otherwise stated $MgSO_4$ was employed as a drying agent. The IR spectra were determined with a Perkin-Elmer, Model 257, infrared recording spectrophotometer fitted with a grating. The UV spectra were determined with a Cary, Model 14, or a Perkin-Elmer, Model 202, recording spectrophotometer. The proton NMR spectra were determined at 60 mHz with a Varian, Model A-60 or Model T-60-A, NMR spectrometer and the ^{13}C NMR spectra were determined at 25 mHz with a JEOL Fourier transform spectrometer, Model PFT-100. The chemical shift values are expressed in δ values (ppm) relative to a Me_4Si internal standard. The mass spectra were obtained with an Hitachi Perkin-Elmer, Model RMU-7, mass spectrometer. All reactions involving strong bases or reactive organometallic intermediates were performed under a nitrogen atmosphere.

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Structural Studies on Juncusol. A Novel Cytotoxic 9,10-Dihydrophenanthrene Derivative from the Marsh Plant *Juncus roemerianus*[1]

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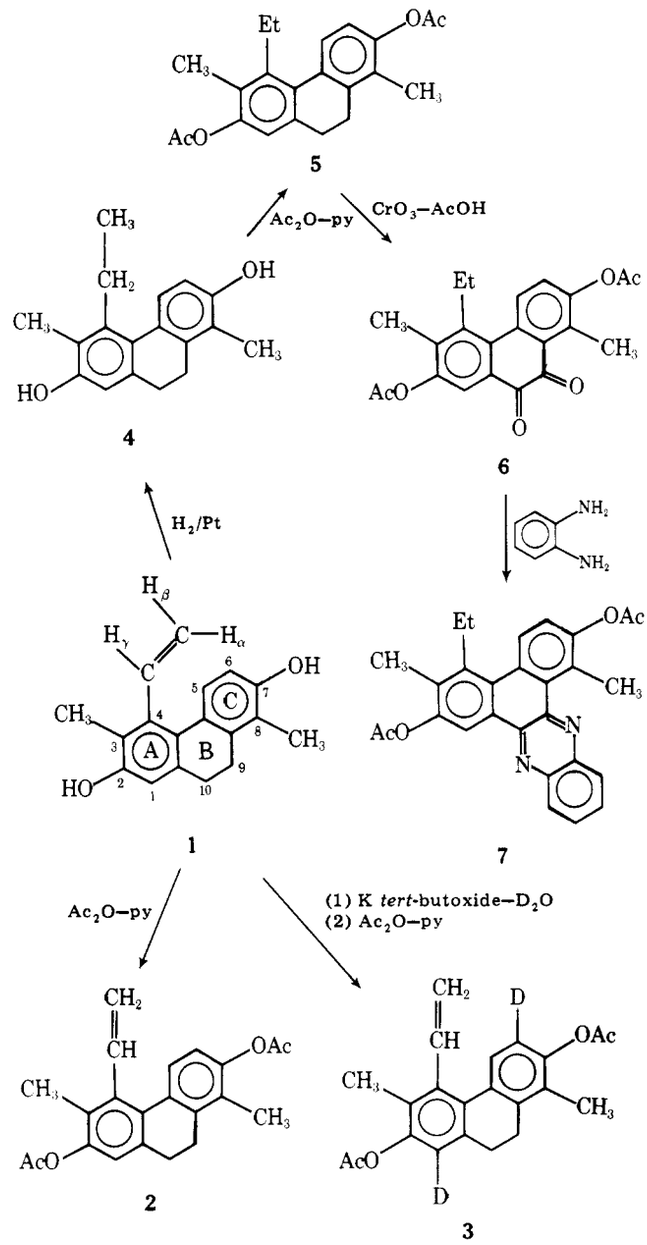
Juncus roemerianus (NO Juncaceae) is the most dominant among a group of plants, commonly known as "marsh grass", which grow abundantly on and near the coastal areas of the southeastern United States. An earlier report[3] indicated that 95% of the organic matter produced in the marsh is not attacked by the marsh herbivores, but on death and decomposition the plants enter the detritus food chain. A 95% ethanolic extract of the tops of *J. roemerianus* showed activity against P 388 lymphocytic leukemia in BDF_1 mice.[4] A preliminary study on the volatile constituents of *J. roemerianus* was reported earlier from our laboratory.[5] To our knowledge no detailed chemical investigation of *J. roemerianus* or any other marsh grass had been reported in the literature prior to our work. The $CHCl_3$ extract of the tops of this plant, upon chromatography over silica gel, yielded, inter alia, the cytotoxic[6] compound juncusol, $C_{18}H_{18}O_2$, mp 175–176 °C. Recently we reported[7] the structure of juncusol diacetate based on an X-ray crystallographic study. We now wish to report an extensive chemical and spectral study of juncusol and its derivatives in support of the structure of juncusol as 1 (Scheme I). Although about 20 of the relatively rare 9,10-dihydrophenanthrene derivatives are known from nature,[8] juncusol is unique in possessing an alkenyl substituent in the ring system in addition to rarely encountered alkyl groups. Juncusol, like all other members of its class, is a phenol, but unlike others it does not contain a methoxyl substituent.

Results and Discussion

The finely ground plant tops (above ground) of *J. roemerianus* were extracted with chloroform. The concentrated chloroform extract was triturated with chloroform–benzene (1:1). Chromatography of the soluble portion on silica gel followed by crystallization from benzene yielded (0.01% dry weight) juncusol, $C_{18}H_{18}O_2$ (M^+ at *m/e* 266), mp 175–176 °C. The IR spectrum of juncusol (1) in KBr exhibits peaks at 3350 (OH), 1603 (aromatic), 930 (vinyl), and 870 and 830 (two adjacent Ar–H) cm^{-1}. The UV spectrum in ethanol shows λ_{max} at 247 sh, 266 sh, 284 sh (log ϵ 4.12), and 318 sh nm, characteristic of the 9,10-dihydrophenanthrenes. A typical 4 H singlet at δ 2.50 in the ^1H NMR spectrum of juncusol confirms[9,10]

Scheme I



its 9,10-dihydrophenanthrene skeleton. The 100 MHz NMR spectrum of juncusol in $CDCl_3$ (with a few drops of acetone-d_6) also shows sharp singlets at δ 2.26 (3 H, Ar–CH_3) and 2.31 (3 H, Ar–CH_3), ABX type of signals for a vinyl group consisting of three sets of "quartets" at δ 5.20 (1 H, J_{AX} = 18 Hz and J_{AB} = 2 Hz), 5.46 (1 H, J_{BX} = 11 Hz and J_{AB} = 2 Hz), and 6.78 (1 H, J_{AX} = 18 Hz and J_{BX} = 11 Hz), two ortho aromatic proton doublets at δ 6.66 (J = 8 Hz) and 7.50 (J = 8 Hz), and an aromatic proton singlet at δ 6.70. The relative low field shift of one of the aromatic proton doublets at δ 7.50 indicates it to be at C-5 (or C-4) of the 9,10-dihydrophenanthrene ring system. Therefore, the ortho aromatic protons must be present at C-5 and C-6 and the remaining proton must be in ring A in juncusol. In the ^1H NMR spectrum of juncusol in pyridine-d_5, the CH_3 groups shift to δ 2.51 ($\delta_{pyridine} - \delta_{chloroform}$ = 0.25 ppm) and 2.62 ($\delta_{pyridine} - \delta_{chloroform}$ = 0.31 ppm), the C-6 aromatic proton shifts to δ 7.07 ($\delta_{pyridine} - \delta_{chloroform}$ = 0.41 ppm), and the ring A aromatic proton singlet shifts to δ 7.10 ($\delta_{pyridine} - \delta_{chloroform}$ = 0.40 ppm). These significantly large pyridine-induced solvent shifts to lower fields can only be attributed to the orientations of the CH_3 groups and the aromatic protons in question being ortho to the OH groups.[11]

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Juncusol is soluble in dilute NaOH solution, and it gives a deep blue color with $FeCl_3$ solution. The reactions of juncusol are shown in Scheme I. Upon treatment with acetic anhydride in pyridine, juncusol yields a diacetate (**2**), $C_{22}H_{22}O_4$ (M^+ at *m/e* 350), mp 110 °C, showing the presence of both the oxygen atoms as phenolic functions. The aromatic protons in the diacetate (**2**) appear, as expected, at lower fields as doublets at δ 7.66 (H-5) and 7.30 (H-6) and as a singlet at δ 6.88 (ring A proton) in the NMR spectrum. Upon deuteration with D_2O in the presence of K *tert*-butoxide followed by acetylation, juncusol gives a dideuterated (demonstrated by mass spectrum) diacetate (**3**) which shows only the aromatic proton at C-5 as a singlet at δ 7.66 in the NMR spectrum. The disappearance of the proton at C-6 and the one in ring A upon deuteration confirms[9] that the former must be ortho and the latter either ortho or para to the hydroxyl groups. Therefore, one of the hydroxyl groups must be present at C-7, and consequently one of the CH_3 groups should be present at C-8 (ortho to OH group). Also, the aromatic proton in ring A, if para to a hydroxyl group, would not be expected to experience a pyridine-induced solvent shift of 0.40 ppm. Moreover, juncusol gives a negative Gibbs test, indicating that there is no proton para to a hydroxyl group in it. Therefore, the ring A proton must be ortho to a hydroxyl group.

Catalytic hydrogenation of juncusol produces a dihydro derivative (**4**), $C_{18}H_{20}O_2$ (M^+ at *m/e* 268), mp 167–168 °C. The absence of the ABX signals and the appearance of a quartet at δ 2.74 (2 H, J = 7 Hz, Ar–C**H**$_2$–CH_3) and a triplet at δ 1.22 (3 H, J = 7 Hz, Ar–CH_2–C**H**$_3$) in the NMR spectrum of the dihydro derivative confirm the presence of a vinyl group in juncusol.

Dihydrojuncusol on treatment with acetic anhydride in pyridine affords a dihydro diacetate (**5**), $C_{22}H_{24}O_4$ (M^+ at *m/e* 352), mp 138 °C. The latter, when oxidized with CrO_3 in acetic acid, gives an orange-yellow quinone (**6**), $C_{22}H_{20}O_6$ (M^+ at *m/e* 380), mp 213–214 °C. The quinone gives a condensation product (**7**) with *o*-phenylenediamine, confirming that it is a 9,10-phenanthrenequinone. The ring A aromatic proton singlet shifts considerably to a lower field at δ 7.55 in the NMR spectrum of the quinone (**6**). This large low field shift can be explained if the ring A aromatic proton is peri to the carbonyl group in the quinone and therefore at C-1 in juncusol. Consequently, the second hydroxyl group must be present at C-2 (ortho to the C-1 proton), and the second CH_3 group is at C-3 (ortho to the C-2 OH). The vinyl group must therefore be present at C-4. The vinyl group at C-4 has a restricted rotation, and consequently the methylene proton (designated by α in **1**) which is trans to the Ar–C–H proton (designated by γ) is expected to be somewhat shielded by the ring current of ring C in orientations where the vinyl group is at right angles to the plain of the 9,10-dihydrophenanthrene ring system. Therefore, the α proton appears at higher field than the methylene proton (designated by β in **1**) which is cis to the Ar–C–H (γ) proton in the NMR spectrum of juncusol. Exactly the reverse is the case of the vinyl protons in styrene, where the vinyl group has free rotation.

As previously reported[7] the structure of juncusol (**1**) was confirmed by a single crystal X-ray diffraction experiment on the diacetate derivative (**2**). Recently, we also reported[12] the carbon-13 NMR analysis of juncusol and its derivatives.

Experimental Section

Nuclear magnetic resonance spectra were obtained using a Jeolco Minimar spectrometer equipped with a spin decoupler and a Varian HA-100 spectrometer. Tetramethylsilane was used as an internal standard, and chloroform-*d* (99.8%) and acetone-d_6 were used as solvents. The hydrogenation was carried out in a Parr pressure reaction apparatus. Mass spectral data were obtained using a Perkin-Elmer Model 270 or a Hewlett-Packard Model 5930 mass spectrometer. Mass spectra were obtained at 70 eV. Infrared spectra were obtained using a Perkin-Elmer Model 137G spectrophotometer. The spectra of solids were obtained by incorporating the sample into a pellet of potassium bromide. The band at 11.035 μm in a polystyrene film (0.05 mm) was used as a reference peak. Column chromatography (wet and dry column chromatography) was performed in glass columns with sintered glass using silica gel, 40–140 mesh, Baker Analyzed Reagent. Thin-layer chromatography (TLC) was performed using E. Merck (Darmstadt) silica gel G and GF-254, Applied Science Laboratories, Inc., coated (20 × 20 cm and 5 × 20 cm) glass plates. Chromatoplates were prepared by using a Desaga spreader with a thickness of 0.25 mm. The plates were activated at 110 °C for 1 h. The solvent system was $CHCl_3$–acetone–diethylamine (5:4:1) or $CHCl_3$–MeOH (95:5) unless otherwise stated. Phosphomolybdic acid reagent (Applied Science) and ultraviolet light were used as detecting agents. Melting points were obtained on a Fisher-Jones apparatus and are uncorrected. Elemental microanalyses were done by Galbraith Laboratories Inc., Knoxville, Tenn. Biological activities were performed by the Cancer Chemotherapy National Service Center, Bethesda, Md.

Isolation of Juncusol (1). The dry ground aerial parts of *Juncus roemerianus* (7000 g), collected from Bay St. Louis, Miss., during the summer of 1972–1973, were extracted with $CHCl_3$ for 24 h in a Soxhlet apparatus. The combined $CHCl_3$ extracts were evaporated in vacuo, the residue was dissolved in a minimum volume of benzene–$CHCl_3$ (1:1), and the undissolved material was collected by filtration. The filtrate was placed in a column (9.5-cm diameter) of silica gel (1000 g). The column was eluted consecutively with benzene–hexane (1:1), benzene–hexane (4:1), benzene, benzene–chloroform (4:1), and benzene–chloroform (1:1). The fractions of 500 mL each were collected and monitored by TLC. Fractions eluted with benzene (25 L) on evaporation gave a dark greenish mass (30.0 g) which was rechromatographed on a column (2.5-cm diameter) of silica gel (185 g), eluting the fractions successively with the same sequence of solvent mixtures used in the previous chromatography. Fractions eluted with benzene–hexane (6 L) and benzene (5 L) were combined and evaporated. The residue was recrystallized several times from benzene–chloroform mixtures. Final recrystallization from benzene gave 1.0 g of juncusol (**1**) as stout colorless needles, mp 175–176 °C. Spectral properties of **1** were the following: UV (EtOH) λ_{max} (log ε) 247 sh, 266 sh, 284 sh (4.12), 318 sh nm; IR ν_{max} (KBr) 3550, 1603, 930, 870, 830 cm^{-1}; ^1H NMR ($CDCl_3$–acetone-d_6) δ 2.26 (3 H, s, Ar–CH_3), 2.31 (3 H, s, Ar–CH_3), 2.50 (s, 4 H), 5.20 (1 H, J_{AX} = 18 Hz, J_{AB} = 2 Hz), 5.46 (1 H, J_{BX} = 11 Hz, J_{AB} = 2 Hz), 6.66 (1 H, d, J = 8 Hz), 6.70 (1 H, s), 6.78 (1 H, J_{AX} = 18 Hz, J_{BX} = 11 Hz), 7.50 (1 H, d, J = 8 Hz). The mass spectrum gave a parent ion peak at *m/e* 266 (M^+) and important peaks at *m/e* 41, 43, 55, 77, 79, 81, 104, 149, 165, 236, 251, and 266 (relative % 70, 63, 54, 40, 50, 47, 44, 48, 90, 56, 100, and 98).

Anal. Calcd for $C_{18}H_{18}O_2$ (mol wt 266): C, 81.20; H, 6.76. Found: C, 81.02; H, 6.92.

Juncusol Diacetate (2). Juncusol (0.1 g) was dissolved in dry pyridine (1 mL), acetic anhydride (0.5 mL) was added to it, and the mixture was stirred at room temperature for 4 h in a round-bottom flask with a drying tube. The resulting mass was freed from excess pyridine in vacuo and poured onto cold water (20 mL). Extraction with chloroform followed by washing of the chloroform layer successively with dilute HCl, H_2O, Na_2CO_3, and H_2O, drying over anhydrous Na_2SO_4, and evaporation of $CHCl_3$ gave a solid product which upon crystallization from benzene gave juncusol diacetate (**2**), mp 110 °C, as white needles (0.1 g). The spectral properties of **2** were the following: IR ν_{max} (KBr) 1750, 1603, 940, 900, 870, 835 cm^{-1}; ^1H NMR ($CDCl_3$) δ 2.18 (3 H, s), 2.20 (3 H, s), 2.36 (6 H, s), 2.72 (4 H, s), 5.3 (1 H, d), 5.44 (1 H, d), 6.7 (1 H, d), 6.88 (1 H, s), 7.30 (1 H, d), 7.66 (1 H, d). The mass spectrum gave a parent ion peak at *m/e* 350 (M^+) and fragmentation at *m/e* 236, 251, 266, 280, and 350.

Anal. Calcd for $C_{22}H_{22}O_4$ (mol wt 350): C, 75.42; H, 6.28. Found: C, 75.66; H, 6.30.

Deuteration of Juncusol. A mixture of juncusol (0.06 g), potassium *tert*-butoxide (0.5 mol equiv), and deuterium oxide (0.5 mL) was heated in a sealed nitrogen-filled tube at 100 °C for 3 days. The solvent was evaporated, and the product was acetylated (acetic anhydride–pyridine at room temperature for 24 h) and purified on TLC plates in the usual way to give the deuterated acetate (**3**) in high yield. The NMR spectrum showed that two aromatic protons exchanged with deuterium. The mass spectrum gave a parent peak at *m/e* 352 (M^+) (calcd for $C_{22}H_{20}D_2O_4$, mol wt 352).

Dihydrojuncusol (4). Juncusol (600 mg) was hydrogenated in methanol solution (25 mL) with 10% Pd/C (10 mg) as a catalyst in the presence of hydrogen gas at 50 psi for 2 h at room temperature. The solution was then filtered. The residue, after evaporation, was dissolved in benzene and filtered again. The mother liquor (15 mL) on

standing yielded transparent crystals, mp 167–168 °C, in quantitative yield. Spectral properties of **4** were the following: IR ν_{max} (KBr) 3550, 1603, 920, 870, 830, 815 cm^{-1}; ^{1}H NMR (Me_2SO-d_6) δ 1.22 (3 H, t, J = 7 Hz, Ar–CH_2–CH_3), 2.25 (6 H, s), 2.6 (4 H, s), 2.74 (2 H, q, J = 7 Hz, Ar–CH_2–CH_3), 6.85 (1 H, s), 7.05 (1 H, d), 7.45 (1 H, d), 9.4 (2 H, s); mass spectrum, m/e 268 (M^+), and fragmentation at m/e 115, 119, 151, 164, 181, 195, 238, 268.

Dihydrojuncusol Diacetate (5). Acetylation of dihydrojuncusol (4) was carried out in pyridine and acetic anhydride in the usual way and the product was crystallized from benzene, mp 138 °C. Compound **5** gave the following spectral data: IR ν_{max} (KBr) 1750, 1603, 920, 870, 830, 815 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.3 (3 H, t), 2.4 (6 H, s), 2.5 (6 H, s), 2.8 (4 H, s), 2.9 (2 H, q), 6.9 (1 H, s), 7.4 (1 H, t), 9.4 (1 H, s). The mass spectrum gave fragmentation at m/e 238, 253, 268, and 310 and a parent peak at m/e 352 (M^+).

Anal. Calcd for $C_{22}H_{24}O_4$ (mol wt 352): C, 75.00; H, 6.81. Found: C, 75.16; H, 6.69.

CrO_3 Oxidation of Dihydrojuncusol Diacetate. Compound **5** (400 mg) was dissolved in glacial acetic acid (10 mL) and gradually added to a solution of CrO_3 (800 mg) in 80% aqueous acetic acid (5 mL), keeping the temperature below 5 °C. After the addition, the mixture was stirred at room temperature for 4 h. The resulting mixture was then poured onto ice water (100 mL), extracted thoroughly with $CHCl_3$, dried over anhydrous Na_2SO_4, and evaporated. The quinone **6** was crystallized from benzene as yellow needles (0.15 g): mp 210–215 °C dec; IR ν_{max} (KBr) 1750, 1650, 1603, 925, 915, 875, 180 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.35 (3 H, t, J = 7 Hz), 2.25 (3 H, s), 2.3 (6 H, s), 2.4 (3 H, s), 2.85 (2 H, q, J = 7 Hz), 7.26 (1 H, d, J = 5 Hz), 7.55 (1 H, s), 7.46 (1 H, d, J = 5 Hz). The mass spectrum gave a parent ion peak at m/e 380 (M^+) and fragmentation at m/e 181, 253, 268, 305, 337, 352, and 380.

Anal. Calcd for $C_{22}H_{20}O_6$ (mol wt 380): C, 69.47; H, 5.26. Found: C, 69.64; H, 5.20.

Reaction of Quinone 6 with *o*-Phenylenediamine. Compound **6** (0.04 g) was refluxed in glacial acetic acid (3 mL) with *o*-phenylenediamine (0.02 g) for 2.5 h. The reaction product was cooled and poured onto ice-cold water, at which time a yellow precipitate separated. The latter was extracted with chloroform, and the chloroform layer was washed with water, dried with anhydrous Na_2SO_4, and evaporated. The dark yellow mass was recrystallized from benzene to give short, fine yellow needles. Compound **7** shrinks at 250–255 °C and finally decomposes at 270 °C. The IR spectrum showed the disappearance of the carbonyl band of the quinone **6** at 1650 cm^{-1}.

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Registry No.—**1**, 62023-90-9; **2**, 62023-91-0; **3**, 67489-25-2; **4**, 64052-93-3; **5**, 64052-94-4; **6**, 67489-26-3; **7**, 67489-27-4; *o*-phenylenediamine, 95-54-5.

References and Notes

(1) (a) Contribution 4 in the series of antineoplastic agents. For part 3, refer to ref 7. (b) Part 7 in the series "Constituents of Marsh Grass."
(2) (a) Department of Chemistry, Mississippi State University. (b) The Institute for Natural Products Research and the Department of Chemistry, University of Georgia. (c) Partly taken from the Ph.D. Thesis of N. V. Mody, Mississippi State University. (d) Boll Weevil Research Laboratory, U.S. Department of Agriculture, Mississippi State, Miss.
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Convenient Synthesis of *N*-Noratropine[1]

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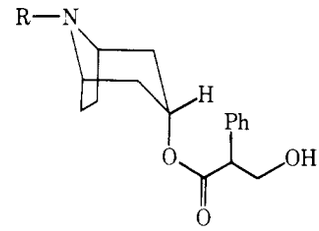
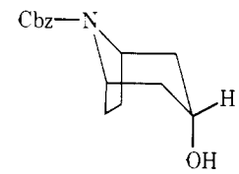
Due to their pronounced biological activities, compounds containing the tropane structure have been of interest for a number of decades.[2–4] Recently, derivatives of *N*-alkylnoratropine, especially the bronchodilator *N*-isopropyl-*N*-methylnoratropinium bromide[5–7] (Ipratropium bromide, Sch 1000), have commanded particular attention.

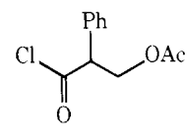
N-Noratropine (**1**) itself, long known as a constituent of various solanaceous plants,[8] was first synthesized by Nádor et al.[9,10] who reacted *N*-carbobenzyloxynortropine (**2**) with *O*-acetyltropic acid chloride (**3**) in the presence of pyridine,



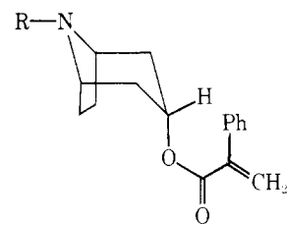
1, R = H
4, R = Cbz
7, R = Me



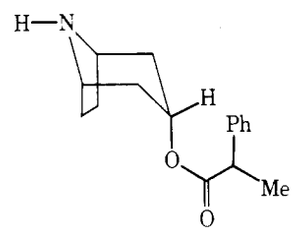
2



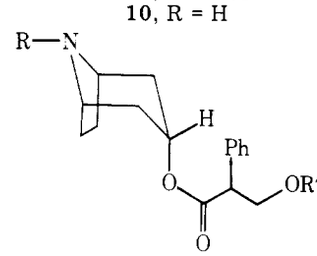
3



5, R = Cbz
10, R = H



6



8, R = Cl_3CCH_2OCO; R′ = H
9, R = R′ = Cl_3CCH_2OCO

followed by acid-catalyzed hydrolysis of the *O*-acetyl group. The resulting *N*-carbobenzyloxynoratropine (**4**) was subjected to hydrogenolytic cleavage to afford noratropine (**1**). However, Bertholdt et al.[11] claimed that under the acylation conditions mentioned above, the acrylate **5** was formed by elimination of AcOH. They conclusively proved that after hydrogenolysis, the phenyl propionate **6** was the final product. The same elimination reaction has also been observed by other workers[12] in a closely related series of compounds. Both groups of investigators pointed out that the desired tropate esters could be obtained in fair yields if the acylation step was carried out in the absence of basic catalysts. Nevertheless, it seemed worthwhile to examine the possibility of N-demethylating commercially available atropine (**7**), which would constitute a much simpler method of synthesizing noratropine.

It has already been shown that the simple bases tropine and tropinone can be demethylated with ethyl chloroformate,[13] but the strongly acidic conditions required for the hydrolysis of the resulting carbamate intermediates were deemed to be

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