Antiprotozoal Quinones. III. Synthesis of 8-Amino-5,6-quinolinediones, 8-Amino-7-chloro-5,6-quinolinediones, and 8-Amino-5,5,7-trichloro-6(5*H*)-quinolones as Potential Antimalarials[1]

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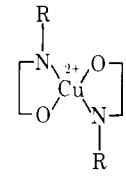
Cu^{2+}-catalyzed oxidation of 6-quinolinol in MeOH with a variety of secondary amines gave a series of 8-dialkylamino-5,6-quinolinediones (**5**). The reaction was useless when attempted with primary amines and other amines which complex cupric ion. Several 8-amino-7-chloro-5,6-quinolinediones (**7**) were obtained by reaction of amines with 7,8-dichloro-5,6-quinolinedione. A number of 8-amino-5,5,7-trichloro-6(5*H*)-quinolones (**10**) were obtained by reaction of amines with 5,5,7,8-tetrachloro-6(5*H*)-quinolone. All the new quinones and quinone derivatives were evaluated against blood-induced *Plasmodium berghei* infections in mice, *P. gallinaceum* in chicks, and *P. gallinaceum* in mosquitoes (*Aedes aegypti*). None of the compounds showed high antimalarial activity. The series **5** in particular is highly toxic in both mice and chicks. 8-Hexylamino-7-chloro-5,6-quinolinedione resulted in increased survival time in *P. berghei* infected mice of 3.1 days at 320 mg/kg and 3.9 days at 640 mg/kg with no toxicity but with no activity in the other screens. These results are part of an increasing body of circumstantial evidence which suggests that the *in vivo* metabolism of the antimalarial 6-methoxy-8-aminoquinolines to quinones may not be contributing to their antimalarial activity.

Interest in 8-amino-5,6-quinolinediones and related compounds was stimulated by their relationship to the presumed active quinonoid metabolite of antimalarials derived from 8-amino-6-methoxyquinoline.[2] We report here the synthesis and results of biological evaluation of further analogs (**5**, **7**, **10**, Scheme I) related to these metabolites as well to 8-amino-5,6-quinolinedione. Previous evaluation of 8-amino-5,6-quinolinediones has been by an indirect means.[3] A 5-methoxy-6-hydroxyquinoline and a 5,6-dihydroxyquinoline, expected to be converted *in vivo* into 8-amino-5,6-quinolinedione, were used. Related carbostyryl analogs have been more thoroughly explored.[4]

8-Dialkylamino-5,6-quinolinediones (5).—Extension of syntheses of Brockman and Havinga[5] and Soviet chemists[6] using Cu^{2+}-catalyzed oxidation of 6-quinolinol in MeOH in the presence of a variety of secondary amines provided facile entry to the series **5**. The particular compounds prepared are given in Table I. We did not attempt to prepare compounds incorporating a common dialkylaminoalkyl moiety in view of the high toxicity encountered with such compounds among the related 1,2-naphthoquinones.[7] Considerable experience with this reaction has helped delineate its scope. No oxidation occurs when an *N*-alkylated ethanolamine or primary aliphatic amine is used, presumably due to the formation of the cupramine complexes **1**[8] and **2**, respectively, in which the cupric ion is catalytically inert. With secondary amines the formation of cupramine complexes such as **2** is apparently less favored and catalytic oxidation uninhibited. Attempts to extend the scope of the reaction by use of various cobalt salts as catalyst failed.



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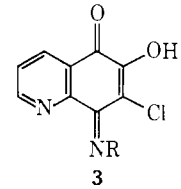
$(RNH_2)_4Cu^{2+}$

2

8-Amino-7-chloro-5,6-quinolinediones (7).—Exhaustive chlorination of 5-amino-6-quinolinol prepared from 6-quinolinol gave 7,8-dichloro-5,6-quinolinedione (**6**),[9] which reacted with primary and secondary amines to give the new quinones **7**. During our work we observed that the pmr spectra of **17** did not support the older formulation[9] of these compounds as the tautomer **3**. The α-CH_2 protons of the Bu group (δ 4.14 ppm from $(CH_3)_4Si$ in $CDCl_3$) appear as a quartet which is analyzed as an overlapping doublet of triplets (J_{CHCH} = 7 Hz, J_{NHCH} = 7 Hz) and which collapses to a clean triplet (J_{CHCH} = 7 Hz) on D exchange of NH. This clearly necessitates that the exchangeable proton be situated on N as indicated in **7** (R^1 = H).



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8-Amino-5,5,7-trichloro-6(5*H*)-quinolones (10).—Exhaustive chlorination of 6-quinolinol in acetic acid[10] gives the pentachloroquinolone 8 which is easily converted into the tetrachloroquinolone **9** by dehydrohalogenation.[10] Reaction of **9** with a variety of amines gave **10**. Extensive secondary reactions can occur during this transformation; for example, the quinone-

(1) This work was supported by the U. S. Army Medical Research and Development Command under Contract No. DA-49-193-MD-2880. This paper is Contribution No. 743 from the Army Research Program on Malaria and was the subject of a preliminary report at the First Northeast Regional Meeting of the American Chemical Society, Boston, Mass., Oct., 1968.

(2) Background information is summarized in our previous paper, F. J. Bullock, J. F. Tweedie, D. D. McRitchie, and M. A. Tucker, *J. Med. Chem.*, **13**, 97 (1970).

(3) N. L. Drake and Y. T. Pratt, *J. Amer. Chem. Soc.*, **73**, 544 (1951).

(4) *Cf.* R. R. Holmes, J. Conrody, J. Guthrie, and R. McKay, *ibid.*, **76**, 2400 (1954); G. R. Pettit, W. C. Fleming, and K. D. Paull, *J. Org. Chem.*, **33**, 1089 (1968).

(5) W. Brockman and E. Havinga, *Rec. Trav. Chim.*, **74**, 937 (1955).

(6) (a) Yu. S. Tsizin and M. V. Rubtsov, USSR Patent 188,495; *Chem. Abstr.*, **67**, 9069s (1967). (b) Yu. S. Tsizin and M. V. Rubtsov, *ibid.*, **79**, 87520m (1969). (c) *ibid.*, **70**, 87516q (1969).

(7) See our paper, "Antiprotozoal Quinones. II," ref 2.

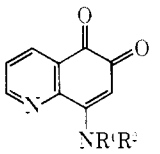
(8) *Cf.* A. E. Martell and M. Calvin, "Chemistry of Metal Chelate Compounds" Prentice-Hall, Inc., Englewood Cliffs, N. J., 1952, p 179.

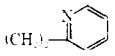
(9) T. Zincke and K. Wiederhold, *Ann. Chem.*, **290**, 359 (1896).

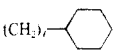
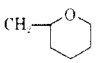
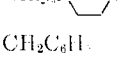
(10) T. Zincke and H. Müller, *ibid.*, **290**, 223 (1891).

TABLE I

8-AMINO-5,6-QUINOLINEDIONES, 8-AMINO-7-CHLORO-5,6-QUINOLINEDIONES, AND 8-AMINO-5,5,7-TRICHLORO-6(5*H*)-QUINOLONES



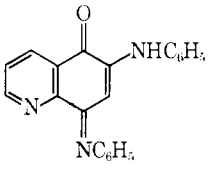
No.	R^1	R^2	Cryst. solvent	Formula[a]	Mp, °C	Yield, %
11	CH_3	$(CH_2)_5CH_3$	C_6H_6–petr. ether	$C_{14}H_{16}N_2O_2$	95–96	16
12	CH_3		C_6H_6–petr. ether	$C_{16}H_{18}N_2O_2$	128–130	25
13	C_2H_5	C_2H_5	C_6H_6–petr. ether	$C_{13}H_{14}N_2O_2$	129–132	21
14	CH_3		C_6H_6–petr. ether	$C_{16}H_{15}N_3O_2 \cdot H_2O$[b]	109–110.5	34
15			$CHCl_3$–petr. ether	$C_{14}H_{14}N_2O_2$	135–137	83
16			C_6H_6–petr. ether	$C_{13}H_{12}N_2O_3$	174–175	61

17	H	$(CH_2)_3CH_3$	EtOH	$C_{13}H_{13}ClN_2O_2$	143.5–144.5	67
18	H	$(CH_2)_5CH_3$	EtOH	$C_{15}H_{17}ClN_2O_2$	127–128	77
19	H	$(CH_2)_{11}CH_3$	EtOH	$C_{21}H_{29}ClN_2O_2$	115–116	75
20	H		EtOH	$C_{15}H_{15}ClN_2O_2$	195–196	64
21	H		EtOH	$C_{16}H_{15}ClN_2O_2$	185–186 dec	66
22	H		EtOH	$C_{16}H_{23}ClN_2O_2$	119–119.5	60
23	H		$CHCl_3$–petr. ether	$C_{15}H_{15}ClN_2O_3$	166.5–168	73
24	H		EtOH	$C_{18}H_{18}ClN_3O_2$	157.5–159	67
25	H	$CH_2C_6H_5$	EtOH	$C_{16}H_{11}ClN_2O_2$	177–178 dec	88
26	C_2H_5	C_2H_5	Et_2O–petr. ether	$C_{13}H_{13}ClN_2O_2$	97–99	80
27	H	C_6H_4-*p*-OCH_3	EtOH	$C_{16}H_{13}ClN_2O_3$	199.5–201.5	34
28	H	C_6H_5[c]	EtOH	$C_{14}H_9ClN_2O_2$	191.5–192.5	32

29	H	$(CH_2)_3CH_3$	EtOH	$C_{13}H_{13}Cl_3N_2O$	160–160.5	91
30	H	$(CH_2)_5CH_3$	EtOH	$C_{15}H_{17}Cl_3N_2O$	75–75.5	37
31	H		EtOH	$C_{15}H_{15}Cl_3N_2O$	179–180	90
32	H		EtOH	$C_{16}H_{15}Cl_3N_2O$	167.5–168.5	77
33	H		EtOH	$C_{14}H_{15}Cl_3N_2O$	167.5–169	78
34	H	$CH_2C_6H_5$	EtOH	$C_{16}H_{11}Cl_3N_2O$	147–148	76
35	H	C_6H_4-*p*-OCH_3	$CHCl_3$–petr. ether	$C_{16}H_{13}Cl_3N_2O$	167–169	73
36	H	C_6H_5[d]	HOAc	$C_{15}H_9Cl_3N_2O$	196–198	73

[a] All compounds were analyzed for C, H, N or C, H, Cl, N and results obtained for these elements were within ±0.4% of the theoretical values unless otherwise indicated. [b] Presence of H_2O of hydration confirmed by nmr ($CDCl_3$). In previous work with a series of 4-amino-1,2-naphthoquinones (ref 2) the quinone bearing the 4-substitution $-N(CH_3)(CH_2)_3N(CH_3)_2$ could also be isolated only as its hydrate. Each of these hydrates has the same number of carbons between basic sites. Calcd: C, 65.58; H, 5.51. Found: C, 66.36, 66.14, 66.08; H, 5.36, 5.69, 5.91. [c] This compound has been prepared independently; see ref 6b. [d] Previously prepared; see ref 9, mp 195°, analysis C, H. [e] These compounds were prepared using a mixture of Et_2O (25 ml) and petroleum ether (250 ml) at 0° instead of EtOH at −30° (see Experimental Section). [f] Previously prepared; see ref 10, mp 200–202°.

imine **4** has also been claimed to be formed on reaction of **9** with aniline.[10] Success in obtaining the series **10** was achieved only under reaction conditions which resulted in the product precipitating from solution essentially as it formed. This was achieved by using



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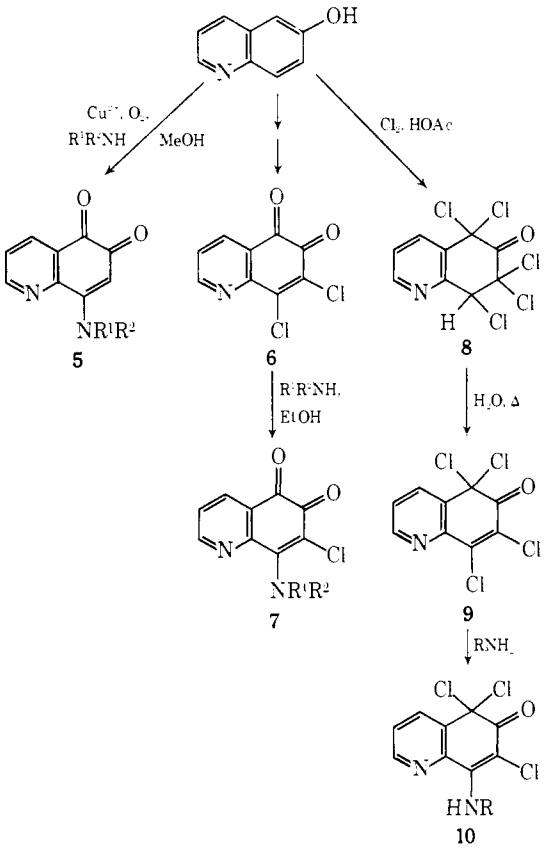
low reaction temperatures or decreasing the polarity of the reaction medium. We also obtained in low yield the compound corresponding with **4** reported by Zincke and Müller[10] but have not yet completed an independent confirmation of the assigned structure. We were unable to isolate such compounds as **4** from reactions of other amines with **9** and it is clear that such a route is not a practical approach to the synthesis of 6-amino-8-imino-5(8H)-quinolones.

Biological Results.—All the compounds of Table I have been evaluated against blood-induced *P. berghei* infections in mice,[11] *P. gallinaceum* in chicks,[12] and *P. gallinaceum* in mosquitoes (*Aedes aegypti*).[13] The 5,5,7-trichloroquinolones (**29–36**) showed no antimalarial activity in any of the screens. The 8-amino-5,6-quinolinediones (**11–16**) showed weak activity but also high toxicity in both mice and chicks. Quinone **16** produced abnormal oocysts at a concentration of 0.01% in the mosquito screen and partial sporozoite suppression at 0.1%. Activity against the sexual phase of the parasite's life cycle was not found with other compounds of this class, however.

Quinone **18** caused an increased mean survival time of *P. berghei* infected mice of 3.1 days at 320 mg/kg and of 3.9 days at 640 mg/kg with no toxicity, but no activity in the other screens. As a class the 8-amino-7-chloro-5,6-quinolinediones are less toxic than the 8-amino-5,6-quinolinediones which in turn, however, appear to be more toxic than the analogous 4-amino-1,2-naphthoquinones.[7]

These studies have failed to produce a compound with potent *in vivo* antimalarial activity, although the quinones prepared are closely related to the presumed active quinoid metabolites of antimalarials derived from 6-methoxy-8-aminoquinolines. In our previous study,[13] an analogous series of 4-amino-1,2-naphthoquinones including several compounds bearing the dialkylaminoalkyl group were also found inactive. It is also of interest that the hydroquinone, 5,6-dihydroxy-8-(5-isopropylaminopentylamino)quinoline trihydrobromide, is essentially inactive against *P. berghei* in mice although 5,6-dimethoxy-8-(5-isopropylaminopentylamino)quinoline has definite activity.[14] Taken together this body of circumstantial evidence suggests that the *in vivo* activity of the 6-methoxy-8-aminoquinolines may not be related to their *in vivo* metabolic conversion to quinones. Their toxicity may be related to this transformation as has been suggested previously.[15]

SCHEME I



Experimental Section[16]

8-Dialkylamino-5,6-quinolinediones (11–16).—These compounds were prepared by the following modification of the procedure of Tsizin and Rubtsov.[6] $Cu(OAc)_2$ (0.1 g) and the dialkylamine (4 ml) were added to a stirred solution of 6-quinolinol[17] (1.45 g, 10 mmol) in 30 ml of MeOH. A steady stream of O_2 was bubbled through this red reaction mixture for 1 hr with external cooling so that the temperature did not exceed 50°.

Quinones **11–14** were isolated in the following manner. The solvent from the reaction mixture was removed under reduced pressure and the oily residue dissolved in $CHCl_3$. The $CHCl_3$ solution was washed with H_2O, dried ($MgSO_4$), and evaporated to a dark red oil. Chromatography on neutral alumina ($CHCl_3$)

(11) T. S. Osdene, P. B. Russell, and L. Rane, *J. Med. Chem.*, **10**, 431 (1967).

(12) Chicks (9–12 days old) were infected (intravenously) with a standard inoculum to produce a disease fatal to 100% of untreated controls within 3–4 days. Candidate compounds were dissolved or suspended in peanut oil and administered either subcutaneously or *per os* immediately after infection. A 100% increase in survival time was considered to be the minimum effective response to the antimalarial activity of the drug. Chicks surviving 30 days are recorded as cured.

(13) E. J. Gerberg, L. T. Richard, and J. B. Poole, *Mosquito News*, **26**, 359 (1966).

(14) Dr. R. E. Strube of Walter Reed Army Institute of Research informs us that this 5,6-dimethoxyquinoline results in an increased survival time in *P. berghei* infected mice of 2.1 days at 40 mg/kg and 5.3 days at 160 mg/kg, and shows toxicity at 640 mg/kg. The analogous 5,6-dihydroxyquinoline in the same test resulted in an increased mean survival time of 0.4 day at 40 mg/kg and showed toxicity at 160 and 640 mg/kg.

(15) B. B. Brodie and S. Udenfried, *Proc. Soc. Exp. Biol. Med.*, **74**, 845 (1950).

(16) Melting points were determined on a Mel-Temp or Fisher–Johns apparatus. The ir and uv spectra were obtained on Perkin-Elmer Model 137 and Beckman DK instruments, respectively. The nmr spectra were taken in $CDCl_3$ on a Varian A-60 instrument using TMS as internal standard. Mass spectra were determined with a CEC Model 11 instrument. Microanalyses were performed by Galbraith Microanalytical Laboratories, Knoxville, Tenn. The ir, uv, and nmr spectra were as expected for the assigned structure.

(17) Prepared in up to 95% yield from 6-methoxyquinoline with refluxing 48% HBr. The yield varied considerably with the source of material which was used as purchased, the best yields being obtained with material from Distillation Products Industries.

gave on evaporation, and trituration with Et_2O, the desired product.

Compound **15** was isolated by dilution of the reaction mixture with H_2O (60 ml) and acidification with HOAc (3 ml). The mixture was extracted with $CHCl_3$, washed several times with H_2O, dried ($MgSO_4$), and evaporated to *ca.* 25 ml. Careful addition of petroleum ether caused crystallization.

Quinone **16** was isolated directly from the reaction mixture after it was chilled to 0°.

8-Amino-7-chloro-5,6-quinolinediones (17–27).—The appropriate amine (30–40 mmol) in a few milliliters of EtOH was added rapidly to a stirred solution of 3 g (13 mmol) of 7,8-dichloro-5,6-quinolinedione[9] in 400 ml of EtOH at 0°. The reaction mixture, which immediately turned deep red, was stirred for 30 min. The red quinones **17–25** were isolated directly by evaporating the solvent *in vacuo* to a volume of 25–50 ml, cooling the residual suspension to 0°, and filtering.

Compound **26** was obtained by evaporating the solvent and dissolving the resulting solid in benzene. Petroleum ether was carefully added to the point of cloudiness and the mixture chilled to 0° and filtered.

Quinone **27** was prepared using the procedure described by Zincke and Wiederhold[9] for the anilido derivative.

8-Amino-5,5,7-trichloro-6(5*H*)-quinolones (29–35).—A solution of 5,5,7,8-tetrachloro-6(5*H*)-quinolone[10] (2.5 g, 9 mmol) in 50 ml of EtOH[18] was cooled to −30° with constant stirring. The amine (27 mmol) in a few milliliters of EtOH was added rapidly. The product began to separate from the yellow-brown reaction mixture within 5 min and after 15 min the mixture was filtered to give the product as a yellow solid.

(18) A few compounds were prepared using a less polar reaction medium, see footnote *c* of Table I.

Preparation and the Results of Antitumor Screening of Some Substituted Amino-, Azido-, Halogeno- and Hydroxy-*p*-benzoquinones[1]

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Synthesis of some amino-, azido-, hydroxyl-, and halo-substituted *p*-benzoquinones are reported. The aminobenzoquinones were prepared by catalytic reduction of the corresponding azide compounds; the latter in turn were obtained by the treatment of halobenzoquinones with NaN_3. Alkylaminoquinones were prepared either by the replacement of methoxyquinones with appropriate amines or by refluxing dialkyl- or diarylbenzoquinones with amines. Some alkyl analogs of 2,5-dihydroxy-3,6-diphenyl-*p*-benzoquinone (polyporic acid) were prepared by free-radical alkylation of 2,5-dihydroxy-*p*-benzoquinone with acyl peroxide. Preliminary screening results of these compounds indicated that 2,5-diazido-3,6-dimethoxy-*p*-benzoquinone, 2,5-dichloro-3,6-diphenyl-*p*-benzoquinone, and 2,5-bis(*p*-ethylphenyl)-3,6-dihydroxy-*p*-benzoquinone possessed moderate activity against Walker carcinosarcoma 256. The last compound also possesses some activity against leukemia L-1210. Marked weight loss in the surviving animals was observed.

The antibacterial activity of many quinone derivatives has long been recognized.[2,3] This property has been attributed mainly to protein binding.[4–8] A number of benzoquinones, naphthoquinones, anthraquinones, and quinoxazines possess activity against protozoa, Gram-positive and Gram-negative bacteria, and *Mycobacterium tuberculosis*.[9] Compounds of this type are also used as antitumor agents.[9] A common *o*-aminoquinonoid unit was noted[10] among some tumor-inhibitory antibiotics such as streptonigrin, actinomycin C, mitomycins, and porfiromycin. Certain 2,5-bis(alkylamino)-3,6-dimethoxy-*p*-benzoquinones are inhibitory to sarcoma 180.[11] Polyporic acid (2,5-dihydroxy-3,6-diphenyl-*p*-benzoquinone) was reported to be active against leukemia L-1210.[12] Some quinones are potent inhibitors of dehydrogenase activity of tumor cells.[13]

It is conceivable that, in addition to the protein binding characteristics, various substituted quinones with different oxidation–reduction potentials may play an important role in the phosphorylation, H transfer, and electron transfer in biological metabolism. Selective inhibition of the tumor cells may be achieved by modification of substituents on the quinone ring system. The present communication involves the synthesis of some amino-, azido-, hydroxyl-, and halo-substituted *p*-benzoquinones and the preliminary structure–activity study in animal tumor systems.

Chemistry.—Although a series of 2,5-diaryl-3,6-dihydroxy-*p*-benzoquinones (I, R = aryl) related to polyporic acid (I, R = C_6H_5) was synthesized and evaluated by Cain,[14] the corresponding diamino analogs II have not yet been studied. In view of the importance of the aminoquinones in oncological studies,[10,11] compounds II were synthesized in this laboratory.

Since the primary amino group is susceptible to both oxidation and hydrolysis, relatively few aminoquinones were reported in the literature. By adaptation of a

(1) (a) This investigation was supported by the Cancer Chemotherapy National Service Center, National Cancer Institute, of the National Institutes of Health, Public Health Service; Contract No. PH-43-65-94; (b) presented in part before the Division of Medicinal Chemistry, 157th National Meeting of the American Chemical Society, Minneapolis, Minn., April 1969 (MEDI 17).

(2) (a) E. A. Cooper, *Biochem. J.*, **7**, 186 (1913); (b) E. A. Cooper, *J. Soc. Chem. Ind.*, **46**, 59 (1927).

(3) S. Hayashi, *Kumamoto Pharm. Bull.*, **1**, 93 (1954).

(4) E. A. Cooper and R. B. Haines, *Biochem. J.*, **22**, 317 (1928).

(5) M. Raciborski, *Anzeiger Akad. Wiss. Krakau*, 553 (1909); *Chem. Zentr.*, **78**, 1595 (1907).

(6) E. Fischer, *Ber.*, **43**, 525 (1910).

(7) R. Cecil, *Advan. Protein Chem.*, **9**, 287 (1959).

(8) S. Hayashi, T. Tanoue, S. Ujioka, and K. Yano, *Kumamoto Pharm. Bull.*, **2**, 62 (1955).

(9) T. V. P. Rao and V. Venkateswarlu, "Symposium on the Synthesis of Heterocyclic Compounds of Physiological Interest," Hyderabad, India, 1964, p 134; *Chem. Abstr.*, **68**, 76551 (1968).

(10) K. V. Rao, K. Biemann, and R. B. Woodward, *J. Amer. Chem. Soc.*, **85**, 2532 (1963).

(11) H. S. Verter and J. Rogers, *J. Org. Chem.*, **31**, 987 (1966).

(12) J. F. Burton and B. F. Cain, *Nature*, **184**, 1326 (1959).

(13) S. Hayashi, H. Ueki, Y. Ueki, H. Aoki, K. Tonaka, J. Fujimoto, K. Katsukawa and M. Mori, *Chem. Pharm. Bull.* (Tokyo), **11**, 948 (1963).

(14) (a) B. F. Cain, *J. Chem. Soc.*, 936 (1961); (b) B. F. Cain, *ibid.*, 356 (1963); (c) B. F. Cain, *ibid.*, 5472 (1964); (d) B. F. Cain, *ibid.*, C, 1041 (1966).