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ISOLATION OF ISOPTEROPODINE FROM THE MARINE MOLLUSK *NERITA ALBICILLA*: ESTABLISHMENT OF THE STRUCTURE VIA TWO DIMENSIONAL NMR TECHNIQUES

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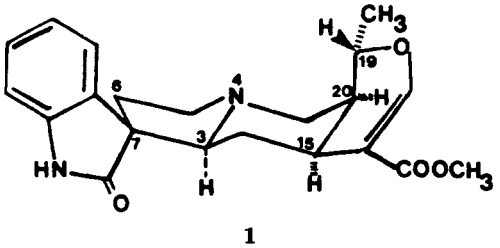
ABSTRACT.—The marine mollusk *Nerita albicilla* was found to contain an oxindole alkaloid that was identified as isoteropodine by two-dimensional nmr spectroscopic techniques.

Marine gastropods have been shown to contain an interesting variety of compounds including steroids, triterpenes, and nitrogenous bases (1). The $CHCl_3$ extract of one such mollusk, *Nerita albicilla* L. (class Gastropoda, family Neritidae), gave a number of Dragendorff (2) positive spots (tlc), which prompted us to initiate a detailed investigation of the chemical nature of the nitrogenous compounds present in this organism. In this paper, we wish to report the isolation and identification of an oxindole alkaloid identified as isopteropodine (**1**) by two-dimensional (2-D) nmr spectroscopic techniques (COSY, ^1H-^{13}C shift correlation, and heteronuclear relayed coherence transfer) and ms (3). This report of isopteropodine represents the first known occurrence of an oxindole alkaloid in a marine invertebrate.

RESULTS AND DISCUSSION

Chromatography of the $CHCl_3$ extract of the defatted residue obtained from the iPrOH extraction of the animal gave a number of fractions exhibiting a positive response to Dragendorff's reagent. The Dragendorff positive fractions were combined and subjected to hplc to give a group of fractions which, on evaporation and recrystallization from MeOH, provided colorless needles of **1**: mp 205°; $[\alpha]^{20}D = -109°$; $C_{21}H_{24}N_2O_4$ (hrms found 368.1728; calcd 368.1736). Examination of the 400 MHz ^1H-nmr spectrum [δ 8.50 (1H, s, N*H*); 7.41 (1H, s, H-17); 7.28 (1H, ddd, J=7.5, 7.6, 1.1 Hz, H-10); 7.16 (1H, dd, J=7.5, 7.6, 1.1, H-11); 7.02 (1H, dd, J=7.5, 1.1 Hz, H-9); 6.84 (1H, dd, J=7.5, 1.1 Hz, H-12)] indicated the presence of an oxindole moiety in **1** (4). This premise was further supported by the ir spectrum which showed a strong NH absorption (3260 cm^{-1}) and two carbonyl absorptions [1720 (ester) and 1630 cm^{-1} (amide)]. Further support was provided by the mass spectral fragmentation of **1** which was consistent with the proposed oxindole structure (5). The 75 MHz ^{13}C-nmr spectrum of **1** [$CDCl_3$, δ 181.6 (C2); 140.3 (C13); 127.6 (C11); 122.4 (C10); 1224.4 (C9), and 109.7 (C12)] supported the existence of the oxindole nucleus as an integral part of the structure (6).

The balance of the structure was derived from the autocorrelated proton (COSY) (7,8), 2-D proton-carbon chemical shift correlation (9-12), and 2-D heteronuclear relayed coherence transfer spectra (13-19). Because applications of these techniques,



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especially the latter, in natural products structure elucidation are still quite limited in number, the strategy employed in the assemblage of the structure of **1** is described in somewhat greater detail below.

Beyond the initial structural inferences drawn above, several other pieces of information regarding the structure of **1** could be garnered from the conventional nmr spectra of the molecule. The observation of the NH resonance and four aromatic proton resonances of the oxindole nucleus necessitated the consideration of a spiro center. This was also consistent with the observation of a quaternary carbon resonance at δ 56.96 in the 75 MHz ^{13}C-nmr spectrum (all ^{13}C resonance multiplicites were established using the APT pulse sequence) (20). Two methylene resonances at δ 54.07 and 53.48 suggested possible attachment to an aliphatic nitrogen, while the two methine resonances at δ 72.12 and 71.20 suggested attachment to an oxygen atom. Finally, the ^{13}C-nmr spectrum also contained a vinyl carbon resonating at δ 154.93 ($^{1}J_{CH}$=208 Hz) strongly suggestive of an oxygen heterocycle (21), in potential conflict with the attribution made in regard to the two methine resonances just mentioned, since three of the four oxygens in the structure must be utilized to account for the oxindole and ester moieties.

When only limited quantities of material are available, the greatest amount of structural information can be extracted from cross-correlated heteronuclear 2-D nmr experiments. Thus, a 2-D proton-carbon chemical shift correlation spectrum (12) was obtained and was used in conjunction with the data from the heteronuclear relayed coherence transfer experiment (Figure 1) (17,19). Because the latter establishes vicinal

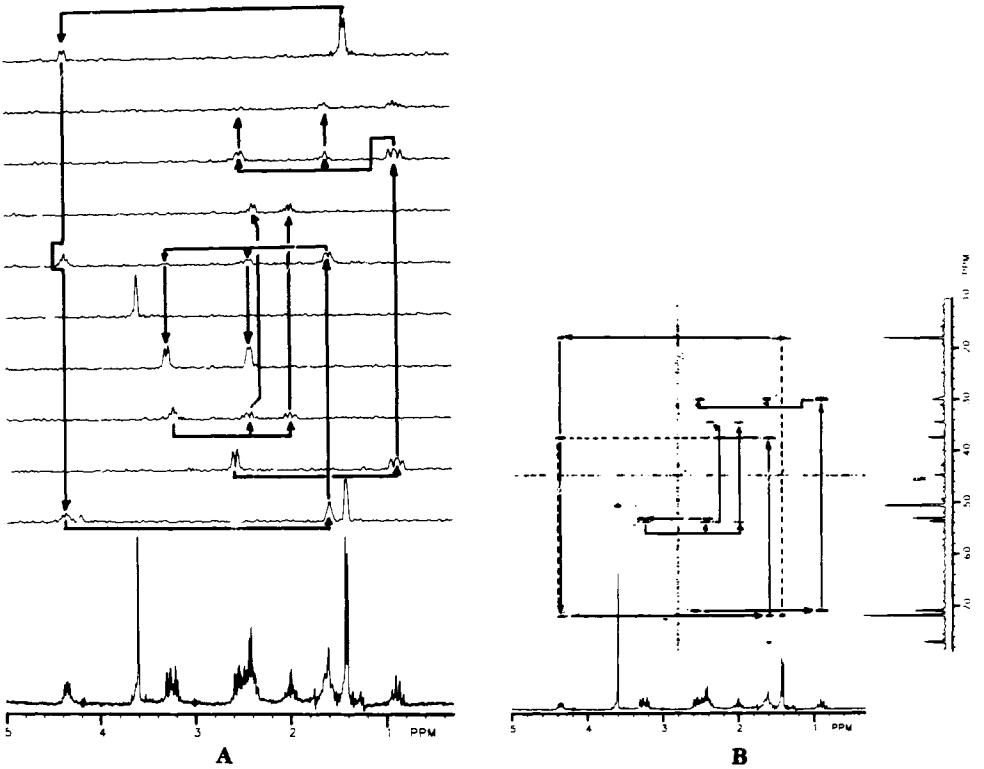
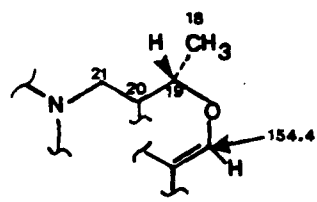


FIGURE 1. Two-dimensional heteronuclear relayed coherence transfer spectrum of isoteropodine in $CDCl_3$ at 75.459/300.068 MHz. **A**: five-level contour plot of the upfield region (F_1=5.0 to 0.25δ). Correlations are shown from resonance to resonance via the solid arrows, with "redundant back correlation" pathways shown for same responses via broken lines. **B**: Slice summary for individual carbon resonances (downfield to upfield shown bottom to top). Correlation pathways are denoted by arrows which correspond to the solid arrows in **A**.

proton connectivities, the autocorrelated proton (COSY) spectrum (8) was acquired last as a check on the consistency of the conclusions drawn from the relayed coherence transfer (RCT2D) spectrum. The proton-carbon chemical shift correlation spectrum provides information of obvious utility and therefore requires no comment on its utilization. In contrast, the RCT2D experiment is relatively new (13-19), with only limited applications having appeared in the literature (17-19), and is thus worthy of some comment.

The RCT2D experiment employs a pulse sequence that is essentially a combination of the COSY and heteronuclear chemical shift correlation experiments to which has been added a mixing interval, τ_m, which is normally set as a function of $1/20\ ^3J_{HH}$. The net result of this pulse sequence is thus to provide carbon resonance responses in the 2-D matrix at the chemical shift of the directly coupled proton(s) and at the shift of the vicinally attached proton(s).

A convenient starting point for the interpretation and utilization of the RCT2D spectrum of **1** is provided by the methyl carbon resonance (δ 18.60, C18) which is associated with a doublet in the proton spectrum (Figure 1). The H-18 methyl doublet correlates vicinally with a proton resonating at δ 4.34 (H-19) which is attached to a carbon resonating at δ 72.12 (C19). This correlation pathway is shown by the solid arrows in Figures 1A and 1B. (The broken line in Figure 1A shows the back correlation pathway which returns to the starting point.) The proton-carbon network is extended to a proton resonating at δ 1.57 (H-20) which is directly bound to the carbon resonating at δ 37.88 (C20). Finally, the trace shown in Figure 1A and 1B for the C20 resonance shows a further vicinal coupling to a proton which correlates H20 to one of the H21 resonances (H21α, δ 2.43), the directly attached carbon (C21) resonating at δ 53.48. The proton-carbon chemical shift correlation spectrum showed the two H-21 resonances to be anisochronous, the response for the H-21β resonance not visible in the contour plot (Figure 1A) but weakly visible in the slice summary (δ 3.28, Figure 1B). Consideration of the connectivity network just described in conjunction with the inferences drawn from the conventional spectra above permits structural segment **2** to be assembled. Finally, having assigned C19, the remaining methine carbon, δ 71.20, may be assigned as C3 which is consistent with the assignments made for several other spiroxindole alkaloids (22,23).



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Repeating the operation just described, beginning with the resonance at δ 71.20 (C3) (^{1}H, δ 2.54), vicinal correlation to the proton at δ 0.86 (H-14) is established, this correlating with the carbon at δ 30.45 (C14). Proton responses extend this correlation to the resonance at δ 30.05 (C15) but no further. The failure of the experiment to link the C15 and C20 resonances may be ascribed to several factors. First, the protons have nearly identical chemical shifts which would tend to make the relay response overlap the normal proton response, thus making it difficult to observe. Second, the H-15 and H-20 resonances are located *cis* to one another, and, hence, the vicinal coupling between them may be well outside of the optimal range for the mixing interval (τ_m) employed in the data acquisition. Circumvention of the former limitation is provided,

however, by 2-D proton double quantum relayed coherence transfer (24) should such a connectivity be critical in establishing the structure.

Finally, beginning from the remaining heteroatom bearing methylene resonance at δ 54.07 (C5), the vicinal relay responses correlate it with the resonance at δ 34.72 (C6), completing the ethylene bridge linking the nitrogen to the C7 spiro position (δ 56.96) of the oxindole nucleus, simultaneously completing the assemblage of the structure.

Connectivities between protons which were established by the RCT2D experiment were in every case confirmed by the acquisition of an autocorrelated ^{1}H (COSY) (8) spectrum. Importantly, RCT2D experiments provide a powerful means of assembling relatively large structural fragments with reasonably good experimental sensitivity when only modest quantities of material are available for study. The experiment has limitations: it will not span quaternary carbons, unlike the less sensitive 2-D ^{13}C-^{13}C double quantum homonuclear experiments; it may fail when the vicinally coupled resonances have nearly identical ^{1}H chemical shifts, although this problem may be circumvented by proton double quantum relayed coherence transfer (24); vicinal relay responses may be weak when the vicinal coupling constant differs significantly from that for which the experiment was optimized. Nonetheless, RCT2D does provide a powerful new tool for natural product structure elucidation.

Compound **1** was finally identified as isopteropodine, rather than pteropodine, on the basis of the above arguments and its spectral and physical data [mp (isopterpodine 209°; pteropodine 217°) rotation (isoteropodine $[\alpha]^{20}D = -111$, pteropodine $[\alpha]^{20}D = -102$) (3), ^{1}H-nmr: the H-19 in pteropodine is more deshielded than in isopteropodine (δ 4.39 and 4.31, respectively), while the converse is true for C18-CH_3 (δ 1.35 and 1.38, respectively), (30) and its derivative (picrate mp 145°)].

Finally, having identified **1** as isopteropine on the basis of the 2-D nmr and other spectral studies, we may compare the ^{13}C-nmr chemical shift assignments made in this study with those made earlier by Borges and co-workers (23). Resonance assignments made in both studies are presented in Table 1 and, with the exception of the methylene

TABLE 1. ^{13}C-nmr Chemical Shift Assignments of Isopteropodine in $CDCl_3$

Position	Previous work (23) (25 MHz, δ)	Present Work (75 MHz, δ)
C2	180.7	181.66
C3	70.9	71.20
C5	53.8	54.07
C6	30.0	34.72
C7	56.7	56.96
C8	133.2	133.74
C9	124.0	124.47
C10	121.0	122.44
C11	127.2	127.64
C12	109.3	109.70
C13	139.5	140.31
C14	34.7	30.45
C15	30.7	30.05
C16	109.5	109.83
C17	154.4	154.93
C18	18.5	18.60
C19	71.8	72.12
C20	37.8	37.88
C21	53.3	53.40
C22	166.9	167.58
C23	50.7	50.93

resonances represented by C6 and C14, can be seen to be in complete agreement. The assignments in the present work were made on the basis of the results obtained from the RCT2D nmr experiment. From this observation, it is quite probable that the C6 and C14 resonance assignments for palmirine (10-methoxyisopteropodine) should also be reversed (23).

In conclusion, the occurrence of an oxindole alkaloid in the marine mollusc *N. albicalla* has been demonstrated. This is believed to be the first report of such a system from a marine source. We suspect that **1** is of dietary origin since the other Dragendorff positive spots were due to phospholipids.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined on a Fisher-Johns apparatus and are uncorrected. Spectra were recorded on the following instruments: ir; Perkin-Elmer model 283; uv: Perkin-Elmer model 200; ^{1}H- and ^{13}C-nmr Nicolet NT-300, Bruker WP-400 and/or WM-500 spectrometers; hplc: Waters Associates model LC 1200 equipped with a model 401 differential refractometer and RCM-100 module with a silica gel cartridge (10μ). Optical Rotation $[\alpha]^{20}$D was recorded on a Perkin-Elmer model 141 Polarimeter. All of the 2-D nmr experiments reported were performed on a Nicolet NT-300 wide bore spectrometer utilizing a 5 mm dual ^{1}H/^{13}C probe at an ambient temperature of 18°. The 300 MHz high resolution spectrum plotted along with axis was obtained on a sample containing 2 mg of **1** dissolved in 0.4 ml of $CDCl_3$ degassed by entrainment with zero grade argon for 20 min. The COSY spectrum (8) was obtained with a sample containing 15 mg of **1** in 0.4 ml of $CDCl_3$ with an acquisition time of 6 h. The data was processed using a sinusoidal multiplication in both frequency domains, after which it was symmetrized (27) to afford the 512×512 real data point contour plot. Both the proton-carbon chemical shift correlation spectrum (12) and the two-dimensional heteronuclear relayed coherence transfer spectrum (17,19) were obtained using a sample containing 80 mg of **1** dissolved in 0.4 ml of $CDCl_3$. Both spectra were collected as 256×1K complex data point matrices and were processed using double exponential apodization in both frequency domains to provide the 256×512 point contour plot of the latter shown in Figure 1. The proton-carbon chemical shift correlation spectrum was acquired in 12 h while the RCT2D spectrum was collected in 18 h. Parameters for the proton-carbon chemical shift correlation spectrum were those routinely employed as described previously (27) while those for the RCT2D spectrum were as follows: $\tau_m/2=14.28$ msec; $\Delta_1/2=1.52$ msec; $\Delta_2=2.0$ msec. Proton pulses were applied using the decoupler coils and were calibrated as $\frac{1}{4}(\gamma H_2/2\pi)=90°$ pulse=32 μsec. Phase cycling was employed in all of the two-dimensional nmr experiments to provide the equivalent of quadrature detention in both frequency domains.[1]

FRACTIONATION AND ISOLATION.—*N. albicilla* (5 kg), collected from the waters of One Tree Island, Queensland, Australia (collected and identified by Dr. R.E. Schroeder; voucher specimen deposited in the Department of Medicinal Chemistry and Pharmacognosy, University of Houston), was soaked in iPrOH before shipment. At the time of processing, the iPrOH was decanted, and the animals were removed after breaking the shell. The specimens (215.9 g) were homogenized in a blender followed by centrifugation to separate the supernatant. The iPrOH extracts were combined, concentrated, and finally lyophilized to give a residue (57 g) which was subjected to flash chromatography on a silica gel 60 column (7.5×20 cm). The eluting solvents were hexane, $CHCl_3$, and increasing concentrations of MeOH (0-10%) in $CHCl_3$. The brownish residue from the $CHCl_3$ fraction (8.5 g) was chromatographed using a silica gel 60 (1.5×60 cm column), which was eluted with a linear gradient of MeOH in CH_2Cl_2 (0-3% v/v). Fractions 8-29 (20 ml each) were combined, evaporated to yield a residue (309 mg) which showed the presence of one major compound with a small impurity (tlc on silica gel 60, solvent: 2.5% MeOH in $CHCl_3$, location reagent: Dragendorff). The residue was subjected to hplc (10μ silica gel cartridge, eluting solvent 3% MeOH in $CHCl_3$) to afford 138.5 mg (0.064% of the animal excluding shell) of chromatographically pure **1**. Crystallization from MeOH gave colorless needles, mp 205-206°, $C_{21}H_{24}N_2O_4$ (hrms: found 368.17284, calcd 368.17361); uv λ max (MeOH) 242 and 292 nm; ir ν max (CCl_4): 3260 (NH), 1720 (ester C=O), 1630 (amide C=O) cm^{-1}, ^{1}H nmr (500 MHz, $CDCl_3$): δ 8.500 (1H, broad s, N*H*); 7.410 (1H, s, H-17); 7.282 (1H, ddd, J=7.5, 1.1 Hz, H-10); 7.167 (1H, ddd, J=7.5, 7.6, 1.1 Hz, H-11); 7.024 (1H, dd, J=7.5, 1.1 Hz, H-9); 6.845 (1H, dd, J=7.5, 1.1 Hz, H-12); 4.341 (1H, dq, J=12.5, 6.2 Hz, H-19); 3.602 (3H, s, H-23); 3.285 (1H, dd, J=10.7, 1.6 Hz, H-21β); 3.222 (1H, m, $\omega\frac{1}{2}$=8.6 Hz, H-5β); 2.548 (1H, dd, J=12.0, 2.7 Hz, H-3); 2.434 (4H, m, $\omega\frac{1}{2}$=39.7 Hz, H-5α, H-6β, H-

[1]Details of the phase cycling scheme utilized in the RCT2D experiment may be obtained directly from the authors.

14α, H-21α); 1.988 (1H, m, ω½=14.5 Hz, H-6α); 1.565 (2H, m, ω½=65.4 Hz, H-15α, H-20α); 1.410 (3H, d, J=6.2 Hz, H-18); and 0.861 (1H, nearly sym. q, J=12 Hz, H-14α); ^{13}C-nmr see Table 1. Hrms: 368.1728 (M^+, $C_{21}H_{24}N_2O_4$, 29%), 353 ($C_{20}H_{21}N_2O_4$, 10%), 351 ($C_{21}H_{23}N_2O_3$, 8%), 337 ($C_{20}H_{21}N_2O3$, 12%), 309 ($C_{19}H_{21}N_2O_2$, 5%), 281 ($C_{15}H_{23}NO_4$, 6%), 267 ($C_{17}H_{19}N_{20}$, 4%), 223 ($C_{12}H_{17}NO_3$, 18%), 208 ($C_{11}H_{14}NO_3$, 10%), 180 ($C_{10}H_{12}O_3$, 15%), 146 (C_9H_8NO, 8%), 130 (C_9H_8N, 22%), 103 (C_8H_7, 5%), 94 (C_5H_8N), 80 (C_5H_6N, 4.5%), 70 (C_4H_8N, 37%), and 69 (C_4H_7N, 100%).

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LITERATURE CITED

1. P.J. Scheuer (ed.), "Marine Natural Products," vol. 1, Academic Press, New York, 1978 (and subsequent volumes).
2. E. Stahl (ed.), "Thin Layer Chromatography," Springer Verlag, New York, 1969, p. 873.
3. K.C. Chan, F. Morsingh, and G.B. Yeoh, *J. Chem. Soc., C.*, 2245 (1966) (and references therein).
4. J.J. Batterhan, "NMR Spectra of Simple Heterocycles," John Wiley and Sons, New York, 1973, p. 257.
5. H. Budzikiewicz, C. Djerassi, and D.H. Williams, "Structure Elucidation of Natural Products by Mass Spectrometry," vol. 1, "Alkaloids," Holden-Day, San Francisco, 1964, pp. 150-157.
6. R.J. Abraham and P. Loftus, "Proton and Carbon-13 NMR Spectroscopy," Heyden and Son Ltd., London, 1979, p. 31.
7. W.P. Aue, E. Bartholdi, and R.R. Ernst, *J. Chem. Phys.*, **64**, 2229 (1976).
8. A. Bax, R. Freeman, and G.A. Morris, *J. Magn. Reson.*, **42**, 164 (1981).
9. A.A. Maudsley, and R.R. Ernst, *Chem. Phys. Lett.*, **50**, 368 (1977).
10. G. Bodenhausen and R. Freeman, *J. Magn. Reson.*, **28**, 471 (1977).
11. R. Freeman and G.A. Morris, *J. Chem. Soc., Chem. Commun.*, 684 (1978).
12. A. Bax and G.A. Morris, *J. Magn. Reson.*, **42**, 501 (1981).
13. P.H. Bolton, *J. Magn. Reson.*, **48**, 336 (1982).
14. P.H. Bolton and G. Bodenhausen, *Chem. Phys. Lett.*, **89**, 139 (1982).
15. G. Eich, G. Bodenhausen, and R.R. Ernst, *J. Am. Chem. Soc.*, **104**, 3731 (1982).
16. A. Bax, *J. Magn. Reson.*, **53**, 149 (1983).
17. H. Kessler, M. Bernd, H. Kogler, J. Zarbock, O.W. Sørensen, G. Bodenhausen, and R.R. Ernst, *J. Am. Chem. Soc.*, **105**, 6944 (1983).
18. P. Bigler, W. Ammann, and R. Richarz, *Org. Magn. Reson.*, **22**, 109 (1984).
19. M.J. Musmar, G.E. Martin, M.L. Tedjamulia, H. Kudo, R.N. Castle, and M. L. Lee, *J. Heterocyclic Chem.*, **21**, 929 (1984).
20. S.L. Patt and J.N. Shoolery, *J. Magn. Reson.*, **46**, 535 (1984).
21. G.E. Martin, R. Sanduja, and M. Alam, *J. Org. Chem.*, **50**, 2383 (1985).
22. M. Shamma and D.M. Hindenlang, "Carbon-13 NMR Shift Assignments of Amines and Alkaloids," Plenum Press, New York, 1979, p. 221.
23. J. Borges, M.T. Manresa, J.L.M. Ramon, C. Pascual, and A. Rumbero, *Tetrahedron Lett.*, 3197 (1979).
24. P.H. Bolton, *J. Magn. Reson.*, **54**, 333 (1983).
25. M. Shamma, R.J. Shine, I. Kompis, T. Sticzay, F. Morsing, J. Poisson, and J.L. Ponsser, *J. Am. Chem. Soc.*, **87**, 1739 (1967).
26. R. Bauman, G. Wider, R.R. Ernst, and K. Wuthrich, *J. Magn. Reson.*, **44**, 402 (1981).
27. R.T. Gampe, Jr., M. Alam, A.J. Weinheimer, G.E. Martin, J.A. Matson, M.R. Willcott, III, R.R. Inners, and R.E. Hurd, *J. Am. Chem. Soc.*, **106**, 1823 (1984).

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