trate was concentrated under reduced pressure at a temperature below 40° to a volume of about 5 liters. The fats were extracted from this concentrate with two 4-l. portions of ether. The aqueous layer was concentrated *in vacuo* to a thick, dark brown sirup. This sirup was shaken vigorously with a liter of ethanol. The alcohol was decanted, and a small amount of water added to the residue to reform a thick sirup. This extraction was repeated three times with one-liter portions of ethanol. The alcoholic layers were combined and stored in a cold room. This solution contained 90 mg. of solids/ml. and exhibited intravenous toxicity at 12.5 mg./20-g. mouse. One-liter portions of this solution were concentrated again to a sirup and extracted with 250 ml. of ethanol. The solution was decanted and concentrated *in vacuo*. The thick sirup from the second alcohol extraction was dissolved in 200 ml. of water containing 10 g. of Darco and stirred under an atmosphere of nitrogen. After removal of the charcoal, the yellow filtrate was lyophilized. The sticky residue, weighing 25–30 g., was found to be toxic at 3.0 mg./mouse.[12]

Ion Exchange on IRC-50 Resin.—A solution of 25 g. of the second alcoholic extract in 100 ml. of water was percolated through a column (1.8 × 65 cm.) containing 180 ml. of Amberlite IRC-50 resin in the hydrogen form. The column was washed with water and eluted with 0.1 *N* acetic acid at a rate of 1–2 ml./min. Fractions of 100-ml. volume were collected and assayed. The active fractions, which were found in the range between 700 and 1500 ml. of eluate, exhibited toxicity at 30 to 250 μg./mouse. The product active at 30–100 μg./mouse was dissolved in water and treated with Darco under an atmosphere of nitrogen. After filtration, the solution was adjusted to *p*H 3 with dilute hydrochloric acid and lyophilized. The residue was extracted with absolute ethanol. The yellow oil remaining after concentration *in vacuo* was dissolved in 10 ml. of water and passed again through the column of Amberlite IRC-50 (H^+). The column was washed with water and eluted with 0.1 *N* acetic acid. Successive 100-ml. fractions were collected, and the product, showing toxicity of 15–40 μg./mouse, was found in fractions 2–8. The less toxic products (100–250 μg./mouse) were purified separately with IRC-50 resin to afford material toxic at 20–40 μg./mouse. The overall recovery of activity was 70%.

Paper Strip Analysis.—The strips were run by the chromatocoil technique,[13] using the following solvent mixtures: 4 parts of *n*-butanol plus 1 part of dioxane, saturated with water; *n*-butanol, acetic acid and water in ratios of 4:1:5 (upper phase); methyl ethyl ketone, water and ethyl cellosolve in ratios of 300:706:15; and 95% ethanol and concentrated aqueous ammonia in a ratio of 19:1. In these systems the R_f values for muscarine chloride are, respectively, 0.35, 0.49, 0.19 and 0.50; for choline chloride 0.20, 0.11, 0.11 and 0.44.

Partition Chromatography.—The solvent system used was 4:5:1 *n*-butanol, water and glacial acetic acid. To a well-stirred mixture of 30 g. of Super-Cel and 200 ml. of upper phase, 30 ml. of the lower phase was slowly added. The equilibrated Super-Cel was slurried into a column (1.5 o.d. × 84 cm.). A mixture of 100 mg. of crude muscarine from ion exchange (toxic at 15–25 μg.), 0.5 ml. of water, 0.4 ml. of *n*-butanol and 0.1 ml. of glacial acetic acid and 0.5 g. of Super-Cel was equilibrated with shaking and then slurried with the aid of an excess of the upper phase into the top of the column. The upper phase of the solvent system was passed through the column at a rate of 10 ml./hour. The first 120 ml. of eluate was discarded and then 20-ml. fractions were collected. Each fraction was diluted with water and lyophilized. The residue was dissolved in 3 ml. of 0.02 *N* hydrochloric acid and lyophilized again. Muscarine exhibiting a toxicity of 7–10 μg./mouse was found in the eluates from 160–220 ml. Muscarine chloride was crystallized from a solution in absolute ethanol by treatment with Darco, followed by the addition of acetone. Stout prisms formed on recrystallization from ethanol–acetone mixtures.

Anal. Calcd. for $C_9H_{20}NO_2Cl$: C, 51.54; H, 9.61. Found: C, 50.94; H, 9.79.

Muscarine Chloroaurate.—This salt was prepared from the crystalline chloride. Muscarine chloroaurate melted at 116–119°.

Anal. Calcd. for $C_9H_{20}NO_2 \cdot AuCl_4$: C, 21.06; H, 3.92. Calcd. for $C_8H_{18}NO_2 \cdot AuCl_4$: C, 19.24; H, 3.63. Found: C, 20.98; H, 3.41.

Muscarine Reineckate.—This salt was prepared in the usual manner. No satisfactory analytical data could be obtained on this salt despite repeated recrystallizations from aqueous acetone.

Anal. Calcd. for $C_9H_{20}NO_2 \cdot [Cr(NH_3)_2(SCN)_4]$: C, 31.69; H, 5.32; N, 19.90. Calcd. for $C_8H_{18}NO_2 \cdot [Cr(NH_3)_2(SCN)_4]$: C, 30.11; H, 5.05; N, 20.48. Found: C, 32.50; N, 21.42.

Acetylmuscarine Chloride.—A suspension of 8.2 mg. of muscarine chloride in 0.8 ml. of pyridine and 0.4 ml. of acetic anhydride was agitated at room temperature for 18 hours, during which time the muscarine went into solution. The product was then precipitated by the addition of 10 volumes of ether. The precipitate was washed with ether and dissolved in hot ethyl acetate to which a few drops of ethanol had been added. As the solution cooled, white hygroscopic plates were deposited. This acetyl derivative is soluble in ethanol, acetone and chloroform. An R_f value of 0.35 was found for acetylmuscarine chloride upon paper strip analysis (Whatman No. 1 paper) using the solvent system: 300 ml. of methyl ethyl ketone, 706 ml. of water and 15 ml. of ethyl cellosolve. An O-acetyl determination was carried out by the method of Kunz and Hudson.[14]

Anal. Calcd. for $C_9H_{19}NO_2Cl \cdot Ac$: acetyl, 17.08. Found: acetyl, 17.44.

Hofmann Degradation of Muscarine. A.—A solution of 2.5 mg. of muscarine chloride in 1.0 ml. of water was shaken with 26 mg. of silver oxide for 3 hours and allowed to stand overnight. The excess silver oxide and silver chloride were removed by filtration. The filtrate and the water washings were distilled through a micro apparatus through which a stream of nitrogen was allowed to pass. The distillate was collected in a cooled trap containing 5.0 ml. of 0.1 *N* hydrochloric acid. The distillation was continued until the residue in the flask was concentrated almost to dryness. This residue was acidified with hydrochloric acid and dissolved in methanol. After concentration to dryness, 3.8 mg. of an oil resulted which on crystallization from acetone–ethanol yielded muscarine chloride. No evidence of any volatile amine could be detected by examination of the collection trap.

B.—In a second experiment 14.9 mg. of muscarine chloride was converted to muscarine hydroxide by percolation through Dowex 1 resin in the hydroxyl form. The distillation was carried out in the manner described above except that the residue was dry distilled at 130–140° for one hour. The residue in the distillation flask was shown by paper strip analysis to be muscarine.

Acknowledgment.—The authors are indebted to Mr. Robert Walker for infrared analysis, Mr. R. N. Boos for microanalyses, and Mr. Charles Butz of the Merck Institute for Therapeutic Research for the toxicity studies. The authors are also indebted to Dr. Alfred C. Haven, Jr., and to Mr. Edward Acton for their assistance in some of the early work on this problem.

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(12) The isolation procedure to this point is a modification of that described by H. King, *J. Chem. Soc.*, 1743 (1922).

(13) V. Schwartz, *Chemistry and Industry*, 102 (1953).

(14) Kunz and Hudson, THIS JOURNAL, **48**, 1982 (1926).

The Synthesis of 9α-Substituted-Δ⁴-androstene-11β-ol-3,17-diones

BY ROBERT H. LENHARD AND SEYMOUR BERNSTEIN

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Fried and co-workers[1] recently have announced certain important observations on the biological activities of the 9α-halogenated derivatives of a number of 11-oxygenated steroids. One of the out-

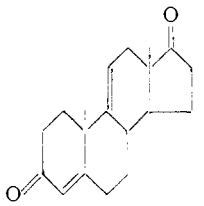
(1) J. Fried and E. F. Sabo, THIS JOURNAL, **75**, 2273 (1953); **76**, 1455 (1954); J. Fried, J. E. Herz, E. F. Sabo, A. Borman, F. M. Singer and P. Numerof, *ibid.*, **77**, 1069 (1955).

standing findings was that 9α-fluorohydrocortisone acetate displayed considerable activity both as a glucocorticoid and mineralocorticoid. This compound possessed an activity about 11 times that of cortisone acetate in the rat liver glycogen assay, and also a higher activity than DOCA in the sodium retention assay in the adrenalectomized rat.

Subsequent to the second Squibb communication it became of interest to us to investigate the effect of 9α-substitution (both halogen and hydroxyl) on the biological activity of Δ^4-androstene-11β-ol-3,17-dione, a weak androgen. Four such 9α-substituted-Δ^4-androstene-11β-ol-3,17-diones now have been synthesized.

The synthetic pathway employed was essentially that outlined by Fried and co-workers.[1] $\Delta^{4,9(11)}$-Androstadiene-3,17-dione (I)[2] in aqueous dioxane on treatment with N-bromoacetamide and perchloric acid was converted into the bromohydrin IIa in 80% yield. Reaction of IIa in absolute alcohol with potassium acetate gave Δ^4-androstene-3,17-dione-9β,11β-oxide (III) in 84% yield. The chlorohydrin IIb was obtained in 77% yield by opening the oxide ring with hydrogen chloride, and the fluorohydrin IIc in 30% yield with hydrogen fluoride. Finally, treatment of the oxide III in tetrahydrofuran with 3 *N* perchloric acid gave in 55% yield Δ^4-androstene-9α,11β-diol-,317-dione (IId).

Bioassays.[3]—In the chick comb test (inunction method; *ca.* 20 μg. per day for 7 days, propylene glycol) both Δ^4-androstene-9α-bromo-11β-ol-3,17-dione (IIa) and Δ^4-androstene-3,17-dione-9β,11β-oxide (III) gave a significantly positive response, whereas Δ^4-androstene-9α-chloro-11β-ol-3,17-dione (IIb), Δ^4-androstene-9α-fluoro-11β-ol-3-17-dione (IIc) and Δ^4-androstene-9α,11β-diol-3,17-dione (IId) were negative. At the same dose level both dehydroisoandrosterone and testosterone produced a strong positive response.



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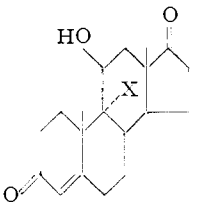
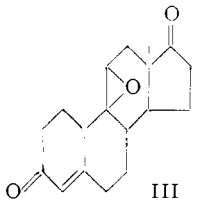


IIa, X = Br
IIb, X = Cl
IIc, X = F
IId, X = OH



III

Experimental

Melting Points.—All melting points are uncorrected, and were determined with uncalibrated Anschütz thermometers.

Optical Rotations.—The rotations are for chloroform solution.

Absorption Spectra.—The ultraviolet absorption spectra were determined in absolute alcohol with a Beckman spectrophotometer (model DU). The infrared absorption spectra (pressed potassium bromide) were determined with a Perkin–Elmer spectrophotometer (model 21).

Petroleum Ether.—The fraction used had a b.p. 60–70° (Skellysolve B).

All evaporations were carried out under reduced pressure.

Δ^4-Androstene-9α-bromo-11β-ol-3,17-dione (IIa). A.—A solution of I (1.0 g.) in dioxane (50 ml.) and water (10 ml.) was cooled to 15°, treated with N-bromoacetamide (1.07 g.) and 71% perchloric acid (10 drops), and allowed to stand at room temperature (25°) for one hour. Excess aqueous sodium sulfite and water were added to the yellow solution, and, after chilling, the product was collected by filtration, and washed with water; 1.07 g. (80% yield), m.p. 168–168.5° dec., with previous browning.

B.—In another run, the product was purified for characterization by crystallization from acetone–petroleum ether, m.p. 171.5–172° dec., with previous browning; λ_{max} 242.5 mμ (ε 16,200); ν_{max} 3333, 1745, 1650 cm.$^{-1}$; $[\alpha]^{24}D$ +190° (*c* 0.721, α_D +1.36°).

Anal. Calcd. for $C_{19}H_{25}BrO_3$ (381.31): C, 59.84; H, 6.61; Br, 20.96. Found: C, 60.12; H, 6.86; Br, 21.21.

Δ^4-Androstene-9α-chloro-11β-ol-3,17-dione (IIb).—A solution of the oxide III (300 mg.) in alcohol-free chloroform (3 ml.) was treated with a saturated solution of anhydrous hydrogen chloride in alcohol-free chloroform (25 ml.), and the mixture was allowed to stand at 0° for 4.5 hours. The yellow solution was evaporated at 0°. Crystallization of the residue from acetone–petroleum ether gave 258 mg. (77% yield) of crude product, m.p. 237.5–240° dec. with previous softening. Three crystallizations from acetone–petroleum ether gave 161 mg. of pure chlorohydrin IIb, m.p. 243.5–245.5° dec. with previous softening; λ_{max} 240 mμ (ε 17,200); ν_{max} 3375, 1750, 1655 cm.$^{-1}$; $[\alpha]^{25}D$ +194° (*c* 0.733, α_D +1.42°).

Anal. Calcd. for $C_{19}H_{25}ClO_3$ (336.85): C, 67.74; H, 7.48; Cl, 10.53. Found: C, 67.97; H, 7.79; Cl, 10.49.

Δ^4-Androstene-3,17-dione-9β,11β-oxide (III).—A solution of the bromohydrin IIa (100 mg.) in absolute alcohol (20 ml.) containing anhydrous potassium acetate (100 mg.) was refluxed for 46 hours. The reaction mixture was evaporated, and the residue dissolved in ethyl acetate. The solution was then washed with water, dried, and evaporated. Crystallization of the residue from acetone–petroleum ether afforded 65 mg. (84% yield) of the oxide II, m.p. 178–180° with previous softening. Three crystallizations from acetone–petroleum ether gave 51 mg. of pure oxide III, m.p. 181–182° with previous softening; λ_{max} 242.5–243 mμ (ε 14,000); ν_{max} 1740, 1670, 1626 cm.$^{-1}$; $[\alpha]^{24}D$ +38° (*c* 0.525, α_D +0.20°).

Anal. Calcd. for $C_{19}H_{24}O_3$ (300.38): C, 75.97; H, 8.05. Found: C, 75.69; H, 8.31.

Δ^4-Androstene-9α-fluoro-11β-ol-3,17-dione (IIc).—A solution of the oxide III (300 mg.) in alcohol-free chloroform (25 ml.) was treated with anhydrous hydrogen fluoride (*ca.* 4 ml.) and allowed to stand at 0° for 4.5 hr. The solution was evaporated at room temperature, and the residue was dissolved in ethyl acetate. The solution was washed with dilute sodium bicarbonate and water, dried, treated with Norite, and filtered through Celite. Evaporation afforded a slightly yellow solid which was crystallized from acetone–petroleum ether to yield 120 mg. of crude product, m.p. 206–207° dec. Four crystallizations from acetone–petroleum ether gave 97 mg. (30% yield) of pure fluorohydrin IIc, m.p. 249.5–250° dec., λ_{max} 238 mμ (ε17,200); ν_{max} 3375, 1745, 1650, 1625 (shoulder) cm.$^{-1}$; $[\alpha]^{25}D$ +184° (*c* 0.593, α_D +1.09°).

Anal. Calcd. for $C_{19}H_{25}FO_3$ (320.39): C, 71.22; H, 7.87; F, 5.93. Found: C, 70.97; H, 8.01; F, 5.75.

Δ^4-Androstene-9α,11β-diol-3,17-dione (IId). A.—A solution of the oxide III (300 mg.) in tetrahydrofuran (6 ml.) and 3 *N* perchloric acid (2 ml.) was allowed to stand at room temperature for 5.5 hr. After the addition of water and salt to the yellow reaction mixture, the product was extracted with ethyl acetate. The extract was washed with saturated sodium bicarbonate solution and saline, dried and evaporated. The residue was crystallized from acetone–petroleum

(2) T. Reichstein, U. S. Patent 2,409,798 (October 22, 1946); S. Bernstein, R. H. Lenhard and J. H. Williams, *J. Org. Chem.*, **19**, 41 (1954); and F. W. Heyl and M. E. Herr, THIS JOURNAL, **77**, 488 (1955).

(3) The androgen assays were carried out by Drs. F. I. Dessau and E. DeRenzo and their associates of this Laboratory. A detailed report on the biological activities of Δ^4-androstene-9α-fluoro-11β-ol-3,17-dione (IIc) will be published elsewhere by Drs. E. Rosenberg and R. I. Dorfman of the Worcester Foundation for Experimental Biology.

ether to give 174 mg. (55% yield) of crude product, m.p. 242.5–246.5° with previous softening. Two crystallizations from acetone–petroleum ether gave 158 mg. of practically pure diol IId, m.p. 248.5–250.5° with previous softening.

B.—In another run, the product was purified for characterization by crystallization from acetone–petroleum ether, m.p. 249.5–250.5° with previous softening; λ_{max} 241–242 mμ (ε 15,500); ν_{max} 3460, 1735, 1664, 1622cm.$^{-1}$; $[\alpha]^{25}D$ +223° (*c* 0.242, α_D +0.54°).

Anal. Calcd. for $C_{19}H_{26}O_4$ (318.40): C, 71.67; H, 8.23. Found: C, 71.73; H, 8.27.

Acknowledgment.—We are indebted to Messrs. Louis M. Brancone, Samuel S. Modes, Frank C. Geronimo, Alexander N. Prezioso and Harold J. Ferrari for the microanalytical data, and to Messrs. William Fulmor and George Morton and Miss Anne Callaghan for the optical rotation data and the infrared absorption spectra.

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Some 1-Alkyl-2-imidazolidinethiones

BY CHIEN-PEN LO

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This note reports the synthesis of the 1-*n*-octyl-1-*t*-octyl-[1] and 1-*n*-octadecyl-2-imidazolidinethiones, the fungicidal properties of which have been discussed elsewhere by Rich and Horsfall.[2] The preparation of a series of 1-alkyl-2-imidazolidinethiones has just been reported by Thorn.[3]

Our 1-alkyl-2-imidazolidinethiones were prepared by a method similar to that used by Thorn.[4] N-Alkylethylenediamines were allowed to react with one mole of carbon disulfide to give the corresponding dithiocarbamic acids (inner salts)[5] which were isolated and characterized. These intermediate dithiocarbamic acids yielded the desired 1-alkyl-2-imidazolidinethiones upon heating above their melting points.

Experimental[6]

N-β-Aminoethyl-N-*n*-octyldithiocarbamic Acid (inner salt).—N-*n*-Octylethylenediamine (172 g.) was treated with carbon disulfide (76 ml.) in acetone (500 ml.) to give 192 g. (77.5%) of a white solid melting at 116–118° (with effervescence).

Anal. Calcd. for $C_{11}H_{24}N_2S_2$: N, 11.3; CS_2, 30.6. Found: N, 10.8; CS_2, 30.3.

1-*n*-Octyl-2-imidazolidinethione.—The above dithiocarbamic acid (161 g.) was heated at 120–140° for two hours and the residue was crystallized from ethanol to give 122.5 g. (88%) of a white solid melting at 54–55°.[7]

Anal. Calcd. for $C_{11}H_{22}N_2S$: N, 13.1; S, 14.9. Found: N, 12.8; S, 15.0.

1-*n*-Octyl-2-imidazolidinethione was also prepared directly from N-*n*-octylethylenediamine and carbon disulfide by omitting the use of solvent and the isolation of the intermediate dithiocarbamic acid. The yield of the crude 1-*n*-octyl-2-imidazolidinethione (m.p. 42–43°) was 90%, based on the amine used.

N-β-Aminoethyl-N-*t*-octyldithiocarbamic Acid (inner salt) was obtained from N-*t*-octylethylenediamine and carbon disulfide in ethanol as a white solid, m.p. 144–146°, yield 57%.

Anal. Calcd. for $C_{11}H_{24}N_2S_2$: N, 11.3; CS_2, 30.6. Found: N, 11.0; CS_2, 30.3.

1-*t*-Octyl-2-imidazolidinethione was prepared by heating the above dithiocarbamic acid at 140–150° for three hours and obtained as a white solid (free ethanol); m.p. 164–165°, yield 66.5%.

Anal. Calcd. for $C_{11}H_{22}N_2S$: N, 13.1; S, 14.9. Found: N, 13.0; S, 15.1.

N-β-Aminoethyl-N-*n*-octadecyldithiocarbamic acid (inner salt) was obtained from N-octadecylethylenediamine and carbon disulfide in ethanol as a white solid, m.p. 106–108° (with effervescence), yield 83.5%.

Anal. Calcd. for $C_{21}H_{44}N_2S_2$: N, 7.2; CS_2, 19.6. Found: N, 6.8; CS_2, 19.2.

1-*n*-Octadecyl-2-imidazolidinethione was prepared by heating the above dithiocarbamic acid at 130–140° for 2.5 hours and obtained as a white solid (from ethanol), m.p. 80–81°, yield 84%.

Anal. Calcd. for $C_{21}H_{42}N_2S$: N, 7.9; S, 9.1. Found: N, 7.8; S, 9.1.

Acknowledgment.—The author wishes to thank Dr. W E Craig for helpful advice and Mrs. Rose Alberts for technical assistance.

(1) In the present work, *t*-octyl denotes 1,1,3,3-tetramethylbutyl.

(2) S. Rich and J. G. Horsfall, *Science*, **120**, 122 (1954). In this article these compounds were named as derivatives of ethylenethiourea.

(3) G. D. Thorn, *Can. J. Chem.*, **33**, 1278 (1955).

(4) For references on this general method of preparation of 2-imidazolidinethiones, see ref. 3.

(5) Because of the non-symmetry of the N-alkylethylenediamine molecule, there are two possible structures for the inner salt of the dithiocarbamic acid: $RN(CSSH)CH_2CH_2NH_2$ and $RNHCH_2CH_2NHCSSH$. Judging from the rather sharp melting point of the crude product, it appeared that one product was formed exclusively or predominantly in this reaction. In this work, the former structure is tentatively assigned to the product pending further structural proof. It is to be noted here that either of the structures would lead to the formation of the same 1-alkyl-2-imidazolidinethione by losing hydrogen sulfide.

(6) All melting points are uncorrected. Analyses were performed under the direction of Mr. Thomas Callan. Carbon disulfide was determined by the method of D. G. Clarke, H. Baum, E. L. Stanley and W. F. Hester, *Anal. Chem.*, **23**, 1842 (1951).

(7) Thorn (ref. 3) reported a melting point of 52–53°.

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Acid-catalyzed Condensations. II.[1] The Condensation of Benzaldehyde with Substituted Acetophenones

BY ROBERT E. LYLE AND LEO P. PARADIS[2]

RECEIVED AUGUST 18, 1955

The hydrogen chloride-catalyzed condensation of benzaldehyde with substituted acetophenones yields the hydrogen chloride addition compounds rather than the chalcones themselves.[3] In view, however, of the successful formation of 1,3,5-triarylbenzenes and substituted dypnones[1] by the hydrogen chloride-catalyzed self-condensation of substituted acetophenones, the application of similar conditions to the synthesis of chalcones appeared worthy of investigation.

The reaction of seventeen monosubstituted acetophenones with benzaldehyde in methanolic hydrogen chloride gave the corresponding chalcones

(1) The first paper in this series is 1,3,5-Triarylbenzenes, R. E. Lyle, E. J. DeWitt, N. M. Nichols and W. Cleland, THIS JOURNAL, **75**, 5959 (1953).

(2) Abstracted from the thesis of Leo P. Paradis presented to the Graduate School of the University of New Hampshire in partial fulfillment of the requirement of the degree of Master of Science.

(3) L. Claisen and A. Claperde, *Ber.*, **14**, 2463 (1881); W. Dilthey, L. Neuhaus, E. Reis and W. Schommer, *J. prakt. Chem.*, **124**, 81 (1930); F. Bergel and A. L. Morrison and N. Rinderknecht, *J. Chem. Soc.*, 659 (1950); A. Bell, *ibid.*, 2834 (1953).