Synthesis and Cytotoxic Activity of 1-Alkoxo- and 1-Amino-2-hydroxy-1,2-dihydroacronycine Derivatives

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Sixteen new derivatives of the natural alkaloid acronycine, bearing 1-alkoxy or 1-amino and 2-hydroxy groups, were synthesized in order to clarify the role of the C-1 substitution. Studies on the cytotoxic activity of compounds 4—19 were carried out in vitro on L-1210 cells. Structure–activity relationships are discussed.

Key words acronycine; cytotoxicity; alkaloid; acridone

The acridone alkaloid acronycine (1), first isolated from Acronychia baueri Schott (Rutaceae) in 1948 a,b exhibits a broad spectrum of activity against numerous solid tumors including sarcoma, myeloma, carcinoma and melanoma.3—5 Nevertheless, clinical trials gave only poor results,6 most probably due to the moderate potency of acronycine and its very low water-solubility which excludes parenteral formulation of the drug. Early structure–activity studies clearly indicated that the 1,2-double bond on the pyran ring was an essential requirement to observe cytotoxic activity in this series.3,7–11 Based on those data, we formulated a hypothesis of bioactivation of acronycine by transformation of the 1,2-double bond into the corresponding epoxide a,b which probably results from epimerization at C-1.12 As expected, these compounds exhibited promising antitumor properties, with a broadened spectrum of activity and an increased potency when compared with acronycine itself on several tumor strains in vitro and in vivo.12,13 In a continuation of our studies on structure–activity relationships in the acronycine series, and with the aim to clarify the role of the C-1 substitution, we report here the synthesis and cytotoxic activity of 1-alkoxy and 1-amino-2-hydroxy-1,2-dihydroacronycine derivates.

The high reactivity of the benzylic position of cis-1,2-dihydroxy-1,2-dihydroacronycine (2) and its diacetate (3) b towards nucleophilic agents led us to consider these two compounds as suitable candidates for substitution reactions at C-1.

Treatment of cis-diol 2 with excess methanol in the presence of hydrochloric acid led to a 1:1 mixture of cis-2-hydroxy-1-methoxy-1,2-dihydroacronycine (4) and trans-2-hydroxy-1-methoxy-1,2-dihydroacronycine (5), which could be separated by column chromatography. These two methoxy compounds gave rise to the corresponding esters at the 2-position, 6—9, upon treatment with acetic anhydride or chloroacetyl chloride in the presence of pyridine. It should be noted that cis-2-chloroacetoxy-1-methoxy-1,2-dihydroacronycine (8) is particularly unstable, most probably due to steric hindrance. For instance, it spontaneously reacts with trace amounts of water to give quantitatively alcohol 4.

In a similar way, reaction of cis-1,2-dihydroxy-1,2-dihydroacronycine (2) with benzyl alcohol, carried out in tetrahydrofuran containing a catalytic amount of boron trifluoride, afforded a 3:7 isomeric mixture of cis-1-benzyloxy-2-hydroxy-1,2-dihydroacronycine (10) and trans-1-benzyloxy-2-hydroxy-1,2-dihydroacronycine (11). The solvent plays a crucial role in the course of this latter reaction. When dimethylformamide or acetonitrile are used instead of tetrahydrofuran, 2-oxo-1,2-dihydroacronycine (12) is the only product which can be isolated from the reaction mixture. Esterification reactions, using either acetic anhydride or chloroacetyl chloride could be successfully performed on the trans-isomer 11, leading to trans-2-acetoxy-1-benzyloxy-1,2-dihydroacronycine (13) and to trans-1-benzyloxy-2-chloroacetoxy-1,2-dihydroacronycine (14), respectively. In contrast, acylation of the cis-isomer 10 seemed, like in the case of 4, more difficult. Indeed, treatment of 10 with chloroacetyl chloride, even when the reaction was carried out at low temperature led to a complicated mixture from which only the unexpected trans-ester 14 could be isolated and identified (which probably results from epimerization at C-1).

Amination reactions at position-1 of the 1,2-dihydroacronycine system could be easily performed using cis-1,2-diacetoxy-1,2-dihydroacronycine (3).13 Indeed, treatment of 3 with methyamine in ethanolic solution at reflux led to trans-2-acetoxy-1-methylamino-1,2-dihydroacronycine (15), accompanied by its saponification product trans-2-hydroxy-1-methylamino-1,2-dihydroacronycine (16). Treatment of the latter with excess acetic anhydride gave access to trans-2-acetoxy-1-methylacetamido-1,2-dihydroacronycine (17). Finally, reaction of 3 with hydrazine in ethanol gave trans-1-hydrazino-2-hydroxy-1,2-dihydroacronycine (18).

The formation of two epimeric ethers upon treatment of diol 2 by methanol or benzyl alcohol is a consequence of the Sx1 type reaction previously known to occur at the benzylic position of various pyranoacumarins.15,16 In contrast, amination of diester 3 proceeds with complete inversion of stereochemistry at C-1 via an Sx2 type reaction. It is of interest to point out that treatment of diacetate 3 with excess of methanol gave the expected 1-alkoxy derivatives 6 and 7 in poor yield. The major compound of this reaction was 2-acetoxy-acronycine (19). The formation of compound 19, in this case, could be explained by an elimination reaction via an E1 mechanism.

The study of the cytotoxic properties of the new acronycine derivatives was carried out in vitro on L-1210...
leukemia cells. The results (IC₅₀) are summarized in Table 1. In contrast with *cis*- and *trans*-1,2-dihydroxy-1,2-dihydroacronycine diesters, most 1-alkoxy and 1-amino-2-hydroxy-1,2-dihydroacronycine derivatives only exhibit marginal cytotoxic activity. This lack of significant activity confirms our previous hypothesis that cytotoxicity in this series is correlated with the presence of a good leaving group at the benzylic position, able to ensure sufficient reactivity toward nucleophilic agents. It should nevertheless be noted that 1-alkoxy derivatives bearing a chloroacetyl ester group at position-2 such as 9 and 14 exhibit significant cytotoxic activity, within the same range of magnitude as the corresponding *cis* and *trans* diesters.

**Table 1. Cytotoxic Activity**

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC₅₀ (µM)</th>
<th>Compound</th>
<th>IC₅₀ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>44.6</td>
<td>12</td>
<td>43.3</td>
</tr>
<tr>
<td>5</td>
<td>50.1</td>
<td>13</td>
<td>&gt;100</td>
</tr>
<tr>
<td>6</td>
<td>43.6</td>
<td>14</td>
<td>5.2</td>
</tr>
<tr>
<td>7</td>
<td>54.0</td>
<td>15</td>
<td>61.3</td>
</tr>
<tr>
<td>8</td>
<td>NT</td>
<td>16</td>
<td>56.6</td>
</tr>
<tr>
<td>9</td>
<td>14.7</td>
<td>17</td>
<td>75.8</td>
</tr>
<tr>
<td>10</td>
<td>40.5</td>
<td>18</td>
<td>&gt;100</td>
</tr>
<tr>
<td>11</td>
<td>48.2</td>
<td>19</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

*Inhibition of L1210 cell proliferation measured by the MMT assay (mean of 2 values obtained in independent experiments). NT: non tested.*
Experimental

General Experimental Procedures

Spectra were recorded on the following apparatus: MS, Nermag R10-10C in dispersion-chemical ionization, using NH₃ as reagent gas. NMR, Bruker AC 200, ¹H-NMR (200 MHz), ¹³C-NMR (50 MHz) and a Bruker DRX400, ¹H-NMR (400 MHz). Chemical shifts are given in δ with tetrakis(dimethylamino)phosphonium hexafluorophosphate (TDF) as an internal standard. Coupling constants (J) are given in Hz. The signals of ¹H and ¹³C spectra were unambiguously assigned by using 2D NMR techniques: ¹H⁻¹H correlation spectroscopy (COSY), ¹³C-¹H HETCORR and heteronuclear multiple bond correlation (HMBC). These 2D experiments were performed using standard Bruker microprograms. Column chromatographies were conducted using flash silica gel 60 Merck (40—63 μm), with an overpressure of 300 mbar. All new compounds gave satisfactory combustion analyses (C, H, N, with calculated values).

(±)-trans-2-Hydroxy-1-methoxy-1,2-dihydroacronycine (5) To a solution of 2 (200 mg, 0.56 mmol) in MeOH (5 ml) was added HCl (10%, 0.1 ml) and the reaction mixture was stirred for 48 h at 0°C. Then the mixture was neutralized with resin IR-45 and the solvent was removed under reduced pressure. The remaining residue was purified by flash chromatography on Si gel with CHCl₃/MeOH (99:1) to give compound 5 (60 mg, 29%) and 4 (60 mg, 29%). ¹H-NMR (CDCl₃, 200 MHz): δ 8.36 (1H, dd, J = 8, 1.5 Hz, H-8), 7.62 (1H, td, J = 8, 1.5 Hz, H-10), 7.39 (1H, J = 8, 1.5 Hz, H-11), 7.24 (1H, J = 8, 1.5 Hz, H-9), 6.26 (1H, H-7, H-1), 4.97 (1H, J = 8, 1.5 Hz, H-9), 3.98 (3H, s, MeO), 3.82 (1H, J = 8, 1.5 Hz, H-2), 3.81 (3H, s, NMe), 2.75 (3H, s, OMe-C₁), 1.59 (3H, s, Me-1), 1.43 (3H, s, Me-4). ¹³C-NMR (CDCl₃, 50 MHz): δ 177.42 (C-7), 162.53 (C-6), 160.08 (C-4a), 148.21 (C-12a), 144.04 (C-11a), 132.48 (C-10), 126.39 (C-5), 125.17 (C-1a), 126.61 (C-9), 115.82 (C-11), 110.77 (C-6a), 99.17 (C-12b), 94.31 (C-5), 78.18 (C-3), 76.11 (C-11), 69.93 (C-5), 52.69 (OMe-C₆), 49.92 (OMe-C₁), 41.83 (Me), 26.60 (Me), 17.48 (Me), 14.04 (Me).

(±)-cis-2-Hydroxy-1-methoxy-1,2-dihydroacronycine (4) ¹H-NMR (CDCl₃, 200 MHz): δ 8.36 (1H, dd, J = 8, 1.5 Hz, H-8), 7.62 (1H, td, J = 8, 1.5 Hz, H-10), 7.39 (1H, J = 8, 1.5 Hz, H-11), 7.24 (1H, J = 8, 1.5 Hz, H-9), 6.26 (1H, H-7, H-1), 4.97 (1H, J = 8, 1.5 Hz, H-9), 3.98 (3H, s, MeO), 3.82 (1H, J = 8, 1.5 Hz, H-2), 3.81 (3H, s, NMe), 2.75 (3H, s, OMe-C₁), 1.59 (3H, s, Me-1), 1.43 (3H, s, Me-4). ¹³C-NMR (CDCl₃, 50 MHz): δ 177.43 (C=7), 162.54 (C=6), 160.09 (C=4a), 148.21 (C=12a), 144.04 (C=11a), 132.48 (C=10), 126.39 (C=5), 125.17 (C=1a), 126.61 (C=9), 115.82 (C=11), 110.77 (C=6a), 99.17 (C=12b), 94.31 (C=5), 78.18 (C=3), 76.11 (C=11), 69.93 (C=5), 52.69 (OMe-C₆), 49.92 (OMe-C₁), 41.83 (Me), 26.60 (Me), 17.48 (Me), 14.04 (Me). MS-DCI m/z: 370 (M⁺). ¹H-NMR (CDCl₃, 200 MHz): δ 8.36 (1H, dd, J = 8, 1.5 Hz, H-8), 7.62 (1H, td, J = 8, 1.5 Hz, H-10), 7.39 (1H, J = 8, 1.5 Hz, H-11), 7.24 (1H, J = 8, 1.5 Hz, H-9), 6.26 (1H, H-7, H-1), 4.97 (1H, J = 8, 1.5 Hz, H-9), 3.98 (3H, s, MeO), 3.82 (1H, J = 8, 1.5 Hz, H-2), 3.81 (3H, s, NMe), 2.75 (3H, s, OMe-C₁), 1.59 (3H, s, Me-1), 1.43 (3H, s, Me-4). ¹³C-NMR (CDCl₃, 50 MHz): δ 177.71 (C=7), 162.53 (C=6), 160.49 (C=4a), 149.49 (C=12a), 144.19 (C=11a), 132.47 (C=10), 127.11 (C=5), 125.25 (C=1a), 126.71 (C=9), 115.41 (C=11), 111.64 (C=6a), 98.14 (C=12b), 94.17 (C=5), 76.57 (C=3), 74.40 (C=10), 66.65 (C=2), 56.73 (OMe-C₆), 55.45 (OMe-C₁), 41.31 (Me), 25.14 (Me), 21.73 (Me). MS-DCI m/z: 370 (M⁺).
7.00 (2H, t, J=7.8 Hz, H-3", 5") , 6.72 (2H, d, J=8 Hz, H-2", 6") , 6.35 (1H, s, H-5), 5.55 (1H, d, J=8.0 Hz, H-2), 5.28 (1H, d, J=8 Hz, H-1) , 4.20 (1H, d, J=9 Hz, CH₂-C₄H₅), 4.20 (2H, s, COCH₃), 4.02 (3H, s, OMe-C₆), 3.80 (3H, s, NMe), 3.52 (1H, d, J=9 Hz, CH₂-C₄H₅), 1.52 (3H, s, Me), 1.51 (3H, s, Me). ¹³C-NMR (CDCl₃, 50 MHz) : 177.59 (C-7), 166.59 (COCH₃), 162.90 (C-6), 159.62 (C-4a), 148.17 (C-12a), 144.55 (C-11a), 136.66 (C-11), 132.55 (C-10), 128.60 (C-2", 2'), 128.09 (C-3", 3'), 7.126 (C-1, 2₁, 2₂, 2₃, 2₄), 126.83 (C-8), 126.50 (C-7a), 121.95 (C-9), 116.14 (C-11), 111.00 (C-6a), 98.83 (12b), 94.37 (C-5), 97.76 (C-3), 73.54 (C-2), 72.51 (C-2), 65.38 (CH₂-C₄H₅), 56.31 (OMe-C₆), 41.96 (NMe), 40.73 (COCH₃), 26.26 (Me), 21.04 (COCH₃), 18.53 (Me). MS-DCI m/z: 512 (M⁺).

(±)-trans-2-Hydroxy-1-methylenamino-1,2-dihydroacronycine (16)
To a solution of 3 (62 mg, 0.14 mmol) in EtOH (2 ml) was added CH₃NH₂ (0.25 ml, 40% soln. in water). The reaction mixture was stirred for 16 h at 80 °C and then the reagents were removed under reduced pressure. The remaining residue was purified by flash chromatography on Si gel with CH₃Cl- MeOH (99:1) to give compound 16 (20 mg, 38%) and 14 (6 mg, 10%). ¹H-NMR (CDCl₃, 200 MHz) : δ: 8.32 (1H, dd, J=8, 1.5 Hz, H-8), 7.60 (1H, td, J=8, 1.5 Hz, H-10), 7.31 (1H, d, J=8 Hz, H-11), 7.22 (1H, t, J=8 Hz, H-9), 6.23 (1H, s, H-5), 4.02 (1H, d, J=8 Hz, H-2), 3.93 (3H, s, OMe-C₆), 3.69 (3H, s, NMe), 3.59 (1H, d, J=8 Hz, H-1), 1.61 (3H, s, NMe-C₁), 1.54 (3H, s, Me), 1.33 (3H, s, Me). ¹³C-NMR (CDCl₃, 50 MHz) : 177.72 (C-7), 161.82 (C-6), 159.74 (C-4a), 148.99 (C-12a), 132.71 (C-9), 127.21 (C-8), 125.53 (C-7a), 120.06 (C-9), 118.16 (C-11), 111.00 (C-6a), 100.92 (C-12b), 98.40 (C-5), 77.00 (C-3), 70.72 (C-2), 57.66 (C-11), 56.14 (OMe-C₆), 43.29 (NMe), 28.15 (NMe-C₁), 26.70 (Me), 16.98 (Me). MS-DCI m/z: 369 (M⁺).

(±)-trans-2-Acetoxy-1-methylenamino-1,2-dihydroacronycine (15)
¹H-NMR (CDCl₃, 200 MHz) : δ: 8.32 (1H, dd, J=8, 1.5 Hz, H-8), 7.61 (1H, td, J=8, 1.5 Hz, H-10), 7.23 (1H, d, J=8 Hz, H-11), 7.23 (1H, t, J=8 Hz, H-9), 6.28 (1H, s, H-5), 5.96 (1H, d, J=3 Hz, H-9), 4.11 (1H, d, J=3 Hz, H-10), 3.95 (3H, s, OMe-C₆), 3.63 (1H, dd, J=8, 3 Hz, H-1), 3.61 (3H, s, NMe), 2.71 (3H, s, OMe-C₁), 2.01 (3H, s, CH₂-C₄H₅), 1.99 (3H, s, NMe-C₁), 1.54 (3H, s, Me), 1.52 (3H, s, Me). ¹³C-NMR (CDCl₃, 50 MHz) : 177.34 (C-7), 175.51 (CH₂-C₄H₅), 162.55 (C-6), 160.44 (C-4a), 148.58 (C-12a), 144.97 (C-11a), 133.08 (C-10), 127.19 (C-8), 125.53 (C-7a), 122.75 (C-9), 116.18 (C-11), 115.69 (C-11), 110.00 (C-6a), 98.55 (C-12b), 94.99 (C-3), 78.10 (C-3), 75.38 (C-2), 56.28 (OMe-C₆), 55.71 (C-1), 42.66 (NMe), 31.34 (NMe-C₁), 26.12 (Me), 22.52 (CH₂-C₄H₅), 16.80 (Me). MS-DCI m/z: 411 (M⁺+H).

(±)-trans-2-Acetoxy-1-methylacetamido-1,2-dihydroacronycine (17)
Treatment of 16 under conditions essentially the same as those described for the preparation of afforded compound 17. ¹H-NMR (CDCl₃, 200 MHz) : δ: 8.30 (1H, dd, J=8, 1.5 Hz, H-8), 7.58 (1H, td, J=8, 1.5 Hz, H-10), 7.20 (1H, t, J=8 Hz, H-9), 6.28 (1H, s, H-5), 6.20 (1H, d, J=8 Hz, H-1), 4.93 (3H, s, H-2), 3.96 (3H, s, OMe-C₆), 3.63 (3H, s, NMe), 2.10 (3H, s, CH₂-C₄H₅), 2.03 (3H, s, NMe-C₁), 1.89 (3H, s, CH₂-C₄H₅), 1.58 (3H, s, Me), 1.41 (3H, s, Me). ¹³C-NMR (CDCl₃, 50 MHz) : 177.33 (C-7), 171.69 (CH₂-C₄H₅), 170.73 (CH₂-C₄H₅), 162.50 (C-6), 161.14 (C-4a), 148.74 (C-12a), 144.99 (C-11a), 132.96 (C-10), 127.04 (C-8), 125.44 (C-7a), 122.13 (C-9), 116.10 (C-11), 112.00 (C-6a), 99.35 (C-12b), 94.55 (C-3), 77.00 (C-3), 72.78 (C-2), 56.27 (OMe-C₆), 52.67 (C-1), 42.97 (NMe), 30.83 (NMe-C₁), 26.06 (Me), 22.60 (CH₂-C₄H₅), 20.99 (CH₂-C₄H₅), 18.15 (Me). MS-DCI m/z: 453 (M⁺+H).