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# New Binary Coumarins from *Citrus* Plants

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**Two new binary coumarins, khelmarin-A (1) and -B (2), were isolated from root bark of *Poncirus trifoliata* which had been used as a rootstock of *Citrus Hassaku* (hassaku), and roots of original seedlings of *Citrus canariculata* (kiku-daidai), respectively. The structures of the new binary coumarins 1 and 2 contain *cis*-khellactone (3) as a common structural component, which is linked with a nordentatin (5) or a xanthoxyletin (6) unit, respectively.**

**Keywords** coumarin; khelmarin-A; khelmarin-B; *Poncirus*; *Citrus*; Rutaceae; *cis*-khellactone; nordentatin; dentatin; xanthoxyletin

In continuing our phytochemical investigations of *Citrus* plants (Rutaceae),[1)] we isolated two novel binary coumarins named khelmarin-A (1) and khelmarin-B (2) from root bark of karatachi [*Poncirus trifoliata* (L.) RAF.] rootstock of *Citrus Hassaku* HORT. *ex.* Y. TANAKA (hassaku) and roots of original seedlings of *Citrus canariculata* HORT. *ex.* Y. TANAKA (kiku-daidai), respectively. The structures of these new binary coumarins were concluded to be as shown by formulae **1** and **2**, respectively, based on the results of spectrometric studies. Many kinds of binary coumarins have been isolated from natural sources[2)] so far and khelmarin-A (1) and -B (2) are members of a new group among them.

## Results and Discussion

**Structure of Khelmarin-A (1)** Khelmarin-A (1) was isolated as an optically active pale yellow oil, $[\alpha]_D$ $-10.6°$ (chloroform), from the acetone extract of root bark of *Poncirus trifoliata* (karatachi) which had been cultivated as a rootstock of *C. Hassaku* (hassaku). The high resolution mass spectrum (HR-MS) showed the molecular formula of this compound to be $C_{33}H_{32}O_8$. The ultraviolet (UV) and infrared (IR) spectra (see Experimental) suggested the presence of a coumarin skeleton in the molecule.[3)] The proton nuclear magnetic resonance ($^1H$-NMR) spectrum (Table I) showed the presence of four methyls attached to oxygen-linked carbons and a 1,1-dimethyl-2-propenyl (dimethylallyl) moiety. Furthermore, in the $^1H$-NMR spectrum using $^1H$–$^1H$ correlation spectroscopy (COSY), four AB-

TABLE I. Chemical Shifts in the $^1H$-NMR Spectra of Khelmarin-A (**1**) and Khelmarin-B (**2**)

| | 1 | 2 |
|---|---|---|
| H-3 | 5.95 (d, $J$=9.5) | 5.96 (d, $J$=9.5) |
| H-4 | 7.41 (d, $J$=9.5) | 7.43 (d, $J$=9.5) |
| H-5 | 7.30 (d, $J$=8.7) | 7.32 (d, $J$=8.7) |
| H-6 | 6.79 (d, $J$=8.7) | 6.80 (d, $J$=8.7) |
| 8-Me | 1.64 (3H, s), 1.68 (3H, s) | 1.65 (3H, s), 1.69 (3H, s) |
| H-9 | 4.10 (dd, $J$=4.4, 11) | 4.11 (dd, $J$=4.4, 11) |
| 9-OH | 3.04 (d, $J$=11) | 2.98 (d, $J$=11) |
| H-10 | 5.61 (d, $J$=4.4) | 5.67 (d, $J$=4.4) |
| H-3′ | 5.81 (d, $J$=9.5) | 5.85 (d, $J$=9.5) |
| H-4′ | 7.40 (br) | 7.38 (br d, $J$=9.5) |
| H-6′ | 6.75 (d, $J$=10.3) | 6.73 (br d, $J$=10.3) |
| H-7′ | 5.64 (d, $J$=10.3) | 5.67 (d, $J$=10.3) |
| 8′-Me | 1.42 (3H, s), 1.54 (3H, s) | 1.44 (3H, s), 1.55 (3H, s) |
| H-10′ | | 6.57 (s) |
| 1″-Me | 1.65 (6H, s) | |
| H-2″ | 6.31 (dd, $J$=10.5, 17.3) | |
| H-3″ | 4.94 (d, $J$=17.3), 4.86 (d, $J$=10.5) | |

Spectra were taken in $CDCl_3$ on a 400 MHz NMR instrument. All signals except for methyls correspond to 1H. Values are in ppm (δ). Multiplicities are indicated by the usual symbols: s, singlet; d, doublet; dd, double-doublets; br, broad. The coupling constants ($J$ values) in parentheses are in Hz.

TABLE II. Chemical Shifts in the $^{13}C$-NMR Spectra of Khelmarin-A (**1**), Khelmarin-B (**2**), *cis*-Khellactone (**3**), Dentatin (**4**), Nordentatin (**5**), and Xanthoxyletin (**6**)

| | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|
| 2 | 158.50 | 158.54 | 160.82 | | | |
| 3 | 112.55 | 112.79 | 112.15 | | | |
| 4 | 140.81 | 142.71 | 144.23 | | | |
| 4a | 111.72 | 111.77 | 114.86 | | | |
| 5 | 130.10 | 130.17 | 128.61 | | | |
| 6 | 114.45 | 114.52 | 114.86 | | | |
| 6a | 156.49 | 156.62 | 156.47 | | | |
| 8 | 79.16 | 79.31 | 79.00 | | | |
| 8-Me | 21.68 | 21.73 | 21.71 | | | |
| | 27.26 | 27.93 | 24.98 | | | |
| 9 | 72.43 | 72.49 | 70.99 | | | |
| 10 | 73.22 | 73.60 | 61.28 | | | |
| 11 | 107.11 | 107.27 | 110.80 | | | |
| 11a | 153.84 | 153.95 | 154.63 | | | |
| 2′ | 160.67 | 160.86 | | 160.47 | 161.23 | 160.75 |
| 3′ | 110.69 | 111.61 | | 111.49 | 110.46 | 112.41 |
| 4′ | 138.65 | 138.18 | | 138.78 | 138.98 | 138.42 |
| 4′a | 108.26 | 108.12 | | 107.40 | 103.87 | 107.38 |
| 5′ | 148.66 | 150.25 | | 151.19 | 146.47 | 152.90 |
| 5′a | 111.97 | 111.87 | | 111.49 | 106.12 | 111.32 |
| 6′ | 116.14 | 115.72 | | 116.22 | 114.87 | 115.84 |
| 7′ | 130.81 | 131.06 | | 130.23 | 130.06 | 130.57 |
| 8′ | 77.00 | 77.00 | | 77.26 | 77.13 | 77.51 |
| 8′-Me | 28.15 | 28.59 | | 27.42 | 27.35 | 28.15 |
| | 28.56 | 28.62 | | 27.42 | 27.35 | 28.15 |
| 9′a | 155.90 | 155.57 | | 155.90 | 155.90 | 157.60 |
| 10′ | 119.94 | 101.78 | | 119.00 | 116.36 | 100.86 |
| 10′a | 153.80 | 158.02 | | 153.87 | 154.27 | 155.68 |
| 1″ | 41.27 | | | 41.04 | 41.07 | |
| 1″-Me | 29.01 | | | 29.30 | 29.60 | |
| | 29.42 | | | 29.30 | 29.60 | |
| 2″ | 150.12 | | | 149.70 | 150.08 | |
| 3″ | 107.71 | | | 108.23 | 108.09 | |
| $OCH_3$ | | | | 63.27 | | 63.59 |

Spectra were recorded in $CDCl_3$ on a 100 MHz NMR instrument. Values are in ppm (δ). Assignments were determined by H–C COSY and H–C long-range COSY spectrometries.

type signals were observed. Two of them having $J=9.5$ Hz were assigned to two $\alpha,\beta$-protons on unsaturated lactones in coumarin nuclei, one pair of protons having $J=8.7$ Hz to *ortho*-located aromatic protons, and the remaining pair ($J=10.3$ Hz) to olefinic protons on a pyran ring. The remaining ABC-type signals at $\delta$ 4.10 (1H, dd, $J=4.4$ and 11 Hz), 3.04 (1H, d, $J=11$ Hz), and 5.61 (1H, d, $J=4.4$ Hz) were transformed into a pair of doublets ($J=4.4$ Hz) at $\delta$ 4.10 and 5.61 by exchanging a hydrogen of the hydroxy group at $\delta$ 3.04 with $D_2O$, suggesting the presence of a hydroxy group located *vicinal* to an ether linkage in the molecule.

In a comparison of the $^{13}$C-NMR spectrum of khelmarin-A (**1**) with those of *cis*-khellactone (**3**)[4] and dentatin (**4**)[5] or nordentatin (**5**)[6] as shown in Table II, marked similarities, except for a significant difference of the chemical shift of C-10, indicated that the structural components of khelmarin-A (**1**) were the previously known monomeric coumarins, *cis*-khellactone (**3**) and nordentatin (**5**). Thus, khelmarin-A (**1**) was proposed to have the structure corresponding to khellactone (**3**) linked at C-10 through an ether oxygen to C-5 of nordentatin (**5**).

The location of the ether linkage in khelmarin-A (**1**) between C-10 in *cis*-khellactone (**3**) and C-5 in nordentatin (**5**) was also supported by the results of nuclear Overhauser effect (NOE) and $^1$H–$^{13}$C correlation spectroscopy *via* long-range couplings (COLOC)[7] experiments as follows. Irradiations of the doublets at $\delta$ 6.75 (H-6′) and at $\delta$ 7.40 (H-4′) gave 4 and 2% enhancements of the signal at $\delta$ 5.61 (H-10), respectively. In the $^1$H–$^{13}$C COLOC experiment with $J=3$ Hz, a significant correlation peak between the proton signal at $\delta_H$ 5.61 (H-10) in the upper coumarin unit and the carbon signal at $\delta_C$ 148.66 (C-5′) on the lower one was observed.

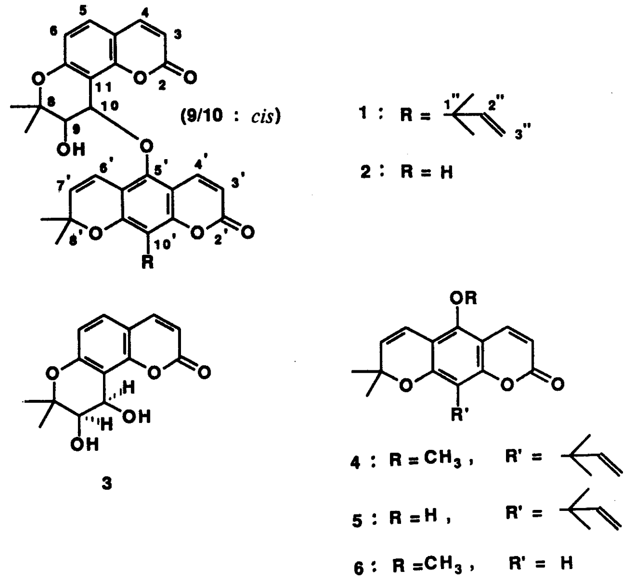
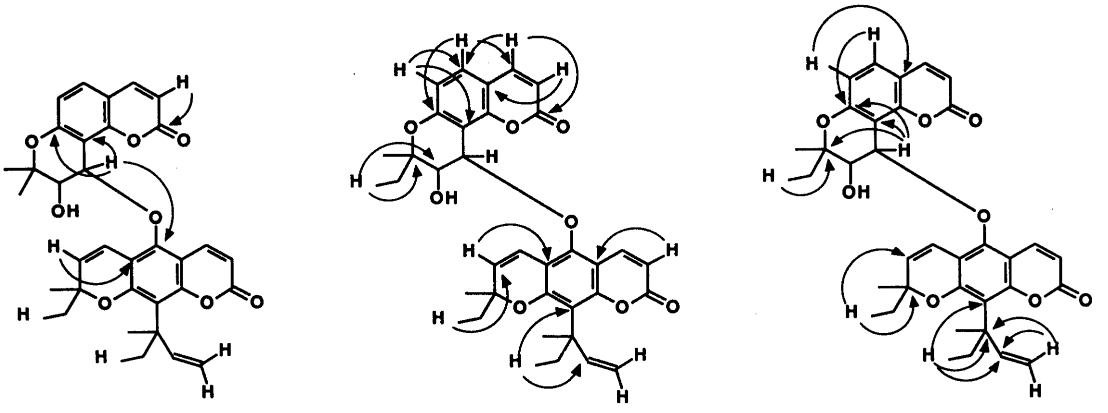


Chart 1

In the electron impact mass spectrum (EI-MS), the observation of two significant fragments at $m/z$ 244 and 312 corresponding to the loss of $H_2O$ from khellactone (**3**) and nordentatin (**5**), respectively, suggested a facile cleavage of the ether linkage at the benzylic carbon in the upper khellactone (**3**) unit. The *cis* orientation of the hydroxy group and the ether linkage was proposed from the value of the coupling constant ($J=4.4$ Hz) between H-9 ($\delta$ 4.10) and H-10 ($\delta$ 5.61), and the presence of 8 or 11% NOE enhancements between these protons in the $^1$H-NMR spectrum. These observations together with other results of $^1$H–$^{13}$C COLOC experiments shown by arrows in the formulae in Fig. 1 indicated the structure of khelmarin-A to be as presented by formula **1** except for the absolute stereochemistry.

**Structure of Khelmarin-B (2)** Khelmarin-B (**2**) was obtained as a pale yellow oil, $[\alpha]_D$ $+11.2°$ (chloroform), from original seedlings of *Citrus canariculata* HORT. *ex.* Y. TANAKA (kiku-daidai). The molecular formula $C_{28}H_{24}O_8$ was deduced by HR-MS. The similarity of the UV and IR spectra of this compound to those of **1**, the similarity of the $^{13}$C-NMR spectrum to those of **3** and **6**, and the appearance of mass fragment peaks at $m/z$ 244 due to fragments corresponding to a half of the molecule suggested a binary coumarin structure of khelmarin-B (**2**) containing *cis*-khellactone (**3**)[4] and xanthoxyletin (**6**)[8] units as structural components. In the $^1$H- and $^{13}$C-NMR spectra of khelmarin-B (see Tables I and II), the signal patterns were found to be analogous with those of **1**, except for the appearances of a 1H-singlet at $\delta_H$ 6.57 (H-10′) and a carbon signal at $\delta_C$ 101.78 (C-10′) as a doublet, with the loss of signals due to the 1,1-dimethyl-2-propenyl moiety in the spectra of **1**. Furthermore, in NOE experiments, 2 and 4%



$J=3$ Hz $J=5$ Hz $J=10$ Hz

Fig. 1. $^1$H–$^{13}$C Correlations in the COLOC Spectra of Khelmarin-A (**1**)

enhancements of the signal at $\delta_H$ 5.67 (H-10) were observed on irradiation of the signals at $\delta_H$ 7.38 (H-4′) and at $\delta_H$ 6.73 (H-6′), respectively. The presence of NOE enhancements between the signals at $\delta_H$ 4.11 (H-9) and 5.67 (H-10) revealed the *cis* stereochemistry of H-9 and H-10. Thus, the ether linkage in the khelmarin-B (**2**) molecule was concluded to involve C-10 and C-5′ in the *cis*-khellactone (**3**) and xanthoxyletin (**6**) units, respectively, and the structure of khelmarin-B was proposed to be as shown by **2** except for the absolute stereochemistry.

## Experimental

IR spectra were recorded on a Jasco IR-810 infrared spectrophotometer in $CHCl_3$, UV spectra on a Jasco UVIDEC-610C double beam spectrophotometer in MeOH, optical rotations on a DIP-181 polarimeter (JASCO) in $CHCl_3$ at 20 °C, and $^1H$- and $^{13}C$-NMR spectra on a JEOL GX-400 NMR spectrometer (with $SiMe_4$ as an internal standard) in $CDCl_3$. EI- and HR-MS spectra were measured on Hitachi M-80 or JMS-HX-110 mass spectrometer, respectively. NOE enhancements were determined by differential NOE techniques, and $^1H$–$^1H$ and $^1H$–$^{13}C$ COSYs and COLOC spectra were recorded on a JEOL GX-400 spectrometer. All thin layer chromatography (TLC) and preparative TLC procedures were done on Kieselgel 60 $F_{254}$ (Merck).

**Isolation of Khelmarin-A (1)** The dried root bark (193 g) of *Poncirus trifoliata* (L.) RAF. (karatachi) which had been cultivated as a rootstock of *Citrus Hassaku* HORT. *ex.* Y. TANAKA (hassaku) in Wakayama prefecture was extracted with acetone at room temperature. The solvent was evaporated off *in vacuo* to give a brown oil (20 g). This extract (5 g) was subjected to silica gel column chromatography and eluted with hexane, benzene, benzene + isopropyl ether (20 : 1 to 1 : 3), benzene + acetone (3 : 1), acetone, and methanol, successively. The benzene + isopropyl ether (1 : 3) and benzene + acetone (3 : 1) fractions were subjected repeatedly to preparative silica gel TLC to give **1** (8 mg) as a pale yellow oil. As other components, clausarin, dentatin, xanthoxyletin, luvangetin, nordentatin, junosmarin, and 5-hydroxyacronycine were isolated and characterized.

Khelmarin-A (**1**): A pale yellow oil, $[\alpha]_D$ −10.6° ($c$=0.123, $CHCl_3$). HR-MS: Calcd for $C_{33}H_{32}O_8$ $m/z$ 556.2095. Found: $m/z$ 556.2122. UV $\lambda_{max}$ nm: 208, 221 (sh), 265, 275, 328, 348 (sh). IR $\nu_{max}$ cm$^{-1}$: 3400 (br), 1730, 1720, 1605, 1590. EI-MS $m/z$ (%): 556 ($M^+$, 0.6), 313 (14), 312 (33), 298 (20), 297 (100), 269 (12), 244 (25), 241 (16), 229 (15), 203 (11), 188 (42). NOE: irradiation of the signal at $\delta$ 7.40 (H-4′) gave 2% enhancement of the signal at $\delta$ 5.61 (H-10); irradiation at $\delta$ 6.75 (H-6′) gave 4% enhancement at $\delta$ 5.61 (H-10); irradiation at $\delta$ 5.61 (H-10) gave 8% enhancement at $\delta$ 4.10 (H-9); irradiation at $\delta$ 4.10 (H-9) gave 11% enhancement at $\delta$ 5.61 (H-10). $^1H$-NMR: see Table I. $^{13}C$-NMR: see Table II. The results of $^1H$- $^{13}C$ COLOC: see Fig. 1.

**Isolation of Khelmarin-B (2)** The dried roots (150 g) of original seedlings of *Citrus canariculata* HORT. *ex.* Y. TANAKA (kiku-daidai) grown in the orchard of Okitsu Branch, Fruit Tree Research Station, Ministry of Agriculture, Forestry and Fisheries, Shimizu, Shizuoka, and collected on September, 1987 were extracted with acetone at room temperature. The acetone extract was concentrated to dryness to give a residue (7.6 g), which was subjected to silica gel column chromatography, and eluted with benzene, benzene + isopropyl ether (3 : 1 to 1 : 3), benzene + acetone (3 : 1), acetone, and methanol. The benzene + acetone (3 : 1) fraction was subjected repeatedly to preparative TLC to give **2** (5 mg) as a pale yellow oil.

From other fractions, known coumarins, seselin, suberosin, osthenol, xanthyletin, angelical, xanthoxyletin, demethylsuberosin, osthenone, junosmarin, 7-methoxy-8-formylcoumarin, and suberenol were obtained and characterized.

Khelmarin-B (**2**): A pale yellow oil, $[\alpha]_D$ +11.2° ($c$=0.170, $CHCl_3$). HR-MS: Calcd for $C_{28}H_{24}O_8$: 488.1469. Found: 488.1453. UV $\lambda_{max}$ nm: 207, 221, 262, 272, 289, 326. IR $\lambda_{max}$ cm$^{-1}$: 3450 (br.), 1735, 1615. EI-MS $m/z$ (%): 488 ($M^+$, 7), 473 (5), 245 (19), 244 (39), 230 (14), 229 (100), 203 (23), 201 (22), 188 (41). NOE: irradiation of the signal at $\delta$ 7.38 (H-4′) gave 2% enhancement of the signal at $\delta$ 5.67 (H-10); irradiation at $\delta$ 6.73 (H-6′) gave 4% enhancement at $\delta$ 5.67 (H-10); irradiation at $\delta$ 5.67 (H-10) gave 5% enhancement at $\delta$ 4.11 (H-9); irradiation at $\delta$ 4.11 (H-9) gave 13% enhancement at $\delta$ 5.67 (H-10). $^1H$-NMR: see Table I. $^{13}C$-NMR: see Table II.

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## References

1) T.-S. Wu, H. Furukawa, and C.-S. Kouh, *Heterocycles*, **19**, 273 (1982); T.-S. Wu, C.-S. Kouh, and H. Furukawa, *Chem. Pharm. Bull.*, **31**, 895 (1983); T.-S. Wu and H. Furukawa, *ibid.*, **31**, 901 (1983); T.-S. Wu, C.-S. Kouh, and H. Furukawa, *Phytochemistry*, **22**, 1493 (1983); M. Ju-ichi, M. Inoue, Y. Fujitani, and H. Furukawa, *Heterocycles*, **23**, 1131 (1985); A. T. McPhail, M. Ju-ichi, Y. Fujitani, M. Inoue, T.-S. Wu, and H. Furukawa, *Tetrahedron Lett.*, **26**, 3271 (1985); T.-S. Wu, S.-C. Huang, T.-T. Jong, J.-S. Lai, and H. Furukawa, *Heterocycles*, **24**, 41 (1986); M. Ju-ichi, M. Inoue, K. Aoki, and H. Furukawa, *ibid.*, **24**, 1595 (1986); T.-S. Wu, R.-J. Cheng, S.-C. Huang, and H. Furukawa, *J. Nat. Prod.*, **49**, 1154 (1986); H. Furukawa, M. Ju-ichi, I. Kajiura, and M. Hirai, *Chem. Pharm. Bull.*, **34**, 3922 (1986); M. Ju-ichi, M. Inoue, R. Tsuda, N. Shibukawa, and H. Furukawa, *Heterocycles*, **24**, 2777 (1986); M. Ju-ichi, M. Inoue, K. Sakiyama, M. Yoneda, and H. Furukawa, *ibid.*, **26**, 2077 (1987); M. Ju-ichi, M. Inoue, I. Kajiura, M. Omura, C. Ito, and H. Furukawa, *Chem. Pharm. Bull.*, **36**, 3202 (1988); M. Ju-ichi, M. Inoue, M. Ikegami, I. Kajiura, M. Omura, and H. Furukawa, *Heterocycles.*, **27**, 1451 (1988); M. Ju-ichi, M. Inoue, C. Ito, M. Matsuoka, H. Furukawa, and I. Kajiura, *ibid.*, **26**, 1873 (1987); M. Ju-ichi, H. Kaga, M. Muraguchi, M. Inoue, I. Kajiura, M. Omura, and H. Furukawa, *ibid.*, **27**, 2197 (1988); C. Ito, T. Mizuno, M. Matsuoka, Y. Kimura, K. Sato, I. Kajiura, M. Omura, M. Ju-ichi, and H. Furukawa, *Chem. Pharm. Bull.*, **36**, 3292 (1988); C. Ito, M. Matsuoka, T. Mizuno, K. Sato, Y. Kimura, M. Ju-ichi, M. Inoue, I. Kajiura, M. Omura, and H. Furukawa, *ibid.*, **36**, 3805 (1988).
2) S. C. Basa, *Phytochemistry*, **27**, 1933 (1988).
3) R. D. H. Murray, J. Mendez, and S. A. Brown, "The Natural Coumarins," John Wiley & Sons, Inc., New York, 1982.
4) Y. Yamada, C.-S. Hsu, K. Iguchi, M. Suzuki, H.-Y. Hsu, and Y.-P. Chen, *Tetrahedron Lett.*, **1974**, 2513.
5) T. Tomimatsu, M. Hashimoto, T. Shingu, and K. Tori, *Tetrahedron*, **28**, 2003 (1972); T. Tomimatsu, H. Hashimoto, and K. Tori, *ibid.*, **30**, 939 (1974).
6) T. R. Govindachari, B. P. Pai, P. S. Subramaniam, and N. Muthukumaraswamy, *Tetrahedron*, **24**, 753 (1968); H. Fuhrer, T. R. Govindachari, B. S. Joshi, and B. R. Pai, *Indian J. Chem.*, **8**, 198 (1970).
7) H. Kessler, C. Griesinger, and J. Lautz, *Angew. Chem. Int. Ed. Engl.*, **23**, 444 (1984).
8) A. K. Ganguly, B. S. Joshi, V. N. Kamat, and A. H. Manmade, *Tetrahedron*, **23**, 4777 (1967).