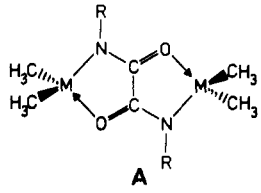
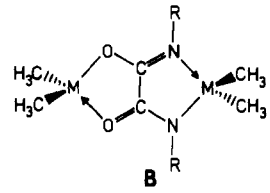

A


B

the expected identical environment for the two Al atoms (the pertinent crystallographic data are presented in Table I). IR/Raman and $^1H/^{13}C$ NMR data in solution are in accord with the crystal structure (Figure 1a). The gallium complexes **2** and **3**, on the other hand, display *two* complete sets of $^1H/^{13}C$ resonances, one with a widely spaced *double* Ga-CH_3 signal (see Figure 2, lower trace). This spectral pattern, indicating the presence of two isomeric forms, proved peculiar to a whole series of oxamide complexes of dialkylgallium and -indium.[4] As vibration spectral evidence seemed to demand a centrosymmetric molecular geometry,[5] a nonplanar bicyclic structure with conformational isomerism was first proposed;[2] this explanation was in conflict, though, with both chemical experience and other NMR findings.[4,6] To resolve the problem, we extended our crystal structure analyses to the *N*-H and *N*-CH_3 gallium complexes.

The space group of **2** proved not to be uniquely determinable on the basis of the proposed chemical structure, Laue symmetry, and systematic extinctions, the ambiguities being *Cm*, *C*2, and *C*2/*m*. Crystal structure analysis subsequently established the space group as *Cm* and molecular symmetry as C_s, with the mirror symmetry plane, unexpectedly, *perpendicular* to the oxamide C-C bond. The high resolution of the data set and the magnitude of the partially refined thermal parameters strongly indicated that the crystal which we investigated was well ordered; thus, only the cis configuration, **2B**, for the diamide ligand was consistent with both the crystallographic symmetry and its position in the unit cell. To date, the amide hydrogen atoms have not been located by difference Fourier techniques; the necessity for such additional confirmation of structure **2B** was obviated, though, by the subsequent sucessful crystal structure determination for the *N*-methyl compound **3** initiated earlier.

The lattice parameter and Laue symmetry observed for **3** were clearly indicative of a triclinic cell with $Z = 2$, and prompted us to assume $P\bar{1}$ as the space group. In the early stages of analysis the major part of one molecule which had an apparent crystallographic inversion center analogous to that observed for **1** was found. Subsequent difference Fourier maps based on this fragment presented peak distributions not interpretable in terms of a second oxamide complex; furthermore, attempts to refine the initial fragment produced results inconsistent with a correct structural model. The establishment of a noncentrosymmetric chemical structure for **2** raised the strong possibility that the crystal of the *N*-CH_3 derivative contained two symmetry-independent molecules; the associated lower crystallographic symmetry raised the possibility that one molecule each of cis and trans configuration might be present in the crystal. By pursuing the crystal structure in space group *P*1, a successful analysis was readily accomplished; however, both symmetry-independent molecules in the unit cell were demonstrated to display the same chemical structure, that is the *cis*-oxamide configuration, **3B** (Figure 1b).

In establishing this configuration, crystallography satisfactorily accounted for the *double* Ga-CH_3 together with one *N*-CH_3 and C=O NMR signal; the presence of a second set of resonances in the ^1H and ^{13}C spectra indicated that the bulk sample was in fact inhomogeneous. This could subsequently be proven by NMR spectroscopy: the upper trace in Figure 2 represents the proton spectrum of a solution of a single crystal of **3**, a transparent needle of exactly the type selected for crystal structure analysis (100-200 μg in 0.3 mL of C_6D_6); clearly, only one form, **3B**, is present in this crystal, with *two* Ga-CH_3 signals and the lower field *N*-CH_3 resonance. The (inverted) lower trace gives the spectrum of a 50-mg random sample from the same batch of **3** from which the above crystal was selected, and shows the presence also of the second set of NMR signals (ratio 30:70). A pure crystal of the other modification to which we assign the trans configuration, **3A**, on the basis of IR/Raman and NMR spectral data, could so far not be isolated. In each case, sublimation also yielded poorly formed, scale-like crystals containing, however, both isomers in about the same ratio as the whole sample. Quite obviously, in selecting the single crystals for x-ray analysis, only the clear needles with pure cis configuration were picked out for both **2** and **3**.

As preliminary investigations have shown,[4,6] the **A**/**B** isomer ratio is determined primarily by the temperature at which oxamide and gallium alkyl are brought to reaction (e.g., 20:80 at 0 °C, 70:30 at 138 °C). It also depends upon both size and nature of the substituents at the oxamide N and gallium: thus, for the *N*-*tert*-butyldimethylgallium derivative, no cis isomer **B** could be detected by either ^1H or ^{13}C NMR.[4] Attempts at thermal isomerization failed owing to competitive decomposition; however, if benzene solutions of **3A**/**B** with varying isomer ratio are kept at 20 °C for 10-30 days (argon atmosphere), a 1:1 equilibrium state can be obtained from either side. The rate of equilibration is enhanced (though with increasing decomposition) by addition of tertiary amines.[4]

To date, the reasons for the appearance of this unusual configurational isomerism for the gallium compounds are not clearly understood. By a detailed study of the conditions governing the cis/trans ratio, we hope to throw some new light on the complexation behavior of group 3B metals, especially with respect to their use as Friedel-Crafts catalysts.

Acknowledgment. For generous support of this work, we are indebted to the Deutsche Forschungsgemeinschaft.

References and Notes

(1) J. Weidlein, *J. Organomet. Chem.*, **49**, 257 (1973).
(2) H. U. Schwering, J. Weidlein, and P. Fischer, *J. Organomet. Chem.*, **84**, 17 (1975).
(3) H. D. Hausen, K. Mertz, and J. Weidlein, *J. Organomet. Chem.*, **67**, 7 (1974).
(4) R. Gräf, Diplomarbeit University, Stuttgart, 1976.
(5) R. Gräf, P. Fischer, and J. Weidlein, *J. Organomet. Chem.*, in press.
(6) P. Fischer, R. Gräf, and J. Weidlein, unpublished results.

Peter Fischer,* Rainer Gräf, John J. Stezowski,* Johann Weidlein
Fachbereich Chemie, Universität Stuttgart
Pfaffenwaldring 55, 7000 Stuttgart 80, West Germany
Received March 11, 1977

Application of Deuterium Magnetic Resonance to Biosynthetic Studies. 1. Biosynthesis of Ovalicin

Sir:

In studying the biosynthesis of a given metabolite, one is interested in not only the biogenesis of the carbon skeleton but the metabolic fate of the hydrogens which are attached to that skeleton. The recognition of protonation, deprotonation, and hydride shift processes is essential to the clarification of mechanistic and stereochemical details. When tritium is used as a tracer, alone or in combination with carbon-14, positions and stereochemistry of labeling must be determined by extensive degradations. While ^{13}C NMR techniques are now a viable alternative to experiments with carbon-14 for studying the biosynthesis of a given carbon skeleton, to date no comparably general substitute for tritium has been exploited. The use of deuterium and deuterium magnetic resonance is ap-

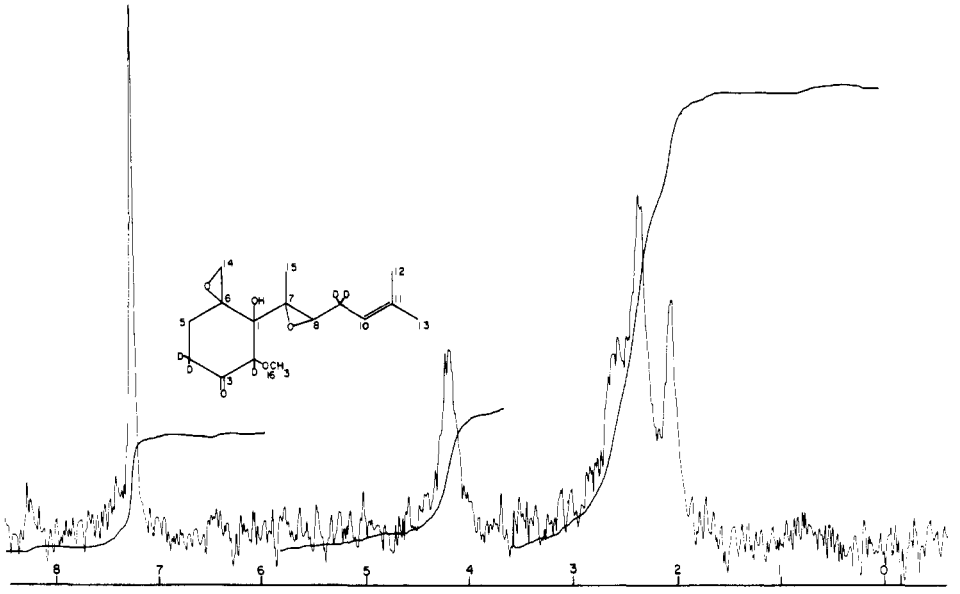


Figure 1. Proton decoupled ^2H NMR spectrum of 0.06 mmol of ovalicin, 7.9% deuterium at each site; 2084 transients, 4K data points.

pealing for a number of reasons. (1) The natural abundance of deuterium is only 0.015%. An enrichment with deuterium of only 1% therefore would lead to a sixtyfold enhancement over the natural abundance signal. (2) Shielding of deuterium is the same as for protons in a given compound. Shift assignments may therefore be made from the ^1H NMR spectrum on micromole quantities of material if necessary. (3) In proton-decoupled ^2H NMR there is no NOE. Also relaxation times T_1 for ^2H are relatively short, minimizing the possibility of partial saturation. Both these factors make integration of deuterium spectra meaningful. (4) As with ^{13}C NMR, laborious degradative sequences can be avoided. (5) Deuterated substrates and reagents are relatively inexpensive. Moreover there are no special problems associated with handling highly enriched materials, in contrast to radioisotopically labeled substances. We have been exploring the application of ^2H NMR to biosynthetic problems and our results are reported below.[1,2]

We have previously presented evidence, based on incorporation of [3,4-$^{13}C_2$]mevalonate, that the biosynthesis of the antibiotic ovalicin[11] (**1**) by *Pseudeurotium ovalis* involves 1,3 migration of the eight-carbon side chain of a bisabolyl cation generated by cyclization of farnesyl pyrophosphate.[12] In support of this scheme we recently reported isolation of the sesquiterpene *β-trans*-bergamotene (**2**) a postulated intermediate, from the mycelial extracts of *P. ovalis*[13,14] (Scheme I).

A number of possibilities exist for the oxidative cleavage of

Scheme I

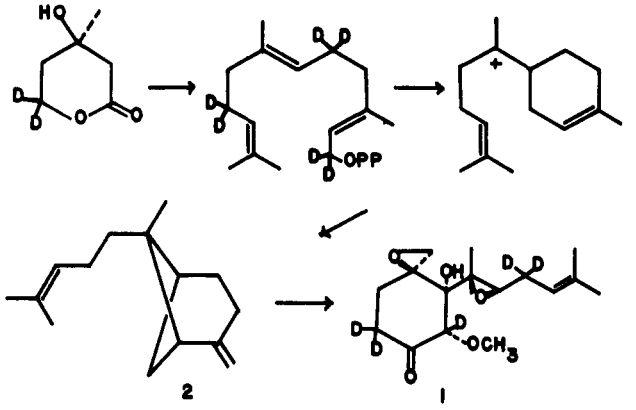


Table I. Proton-Proton Decoupling Experiments[a]

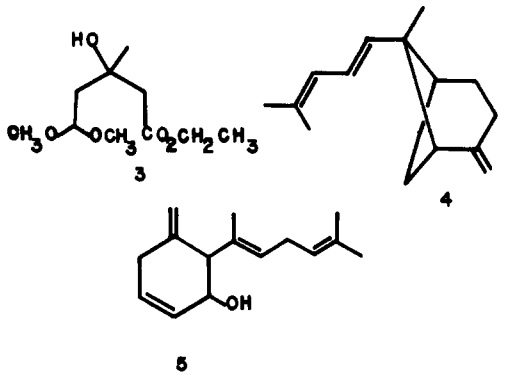
Irradn	H-8	H-9a	H-9b	H-10
	2.87	2.12	2.37	5.15
	dd (6.5, 7.0)	ddd (7.0, 12, 7.5)	ddd (7.5, 6, 12)	t (7.5)
5.15	dd (6.5, 7)	dd (7, 12)	dd (6, 12)	
2.87		dd (7.5, 13)	dd (7.5, 13)	t (7.5)
2.12	d (6)			d (7)

[a] In δ (*J*, hertz); d = doublet, t = triplet.

bergamotene to the *o*-menthane framework of ovalicin. In order to distinguish among the various mechanisms experiments with specifically deuterated mevalonate have been carried out.

In determining the structure of ovalicin, the Sandoz group assigned the ^1H NMR spectrum without distinguishing among resonances due to the three pairs of methylene protons at C-4, C-5, and C-9.[11] We have now assigned these latter signals by a series of proton-proton decoupling experiments (270 MHz)[15] and by chemical deuteration of C-4. As summarized in Table I, the diastereotopic protons H-9a and H-9b were assigned to the signals at δ 2.12 and 2.37 by their coupling to the H-8 epoxide methine and H-10 olefinic proton. The protons at C-4 and C-5 constitute an ABCD spin system and appear as a pair of complex multiplets at ca. δ 2.4–2.55 and 2.6–2.7. In order to assign the H-4 resonances, ovalicin was treated with 3 equiv of potassium *tert*-butoxide in *tert*-butyl alcohol-*O*-*d*/tetrahydrofuran (0 °C, 30 min). The ^2H NMR spectrum[16] of the resulting deuterated ovalicin (7% d_3, 73% d_2, 14% d_1, 6% d_0) showed two peaks, δ 2.38 and 2.62, assigned to the exchangeable C-4 methylenes, and a much smaller peak, δ 4.21, due to exchange at C-2.

[5,5-$^2H_2$]Mevalonate (0.346 g, 2.62 mmol), synthesized by lithium aluminum deuteride reduction of the ester acetal **3** followed by acetylation and performic acid oxidation as previously described,[12] was administered to 7-day-old shaken cultures of *P.* ovalis (24 500-ml flasks, 100 ml of nutrient per flask). The deuterated mevalonate contained (3*RS*,5*RS*)-[2-^{14}C,5-$^3H_2$]mevalonate (4.0×10^6 dpm ^{14}C, $^3H/^{14}C = 1.98$) as an internal standard. After an additional 7 days, the cultures were harvested and the labeled ovalicin was extracted and purified in the usual manner,[12] to give 20 mg of ovalicin, mp 94–95 °C (lit.[11] mp 95–96 °C). Measurement of the ^{14}C activity indicated a total incorporation of 4.5% (based on (3*R*)-mevalonate) and an enrichment of 7.9% at each site. The



$^3H/^{14}C$ ratio (1.58) indicated retention of 5/6 of the tritium isotope.

In order to determine the positions of deuteration, the labeled ovalicin sample was analyzed directly by ^2H NMR. The resulting spectrum showed four signals: δ 4.21 ($CDOCH_3$, 1 D), 2.63 (CD_2CO, 1 D), 2.4 (CD_2CO, $CD_2CH=$, 2 D), and 2.09 ($CD_2CH=$, 1 D) (Figure 1).

The ^2H NMR results are consistent with the observed $^3H/^{14}C$ ratio and reconfirm previous experiments on the specific incorporation of mevalonate. Furthermore the retention of five of six deuteriums derived from C-5 of mevalonate sets strict boundary conditions on any mechanistic proposals for the oxidative cleavage of a bergamotene intermediate. Specifically such intermediates as the dehydrobergamotene (**4**), suggested by Birch,[17] or a monocyclic structure **5** related to carquejyl acetate[18] are ruled out, as is oxidation of the methylene bridge of bergamotene to a ketone.

The experiments described above illustrate the utility of ^2H NMR as a biosynthetic tool while providing important information bearing on the biosynthesis of ovalicin. Further biosynthetic applications of ^2H NMR will be reported in due course.[19,20]

References and Notes

(1) ^2H NMR has been used recently in a number of mechanistic studies: cyclohexadiene dimerizations,[3] the Diels–Alder dimerization of pentadiene,[4] the homoenolization of fenchone,[5] and the synthesis and rearrangement of benzvalene.[6] Except for the work of Stothers, these studies involved rather high levels of enrichment (>90%). Indirect detection of deuterium is possible. Broad-band deuterium decoupling of $^{13}CD_3$-enriched corrins has been used to demonstrate the absence of protium in any of the methionine-derived methyl groups.[7] A study of scytalone biosynthesis utilized ^{13}C–^2H coupling to assign the positions of deuterium enrichment.[8] There have been only two reports of the direct application of ^2H NMR to biosynthetic investigations. Sato has recently described a ^2H NMR study of griseofulvin biosynthesis[9] and Bycroft has detected deuterium in penicillin G derived from feeding of labeled cystine.[10]

(2) Cf. P. Diehl in "Nuclear Magnetic Resonance Spectroscopy of Nuclei Other than Protons", T. Axenrod and G. A. Webb, Ed., Wiley, New York, N.Y., 1974.

(3) W. R. Moore, P. D. Mogolesko, and D. D. Traficante, *J. Am. Chem. Soc.*, **94**, 4853 (1972).

(4) J. A. Berson and R. Malherbe, *J. Am. Chem. Soc.* **97**, 5910 (1975).

(5) A. L. Johnson, J. B. Stothers, and C. T. Tan, *Can. J. Chem.*, **53**, 212 (1975).

(6) U. Burger and F. Mazenod, *Tetrahedron Lett.*, 2881, 2885 (1976).

(7) M. Imfeld, C. A. Townsend, and D. Arigoni, *J. Chem. Soc., Chem. Commun.*, 541 (1976); A. Battersby, R. Hollenstein, E. McDonald, and D. C. Williams, *ibid.*, 543 (1976). See also A. I. Scott, M. Kajiwara, T. Takahashi, I. M. Armitage, P. Demou, and D. Petrocine, *ibid.*, 544 (1976).

(8) U. Sankawa, H. Shimada, T. Sato, T. Kinoshita, and K. Yamasaki, *Tetrahedron Lett.*, 483 (1977).

(9) Y. Sato and T. Oda, *Tetrahedron Lett.*, 2695 (1976).

(10) B. W. Bycroft, C. M. Wels, K. Corbett, and D. A. Lowe, *J. Chem. Soc., Chem. Commun.*, 123 (1975).

(11) H. P. Sigg and H. P. Weber, *Helv. Chim. Acta*, **51**, 1395 (1968); P. Bollinger, H. P. Sigg, and H. P. Weber, *ibid.*, **56**, 819 (1973); T. Sassa, H. Kaise, and K. Munakata, *Agric. Biol. Chem.*, **34**, 649 (1970).

(12) D. E. Cane and R. H. Levin, *J. Am. Chem. Soc.*, **97**, 1282 (1975); D. E. Cane and R. H. Levin, *ibid.*, **98**, 1183 (1976). See also M. Tanabe and K. T. Suzuki, *Tetrahedron Lett.*, 4417 (1974).

(13) D. E. Cane and G. G. S. King, *Tetrahedron Lett.*, 4737 (1976).

(14) Bergamotene has also been isolated from *Asperigillus fumigatus*, a fungus which produces fumagillin, a substance closely related to ovalicin; cf. S. Nozoe, H. Kobayashi, and N. Morisaki, *Tetrahedron Lett.*, 4625 (1976).

(15) ^1H NMR spectra (270 MHz) were obtained in $CDCl_3$ solutions with $CHCl_3$ as internal standard (δ 7.24) using a Bruker HX 270 operated in the FT mode, spectral width 3000 Hz, 16K. Reported coupling constants are ±1.0 Hz and chemical shifts are ±0.01 ppm.

(16) Proton decoupled ^2H NMR spectra were obtained at 41.44 MHz on degassed $CHCl_3$ solutions with $CDCl_3$ as internal standard (δ 7.24) in 10-mm sample tubes. A pulse angle of 90° was employed, spectral width 500 Hz, using 2K or 4K data points. Chemical shifts are ±0.02 ppm.

(17) A. J. Birch and S. F. Hussain, *J. Chem. Soc. C*, 1473 (1969).

(18) G. Snatzke, A. F. Thomas, and G. Ohloff, *Helv. Chim. Acta*, **52**, 1253 (1969). The structure for carquejyl acetate given in ref 12 is incorrect.

(19) In a separate study of rosenonolactone biosynthesis we have recently obtained spectra with S/N = 30 in 4 hours (2K data points) on a 0.05-mmol sample enriched with 3% deuterium (D. E. Cane and P. N. Murthy, unpublished work). Such combinations of enrichment level and quantity of metabolite are well within the range usually achievable with microorganisms.

(20) This work was supported by the National Institutes of Health (GM 22172) and by a grant from the Merck Foundation. The Bruker HX 270 facility is supported by NIH Grant No. 1-P07-PR00798 from the Division of Research Resources.

David E. Cane,* Stephen L. Buchwald

Department of Chemistry, Brown University
Providence, Rhode Island 02912

Received May 9, 1977

Unusual Stability Characteristics in Methanol of the Complexes of a New Pyridine-Substituted Cyclic Polyether–Ester Compound with Na^+, K^+, Ag^+, and Ba^{2+}—Comparison with Oxygen, Sulfur, and Nitrogen Analogues

Sir:

In two recent communications, van Bergen and Kellogg[1,2] report the synthesis of an unusual macrocycle **1** incorporating into the ring Hantzsch esters. These esters may be used as a "mimic" of NAD(P)H because of their hydride-donating properties. Their goal was to enhance hydride transfer by adding a crown ether ring onto the ester in hopes of holding metal cations, which are known to catalyze this transfer, in close proximity to the hydride donor. We report here the results of a calorimetric study of the cation binding properties of two ligands similar to **1**, namely **2** and **3**. Complexing data for other macrocyclic compounds **4–8** are also included to allow comparisons between these ester-containing systems and cyclic polyethers.

Like the Hantzsch ester crown, the macrocycles under consideration in this study differ from simple crown ethers in