$$\text{PBN} + N_3\cdot \xrightarrow{k_3} \text{adduct} \quad r_3 = k_3[\text{PBN}][N_3\cdot] \qquad (3)$$

$$N_3\cdot + O_2^- \xrightarrow{k_4} N_3^- + {}^3O_2 \quad r_4 = k_4[N_3\cdot][O_2^-] \qquad (4)$$

Variation of N_3^- Concentration. In this experiment, the concentration of PBN was fixed at 1×10^{-3} M with the sensitizer MB at 1×10^{-4} M. The ESR intensity of the azide adduct was then determined for N_3^- concentrations of 1×10^{-4} and 4×10^{-3} M. Since $k_1 \equiv 10k_2$, $r_1 = r_2$ when the concentration of N_3^- equals 1×10^{-4}. In this case, 50% of the $^1O_2$ molecules generated will be quenched by N_3^-. However, when the $[N_3^-]$ is increased by 40 times, essentially all of the $^1O_2$ molecules will be quenched and the amount of radicals observed should double. In fact, an increase of 2.5 was observed.

Variation of PBN Concentration. In this case, the $[N_3^-]$ was fixed at 1×10^{-3} M with [MB] = 1×10^{-4} M and [PBN] was 1×10^{-3} vs 1×10^{-2} M. For 1×10^{-2} M, $r_1 = r_2$, and 50% of the $^1O_2$ molecules would react with N_3^-. However, at 1×10^{-3} M PBN, $r_1 = 10r_2$ and ~90% of the $^1O_2$ molecules would be quenched by N_3^-. The ESR predicted outcome depends on the lifetime of the $N_3\cdot$ radical and the rate constants k_3 and k_4. If essentially all the $N_3\cdot$ radicals are trapped, then one would expect that the ESR intensity would be halved by the order of magnitude increase in [PBN]. If, however, $N_3\cdot$ is relatively short lived, r_3 will be increased due to the increase in [PBN], and an increase of ~5 in ESR intensity would be predicted. The experimentally observed change was an increase in signal intensity of 2. This suggests that the $N_3\cdot$ radical is relatively short lived as well as demonstrating that the observed change is within the predicted limits.

Variation of $^1O_2$ Lifetime. In this experiment, [PBN] = 1×10^{-3}, $[N_3^-] = 1 \times 10^{-3}$, and [MB] = 1×10^{-4} M. The lifetime of the $^1O_2$ was varied by using D_2O vs. H_2O. This increases the lifetime of $^1O_2$ from 2 to 20 μs,[14] provided of course there is no other species present which quenches $^1O_2$. However, this is not the case since both N_3^- and PBN are present and reactive to $^1O_2$, thereby preventing $^1O_2$ from achieving its natural lifetime. With H_2O as the solvent, $r_1 = 10r_2$ and ~90% of the $^1O_2$ produced will react according to eq 1. Replacing H_2O by D_2O will not change this ratio; yet, the number of available $^1O_2$ molecules will increase because of the longer inherent lifetime. Previous work has shown[4] that at a quencher concentration of 10^{-3} M, a 2.3 increase in rate is observed (this value approaches 10 as the [quencher] is reduced). Therefore, a 2.3 increase in radical concentration is predicted while experimentally we observed a 3-fold increase.

These three experiments are all consistent with the predicted change in ESR intensity of the $N_3\cdot$ radical adduct if in fact it arises during the quenching of $^1O_2$ by N_3^-. Additionally, these results are quantitively consistent within reasonable limits of errors and are in agreement with a charge-transfer mechanism of physical quenching in which the charge-transfer complex dissociates into free $N_3\cdot$ and O_2^- radicals.

$$^1O_2 + N_3^- \rightarrow \{O_2^- \cdots N_3\cdot\} \rightarrow O_2^- + N_3\cdot \qquad (5)$$

The PBN then traps the $N_3\cdot$ radical to produce the ESR spectrum observable. It is feasible that the spin trap interacts with the complex in such a way as to cause dissociation. The O_2^- adduct is not observed in this system which is not unexpected due to difficulties of trapping O_2^- in aqueous media.[15] In addition, O_2^- disproportionates at these pH's to O_2 and H_2O_2 with a significant rate.[16] In the case where PBN is not present, this N_3^- quenching mechanism with dissociation may still occur but then reacts by eq 4 to be consistent with physical quenching. Finally, these results suggest that at least 15% of the charge-transfer complexes formed dissociate into free ions prior to recombination. The fact that PBN may not react with all the $N_3\cdot$ radicals formed (eq 3) makes this a minimum value. Spin trapping thus gives the first spectroscopic evidence for participation of N_3 radicals in the quenching of $^1O_2$ by N_3^- in H_2O.[17]

Registry No. $N_3\cdot$, 12596-60-0; O_2, 7782-44-7; N_3^-, 14343-69-2; PBN spin adduct, 58200-47-8; DMPO spin adduct, 80387-88-8; 4-PyOBN spin adduct, 80387-89-9; PBN, 3376-24-7.

(14) Kearns, D. R. *Org. Chem.* (*N.Y.*) **1979**, *40*, 115–137.
(15) Finkelstein, E.; Rosen, G. M.; Rauckman, E. J.; Paxton, *J. Mol. Pharmacol.* **1979**, *16*, 676–687.
(16) Bielski, B. H. *Photochem. Photobiol.* **1978**, *28*, 645–649.
(17) It is interesting to note that azido radicals are produced during the formation of azidohydroperoxides in the sensitized photooxygenation of olefins quenched by N_3^-. Foote, C. S.; Fujimoto, T. T.; Chang, Y. C. *Tetrahedron Lett.* **1975**, 45–47.

Structure of the Cyclic Peptide Dolastatin 3 from *Dolabella auricularia*[1]

George R. Pettit,* Yoshiaki Kamano, Peter Brown,[2] Devens Gust, Masuo Inoue, and Cherry L. Herald

Cancer Research Institute and Department of Chemistry Arizona State University, Tempe, Arizona 85287
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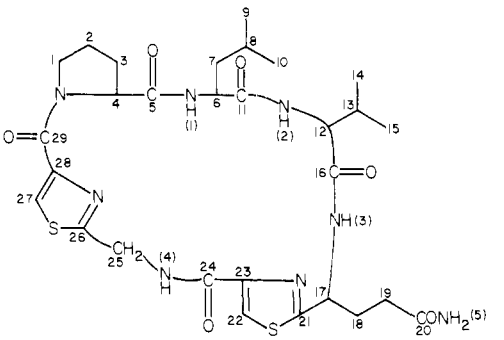
The Indian Ocean sea hare *Dolabella auricularia*[3] has been found to be an exceptionally productive source of new anticancer biosynthetic products.[4,5] Recently, we recorded preliminary observations concerning our discovery of nine antineoplastic and/or cytotoxic substances in *D. auricularia*.[4a] Dolastatins 1–9 were obtained in about 1-mg amounts each from 100 kg of the wet sea hare. Because of the 1-mg quantities, and lack of crystallinity, structural elucidation of the dolastatins has presented an ample challenge.

We are now pleased to report assignment of unique cyclic peptide[6] structure **1** to the powerful cell growth inhibitory (murine P388 lymphocytic leukemia cell line $ED_{50} < 1 \times 10^{-4} - 1 \times 10^{-7}$ μg/mL)[7] dolastatin 3: colorless amorphous solid from methylene



1, *cyclo*[Pro-Leu-Val-(gln)Thz-(gly)Thz], dolastatin 3

(1) Part 83 of "Antineoplastic Agents". For part 82, refer to: Pettit, G. R.; Cragg, G. M.; Gust, D.; Brown, P.; Schmidt, J. M. *Can. J. Chem.*, submitted for publication.
(2) Dedicated to the memory of our friend and colleague Professor Peter Brown who expired on March 25, 1981.
(3) This species (Mollusca phylum, Aplysiomorpha order, Aplysiidae family) designation also includes, e.g., *D. andersoni*, *D. californica*, *D. ecaudata*, and *D. scapula*. See ref 4a, footnote 6.
(4) (a) Pettit, G. R.; Kamano, Y.; Fujii, Y.; Herald, C. L; Inoue, M.; Brown, P.; Gust, D.; Kitahara, K.; Schmidt, J. M.; Doubek, D. L.; Michel, C. *J. Nat. Prod.* **1981**, *44*, 482. (b) Pettit, G. R.; Ode, R. H.; Herald, C. L.; Von Dreele, R. B.; Michel, C. *J. Am. Chem. Soc.* **1976**, *98*, 4677–4678.
(5) Some of the *D. auricularia* constituents such as dolatriol[4b] and related dolastanes may originate from dietary utilization of certain brown algae (particularly from the semitropical *Dictyota* genus, Dictyotaceae family). Evidence for such a chain of biological events has been obtained by Fenical and colleagues: Sun, H. H.; McConnell, O. J.; Fenical, W.; Hirotsu, K.; Clardy, J. *Tetrahedron* **1981**, *37*, 1237–1242. See also: Amico, V.; Oriente, G.; Piattelli, M.; Tringali, C.; Fattorusso, E.; Magno, S.; Mayol, L. *Ibid.*, **1980**, *36*, 1409–1414.
(6) Five other interesting cyclic peptides have recently been isolated from marine tunicates: (a) Ireland, C.; Scheuer, P. J. *J. Am. Chem. Soc.* **1980**, *102*, 5688–5691. (b) Rinehart, K. L., Jr.; Gloer, J. B.; Cook, J. C., Jr.; Mizsak, S. A.; Scahill, T. A. *Ibid.* **1981**, *103*, 1857–1859. (c) Rinehart, K. L., Jr.; Gloer, J. B.; Hughes, R. G., Jr.; Renis, H. E.; McGovern, J. P.; Swynenberg, E. B.; Stringfellow, D. A.; Kuentzel, S. L.; Li, L. H. *Science* (*Washington D.C.*) **1981**, *212*, 933–935.

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Table I. Structurally Important HREI Fragmentation Ions Derived from Dolastatin 3 (1)

weight		
obsd	calcd	interpretation
660.2767	660.2512	M
632.2554	632.2563	M − CO
562.1992	562.1906	Leu-Pro-(gly)Thz-(gln)Thz + H or Leu-Val-(gln)Thz-(gly)Thz − H
548.1740	548.1749	Pro-(gly)Thz-(gln)Thz-Val + H
450.1170	450.1144	Val-(gln)Thz-(gly)Thz
449.1173	449.1065	Val-(gln)Thz-(gly)Thz − H
422.1202	422.1194	Val-(gln)Thz-(gly)Thz − CO
351.0789	351.0459	(gly)Thz-(gln)Thz
322.0230	322.0432	(gly)Thz-(gln)Thz − H − CO
310.1306	310.1099	Val-(gln)Thz
281.1875	281.2103	Pro-Leu-Val − CO
213.1621	213.1603	Val-Leu + H
210.1351	210.1368	Pro-Leu
185.1675	185.1654	Val-Leu + H − CO
184.1557	184.1575	Val-Leu − CO
183.1516	183.1497	Pro-Leu + H − CO

chloride–methanol (or ethyl acetate–ethanol); mp 133–137 °C; TLC R_f 0.27 (96:4 ethyl acetate–ethanol); $[\alpha]^{26}_D$ −35.5° (c = 0.09, CH_3OH); $C_{29}H_{40}N_8O_6S_2$ (by high-resolution EI mass spectroscopy, see Table I);[8a] $\lambda_{max}^{CH_3OH}$ 206 (ε 13 940, thiazole K band) and 238 (ε 8960, thiazole B band) nm; IR (KBr) 3427, 3379, 3330 (NH), 3090 (NH), 3020 (NH), 1670 (–CONH–), 1629 (–CONH–), 1544 (–CONH–), 1501, 1494, 1445 (thiazole), 1390, 1370, 1310, 1240 (thiazole), 1065 (thiazole), 823, 760, and 620 cm^{-1}.

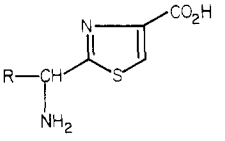
Solution of the dolastatin 3 structural problem with approximately 1 mg required a carefully selected series of microchemical and spectroscopic experiments. The chromatographic behavior, physical appearance, infrared, and high-resolution (400-MHz) ^1H and ^{13}C NMR spectra (Table II) suggested the possibility of a peptide-like substance. Results of amino acid analyses[8b] (25–100 μg quantities of dolastatin 3, 6.1 N HCl, 105 °C, 24 h) indicated 1:1:1 molar ratios of proline, valine, and leucine plus two unknown amino acid components. That five amino acids primarily constituted dolastatin 3 was established by results of separate acid hydrolyses (6.1 N HCl, as above) followed by methylation (diazomethane, 1:1 ethyl ether-methanol), acetylation (acetic anhydride–pyridine), and gas chromatographic–mass[8c] spectral analyses of the products. The N-Ac-Leu-OMe, N-Ac-Pro-OMe, and N-Ac-Val-OMe produced by these reactions gave GC-mass spectral fragmentation sequences that were identical with authentic specimens. By GC–MS techniques two unknown N-acetylated amino acid methyl esters (**3** and **4**, Scheme I) were readily exposed, but no other hydrolysis product was detected. The same results were obtained by subjecting dolastatin 3 to reaction (30 min) with hydrogen chloride in refluxing methanol followed by acetylation of the products.

When it became necessary to sacrifice nearly 200 μg of dolastatin 3 for element analyses, the presence of sulfur was already suspected from study of the ultraviolet, infrared,[9] ^1H and ^{13}C NMR[8d] spectra. Especially helpful were the two sharp ^1H NMR signals at δ 8.070 and 8.082 attributed to thiazole ring (cf. also ref 6a) protons. Two thiazole amino acid units were further revealed by subtracting ^1H and ^{13}C NMR signals due to Leu, Pro, and Val. Detection and estimation of sulfur content by elemental analysis, interpretation of the two new N-acetyl methyl esters (**3**, **4**) mass spectral fragmentation pathways (Scheme I), and analysis (Table II)[10] of the dolastatin 3 400-MHz ^1H - and ^{13}C NMR data

Table II. Dolastatin 3 ^1H and ^{13}C NMR Assignments Relative to Tetramethylsilane in Deuteriochloroform Solution

structure 1 assignment no.	chemical shift, ppm ^{13}C	chemical shift, ppm ^1H	^1H multiplicity (J, Hz)
1	48.3	3.69	1 H m
		3.85	1 H m
2	25.5	1.9-2.3	2 H m
3	29.7	1.9-2.3	2 H m
4	62.6	3.975	1 H dd (J = 7.9)
5	169.5		
6	48.6	3.85	1 H m
7	41.0	2.14	1 H m
8	25.5	1.53	1 H m
9	23.3	0.957	3 H d (J = 6.59)
10	21.2	0.905	3 H d (J = 6.59)
11	171.9		
12	55.7	4.758	1 H dd (J = 7.4, 9.2)
13	31.8	2.06	1 H m
14	18.6	1.048	3 H d (J = 6.59)
15	19.6	1.161	3 H d (J = 6.84)
16	171.1		
17	55.0	5.542	1 H ddd (J = 9.0, 10.6, 4.2)
18	29.7	2.54 2.54	2 H m
19	33.3	2.30 2.30	2 H m
20	165.8		
21	149.1		
22	124.4	8.082	1 H
23	161.0		
24	174.8		
25	37.7	5.249	1 H dd (J = 7.3, 18.1)
		4.661	1 H dd (J = 2.2, 18.3)
26	148.3		
27	123.8	8.070	1 H
28	160.2		
29	171.2		
(1)		5.992	1 H d (J = 6.8)
(2)		8.318	1 H d (J = 9.3)
(3)		7.855	1 H d (J = 9.0)
(4)		8.755	1 H dd (J = 5.4, 1-2)
(5)		6.305	1 H br s
		5.422	1 H br s

confirmed the presence of two thiazole amino acids, (gly)Thz (**2a**) and (gln)Thz (**2b**).[11]

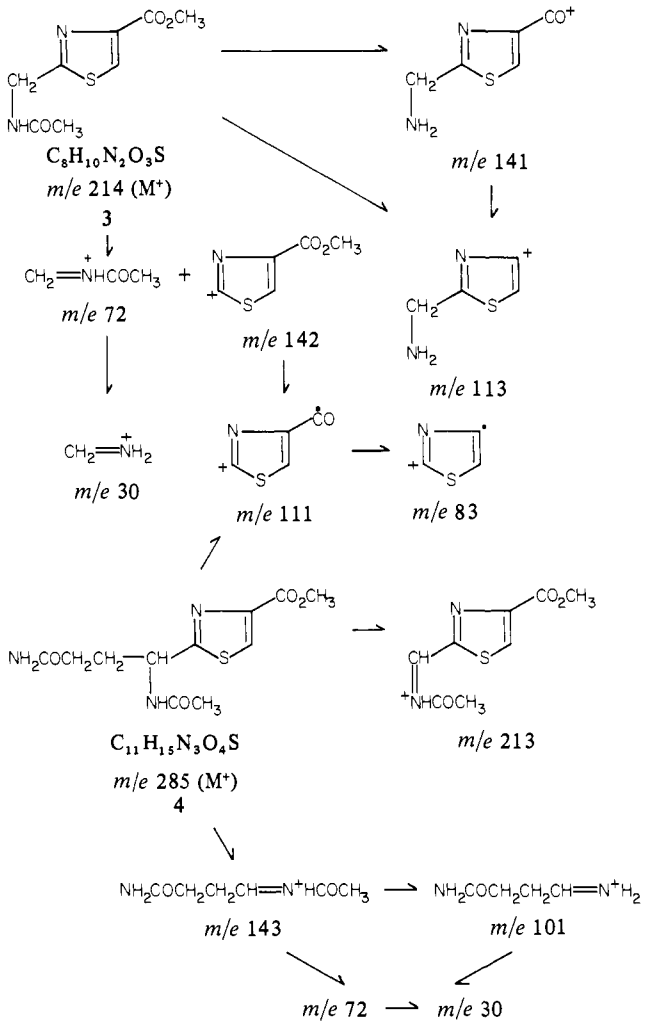


2a, R = H
b, R = $CH_2CH_2CONH_2$

After the amino acid composition of dolastatin 3 was established, sequencing experiments were undertaken. The complete lack of reaction between dolastatin 3 and acetic anhydride suggested a cyclic arrangement. While attempts at enzymatic or selective acid-catalyzed cleavage to obtain fragments for mass

(7) Schmidt, J. M.; Pettit, G. R. *Experientia* **1978**, *34*, 659–660.
(8) Instruments employed included (a) MAT 731 mass spectrometer (b) Beckman Model 121, (c) MAT 312 mass spectrometer, and (d) Bruker WH-90 and WH-400 NMR spectrometers.
(9) Thorn, G. D. *Can. J. Chem.* **1960**, *38*, 2349–2358.
(10) For related NMR assignments refer to ref 6a. Also see: Hindenlang, D. M.; Shamma, M.; Miana, G. A.; Shah, A. H.; Cassels, B. K. *Liebigs Ann. Chem.* **1980** 447–450. Pook, K.-H.; Nassal, M.; Birr, C.; Wieland, T. *Int. J. Pept. Protein Res.* **1980**, *15*, 32–40.
(11) The two thiazole amino acids appear to have been formed by biosynthetic condensation: glycine + cysteine → **2a**; glutamine + cysteine → **2b**. Eventually, the complete series of common protein amino acids each condensed with cysteine will probably be found in a natural peptide and/or synthesized. Therefore, a simplified peptide nomenclature for describing such naturally occurring amino acids seemed necessary. We propose describing the product as a 4-carboxythiazole (Thz) with an amine-containing side chain at position 2 derived from a natural amino acid. By this method, e.g., (trp)Thz would be the product of Trp + Cys and (tyr)Thz the result of Tyr + Cys. Additional evidence for the necessity of such a new system will be apparent from reviewing: Pettit, G. R. "Synthetic Peptides"; Elsevier: Amsterdam, The Netherlands, 1980. To date, (ala)Thz and (leu)Thz have been found in ulicyclamide and ulithiacyclamide, respectively.[6a] Bacitracin A from *Bacillus licheniformis* contains a 4-carboxythiazoline derived from Ile. Such dihydrothiazoles can be readily simplified to, e.g., (ile)Thzl.

Scheme I. EI fragmentation of N-Ac-(gly)Thz-OMe and N-Ac-(gln)Thz-OMe



spectral analysis were unsuccessful, it was found possible to proceed in a quite workable manner by using the intact molecule. Detailed analysis of the dolastatin 3 electron-impact high-resolution mass spectrum led to the observations and assignments summarized in Table I. All of the evidence obtained from dolastatin 3 by chemical and spectroscopic techniques as well as biosynthetic considerations led to *cyclo*[Pro-Leu-Val-(gln)Thz-(gly)Thz] as the structure (**1**)[12] of this new P388 cell growth inhibitor.

Isolation[13] of the potentially important dolastatins in larger quantities combined with investigations directed at structural determinations and biological evaluations (U.S. National Cancer Institute) are currently in progress. Now it appears very likely that some of the dolastatins (such as **1**) will become readily available by total syntheses.

Acknowledgment. For very necessary financial assistance we are pleased to acknowledge support provided by Contract N01-CM-97297 with the Division of Cancer Treatment, NCI, National Institutes of Health, DHW, Grants CA16049-01-CA16049-07 awarded by the National Cancer Institute, DHW, Mary Dell Pritzlaff, the Olin Foundation (Spencer T. and Ann W.), the Fannie E. Rippel Foundation and the National Science Foundation Regional Facility at the University of South Carolina (CH78-18723). For other assistance we are also pleased to thank Drs. M. I. Suffness, J. M. Schmidt, J. A. Hasler, P. Lohavanijaya, J. A. McCloskey, D. Smith, J. R. Cronin, A. M. Yates, R. R. Inners, and J. Witschel, Jr.

Registry No. 1, 80387-90-2; **2a**, 25438-22-6; **2b**, 80387-91-3; **3**, 80387-92-4; **4**, 80387-93-5.

(12) Presumably, each amino acid unit bears the usual L configuration. But the resistance of dolastatin 3 to enzymatic cleavage suggests that the absolute configuration of each amino acid needs to be definitely established by chiral chromatographic or other methods. Eventually this point and the reverse order of bonding possibility (less likely by biosynthetic precedent) *Cyclo*[Val-Leu-Pro-(gly)Thz-(gln)Thz] can be resolved by the increased availability of dolastatin 3 for degradation and/or by total synthesis. The latter approach is quite feasible and a synthesis of (gly)Thz (**2a**) has already been described: Cross, D. F. W.; Kenner, G. W.; Sheppard, R. C.; Stehr, C. E. *J. Chem. Soc.* **1963**, 2143-2150.

(13) The sensitivity exhibited by some of the dolastatins suggests it is the presence of dehydroamino acid and/or thiazoline units that complicate purification procedures.

^{13}C NMR Spectra of Carbonium Ions in the Solid State: The 2-Norbornyl Cation

Costantino S. Yannoni* and Volker Macho†

IBM Research Laboratory
San Jose, California 95193

Philip C. Myhre*

Department of Chemistry, Harvey Mudd College
Claremont, California 91711

Received September 21, 1981

Studies of the structure and dynamics of the norbornyl cation are legion, but the interpretation of collected data remains a subject of controversy.[1] We report here studies of the norbornyl cation in the solid state over the 77–200 K range using cross-polarization magic-angle spinning (CPMAS) ^{13}C NMR spectroscopy.[2] The results will not resolve contentious issues, but they do provide important additional information that serves to bound speculation. Further, these results illustrate the potential power of MAS ^{13}C NMR spectroscopy to probe dynamic and structural characteristics of reactive intermediates. Results include (1) failure to find evidence for a "classical" norbornyl cation at 77 K, (2) the first solid-state NMR line-shape analysis of a chemical exchange process, the 6,1,2-hydride shift of the norbornyl cation.

exo-Norbornyl-^{13}C chloride, enriched at C-1, C-2, and C-6, was prepared by the route and in yields shown in Scheme I.[3] The 2-norbornyl-^{13}C acetate formed by buffered acetolysis (70 °C) of 2-(4-cyclopentenyl)ethyl-*1*-^{13}C *p*-nitrobenzenesulfonate (0.05 M) gave the ^{13}C distribution pattern shown in Scheme I.[4] Treatment of the acetate with Lucas reagent at 25 °C gave norbornyl-^{13}C chloride with additional scrambling of label at the indicated three carbons, but there was no detectable migration of label into other positions.[5]

An intimate solid mixture of the labeled norbornyl chloride and SbF_5 was prepared by vapor-phase codeposition.[6] Initial spectra of the solid codeposit at 190 K indicated only partial conversion to the cation. After 4 days at dry ice temperature, conversion was complete, and the label appeared to be statistically scrambled.

^{13}C spectra of the solid sample of the norbornyl cation at various temperatures were obtained with the use of cross-polarization,

† Institut fur Atom-und Festkoerperphysik, Koenigin-Luise Str. 34a, D1000 Berlin 33, West Germany.

(1) (a) Olah, G. A. *Acc. Chem. Res.* **1976**, *9*, 41. (b) Brown, H. C.; von R. Schleyer, P. "The Non-Classical Ion Problem"; Plenum Press: New York, 1977.

(2) Schaefer, J.; Stejskal, E. O. Top. Carbon-13 NMR Spectrosc. **1979**, *3*, 281.

(3) The method of synthesis follows from work reported by (a) Lawton, R. G. *J. Am. Chem. Soc.* **1961**, *83*, 2300. (b) Hooz, J.; Gilianni, S. S. H *Can. J. Chem.* **1968**, *46*, 86. (c) Schmid, G. H.; Wolkoff, A. W. *J. Org. Chem.* **1967**, *32*, 239. (d) Murdock, K. C.; Angier, R. B. *Ibid.* **1962**, *27*, 2359.

(4) This result may be compared with related ^{14}C studies of: Lee, C. C.; Hahn, B. J. *J. Am. Chem. Soc.* **1969**, *91*, 6420.

(5) ^{13}C distributions were obtained from integrations of liquid-state spectra.

(6) Myhre, P. C.; Yannoni, C. S. *J. Am. Chem. Soc.* **1981**, *103*, 230.

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