1.08, CHCl₃).

To a suspension of CuBr (13 mg, 0.1 mmol) in ether (1 mL) at 0 °C was added allylmagnesium bromide (2.2 mL, 0.48 mmol), followed by the above-obtained tosyl-triflate 15 in ether (3 mL), and the reaction mixture was stirred at the same temperature for 3.5 h. Then n-Bu₂CuLi (1.4 mmol) in ether (3 mL) was introduced and the mixture was stirred at room temperature for 12 h. usual workup followed by column chromatography gave 62 mg (58%) of 14.

Preparation of L-Factor [(4S,5S)-(+)-5-Hydroxy-4-deca-

Results and Discussion

Relative Stereochimetry of 2. The ¹H NMR data acquired by us for LL-C10037a were identical to that reported in the literature¹ (Table I). The only difference was in the interpretation of the chemical shifts of the two

| Table I |
|-----------------|-----------------|
| H   | ¹H NMR (400 MHz, DMSO-d₆) | ¹³C NMR (100.6 MHz, DMSO-d₆) |
|     | δ multiplicity, J, Hz | δ     |
| 1   | 7.04 dd, J = 2.5, 2.7 | 189.6  |
| 2   | 7.18 dd, J = 2.5, 2.7 | 128.3  |
| 3   | 7.16 dd, J = 3.1, 2.7 | 192.5  |

| 2' (CH₃) | 2.04 s |
| OH       | 5.79 d, J = 6.4 |
| NH       | 9.04 br s |

Introduction

The antitumor metabolite LL-C10037α was isolated from Streptomyces LL-C10037 by researchers at Lederle Laboratories, and its gross structure was reported as ¹. This structure represented the first reported occurrence of a γ-aminooxyepoxymiquinone, and we wanted to explore its bioorganic implications. As the initial part of our effort we repeated the spectral analysis with an authentic sample of LL-C10037α in order to confirm the structural assignment made earlier and to determine the relative configuration. In this paper data are presented for revising the structure of LL-C10037α, for establishing the structures of two additional metabolites produced by the same organism, and for defining the absolute stereochemistry of each metabolite.

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Supplementary Material Available: Experimental data for 5, 6, 8-10, and 14 (3 pages). Ordering information is given on any current masthead page.
acidic protons in the spectrum acquired in DMSO-d$_6$. Whereas we assigned the doublet at 5.8 ppm ($J = 6.4$ Hz) to the OH proton and the broad singlet at 9.0 ppm to the NH proton, the previous workers made the reverse assignments. Based on our interpretation of the NMR data, LL-C10037$\alpha$ should be 2-acetamido-4-hydroxy-5,6-epoxyquinol, 2. This correction was confirmed by X-ray crystallography, which also indicated that the atoms C-2, C-3, C-5, and C-6 are roughly in a plane and C-1 and C-4 are both displaced to the same side of this plane. The hydroxyl group extended in an equatorial direction and bore a cis relationship with the oxygen of the oxirane ring. Thus, 2 would be the cis equatorial stereoisomer shown, or its mirror image. Remarkably, therefore, 2, $[\alpha]^{D0}_{20}$ $-$202$^\circ$ (c 0.334, MeOH), has the same gross structure and relative stereochemistry as (+)-MT35214, $[\alpha]^{D0}_{20}$ +104$^\circ$ (c 1, MeOH),$^5$ obtained by acetylation of antibiotic MM14201 produced by Streptomyces sp. NCIB 11813.$^5$ The difference in the sign of their specific rotations indicates that they apparently form an enantiomeric pair.$^4$

2 was oxidized to the epoxyquinone 3, $[\alpha]^{D0}_{20}$ +115.6$^\circ$ (c 0.5, MeOH), and its specific rotation compared to that which was reported for the corresponding epoxyquinone MT36531, $[\alpha]^{D0}_{20}$ $-$99$^\circ$ (c 0.5, MeOH).$^5$ The same trend was observed,$^4$ providing further evidence in support of the enantiomeric relationship.

![Diagram of compounds](image)

**Absolute Stereochemistry of 2.** A number of other epoxyquinols have been isolated from natural sources: (+)-epoxydon,$^8$ isoeoxydon,$^6,7$ panepoxydon,$^6$ (+)-terremutin,$^4$ desoxypiepoxydon,$^4$ and chaloxone.$^9$ Generally, the absolute stereochemistry of the epoxide ring has been determined from circular dichroism (CD) data. An empirical correlation of the sign of the R band at approximately 340 nm of an epoxyquinol with compounds of known absolute stereochemistry resulted in formulation of an "inverse quadrant" rule, with the sign of the R band dictated by the oxirane oxygen atom lies.$^{11}$ In Table II the CD data for 2 and some of these other metabolites are given. Since the R band of 2 is negative ($\Delta\varepsilon_{341} -5.59$), similar to terremutin, 4 ($\Delta\varepsilon_{341} -1.64$), 2 should have the oxirane oxygen lying below the plane of the cyclohexenone ring, as shown. Having previously established that 2 is a cis stereoisomer, its absolute stereochemistry would be 4S,5S,6S. However, although numerous epoxyquinols have been assigned in this manner, we were not content to rely solely on such an empirical correlation. The epoxyquinone 3 was also analyzed by CD, and the data were compared with that of other epoxyquinones in the literature. A standard has been terreic acid, 5, whose absolute stereochemistry was established by chemical correlation with 4.$^{12}$ Such compounds exhibit two Cotton effects for n $\rightarrow$ $\pi^*$ transitions between 300 and 400 nm. These transitions have been associated with the two individual C-O chromophores, and the difference in the band positions has been ascribed to the transitions from the energetically higher n orbital of the two carbonyl

![Figure 1.](image)
groups into the π* orbital. Additionally, there may be some intramolecular hydrogen bonding at one end of the quinone system. The sign of the CD spectrum of 3 (Table III) is opposite that of 5, indicating that the former has 5R,6S stereochemistry, consistent with that assigned to 2. However, both assignments are based on the same underlying empirical rule since 5 was assigned by comparison with 4. We therefore chose to establish the absolute stereochemistry unequivocally with a nonempirical method: either the exciton chirality method, pioneered by Nakanishi, or single-crystal heavy-atom X-ray crystallographic analysis.

The p-bromobenzoate 6 was prepared in 75% yield by treating 2 with p-bromobenzoyl chloride, triethylamine, and a catalytic amount of (dimethylamino)pyridine (DMAP) in tetrahydrofuran (THF). Unfortunately, an acceptable crystal for the X-ray analysis could not be obtained. However, the CD spectrum of 6 (Figure 1a) showed a split Cotton effect (ΔE240 = -9.2 and ΔE300 = +1.9), which is due to the interaction between the bromobenzoate and the enone chromophores. Applying the exciton chirality rule, this negative first Cotton effect corresponds to a negative chirality, and the projection of the two chromophores should be counterclockwise; the resulting absolute stereochemistry is that shown.

Simultaneously, we prepared a number of urethanes of 2 with optically active isocyanates. One of these, 7, obtained by reaction with (S)-(−)-α-methylbenzyl isocyanate in THF at reflux was carefully recrystallized from toluene. This yielded a crystal suitable for X-ray analysis. The ORTEP drawing in Figure 2 clearly shows the same 4S,5S,6S,10S stereochemistry.

Structure and Absolute Stereochemistry of LL-C10037α and LL-C10037γ. Two additional metabolites of Streptomyces LL-C10037 have been isolated and purified by column chromatography on silica gel. One, more polar than 2, has been named LL-C10037β, and the other, slightly less polar than 2, has been named LL-C10037γ. The UV and IR spectra indicated that each was also a 2-acetamidocyclohexenone. From the presence of one methylene adjacent to the ketone carbonyl (δ 2.00 and 2.78), three exchangeable hydrogens, and two carbonyl protons (δ 4.17 and 4.58), the diol structure 8 was assigned to the more polar metabolite. The cis stereochemistry was derived from the H-4/H-5 coupling constant (0.4 Hz). The proton NMR spectrum of the third compound contained resonances from two adjacent methylenes, one next to the ketone, and structure 9 was therefore assigned. The 1H/1H COSY spectra of each contained all cross peaks consistent with these structures.

In order to determine the absolute stereochemistry of each of these new metabolites, the p-bromobenzoates 10 and 11 were prepared. The CD spectra of each (Figure 1, parts b, c) displayed the same negative split Cotton effect that had been observed for 6. Therefore, applying the exciton chirality rules confirmed the biogenetic expectation that these compounds have the same absolute stereochemistry as 2.

Conclusions

Antibiotic LL-C10037α has now been shown to have the epoxyquinol structure 2. Two additional related metabolites of Streptomyces LL-C10037 were isolated and characterized as 8 and 9.

While numerous other naturally occurring epoxyquinols have been reported over a 22-year period, until recently—while our work was in progress—in no case had the absolute stereochemistry of any of these been established by an unambiguous, nonempirical analysis. Absolute stereochemistry for the epoxide carbons had only been inferred from empirical correlations of the signs of Cotton effects in the circular dichroism spectra ("inverse quadrant rule"). We have prepared the carbamate 7 of 2 and (S)-(−)-α-methylbenzyl isocyanate and analyzed a single-crystal by X-ray diffraction, unambiguously yielding the absolute stereochemistry as 2.
stereochirality of 2 as 4S,5S,6S. We also prepared the p-bromobenzoate 6 of 2, as well as those—10 and 11—of 8 and 9, respectively. The CD spectra of these derivatives were analyzed for the interactions of the enone and benzoate transition moments; this nonempirical use of circular dichroism also yielded 4S absolute stereochirality for all three.

In this study as well as Scheuer's \cite{16} application of the "inverse quadrant rule" to the CD spectra of the parent epoxides yielded the correct absolute configuration for C-5/C-6. Thus, this rule may now be more reliably invoked.

Work is now proceeding toward isolation of the epoxides from Streptomyces LL-C10037 and NCIB 11813 that generate the enantiomeric epoxides of 2 and (+)-MT85214, respectively.

**Experimental Section**

**General Procedures.** $^1$H NMR spectra (400 MHz) and $^{13}$C NMR spectra (100.6 MHz) were taken on a Bruker AM 400 spectrometer. All $^{13}$C NMR spectra were broadband decoupled. Five-millimeter NMR tubes were used for all NMR measurements. $^1$H and $^{13}$C NMR samples were referenced with TMS or t-BuOH. IR spectra were recorded on a Nicolet 5DX FTIR spectrometer. Melting points were taken on a Buchi melting point apparatus (19600), 246 nm (14700); $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 2.21 (e, $J$ = 4.3 Hz), 2.13 (e, $J$ = 4.6 Hz), 1.2, 2.9 Hz); $^1$C NMR (100.6 MHz, methanol-d$_6$) $\delta$ 189.3, 172.2, 133.7, 129.8, 70.1, 68.6, 42.9, 23.9; low-resolution mass spectrum (EI) $m/z$ 265 (51700), 265 (52700), 209 (81 800); $^1$H NMR (400 MHz, methanol-d$_6$) $\delta$ 2.1 (s, 1 H), 2.67 (dd, 1 H, $J$ = 2.6, 2.9, 4.3 Hz), 6.25 (dd, 1 H, $J$ = 2.9, 3.0 Hz), 7.40 (dd, 1 H, $J$ = 2.9, 3.0 Hz), 8.02, 8.13 (AA'BB', 4 H, $J$ = 8.7 Hz), 8.4 (bs, 1 H); $^{13}$C NMR (100.6 MHz, acetone-d$_6$) $\delta$ 183.9, 170.3, 165.6, 132.9, 132.4, 131.2, 128.7, 128.9, 125.0, 65.8, 52.8, 52.1, 24.2; $^1$H NMR (acetone-d$_6$) $\delta$ 129.0, 128.6, 121.9, 125.0, 114.0, 60.9, 52.8, 52.1, 24.2; $^1$H NMR (CDCl$_3$) $\delta$ 135-136 $^\circ$C; $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.89 (1 H, bs), 7.51 (1 H, $J$ = 7.2 Hz), 3.91 (1 H, d, $J$ = 3.7 Hz), 3.83 (1 H, dd, $J$ = 3.7, 2.2 Hz), 2.22 (3 H, s); $[\alpha]_2^D$ = +115.6$^\circ$ (c, 0.5 in MeOH).

**p-Bromobenzoate of 6.** To a cold ($0^\circ$C) stirred solution of 2 (40.0 mg, 0.219 mmol) and triethylamine (24.3 mg, 0.24 mmol) in THF (2.0 mL) was slowly added p-bromobenzoyl chloride (60.0 mg, 0.273 mmol) in THF (1.0 mL). DMAP (1.4 mg, 0.012 mmol) in THF (0.7 mL) was then added, and the reaction solution was allowed to warm to room temperature. After 17 h, the reaction was quenched and extracted with CHCl$_3$ (5 x 3.0 mL). The combined organic solution was washed with H$_2$O (2 x 1.0 mL), dried over Na$_2$SO$_4$, and evaporated to give a white solid (85.0 mg). Recrystallization from hexane/THF afforded 70 mg of (+)-p-bromobenzoate of 6 (50%).

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*References*

The reaction solution was allowed to warm to room temperature. Solvent to afford 12.0 mg (20% yield) of a colorless solid mp 96-98. triethylamine (18.9 mg, 0.20 mmol), p-bromobenzoyl chloride was chromatographed again with 25% hexane/EtOAc as eluting purified on a silica gel 60 column (10 cm). The concentrated in vacuo to afford 64.0 mg of a solid, which was further remaining aqueous solution was extracted with CH$_2$Cl$_2$ (3 mL). The combined organic solution was washed with HzO (3 mL). The solution of two-necked round-bottomed flask, equipped with a condenser and a septum cap, a solution of (S)-(-)-a-Methylbenzylurethane (28.0 mg, 0.167 mmol) in CH$_2$Cl$_2$ (5.0 mL) were added dry. The ethanol was stirred at reflux under a nitrogen atmosphere for 24 h. The wine-colored material was chromatographed with 25% hexane/EtOAc to give 24.0 mg of partially pure POM bromobenzyl chloride (28.0 mg, 0.167 mmol) in THF (1.0 mL), and DMAP (1.0 mg). The reaction solution was allowed to warm to room temperature. After 30 h, the reaction mixture was cooled and quenched by adding crushed ice. The organic layer was separated, and the remaining aqueous solution was extracted with CH$_2$Cl$_2$ (3 x 2 mL). The combined organic solution was washed with HzO (3 x 1.0 mL) and dried over Na$_2$SO$_4$. The dried extract was concentrated in vacuo to afford 64.0 mg of a solid, which was further purified on a silica gel 60 column (10 x 1.5 cm), eluting with 50% hexane/EtOAc to give 24.0 mg of partially pure (11). The material was chromatographed again with 25% hexane/EtOAc as eluting solvent to afford 12.0 mg (20% yield) of a colorless solid: mp 96-98°C; IR (KBr) 3349, 1718, 1684, 1667, 1591, 1508, 1233, 1289, 1245, 1118, 1055, 897, 547, 757 cm$^{-1}$; UV max (e) 240 (30600), 246 nm (24800); $^1$H NMR (400 MHz, acetone-d$_6$) $\delta$ 7.9 (1 H, d, J = 7.9 Hz), 7.2-7.4 (6 H, m), 6.0 (1 H, d, J = 7.4 Hz, exch), 5.8 (1 H, m), 4.9 (1 H, d, J = 7.2 Hz), 4.0 (1 H, m), 3.6 (1 H, d, J = 3.8 Hz), 2.1 (3 H, s), 1.5 (3 H, d, J = 6.8 Hz); $^{13}$C NMR δ (100.6 MHz, CDC$_1$i) 183.0, 163.0, 154.3, 142.9, 129.2, 128.8, 127.6, 125.9, 121.3, 66.5, 51.6, 51.4, 51.1, 24.6, 22.4; [a]$_{D}^{20} = -125.6^\circ$ (c 0.05, in MeOH). Anal. Calc'd: C, 61.81; H, 5.49; N, 8.48. Found: C, 61.56; H, 5.44; N, 8.18.

X-ray Work on C$_{10}$N$_2$H$_7$(7). A crystal of dimensions 0.20 x 0.10 x 0.05 mm was used for collection of data. Unit cell parameters were refined from a least-squares analysis of the angle settings of 13 reflections in the range 22° < 2θ < 35°. Intensity data were collected with the ω-2θ scan technique and a scan speed of 2° min$^{-1}$ in ω. The intensities of three standard reflections monitored throughout the data collection exhibited an average fluctuation of 2.1%. From 1802 reflections measured to (sin θ/λ)$_{max}$ = 0.5947 Å$^{-1}$ with the range of indices 0 ≤ 0 ≤ 8 ≤ k ≤ 43, and 0 ≤ l ≤ 5, 1235 unique data having F$_{o}$ > 3σ(F$_{o}$) were obtained.

All calculations were performed on a VAX II computer with programs from the TEXRAY crystallographic software package. Atomic positions for all non-hydrogen atoms were derived from the direct methods program MITHRIL. Hydrogen atoms attached to C atoms were placed in calculated positions (C–H = 0.95 Å) and of the attached C atom. Following two cycles of least-squares refinement, the remaining hydrogen atoms, H(9) and H(11), were located from a difference electron density map; the positional parameters and an isotropic thermal parameter were subsequently refined for each of these atoms. Final refinement of F$_{o}$ with 224 variable, 1235 observations, and F$_{c}$ > 3σ(F$_{o}$) affords the residuals R = 0.035 and R$_{w}$ = 0.049, where the weights are derived from counting statistics and a value of p = 0.05. In the final cycle Δ/σ = 0.01 and the maximum excursion in the final difference electron density map = 0.32 e Å$^{-3}$. The data were not corrected for absorption.

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Kuanonamines A, B, C, and D: Pentacyclic Alkaloids from a Tunicate and Its Prosobranch Mollusk Predator Chelynotus semperi

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From a Micronesian tunicate and its predator, a prosobranch mollusk Chelynotus semperi, we have isolated five alkaloids, the known shermilamine B (1) and four new pentacyclic compounds, kuanonamines A-D (2-5).

The structures were established by extensive NMR analysis and correlations. Cytotoxicity (IC$_{50}$) against KB cells ranged from >10 μg/mL for 3 to 5 μg/mL for 1 and 5 to 1 μg/mL for 2.