Keyphrases

2-o-Tolylcyclohexanols, *trans* and *cis*
Optical rotatory dispersion—absolute configuration
Circular dichroism—absolute configuration
Column chromatography—separation
NMR spectrometry—identity
Specific rotation—identity
IR spectrophotometry—structure
GLC—analysis

Specific Assay Methods for Droperidol and Fentanyl Citrate in a Pharmaceutical Combination

By CASIMIR A. JANICKI, RONALD J. BRENNER, and BARBARA E. SCHWARTZ

In acid solution, droperidol, 1-{1-[3-(*p*-fluorobenzoyl)propyl]-1,2,3,6-tetrahydro-4-pyridyl}-2-benzimidazolinone, is decomposed to form 4′-fluoro-4-(4-oxopiperidino)butyrophenone and 2-benzimidazolinone. Fentanyl, *N*-(1-phenethyl-4-piperidyl)propionanilide, is decomposed in acid solution to form 4-anilino-1-(2-phenylethyl)piperidine. A procedure to assay droperidol in the presence of its hydrolysis products, fentanyl, and its hydrolysis product is given. Droperidol is analyzed by its UV absorption after being separated from interfering products. A procedure to assay fentanyl in the presence of its hydrolysis products, droperidol, and its hydrolysis products is given. Droperidol and 4-anilino-1-(2-phenylethyl)-piperidine are separated from fentanyl through the formation of a reineckate derivative and fentanyl is subsequently determined by a methyl orange procedure. Data are presented to show the accuracy and precision of the methods.

DROPERIDOL, 1 - {1 - [3 - (*p* - fluorobenzoyl)-propyl]-1,2,3,6 - tetrahydro - 4 - pyridyl} - 2-benzimidazolinone, was first synthesized by Janssen *et al.* and has been shown to be a sedative or tranquilizer (1, 2). Fentanyl citrate [*N*-(1-phenethyl-4-piperidyl) propionanilide dihydrogen citrate] was also synthesized by Janssen *et al.*, and has been shown to be a potent narcotic analgesic (2). It has been reported to be 100 times more potent on a weight basis than morphine as an analgesic (3).

A combination of droperidol and fentanyl citrate in solution has been employed for neuroleptanalgesia or as an adjunct to nitrous oxide for general anesthesia in man (4). It has also been successfully employed in veterinary medicine as an analgesic tranquilizer[1] for use in surgery in dogs (5).

The specific assays for each active ingredient in the presence of the other was essential to study the stability of the various combinations prepared in these laboratories. The usual ratio of droperidol to fentanyl in solution was 50:1. The fentanyl content varied from 20 mcg./ml. to the concentration used in the veterinary product 400 mcg./ml.

Fentanyl, because of its low concentration and its low ratio with respect to droperidol, presented a formidable analytical problem. For the assay of fentanyl to be specific, fentanyl had to be separated not only from its own hydrolysis product, but also from a large amount of droperidol and hydrolysis products of droperidol.

The proposed assay methods for droperidol and fentanyl, alone or in combination, are specific for the intact drug.

EXPERIMENTAL

Hydrolysis Studies—Droperidol (I) was refluxed overnight in 1 *M* hydrochloric acid and was found to undergo complete hydrolysis. Two major products were isolated, 4′-fluoro-4-(4-oxopiperidino)butyrophenone (II) and 2-benzimidazolinone (III) (Scheme I).

A solution of droperidol in monoglyme was refluxed overnight in 2 *M* sodium hydroxide and was recovered unchanged.

Fentanyl (IV), prepared from its citrate salt, was refluxed overnight in 1 *M* hydrochloric acid and was found to undergo complete hydrolysis. One

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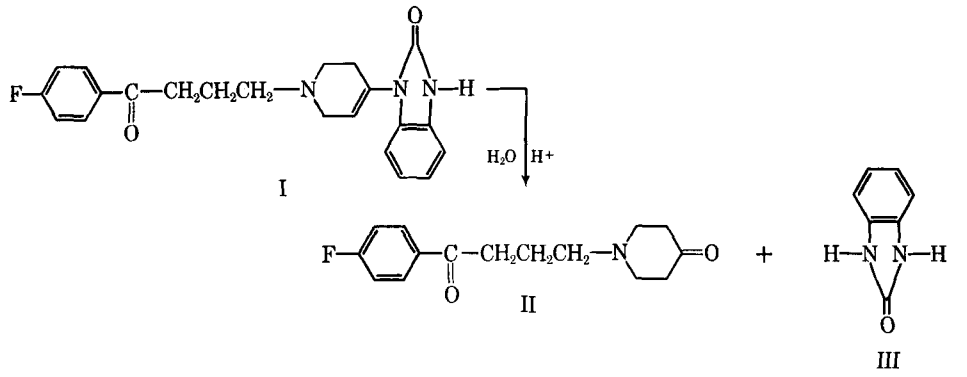
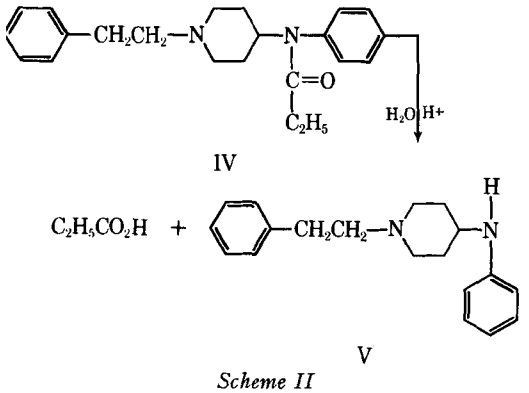
The authors thank Dr. Janis Plostnieks, Chemical Research Department, for the hydrolysis studies of droperidol and fentanyl.

[1] Marketed as Innovar-Vet by McNeil Laboratories, Inc., Fort Washington, Pa.



Scheme I



Scheme II

major product was isolated, 4-anilino-1-(2-phenylethyl)piperidine (V) (Scheme II).

A monoglyme solution of fentanyl was recovered unchanged after refluxing in 2 *M* sodium hydroxide overnight.

Method Development—*Droperidol Assay*—Droperidol exhibits a strong ultraviolet absorption spectrum and an assay method was chosen to utilize this property. The two major hydrolysis products have ultraviolet absorption spectra which would interfere in a direct ultraviolet assay for droperidol. The ultraviolet absorption spectra of droperidol ($\epsilon_{max.}$ 276 mμ = 7100; $\epsilon_{max.}$ 247 mμ = 14,500), II ($\epsilon_{max.}$ 248 mμ = 12,500), and III ($\epsilon_{max.}$ 276 mμ = 6,800) are given in Fig. 1.

Fentanyl ($\epsilon_{max.}$ 258 mμ = 465) and its hydrolysis product (V) ($\epsilon_{max.}$ 257 mμ = 440) have ultraviolet absorption spectra which are of low intensity. The spectra are presented in Fig. 2. Unless they were present in solution at very high concentrations, fentanyl and its hydrolysis product would not be expected to interfere in an ultraviolet assay method for droperidol.

A reaction, which is characteristic of aldehydes and some ketones, is the addition of sodium bisulfite to form water-soluble bisulfite addition products. One of the hydrolysis products of droperidol (II) quantitatively forms such an addition product very readily. The addition of bisulfite would be expected to add first to the piperidino carbonyl group. Sodium bisulfite may also react with droperidol under the proper conditions. When mixtures of droperidol and II were extracted with chloroform from 0.3% aqueous sodium bisulfite solution buffered at pH 6, droperidol was quantitatively found in the chloroform layer, while II was

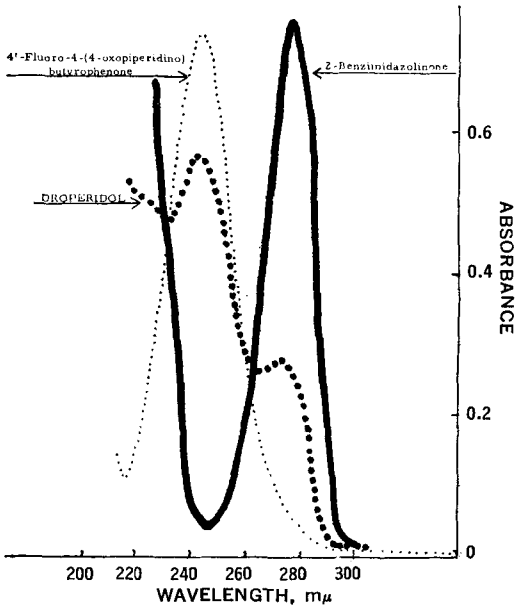


Fig. 1—The UV absorption spectra of droperidol (0.0147 mg./ml.), 4'-fluoro-4-(4-oxopiperidone)butyrophenone (0.0155 mg./ml.), 2-benzimidazolinone (0.0151 mg./ml.) in 0.1 M hydrochloric acid, 1-cm. cells.

quantitatively found in the aqueous layer. The concentration of sodium bisulfite is critical. Increasing the sodium bisulfite concentration from 0.3 to 1.2% resulted in losses of droperidol in the chloroform layer. At a concentration of 0.9% sodium bisulfite, there was a 3% loss of droperidol and a 40% loss at 1.2%. No losses of droperidol were encountered using the conditions described in the proposed assay. Lowering the pH resulted in incomplete extraction of droperidol into the chloroform. The 2-benzimidazolinone is unaffected by sodium bisulfite and is found essentially in the pH 6 layer having a partition coefficient of 0.15 with chloroform.

The droperidol in the chloroform layer was now separated from II and about 96% of III. Droperidol was quantitatively extracted from chloroform into citric acid. The III present in the chloroform was also extracted into the citric acid. Since III has no ultraviolet absorption at 246 mμ, the absorbance at 246 mμ is entirely due to the droperidol (Fig. 1). Mixtures of droperidol and III were assayed for droperidol by the use of the proposed assay. No

TABLE I—ASSAY OF DROPERIDOL IN PRESENCE OF 2-BENZIMIDAZOLINONE

Droperidol, mg.	2-Benzimidazolinone, mg.	Droperidol Found, mg.
2.00	0	2.00
1.80	0.07	1.79
1.60	0.14	1.58
1.40	0.21	1.37
1.00	0.35	0.98
0.00	0.70	0.00

TABLE II—THIN-LAYER CHROMATOGRAPHIC[a] R_f VALUES FOR COMPOUNDS

Compd.	R_f
Droperidol	0.28
4′-Fluoro-4-(4-oxopiperidino)-butyrophenone	0.47
2-Benzimidazolinone	0.22
Fentanyl	0.71
4-Anilino-1-(2-phenylethyl)piperidine	0.40

[a] Silica Gel GF, 5% v/v methanol in chloroform, no paperwick in tank.

interference was found from the hydrolysis product as indicated from the data presented in Table I.

The fate of each component of known mixtures of droperidol and its two hydrolysis products was followed by a thin-layer chromotographic method. Samples spotted on a fluorescent silica plate[2] were developed once, in a tank without a paper wick, *with 5% v/v methanol in chloroform. After air-drying* the plate, droperidol and its two hydrolysis products could be detected under short wavelength UV light. The spots appeared dark against a fluorescent background. However, when mixtures of all three compounds were chromotographed only 2 spots appeared under short wavelength UV light. The droperidol spot overlapped the III spot. When the plate was sprayed with a 0.2% alcoholic solution of dichlorofluorescein, dried at 60° for 15 min., and viewed under long wavelength UV light, only the spot of III could be observed. Under short wavelength UV light, two spots appeared, the II spot and the droperidol–III combined spot. With the dichlorofluorescein spray, as little as 1 mcg. of III could be detected in large amounts (100 mcg.) of droperidol. The thin-layer study confirmed that II remained completely in the aqueous bisulfite solution, while droperidol could not be detected in any significant amounts in the aqueous layer.

The R_f values obtained for droperidol and its hydrolysis products and for fentanyl and its hydrolysis product are given in Table II.

In quantitative TLC work it was necessary to prepare the fluorescent silica plates in the laboratory, using Silica Gel GF_{254}.[3] Impurities in the commercial precoated plates made this necessary.

When the samples were in acidic media it was necessary to obtain the free bases for the TLC study. The aqueous solution was made neutral by the addition of 0.1 *N* sodium hydroxide and evaporated to dryness without the aid of heat. The residue was taken up in methanol or acetone and streaked onto a fluorescent silica plate and developed as previously described. An alternate procedure that can be used is to make the aqueous solution slightly alkaline with 0.1 *N* sodium hydroxide and extract with chloroform. The chloroform solution is then streaked onto the fluorescent silica plate and developed as previously described.

Fentanyl Assay—An ultraviolet assay method for fentanyl was not suitable because of its low $\epsilon_{max.}$ value and its usually low concentrations found in pharmaceutical dosage forms. The ultraviolet absorption spectra for fentanyl and its major hydrolysis product are given in Fig. 2. Fentanyl, because of the basic nitrogen in the piperidine ring, was found to form a chloroform-soluble methyl orange complex. The hydrolysis product of fentanyl also forms a chloroform-soluble methyl orange complex. However, V forms a chloroform-insoluble reineckate in sulfuric acid, while fentanyl under the same conditions is quantitatively extracted in chloroform. The thin-layer method described previously, confirmed that V was not extracted into the chloroform with fentanyl.

Droperidol also forms an insoluble reineckate precipitate in sulfuric acid. Therefore, the use of Reinecke salt quantitatively removes both droperidol and the hydrolysis product of fentanyl. On silica thin-layer plates, no trace of droperidol could be found in the chloroform layer. II also forms an insoluble reineckate under the same conditions while III does not. III does not form a chloroform-soluble methyl orange complex, and therefore does not interfere in the proposed fentanyl assay.

The use of 2% methanol in chloroform was used instead of pure chloroform as a precaution to prevent any possible adsorption of fentanyl by the glassware. The concentration of fentanyl in the chloroform solution in the proposed assay is low, being 0.004 mg./ml.

PROPOSED METHODS

Reagents—Hydrochloric acid, 1 *M;* citric acid, 0.1 *M;* sulfuric acid, 20% v/v methanol in chloroform; sodium bisulfite solution, 1%, prepare fresh daily; methyl orange (Fisher Scientific Co.), aqueous solution; sodium phosphate solution,

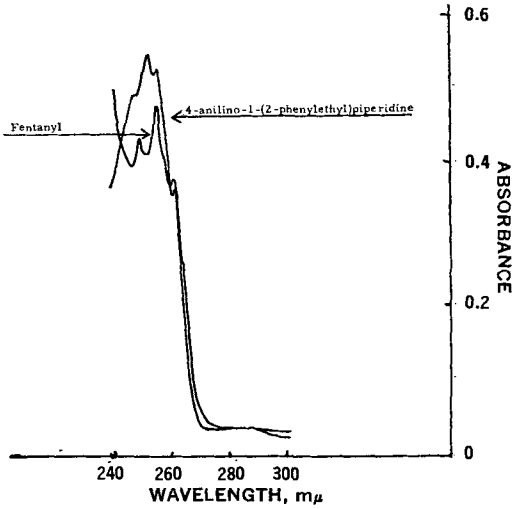


Fig. 2—The UV absorption spectra of fentanyl (0.342 mg./ml.) and 4-anilino-1-(2-phenylethyl)piperidine (0.348 mg./ml.) in 0.1 M hydrochloric acid, 1-cm. cells.

[2] Uniplate, precoated thin-layer chromatography plates from Analtech, Inc., Wilmington, Del.
[3] Brinkmann Instruments, Westbury, N. Y.

0.2 *M;* pH 5 buffer and pH 6 buffer, adjust the 0.2 *M* sodium phosphate solution to pH with 1 *M* sodium hydroxide; lactic acid USP; analytical reagent grades of chloroform, ether, anhydrous methanol.

One percent Reinecke solution, 1 Gm. of Reinecke salt (ammonium tetrathiocyanodiammonochromate, Fisher Scientific Co.) added to 100-ml. water shaker for 15 min., and filtered; prepare this solution immediately before use.

Apparatus—A Beckman DK-2A recording spectrophotometer and a Burrell wrist-action shaker were used. Boston round flint bottles, 30 ml., 60 ml., and 120 ml., fitted with plastic caps having a conical polyethylene liner were obtained from Erno Products Co., Philadelphia, Pa.

Assay for Droperidol—Dilute the sample of droperidol, or the sample of droperidol in combination with fentanyl, with water to obtain a 0.15 mg./ml. solution of droperidol. Pipet 15 ml. of the diluted sample into a 60-ml. bottle containing 10 ml. of the pH 6 buffer, 10 ml. of a 1% sodium bisulfite solution, and exactly 15 ml. of chloroform. Shake for 15 min. on a mechanical shaker, centrifuge, aspirate, and discard the aqueous layer. Pipet 5 ml. of the clear chloroform layer to a 120-ml. bottle containing exactly 50 ml. of 0.1 *M* citric acid. Shake for 30 min. on a mechanical shaker. Centrifuge for a couple of minutes and decant the clear citric acid solution to a clean container. Record the ultraviolet absorption spectrum from 300 to 230 mμ *versus* 0.1 *M* citric acid in 1-cm. cells.

An 0.15 mg./ml. solution of droperidol standard in chloroform is prepared, and taken through the proposed assay. The calculation is then:

$$\frac{\text{absorbance at 246 m}\mu \text{ for the sample}}{\text{absorbance at 246 m}\mu \text{ for the std.}} \times 100 = \%\ \text{droperidol}$$

If the sample contains a preservative such as methyl- or propylparaben, the UV spectrum of droperidol in 0.1 *M* citric acid will be distorted. The following step will remove the preservatives from citric acid solution. The droperidol standard should also be taken through this step.

Extract 20 ml. of the citric acid solution of droperidol with two 70-ml. portions of ether. Discard the ether extracts and record the UV spectrum of the sample as directed.

Assay for Fentanyl—Dilute the fentanyl sample or sample of fentanyl in combination with droperidol with water to obtain a 0.02 mg./ml. solution of fentanyl. Pipet 5 ml. of the diluted solution (rinsing the pipet with the solution once before the sample to be analyzed is taken) into a 60-ml. bottle containing exactly 2 ml. of 20% sulfuric acid. Add exactly 25 ml. of the 2% methanol in chloroform solution and 10 ml. of the Reinecke solution to the sample. Shake the bottle for 15 min. on a mechanical shaker. Do not centrifuge the sample but aspirate and discard the aqueous layer. Filter the chloroform layer through a cotton pledget to remove any traces of the precipitate.

Pipet 15 ml. of the chloroform layer to a 60-ml. bottle containing 10 ml. of pH 5 buffer. Add 1 ml. of the methyl orange reagent and shake the bottle on a mechanical shaker for 15 min. Centrifuge, aspirate, and discard the aqueous layer. Pipet 10 ml. of the chloroform layer to a bottle containing exactly 10 ml. of 1 *M* hydrochloric acid. Shake for 5 min. on a mechanical shaker, centrifuge, and carefully decant the acid layer. Determine the absorbance of the acid layer in 1-cm. cells at the maximum near 505 mμ.

A standard is prepared by dissolving fentanyl citrate in water and diluting it with water to obtain a 0.020 mg./ml. solution expressed as the free base. If the sample contains droperidol, the standard should contain the same amount of droperidol as present in the sample. Droperidol is insoluble in water. It may be solubilized by adding a few drops of lactic acid and heating on a steam bath until dissolved. Upon solution, add hot water and dilute to volume with water after the solution has reached room temperature.

Table III—Precision of the Proposed Assay Method Calculated as the Standard Deviation

Sample	No. of Assays	Droperidol Assay, %	Fentanyl Assay, %
Droperidol	16	1.53	...
Droperidol and fentanyl	118	2.40	3.98
Fentanyl	34	...	2.87

RESULTS AND CONCLUSIONS

The precision of the proposed assays was tested on actual samples that were being tested for their chemical-physical stability. The standard deviations calculated are those which would be normally expected in the laboratory. The standard deviations are presented in Table III.

The proposed assays are stability assays. Each hydrolysis product of droperidol, fentanyl, and the hydrolysis product of fentanyl were taken through the droperidol assay and found not to interfere in the assay. Droperidol, both hydrolysis products of droperidol, and the hydrolysis product of fentanyl were taken through the fentanyl assay and found not to interfere in the assay. The fate of the compounds was followed by a thin-layer chromatographic study of each phase of each extraction procedure.

The assay for droperidol does not appear to have any critical steps. The sodium bisulfite solution should be made fresh daily.

The assay for fentanyl, once started, should be carried to completion without undue delay. Colorimetric reading variation is high when the samples are left in contact with the Reinecke solution for longer than 1 hr. The fentanyl–methyl orange complex in the chloroform decomposes slowly on standing. This does not present a major problem since the chloroform solution is next extracted with 1 *M* hydrochloric acid. The red colored acid solution is stable for days.

If the ratio of droperidol to fentanyl in solution is varied from 50:1 to 25:1; no problems are encountered in either the droperidol or fentanyl assays. However, if the ratio of droperidol to fentanyl is lowered to 15:1, high fentanyl results are encountered. The high results are due to droperidol. From 1 to 3% of the droperidol is not completely precipitated by the Reinecke solution and is extracted into chloroform along with the fentanyl. At this point it is possible to do a TLC separation of fentanyl from droperidol using the TLC method previously described. The fentanyl band is scraped

off and extracted with chloroform and the chloroform solution treated with methyl orange as described. However, it was found that simply by adding sufficient droperidol (in lactic acid solution) to the sample during dilution to raise the ratio of droperidol to fentanyl to 50:1 in the final solution (0.02 mg./ml. fentanyl) complete precipitation of the droperidol was achieved.

The reineckate precipitate of droperidol can be used as a quantitative measure of droperidol. Once 4′-fluoro-4-(4-oxopiperidino)butyrophenone is removed by the addition of sodium bisulfite as directed in the proposed assay for droperidol, the chloroform solution of droperidol can be treated with the Reinecke solution and sulfuric acid. The droperidol-Reinecke precipitate is collected, dissolved in acetone, and the absorbance of the red colored solution read at about 525 mμ. The interference of 4-anilino-1-(2-phenylethyl)piperidine in the Reinecke precipitation of droperidol is small for the droperidol–fentanyl pharmaceutical preparations. The fentanyl would have to be completely hydrolyzed to affect the droperidol reineckate precipitate by 2%.

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Keyphrases

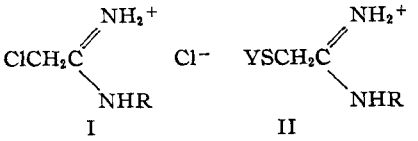
Droperidol, fentanyl citrate solution—analysis
Fentanyl citrate, droperidol—hydrolysis products
Chloroform extraction—droperidol
UV spectrophotometry—analysis
Methanol–chloroform extraction—fentanyl citrate
Colorimetric analysis—methyl orange reagent
TLC—identity

Synthesis of α-Mercaptoacetamidinium Chlorides *via* the Corresponding Phosphorothioates

By TERRY T. CONWAY, ABOO SHOEB, and LUDWIG BAUER

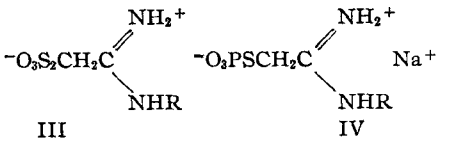
A facile synthesis of α-mercaptoacetamidinium chlorides is described. The procedure consists of treating α-chloroacetamidinium chlorides with trisodium phosphorothioate that formed the corresponding *S*-alkyl phosphorothioates. The latter were not isolated, but were hydrolyzed in the reaction medium by hot dilute acid to furnish the required products.

THE SYNTHESIS of α-mercapto amidines and derivatives as potential antiradiation agents has been under study in this laboratory for some time (1–3). The general approach consisted of treating an α-chloroacetamidinium chloride, I, with a sulfur bearing nucleophile, YS^-, to obtain a substituted acetamidinium cation, II. These



I II

reactions were successful since the substitution reaction of the halo group of the free α-chloroacetamidine was considerably faster than any reaction involving nucleophilic attack at the sp^2 amidine carbon. It was particularly important to exclude the latter type of reaction in an aqueous basic medium since displacement of the halo group by YS^- could easily be accompanied by hydrolysis of the amidine.[1] It was found that a relatively weakly basic, but excellent nucleophile like thiosulfate ion reacted quickly with I to form a series of α-amidinium Bunte salts, III. Due to the relatively easy preparation of



III IV

compounds of type III, it was of interest to explore the reaction of I with phosphorothioate ion, $^-O_3PS^-$, in the endeavor to synthesize a series of α-amidinium phosphorothioates, shown as the sodium salt, IV. It was intended to test a

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[1] The basic hydrolysis of amidines is a well-documented reaction. [For a recent reference see DeWolfe, R. D., and Keefe, J. R., *J. Org. Chem.*, **27**, 493(1962).]