THE USE OF THE ACETONIDE DERIVATIVE IN THE PREPARATION OF D-THREO-CHLORAMPHENICOL-1-^{3}H AND ITS NITROSO DERIVATIVE

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SUMMARY

An efficient synthesis of millimolar quantities of high specific activity D-threo-chloramphenicol-1-^{3}H is described. The key step is the chromatographic separation of the compound from its 1-epimer, produced in the $Ca(B^3H_4)_2$ reduction of chloramphenicol ketone (D-α-dichloroacetylamino-β-hydroxy-p-nitropropiophenone), by use of the acetonide derivative. Stereochemical aspects of the N-bromosuccinimide oxidation of chloramphenicol to chloramphenicol ketone have been investigated, and the previously reported [1] specific rotation of chloramphenicol ketone has been corrected. The synthesis of nitrosochloramphenicol-1-^{3}H is described.

Key Words: Chloramphenicol-1-^{3}H, Nitrosochloramphenicol-1-^{3}H, Epimer separation

INTRODUCTION

Among the side effects of D-threo-chloramphenicol (CAP) (1a) administration to humans, the rare but devastating occurrence of bone marrow aplasia, as distinct from the more common reversible hematopoietic depression, was delineated by Yunis and Bloomberg [2] and is now well-recognized [3]. The lack of observations of aplastic anemia in connection with the close analog thiamphenicol (which differs from CAP by the substitution of the p-nitro group by a methylsulfonyl moiety) has been taken as evidence of the involvement of the nitro group in this irreversible toxicity [4]. Recent efforts have been aimed at the elucidation of metabolic pathways of CAP which may lead to such an effect [5], and the nitroso derivative (NO-CAP, 2a) [6] has been implicated as a possible activated intermediate. NO-CAP has recently been shown to be a much more potent inhibitor of DNA synthesis than is CAP, and it causes irreversible inhibition of cell growth, and cell death, at low concentrations in bone marrow cell culture [7].

To facilitate studies of the mechanism of toxicity and of the mechanism of action of CAP, the synthesis of relatively large quantities of tritium-labeled CAP and NO-CAP of high specific activity was undertaken.

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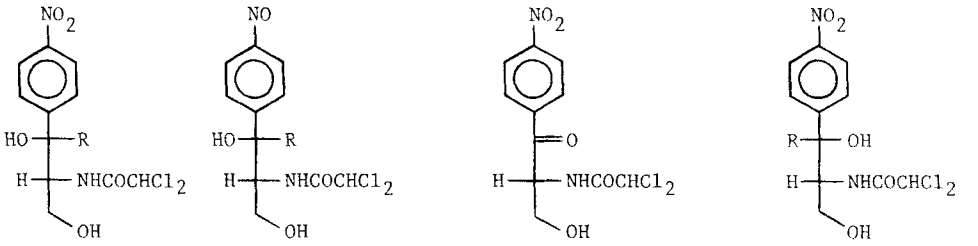
RESULTS AND DISCUSSION

Tritiated CAP has usually been procured by the reduction of D-α-dichloroacetylamino-β-hydroxy-p-nitropropiophenone (CAP ketone, 3) with a tritiated hydride source. This produces a mixture of CAP-1-^{3}H (1b) and epi-CAP-1-^{3}H (4b),



1 2 3 4

a: R = H b: R = ^{3}H

from which 1b has been separated by CAP-seeded fractional recrystallizations [5], reverse phase HPLC of small quantities [8], or preparative TLC separation of derivatives obtained by hydrolysis of the dichloroacetyl group followed by triacetylation [9]. These methods were unsuitable for the present work because of unwanted dilution of label, the limited amounts of product which could easily be produced, or the large number of synthetic steps required. In addition, no previously reported procedure made efficient use of labeled starting material.

In the present work it was found that a mixture of acetonides--1,3-dioxanes, often used in the protection of 1,3-glycol systems [10]--could be formed from the mixture of 1 and 4 produced in a $Ca(BH_4)_2$ reduction of CAP ketone. The large chromatographic difference between them allowed for their easy separation by chromatographic means. The separated acetonides can be quantitatively deprotected under proper conditions to return the separated epimers 1 and 4 in high purity.

The synthesis scheme developed for preparation of CAP-1-^{3}H and NO-CAP-1-^{3}H is shown in Figure 1.

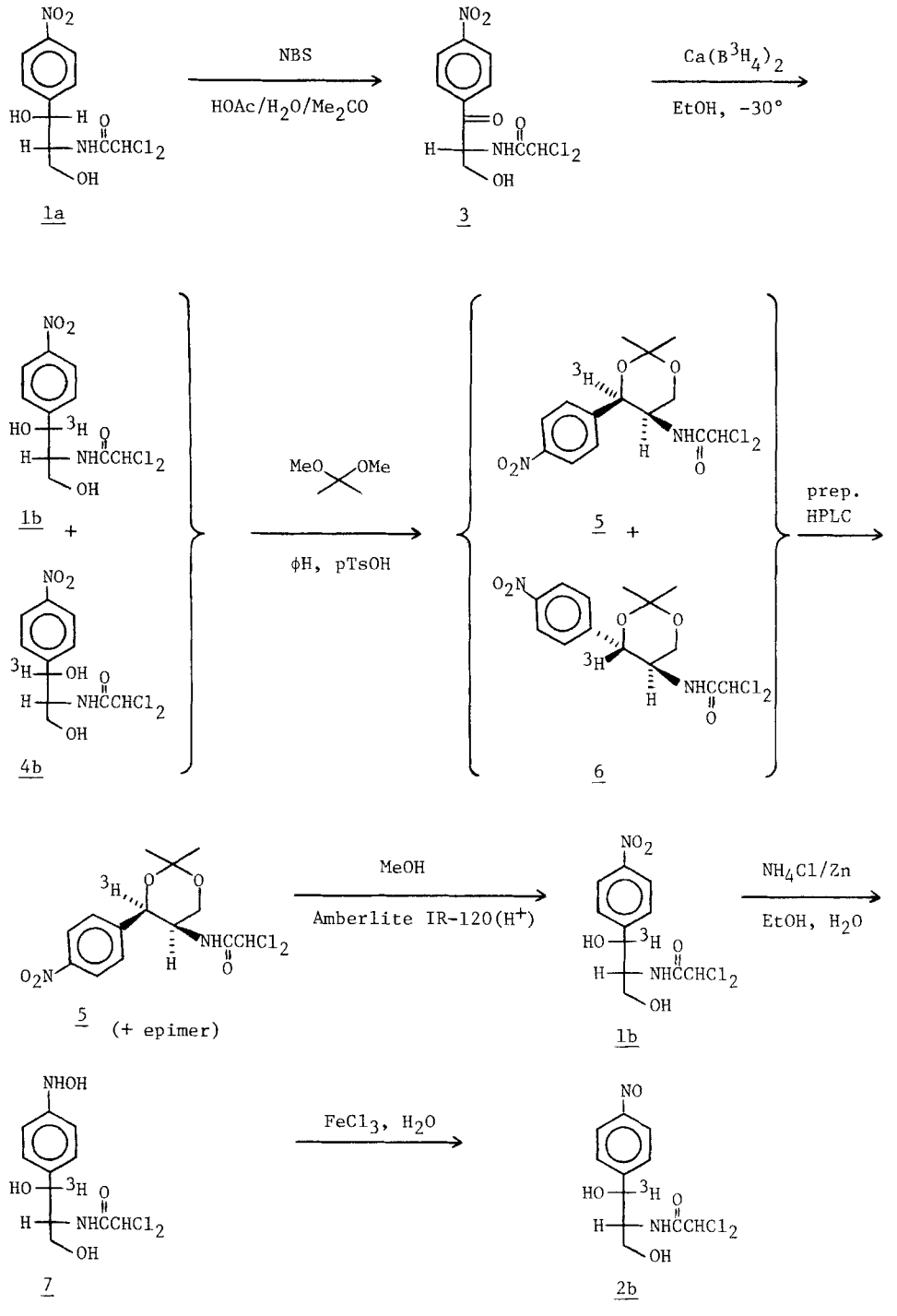


Figure 1. Synthesis of D-threo-Chloramphenicol-1-^{3}H and D-threo-Nitrosochloramphenicol-1-^{3}H

Initially, the N-bromosuccinimide oxidation of commercial CAP ($[\alpha]_D^{25}$ = +18.4° (c = 2.5, EtOH)) using the procedure of Kutter and Machleidt [1] yielded CAP-ketone (3) possessing a specific rotation of near zero, contrary to the value reported by these authors (+20.8° (c = 2.5, EtOH)). Variations on this oxidation procedure were made in efforts to speed up the reaction (higher temperatures or exposure to UV light) or to remove the HBr formed in the reaction (two-phase reaction over $NaHCO_3$ or continuous argon bubbling) in attempts to minimize possible acid-catalyzed enolization (and therefore racemization) of the product ketone. In eight batches of CAP-ketone produced under various conditions, however, the specific rotations were never observed to exceed +11.2° (c = 2.5, EtOH).

Because the reported specific rotation of CAP-ketone now seemed in doubt, one sample of the ketone, having $[\alpha]_D^{25}$ = +9.8° (c = 2.5, EtOH), was subjected to $CaBH_4$ reduction as shown in Figure 1 using unlabeled $Ca(BH_4)_2$, and the synthetic D-threo-CAP was isolated through the acetonide derivatization procedure (discussed below), was purified, and its specific rotation measured. The ratio of this figure, +15.0°, to that (+18.4°) of the starting D-threo-CAP, indicated the degree of racemization which occurred during the synthetic sequence. It has been demonstrated in these laboratories that the protection-deprotection sequence (1 → 5 → 1) causes insignificant (< 2%) isomerization of a pure CAP standard. Moreover, it has been reported [1] that the $Ca(BH_4)_2$ reduction of CAP-ketone under these conditions does not result in significant isomerization at the position α to the original carbonyl group (C2). Therefore, it is concluded here that the reduction of optical purity observed in the synthetic CAP relative to starting CAP (15.0 ÷ 18.4 = 0.82) occurred during the oxidation of CAP to CAP-ketone, and thus that the maximum specific rotation of CAP-ketone, as calculated from these results, cannot exceed +9.8° ÷ 0.82 = +12.0°. Indeed, if some isomerization had taken place during the $Ca(BH_4)_2$ reduction, then the maximum specific rotation of CAP-ketone must be even less than +12.0°.

Attempts were made, without success, to carry out the benzylic oxidation of CAP to CAP-ketone under neutral conditions, using MnO_2 in acetone [11], and DDQ [12] and o-chloranil in both benzene and benzene/methanol. The procedures which

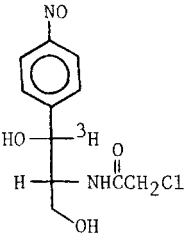
resulted in product of highest optical rotation involved reaction with the NBS/ $HOAc/H_2O/Me_2CO$ system exposed to a weak ultraviolet source for 2-4 h at room temperature, followed by workup in the absence of strong base. It was observed that the optical purity of individual batches varied considerably with small changes in reaction and workup conditions. Failure to confirm the optical purity of this product before its use in the synthesis of labeled CAP may thus result in material which is partially or totally racemized, and it is conceivable that perturbations of experimental data may result from the use of such material in biochemical experiments.

Reduction of CAP-ketone samples using $Ca(BD_4)_2$ prepared from 0.25 molar equivalent (based on ketone) of $NaBD_4$ (98% D) produced high yields of CAP-1-D and epi-CAP-1-D mixtures. Derivatization of these isomer mixtures using acid-catalyzed exchange with 2,2-dimethoxypropane [13] proceeded quantitatively to give a mixture of epimeric acetonides 5 and 6 (substitute ^2H for ^3H). Gram quantities of such an epimeric mixture can easily be separated by open column chromatography (Grade IV silica gel eluted with $CHCl_3$). Alternatively, HPLC separation of the deuterated acetonide mixture (SiO_2, 2:3 ethyl acetate/hexane) and NMR analysis of the individual isomers showed ≧ 95 atom % deuterium at the benzylic position, establishing the efficient incorporation of label into the substrate.

A portion of CAP-ketone of $\alpha_D^{25} = 11.2°$ (c = 2.5, EtOH) (≧ 97% R,R isomer) was treated at -30° with $Ca(B^3H_4)_2$ produced from 0.017 molar equivalent (based on ketone) of NaB^3H_4, specific activity 18 Ci/mmole. After completing the reduction by addition of excess unlabeled $NaBH_4$, 4.65 Ci of a mixture of 1b and 4b was obtained from 5 Ci of NaB^3H_4. The derivatization of this isomer mixture as described above proceeded quantitatively to give a mixture of epimeric acetonides 5 and 6 which were separated easily by HPLC to give 2.61 Ci of 5 and 1.26 Ci of 6. Deprotection of the former using Amberlite IR-120(H^+) in methanol by the procedure of Bongini et al. [14], yielded 2.6 Ci (2.60 Ci/mmole) of D-threo-CAP-1-^{3}H (1b) of ≧ 97% purity, without dilution of label from NaB^3H_4 and in an overall radiochemical yield of 52%. This procedure represents by far the most efficient synthesis of labeled D-threo-chloramphenicol reported, and is the only practical

procedure for the preparation of multiCurie quantities of high specific activity material.

Portions of the D-threo-CAP-1-^{3}H (diluted to a specific activity of 1.12 Ci/mmole), as well as unlabeled CAP, were converted to NO-CAP (2) (see Figure 1) using the procedure of Corbett and Chipko [6]. These reactions as performed in this laboratory, in addition to providing a 10-20% yield of NO-CAP when purified by SiO_2 column chromatography using ethyl acetate, gave a by-product in 30-50% yield which was determined by its blue color in solution and its positive pentacyanoammine ferroate reaction to contain a C-nitroso group. Its UV-visible and infrared spectra were identical to NO-CAP [6]; however, its mass spectrometric fragmentation suggests the structure 8, 1-(p-nitrosophenyl)-2-chloroacetylamino-1,3-propanediol. The material may arise in the first step in the conversion of CAP to NO-CAP, the Zn/NH_4Cl reduction to NHOH-CAP, 7.



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EXPERIMENTAL

Chemicals

D-threo-Chloramphenicol was supplied by Aldrich Chemical Company. Sodium boro[^{3}H]hydride was obtained from the Radiochemical Centre, Amersham, England.

Apparatus

Radioactivity measurements were made using a Packard Model 2425 liquid scintillation counter, with Liquifluor scintillant (New England Nuclear). Radiochromatogram scans were accomplished with a Packard Model 7201 scanner, and autoradiograms using LKB Ultrofilm (LKB-Produkter AB, Bromma, Sweden). Preparative HPLC was performed on a Waters System 500, and peak detection was by differential

refractive index. NMR analyses were performed on a Varian EM360-A, IR analyses on a Beckman Acculab I, and UV analyses on a Varian SuperScan 3 instrument. Mass spectral thermograms were recorded at 70 ev using a Finnigan 4000 interfaced with an Incos 2300 data system. Optical rotations were measured using a Perkin-Elmer Model 141 with a 1-dm pathlength cell at 589 nm wavelength.

D-α-Dichloroacetylamino-β-hydroxy-p-nitropropiophenone (3)

The basic method of Kutter and Machleidt [1] was used. The product of maximum optical purity was obtained by the following procedure. To a solution of chloramphenicol (5.0 g, 15.4 mmoles) in 125 ml acetone were added 12 ml water and 5 ml acetic acid. This solution, at room temperature and with continuous argon bubbling, was treated with 4.0 g (22.5 mmoles) N-bromosuccinimide freshly recrystallized from water. The reaction mixture was exposed to a weak ultraviolet source until initiation of reaction was indicated by development of a yellow color (ca. 30 min). After 2.5 h the organic solvent was removed in vacuo at ≦ 25°, and the residue taken up in ethyl acetate and washed successively with water, saturated $NaHCO_3$, water, and saturated NaCl (2X). After drying over Na_2SO_4, the solution was stripped of solvent in vacuo, and the product was recrystallized three times from ether. Resulting was 1.7 g (34%) CAP-ketone, m.p. 123.5-124.5° (lit. [1] 124-125°), $[\alpha]_D^{25}$ = +11.2° (c = 2.5 g/100 ml EtOH) (lit. [1] +20.8°). Other spectral data were consistent with the proposed structure.

In other experiments it was noted that the optical purity of the product generally decreased with longer reaction times or higher reaction temperatures. Elimination of the base extraction during workup had no effect on the product's specific rotation. Attempted oxidations of chloramphenical by the use of manganese dioxide [11], DDQ [12] and o-chloranil under various conditions gave no reaction.

$Ca(B^3H_4)_2$ Reduction of CAP-Ketone

The reaction was carried out using essentially the procedures of Pohl and Krishna [5], derived from Levai et al. [15], and modified as follows: Sodium boro[^{3}H]hydride (5 Ci, 18 Ci/mmole, 0.28 mmole) was transferred into a flask containing an argon atmosphere, using 2.5 ml absolute ethanol, and the flask was

quickly cooled to -30° using a dry ice/bromobenzene bath. The stirred suspension was treated with a solution of $CaCl_2 \cdot 2H_2O$ (20.6 mg, 0.14 mmole) in 1 ml ethanol for 1 h at -30°. A solution of CAP-ketone (6 equiv., 1.68 mmoles, 543 mg, $[\alpha]_D^{25}$ =11.2° (c = 2.5, EtOH)) in 8 ml ethanol was added dropwise. After continued stirring for 1.5 h, TLC analysis (SiO_2, EtOAc) indicated reduction of about two-thirds of the starting ketone. Solid $NaBH_4$ (20 mg) was then added, and after stirring a further 30 min all ketone had been consumed (TLC analysis). The cooling bath was removed and 1 ml acetone was added, followed after 30 min with 1 ml 1 N HCl. Solvent was removed under reduced pressure, and the residue was taken up twice with ethanol and the solvent evaporated to remove any labile tritium. Finally, the product was dissolved in ethyl acetate and the solution washed, dried (Na_2SO_4) and evaporated to give 660 mg, 4.65 Ci, of a mixture of D-threo-CAP-1-^{3}H and its 1-epimer as a slightly yellow oil.

D-threo-Chloramphenicol-1-^{3}H (1b)

To the mixture of tritiated chloramphenicol epimers was added 22 ml benzene and 3 ml 2,2-dimethoxypropane [14]. The suspension was warmed to 70°, and 2 mg solid p-toluenesulfonic acid was added. Immediate vigorous reflux and rapid dissolution of the substrate indicated acetonide formation, and after 3 min the solution was cooled, washed with saturated $NaHCO_3$, then saturated NaCl, dried over Na_2SO_4, and evaporated to yield 745 mg, 4.68 Ci, of slightly yellow oil. Thin layer chromatographic analysis (SiO_2, EtOAc) revealed two components: one (in slight excess as visualized by UV) corresponded to a standard of the acetonide of D-threo-chloramphenicol (R_f = 0.53) and the other appeared at R_f 0.58 (SiO_2, 4:96 EtOH/$CHCl_3$, R_f's are 0.42 and 0.27, respectively.) Preparative HPLC separation of the mixture was accomplished using one Waters PrepPak 500/Silica cartridge and the solvent system ethyl acetate/hexane (2:3) at a flow rate of 100 ml/min to give epi-CAP-1-^{3}H-acetonide (6) (R_v = 4.6 column volumes, 1.26 Ci) and CAP-1-^{3}H-acetonide (5) (R_v = 8.4 column volumes, 2.61 Ci). These products were chromatographically indistinguishable from unlabeled samples prepared in the same manner.

cis-5-Dichloroacetylamino-2,2-dimethyl-4-(p-nitrophenyl)-1,3-dioxane (CAP acetonide); m.p. 149-150°; NMR (CD_3OD): δ 1.61 (s, 3H, methyl), 1.64 (s, 3H,

methyl), 4.12 (ddd, J = 30, 12 and 2, 2H, C3 methylene), 4.26 (∿ quint, 1H, C2H), 5.50 (d, J = ½, 1H, C1H), and 7.89 (dd, 4H, ArH); mass spectrum (70 ev): m/e 347 (m - 15), 211 ($C_7H_{11}Cl_2NO_3$), 153 ($C_4H_5Cl_2NO$), 70 (C_3H_6O), 43 (C_2H_2O); IR (nujol): cm^{-1} 3420 (NH), 1700 (C=O), 1505, 1370 (NO_2); UV max 285 (ε = 10,543). Analysis: Calc. for $C_{14}H_{16}Cl_2N_2O_5$: C, 46.30%; H, 4.44%; Cl, 19.52%; Found: C, 46.27%; H, 4.42%; Cl, 19.64%.

trans-5-Dichloroacetylamino-2,2-dimethyl-4-(p-nitrophenyl)-1,3-dioxane (epi-CAP acetonide); m.p. 177.5-178.5°; NMR (CD_3OD): δ 1.45 (s, 3H, methyl), 1.62 (s, 3H, methyl), 3.89 (d, J = 3, 2H, C3 methylene), 3.94 (m, 1H, C2H), 5.00 (dd, J = 6 and 3, 1H, C1H), 7.89 (dd, 4H, ArH); mass spectrum (70 ev): m/e 347 (m - 15), 211 ($C_7H_{11}Cl_2NO_3$), 153 ($C_5H_5Cl_2NO$), 70 (C_3H_6O), 43 (C_2H_2O); IR (nujol): cm^{-1} 3260, 3080 (amide), 1660 (C=O), 1550, 1370 (NO_2); UV max 285 (ε = 11,018). Analysis: Calc. for $C_{14}H_{16}Cl_2N_2O_5$: C, 46.30%; H, 4.44%; Cl, 19.52%; Found: C, 46.45%; H, 4.53%; Cl, 19.61%.

The CAP-1-^{3}H acetonide sample (383 mg, 2.61 Ci, 2.47 Ci/mmole) was stirred [15] in 8 ml methanol with 0.5 meq methanol-washed Amberlite IR-120(H^+). After 15 h the mixture was filtered through a Celite pad and the filtrate evaporated to yield 324 mg, 2.60 Ci (52% overall radiochemical yield) (2.6 Ci/mmole) D-threo-chloramphenicol-1-^{3}H as a slightly yellow solid, which by TLC-radiochromatogram scanning and autoradiography in three solvent systems was ≧ 97% pure.

(1R,2R)-1-p-Nitrosophenyl-2-dichloroacetylamino-1,3-propanediol-1-^{3}H (2b)

The basic procedure of Corbett and Chipko [6] on unlabeled material was utilized. D-threo-CAP-1-^{3}H (328 mg, 1.12 Ci) was suspended in 1 ml EtOH; solid NH_4Cl (110 mg, 2.04 mmoles) was added, and water (3 ml) was added dropwise to the vigorously stirred mixture. Powdered zinc (260 mg, 4 mmoles) was added in small portions over 15 min, and the reaction mixture was stirred an additional 15 min. The mixture was filtered and the filter cake washed with 3 ml water. TLC analysis (SiO_2, EtOAc) of the filtrate showed complete consumption of starting material. The filtrate was added to a cooled (0°) solution of $FeCl_3 \cdot 6H_2O$ (4.1 mmoles) in 5 ml water. After 30 min stirring the solution was extracted four times with ethyl acetate, and the combined organics were dried (Na_2SO_4) and evaporated *in vacuo* to

yield 247 mg (704 mCi) dark green oil. The product was applied to a column (2 x 24 cm) of silica gel and eluted with ethyl acetate to yield 111 mCi of nitroso-chloramphenicol-1-^{3}H (1.12 Ci/mmole) as a light blue solid (chromatographic and spectral characteristics as previously reported for unlabeled material [6]) and 381 mCi of a later eluting blue compound tentatively identified as 1-p-nitroso-2-chloroacetylamino-1,3-propanediol-1-^{3}H (1.13 Ci/mmole), UV (EtOH): 287 nm (ε = 79,400), 315 nm (ε = 98,700); IR (nujol): cm^{-1} 3400 (OH), 1690 (C=O), 1540 (N=O); mass spectrum (70 ev): m/e 288 (M^+), 270 (m - 18), 257, 259 (m-CH_2OH), 153 (O_2NArCH_2OH), 136, 138 ($HOCH_2C^+HNHCOCH_2Cl$), 119, 121 (CH_2^+-$^\cdot$$CHNHCOCH_2Cl$).

Repetitions of this procedure on unlabeled material gave similar results; the two products are easily separable on TLC (Merck Silica Gel 60, F254) developed in ethyl acetate: nitroso-CAP, R_f = 0.25; dechloro-nitroso-CAP, R_f = 0.17.

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