Synthesis and Anti-proliferative Activity of 2-Hydroxy-1,2-dihydroacronycine Glycosides

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Purpose. Combination of the acronycine pharmacophore with various sugar units appeared of interest, since numerous anticancer agents possess a sugar moiety, which strongly influence both their bioavailability and their selective toxicity towards tumor cells.

Methods. A series of 2-hydroxy-1,2-dihydroacronycine glycosides were synthetized, by condensation of the racemic aglycone with appropriate glycoside donors. Their effect on the inhibition of L1210 cell proliferation were evaluated.

Results. Compounds **6a, 6b, 11a, 11b,** and **12a, 12b,** including a halogenated sugar moiety displayed activities of the same order of magnitude as acronycine itself. Compounds **7a, 7b,** and **8a, 8b,** bearing a 2,3,6-trideoxy-3-azido-L-*lyxo-* and L-*arabino*-hexopyranose unit respectively, were significantly more potent than acronycine in inhibiting cell proliferation.

Conclusions. The activity of 2-hydroxy-1,2-dihydroacronycine glycosides seems to be related to the lipophilicity of the sugar unit.

KEY WORDS: acronycine; glycosides; 2-hydroxy-1,2-dihydroacronycine; 2-glycosyloxy-1,2-dihydroacronycine; cytotoxicity.

INTRODUCTION

Acronycine (1) is an acridone alkaloid, which has been first isolated from the shrub *Acronychia baueri* Schott. (Rutaceae). It exhibits a broad spectrum of antitumor properties. Nevertheless, its clinical trials have been so far hampered by its very low water solubility, its weak absorption after oral administration, and its poor tolerance (1–3). Numerous anticancer agents currently used in therapeutics, e.g. anthracyclines, bleomycine and epipodophyllotoxin glycosides, possess a sugar moiety, which strongly influence their bioavailability and also their selective toxicity towards tumor cells. It was therefore of interest to combine the acronycine pharmacophore with various sugar units. We describe here the synthesis and the cytotoxic and antitumor activities of some 2-glycosyloxy-1,2-dihydroacronycine derivatives.

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MATERIALS AND METHODS

General Experimental Procedures

Optical rotations were measured on a Perkin-Elmer 257 polarimeter. Spectra were recorded on the following apparatus: cd, Jouan-Roussel type IV; ms, Nermag R-10-10C in desorption-chemical ionization (reagent gas: NH_3) (dci); ^{1}H-nmr, Bruker AC 300 (300MHz); ^{13}C-nmr, Bruker AC 300 (75 MHz) or Bruker AM 500 (125 MHz). Chemical shifts are reported in δ values (ppm) relative to TMS as internal standard. The following abbreviations are used: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. The signals of ^{1}H and ^{13}C spectra were unambiguously assigned by using 2D nmr techniques: ^{1}H^{1}H-COSY, ^{13}C-^{1}H HETCORR and COLOC. These 2D experiments were performed using standard Bruker microprograms. Column chromatographies were conducted using silica gel 60H Merck (5–40 μm), silica gel C60 Sorbsil (20–40 μm), and flash silica gel 60 Merck (40–63 μm), with an overpressure of 300 mbars. All new compounds gave satisfactory combustion analyses (C, H, N, within ± 0.4% with calculated values).

Chemistry

*3,4,6,-Tri-*O*-acetyl-1,2-*O*-(1(S)-methyl-(1,2-dihydroacronycin -2(R and S)-yl)-ethylidene)-α-D-glucopyranose (**4a** and **4b**)*

A mixture of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (0.411 g, 1 mmol), 2-hydroxy-1,2-dihydroacronycine (**3**) (0.081 g, 0.24 mmol), yellow mercuric oxide (0.450 g, 2.7 mmol), mercuric bromide (0.225 g, 0.625 mmol) and granular molecular sieve 4 Å (0.500 g) in anhydrous dichloromethane (15 ml) was stirred for 1 h at room temperature. After filtration over celite, the reaction mixture was diluted with dichlormethane (30 ml), washed with saturated sodium hydrogenocarbonate solution (2 × 25 ml) and dried over anhydrous sodium sulfate. The solvent was removed under reduced pressure. Column chromatography (solvent: dichloromethane-methanol 98:2) afforded 4 (0.096g, 60%) as a 1:1 unseparable mixture of the two diastereoisomers **4a, 4b;** ms (dci) m/z: 670 $(M + H)^+$, 366, 331; ^{1}H nmr ($CDCl_3$) δ: 8.26 (d, J = 8, H-8), 7.45 (m, H-10), 7.43 and 7.31 (d, J = 8, H-11), 7.10 (t, J = 8, H-9), 6.16 and 6.15 (s, H-5), 5.64 and 5.58 (d, J = 5, H-1′), 5.08 (m, H-3′), 4.83 (dd, J = 9, 3, H-4′), 4.29 (dd, J = 5, 3, H-2′), 4.16 (m, CH_2-6′), 3.89 (s, OCH_3), 3.71 (s, NCH_3), 3.60 (m, H-2), 3.10 − 2.95 (m, CH_2-1), 2.10, 2.00, 1.90 (3 s, 3 $OCOCH_3$), 1.76 (s, CH_3-C-1″), 1.44, 1.36 (2 s, $(CH_3)_2$-C-3).

*1,2-Dihydroaconycin-2-yl 3,4-di-*O*-Acetyl-2,6-Dideoxy-α - L- Arabino-Hexopyranosides (**5a** and **5b**)*

Method A (Königs-Knorr conditions): Reaction of 2-hydroxy-1,2-dihydroacronycine (**3**) (0.081 g, 0.24 mmol) and 3,4-di-*O*-acetyl-2,6-dideoxy-α-L-*arabino*-hexopyranosyl bromide (0.295 g, 1 mmol) under conditions identical with those described for the synthesis of **4a** and **4b,** followed by column chromatography (solvent:dichloromethane-methanol 98:2) afforded the α-L-glycosides **5a, 5b,** accompanyied by trace amounts of the corresponding β anomers (isomer ratio α:β 96:4), as a diastereoisomeric mixture (0.083g, overall yield

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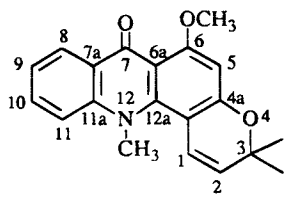

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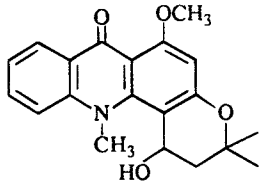

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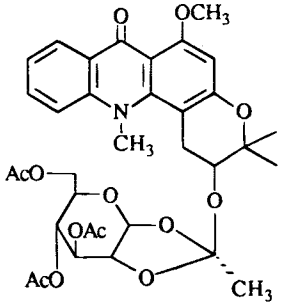

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4a 2 *R*

4b 2 *S*

60%). A second column chromatography (solvent:toluene-ethyl acetate 80:20) permitted to isolate **5a** (0.034g) and **5b** (0.031g).

Method B (Lewis acid catalyzed conditions):Tin (IV) chloride (0.2 ml, 1.73 mmol) and molecular sieve 3 Å (1.00g) were added to a solution of 1,3,4-tri-*O*-acetyl-2,6-dideoxy-α-L-*arabino*-hexopyranose (0.274 g, 1mmol) and 2-hydroxy-1,2-dihydroacronycine (**3**) (0.129 g, 0.38 mmol) in anhydrous acetonitrile (5ml). The mixture was stirred for 4 h at room temperature, neutralized with concentrated aqueous ammonia, filtered and evaporated under reduced pressure. Sugar side products were removed by flash chromatography (solvent:hexane- ethyl acetate 70:30). Compounds **5a** and **5b** were obtained as a 1:1 diastereoisomeric mixture (122 mg, overall yield 58%). A second column chromatography, using the conditions described in method A permitted to isolate **5a** and **5b.**

*1,2-Dihydroaconycin-2(R)-yl 3,4-di-*O*-acetyl-2,6-dideoxy-α -L- arabino-hexopyranoside (**5a**)*

$[\alpha]^{25}D$ − 20° (*c* = 0.25, methanol); ms (dci) m/z:554 (M + H)$^+$; CD:λnm (Δε): 243 (+1.20), 251 (+0.09), 254 (+0.21), 264 (−0.09), 276 (+0.39); ^{1}H nmr ($CDCl_3$) δ:8.38 (dd, J = 8, 2, H-8), 7.62 (td, J = 8, 2, H-10), 7.42 (d, J = 8, H-11), 7.30 (t, J = 8, H-9), 6.33 (s, H-5), 5.28 (ddd, J = 13, 10, 6, H-3′), 5.15 (dd, J = 3.5, 1, H-1′), 4.81 (t, J = 10, H-4′), 4.02 (s, OCH_3), 3.93 (dq, J = 10, 5, H-5′), 3.84 (s, NCH_3), 3.75 (dd, J = 8, 5, H-2), 3.11 (dd, J = 15, 5, H-1a), 2.91 (dd, J = 15, 8, H-1b), 2.22 (ddd, J = 13, 6, 1, H-2′eq), 2.11 (s, $OCOCH_3$), 2.06 (s, $OCOCH_3$), 1.88 (td, J = 13, 3.5, H-2′ax), 1.58 (s, C-CH_3), 1.52 (s, C-CH_3), 1.20 (d, J = 5, CH_3-6′); ^{13}C nmr ($CDCl_3$) δ: 177.7 (C-7), 170.2 (*C*OCH_3), 170.0 (*C*OCH_3), 160.9 (C-6), 158.3 (C-4a), 150.2 (C-12a), 145.7 (C-11a), 132.4 (C-10), 126.8 (C-8), 125.8 (C-7a), 121.7 (C-9), 116.3 (C-11), 111.2 (C-6a), 98.7 (C-12b), 94.9 (C-5), 92.9 (C-1′), 76.6 (C-3), 74.4 (C-4′), 72.2 (C-2), 68.8 (C-3′), 66.4 (C-5′), 56.0 (O*C*H_3), 44.3 (N*C*H_3), 35.2 (C-2′), 29.7 (C-1), 26.3 (C-*C*H3), 21.2 (C-*C*H3), 20.8 (CO*C*H3), 20.5 (CO*C*H3), 17.4 (*C*H3-6′).

*1,2-Dihydroaconycin-2(S)-yl, 3,4-di-*O*-acetyl-2,6-dideoxy-α -L-* arabino-*hexopyranoside (**5b**):*$[\alpha]^{25}D$ − 83° (*c* = 0.1, methanol); ms (dci) m/z:554 (M + H)$^+$; CD:λnm (Δε):243 (−2.58), 253 (0), 264 (−3.31), 276 (+1.88);^{1}H nmr ($CDCl_3$) δ:8.37 (dd, J = 8, 2, H-8), 7.65 (td, J = 8, 2, H-10), 7.37 (d, J = 8, H-11), 7.26 (t, J = 8, H-9), 6.32 (s, H-5), 5.22 (ddd, J = 13, 10, 6, H-3′), 5.06 (dd, J = 3.5, 1, H-1′), 4.71 (t, J = 10, H-4′), 4.00 (s, OCH_3), 3.80 (s, NCH_3), 3.66 (dq, J = 10, 5, H-5′), 3.65 (m, H-2), 3.10 (m, H-1a, H-1b), 2.27 (ddd, J = 13, 6, 1, H-2′eq), 2.04 (s, $OCOCH_3$), 2.02 (s, $OCOCH_3$), 1.81 (td, J = 13, 3.5, H-2′ax), 1.48 (s, C-CH_3), 1.46 (s, C-CH_3), 0.96 (d, J = 5, CH_3-6′); ^{13}C nmr ($CDCl_3$) δ:177.8 (C-7), 170.1 (*C*OCH_3), 170.0 (*C*OCH_3), 160.8 (C-6), 158.4 (C-4a), 150.1 (C-12a), 145.7 (C-11a), 132.5 (C-10), 126.9 (C-8), 126.1 (C-7a), 121.7 (C-9), 116.1 (C-11), 111.2 (C-6a), 99.0 (C-12b), 98.9 (C-1′), 95.1 (C-5), 78.6 (C-2), 76.6 (C-3), 74.5 (C-4′), 68.7 (C-3′), 66.3 (C-5′), 56.1 (O*C*H_3), 44.1 (N*C*H_3), 35.0 (C-2′), 29.9 (C-1), 29.7 (C-*C*H3), 25.8 (C-*C*H3), 20.9 (CO*C*H3), 20.8 (CO*C*H3), 17.3 (*C*H3-6′).

*1,2-Dihydroaconycin-2-yl 2-iodo-3,4-di-*O*-acetyl-2,6-dideoxy-α -L- arabino-hexopyranosides (**6a** and **6b**)*

N-iodosuccinimide (0.067 g, 0.3 mmol) and molecular sieve 3 Å (1.00g) were added to a solution of 3,4-di-*O*-acetyl-L-rhamnal (0.064 g, 0.3 mmol) and 2-hydroxy-1,2-dihydroacronycine (**3**) 0.021 g, 0.06 mmol) in acetonitrile (10 ml). The mixture was stirred for 7 h at room temperature under argon, filtered, diluted with dichloromethane (30 ml), washed successively with saturated sodium thiosufate solution (25 ml) and water (2 × 25 ml), dried over anhydrous sodium sulfate and concentrated under reduced pressure. Column chromatography (solvent:toluene-ethyl acetate 80:20) afforded successively 6a (0.008 g) and 6b (0.007 g) (overall yield:38%). *1,2-Dihydroaconycin-2(R)-yl 2-iodo-3,4-di-*O*-acetyl-2,6-dideoxy-α -L- arabino-hexopyranoside (**6a**):*$[\alpha]^{25}D$ − 26° (*c* = 0.4, chloroform); ms (dci) m/z:680 (M + H)$^+$;^{1}H nmr ($CDCl_3$) δ: 8.23 (d, J = 8, H-8), 7.53 (t, J = 8, H-10), 7.30 (d, J = 8, H-11), 7.16 (t, J = 8, H-9), 6.17 (s, H-5), 5.21 (d, J = 1, H-1′), 5.06 (t, J = 9, H-4′), 4.50 (dd, J = 9, 4, H-3′), 4.45 (dd, J = 4, 1, H-2′), 3.87 (s, OCH_3), 3.71 (s, NCH_3), 3.70 (m, H-5′), 3.65 (dd, J = 7, 5, H-2), 2.98 (dd, J = 16, 5, H-1a), 2.81 (dd, J = 16, 7, H-1b), 2.05 (s, $OCOCH_3$), 2.01 (s, $OCOCH_3$), 1.47 (s, C-CH_3), 1.40 (s, C-CH_3), 0.81 (d, J = 6, CH_3-6′).

*1,2-Dihydroaconycin-2(S)-yl 2-iodo-3,4-di-*O*-acetyl-2,6-dideoxy-α -L-* arabino-*hexopyranoside (**6b**):*$[\alpha]^{25}D$ − 6° (*c* = 0.4, chloroform); ms (dci) m/z: 680 (M + H)$^+$;^{1}H nmr ($CDCl_3$) δ: 8.21 (d, J = 8, H-8), 7.50 (t, J = 8, H-10), 7.27 (d, J = 8, H-11), 7.11 (t, J = 8, H-9), 6.17 (s, H-5), 5.16 (d, J = 1, H-1′), 4.91 (t, J = 9, H-4′), 4.55 (dd, J = 4, 1, H-2′), 4.32 (dd, J = 9, 4, H-3′), 3.90 (s, OCH_3), 3.69 (s, NCH_3), 3.68 (m, H-2), 3.34 (qd, J = 9, 6, H-5′), 3.03 (m, H-1a, H-1b), 1.99 (s, $OCOCH_3$), 1.90 (s, $OCOCH_3$), 1.43 (s, C-CH_3), 1.41 (s, C-CH_3), 0.72 (d, J = 6, CH_3-6′).

*1,2-Dihydroaconycin-2(R and S)-yl 3-azido-4-O-acetyl-2,3,6-trideoxy-α -L-*lyxo-*hexopyranosides (**7a** and **7b**)*

Reaction of 2-hydroxy-1,2-dihydroacronycine (**3**) (0.129 g, 0.38 mmol) and 1,4-di-*O*-acetyl-3-azido-2,3,6-trideoxy-L-

lyxo-hexopyranose (0.257 g, 1 mmol) under conditions identical with those described for the synthesis of **5a** and **5b** (method B), followed by column chromatography (solvent: hexane- ethyl acetate 70:30) afforded **7a** and **7b** as a 1:1 unseparable diastereoisomeric mixture (0.116 g, overall yield 57%); ms (dci) m/z:537 $(M + H)^+$, 382;^{1}H nmr ($CDCl_3$) δ: 8.27 (d, J = 8, H-8), 7.55 (m, H-10), 7.27 (m, H-11), 7.11 (m, H-9), 6.18 (s, H-5), 5.11 (m, H-1′), 5.09 (t, J = 1, H-4′R), 4.96 (t, J = 1, H-4′S), 3.95 (s, OCH_3), 3.85 – 3.55 (m, H-5′, H-3′, H-2aS), 3.75 (s, NCH_3), 3.06 (m, H-1aS, H-1aR, H-1bS, H-1bR), 2.79 (dd, J = 16, 7, H-2bR), 2.16 (s, $OCOCH_3$), 1.92 (td, J = 13, 3, H-2′ax), 1.87 (ddd, J = 13, 6, 1, H-2′eq), 1.46 (s, C-CH_3), 1.42 (s, C-CH_3), 1.08 (d, J = 6, CH_3-6′R), 0.82 (d, J = 6, CH_3-6′S).

*1,2-Dihydroaconycin-2(R and S)-yl 3-azido-4-O-acetyl-2,3,6-trideoxy-α -L- arabino- hexopyranosides (**8a** and **8b**)*

Reaction of 2-hydroxy-1,2-dihydroacronycine (**3**) (0.129 g, 0.38 mmol) and 1,4-di-*O*-acetyl-3-azido-2,3,6-trideoxy-*L-arabino*-hexopyranose (0.257 g, 1 mmol) under conditions identical with those described for the synthesis of **5a** and **5b** (method B), followed by column chromatography (solvent: hexane- ethyl acetate 70:30) afforded **8a** and **8b** as a 1:1 unseparable diastereoisomeric mixture (0.120 g, overall yield 59%); ms (dci) m/z: 537 $(M + H)^+$, 382;^{1}H nmr ($CDCl_3$) δ: 8.23 (d, J = 8, H-8), 7.50 (m, H-10), 7.25 (m, H-11), 7.18 (m, H-9), 6.21 (s, H-5S), 6.20 (s, H-5R), 5.02 (dd, J = 3, 1, H-1′R), 4.92 (dd, J = 3, 1, H-1′S), 4.61 (t, J = 10, H-4′R), 4.48 (t, J = 10, H-4′S), 3.91 (s, OCH_3), 3.89 (s, OCH_3), 3.79 (m, H-5′R), 3.71 (s, NCH_3), 3.68 (m, H-3′), 3.61 (dd, J = 8, 5, H-2R), 3.52 (m, H-2S), 3.35 (qd, J = 10, 6, H-5′S), 3.05 (m, H-1aS, H-1bS), 3.01 (dd, J = 14, 5, H-1aR), 2.81 (dd, J = 14, 7, H-1bR), 2.10 (s, $OCOCH_3$), 2.08 (m, H-2′eq), 1.99 (s, $OCOCH_3$), 1.75 (m, H-2′ax), 1.48 (s, C-CH_3), 1.42 (s, C-CH_3), 1.11 (d, J = 6, CH_3-6′R), 0.81 (d, J = 6, CH_3-6′S).

*1,2-Dihydroaconycin-2(R and S)-yl 3-amino-4-O-acetyl-2,3,6-trideoxy-α -L- lyxo-hexopyranosides (**9a** and **9b**)*

A solution of glycosides **7a, 7b** (0.050 g, 0.09 mmol) in methanol (3 ml) was hydrogenated (H_2, 5% Pd-C, 1 Atm) at 20° for 7 h. The catalyst was removed by filtration over celite and the solvent evaporated under reduced pressure. Flash column chromatography (solvent: dichloromethane-methanol 98:2) afforded **9** (0.030g, 59%) as an unseparable mixture of the two diastereoisomers **9a, 9b**; ms (dci) m/z: 511 $(M + H)^+$, 172;^{1}H nmr ($CDCl_3$) δ:8.35 (d, J = 8, H-8), 7.61 (m, H-10), 7.34 (m, H-11), 7.23 (m, H-9), 6.28 (s, H-5S), 5.14 (t, J = 1, H-4′R), 5.05 (m, H-1′), 4.87 (t, J = 1, H-4′S), 3.96 (m, H-5′R), 3.94 (s, OCH_3), 3.77 (s, NCH_3), 3.67 (m, H-1, H-3′), 3.41 (dq, J = 6, 1, H-5′S), 3.04 (m, H-1aS, H-1bS, H-1aR), 3.00 (m, NH_2), 2.84 (dd, J = 14, 7, H-1bR), 2.17 (s, $OCOCH_3$), 2.10 (s, $OCOCH_3$), 1.82 (m, H-2′ax, H-2′eq), 1.44 (s, C-CH_3), 1.41 (s, C-CH_3), 1.08 (d, J = 6, CH_3-6′R), 0.81 (d, J = 6, CH_3-6′S).

*1,2-Dihydroaconycin-2-yl 3-amino-2,3,6-trideoxy-α- L-arabino-hexopyranosides (**10a** and **10b**)*

A solution of glycosides **8a, 8b** (0.54 g, 1.0 mmol) in methanol (10 ml) was hydrogenated (H_2, 5% Pd-C, 1 Atm) at 20° for 5 h. The catalyst was removed by filtration over celite and the solvent evaporated under reduced pressure. The residue was dissolved in 0.1 *M* sodium methoxide in methanol (5 ml) and the mixture was stirred for 2 h at 20°. After neutralization by addition of Amberlite IR-50 (H^+) ion exchange resin and filtration, the solvent was evaporated under reduced pressure. Purification by flash column chromatography (solvent: dichloromethane-methanol-concentrated aqueous ammonia 95:4.5:0.5) afforded 10 (0.29g, 62%) as a mixture of the two diastereoisomers **10a, 10b**. Repeated column chromatography of the above mixture on silica gel 60H using the same solvent, permitted to obtain pure anlytical samples of **10a** and **10b.**

*1,2-Dihydroaconycin-2(R)-yl 3-amino-2,3,6-trideoxy-α-L-arabino-hexopyranoside (**10a**)*:$[\alpha]^{25}D$ − 14° (*c* = 0.35, chloroform); ms (dci) m/z: 469 $(M + H)^+$;^{1}H nmr ($CDCl_3$) δ: 8.24 (d, J = 8, H-8), 7.55 (t, J = 8, H-10), 7.30 (d, J = 8, H-11), 7.28 (t, J = 8, H-9), 6.22 (s, H-5), 4.99 (dd, J = 3, 1, H-1′), 3.88 (s, OCH_3), 3.71 (s, NCH_3), 3.68 (m, H-2, H-5′), 3.28 – 2.90 (m, H-1a, H-1b, H-3′, H-4′), 2.10 (br. s, D_2O exch., NH_2), 1.90 (ddd, J = 13, 6, 1, H-2′eq), 1.56 (td, J = 13, 3, H-2′ax), 1.50 (s, C-CH_3), 1.45 (s, C-CH_3), 1.22 (d, J = 6, CH_3-6′); ^{13}C nmr ($CDCl_3$) δ: 177.8 (C-7), 160.9 (C-6), 158.7 (C-4a), 150.4 (C-12a), 145.8 (C-11a), 132.6 (C-10), 126.8 (C-8), 125.7 (C-7a), 121.8 (C-9), 116.4 (C-11), 111.1 (C-6a), 99.2 (C-12b), 95.2 (C-5), 92.7 (C-1′), 76.9 (C-3), 76.8 (C-4′), 71.3 (C-2), 69.3 (C-5′), 56.1 (OCH_3), 49.9 (C-3′), 44.3 (NCH_3), 38.3 (C-2′), 26.8 (C-1), 26.2 (C-*C*H3), 21.1 (C-*C*H3), 17.8 (*C*H3-6′).

*1,2-Dihydroaconycin-2(S)-yl 3-amino-2,3,6-trideoxy-α-L-arabino-hexopyranoside (**10b**)*:$[\alpha]^{25}D$ − 104° (*c* = 0.8, chloroform); ms (dci) m/z:469 $(M + H)^+$;^{1}H nmr ($CDCl_3$) δ:8.28 (d, J = 8, H-8), 7.57 (t, J = 8, H-10), 7.30 (d, J = 8, H-11), 7.16 (t, J = 8, H-9), 6.20 (s, H-5), 4.93 (dd, J = 3, 1, H-1′), 3.93 (s, OCH_3), 3.74 (s, NCH_3), 3.56 (m, H-2, H-5′), 3.03 (m, H-1a, H-1b), 2.90 (m, H-3′, H-4′), 2.09 (br. s, D_2O exch., NH_2), 2.06 (ddd, J = 13, 6, 1, H-2′eq), 1.53 (td, J = 13, 3, H-2′ax), 1.44 (s, C-CH_3), 1.39 (s, C-CH_3), 1.15 (d, J = 6, CH_3-6′); ^{13}C nmr ($CDCl_3$) δ: 177.9 (C-7), 160.9 (C-6), 158.6 (C-4a), 150.2 (C-12a), 145.8 (C-11a), 132.6 (C-10), 126.9 (C-8), 125.7 (C-7a), 121.8 (C-9), 116.3 (C-11), 111.1 (C-6a), 99.8 (C-12b), 99.6 (C-1′), 95.1 (C-5), 78.2 (C-2), 77.8 (C-4′), 76.9 (C-3), 69.1 (C-5′), 56.1 (OCH_3), 49.8 (C-3′), 44.1 (NCH_3), 39.3 (C-2′), 30.4 (C-1), 25.9 (C-CH3), 20.6 (C-*C*H3), 17.9 (*C*H3-6′).

*(−)-2-Hydroxy-1,2-dihydroacronycine (**3a**)*

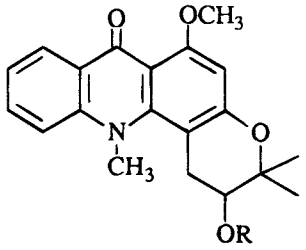
A solution of **10a** (0.047 g, 0.1 mmol) in methanol (2 ml) and 0.25 *N* aqueous hydrochloric acid (3 ml) was heated under reflux for 2 h. After cooling, the mixture was extracted with dichloromethane (5 × 5 ml). The organic layer was dried over anhydrous sodium sulfate, fitered and evaporated under reduced pressure. Column chromatography (solvent:dichloromethane-methanol 98:2) afforded **3a** as a yellow amorphous solid (0.031g, 90%); $[\alpha]^{20}D$ − 15° (*c* = 0.4, chloroform).

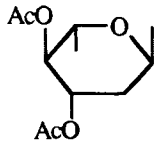
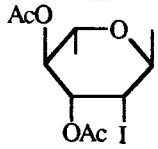
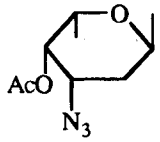
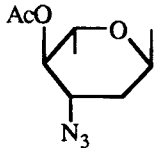
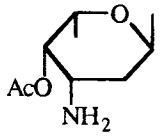
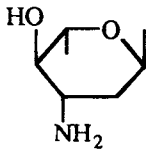
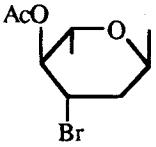
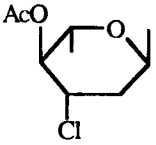
*(+)-2-Hydroxy-1,2-dihydroacronycine (**3b**)*

Hydrolysis of **10b** under the conditions described for that of **10a** yielded 3b; $[\alpha]^{20}D$ + 15° (*c* = 0.4, chloroform).

Biology

The murine leukemia L1210 was from the American Type Culture Collection (Rockville Pike, MD). Cells were grown in



3 R = H	**3a** 2*R* **3b** 2*S*
5 R = 	**5a** 2*R* **5b** 2*S*
6 R = 	**6a** 2*R* **6b** 2*S*
7 R = 	**7a** 2*R* **7b** 2*S*
8 R = 	**8a** 2*R* **8b** 2*S*
9 R = 	**9a** 2*R* **9b** 2*S*
10 R = 	**10a** 2*R* **10b** 2*S*
11 R = 	**11a** 2*R* **11b** 2*S*
12 R = 	**12a** 2*R* **12b** 2*S*

RPMI medium supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin and 10 mM HEPES buffer (pH 7.4). The cytotoxicity was measured by the Microculture Tetrazolium assay essentially as previously described (4). Cells were exposed for 48 hours to nine graded concentrations in triplicate of the cytotoxic drug. Results are expressed as IC 50 (mean ± sem, n = 3), which is defined as the drug concentration inhibiting the absorbance by 50% with respect to untreated cells.

RESULTS AND DISCUSSION

We had previously described the syntheses of both 1- and 2-hydroxy-1,2-dihydroacronycine (5). These alcohols, resulting from the addition of H_2O to the double-bond at the pyran ring of acronycine, could be considered as suitable candidates for glycosidation reactions. Unfortunately, 1- hydroxy-1,2-dihydroacronycine (**2**) proved rapidly to be very unstable in organic solutions, and all our attempts towards its glycosidation have remained unsuccessful up to now. In contrast, 2-hydroxy-1,2-dihydroacronycine (**3**) is stable enough to give satisfactory glycosidation reactions.

Condensation of **3** with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide under Königs-Knorr conditions (6) did not permit to obtain the corresponding glucosides but led to the diastereoisomric orthoesters **4a, 4b** which could not be separated. The use of 3,4-di-*O*-acetyl-2,6-dideoxy-α-L-*arabino*-hexopyranosyl bromide (7) as glycosylating reagent under the same conditions, led to the expected α-L-glycosides **5a, 5b,** accompanyied by trace

Table 1. Inhibition of L1210 Cell Proliferation by 2-hydroxy-1,2-Dihydroacronycine Glycosides

Compound	IC_{50}, μM
Acronycine (**1**)	24.1 ± 4.0
3	23.5 ± 3.5
5 a	20.8 ± 2.9
5 b	82.5 ± 17.6
6 a, b	5.6 ± 0.7
7 a, b	2.8 ± 0.5
8 a, b	3.0 ± 0.4
9 a, b	77.2 ± 22.8
10 a, b	40.7 ± 4.1
11 a	11.3 ± 1.0
11 b	19.8 ± 3.2
12 a, b	13.4 ± 1.5

amounts of the corresponding β anomers (isomer ratio α:β 96:4, as determined by ^{1}H NMR on the reaction mixture). Di-*O*-acetyl-L-rhamnal (7) condensation with **3**, in the presence of N-iodosuccinimide (8), furnished the two diastereoisomeric iodoglycosides **6a, 6b.** In contrast, when 1,3,4-tri-*O*-acetyl-2,6-dideoxy-α-L-*arabino*-hexopyranose was used, in the presence of tin tetrachloride in acetonitrile (9), only the α-isomers **5a, 5b** were obtained. Under these latter conditions, 1,4-di-*O*-acetyl-3-azido-2,3,6-trideoxy-L-*lyxo*- and L-*arabino*-hexopyranose (10,11) afforded **7a, 7b,** and **8a, 8b,** respectively. Catalytic hydrogenation of the azidoglycosides **7a, 7b** gave the corresponding amino-glycosides **9a, 9b,** whereas catalytic hydrogenation of **8a, 8b,** followed by deacetylation led to the fully deprotected aminoglycosides **10a, 10b.** The diastereoisomeric glycosides (2R and 2S) could be separated, in the cases of **5a, 5b, 6a, 6b,** and **10a, 10b,** using repeated column chromatography. The absolute configuration at C-2 on the aglycone part of each glycoside was deduced from ^{1}H and ^{13}C NMR data, in comparison with those of related angular hydroxydihydro-pyranocoumarin glycosides of known configuration (12). Finally, acidic hydrolysis of **10a** and **10b,** afforded respectively the aglycones **3a** and **3b,** which had not been previously reported in their optically active forms. The above glycosides, and their analogs **11a, 11b, 12a,** and **12b,** previously synthesized in our laboratory (13) were tested against L1210 murine cells *in vitro,* in comparison with acronycine (table I). Compounds **6a, 6b, 11a, 11b,** and **12a, 12b,** including a halogenated sugar moiety displayed activities of the same order of magnitude as acronycine itself. Compounds **7a, 7b** and **8a, 8b,** bearing a 2,3,6-trideoxy-3-azido-L-*lyxo*- and L-*arabino*-hexopyranose unit respectively, were significantly more potent than acronycine in inhibiting cell proliferation. In contrast, more hydrophilic glycosides **9a, 9b,** and **10a, 10b** exhibited only marginal activity. The activity of 2-hydroxy-1,2-dihydroacronycine glycosides seems therefore to be related to the lipophilicity of the sugar unit (14,15).

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