Pergamon

Tetrahedron Vol. 51, No. 28, pp. 7667-7672, 1995
Copyright © 1995 Elsevier Science Ltd
Printed in Great Britain. All rights reserved
0040-4020/95 $9.50+0.00

0040-4020(95)00405-X

Brominated Acetylenic Fatty Acids from *Xestospongia* sp., a Marine Sponge-Bacteria Association

Sarah E. Brantley and Tadeusz F. Molinski*

Department of Chemistry, University of California, Davis, CA 95616

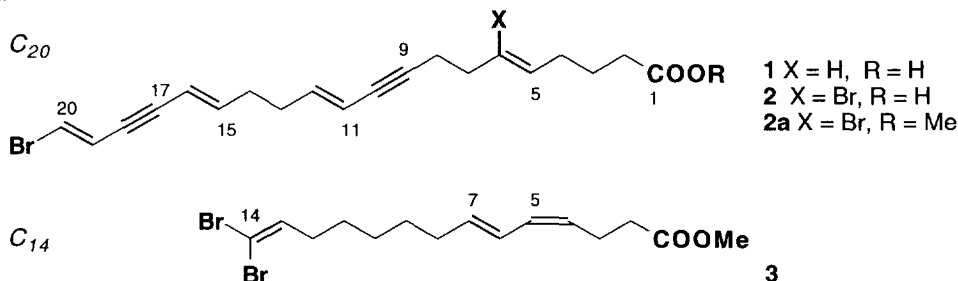
and

Chris M. Preston and Edward F. DeLong

Marine Science Institute, University of California, Santa Barbara, CA 93106

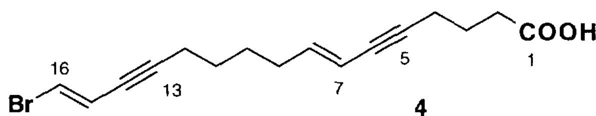
Abstract: Three new brominated fatty acids, (5*E*, 11*E*, 15*E*, 19*E*)-20-bromoeicosa-5,11,15,19-tetraene-9,17-diynoic acid (**1**), (5*Z*, 11*E*, 15*E*, 19*E*)-6,20-dibromoeicosa-5,11,15,19-tetraene-9,17-diynoic acid (**2**) and (*Z E*)-14,14-dibromo-4,6,13-tetradecatrienoate, characterized as the methyl ester, **3**, were isolated from *Xestospongia* sp. from the Indian Ocean. Compounds **1 -2** (C_{20}) and **3** (C_{14}) are brominated fatty acids of unusual carbon number that have not been encountered previously in marine invertebrates. *Xestospongia* sp. is an association of sponge cells and bacteria, the latter accounting for *ca.* 50% of cellular mass.

Introduction: Brominated acetylenic fatty acids are characteristic of natural products produced by marine sponges (Porifera) of the genera *Xestospongia*, *Petrosia* and *Oceanapia* in the family Nepheliospongidae.[1-9] The known brominated acetylenic acids are usually based on C_{16} and C_{18} chain lengths, with the exception of one C_9 acid.[4] We wish to report three new brominated fatty acids of unusual chain length; the C_{20} acetylenic acids, **1** and **2**, and a ω,ω-dibromovinylidene C_{14} fatty acid ester **3** from an Indian Ocean sponge *Xestospongia* sp., an invertebrate containing a high proportion of eubacteria in its tissues (as much as 56% of the total sponge biomass, Table 1). Free C_{20} acids are rarely found as marine natural products while myristic acid (C_{14}), more commonly seen in bacteria, has been found only in the esterified form in the sponge-derived alkaloids bengazole A and bengamide A.[10,11]



Purification of an extract of one sample of the sponge *Xestospongia* sp. by flash chromatography (silica gel, *n*-hexane: EtOAc) gave a mixture of unsaturated fatty acids which were separated by HPLC (85:15 MeOH/H_2O) to provide pure **1** and **2** in addition to the known C_{16} carboxylic acid, **4**.[4]

Mass spectrometric measurements of **1** using FABMS and EIMS were unsuccessful, however, chemical ionization (NH_3) showed a prominent pseudomolecular ions MH^+ (*m/z* 375/377) and $M+NH_4^+$ (*m/z* 392/394) showing the presence of one bromine and a formula of $C_{20}H_{23}BrO_2$ (HRCIMS; *m/z* $M+H^+$ 394.1212, Δ mmu 0.7). The UV spectrum of **1** revealed chromophores (λ_{max} 270 (ε 27,500), 288 (22,200)) due to conjugated unsaturated bonds while the IR (ν 2214) suggested the presence of acetylenic unsaturation. Interpretation of the ^1H NMR, ^{13}C NMR spectra and comparison with published data of known compounds identified two conjugated unsaturated groups (an ene-3,4-yne moiety and an ene-3,4-yne-5,6-ene system) and an isolated carbon-carbon double bond. The bromine was located at the C20 terminus as shown by the low field chemical shift of H19 (6.31, dd, 1H, J = 14, 2.2 Hz). A COSY experiment revealed homo-propargylic coupling (J= 2.2 Hz) between H19 and H16 (δ 5.58, bdt, 1H, J = 15.9, 2.2, 1.7 Hz) in addition to sequential vicinal and allylic correlations from H16 to H2. HMQC and HMBC experiments showed correlations from H3 (δ 1.68, quin., 2H, J = 7.4 Hz) to both C5 (δ 130.8, d) and C1 (δ 177.2, s) and established that three contiguous methylenes (C2 to C4) were adjacent to the carboxyl terminus. The molecular structure **1** was assembled by interpretation of COSY, in particular allylic and homoallylic correlations, and additional HMBC correlations which also furnished full assignments of the ^{13}C NMR spectrum. All double bonds have *E* geometry as shown by the magnitudes of vinylic vicinal coupling constants ranging from J = 14-15.9 Hz.



Compound **2** differs from **1** only by the substitution of bromine for hydrogen at C6. The free acid failed to show a pseudomolecular ion in the FAB, EI or CI MS. Treatment of **2** with diazomethane gave a methyl ester **2a** which provided a formula of $C_{20}H_{22}Br_2O_2$ for **2** (*m/z* at 484/486/488, $M+NH_4^+$, intensity ratio 1:2:1). The UV spectrum of **2** (λ 271, 286 nm) was unchanged over that of **1** and the ^1H NMR and ^{13}C NMR spectra of **2** were also very similar. The vinyl proton signal of H5 (δ 5.93, t, J = 7.7 Hz) appeared now as triplet suggesting the additional bromine was now present at C6. The calculated chemical shift of H5 for both the *E* - isomer (δ 5.36 ppm) and *Z* - isomer (δ 5.98 ppm) is more consistent with the 5,6-*Z* configuration for **2** (δ 5.93, t, H5, J = 7.7 Hz) as shown.[12] All other ^1H NMR and ^{13}C NMR signals were essentially the same as those of **1**.

Extraction and purification of a second specimen from the same collection of *Xestospongia* sp., failed to yield **1** and **2**. Instead, purification of the fatty acid fraction as before provided a new C_{14} fatty acid, (*Z*, *E*) 14,14-dibromotetradec-4,6,13-trienoic acid, isolated as its methyl ester **3**, after treatment with diazomethane and HPLC separation. The HRCI mass spectrum of **3** provided a formula

of $C_{15}H_{22}Br_2O_2$ ($M+NH_4^+$, at *m/z* 410.0310, Δmmu 2). The UV spectrum (λ_{max} 231 nm, ε 7900) and ^{13}C NMR spectrum (δ 134.9 d, C7; 129.4, d, C5; 126.6, d, C4; 124.9, d, C6) revealed a conjugated 4*Z*, 5*E* diene ($J_{4,5}$ = 11.5 Hz; $J_{6,7}$ = 15.4 Hz) while the remaining double bond (δ 138.2, d, C13; 88.1, s, C14) was assigned to a 14,14-dibromovinylidene group. The exceptionally high field ^{13}C signal observed for C14 in **3** (δ 88.1, s) is in disagreement with the C9 assignment (δ 118.8, s) for the known natural product 9,9-dibromo-8-nonenoic acid.[4] 1,1-Dibromo-1-nonene, prepared from *n*-octanal (CBr_4, Ph_3P)[13] showed ^{13}C signals due to sp^2 carbons at δ 88.6 (s, C1) ppm and 138.7 (d, C2), in agreement with the assignments for **3** (δ 138.2, d, C13; 88.1, s, C14). The high field ^{13}C shift for C14 is due to the 'heavy atom' effect caused by substitution with two bromine atoms.[14]

The relative proportion of eubacterial, eukaryotic and archaebacterial ribosomal ribonucleic acid (rRNA) in *Xestospongia* sp. was determined by measuring the proportion of each radiolabeled group-specific oligonucleotide bound to total rRNA extracted from the sponge.[15-17] Eubacterial rRNA accounted for an average of 46% of the total sponge rRNA, in three *Xestospongia* specimens examined (Table 1). Nucleic acid sequence analyses of the sponge-associated eubacterial rRNAs indicated the presence of several bacterial phyla, including cyanobacterial types (C.M. Preston and E.F. Delong, unpublished data).

	#	Eubacterial	Eukaryotic	Archaebacterial
Specimen	1	37	63	nd
	2	56	44	nd
	3	46	54	nd
Mean ± SEM		46%±5.5	54%±5.5	-

Table 1: Relative percentage of ribosomal RNA in *Xestospongia* sp. tissue (random specimens)

Discussion. The first and second samples of *Xestospongia* sp examined in this study showed surprising variability in chemical composition, even though the two specimens appeared morphologically identical and were collected at the same site. The high bacterial content of *Xestospongia* sp. is reminiscent of the sponge *Dysidea herbacea* which contains up to 40% by weight of cyanobacteria and satisfies one criterion proposed for symbiotic bacterial association in marine sponges.[18] The cyanobacterium *Oscillatoria spongiella* is responsible for production of trichloroleucine metabolites in *D. herbacea*.[19,20] The variability of chemical structure in unsaturated fatty acids isolated from individual specimens of *Xestospongia* (differing chain length, bromine substitution, degree of unsaturation) parallels the heterogeneity of metabolite distribution found in individual specimens of *D. herbacea*.[21] If bacteria associated with *Xestospongia* sp. are responsible for production of brominated fatty acids, the variability of their structures may be a consequence of changes in bacterial speciation or metabolic activity between individual specimens. Further work is under way to clarify the role of bacteria in secondary metabolite production within *Xestospongia* sp.

Acknowledgments: We are grateful to M.K. Harper, Scripps Institution of Oceanography, for identification of the sponge and to Dr. Jeff de Ropp for assistance with HMBC and HMQC

experiments. The 500 MHz NMR spectrometer was partially funded through NIH ISIO-RR04795 and NSF BBS88-04739. Support from the Office of Research, UC Davis is gratefully acknowledged. This paper is funded in part by a grant from the National Sea Grant College Program, National Oceanic and Atmospheric Administration, U.S. Department of Commerce, under grant number NOAA NA36RG0537, project number R/MP-57A through the California Sea Grant College Program, and in part by the California State Resources Agency. The views expressed herein are those of the authors and do not necessarily reflect the views of NOAA or any of its sub-agencies. The U.S. Government is authorized to reproduce and distribute for governmental purposes.

Experimental Section:

^{1}H NMR spectra were recorded at 300 MHz or 500 MHz and ^{13}C NMR spectra were recorded at 75 MHz or 125 MHz. ^{13}C NMR multiplicities were determined by DEPT or HMQC experiments. Signals are referenced to TMS, δ = 0.00 ppm (^{1}H) or solvent signal (^{13}C; CD_3OD, δ 49.00 ppm, $CDCl_3$, 77.00 ppm,). General procedures can be found elsewhere.[22]

Collection and Extraction of *Xestospongia* sp.; Isolation of 1-4 The sponge was collected from Bennett Shoal, Exmouth Gulf, Western Australia (-5 m; 22° 06' S 114° 08' E), in January 1993 and frozen immediately. The sponge has an erect conical form (ca. 20 cm diameter) with a tan-pink ectoderm. The A portion of the sponge (268 g) was freeze-dried, covered with methanol (2 L), and stored overnight. The solvent was filtered and the sponge was extracted a second time with methanol in the same manner. The combined extracts were concentrated to 1L and separated using a modified Kupchan partition[23] as follows. The water content (% v/v) of the MeOH extract was adjusted prior to sequential partitioning against *n*-hexane (10% v/v), CCl_4 (20% v/v), and $CHCl_3$ (40% v/v). The CCl_4 and $CHCl_3$ extracts were combined (3.94 g) and separated by vacuum flash chromatography using TLC grade silica gel H (5-40 µm) and 5-100% EtOAc/*n*-hexane solvent system. The acids eluted with 50-100% EtOAc. Further purification by reverse phase HPLC (Dynamax-60A RP18; 85% MeOH / H_2O) gave two new C_{20} acids, **1** (0.0015% dry weight) and **2** (0.0027%), in addition to the known C_{16} acid, **4** (0.0013%).

(5*E*, 11*E*, 15*E*, 19*E*) 20-Bromoeicosa-5,11,15,19-tetraen-9,17-diynoic acid (1): $C_{20}H_{23}BrO_2$; amorphous powder, UV (MeOH) λ_{max} 270 nm (ϵ 27500), 288 (22200); IR (NaCl) 2214 (w), 2190 (w), 1697 cm^{-1}; ^{1}H NMR (10:1 $CDCl_3$: CD_3OD, 0.01% TMS) δ 6.66 (d, 1 H, J = 14 Hz, H20), 6.31 (dd, 1 H, J = 14, 2.2 Hz, H19), 6.17 (dt, 1 H, J = 15.9, 6.7 Hz, H15), 6.02 (dt, 1 H, J = 15.8, 6.6 Hz, H12), 5.58 (ddt, 1 H, J = 15.9, 2.2, 1.7 Hz, H16), 5.48 (m, 3 H, H5, 6, 11), 2.31 (m, 2 H, H8), 2.22 (t, 2 H, H2), 2.20 (m, 4 H, H13 & H14), 2.19 (t, 2 H, H7), 2.06 (m, 2 H, H4), 1.68 (quin., 2 H, J = 7.4 Hz, H3); ^{13}C NMR (10:1 $CDCl_3$: CD_3OD, 0.01% TMS) δ 177.2 (s, C1), 144.5 (d, C15), 141.4 (d, C12), 130.8 (d, C5), 129.5 (d, C6), 117.8 (d, C19, 20), 111.1 (d, C11), 110.1 (d, C16), 90.5 (s, C17), 89.0 (s, C9), 85.0 (s, C18), 79.4 (s, C10), 33.9 (t, C2), 32.6 (t, C7 or 4), 32.1(t, C13, 14, 4 or 7), 25.0 (t, C3), 19.9 (t, C8); HRCIMS (NH_3) obsd m/z 392.1218 ($M+NH_4^+$), $C_{20}H_{27}BrNO_2$ requires 392.1221.

(5*Z*, 11*E*, 15*E*, 19*E*) 6,20-Dibromoeicosa-5,11,15,19-tetraen-9,17-diynoic acid (2): $C_{20}H_{22}Br_2O_2$; amorphous powder; UV (MeOH) 271, 286 nm; IR (NaCl) 2214 (w), 2187 (w), 1716 cm^{-1}; ^1H NMR (10:1 $CDCl_3$: CD_3OD, .01% TMS) δ 6.67 (d, 1 H, J = 14.0 Hz, H20), 6.31 (dd, 1 H, J = 14.0, 2.3 Hz, H19), 6.16 (dt, 1 H, J = 15.9, 6.8 Hz, H15), 6.01 (dt, 1 H, J = 15.8, 6.6 Hz, H12), 5.93 (t, 1 H, J = 7.7 Hz, H5), 5.58 (dd, 1 H, J = 15.9, 1.9 Hz, H16), 5.47 (br d, 1 H, J = 15.8 Hz, H11), 2.64 (m, 2H, H7), 2.48 (td, 2 H, J = 7.3, 2.0 Hz, H8), 2.30 (t, 2 H, J = 7.5 Hz, H2), 2.16 (m, 4H, H13, 14), 2.13 (q, 2 H, J = 7.5 Hz, H4), 1.71 (quin., 2 H, J = 7.5 Hz, H3); ^{13}C NMR (10:1 $CDCl_3$: CD_3OD, 0.01% TMS) δ 176.5 (s), 144.4 (d), 141.4 (d), 133.2(d), 124.3 (s), 117.9 (d), 117.8 (d), 110.9 (d), 110.2 (d), 90.5 (s), 87.4 (s), 85.0 (s), 79.8 (s), 35.1 (t), 33.8 (t), 32.5 (t), 32.0 (t), 29.2 (t), 24.7 (t), 18.8 (t); LRCIMS (NH_3) obsd m/z 484/486/488 ($M+NH_4^+$), $C_{21}H_{28}Br_2NO_2$

16-Bromo-(7*E*, 15*E*)-hexadeca-7,15-dien-5,13-diynoic acid (4)[4]: $C_{16}H_{19}BrO_2$; amorphous powder; IR (NaCl) ν 2215 (w), 1700 cm^{-1}; ^1H NMR (10:1 $CDCl_3$: CD_3OD, 0.01% TMS) δ 6.58 (d, 1 H, J = 14.0 Hz), 6.18 (dt, 1 H, J = 14.0, 2.3 Hz), 6.04 (dt, 1 H, J = 15.8, 7.0 Hz), 5.46 (dt, 1 H, J = 15.8, 1.7 Hz), 2.43 (t, 2H, J = 7.5 Hz), 2.38 (td, 2H, J = 7.0, 1.7 Hz), 2.27 (td, 2H, J = 6.7, 2.3 Hz), 2.11 (qd, 2H, J = 7.0, 1.0 Hz), 1.83 (sex., 2H, J = 7.5 Hz), 1.52 (m, 4H); ^{13}C NMR (10:1 $CDCl_3$: CD_3OD, 0.01% TMS) δ 143.0 (d), 118.1 (d), 117.2 (d), 110.4 (d), 92.2 (s), 87.8 (s), 80.0 (s), 33.6 (t), 32.5 (t), 28.1 (t), 27.9 (t), 24.4 (t), 19.4 (t), 19.0 (t). C1 signal below level of detection. C14 obscured by $CDCl_3$.

Methyl (*Z*, *E*) -14,14-dibromo-4,6,13-tetradecatrienoate (3) A second specimen of *Xestospongia* sp. was extracted as follows. The sponge (27.18 g) was lyophilized and extracted twice with methanol (2 X 500 ml; first for 4 hours and secondly, 16 hours). The combined extracts were evaporated to one third volume and solvent partitioned as described for compounds **1-4**.[23] The $CHCl_3$ soluble extract extract (672.8 mg) was flash chromatographed over silica with a gradient solvent system of 5-100% EtOAc/*n*-hexane. The fifth fraction (61.5 mg) was passed through a short C_{18} cartridge (Varian Mega Bond Elute, 60-100% MeOH/ H_2O.) and the eluate (44.5 mg) was treated with diazomethane to give a mixture of methyl esters. This material was separated by reversed phase HPLC (Dynamax C_{18}, 87% MeOH/H_20) to give pure **3** (2.7 mg, 0.0099% of dry weight) as a colorless oil. **3**: $C_{15}H_{22}Br_2O_2$, oil; UV (MeOH) 231 nm (ε 7900); IR (NaCl) 1739, 1437, 1169 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.40 (t, 1H, J = 7 Hz, H13), 6.33 (dd, 1H, J = 15.2, 11.5 Hz, H6), 6.02 (t, 1H, J = 11.5 Hz, H5), 5.70 (dt, 1H, J = 15.2, 7.4 Hz, H7), 5.26 (dt, 1H, J = 11.5, 7.4 Hz, H4), 3.72 (s, 3H, OCH_3), 2.51 (bt, 2H, J = 7.6 Hz, H3), 2.45 (bt, 2H, H2), 2.15 (m, 4H, H8, H12), 1.4 (m, 6H, H9, 10, 11); ^{13}C NMR ($CDCl_3$) δ 173.8 (C1), 138.2 (C13), 134.9 (C7), 129.4 (C5), 126.6 (C4), 124.9 (C6), 88.1, (C14), 51.0 (OCH_3), 33.6 (C2), 32.2, 32.4 (C8, C12), 28.1-28.5 (C9, C11), 27.1 (C10), 22.7 (C3); HRCIMS (NH_3) obsd m/z 410.0310 ($M+NH_4^+$), $C_{15}H_{25}Br_2NO_2$ requires 410.0330.

1,1-Dibromo-1-nonene: *n*-Octanal (2.16g, 17 mmol) was added to a stirred mixture of triphenylphosphine (9.8 g, 37 mmol) and carbon tetrabromide (6.4 g, 19 mmol) in CH_2Cl_2 (55 mL) at 0° according to Seebach *et al.*[13] and allowed to warm to 23° with stirring (30 h). The crude yellow oil obtained after work-up was purified by silica flash chromatography (1:1 hexane/EtOAc) to give pure

1,1-dibromo-1-nonene (2.16g, 72%). ^{1}H NMR identical to lit.[13] ^{13}C NMR ($CDCl_3$) 138.7 (d, C2), 88.6 (s, C1), 33.0 (t), 31.8 (t), 29.0 (t), 27.8 (t), 22.7 (t), 14.1 (t, C9).

Ribosomal RNA Analysis: Three specimens were randomly selected from the same collection of *Xestospongia* sp. that was used in the above extraction. Nucleic acid was extracted and probed against a group specific oligodeoxynucleotide library using standard procedures described elsewhere.[15]

References:

(1) Bourguet-Kondraki, M. L.; Rakotoarisoa, M. T.; Martin, M. T.; Guyot, M. *Tetrahedron Lett.* **1992**, *33(2)*, 225-226.
(2) Carballeira, N. M.; Shalabi, F. *J. Nat. Prod.* **1993**, *56(5)*, 739-746.
(3) Fusetani, N.; Li, H.-y.; Tamura, K.; Matsunaga, S. *Tetrahedron* **1993**, *49(6)*, 1203-1210.
(4) Hirsh, S.; Carmely, S.; Kashman, Y. *Tetrahedron* **1987**, *42(14)*, 3257-3261.
(5) Ichiba, T.; Scheuer, P. J. *Helv. Chim. Acta* **1994**, *76*, 2814-2816.
(6) Patil, A. D.; Kokke, W. C.; Cochran, S.; Francis, T. A.; Tomszek, T.; Westley, J. W. *J. Nat. Prod.* **1992**, *55(9)*, 1170-1177.
(7) Quinn, R. J.; Tucker, D. J. *Tetrahedron Lett.* **1985**, *26(13)*, 1671-1672.
(8) Quinn, R. J.; Tucker, D. J. *J. Nat. Prod.* **1991**, *54(1)*, 290-294.
(9) Schmitz, F. J.; Gopichand, Y. *Tetrahedron Lett.* **1978**, *39*, 3637-3640.
(10) Molinski, T. F. *J. Nat. Prod.* **1993**, *56(1)*, 1-8.
(11) Adamczeski, M.; Quinoà, E.; Crews, P. *J. Am. Chem. Soc.* **1988**, *110(5)*, 1598-1602.
(12) Pascual, J.; Meier, J.; Simon, W. *Helv. Chim. Acta* **1966**, *49*, 164.
(13) Seebach, D.; Hässig, R.; Gabriel, J. *Helv. Chim. Acta* **1983**, *66(27)*, 308-334.
(14) Wehrli, F. W.; Marchand, A. P.; Wehrli, S. *Interpretation of Carbon-13 NMR Spectra*; John Wiley & Sons: Chichester, 1983.
(15) DeLong, E. F. *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 5685-5689.
(16) Stahl, D. A.; Flesher, B.; Mansfield, H. R.; Montgomery, L. *Appl. Environ. Microbiol.* **1988**, *54*, 1079-1084.
(17) Stahl, D. A.; Amann, R. I. *Development and Application of Nucleic Acid Probes in Nucleic Acid Techniques in Bacterial Systematics*; J.W. Wiley: New York, 1991, pp 205-248.
(18) Unson, M. D.; Holland, N. D.; Faulkner, D. J. *Mar. Biol.* **1994**, *119*, 1-11.
(19) Unson, M. D.; Faulkner, D. J. *Experientia* **1993**, *49*, 349-353.
(20) Dumdei, E.; Gehrmann, J.; Dexter, A. F.; Field, K.; Garson, M. J.; Molinski, T. F. unpublished results.
(21) Searle, P. A.; Jamal, N. M.; Lee, G. M.; Molinski, T. F. *Tetrahedron* **1994**, *50(13)*, 3879-3888.
(22) Searle, P. A.; Molinski, T. F. *J. Org. Chem.* **1993**, *58(26)*, 7578-7580.
(23) Kupchan, S. M.; Britton, R. W.; Ziegler, M. F.; Sigel, C. W. *J. Org. Chem.* **1973**, *38(1)*, 178-179.

(*Received in USA* 24 *January* 1995; *revised* 14 *May* 1995; *accepted* 16 *May* 1995)