pyrolysis of **12** for 46 h at 350 °C gives a 50% yield of 2,4-dimethylisopropylbenzene, which most likely arises via dehydration and dehydrogenation of the ene adduct **13**.[19]

The cyclization of **16** was investigated as a model study for the synthesis of steroids by annealation of the D ring. Treatment of **16** with 2 equiv of $MeAlCl_2$ for 24 h at 0 °C gives a 50% yield of *trans*-hydrindanone **18**[20] via the intermediate **17**. Baldwin and Lusch have reported the cyclization of ketones, but only at 100–140 °C in the presence of $AlCl_3$.[9c]

The reactions of **19** and **20** were explored to determine the effect of double-bond stereochemistry on the stereochemistry of the ene adduct, and if the less nucleophilic 1,2-disubstituted double bond could be used as the ene. The *Z* isomer **19**[21] gives exclusively the cis-substituted adduct **21** with 1 equiv of Me_2AlCl for 2 h at 0 °C, while the *E* isomer gives mainly the trans-substituted isomers **22** and **23** (see Scheme III). Due to the less nucleophilic double bond of **19** and **20**, reaction is much slower than with **10** and methyl addition to the aldehyde competes, giving 15–20% of 7-decen-2-ol. The exclusive formation of **21** from **19** is due to geometrical constraints on the transition state.[6] The ene reaction thus offers a promising route to 2-alkenylcyclohexanols with control of stereochemistry.

The above examples indicate that alkylaluminum halides are Lewis acids with many unique properties which make them attractive reagents for organic synthesis.

Acknowledgment. We thank the Research Corp., the National Institutes of Health, and the Mobil Foundation for financial support. The synthesis of **18** was carried out by Ketih McDaniel.

Supplementary Material Available: Physical data for all products (4 pages). Ordering information is given on any current masthead.

(19) Rouessac, F.; Le Perchec, P.; Conia, J.-M. *Bull. Soc. Chim. Fr.* **1967**, 818.

(20) Johnson, W. S. *J. Am. Chem. Soc.* **1944**, *66*, 215. Lansbury, P. T.; Briggs, P. C.; Demmin, T. R.; Du Bois, G. E. *Ibid.* **1971**, *93*, 1311. Zeeh, B.; Jones, G.; Djerassi, C. *Chem. Ber.* **1967**, *100*, 3204. Three minor products in a total of 25% yield are also formed in the cyclization of **16**.

(21) We thank Bedoukian Research Inc. for a generous gift of **19**.

(22) Fellow of the Alfred P. Sloan Foundation, 1979–1981.

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Myricoside, an African Armyworm Antifeedant: Separation by Droplet Countercurrent Chromatography

Sir:

Our continuing search for insect antifeedant compounds from natural sources[1] has led us to examine the active constituents from the roots of *Clerodendrum myricoides* (Verbenaceae). This shrub was collected in East Africa, mainly on the basis of information provided by "Bwana Mganga", the local medicine man.[2] Extracts from the roots revealed potent insect antifeedant activity when tested against the African armyworm *Spodoptera exempta*, using the leaf disk bioassay with *Zea* mays.[3] Separation of the active material was monitored by this antifeedant bioassay,[1a,c] using a combination of polyamide chromatography and droplet countercurrent chromatography (DCCC).[4] This latter technique proved to be extremely efficient for the separation of the desired bioactive compound; all other semipreparative-scale methods failed or led to decomposition of material. We now report the structure of the active component, myricoside, as 3,4-dihydroxy-β-phenethyl-*O*-β-D-apiofuranosyl-(1→3)-α-L-rhamnopyranosyl-(1→3)-4-*O*-caffeoyl-β-D-glucopyranoside (**1**) (Figure 1). It is a potent antifeedant against *S. exempta*, the 10-ppm activity level being comparable to that of ajugarin.[5,6]

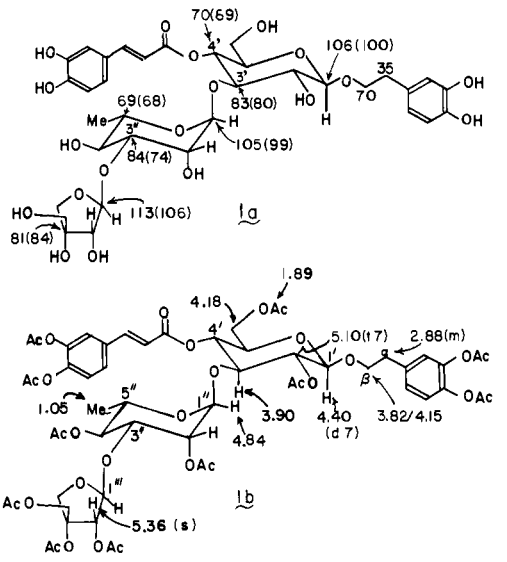

Figure 1. (a) Myricoside. Pertinent ^{13}C NMR peaks are shown; values in parentheses are chemical shifts in the peracetate. (b) Myricoside peracetate. Pertinent ^1H NMR peaks are shown.

Addition of ether to the aqueous methanolic extract of the roots (500 g) gave a precipitate (1 g) which was eluted from polyamide (Woelm) with H_2O. Further fractionation was carried out by DCCC. The active fraction (125 mg) was partitioned between a $CHCl_3$–MeOH–H_2O (7:13:8) equilibrated solvent mixture, with passage of the upper aqueous phase as mobile ascending droplets (flow rate 5 mL/h) through the stationary organic phase.[4c] Base-line separation into four fractions (I–IV) was achieved after 12 h, using a total of only 60 mL of solvent (collected in 1-mL aliquots, 254-nm detection). The bioactive fraction II was finally passed through polyamide (H_2O) to yield 10 mg of myricoside (**1**): mp 165–167 °C (aqueous MeOH); IR (Nujol) 3400 (br, OH), 1705 (conjugated ester), 1600 cm^{-1} (aromatic); UV (MeOH) 216 (ε 19 900), 246 sh (ε 11 000), 288 (ε 13 700), 300 (ε 14 400), 330 nm (ε 20 600; shifts to 375 nm (ε 21 200) on addition of base). These data suggested that **1** contained caffeate and catechol moieties as chromophores in a 1:1 ratio; this was corroborated by actual simulation experiments with a 1:1 mixture of methyl caffeate and catechol carried out in the pH range 2–9.

^1H and ^{13}C NMR showed **1** to have aromatic and sugar moieties. Acid hydrolysis of myricoside (1 mg) in refluxing aqueous 2 N HCl/MeOH (1:1) yielded D-apiose, L-rhamnose, and D-glucose[7] as well as caffeic acid. Mild hydrolysis of **1** in refluxing aqueous 0.1 N HCl for 20 min gave apiose as the only detectable sugar, suggesting this sugar to be the terminal unit. The following

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(2) Taniguchi, M.; Chapya, A.; Kubo, I.; Nakanishi, K. *Chem. Pharm. Bull.* **1978**, *26*, 2910.

(3) The *S. exempta* bioassays were carried out at the International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya.

(4) (a) Tanimura, T.; Pisano, J. J.; Ito, Y.; Bowman, R. L. *Science* **1971**, *169*, 54. (b) Hostettmann, K.; Hostettmann-Kaldas, M.; Nakanishi, K. *Helv. Chim. Acta* **1978**, *61*, 1990. (c) Hostettmann, K.; Hostettmann, M.; Nakanishi, K. *J. Chromatogr.* **1979**, *170*, 355. (d) The apparatus is handled by Tokyo Rikakikai Co. Ltd., Nishikawa Bldg., Toyama-cho, Kanda, Tokyo.

(5) Kubo, I.; Lee, W.-W.; Balogh-Nair, V.; Nakanishi, K.; Chapya, A. *J. Chem. Soc., Chem. Commun.* **1976**, 949.

(6) In tests carried out at Columbia University, myricoside is not active against the Southern armyworm *S. eridania* or the Mexican bean beetle *Epilachna varivestis*.

(7) The sugars were detected in the free form by TLC (silica gel, 4:1:5 *n*-BuOH-AcOH-H_2O), and as their dansylhydrazone derivatives: Avigad, G. *J. Chromatogr.* **1977**, *139*, 343.

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Table I. ^{1}H NMR Peaks (ppm) of the Sugar Moiety of Myricoside Peracetate (1b)[a]

H	glucose	rhamnose	apiose
1	4.40 (d, 7)	4.84 (br s)	5.0[c]
2	5.10 (t, 7)	5.0[c]	5.36 (s)
3	3.90 (t, 7)[b]	3.90 (t, 8)[b]	
4	5.0[c]	4.90 (t, 8)	4.65 (AB q, 10)
5	3.65 (m)	3.90 (m)[b]	3.77 (m)[d]
6	4.18 (CH_2, d, 6.5)	1.05 (CH_3, d, 6.5)	

[a] In $CDCl_3$; 220 MHz. Splitting patterns and J values (Hz) are given in parentheses. [b] Overlapping signals. J values are estimated from adjacent protons. [c] Mutually overlapping. [d] J values of the "AB q" could not be measured due to overlap with other protons, including the aglycone side-chain proton.

microscale derivatization studies showed that **1** contained four aromatic and seven aliphatic hydroxyl groups. Methylation of **1** ($NaH/Me_2SO/CH_3I$)[8] gave myricoside permethylate (CIMS, m/e 911 (M + 1)), whereas reaction with $(CH_3)_2SO_4$ followed by acetylation showed the presence of four aromatic methoxyl and seven aliphatic acetoxyl groups (^{1}H NMR). Acetylation of **1** with Ac_2O/Pyr overnight led to a *fully* acetylated derivative, mp 85–86 °C, the ^{1}H NMR spectrum[9] of which revealed the presence of eleven distinct acyl signals (four aromatic, δ 2.3–2.4, and seven aliphatic, δ 1.8–2.1).

The following FD-MS data established the sequence to be apiose (terminal)–rhamnose–glucose–aglycone: myricoside (**1**), m/e 779 ($M^+ + {}^{23}Na$, 20%), 647 (799 − api, 100%), 501 (779 − api − rha, 20%), 484[10] (777 − api − caf, 16%), 335 (647 + ^{23}Na; double cation, 10%); m/e 1241 ($M^+ + {}^{23}Na$, 100%), 259 (terminal api, 4%).

The ^{13}C NMR peaks for **1** and **1**-peracetate were assigned on the basis of chemical shift considerations, off-resonance decoupled data, and comparison with data of methyl caffeate, glucose, rhamnose, and apiose.[11] Pertinent ^{13}C NMR peaks are shown in structure **1a**. The interglycosidic linkages were established in the following manner: (a) The 2′-, 2″-, and 2‴-oxygen functions are free OH's since all three anomeric carbons are shifted upfield in the peracetate (see **1a**). This is in agreement with known upfield shifts accompanying acetylation at C-2 carbons due to a β effect from neighboring acyl groups.[12] (b) The rhamnose and caffeate moieties are attached to the glucose C-3′ and C-4′, respectively. Namely, the C-3′ signal undergoes a −3-ppm shift (83 to 80 ppm) upon acetylation as a result of the β effect from the adjacent 2′-acetyl group. In addition, the C-3′ peak is located at 5-ppm higher field than the corresponding C-3′ peak (88 ppm) of *O*-β-D-glucopyranosyl-(1→3)-*O*-β-D-glucopyranoside;[13] this again is due to the β effect from the neighboring caffeate group. (c) Apiose is atached to C-3″ since in the peracetate the C-3″ peak undergoes a −10-ppm shift (84 to 74 ppm) corresponding to acetylation on both adjacent hydroxyls (2″ and 4″).

The ^{1}H NMR spectrum of **1**-peracetate, despite the great overlap (20 protons within a 1.8-ppm spread), was in full agreement with structure **1b** (Table I). The α and β protons of the aglycone (see **1b**) constituted a complex pattern; thus the α protons appeared as a complex multiplet at ca. 2.88 ppm, whereas

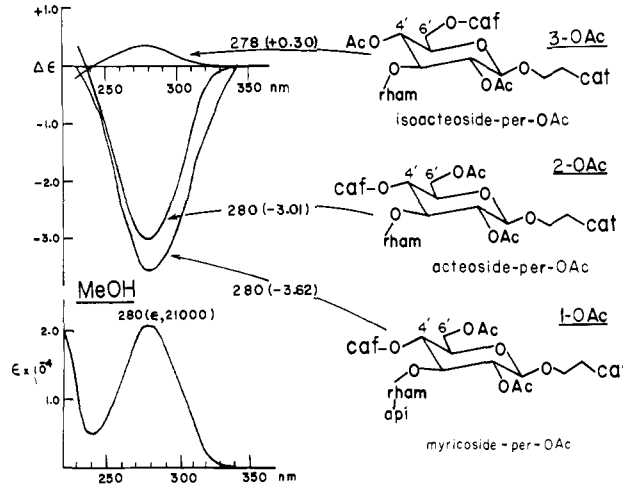

Figure 2. UV and CD (in MeOH) of peracetates of myricoside (**1**), acteoside (**2**), and acteoside isomer **3**.

the two β protons absorbed at 3.82 and 4.15 ppm as multiplets. The nonequivalence of the β-H's is presumably due to both the influence of the 1′-chiral center and the exoanomeric effect.[14,15] Since the 360-MHz ^{1}H NMR spectrum of the minor fraction III (1.5 mg) from the DCCC showed this to still be a mixture, it was separated as their peracetates, 1 and 0.5 mg, by high-performance LC, Whatman Partisil, CH_2Cl_2–EtOAc–MeOH (92:8:0.07).[16] The ^{1}H NMR and FD-MS data indicated that the two acetates were the peracetates of the known compounds acteoside (**2**) and its isomer **3**,[17] respectively (see Figure 2 for structures).[18] Compounds **2** and **3** differ only in the attachment of the caffeate group; in **2** it is attached to 4′ as in **1**, whereas in **3** it is attached to 6′. In agreement with this, the 5″-Me signal of the rhamnose moieties in **1**- and **2**-peracetates both have their ^{1}H NMR peaks at 1.05 ppm (see **1b**), while in **3**-acetate it is at 1.16 ppm. The UV spectra of all three peracetates are virtually superimposable (Figure 2). However, the intensities of the CD Cotton effect are ca. 10 times stronger for **1**- and **2**-peracetates in comparison to **3**-peracetate (Figure 2). This is in agreement with the fact that in **1** and **2** the caffeate group is attached directly to the 4′-chiral center; in **3**, however, it is one carbon removed from the 5′-chiral center.

The phenolic glycosides **1** and **2** have also been isolated from the related species *Clerodendrum johnstonii*.[19] We believe myricoside to be the first reported phenolic glycoside exhibiting insect antifeedant activity.

Acknowledgment. We are grateful to Mr. H. Naoki, Suntory Institute for Bioorganic Research, for FD-MS measurements of myricoside (**1**) and to J. Termini for experimental assistance. The studies carried out by P.H.S. were partly supported by the Pe-

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(9) Recorded at 200 MHz on a Varian XL-200 instrument.
(10) The FD MS of saponins usually give odd-numbered fragments from the odd-numbered $M^+ + Na$ peaks due to cleavage of the sugar moiety and a hydrogen migration: Schulten, H. R.; Komori, T.; Nohara, T.; Higuchi, R.; Kawasaki, T. *Phytochemistry* **1978**, *34*, 1003. It is conceivable that they arise from hydrolytic elimination of sugar moieties in the condensed phase and subsequent cationization. The genesis of the m/e 484 peak could be a direct fragmentation of the ester group in the m/e 647 fragment.
(11) Ishii, H.; Tori, K.; Tozyo, T.; Yoshimura, Y. *Chem. Lett.* **1978**, 719.
(12) Eliel, E. L.; Bailey, W. F.; Kopp, L. D.; Willer, R. L.; Grant, D. M.; Bertrand, R.; Christensen, K. A.; Dalling, R. K.; Duch, M. W.; Wenkert, E.; Schell, M.; Cochran, D. W. *J. Am. Chem. Soc.* **1975**, *97*, 322. Wehrli, F. W.; Wirthlin, T. "Interpretation of Carbon-13 NMR Spectra"; Heyden: London, 1978; p 37.
(13) Tori, K.; Seo, S.; Yoshimura, Y.; Arita, H.; Tomita, Y. *Tetrahedron Lett.* **1977**, 179.
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(15) The complex patterns of the α and β protons were ascertained in the model compound 3,4-dimethoxy-β-phenethyl-*O*-β-D-(2,3,4,6-*O*-tetraacetyl)-glucopyranoside, mp 95–97 °C, which was prepared from bromo-*O*-2-D-(2,3,4,6-*O*-tetracetyl)glucopyranoside and 3,4-dimethoxyphenethyl alcohol by a standard Koenigs–Knorr-type reaction: Reynolds, D. D.; Evans, L. *J. Am. Chem. Soc.* **1938**, *60*, 2559.
(16) Addition of this trace amount (0.07%) of the MeOH was essential for base-line separation, 1 mL/min flow rate. The peracetates of **2** and **3** were eluted after 23 and 27 min, respectively.
(17) (a) Birkofer, L.; Kaiser, C.; Thomas, V. Z. Z. *Naturforsch.* **1968**, 1051. (b) Nonaka, G.; Nishioka, I. *Phytochemistry* **1977**, *16*, 1265.
(18) Extensive ^{1}H NMR studies were not carried out on the known acteoside **2** and isoacteoside **3** because of the limited amount. They were both isolated and characterized in the final stage of the studies and therefore were not used as references. However, the ^{1}H NMR spectrum of **2**-peracetate was found to be very similar to that of **1**-peracetate (except for apiose) and hence corroborated its structure.
(19) Cozart, D., unpublished data.

troleum Research Fund, administered by the American Chemical Society. The work was supported by NIH Grant AI-10187.

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Received August 11, 1980

Use of Kinetic Isotope Effects in Mechanism Studies. 3. Measurement of Hydrogen Isotope Effects on the Primary Chlorine Isotope Effect during Elimination Reactions[1]

Sir:

We have recently reported[2] that the excellent experimental data for the temperature dependence of the primary kinetic isotope effects measured for dehydrobromination of $C_6H_5C^lH(CH_3)CH_2Br$ (I-Br)[3,4] can be computer simulated by assuming that reaction proceeds via an internal-return mechanism, Scheme I. Since this elimination reaction is considered to be an example of a concerted E2 mechanism,[5] we initiated studies designed to help distinguish between the two possible interpretations of such isotope data. An elimination reaction offers additional methods which can be employed to help determine if reaction proceeds by a one-step or a two-step pathway, namely, leaving group isotope effects[6] and element effects.[7] We have questioned the validity of using the element effect when studying dehydrohalogenation reactions, and therefore wish to report a novel approach which measures the hydrogen isotope effect on a primary chlorine isotope effect to aid in assigning mechanisms for these eliminations.

The Arrhenius behavior and primary kinetic hydrogen isotope effects observed for ethoxide-promoted dehydrochlorination of $C_6H_5C^lH(CH_3)CH_2Cl$ (I-Cl) are similar to those reported for I-Br.[8] Both an E2 mechanism and the E1cb pathway with internal return should give measurable $^{35}Cl/^{37}Cl$ leaving group isotope effects;[9] however, measuring k^{35}/k^{37} for both the protio and deuterio compounds should in principle allow for experimental differentiation between the two mechanisms. Experimental results are summarized in Table I.

If the elimination of I-Cl went by an E1cb mechanism with a moderate amount of internal return, there should be a dramatic difference in the observed k^{35}/k^{37} for I-Cl-*h* vs. I-Cl-*d*. When the same internal-return parameters needed to model the Arrhenius behavior of I-Br were used, we calculated an expected k^{35}/k^{37} of 1.00370 for I-Cl-*h*.[10] In this model, the forward step, k_2, is favored by a factor of 2.5 over the return step, k_{-1}, and it is not surprising to expect a measurable k^{35}/k^{37}. However, when reaction occurs with I-Cl-*d*, k_2 is now favored by a factor of 25, and a rather large drop in the chlorine isotope effect is expected, $k^{35}/k^{37} = 1.00050$. These two values are readily distinguished from each other since measurements of $^{35}Cl/^{37}Cl$ are accurate enough to calculate k^{35}/k^{37} to better than 50 parts per 10^5.[9] Intuitively, we felt that k^{35}/k^{37} should be about the same for I-Cl-*h* and I-Cl-*d* if reaction proceeded by an E2 mechanism. This expectation was substantiated by model calculations of isotope effects for I-Cl-*h* and I-Cl-*d* by using methods described elsewhere.[11] The results from these calculations will be reported in the full paper.

Scheme I

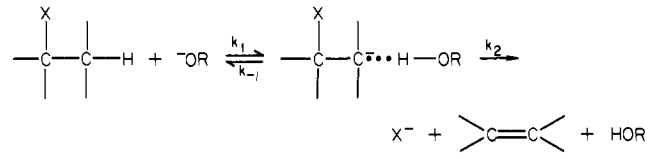

Table I. Chlorine Isotope Effects for Sodium Alkoxide Promoted Dehydrochlorination Reactions in Alcohol

compound[a]	solvent	k^{35}/k^{37} [b]	temp, °C
I-Cl-*h*	EtOH	1.00590 ± 0.00013	75
I-Cl-*d*	EtOH	1.00507 ± 0.00036	75
V	EtOH	1.00580 ± 0.00034	75
IV-*h*	EtOH	1.00908 ± 0.00008	24
IV-*d*	EtOH	1.00734 ± 0.00012	24
IV-*h*	MeOH	1.00978 ± 0.00020	21
IV-*d*	MeOH	1.00776 ± 0.00020	21
III-*h*	EtOH	1.01229 ± 0.00047	0
III-*d*	EtOH	1.01003 ± 0.00024	0
III-*h*	MeOH	1.01255 ± 0.00048	20
III-*d*	MeOH	1.01025 ± 0.00043	20

[a] I-Cl = $C_6H_5C^lH(CH_3)CH_2Cl$, III = $C_6H_5C^lHClCF_2Cl$, IV = $C_6H_5C^lHClCH_2Cl$, V = $C_6H_5CH_2CH_2Cl$. [b] k^{35}/k^{37} values were calculated as described by Hill, J. W.; Fry, A. *J. Am. Chem. Soc.* 1962, *84*, 2763.

The observed $k^{35}/k^{37} = 1.00590$ at 75 °C for the ethoxide-promoted elimination of I-Cl-*h* coupled with a $k^{35}/k^{37} = 1.00507$ for I-Cl-*d* provides experimental evidence that strongly favors an E2 pathway for this dehydrochlorination reaction. The rather large element effect, I-Cl:I-Br = 1:64 at 50 °C, coupled with substantial isotope effects, k^H/k^D greater than 6 at 50 °C, should be convincing evidence that there is cleavage of both C–H and C–X bonds in what would be an E2 transition state;[5a] however, we have measured k^H/k^D values of 2–4 for the alkoxide-promoted dehydrohalogenations of $ArCHXCF_2X$ which appear not to proceed by a simple E2 mechanism and yet give k^{Br}/k^{Cl} ratios of 25–50.[12] This has led us to doubt the validity of using element effects to assign mechanisms to elimination reactions.

Kaldor and Saunders[13] cite our temperature-independent isotope effects for the ArC^lHXCF_2X systems as the only cases where observed Arrhenius behavior of an elimination reaction differs markedly from the E2 pattern as exemplified by the temperature dependence of k^H/k^D for I–Br and their own results. Using an internal-return mechanism as a model, it is possible to predict

(1) (a) Other papers in this series are ref 12 (part 1) and ref 2 (part 2). (b) Presented in part at the IUPAC 5th International Symposium on Physical Organic Chemistry, Santa Cruz, CA, Aug 17–22, 1980.
(2) Koch, H. F.; Dahlberg, D. B. *J. Am. Chem. Soc.* **1980**, *102*, 6102.
(3) (a) Shiner, V. J., Jr.; Smith, M. C. *J. Am. Chem. Soc.* **1961**, *83*, 593. (b) Shiner, V. J., Jr.; Martin, B. *Pure Appl. Chem.* **1964**, *8*, 371.
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(5) (a) Bordwell, F. G. *Acc. Chem. Res.* **1972**, *5*, 377. (b) Saunders, W. H., Jr. *Ibid.* **1976**, *9*, 21.
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(7) Bunnett, J. F.; Garbisch, E. W.; Pruitt, K. M. *J. Am. Chem. Soc.* **1957**, *79*, 385.
(8) S. K. Sweinberg, unpublished results.
(9) Grout, A.; McLennan, D. J.; Spackman, I. H. *J. Chem. Soc., Perkin Trans. 2* **1977**, 1758.
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