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# STUDIES ON THE CONSTITUENTS OF THE GREEN ALGA *Ulva lactuca*

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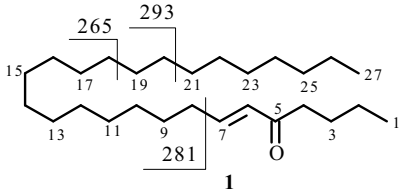
*Phytochemical investigations on a marine green alga* Ulva lactuca *led to the isolation of two new compounds (*E*)-6-heptacosen-5-one (**1**) and (*E*)-6-octadecen-5-ol (**2**), along with four known compounds, (Z)-10-hexacosene (**3**), docosanoic acid (**4**), palmitic acid (**5**), and isofucosterol (**6**). Compounds **3** and **4** were isolated for the first time from this species. The structures of the compounds were deduced with the help of modern spectroscopic techniques.*

**Keywords**: *Ulva lactuca*, Mediterranean Sea, (*E*)-6-heptacosen-5-one, (*E*)-6-octadecen-5-ol, (*Z*)-10-hexacosene, docosanoic acid.

*Ulva lactuca* (Linnaeus), commonly known as sea lettuce, is a marine green seaweed having broad leaf-like fronds. The nutritional composition of *U. lactuca* includes dietary fiber, amino acid, and tocopherol compounds [1]. It contains 16.5% of water-soluble and 13.3% insoluble dietary fibers [2]. Some substances isolated from this alga have shown pharmacological activities [3, 4]. The polysaccharide showed activity against a number of human and avian influenza viruses *in vitro* [5]. Other studies revealed that the heteropolysaccharide isolated from *U. lactuca* can stimulate macrophage $\beta$- and T-cells in mice [6]. Moreover, several new bioactive compounds have been isolated from marine sources [7–11]. We report herein the characterization of products extracted from *U. lactuca* collected from the Alexandria coast of the Mediterranean sea.

The petroleum ether–soluble fraction of the methanolic extract of the whole alga *U. lactuca* was subjected to column chromatography over flash silica gel and eluted with different mobile phases. The fraction F-2, obtained on elution with petroleum ether–$Me_2CO$ (9.9:0.1), was subjected to column chromatography using petroleum ether to obtain a new compound, (*E*)-6-heptacosen-5-one (**1**), as a yellow oil. Its molecular formula was determined as $C_{27}H_{52}O$, based on the EI-MS, CI-MS, and $^{13}C$ NMR spectra. CI-MS showed a molecular ion $[M]^+$ peak at *m/z* 392 in addition to significant fragment ions at *m/z* 293, 281, and 265 due to the losses of ($C_7H_{15}$), ($C_7H_{11}O$), and ($C_9H_{19}$), respectively. The fragment ion at *m/z* 111 was due to the loss of $C_{20}H_{41}$ from the $M^+$, suggesting the presence of a double bond at C-6 (Fig. 1). The $^1H$ NMR spectrum of **1** showed two triplets at $\delta$ 0.86 ($J_{1,2}$ = 7.3 Hz) and 0.83 ($J_{27,26}$ = 6.6 Hz), along with a broad singlet in the range of $\delta$ 1.25–1.30, typical of a straight-chain hydrocarbon. It also showed signals at $\delta$ 6.80 (1H, m, H-7) and 6.06 (1H, d, $J_{6,7}$ = 15.9 Hz, H-6) for olefinic protons. These protons showed HMQC interactions at $\delta$ 147.4 and 130.0. Furthermore, a two- proton triplet at $\delta$ 2.49 ($J_{4,3}$ = 7.4 Hz), a multiplet at $\delta$ 2.17 (2H), and a broad singlet at $\delta$ 1.56 (4H) were assigned to H-4, H-8, and H-3/H-9, respectively.




**1**

Fig. 1. Mass fragments in the mass spectrum of compound **1**.

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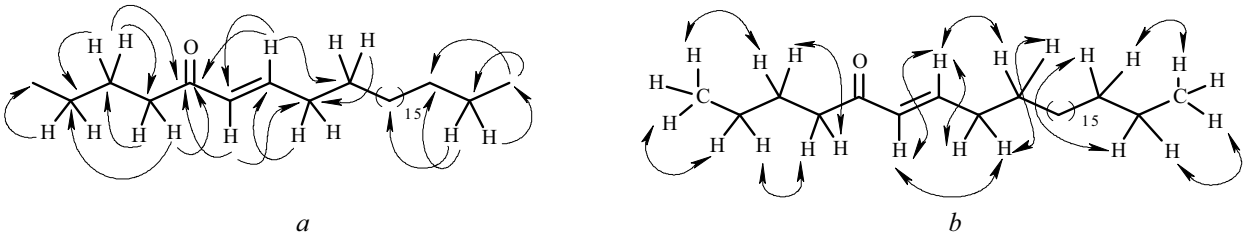
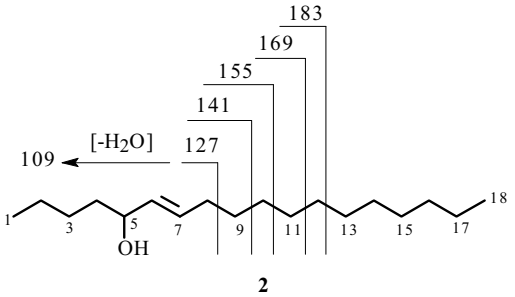


*a* *b*

Fig. 2. Key HMBC (*a*) and TOCSY (*b*) correlations of compound **1**.

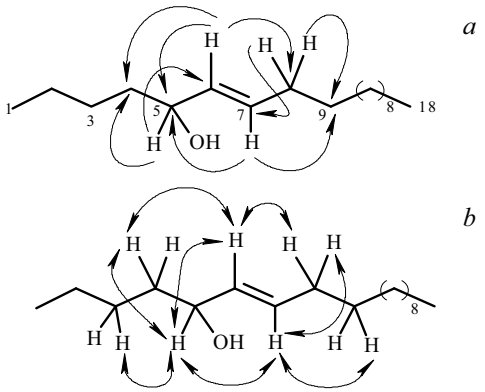
The $^{13}$C NMR spectrum showed a downfield signal at δ 201.1, which could be assigned to the carbonyl carbon. The signals observed in the range δ 22.4–32.4 indicated the presence of a long alkenyl chain. H-4 (δ 2.49) displayed HMBC interaction with C-5 (δ 201.1), whereas H-7 (δ 6.80) showed HMBC connectivities with C-6 (δ 130.3), C-5 (δ 201.1), and C-9 (δ 24.4) (Fig. 2a). This data indicated the presence of a *trans* $\alpha,\beta$-unsaturated double bond conjugated with a carbonyl group. The stereochemistry of the double bond was determined, based on the large coupling constant ($J_{6,7}$ = 15.9 Hz) for H-6 and H-7, as *trans*. The $^{13}$C NMR spectrum showed an allylic carbon resonating at δ 32.5, typical of methylenes adjacent to a *trans* double bond [12, 13]. The position of the carbonyl and the double bond was inferred by the HMBC and TOCSY experiments (Fig. 2a,b).

Moreover, fraction F-2 furnished a new compound, (*E*)-6-octadecen-5-ol (**2**), as a yellow oil. The molecular formula was determined as $C_{18}H_{35}O$, based on the EI-MS, CI-MS, and $^{13}$C NMR spectra. CI-MS showed an $[M + H]^+$ ion peak at *m/z* 269 ($C_{18}H_{36}O$). The mass spectrum exhibited an ion at *m/z* 251 due to the loss of $H_2O$, indicating the presence of a hydroxyl group. The mass spectrum also showed fragments with a difference of 14 mass units up to *m/z* 127 ($C_8H_{15}O$), which was followed by a fragment at *m/z* 109 ($C_8H_{13}$) instead of *m/z* 113 ($C_7H_{13}O$), indicating the presence of a double bond in the molecule attached to an oxymethine (Fig. 3). The IR spectrum showed absorption bands at 3381 (OH), 2956 (C=C), and 1128 (C-O) cm$^{-1}$. The double bond was deduced by the presence of two signals at δ 5.60 (1H, dt, $J_{7,6}$ = 15.4, $J_{7,8}$ = 6.9 Hz, H-7) and 5.42 (1H, dd, $J_{6,7}$ = 15.4, $J_{6,5}$ = 6.9 Hz, H-6) in the $^1$H NMR spectrum. The quartet at δ 4.01 (1H, $J_{5,4a} = J_{5,4b} = J_{5,6}$ = 6.9 Hz, H-5) was due to the oxymethine proton. The position of the hydroxyl group and the double bond was also deduced by the HMBC and TOCSY experiments (Fig. 4a,b). On the basis of HMQC, these protons have cross links with carbons at δ 132.2 (C-7), 133.0 (C-6), and 73.3 (C-5). In the HMBC spectrum, H-6 showed interactions with C-5 and C-4 resonating at δ 73.3 and 37.3, respectively, whereas H-7 did not show any interaction with C-4. It was therefore concluded that OH is linked to C-5, whose proton resonated at δ 4.01. These connectivities were also confirmed by COSY spectrum. Furthermore, the $^1$H NMR spectrum showed a quartet at δ 2.00 (2H, $J_{8,9a} = J_{8,9b} = J_{8,7}$ = 6.9 Hz, H-8), along with a multiplet at δ 1.40–1.51 (2H, H-4), and their respective $^{13}$C NMR values were observed at δ 32.2 and 37.3. It also showed two three-proton triplets at δ 0.84 ($J_{1,2}$ = 6.9 Hz, H-1) and 0.85 ($J_{18,17}$ = 6.9 Hz, H-18), and a broad singlet at δ 1.25 characteristic of a straight chain hydrocarbon. The signals appearing in the range δ 29.2–31.9 in the $^{13}$C NMR spectrum were due to long alkenyl chains. The stereochemistry of the double bond was inferred from the $^{13}$C NMR spectrum. The C-8 appearing at δ 32.2 was due to the absence of the *cis* shielding effect of the double bond. This indicated a *trans* double bond [12].



**2**

Fig. 3



*a* *b*

Fig. 4

Fig. 3. Mass fragments in the mass spectrum of compound **2**.

Fig. 4. Selected HMBC (*a*) and TOCSY (*b*) interactions for compound **2**.

Other constituents, (*Z*)-10-hexacosene (**3**) [14], docosanoic acid (**4**) [15], palmitic acid (**5**) [16], and isofucosterol (**6**) [17, 18], have been isolated from this alga. Their structures were identified by comparison of their spectral data with the reported literature values.

## EXPERIMENTAL

**General Experimental Procedures**. Melting points were measured on a Buchi-535 apparatus (uncorrected). The IR spectra were recorded on a Jasco A-302 spectrometer. EI-MS (ionization voltage 70 eV), HR-EI-MS, and CI-MS were recorded on JEOL (JMS HX-110) and Finnigan (MAT312) Instruments. $^1$H NMR spectra were recorded with a Bruker Avance spectrometer at 300, 400, or 500 MHz, whereas $^{13}$C NMR were done on the same instrument at 75, 125, or 150 MHz in $CDCl_3$. Chemical shifts are recorded relative to TMS ($\delta = 0$) internal standard, and coupling constants J are reported in Hz. Column chromatography was performed on silica gel (E-Merck, 70–230 and 230–400 mesh). Aluminum sheets precoated with silica gel 60 $GF_{254}$ (20 × 20 cm, 0.5 mm thick, E-Merck) were used for thin layer chromatography; the spots were detected with ceric sulfate followed by heating.

**Plant Material**. The green alga, *U. lactuca* (Linnaeus), was collected from sublittoral rocks at Abu Qir Bay in the Mediterranean Sea, Alexandria, Egypt during mid July 2005, and identified by Prof. Samy Ahmad Shaalan, Botany Department, Faculty of Science, Alexandria University. The freshly collected alga was washed with freshwater to remove sand and epiphytes, air dried, and then finely powdered.

**Extraction and Isolation**. The dried whole alga (1.34 kg, dry weight) was extracted with MeOH for two weeks at room temperature. The MeOH extract was filtered and evaporated under vacuum to give a dark green residue (70 g). The residue was partitioned between petroleum ether and $H_2O$. The water-soluble fraction was further extracted with EtOAc and *n*-BuOH. The PE-soluble fraction (53.93 g) was loaded onto a silica gel column and then subjected to gradient elution with petroleum ether–$CH_2Cl_2$, petroleum ether–$Me_2CO$, and $CH_2Cl_2$ to give four main fractions F-1, F-2, F-3, and F-4. The fraction F-1 obtained on elution with petroleum ether–$CH_2Cl_2$ (9.9:0.1) yielded compound **3** (100 mg). The fraction F-2 obtained on elution with petroleum ether–$Me_2CO$ (9.9:0.1) was subjected to another flash column chromatography over silica gel using petroleum ether as eluting solvent to afford compounds **1** (3.8 mg), **2** (4.1 mg), and **4** (6.2 mg). The fraction F-3 obtained on elution with petroleum ether–$Me_2CO$ (9.8:0.2) yielded a light green colored amorphous residue. Repeated recrystallization of this residue from MeOH afforded a white solid **6** (9.0 mg). The fraction F-4 obtained on elution with petroleum ether–$Me_2CO$ (9.8:0.2) was subjected to flash column chromatography over silica gel using petroleum ether and petroleum ether–$Me_2CO$ as eluting systems. The subfraction eluted with petroleum ether (100%) gave pure compound **5** (30 mg).

**(*E*)-6-Heptacosen-5-one (1)**: $C_{27}H_{52}O$, oily compound. IR ($CHCl_3$, ν, $cm^{-1}$): 723, 1073, 1124 (C-O), 1272, 1379, 1462, 1732 (C=O), 2854, 2925 (C-H), 2956. CI-MS *m/z*: 392 $[M]^+$ ($C_{27}H_{52}O$), 293 ($C_{20}H_{37}O$), 281 ($C_{20}H_{41}$), 265 ($C_{18}H_{33}O$); EI-MS, *m/z* (%): 181 (6), 167 (6), 140 (9), 125 (17), 111 (3), 97 (18), 83 (16), 69 (20), 55 (100). $^1$H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 6.80 (1H, m, H-7), 6.06 (1H, d, $J_{6,7} = 15.9$, H-6), 2.49 (2H, t, $J_{4,3} = 7.4$, H-4), 2.17 (2H, m, H-8), 1.56 (4H, br.s, H-3/H-9), 1.25–1.30 (36H, br.s, H-2/H-10–H-26), 0.86 (3H, t, $J_{1,2} = 7.3$, H-1), 0.83 (3H, t, $J_{27,26} = 6.6$, H-27). $^{13}$C NMR (125 MHz, $CDCl_3$, δ): 14.0 (C-1), 22.7 (C-2), 24.4 (C-3, C-9), 40.1 (C-4), 201.1 (C-5), 130.3 (C-6), 147.4 (C-7), 32.5 (C-8), 22.4–32.4 (C-10–C-26), 14.1 (C-27).

**(*E*)-6-Octadecen-5-ol (2)**: $C_{18}H_{35}O$, oily compound. IR ($CHCl_3$, ν, $cm^{-1}$): 723, 969, 1128 (C-O), 1377, 1462, 2855, 2925 (C-H), 3381 (O-H). CI-MS *m/z*: 269 $[M + H]^+$ ($C_{18}H_{36}O$), 251 ($C_{18}H_{35}$), 250 ($C_{18}H_{34}$); EI-MS, *m/z* (%): 183 (3), 169 (7), 155 (3), 141 (6), 127 (18), 109 (17), 95 (19), 81 (21), 67 (24), 57 (100). $^1$H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 5.60 (1H, dt, $J_{7,6} = 15.4$, $J_{7,8} = 6.9$, H-7), 5.42 (1H, dd, $J_{6,7} = 15.4$, $J_{6,5} = 6.9$, H-6), 4.01 (1H, q, $J_{5,4a} = J_{5,4b} = J_{5,6} = 6.9$, H-5), 2.00 (2H, q, $J_{8,9a} = J_{8,9b} = J_{8,7} = 6.9$, H-8), 1.40–1.51 (2H, m, H-4), 1.25 (22H, br.s, H-2/H-3/H-9–H-17), 0.85 (3H, t, $J_{18,17} = 6.9$, H-18), 0.84 (3H, t, $J_{1,2} = 6.9$, H-1). $^{13}$C NMR (125 MHz, $CDCl_3$, δ): 14.1 (C-1, C-18), 25.5 (C-2), 28.9 (C-3), 37.3 (C-4), 73.3 (C-5), 133.0 (C-6), 132.2 (C-7), 32.2 (C-8), 31.9 (C-9), 29.2–29.7 (C10–C-15), 31.8 (C-16), 22.7 (C-17).

**(*Z*)-10-Hexacosene (3)**: $C_{26}H_{51}$, oily compound. IR ($CHCl_3$, ν, $cm^{-1}$): 722, 1379, 1462, 1652, 2854, 2925, 2956. CI-MS *m/z* 365 $[M + H]^+$ ($C_{26}H_{52}$); EI-MS, *m/z* (%): 238 $[M + H - C_9H_{19}]^+$ (38), 210 $[M - C_{11}H_{21}]^+$ (3), 182 (2), 168 (3), 154 (4), 140 (7), 125 (19), 111 (42), 97 (83), 83 (99), 69 (94), 55 (100), 43 (77). $^1$H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 5.33 (2H, t, J = 4.6, H-10/H-11), 2.00 (4H, q, J = 6.1, H-9/H-12), 1.24 (40H, br.s, H-2–H-8/H-13–H-25), 0.86 (3H, t, $J_{26,25} = 6.5$, H-26), 0.84 (3H, t, $J_{1,2} = 7.2$, H-1). $^{13}$C NMR (150 MHz, $CDCl_3$, δ): 14.1 (C-1, C-26), 22.7 (C-2), 31.9 (C-3), 28.9–30.0 (C-4–C-8, C-13–C-23), 27.2 (C-9, C-12), 129.9 (C-10, C-11), 31.8 (C-24), 22.7 (C-25).

**Docosanoic acid (4)**: $C_{22}H_{44}O_2$, white amorphous powder; mp 71–72°C. FTIR (ν, $cm^{-1}$): 758, 1298, 1467, 1701, 2848, 2916. HR-EI-MS, *m/z*: 340.3338 $[M]^+$ (calcd 340.3341 $[M]^+$), 297.2788 ($C_{19}H_{37}O_2$), 241.2180 ($C_{15}H_{29}O_2$), 185.1535 ($C_{11}H_{21}O_2$), 129.0910 ($C_7H_{13}O_2$), 73.0287 ($C_3H_5O_2$), 57.0696 ($C_4H_9$); EI-MS, *m/z* (%): 340 (11), 297 (2), 241 (1), 185 (1), 129 (12), 73 (61), 57 (100). $^1$H NMR (300 MHz, $CDCl_3$, δ, ppm, J/Hz): 2.33 (2H, t, $J_{2,3}$ = 7.4, H-2), 1.61 (2H, m, H-3), 1.23 (36H, br.s, H-4–H-21), 0.86 (3H, t, $J_{22,21}$ = 6.4, H-22). $^{13}$C NMR (75 MHz, $CDCl_3$, δ): 178.4 (C-1), 33.8 (C-2), 24.7 (C-3), 29.1–29.7 (C-4–C-19), 31.9 (C-20), 22.7 (C-21), 14.1 (C-22).

**Palmitic acid (5)**: $C_{16}H_{32}O_2$, white amorphous powder; mp 62–63°C. IR (KBr, ν, $cm^{-1}$): 670, 722, 1098 (C–O), 1468, 1699 (C=O), 2850, 2918, 2955 (C–H), 3443 (O–H). HR-EI-MS *m/z* 256.2407 $[M]^+$ (calcd 256.2402, $[M]^+$). $^1$H NMR (400 MHz, $CDCl_3$, δ, ppm, J/Hz): 2.32 (2H, t, $J_{2,3}$ = 7.5, H-2), 1.61 (2H, m, H-3), 1.28 (24H, br.s, H-4–H-15), 0.86 (3H, t, $J_{16,15}$ = 6.3, H-16). $^{13}$C NMR (75 MHz, $CDCl_3$, δ): 178.6 (C-1), 33.8 (C-2), 24.7 (C-3), 29.1–29.7 (C-4–C-13), 31.9 (C-14), 22.7 (C-15), 14.1 (C-16) [16].

**Isofucosterol (6)**: $C_{29}H_{48}O$, white solid; mp 120–121°C (MeOH); $[\alpha]_D^{26}$ –10.9° (*c* 0.06, $CHCl_3$). IR ($CHCl_3$, ν, $cm^{-1}$): 742, 1092 (C–O), 1378, 1441 (C–H), 1663 (C=C), 2932 (C–H), 3380 (O–H). HR-EI-MS *m/z* 412.3727 $[M]^+$ (calcd 412.3705, $[M]^+$). $^{13}$C NMR (150 MHz, $CDCl_3$, δ): 37.3 (C-1), 31.7 (C-2), 71.8 (C-3), 42.3 (C-4), 140.8 (C-5), 121.7 (C-6), 31.9 (C-7, C-8), 50.1 (C-9), 36.5 (C-10), 21.1 (C-11), 39.8 (C-12), 42.4 (C-13), 56.8 (C-14), 24.3 (C-15), 28.3 (C-16), 56.0 (C-17), 11.8 (C-18), 19.4 (C-19), 36.2 (C-20), 18.8 (C-21), 35.9 (C-22), 27.9 (C-23), 145.9 (C-24), 28.6 (C-25), 21.0 (C-26, C-27), 116.4 (C-28), 12.8 (C-29) [17].

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## REFERENCES

1. J. Ortiz, N. Romero, P. Robert, J. Araya, J. Lopez-Hernandez, C. Bozzo, E. Navarrete, A. Osorio, and A. Rios, *Food Chem*., **99**, 98 (2006).
2. C. Bobin-Dubigeon, M. Lahaye, and J. L. Barry, *J. Sci. Food Agric.*, **73**, 149 (1997).
3. A. M. M. Ibrahim, M. H. Mostafa, M. H. El-Masry, and M. M. A. El-Naggar, *Egypt. J. Aquat*. *Res*., **31**, 146 (2005).
4. N. E. Awad, *Phytother. Res.*, **14**, 641 (2000).
5. V. Ivanova, R. Rouseva, M. Kolarova, J. Serkedjieva, R. Rachev, and N. Manolova, *Prep. Biochem*., **24**, 83 (1994).
6. E. Ivanova, R. Toshkova, V. Ivanova, R. Rouseva, and M. Kolarova, *Dokl. Bulg. Akad. Nauk.*, **47**, 109 (1994).
7. Atta-ur-Rahman, M. I. Choudhary, A. Hayat, A. M. Khan, A. Ahmad, and S. Malik, *Phytochemistry*, **52**, 495 (1999).
8. S. V. Khotimchenko, V. E. Vaskovsky, and T. V. Titlyanova, *Bot. Mar.*, **45**, 17 (2002).
9. J. W. Blunt, B. R. Copp, M. H. G. Munro, P. T. Northcote, and M. R. Prinsep, *Nat. Prod. Rep*., **23**, 26 (2006).
10. K. B. Glaser and A. M. S. Mayer, *Biochem. Pharmacol*., **78**, 440 (2009).
11. K.-B. Oh, J. H. Lee, S.-C. Chung, J. Shin, H. J. Shin, H.-K. Kimd, and H.-S. Leeb, *Bioorg. Med. Chem. Lett*., **18**, 104 (2008).
12. S. B. Khan, A. U. Haq, N. Afza, A. Malik, M. T. H. Khan, M. R. Shah, and M. I. Choudhary, *Chem. Pharm. Bull*., **53**, 86 (2005).
13. I. Yasuda, K. Takeya, and H. Itokawa, *Chem. Pharm. Bull*., **29**, 564 (1981).
14. R. Lecuona, G. Riba, P. Cassier, and J. L. Clement, *J. Invertebr. Pathol*., **58**, 10 (1991).
15. U. R. Joshi and A. H. Kapadi, *Synth. Commun*., **14**, 681 (1984).
16. M. S. Ali, M. Saleem, V. U. Ahmad, and S. Shameel, *Z. Naturforsch.*, **56B**, 837 (2001).
17. S. De Rosa, A. De Giulio, and G. Tommonaro, *Phytochemistry*, **44**, 861 (1997).
18. A. K. Siddhanta, A. M. Goswami, M. Shanmugam, K. H. Mody, and B. K. Ramavat, *J. Indian Chem. Soc*., **79**, 294 (2002).