light-tan broth were discarded (t.l.c. disclosed negligible amount of **5**). Those with dark-brown broth were combined, filtered, and the air-dried mycelium, in a Soxhlet apparatus, extracted 18 h with hexane. The mycelium was then extracted 15–30 times with warm pyridine and the combined pyridine extracts assayed spectrophotometrically at 440 mμ. Products in the concentrated pyridine solution were separated by t.l.c. in solvent *d*. The orange bands, $R_f = 0.2$, from 30 plates were combined and eluted with solvent *h*. The residue from the eluate was dissolved in a small amount of pyridine and applied to a 20 g silica column which had been prepared in ethyl acetate. The column was eluted with ethyl acetate and the main orange fraction furnished crystals which did not melt under 330°. The crystals were identical with synthetic **5** and with the major component of natural **5** by chromatography in 6 solvent systems. The acetate derivatives were also identical. The yields varied from 2–6 g of dry mycelium and 2–10 mg of **5** from 4 l of whole broth.

Acknowledgments

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N-alkyne-*N*-bis(2-chloroethyl)amines[1,2]

GEORGE R. PETTIT AND B. J. DANLEY
Department of Chemistry, Arizona State University, Tempe, Arizona, 85281

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A reaction employing copper(II) chloride has been developed for condensing propargyl alcohol with formaldehyde and bis(2-chloroethyl)amine. The modified Mannich reaction was extended to include condensation of several other α-hydroxyacetylenes of the terminal alkyne class with bis(2-chloroethyl)amine.

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Preparation of actidione acetate and estrone nitrogen mustards (1) has been used to illustrate utility of the Mannich reaction as a mild route to bis(2-chloroethyl)amine derivatives of certain biologically important natural products. A series of model experiments preceding this study clearly emphasized that such reactions involving bis(2-chloroethyl)amine required carefully defined experimental conditions for each broad class of active-hydrogen component. At that time, procedures were developed for Mannich reactions between bis(2-chloroethyl)amine and various ketones (2*a*) or amides (2*b*).[3] Extension to acetylene derivatives of the propargyl alcohol type became the next objective. Encouraging results here should allow extension to several important ethynyl steroids.

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[1] Antineoplastic Agents. XXI. For Part XX, see G. R. Pettit and S. K. Gupta, Can. J. Chem. **45**, 1561 (1967). The present contribution was abstracted in part from the M.S. thesis submitted by B. J. Danley to the Graduate School, Arizona State University, October 1967.

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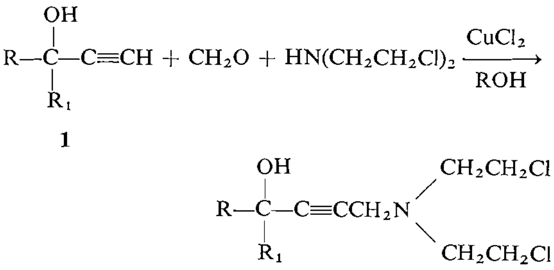
[3] See also reference 3.

In 1933 Mannich and Chang (4) succeeded in condensing phenylacetylene with formaldehyde and diethyl amine in dioxane solution. Subsequently, a number of similar transformations were recorded (5). However, propargyl alcohol and related α-hydroxyacetylenes proved refractory in the usual Mannich reaction and could only be successfully employed as ester or ether derivatives (6). While I. G. Farbenindustrie patents in 1939 (7) disclosed a reaction between acetylene, formaldehyde, and secondary amines in acetic acid containing small amounts of cuprous acetate or cuprous chloride as catalyst, this modification of the Mannich reaction appears to have remained unexplored with α-hydroxyacetylenes until Libman and Kuznetsov (8) described successful Mannich reactions with α-hydroxyacetylenes in the presence of cuprous acetylide or cuprous chloride catalysts. The following year a patent (9) with similar observations was recorded. More recently a study of propargyl alcohol in the Mannich reaction with copper(II) sulfate as catalyst was summarized (10).[4]

No example of a Mannich reaction between bis(2-chloroethyl)amine and a terminal alkyne appears to have been described.[5] A considerable number of experiments (without addition of copper salts) directed at obtaining the Mannich base derived from propargyl alcohol (**1***a*), formaldehyde, and bis(2-chloroethyl)amine were without exception unsuccessful. Those reaction conditions commonly employed in Mannich reactions involving terminal alkynes which seemed feasible[6] with bis(2-chloroethyl)amine and those already known to be useful with this amine in similar condensation reactions (2,3) proved of no value.[7] Substitution of propargyl acetate gave no improvement.

The required transformation (**1** → **2**) was finally achieved as follows. An equimolar mixture of propargyl alcohol and cupric chloride dihydrate in ethyl alcohol was heated at reflux approximately 30 min. The reaction mixture changed in color from dark green to light yellow and a colorless solid separated.[8] Next, 37% formalin (1.25 mole) and bis(2-chloroethyl)amine hydrochloride (0.5 mole) were added and heating at reflux continued 1 h. An extended cooling period resulted in separation of amine **2***a* hydrochloride accompanied by copper containing products. Dissolution of the solid phase in ammonium hydroxide and subsequent reconversion of amine **2***a* to the hydrochloride salt provided 62% of the required Mannich base. Application of a similar (*t*-butyl alcohol as solvent) technique

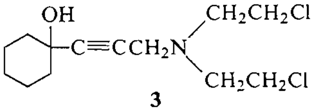


$$\mathrm{R{-}\overset{OH}{\underset{R_1}{C}}{-}C{\equiv}CH + CH_2O + HN(CH_2CH_2Cl)_2 \xrightarrow[ROH]{CuCl_2}}$$

1

$$\mathrm{R{-}\overset{OH}{\underset{R_1}{C}}{-}C{\equiv}CCH_2N(CH_2CH_2Cl)_2}$$

2*a*, $\mathrm{R = R_1 = H}$
2*b*, $\mathrm{R = R_1 = CH_3}$
2*c*, $\mathrm{R = CH_3CH_2—, R_1 = CH_3}$



3

with three tertiary alcohols (eg., 1-ethynylcyclohexanol) provided Mannich base nitrogen mustards **2***b*, **2***c*, and **3** in 85, 75, and 49% conversion,

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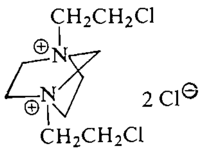
[4] A useful mechanistic interpretation of the Mannich reaction with propargyl alcohol and rationale for use of copper salts in such reactions is also summarized in ref. 10.

[5] However, one such amine (cf. *i*) has been obtained by chlorination of the diol derived from propargyl bromide and diethanol amine (11).

$$\mathrm{RC{\equiv}CCH_2N(CH_2CH_2Cl)_2}$$

i

[6] For example, condensation of formaldehyde with bis-(2-chloroethyl)amine readily leads to 1,4-diazabicyclo-[2·2·1]heptane (*ii*) (12). Additional evidence for the structure of quarternary salt *ii* has recently been provided by Böhme and Orth (13).



ii

[7] The same result was obtained by Dr. J. A. Settepani with 1-ethynylcyclohexanol.

[8] Reduction of copper(II) to copper(I) under similar conditions has been proposed previously (8). The Russian chemists (8) noted that when the Mannich reaction with α-hydroxyacetylenes was performed with the free amine, copper(I) or copper(II) gave similar yields of Mannich base but under acidic conditions, copper(II) resulted in smaller conversion. The latter observation suggested that before a Mannich reaction could occur, copper(I) was necessary. Although the carbanion derived from a cuprous acetylide seemed an attractive intermediate in the present study, a more elaborate mechanism involving a π-bonded copper complex (14) was not excluded.

respectively. Purity of the Mannich base hydrochlorides was ascertained by thin-layer chromatography. The assigned structures received support from elemental analyses and interpretation of their proton magnetic resonance spectra.

In summary, the copper salt modification of the Mannich reaction seems particularly suited to a 1-alkyne bearing electron releasing substituents at position-3.

Experimental

The acetylene intermediates with the exception of propargyl alcohol (from J. T. Baker) were supplied (we wish to thank Dr. R. Tedeschi) by Airco Chemical, Middlesex, New Jersey. Each reaction was performed with magnetic stirring under nitrogen. Solvent extracts of aqueous solutions were dried over magnesium sulfate. Analytical specimens were colorless and exhibited one spot on a thin-layer chromatogram (95:5 chloroform–ethanol mobile phase). The thin-layer chromatographic determinations were executed using microscrope slides coated with silica gel HF_{254} (E. Merck, A. G. Darmstadt, Germany). Plates used to detect bis(2-chloroethyl)amine hydrochloride were eluted with the upper phase from a mixture of 1:4:5 acetic acid-*n*-butyl alcohol-water and developed with iodine vapor.

Melting points were determined using a Thomas Hoover capillary melting point apparatus and are uncorrected. The proton magnetic resonance (Varian A-60 spectrometer, deuterium oxide as solvent and tetramethylsilane as external standard) and infrared (Beckman IR-12 spectrophotometer) spectra were recorded by Miss K. Reimer of these laboratories. The atomic absorption spectra (Perkin-Elmer model 303 instrument) were determined (sample dissolved in concentrated nitric acid) by David Nava of this Department. Elemental microanalyses were provided by Dr. A. Bernhardt, Max Planck Institut, Mülheim, Germany.

4-[Bis(2′-chloroethyl)amino]-2-butyn-1-ol (2a) Hydrochloride

A general study of the sequence of adding reactants, time and temperature, mole ratios of reactants, and 37% formalin vs. trioxymethylene led to the following choice of experimental conditions. A mixture of propargyl alcohol (9.0 g, 0.16 mole) and cupric chloride dihydrate (27.3 g, 0.16 mole) in absolute ethanol (120 ml) was heated at reflux approximately 30 min. During heating the solution changed from dark green to light yellow and a colorless solid separated. Next, 37% formalin (16.2 g, 0.20 mole) and bis(2-chloroethyl)amine hydrochloride (14.3 g, 0.08 mole, ref. 15) were added to the mixture. Following a 1 h period at reflux, the solution was placed in a refrigerator 14 days while the crude crystalline product (26.5 g) slowly separated. A 25.2 g portion of the solid was added to cold concentrated ammonium hydroxide (240 ml). The basic solution was extracted with diethyl ether (4 × 100 ml) and the extract was washed with water (100 ml). The dry ethereal extract was treated with hydrogen chloride and the oil which separated was crystallized from acetone-diethyl ether; yield 11.8 g, m.p. 86.0–88.5°. Based upon expected recovery of product from the original 26.5 g of crude solid, the yield was 62% from propargyl alcohol. Three recrystallizations from acetone gave a pure specimen melting at 88.2–88.9°. In another experiment an analytical sample[9] was recrystallized (4 times) from *n*-butyl alcohol to provide crystals melting at 88.5–89.0°; ν_{max} (KBr) 3310 cm^{-1}; p.m.r. response at δ 3.70–4.21 (multiplet, 8 CH_2CH_2 protons), 4.37 (singlet, 4 protons, O—CH_2—C and C—CH_2—N).

Anal. Calcd. for $C_8H_{14}Cl_3NO$: C, 38.96; H, 5.73; Cl, 43.14; N, 5.68. Found: C, 38.85; H, 5.64; Cl, 43.09; N, 5.57.

Examination of the amine components from the liquid phase of the original reaction mixture by thin-layer chromatography indicated that very little, if any, amine 2*a* remained in solution at end of the 14 day cooling period. However, a substantial amount of bis(2-chloroethyl)amine was detected. A similar study of the solid reaction product indicated amine 2*a* hydrochloride as major nitrogen-containing component. The solid products which separated from four successive reaction mixtures were examined (atomic absorption spectroscopy), and found to contain 18.4, 18.7, 18.7, and 20.4% copper, respectively.

5-[Bis(2′-chloroethyl)amino]-2-methyl-3-pentyn-2-ol (2b) Hydrochloride

The following procedure was found satisfactory for the alkyne tertiary alcohols. A mixture composed of 2-methyl-3-butyn-2-ol (13.5 g, 0.16 mole), cupric chloride dihydrate (27.3 g, 0.16 mole), and *t*-butyl alcohol (120 ml) was heated at reflux 1 h. The solution changed from dark green to a medium yellow-green and a substantial amount of colorless solid separated. To the cooled mixture was added 37% formalin (16.2 g, 0.20 mole) and bis(2-chloroethyl)amine hydrochloride (14.3 g, 0.08 mole). Heating at reflux was continued 1 h and during this period a major portion of the colorless solid redissolved. The cool mixture was concentrated to dryness *in vacuo*. Water (100 ml) was added and evaporated (*in vacuo*). The residue was dissolved in cold concentrated ammonium hydroxide (240 ml) and the solution extracted with diethyl ether. The ethereal extract was washed with cold water, dried, and treated with hydrogen chloride. After cooling (2 days), the solid hydrochloride (18.7 g, 85% yield, m.p. 123.5–127.5°) was collected. Three recrystallizations from acetone provided an analytical sample as needles; m.p. 134.5–135°; ν_{max} (KBr) 3370 and 2570 cm^{-1}; and p.m.r. δ 1.54 (singlet, 6 methyl protons), 3.66–4.17 (multiplet, 8 CH_2CH_2 protons), and 4.32 (singlet, 2 protons, C—CH_2—N).

Anal. Calcd. for $C_{10}H_{18}Cl_3NO$: C, 43.73; H, 6.61; Cl, 38.73; N, 5.10. Found: C, 43.61; H, 6.53; Cl, 38.66; N, 5.30.

6-[Bis(2′-chloroethyl)amino]-3-methyl-4-hexyn-3-ol (2c) Hydrochloride

The preceding experiment was repeated employing 3-methyl-1-pentyn-3-ol (15.7 g, 0.16 mole). In this case the crude crystalline amine (2*c*) hydrochloride weighed 17.3 g (75% yield) and melted at 114–118° (sintering from 112°). Three recrystallizations from acetone led to a pure sample

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[9] The analytical sample was dried to constant weight.

of needles; m.p. 137–138.5°; ν_{max} (KBr) 3380 and 2550 cm^{-1}; and p.m.r. δ 1.01 [triplet, 3 methyl (C-1) protons], 1.47–2.00 (partially masked quartet, 2 protons, C-2 methylene, $J = 7$ c.p.s.), 1.51 [singlet, 3 methyl (C-3) protons], 3.64–4.18 (multiplet, 8 CH_2CH_2 protons), and 4.34 (singlet, 2 protons, C—CH_2N).

Anal. Calcd. for $C_{11}H_{20}Cl_3NO$: C, 45.77; H, 6.98; Cl, 36.85; N, 4.85. Found: C, 45.63; H, 6.99; Cl, 36.78; N, 5.05.

1-(1′-Hydroxycyclohexane)-3-[bis(2′-chloroethyl)amino]-1-propyne (3) Hydrochloride

Starting with 1-ethynylcyclohexanol (19.9 g, 0.16 mole) the preceding experiment was repeated. Crystallization of the crude oily amine (3) hydrochloride from acetone provided 12.3 g (49% yield) of needles melting at 116–117.5° (sintering from 114°). Four recrystallizations from ethyl acetate gave an analytical sample as needles; m.p. 129.7–130.6°. After drying at 80° (2 mm) for 48 h, the specimen melted at 125–128°; ν_{max} (KBr) 3370 and 2560 cm^{-1}; p.m.r., δ 1.2–2.2 (broad multiplet, 10 methylene protons), 3.67–4.21 [multiplet, 8 protons, $N(CH_2CH_2Cl)_2$], and 4.38 (singlet, 2 protons, C-CH_2-N).

Anal. Calcd. for $C_{13}H_{21}Cl_3NO$: C, 49.62; H, 7.05; Cl, 33.80; N, 4.45. Found: C, 49.47; H, 7.13; Cl, 33.70; N, 4.55.

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The molecular structure of ryanodol-*p*-bromo benzyl ether[1]

Surendra Nath Srivastava and Maria Przybylska
Division of Pure Chemistry, National Research Council of Canada, Ottawa, Canada

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The molecular structure of ryanodol-*p*-bromo benzyl ether was solved without any chemical assumptions, using three dimensional data obtained by the heavy atom technique. It was found to be identical with the structure proposed by Dr. K. Wiesner, except that the configuration was reversed at one carbon atom. The refinement of atomic parameters with a least-squares method is still in progress. The *R* factor is at present 0.12.

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The structure of ryanodine, isolated from *Ryania speciosa* Vahl, has been deduced from extensive chemical study by Wiesner, Valenta, and Findlay (1). Ryanodine is an ester of pyrrole-α-carboxylic acid with ryanodol. Since this alkaloid has heavy oxygen substitution and complex structure, Dr. K. Wiesner suggested an *x*-ray analysis be done and he supplied authentic crystals of ryanodol-*p*-bromo benzyl ether, $C_{27}H_{37}O_8Br$.

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