Relative and Absolute Stereochemistry of Pyripyropene A, A Potent, Bioavailable Inhibitor of Acyl-CoA:Cholesterol Acyltransferase (ACAT)

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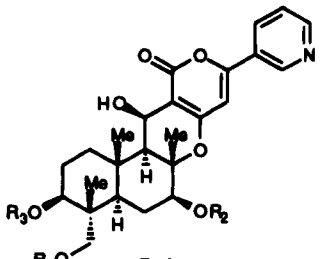
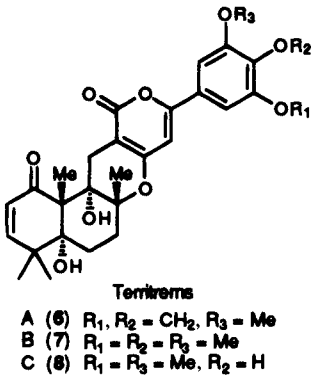
Acyl-CoA:cholesterol acyltransferase (ACAT), the enzyme responsible for intracellular esterification of cholesterol, plays a critical role in three events believed to contribute significantly to atherosclerosis: absorption of dietary cholesterol in the gut, lipoprotein synthesis in the liver, and accumulation of oily cholesteryl esters within the macrophages and smooth muscle cells of developing arterial lesions.[1-3] Inhibitors of ACAT therefore hold great promise as antiatherosclerotic agents. Recently we disclosed the isolation, biological properties, and planar structures of the pyripyropenes A–D (**1**–**4**),[4] novel polyoxygenated metabolites of *Aspergillus fumigatus* FO-1289 which strongly inhibit ACAT with IC_{50} values of 58, 117, 53, and 268 nM, respectively. The pyripyropenes apparently



represent the most potent naturally occurring ACAT inhibitors reported to date, and as such they represent excellent lead compounds.[5] Importantly, pyripyropene A (**1**) proved to be orally active in hamsters, reducing cholesterol absorption by 32–46% after single doses of 25–75 mg/kg.[4c] Interestingly, structures **1**–**4** resemble the territrems A–C (**6**–**8**), mycotoxin tremorgens from *Aspergillus terrus* which inhibit acetylcholinesterase[6] and ADP-fibrinogen-induced platelet aggregation.[7] As a prelude to total synthesis, we describe herein the determination of the complete relative and absolute stereochemistry of pyripyropene A (**1**).



The relative stereochemistry at C(4), C(6), C(7), and C(10) in **1** initially emerged from nuclear Overhauser effect (NOE) measurements[8] which established the syn dispositions of Me(12), Me(14), Me(15), and the C(7) OAc (Figure 1a). Thus, irradiation of the Me(15) resonance at δ 0.89 led to a 3.0% enhancement of Me(12) (δ 1.44). Irradiation of Me(12) likewise gave NOE enhancements of 3.9% and 3.4% for Me(14) (δ 1.69) and Me(15), respectively, and irradiation of Me(14) enhanced both Me(12) (3.4%) and OAc(7) (δ 2.15 ppm, 1.6%). Unfortunately, irradiation at H(1) (δ 4.79) was inconclusive, and overlap of H(9) with H(8a) and of H(7) with H(13) precluded analysis of the relative configurations at C(1), C(9), and C(13).

The preparation of tris(desacetyl)pyripyropene A (**5**)[4b,9] allowed us to establish the syn relationships among H(1), H(7), and H(9) (Figure 1b). Irradiation of H(1) at δ 3.61 gave an NOE enhancement of H(9) (δ 1.46) of 3.8%. Irradiation at H(7)

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(2) Heider, J. G. In *Pharmacological Control of Hyperlipidaemia*; Fears, R., Ed.; J. R. Prous Science Publishers: Barcelona, Spain, 1986; pp 423–438.

(3) Sliskovic, D. R.; White, A. D. *Trends Pharmacol. Sci.* **1991**, *12*, 194–199.

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(5) Other natural ACAT inhibitors include beauvericin, a cyclodepsipeptide, and AS-183, (IC_{50} 3.0 and 0.94 μM, respectively). Currently, the best synthetic inhibitors are DuP-128 (Billheimer, J. T.; Cromley, D. A.; Higley, C. A.; Wexler, R. R.; Robinson, C. S.; Gillies, P. J. *Abstracts of the 9th International Symposium on Atherosclerosis*; Rosemont, IL, 1991; p 94), CP-113,818 (Chang, G.; Hamanaka, E. S.; McCarthy, P. A.; Walker, F. J.; Diaz, T. L.; Johnson, D. A.; Kraus, K. G.; Maloney, M. E.; Martingano, R. J.; Wint, L. T.; Marzetta, C. A.; Goldberg, D. I.; Freeman, A. M.; Long, C. A.; Pettini, J. L.; Savoy, Y. E. *Abstracts of Papers*, 206th National Meeting of the American Chemical Society, Chicago, IL; American Chemical Society: Washington, DC, 1993; MEDI 46), PD-129,337 (Trivedi, B. K.; Holmes, A.; Stoeber, T. L.; Blankley, C. J.; Roark, W. H.; Picard, J. A.; Shaw, M. K.; Essenburg, A. D.; Stanfield, R. L.; Krause, B. K. *J. Med. Chem.* **1993**, *36*, 3300–3307), and KF-17,828 (Kumazawa, T.; Yanse, M.; Harakawa, H.; Obase, H.; Shirakura, S.; Ohishi, E.; Oda, S.; Kubo, K.; Yamada, K. *J. Med. Chem.* **1994**, *37*, 804–810), with IC_{50} values of 18, 22, 17, and 23 nM, respectively.

(6) (a) Isolation of territrems A and B: Ling, K.-H.; Yang, C.-K.; Peng, F.-T. *Appl. Environ. Microbiol.* **1979**, *37*, 355–357. Territrem C: Ling, K.-H.; Liou, H.-H.; Yang, C.-M.; Yang, C.-K. *Appl. Environ. Microbiol.* **1984**, *47*, 98–100. (b) Crystal structure of territrem B: Hseu, T. H.; Yang, C.-K.; Ling, K.-H.; Wang, C. J.; Tang, C. P. *Cryst. Struct. Commun.* **1982**, *11*, 199–206. (c) Bioactivity: Arvanov, V. L.; Ling, K.-H.; Chen, R.-C.; Tsai, M.-C. *Neurosci. Lett.* **1993**, *152*, 69–71.

(7) Lee, C.-Y.; Ling, K.-H.; Wang, C.-T. *Thromb. Haemostasis* **1991**, *65*, 1078.

(8) In the steady-state NOE difference studies we employed the Kinns–Sanders frequency cycling method, typically with 4–5 s total irradiation time per multiplet. Kinns, M.; Sanders, J. K. M. *J. Magn. Reson.* **1984**, *56*, 518–520.

(9) Tris(desacetyl)pyripyropene A (**5**) (1.1 mg) was reconverted to semisynthetic pyripyropene A (**1**) by treatment with acetic anhydride (2.5 μL, 3.7 equiv), triethylamine (5.0 μL, 5 equiv), and DMAP (1 crystal) in THF (150 μL) at room temperature for 2 h. Extractive workup (H_2O/CH_2Cl_2) followed by preparative TLC (0.25 mm × 20 × 20 cm; 5% methanol/chloroform eluant) furnished **1**, identical with natural pyripyropene A (^1H NMR, FAB-HRMS, and TLC in two solvent systems), in 79% yield.

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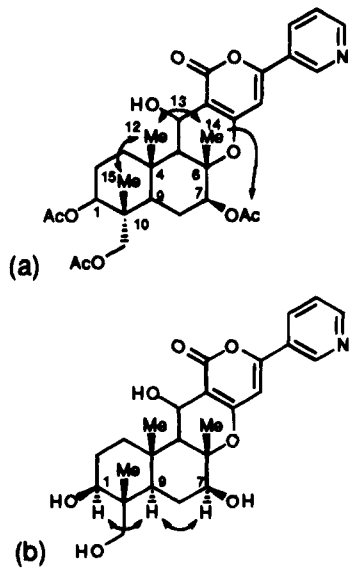


Figure 1. NOE enhancements for (a) pyripyropene A (**1**) (250 MHz in $CDCl_3$) and (b) tris(desacetyl)pyripyropene A (**5**) (500 MHz in CD_3OD).

(δ 3.73) in turn afforded a 4.7% enhancement of H(9), and irradiation at H(9) led to 6.4% and 6.7% enhancements for H(1) and H(7), respectively. All of these findings were ultimately verified and the relative conformations at C(5) and C(13) elucidated via single-crystal X-ray analysis of **1** (Figure 2).[10]

Having defined the complete relative stereochemistry of **1**, we next sought to determine the absolute configuration via a modification of the Mosher NMR method.[11,12] The tris-Mosher ester derivatives **9** and **10** were prepared by treatment of **5** with (*S*)-(−)- and (*R*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetic (MTPA) acid in the presence of DCC and DMAP (THF, room temperature).[11] The ^{1}H NMR spectra of **9** and **10** could be completely assigned via selective ^{1}H decoupling (see supplementary material for details). Comparison of the ^{1}H chemical shifts in **9** and **10** (Figure 3) and application of the Kakisawa–

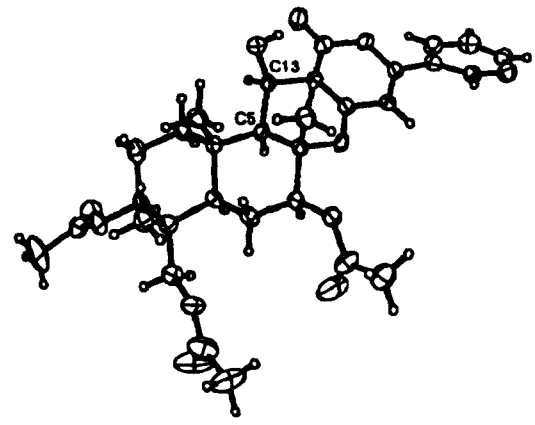
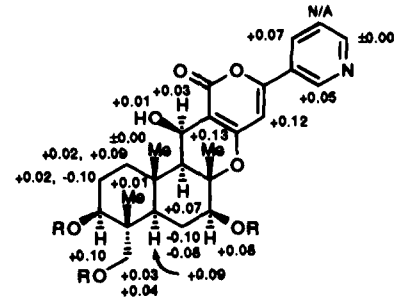


Figure 2. ORTEP plot for crystalline pyripyropene A (**1**).



9 R = *(S)*-MTPA
10 R = *(R)*-MTPA

Figure 3. Absolute stereochemistry determination: $\Delta\delta$ values (ppm, 500 MHz, $\Delta\delta = \delta_S - \delta_R = \delta_9 - \delta_{10}$) for the tris-Mosher ester derivatives **9** and **10**.

Kashman test[12d,e] revealed that the absolute configurations at C(1) and C(7) are *S*.

In view of the common biosynthetic origin of the pyripyropenes, we presume that congeners B–D (**2**–**4**) share the relative and absolute stereochemistry of **1**; the structurally related territrems A–C (**6**–**8**) may embody the same absolute stereochemistry as well. Studies directed toward the total synthesis of the pyripyropenes will be reported in due course.

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Supplementary Material Available: Tables of X-ray and NMR data and procedures for the preparation of **9** and **10** (10 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

(10) The crystal of **1** (0.06 × 0.30 × 0.36 mm, from methanol/water) belonged to the $P2_12_12_1$ space group with a = 7.430(1) Å, b = 10.528(2) Å, and c = 38.639(8) Å and four $C_{31}H_{37}NO_{10}$ molecules in the unit cell. A 1-Å data set (maximum sin θ/λ = 0.5) was collected at room temperature. The structure was solved and refined for 1846 nonzero reflections ($I > 3.0\sigma$) using the SHELXTL series of programs. The methyl and hydroxyl hydrogens were located via difference Fourier techniques. The hydrogen parameters were included in the structure factor calculations but not refined. The final model was refined to an R index of 5.27%. The absence of a suitable heavy atom precluded the determination of absolute configuration. Additional crystallographic data are included as supplementary material.

(11) Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543–2549. Dale, J. A.; Mosher, H. S. *J. Am. Chem. Soc.* **1973**, *95*, 512–519.

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