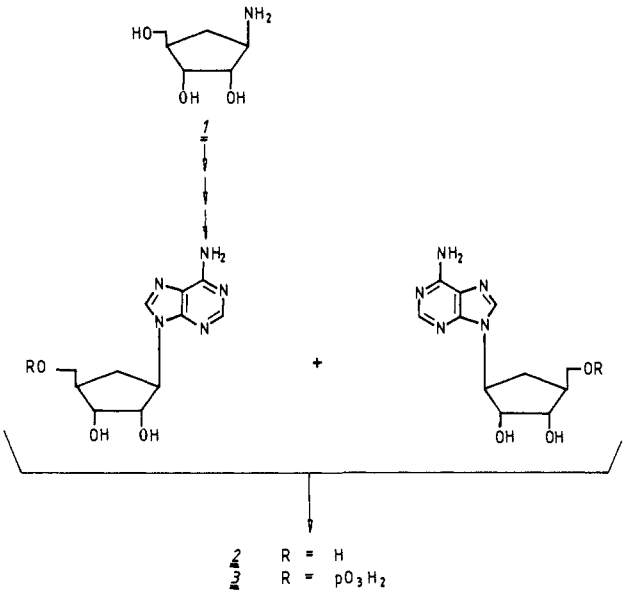
Resolution of Aristeromycin Enantiomers

Sir:

The optical enantiomers of (±)-C-Ado could efficiently and conveniently be separated after a selective enzymatic degradation of (−)-C-AMP to (−)-C-Ado in a reaction mixture containing (±)-C-AMP and 5′-ribonucleotide phosphohydrolase. (+)-C-Ado could subsequently be obtained upon treatment of the remaining (+)-C-AMP with alkaline phosphatase. Of the two enantiomers, only the (−) form showed significant cytostatic and antiviral activity. The (+) enantiomer was totally inactive.

The carbocyclic analogue of adenosine (C-Ado, **2**) was first synthesized chemically as its racemates by Shealy et al. in 1966.[1] Biological and biochemical studies of (±)-C-Ado[2] showed that it is highly cytotoxic (ED_{50} = 0.7 μM) for Hep-2 cells in culture and that it is a substrate for adenosine kinase and adenosine deaminase. Aristeromycin, one of the enantiomers of (±)-C-Ado, was reported as a natural product in 1967. It was isolated from the culture filtrate of *Streptomyces citricolor* by Kusaka et al.[3] and they demonstrated that aristeromycin displays strong inhibitory activities against some phytopathogenic bacteria and fungi. The absolute configuration was established as (1′*R*,2′*S*,3′*R*,4′*R*)-9-[β-2′α,3′α-dihydroxy-4′β-(hydroxymethyl)cyclopentyl]adenine by the same group.[4,5] Later, different synthetic routes to (±)-C-Ado were reported by other investigators,[6-8] and the racemate was resolved into its isomers by chromatography on cellulose.[9] However, the melting points of the resolved isomers[9] (120–121 and 142–144 °C) differed from that of the natural (−)-aristeromycin (213–215 °C).[4] These differences also existed for the melting points of (±)-C-Ado and (±)-carbauridine synthesized by Holý[9] (255–256 and 130–132 °C) and the melting points of the same products synthesized by Shealy et al. (241–243[10] and 176–179 °C[11]). The CD spectra of the resolved forms and the naturally occurring aristeromycin differed significantly.[9] Shealy et al.[12] compared their racemic C-Ado with the natural material obtained by Kishi et al.,[4,5] and except for the melting point and optical rotation, both products were found to be identical. Buchanan and Wightman[13] suggested that the amine 1 synthesized by Holý may have the all-cis structure. Here, we present a convenient enzymatic method for the resolution of the optical enantiomers of (±)-C-Ado. Of the two enantiomers, only the (−) form proved biologically active in inhibiting tumor cell growth and virus replication. The (+) enantiomer was totally inactive.

The carbocyclic analogue of adenosine was synthesized according to the procedure described by Shealy et al.[10] However, the aminocyclopentane derivative 1, used as the



starting material, was prepared by the stereospecific route from cyclopentadiene, developed by Vince et al.,[8,14] rather than by the much longer and low-yield norbornadiene route. The unblocked nucleoside was phosphorylated at the 5′-position according to the method described by Sowa et al.[15] However, instead of the classical but time-consuming procedure with charcoal followed by purification on a DEAE (HCO_3^-) column, the reaction mixture was neutralized with ammonia, concentrated to a small volume, applied onto a silica gel column, and eluted with $MeOH/H_2O/NH_3$ (33% 60:20:10). This chromatographic procedure gave a clean separation between the reaction products, and starting from the nucleoside, pure 5′-monophosphate **3** was obtained as its ammonium salt within 8 h (81% yield) (reaction time 4 h). Thin-layer chromatography of (±)-C-AMP (carbocyclic AMP) was performed on silica gel plates with $MeOH/H_2O/NH_3$ (33% 60:10:5) as eluent (R_f 0.51). In order to obtain a clean separation, the TLC plate should be presaturated with wet ammonia vapors (20 min) and the eluent should be renewed just before the TLC plate is developed. the diammonium salt of (±)-C-AMP, which is very hygroscopic, was converted into its sodium salt by passing an aqueous solution through a Whatman P11 (Na^+) column. The enzymatic hydrolysis of the nucleotide was performed with 5′-ribonucleotide phosphohydrolase (5′-nucleotidase) from Crotalus atrox venom (Grade IV, 70 units/mg, Sigma Chemical Co.). (±)-C-AMP (1 mmol) was dissolved in glycine buffer (0.07 M, pH 9) containing $MgCl_2$ (0.02 M), and the reaction was initiated by the addition of enzyme (1 mg) to the reaction mixture at 37 °C. Two controls were included: one without any enzyme and one with enzyme, but with AMP as substrate. Complete hydrolysis of AMP was obtained after 30 min, while approximately 5 h of incubation was necessary to release half of the carbocyclic adenosine. Longer reaction time did not change the ratio between C-Ado and C-AMP. The reaction mixture was neutralized, evaporated, and applied onto a silica gel column (50 g). Carbocyclic adenosine was eluted first (EtOAc/MeOH (5:5)), followed by carbocyclic AMP ($MeOH/H_2O/NH_3$ (33%, 75:20:10)). Fractions of carbocyclic adenosine, contaminated with glycine, were purified

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0022-2623/85/1828-1385$01.50/0

Table I. Antiviral, Cytotoxic, and Antitumor Properties of the Aristeromycin Enantiomers

	MIC_{50},[a] μg/mL				
assay syst	(±)-C-Ado	(±)-C-AMP	(−)-C-Ado	(+)-C-AMP	(+)-C-Ado
Vaccinia virus/PRK	2	2	2	>400	400
Herpes simplex-1 virus/PRK	4	7	2	200	≥200
Herpes simplex-2 virus/PRK	4	7	2	≥400	>400
Vesicular stomatitis virus/PRK	3	2	2	>400	300
cytotoxicity for PRK	10	20	4	>400	>400
Reo-1 virus/Vero	2	≥10	2	>200	>400
Parainfluenza-1 virus/Vero	3	2	2	>200	≥200
Coxsackie B4 virus/Vero	2	≥10	2	>200	>400
Sindbis virus/Vero	3	>10	2	>200	>400
Semliki forest virus/Vero	2	≥10	2	>200	>400
Measles virus/Vero	3	≥10	2	>200	≥200
cytotoxicity for Vero	4	10	4	>400	>400
Vesicular stomatitis virus/HeLa	4	7	2	>400	300
Coxsackie B4 virus/HeLa	4	>10	>4	>400	>400
Polio-1 virus/HeLa	8	>10	>4	>400	>400
cytotoxicity for HeLa	10	20	4	>400	>400
L1210 proliferation	3.0	3.1	2.7	>100	>400

[a] Minimum inhibitory concentration required to inhibit virus cytopathogenicity [in PRK (primary rabbit kidney), Vero, or HeLa cells] or murine leukemia L1210 cell growth by 50% or to cause a microscopically detectable alteration of normal cell morphology (PRK, Vero, HeLa).

by chromatography on a Dowex AGl X-4 (OH^-) column. Crystallization from MeOH yielded 0.122 g (92%) of (−)-aristeromycin:mp 214 °C (dec); $\lambda_{max}(H_2O)$ 262 nm; $[\alpha]^{24}_D$ −51.1° (*c* 0.5, DMF). Literature[4] values: mp 213–215 °C (dec); $\lambda_{max}(H_2O)$ 262 nm; $[\alpha]^{25}_D$ −52.5° (*c* 1.0, DMF). In order to compare the physical data published by Imai et al.[16] for (−)-C-AMP with those of (+)-C-AMP, obtained here, the (+)-aristeromycin monophosphate ammonium salt was applied onto a column of Dowex AGl X–4 ($HCOO^-$, 200–400 mesh). The column was washed with water, and (+)-C-AMP was eluted with 0.1 N HCOOH. The UV-absorbing fractions were collected and evaporated, yielding 133.5 mg (78%) of (+)-aristeromycin 5′-monophosphate: mp 187 °C (soften); λ_{max}(pH 2) 260.5 nm, λ_{min}(pH 2) 235 nm; $[\alpha]^{24}_D$ +34.8° (*c* 0.5, H_2O). Literature[16] values for (−)-C-AMP: mp 186–188 °C (incor); λ_{max}(0.1 N HCl) 260.5 nm, λ_{min}(0.1 N HCl) 234 nm; $[\alpha]^{24}_D$ −34.6° (*c* 1.0, H_2O). Thus, an excellent correlation was found between the natural occurring aristeromycin and the resolved enantiomers.

The (+)-aristeromycin 5′-monophosphate was then treated with calf intestinal alkaline phosphatase (Boehringer, Mannheim). The assay mixture (1 mL) contained 4 mg of (+)-C-AMP, 28 units of alkaline phosphatase, and 0.25 mM $MgCl_2$ in 50 mM Tris-HCl, pH 8.8. Following 2.5 h of incubation at 37 °C, (+)-C-AMP was totally converted to (+)-C-Ado, as demonstrated by thin-layer chromatography with *n*-BuOH/HOAc/H_2O (2:1:1) [R_f 0.11 for (+)-C-AMP as compared to R_f 0.59 for (+)-C-Ado]. Under the same reaction conditions, (±)-C-AMP was totally converted to (±)-C-Ado; (±)-C-AMP and (±)-C-Ado showed similar R_f values as (+)-C-AMP and (+)-C-Ado, respectively.

Table I presents the antiviral and antitumor properties of the carbocyclic adenosine isomers. The results are expressed as the minimum inhibitory concentrations (MIC_{50}) required to inhibit viral cytopathogenicity[17] or tumor cell proliferation[18] by 50%. Also recorded was the minimum cytotoxic concentration required to cause a microscopically detectable alteration of normal cell morphology. The racemate (±)-C-Ado showed antiviral activity and cytotoxicity at the same concentration as reported previously with a C-Ado preparation obtained from J. A. Montgomery.[17] It inhibited L1210 cell growth at a concentration of 3 μg/mL. The racemic (±)-C-AMP was biologically active at roughly the same concentrations as (±)-C-Ado. Of the two enantiomers, only (−)-C-Ado showed antitumor and antiviral activity. However, (−)-C-Ado was not selective in its antiviral activity since it inhibited virus replication at a concentration that was equal to or only slightly lower than the cytotoxic concentration. Whereas (−)-C-Ado was active at a concentration of 2–4 μg/mL, neither (+)-C-Ado nor (+)-C-AMP showed antiviral, cytotoxic, or antitumor potential at a concentration up to 200–400 μg/mL. The (±)-C-Ado obtained by enzymatic cleavage of (±)-C-AMP had similar biological activities as the chemically prepared (±)-C-Ado (data not shown). Thus, the biologically inactive product obtained upon enzymatic hydrolysis of (+)-C-AMP could be identified as (+)-C-Ado.

In conclusion, the biological activity of (±)-C-Ado and (±)-C-AMP can be attributed exclusively to the presence of (−)-C-Ado and (−)-C-AMP in the racemic mixtures.

Acknowledgment. This study was supported by the Belgian NFWO (Nationaal Fonds voor Wetenschappelijk Onderzoek). We thank Anita Van Lierde for excellent technical assistance. P.H. is a Senior Research Assistant of the Belgian NFWO.

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Received May 6, 1985