Chiral Dihydroxylation of Acronycine: Absolute Configuration of Natural *cis*-1,2-Dihydroxy-1,2-dihydroacronycine and Cytotoxicity of (1*R*,2*R*)- and (1*S*,2*S*)-1,2-Diacetoxy-1,2-dihydroacronycine

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Sharpless asymmetric dihydroxylation of acronycine (**1**) gave (1*R*,2*R*)-1,2-dihydroxy-1,2-dihydroacronycine (**2**) and (1*S*,2*S*)-1,2-dihydroxy-1,2-dihydroacronycine (**3**), which allowed determination of the absolute configuration of natural *cis*-1,2-dihydroxy-1,2-dihydroacronycine as 1*R*,2*R*. The cis isomer had been previously isolated from various *Sarcomelicope* species. Benzylic reduction of isomers **2** and **3** gave the alcohols **4** (2*R*) and **5** (2*S*), respectively. Acetylation of **2** and **3** afforded the corresponding esters **6** and **7**. No significant difference of cytotoxicity was observed between these (1*R*,2*R*)- and (1*S*,2*S*)-enantiomers and the recently described, highly active racemic *cis*-1,2-diacetoxy-1,2-dihydroacronycine, when tested against L-1210 cells in vitro.

The acridone alkaloid acronycine (**1**), first isolated from *Acronychia baueri* Schott (Rutaceae),[1] was found to be active against numerous solid tumors.[2−5] Nevertheless, clinical trials gave only poor results, due to both the low water solubility and the moderate potency of acronycine.[6] We have recently reported the synthesis of some (±)*cis*-1,2-dihydroxy-1,2-dihydroacronycine esters that were more active in vivo than acronycine as antitumor drugs.[7] Indeed, these new acronycine derivatives have shown promising antitumor activity, with a broadened spectrum and an increased potency when compared with acronycine itself on several tumor strains both in vitro and in vivo. Among these esters, racemic *cis*-1,2-diacetoxy-1,2-dihydroacronycine seems of particular interest because of its high activity in vivo against P-388 leukemia and against the highly resistant tumor C-38 colon carcinoma. Therefore, it was of great interest, in a continuation of our work on the acronycine series, to study the influence of the absolute configuration on the biological activity of this compound. In addition, the chiral synthesis of the two enantiomers of *cis*-1,2-dihydroxy-1,2-dihydroacronycine should allow us to determine the absolute configuration of this alkaloid, which had been previously isolated in its optically active (−)-form from several *Sarcomelicope* species.[8,9] In this paper, we report the enantioselective preparation of (1*R*,2*R*)-1,2-dihydroxy-1,2-dihydroacronycine and (1*S*,2*S*)-1,2-dihydroxy-1,2-dihydroacronycine, according to the Sharpless methodology,[10] which gives access to the corresponding diacetates. The biological activity of these latter derivatives is evaluated.

Following the Sharpless methodology, osmium-catalyzed asymmetric dihydroxylation (AD) of acronycine (**1**) was performed using diphenylpyrimidine ligands involving either dihydroquinine [$(DHQ)_2$-PYR] or dihydroquinidine [$(DHQD)_2$-PYR] (Scheme 1).[10] From the stereoselectivity rules established by Sharpless for the AD reaction, it was concluded that the use of the $(DHQ)_2$-PYR ligand should give an enantiomeric excess of (1*R*,2*R*)-1,2-dihydroxy-1,2-dihydroacronycine (**2**) and that $(DHQD)_2$-PYR ligand should give an excess of the corresponding enantiomer (1*S*,2*S*)-1,2-dihydroxy-1,2-dihydroacronycine (**3**).

The enantiomeric excess of the chiral diols **2** and **3**, determined by chiral HPLC, was 40% and 70%, respectively. This technique was also used for the purification of the enantiomers on a preparative scale.

To ensure that the absolute configuration of each diol matched that predicted by Sharpless, we carried out a benzylic reduction of the chiral diols **2** and **3** to the corresponding homobenzylic alcohols, respectively, **4** and **5**. This was achieved by the use of $NaBH_3CN$ in the presence of ZnI_2.[11] The absolute configuration at C-2 of each 2-hydroxy-1,2-dihydroacronycine enantiomer was previously assigned in our laboratory by comparison of their ^{1}H and^{13}C NMR spectra with published data for the related diastereoisomeric glycosides of the angular hydroxydihydropyranocoumarins (+)- and (−)-lomatin.[12−14]

Furthermore, the enantioselective synthesis of acronycine diols enabled us to determine the absolute configuration of the natural (−)*cis*-1,2-dihydroxy-1,2-dihydroacronycine, previously isolated from the bark of *Sarcomelicope glauca* Hartley[15] and from the bark of *Sarcomelicope dogniensis* Hartley,[16] as 1*R*,2*R*.

Finally, the desired (1*R*,2*R*)-1,2-diacetoxy-1,2-dihydroacronycine (**6**) and (1*S*,2*S*)-1,2-diacetoxy-1,2-dihydroacronycine (**7**) were obtained in a good yield by treatment of *cis*-diols **2** and **3** with excess acetic anhydride in pyridine.

The study of the biological properties of the enatiomerically pure acronycine derivatives **6** and **7** was carried out in vitro on L-1210 leukemia. The results (IC_{50}), reported in Table 1, indicate there is no significant difference in cytotoxicity between the two enantiomers (1*R*,2*R*)- and (1*S*,2*S*)-1,2-diacetoxy-1,2-dihydroacronycine and the racemic *cis*-1,2-diacetoxy-1,2-dihydroacronycine.

Experimental Section

General Experimental Procedures. Optical rotations were obtained on a Perkin−Elmer 241 polarimeter. ^{1}H NMR [chemical shifts δ (ppm), J (Hz)] and ^{13}C NMR spectra were recorded at 300 and 75 MHz, respectively, using a Bruker AC-300 spectrometer. Chemical ionization mass spectra (DICMS; NH_3, positive ion mode) and electronic impact mass spectra (EIMS) were recorded on a Nermag R 10-10C spectrometer.

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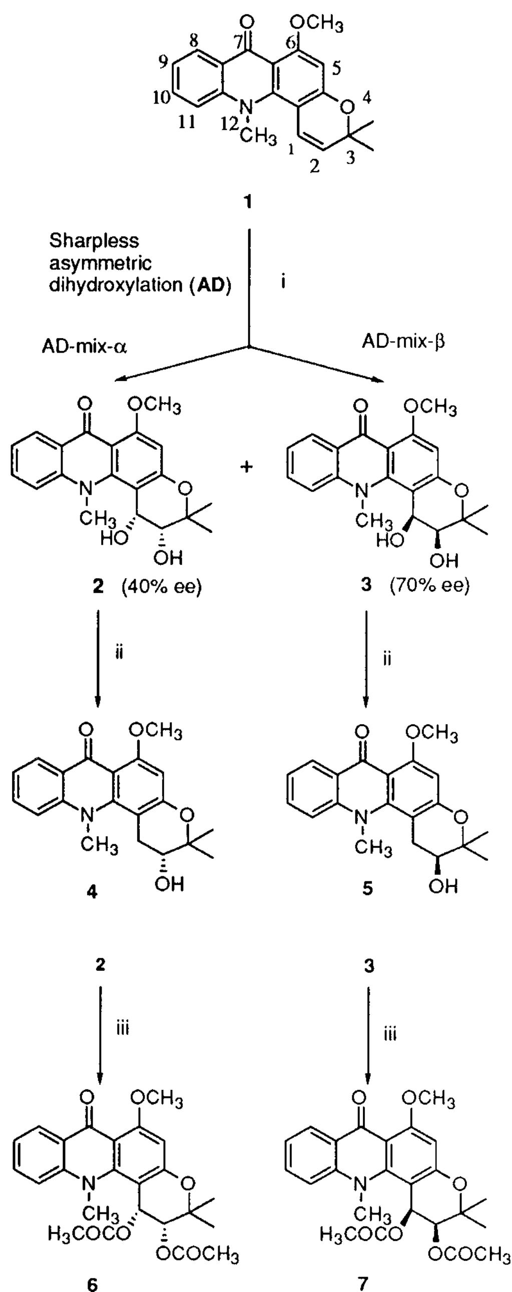
Scheme 1[a]



[a] Key: (i) K_2OsO_2, $2H_2O$, chiral ligand, $K_3Fe(CN)_6$, K_2CO_3, t-BuOH−H_2O, rt; (ii) $NaBH_3CN/ZnI_2$, $ClCH_2CH_2Cl$, rt; (iii) Ac_2O, Py, rt.

Table 1. Cytotoxic Activity[a]

compound	IC_{50} (μM)
acronycine	10.4
racemic *cis*-diacetate	3.4
6	3.1
7	3.7

[a] Inhibition of L-1210 cell proliferation measured by the MMT assay (mean of two values obtained in independent experiments).

HRMS were recorded on a Micromass ZAB2-SEQ spectrometer. Flash chromatography was performed on Si gel, type 60 Å column chromatograph Chromagel, 35−70 μm, with an overpressure of 300 mBars. Chromatography was performed on Si gel, type 60 Å C. C. Chromagel, 20−45 μm. Acronycine was prepared according to the method of Hlubucek.[17]

(1*R*,2*R*)-1,2-Dihydroxy-1,2-dihydroacronycine (2) and (1*S*,2*S*)-1,2-Dihydroxy-1,2-dihydroacronycine (3). Preparation of the Chiral Diols. Potassium osmate dihydrate (K_2OsO_2), $2H_2O$ (5 mg, 1 mol % of olefin), and $(DHQ)_2$-PYR for AD-mix-α or $(DHQD)_2$-PYR for AD-mix-β (13.65 mg, 1 mol %) were ground together to give a fine powder, then blended into the bulk powdered ingredients (99.4% by wt) $K_3Fe(CN)_6$ (1.5 g, 3 equiv/mole of olefin) and K_2CO_3 (640 mg, 3 equiv), producing a fine yellow powder. This powder was added rapidly to a stirred mixture of 1:1 *tert*-butyl alcohol−H_2O (18 mL) at room temperature. After 15 min, methanesulfonamide (CH_3SONH_2) (295 mg, 2 equiv) and acronycine (500 mg, 1.55 mmol) were added. The reaction mixture was maintained under vigorous agitation over 48 h. Then, solid $Na_2S_2O_5$ (932 mg) was slowly added and the mixture stirred for 1 h. CH_2Cl_2 (40 mL) was added to the reaction mixture, and after separation of the layers, the aqueous phase was further extracted with the organic solvent (3 × 40 mL). The combined organic extracts were washed with 2N KOH (50 mL), dried over anhydrous sodium sulfate, and concentrated. The crude product obtained was purified by flash chromatography; elution with CH_2Cl_2 gave unreacted acronycine, while further elution with CH_2Cl_2−MeOH, 97:3, provided *cis*-1,2-dihydroxy-1,2-dihydroacronycine as a yellow powder (222 mg, 40%).

HPLC Conditions for the Analytical Determination of the Enantiomeric Excesses and for the Preparation of Enantiomerically Pure Samples on a Preparative Scale. Column PHENOMENEX: CHIREX (*S*)-indoline carboxylic acid and (*R*)-1-α-naphthylethylamine (1/4″ × 25 cm). Int. diam, 4.6 mm; eluent, *n*-heptane−CH_2Cl_2−EtOH: 130/40/30; flow rate, 1 mL/min; solutions, 1 mg/mL in tetrahydrofuran (1 μL injected); detection, 275 nm.

Using chiral ligands $(DHQ)_2$-PYR and $(DHQD)_2$-PYR, the ee of diols **2** (1*R*,2*R*) and **3** (1*S*,2*S*) was determined to be 40% and 70%, respectively. Enantiomerically pure samples of **2** and **3** were obtained by repetitive semipreparative HPLC, performed on the diol mixtures obtained from reaction with $(DHQ)_2$-PYR and $(DHQD)_2$-PYR, respectively. HPLC purified samples of **2**, $[\alpha]^{20}_D$ −37.8° (*c* 0.5, MeOH), and **3**, $[\alpha]^{20}_D$ +37.8° (*c* 0.5, MeOH), were recrystallized from MeOH to afford yellow needles: mp 232−234 °C; ^1H NMR (DMSO-d_6, 300 MHz) δ 8.04 (1H, dd, *J* = 8, 2 Hz, H-8), 7.66 (1H, td, *J* = 8,2 Hz, H-10), 7.41 (1H, dd, *J* = 8, 1 Hz, H-11), 7.18 (1H, td, *J* = 8,1 Hz, H-9), 6.16 (1H, s, H-5), 5.05 (2H, m, H-1, OH-2), 4.55 (1H, d, *J* = 9 Hz, OH-1), 3.80 (3H, s, OMe), 3.77 (3H, s, NMe), 3.64 (1H, t, *J* = 5 Hz, H-2), 1.42 (3H, s, Me), 1.39 (3H, s, Me); ^{13}C NMR (DMSO-d_6, 75 MHz) δ 176.7 (C-7), 162.1 (C-6), 160.2 (C-4a), 150.1 (C-12a), 145.3 (C-11a), 133.7 (C-10), 126.7 (C-8), 125.5 (C-7a), 121.1 (C-9), 117.7 (C-11), 111.6 (C-6a), 104.4 (C-12b), 95.0 (C-5), 78.6 (C-3), 71.2 (C-2), 65.1 (C-1), 56.8 (OCH_3), 42.6 (NCH_3), 26.2 [C-3(CH_{3b})], 23.3 [C-3(CH_{3a})]; EIMS *m*/*z* 355 [$M^{\bullet+}$], 337.

(2*R*)-2-Hydroxy-1,2-dihydroacronycine (4) and (2*S*)-2-Hydroxy-1,2-dihydroacronycine (5). To a stirred solution of **2** (60 mg, 0.17 mmol) in 1,2-dichloroethane (15 mL) at room temperature were added solid zinc iodide (81 mg, 0.26 mmol) and sodium cyanoborohydride (80 mg, 1,29 mmol). The reaction mixture was stirred at room temperature for 48 h, filtered through Celite, and washed with CH_2Cl_2 (40 mL). The combined filtrates were evaporated to dryness, and the residue was chromatographed (eluent: CH_2Cl_2−MeOH, 98:2, v/v) to give unreacted diol **2** and **4** (20 mg, 35%): $[\alpha]^{20}_D$ −14.9° (*c* 0.4, $CHCl_3$) [lit.[14] $[\alpha]^{20}_D$ −15° (*c* 0.4, $CHCl_3$)] as a yellow amorphous solid; ^1H NMR ($CDCl_3$, 300 MHz) δ 8.30 (1H, dd, *J* = 8, 2 Hz, H-8), 7.58 (1H, td, *J* = 8, 2 Hz, H-10), 7.30 (1H, dd, *J* = 8, 2 Hz, H-11), 7.20 (1H, td, *J* = 8, 2 Hz, H-9), 6.26 (1H, s, H-5), 3.93 (3H, s, OMe), 3.82 (1H, dd, *J* = 6, 5 Hz, H-2), 3.75 (3H, s, NMe), 3.12 (1H, dd, *J* = 16, 5 Hz, H-1a), 2.88 (1H, dd, *J* = 15, 6 Hz, H-1b), 2.11 (1H, br s, D_2O exch, OH-2), 1.62 (3H, s, Me), 1.46 (3H, s, Me); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 177.55 (C-7), 160.64 (C-4a), 158.45 (C-6), 150.31 (C-12a), 145.55 (C-11a), 132.44 (C-10), 126.62 (C-8), 125.37 (C-7a), 121.51 (C-9), 116.18 (C-11), 110.92 (C-6a), 98.91 (C-12b), 94.90 (C-5), 77.42 (C-3), 69.23 (C-2), 55.87 (OCH_3), 44.02 (NCH_3), 31.44 (C-1), 25.19 [C-3(CH_{3b})], 21.43 [C-3(CH_{3a})]; DCIMS *m*/*z* 340 $[M + H]^+$. In a similar way, benzylic reduction of **3** gave **5** (22 mg, 38%); $[\alpha]^{20}_D$ +15.2° (*c* 0.4, $CHCl_3$) [lit.[14] $[\alpha]^{20}_D$ +15° (*c* 0.4, $CHCl_3$)].

(1*R*,2*R*)-1,2-Diacetoxy-1,2-dihydroacronycine (6) and (1*S*,2*S*)-1,2-Diacetoxy-1,2-dihydroacronycine (7). A cooled

mixture of Ac_2O (0.5 mL, 5 mmol) and dry pyridine (0.5 mL) was added to **2** (100 mg, 0.28 mmol) and the reaction mixture stirred at room temperature for 24 h and poured into cold H_2O (10 mL). The precipitate obtained was filtered, washed with H_2O, and dried in vacuo under P_2O_5 to afford **6** (110 mg, 90%): $[\alpha]^{20}_D$ −67.2° (*c* 0.16, MeOH); ^1H NMR ($CDCl_3$, 300 MHz) δ 8.85 (1H, dd, *J* = 8, 2 Hz, H-8), 7.64 (1H, td, *J* = 8, 2 Hz, H-10), 7.26 (2H, m, H-9 and H-11), 6.55 (1H, d, *J* = 5 Hz, H-1), 6.31 (1H, s, H-5), 5.48 (1H, d, *J* = 5 Hz, H-2), 4.00 (3H, s, OMe), 3.63 (3H, s, NMe), 2.04 (3H, s, $CH_3CO-O-C_1$), 1.97 (3H, s, $CH_3CO-O-C_2$), 1.56 (3H, s, Me), 1.47 (3H, s, Me); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 177.4 (C-7), 170. 9 ($CH_3\mathit{C}O-O-C_2$), 165.3 ($CH_3\mathit{C}O-O-C_1$), 162.7 (C-6), 159.8 (C-4a), 149.3 (C-12a), 144.9 (C-11a), 132.8 (C-10), 126.9 (C-8), 125.6 (C-7a), 121.9 (C-9), 115.7 (C-11), 111.9 (C-6a), 97.5 (C-12b), 94.7 (C-5), 76.2 (C-3), 69.3 (C-2), 65.7 (C-1), 56.2 ($C_6-O\mathit{C}H_3$), 42.4 ($N\mathit{C}H_3$), 24.4 [C-3($\mathit{C}H_{3b}$)], 23.4 [C-3($\mathit{C}H_{3a}$)], 20.9 ($\mathit{C}H_3CO-O-C_2$), 20.6 ($\mathit{C}H_3$-$CO-O-C_1$); EIMS *m*/*z* 439 [$M^{\bullet+}$]; HREIMS *m*/*z* 439.1635 (calcd for $C_{24}H_{25}NO_7$, 439.1631). Acetylation of **3** (100 mg, 0.28 mmol) under the same conditions afforded **7** (117 mg, 95%): $[\alpha]^{20}_D$ +67.2° (*c* 0.16, MeOH) HREIMS *m*/*z* 439.1633.

Cell Culture and Cytotoxicity. L-1210 cells were cultivated in RPMI 1640 medium (Gibco) supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 units/mL penicillin, 100 μg/mL streptomycin, and 10 mM HEPES buffer (pH 7.4). Cytotoxicity was measured by the microculture tetrazolium assay as described previously.[18] Cells were exposed to graded concentration of drug (nine serial dilutions in triplicate) for 48 h. Results are expressed as IC_{50} determined by the concentration that reduced by 50% the optical density of treated cells with respect to the optical density of untreated controls.

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