Anal. Calcd. for $C_{28}H_{26}N_4O_9$: C, 54.97; H, 5.22; N, 11.15. Found: C, 54.94; H, 5.23; N, 11.29.

p-Nitrocarbobenzyloxyglycyl-L-tyrosylglycine.—To 1.86 g. (3.7 mmoles) of p-nitrocarbobenzyloxyglycyl-L-tyrosylglycine ethyl ester was added 1 *N* NaOH (9.5 ml.) with stirring at such a rate that a pH of about 12 was maintained. After 1.5 hr. the solution was clarified by filtration, and the filtrate was acidified (congo red) with 4 *N* HCl, then left at 4° for a few hours. The crystals formed were washed with water and dried yielding 1.19 g. (68%), m.p. 218–220° dec.

Anal. Calcd. for $C_{21}H_{22}N_4O_9$: C, 53.16; H, 4.67; N, 11.81. Found: C, 53.07; H, 4.93; N, 11.81.

Glycyl-3,5-diiodo-L-tyrosine.—A solution of 5.1 g. (31 mmoles) of iodine monochloride in 11.3 ml. of 20% HCl was added with stirring to a solution of 3.6 g. (15 mmoles) of glycyl-L-tyrosine[15] in 25 ml. of 1 *N* HCl. After 1.5 hr. an aqueous solution of sulfur dioxide was added until the reaction mixture became pale yellow. The precipitate formed on adjusting the pH to 4 with 6 *N* NH_4OH was washed with water, and purified by reprecipitation (pH 7) from its solution in 6 *N* NH_4OH yielding 5.1 g. (69%) of needles, m.p. 222–224° dec.; ref. 16 gives a sintering point of 122–125° and a melting point of 290–292° dec. For paper chromatography, see Table I.

Anal. Calcd. for $C_{11}H_{12}I_2N_2O_4$: C, 26.96; H, 2.47; I, 51.79; N, 5.72. Found: C, 27.08; H, 2.69; I, 51.89; N, 5.82.

3,5-Diiodo-L-tyrosylglycine.—Dowex 50 × 12, H-form (about 20 ml. of wet resin) was added to a solution of 1.42 g. (6 mmoles) of L-tyrosylglycine[17] in 60 ml. of water until the pH of the mixture was 3.2. (Addition of more resin did not cause a further change in pH). Then 3 ml. of a 4 *M* solution of iodine monochloride (12 mmoles) in 1 *N* HCl was added with stirring over a period of about 5 min. After stirring for another 30 min. the resin was collected by filtration and washed with water, then with 60 ml. of 2 *N* NH_4OH, and again with water. The combined alkaline filtrates were concentrated to about 60 ml. When the pH was adjusted to 4, a precipitate formed which was collected after cooling and washed with ice-cold water yielding 1.66 g. (57%), m.p. 195.5–196.5° dec. Dissolution in 2 *N* NH_4OH and reprecipitation with 2 *N* HCl gave 1.20 g. of small crystals (m.p. unchanged) which were dried *in vacuo* at room temperature. A second crop, m.p. 184–185° dec. (0.50 g.), was obtained from the filtrate. For paper chromatography, see Table I.

Anal. Calcd. for $C_{11}H_{12}I_2N_2O_4 \cdot 0.5H_2O$: C, 26.47; H, 2.63; I, 50.86; N, 5.61. Found: C, 26.68; H, 2.64; I, 50.83; N, 5.53.

Glycyl-3,5-diiodo-L-tyrosylglycine.—A solution of 1.7 g. (6.7 mmoles) of iodine and 1.6 g. of potassium iodide in 10 ml. of water was added slowly (1 hr.) to a stirred solution of 0.89 g. (3.0 mmoles) of glycyl-L-tyrosylglycine[18] in 10 ml. of a 2% aqueous solution of methylamine. A 20% aqueous solution of methylamine was added dropwise as needed in order to maintain the pH of the reaction mixture between 7.5 and 9. After stirring for another hour the excess of iodine was reduced with an aqueous solution of sulfur dioxide and the pH was brought to 6.5 with 4 *N* HCl. The microcrystalline precipitate was washed with a small amount of ice-cold water and dried *in vacuo* at room temperature yielding 1.21 g., m.p. 211–212° dec. A second crop was obtained from the mother liquors; total yield, 1.34 g. (80%). For paper chromatography, see Table I.

Anal. Calcd. for $C_{13}H_{15}I_2N_3O_5 \cdot 0.5H_2O$: C, 28.08; H, 2.90; I, 45.64; N, 7.56. Found: C, 28.06; H, 3.13; I, 45.49; N, 7.47.

Glycyl-L-thyroxine.—The reaction of glycyl-3,5-diiodo-L-tyrosine (2.45 g., 5.0 mmoles) with 4-hydroxy-3,5-diiodophenylpyruvic acid (2.59 g., 6.0 mmoles) was carried out essentially as described previously[10] for the reaction of 3,5-diiodo-L-tyrosine-I^{131} with the same keto acid. The solution of the keto acid[19] was freshly prepared and the extraction with 1-butanol was omitted. The reaction mixture was evaporated and the residue was washed with small amounts of a saturated solution of sodium sulfate and of ice-cold water. The crude product was purified by dissolution in 1 *N* NH_4OH and reprecipitation (pH 5) with 4 *N* HCl. The substance was dried *in vacuo* at room temperature yielding 0.46 g. (11%), m.p. 183–185° dec. For paper chromatography, see Table I.

Anal. Calcd. for $C_{17}H_{14}I_4N_2O_5 \cdot 2H_2O$: C, 23.47; H, 2.09; I, 58.35; N, 3.22. Found: C, 23.23; H, 2.14; I, 58.11; N, 3.36.

L-Thyroxylglycine.—Solid 4-hydroxy-3,5-diiodophenylpyruvic acid (1.73 g., 4.0 mmoles) was added in small portions to a buffered solution (pH 6.7) of 3,5-diiodo-L-tyrosylglycine hemihydrate (1.47 g., 2.9 mmoles). Other reaction conditions were similar to those described previously (*cf.* glycyl-L-thyroxine). After reprecipitation at pH 7 of the crude product, the peptide was dried *in vacuo* at room temperature yielding 0.23 g. (9%), m.p. 207–210° dec. For paper chromatography, see Table I.

Anal. Calcd. for $C_{17}H_{14}I_4N_2O_5 \cdot H_2O$: C, 23.96; H, 1.89; I, 59.58; N, 3.29. Found: C, 23.77; H, 1.87; I, 59.10; N, 3.54.

Glycyl-L-thyroxylglycine.—Reaction conditions were similar to those described in the preceding paragraph. Starting materials were 1.73 g. (4.0 mmoles) of 4-hydroxy-3,5-diiodophenylpyruvic acid and 1.64 g. (2.9 mmoles) of glycyl-3,5-diiodo-L-tyrosylglycine hemihydrate. The crude reaction product was washed by centrifugation, then reprecipitated at pH 4.5, and dried *in vacuo* at room temperature yielding 0.18 g. (7%) of fine crystals, m.p. 198–201° dec.

Anal. Calcd. for $C_{19}H_{17}I_4N_3O_6 \cdot 2H_2O$: C, 24.62; H, 2.28; I, 54.76; N, 4.53. Found: C, 24.89; H, 2.29; I, 54.76; N, 4.76.

Inspection of paper chromatograms of the substance in short wave ultraviolet light revealed the presence of a faintly fluorescent spot. The fluorescent impurity was removed by extracting a solution of the tripeptide in 1 *N* NaOH several times with 1-butanol, washing the combined butanol extracts with water, then removing the butanol by evaporation under reduced pressure. Recrystallization of the residue gave a chromatographically pure product. For R_f values, see Table I.

(15) Nutritional Biochemicals Corp., Cleveland, Ohio.

(16) E. Abderhalden and M. Guggenheim, *Ber.*, **41**, 1237 (1908).

(17) M.p. 259–264° dec.; after recrystallization from acetone–water, 282–283° dec.; *cf.* E. Abderhalden, R. Abderhalden, H. Weidle, E. Baertich and W. Morneweg [*Fermentforschung.*, **16**, 98 (1938)] and H. Zahn and K. Ziegler [*Ann.*, **610**, 132 (1957)].

(18) M.p. 239–242° dec.; *cf.* ref. 17 and T. Yamashita, *J. Biochem.* (Tokyo), **48**, 651 (1960).

(19) Commercially available from Osaka Laboratory of Synthetic Organic Chemicals, Nishinomiya, Japan.

Synthesis of 2-(1-Nonenyl)-4-quinolinol (Pyo III) and Some Related 2,4-Disubstituted Quinolines

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In 1945 Hays and co-workers[2] reported the isolation of five closely related antibiotic metabolites of *Pseudomonas aeruginosa*. These substances were designated Pyo Ib, Pyo Ic, Pyo II, Pyo III, and Pyo IV. In 1952 Wells[3] reported the structural elucidation and synthesis of Pyo Ib, Pyo Ic, and Pyo III. Pyo Ib and Pyo Ic were shown to be the homologous 2-heptyl-4-quinolinol and 2-nonyl-4-quinolinol, respectively. Application of the Conrad–Limpach reaction[4] afforded synthetic Pyo Ib and Pyo Ic in yields above 25%. Structural studies featuring hydrogenation and ozonization[5] provided convincing evidence that the structure of Pyo III was 2-(1-nonenyl)-4-quinolinol. By use of chromatography an 0.8% yield of Pyo III was obtained by

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(2) E. E. Hays, *et al.*, *J. Biol. Chem.*, **159**, 725 (1945).

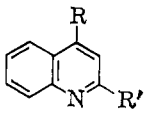
(3) I. C. Wells, *ibid.*, **196**, 331 (1952).

(4) N. J. Leonard, H. F. Herbrandson, and E. M. Van Heyningen, *J. Am. Chem. Soc.*, **68**, 1279 (1946).

(5) I. C. Wells, W. H. Elliott, S. A. Thayer, and E. A. Doisy, *J. Biol. Chem.*, **196**, 321 (1952).

TABLE I

SOME HOMOLOGS OF 2-(1-NONENYL)-4-QUINOLINOL AND INTERMEDIATES

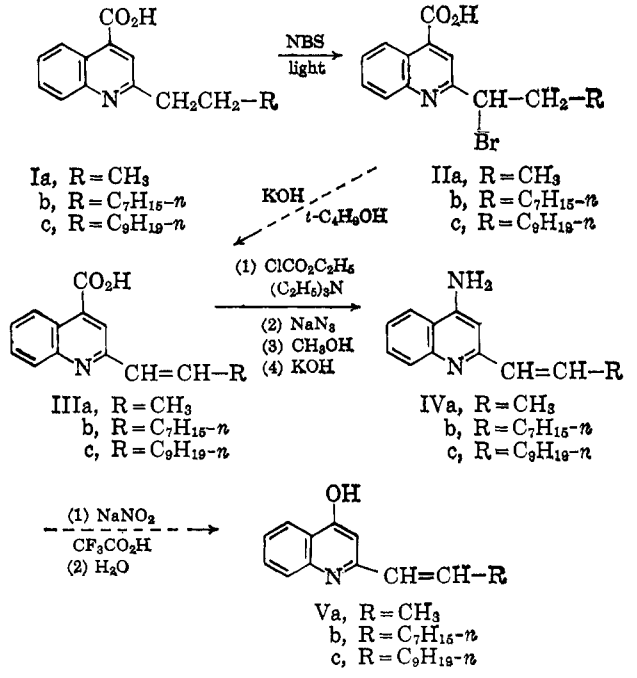


No.	R	R′	Formula	Calcd., % C	Calcd., % H	Calcd., % N	Found, % C	Found, % H	Found, % N	M.p.; °C.	Yield, %	Recrystn. solvent
Ia	CO_2H	$CH_2CH_2CH_3$[a]	$C_{13}H_{13}NO_2$							155–158	56	Methanol
Ib	CO_2H	C_9H_{19}-*n*[b]	$C_{19}H_{25}NO_2$	76.22	8.42		76.28	8.44		124–125	90	Methanol
Ic	CO_2H	$C_{11}H_{23}$-*n*	$C_{21}H_{29}NO_2$	77.02	8.93	4.28	77.10	8.84	4.12	124–127	78	Methanol
IIa	CO_2H	$C(Br)HCH_2CH_3$	$C_{13}H_{12}BrNO_2$	53.08	4.11	4.76	53.25	4.26	4.95	164–166	48	Methanol
IIc	CO_2H	$C(Br)HC_{10}H_{21}$-*n*	$C_{21}H_{28}BrNO_2$	62.06	6.95	3.45	61.75	6.78	3.05	116–117	61	Methanol
IIIa	CO_2H	$CH{=}CHCH_3$[c]	$C_{13}H_{11}NO_2$	73.22	5.20		73.24	5.43		206–208	68	Dimethylformamide–water
IIIc	CO_2H	$CH{=}CHC_9H_{19}$-*n*	$C_{21}H_{27}NO_2$	77.50	8.36	4.30	77.35	8.39	4.28	138–139	47	Methanol
IVa	$-NH_2$	$CH{=}CHCH_3$	$C_{12}H_{12}N_2 \cdot HCl$	65.30	5.94		65.50	6.10		238 dec.	27	Acetone
IVc	$-NH_2$	$CH{=}CHC_9H_{19}$-*n*	$C_{20}H_{28}N_2 \cdot HNO_3$	66.82	8.13	11.69	66.80	8.12	11.78	173–175	44	Methanol
Va	$-OH$	$CH{=}CHCH_3$	$C_{12}H_{11}NO$	77.81	5.99		77.39	6.17		210 dec.	20	Acetone
Vc	$-OH$	$CH{=}CHC_9H_{19}$-*n*	$C_{20}H_{27}NO$	80.76	9.15		80.45	8.81		144–145	40	Acetone

[a] Lit.[7] m.p. 156° (water). [b] Lit.[7] m.p. 145° (ethanol). [c] Lit.[9] m.p. 153–155° trihydrate (ethanol).

cyclization of the anil derived from crude methyl 3-oxo-4-dodecenoate in boiling diphenyl ether. Although this synthesis served to confirm the assigned structure, attempts to improve the yield were not encouraging. Moreover, the other known routes to 4-quinolinols are likewise rendered inappropriate for a practical synthesis of Pyo III by virtue of its conjugated double bond which is the most distinguishing feature of the metabolite. It is noteworthy that no other examples of 2-(1-alkenyl)-4-quinolinols have been found in the literature.

In the reaction sequence Ib to Vb is portrayed a new general four-step synthesis of Pyo III (Vb). The preparation of lower and higher homologs (Ia and c to Va and c) attests to the general nature of the method, which has the added advantages of convenience, inexpensive starting materials, satisfactory over-all yields, and relatively mild operating conditions.



(6) R. C. Elderfield "Heterocyclic Compounds," Vol. 4, John Wiley and Sons, Inc., New York, N. Y., 1952, Chapter 1.

2-Nonylcinchoninic acid (Ib) was readily prepared in yields above 90% from isatin and 2-undecanone by the Pfitzinger reaction.[7] Ziegler bromination of Ib with N-bromosuccinimide produced 2-(1-bromononyl)cinchoninic acid (IIb) which was dehydrohalogenated with potassium hydroxide in *t*-butyl alcohol to obtain 2-(1-nonenyl) cinchoninic acid (IIIb).

The Weinstock[8] modification of the Curtius reaction was used to advantage for the conversion of acid IIIb into the corresponding amine IVb in a unit operation. Treatment of the carboxylic–carbonic mixed anhydride prepared from IIIb, ethyl chloroformate, and triethylamine with aqueous sodium azide followed by the introduction of methanol produced the corresponding methyl carbamate. The latter was hydrolyzed *in situ* with alkali, and the amine (IVb) was isolated as the nitrate salt.

Diazotization of IVb in trifluoroacetic acid and subsequent hydrolysis of the diazonium salt *in situ* gave 2-(1-nonenyl)-4-quinolinol (Pyo III, Vb) in a yield of 50–60%.

The representative homologs of Pyo III and their intermediates selected for Table I were prepared by the same general procedures described in detail for the synthesis of compounds Ib to Vb inclusive.

It is noteworthy that 2-propenylcinchoninic acid (IIIa)[9] was the only example of the 2-(1-alkenyl)cinchoninic acids found in the literature. It was isolated as a trihydrate in 9.8% yield as a product of the interaction of pyruvic acid, crotonaldehyde and aniline under the conditions of the Doebner–Miller reaction. Although many derivatives of 4-aminoquinoline and 4-aminoquinaldine are known, none of the 2-alkenyl-4-aminoquinolines has been hitherto reported.

Experimental[10]

2-(1-Bromononyl)cinchoninic Acid (IIb).—To a mixture of 29.8 g. (0.1 mole) of 2-nonylcinchoninic acid[7] and 17.8 g. (0.1

(7) N. P. Buu-Hoï and R. Royer, *J. Chem. Soc.*, 106 (1948).
(8) J. Weinstock, *J. Org. Chem.*, **26**, 3511 (1961).
(9) M. Richter and P. Boyde, *J. prakt. Chem.*, **9**, 124 (1959); *Chem. Abstr.*, **54**, 6715 (1960).
(10) All melting points are uncorrected.

mole) of N-bromosuccinimide in 500 ml. of carbon tetrachloride there was added 100 mg. of benzoyl peroxide. The mixture was stirred and heated under reflux on the steam bath for 1 hr. in the presence of two No. 2 Photoflood lamps. The succinimide was collected by filtration. Evaporation of the solvent from the filtrate left a tan solid residue which was recrystallized from methanol to yield 15 g. (39%) of pale yellow crystals, m.p. 125–126°.

Anal. Calcd. for $C_{19}H_{24}BrNO_2$: C, 60.32; H, 6.40; N, 3.70. Found: C, 60.28; H, 6.35; N, 3.61.

2-(1-Nonenyl)cinchoninic Acid (IIIb).—To 25 g. (0.066 mole) of 2-(1-bromononyl)cinchoninic acid there were added 50 g. of finely ground potassium hydroxide and 125 ml. of *t*-butyl alcohol. The mixture was stirred and heated under reflux for 1 hr., during which time a viscous mass of salt had formed on the bottom and sides of the flask. The mixture was diluted with 100 ml. of water and acidified to pH 2 with dilute sulfuric acid. The solid was collected by filtration and washed well with water to remove the residual salts. Recrystallization from methanol gave 16.2 g. (82%) of light yellow crystals, m.p. 137–138°.

Anal. Calcd. for $C_{19}H_{23}NO_2$: C, 76.73; H, 7.80. Found: C, 77.00; H, 7.94.

4-Amino-2-(1-nonenyl)quinoline (IVb).—To a solution of 2.97 g. (0.01 mole) of 2-(1-nonenyl)cinchoninic acid and 1.38 ml. (0.01 mole) of triethylamine in 25 ml. of tetrahydrofuran at 5° there was added dropwise 1.4 g. (0.013 mole) of ethyl chloroformate. The mixture was stirred for 0.5 hr. while a solution of 0.86 g. (0.013 mole) of sodium azide in 20 ml. of water was added dropwise. After stirring an additional hour at 5°, the solution was poured onto 100 g. of cracked ice and the azide was extracted into ether. A few milliliters of saturated sodium chloride solution was added to break up the resulting emulsion. The organic layer was separated and dried over anhydrous magnesium sulfate. Anhydrous methanol (10 ml.) was added to the filtered solution and the ether was removed under reduced pressure. The residue was boiled for 2 hr. with 10 ml. of anhydrous methanol in 35 ml. of benzene. The benzene was removed under reduced pressure and the carbamate was heated under reflux for 5 hr. in aqueous methanolic potassium hydroxide prepared from 2 g. of potassium hydroxide pellets, 20 ml. of methanol, and 5 ml. of water. The mixture was diluted with 50 ml. of water and the amine was extracted into ether. The ether was removed under reduced pressure and the residue was dissolved in 20 ml. of acetone. The pH was adjusted to 2 by the addition of dilute (1:1) nitric acid. The amine salt was collected by filtration and washed with acetone to yield 1.07 g. (33%) of off-white crystalline 4-amino-2-(1-nonenyl)quinoline nitrate, m.p. 163–164°.

Anal. Calcd. for $C_{18}H_{25}N_3O_3$: C, 65.23; H, 7.60; N, 12.68. Found: C, 65.58; H, 7.70; N, 12.63.

2-(1-Nonenyl)-4-quinolinol (Vb, Pyo III).—To a light yellow solution of 2.64 g. (0.08 mole) of 4-amino-2-(1-nonenyl)quinoline nitrate dissolved in 20 ml. of trifluoroacetic acid at 5° there was added 0.52 g. (0.08 mole) of sodium nitrite. After the deep red solution was stirred for 0.5 hour at 5°, 7 ml. of water was added all at once, and the solution was stirred for an additional 0.5 hour. The mixture was poured into 50 ml. of water and extracted with ether. The ether layer was separated, washed with water, and evaporated under reduced pressure to obtain a red oil. The oil was dissolved in 25 ml. of acetone and diluted with a saturated aqueous sodium carbonate solution to pH 9. The tan crystals were collected by filtration, washed with water, and dissolved in 20 ml. of methanol. After a carbon treatment, the filtrate was evaporated to obtain a yellow crystalline solid. Recrystallization from acetone yielded 1.2 g. (56%) of pale yellow needles, m.p. 151–152°.

Anal. Calcd. for $C_{18}Y_{23}NO$: C, 80.25; H, 8.61; N, 5.20. Found: C, 80.05; H, 8.60; N, 5.08.

The infrared spectrum was identical with the spectrum of authentic 2-(1-nonenyl)-4-quinolinol (Pyo III).[2] The *trans* character of the double bond in the nonenyl side chain was clearly shown by the strong band at 965 cm.$^{-1}$ which is characteristic of a *trans*-disubstituted ethylene.[11] Likewise the other compounds bearing alkenyl groups showed a similar absorption band. Lack of absorption in the region of 690 cm.$^{-1}$ indicated the absence of *cis* isomer in every case.

(11) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," 2nd Ed., John Wiley and Sons, Inc., New York, N. Y. 1958, p. 45.

Acknowledgment.—The authors wish to thank Richard M. Downing for the microanalyses and Donald Evans and David Whitehead for the infrared spectra.

A Convenient Synthesis of Tricyclic 2-Quinolizidones[1]

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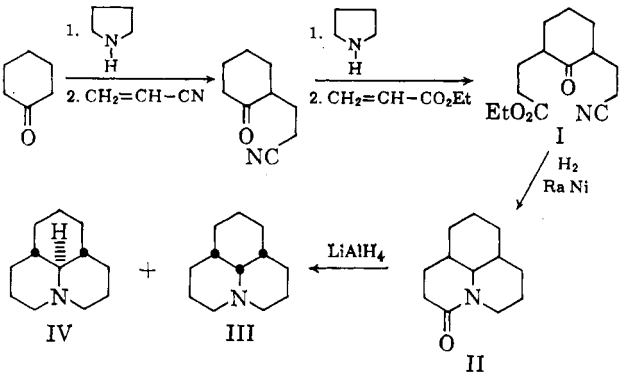
We have previously shown the utility of our quinolizidine synthesis in the preparation of matrine,[2] matridine,[3] and various tricyclic systems with nitrogen at a bridgehead.[4] This work reports a modification of that scheme which allows its application to the synthesis of 2-quinolizidones typified by II.

This new route is outlined below.



The steps are all unexceptional and follow closely the basic pathway we investigated earlier. Key in the synthesis is the Stork enamine synthesis[5] for the preparation of I and the reductive cyclization of I to provide II. We have discussed the latter reaction previously.[4]

The structure and stereochemistry of the quinolizidone (II) were established by lithium aluminum hydride reduction of II to a mixture of hexahydrojulolidines, which consisted of 60% III and 40% IV.

Experimental[6]

Ethyl β-[2-(1-Oxo-6-cyanoethyl)]cyclohexylpropionate (I).—The pyrrolidine enamine was prepared by refluxing 35 g. (0.23 mole) of the above ketone with 57 g. (0.81 mole) of pyrrolidine in 200 ml. of anhydrous benzene. After 18 hr. the theoretical amount of water had been collected in an azeotrope separator. The excess pyrrolidine and benzene were removed *in vacuo* and

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(2) L. Mandell, K. P. Singh, J. T. Gresham, and W. Freeman, *J. Am. Chem. Soc.*, **85**, 2682 (1963).

(3) L. Mandell and K. P. Singh, *ibid.*, **83**, 1766 (1961).

(4) L. Mandell, J. U. Piper, and K. P. Singh, *J. Org. Chem.*, **28**, 3440 (1963).

(5) G. Stork, A. Brizzolera, H. Landesman, J. Szmuszkovicz, and R. Terrell, *J. Am. Chem. Soc.*, **85**, 207 (1963).

(6) Melting points and boiling points are uncorrected. Elemental analyses were done by Drs. G. Weiler and F. B. Strauss, Oxford, England.