Synthesis of ^{3}H-Labelled Indicine N-Oxide

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SUMMARY

Indicine N-oxide, an antitumor agent possessing an unusual type of potential alkylating capacity, was recently selected for clinical trial. Radioactive-labelled indicine N-oxide was sought for studies of its biological properties relating to antitumor activity and toxicity. Available indicine N-oxide was reduced to indicine, and the alkaloid ester was hydrolyzed to its component compounds, retronecine and (-)-trachelanthic acid. The radioactive label was then introduced into the hydroxymethyl grouping of retronecine by a previously reported procedure involving oxidation of retronecine to methyl 1,2-dehydro-7β-hydroxy-8α-pyrrolizidine-1-carboxylate followed by reduction of the methoxycarbonyl grouping with $LiAl^{3}H_{4}$. Recombination of the alcohol and the carboxylic acid, a heretofore challenging problem in syntheses of pyrrolizidine alkaloid esters, was achieved using an adaptation of the mild esterification procedure recently reported by Hassner and Alexanian. By this method, indicine was obtained following reaction of retronecine, the isopropylidene derivative of (-)-trachelanthic acid, and N,N'-dicyclohexylcarbodiimide in the presence of 4-(dimethylamino)pyridine in toluene. Tritium-labelled indicine thus formed was then treated with m-chloroperoxybenzoic acid to give labelled indicine N-oxide.

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INTRODUCTION

Several compounds from the pyrrolizidine alkaloid group exhibit antitumor activity (1,2), but the toxicities of all but one of these compounds have precluded their clinical use. The relatively limited toxicity of indicine N-oxide (I), which occurs along with indicine (II) in Heliotropium indicum (3), allowed it to be chosen for clinical trials (4). We wish to describe a process whereby available indicine N-oxide was converted to ^{3}H-labelled material for use in biological studies (5).

RESULTS AND DISCUSSION

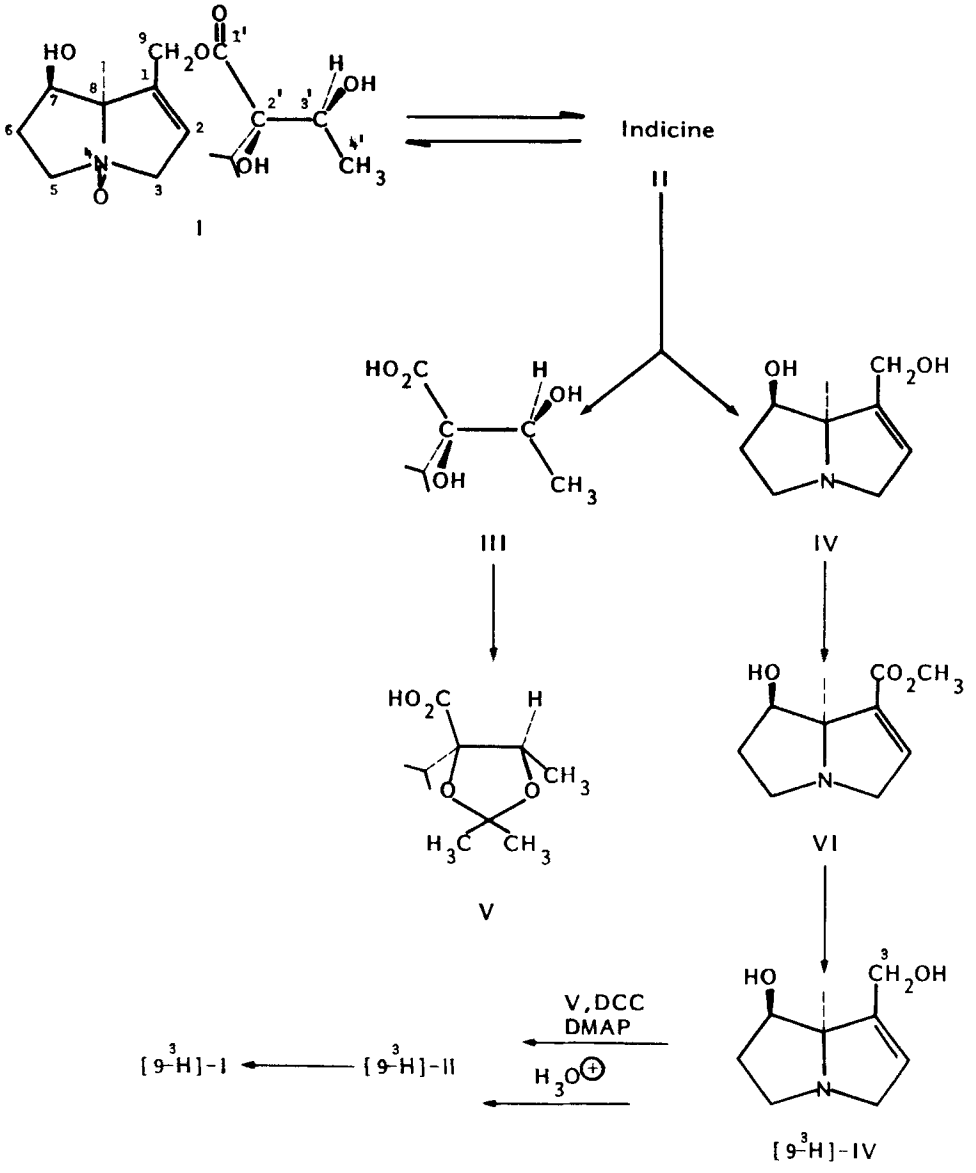
The basic synthetic steps used consisted of, first, the hydrolysis of indicine to its fundamental moieties (-)-trachelanthic acid (III) and retronecine (IV), followed by introduction of a label into retronecine, and finally, the reassembly of constituents (III and IV) to produce labelled indicine (see Scheme 1). The recombination was the key step in the task since methods for converting retronecine to [9-^{3}H]-retronecine are known (6,7) and, furthermore, the total synthesis of retronecine (8) offers opportunities to introduce a radioactive label. The ester was reformed using an adaptation of a recently reported method which involves reaction of an alcohol with an in situ-generated carboxylic anhydride [from the acid and N, N'-dicyclohexylcarbodiimide (DCC)] in the presence of a 4-aminopyridine [for example, 4-(dimethylamino)pyridine (DMAP)] as a catalyst (9). This esterification method proved in several ways to be well suited for carrying out the task at hand--the reaction occurs readily at room temperature, bulky substituents in both alcohol and acid are well tolerated, the alcohol and acid may be used in equimolar amounts, and O-protective groupings are unaffected. The acid III

was first converted to its isopropylidene derivative V in order to protect the vicinal hydroxyl groupings. The esterification was then carried out in toluene, and the isopropylidene derivative of indicine thus formed was extracted from the reaction solution with dilute hydrochloric acid. The acidic aqueous solution was then simply kept overnight at room temperature while the acid-labile protective grouping was completely removed. Subsequent isolation steps led to a homogeneous product in 50% yield. The identity of the ^{1}H and ^{13}C NMR spectra of this product with those of authentic indicine attests retention of the original stereochemical configuration. Indicine N-oxide was then readily formed by treatment of indicine with m-chloroperoxybenzoic acid in acetone at room temperature. This procedure for preparing the N-oxide proved simpler than that reported previously in which indicine was treated with excess hydrogen peroxide in ethanol over a three-day period (3).

Syntheses of both retronecine and (-)-trachelanthic acid have been reported (8, 10), but their synthetic union to produce indicine has not heretofore been described. Other 9-substituted monoesters of retronecine (and stereoisomeric heliotridine) have been prepared from the pyrrolizidine alcohol via the corresponding chloromethyl compound and the sodium salt of the appropriate acid (11-14). Reported examples utilize widely divergent isolation efforts; some products apparently were isolated readily, but the countercurrent distribution technique was used for others. Reported yields range from 13% (for 9-heliotrylretronecine) (14) to about 50% (for heliotrine) (11). A diastereomer of indicine, intermedine (an ester of retronecine and (+)-trachelanthic acid), was prepared through the chloromethyl intermediate and purified by countercurrent separation (13), but the yield was not reported (13). We were unable to develop a satisfactory procedure for the preparation of indicine from the chloromethyl intermediate, and a search for a more effective method led to the approach described in this report.

Scheme 1

Synthesis of ^{3}H-Labelled Indicine N-Oxide



EXPERIMENTAL

Materials And Methods

Indicine N-oxide used in this investigation was supplied by Dr. J. A. R. Mead, Developmental Therapeutics Program, Division of Cancer Treatment, National Cancer Institute, Department of Health and Human Services, Bethesda, Maryland. $LiAl^{3}H_4$ was purchased from New England Nuclear Corporation, Boston, Massachusetts, and supplied in glass ampoules, each containing 25.0 mCi in 2.63 mg (9.51 mCi/mg). Activated MnO_2 was purchased from Winthrop Laboratories, Division of Sterling Drug Inc., New York, New York.

Thin-layer chromatography (TLC) used to monitor reactions and to examine products for homogeneity was done on Analtech precoated (250 μm) silica gel G(F) plates. MeOH was used as the developing solvent unless another solvent is specified. Chromatograms involving solutions containing UV-absorbing materials in the presence of pyrrolizidine types were viewed under UV lamps before they were sprayed with a solution prepared by dissolving $(NH_4)_2SO_4$(150 g) in dilute H_2SO_4 solution (30 ml concentrated H_2SO_4 in 750 ml H_2O). The sprayed plates were then heated on a hot plate to cause charring of the pyrrolizidine compounds leaving dark-brown to black spots. Radiochemical purity assessments of [9-^{3}H]-I were done by liquid scintillation counting on 0.5 cm zonal cuts of types SA and SG Gelman ITLC plates; type SA plates were developed in MeOH-0.1M phosphate buffer (pH 7)-H_2O (90:1:9, v/v/v) and the SG type in n-BuOH-H_2O (43:7, v/v). The respective R_f values for [9-^{3}H]-I in these systems were 0.72 and 0.86. Detection by I_2 vapor was also possible but was best done using the Gelman plates. Elemental analysis, spectral determinations, and specific rotation measurements were performed in the Molecular Spectroscopy Section of Southern Research Institute under the direction of Dr. W. C. Coburn, Jr. The ^{1}H NMR spectra were

determined with a Varian XL-100-15 spectrometer, and the ^{13}C NMR spectra were determined with a Bruker WH 400 spectrometer operating at 100.6 MHz. All NMR spectra were determined in Me_2SO-d_6 using Me_4Si as internal reference. Chemical shifts (δ in ppm) listed for multiplets were measured from the approximate centers. Chemical shift assignments were aided by spin-decoupling and by addition of D_2O. Mass spectra were recorded on a Varian MAT 311A mass spectrometer equipped with a combination electron impact/field ionization/field desorption ion source. Specific rotations were measured with a Rudolph polarimeter. Melting points were observed on a Mel-Temp apparatus.

Indicine (II). The N-oxide I was converted to II using a straightforward adaptation of a reported procedure for the preparation of 9-angelylretronecine from its N-oxide by reduction with Zn dust and 2N H_2SO_4 (15). The II thus obtained as a colorless sirup (16) in 98% yield (4.65 g from 5.00 g of I) was homogeneous by TLC. Spectral data: ^{13}C NMR (in Me_2SO-d_6), δ 16.65 (4'-CH_3), 17.15 and 17.28 (-CH($\underline{C}H_3$)$_2$), 31.97 (-$\underline{C}H(CH_3)_2$), 36.17 (6-CH_2), 53.42 (5-CH_2), 61.56, 62.41 (3-CH_2, 9-CH_2), 68.44, 69.83 (3'-CH, 7-CH), 77.34 (8-CH), 82.49 (2'-CH), 126.34 (2-CH), 134.64 (1-C), 174.00 (1'-C); ^1H NMR (in Me_2SO-d_6), δ 0.85 [d, 6, ($C\underline{H}_3$)$_2$CH], 1.05 (d, 3, 4'-CH_3), 1.8 [m, 2 (probably nonequivalent), 6-CH_2], 2.00 [m, 1, (CH_3)$_2$C$\underline{H}$], 2.6 and 3.1 [m, 2 (nonequivalent), 5-CH_2], 3.3 and 3.8 [m, 2 (nonequivalent), 3-CH_2], 3.9 (m, 1, 3'-C$\underline{H}$OH), 4.0 (m, 1, 8-CH), 4.13 (m, 1, 7-C$\underline{H}$OH), 4.37 (d, 1, 7-CHO$\underline{H}$), 4.46 (s, 1, 2'-COH), 4.6 (m, 1, 3'-COH), 4.74 [m, 2 (nonequivalent), 9-CH_2], 5.79 (m, 1, 2-CH). The NMR data is consistent with that to be expected from the assigned structure and is in accord with reported data for analogs (14,17).

(-)-Trachelanthic Acid (III) and Retronecine (IV). Hydrolysis of II in $Ba(OH)_2$ solution was carried out as described by Adams and Rogers for the hydrolysis of monocrotaline (18). Crystalline III, mp 90-92° and $[\alpha]_D^{24}$ -4.8±0.2° (c=2.0, EtOH) [lit. mp 94° (3) and 89° (10a); lit. $[\alpha]_D^{25}$ -3.4° (c=2.5, H_2O)(10a)], remained

in 83% yield (1.53 g from 3.40 g of II) following removal of Et_2O after isolation as described for monocrotic acid. Workup of the aqueous solution obtained after the extraction with Et_2O gave IV HCl, mp 161-162° [lit. mp 161-162° (18)], in 88% yield (1.92 g) after recrystallization from EtOH. In order to obtain the free base, IV HCl (1.92 g, 10.0 mmoles) was treated with 1N NaOH (10 ml), and the resulting solution was treated with Norite, filtered (Celite mat), and lyophilized. The solid residue was extracted with $CHCl_3$, and evaporation of the filtered $CHCl_3$ solution left crystalline III, mp 120-121° [lit. mp 121° (18)], in 94% yield (1.46g).

4(R)-(1-methylethyl)-2,2,5(S)-Trimethyl-1,3-dioxolane-4-carboxylic Acid (V). A solution of III (1.6 g, 10 mmoles) in 2,2-dimethoxypropane (16 ml) was treated with concentrated HCl (0.025 ml). After 1.5 h at 25°, the solution was evaporated under reduced pressure (H_2O aspirator, bath to 40°) to give a tan solid which was sublimed in vacuo (< 1mm, bath to 55°) to give pure V, mp 51-53° and $[\alpha]_D^{25}$ +35.9±0.5°(c=1, EtOH), in 85% yield (1.7 g). Anal. Calcd for $C_{10}H_{18}O_4$: C, 59.39; H, 8.97. Found: C, 59.70; H, 9.11.

Methyl 1,2-Dehydro-7β-hydroxy-8α-pyrrolizidine-1-carboxylate (VI). The procedure that follows is similar to those reported by Aasen and Culvenor (19) and by Hsu and Allen (6). A solution of IV (2.53 g, 16.3 mmol) in MeOH (25 ml) was added to a mechanically stirred mixture of activated MnO_2 (110 g), KCN (10 g), glacial AcOH (6.5 ml), and MeOH (225 ml). The mixture was stirred 64 h at 20-25° and then filtered. The insoluble was removed from the funnel and stirred with MeOH (200 ml) until a uniform suspension resulted. This mixture was filtered, and, after the filter cake had been pressed, the washing with MeOH was repeated once more. The original filtrate and the two washings were combined and evaporated under reduced pressure to give a yellow-orange oil. This residue was stirred with 2N HCl (40 ml). Orange amorphous solid (polymerized pyrrolic matter) that formed was removed by treatment with Norite and filtration through

Celite. The pale-yellow filtrate was made basic by treatment with concentrated NH_4OH solution. NaCl was then added with stirring until the solution was saturated. Four extractions with $CHCl_3$ (50-ml portions) followed, and the $CHCl_3$ solution was dried (Na_2SO_4) and evaporated in vacuo to give VI as a white crystalline solid, mp 119-121° [lit. mp 122° (19)] and homogeneous by TLC, in 35% yield (1.04 g). Spectral data: mass, m/e 183 (M^+). The VI thus obtained was of suitable purity for conversion to [9-^{3}H]-IV. In the two earlier reports of the conversion of IV to VI by the described method, the yields of VI were 14% (19) and 10% (6) after purification by column chromatography.

[9-^{3}H]-Retronecine. Tritiated $LiAlH_4$ (7.9 mg, 0.21 mmole; 75 mCi) was combined with ordinary $LiAlH_4$ (82.0 mg, 2.16 mmoles) in dry tetrahydrofuran (THF, 15 ml), and the mixture was stirred for 30 min before a solution of VI (300 mg, 1.64 mmole) in THF (8 ml) was added dropwise during 10 min. This mixture was stirred at 25° overnight (about 16 h), then refluxed 4 h, cooled to 25°, treated dropwise with a solution of EtOAc (1.5 ml) in THF (6 ml), refluxed 10 min, cooled, treated with 10% NaOH (1.5 ml), diluted with more THF (8 ml), and left to stir at 25° for about 18 h. H_2O (5 ml) was then added, and, after about 10 min, the nearly clear supernatant was removed by decantation from a gel-like precipitate. The gel was treated with a few drops of H_2O until it could be stirred into a uniform suspension. More THF was added, and the resulting mixture was filtered through Celite. The filtrate was combined with the supernatant removed earlier, and the solution was evaporated under reduced pressure (H_2O aspirator, bath at 25°) until THF had been removed. The cloudy aqueous mixture that remained was treated with 1N HCl (3 ml) to produce pH 2. A small amount of Norit was added, and the solution was left in a refrigerator overnight. Filtration (Celite mat) gave a colorless filtrate which was treated with 1N NaOH to pH 11. This solution was then lyophilized. The dry residue was stirred with four portions of $CHCl_3$

(totalling 100 ml), and evaporation of the combined and filtered solution left a viscous, pale-yellow oil, homogeneous on TLC and identical with authentic retronecine; yield 92% (234 mg). The product crystallized on standing overnight in a refrigerator and was used directly for conversion to [9-^{3}H]-II.

Indicine (II) From IV and V. A mixture of IV (218 mg, 1.406 mmole), V (463 mg, 2.29 mmoles), N,N'-dicyclohexylcarbodiimide (472 mg, 2.29 mmoles), 4-(dimethylamino)pyridine (DMAP, 17 mg, 0.14 mmole) and dry toluene (6 ml) was stirred in a closed flask. Examination of the reaction solution by TLC after 68 h revealed absence of IV, a spot of $R_f \sim 0.2$ due to the isopropylidene derivative of II, a UV-absorbing spot due to DMAP of $R_f \sim 0.15$ touching that due to the desired product, and a very weak spot of $R_f \sim 0.6$, apparently due to the product of esterification of both hydroxyl groupings of IV[1]. Precipitated N,N'-dicyclohexylurea was removed by filtration with the aid of a little toluene, and the filtrate was extracted with 0.6 N HCl (three times with 6-ml portions). The acidic aqueous solution was washed twice with Et_2O (10 ml), and the washings were discarded. The aqueous layer was filtered, left overnight (18 h) at 25°, made basic by treatment with 28% NH_4OH solution, treated with solid NaCl until saturation was reached, then extracted with Et_2O (four times with 30-ml portions). The dried (Na_2SO_4) Et_2O solution was evaporated to give a foamy glass (0.32 g). Examination by TLC revealed II ($R_f \sim 0.15$) to be the major component. A very weak spot of $R_f \sim 0.55$, probably due to the diester, was also present. The presence of DMAP was evidenced by its UV-absorbing spot of almost the same R_f as II.

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[1] The mass spectrum produced by the product mixture showed the desired isopropylidene derivative of II to be the major component with peaks of m/e 339 (M), 324 (M-CH_3), and 322 (M-OH). A minor product gave weaker peaks of m/e 523 and 508 which correspond to M and M-CH_3 for the product of diacylation of IV by V.

Using another solvent system, $CHCl_3$-MeOH- 28% NH_4OH (85:14.5:0.5, v/v/v), II and the probable diester travelled nearly the same as when MeOH was used, but DMAP moved further, giving an R_f nearly the same as that of the slight impurity believed to be the diester. The crude product was then applied in MeOH solution to two 20 x 20-cm Analtech 2-mm silica gel GF plates and chromatographed using the $CHCl_3$-MeOH-NH_4OH system defined above. The plates were developed twice, and the silica gel below the UV-absorbing band due to DMAP was removed from the plates, pulverized, then stirred with EtOH (225 ml) at 25° for 16 h. The filtered solution was evaporated in vacuo to give TLC-homogeneous II as a viscous colorless oil in 50% yield (210 mg). The ^1H and ^{13}C NMR spectra of II thus prepared are virtually identical with those of II prepared from I as described above. This procedure was successfully applied to the conversion of [9-^{3}H]-IV to [9-^{3}H]-II.

Indicine N-Oxide (I) From II. In parallel runs, 70 mg (0.234 mmole) each of [9-^{3}H]-II and ordinary II were dissolved in dry Me_2CO (4 ml) containing m-chloroperoxybenzoic acid (48 mg of 85% purity, 0.236 mmole). After 1 hr, examination of the reaction solutions by TLC indicated all II ($R_f \sim 0.2$) had been converted to I ($R_f \sim 0.35$), and tests with KI-starch paper for peroxy acid were negative. The UV-absorbing spot due to m-chlorobenzoic acid had $R_f \sim 0.8$. A 20 x 20-cm Analtech 2-mm silica gel GF plate was divided in half by removing the silica gel along a center line about 5 mm in width. The reaction solutions were applied to the plate, each to one side of the division line, and the plate was developed once with MeOH. The side of the plate containing the radioactive-labelled material and most of the side containing the ordinary material were covered with a glass plate, then the exposed part was treated with $(NH_4)_2SO_4$-H_2SO_4 spray reagent. The treated area was heated on the edge of a hot plate until a charred band due

to I was evident. The band on the radioactive-labelled side of the plate on line with the charred band was well separated from the UV-absorbing band due to m-chlorobenzoic acid. The band containing labelled I was cut from the plate, pulverized, and stirred with EtOH (250 ml) at 25° for 4 hr. Filtration was followed by a second extraction of the silica gel with EtOH. Evaporation of the filtrates followed by drying in vacuo (over NaOH pellets and P_2O_5) gave [9-^{3}H]-I as a glassy residue in 75% yield (49 mg and 6 mg). This material was chemically and radiochemically homogeneous according to TLC; specific activity 37.7 μCi/mg or 11.9 mCi/mmole[2]. To avoid loss of material, no effort was made to cause crystallization. The glassy film was dissolved in H_2O to give a stock solution used in the biological studies.

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[2]Since a ratio of two moles of ester per mole $LiAlH_4$ is required for reduction to a primary alcohol, the maximum radioactivity that could be incorporated in [9-^{3}H]-I in the operations described is 26 mCi or 50 μCi/mg.

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