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*J. Nat. Prod.*, **1994**, 57 (1), 84-89• DOI:
10.1021/np50103a012 • Publication Date (Web): 01 July 2004

*Journal of Natural Products*
*Vol. 57, No. 1, pp. 84-89, January 1994*

# POLAR METABOLITES FROM THE SEA CUCUMBER *CUCUMARIA FRONDOSA*

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ABSTRACT.—The structures of a new glyceryl phosphoryl ether [**1**] and an isocaffeine derivative [**4**] from the sea cucumber, *Cucumaria frondosa*, were elucidated by spectroscopic means and chemical transformations. Compound **1** is 1-*O*-[(11*Z*)-octadecenyl]-*sn*-glyceryl-3-phosphorylcholine and compound **4** is 1,3,9-trimethyl-8-nitrosoisoxanthine. The dinucleotides thymidylyl-(3′-5′)-thymidine [**7**], 2′-deoxyadenylyl-(3′-5′)-thymidine [**8**] and thymidylyl-(3′-5′)-2′-deoxyadenosine-3′-monophosphate [**9**], in the form of their sodium salts, were also isolated from this same source.

Recently we reported the isolation of novel sulfated hydrocarbons (1) and sulfated oligosaccharides (2) from the sea cucumber *Cucumaria frondosa* Gunnerus (Class Holothuroidea). In our continuing investigation of the polar constituents of this organism, we have encountered a novel glyceryl phosphoryl ether, **1**, an isocaffeine derivative, **4**, and three dinucleotides, **7**, **8**, and **9**.

The $^1$H-nmr spectrum (pyridine-$d_5$/$D_2O$) of compound **1** closely resembles those of known glyceryl phosphoryl ethers. The chemical shift assignments (Table 1) were facilitated by comparison with literature data (3–5) and reference to the 2D COSY nmr spectrum. The $^{15}$N-nmr spectrum shows a signal at δ 0.8 ppm confirming the presence of a nitrogen atom in the molecule (6). The $^{13}$C-nmr chemical shift assignments were made on the basis of DEPT and HETCOR spectra and comparison with data from known homologs (3–6). Thus, apart from the location of the double bond and the precise length of the side-chain, the structure of compound **1** was well defined by nmr methods.

The positive fabms (glycerol) of **1** displayed a substantial ion at *m/z* 508 corresponding to $M^+$ $(C_{26}H_{54}NO_6P)+H$, while significant fragments at *m/z* 256 and 166 are readily accounted for via fission at the ethereal bonds of the hydrocarbon and phosphoryl choline side-chains, respectively.

The presence of a cis double bond in **1** is indicated by the $^{13}$C-nmr spectrum, which shows signals at δ 28.2 and 28.8 ppm corresponding to carbons allylic to a cis olefin (7). The location of the double bond was determined by ozonolysis to the aldehyde **2** which displayed in the eims a significant ion at *m/z* 407, corresponding to $M^+$ $(C_{19}H_{40}NO_7P)-H_2O$. The same product **2** was also obtained by $OsO_4$-$NaIO_4$ (8) cleavage of **1**. Treatment of **1** with dimethyldisulfide (9) gave the derivative **3** whose positive fabms (glycerol) afforded further support for the location of the double bond at C-11 by displaying an ion *m/z* 146 corresponding to $CH_3(CH_2)_5CHSCH_3+H$ resulting from cleavage of the C-11, C-12 bond. The $^{13}$C-nmr chemical shift assignments (Table 1) are entirely consistent with the location of the double bond at C-11.

The optical rotation, $[\alpha]^{20}D$ −2.9°, of **1** is in good agreement with reported data (3,4) for homologs possessing the absolute configuration as in **1**, hence its structure is 1-*O*-[(11*Z*)-octadecenyl]-*sn*-glycerol-3-phosphorylcholine. While compound **1** has not been reported previously, glyceryl phosphoryl ethers are widespread among marine animals and are generally found in the neutral polar lipids of echinoderms. Some glyceryl phosphoryl ethers isolated from a hydroid have been reported by Fusetani *et al.* (3) to possess hemolytic activity.

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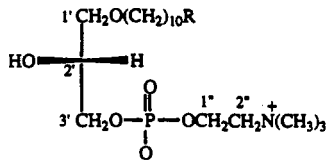
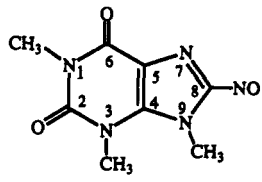
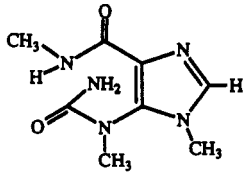


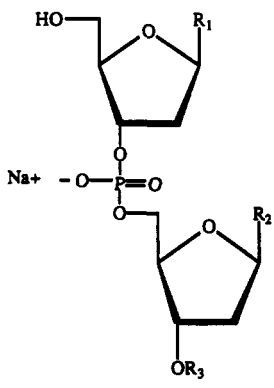
**1** R=CH=CH(CH$_2$)$_5$CH$_3$
**2** R=CHO
**3** R=CH(SCH$_3$)—CH(SCH$_3$)(CH$_2$)$_5$CH$_3$



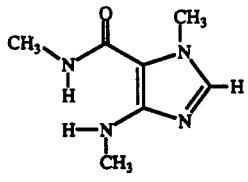
**4**



**5**



**7** R$_1$, R$_2$=*thymine*, R$_3$=H
**8** R$_1$=adenine, R$_2$=thymine, R$_3$=H
**9** R$_1$=thymine, R$_2$=adenine, R$_3$=PO$_3$Na$_2$



**6**

The $^1$H-nmr spectrum ($CDCl_3$-$CD_3OD$, 1:1) of compound **4** shows three methyl singlets at δ 3.49, 3.82, and 4.16 ppm. This information, together with uv and fabms data (*vide infra*) suggested a caffeine or isocaffeine derivative substituted at C-8. Comparison of the $^1$H-nmr data with literature values (10–13) indicated that **4** is an isoxanthine derivative and this was confirmed in an nOe experiment which demonstrated a 1.7% nOe between the 9-$CH_3$ and 3-$CH_3$ signals (see Table 2). Support for the structure **4** was obtained from the positive fabms (glycerol) which shows substantial ions corresponding to $M^+$ ($C_8H_9N_5O_3$)+matrix (315), $M^+$+H (224), and $M^+$ (223), as well as major ions for $M^+$−O (207) and $M^+$−NO+H (194) characteristic of the nitroso group (14).

Chemical corroboration for the structure of compound **4** comes from its transformation to compound **5** involving substitution of the nitroso group by hydrogen and ammonolytic cleavage of the 1,2 bond. In 1931, Biltz and Sauer (15) reported the preparation of 1,3,9-trimethyl-8-nitrosoisoxanthine and noted the conversion of 8-nitrosoisoxanthines to 8-bromoisoxanthines by bromine water. Thus, the ready reverse electrophilic aromatic substitution at C-8 of **4** is precedented. In addition, Biltz and Rakett (16) reported that basic hydrolysis of caffeine gives rise to products corresponding to cleavage of the 1-2 bond of the purine ring.

Using this procedure we have prepared the caffeine hydrolysis product **6** and characterized it by $^1$H nmr to provide comparative chemical shift data for **5**. The structure of the latter is further supported by the eims which displays abundant ions corresponding to $M^+$ ($C_8H_{13}N_5O_2$) (211), M-CO (183, base peak) and M-CO-$CH_3$ (168).

TABLE 1. $^{1}H$- and $^{13}C$-nmr Data of **1** (pyridine-$d_5$/$D_2O$, 5:1) in ppm.[a]

| Position | $^{1}H$ | $^{13}C$ (*J*, Hz) |
|---|---|---|
| 1 | 3.64 m | 71.5 |
| 2 | 1.64 m | 31.6 |
| 3 | 1.25 bs | 26.0 |
| 4–7 | 1.25 bs | 29.2 |
| 8 | 1.25 bs | 29.6 |
| 9 | 1.25 bs | 27.1 |
| 10 | 1.9 bs | 28.8 |
| 11 | 5.5 bs | 130.0 |
| 12 | 5.5 bs | 130.0 |
| 13 | 2.1 bs | 28.2 |
| 14 | 1.25 bs | 27.1 |
| 15 | 1.25 bs | 29.6 |
| 16 | 1.25 bs | 31.7 |
| 17 | 1.25 bs | 22.6 |
| 18 | 0.9 bs | 14.0 |
| 1′ | 3.9 bs | 72.4 |
| 2′ | 4.5 m | 70.1 d (*J*=7.1)[b] |
| 3′ | 4.5 m | 67.7 d (*J*=5.5)[b] |
| 1″ | 4.1 m | 59.6 d (*J*=4.8)[b] |
| 2″ | 4.8 bs | 66.6 d (*J*=2.9)[b] |
| $NCH_3$ | 3.58 s | 54.1 |

[a]Assignments based on 2D-COSY, HETCOR, DEPT spectra and literature data (3–5).

[b]Coupling with $^{31}P$.

Thus, we conclude that **4** is 1,3,9-trimethyl-8-nitrosoisoxanthine and appears to be the first isoxanthine (isocaffeine) derivative reported from a natural source.

In addition to compounds **1** and **4**, we have also isolated three dinucleotides in the form of their sodium salts. Their structures were determined by $^{1}H$-nmr, uv and mass spectral analysis and by comparison with the available literature data. Thus the sodium salts of 2′-deoxythymidylyl-(3′-5′)-2′-deoxythymidine [d(TpT)], **7** (17-19), 2′-deoxyadenylyl-(3′-5′)-2′-deoxythymidine [d(ApT)], **8** (20-21), and the trisodium salt of 2′-deoxythymidylyl-(3′-5′)-2′-deoxyadenosine-3′-monophosphate [d(TpAp)], **9** (19) have all been isolated from the aqueous methanolic extract of *C. frondosa*. This is the first report of these dinucleotides being isolated in the free form.

TABLE 2. $^{1}H$-nmr Data of Compounds **4–6**.

| Position | Compound | | |
|---|---|---|---|
| | **4**[a] | **5**[b] | **6**[b] |
| 1-$CH_3$ | 3.49 s | 3.07 d (*J*=4.7 Hz) | 3.10 d[c] (*J*=4.7 Hz) |
| 3-$CH_3$ | 3.82 s | 3.24 s | 2.94 d[c] (*J*=5.3 Hz) |
| 9-$CH_3$/7-$CH_3$ | 4.16 s | 3.60 s | 3.80 s |
| 1-NH | — | 7.11 bs | 7.10 bs |
| 2-$NH_n$ | — | 8.11 bs (n=2) | 5.95 bs (n=1) |
| 8-H | — | 8.50 s | 7.42 s |

[a]In $CDCl_3$-$CD_3OD$ (1:1).

[b]In pyridine-$d_5$.

[c]Values may be interchanged.

## EXPERIMENTAL

Nmr spectra were recorded on a Varian XL-200 spectrometer using tetramethylsilane as internal reference. Fab and ei mass spectra were recorded on a Kratos MS50 instrument. Optical rotations were measured on a Perkin-Elmer 241 polarimeter. Infrared spectra were measured on a Bruker IFS-25 spectrometer; ultraviolet spectra were measured on a Pye Unicam PU 990 uv/vis spectrometer.

Extraction procedures and vouchering were as described previously (2).

ISOLATION OF COMPOUND **1**.—Evaporation of fractions 22–28 gave an amorphous material (ca. 600 mg) that was rechromatographed over a Kieselgel 60 (60 g, 230–400 mesh) column eluting with a discontinuous gradient of $CHCl_3$-$CH_3OH$-$H_2O$ (6.5:3:0.5→4:3:3) to give 85 fractions (10–15 ml each) which were combined on the basis of Si gel tlc to provide three subfractions: a (1–16, 250 mg), b (17–75, 150 mg), and c (76–85, 17 mg). Fraction c was compound **1**, an oil [$R_f$=0.32, $CHCl_3$-$CH_3OH$-$H_2O$ (6.5:3.8:1.1)]; $[\alpha]_D$ −2.9° [$c$=0.1, $CHCl_3$-$CH_3OH$ (4:1)]; fabms (glycerol) $m/z$ (%) 508 $[M+H]^+$ (17.7), 256 (5.6), 184 (100.0), 166 (47.0).

ISOLATION OF COMPOUND **4**.—Subfraction a (150 mg) from the previous column was rechromatographed over a column (10 g, LiChroprep RP-18) eluting with a discontinuous gradient of $(CH_3)_2CO$-$H_2O$ (3:3→4:3) under pressure to give 29 fractions (ca. 5–10 ml each). Evaporation of fractions 5–6 [$R_f$=0.95, C-18 tlc, $CH_3OH$-$H_2O$ (1:1)] gave amorphous compound **4** (7.6 mg) which was further purified by Si gel preparative tlc [0.5 mm, 20×20 cm (1 plate)] using $CHCl_3$-$CH_3OH$-$H_2O$ (4:3:0.5). The recovered band ($R_f$=0.7) was amorphous compound **4** (5.6 mg): colorless powder, mp 273° (dec); λ max [EtOH-$H_2O$ (4:1)] 300 nm (log ε 5.43); Ft-ir ($cm^{-1}$) 3360, 3300, 1706, 1701, 1648, 1617, 1200; fabms (glycerol) $m/z$ (%) 351 $[259+matrix]^+$ (6.9), 315 $[M+matrix]^+$ (7.2), 259 $[M+2H_2O]^-$ (32.3), 224 $[M+H]^+$ (12.6), 223 $[M]^+$ (66.4), 207 $[M-O]^+$ (41.0), 194 $[M-NO+H]^+$ (94.4).

ISOLATION OF COMPOUNDS **7**, **8**, **9**.—The crude methanolic extract (ca. 2.5 g) of *C. frondosa*, obtained as previously described (2), was chromatographed over a Si gel 60 (100 g, 230–400 mesh) column eluting with discontinuous gradient of $CHCl_3$-$CH_3OH$ (4:1→4:4) and $CHCl_3$-$CH_3OH$-$H_2O$ (4:3:1) to give 26 fractions (ca. 150–200 ml each) which were combined on the basis of tlc to provide four subfractions a–d.

Fraction c (9–13, 410 mg) was rechromatographed over a column (6 g, LiChroprep RP-18) eluting with a discontinuous gradient of $(CH_3)_2CO$-$CH_3OH$-$H_2O$ (2:2:3→2:2:4) solvent system, to give 42 fractions (1–3 ml each) that were combined on the basis of tlc to provide five subfractions. Subfraction cc (9–19, 0.320 g) was further purified on a column (6 g, LiChroprep RP-18) eluting with $(CH_3)_2CO$-$CH_3OH$-$H_2O$ (2:2:4) to give 74 fractions (1–3 ml each), which were combined on the basis of tlc to provide seven subfractions. Subfraction ccb (9–15, 127 mg, wet) was finally purified by Si gel preparative tlc (1 mm, 20×20 cm, 127 mg/2 plates) using $CHCl_3$-$CH_3OH$-$H_2O$ (4:3:0.5) as solvent system to give compound **7** (6.2 mg, $R_f$=0.6).

COMPOUND **7**.—A colorless powder, mp 238–242° (dec); $[\alpha]_D$ +6.31° ($c$=0.95, $H_2O$); λ max ($H_2O$) 290 nm (log ε 5.48); fabms (magic bullet) $m/z$ (%) 591 $[M+Na]^+$ (0.7), 590 $[M+Na-H]^-$ (1.7), 568 $[M]^+$ (2.6), 328 $[327+H]^-$ (7.5); fabms (glycerol) $m/z$ (%) 590 $[M+Na+H]^-$ (1.0), 320 $[343-Na]^-$, 262 $[241+Na-2H]^-$ (34.0), 149 $[125+Na+H]^-$ (100.0).

Fraction d (14–25, 150 mg) was rechromatographed over a column (6 g, LiChroprep RP-18) eluting with $(CH_3)_2CO$-$CH_3OH$-$H_2O$ (2:2:3) as solvent system to give 47 fractions (ca. 1–2 ml each) which were combined on the basis of tlc to provide six subfractions.

Subfraction db (9–14, 28 mg) was further purified by a column (5 g, LiChroprep RP-18) eluting with $(CH_3)_3CO$-$CH_3OH$-$H_2O$ (2:2:4) to give 39 fractions (0.5–1.5 ml each). Again, on the basis of tlc, a certain number of fractions were combined to provide two subfractions. Subfraction dba (4–10, 17.2 mg) was finally rechromatographed on Si gel preparative tlc (0.5 mm, 20×20 cm, 17.2 mg/plate) using $CHCl_3$-$CH_3OH$-$NH_4OH$ aqueous (3:3:0.5) to give three bands. From a band ($R_f$=0.7) amorphous compound **8** (12.8 mg) was recovered; colorless powder, mp 211–214° (dec); $[\alpha]_D$ −62.1° ($c$=0.95, $H_2O$), λ max ($H_2O$) 290 nm (log ε 5.48); fabms (magic bullet) $m/z$ (%) 579 $[M+H]^-$ (3.7), 559 (3.2); fabms (TEA) $m/z$ (%) 599 $[M+Na-H]^-$ (1.1), 559 $[M-H_2O]^-$ (2.3), 321 $[343-Na+H]^-$ (92.3), 149 $[125+Na+H]^-$ (100.0).

A second band ($R_f$=0.6) afforded amorphous compound **9** (4.3 mg); colorless powder, mp 237–244°; $[\alpha]_D$ −1.82° ($c$=0.1, $H_2O$); λ max ($H_2O$) 290 nm (log ε 5.42); fabms (magic bullet) $m/z$ (%) 499 $[M-125+H+Na]^+$ (16.1), 341 $[340-(225+134)-H]^+$ (16.2), 174 $[134+NaO+H]^+$ (100.0); fabms (glycerol) $m/z$ (%) 320 $[343-Na]^-$ (94.1), 262 $[241+Na-2H]^-$ (48.0), 172 $[134+NaO-H]^-$ (1.8), 149 $[125+Na+H]^-$ (100.0).

COMPOUND **2** BY OZONOLYSIS OF **1**.—Compound **1** (4 mg) in $CHCl_3$-$CH_3OH$ (3 ml, 1:1), cooled at −78° in dry ice/$Me_2CO$ was treated with ozone for four min. Then $CH_3COOH$-$H_2O$ (2 ml, 1:1) and granular zinc (50 mg) were added and the mixture stirred at room temperature for 12 h and filtered. The solvent was

evaporated *in vacuo* and the product was purified with a Si gel column (8 g, 230–400 mesh) eluting with $CHCl_3$, $CHCl_3$-$CH_3OH$ (3:1) and $CHCl_3$-$CH_3OH$-$H_2O$ (3:2:1), to give compound **2** [2.1 mg, oily; $R_f$=0.3, $CHCl_3$-$CH_3OH$-$H_2O$ (3:2:0.5)]; $^1H$ nmr [$CDCl_3$-$CD_3OD$ (1:1)] δ (ppm) 9.75 (bs, 1H) 3.6–4.2 (11H), 3.3 (9H), 2.4 (2H), 1.2–1.4 (16H); eims *m/z* (%) 407 $[M-H_2O]^+$ (7); fabms (glycerol) *m/z* (%) 408 $[M-H_2O+H]^+$ (8.7).

COMPOUND **2** BY $OsO_4/NaIO_4$ TREATMENT FROM **1**.—To compound **1** (4.3 mg) in THF (3 ml) was added a solution (3 ml) of $NaIO_4$ (100 mg, excess) and a catalytic amount of $OsO_4$. The reaction mixture was stirred at room temperature for 9 h. Then the THF was evaporated in vacuo (30–35°) and the aqueous solution extracted with $CHCl_3$ (3×10 ml each) which was dried over anhydrous $Na_2SO_4$, filtered and evaporated *in vacuo*. The $^1H$-nmr spectrum of this sample (0.9 mg) showed no significant signal due to an aldehyde proton. The aqueous layer was evaporated to dryness and the product was purified over a column (3 g, LiChroprep RP-18) eluted with $CHCl_3$-$CH_3OH$ (1:4) and $CHCl_3$ to give compound **2** (3.1 mg, oily).

COMPOUND **3**.—A solution of compound **1** (2 mg) in $CHCl_3$-$CH_3OH$ (2 ml, 1:1) was treated with dimethyldisulfide (0.35 ml) and iodine (10 mg) in $CHCl_3$ (1 ml) and stirred at 50° for 24 h. The reaction was monitored to completion by tlc. The solvent was evaporated *in vacuo* and the product dried under reduced pressure. The product **3** [(2.3 mg) exhibited: oil; $R_f$=0.85, $CHCl_3$-$CH_3OH$-$H_2O$ (3:2.5:0.5)]; fabms (glycerol) *m/z* (%) 506 $[M(601)-2CH_3SH+H]^+$ (2.8), 480 $[M-145+Na+H]^+$ (23.0), 330 $[M-C_2H_{41}S_2+Na]^+$ (16.0), 183 $[M-C_{23}H_{47}O_2S_2+H]^+$ (100.0), 146 $[C_8H_{17}S+H]^+$ (13.0).

COMPOUND **5**.—Compound **4** (3.4 mg) in HOAc (0.5 ml) and $H_2O$ (1 ml) was treated with aq. $NH_4OH$ (1 ml) and NaOH (2N, 2 ml) for 1 h and evaporated to dryness at 35–40° in vacuo. The compound was purified by Si gel preparative tlc (0.5 mm, 20×20 cm) using $CHCl_3$-$CH_3OH$-$H_2O$ (3:2:0.5) as solvent system and the band ($R_f$=0.6) provided compound **5** (1.2 mg, light brown sticky solid); λ max ($H_2O$) 280 nm (log ε 5.74); eims *m/z* (%) 211 $[M]^+$ (31.2), 183 $[M-CO]^+$ (100.0), 168 $[183-CH_3]^+$ (78.2), 154 (8.7), 141 (11.2), 127 (20.2).

COMPOUND **6**.—Caffeine (50 mg, 0.25 mmol), $NH_4OH$ (aqueous, 10 ml) and NaOH (2N, 5 ml) were stirred at 40–50° for 1.75 h. Then the reaction solution was extracted with $CHCl_3$ (3×20 ml), dried over anhydrous $Na_2SO_4$, filtered and evaporated *in vacuo*. The product was purified by Si gel prep. tlc (1 mm, 20×20 cm) to provide amorphous compound **6** [13 mg, 23%, $R_f$=0.5, $CHCl_3$-$CH_3OH$ (5:0.5)]; $^1H$-nmr data, see Table 1.

## ACKNOWLEDGMENTS

This study was supported by a grant from the Natural Sciences and Engineering Research Council of Canada and the Turkish Government through a grant via Karadeniz Technical University to N.Y. Thanks are due to Mr. Dan Drummond for assistance with mass spectra. The contribution of the Huntsman Marine Laboratory in collecting organisms is gratefully acknowledged.

## LITERATURE CITED

1. J.A. Findlay, N. Yayli, and L.A. Calhoun, *J. Nat. Prod.*, **54**, 302 (1991).
2. J.A. Findlay, N. Yayli, and L. Radics, *J. Nat. Prod.*, **55**, 93 (1992).
3. N. Fusetani, K. Yasukawa, S. Matsunaya, and K. Hashimoto, *Comp. Biochem. Physiol.*, **83B**, 511 (1986).
4. M. Iorizzi, L. Minale, and R. Riccio, *J. Nat. Prod.*, **54**, 1254 (1991).
5. G. Hirth, H. Saroka, W. Bannwarth, and R. Barner, *Helv. Chim. Acta*, **66**, 1210 (1983).
6. R. Murari, Abd. El-Rahman, M.A. Mohamed, Y. Wedmid, S. Parthawarathy, and W.J. Baumann, *J. Org. Chem.*, **47**, 2158 (1982).
7. R.M. Silverstein, G.C. Bassler, and T.C. Morrill, "Spectroscopic Identification of Organic Compounds," John Wiley and Sons, Inc., New York, 1991, p. 238.
8. R. Pappo, D.S. Allen, R.U. Lemieux, and W.S. Johnson, *J. Org. Chem.*, **21**, 478 (1956).
9. E. Dunkelblum, S.H. Tan, and P.J. Silk, *J. Chem. Ecol.*, **11**, 265 (1985).
10. D. Lichtenberg, F. Bergmann, and Z. Neiman, *J. Chem. Soc., Perkin Trans. II*, 1676 (1972).
11. F. Bergmann, M. Rahat, and D. Lichtenberg, *J. Chem. Soc., Perkin Trans. I*, 1225 (1973).
12. M. Rahat, F. Bergmann, and I. Tamir, *J. Chem. Soc., Perkin Trans. II*, 35 (1979).
13. Y. Yanuka and F. Bergmann, *Tetrahedron*, **42**, 5991 (1986).
14. J. Charalambous, R.R. Fysh, C.G. Herbert, M.H. Johri, and W.M. Shutie, *Org. Mass Spectrom.*, **15**, 221 (1980).
15. H. Biltz and J. Sauer, *Chem. Ber.*, **64B**, 752 (1931).
16. H. Biltz and H. Rakett, *Chem. Ber.*, **61B**, 1409 (1928).

17. D.J. Wood, F.E. Hruska, and K.K. Ogilvie, *Can. J. Chem.*, **52**, 3353 (1974).
18. F.-T. Liu and N.C. Yang, *Biochemistry*, **23**, 4865 (1978).
19. M.M. Kabachnik, N.G. Timofeeva, M.V. Budanov, V.K. Potapov, and Z.A. Shabarsova, *Zh. Obshch. Khim.*, **43**, 379 (1973).
20. C.A. Belfi, A.V. Arakali, C.R. Paul, and C.H. Box, *Radiat. Res.*, **106**, 17 (1986).
21. G.W. Buchko, F.E. Hruska, and K.L. Sadana, *Can. J. Chem.*, **68**, 2011 (1990).

*Received 17 June 1993*