are greatly appreciated. We gratefully acknowledge the receipt of an NIH Research Career Development Award (K.S.S.), an NSF Presidential Young Investigator Award (R.A.S.), and Sloan Foundation Research Fellowships (R.A.S. and K.S.S.).

Absolute Configuration of (−)-β-*trans*-Bergamotene

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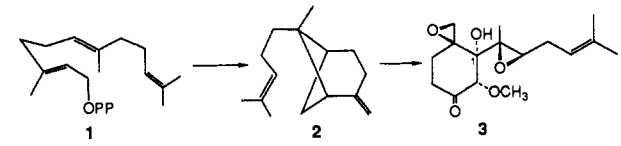
We recently reported that a cell-free preparation from the fungus *Pseudeurotium ovalis* can catalyze the conversion of farnesyl pyrophosphate (FPP, **1**) to the bicyclic sesquiterpene hydrocarbon β-*trans*-bergamotene (**2**) and further that the cyclization proceeded with net retention of configuration at C-1 of FPP.[1] The latter conclusion was based upon the reasonable assumption that (-)-β-bergamotene, which we have isolated from mycelial extracts of *P. ovalis*,[2] has the (1*S*,5*S*,7*R*)-configuration illustrated, taking into account the demonstrated conversion of β-bergamotene to ovalicin (**3**),[4] a metabolite of known absolute configuration,[5] and assuming that the introduction of oxygen at C-1 of ovalicin has proceeded with retention of configuration.[6] We now report results that establish the absolute configuration of (−)-β-bergamotene based on a novel combination of enzymatic and NMR spectroscopic techniques.

The approach we chose was to prepare samples of chirally deuterated FPP, of known absolute configuration, which would be labeled at a site, C-4, that would be unaffected by the enzymatic cyclization. Incubation of the deuterated FPP with bergamotene synthase and analysis of the resulting bergamotene by ^{2}H NMR would establish the relative configuration of deuterium label in the product, leading to the unambiguous assignment of the absolute configuration of **2**. A combination of ^{1}H–^{1}H COSY and ^{1}H{^{13}C} heteronuclear shift correlation was used to identify the signals corresponding to the relevant protons attached to C-3 of bergamotene. Irradiation of the H-14 methyl protons then gave rise to a 0.6% NOE enhancement of the signal at δ 2.24, which was therefore assigned to H-3$_{exo}$. In confirmation of this assignment, irradiation at δ 1.43, previously assigned to H-6$_{exo}$,[1] resulted in a 2.6% enhancement of the H-3$_{endo}$ signal at 2.55.

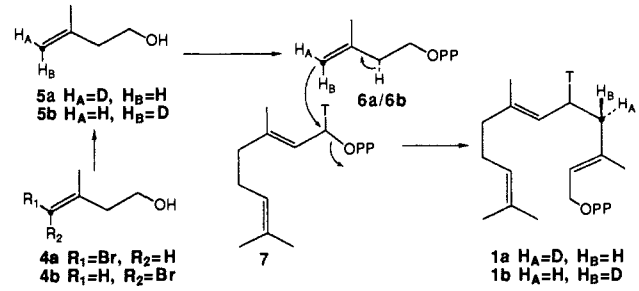
To prepare the requisite labeled samples of (4*S*)- and (4*R*)-(4-^{2}H)FPP, (4*E*)- and (4*Z*)-4-bromoisopentenol[7a] (**4a** and **4b**) were each metalated by an adaptation of the method of Ogura[8] (*s*-BuLi, Et_2O, 1.5 h, −90 °C). Quenching of the individual lithio anions with CF_3CO_2D gave (4*E*)- and (4*Z*)-[4-^{2}H]isopentenol (**5a** and **5b**), which were each converted to the corresponding isopentenyl pyrophosphate (IPP) esters **6a** and **6b** by tosylation (TsCl, Py, 1 h, 25 °C) and displacement with tris(tetra-*n*-butylammonium)pyrophosphate (CH_3CN, 12 h, 25 °C).[9] Prenyl transferase[10] mediated coupling of **6a** and **6b**, containing [4-^{14}C]-IPP as internal standard, with [1-^{3}H]geranyl pyrophosphate (GPP, **7**) gave (4*S*)- and (4*R*)-[4-^{2}H,5-^{3}H,4-^{14}C]FPP (**1a** and **1b**).[7,11]

Incubation of 1.05 μmol of (4*S*)-[4-^{2}H]FPP (**1a**) with 250 mL of a cell-free preparation from *P. ovalis* containing bergamotene synthase[1] for 4 h at 30 °C gave 599 nmol of β-bergamotene (**2a**) which was analyzed by 61.4 MHz ^{2}H NMR spectroscopy after being mixed with 5 mg of synthetic (±)-bergamotene[14] and purified by SiO_2 column chromatography. The ^{2}H NMR spectrum of **2a** displayed a single peak at δ 2.53 corresponding to deuterium in the H-3$_{endo}$ (H-3*si*) position. Similarly, incubation of 2.25 μmol of (4*R*)-[4-^{2}H]FPP (**1b**) with bergamotene synthase yielded 1.2 μmol of β-bergamotene (**2b**), which gave rise to a ^{2}H NMR signal at δ 2.24 corresponding to deuterium in the complementary H-3$_{exo}$ (H-3*re*) position. Since the configuration at the deuterated carbon

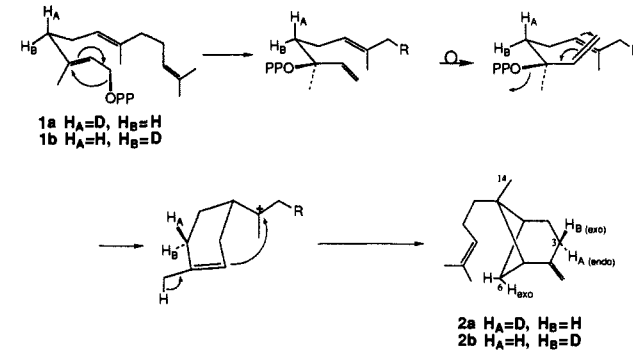
Scheme I



Scheme II



Scheme III



(1) Cane, D. E.; McIlwaine, D. B.; Harrison, P. H. M. *J. Am. Chem. Soc.* **1989**, *111*, 1152.
(2) Cane, D. E.; King, G. G. S. *Tetrahedron Lett.* **1976**, 4737. β-*trans*-Bergamotene has also been isolated from *Aspergillus fumigatus*, which produces the antibiotic fumagillin.[3]
(3) Nozoe, S.; Kobayashi, H.; Morisaki, N. *Tetrahedron Lett.* **1976**, 4625. Turner, J. R.; Tarbell, D. S. *Proc. Natl. Acad. Sci., U.S.A.* **1962**, *48*, 733. McCorkindale, N. J.; Sime, J. G. *Proc. Chem. Soc.* **1961**, 331.
(4) Cane, D. E.; McIlwaine, D. B. *Tetrahedron Lett.* **1987**, *28*, 6545.
(5) Sigg, H. P.; Weber, H. P. *Helv. Chim. Acta* **1968**, *51*, 1395.
(6) Hayaishi, O. *Oxygenases*; Academic Press: New York, 1962. Gautier, A. E. Dissertation; ETH Zurich, 1980; No. 6583.
(7) (a) Cornforth, J. W.; Cornforth, R. H.; Popjak, G.; Yengoyan, L. *J. Biol. Chem.* **1966**, *241*, 3970. (b) Donninger, C.; Popjak, G. *Proc. R. Soc. London, Ser. B* **1966**, *163*, 465. (c) Cornforth, J. W.; Cornforth, R. H.; Donninger, C.; Popjak, G. *Proc. R. Soc. London, Ser. B* **1966**, *163*, 492. (d) Popjak, G.; Cornforth, J. W. *Biochem. J.* **1966**, *101*, 553.
(8) Ogura, K.; Koyama, T.; Kobayashi, M.; Ito, M. *Biochemistry* **1987**, *26*, 4745.
(9) Davisson, V. J.; Poulter, C. D.; Woodside, A. B.; Stremler, K. E.; Neal, T. R.; Muelbacher, M. *J. Org. Chem.* **1986**, *51*, 4768.
(10) Avian prenyl transferase was purified from chicken livers (Pel-Freeze Biologicals), as described, to the hydroxylapatite step to a specific activity of 14 nmol/min/mg protein (0.014 U/mg): Reed, B. C.; Rilling, H. C. *Biochemistry* **1975**, *14*, 50.
(11) The ionization–condensation–elimination reaction has been shown to take place on the *re* face of the IPP double bond.[7] A typical incubation involved 2 μmol (4*E*)-[4-^{2}H]IPP, 0.1 μCi [4-^{14}C]IPP, and 4 μmol [1-^{3}H]GPP in 3.5 mL of 10 mM HEPES buffer (pH 7.0) containing 1.0 mM $MgCl_2$, 200 μL of 50 mM DTE, and 0.14 U of prenyl transferase. After 3 h at 30 °C, the resulting **1a** was purified by a combination of Sephadex G-25 gel filtration, C_{18} reverse phase ion pairing, and ion-exchange chromatography.[12,13]
(12) Beyer, P.; Kreuz, K.; Kleinig, H. In *Methods in Enzymology*; Law, J. H., Rilling, H. C., Eds.; Academic Press: New York, 1985; Vol. 111, pp 248–252.
(13) Cane, D. E.; Oliver, J. S.; Harrison, P. H. M.; Abell, C.; Hubbard, B. R.; Kane, C. T.; Lattman, R. *J. Am. Chem. Soc.* Submitted for publication.
(14) Corey, E. J.; Desai, M. C. *Tetrahedron Lett.* **1985**, *26*, 3535. Snider, B.; Kulkarni, Y. S. *J. Org. Chem.* **1985**, *50*, 2809.

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is not perturbed by the enzymatic cyclization, the enzymatically generated product is shown to be (1*S*,5*S*,7*R*)-β-*trans*-bergamotene.[15]

Acknowledgment. This work was supported by a grant from the National Institutes of Health, GM30301, and by an N.I.H. Fogarty Center Senior International Fellowship to D.E.C.

(15) For a related example of the assignment of the absolute configuration of a natural product by ^2H NMR analysis of the diterpene pleuromutilin derived from a chirally deuterated precursor see: Hasler, H.; Dissertation; ETH Zurich, 1979; No. 6359.

Chemoselective Reduction of Oxiranes by Methylmetals in the Presence of the Copper(I)–Phosphine Complex

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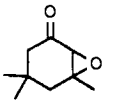
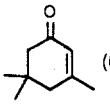
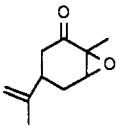
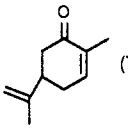
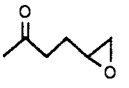
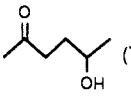
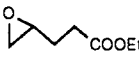
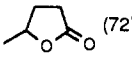
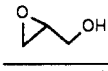
The organocopper "ate" complexes such as homocuprates[1] and mixed cuprates[2] have been known to perform the alkylative ring opening of oxiranes, whereas such competing pathways as rearrangement and halohydrin formation occur during the reaction of oxiranes with other organometallic compounds, particularly Grignard reagents.[3] In this investigation, we have found that methylmetal compounds react with oxiranes in the presence of the copper(I)–phosphine complex to afford the reductive ring opening. The reduction of oxiranes to alcohols has been known to be attained by a variety of reducing agents including metal hydrides. However, the preferential reduction of the epoxide over such readily reducible functionalities as a carbonyl group has, up to now, been difficult.[4]

Our results are indicated in Table I. Cyclohexene oxide in ether was added dropwise to an equimolar amount of methylmagnesium iodide in an ether solution containing 10 mol % of $CuBr(PBu_3)_2$ at 0 °C under nitrogen. The reaction was stopped after 2 h by the addition of aqueous NH_4Cl to give cyclohexanol in 73% yield. The reaction of 1,2-epoxybutane with methylmagnesium iodide led to halohydrin formation as the main pathway (67%), and butanols were obtained only as minor products (2-butanol, 14%; 1-butanol, 4%). The addition of an equimolar amount of $CuBr(PBu_3)_2$ enhanced the reduction reaction (2-butanol, 16%; 1-butanol, 8%). However, the use of methyllithium instead of the Grignard reagent afforded 2-butanol as the sole volatile product in 86% yield. Carbonyl, carbalkoxy, and cyano functionalities were compatible with these reaction conditions, i.e., acetophone, cyclohexanone, 4-heptanone, ethyl benzoate, methyl propionate, and benzonitrile were recovered intact after treatment with methylmagnesium iodide or methyllithium in the presence of $CuBr(PBu_3)_2$. Thus, when oxiranes containing a carbonyl or carbalkoxy functionality elsewhere in the molecule, such as isophorone oxide, carvone oxide, 5,6-epoxy-2-hexanone, and ethyl 4,5-epoxypentanoate, were subjected to the reaction with methyllithium in the presence of $CuBr(PBu_3)_2$, the products based on the chemoselective reduction of the epoxy ring were obtained. While the reaction of styrene oxide with methylmagnesium iodide in the presence of $CuBr(PBu_3)_2$ gave preferentially the methyl addition products (**7**, 42%; **8**, 30%) and styrene (which was probably formed by the dehydration of 1- and/or 2-phenylethyl alcohol, the primary reduction product) as the minor product (14%), the use of $CuBrPBu_3$ instead of $CuBr(PBu_3)_2$ resulted in the preferential formation of the reduction products (styrene, 25%; 2-phenylethyl alcohol, 48%). The methylmetal seems to be more effective for the reduction of oxiranes than other alkylmetals as revealed by the observation that, in the reaction with 1,2-epoxybutane in the presence of 10 mol % of $CuBr(PBu_3)_2$, the methyl, *n*-butyl, and *tert*-butyl Grignard reagents gave 18, 8,[5] and 0% of butanols, respectively.

With respect to the mechanism for this reduction reaction of oxiranes, a pathway via the intermediary generation of the methylcopper–phosphine complex may be first envisaged since it has been well-known that the reaction of alkylmetals with cuprous halides generates the alkylcopper species. However, the me-

Table I. Reactions of Epoxides with $(RM)CuBr(PBu_3)_n$ Complexes

epoxide	RM	RM/CuBr/PBu_3 molar ratio	temp, °C	product (yield,[a] %)
	MeMgI	1/0.1/0.2	0	c-$C_6H_{11}OH$ (73)
 1	MeMgI	1/0.1/0.2	0	2-BuOH (2) (14), 1-BuOH (3) (4), $EtCH(OH)CH_2I$ (4) (67)
1	MeMgI	1/1/2	0	2 (16), 3 (8), 4 (60)
1	*n*-BuMgI	1/0.1/0.2	0	3 (8), 3-octanol (30), $EtCH(OH)CH_2Br$ (22)
1	*t*-BuMgCl	1/0.1/0.2	0	-
1	MeLi	1/1/2	-50	2 (86)
	MeLi	1/1/2	-50	 (65)
	MeLi	1/1/2	-50	 (73)
	MeLi	1/1/2	-50	 (70[b])
	MeLi	1/1/2	-50	 (72[b])
 5	MeMgI	1/1/2	0	$PhCH{=}CH_2$ (6) (14), PhCH(OH)Et (7) (42), $PhCH_2CH(OH)$-Me (8) (30)
5	MeMgI	1/1/1	0	6 (25), $PhCH_2CH_2OH$ (48), 8 (8)
	MeLi[c]	1/1/1	-50	$MeCH(OH)CH_2OH$ (85)

[a] Determined by VPC analysis unless stated otherwise. [b] Yield after isolation by silica gel column chromatography. [c] Two equivalents of methyllithium was used.

(1) (a) Herr, R. W.; Wieland, D. M.; Johnson, C. R. *J. Am. Chem. Soc.* **1970**, *92*, 3813. (b) Johnson, C. R.; Herr, R. W.; Wieland, D. M. *J. Org. Chem.* **1973**, *38*, 4263. (c) Hartman, B. C.; Livinghouse, T.; Rickborn, B. *J. Org. Chem.* **1973**, *38*, 4346.

(2) (a) Acker, R. D. *Tetrahedron Lett.* **1977**, 3407. (b) Lipshutz, B. H.; Kozlowski, J.; Wilhelm, R. S. *J. Am. Chem. Soc.* **1982**, *104*, 2305. (c) Lipshutz, B. H.; Wilhelm, R. S.; Kozlowski, J. A.; Parker, D. *J. Org. Chem.* **1984**, *49*, 3928.

(3) Herr, R. W.; Johnson, C. R. *J. Am. Chem. Soc.* **1970**, *92*, 4979.

(4) A chemoselective reduction of epoxides in the presence of such modestly reducible groups as carbamoyl and carboxyl has been performed with $NaBH_4$ in a mixed solvent containing MeOH. Ookawa, A.; Hiratsuka, H.; Soai, K. *Bull. Chem. Soc. Jpn.* **1987**, *60*, 1813.

(5) It is interesting that the *n*-butyl Grignard reagent gave only 1-BuOH as the reduction product, whereas the methyl Grignard reagent afforded a mixture of 2- and 1-butanols, the former being major and the latter being minor.

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