labeled sodium [1-^{13}C,$^{18}O_2$]acetate (isotopic purity: 90% ^{13}C, 18% $^{18}O_1$, 81% $^{18}O_2$)[8g] into ravenelin (**1**) with growing cultures of *Drechslera ravenelii* CBS 200.29.[2]

Expansion of the 100.6-MHz ^{13}C NMR spectra of **1** showed that isotopically shifted signals were present at C-1, C-8, C-9, and C-10a (Table I), but the peaks were disappointingly broad and poorly resolved. Since previous experience had demonstrated that attachment of electron-withdrawing groups on hydroxyl oxygens increases ^{18}O isotope shift magnitude,[6d,8d] the [^{13}C, ^{18}O] ravenelin (**1**) was converted to its triacetate, **7**.[1] The ^{13}C NMR spectra of **7** confirmed[17] the presence of ^{18}O at the four sites, but the signals were still complicated due to long-range carbon–carbon couplings. The cause of this common problem, which often obscures the small ^{18}O isotope shifts, is excess incorporation of [^{13}C, ^{18}O] precursor.[8e-g] This results in multiple ^{13}C labels within a single molecule. Fortunately this difficulty can be overcome by inverting only the uncoupled ^{13}C–^{16}O and ^{13}C–^{18}O singlets with a spin-echo Fourier transform (SEFT) NMR sequence: $(90°-\tau-180°-\tau-\text{acquisition}-T)_N$, where $\tau = 1/(2J_{CC})$ and J_{CC} is the estimated long-range coupling constant.[8g,18] Application of SEFT ^{13}C NMR spectrometry to **7** eliminated interference from coupling and permitted exact measurement of isotope shift magnitudes. Although the isotope ratios in ^{13}C-coupled spectra and in SEFT experiments must be viewed with caution,[8g] comparison of all results showed that the $^{16}O/^{18}O$ ratios in Table I are approximately correct (±5–10%).

The low natural abundance of ^{13}C (1.1%) and the relatively limited chance of incorporating two neighboring ^{13}C labels into the same molecule enable detection of intact carbon–oxygen bonds by ^{13}C NMR spectrometry through use of doubly labeled [^{13}C,^{18}O] precursor. In contrast, such information is not easily accessible through mass spectrometry. Appearance of a significant isotope shift at C-10a, but not at C-4a, which is also derived from the carboxyl of acetate, demonstrates that ravenelin **1b** (Scheme II) is present. Since the two carbon labeling patterns in ring A of **1** occur in about 1:1 ratio,[3b] the total amount of ^{18}O at C-10a and C-8 (Table I) indicates that the hydroxyl group in ring A of possible intermediates[19] **4** or **5** must have had a ^{16}O:^{18}O ratio close to 30:70. Comparing this value to the amounts of ^{18}O present in ravenelin (**1**) at C-1 and C-9 (which represent maximal oxygen labeling except for exchange during biosynthesis[20]) suggests xanthone ring closure proceeds by nucleophilic attack of the ring A hydroxyl group of **6a** or **6b** on the ortho position of ring B. The eliminated ring B ortho substituent need not be hydroxyl, but **6** is a chemically reasonable[10] hypothetical intermediate. Its potential precursors, chrysophanol (**4**) and islandicin (**5**), have not yet been proven to be on the direct biogenetic pathway; however, carbon[21] and oxygen[22] labeling studies on **5** agree with their possible involvement.[19]

Although our results do not exclude a small proportion of cyclization in the opposite sense, they do demonstrate that **1b** and **1c** represent the major acetate-derived carbon–oxygen labeling patterns of ravenelin. Possible participation of deoxygenated intermediates that undergo aerobic aromatic hydroxylation (except at C-4 of **1**) is also eliminated. This methodology is applicable not only to investigations of xanthone biosynthesis but, more generally, to studies on cyclization mechanisms involving attack by one oxygen-bearing carbon on another.

Acknowledgment. We thank the Stable Isotopes Resource of Los Alamos National Laboratory for generous gifts of [^{18}O]water and the Natural Sciences and Engineering Research Council of Canada for financial support (Grant A0845 and NSERC summer studentship to J.G.H.). We are very grateful to Professor M. A. Pickard (University of Alberta) for advice on culturing *D. ravenelii*.

Registry No. 1, 479-44-7; triacetate **7**, 80754-76-3.

(17) The ^{13}C NMR spectra of **7** was assigned by comparison to **1** by using substituent effects in xanthones and by examination of ^1H-coupled spectra of unlabeled **7** (Westerman, P. W.; Gunasekera, S. P.; Uvais, M.; Sultanbawa, S.; Kazlauskas, R. *Org. Magn. Reson.* **1977**, *9*, 631–636. Sundholm, E. G. *Acta Chem. Scand., Ser. B* **1978**, *B32*, 177–181). The assignments ($CDCl_3$, ppm relative to Me_4Si) are 173.9 (C-9), 169.6 (Ac), 169.5 (Ac), 168.0 (Ac), 156.2 (C-8), 150.2 (C-10a), 148.6 (C-4a), 146.8 (C-1), 138.2 (C-3), 135.3 (C-4), 134.4 (C-6), 119.8 (C-2), 118.9 (C-7), 115.9 (C-5), 115.4 (C-8a), 114.3 (C-9a), 21.1 (2 × Ac), 20.2 (Ac), 16.5 (C-11).

(18) (a) Rabenstein, D. L.; Nakashima, T. T. *Anal. Chem.* **1979**, *51*, 1465A–1474A and references therein. (b) Brown, D. W.; Nakashima, T. T.; Rabenstein, D. L. *J. Magn. Reson.* **1981**, *45*, 302–314.

(19) Dr. Thomas J. Simpson (Chemistry Department, University of Edinburgh) has isolated trace amounts of islandicin (**5**) along with ravenelin (**1**) (personal communication). After submission of this manuscript chrysophanol (**4**) was shown to co-occur with ravenelin (**1**) (Van Eijk, G. W.; Roeymans, H. J. *Exp. Mycol.* **1981**, *5*, 373–375).

(20) Normalization of ^{13}C NMR spectra of [^{13}C,^{18}O]ravenelin (**1**) to unlabeled **1** shows about 10-fold total ^{13}C enhanced at each labeled carbon. The ^{16}O:^{18}O isotope ratios indicate a little less than 50% loss of ^{18}O to the medium after accounting for precursor isotopic purity and removal of one of the acetate oxygens in the biogenetic process.

(21) Casey, M. L.; Paulick, R. C.; Whitlock, H. W. *J. Org. Chem.* **1978**, *43*, 1627–1634.

(22) Gatenbeck, S. *Acta Chem. Scand.* **1960**, *14*, 296–302.

Nocardicin A Biosynthesis: Stereochemical Course of Monocyclic β-Lactam Formation

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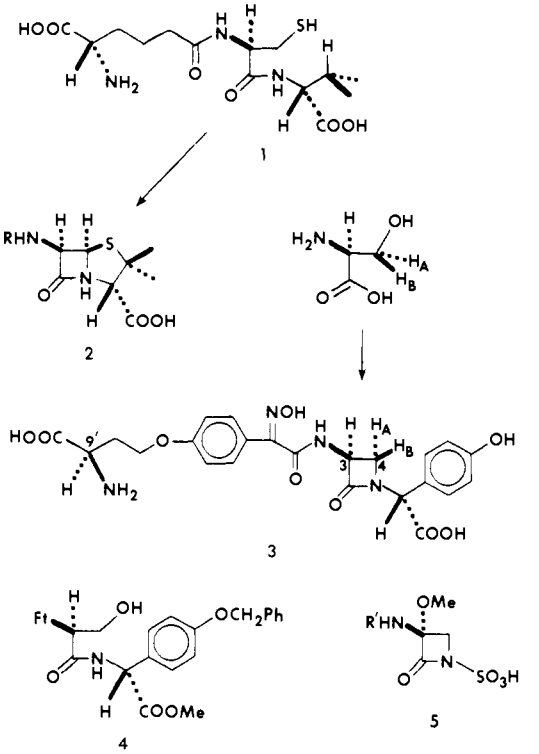
Unlike the complex and poorly understood sequence of oxidative reactions that occur in vivo to convert the Arnstein tripeptide **1**



to isopenicillin N (**2**) [R = δ-(L-α-aminoadipyl)],[1] biosynthetic results obtained in these laboratories[2] have clearly shown that the amino acid precursors of nocardicin A (**3**), the L isomers of methionine, (*p*-hydroxyphenyl)glycine, and serine, are assembled

(1) O'Sullivan, J.; Bleaney, R. C.; Huddleston, J. A.; Abraham, E. P. *Biochem. J.* **1979**, *184*, 421–26. Konomi, T.; Herchen, S.; Baldwin, J. E.; Yoshida, M.; Hunt, N. A.; Demain, A. L. *Ibid.* **1979**, *184*, 427–30. For a review see: O'Sullivan, J.; Abraham, E. P. In "Antibiotics: Biosynthesis"; Corcoran, J. W., Ed.; Springer Verlag: Berlin, 1981; Vol. 4, pp 101–22.

(2) Townsend, C. A.; Brown, A. M. *J. Am. Chem. Soc.* **1981**, *103*, 2873–74. See also: Hosoda, J.; Tani, N.; Konomi, T.; Ohsawa, S.; Aoki, H.; Imanaka, H. *Agric. Biol. Chem.* **1977**, *41*, 2007–12.

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Table I

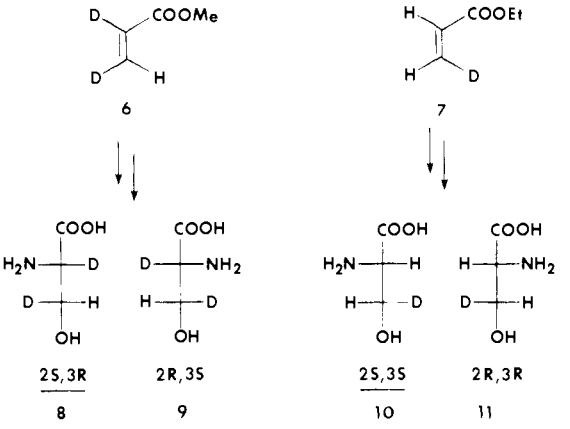
amino acid[a]	^{14}C spec incorp	$^{3}H/^{14}C$ amino acid	$^{3}H/^{14}C$ 3	% ^{3}H retained
L-[3-^{3}H, U-^{14}C] ser	8.2	4.87	4.19	86
L-[3-^{3}H, 3-^{14}C] ser	5.8	3.74	3.67	98
L-[2-^{3}H,1-^{14}C] ser	5.2[b]	4.96	0.93	19
D-[2-^{3}H,1-^{14}C] ser	0.24[b]	4.94		[c]

[a] Specific activities and $^{3}H/^{14}C$ ratios were determined for the respective *N*-tosyl serines. [b] The optical purities of the L- and D-serines were 96% and 85% respectively. These precursors were fed in the additional presence of 0.4 mM L-methionine to maximize production of 3 (note: 1 mM L-methionine described in ref 2 is in error). [c] The level of radioactivity was too low to obtain a meaningful $^{3}H/^{14}C$ ratio.

at the proper oxidation state for β-lactam formation. In double-label experiments where tritium was situated at the serine β carbon, incorporation into nocardicin A (**3**) unambiguously revealed that no change in oxidation state took place at this center during the course of four-membered ring formation (Table I).[2] Generation of a peptide precursor prior to β-lactam formation being presumed, as is now known[1] to be the case in penicillin biosynthesis (cf. **1**), the most direct mechanistic rationale for the cyclization process was taken to be nucleophilic displacement by amide nitrogen of the presumably activated seryl hydroxyl.[3] Phosphorylation, while not the only potential means for such activation in vivo,[6] has received support[7] in vitro in the cyclization under Mitsunobu conditions[8] of optically active dipeptide **4** (Ft = phthalimido). The expectation of configurational inversion at the serine β carbon in the course of a single nucleophilic displacement is borne out in the known stereochemical course of the Mitsunobu reaction at primary and secondary carbinol centers.[8,9] As reported in this communication, chiral labeling studies demonstrate inversion of configuration at the serine β center on incorporation into nocardicin A (**3**), an observation in keeping with the proposed role of amide nitrogen as the sole nucleophile involved in β-lactam formation in vivo. By contrast, in the oxidative cyclization of **1** to isopenicillin N (**2**) the β-lactam ring is formed with net stereochemical retention.[10]

Prior to carrying out the envisioned incorporations of diastereotopically labeled [3-^{2}H]serines, the fate of the α hydrogen of this amino acid was investigated. D,L-[2-^{3}H]Serine was prepared according to a known procedure[11] and admixed with a ^{14}C internal standard. Resolution of the *N*-acetyl derivative[12] afforded the L and D doubly labeled antipodes, which were administered to growing cultures of *Nocardia uniformis* subsp. *tsuyamanensis* (ATCC 21806) as before.[2] As shown in Table I, incorporation of carbon label was at least[13] 20 times more efficient for the L isomer. Extensive but not complete loss (81%) of α tritium from this isomer was observed, most probably owing to reversible transamination to hydroxypyruvate prior to incorporation into the antibiotic.[14]

Methyl (*E*)-[2,3-$^{2}H_2$]acrylate (**6**) and ethyl (*Z*)-[3-^{2}H]acrylate



(**7**) were prepared essentially according to the method of Hill.[15] The deuterated esters were separately converted largely to racemic pairs of the diastereomers **8**/**9** and **10**/**11** by the Walsh–Cheung procedure[16] as modified by Benkovic and Slieker.[17] Interpretation of mass spectra of appropriate synthetic intermediates and the 300-MHz ^{1}H NMR spectra of the racemic serines at pD 12.4[18] indicated diastereomeric purities at the respective β carbons of 86 ± 3%.

Before we proceed to the incorporation of the deuterium labeled serines, several points merit attention. First, the high retention of tritium at the β carbon of serine on incorporation into nocardicin A (**3**) (Table I) gave grounds for the hope that serine hydroxymethylase would not significantly reduce the diastereotopic purities of the ^{2}H-labeled serines **8**/**9** and **10**/**11** during the course of their incorporation into **3**. Second, the markedly lower specific incorporation of D-serine (Table I, cf. ref 13) made it possible to administer the racemates **8**/**9** and **10**/**11** without prior resolution. Third, the hydrogens bound to the β-lactam carbons form an AMX spin system, H-3, H-4A, and H-4B having chemical shifts in D_2O relative to DSS (sodium 4,4-dimethyl-4-silapentanesulfonate) at 25 °C of 4.99, 3.83, and 3.18 ppm, respectively,[19] fortuitously well-spaced resonances eminently suited to the application of ^{2}H NMR spectroscopy.

Therefore, the racemic ^{2}H-labeled serines **8**/**9** and **10**/**11** were separately administered to cultures of *Nocardia uniformis* after 3 days of growth. The nocardicin A (**3**) produced was isolated, purified, and crystallized as previously described.[2] The sites of deuterium enrichment were determined by ^{2}H NMR spectroscopy

(3) Relevant to this problem are very recently reported biosynthetic results for monobactam **5** (R′ = acetyl or D-glutamyl-D-alanyl, the latter also known as sulfazecin) which suggests that like nocardicin A, its β-lactam carbons are derived from serine[4,5] and that the cyclization process proceeds without change in oxidation state at the seryl β position.[5]

(4) Imada, A. "Symposium: Biosynthesis of β-Lactam Antibiotics", 21st Interscience Conference on Antimicrobial Agents and Chemotherapy, American Society for Microbiology, Chicago, IL, November 1981. See also: Imada, A.; Kitano, K.; Kinotaka, K.; Muroi, M.; Asai, M. *Nature* **1981**, *289*, 590–1.

(5) O'Sullivan, J.; Aklonis, C. A.; Gillum, A. M.; Souser, M. L.; Sykes, R. B. 21st Interscience Conference on Antimicrobial Agents and Chemotherapy, American Society for Microbiology, Chicago, IL, November, 1981, abstract 877. See also: Sykes, R. B.; Cimarusti, C. M.; Bonner, D. P.; Bush, K.; Floyd, D. M.; Georgopapadakou, N. H.; Koster, W. H.; Liu, W. C.; Parker, W. L.; Principe, P. A.; Rathnum, M. L.; Slusarchyk, W. A.; Trejo, W. H.; Wells, J. S. *Nature* **1981**, *291*, 489–91.

(6) In view of the structures of the various isolated monobactams,[5] sulfation is an interesting possibility for hydroxyl activation and inter alia protonation or acylation cannot be ignored.

(7) Townsend, C. A.; Nguyen, L. T. *J. Am. Chem. Soc.* **1981**, *103*, 4582–83.

(8) Mitsunobu, O. *Synthesis* **1980**, 1. Either triphenylphosphine or triethylphosphite could be used with equal success in this cyclization.

(9) For example: Townsend, C. A.; Neese, A. S.; Theis, A. B. *J. Chem. Soc., Chem. Commun.* **1982**, 116–8. Miller, M. J.; Mattingly, P. G.; Morrison, M. A.; Kerwin, J. F., Jr. *J. Am. Chem. Soc.* **1980**, *102*, 7026–32.

(10) Aberhart, D. J.; Liu, L. J.; Chu, J. Y. R. *J. Chem. Soc. Perkin Trans. 1* **1975**, 2517–23. Morecombe, D. J.; Young, D. W. *J. Chem. Soc., Chem. Commun.* **1975**, 198–199. Young, D. W.; Morecombe, D. J.; Sen, P. K. *Eur. J. Biochem.* **1977**, *75*, 133–47.

(11) Miles, E. W.; McPhie, P. *J. Biol. Chem.* **1974**, *249*, 2852–57.

(12) Greenstein, J. P.; Winitz, M. "Chemistry of the Amino Acids"; Wiley: New York, 1961; pp 2229–34.

(13) The low but positive incorporation of D-serine parallels earlier observations with D-(*p*-hydroxyphenyl)glycine and is almost certainly due in part if not totally to incorporation of the L isomer present (see footnote *b*, Table I). Evidently enzymic racemization and/or possibly transport proceeds at a very low rate.

(14) In penicillin biosynthesis, analogous evidence against an α,β-dehydrocysteine-containing intermediate has been obtained: Arnstein, H. R. V.; Crawhall, J. C. *Biochem. J.* **1957**, *67*, 180–87. Bycroft, B. W.; Wels, C. M.; Corbett, K.; Lowe, D. A. *J. Chem. Soc., Chem. Commun.* **1975**, 123–25.

(15) Hill, R. K.; Newkome, G. R. *J. Org. Chem.* **1969**, *34*, 740–1. Vaughan, W. R.; Milton, K. M. *J. Am. Chem. Soc.* **1952**, *54*, 5623–30.

(16) Cheung, Y.-F.; Walsh, C. *J. Am. Chem. Soc.* **1976**, *98*, 3397–8.

(17) Slieker, L.; Benkovic, S. J. *J. Labelled Compd. Radiopharm.*, in press.

(18) Ogura, H.; Arata, Y.; Fujiwara, S. *J. Mol. Spectrosc.* **1967**, *23*, 76–85.

(19) Extensive homonuclear decoupling experiments of **3** in D_2O at 300 MHz, 25 °C, and pD 8 revealed that H-4A and H-9′ were incorrectly assigned[9] and should be reversed, i.e., H-4A at δ 3.83 and H-9′ at δ 3.94.

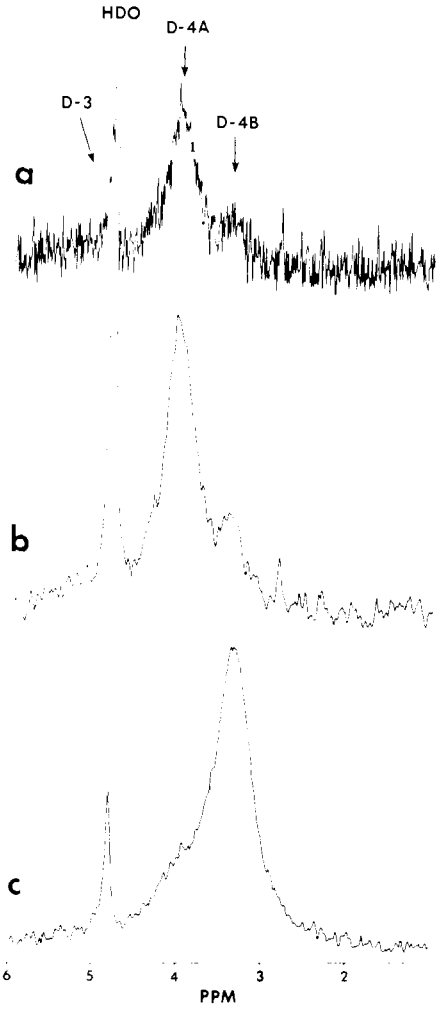


Figure 1. ^2H NMR spectra of nocardicin A (**3**) obtained from incorporations of diastereotopically ^2H labeled serines **8/9** and **10/11** acquired under the following conditions: Bruker WM-300, 46.1 MHz; spectral width 2000 Hz, 4K points, acquistion time 4.096 s; 90° pulse. (a) A 55-mg sample in 2.5 mL of deuterium-depleted water;[20] 52 250 transients, zero filling the FID by successive transfers into 8K and 16K of zeros prior to Fourier transformation. (b) As in (a), 51 715 transients, sensitivity enhancement achieved by treatment of FID with 1.5-Hz line broadening. (c) A 150 mg sample in 2.5 mL of deuterium-depleted water;[20] 23 505 transients, FID treated as in (a) and (b) but with 1.0-Hz line broadening.

at 46.1 MHz in deuterium-depleted water (Aldrich) at 45 °C, pH 7.6.[20] The spectral data obtained under conditions of broad-band proton decoupling are depicted in Figure 1.

A sample of nocardicin A derived from fermentation in the presence of the (2*S*,3*R*)/(2*R*,3*S*)-serines **8/9** gave spectrum a. As was hoped for (cf. Table I), a degree of deuterium enrichment at C-3 was detectable as a weak signal on the downfield side of the HDO resonance. Application of 1.5-Hz line broadening to the FID that gave rise to spectrum a generated spectrum b. The D-3 resonance was now merged with the comparatively intense HDO signal but the distribution of deuterium label at C-4 was clearly discernible, the A position bearing approximately 85% of the heavy isotope. An entirely complementary result was obtained from a second specimen of nocardicin A derived from the diastereomeric (2*S*,3*S*)/(2*R*,3*R*)-serines **10/11**, whose ^2H NMR analysis is displayed as spectrum c. In summary, therefore, L-serine bearing a label at the (3*R*)-locus gave rise to enhanced ^2H content at position 4A while a (3*S*)-label specifically enriched position 4B; that is, within the accuracy of the method, the stereochemical course of β-lactam formation in vivo is substantially, if not exclusively, inversion.

The most direct interpretation of the stereochemical results is a simple nucleophilic displacement of presumably activated seryl hydroxyl by amide nitrogen in an at present hypothetical peptide intermediate to form the critical β-lactam ring. Alternative mechanisms involving intermediate participation of an enzyme nucleophile, whether by displacement or elimination/addition (loss of serine α hydrogen) are of diminished likelihood.

Acknowledgment. We thank Y.-F. Cheung (Merck) and S. J. Benkovic (Penn State) for generously providing experimental details for the serine syntheses, S. B. Christensen of these laboratories for obtaining the ^2H NMR spectra, W. G. Egan (Bureau of Biologics, FDA) and D. E. Cane (Brown University) for helpful technical suggestions regarding ^2H NMR spectroscopy, and M. Koreeda (University of Michigan) for mass spectral analyses. The National Institutes of Health are gratefully acknowledged for financial support (Grants A1 14937 and RR 07041) 07041) and for providing partial funding to acquire the Bruker WM-300 used (Grant GM 27512).

Registry No. 3, 39391-39-4; (±)-**8**, 80612-42-6; (±)-**10**, 80612-43-7; L-serine, 56-45-1.

(20) Line widths at half height for the C-4 deuterons were approximately 30 Hz at 25 °C, broader than expected presumably owing to aggregation (spectrum c was recorded in a solution close to saturation at 25 °C, 0.12 M). So that the rotational correlation times could be reduced, the samples were heated to 45 °C, giving line widths of about 15 Hz. For a demonstrated case of intermolecular association in a species of similar molecular weight investigated by ^2H NMR see: Egan, W. *J. Am. Chem. Soc.* **1976**, *98*, 4091-93. The deuterium-depleted water had a ^2H content 3.3×10^{-3} times natural abundance.

Irreversible Inhibition of the Enzymic Oxidation of Arachidonic Acid to 15-(Hydroperoxy)-5,8,11(*Z*),13(*E*)-eicosatetraenoic Acid (15-HPETE) by 14,15-Dehydroarachidonic Acid

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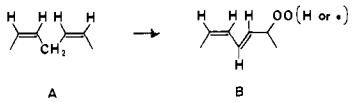
The biosynthesis of physiologically important eicosanoids such as prostaglandins and leukotrienes from arachidonic acid involves an initial oxidation of the lipoxygenase type in which a *cis,cis*-1,4-pentadiene unit (**A**) is transformed into a 1-oxygenated *cis,trans*-2,4-pentadiene moiety (**B**).



Aspirin, indomethacin, and a number of other nonsteroidal antiinflammatory agents (NSIA's) have been found to inhibit the biosynthesis of prostaglandins (PG's) by blocking the initial step, a lipoxygenase reaction of arachidonic acid at C-11 catalyzed by the so-called cyclooxygenase (CO) enzyme.[1,2] Although the mechanism of action of these empirically discovered and therapeutically valuable PG biosynthesis inhibitors is unclear, they seem to block only the 11-lipoxygenase and to be ineffective as inhibitors of other biooxidation modes of arachidonic acid, for example, the 5-lipoxygenase (leukotriene) pathway.[3] In contrast, eicosa-5,8,11,14-tetraynoic acid (ETYNA) inhibits 11- and 12- but not 5-lipoxygenase reactions of arachidonic acid when used at sufficiently high concentration.[2] Because of the significant biological role of eicosanoids formed by the lipoxygenation of arachidonic acid at different positions (5, 11, 12, and 15) we have been attempting to develop position-selective inhibitors that can selectively block any of the arachidonate oxidation pathways. This com-

(1) Schaaf, T. K. *Annu. Rep. Med. Chem.* **1977**, *12*, 182.
(2) Bailey, D. M.; Chakrin, L. W. *Annu. Rep. Med. Chem.* **1981**, *16*, 213.
(3) Borgeat, P.; Samuelsson, B. *Proc. Natl. Acad. Sci. U.S.A.* **1979**, *76*, 3213.

0002-7863/82/1504-1750$01.25/0