Complete regio- and stereospecificity in the Lewis acid catalyzed Diels-Alder reactions of (Z)-2-methoxy-1-(phenylthio)-1,3-butadienes. Conversion of the CS configuration of an add...

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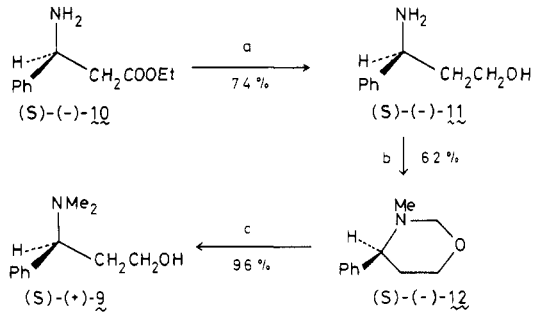
Scheme III



[a] $LiAlH_4$-Et_2O, 0 °C. [b] CH_2=O-HCOOH. [c] $LiAlH_4$-$AlCl_3$-Et_2O, 0 °C.

indicating that both compounds possess the same relative stereochemistry[10] with respect to the C-3 and C-4 substituents, and hence they are diastereomeric due to S=O chirality. The reaction of *C*-phenyl-*N*-methylnitrone (**3**) with the sulfoxide (+)-**1** in refluxing benzene for 15–20 h also yielded 4-(*p*-tolylsulfinyl)isoxazolidines **6a** and **6b**[11] in 36% and 4% yields, respectively,[12] which were again diastereomeric for S=O.

For determination of the degree of the chiral induction in the above cycloadditions, the mixture of **4a,b** was subjected to reductive N–O bond cleavage followed by the reductive removal of *p*-tolylsulfinyl group to afford in 47% overall yield 3-anilino-3-phenyl-1-propanol (**7**): mp 81 °C;[13] $[\alpha]^{25}_D$ + 41° (*c* 0.25, $CHCl_3$). The amino alcohol **7** was then N-methylated with CH_2=O and $NaBH_3CN$ to give in 89% yield 3-(*N*-methylanilino)-3-phenyl-1-propanol (**8**): bp 165–175 °C (0.3 torr); $[\alpha]^{23}_D$ +209° (*c* 0.43, $CHCl_3$). The enantiomeric excess of **8** was determined as more than 90% by using the chiral NMR shift reagent Eu(hfc)$_3$.

A mixture of **6a,b** was converted to (–)-3-(dimethylamino)-3-phenyl-1-propanol [**9**: bp 110–120 °C (13 torr); $[\alpha]^{25}_D$ –40° (*c* 0.40, $CHCl_3$)] by N-methylation, reductive N-O cleavage, and desulfurization as sequence of reactions shown in Scheme II. The optical purity of **9** was determined as no less than 80% by use of Eu(hfc)$_3$. The absolute configuration of (–)-**9** was determined as *R* by the comparison of the optical rotation with (*S*)-(+)-**9** which was obtained from (–)-β-phenyl-β-alanine ethyl ester[14] by the reactions shown in Scheme III.[15] Thus the absolute configuration at C-3 in **6a** and probably in **4a** was assigned as the *S* configuration.

Although the present study is limited to the reactions with only two acyclic nitrones,[17] the remarkably high chiral induction observed is sufficient to suggest the potential use of **1** as the chiral inducing agent in cycloadditions. Mechanistic studies concerning the steric course of the 1,3-dipolar cycloaddition and the application of **1** to other cycloadditions are now in progress in this laboratory.

Registry No. **1**, 54828-68-1; **2**, 1137-96-8; **3**, 3376-23-6; **4** (isomer 1), 82769-67-3; **4** (isomer 2), 82769-68-4; **5**, 82769-69-5; **6** (isomer 1), 82769-70-8; **6** (isomer 2), 82769-71-9; **7**, 82769-72-0; **8**, 82769-73-1; (*R*)-(–)-**9**, 82769-74-2; (*S*)-(+)-**9**, 82769-75-3; (*S*)-(–)-**10**, 3082-69-7; (*S*)-(–)-**11**, 82769-76-4; (*S*)-(–)-**12**, 82769-77-5; 3-anilino-2-[(4-methylphenyl)sulfinyl]-3-phenyl-1-propanol, 82769-78-6; 3-(dimethylamino)-2-[(4-methylphenyl)sulfinyl]-3-phenyl-1-propanol, 82769-79-7; 3-(dimethylamino)-2-[(4-methylphenyl)thio]-3-phenyl-1-propanol, 82769-80-0.

(9) MCPBA oxidation of **5** gave a mixture of **4a,b**.

(10) Although NMR data for both **4** and **6** ($J_{3,4}$ = 3–6 Hz) suggest the trans configuration, the chemical confirmation should be the subject of further investigation. Base-catalyzed isomerization experiments (e.g., *n*-BuLi, LDA) failed due to the decomposition of the isoxazolidines.

(11) **6a**: mp 100–101 °C (*n*-hexane): TLC R_f 0.25(AcOEt-n-hexane 1:1); $[\alpha]^{25}_D$ +227° (*c* 0.21, $CHCl_3$); ^1H NMR ($CDCl_3$, 200 MHz) δ 2.36 (3 H, s), 2.66 (3 H, s), 3.70 (1 H, m), 4.0 (1 H, d, *J* = 6 Hz), 4.25 (2 H, m), 7.0–7.7 (9 H). **6b**: mp 112–113 °C (*n*-hexane): TLC R_f 0.33 (AcOEt–*n*-hexane, 1:1); $[\alpha]^{23}_D$ +47.7° (*c* 1.43, $CHCl_3$); ^1H NMR ($CDCl_3$, 200 MHz) δ 2.38 (3 H, s), 2.62 (3 H, s), 3.60 (m, 2 H), 4.10 (1 H, m), 4.5 (1 H, dd, *J* = 9, 3 Hz), 7.2–7.6 (9 H).

(12) 2-Methyl-3-phenyl-4-isoxazoline was isolated as a very minor product.

(13) M. E. Speeter and W. H. Maroney, *J. Am. Chem. Soc.*, **76**, 5810 (1954).

(14) (a) E. Graf and H. Boeddeker, *Justus Liebigs Ann. Chem.*, **613**, 111 (1958). (b) M. Pais, R. Sarfati, and F.-X. Jarreau, *Bull. Soc. Chim. Fr.*, **1973**, 331. (c) Hydrolysis of (–)-β-phenyl-β-alanine ethyl ester afforded (*S*)-(–)-β-phenyl-β-alanine: mp 232 °C; $[\alpha]^{25}_D$ –9.6° (*c* 0.82, H_2O).

(15) (–)-β-Phenyl-β-alanine ethyl ester (**10**): bp 140–150 °C (16 torr) [lit.[14a] bp 148–149 °C (13 torr)]; $[\alpha]^{22}_D$ –2.4° (*c* 0.13, EtOH) (lit.[14a] –3.6°). (–)-3-Amino-3-phenyl-1-propanol (**11**): bp 90–100 °C (0.13 torr); $[\alpha]^{23}_D$ –2.8° (*c* 0.57, EtOH). (–)-3-Methyl-4-phenyltetrahydro-1,3-oxazine (**12**): bp 110–120 °C (14 torr); $[\alpha]^{24}_D$ –49° (*c* 0.11, $CHCl_3$). (+)-3-(Dimethylamino)-3-phenyl-1-propanol (**9**): bp 115–125 °C (14 torr); $[\alpha]^{24}_D$ + 24.2° (*c* 0.07, $CHCl_3$).

(16) It should be noted that **4a** and **6a** have the *S* notation because of the change in priority of the substituents on the chiral carbon atom.

(17) As preliminary experiments, the reaction of several *N*-methyl-*C*-arylnitrones have been performed, and a comparable order of diastereoselectivity was observed. The reactions of **1** with some cyclic nitrones are now underway. All combined results will be reported elsewhere in the near future.

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Received May 13, 1982

Complete Regio- and Stereospecificity in the Lewis Acid Catalyzed Diels–Alder Reactions of (*Z*)-2-Methoxy-1-(phenylthio)-1,3-butadienes. Conversion of the CS Configuration of an Adduct to the CC Configuration at the Allylic Position by a [2,3] Sigmatropic Rearrangement[1]

Summary: Three dienes of the indicated type react with methyl vinyl ketone (MVK) and with 2-cyclohexen-1-one under catalysis by magnesium bromide or ethylaluminum dichloride to provide good yields of only the endo adducts in which the regiochemistry is completely controlled by the sulfur atom; the acetyl group of one of the adducts with MVK has been converted to an isopropenyl group and the product treated with diethylzinc/methylene iodide to give, by a [2,3] sigmatropic rearrangement, a material in which a quarternary carbon atom has been generated in a predictable configuration at the allylic position with respect to the phenylthio group.

Sir: Since its introduction in 1974,[2a] Danishefsky's diene, the readily prepared *trans*-1-methoxy-3-[(trimethylsilyl)oxy]-1,3-butadiene, has proved to be particularly useful in synthesis.[2b] Other dienes bearing two heteroatom substituents[3] which have recently been added to the repertoire of the synthetic chemist include the (*E,E*)-1-oxygenated-4-(phenylthio)- and the 2-oxygenated-3-(phenylthio)-1,3-butadienes of Trost[4] and the (*Z*)-2-methoxy-

(1) Taken in part from the Ph.D. Thesis of Z.K., University of Pittsburgh, 1981.

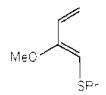
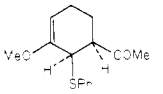
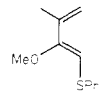
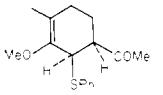
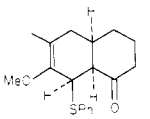
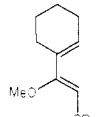
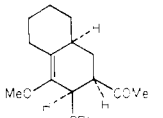
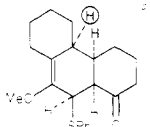
(2) (a) Danishefsky, S.; Kitahara, T. *J. Am. Chem. Soc.* **1974**, *96*, 7807. (b) Danishefsky, S. *Acc. Chem. Res.* **1981**, *14*, 400.

(3) Review of Diels–Alder reactions of heterosubstituted 1,3-dienes: Petrzilka, M.; Grayson, J. I. *Synthesis* **1981**, 753.

(4) Trost, B. M.; Ippen, J.; Vladuchick, W. C. *J. Am. Chem. Soc.* **1977**, *99*, 8116. Trost, B. M.; Vladuchick, W. C.; Bridges, A. J. *Ibid.* **1980**, *102*, 3548, 3554.

0022-3263/82/1947-4005$01.25/0

Table I. Lewis Acid Catalyzed Diels-Alder Reactions

entry	diene	dienophile	catalyst	conditions[a]	product	yield,[b] %
1	 1		$MgBr_2$	25 °C, 6.5 h	 4	85
2	 2		$MgBr_2$	25 °C, 30 min	 5	90
3	2		$EtAlCl_2$	25 °C, 10 min	 6	91
4	 3		$EtAlCl_2$	−78 °C, 4.5 h	 7	80
5	3		$EtAlCl_2$	0 °C, 6 h	 8	85

[a] The diene was added to an equimolar CH_2Cl_2 solution of the acid and dienophile which had been stirred for at least 20 min. The reaction was terminated when TLC indicated complete consumption of the diene. [b] Of purified adduct. [c] The orientation of the circled proton is assumed (see text).

1-(phenylthio)-1,3-butadienes **1–3**,[5,6] the use of which in Diels–Alder reactions is the subject of this paper. A considerable virtue of the latter dienes for practical synthetic use is that both acyclic dienes such as **1** and **2** and the cyclic variety such as **3** are readily available in high yields by completely stereospecific two-step syntheses.[5,6] We now disclose the additional extremely attractive features that these dienes undergo facile Lewis acid catalyzed Diels–Alder reactions[7] with enones, that these catalyzed reactions provide completely regiospecifically the adducts in which the enol ether function, a readily unmasked ketone equivalent, is oriented meta to the carbonyl group of the dienophile, the opposite orientation to that observed in the absence of the phenylthio group,[3] and that in all cases studied, the reaction catalyzed by 1 molar equiv of Lewis acid proceeds with complete stereospecificity, providing the product of endo addition. We also demonstrate one of many conceivable uses of a valuable feature of the adducts, namely, the presence of an allylic phenylthio group of defined stereochemistry; the carbon–sulfur configuration of this center can be converted to a corresponding carbon–carbon configuration at the allylically related position via a [2,3]sigmatropic rearrangement.[11]

The results of the Lewis acid catalyzed reactions are shown in Table I.[12,13] The structures and configurations of the adducts were determined mainly by 300-MHz NMR spectroscopy with appropriate decouplings. The key features of **5** were similar to those already reported for **4**.[5a] In the case of **6**, a small quantity of the exo adduct, epimeric at the carbon atom bearing the phenylthio group, was also available as the minor product from the noncatalyzed reaction (12 h, 110 °C). The proton on the sulfur-bearing carbon atom of **6** appears as a doublet (J = 4.2 Hz) at δ 3.75, significantly upfield of the broad singlet (half-width = 4.2 Hz) at δ 4.27 for the corresponding proton of the epimeric exo adduct, a difference which is general[1] for endo (δ 3.75–4.10) and exo (δ 4.10–4.27) adducts and

(5) (a) Cohen, T.; Mura, A. J., Jr.; Schull, D. W.; Fogel, E. R.; Ruffner, R. J.; Falck, J. R. *J. Org. Chem.* **1976**, *41*, 3218. (b) Cohen, T.; Ruffner, R. J.; Shull, D. W.; Fogel, E. R.; Falck, J. R. *Org. Synth.* **1980**, *59*, 202. (c) Cohen, T.; Kosarych, Z. *Tetrahedron Lett.* **1980**, *21*, 3955.

(6) Further improvements have been made in the preparation of the parent diene, (*Z*)-2-methoxy-1-(phenylthio)-1,3-butadiene (**1**).[1] The bis(phenylthio)methane, which is now commercially available (Alfa, Parish, Watree), is very conveniently prepared in nearly quantitative yield by passing dry HCl into a solution of 0.50 mol of dimethoxymethane in 1.50 mol of thiophenol at 0 °C for 20 min and then heating the solution at reflux for 12 h; the methanol phase is separated, and the remaining liquid is washed with aqueous base, diluted with ether, dried ($MgSO_4$), and evaporated to provide a light yellow oil which crystallizes upon standing. The diene is best purified by washing its solution with an ammonium chloride buffer solution (pH ~8) rather than filtering through silica gel; with this modification, the elimination step proceeds routinely in 85% yield.

(7) Except in the case of reaction with aldehydes,[8] *trans*-1-methoxy-3-[(trimethylsilyl)oxy]-1,3-butadiene has not been successfully coupled with dienophiles under acid catalysis.[9] However, mild catalysis may have been manifested by zinc chloride in the reaction of the 2-methyl analogue with a quinone.[10]

(8) Danishefsky, S.; Kerwin, J. F., Jr.; Kobayashi, S. *J. Am. Chem. Soc.* **1982**, *104*, 358.

(9) Harayama, T.; Cho, H.; Inubushi, T. *Chem. Pharm. Bull.* **1978**, *26*, 1201.

(10) Kozikowski, A. P.; Sugiyama, K.; Springer, J. P. *J. Org. Chem.* **1981**, *46*, 2426.

(11) Evans was the first to demonstrate that Diels–Alder reactions with dienes possessing a sulfur substituent at the 1-position could be advantageously followed by such a rearrangement, in that case to introduce an oxygen atom at the allylic position in a stereospecific manner. Evans, D. A.; Bryan, C. A.; Sims, C. L. *J. Am. Chem. Soc.* **1972**, *94*, 2891.

(12) All products were characterized by ^1H NMR, IR, and mass spectroscopy. Elemental compositions were determined for new compounds by exact mass determination. The complete spectral data are available in ref 1.

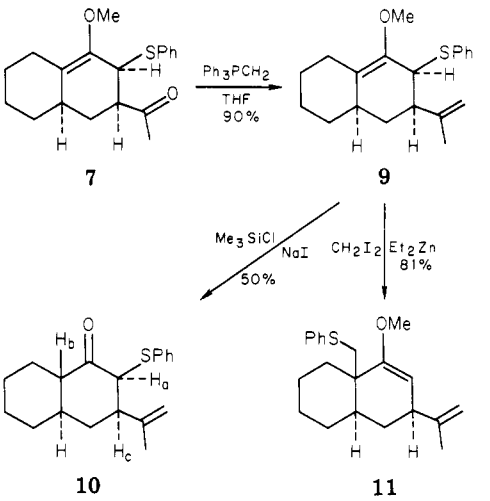
(13) The dienes used in this study contained a trace of radical inhibitor with which they had been stored.[5b]

is attributed to deshielding of the proton by the cis acyl group in the exo adduct. By irradiation of this proton with observation of the signal for the proton on the adjacent carbon atom bearing the acyl group, the latter proton in neither isomer was found to be involved in diaxial coupling with the other adjacent bridgehead proton. Thus the ring junction, as expected, is cis in both cases, and the configurations are established. By similar experiments, the adduct **7** (mp 127 °C) was shown to have an axial proton (δ 2.38, ddd, J = 12.9, 2.6, 2.4 Hz) on the carbon atom bearing the acetyl group and an equatorial proton (δ 4.10, d, J = 2.6 Hz) on the sulfur-bearing carbon atom; further confirmation of the configuration assigned to **7** is derived below from the NMR spectra of its transformation products. Adduct **8** was assigned a cis relationship between the phenylthio and acyl groups on the basis of (1) the chemical shift (δ 3.77) of the proton on the sulfur-bearing carbon atom (see above), (2) the determination that this proton is coupled to that on the neighboring carbon atom by $J \simeq 2.4$ Hz, and (3) the fact that the latter is also coupled to the proton on the other neighboring carbon atom by a small (but not precisely determinable) coupling constant; the configuration of the circled proton is assumed on the basis of the well-known suprafacial addition that occurs in Diels–Alder reactions, a mode which was shown to occur in entry 4.

Earlier work[5a] had shown that the uncatalyzed reaction of the parent diene (**1**) with methyl vinyl ketone (MVK) at 25 °C for 71 h provides 66% of the endo adduct **4** and none of the exo isomer; it is now seen (entry 1) that acid catalysis gives the same product more rapidly and in higher yield. At a higher temperature (110 °C), diene **2** undergoes an uncatalyzed reaction with the same dienophile to give after 12 h 50% of an 84:16 mixture of the endo (**5**) and exo (trans) isomers; the catalyzed reaction (entry 2) is again far faster, and it gives only the endo adduct (**5**). Similarly, Lewis acid catalysis of the addition of the same diene to 2-cyclohexen-1-one (entry 3) greatly increases the stereoselectivity over that observed in the low-yield thermal process in which some of the exo adduct is also formed. In entries 3–5, ethylaluminum dichloride,[14] which is valuable in such reactions because its Brønsted basicity[15] helps prevent proton-initiated polymerization of the diene, was used. The cyclic diene **3** also undergoes smooth, acid-catalyzed, stereospecific endo addition to both MVK and cyclohexenone; in both cases, no uncatalyzed process occurred below the decomposition temperature of the diene (~150 °C).

In the addition of the parent diene **1** to MVK at room temperature, the complete control of regiochemistry by the sulfur substituent has been attributed[16] to a combination of frontier and secondary orbital interactions, the latter also being responsible for the endo specificity. Evidence for this point of view has been gleaned from the product ratios of the addition of **1** to the weak dienophile acrylonitrile.[17,18] Calculations indicate that these tendencies would be strengthened in the acid-catalyzed process.[19] The results reported here are in sharp contrast to the behavior of the isomeric 2-methoxy-3-(phenylthio)-1,3-butadiene in which the weak regiocontrol exerted by sulfur in the thermal reaction is further weakened by the use of Lewis acids; while very little regioselectivity was observed by Trost in the case of the above diene, control by sulfur was substantial in the cases of 1,3-butadienes substituted at the 2,3- or the 1,4-positions with acetoxy and phenylthio groups, especially in the presence of Lewis acids.[4]

Scheme I



Because of the great synthetic utility of allylic phenylthio groups, the complete regio- and stereospecificity of the Diels–Alder reactions reported herein bodes well for the synthetic promise of dienes such as **1–3**.[20] Scheme I demonstrates that the CS bond of an adduct can be stereospecifically transformed into a CC bond at the allylic position, in this case with creation of a tetrasubstituted carbon atom.[22] When **7** is treated with 3 equiv of Wittig reagent, generated by treatment of the phosphonium bromide with lithium diisopropylamide in THF, the acetyl group is converted to an isopropenyl group. That no epimerization occurs during this transformation is evident from the ^{1}H NMR profile of the ring protons of the product (**9**), which exhibits a singlet at δ 3.84 (half-width = 6.4 Hz) for the proton on the sulfur-bearing carbon atom and a doublet at δ 2.44 (J = 12.9 Hz, half-width of each peak = 6.4 Hz) for the allylic proton on the carbon atom bearing the isopropenyl group. The structures of both **7** and **9** are confirmed by conversion[23] of the enol ether **9** to the ketone **10** (mp 138 °C), the ^{1}H NMR spectrum of which exhibits key peaks at δ 3.90 (d, J = 3.23 Hz, 1 H, H_a), 2.89 (td, J = 11.3, 3.23 Hz, 1 H, H_b), and 2.72 (dt, J = 12.94, 3.23 Hz, 1 H, H_c) which were assigned with the aid of decoupling experiments; the downfield position of the peak for H_b is due to deshielding by both the adjacent carbonyl group and the axially oriented sulfur atom.[24] Treatment of **9** with a zinc carbenoid[25] provides a good yield of **11** in which the three chiral centers generated completely stereospecifically have the same relative configurations as those in the eudesmane sesquiterpenes.[26]

(14) Furukawa, J.; Kobayashi, E.; Haga, K.; Iseda, Y. *Polym. J.* **1971**, *2*, 475. Roush, W. R.; Gillis, H. R. *J. Org. Chem.* **1980**, *45*, 4267.
(15) Snider, B. B. *Acc. Chem. Res.* **1980**, *13*, 426.
(16) Alston, P. V.; Ottenbrite, R. M.; Cohen, T. *J. Org. Chem.* **1978**, *43*, 1864.
(17) Cohen, T.; Ruffner, R. J.; Shull, D. W.; Daniewski, W. M.; Ottenbrite, R. M.; Alston, P. V. *J. Org. Chem.* **1978**, *43*, 4052.
(18) However, the importance of secondary orbital interactions in determining regiochemistry in Diels–Alder reactions has been questioned. Fleming, I.; Michael, J. P.; Overman, L. E.; Taylor, G. P. *Tetrahedron Lett* **1978**, 1313.
(19) Alston, P. V., private communication.
(20) Kozikowski has recently reported regiospecificity of **2**[10] and close analogues[21] in acid-catalyzed reactions with a quinone.
(21) Kozikowski, A. P.; Sugiyama, K.; Huie, E. *Tetrahedron Lett.* **1981**, *22*, 3381. Kozikowski, A. P.; Huie, E.; Springer, J. P. *J. Am. Chem. Soc.* **1982**, *104*, 2059.
(22) Review of construction of quarternary carbon centers: Martin, S. F. *Tetrahedron* **1980**, *36*, 419.
(23) Kosarych, Z.; Cohen, T. *Tetrahedron Lett.* **1980**, *21*, 3959. This reference also describes the similar conversion of **5** to the diketone in quantitative yield.
(24) Trost, B. M.; Salzmann, T. N.; Hiroi, K. *J. Am. Chem. Soc.* **1976**, *98*, 4887.
(25) Kosarych, Z.; Cohen, T. *Tetrahedron Lett.* **1982**, *23*, 3019.

Kozikowski has demonstrated other interesting uses for Diels–Alder adducts of **2**[10] and analogues,[21] and many other uses can be envisioned.

Acknowledgment. We thank the National Institutes of Health for providing financial support (Grant No. GM 22760) and the National Science Foundation for funds to purchase the 300-MHz Bruker NMR instrument used in this study (Grant No. CHE 7905185). We also thank Dr. Alvin Marcus for determining the mass spectral data.

Registry No. 1, 60466-66-2; 2, 77003-92-0; 3, 77004-00-3; 4, 60466-71-9; 5, 77004-04-7; 6, 82798-92-3; 7, 82798-93-4; 8, 82798-94-5; 9, 82808-06-8; 10, 82798-96-7; 11, 82798-95-6; methyl vinyl ketone, 78-94-4; 2-cyclohexen-1-one, 930-68-7.

(26) Devon, T. K.; Scott, A. I. "Handbook of Naturally Occurring Compounds"; Academic Press: New York, 1972; Vol. 2, pp 137–146. The assignment of configuration at the quarternary center of 11 is assumed on the basis of the mechanism of the [2,3]sigmatropic rearrangement.[27]

(27) Trost, B. M.; Melvin, L. S., Jr. "Sulfur Ylides"; Academic Press: New York, 1975; Chapter 7. Block, E. "Reactions of Organosulfur Compounds"; Academic Press, New York, 1978; Chapter 7.

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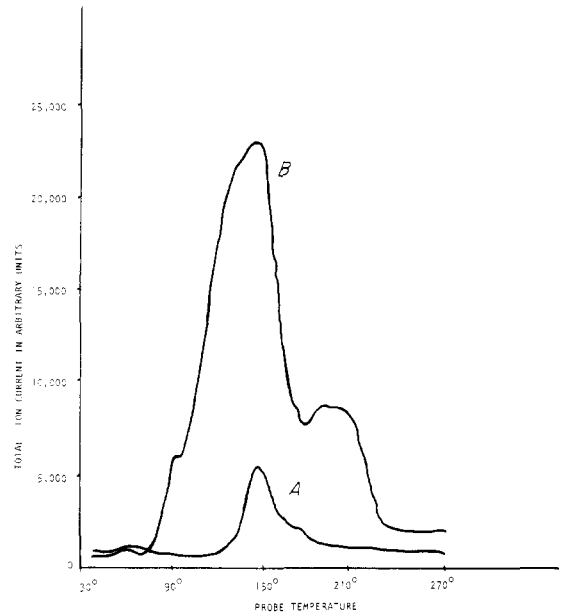

Figure 1. Total ion current profile of the potassium salt of penicillin G. A total of 40 scans was recorded while the temperature of the probe was raised from 50 to 300 °C over a period of 5 min. Since the mass spectrum of NH_4Cl by itself showed no ions at m/z >70, the region scanned was 70–500 amu. The shoulder appearing in the profile curves appears to indicate extensive thermal decomposition above 170 °C: (A) potassium salt of penicillin G, (B) potassium salt of penicillin G with NH_4Cl added.

Facile Ionization Induced by Ammonium Salts: Mass Spectra of Nonvolatile Compounds Using Unmodified Electron-Impact Mass Spectrometers[1]

Summary: A simple technique is described for obtaining meaningful spectra from nonvolatile and/or thermolabile compounds, using a direct probe in commercially available, low-resolution or high-resolution, electron-impact mass spectrometers.

Sir: Many biologically active compounds are nonvolatile and/or thermolabile and fail to give satisfactory mass spectra under electron-impact conditions.[2] We report here a simple technique for obtaining meaningful spectra from many such compounds using a direct probe in commercially available, low-resolution or high-resolution, electron-impact mass spectrometers (EIMS). Unlike other techniques[3] (field desorption, plasma desorption, laser desorption, flash desorption, fast atom bombardment, "in beam", etc.) our method does not require any modification of the conventional spectrometer or addition of expensive accessories.

This technique is based on our previous observation[4,5] that the addition of ammonium salts (NH_4X) to a solid sample in a chemical-ionization mass spectrometer produces abundant $(M + H)^+$ and/or $(M + NH_4)^+$ ions as well as $(M + X)^-$ and $(M - 1)^-$ ions.

We extended the ammonium chloride technique to electron-impact mass spectrometry and observed that ionization of sample compounds is remarkably facilitated (as shown by increase in the ion current in the higher molecular weight region of the spectrum; see Figure 1). Our technique for EI mass spectrometry involves using a conventional solids probe while adding a trace of ammonium chloride[6] as a solid (or a solution of ammonium iodide in methanol) to a solid sample, which may even be in the form of a salt.

To obtain some insight into the ionization enhancement in the presence of ammonium chloride we have studied the spectra of penicillin G in detail. Under electron-impact conditions, the potassium or sodium salt of penicillin G produces fragment ion that are much smaller than the whole molecule and therefore do not provide much structural information. Being thermolabile, even free penicillanic acid gave a poor spectrum—a weak molecular ion was seen very briefly.

By using the ammonium salt addition technique we observed much improved spectra: the molecular ion M^+ of the *free acid* corresponding to the salt was observed as the highest molecular weight peak (see Figure 2). That the observed molecular ion at m/z 334 was not $(M + NH_4^+ - H_2O)$[7] was established easily: the position of this peak remains unchanged upon the substitution of $^{15}NH_4Cl$ for

(1) Mass Spectral Studies Part 12. For part 11, see Shefer, S.; Salen; G.; Cheng, F. W.; Dayal, B., Batta, A. K.; Tint, G. S.; Bose, A. K.; Pramanik, B. N. *Anal. Biochem.*, **1982**, *121*, 23.

(2) For recent reviews on mass spectrometry of nonvolatile and thermally unstable compounds, see: (a) Cotter, R. J. *Anal. Chem.* **1980**, 52, 1589A. (b) Daves, G. D. *Acc. Chem. Res.* **1979**, *12*, 359.

(3) For some recent publications, see below and references cited therein: (a) Zakett, D.; Schoen, E. E.; Cooks, R. G.; Hemberger, P. H. *J. Am. Chem. Soc.* **1981**, *102*, 1295. (b) Takeda, N.; Umemura, M.; Harada, K.; Suzuki, M.; Tatematsu, A. *J. Antibiot.* **1981**, *34*, 617. (c) Linscheid, M.; D'Angona, J.; Burlingame, A. L.; Dele, A., Ballou, C. E. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 1471.

(4) Bose, A. K.; Fujiwara, H.; Pramanik, B. N.; Lazaro, E.; Spillert, C. R. *Anal. Biochem.* **1978**, *89*, 284.

(5) Bose, A. K.; Pramanik, B.; Tabei, K.; Bates, A. D. Presented at the 28th Annual Conference on Mass Spectrometry and Allied Topics, New York, May 25–30, 1980.

(6) Nonhalide salts such as $(NH_4)_2SO_4$ can also be used. Optimum results were obtained by adding 50–150 μg of NH_4Cl and about 50 μg of the sample compound. The lower limit of detection was 1–5 μg, depending on the sample.

(7) One of the reviewers had suggested that the apparent M^+ could be in reality be a CI-type ion $(M + NH_4^+ - H_2O)$; such, however, is not the case.

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