ml Gold Shield tetrahydrofuran (Aldrich Chemical Co., Milwaukee, Wi.) in a stainless steel container with constant stirring. Five grams (.09 mol) pyrolitically prepared active MnO_2 (by heating $MnCO_3$ overnight at 220°) was slowly added over the course of 30 minutes. The reaction was monitored with TLC. After disappearance of the retronecine (Rf=0, dehydroretronecine aldehyde Rf=.68, acetone) the mixture was filtered under reduced pressure through a sintered glass funnel to remove the MnO_2 and tetrahydrofuran was removed under reduced pressure. The crude product was purified by column chromatography on silica gel using ether as the eluting solvent. The chromatographically pure material was dried under high vacuum for 6 hr to give 270 mg (1.81 mmol) (28% yield) of colorless plate-like crystals. IR (KBr): 3460, 3120 (O-H), 1680 (C=O), and 1640 cm^{-1} (C=C); NMR ($CDCl_3$):9.81 (singlet, 1H, -CHO), 6.68 (doublet, 1H, J= 3 Hz, C-3), 6.52 (doublet, 1H, J=3 Hz, C-2), 5.40 (multiplet, 1H, C-7), 4.10 (multiplet, 2H, C-5) and 2.94 δ (multiplet, 2H, C-6); MS m/e: 151 (M+), 134,

122, 106, 105, 104, 95, 94 (Base), 80, 79, 78, and 66.

^{3}H-dehydroretronecine (III)

330 mg (2.21 mmol) of (II) was dissolved in 5 ml of anhydrous ethanol with constant stirring. To this was added 8.5 mg (.22 mmol) (25 mCi) of NaB^3H_4 (New England Nuclear, Boston, Mass.). After stirring at room temperature for 12 hr, 200 mg $NaBH_4$ (5.26 mmol) was added to insure a complete reduction. The ethanol was evaporated under a stream of N_2. Ten ml of saturated aqueous K_2CO_3 was added and (III) was extracted into $CHCl_3$ (25 ml x 4). The pyrrole-$CHCl_3$ was dried over anhydrous $MgSO_4$ and the $CHCl_3$ removed under reduced pressure. Labeled DR was recrystallized from acetone-hexane (253 mg, 1.68 mmol, 76% yield, 55 μCi/mg). This material is identical with respect to TLC and IR to authentic dehydroretronecine prepared by the method of Culvenor et al. (10).

^{3}H-senecioyl dehydroretronecine (IV and V)

With constant stirring, 110 mg (.73 mmol) of cold DR and 9.7 mg (.06 mmol) ^{3}H-DR (III) (530.5 μCi) were dissolved in 4 ml of anhydrous tetrahydrofuran. The solution was subsequently cooled in an EtOH-dry ice bath. 0.9 g (9 mmol) of senecioyl chloride (Aldrich Chemical Co., Milwaukee, Wi.) in 1 ml benzene was added dropwise. After 15 min the reaction mixture was added to 75 ml ice cold $CHCl_3$. The excess senecioyl chloride was destroyed by shaking the solution with ice cold 1N NaOH (30 ml x 2). The pyrrole solution was dried with anhydrous $MgSO_4$ and the solvent removed under reduced pressure. The resultant oil (112 mg, 46% yield, based on recoverable radioactivity associated with senecioyl dehydroretronecine, 2.2 μCi/mg) rapidly decomposed to a red precipitate when exposed to air at room temperature. TLC (under N_2, in the dark) of the product when developed in either $CHCl_3$:MeOH:NH_4OH or $CHCl_3$ showed it was composed of two radiochromatically pure spots (Rfs = .29(MSDR) and .46(DSDR), $CHCl_3$). These were positive for Ehrlich's pyrrole reagent (11). The oil was intractible to recrystallization attempts in various solvents. NMR and IR data from the crude material indicate it is a mixture of monosenecioyl and disenecioyl dehydroretro-

necine. IR showed the typical α,β-unsaturated carbonyl absorption at 1690 cm^{-1} for the senecioyl ester side chain. NMR spectra revealed the characteristic pyrrole protons as two doublets at 6.17 and 6.53 δ, in addition to absorption due to the dimethyl groups in the senecioyl side chain at 1.93 and 2.20 δ. Attempts to separate the two esters via column chromatography (silica gel, $CHCl_3$) or preparative TLC resulted in breakdown of a large percentage of the pyrrole on the column or plate. However, when 75 mg of cold SDR was chromatographed on a 150 μ preparative TLC plate and the pyrrole from the lower portion of the pyrrole positive band isolated, approximately 4 mg of material enriched in MSDR was isolated. The point of attachment of the senecioyl ester side chain is tentatively assigned to the 9-C (IV) on the basis of NMR data. The C-7 proton, a multiplet at 5.17δ in DR, is only slightly shifted to 5.10δ. However, the C-9 protons on the MSDR, a singlet at 4.56δ in DR, shifted downfield to 5.70δ, which is consistent with the expected deshielding effect from the attached senecioyl group.

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