Synthesis and Cytotoxic and Antitumor Activity of Esters in the 1,2-Dihydroxy-1,2-dihydroacronycine Series

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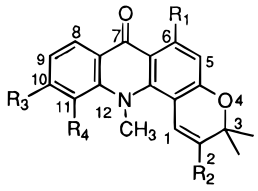
Seven 1,2-dihydroxy-1,2-dihydroacronycine and 1,2-dihydroxy-1,2-dihydro-6-demethoxyacronycine esters and diesters were synthesized *via* osmic oxidation of acronycine or 6-demethoxyacronycine followed by acylation. The 6-demethoxyacronycine derivatives were found to be inactive, whereas in contrast, all of the acronycine derivatives were more potent than acronycine itself when tested against L1210 cells *in vitro*. Four selected acronycine derivatives (**17**, **19**, **21**, and **22**) were evaluated *in vivo* against murine P388 leukemia and colon 38 adenocarcinoma implanted in mice. All compounds were markedly active against P388 at doses 4−16-fold lower than acronycine itself. Against the colon 38 adenocarcinoma, the three compounds **17**, **21**, and **22** were highly efficient. 1,2-Diacetoxy-1,2-dihydroacronycine (**17**) was the most active, all the treated mice being tumor-free on day 23.

Introduction

The acridone alkaloid acronycine (**1**), first isolated from *Acronychia baueri* (Rutaceae)[1] was found active against numerous solid tumors including sarcoma, myeloma, carcinoma, and melanoma.[2] Nevertheless, clinical trials[3] gave poor results, both due to the very low water solubility and to the moderate potency of acronycine.

Despite the broad antitumor spectrum of acronycine, the mechanism of its action at both cellular and molecular level has not yet been clearly established. Early experiments indicated that this alkaloid acted primarily by alteration of membranous organelles and that its delayed effects were due at least in part to interference with the structure and function of cell-surface components.[4] Recent *in vitro* experiments suggested that acronycine did not interact with nucleic acids, but was an effective inhibitor of DNA synthesis.[5] These results were not in agreement with a previous investigation of the DNA-binding property of acronycine by Dorr and Liddil which demonstrated that this alkaloid should interact with DNA, either by intercalation or by some other noncovalent process able to stabilize the double helix against thermal denaturation.[6]

Structure−activity relationships in the acronycine series clearly indicate that the 1,2-double bond on the pyran ring is an essential structural requirement to observe a cytotoxic activity. Indeed, 3,12-dihydro-6-[[(dimethylamino)ethyl]oxy]-3,3,12-trimethyl-7*H*-pyrano-[2,3-*c*]acridin-7-one (**2**),[7] 6-demethoxyacronycine (**3**),[8] 2-nitroacronycine (**4**),[5] 11-methoxyacronycine (**5**),[9] and 10,11-dimethoxyacronycine (**6**)[9] exhibit cytotoxic and/or antitumor activities whereas 1,2-dihydroacronycine (**7**)[2a] and 1,2-dihydro-2-nitroacronycine (**8**)[2a] are inactive compounds.



1 : Acronycine $R_1 = OCH_3$; $R_2 = H$; $R_3 = H$; $R_4 = H$

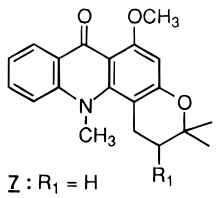
2 : $R_1 = OCH_2CH_2N(CH_3)_2$; $R_2 = H$; $R_3 = H$; $R_4 = H$

3 : $R_1 = H$; $R_2 = H$; $R_3 = H$; $R_4 = H$

4 : $R_1 = OCH_3$; $R_2 = NO_2$; $R_3 = H$; $R_4 = H$

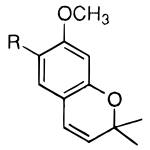
5 : $R_1 = OCH_3$; $R_2 = H$; $R_3 = H$; $R_4 = OCH_3$

6 : $R_1 = OCH_3$; $R_2 = H$; $R_3 = OCH_3$; $R_4 = OCH_3$



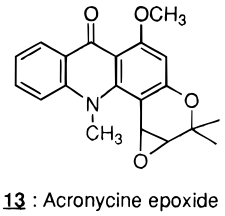
7 : $R_1 = H$

8 : $R_1 = NO_2$

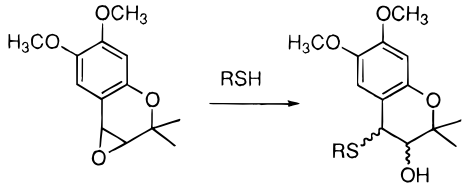


9 : Precocene I (R = H)

10 : Precocene II (R = OCH_3)



13 : Acronycine epoxide



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These observations led us to remark a striking similarity between acronycine derivatives and the insecticidal chromenes precocenes, as far as alteration of membranes at the subcellular level and structure–activity relationships were concerned.[10] Actually, precocenes I (**9**) and II (**10**) act on the mitochondrial and nuclear membranes of the *corpora allata* of sensitive insects.[11] The pyran double bond plays a crucial role in the insecticidal activity of these compounds since the corresponding epoxides, formed *in situ* through cytochrome P-450 mediated oxidation, are the active metabolites of precocenes.[12] Those extremely reactive epoxides are responsible for the alkylation of nucleophiles present in biological matrices such as cellular proteins and ultimately for irreversible damages and cell death.[13] From a chemical point of view, the alkylating properties of epoxyprecocene II (**11**) toward nucleophilic agents were unambiguously demonstrated through its reaction with various thiols, leading to adducts (**12**) with the sulfur atom linked to the benzylic position of the benzopyranyl skeleton.[14]

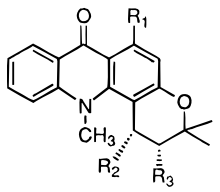
The recent isolation in our laboratory of acronycine epoxide (**13**) from several New-Caledonian *Sarcomelicope* species led us to consider this compound as a possible active metabolite of acronycine *in vivo*.[10] The high reactivity of that epoxide and the difficulties encountered by us[15] and by others[16] in the attempts toward its synthesis are consistent with this hypothesis. In addition, this assumption should explain the importance of the 1,2-double bond on the pyran ring to observe cytotoxic activty in the acronycine series. Nevertheless, the great chemical unstability of acronycine epoxide and its fast reaction with water to yield the corresponding diols[15] exclude its possible use as an anticancer agent. With the goal of finding new candidates having better stability than and, to a lesser extent, similar reactivity toward nucleophilic agents as acronycine epoxide we synthesized and evaluated the biological activities of various *cis*-1,2-dihydroxy-1,2-dihydroacronycine and *cis*-1,2-dihydroxy-1,2-dihydro-6-demethoxyacronycine esters.

Chemistry

cis-1,2-Dihydroxy-1,2-dihydroacronycine had been previously obtained by oxidation of acronycine with 1 equiv of osmium tetroxide in pyridine.[17] This reaction was difficult to apply for a large-scale preparation of **14**, due to the cost and toxicity of osmium tetroxide and to the difficulties encountered during the workup under such conditions. *Cis*-diols **14** and **15** were therefore more conveniently obtained by catalytic osmium oxidation of **1** and **3**, respectively, using *N*-methylmorpholine *N*-oxide to regenerate the oxidizing agent.

Treatment of *cis*-diols **14** and **15** with excess acetic anhydride in pyridine afforded the corresponding diesters **17** and **18**, respectively. When 1 equiv of anhydride was used, monoesters at the less hindered 2-position exemplified by acetate **16** and benzoate **21** were obtained. Treatment of monobenzoate **21** with excess acetic anhydride led to the mixed ester **22**. Finaly treatment of diols **14** and **15** with *N*,*N*′-carbonyldiimidazole in 2-butanone under reflux yielded cyclic carbonates **19** and **20**, respectively.

In order to ensure that the reactivity of 1,2-dihydroxy-1,2-dihydroacronycine diesters toward nucleophilic agents



Relative configuration

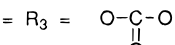
14 : $R_1 = OCH_3$; $R_2 = OH$; $R_3 = OH$

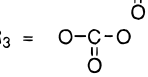
15 : $R_1 = H$; $R_2 = OH$; $R_3 = OH$

16 : $R_1 = OCH_3$; $R_2 = OH$; $R_3 = OCOCH_3$

17 : $R_1 = OCH_3$; $R_2 = OCOCH_3$; $R_3 = OCOCH_3$

18 : $R_1 = H$; $R_2 = OCOCH_3$; $R_3 = OCOCH_3$

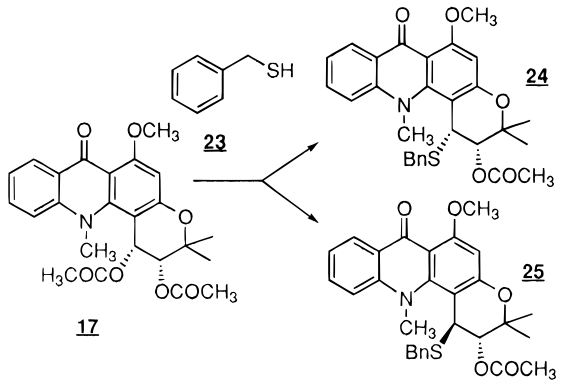
19 : $R_1 = OCH_3$; $R_2 = R_3 =$ 

20 : $R_1 = H$; $R_2 = R_3 =$ 

21 : $R_1 = OCH_3$; $R_2 = OH$; $R_3 = OCOC_6H_5$

22: $R_1 = OCH_3$; $R_2 = OCOCH_3$; $R_3 = OCOC_6H_5$

was similar with that of acronycine and precocene epoxides, compound **17** was allowed to react in acidic medium with benzyl mercaptan (**23**). *Cis* and *trans*



adducts **24** and **25** with the sulfur atom linked to the benzylic position of the pyranyl ring were obtained in almost quantitative yield within 24 h.

Results and Discussion

The study of the biological properties of the new acronycine derivatives was carried out first *in vitro* on L1210 leukemia. The results (IC_{50}) are reported in Table 1. Compounds **16**, **17**, **21**, and **22** were slightly more potent (3–5-fold) than acronycine, while the most cytotoxic derivative, compound **19**, was 75-fold more potent than acronycine in inhibiting L1210 cell proliferation. All these cytotoxic compounds bear a methoxy group at position 6 and at least one ester at position 2. The presence of the methoxy group markedly increased the cytotoxicity of the derivatives, as shown by **19** which is >100-fold more potent than **20**. The presence of two esters instead of two hydroxyl groups in the 1 and 2 positions also increased the cytotoxicity, but to a lesser extent (compare **14** and **17**).

The perturbation of the cell cycle induced by these compounds was studied on the same cell line. Acronycine induced a partial accumulation of cells in the

Table 1. Cytotoxicity and Antitumor Activity of the Compounds

		Antitumor activity	
compd	cytotoxicity IC_{50} L1210 (μM)[a]	P388 leukemia[b] dose,[d] T/C, % (survival)	colon 38 adenocarcinoma[c] dosed,[d] T/C, % (tumor volume)
1	24.1 ± 1.5	200 mg/kg ip, 125	200 mg/kg ip, 4
3	29.7 ± 4.6	NT	NT
7	34.4 ± 9.0	NT	NT
14	80.6 ± 19.5	NT	NT
15	>50	NT	NT
16	8.9 ± 1.6	NT	NT
17	5.8 ± 0.8	25 mg/kg ip, 289 25 mg/kg iv, 220	12.5 mg/kg ip, 0
18	>50	NT	NT
19	0.32 ± 0.08	12.5 mg/kg ip, 202	6.25 mg/kg ip, 68
20	>50	NT	NT
21	7.1 ± 0.4	12.5 mg/kg ip, 258	6.25 mg/kg ip, 18
22	5.0 ± 0.2	50 mg/kg ip, 201	12.5 mg/kg ip, 13

[a] Inhibition of L1210 cell proliferation measured by the MTT assay (mean ± standard error mean of at least 3 values obtained in independent experiments). NT: not tested. [b] Mice were inoculated ip on day 0 with 10^6 P388 cells and the compounds were administered ip or iv on day 1. [c] Tumor fragments were implanted sc on day 0, and the compounds were administered ip on days 2 and 9. The tumor volume was measured on day 23. [d] Dose (mg/kg) giving the optimal therapeutic effect without toxicity.

G2+M phase of the cell cycle (Figure 1), as already described.[18] In contrast, the cytotoxic derivatives modified the DNA distribution in a different manner, inducing a marked accumulation of cells in the S phase: 80% of the cells were blocked in this phase after 21 h of exposure. Moreover, a good relation was found in this series between cytotoxicity and potency in accumulating cells in the S phase, **19** being the most potent (not shown). This relationship suggests that the cytotoxicity is the consequence of the accumulation of cells in the S phase. Interestingly, the fact that these compounds induced a perturbation of the cell cycle different from that of acronycine suggests some differences in their mechanism of action at the molecular level. The modification of the DNA distribution induced by our compounds is similar to that observed with inhibitors of nucleic acid synthesis. *In vivo*, we used two standard experimental models, the ip P388 leukemia and the sc colon 38 adenocarcinoma. Table 1 shows the results, in terms of percent T/C (survival or tumor volume) obtained at the optimal dosage, i.e. the dose giving the best therapeutic effect without toxicity. Against P388 leukemia, acronycine was moderately active, while compounds **17**, **19**, **21**, and **22** were markedly active, and at doses 4−16-fold lower. Compound **17** administered by the iv route was slightly less active than by the ip route (T/C 289%), against this ip tumor, inducing a T/C of 220%. This result demonstrates a good distribution of this compound. Against the colon 38 adenocarcinoma, compounds **17**, **21**, and **22** were highly efficient, inhibiting by more than 82% the tumor growth. The derivative **17** was the most active, all the treated mice being tumor-free on day 23. As previously described,[2c] acronycine was also markedly active on this model, but less active than **17** and at a 16-fold higher dose.

In conclusion, for the first time since the discovery of acronycine, compounds more active *in vivo* than acronycine itself as antitumor drugs have been synthesized. Indeed, 1,2-dihydroxy-1,2-dihydroacronycine diesters exhibit promising antitumor properties, with a broadened spectrum of activity and an increased potency

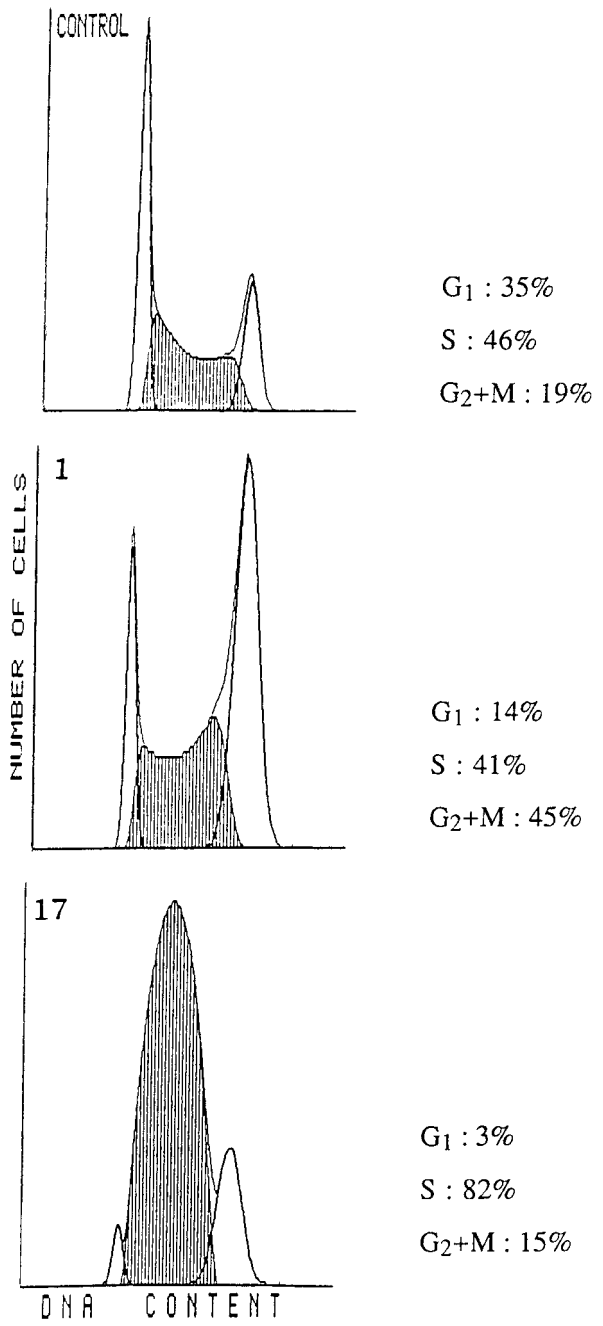


Figure 1. Modification of the typical DNA histogram and distribution into the different phases of the cell cycle of L1210 cells treated for 21 h with 50 μM compound **1** or 10 μM compound **17**.

when compared with acronycine on several tumor strains *in vitro* and *in vivo*. *cis*-1,2-Diacetoxy-1,2-dihydroacronycine seems of particular interest in this respect, due to its high activity *in vivo* against P388 leukemia and against the highly resistant solid tumor C-38 colon carcinoma.

Experimental Section

Chemistry. Mass spectra were recorded with a Nermag R-10-10C spectrometer using electron impact (EIMS) and/or chemical ionization (CIMS; reagent gas, NH_3) techniques. UV spectra (λ_{max} in nm) were determined in spectroscopic grade MeOH on a Beckman Model 34 spectrophotometer. IR spectra (ν_{max} in cm^{-1}) were obtained in potassium bromide pellets on a Perkin-Elmer 257 instrument. ^{1}H-NMR (δ [ppm], J [Hz]) and ^{13}C-NMR spectra were recorded at 300 and 75 MHz, respectively, using a Bruker AC-300 spectrometer. Column

chromatographies were conducted using flash silica gel 60 Merck (40−63 μm) with an overpressure of 300 mbars.

Biological Materials. Cell Culture and Cytotoxicity. L1210 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.[19] Cells were exposed to graded concentrations of drug (nine serial dilutions in triplicate) for 48 h. Results are expressed as IC_{50}, the concentration which reduced by 50% the optical density of treated cells with respect to the density of untreated controls.

For the cell cycle analysis, L1210 cells (5×10^5 cells/mL) were incubated for 21 h with various concentrations of drugs. Cells were then fixed by 70% ethanol (v/v), washed, and incubated in PBS containing 100 μg/mL RNAse and 25 μg/mL propidium iodide for 30 min at 20 °C. For each sample, 10 000 cells were analyzed on an ATC3000 flow cytometer (Bruker, Wissembourg, France).

Antitumor Activity. The antitumor activity of the compounds was evaluated on two experimental murine models: the P388 leukemia and the colon 38 adenocarcinoma. P388 cells (NCI, Frederick) were inoculated ip (10^6 cells/mouse) into B6D2F1 mice (Iffa credo) on day 0. The drugs were dissolved in 1% tween 80 in water and injected ip or iv on day 1. The results are expressed in terms of percent T/C (median survival time of treated animals divided by median survival time of controls, ×100). The colon adenocarcinoma 38 (NCI, Frederick) was introduced by sc implantation of a tumor fragment into the dorsal flank. The drugs were administered by ip injection on days 2 and 9. The tumor volume was measured on day 23, and the results are expressed as percent T/C (median tumor volume in treated animals divided by median tumor volume of controls, ×100).

(±)-*cis*-1,2-Dihydroxy-1,2-dihydroacronycine (14). To a solution of osmium tetroxide (2.5% in 2-methyl-2-propanol) (6.4 mL) and *N*-methylmorpholine *N*-oxide dihydrate (1.68 g, 11 mmol) in *t*-BuOH−THF−H_2O (10:3:1, 45 mL) was added acronycine (**1**) (3.21 g, 10 mmol). The reaction mixture was stirred at room temperature for 2 days. Saturated aqueous $NaHSO_3$ was added, and the mixture was stirred at room temperature for 1 h and extracted with CH_2Cl_2 (5 × 80 mL) to give a yellow solid (4 g) which was purified by flash chromatography. Elution with CH_2Cl_2/MeOH, 98:2, gave unreacted **1** (0.7 g), while further elution with CH_2Cl_2/MeOH, 95:5, provided **14** (2.66 g, 75%).

(±)-*cis*-1,2-Dihydroxy-1,2-dihydro-6-demethoxyacronycine (15). Dihydroxylation of 6-demethoxyacronycine (**3**) (0.582 g, 2 mmol) in conditions essentially similar to these described for the preparation of **14** afforded **15** (0.422 g, 65%): ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.39, 1.44 (2s, 2 × 3H), 3.68 (t, J = 5 Hz, 1H, → d, J = 5 Hz on D_2O addition, H-C2), 3.94 (s, 3H), 4.75 (d, J = 9 Hz, 1H exchange D_2O, HO-C1), 5.11 (d, J = 5 Hz, 1H exchange D_2O, HO-C2), 5.19 (dd, J = 9, 5 Hz, 1H, → d, J = 5 Hz on D_2O addition, H-C1), 6.67 (d, J = 9 Hz, 1H, H-C5), 7.26 (td, J = 8, 1 Hz, 1H), 7.65 (dd, J = 8, 1 Hz, 1H), 7.73 (td, J = 8, 2 Hz, 1H), 8.06 (d, J = 9 Hz, 1H), 8.16 (dd, J = 8, 2 Hz, 1H); ^{13}C-NMR (75 MHz, DMSO-d_6) 23.8, 27.0, 42.9, 66.1, 71.7, 79,7, 112.2, 115.0, 119.3, 120.4, 123.6, 123.9, 127.8, 129.3, 135.9, 147.2, 148.6, 161.2, 178.9; EIMS m/z 325 ($M^{\bullet+}$), 307, 251, 236; IR (KBr) 3450, 3290, 3000, 2985, 1605, 770, 650; UV λ nm (MeOH) (log ϵ) 259 (4.61), 275 (4.55), 282 (4.57), 325 (3.86), 392 (3.94). Anal. ($C_{19}H_{19}NO_4$) C, H, N.

(±)-*cis*-1-Hydroxy-2-acetoxy-1,2-dihydroacronycine (16). To a solution of **14** (0.100 g, 0.28 mmol) in dry pyridine (2 mL) was added 1 equiv of acetic anhydride (0.013 mL, 0.28 mmol). The reaction mixture was stirred at room temperature during 24 h and then diluted with water. The precipitate was filtered, washed with water, and dried *in vacuo* under P_2O_5. The obtained residue was chromatographed on a silica gel (40−60 μm) column (eluent CH_2Cl_2/MeOH, 99:1, v/v) to provide **16** (0.062g, 81%): ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.50, 1.55 (2s, 2 × 3H), 2.15 (s, 3H), 2.85 (s, 1H exchange D_2O, HO-C1), 3.82 (s, 3H), 3.83 (s, 3H), 5.32 (d, J = 6 Hz, 1H), 5.40 (d, J = 6 Hz, 1H), 6.07 (s, 1H), 7.15 (td, J = 8, 1 Hz, 1H), 7.31 (dd, J = 8, 1 Hz, 1H), 7.57 (td, J = 8, 2 Hz, 1H), 8.25 (dd, J = 8, 2 Hz, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$) 20.8, 22.3, 25.3, 41.7, 55.8, 63.7, 71.8, 76.4, 93.9, 100.7, 115.9 (C-11, C-6a), 121.5, 125.1, 126.9, 132.5, 144.5, 148.8, 158.7, 162.1, 171.0, 177.7; CIMS m/z 398 $(M + H)^+$; IR (KBr) 3350, 3010, 2990, 1730, 1620, 1395, 1210, 775; UV λ nm (MeOH) (log ϵ) 229 (4.07), 275 (4.53), 298 (3.85), 384 (3.76). Anal. ($C_{22}H_{23}NO_6$) C, H, N.

(±)-*cis*-1,2-Diacetoxy-1,2-dihydroacronycine (17). In a typical experiment, a cooled mixture of acetic anhydride (5 mL, 50 mmol) and dry pyridine (5 mL) was added to **14** (1.775 g, 5 mmol). The reaction mixture was first stirred at room temperature for 24 h and after was poured on cold H_2O (50 mL). The obtained precipitate was filtered, washed with water, and dried *in vacuo* under P_2O_5 to afford **17** (2.034 g, 92%): ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.47, 1.56 (2s, 2 × 3H), 1.97 (s, 3H), 2.04 (s, 3H), 3.63 (s, 3H), 4.00 (s, 3H), 5.48 (d, J = 5 Hz, 1H), 6.31 (s, 1H), 6.55 (d, J = 5 Hz, 1H), 7.26 (m, 2H), 7.64 (dd, J = 8, 1 Hz, 1H), 8.35 (dd, J = 8, 2 Hz, 1H); ^{13}C-NMR (75 MHz, $CDCl_3$) 20.6, 20.9, 23.4, 24.4, 42.4, 56.2, 65.7, 69.3, 76.2, 94.7, 97.5, 111.9, 115.7, 121.9, 125.6, 126.9, 132.8, 144.9, 149.3, 159.8, 162.7, 165.3, 170.9, 177.4; EIMS m/z 429 ($M^{\bullet+}$); IR (KBr) 3000, 2950, 2870, 1752, 1639, 1590, 1245, 1160, 772; UV λ nm (MeOH) (log ϵ) 228 (4.23), 274 (4.67), 298 (3.96), 3,80 (3.89). Anal. ($C_{24}H_{25}NO_7$) C, H, N.

(±)-*cis*-1,2-Diacetoxy-1,2-dihydro-6-demethoxyacronycine (18). Diacetylation of **15** (0.072 g, 0.22 mmol) in conditions essentially similar to those described for the preparation of **17** afforded **18** (0.082 g, 91%): ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.49, 1.59 (2s, 2 × 3H), 2.03 (s, 6H), 3.71 (s, 3H), 5.49 (d, 1H, J = 5 Hz), 6.63 (d, 1H, J = 5 Hz), 6.86 (d, 1H, J = 9 Hz), 7.29 (td, 1H, J = 8, 1 Hz), 7.35 (dd, 1H, J = 8, 1 Hz), 7.70 (td, 1H, J = 8, 2 Hz), 8.40 (dd, 1H, J = 8, 2 Hz), 8.41 (d, 1H, J = 9 Hz); ^{13}C-NMR (75 MHz, $CDCl_3$) 20.6, 20.9, 23.3, 24.5, 41.7, 65.9, 69.1, 76.0, 104.3, 113.5, 116.5, 119.9, 121.9, 123.5, 127.0, 129.6, 133.5, 146.0, 146.6, 159.6, 170.5, 170.8, 177.5; EIMS m/z 409 ($M^{\bullet+}$) 308, 290, 236; IR (KBr) 3010, 3000, 2970, 1738, 1625, 1595, 1230, 765 cm^{-1}; UV λ nm (MeOH) (log ϵ) 388 (3.85), 319 (3.91), 280 (4.53), 271 (4.57), 258 (4.58), 226 (4.26), 210 (4.29). Anal. ($C_{23}H_{23}NO_6$) C, H, N.

(±)-*cis*-1,2-Di-*O*-carbonyl-1,2-dihydroxy-1,2-dihydroacronycine (19). *N*,*N*′-Carbonyldiimidazole (1.62 g, 10 mmol) was added to a solution of **14** (0.710 g, 2 mmol) in 2-butanone (50 mL). The reaction mixture was refluxed for 3 h under argon, and then 5% aqueous $NaHCO_3$ (60 mL) was added. The solution was extracted with EtOAc (3 × 40 mL), and the organic layers were separated, dried over anhhydrous Na_2SO_4, and evaporated under reduced pressure. Crystallization overnight from CH_2Cl_2 gave **19** (0.5 g, 65%) as light-yellow needles: mp 197 °C (CH_2Cl_2); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.45, 1.63 (2s, 2 × 3H), 3.89 (s, 3H), 3.99 (s, 3H), 4.81 (d, 1H, J = 8 Hz), 6.28 (d, 1H, J = 8 Hz), 6.30 (s, 1H), 7.26 (td, 1H, J = 8, 1 Hz), 7.35 (dd, 1H, J = 8, 1 Hz), 7.43 (td, 1H, J = 8, 2 Hz), 8.29 (dd, 1H, J = 8, 2 Hz); ^{13}C-NMR (75 MHz, $CDCl_3$) 21.9, 24.3, 43.8, 56.4, 71.0, 74.0, 78.8, 95.5, 97.3, 112.5, 116.4, 122.5, 126.1, 126.7, 132.9, 145.0, 148.7, 153.5, 159.3, 163.7, 177.7; CIMS m/z 382 $(M + H)^+$, 338, 322; IR (KBr) 3015, 3000, 2990, 1805, 1635, 1610, 1590, 770; UV λ nm (MeOH) (log ϵ) 227 (4.18), 273 (4.62), 296 (3.85), 377 (3.86). Anal. ($C_{21}H_{19}NO_6$) C, H, N.

(±)-*cis*-1,2-Di-*O*-carbonyl-1,2-dihydroxy-1,2-dihydro-6-demethoxyacronycine (20). Treatment of **15** (0.05 g, 0.15 mmol) in conditions essentially similar to those described for the preparation of **19** afforded **20** (0.034 g, 61%): ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.45, 1.65 (2s, 2 × 3H), 4.00 (s, 3H), 4.86 (d, 1H, J = 8 Hz), 6.39 (d, 1H, J = 8 Hz), 6.87 (d, 1H, J = 9 Hz), 7.32 (td, 1H, J = 8, 1 Hz), 7.47 (dd, 1H, J = 8, 1 Hz), 7.72 (td, 1H, J = 8, 2 Hz), 8.39 (dd, 1H, J = 8, 2 Hz), 8.48 (d, 1H, J = 9 Hz); ^{13}C-NMR (75 MHz, $CDCl_3$) 21.9, 25.5, 43.6, 71.8, 75.3, 79.4, 106.6, 114.5, 119.0, 120.4, 123.4, 123.7, 126.8, 131.0, 135.0, 148.8, 148.9, 154.5, 159.4, 177.2; CIMS m/z 352 $(M + H)^+$, 310, 308, 292; IR (KBr) 2920, 1795, 1595, 1340, 1215, 1030, 775; UV λ nm (MeOH) (log ϵ) 388 (3.68), 266 (4.43), 224 (4.14), 209 (4.13). Anal. ($C_{20}H_{17}NO_5$) C, H, N.

(±)-*cis*-1-Hydroxy-2-(benzoyloxy)-1,2-dihydroacronycine (21). To a solution of **14** (0.178 g, 0.5 mmol) in dry pyridine (3 mL) was added benzoic anhydride (0.125 g, 0.5 mmol). The reaction mixture was stirred at room temperature

during 36 h and then evaporated under reduced pressure (T < 40 °C). The residue was chromatographed on a silica gel column (20−40 μm) (eluent: ethyl acetate/toluene, 70:30, v/v) and **21** (0.092 g, 40%) was obtained: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.44, 1.54 (2s, 2 × 3H); 2.20 (br s, 1H), 3.83 (s, 3H), 3.96 (s, 3H), 5.37 (m → d J = 5 Hz on exchange D_2O, H-C1), 5.56 (d, 1H, J = 5 Hz), 6.24 (s, 1H), 7.13 (t, 1H, J = 8 Hz), 7.25 (m, 3H), 7.42 (td, 1H, J = 8, 2 Hz), 7.50 (td, 1H, J = 8, 2 Hz), 7.79 (dd, 2H, J = 8, 2 Hz), 8.24 (dd, 1H, J = 8, 2 Hz); ^{13}C-NMR (75 MHz, $CDCl_3$) 22.5, 25.3, 41.7, 56.1, 64.1, 72.5, 76.5, 93.9, 101.5, 111.7, 115.9, 121.6, 125.3, 126.7, 128.4 (C-2′ + C-6′), 128.8, 129.7 (C-3′ + C-5′), 132.6, 133.4, 144.5, 148.6, 159.0, 162.3, 166.1, 177.4; EIMS m/z 459 ($M^{\bullet+}$), 441, 337, 308, 122, 105, 77; IR (KBr) 3350, 3100, 3000, 2950, 1720, 1630, 1600, 1270, 1220, 770, 720. Anal. ($C_{27}H_{25}NO_6$) C, H, N.

(±)-*cis*-1-Acetoxy-2-(benzoyloxy)-1,2-dihydroacronycine (22). To a cold solution of **21** (0.092 g, 0.2 mmol) in dry pyridine (2.5 mL) was added acetic anhydride (2.5 mL, 25 mmol). The reaction mixture was left overnight at room temperature and evaporated carefully at low temperature (T < 40 °C). The residue obtained was chromatographed on a silica gel column (eluent: CH_2Cl_2) and gave **22** (0.1 g, 95%): ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.45, 1.57 (2s, 2 × 3H), 1.78 (s, 3H), 3.55 (s, 3H), 3.95 (s, 3H), 5.64 (d, 1H, J = 5 Hz), 6.28 (s, 1H), 6.51 (d, 1H, J = 5 Hz), 7.10 (m, 2H), 7.25 (t, 2H, J = 8 Hz), 7.39 (tt, 1H, J = 8, 2 Hz), 7.47 (td, 1H, J = 8, 2 Hz), 7.75 (dd, 2H, J = 8, 2 Hz), 8.24 (dd, 1H, J = 8, 2 Hz); ^{13}C-NMR (75 MHz, $CDCl_3$) 21.0, 23.2, 24.9, 42.3, 56.3, 66.1, 69.6, 77.4, 94.5, 97.4, 112.0, 115.6, 122.0, 125.6, 127.0, 128.5 (C-2′ + C-6′), 129.0, 129,7 (C-3′ + C-5′), 132.9, 133.5, 144.8, 149.4, 160.0, 162.9, 165.9, 171.1, 177.5; EIMS m/z 501 ($M^{\bullet+}$) 122, 105; IR (KBr) 3100, 3005, 2980, 1730, 1630, 1600, 1285, 1225, 770, 720; UV λ nm (MeOH) (log ϵ) 229 (4.40), 274 (4.61), 298 (3.96), 380 (3.80). Anal. ($C_{29}H_{27}NO_7$) C, H, N.

(±)-*cis*-2-Acetoxy-1-(benzylthio)-1,2-dihydroacronycine (24) and (±)-*trans*-2-Acetoxy-1-(benzylthio)-1,2-dihydroacronycine (25). To a solution of **17** (0.05 g, 0.12 mmol) in methanol (2 mL) were added 0.1 mL of 1 N HCl and benzyl mercaptan (0.015 mL, 0.12 mmol). The reaction mixture was stirred at room temperature during 24 h and evaporated carefully at low temperature (T < 40 °C). Purification by flash column chromatography on silica gel (eluent: CH_2Cl_2/MeOH, 95:5, v/v) afforded **24** and **25** (0.054 g, 90% overall yield) as a 1:1 (NMR) diastereoisomeric mixture. Repeted column chromatography of the above mixture on silica gel 60H (eluent: ethyl acetate/toluene, 70:30 v/v) gave pure analytical samples of **24** and **25**.

24: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.43, 1.49, (2s, 2 × 3H), 2.28 (s, 3H), 2.68 (d, 1H, J = 12 Hz), 3.17 (d, 1H, J = 12 Hz), 3.68 (s, 3H), 3.95 (s, 3H), 4.30 (d, 1H, J = 6 Hz), 5.50 (d, 1H, J = 6 Hz), 6.35 (s, 1H), 6.48 (dd, 2H, J = 8, 1 Hz), 6.80 (dd, 2H, J = 8, 1 Hz), 6.95 (tt, 1H, J = 8, 1 Hz), 7.30 (td, 1H, J = 8, 2 Hz), 7.35 (dd, 1H, J = 8, 2 Hz), 7.63 (td, 1H, J = 8, 2 Hz), 8.50 (dd, 1H, J = 8, 2 Hz); CIMS m/z 504 $(M+H)^+$. Anal. ($C_{29}H_{29}NO_5S$) C, H, N, S.

25: ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.49, 1.58 (2s, 2 × 3H), 2.22 (s, 3H), 2.68 (d, 1H, J = 12 Hz), 3.24 (d, 1H, J = 12 Hz), 3.68 (s, 3H), 3.85 (s, 3H), 3.95 (d, 1H, J = 9 Hz), 5.28 (d, 1H, J = 9 Hz), 6.26 (s, 1H), 6.48 (dd, 2H, J = 8, 1 Hz), 6.80 (dd, 2H, J = 8, 1 Hz), 6.95 (tt, 1H, J = 8, 1 Hz), 7.30 (td, 1H, J = 8, 2 Hz), 7.35 (dd, 1H, J = 8, 2 Hz), 7.63 (td, 1H, J = 8, 2 Hz), 8.38 (dd, 1H, J = 8, 2 Hz); CIMS m/z 504 $(M + H)^+$. Anal. ($C_{29}H_{29}NO_5S$) C, H, N, S.

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