2 mg.).

From the products eluted with benzene-BuOH (94:4)—benzene-BuOH (6:1), 2 mg. of crystalline substance, m.p. 240~250° which showed only one spot on the chromatogram was crystallized from acetone +H_2O. A mixed melting point with an authentic specimen, kindly supplied by Prof. Reichstein, showed no depression. The other component could not be purified by chromatography.

We wish to express our thanks to Prof. T. Reichstein, Basel, for his kind donation of utendin. We are also indebted to Mrs. T. Toma and Miss A. Maeda for the elemental analysis.

Summary

The roots of *Metaplexis japonica* Makino contain a complex glycoside mixture.

The alkaline hydrolysate of the crude ester-aglycone mixture showed the presence of sarcostin (I), deacylmetaplexigenin (Ⅱa), deacylcynanchogenin (Ⅳa), utendin (Ⅵ), and three other new aglycones. The structure of one of these new aglycones, named pergularin, was found to be closely related to utendin.

(Received November 11, 1965)

[Chem. Pharm. Bull. 14(7) 726~731 (1966)]

UDC 582.932 : 581.19 : 547.92

99. Hiroshi Mitsuhashi, Taro Nomura, and Masamichi Fukuoka :
Studies on the Constituents of Asclepiadaceae Plants. XX.[*1] Epimerization at C-17 and Optical Rotatory Dispersion Study of C/D *cis* Pregnan-20-one Derivatives. 2.[*2]

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It has already been shown that a series of polyhydroxy pregnane compounds occur in the plant family Asclepiadaceae.1~7)

All these compounds possess $12\beta,14\beta$-hydroxy groups and an 20-oxygen group. When these C/D *cis*-pregnan-20-one compounds, for example, ramanone (Ⅳa) and deacylcynanchogenin (Ⅴa) were treated with an alkaline solution, an equlibrium mixture of 17β-H-20-keto and 17α-H-20-keto compounds was produced.

The 17β-H-epimer is more stable than the 17α-H-epimer and is obtained as the main product.*2,2,6,7) The optical rotatory dispersion curves of the 17β-H-epimers showed a negative Cotton effect and the curves of the 17α-H-epimers showed a positive Cotton effect.*2,2,6) In C/D *trans*-17-H-20-keto steroids, the 17α-H-20-keto compound

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[*1] Part XIX. H. Mitsuhashi, T. Nomura, M. Hirano : This Bulletin, **14**, 717 (1966). Part of this work was reported at the Hokkaido Branch Meeting of Pharmaceutical Society of Japan. Feb. 27, 1965.

[*2] Epimerization at C-17 and Optical Rotatory Dispersion Study of C/D *cis*-Pregnan-20-one Derivatives. 1. H. Mitsuhashi, T. Nomura, M. Fukuoka : Steroids, **4**, 483 (1964).

[*3] Kita-15-jo, Nishi-7-chome, Sapporo (三橋 博, 野村太郎, 福岡正道).

1) K. A. Jaeggi, E. K. Weiss, T. Reichstein : Helv. Chim. Acta, **46**, 694 (1963).
2) H. Mitsuhashi, Y. Shimizu : Steroids **2**, 373 (1963).
3) H. Mitsuhashi, T. Nomura : This Bulletin, **12**, 1523 (1964).
4) H. Mitsuhashi, T. Sato, T. Nomura, I. Takemori : *Ibid.*, **13**, 267 (1965).
5) H. Mitsuhashi, T. Nomura : *Ibid.*, **13**, 274 (1965).
6) *Idem* : Steroids, **3**, 271 (1964).
7) H. H. Sauer, E. K. Weiss, T. Reichstein : Helv. Chem. Acta, **48**, 860 (1965).

is the more stable form[8,9,10] and shows a positive Cotton effect. The corresponding 17β-H-epimer shows a negative Cotton effect.[11] Combination of the above evidences, the fusion of C/D ring juncture and configuration of C-17 side chain would be presumed. In considering the above data, the fusion of the C/D ring juncture and the configuration of the C-17 side chain is well established. The ratio of these two isomers, in the C/D *trans*-pregnan-20-one series, was reported by Butenandt,[11] Rubin[10] and other workers,[12,13] Rubin has summarized this data in his review.[10] Usually, a value of $75\sim80\%$ for the 17α-H-20-keto compounds was obtained by based catalyzed equilibration, but slightly higher amounts of the 17β-H-isomer at equilibration resulted in the case of 11α-hydroxylated and 11-keto steroids.[13] More striking variations were reported

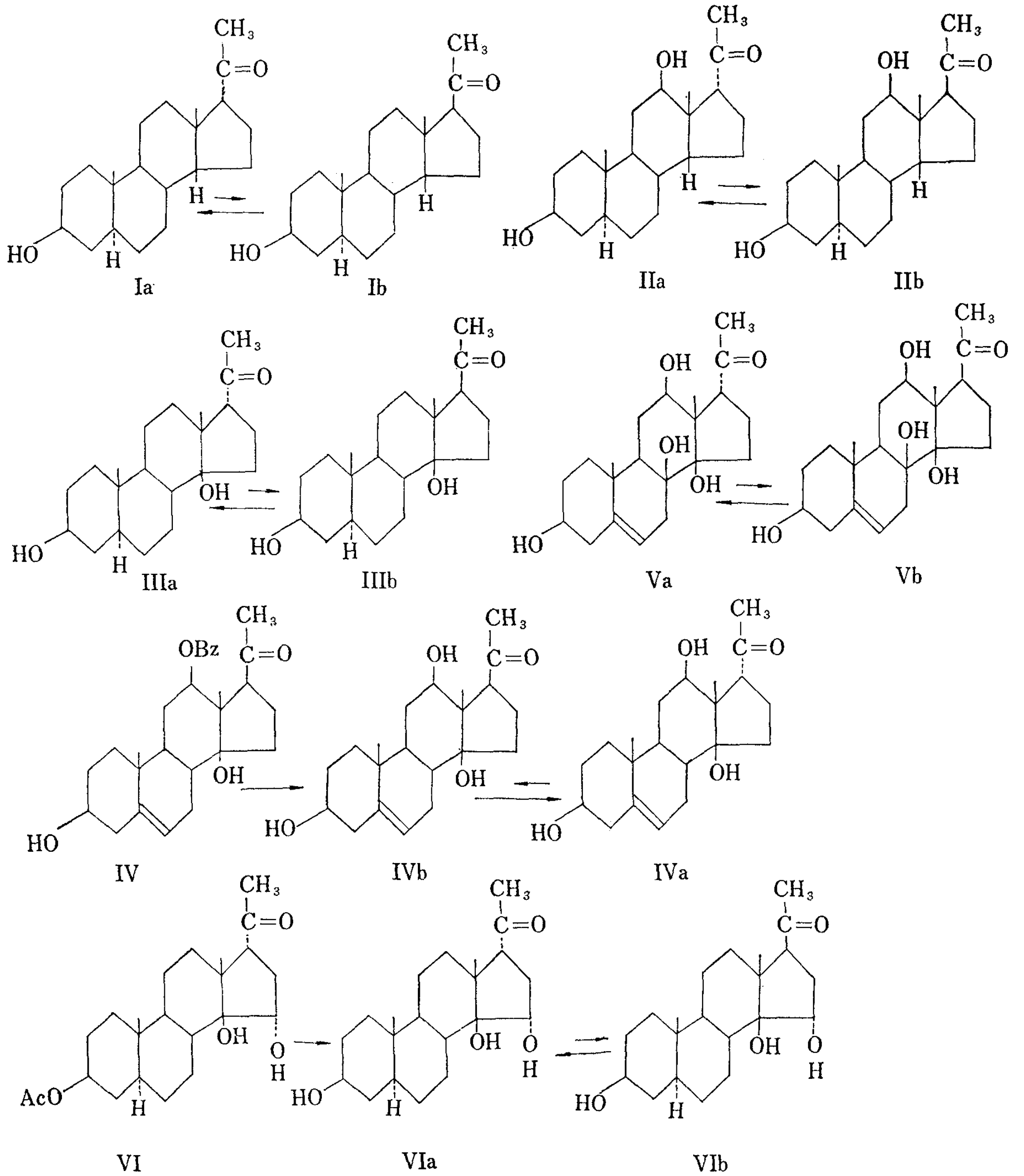

Chart 1.

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[8] A. Lardon : Helv. Chim. Acta, **32**, 1517 (1949).

[9] A. Butenandt, L. Mamoli : Ber., **68**, 1847 (1935); A. Butenandt, G. Fleisher : Ber., **70**, 96 (1937).

[10] M. B. Rubin : Steroids, **2**, 561 (1963).

[11] C. Djerassi : "Optical Rotatory Dispersion, Application to Organic Chemistry," McGraw-Hill Book Co., 1960.

[12] R. B. Moffett, W. M. Hoehn : J. Am. Chem. Soc., **66**, 2098 (1944).

[13] C. W. Marchall, T. F. Gallagher : J. Biol. Chem., **179**, 1265 (1949); W. F. Johns, R. M. Lukes, L. H.

in the case of 16β-substituted-20-keto pregnanes. The interration of the 16β and 17β-side chain gave complete specificity and no detectable amounts of the 17α-H-isomer could be found at equilibrium.10,14)

A series of pregnane compounds isolated from Asclepiadaceae family, has 12β,14β-hydroxy groups and we have examined the effect of these hydroxy group on the equilibrium mixture. As these hydroxy group are located near the 20-keto group, it would be expected that these hydroxyl groups would have an effect on the isomer ratio under equilibrating conditions. This report is an attempt to summarize the effects of these neighboring groups in a series of C/D *cis*-pregnan-20-one compounds on the isomer ratio. The rotational differences can be used for determination of the isomer ratio in an equilibrium mixture, but as Djerassi has pointed out11,15) the rotatory dispersion measurement is a more useful method. We have used rotatory dispersion curves to determine the C-17 isomer ratio, 3β-hydroxy-5α,14β,17α-pregnan-20-one (Ia)*2,16) has been taken as the simplest case for this purpose in the C/D *cis*-steroids. This compound (I) was treated with 3% potassium hydroxide and the reaction mixture examined by thin-layer chromatography (Al_2O_3, 2% EtOH/$CHCl_3$). Only two spots (Ia, Ib) were detected and the optical rotatory dispersion curve was measured. The composition of these two isomers in the mixture was calculated at 304 and 315 mμ by comparison with the curves*2 of Ia and Ib. The results are shown in Table I.

Table I.

	Ia*2	Ib*2	Eqil. mix.	% 17β-H	
				Calcd.	average
$[\alpha]_{304}$	−945°	+1120	−355	71	69
$[\alpha]_{315}$	−905°	+1013	−244	66	

To investigate the effect of 12β-OH in the equilibration, 3β,12β-dihydroxy-5α,14β,17α-pregnan-20-one (IIa)*2 was treated as described above. The reaction mixture was examined by thin-layer chromatography. Two spots (IIa, IIb) were located as reported in the previous paper.*2 The rotatory dispersion curve of this mixture gave the equilibrium ratio by the comparison with curves*2 of IIa and IIb. The results are shown in Table II. To show the effect of a 14β-OH group on the equilibrium, 3β,14β-dihydroxy-5α,17α-pregnan-20-one (IIIa)*2,16) was treated as described above. After a check by thin-layer chromatography, the rotatory dispersion measurement of this equilibrium mixture was carried out. The ratio of these two epimers was calculated, as shown in Table III.

Table II.

	IIa2*	IIb*2	Eqil. mix.	% 17β-H isomer	
				Calcd.	average
$[\alpha]_{304}$	−460°	+1760°	+414°	60	
$[\alpha]_{315}$	−240	+1280	+385	59	61
$[\alpha]_{315}$	− 20	+ 900	+314	63	

Sarett : J. Am. Chem. Soc., **76**, 5027 (1954); V. R. Mattox : J. Am. Chem. Soc., **74**, 4340 (1952).

14) P. Grabbe, L. M. Guerrero, J. Romo, F. Sanchez-Viesca : Tetrahedron, **19**, 25 (1963); J. C. Danilewicz, W. Klyne : J. Chem. Soc., **1965**, 1303.

15) N. L. Allinger, R. B. Hermann, C. Djerassi : J. Am. Chem. Soc., **25**, 922 (1960).

16) A. Plattner, L. Ruzicka, H. Heusser, E. Angliker : Helv. Chim. Acta, **30**, 385, 395 (1947).

Table Ⅲ.

	Ⅲa*2	Ⅲb*2	Eqil. mix.	% 17β-H isomer	
				Calcd.	average
$[\alpha]_{303}$	−1463°	+480°	−1320°	93	
$[\alpha]_{315}$	−1103°	+293	−1020	94	94
$[\alpha]_{325}$	− 715	+213	− 680	96	

Other polyhydroxy pregnane compounds, benzoylramanone (Ⅳ),[6] deacylcynanchogenin (Ⅴa)[2] and 3β,14β,15α-trihydroxy-5α-pregnan-20-one (Ⅵa)[17] were treated in the same manner, and the ratio of the two C-17 isomers determined. The results are shown in Tables Ⅳ～Ⅵ.

Table Ⅳ.

	Ⅳa*2	Ⅳb*2	Eqil. mix.	% 17β-H isomer	
				Calcd.	average
$[\alpha]_{305}$	−1250°	+895°	−740°	76	
$[\alpha]_{315}$	− 860	+670	−490	〃	76
$[\alpha]_{325}$	− 580	+480	−330	〃	

Table Ⅴ.

	Ⅴa*2	Ⅴb*2	Eqil. mix.	% 17β-H isomer	
				Calcd.	average
$[\alpha]_{301}$	−700°	+1040°	−290°	76	
$[\alpha]_{315}$	−440	+ 780	−170	77	77
$[\alpha]_{325}$	−260	+ 550	− 90	79	

Table Ⅵ.

	Ⅵa	Ⅵb	Eqil. mix.	% 17β-H isomer	
				Calcd.	average
$[\alpha]_{304}$	−1351°	+1115°	−967°	84	
$[\alpha]_{315}$	−1162	+ 807	−709	81	83
$[\alpha]_{325}$	− 621	+ 577	−419	83	

From these results, one can draw the following conclusions:

1. **Effect of a 12β-Hydroxyl Group**——In the case of C/D *cis*-20-keto series, slightly larger amounts of 17α-H-20-keto isomer appear to be present at equilibrium in 12β-hydroxylated steroids. (compare, Table Ⅰ—Table Ⅱ, Table Ⅲ—Table Ⅳ and Ⅴ).
2. **Effect of 14β-Hydroxyl Group**——In the C/D *cis*-20-keto series, the presence of 14β-hydroxyl group causes slightly larger amunts of 17β-H-20-keto isomer to be present at equilibrium (compare, Table Ⅰ—Ⅲ, Table Ⅱ—Ⅳ and Ⅴ).

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[17] H. Mitsuhashi, M. Fukuoka: This Bulletin, in press.

[18] H. Mitsuhashi, Y. Shimizu, E. Yamada, I. Takemori, T. Nomura: This Bulletin, **10**, 808 (1962).

Experimental

The optical rotatory dispersion curves were measured with an automatic recording ORD-UV-5-Niphon Bunko.

Materials——3β-hydroxy-5α,14β,17α-pregnan-20-one (Ia), 3β,12β-dihydroxy-5α,14β,17α-pregnan-20-one (Ⅱa), 3β,14β-dihydroxy-5α,17α-pregnan-20-one (Ⅲa), 3β-acetoxy-14β,15α-dihydroxy-5α,17α-pregnan-20-one (Ⅵ), were prepared as previously reported.*2,17) Benzoylramanone (Ⅳ) and deacylcynanchogenin (Va) were isolated from *Metaplexis japonica* Makino6) and *Cynanchum caudatum* Max.2)

Isomerization of 3β-Hydroxy-5α,14β,17α-pregnan-20-one (Ia)*2——3.6 mg. of Ia was dissolved in 2 ml. of 3% KOH and allowed to stand for 24 hr. at 30°. The thin-layer chromatographic analysis (2% EtOH-$CHCl_3$) was carried out. It gave only two spots (Ia, Ib), as reported in the previous paper.*2 The rotatory dispersion curve of this reaction mixture was measured and the ratio of these two isomers (Ia, Ib) was calculated by comparison with the curves of Ia and Ib.*2 The results are shown in Table I. When the reaction time extended to 48 hr., the same curve was obtained. The measuring conditions were : C= 0.18, l= 0.5, in 3% KOH-MeOH, 28°.

Isomerization of 3β,12β-Dihydroxy-5α,14β,17α-pregnan-20-one (IIa)*2——5.6 mg. of Ⅱa was dissolved in 2 ml. of 3% KOH-MeOH, and treated as with Ia. Thin-layer chromatographic analysis (Al_2O_3, 3.5% MeOH-benzene) gave only two spots (Ⅱa, Ⅱb). The rotatory dispersion measurement of this reaction mixture was carried out and the composition of these two isomers (Ⅱa, Ⅱb) was calculated by comparison with the curves of Ⅱa, Ⅱb.*2 The results are shown in Table Ⅱ. Measuring conditions as follows : C=0.28, l= 0.5, in 3% KOH-MeOH, 28°.

Isomerization of 3β,14β-Dihydroxy-5α,17α-pregnan-20-one(IIIa)*2——4.91 mg. of Ⅱa was dissolved in 2 ml. of 3% KOH-MeOH, and treated as Ia. A thin-layer chromatographic analysis (Al_2O_3, 2% MeOH-benzene) gave only two spots (Ⅲa, Ⅲb). The rotatory dispersion curve of this mixture was measured and the composition of these two isomers (Ⅲa, Ⅲb) was calculated. The results are shown in Table Ⅲ. Measuring conditions as follows : C=0.246, l= 0.5, in 3% KOH-MeOH, 27°.

Alkaline Treatment of Benzoylramanone (IV)6)——10.4 mg. of Ⅳ was dissolved in 2 ml. of 3% KOH-MeOH and treated as Ia. Thin-layer chromatographic analysis (Al_2O_3, 30% EtOAc-benzene) and paper chromatography ($CHCl_3$-H·$CONH_2$)18) showed that the spot of Ⅳ could not be found, but only two spots (Ⅳa, Ⅳb) were found. The rotatory dispersion measurement of this mixture was carried out and the composition of these two isomers (Ⅳa, Ⅳb) was calculated by the comparison of the curves of Ⅳa and Ⅳb) The results are shown in Table Ⅳ. Measuring conditions as follows : C= 0.40, l= 0.5, in 3% KOH, 28°.

Isomerization of Deacylcynanchogenin (Va)2)——8.0 mg. of Va was dissolved in 2 ml. of 3% KOH-MeOH, and treated as Ia. Thin-layer chromatography, (Al_2O_3 3% MeOH-benzene), and paper chromatographic analysis ($CHCl_3$-H·$CONH_2$)18) were carried out, only two spots (Va, Vb) were found. The optical rotatory dispersion curve of this mixture was measured and the composition of these isomers calculated. The results are shown in Table V. Measuring conditions as follows : C= 0.40, l= 0.5, in 3% KOH-MeOH, 28°.

Alkaline Treatment of 3β-Acetoxy-14β,15α-dihydroxy-5α,17α-pregnan-20-one (VI)17)——37 mg. of Ⅵ was dissolved in 4 ml. of 3% KOH-MeOH and allowed to stand for 24 hr. at 30°. After treatment as usual, the reaction mixture was extracted continuously with ether, thin-layer chromatographic analysis (Al_2O_3, 30% EtOAc-benzene) of this extract (32 mg.) showed two spots. This mixture was submitted to column chromatography over Al_2O_3 using benzene-MeOH mixture. Ⅵb, m.p. 220～227°, 2 mg. was eluted with 0.8% MeOH-benzene, and Ⅵa, m.p. 197～200°, 15 mg. with 2% MeOH-benzene. Mixed melting point with authentic specimen of Ⅵa17) showed no depression. The rotatory dispersion curves of Ⅵa and Ⅵb were measured with results as follows : Ⅵa, trough $[\alpha]_{304}$ − 1324°, peak $[\alpha]_{264}$ +2027, C= 0.19, l= 0.2, in MeOH, 20°. Ⅵb, peak $[\alpha]_{304}$ +1115°, trough $[\alpha]_{263}$ −679°, C= 0.078, l= 0.2, 20°.

Isomerization of 3β,14β,15α-Trihydroxy-5α,17α-pregnan-20-one (VIa)——3.1 mg. of Ⅵa was dissolved in 2 ml. of 3% KOH-MeOH and treated as in Ia. A thin-layer chromatographic study (30% EtOAc-benzene) of this reaction mixture showed only two spots (Ⅵa, Ⅵb). The rotatory dispersion curve of this mixture was measured, and the composition of these two isomers (Ⅵa, Ⅵb) was calculated, with results as shown in Table Ⅵ.

The authors are grateful to Dr. K. Takeda, Director of the Shionogi Research Laboratories, for the supply of the starting material. They are also indebted to Dr. K. Kuriyawa for kind suggestion.

Summary

Alkaline treatment of C/D *cis*-pregnan-20-one derivatives produced an equilibrium mixture of 17α- and 17β-H-20-keto compounds. The 17β-H-epimers are the more

stable form and their rotatory dispersion curves show negative Cotton effects. The 17α-H-20-keto compounds, the less stable form, show positive Cotton effects. The ratios of these two epimers were calculated from the optical rotatory dispersion curves and varied with the influence of neighboring groups.

(Received December 5, 1965)

[Chem. Pharm. Bull. 14(7) 731~735 (1966)]

UDC 547.546.04 : 547.568.1.04

100. **Seiji Miyano, Nobuhiro Abe, and Akiko Uno** : Reductive Benzylation of Aromatic Nitro Compounds by Means of Benzyl Alcohol.

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It has been known for a long time that nitrobenzene was reduced to azoxybenzene with methanol or ethanol in the presence of caustic alkali.[*2,1,2] Although the same reduction was also effected[3,4] by benzyl alcohol and potassium hydroxide at an elevated temperature,[*3] no work to achieve further reduction leading to amines by means of benzyl alcoholic potassium hydroxide has appeared so far.

More recently Sprinzak[*4,5] reported that a number of aromatic primary amines can be benzylated readily with benzyl alcohol in the presence of potassium hydroxide to give N-benzylanilines at a temperature between 250~280°.[*5]

The Sprinzak's report prompted us to reinvestigate the reduction of aromatic nitro compounds by means of benzyl alcoholic potassium hydroxide with the expectation that successful reduction to amines might be followed by Sprinzak's benzylation to yield N-benzylamines (I) as final products. Thus an attempt to combine (1) reduction of nitrobenzene and (2) benzylation of the resulting aniline into a single operation was made. (Chart 1).

$$C_6H_5NO_2 \xrightarrow[(1)]{C_6H_5CH_2OH\text{-}KOH} [C_6H_5NH_2] \xrightarrow[(2)]{C_6H_5CH_2OH\text{-}KOH} \underset{\text{I}}{C_6H_5NHCH_2C_6H_5}$$

Chart 1.

In this investigation modified conditions, *e. g.* use of large excess of the reagent, removal of water as it was formed and higher reaction temperature[*6] (225~260°)

[*1] Nanakuma, Fukuoka (宮野成二, 安倍宣博, 宇野昭子).

[*2] Isopropyl alcohol is capable of reducing nitrobenzene to aniline in the presence of sodium hydroxide : R. Lyons, M. Pleasant : Ber., **62B**, 1723 (1929).

[*3] The reduction was carried out in boiling xylene.

[*4] An earlier paper also revealed that U. O. P. nickel catalyst was capable of N-benzylation : E. F. Pratt, E. J. Frazza : J. Am. Chem. Soc., **76**, 6174 (1954).

[*5] At this temperature range it is to be considered that potassium hydroxide would be converted to potassium benzylate. See ref. 5.

[*6] The previously reported reductions of nitrobenzene by the reagent were run at much lower temperatures. See ref. 1~4.

1) Houben-Weyl : "Die Methoden der Organische Chemie," 3 Aufl., Band 2, 342 (1922).

2) W. Schraube : Ber., **8**, 619 (1875).

3) H. Fry, J. Cameron : J. Am. Chem. Soc., **49**, 864 (1928).

4) K. Ino, R. Oda : J. Soc. Chem. Ind. Japan, **46**, 1182 (1943); C. A., **42**, 6334 (1948).

5) Y. Sprinzak : J. Am. Chem. Soc., **78**, 3207 (1956); Org. Syntheses, Coll. Vol., **IV**, 92 (1958).