SYNTHESIS OF CARBON-14 LABELED CI-926 AND CI-927, NEW ANTIHYPERTENSIVES[1]

Sheryl J. Hays
Warner-Lambert/Parke-Davis Pharmaceutical Research
Ann Arbor, Michigan 48105

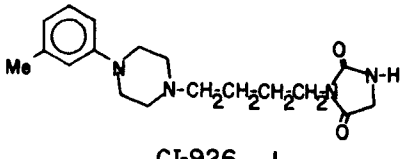
SUMMARY

3-[4-[4-(3-Methylphenyl)-1-piperazinyl]butyl]-2,4-imidazolinedione, CI-926 (1), and 3-[[4-4-(3-ethoxyphenyl)-1-piperazinyl]butyl]amino]-5,5-dimethyl-2-cyclohexen-1-one, CI-927 (2) are new antihypertensive agents which have been carbon-14 labeled in three steps starting from ethylene-$^{14}C_2$ oxide (5). The overall radiochemical yields for 1 and 2 were 69% and 66% respectively. The specific activity for both compounds was initially 22.6 mCi/mmol before final dilution.

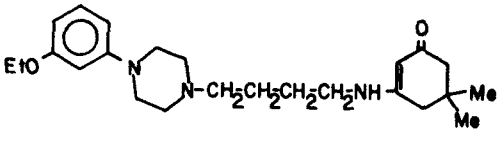
Key Words: Carbon-14, CI-926, CI-927, Antihypertensive

INTRODUCTION

3-[4-[4-(3-Methylphenyl)-1-piperazinyl]butyl]-2,4-imidazolinedione, CI-926 (1), and 3-[[4-[4-(3-ethoxyphenyl)-1-piperazinyl]butyl]amino]-5,5-dimethyl-2-cyclohexen-1-one CI-927 (2), are novel antihypertensive agents which function in part through interaction with alpha adrenoreceptors.[2-4] To facilitate the preclinical pharmacokinetic and metabolic studies[5] of these drugs in animals, the carbon-14 labeled compounds were synthesized. The synthesis of the labeled compounds is described herein.



CI-926 1



CI-927 2

RESULTS AND DISCUSSION

The initial unlabeled synthesis of both CI-926 (1) and CI-927 (2)[6] were not amenable for the preparation of the radiolabeled material and new

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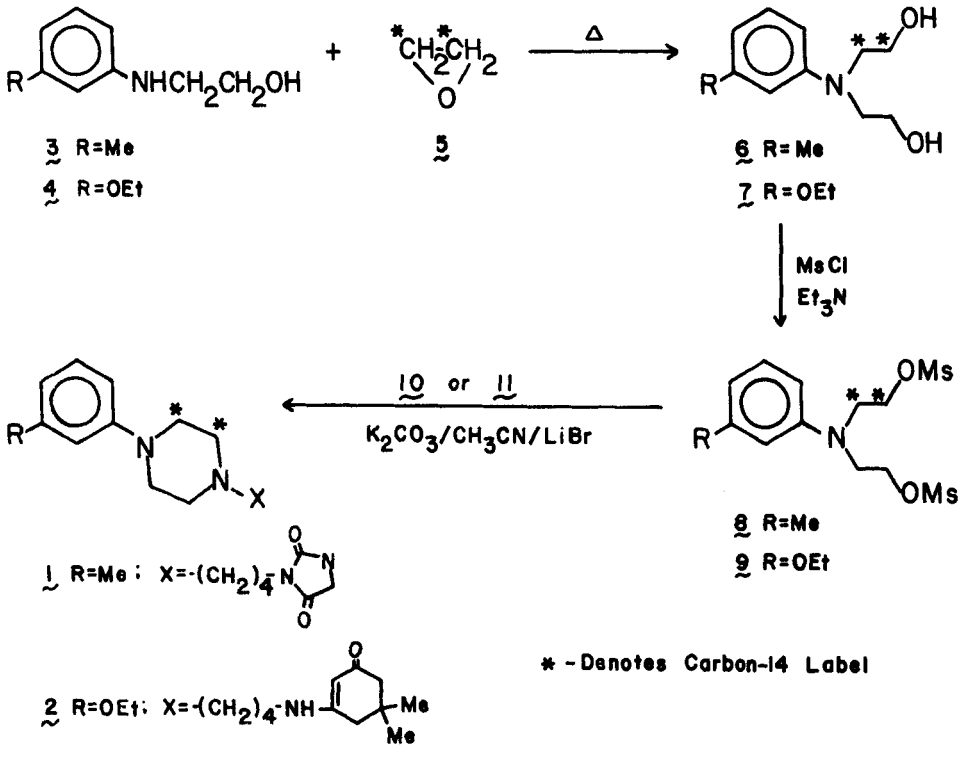
syntheses were designed as shown in Scheme 1. The appropriate unlabeled 2-phenylaminoethanol 3 or 4 and carbon-14 labeled ethylene oxide (5) were combined and heated. The intermediate diols 6 or 7 were produced in radiochemical yields of 77% and 85% respectively. The diols 6 or 7 were treated with methanesulfonyl chloride to produce the dimesylates 8 or 9.

Scheme 1

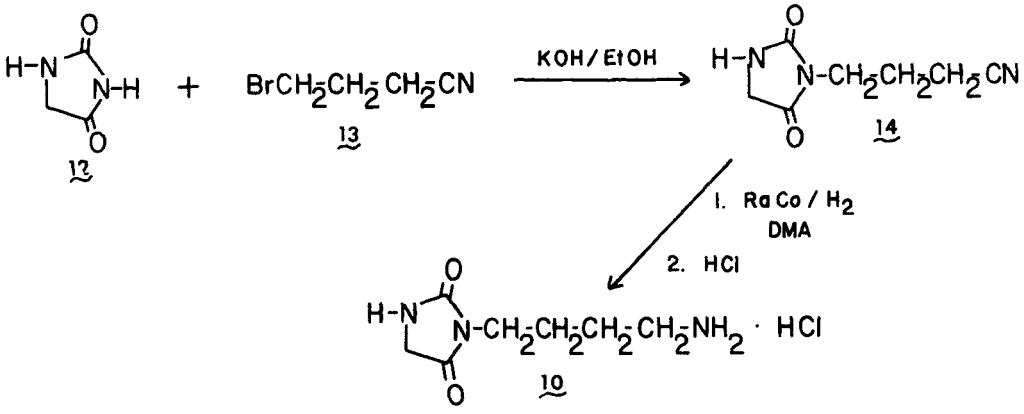


The radiochemical yield for the dimesylate 8 was 96%, while dimesylate 9 was produced in 91% yield. Addition of the dimesylate 8 to lithium bromide, potassium carbonate and the 3-(4-aminobutyl)-2,4-imidazolidinedione (10) in refluxing acetonitrile afforded a 93% yield of CI-926 (1). Similar reaction conditions utilizing dimesylate 9 and the primary amine, 3-[(4-aminobutyl)-amino]-5,5-dimethyl-2-cyclohexen-1-one (11) resulted in an 85% radiochemical yield of CI-927 (2). Anhydrous sodium sulfate was added to this last step of the CI-927 synthesis. This reduced the hydrolysis of the otherwise stable enamine functionality of 2. The overall radiochemical yields for CI-926 and

CI-927 were 69% and 66% respectively. The specific activity for both compounds was approximately 22.6 mCi/mmol before final dilution.

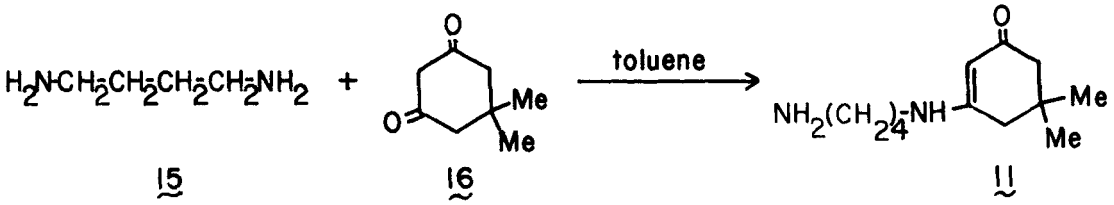
The unlabeled primary amine 10 for the synthesis of CI-926 (1) was synthesized by alkylation of hydantoin (12) to produce 4-bromobutanenitrile (13) as the only product. The nitrile 14 was subsequently hydrogenated with Raney cobalt and converted to the hydrochloride salt as shown in Scheme 2. The three step process produced the desired intermediate 10 in an overall 4.2% yield. No efforts were made to optimize the yields for these steps.

Scheme 2



Addition of 1,4-butanediamine (15) and 5,5-dimethyl-1,3-cyclohexanedione (16) in refluxing toluene produced the unlabeled primary amine 11 in an 81% yield as shown in Scheme 3. This intermediate was used for the synthesis of CI-927 (2).

Scheme 3



EXPERIMENTAL

Melting points were determined on a Thomas Hoover capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were determined on a Varian XL-300 (300 MHz) spectrometer. Chemical shifts were reported in (ppm) downfield from tetramethylsilane. Liquid scintillation counting was performed

with a Packard Tricarb 4530 liquid scintillation counter using Mallinckrodt Handifluor liquid scintillation cocktail. Ethylene-$^{14}C_2$ oxide (22.6 mCi/mmol) was purchased from Amersham. The unlabeled chemicals, 2,4-imidazolidinedione (hydantoin), 4-bromobutanenitrile, 1,4-butanediamine (putrescine) and 5,5-dimethyl-1,3-cyclohexanedione were purchased from Aldrich Chemical Company. 1-Octanesulfonic acid sodium salt was purchased from Eastman Chemical Company. Thin layer chromatography plates were radiochemically analyzed using a Berthold LB 2832 automatic TLC-linear analyzer. High pressure liquid chromatography (HPLC) was performed using a Spectra Physics SP 8700 solvent delivery system, Kratos Spectroflow 773 variable wavelength UV detector, Hewlett-Packard 3390A integrator and Packard Tricarb RAM 7500 radioactivity monitor.

2,2'-[(3-Methylphenyl)imino]bisethanol-1,2-$^{14}C_2$ (6).--2-[(3-Methylphenyl)-amino]ethanol (3) was synthesized according to the method of Yur'ev, et al.[7] The alcohol 3 (360 mg, 2.38 mmol) was placed in a 1 mL Wheaton V-vial with stirring bar and adapted to a stopcock via a Wheaton (13-425 to 13-425 screw thread) connector. The stopcock was attached to a vacuum manifold. The ethylene-$^{14}C_2$ oxide (21.0 mCi, 22.6 mCi/mmol, 0.93 mmol) was attached to the manifold in a breakseal tube and the ethylene oxide was transferred to the reaction vessel by static vacuum distillation using liquid nitrogen cooling. The stopcock was closed after the transfer and the reaction was warmed to 25°C. The stopcock and vial were removed from the manifold and inverted several times to mix the two components. The reaction was heated to 105°C for 4 h, cooled to 25°C and stirred an additional 18 h. The crude reaction product was separated from remaining starting material by flash chromatography on a silica gel column (20 x 3 cm) eluted with toluene/EtOAc/Et_3N (50/50/1). The product was eluted from the column in 260-660 mL of solvent. The eluant was concentrated under reduced pressure and the clear oil (17.1 mCi, 276 mg, 77.0% radiochemical yield) was dried for 18 hours at 25°C under vacuum. A TLC analysis of the diol 6 on silica gel eluted with toluene/EtOAc/Et_3N (250:250:1) indicated that the product was greater than 99% radiochemically pure.

2,2'-[(3-Methylphenyl)imino]bisethanol-1,2-$^{14}C_2$, bis(methanesulfonate) (8).--The diol 6 (17.1 mCi, 276 mg, 0.79 mmol) was dissolved in CH_2Cl_2 (9 mL) and Et_3N (283 mg, 390 µL, 2.8 mmol). Methanesulfonyl chloride (320 mg, 216 µL, 2.79 mmol) was added dropwise over a 2 min period and the reaction was stirred at 25°C for 2 h. The reaction solution was transferred to a separatory funnel and the CH_2Cl_2 solution was washed with ice water (15 mL), 10% $NaHCO_3$ (15 mL) and saturated NaCl (15 mL). The CH_2Cl_2 layer was dried ($MgSO_4$), filtered and concentrated under reduced pressure. The crude material was chromatographed on a BondElut (2.8 mL) silica column eluted with 10 mL of toluene/EtOAc (1:1). The solvent was removed under reduced pressure. A TLC analysis of the purified yellow oil 8 (16.5 mCi, 476 mg, 96.2% radiochemical yield) on silica gel eluted with toluene/EtOAc/Et_3N (5:5:0.1) showed that the dimesylate 8 was 87% radiochemically pure.

3-[4-[4-(3-Methylphenyl)-1-piperazinyl-2,3-$^{14}C_2$]butyl]-2,4-imidazolidinedione (1).--The dimesylate 8 (16.5 mCi, 476 mg, 1.36 mmol), LiBr (245 mg, 2.82 mmol), K_2CO_3 (594 mg, 4.30 mmol), $MgSO_4$ (1.0 g), CH_3CN (25 mL) and 1-(4-aminobutyl)-2,4-imidazolidinedione monohydrochloride (10, 597 mg, 2.87 mmol) were combined in a 50 mL round bottom flask with stirring bar under an argon atmosphere. The reaction was refluxed for 40 h and cooled to 25°C. The solid was collected by vacuum filtration and washed with excess CH_3CN. The filtrate was evaporated to dryness under reduced pressure to produce a yellow oil. This crude residue was flash chromatographed on a silica gel column (22.5 x 2.5 cm) eluted initially with CH_2Cl_2 (200 mL) and then CH_2Cl_2/MeOH (9:1). The solvent was removed under reduced pressure to yield a white solid (15.3 mCi, 240 mg, 92.7% radiochemical yield) with a specific activity of 22.6 mCi/mmol. A TLC analysis on silica gel eluted with CH_2Cl_2/MeOH (9:1) indicated that the product was greater than 99% radiochemically pure. Unlabeled 1 (318 mg) was added to the carbon-14 labeled product and the solid was recrystallized from toluene/hexane (1:1). The solid was collected by filtration and was dried at 25°C in a vacuum oven for 3 days. The final product (9.93 mCi, 412 mg) [mp 104-105°C, unlabeled authentic sample mp 104-105°C] had a specific activity of 8.21 mCi/mmol. Thin layer

chromatography (TLC) was performed using 3 solvent systems described as follows: System 1, Silica gel (E. Merck, 0.25 mm) CH_2Cl_2/MeOH (9:1) R_f=0.42; System 2, Silica gel (E. Merck, 0.25 mm) EtOAc/EtOH/Et_3N (8:2:0.1) R_f=0.24; System 3, LKC 18 F reversed phase (Whatman, 0.20 mm) EtOH/H_2O/Et_3N (7:3:0.1) R_f=0.65. HPLC analysis of the product utilized an Alltech 5 μ C-18 column with the following solvent system: CH_3CN/0.05 M $(NH_4)_2HPO_4$ pH 3.0 (20:80) at a flow rate of 1.0 mL/min. The retention time for the compound was 11.3 minutes using sequential monitoring with UV detector at 214 nm and radioactivity detector. Both radiochemical and chemical purity of 1 were found to be greater than 98% by TLC and HPLC. NMR and IR spectra were identical to those obtained with an authentic sample. IR (KBr): 3270 cm^{-1} (NH); 1760, 1700 cm^{-1} (C=0). ^1H NMR (90 MHz) ($CDCl_3$) 1.62 (m, 4H, C-CH_2-CH_2-C), 2.35 (s, 3H, arom-CH_3), 2.57 (m, 6H, C-N-(CH_2-C)$_2$, N-CH_2-C), 3.20 (m, 4H, Ar-N-(CH_2-C)$_2$)$_2$, 3.59 (m, 2H, C-CH_2-N-C=0), 3.98 (s, 2H, NH-CH_2-C=0), 5.63 (b, 1H, NH), 6.77 (m, 3H, arom. 2H, 4H, 6H), 7.18 (d/d, 1H, arom. 5H).

Anal. calculated for $C_{18}H_{26}N_4O_2$: C, 65.42; H, 7.93; N, 16.96. Found: C, 65.26; H, 8.01; N, 16.86.

2,2'[(3-Ethoxyphenyl)imino]bisethanol-1,2-$^{14}C_2$ (7).--2-[(3-Ethoxyphenyl)-amino]ethanol (4) was synthesized according to the method of Yur'ev et al.[1] The alcohol 4 (430 mg, 2.38 mmol) and ethylene-$^{14}C_2$ oxide (21.0 mCi, 22.6 mCi/mmol, 0.93 mmol) were reacted and worked up as described for compound 6. The crude reaction product was purified from remaining starting material by flash chromatography on a silica gel column (20 x 3 cm) eluted with toluene/EtOAc/Et_3N (250:250:1). The product was eluted from the column in 320-580 mL of solvent. The eluant was concentrated under reduced pressure and the clear oil (19.2 mCi, 191 mg, 0.85 mmol, 85.0% radiochemical yield) was dried for 18 hours at 25°C under vacuum. A TLC analysis of the diol 7 on silica gel eluted with toluene/EtOAc/Et_3N (250:250:1) indicated that the product was greater than 99% radiochemically pure.

2,2'-[(3-Ethoxyphenyl)imino]bisethanol-1,2-$^{14}C_2$, bis(methanesufonate) (9).--The diol 7 (19.2 mCi, 190 mg, 0.85 mmol) was dissolved in CH_2Cl_2 (9 mL)

and Et_3N (244 mg, 336 μL, 2.4 mmol). The methanesulfonyl chloride (197 mg, 134 μL, 1.72 mmol) was added dropwise over a 2 min period. The reaction was run, worked up and purified as described for compound 8. A TLC of the purified yellow oil 9 (17.4 mCi, 292 mg, 90.7% radiochemical yield) on silica gel eluted with toluene/EtOAc/Et_3N (5:5:0.1) showed that the dimesylate was 91% radiochemically pure.

3-[[4-[4-(3-Ethoxyphenyl)-1-piperazinyl-2,3-$^{14}C_2$]butyl]amino]-5,5-dimethyl-2-cyclohexen-1-one (2).--The dimesylate 9 (17.4 mCi, 292 mg, 0.77 mmol), LiBr (143 mg, 1.65 mmol), K_2CO_3 (246 mg, 1.78 mmol), $MgSO_4$ (1.78 g), CH_3CN (25 mL) and 3-[(4-aminobutyl]amino]-5,5-dimethyl-2-cyclohexen-1-one (11, 302 mg, 1.44 mmol) were combined in a 50 mL round bottom flask with stirring under an argon atmosphere. The reaction was refluxed for 31 h and cooled to 25°C. The solid was collected by vacuum filtration and washed with excess CH_3CN. The filtrate was evaporated to dryness under reduced pressure to produce a yellow oil. This crude product was flash chromatographed on a silica gel column (22 x 2.5 cm), eluted for 200 mL initially with CH_2Cl_2 and then CH_2Cl_2/MeOH (9:1). The product eluted in volumes 360-500 mL. The solvent was removed under reduced pressure to yield a white solid (14.9 mCi, 262 mg, 85.6% radiochemical yield) with a specific activity of 22.6 mCi/mmol. Unlabeled 2 (391 mg) was added to the carbon-14 labeled product and the solid was recrystallized from toluene/cyclohexane (1:1). The solid was collected by vacuum filtration and was dried at 43°C in a vacuum oven for 24 hours. The final product (13.1 mCi, 610 mg) [mp 116-117°C, unlabeled authentic sample mp 116-117°C] had a specific activity of 8.54 mCi/mmol. Thin layer chromatography was performed using 3 TLC systems described as follows: System 1, Silica gel (E. Merck 0.25 mm) CH_2Cl_2/MeOH (9:1) R_f=0.13; System 2, Silica gel (E. Merck 0.25 mm) toluene/EtOH (5:5) R_f=0.13; System 3, LKC 18 F reversed phase (Whatman, 0.20 mm) EtOH/H_2O/Et_3N (7:3:0.1) R_f=0.49. HPLC analysis of the product utilized an Alltech 5 μ C-18 column with the following solvent system: CH_3CN/[H_2O (600 mL), HOAc (20 mL), 1-octanesulfonic acid sodium salt (PIC B-8) (23.4 g)] (47:53) at a flow rate of 2.0 mL/min. The retention time for the compound was 4.7 min using sequential monitoring with UV detector at 280 nm and

radioactivity detector. Both radiochemical and chemical purity of 2 were found to be greater than 99% by TLC and HPLC. NMR and IR spectra were identical to those obtained with an authentic sample. IR (KBR): 3250 cm^{-1} (NH); 1550 cm^{-1} (arom). ^1H NMR (300 MHz) ($CDCl_3$): δ 1.05 (s, 6H, CH_3), 1.40 (t, 3H, O-CH_2-CH_3), 1.66 (m, 4H, C-CH_2-CH_2-C), 2.14 (s, 1H, O=C-CH or CH_2-C=C), 2.17 (s, 1H, O=C-CH or CH_2-C=C), 2.41 (t, 2H, N-CH_2-C), 2.58 [t, 4H, C-N-(CH_2-C)$_2$], 3.10 (m, 2H, CH_2-NH), 3.20 [m, 4H, Ar-N-(CH_2-C)$_2$], 4.02 (q, 2H, O-CH_2-C), 5.08 (s, 1H, CH-C=C), 5.17 (s, 1H, NH), 6.45 (m, 3H, arom. 2H, 4H, 6H), 7.15 (t, 1H, arom. 5H).

Analysis calculated for $C_{24}H_{37}N_3O_2$: C, 72.18; H, 9.53; N, 10.27. Found: C, 72.58; H, 9.18; N, 10.38.

3-(4-Aminobutyl)-2,4-imidazolidinedione monohydrochloride (10).--2,4-Imidazolinedione (12) (3.5 g, 35 mmol) was added to absolute ethanol (65 mL) containing KOH (35 mmol). The solution was heated to reflux and the potassium salt began to precipitate out. 4-Bromobutanenitrile (13) (7.15 g, 48 mmol) was added dropwise over 20 min and the solution was refluxed for 2.5 h. The reaction was cooled, the KBr separated by filtration and the solution concentrated under reduced pressure. The crude residue containing starting material and alkylated product was dissolved in CH_2Cl_2 and the solid starting hydantoin 12 was filtered. The CH_2Cl_2 was concentrated under reduced pressure to yield the nitrile 14 (1.60 g, 27.4%) (mp 110-112°C). This alkylated product 14 (1.52 g, 9.1 mmol) was dissolved in dimethylacetamide (DMA) (100 mL) containing Et_3N (0.5 mL) and Raney cobalt (0.5 g). The compound was hydrogenated at 1500 psi at 100°C for 17 h. The DMA was removed under reduced pressure at 60°C and the residue (1.5 g) was placed on a Dowex 50 W (H^+ form) ion exchange column and was eluted with 0.1 M HCl (440 mL followed by 0.5 M HCl). The product was eluted in volumes 500-800 mL, concentrated under reduced pressure, and recrystallized from EtOH to yield 10 as a white solid (239 mg, 15.4%) (4.2% for the 2 steps) (mp 180-181°C) IR (KBr): 1710 (C=O). ^1H NMR (D_2O) (90 MHz): δ 1.70 (m, 4H, CH_2), 1.93 (s, 2H, N-CH_2-C=O), 2.95 (m, 2H, N-CH_2), 3.50 (m, 2H, N-CH_2).

Analysis calculated for $C_7H_{13}N_3O_2 \cdot HCl$: C, 40.49; H, 6.79; N, 20.24. Found: C, 40.66; H, 7.00; N, 19.99.

3-[(4-Aminobutyl)amino]-5,5-dimethyl-2-cyclohexen-1-one (11).--1,4-Diaminobutane (15) (19.4 g, 0.22 mol) and 5,5-dimethyl-1,3-cyclohexanedione (16) (2.8 g, 0.02 mol) were dissolved in 50 mL of toluene in a 100 mL round bottom flask fitted with a Dean Stark trap and condenser. The solution was refluxed for 5 h and the reaction was cooled and filtered. The solid (0.17 g) was identified by NMR and IR as the disubstituted adduct, 3,3'-[1,4-butanediylbis(imino)bis-[5,5-dimethyl-2-cyclohexen-1-one. The toluene filtrate was extracted with 0.5 M HCl (4 x 50 mL). The aqueous extracts were made alkaline with 10% NaOH and the H_2O layer was extracted with CH_2Cl_2 (4 x 50 mL). The CH_2Cl_2 layer was dried ($MgSO_4$), filtered and concentrated under reduced pressure to yield a crude mixture of 1,4-diaminobutane and product 11. The mixture was flash chromatographed on a silica gel (21 x 2.5 cm) column eluted with CH_2Cl_2/MeOH/Et_3N (450:50:2.5). The pale yellow solid (3.4 g, 81%) (mp 79-81°C) could be recrystallized from toluene. IR (KBr): 3230 (NH_2) cm^{-1}. ^1H NMR ($CDCl_3$) (90 MHz): δ 1.07 (s, 6H, CH_3), 1.60 (m, 6H, C-CH_2-CH_2-C, NH_2), 2.16 (s, 4H, O=C-CH_2-C, C=C-CH_2-C), 2.72 (t, 2H, C-CH_2-N), 3.07 (q, 2H, NH_2-CH_2), 5.02 (s, 1H, C=CH), 5.45 (b, 1H, C-NH-C). Mass spect. (EI) m/e 210 (M^+, 100), 166 (84).

Analysis calculated for $C_{12}H_{22}N_2O \cdot 0.5\ H_2O$: C, 65.66; H, 10.48; N, 12.77. Found: C, 65.64; H, 10.19; N, 12.61.

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