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121. Nobuko Kataoka, Akira Imamura, Yutaka Kawazoe, Goro Chihara, and Chikayoshi Nagata : Electron Spin Resonance Study on the Photoinduced Radicals from Carcinogenic 4-Nitroquinoline 1-Oxide.*[1]

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Since strong carcinogenic activity of 4-nitroquinoline 1-oxide was demonstrated by Nakahara, *et al.*,[1] many studies have been done in this connection on its chemical reactivities and its modes of biological action.[2~4]

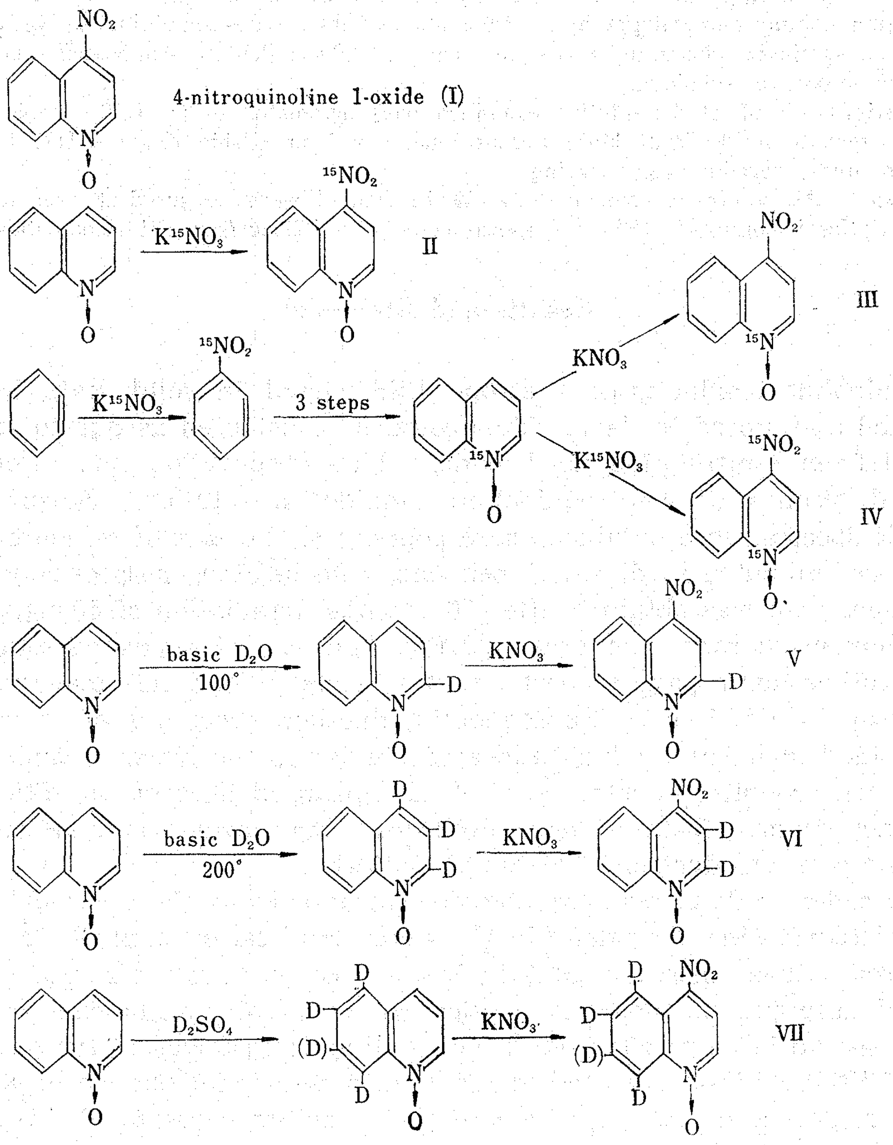

Chart 1. Preparation of Labeled Compounds

[*1] This paper constitutes part I of a series entitled "ESR Studies on 4-Nitroquinoline 1-Oxide and its Related Compounds." This work has been reported at the third ESR symposium in Tokyo (Nov., 1964).

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1) W. Nakahara, F. Fukuoka, T. Sugimura : Gann, **48**, 129 (1957).
2) H. Endo : *Ibid.*, **49**, 151 (1958).
3) W. Nakahara, F. Fukuoka : *Ibid.*, **50**, 1 (1959).
4) W. Nakahara : Arznei. Forsch., **14**, 842 (1964).

On the course of our serial studies on the carcinogenesis with chemical carcinogens, strong electron spin resonance (ESR) signals were observed by UV-irradiation of 4-nitroquinoline 1-oxide in solid state or in solutions in several kinds of organic solvents. Of interest, is that three kinds of spectra with different fine structures were observed depending on the sort of the solvents used, that is, dioxane type-, benzene type-, and hexane type-spectra, respectively. Our present work concerns the ESR study of 4-nitroquinoline 1-oxide from photochemical point of view and general features of UV-irradiation of this compound and structural characterizations of the photoinduced radicals are presented in this paper.

Experimental

Compounds——4-Nitroquinoline 1-oxide was synthesized by the authentic preparative method,[5] and purified by alumina column chromatography. Preparation of its isotope-containing derivatives were carried out according to a synthetic scheme as shown in Chart 1,[6] these deutero compounds being identified by nuclear magnetic resonance technique.

UV-Irradiation——1 ml. of the solution examined were irradiated in an ESR sample tube of quartz (0.5 cm. in diameter), by a 100 Watt high pressure mercury lamp (Ushio Kogyo's UM 100 type) from a distance of 6 cm. under running water-cooling.

ESR Spectra——All spectra of compounds (I～VII) in Chart 1 were measured at room temperature by a Japan Electron Optics Laboratory's JES-3BX spectrometer with 100 Kc./sec. field modulation.

Results and Discussion

4-Nitroquinoline 1-oxide gave a strong ESR signal in solid state unless it was highly purified and stored in dark. This could be considered as due to stable radicals photoinduced from 4-nitroquinoline 1-oxide. This observation has interested us in photochemical studies of 4-nitroquinoline 1-oxide in solution. Surprisingly, three different ESR spectra were obtained corresponding to the sort of solvents used, when this oxide was irradiated in dioxane, benzene, and hexane, respectively. Thus, dioxane-type spectrum was obtained after 30 minutes' irradiation of 50 mg. of this oxide in 1 ml. of dioxane at room temperature[*3] (Fig. 1, a-I). Its saturated solution in benzene, on the other hand, gave a spectrum of a triplet under the same irradiation condition as shown in Fig. 1, b-1. Subsequently, another spectrum of a triplet (Fig. 1, c-1) was obtained in hexane, which appeared similar to the benzene type but not the same, since their coupling constants are 27.25 gauss and 14.36 gauss, differing remarkably from each other. Such a large difference can hardly be expected as caused merely by the solvent effect on one sort of radical.

Then, in order to determine the chemical structures of these radicals, ^{15}N- or D-replaced 4-nitroquinoline 1-oxides (II～VII) were synthesized and their ESR spectra were measured. Since nitrogen and hydrogen isotopes have different nuclear spin numbers and different magnetogyric ratios (γ) from the non-labeled nuclei, isotope replacement should be very effective in elucidating the radical structure. Thus, replacement of ^{14}N by ^{15}N is expected to result in a significant change in signal pattern (triplet to doublet) and in magnitude of the coupling constant A ($A_{^{15}N}=1.403\,A_{^{14}N}$).

[*3] Radical production seems to be strongly dependent on concentration of solute, irradiating period, with or without oxygen, kind of solvents and many other experimental factors. The conditions described in this paper was chosen as one of the optimum condition. Under other conditions with lesser concentrations or shorter irradiation time, radical productions were also observable.

5) E. Ochiai, M. Ishikawa, Z. Sai: Yakugaku Zasshi, **63**, 280 (1943).

6) Details will be found in the paper submitted to the Chemical and Pharmaceutical Bulletin by some of us.[7,8]

7) Y. Kawazoe, M. Ohnishi, N. Kataoka: This Bulletin, **13**, 396 (1965).

8) Y. Kawazoe, M. Ohnishi: in press.

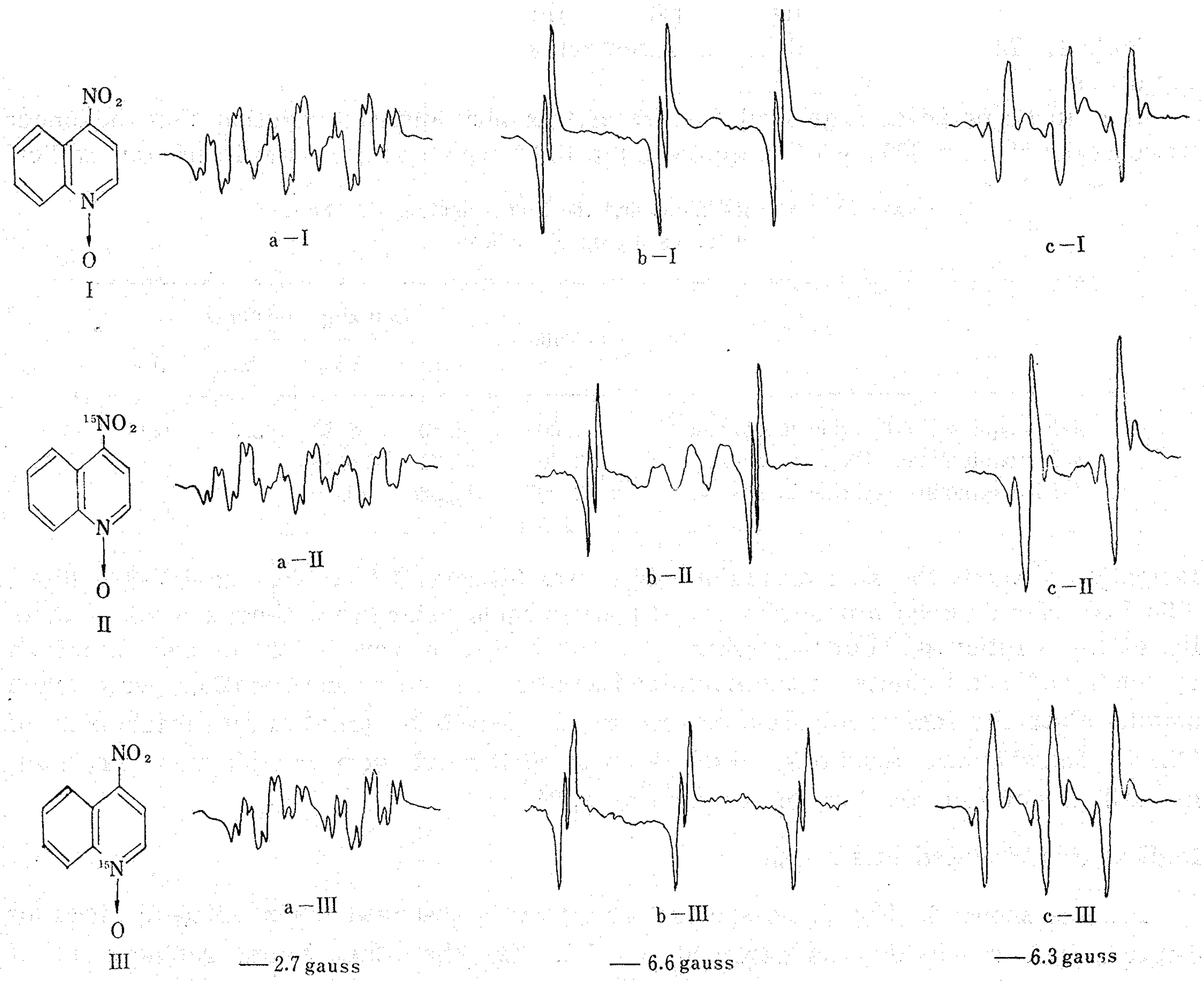

Fig. 1. The Electron Spin Resonance Spectra of Radicals induced by Photoirradiation of 4-Nitroquinoline 1-Oxide and the Same Compound replaced by ^{15}N in the Nitro Group or in the Aromatic Ring in Different Kinds of Solvents; (a) in Dioxane, (b) in Benzene and (c) in Hexane Solutions, respectively

Replacement of H by D must also cause spectral changes, that is, from doublet to triplet and from A_H to $0.154A_H$[9].

As expected, spectra of labeled compounds gave useful informations on the radical structures as shown in Fig. 1 and Fig. 2.

The three kinds of radicals were classified into two groups by considering which of the nitrogen nuclei interacted more with the unpaired electron; aromatic ring nitrogen or nitro group nitrogen.

Thus, the fact that no spin density existed on the nitro-nitrogen was unequivocally proved for the dioxane type radical by ^{15}N-replacement of nitro-nitrogen, since no spectral change occurred at all (Fig. 1, a-II). With regard to the benzene and the hexane type radicals, their triply split spectra did become a doublet by ^{15}N-replacement at the same position (Fig. 1, b-II, c-II). ^{15}N-Replacement of the aromatic ring nitrogen, on the other hand, caused a profound spectral change only of the dioxane type but not of the others (Fig. 1, a-III, b-III, c-III). As a result, it can be concluded that either one of nitrogens in the molecule may significantly interact with an odd electron in these radicals, but not both.

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[9] $\gamma_D/\gamma_H=0.154$, $\gamma_{^{15}N}/\gamma_{^{14}N}=1.403$

g-Values and spin coupling constants for three types of radicals are summarized in Table I. More details will be discussed for each radical structures in the following section.

It is to be noted as a general feature of this photoinduced reaction that the longer wave region[*4] than 300 mμ of mercury lamp light was effective by itself for radical formation without the shorter region which was filtered off by a Toshiba-UV-D31 filter. The ESR signals once produced were still observable after more than a week's standing of the solution without degassing. No consideration was given to contamination by other radicals induced in the examined solution as their concentrations were much smaller than the main radicals described here. Details on kinetics and mechanism of These photochemical reactions, which seem to go through very complicated processes, are still under investigation in our laboratory.[*5]

Table I. The g-Values and the Spin Coupling Constants of Three Types of Radicals

	g-Values	Coupling constants			
		$A_{^{14}N}$	$A_{^{15}N}$	A_{Ha}	A_{Hb}
4-Nitroquinoline 1-Oxide in dioxane	2.005	6.15	8.29	2.33	0.67
4-Nitroquinoline 1-Oxide in benzene	2.004	27.25	38.38		
4-Nitroquinoline 1-Oxide in hexane	2.005	14.36	19.80		

Radical Photoinduced in Dioxane

As was shown in Fig. 1, no spectral change was observed when nitro-nitrogen of 4-nitroquinoline 1-oxide was replaced by ^{15}N. On the other hand, replacement of aromatic ring nitrogen by ^{15}N caused a profound spectral change.

This was further confirmed by the spectrum of compound (Ⅳ) in which both nitrogens were replaced by ^{15}N (Fig. 2, Ⅳ).

Then, it was somewhat surprising that no change appeared onthe spectrum when both of 2- and 3-H of quinoline skeleton were replaced by deuterium (Fig. 2, V, Ⅵ). It may, as a result, be suggested that further fine splitting besides by ring nitrogen was associated with protons on benzene moiety, and this was conclusively confirmed by a spectral change caused by deuteration of benzene ring protons (Fig. 2, Ⅶ). These findings led us, with no doubt, to a conclusion that electron spin delocalization must be limited to benzene moiety and ring nitrogen only.

Thus, the spectrum of compound (I) was analyzed so that each of three parts separated by nitrogen coupling was triply split due to two equivalent protons (denoted as Ha), and each of them being further split into a finer triplet due to other two equivalent protons (denoted as Hb). The hyperfine structures of compounds (I and Ⅲ) were now to be reconstructed based on the experimentally determined coupling constants, and reasonable agreement was obtained in both cases as shown in Fig. 3 and Fig. 4, respectively.

[*4] 4-Nitroquinoline 1-oxide shows absorption maxima at 258 mμ and 370 mμ in ethanol.

[*5] Recently, Kosuge, *et al.* reported that irradiation of 4-nitroquinoline 1-oxide in benzene led to its deoxygenation to yield 4-nitroquinoline.[10)]

10) T. Kosuge, M. Yokota: Yakugaku Zasshi, **85**, 69 (1965).

From above considerations, we propose the following formula (Chart 2) as the radical structure of dioxane type with two sets, each of two approximately equivalent aromatic protons, where spin delocalization on X and Y parts is as little as not to be observed on ESR spectrum.

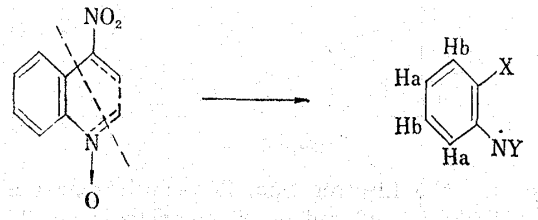

Chart 2.

Radicals Photoinduced in Benzene and in Hexane

A main triplet pattern was obtained both in benzene and in hexane solutions. A change from triplet to doublet was observed in both cases when nitro-nitrogen was replaced by heavy nitrogen (Fig. 1). In order to eliminate the so-called solvent effect from possibilities causing their spectral difference, a considerable amount of benzene was added to the hexane solution irradiated, but no substantial change in spectrum was observed, retaining a hexane type triplet. This fact led us to a conclusion that the radicals generated in benzene and in hexane were different from each other, both of which were associated mainly with nitro group nitrogen but not with aromatic ring nitrogen.

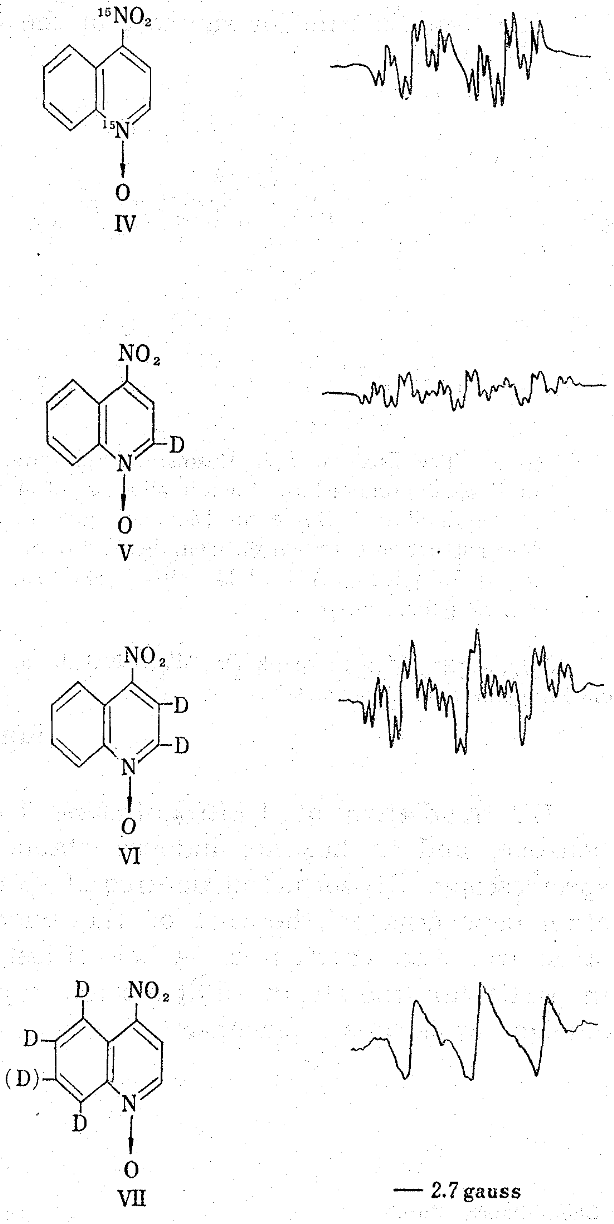

Fig. 2. The Electron Spin Resonance Spectra of Radicals in Dioxane produced by Photoirradiation of the Isotopically labelled 4-Nitroquinoline 1-Oxide

D-Replacement was unfortunately not so effective in elucidating radical structure of hexane type since fine structures in its spectrum was associated mainly with a nitrogen nucleus but scarcely with any of protons. But following postulations were tentatively proposed regarding its structure; one was • NO radical produced by the breakage of the bond between nitro group and aromatic nucleus, and the other was -NO$_2$ twisted nearly perpendicularly from a plane of aromatic ring. The possibility of • NO radical may probably be excluded in comparison with the reported ESR spectrum of • NO radical.[11] The twisted model may also be eliminated because it can hardly be expected to diminish the spin coupling completely. Although other possibilities such that the unpaired electron on nitro-nitrogen is isolated from the conjugated system may be considered, more refined experiments are needed to derive

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[11] R. Beringer, J. G. Castle: Phys. Rev., 78, 581 (1950).

the final conclusion on this problem.

The same is true for the case of the radical in benzene solution.

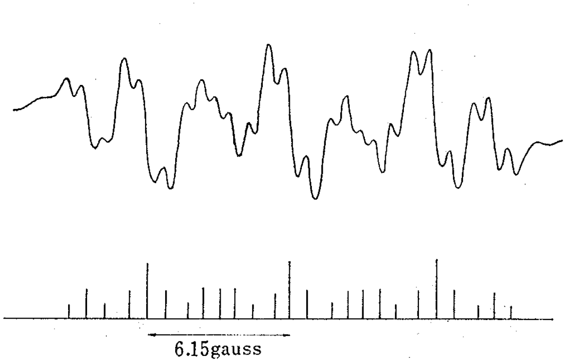

Fig. 3. The Electron Spin Resonance Spectrum of Radical produced by Photoirradiation of 4-Nitroquinoline 1-Oxide in Dioxane and the Reconstruction based on the Coupling Constants, $A_N=6.15$ gauss, $A_{H_a}=2.33$ gauss, and $A_{H_b}=0.67$ gauss, respectively.

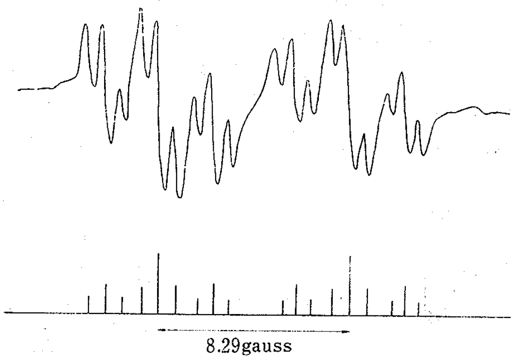

Fig. 4. The Electron Spin Resonance Spectrum of Radical produced by Photoirradiation of 4-Nitroquinoline 1-Oxide (^{15}N) in Dioxane and the Reconstruction based on the Coupling Constants, $A_{^{15}N}=8.29$ gauss, $A_{H_a}=2.33$ gauss, and $A_{H_b}=0.67$ gauss, respectively.

The authors wish to thank Dr. Waro Nakahara, Director of this institute, for his continued interest and encouragement in this work.

Summary

UV-irradiation of 4-nitroquinoline 1-oxide in solutions, that is, in dioxane, in benzene, and in hexane, induced stable radicals which were investigated by ESR spectroscopy. Hyperfine structures of spectra thus obtained are different from each other depending on the sort of the solvents used. Determination of these radical structures were carried out by help of isotope-containing 4-nitroquinoline 1-oxides and, in particular, the structure of dioxane type radical was proposed, based on spectral changes by isotope replacement.

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122. **Shigeharu Inouye**: Syntheses of Methyl 3,6-Diamino-3,6-dideoxy-α-D-glucopyranoside and Methyl 3,6-Diamino-3,6-dideoxy-α-D-mannopyranoside.

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The synthesis of a diamino-sugar is one of the subjects of current research in the amino-sugar chemistry. Among the 3,6-diamino-3,6-dideoxy-hexose family, the syntheses of methyl 3,6-diamino-3,6-dideoxy-α-D-altropyranoside[1] and 3,6-diamino-3,6-dideoxy-D-idose[2] were reported.

[*1] Morooka, Kohoku-ku, Yokohama-shi (井上重治).

[1] *a*) M.I. Wolfrom, Yen-Lung Hung, D. Horton: J. Org. Chem., **30**, 3394 (1965). *b*) S. Inouye: This Bulletin, in press.

[2] S. Hanessian, T.H. Haskell: J. Org. Chem., **30**, 1080 (1965).